WORK/QUALITY ASSURANCE PROJECT PLAN
FOR
CHESAPEAKE BAY MAINSTEM
WATER QUALITY MONITORING PROGRAM

Prepared by
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1.0 Project Name

CHESAPEAKE BAY MAINSTEM
WATER QUALITY MONITORING PROGRAM

2.0 Project Requested By

COMMONWEALTH OF VIRGINIA
DEPARTMENT OF ENVIRONMENTAL QUALITY
PO BOX 10009
RICHMOND, VA 23240-0009

3.0 Date of Request

OCTOBER 19, 1995

4.0 Date of Project Initiation

JANUARY 1, 1996

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7.0 Project Description

Project Background

The Chesapeake Bay Mainstem Monitoring Program is part of the long-term Chesapeake Bay Monitoring Program (CBMP) which was developed from recommendations in an Environmental Protection Agency (USEPA) report entitled "A Framework for Action." The Chesapeake Bay Mainstem and Tributary Monitoring Program was initiated in 1984 to address informational gaps and duplication of effort in existing state and federal monitoring programs. This monitoring program was spatially extended to include the Elizabeth River in 1989, and beginning in 2010 Old Dominion University no longer collects data on the Elizabeth River for this program. Although the current program is contractually administered on an annual basis, it is expected to be a long-term effort with no specific completion date.

7.1 Objective and Scope Statement

Project Objectives

The general objectives of the CBP Water Quality Monitoring Program are to characterize water quality conditions in the Bay, to provide environmental managers with information and guidance for evaluating the effectiveness of nutrient reduction strategies and achievement of the goals of the multijurisdictional Chesapeake Bay Agreement, and to enable a more comprehensive understanding of water quality processes and the relationships between water quality and living resources.

7.2 Data Usage

Additional data usage which will be supported by this monitoring program include:

- To characterize the current state of the Chesapeake Bay and Bay tributaries (baseline conditions), using water quality indicators;
- To provide synoptic evaluations and to determine patterns of water quality in the Bay, including statistical determinations of temporal and spatial variability;
- To determine long-term trends or changes in key water quality parameters to measure the effectiveness of pollution control programs;
- To provide data for inclusion in and calibration of the Chesapeake Bay watershed and ecological models; and

- To integrate the information collected in all components of the monitoring program to provide a more comprehensive understanding of water quality processes and the relationships between water quality and living resources.

7.3 Monitoring Network Design and Rationale

Rationale for the Monitoring Network:

The design of the water quality monitoring program has been largely a collaborative effort between USEPA, the designated committees/subcommittees, environmental managers from the participating states and scientists from academic research institutions. Scientists from ODU are active members of a number of the USEPA committees, subcommittees, and workgroups (e.g., the Integrated Monitoring Networks Workgroup, the Data Integrity Workgroup, the Scientific and Technical Advisory Committee). Therefore, they are intimately involved with the development of the program design and have demonstrated a dedication to its close coordination. In fact, many related projects including those involving water quality trend analysis, assessments of the power and robustness of the program design, and coordination with other monitoring efforts have or are currently being conducted by the project scientists. Issues involving water quality monitoring, biological monitoring, other research programs in the Bay, or those of general technical or QA concern are identified to those committees. Likewise, any information coming out of these committees concerning future changes in monitoring design or program direction are transferred to project personnel in a like manner.

Design of the Monitoring System

Originally, water quality was monitored in the lower Bay mainstem bi-monthly from March to October and monthly from November to February. Due to funding limitations, one cruise (during March) was eliminated in 1988. One of the October cruises was eliminated in 1989 due to additional funding constraints. A monitoring refinement effort has demonstrated that a reduction to fourteen cruises per year would allow sufficient power for detecting long-term trends, as well as accommodating concerns for living resource monitoring. The 14 cruise collection regime described in RFP# 96-004-BS is the result of this refinement. It states as follows:

Fourteen (14) sampling events per year (monthly sampling with twice per month in July and August). Sampling dates shall be established by the DEQ to insure the bay-wide sampling occurs within the same reasonable time period and that water quality sampling occurs concurrently with sampling by the plankton monitoring component. All group 1 stations must be sampled within a four day period during each sampling event. Exceptions to this shall be allowed in cases where weather causes unsafe sampling conditions. The contractor shall ensure compliance with the sampling schedule through the development and use of contingency plans.

In 2004 two more sampling cruises were added to the lower Bay mainstem cruise sampling schedule, to bring the total to 16 sampling events. These additional sampling events were added to the months of June and September, so that there will be bi-monthly sampling events for June through September. These were added to help assess the 30 day mean Dissolved Oxygen criterion in the summer sampling events. In 2006 the two additional cruises were dropped due to funding limitations. In 2006 the February cruise was not completed except for station LE5.5-W, so there were two cruises completed in June for a total of 14 sampling events. In 2007 the second cruise in June was added back into the cruise sampling schedule for a total of 15 sampling events. In 2008 the second June cruise was dropped due to funding limitations. In 2010 the second July sampling was dropped and a second sampling was added to June to try to capture the onset of hypoxic conditions in the Bay. In 2012 a second sampling only collecting physicochemical profile data was added to July to capture the entire summer of hypoxic conditions in the Bay for a total of 15 sampling events. In 2013 one winter cruise (January 2014) and two summer nutrient cruises (August 2013 and June 2014) were dropped due to funding limitations. In 2014 these cruises were restored to the 2012 cruise scenario.

The WQL has monitored the lower Bay mainstem water quality stations since 1984. The eight stations are each located along one of the three main shipping channels: the Thimble Shoal Channel, extending from the confluence of the Bay and Hampton Roads Harbor to Cape Henry (Sites LE5.5, CB8.1, and CB8.1E); Chesapeake Channel, extending roughly along the Bay mainstem (Sites CB7.4, CB7.3, and CB6.4); and the North Channel, along the Eastern Shore to off Cape Charles (Sites CB7.4N, and CB7.3E). The WQL has also monitored the remaining Virginia mainstem stations a total of 14 - 16 times per year starting in 1996 as identified in RFP#96-004-BS for a total of 27 mainstem stations. In September 1996 the LE5.5 station was moved to a location 0.6 miles west of the original location, and the station name was changed to LE5.5-W. Station LE5.5 had an average depth of approximately 20 meters and the new station location LE5.5-W is approximately 7 meters. The locations of these sites are identified in Table 7.1 and shown in Figure 7.1. The site locations were determined and specified by the USEPA in their Bay segmentation scheme. For the Bay mainstem stations that have phytoplankton and picoplankton samples collected, these samples will be collected by the Water Quality field personnel.

Table 7.1 Station designation, geographic location and description for 27 Group 1 Stations.

Station Name	Latitude/ Longitude (NAD83 datum)	Location	Region Characterized/Sampling Coordination	Annual Sampling Frequency x No. of Depths Sampled
CB5.4	37.8000° 76.1750°	Deep Main Channel	Segment CB5, Upper VA Bay, Deep Segment, Depth ~26m	15x4
CB5.4W	37.8133° 76.2951°	Mouth of Great Wicomico	Segment CB5, Upper VA Bay, Shallow Segment, Depth ~5m	15x2
CB5.5	37.6919° 76.1903°	Main Channel	Segment CB5, Upper VA Bay, Deep Segment, Depth ~18m	15x4
CB6.1	37.5883° 76.1625°	Main Channel, Lower End off of Rappahannock River	Segment CB6, Upper VA Bay, Medium Depth Segment, Depth ~12m, Plankton Station	15x4
CB6.2	37.4867° 76.1567°	Central Bay	Segment CB6, Medium Depth Segment, Depth ~10m	15x4
CB6.3	37.4124° 76.1578°	Central Bay Channel (Wolftrap)	Segment CB6, Medium Depth Segment, Depth ~11m	15x4

CB6.4	37.2364° 76.2083°	Central Bay, Offshore from the York River mouth	Possible reducing substrate, Segment CB6, Medium Depth Segment, Depth ~11m, Plankton Station	15x4
CB7.1	37.6835° 75.9900°	Eastern Shore Channel	Segment CB7, Deep Segment, Depth ~21m	15x2
CB7.1N	37.7751° 75.9749°	Tangier Sound Channel	Segment CB7. Deep Segment, Depth ~24m	15x2
CB7.1S	37.5812° 76.0583°	Eastern Shore Channel	Segment CB7, Medium Depth Segment, Depth ~13m	15x2
CB7.2	37.4115° 76.0806°	Eastern Shore Channel	Segment CB7, Deep Segment, Depth ~21m	15x2
CB7.2E	37.4114° 76.0251°	Eastern Shore, Side Channel	Segment CB7, Medium Depth Segment, Depth ~13m	15x2
CB7.3	37.1167° 76.1253°	Mainstem York Spit Channel	Historical station (AESOP7070), Segment CB7, Lower Bay Channel, Medium Depth Segment, Depth ~13m	15x4
CB7.3E	37.2285° 76.0542°	Lower Eastern shore Channel area, Southern End, Off old Plantation Fl	Area that receives influx of more dense, higher salinity shelf water from North Channel, Segment CB7, Deep Segment, Depth ~17m, Plankton Station	15x2

CB7.4	36.9955° 76.0208°	Baltimore Channel at Bay Bridge, Bay Mouth	Bay/Ocean transition area at Mid Bay mouth channel, Segment CB7, Medium Depth Segment, Depth ~14m, Plankton Station	15x4
CB7.4N	37.0622° 75.9833°	North Channel at Bay Bridge, Bay Mouth	Bay/Ocean transition area, North Channel-dense, higher salinity shelf water, Segment CB7, Medium Depth Segment, Depth ~13m	15x2
CB8.1	36.9952° 76.1783°	Lower Bay between James River mouth and Thimble Shoals Channel	Thimble Shoals Channel at Lower Bay, Segment CB8, Shallow Segment, Depth ~9m	15x2
CB8.1E	36.9472° 76.0352°	Thimble Shoals Channel at Bay Bridge, Bay Mouth	Bay/Ocean transition area at lower Bay mouth channel, Segment CB8, Deep Segment, Depth ~19m	15x2
EE3.4	37.9083° 75.7917°	Pocomoke Sound	Shallow Segment, Depth ~ 4m	15x2
EE3.5	37.7964° 75.8447°	Pocomoke Sound Channel	Deep Segment, Depth ~ 20m	15x2
LE3.6	37.5969° 76.2853°	Mouth of Rappahannock	Part of Rappahannock Segmentation, Shallow Segment, Depth ~ 9m, Plankton Station	15x2
LE3.7	37.5307° 76.3071°	Mouth of Piankatank	Part of Rappahannock Segmentation, Shallow Segment, Depth ~ 7m	15x2

LE5.5-W	36.9988° 76.3135°	Mouth of James River	VIMS historical station (JA0.0), James River/Bay transition, Part of James Segmentation, Shallow Segment, Depth ~ 7m, Plankton Station From June 1984 to August 1996 this station was sampled at a site approximately 0.6 miles east of the current location and named LE5.5.	15x2
WE4.1	37.3117° 76.3467°	Mobjack Bay	Segment WE4, Shallow Segment, Depth ~ 7m	15x2
WE4.2	37.2417° 76.3867°	Mouth of York River	Segment WE4, Medium Depth Segment Depth ~13m, Plankton Station	15x2
WE4.3	37.1767° 76.3733°	Mouth of Poquoson River	Segment WE4, Shallow Segment, Depth ~ 4m	15x2
WE4.4	37.1100° 76.2933°	Mouth of Back River	Segment WE4, Shallow Segment, Depth ~ 4m	15x2

Note: 15 cruises are scheduled for July 2018 to June 2019, but nutrient samples will only be collected on 14 cruises.

The sampling schedule for the contractual period is predetermined by the USEPA and the Scientific and Technical Analysis and Reporting (STAR) committee or another CBP committee or subcommittee and is passed along to Virginia's participating monitoring laboratories through the Virginia Department of Environmental Quality (VA DEQ) Project Officer. Sampling dates are coordinated with the State of Maryland, to ensure that samples are collected within reasonably the same time period. Weather and sea conditions permitting, all field monitoring and sample collections for each cruise are completed during a specific time period. In the case of weather delays, field monitoring and sampling is attempted when weather conditions permit. If there is any delay, the VA DEQ Project Officer is notified. The VA DEQ then advises participating institutions and agencies of the desired contingency sampling schedule.

7.4 Monitoring Parameters and Frequency of Collection

Parameters Monitored

As stated in RFP #96-0040BS:

The Water Quality Monitoring Component consists of water quality sampling at twenty-seven stations within the mainstem Chesapeake Bay. The objectives are to: characterize the health of regional areas of the lower Chesapeake Bay as indicated by water quality conditions; identify temporal and spatial trends; improve understanding of the relationship between water quality and living resources, and; warn of environmental degradation by producing a database that will allow evaluations of water quality impacts due to anthropogenic factors.

The parameters for which physicochemical data are collected and chemical analysis are performed is presented in Table 7.4. More detailed field collection and sample preparation procedures are described in Section 12.0 and Appendix 2.

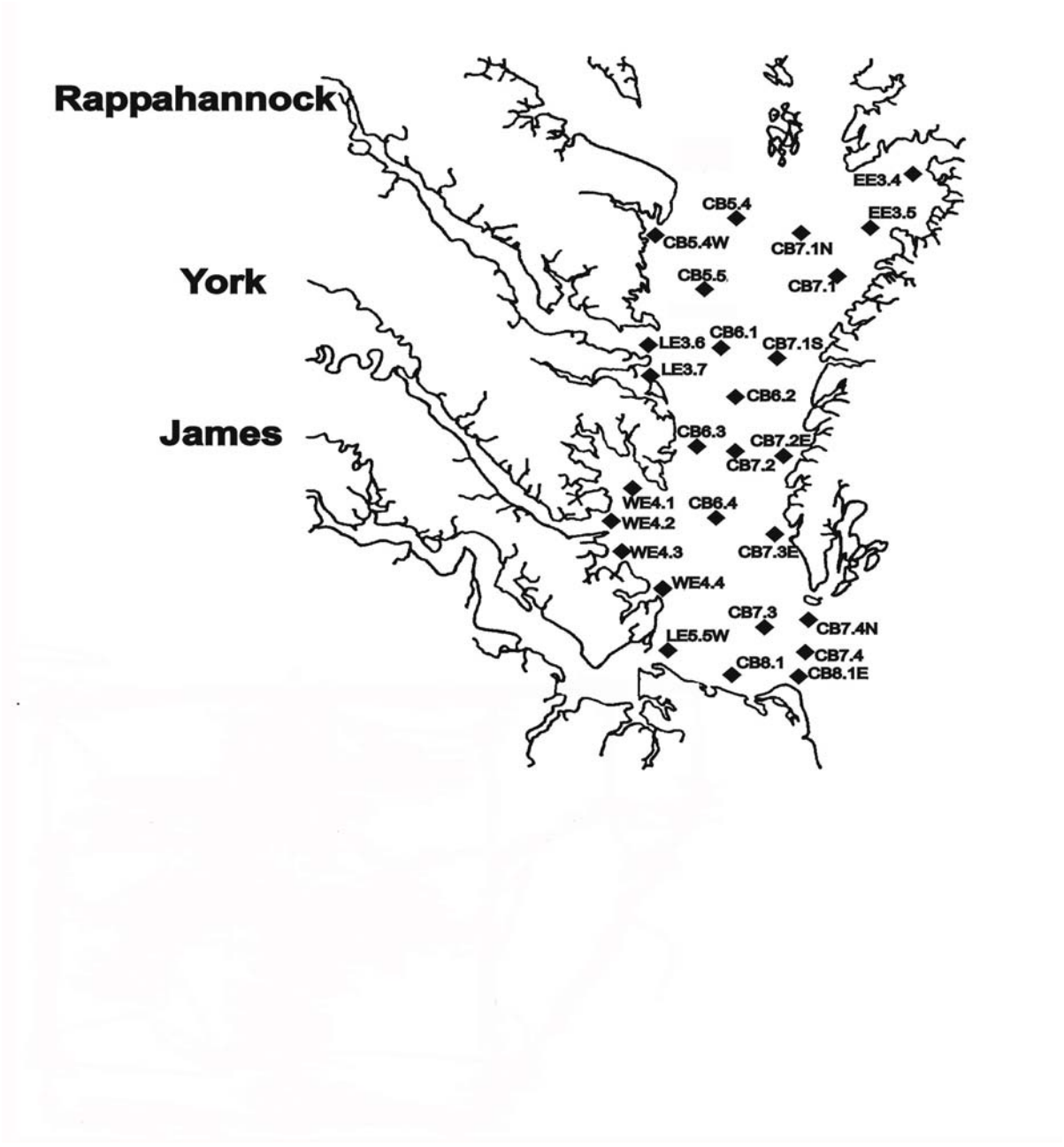


Figure 7.1 Locations of Virginia Chesapeake Bay Monitoring Program Mainstem Stations

Table 7.4 Field and Laboratory Parameters analyzed for the Chesapeake Bay Mainstem Water Quality Monitoring Program.

At every station, measure every meter until 15 meters then every 2 meters until 1 meter above bottom.

Temperature, pH, Salinity, Specific Conductance, and Dissolved Oxygen.
***Exception June - September sample every meter if a pycnocline is detected or the oxygen levels change by 1.0 mg/L or more.**

At every station:

Secchi Disk Depth: (20 cm disk)

At every station, the depths are determined using the guidelines set forth in the standard operating procedure based on the secchi depth. If there is a reading less than 1% of the surface reading with the underwater probe before reaching bottom the light profile is terminated:

Light Attenuation

At every station, from 1 m below the surface and 1 m above the bottom, except that at stations CB5.4, CB5.5, CB6.1, CB6.2, CB6.3, CB6.4, CB7.3 and CB7.4, two additional samples shall be taken, one just above and one just below the pycnocline, for each indicated parameter. Actual depth of sample shall be determined by calculations provided by VA DEQ. When a pycnocline is not detected, samples shall be collected at one third and two thirds of the depth of the water column.

***Exception one cruise in July water samples will not be collected.**

Silicate (filtered)
Particulate Carbon
Total Suspended Solids
Fixed Suspended Solids
Chlorophyll a and Phaeophytin (report at all wavelengths)
Particulate Nitrogen and **Total Dissolved Nitrogen**
Nitrate + Nitrite (filtered), Nitrite (filtered), and Ammonia (filtered)
Particulate Phosphorus, **Total Dissolved Phosphorus**,
Dissolved Orthophosphate
Dissolved Organic Carbon-surface samples only at living resources stations when they are sampled

In-Vivo Fluorescence measurements:

Vertical Profiling: At each station, a vertical profile of in-vivo fluorescence (IVF) is collected. IVF readings are taken at 1 m, 2 m, 3 m and at 3 m intervals thereafter to 1 meter above bottom. As appropriate, water from the discharge hose of the fluorometer is collected for calibration purposes. When the fluorometric probe is utilized, readings are begun at 1 meter and collected at the same depths as the probe data. When the fluorometric probe is utilized, water collected for the stations chlorophyll *a* samples are used for calibration purposes.

Horizontal Profiling: Between stations, a horizontal profile of chlorophyll fluorescence is collected from a hull pump located on the hull of the sampling vessel. The fluorescence is recorded as an average over 30 seconds on a digital recorder and/or a computer. GPS coordinates are recorded on a computer simultaneously with the fluorescence profile. Beginning station location and time is recorded as a contingency if there is a problem with the GPS computer file. As much as practical, the boat proceeds on a straight path and at a constant speed to the next station. Ending station location and time is recorded. This process is carried out between all feasible stations dependent upon weather, time, vessel capabilities and logistical constraints.

Fluorescence calibration: Calibration are performed by collecting and filtering water passing through the fluorometer and subsequently analyzing the filtered material for Chlorophyll a. Calibration samples are collected at times encompassing all fluorometer ranges encountered in the field. These procedures are contained in the Standard Operating Procedure for Chlorophyll: Spectrophotometeric Method and the Standard Operating Procedure for the Measurement of in vivo Fluorometry in Water and Seawater using a Model 10-AU Turner Fluorometer. Refer to the database manual for the procedures to calculate the horizontal and vertical fluorometry regressions.

Sea conditions and vessel size permitting, all sample filtration and preservation analysis are performed on board the research vessel immediately following collection in order to minimize potential changes in chemical speciation, partitioning (e.g. particulate or dissolved), and cell lysis. When conditions do not permit filtering on-board, the samples are transferred to the laboratory which is located near the Lafayette River thus minimizing time delays in the filtering process.

Coordinated Split Sampling Program:
A mainstem split sampling program between ODU, Chesapeake Biological Laboratory (CBL), Virginia Division of Consolidated Laboratory Services (DCLS), and Virginia Institute of Marine Science (VIMS) will be conducted to ensure comparability of methods and data. Samples are collected by the State of Maryland Department of Natural Resources (MDNR) and split immediately upon collection. This split sampling program is performed quarterly. Parameters which are analyzed are: total suspended solids; fixed suspended solids, orthophosphate-P; dissolved phosphate-P; particulate phosphate-P; dissolved nitrogen; particulate nitrogen; nitrate-N+nitrite-N; ammonium-N; nitrite-N; silicate-Si; particulate carbon; chlorophyll and phaeophytin.

A tributary split sampling program between ODU, DCLS, Maryland Department of Health and Mental Hygiene (DHMH), CBL, Virginia Tech Occoquan Laboratory, United States Geological Survey (USGS) and Pennsylvania Department of Environmental Protection (PADEP) will be conducted to ensure comparability of tributary data. Samples are collected by the District of Columbia Water Quality Division and split in the laboratory, and analyzed for the above-mentioned parameters and also dissolved organic carbon. This split sampling program is also performed quarterly.

7.5 Parameter Table

Sampling Protocols, Analytical Methods and Procedures

The selection of analytical procedures has focused upon the utilization of procedures which are appropriate for generating high-quality data in an estuarine water matrix. The methods which are utilized are presented in Table 7.6. Specific analytical procedures referenced in Table 7.6 are presented as SOPs in the indicated attachments to this WQAPP. All procedures used have been previously reviewed by USEPA CBP QAOs during on-site evaluations and meet or exceed the DQIs specified in the SOW for RFP# 96-004-BS and meet the approval of Data Integrity Workgroup. In the event that there is any change in the analytical instrumentation or procedures, a method comparison study will be coordinated with the Data Integrity Workgroup and performed prior to generating data for submittal to the CBMP database.

Table 7.6 Sampling and Analytical methods utilized for the Chesapeake Bay Mainstem Water Quality Monitoring Program.

Standard Operating Procedure for Chesapeake Bay Program Cruise Deployment	15SEP14	Appendix 2
Standard Operating Procedure for YSI EXO2 Water Quality Monitor	15FEB15	Appendix 4
Standard Operating Procedure for Dissolved Organic Carbon in Water and Seawater using Combustive/Non-Dispersive Infrared Gas Analysis	15NOV16	Appendix 7
Standard Operating Procedure for Particulate Carbon and Nitrogen in Water and Seawater Using Flash Combustion/Chromatographic Separation and a Thermal Conductivity Detector	18APR16	Appendix 3
Standard Operating Procedure for Total Suspended Solids Dried at 103-105ºC and Fixed Suspended Solids Ignited at 550˚C	15APR14	Appendix 8
Standard Operating Procedure for Chlorophyll: Spectrophotometric Method	15APR14	Appendix 9
Standard Operating Procedure for Total Dissolved Nitrogen in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 11
Standard Operating Procedure for Nitrate + Nitrite in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 12
Standard Operating Procedure for Nitrite in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 13

Standard Operating Procedure for Measurement of Light Attenuation in Seawater Using Incident Radiation Sensors and a Data Logger	01JUL15	Appendix 6
Standard Operating Procedure for the Measurement of *In Vivo* Fluorometry in Water and Seawater Using a Model 10-AU Turner Fluorometer	15FEB15	Appendix 5
Standard Operating Procedure for Ammonia in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 14
Standard Operating Procedure for Orthophosphate in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 17
Standard Operating Procedure for Particulate Phosphorus in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 15
Standard Operating Procedure for Silicate in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 10
Standard Operating Procedure for Total Dissolved Phosphorus in Seawater and Brackish Water using the LACHAT QuikChem 8500 Series 2 FIA+	01JUL14	Appendix 16

7.6 Contingency Plans

There are several conditions where a contingency plan is required to ensure that sampling events can proceed to successful and timely completion. The following contingency plan has ensured that the WQL has missed only two field sampling events, not including weather, since beginning the CBMP in 1984. When there is any doubt about the safety of a sampling event due to weather conditions, the WQL notifies the VA DEQ Project Officer. This level of coordination is critical to the other participants in the CBMP.

Weather Conditions can have a major influence on the safety of personnel and equipment during sampling events conducted on research vessels operating in the Chesapeake Bay. For the Chesapeake Bay mainstem, the WQL charters the Department of Ocean, Earth and Atmospheric Sciences' 55 foot R/V Fay Slover, 23 foot R/V Beagle II, 23 foot R/V ODU II or R/V Cynoscian. The R/V Fay Slover is equipped with standard oceanographic sampling gear, advanced marine electronics, and has space for eight research staff on overnight trips. It is capable of carrying a larger staff on day cruises, and it has a heated/air conditioned laboratory. The R/V Beagle II is a 23 foot fiberglass Parker with an enclosed V-berth cabin and a partial hardtop which covers the wheelhouse and a portion of the deck. It has a research staff capacity of four people plus the operator. The ODU II is a 23 foot Sea Ox with an enclosed V-berth cabin and a partial hardtop which covers the wheelhouse and a portion of the deck. It has a research staff capacity of four people plus the operator. The R/V Cynoscian is a 21 foot fiberglass Sou'wester modified cutty cabin. It has a research staff capacity of four people plus the operator. In addition, the WQL has contracted commercial vessels. All of these vessels have been used under a variety of adverse conditions and the appropriate vessel will be selected based on the conditions. Should any vessel not be operational, the others are fully capable of and committed to supporting the sampling event.

The same personnel are not always available for every cruise. The WQL has cross-trained members of its staff in the specific sample collection techniques required for the CBMP.

Field instruments and equipment are exposed to salt spray and extreme conditions at sea. Recognizing that there is a probability of failure at sea, the WQL has mandated on-ship redundancy in all instruments and equipment critical to collecting field data and samples as much as feasible. Because of the extensive equipment available within the University, the WQL may be able to deliver additional equipment to the field in the event that backups fail.

8.0 Project Fiscal Information

The cost for providing the research services identified in this WQAPP is identified in the ODU proposal submitted to VA DEQ in response to RFP# 96-004-BS dated October 19, 1995. In as much as the Chesapeake Bay Monitoring Program continues to be a dynamic study and modifications to this WQAPP are anticipated, the WQL concurs fully with the General Terms and Conditions in RFP# 96-004-BS at paragraph IV, sub-paragraph O.

9.0 Schedule of Tasks and Products

This project is expected to begin July 1, 2018 and unless renewed to terminate on June 30, 2019. Table 9.1 provides a comprehensive schedule of events, tasks, and products associated with the CBMP Water Quality Monitoring Component. The WQL is aware that certain dates have yet to be established and these dates will be announced by VA DEQ or the various USEPA CBP committees, subcommittees, and workgroups. Unscheduled meetings are listed but the Due Date is indicated by the month in which it should occur and TBA (To Be Announced).

An example of the Semi-Annual Progress (Status) Report is provided in Reports (Section 19.0). The format and content of the submittal of Water Quality and QA Data is provided in Documentation, Data Reduction and Reporting (Section 15.0). WQL will attend, at a minimum, the Data Integrity Workgroup meetings as indicated in Table 9.1

The approximate dates for the Coordinated Split Sample Program (CSSP) are in Table 9.1. Additionally, the WQL is anticipating on-site inspections by VA DEQ.

Although the scheduled dates for submittal of Water Quality and QA Data are set in Table 9.1, a review process may result in resubmittal of corrected data sets. The full process is described in Documentation, Data Reduction and Reporting (Section 15.0). Briefly, the Chesapeake Bay Program Office (CBPO) reviews the data within 10 days of receipt and will either request corrections or will send a sign-off form for verification. If corrections are requested, the WQL will correct or certify the values and resubmit within two weeks of receipt from CBPO. When the WQL receives the sign-ff form it will be filled out by ODU stating the data is either accepted entirely as is, accepted with limited modifications that are noted or rejected. If the data is modified or rejected, the data submittal process is repeated until the data is accepted as is. Should a discrepancy be discovered by VA DEQ, CBPO or the WQL within two years of the payment for that data set, the WQL will correct or rectify the problem and resubmit within 90 days of notification of the discrepancy.

Table 9.1 Comprehensive Schedule of Events, Tasks and Products for the CBMP Water Quality Monitoring Component. ("TBA" indicates that the date is to be announced in the near future.)

Event, Task or Product	Due Date
CBMP Mainstem Cruise # 724	07/9-12/18
CBMP Mainstem Cruise # 725	07/23-26/18
Mainstem CSSP Sample	08/6/18
CBMP Mainstem Cruise # 726	08/6-9/18
CBMP Mainstem Cruise # 727	08/27-8/30/18
CBMP Data Integrity Workgroup Meeting	09/TBA/18
CBMP Mainstem Cruise # 729	09/17-20/18
CBP Tributary CSSP Sample	09/10/18
Invoice for 07/01/18-09/30/18 (20% of total price)	10/15/18
CBMP Mainstem Cruise # 730	10/15-18/18
Submission of 8/2018 multi-lab CSSP Mainstem field split data	10/31/18
Submission of Jul/Aug 18 Water Quality and QA Data	10/31/18
CBMP Mainstem Cruise # 732	11/12-15/18
Mainstem CSSP Sample	11/13/18
Submission of 9/2018 multi-lab CSSP Tributary field split data	11/30/18
CBMP Data Integrity Workgroup Meeting	12/TBA/18
CBP Tributary CSSP Sample	12/01/18
CBMP Mainstem Cruise # 734	12/10-13/18
Submission of Sep/Oct 18 Water Quality and QA Data	12/31/18
Submission of Jul/Sep 18 Fluorometry Data	12/31/18
CBMP Mainstem Cruise # 736	01/TBA/19
Semi-Annual Progress (Status) Report for 7/01/18-12/31/18	01/15/19
Invoice for 10/01/18-12/31/18 (20% of total price)	01/15/19
Submission of 11/2018 multi-lab CSSP Mainstem field split data	01/31/19
CBMP Mainstem Cruise # 738	02/TBA/19
Mainstem CSSP Sample	02/TBA/19
Submission of 12/2018 multi-lab CSSP Tributary field split data	02/28/19
Submission of Nov/Dec 18 Water Quality and QA Data	02/28/19

Table 9.1 Comprehensive Schedule of Events, Tasks and Products for the CBMP Water Quality Monitoring Component. ("TBA" indicates that the date is to be announced in the near future.) (Continued)

Event, Task or Product	Due Date
CBMP Mainstem Cruise # 740	03/TBA/19
CBP Tributary CSSP Sample	03/TBA/19
CBMP Data Integrity Workgroup Meeting	03/TBA/19
CBMP Mainstem Cruise # 742	04/TBA/19
Submission of Oct/Dec 18 Fluorometry Data	04/15/19
Water Quality Monitoring QAPjP for 7/1/19 through 6/30/19	04/15/19
Invoice for 01/01/19-03/31/19 Period (20% of total price)	04/15/19
Submission of Jan/Feb 19 Water Quality and QA Data	04/30/19
Submission of 2/2019 multi-lab CSSP Mainstem field split data	04/31/19
CBMP Mainstem Cruise #744	05/TBA/19
Mainstem CSSP Sample	05/TBA/19
Submission of 3/2019 multi-lab CSSP Tributary field split data	05/31/19
CBMP Data Integrity Workgroup Meeting	06/TBA/19
CBMP Mainstem Cruise # 746	06/TBA/19
CBP Tributary CSSP Sample	06/TBA/19
CBMP Mainstem Cruise # 747	06/TBA/19
Submission of Mar/Apr 19 Water Quality and QA Data	06/30/19
Semi-Annual Progress (Status) Report for 01/01/19-06/30/19 Period	07/15/19
Invoice for 04/01/19-06/30/19 Period (20% of total price)	07/15/19
Submission of 5/2019 multi-lab CSSP Mainstem field split data	07/31/19
Submission of 6/2019 multi-lab CSSP Tributary field split data	08/31/19
Submission of May/Jun 19 Water Quality and QA Data	08/31/19
Submission of Jan/Jun 19 Fluorometry Data	10/15/19

10.0 Project Organization and Responsibility

Dr. John R. Donat, Director of the Water Quality Laboratory, is the Project Manager for this project. Senior project personnel assigned to this project are described below.

Project Manager (PM), Dr. John R. Donat - is responsible for directing all technical and management activities associated with the project and will ensure that all contractual obligations are met. The PM conducts regular staff meetings with the scientists, Project Coordinator, and Laboratory Supervisors to discuss project status, problems encountered, as well as any major decisions required for the successful completion of the project. The PM approves the quality control (QC) protocols to ensure the quality and veracity of the results. The PM meets on a regular basis with the Quality Assurance Officer (QAO) to ensure that the project is being performed consistent with the WQAPP. The Quality Assurance Officer, Project Coordinator and Water Quality Laboratory Supervisor report to the Project Manager. The PM or his representatives will participate in meetings, workshops, and coordinating sessions with the contracting agency. His qualifications include: over 35 years of laboratory and field environmental and oceanographic research experience. Dr. Donat is an Associate Professor of Environmental and Analytical Chemistry at Old Dominion University.

Project Coordinator (PC), Mr. Michael F. Lane - is responsible for coordinating all tasks and schedules for this project. Under the direction of the PM, the PC will ensure the timely and efficient completion of the project elements. His qualifications include: over 5 years of field experience, 17 years of laboratory experience, and 18 years of project coordination experience.

Water Quality Laboratory Supervisor (WQLS) and WQL Quality Assurance Officer (QAO),, Ms. Suzanne C. Doughten -is responsible for the coordination and supervision of the analysis of nutrients, plant pigments, and other water quality parameters. The WQLS is responsible for implementing all of the appropriate laboratory and field QC procedures as approved by the PM, maintaining supplies and equipment necessary for analyses, the supervision and training of all lab personnel in the WQL, and coordination of interactions between senior personnel and the technical staff. The QAO periodically meets with the technical supervisors to discuss results, QC trends, and any potential analytical or QC problems. The QAO oversees trouble-shooting any methodological problems identified by the QC protocol or personnel, at her discretion. The QAO reports directly to the PM. Her qualifications include: 25 years of field experience, 25 years of laboratory experience, and 22 years of experience in supervising field sampling teams and wet chemistry analytical laboratory operations.

Chief Scientist (CS) - are responsible for the supervision and training of all field personnel. In addition to the more specific duties which are described throughout this WQAPP, the chief scientist ensures that all field activities adhere to WQL policies, guidelines, and protocols (as approved by the PM), and has ultimate decision-making authority over all technical and

logistical matters which arise during sampling events. The chief scientist report to the WQLS.

Lab Specialists (LSs) - are responsible for performing field measurements; sample collection, handling, transport, storage and logging; calibrations and analytical procedures; QC analyses; and data reduction and transmittal. Technicians are responsible for performing all duties within the WQL guidelines and protocols. The LSs report to the WQLS.

Laboratory Assistants (LAs) - are primarily responsible for the cleaning of labware, assist with some aspects of analytical and field collection preparation such as reagent preparation, preparing filters, etc., as needed.

11.0 Data Quality Requirements and Assessments

Quality assurance indicators are quantitative expressions of the acceptable limits in the uncertainty of a measurement and throughout this WQAPP will be referred to as data quality indicators (DQIs). In monitoring environmental parameters, DQIs are expressed in terms of precision, accuracy, completeness, representativeness, and comparability. The purpose of DQIs is to ensure that data produced for the Chesapeake Bay Monitoring Program are of known and consistent quality which meets or exceeds the requirements for the VA DEQ and USEPA CBP to use in evaluating status and trends of water quality in Chesapeake Bay and its tributaries.

DQIs are established by the VA DEQ Project Officer and Quality Assurance Officer (QAO) prior to project initiation, using a knowledge of the CBP and the technical specifications contained within the VA DEQ RFP #96-004-BS, dated October 19, 1995. The following is a description of the DQIs for this WQAPP based on actual performance data from past and present WQL research grants and contracts. In addition the current version of the Quality Manual for ODU WQL is followed.

The following terms apply to this WQAPP:

Quality assurance (QA) refers to a system or program designed to ensure the producer and users of environmental services that the analytical products meet the required standards of quality with pre-determined levels of confidence. In addition, QA documents that the reported results do, indeed, refer to the sample that was submitted to or collected by the laboratory. This WQAPP includes the coordinated activities of quality control, quality assessment, and appropriate documentation of all procedures.

Quality control (QC) refers to those actions taken in a laboratory to ensure and define the quality of the product or service meets the needs of the user and that the measurement system remains in "control." The concept of quality assessment is often included as a component of a comprehensive QC program. Quality assessment involves a protocol that allows a continuing evaluation of the analytical system and its products. Documentation protocols in this WQAPP detail the analytical systems employed, the experience, training and responsibilities of the laboratory staff, and the "chain-of-custody" for all samples and data.

Precision is defined as "a measure of mutual agreement among individual measurements of the same property, usually under prescribed similar conditions." (QAMS 004/80, U.S. USEPA, 1980). The system of overall precision assessments is determined for the combined sampling and analytical process through replicate analysis of samples. A relative percent difference (RPD) of 20% or less is generally considered acceptable for dissolved parameters and an (RPD) of 30% or less is generally considered acceptable for particulate parameters if all other QC conditions are within acceptable limits.

Accuracy is defined as "the degree of agreement of a measurement (or an average of measurements of the same thing), X, with an accepted reference or true value, T, usually expressed as the difference between the two values, X-T, or the difference as a percentage of the reference or true value, 100(X-T)/T, and sometimes expressed as a ratio, X/T. Accuracy is a measure of the bias in a system." (QAMS 004/80, U.S. USEPA, 1980). Percent recovery in the range of 80 to 120% is generally considered to be acceptable providing all other QC conditions are within acceptable limits. A method of estimating accuracy used by the WQL is the use of standard reference materials (where available) and spiked samples.

Completeness is defined as "a measure of the amount of valid data obtained from a measurement system compared to the amount that was expected to be obtained under correct normal conditions" (QAMS 004/80, U.S. USEPA, 1980) and is defined as follows for all measurements:

$$\%Completeness = 100\ (\frac{V}{n})$$

where:

V	=	Number of measurements or analyses judged valid
n	=	Total number of measurements planned, samples collected or necessary to achieve a specified statistical level of confidence in decision making.

Completeness with respect to the CBMP is defined as the percentage of validated data produced by the WQL as compared to the expected or planned validated data that should be produced from the number of samples collected during the 15 Mainstem (field sampling events) in 2018/2019. The expected data set is to be based upon the number of samples collected by the WQL as defined in RFP# 96-004-BS.

Representativeness is defined as "the degree to which data accurately and precisely represent a characteristic of a population, parameter variations at a sampling point, or an environmental condition." (QAMS 004/80, U.S. USEPA, 1980). The study design, sampling plan and analytical methods have been prescribed by the VA DEQ and the USEPA CBP. The WQL confirms that the analytical methods selected for this contract are designed to provide results that are representative of the environmental monitoring methods used in the CBMP. This WQAPP will be modified or revised as necessary based upon guidance received from the CBMP STAR committee and Data Integrity Workgroup when approved by the VA DEQ Project Officer.

Comparability is defined as "a measure of the confidence with which one data set can be compared to another." (QAMS 004/80, U.S. USEPA, 1980). The study design, sampling plan and analytical methods have been prescribed by VA DEQ and the USEPA CBP. The WQL confirms that the sampling protocols and analytical methods selected for this contract are designed to provide results that are comparable to the environmental monitoring methods used in the CBP and are consistent with methods recognized or approved by the Data Integrity Workgroup.

Method Detection Limit is defined as the minimum concentration that can be detected and determined to be statistically different from zero. The method for determining MDLs is based upon the Students' t value multiplied by the standard deviation of not less than seven replicate analyses of the same sample. This MDL will be highly dependent upon the instrument, reagents, SOPs, personnel and, most importantly, the matrix of the media being analyzed.

Relationship between relative range and coefficient of variation for two replicates:

The relative range (RR) is represented by the equation below:

$$RR = \frac{|D_1 - D_2|}{(D_1 + D_2)/2}$$

where,

D_1	=	Measured concentration of duplicate 1,
D_2	=	Measured concentration of duplicate 2,
$\lvert D_1 - D_2 \rvert$	=	Absolute difference between duplicates and
$(D_1 + D_2)/2$	=	Mean

The coefficient of variation (CV) is defined as:

$$\frac{SD}{mean}$$

Where the mean is defined in the above equation for relative range and SD is standard deviation defined in the following equation:

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

where:

SD = Standard deviation
y_i = Measured value of the i^{th} replicate
y = Mean of replicate measurements
n = Number of replicates

When comparing relative range to CV, only two replicates are used. Thus, the denominator in the equation for standard deviation is always one. The equation is then simplified as follows:

$$SD = \sqrt{(D_1 - mean)^2 + (D_2 - mean)^2}$$

where:

SD = Standard deviation
D_1 = Measured concentration of duplicate 1 and
D_2 = Measured concentration of duplicate 2.

When comparing relative range to CV the mean in both equations is the same, thus it can be treated like a constant. The denominators from both equations can be eliminated. This results in the equations being the simplified SD equation shown above for CV, and for relative range the following equation is used:

$$RR = |D_1 - D_2|$$

Since the mean in the standard deviation equation can be treated as a constant when comparing the two equations, a consistent relationship between RR and CV can be generated.

When taking two numbers with a difference of one, the standard deviation is calculated as 0.7071 to four significant figures. Thus, the following equation can be calculated:

$$CV = (RRx0.7071)x100$$

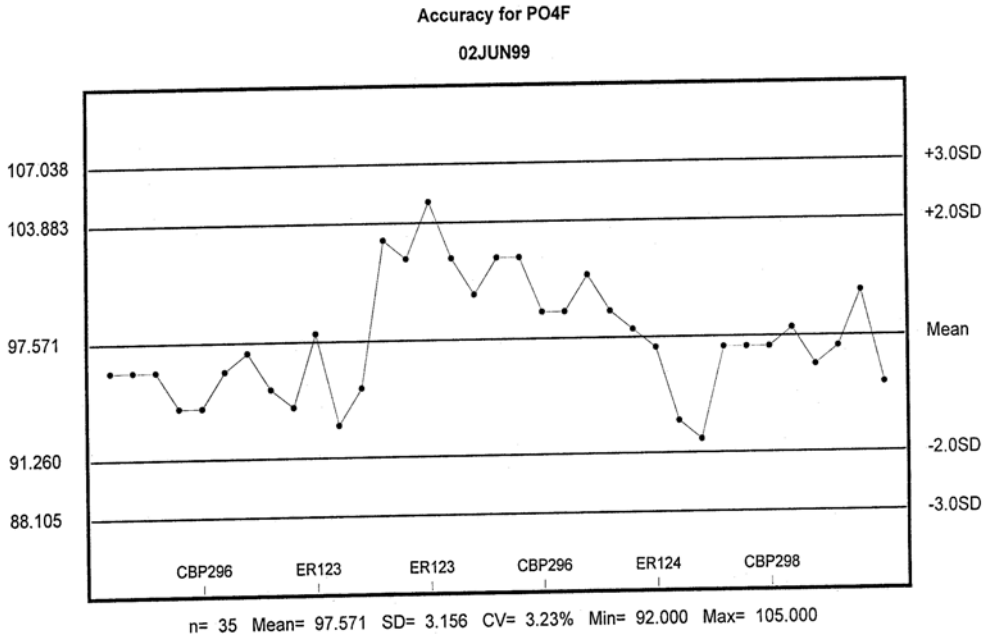
Substitute a RR of .2828 and CV=20.

Procedures for Assessing Accuracy and Precision:

This WQAPP is designed to ensure that accurate and precise data are being generated and transferred to VA DEQ and USEPA CBP. Quality control (QC) measures include those actions which are taken in the laboratory to verify that the measurement system is in control (e.g. instrument calibration; the analysis of reference standards; the analysis of matrix spikes, replicates, and blanks; the maintenance of quality control charts). The QA program is designed to manage sample handling, documentation and custody; proper data generation; and quality control actions. The QA program primarily tracks and monitors the fate of a sample from collection to data submission allowing the Project Manager, technical staff, and VA DEQ to assure proper sample analysis through appropriate methods, and that the necessary QC measures have been taken to ensure that representative data of definable quality have been produced.

Real-time control charts for accuracy and precision are maintained. Quality control (QC) charts are based on the most recent 35 data points produced for a particular analysis and matrix. As new data points are added, the earliest ones are deleted. Quality control charts for accuracy and precision are used by the laboratory specialists in evaluating the performance of the instrumentation for the analyses being performed by the WQL. Determination of accuracy will be accomplished by evaluating a continuing series of spiked samples. Accuracy of analysis is also assessed by analyzing standard reference materials obtained commercially. Determination of precision will be accomplished by evaluating a continuing series of replicated samples. Precision of analysis is also assessed by evaluating the correlation coefficient of the calibration curve.

ACCURACY CHART FOR FILE: ACCPO4F.XLS

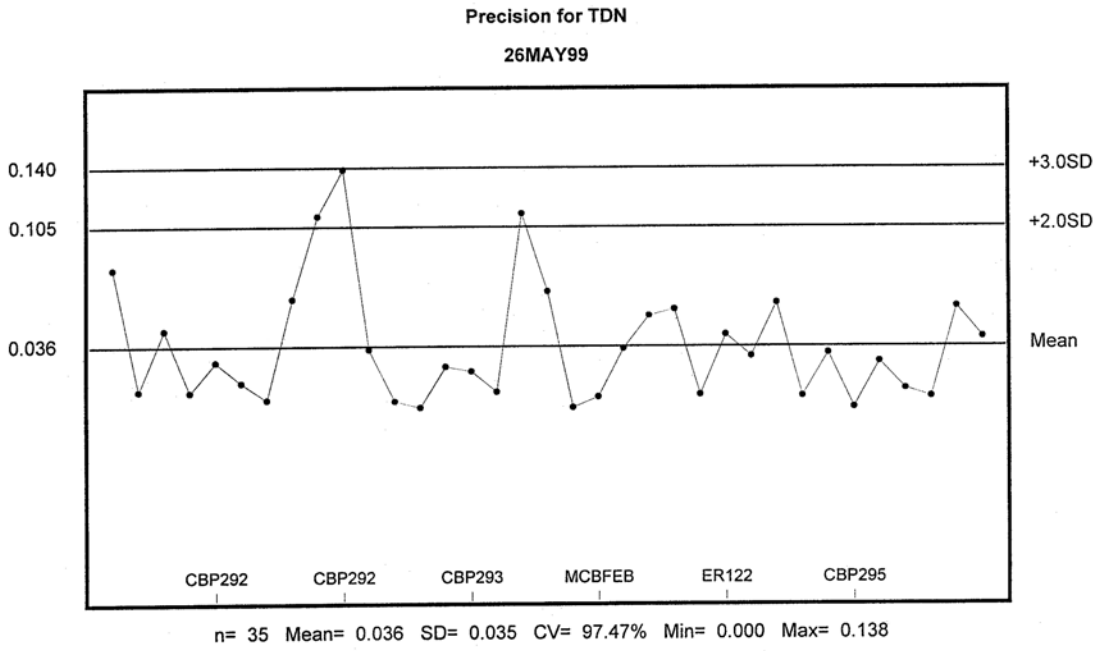


Cruise	Spike	Date Analyzed
CBP296	96	29-Apr-99
CBP296	96	29-Apr-99
CBP296	96	29-Apr-99
CBP296	94	29-Apr-99
CBP296	94	29-Apr-99
CBP296	96	29-Apr-99
CBP296	97	29-Apr-99
CBP296	95	29-Apr-99
ER123	94	29-Apr-99
ER123	98	29-Apr-99
MCBDEC	93	29-Apr-99
MCBFEB	95	29-Apr-99
ER123	103	13-May-99
ER123	102	13-May-99
ER123	105	13-May-99
CBP296	102	13-May-99
CBP296	100	13-May-99
CBP296	102	13-May-99

Cruise	Spike	Date Analyzed
CBP296	102	13-May-99
CBP296	99	13-May-99
CBP296	99	13-May-99
CBP296	101	13-May-99
ER124	99	26-May-99
ER124	98	26-May-99
ER124	97	26-May-99
MCBMAY	93	26-May-99
MCBMAY	92	27-May-99
CBP298	97	27-May-99
CBP298	97	27-May-99
CBP298	97	27-May-99
CBP298	98	27-May-99
CBP298	96	27-May-99
CBP298	97	27-May-99
CBP298	100	27-May-99
CBP298	95	27-May-99

Figure 11.1 Typical Accuracy Control Chart

PRECISION CONTROL CHART FOR FILE: PRECTDN.XLS



Rep 1	Rep 2	Cruise	Rel. Range	Date Analyzed
0.258	0.238	ER120	0.0806	3-Feb-99
0.203	0.205	ER120	0.0098	3-Feb-99
0.218	0.228	CBP292	0.0448	4-Feb-99
0.223	0.225	CBP292	0.0089	4-Feb-99
0.223	0.229	CBP292	0.0265	4-Feb-99
0.207	0.204	CBP292	0.0146	4-Feb-99
0.218	0.217	CBP292	0.0046	4-Feb-99
0.197	0.185	CBP292	0.0628	4-Feb-99
0.136	0.152	CBP292	0.1111	4-Feb-99
0.17	0.148	CBP292	0.1384	4-Feb-99
0.262	0.271	CBP292	0.0338	4-Feb-99
0.265	0.266	CBP293	0.0038	17-Feb-99
0.303	0.303	CBP293	0.0000	17-Feb-99
0.208	0.213	CBP293	0.0238	17-Feb-99
0.187	0.191	CBP293	0.0212	17-Feb-99
0.217	0.219	CBP293	0.0092	17-Feb-99
0.159	0.178	CBP293	0.1128	17-Feb-99
0.138	0.129	CBP293	0.0674	17-Feb-99
0.196	0.196	CBP293	0.0000	17-Feb-99
0.481	0.478	MCBFEB	0.0063	17-Feb-99
0.201	0.208	ER121	0.0342	1-Mar-99
0.458	0.483	ER121	0.0531	1-Mar-99
0.171	0.181	CBP293	0.0568	1-Mar-99
1.082	1.074	CBP293	0.0074	1-Mar-99
0.267	0.256	ER122	0.0421	29-Mar-99
0.231	0.238	ER122	0.0299	29-Mar-99
0.481	0.511	ER122	0.0605	29-Mar-99
0.303	0.305	CBP295	0.0066	29-Mar-99
0.225	0.218	CBP295	0.0316	29-Mar-99
0.221	0.221	CBP295	0.0000	29-Mar-99
0.152	0.148	CBP295	0.0267	29-Mar-99
0.183	0.185	CBP295	0.0109	29-Mar-99
0.163	0.162	CBP295	0.0062	29-Mar-99
0.133	0.141	CBP295	0.0584	29-Mar-99
0.169	0.176	CBP295	0.0406	29-Mar-99

Figure 11.2 Typical Precision Control Chart

Calibration curves generally include a minimum of four standard concentration points which should encompass the expected range of recoveries and a minimum of two matrix blanks. Exceptions are noted in the individual methods SOP. The accuracy of calibration curves are confirmed when acceptable results are obtained for certified reference material (CRM) or standard reference material (SRM) when available. When certified or standard reference material is not available, the accuracy of the stock standard used for an analysis is determined by purchasing commercially available stock standards. The precision of the calibration curve is confirmed by calculating the correlation coefficient (r). A calibration check standard is analyzed at least every 20 samples.

Whenever any aspect of quality control is considered "out-of-control" or otherwise deemed to be problematic, all samples beginning with the last in control situation are re-analyzed. If the "out-of-control" situation persists, intensive troubleshooting of the analysis is commenced until the problem is resolved.

Upon meeting all accuracy and precision criteria, the analytical results are evaluated for representativeness against reasonably expected results whenever historical and ecological information is known on which to base such an evaluation. Analytical results for collocated samples are also evaluated for comparability.

Procedures for Determination of Method Detection Limits (MDLs):

Method detection limits for the CBMP are driven by the need to measure detectable concentrations of the selected parameters in complex matrices (e.g., estuarine and sea water). MDLs are determined and verified annually, or when there is a change in the test method or instruments that affect sensitivity.

For dissolved parameters the most recent version of the EPA Definition and Procedure for the Determination of the Method Detection Limit will be used. For particulate parameters low level samples are utilized. A minimum of 7 particulate samples are filtered and analyzed. The standard deviation of the replicates is multiplied by the student *t* to obtain a MDL.

The MDLs are reported to VA DEQ and USEPA CBPO each time they are determined.

DQIs Specific to this Project

The DQIs identified in Table 11.1 have been met by the WQL in previous years of monitoring Chesapeake Bay and its tributaries and meet or exceed those required by VA DEQ RFP# 96-004-BS.

Table 11.1 WQL Data Quality Indicators for Field Sampling, Field Measurements and Laboratory Analyses. ("f" following parameter name indicates field sampling or field measurements and "ATT" refers to the appropriate Attachment.)

Parameter	Method (SOP)	Precision	Accuracy	Complete	Full Calibration curve frequency	Calibration r
Sampling (f)	ATT: 2	N/A	N/A	90%	N/A	N/A
pH (f)	ATT: 4	N/A	N/A	90%	N/A	N/A
D.O. (f)	ATT: 4	N/A	N/A	90%	N/A	N/A
Secchi (f)	ATT: 2	N/A	N/A	90%	N/A	N/A
Conductivity(f)	ATT: 4	N/A	N/A	90%	N/A	N/A
Salinity (f)	ATT: 4	N/A	N/A	90%	N/A	N/A
Light Attenuation (f)	ATT: 6	N/A	N/A	90%	N/A	N/A
Fluorometry (f)	ATT: 5	N/A	N/A	90%	Daily	r^2=0.8
Water Temp. (f)	ATT: 4	N/A	N/A	90%	N/A	N/A
Depth (f)	ATT: 2,4	N/A	N/A	90%	N/A	N/A
Total Dissolved Phosphorus	ATT: 10,17	≤20% RPD	80-120%	90%	Daily	0.9980
Dissolved Ortho Phosphate	ATT: 10,18	≤20% RPD	80-120%	90%	Daily	0.9980
Particulate Phosphorus	ATT: 10,16	≤30% RPD	80-120%	90%	Daily	0.9980
Nitrite	ATT: 10,14	≤20% RPD	80-120%	90%	Daily	0.9980
Nitrite + Nitrate	ATT: 10,13	≤20% RPD	80-120%	90%	Daily	0.9980
Ammonia	ATT: 10,15	≤20% RPD	80-120%	90%	Daily	0.9980
Total Dissolved Nitrogen	ATT: 10,12	≤20% RPD	80-120%	90%	Daily	0.9980

Table 11.1 (Continued)

Parameter	Method (SOP)	Precision	Accuracy	Complete	Full Calibration curve frequency	Calibration r
Particulate Nitrogen	ATT: 3	≤30% RPD	N/A	90%	Weekly	0.9980
Particulate Carbon	ATT: 3	≤30% RPD	N/A	90%	Weekly	0.9980
Chlorophyll	ATT: 9	≤30% RPD	N/A	90%	N/A	N/A
Total Suspended Solids	ATT: 8	≤30% RPD	N/A	90%	N/A	N/A
Dissolved Organic Carbon	ATT: 7	≤20% RPD	80-120%	90%	Weekly	0.998
Silicates	ATT: 10,11	≤20% RPD	80-120%	90%	Daily	0.9980

Laboratory QC Checks

In order to ensure that the analytical conditions remain "in control" and produce sensitive and dependable data, a number of routine quality control (QC) checks are analyzed with each batch of samples. In general, 10-20% of all samples processed by the Water Quality Laboratory represent these QC checks. At a minimum, for each set of 20 samples, the following QC samples are analyzed:

midpoint standard spike - the analyte is spiked into an aliquot of a random sample. These data are used to assess accuracy (% recovery);
replicate analyses - sample aliquots analyzed separately. These data are used to assess precision.
calibration check standards - standards analyzed to verify maintenance of instrument calibration during analyses.

Unless noted in the relevant standard operating procedure, calibration curves always include at least two blanks and four standards. The accuracy of calibration curves are confirmed when acceptable results are obtained for the analysis of standard or certified reference materials where available. Maintenance of calibration throughout the analysis is determined by analyzing a standard after every 20 samples. The correlation coefficient (r value) of (least squares linear regression) calibration curves is concurrently used to assess precision. A correlation coefficient $\geq$0.9980 is required for most analysis to be acceptable.

The procedures for preparing QC checks and calibration procedures for individual water quality variables are presented in the analytical SOPs.

Field QC Checks

In the field, the following QC checks are made:

field blank - taken at or near the end of each cruise day and carried through the handling and analytical processes to evaluate contamination potential. These samples should be below the lowest standard analyzed for the parameter. Troubleshooting occurs if field blanks are above this threshold.

collocated replicates - replicates taken in rapid succession to estimate field precision as a contrast to laboratory precision.

field splits - samples collected in same sample container but filtered separately to estimate filtration precision
interlaboratory splits - participation in the Chesapeake Bay Split Sampling Program to evaluate and compare accuracy and precision of laboratories throughout the Bay. Field accuracy (apparent bias) and field and laboratory precision are evaluated quarterly (independent evaluation by CBPO contractor) for quadruplicate samples taken from a mainstem station and triplicate samples taken from a tributary station.

Evaluation of QC Check Data

Control charts for accuracy are developed using percent recovery. Control charts for precision are developed using the method outlined in U.S. EPA Report 600/4-9-019, Section 6.3.2. The classic Shewhart technique (U.S. EPA 600/4-79-019, Section 6.3.1) is used to define the upper and lower control limits for the control charts for determining accuracy and precision. Acceptable control limits are determined using Table 11.1 for each analyte. Also if its distribution is normal, approximately 68 percent of the data should fall within the mean ± standard deviation (SD) interval which is defined as follows:

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

where:

SD = Standard deviation
y_i = Measured value of the i^{th} replicate
y = Mean of replicate measurements
n = Number of replicates

"Out-of-control" situations are indicated when test points fall beyond control limits established in Table 11.1. A warning of possible systematic error is indicated if seven successive sample points fall away from the mean on the same side of the percent recovery center line or if a discernable trend develops. For precision, differences between replicates are not considered to be problematic when the difference is less than the sensitivity of the analyses.

Calibration accuracy is assessed by analyzing commercially prepared reference standards (when available) with each analyses. Check standards are analyzed throughout the analytical run.

12.0 Sampling Procedures

All field monitoring, sampling and other project related activities are planned and approved by the appropriate USEPA Chesapeake Bay Program (USEPA CBP) committees/subcommittees (e.g. STAR, Data Integrity Workgroup, etc.) in coordination with the VA DEQ with the goal of meeting project objectives.

Samples are collected at sites which have been pre-determined by the CBP according to the CBP Chesapeake Bay segmentation scheme. Specific sampling sites which are monitored and the sampling frequency are described in Section 7.0.

All field monitoring and sample collections, handling, and processing are performed in accordance with procedures and protocols which have been specified by the USEPA CBP in coordination with the VA DEQ and have been evaluated by USEPA Quality Assurance Officers during on-site evaluations. Specific procedures and protocols which are utilized are described in detail in the attached field deployment Standard Operating Procedures (SOPs) (Appendix 2). It is the policy of ODU that all work performed must be conducted in strict accordance with approved SOPs in order to ensure representativeness, inter-organizational (and inter-institutional) comparability, comparability of the resulting data from cruise-to-cruise, and to ensure that project objectives are met. Any changes in procedures or protocols require the approval of the CBP QAO in coordination with the VA DEQ QAO and Project Officer. The USEPA QAO may require approval of changes by the CBP Data Integrity Workgroup and CBP STAR committee. Any approved changes or additions to procedures or protocols must be implemented by revision of the applicable SOP which is submitted to all individuals on the WQAPP distribution list.

13.0 Sample Custody Procedures

Sample Collection

The location of field monitoring activities and associated sample collections (see Section 7.0) is determined using a Global Positioning System to achieve more accurate positioning than LORAN or by reference to land based objects. Upon reaching the sampling site, the exact sampling location, time and other observations are recorded onto the field data sheet. Samples are then collected, subsampled, processed (to extent possible depending on boat size and weather), and preserved in accordance with the field operations standard operating procedures.

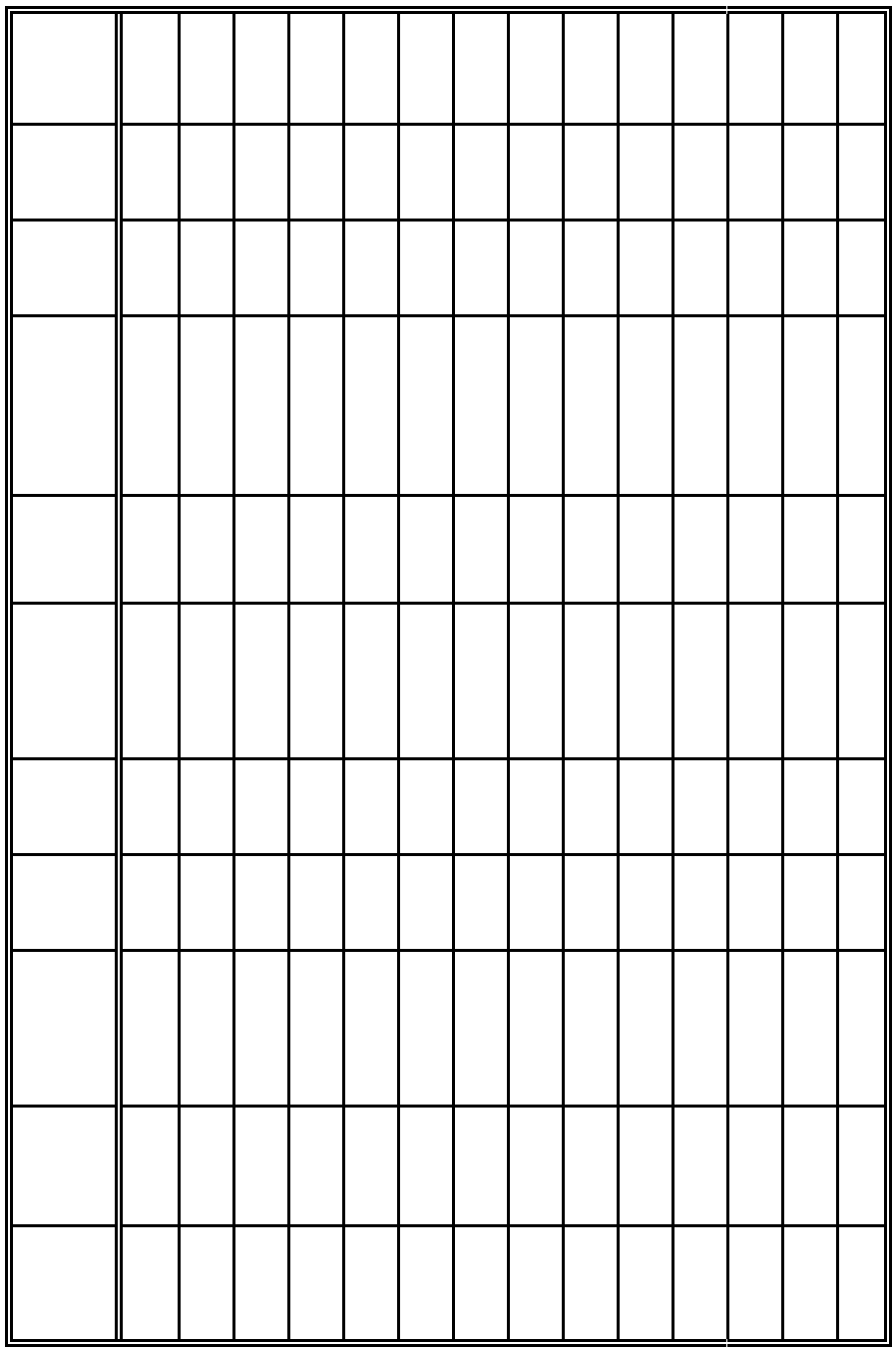
Once sample processing and preservation have been completed, the samples are immediately placed into the freezer (if available) or packed in ice, as appropriate. During the remainder of the sampling cruise, samples are considered to be in the custody of the Chief Scientist and field technicians. Upon completion of the cruise, all samples are transported to the analytical laboratory packed in ice or frozen samples are placed frozen in the cooler.

As soon as possible after arrival at the laboratory, samples are placed in either refrigerators, freezers or ovens, whichever is specified by the applicable SOPs (see appendices). The refrigerators, freezers and ovens are pre-numbered for identification. Appropriate entries are made onto the laboratory chain-of-custody logbook (Figure 13.1).

Anytime a sample is handled for any reason, the chain-of-custody logbook (Figure 13.1) entries are performed, including the reason for sample handling. Samples are replaced into a refrigerator or freezer as soon as possible with chain-of-custody entries made.

After the completion of all required analyses and data validation, sample disposal will occur according to contractual specifications. Only the WQLS may authorize disposal of samples. When this occurs, chain-of-custody logbook entry is made noting disposal, the date and time of disposal, and the initials of the individual who performed this function.

SAMPLE CHAIN-OF-CUSTODY



14.0 Calibration Procedures and Preventive Maintenance

Field Procedures

The CTDs are calibrated before each cruise and are post-calibrated after each cruise according to the manufacturer's instructions.

For each field instrument a maintenance notebook is maintained. It may include all calibration and post-calibration records. Post-cruise calibration check acceptance limits are set by the Chesapeake Bay Program in the Water Quality Sampling and Analysis Chapter IV. Mainstem and Tributary Field Procedures. All data corrections (if applicable) which were necessary following post-calibration and standardization procedures are performed as per manufacturer's instructions. As of January 2008 correction factors will no longer be applied to Chesapeake Bay field data at the request of Data Integrity Workgroup. The standards and reagents used for the calibration and post-calibration for the CTD are prepared according to the manufacturer's instructions. All troubleshooting and maintenance for field instruments are performed according to manufacturer's instructions, and recorded in the maintenance notebook (see Appendix 4, 5 and 6).

All chemicals which are used are obtained from the instrument manufacturer or from vendors of scientific supplies.

Laboratory Procedures

All laboratory instrument calibrations are performed according to the procedures (Appendix 3, 7 through 17). For each instrument, an instrument logbook is used to record all usage, maintenance, calibration, problems with instruments, the troubleshooting which is employed to correct the problem, and when the instrument is serviced. The frequency of instrument calibration is indicated in the Appendices for the applicable procedures.

For each analytical method, the frequency of calibration, the reagents and methods for preparing calibration standards, and acceptable ranges for calibration standards are delineated in the applicable SOPs (See Appendices). The information which is required for data sheets is found in the respective Appendix.

All chemicals are obtained from the instrument manufacturer or from vendors of scientific supplies. If an analysis requires standardization of reagents or calibration standards, the frequency of this standardization is indicated in the applicable procedure. A standardization logbook is maintained for all applicable reagents and calibration standards. In this notebook all raw data necessary for the standardization calculations is recorded. In addition, the data of the standardization, the analyst's initials, and the calculations are recorded.

Preventive Maintenance

Preventative maintenance for all equipment is performed as per manufacturer's instructions and recommended schedule/frequency of performance in order to maintain equipment in good working condition and minimize downtime for all field and laboratory equipment. All preventative maintenance and repairs are performed either by qualified WQL personnel, the manufacturer's service engineers or other qualified personnel.

When the applicable manufacturer's operation or maintenance manual(s) does not specify a maintenance plan and schedule in an organized format (e.g. table), specific maintenance procedures and frequencies are extracted from all available manuals and consolidated into a table. Whenever experience indicates that additional or more frequent maintenance is required, the maintenance schedule and plan is modified to reflect this. An example of an instrument maintenance plan and schedule is presented in the respective Appendices. The maintenance plan and schedule for each instrument are accessible to all laboratory personal. All maintenance and repairs are logged into the applicable instrument usage and/or maintenance logbook.

Spare parts lists for all equipment are located in the applicable manufacturer's maintenance or operating manual. An inventory of spare parts and consumables is maintained to an extent that is sufficient to maintain the operation of all equipment. Except for standard hardware, spare parts are obtained from the manufacturer or their representative or distributor.

To the extent that is possible, a redundancy of all field and laboratory equipment is maintained in operable condition in the event that one piece of equipment becomes inoperable. Redundant field equipment available is calibrated and taken on all field monitoring cruises for availability as needed.

15.0 Documentation, Data Reduction, and Reporting

General Overview of Water Quality and QA Data Submittals

For all applicable data not electronically captured, data are entered separately into two files by two data entry technicians. These data sets are compared and edited until the two copies match exactly. If only one data set is entered or the data is downloaded directly from the instrument, the data are visually inspected and edited. Corrected data sets are imported into an Access® database in DUET format tables. Data are screened with in house QA checks created in Access®. These checks indicate missing values, missing parameters, problematic depth measurements, and identify values outside of the expected range for each of the variables. Finalized data set tables are exported to an Access® database and sent to USEPA Chesapeake Bay Program Database using the CBP Intranet web site. A data submittal letter and metadata files are electronically transferred by email. The DUET format Access® database tables are then checked for discrepancies in format or violations of quality control range checks by personnel at the USEPA Chesapeake Bay Program office. Any problems with the data sets are identified and a checklist indicating these problems is available on the DUET website maintained by the Chesapeake Bay Program. Any problems with data set format are corrected by modifying the Access® database programs. Access® database tables are then regenerated with the proper format and resubmitted. If quality control checks indicate values outside of the expected ranges, the values identified are checked by the WQL supervisor (WQLS) and their validity confirmed. If any values are determined to be invalid, corrections are made to the Access® database tables. The Access® database tables are then recreated and the data are resubmitted. The confirmation process continues until no corrections to the data sets are required. The data submittal letter is created by modifying a word processing format template file. Contents of the letter are reviewed by the WQLS. Any errors identified are corrected and the completed letter sent to the USEPA via electronic mail. Validity of the data submittal letter contents is checked by officials at the USEPA Chesapeake Bay Program. If format discrepancies or content errors are found, the WQL is contacted by electronic mail, and any appropriate changes are made and the data submittal letter is re-transmitted via electronic mail.

This process is fully described in the WQL Data Management Plan for the CBMP Water Quality Monitoring Component (Appendix 1). This process includes: 1) an initial visual screening; 2) double data entry where applicable; 3) computerized screening analyses or visual inspection; 4) formatting of Access® database tables; 5) preparation of data submittal letter; 6) submittal of Access® database tables; 7) submittal of data submittal letter; 8) data validation and signoff; and 9) backup procedures for all data sets.

Additional Data Set Formats

Chesapeake Bay Program data sets can be provided to the DEQ and the USEPA in formats other than the one currently produced if a change order to the contract is provided by the DEQ. The WQL currently has the capability to produce data sets in the following formats:
1) ASCII format and 2) Access® database DUET format tables.

16.0 Data Validation

The reporting units for each water quality variable are indicated in the individual standard operating procedures (SOPs). Calibration curves are generally calculated using ordinary least squares linear regression; all other equations used are specified in the individual SOP's. All bench data sheets are retained in the Water Quality Laboratory (WQL) in notebooks and files. Summary sheets are compiled from bench data sheets, and placed in notebooks or archives.

Data from filtration volumes for applicable parameters are recorded on a bench sheet by the lab or field technician. Data are entered into a database from these bench sheets to calculate the final result. These spreadsheets are visually inspected by laboratory personal who did not perform the data entry. All other data are captured directly from the instrument using computer software provided by the instrument manufacturer. Some of the data which require further calculations (examples are chlorophyll *a* and particulate phosphate) are transferred into a database to calculate the final result. These are visually inspected by laboratory personal who did not perform the data entry. Laboratory specialists review all data to ensure it has passed the required data quality assessments.

Once these steps are completed, all data are given to the WQLS for review. This review consists of checking data for completeness, outlying or suspect values, comparability of nutrient parameters (NO23 > NO2, etc.), and to apply any problem codes necessary to the data.

Validation and Reporting

The complete process is described in detail in Appendix 1. The procedures for QC reviews of analytical data are described in sections 14.0 and 15.0. In general, the quality control and analytical data are entered and validated by the laboratory personnel, as summarized in Figure 16.1. When applicable, double-entry is performed on networked personal computers by two separate data personnel. The process is tracked by forms designed to ensure that all data are reviewed completely (Figure 16.2). Completed data files are compared, individually corrected using the original data sheets, and then re-compared. This process is repeated until the two files match exactly. Once data are verified to have been entered correctly, they are uploaded to the Access® database, and then screened using a Access® database program designed to evaluate the data using several criteria: values below the method detection limit are flagged and values above an ecologically realistic value are also identified. If questionable values are identified, the original bench sheets and QC data are re-examined, and the values are validated or corrected as necessary. Once the entire data set is acceptable, data set tables are exported to an Access® database, and the database is sent to a database maintained by the USEPA (CBCC). Data validation and reporting process is performed by the Water Quality Laboratory Supervisor, Ms. Suzanne Doughten.

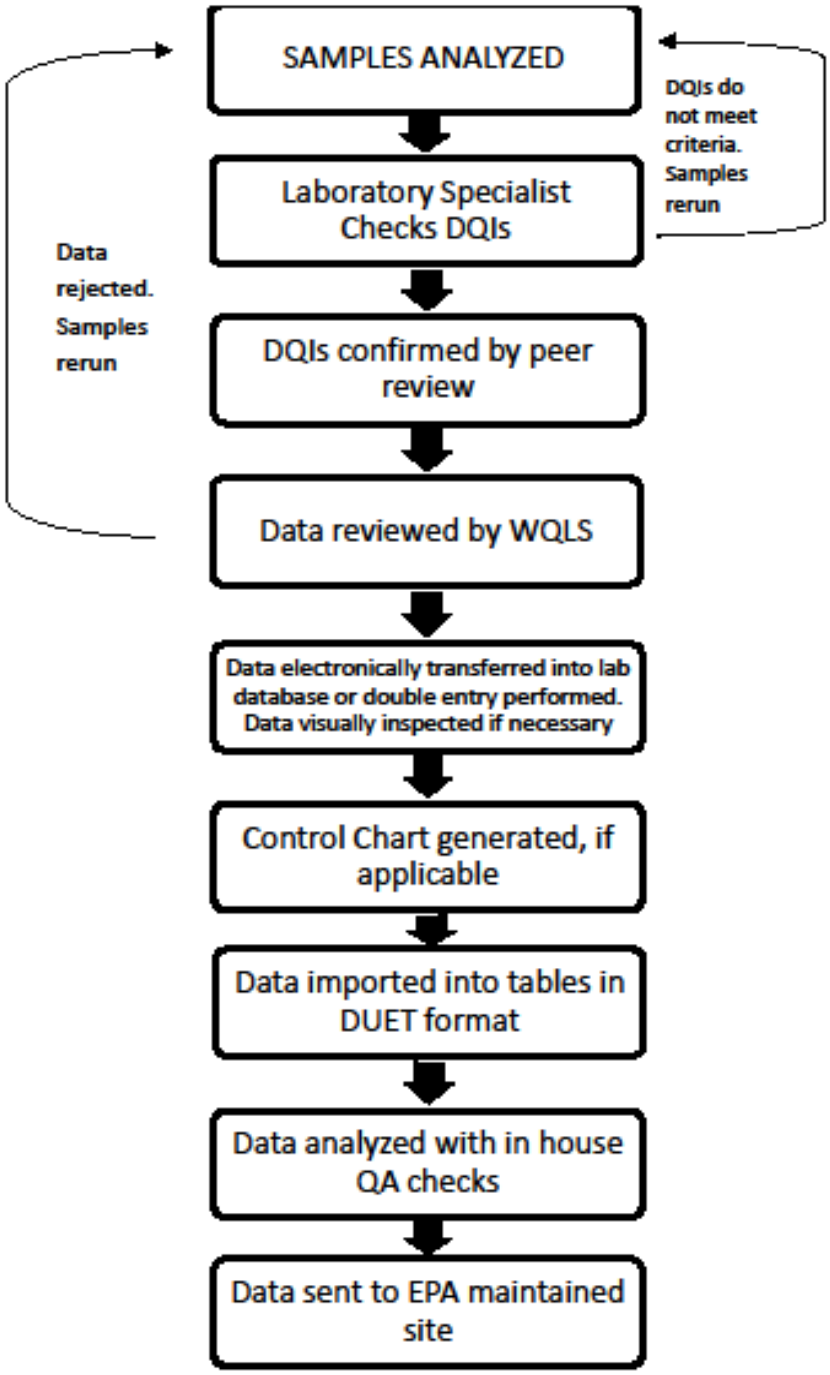


Figure 16.1 Flow of analytical and QC data through the Data Management Program

CHESAPEAKE BAY DATA CHECKOFF SHEET

Cruise_____
Dates Collected_____
Log Numbers _____

Field Sheets:
WQ_EVENT: copy 1:_____
(Front) copy 2:_____
compared:_____
Append to database:_____

Logbook: Copy 1:_____
Copy 2:_____
Compared:_____
Append to database:_____

WQ_DATA_FIELD: In FIELD_YSI:_____
(Back) Run query 7: _____
Appended to database: _____

Light Attenuation:
Entered: _____
Appended: _____

	CHECK HAND ENTERED DATA	IMPORT TO DATABASE	APPEND TO DATABASE	QC IN DATABASE	Control Charts Entered
TSS/FSS					
CHLA/PHEO					
PC/PN					
DOC					
TDN					
TDP					
NO23F					
NH4F					
PO4F					
NO2F					
SIF					
PP					

Figure 16.2 Water Quality Laboratory Data Tracking Form

17.0 Performance and System Audits

An audit is defined as a systematic check to determine the quality of operation of laboratory and field collection activities. It may be internal or external but is generally comprised of the following:

Performance Audit - a check to determine the accuracy of the total or portions of the measurement system. Test samples are analyzed and results evaluated.

Systems Audit - an evaluation of all components of laboratory measurement systems including QC procedures to assess appropriate use.

Internal Audits

Internal performance audits may be conducted by the QAO through the use of blind control samples such as commercially prepared reference materials, standards, spiked samples, and duplicated measurements. Results are compared with "true" values and evaluated for accuracy and/or precision. Audit records maintained by the QAO also include ancillary information:

- verification of adequacy of written procedures
- verification of understanding of analyst
- review of analytical data and calculations

These performance audits may be conducted annually or more frequently if QC data warrants. The DQIs for accuracy and precision represent the acceptance criteria for the audits. If WQL personnel do not achieve acceptance criteria in the internal performance audits, the QAO will work with the WQLS to ensure that the appropriate corrective actions are identified (eg. problem areas identified, SOPs evaluated for revision, personnel retrained and/or counseled, etc.). Should a technician consistently fail performance audits, their job responsibilities will be re-evaluated and they will be reassigned to a less critical position.

Routine systems audits are required to successfully achieve DQIs. The following items are checked daily in the laboratory when it is open, and written records are kept of the results:

- refrigerator/freezer temperatures
- oven temperatures
- pH probe meter calibrated if used
- laboratory water resistivity if used
- balance checked with weights which bracket item being weighed if used
- all samples handled are logged out in chain-of-custody during use and logged in after use

The following items are checked monthly in the laboratory, and written records are kept of the results:

- all balances are checked with weight set
- spectrophotometer is checked with NIST filters

The following items are checked quarterly in the laboratory, and written records are kept of the results:

- autoclave temperature is verified against a thermometer
- pipettes and bottle-top dispensers are checked for precision and accuracy

The following items are checked annually in the laboratory, and written records are kept of the results:

- thermometers are calibrated against a NIST-traceable thermometer
- weight sets are checked for calibration
- balances are checked for accuracy and precision
- centrifuge is checked for timing and rpms
- high temperature oven is checked
- autoclave is checked for correct operation

Field audits consist of field blanks, field split samples and field replicate samples. The results of these analyses are evaluated to determine whether field operations produce contamination or reduction in precision. The QAO may conduct systems audits of field operations to ensure that they conform to the SOPs.

External Audits

The WQL participates in numerous performance and systems audits. The WQL has successfully completed both performance and systems audits for VA DEQ and USEPA CBPO (CBP audits). These extensive systems audits are conducted during on-site visits. Starting in 1998, the WQL participates in the bi-annual CBP Blind Audit program. Starting in Fall 1999, the WQL participates in the Semi-Annual Round-Robin Analytical Evaluation of U. S. Geological Survey Inorganic Standard Reference Samples. In addition, the WQL participates quarterly in the Chesapeake Bay Coordinated Split Sample Program.

The WQL will participate in laboratory inspections conducted by VA DEQ and USEPA CBPO QAO. These on-site inspections will be considered as the only mandatory external audit required for this study. The WQL will also analyze performance evaluation samples, using the same methods and personnel identified in this WQAPP, issued from VA DEQ or USEPA CBPO QAO.

The WQL participates in the Virginia Environmental Laboratory Accreditation Program administered by the Virginia Division of Consolidated Laboratory Services. This includes biennial laboratory inspections, and analysis of commercially prepared performance testing sample annually. As of January 2012, WQL is a certified laboratory under 1VAC30-45 of the Virginia Administrative Code.

Another type of performance evaluation is participation in the USEPA CBP Coordinated Split Sample Program (CSSP). This program was described in Section 7.0 and results from each participating lab will be used to assure comparability of methods used and data generated in this study. As active working members of the USEPA Data Integrity Workgroup, when CSSP data suggest that one or more parameters may not be comparable, Data Integrity Workgroup (and the WQL) will investigate the cause and solution. If this occurs, the WQL will begin internal systems and performance audits to determine whether the problem exists within our facility.

Evaluation reports resulting from each of these external audits are reviewed by the WQLS, the QAO, and the Project Manager to determine whether corrective actions are necessary to improve laboratory performance. If necessary, more frequent internal audits are conducted to ensure that the corrective actions have been effective.

18.0 Corrective Action

An event is considered "out-of-control" when pre-established criteria are not met. "Nonconformance" represents the occurrence of problems in documentation, procedures or characteristics of an event which are sufficient to make the quality indeterminate or not acceptable. An out-of-control event is a subcategory of nonconformance. "Corrective Actions" are a series of intensive methods evaluation/troubleshooting precautions and personnel proficiency evaluations that are taken to correct an out-of-control event and to ensure that nonconformance of a similar nature does not re-occur.

Many of the corrective actions for general analytical problems which could occur in the CBP water quality monitoring effort are presented in the project Corrective Actions Plan (Table 18.1). When the corrective action is listed as “notify WQLS or Chief Scientist”, these individuals will use their training and knowledge of the project to determine the correct coarse of action. They will be responsible for any documentation that may be required.

For problems not directly covered by this plan, the WQLS and the QAO will be informed of the nature of the problem in order to decide upon corrective actions. These documents will be reviewed by the QAO and, if deemed necessary, the Project Manager to evaluate procedural/policy changes which would limit likelihood of reoccurrence. The information is summarized in data submittal letters and/or semi-annual progress reports to the VA DEQ and CBPO (see section 19.0). In addition, if a corrective action results in a procedural change, this will be documented on a DEQ provided procedure modification tracking form (Figure 18.1).

All factors that affect data quality require immediate investigation and corrective action by the WQLS, in collaboration with the QAO. Examples of conditions which initiate corrective actions include:

Failure to meet calibration criteria;

Inadequate record-keeping or deficiencies in the chain-of-custody documentation;

Improper sample preservation, handling, storage; and/or

Analytical errors indicated by failure to meet QC check criteria.

Whenever nonconformance is detected, actions will be initiated to correct problems.

Table 18.1 Corrective Action Plan for out-of-control situations. These will not always result in a corrective action report. These are problems that have been seen in the past and actions which may resolve them without jeopardizing sample quality.

PROBLEM	ACTION
●**General**	
Any member of the study is not confident in their knowledge, skills or ability to conduct their assigned duties or responsibilities	Notify WQLs so training can occur.
Any member of the study observes an unsafe or hazardous action or condition	Individual must immediately correct the problem if it does not place them in danger. Immediately notify the WQLS.
Any member of the study disagrees with selected method, SOP or protocol being used	Notify WQLS and QAO. These can be reviewed, but if they match the QAPP must be used. To change DEQ and/or Data Integrity Workgroup must agree.
Any member of the study observes a violation of the WQAPP and the applicable protocols, methods or SOPs	Notify WQLS in laboratory and Chief Scientist in the field. Depending on severity DEQ and CBP may be notified
Samples are lost due to breakage of containers	Notify WQLS in laboratory and Chief Scientist in the field
The identity of any sample is compromised by absence of labels or records indicating sample identity (i.e. sample numbers)	Notify WQLS. See if identity of sample can be determined or if another sample is available to analyze. If already analyzed and identity uncertain, document that unsure of sample identity.
Methods, protocols or SOPs for analytical methods not approved by the sponsor in the WQAPP are to be used or are being used	Cease analysis and notify WQLS. Depending on severity DEQ and CBP may be notified
During any step in the sample collection, preservation, preparation or analysis the individual becomes confused and is uncertain about the integrity of the sample	Cease the process, return to last step when the individual is certain of the step or process. Notify WQLS or Chief Scientist if uncertainty persists.

PROBLEM	ACTION
●Sample Collection	
Sample containers are inappropriate for the sample or analyte of concern	Get correct container. If no correct containers available notify Chief Scientist
Sample containers appear to be contaminated or become contaminated during sampling	Get another container. If no correct containers available notify Chief Scientist
Sample containers requiring pre-measured preservatives are missing or do not contain the appropriate volume or preservative	Add correct preservative. If no preservative available notify Chief Scientist
The wrong sample has been collected in a pre-labeled container	If container correct except label, re label container with correct information. If incorrect container type get another container and add correct label. If no correct containers available notify Chief scientist
Sample container is lost during sampling effort and insufficient backup containers are available	Notify Chief scientist
Sample container label is illegible or was prepared in pencil or non-waterproof ink	Create a new label

PROBLEM	ACTION
●Sample Transfer and Receipt	
Samples were transported from collection site to the laboratory without proper refrigeration or preservation	Document observations. Notify Chief Scientist and/or WQLS. Determination will be made if samples can be analyzed. Depending on sample type and amount of time that has elapsed VA DEQ, CBP and/or WQL Director may be notified.
Samples were lost, contaminated, or broken during transportation from collection site to the laboratory	Document observations. Notify Chief Scientist and/or WQLS. Determination will be made if samples can be analyzed. VA DEQ, CBP and/or WQL Director may be notified if another sample(s) does not exist that can be analyzed, or it may be noted in sample report.
Samples were received at the laboratory with missing seals or caps and/or with loose caps	Document observations. Notify Chief Scientist and/or WQLS. VA DEQ, CBP and/or WQL Director may be notified if sample(s) determined to be compromised and another sample(s) does not exist that can be analyzed.
Samples were received at the laboratory with missing or illegible labels and/or with invalid sample numbers	Notify Chief Scientist and/or WQLS. See if identity of sample can be determined, or if another sample is available to analyze. VA DEQ, CBP and/or WQL Director may be notified if problem is not fixed.
Sample numbers do not agree with those indicated on chain-of-custody or sample manifest	Notify Chief Scientist and/or WQLS. Determine correct sample numbers and correct them on sample label or chain-of-custody. Document these changes.
At the time samples were received at the laboratory the holding times for the analyte(s) of concern were exceeded	Notify Chief Scientist and/or WQLS. It will be noted that they were analyzed out of holding time.

PROBLEM	ACTION
●Sample Storage	
Samples found in storage are not included on sample storage manifest	Notify WQLS. See if origin of sample can be determined, or if another sample is available to analyze. VA DEQ, CBP and/or WQL Director may be notified if problem is not fixed. Correct sample manifest and document these changes
Samples were stored under incorrect temperature or other conditions	Document observations. Notify WQLS. Determination will be made if samples can be analyzed. Depending on sample type and amount of time that has elapsed VA DEQ, CBP and/or WQL Director may be notified.
Samples or sample sets cannot be located	Notify WQLS.
Samples characteristics have noticeably changed as compared to characteristics when collected	Notify WQLS. Document observations.
Storage container appears to be cracked or broken.	Notify WQLS. See if another sample available to analyze. Document observations.

PROBLEM	ACTION
●Sample Holding Time and Preparation	
Sample holding time has been exceeded for the analyte(s) of concern	Notify WQLS. It will be noted that they were analyzed out of holding time.
Sample holding time is within 24 to 48 hours of expiring	Notify WQLS if sample is not scheduled to be analyzed within this time period, so sample analysis can be scheduled within holding time
Sample appears to have lost volume or there is evidence of leakage during storage	Notify WQLS to determine if sample is compromised.
Sample has been prepared or digested by an incorrect method	Notify WQLS if sample cannot be prepared or digested by correct method.
Sample characteristics are noticeably altered during adjustment to room temperature, upon exposure to air, or when altered for pH, alkalinity, etc	Notify WQLS and document

PROBLEM	ACTION
●Preparation of Standards, Blanks, Duplicates and Splits	
Reagents used in preparing standards are potentially contaminated	Remake reagents and/or standards. Notify WQLS.
A portion or all of the sample is lost during the preparation of spikes or duplicates	Document loss of sample. Notify WQLS
Inadequate sample volume is present for required preparation and analysis	Notify WQLS. If another sample container is not available to analyze, analyze diluted with a note to explain.
Shelf life of reagents used in preparing standards is exceeded	Order new reagents and remake standards. Notify WQLS if samples were analyzed with expired chemicals.
●Analytical Methods	
Methods, protocols or SOPs for analytical methods approved by the sponsor in the WQAPP are not available at the work station and referred to while preforming the analysis	Obtain proper documents. If documents cannot be found notify WQLS
Methods, protocols or SOPs are unclear or do not seem to be applicable	Notify WQLS
Method detection limits will not achieve desired DQIs	Notify WQLS

PROBLEM	ACTION
●Instrumental Analysis	
Logbooks for the instrument or ancillary devices indicates that WQAPP scheduled calibration or maintenance procedures have not been implemented or are not being strictly followed	Notify WQLS. Perform calibration and/or necessary maintenance.
●Data Management and Records Review	
Percent recovery not in compliance with DQIs	Reanalyze effected samples. Notify WQLS if continuing problem or cannot reanalyze samples.
Noncompliance with accuracy DQIs	Reanalyze effected samples. Notify WQLS if continuing problem or cannot reanalyze samples.
Noncompliance with precision DQIs	Reanalyze effected samples. Notify WQLS if continuing problem or cannot reanalyze samples.
Matrix effects evident	Notify WQLS

CHESAPEAKE BAY MONITORING PROGRAM PMTF#____
PROCEDURE MODIFICATION TRACKING FORM APPROVED__ DENIED__

This form is used to request approval for modifications and to document approved modifications made to Chesapeake Bay Program office procedures or methods. It is not a substitute for timely contact with the CBPO Quality Assurance officer or his/her designee who may be reached at 1-800-523-2281. A detailed method description including the proposed modification must be attached to this form prior to submittal to CBPO.

DATE SUBMITTED	DATE APPROVED
REQUESTOR NAME WQL	ORGANIZATION Old Dominion University
NEWLY PROPOSED [ ] MODIFICATION FIELD APPROVED [ ] MODIFICATION APPROVED BY: DATE:	
TYPE OF PROCEDURE/METHOD	SAMPLING [ ] ANALYTICAL [ ] REPORTING [ ] FIELD [ ] OTHER [ ] MEASUREMENT SPECIFY:
DURATION	PERMANENT [ ] EFFECTIVE DATE: TEMPORARY [ ] START DATE: END DATE:
PROCEDURE/METHOD DESCRIPTION	
MODIFICATION DESCRIPTION	
JUSTIFICATION FOR MODIFICATION	
ANALYTICAL PARAMETERS THAT MAY BE AFFECTED BY THIS CHANGE	
AFFECTED QA PLAN(S) (INCLUDE TITLE, REVISION, AND DATE)	QUALITY ASSURANCE PROJECT PLANS FOR THE CHESAPEAKE BAY WATER QUALITY AND BIOLOGICAL MONITORING PROGRAM
AFFECTED CRUISE(S)	
PMTF COMPLETED BY	NAME: DATE:

VA DEQ APPROVAL: NAME_____ TITLE_____

SIGNATURE_____ DATE_____

CBPO APPROVAL: NAME_____ TITLE_____

SIGNATURE_____ DATE_____

Figure 18.1 Procedure Modification Tracking Form

In addition to these ongoing evaluation criteria, the overall performance of the program is periodically evaluated by internal and external performance and system audits conducted by VA DEQ and USEPA (Section 17.0). The WQL program has consistently received extremely favorable comments on these external audits/performance evaluation and analyses/site visits. However, any deficiencies noted in any internal or external systems audit will prompt the corrective action procedures, the associated documentation, reporting requirements (Section 19.0), review by the Project Manager and, if needed, procedural or policy changes. If required by the nature of the problem, the WQL Data Integrity Workgroup representative will introduce the topic for discussion on a CBP program-wide basis.

Whenever indications of nonconformance are detected, all individuals involved in the analysis or other event in question have the following interactive roles and responsibilities:

Water Quality Laboratory Specialists (LS): Must be able to recognize non-conformance and immediately notify the WQLS. The LS work with the WQLS and the QAO to resolve the problem. Each LS is responsible for correcting problems that might affect quality, as well as for providing information to the WQLS that is required for documentation of the corrective actions.

Water Quality Laboratory Supervisor (WQLS): Must review all analytical and QC data for reasonableness, accuracy and clerical errors. The WQLS is responsible for monitoring QC charts, for conducting internal systems audits, for ensuring strict adherence to all quality assurance protocols (e.g. strict adherence to SOPs) as well as for evaluating and responding to the results of external system audits. In an out-of-control event, the WQLS works with the QAO to implement corrective actions. The WQLS ensures that the corrective action documentation is initiated and submitted to the correct persons.

Quality Assurance Officer (QAO): Reviews all aspects of the water quality monitoring effort to ensure that nonconformance events which may have been unnoticed by the LSs or the WQLS are identified for corrective actions. The QAO works with the WQLS to identify the source of the problem and the necessary corrective actions are taken. The QAO ensures that adequate corrective action, documentation and reporting protocols are followed by all personnel. The QAO must review and approve of all corrective action reports and submit them to the Project Manager if appropriate.

Project Manager (PM): Ensures that all reports are reviewed by appropriate parties (i.e. WQLS, QAO, Project Coordinator and/or the project scientists, as appropriate) prior to submittal. The PM ensures that all major corrective actions reported to the PM have been implemented/documented and is responsible for ensuring that major problems and resulting corrective actions are reported to VA DEQ and CBPO.

19.0 Reports

The final product in any environmental study is a comprehensive report which addresses the issues of concern through the use of validated data. The validation process must be included in the final report with the appropriate QA data and observations. There are several times when QA reporting procedures must be implemented.

Corrective Action Reports

The first reporting procedure is when any component of the study is considered out-of-control, and cannot be quickly corrected by the technician and all effected samples reanalyzed with corrected situation. The corrective action for some of these situations is provided in Section 18.0 and must take priority over all other activities. Situations may occur that are not anticipated and are not covered in Section 18.0. The reporting procedure begins with the first individual to observe an out-of-control situation. The individual begins a reporting process with immediately notifying their supervisor. The WQLS, QAO and/or Project Manager determines if the event can be resolved without loss of valid data. If an analysis or observation cannot be reproduced to produce valid and representative data, either the Project Manager or WQLS notifies the VA DEQ Project Officer and CBP Data Manager in the submittal letter about any missing data and the cause, and the remaining data are submitted. If it is a one time occurrence that is not expected to occur again, this will be the only documentation needed. If it is a recurring problem, then corrective action must commence and be documented.

If the analysis or observation can be reproduced, a different scenario is followed. The Project Manager, QAO, and/or the WQLS and the individual technicians involved follow the corrective actions prescribed in Section 18.0 or other appropriate action to resolve the problem. If a corrective action report is needed it will be provided to the WQL Supervisor. If a procedure modification tracking form (Figure 18.1) is needed it will be provided to VA DEQ and the WQL Supervisor. The response report will include the actions taken to resolve the immediate problem and the actions taken to ensure that the problem is permanently resolved.

QAO Data Quality Assessments

The second reporting procedure occurs when data submittal reports are generated. All analytical and QC data are reviewed by the WQLS. When questionable data is identified, the data is submitted to the QAO for review. It is the responsibility of the WQLS, working with all of the personnel assigned to the study, to report the current assessment of data accuracy, data precision and completeness of data acquired to date.

Water Quality and QA Data Reporting

Once questionable data is released by the QAO, the data are returned to the Water Quality Laboratory for reporting as described in Section 15.0. The final data product as well as pertinent QA information are submitted to the VA DEQ and CBPO through a data submittal package consisting of: 1) DUET compatible data files to a database site maintained by CBPO; 2) DUET compatible QC data files to a database site maintained by CBPO; and 3) a Data Submission letter by electronic e - mail. The data submission letter provides all pertinent identifiers and QA information to the funding agencies. The data submittal packages are submitted in 60 day intervals starting in October 2018 and then in December 2018, February 2019, April 2019, June 2019 and August 2019.

Semi-Annual Progress (Status) Report (Figure 19.1)

Semi-Annual Progress Reports represent a third QA reporting event. These reports are submitted to the VA DEQ within fifteen days following the end of each calendar 6 month period, starting in January of 2019. These reports summarize the progress of the monitoring program and the conformance to collection, analytical and data submittal schedules, as well as providing a detailed synopsis of QA information including the CBP QA Checklist. This report will be submitted electronically.

Other Reports

Invoices
Invoices will be submitted to VA DEQ following the format provided by that office and will be submitted in accordance with the schedule provided in Section 9.0 of this WQAPP.

Cruise Reports
Report summarizing the results of and events which occurred during the 15 Mainstem field sampling events (cruises) will be submitted to the VA DEQ in the format provided by that office.

CSSP Data Reports
The results of analyses conducted by the WQL for the CSSP will be reported to the Data Integrity Workgroup electronically.

Data Analysis Reports and Special Studies
The guidance for data analysis reports for interpretation of data collected under this study, including but not limited to format, style, content and direction will be provided by the VA DEQ Project Officer. These reports will be negotiated as amendments to this WQAPP and may require modifications to this WQAPP.

Published Manuscripts
Any published manuscripts which utilize data collected in whole or in part under this monitoring program will be forwarded to DEQ. The WQL intends to coordinate any publication efforts with VA DEQ Project Officer in advance.

CHESAPEAKE BAY WATER QUALITY MONITORING PROGRAM

Semi_Annual Report for the Period of _____

Organization: Water Quality Laboratory
Project Manager: Dr. J. R. Donat
Project Coordinator: Mr. M.F. Lane
Laboratory Supervisor: Ms. S.C. Doughten

I. Summary Statement
II. Conformance to Sample Collection Schedule
III. Analytical Status
IV. Data Submittal
V. Quality Assurance/Quality Control Status
Accuracy, Precision, Internal Performance Audits

VI. Statement of Work Planned for Next Quarter

Table 1. Virginia Mainstem Water Quality Component
Stations, Parameters to be Measured at Each Station,
Frequency of Sampling

Quarterly Report QA Checklist

YES NO

1. Changes in organizational structure.
2. Changes in staffing arrangements.
3. Performance in external audits.
4. Problems with field/lab blanks.
5. Changes in calibration procedures or frequency.
6. Changes in analytical procedures.
7. Changes in data reduction, validation or reporting.
8. Internal quality control.
9. Preventive maintenance problems.
10. Problems with sample acceptance criteria.
11. Changes in procedures to assess data precision and accuracy.
12. Corrective actions applied.
13. Revisions of SOPs, QA manuals, etc.
14. New instrumentation.
15. Training.
16. Change in current percent of samples duplicated.
17. Change in the current percent of samples spiked.

Figure 19.1 Format for Water Quality and QA Data Report

Appendix 1:

CBMP Data Management Plan

CHESAPEAKE BAY PROGRAM
WATER QUALITY MONITORING PROGRAM
DATA MANAGEMENT PLAN

Water Quality Laboratory

College of Sciences
Old Dominion University
Norfolk, Virginia 23529-0456

REVISED January 2015

Version # 3

Revised by Suzanne Doughten

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I. General Overview

All data received that requires data entry is entered twice, compared and edited until the two copies match exactly. Visual inspection of data entry for volume filtered is performed for the total suspended solids, particulate carbon/particulate nitrogen and chlorophyll/phaeophytin, for entering the stations and depths to the light data and vertical fluorometry data, and for the transects for horizontal fluorometry. Corrected data sets are uploaded to the Access®database, and then screened using an Access®database program designed to evaluate the data. The criteria to evaluate the data are as follows: values below the method detection limit and between the detection limit and reporting limit are qualified, and values above an ecologically realistic value are also identified. If questionable values are identified, the original bench sheets and QC data are re-examined, and the values are validated or corrected as necessary. Problem codes are added if needed. Finalized data sets are exported to an Access®database, and the database is sent to a site maintained by the Chesapeake Bay Program Data Center. The DUET (Data Upload Evaluation Tool) format data sets are then checked for discrepancies in format or violations of quality control range checks before the data is accepted into the Water Quality Database at the Chesapeake Bay Program Data Center. Any problems with the data sets are identified and a checklist indicating these problems is forwarded to the WQL by officials from the Chesapeake Bay Program. Any problems with data set format are corrected by modifying the Access®database programs. Data sets are then regenerated with the proper format and resubmitted to DUET. If quality control checks indicate values outside of the expected ranges, the values identified are checked by the WQL laboratory supervisor and their validity confirmed. If any values are determined to be invalid, corrections are made to the Access®database. The data are resubmitted. The confirmation process continues until no corrections to the data sets are required.

Documentation files are no longer required by the Chesapeake Bay Program. Documentation is recorded in the data tables submitted in Access®, and in a data submittal letter which is sent to the Chesapeke Bay Program via electronic mail. Validity of the documentation is checked by officials at the Chesapeke Bay Program. If format discrepancies or content errors are found, the WQL is contacted by electronic mail, and any appropriate changes are made and the effected files are resubmitted.

II. Initial Visual Screening

The Water Quality Laboratory (WQL) generates data as both ASCII format data sets downloaded directly from instruments and photocopies of laboratory and field bench sheets. ASCII format data files are visually inspected for potential errors. If any potential errors are discovered, validity of the data is confirmed, and any required corrections to the ASCII format files are made. Copies of the bench sheets are also visually inspected by the WQL supervisor. If mistakes or problems are found or suspected, the WQL laboratory personnel responsible for the entry is contacted, the errors confirmed and changes made to these copies, if applicable, are made before data entry is performed.

III. Data entry

Data entry from the bench sheets for applicable parameters is performed by qualified personnel using programs and overlay files developed using the Access®database forms developed in the WQL. The water quality data that are double entered are compared for differences between files using queries and macros developed in the Access®database. Differences in the data files are compared against the original data sheets and corrections to the data sets are made accordingly. Comparisons of the data sets are continued until there are no differences found between the two copies of the data set. All data entry is conducted on the ODU Network.

One copy of all of the corrected data sets is transferred to the applicable DUET format table in the Access®database.

IV. Screening Procedures

Screening analyses are run on the Access®database tables by the WQL Supervisor. These programs are designed to augment the initial visual screening and comparisons of the data by checking for repeated observations, values outside of the expected ranges for each water quality variables, and completeness of the data set. Results from the Access®queries are inspected by the WQL Supervisor to determine if changes to the raw data sets are required. Any recommended changes to the data sets are made and the screening programs are run until visual inspection of the screening results indicates that no errors are in the data set. Note that all changes indicated by screening analyses are performed on the data stored in the Access®database.

The screening programs do not identify any observations which do not meet the quality assurance range checks required by the Chesapeake Bay Program Data Center. These are run once the data is submitted using DUET. A QA report is generated by DUET which can be reviewed.

V. Access®Data Tables

Seven separate data tables in Access®are created for each submittal containing all of the required water quality data for the two month submittal period. The split sample analyses are submitted separately in Excel®

The naming scheme for the Access®database submitted is as follows:

O< F> < S> .MDB

where F is the first cruise number of the submittal period, S is the second cruise number of the submittal period.

Data set contents, variable names and formats comply with the data dictionary standards specified in the most recent version of the Chesapeake Bay Program Water Quality Database Design and Data Dictionary or other guidance provided by the Chesapeake Bay Program.

VI. Submittal of DUET Format Database

Once validity of the data tables are confirmed, an Access® database is created which contains the data for the months to be submitted. A data submittal package is prepared and submitted to the Chesapeake Bay Program through DUET and electronic mail. Contents of the submittal package include the following items:

1. A letter stating the purpose and contents of the data submittal package and why there is any missing observations in the data set (Figure 1). This letter contains a table listing the current and all previous data submittals for a given year (Figure 2).

2. The Access®database which contains the DUET format data tables and the quality control tables.

3. A metadata file which contains changes to the monitoring program through the years, data quality information, parameter and method information, and the date ranges the metadata covers.

VII. Data Validation and Signoff

A series of screening analyses are conducted on all data sets received by contracting agencies. These analyses identify inconsistencies in the required CBP data set format, missing values, and range checks. Any errors or range check violations are summarized in a QA report which can be downloaded or viewed in DUET. In addition the Data Set Checklist and Signoff form (Figure 3) is sent via electronic mail to ODU and reviewed by the WQL Supervisor. If the range checks identify erroneous data, modifications are made to the Access®database tables and resubmitted following the procedures described above. If inconsistencies in data set format are identified, modifications to the Access®database are made to correct these errors and the Access® database tables are recreated. The new Access® database tables are then resubmitted to the Chesapeake Bay Program. The validation process is repeated for all resubmitted data and is continued until no errors are found in the submitted data sets. After completion of the validation process, a completed copy of the Data Set Checklist and Signoff is sent to the Chesapeake Bay Program confirming that the WQL has signed off on the data submitted. All communications for this process are conducted via electronic mail.

VIII. Backup Procedures

Finalized Access®database are kept on a directory on the ODU Server Network and are backed up daily by ODU Information Technology Services. Each week a backup is made and stored for one month. Each month a back up is made that is stored for one year.

IX. Additional Data Set Formats

Chesapeake Bay Program data sets can be provided to the Virginia DEQ and the EPA in formats other than the one currently produced if a change order to the contract is provided by the Virginia DEQ. The WQL currently has the capability to produce data sets in the following formats: 1) ASCII format fixed field data sets with specific column locations for each variable 2) ASCII format delimited files with quotation delimiters surrounding character variables and all variables separated by a single comma delimiter, 3) ASCII format delimited files with pipe delimiters and 4) Access®database structure for import into the DUET database management software package.

Enclosed is the summary of the submittal of the field and water quality data for the Chesapeake Bay Mainstem Monitoring Program cruises BAY 632 and BAY 635 for the period of September and October, 2014. The data file specifically referenced is O632635.mdb. This database is in Microsoft Access® in the DUET format. It was uploaded to DUET on the Chesapeake Bay Program web site.

On the September cruise could not sample station CB7.1N due to high winds and seas which made sampling this station unsafe.

We have subjected these data to a thorough QA review and have resolved all problems with the following exceptions:

Cruise #	Parameter	Site	Comment
BAY 632	LIGHT/ KD	ALL STATIONS	There were problems with the light air sensor mounted on the research vessel. Collected an air reading from the underwater sensor on deck at each station before deploying the rosette. Corrected the value in the laboratory for the multiplier in air after the cruise. These values were used for all EPAR_S values in the WQ_KD table.
	LIGHT/ KD	CB5.4W	2.0 meter reading was deleted as invalid.
	LIGHT/ KD	CB7.4 & EE3.5	0.5 meter reading was deleted as invalid.
	LIGHT/ KD	WE4.1, CB5.5 & CB5.4	0.5 and 1.0 meter readings were deleted as invalid.

Also included in this letter is a table summarizing the status of data submittals for the Chesapeake Bay Water Quality Monitoring Program (Table 1). This table gives the time period covered, the CBP cruise number and the name of the database on which the data are located. Please call me at (757) 451-3043 if you have any questions about this data submittal. The data are located in a Microsoft Access® database at the FTP site maintained by the Chesapeake Bay Program.

Figure 1. An example of letter enclosed with the data submittal package listing the cruise numbers, QC data table names, name of the submittal database and unresolved problems for each cruise. Example shown refers to the submittal of the Mainstem collections for period of September and October, 2014 completed on December 18, 2014.

Table 1. Summary of water quality and QA/QC data submittal status for the Chesapeake Bay Program Water Quality Monitoring Program.

Dates	CBP Cruise #'s	Data Set Names	Data Set Type	Method of Data Transfer	Date Submitted	Comments
FEB 14	618	O618.MDB	Water Quality	upload to DUET	04/29/14	
FEB 14	618	0618.MDB	QA/QC	upload to DUET	04/29/14	
MAR to APR 14	621,622	O621622.MDB	Water Quality	upload to DUET	06/30/14	
MAR to APR 14	621,622	O621622.MDB	QA/QC	upload to DUET	06/30/14	
MAY to JUN 14	624,626,627	O624627.MDB	Water Quality	upload to DUET	08/22/14	
MAY to JUN 14	624,626,627	O624627.MDB	QA/QC	upload to DUET	08/22/14	
JUL to AUG 14	628,629,630,631	O628631.MDB	Water Quality	upload to DUET	10/29/14	
JUL to AUG 14	628,629,630,631	O628631.MDB	QA/QC	upload to DUET	10/29/14	
SEP to OCT 14	632,635	O632635.MDB	Water Quality	upload to DUET	12/18/14	
SEP to OCT 14	632,635	O632635.MDB	QA/QC	upload to DUET	12/18/14	

Comments:

Figure 2. Example water quality submittal summary table.

MONITORING DATA STATUS

MEDIA: WQ PROGRAM: VA LOCATION: Mainstem

RECEIVED DATE: 8/22/2014 REPORT DATE: 08/27/2014
STAFF NAME: M. Mallonee LAST ENTRY: 08/27/2014
DATA SHORT NAME: May - June 2014 ODU Mainstem, QC files

DATA SUBMISSION INFORMATION
SUBMITTER NAME: Suzanne Doughten DATA START DATE: 05/12/2014
ORGANIZATION: VA ODU DATA END DATE: 06/25/2014
FILE, TAPE, DISK: Online DUET
DATA FORMAT: ACCESS Database
IN FILE LOCATION: O624627.mdb
COMMENTS:

DATA PROCESSING INFORMATION
STATUS: Accepted
OUT FILE LOCATION: W1SQLP\wq_1.mdb
ACTIVITY LOG: (Keep daily records of activity)

```
Uploaded   8/22/2014 11:50:31 AM
Begin Data Checks 8/22/2014 11:50:41 AM
Passed Data Checks 8/22/2014 11:50:59 AM
Accepted   8/26/2014 2:36:09 PM
Begin Import 8/26/2014 9:01:58 PM
Transferring Water Quality Measured 8/26/2014 9:02:43 PM
Imported into the Water Quality Database 8/27/2014 2:00:56 AM
```

08/29/2014 - Sign off to Peter Tango for VA ODU

The ODU dataset containing May and June 2014 Mainstem monitoring data was processed through DUET, and has been added to the database.

Please call or e-mail me if you have any questions. The data set checklist has been reviewed by the data originator and the dataset is (check one):

_X_ Accepted entirely as is
_____ Accepted with limited modifications (noted below)
_____ Rejected (reason noted below)

Suzanne C. Doughten — August 29, 2014
Signature of data originator — Date

Figure 3. Example Data Set Checklist and Signoff Form for Chesapeake Bay Program Water Quality Submittals.

Appendix 2:

SOP Chesapeake Bay Mainstem Deployment

STANDARD OPERATING PROCEDURE FOR CHESAPEAKE BAY PROGRAM CRUISE DEPLOYMENT

REVISED BY: Heather Wright

FOR:

Water Quality Laboratory
College of Sciences
Old Dominion University
Norfolk, Virginia 23529-0456

DOCUMENT FILE LOCATION: Water Quality Laboratory

EFFECTIVE DATE: September 15, 2014

TERMINATION DATE:

APPROVED BY:

John R. Donat, Ph.D. Date
Water Quality Laboratory Director

Suzanne C. Doughten Date
Water Quality Laboratory Supervisor

DISCLAIMER: This SOP applies to cruise deployment for field monitoring and sample collection in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

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1. LOCATION

This procedure will be used by Water Quality Laboratory (WQL) staff during cruise deployment on the Chesapeake Bay.

2. PURPOSE

This procedure is designed to ensure that all protocols are followed during physical profile measurements, sample collection, and onboard processing of samples during field operations. The intended data user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program (CBMP). These data will be used to assess whether the multi-jurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of the data, and its inclusion in computer models of the Chesapeake Bay. Trend analyses are also performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and its tributaries.

3. APPLICABILITY

This procedure is applicable to field operations conducted on the Chesapeake Bay by the WQL.

4. OVERVIEW

This SOP is based on the procedures developed by Old Dominion University in support of field operations. The physical profile of the station is measured using a sonde calibrated for temperature, salinity, conductivity, pH, depth, and dissolved oxygen. Typically a rosette equipped with go-flow bottles and a fluorometeric probe, along with the WQL sonde and light attenuation meter is used; alternatively, an array with a submersible pump, sonde with fluorometric probe, and light attenuation meter may be used. (Light attenuation readings may be collected separately from the physicochemical profile if needed. Vertical fluorometry may be collected through the fluorometer.). The equipment is positioned in the water column, readings and samples are collected according to each parameter's SOP. The water samples are processed as soon as possible. Continuous measurements of horizontal fluorometry data are obtained from a flow-through water system while underway between stations (when possible on the vessel).

5. DEFINITIONS AND ABBREVIATIONS

DOC:	Dissolved Organic Carbon
NH4:	Ammonia
NO3:	Nitrate
NO2:	Nitrite
OPO4:	Orthophosphorus
PC:	Particulate Carbon
PN:	Particulate Nitrogen
PP:	Particulate Phosphorus
SI:	Silicate
TDN:	Total Dissolved Nitrogen
TDP:	Total Dissolved Phosphorus
TSS:	Total Suspended Solids
L:	Liter(s)
mL:	Milliliter(s)
g:	Gram(s)
mg:	Milligram(s)
mg/L:	Milligram(s) per liter
N:	Normality of the chemical solution (g/L)
M:	Molarity of the chemical solution (mol/L)
CBP:	Chesapeake Bay Program
CBMP:	Chesapeake Bay Monitoring Program
USEPA:	United States Environmental Protection Agency
HDPE:	High-density polyethylene
reagent water:	Resistivity > 10 megohm-cm

6. CHEMICALS USED

All chemicals must be analytical grade or of a higher purity except as noted.
Magnesium carbonate ($MgCO_3$) - (for Chlorophyll preservation)

6.1 Before preparing reagents: wipe counter, balance, and balance table with reagent water.

6.2 On each reagent bottle the following information must be recorded:
Reagent Identification (name) and Concentration:
Date prepared:
Prepared by:
Expiration date: (put N/A if there is no holding time)
Storage requirements: (i.e. ambient temperature, refrigerated)
Reagent log number

6.3 Inspect the reagents required for sufficient quantity, expiration dates, and appearance.

If necessary, remake the following reagent:
Magnesium carbonate suspension: Measure 100mL of reagent water using a graduated cylinder. Add 1g of finely powered magnesium carbonate and shake. Shake immediately before each use. Shelf life N/A.

NOTE: Reagent containers are used only for the intended reagent and are reused. Before fresh reagents are added, the containers are rinsed 3 times with reagent water and 3 times with the fresh reagent.

7. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

8. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

9. ON BOARD PREPARATION

The following is a guide for setting up onboard the R/V Fay Slover but locations may change as necessary. If another research vessel is utilized, equipment is positioned according to need and maximizing efficiency.

9.1 ON DECK:

9.1.1 Sample Carboys - Four carboys for use in phytoplankton sample collection.

9.1.2 Sampling Equipment

9.1.2.1 When using the Submersible pump - *only used if the rosette is unavailable* The barrel containing the submersible pump, hose, and cables is positioned next to the winch. The discharge end of the hose is routed to the lab if collecting there, alternatively the samples can be collected on deck. The pump, light meter, and sonde (with cables) are attached to an array along with a weight.

9.1.2.2 When using the rosette - Mount the field instruments (with cables) on the rosette using duct tape and/or zip ties. See the light attenuation and sonde SOPs for details. *NOTE: Do not use a clamp to attach the sonde, it can cause damage.*

9.1.2.3 Mount the SA light sensor, refer to the current light attenuation SOP for details. *NOTE: One is typically left mounted on a mast.*

9.1.2.4 The cables for the light meters and sonde are routed down into the laboratory area and connected to the light and sonde dataloggers.

9.1.3 Secchi Disk - Placed by the ladder for easy access.

9.1.4 Coolers

9.1.4.1 Place 2 under the covered portion of the deck, by the wind shield, one for WQL -09 samples and the other for phytoplankton.

9.1.4.2 2 Empty, for sample transport, are tied up on the bow for storage.

9.1.4.3 1 (or more) with extra ice, on the stern, out of the way as much as possible.

9.1.5 Phytoplankton sample bottles - placed for easy access by the chief scientist.

9.2 IN THE LABORATORY:

9.2.1 Vacuum pumps - placed on the shelf. A vacu-guard® (or equivalent) is attached to the pump hose to protect the pump from water damage.

9.2.2 Filtration manifolds and waste flasks - placed on the counter with hosing attached to pumps. Ensure the waste water never enters the pump.

9.2.3 Graduated cylinders, filters, bench sheets, vials, and any other necessary equipment are placed for easy access during filtration.

9.2.4 All field and light/fluorometry sheets, field log, sonde datalogger, light datalogger, and any other necessary equipment are placed for easy access by the chief scientist.

9.2.5 A carboy containing reagent water for rinsing is placed for easy access during the cruise, typically on a table by the escape ladder.

9.2.6 All of the sample bottles, redundant equipment, and extra reagent water and supplies are stored forward in the boat.

10. DAY OF THE CRUISE

10.1 The final call to depart or stand down due to weather is made as early as possible if it was in question the previous day.

10.2 The Chief Scientist informs the captain/boat crew of the WQL crew present and readiness to depart.

10.3 Onboard preparation is performed, if not completed earlier.

10.4 The Chief Scientist and Captain confirm planned route, technicians are informed.

10.5 Sonde DO and depth values are verified. If not within specifications, the sonde is either recalibrated or replaced. *NOTE: DO recalibration is only preformed on the vessel if both YSIs are out of calibration.*

10.6 The chief scientist records the following on the Field Summary Sheet: (1/day)

- cruise identification
- date and day count of the cruise
- general weather statement
- names of chief and scientific crew
- station ID, arrival and departure time, any comments
- sonde calibration and post cruise calibration check information (this is done in the laboratory after the cruise)
- storage thermometer and temperature information
- daily DO check
- instruments used and depth check

11. ON-STATION

11.1 DETERMINE THE SECCHI DEPTH

A 20 cm secchi disk is attached to a weighted line marked in 0.1 m increments. Readings are determined on the shady side of the boat. If overcast then at discretion of technician, notify Chief Scientist if no shade present.

11.1.1 Lower the secchi disk until the black and white quadrants are no longer distinguishable. Note this depth.

11.1.2 Slowly retrieve the secchi disk, noting the depth in which the black and white quadrants are once again distinguishable.

11.1.3 The secchi depth is the average of the depths observed in steps 11.1.1 and 11.1.2. This depth is recorded onto the field data sheet to the closest 0.1 m. Report value to the chief scientist.

11.2 COLLECT STATION DATA

The Chief Scientist records the following on the field data sheet at each station:
(See Appendix 2 for a completed sheet example)

- station identification
- Field Chief identification
- research vessel identification
- cruise identification
- date and arrival time on station (in military time)
- GPS location
- station depth
- secchi depth
- weather code (circle pre-defined condition category), record wind direction as N, NE, NNW, etc. Tidal Stage is circled.
- pycnocline and/or biology calculation, also marking the “phytoplankton collected” box when appropriate. (See Appendix 1 for designated stations)
- physicochemical profile: Position sonde at desired depth, allow readings to stabilize, record values both electronically and on the field sheet. Except the 1m reading, collect data from the bottom to the surface, every 2 meters (plus the bottom) for depths greater than 15 meters and every 1 meter for depths less than 15 meters. *NOTE: June to September, collect every meter for depths greater than 15 meters if there is a DO change of more then 1.0 mg/L or a pycnocline.*

11.3 Light attenuation and fluorometry profile

Data are recorded on the Light Attenuation and Fluorometry Field Data Sheet. Refer to current Light Attenuation and Fluorometry SOPs for further instruction.
NOTE: Vertical fluorometry is typically collected using the probe on the rosette, or the probe on the sonde if the rosette is unavailable. If neither probe is available, the submersible pump is attached to the fluorometer for vertical profile collection.

11.4 Collection of ambient water samples

Samples are collected at the surface and bottom, and above pycnocline (or 1/3 depth of station) and below pycnocline (or 2/3 depth of station), at designated stations.

When a submersible pump is used, the "old" water must be flushed prior to collection of sample. Pumping water for ~1 minute is usually sufficient but should be verified periodically, especially when changing hose sizes or pump types. Sample carboys are rinsed twice, including flushing the stopcock, before a sample is collected.

11.4.1 The surface sample: collected 1 m below the surface, into the sample carboy labeled "S" if collecting with a pump, taking note of the go-flow bottle number if using the rosette.

11.4.2 The bottom sample: collected 1m above the bottom, into the sample carboy labeled "B" if collecting with a pump, taking note of the go-flow bottle number if using the rosette.

11.4.3 Calculate the pycnocline threshold for further sample and/or biology collection if necessary. Samples are collected in carboys labeled "AP" & "BP" if collecting with a pump, taking note of the go-flow bottle number if using the rosette.
NOTE: for non-pycnocline stations, carboys AP = "1/3" and BP = "2/3"

$$\left[\left(\left|\frac{}{S_{conductivity}} - \frac{}{B_{Conductivity}}\right|\right) \div \left(\frac{}{B_{Depth}} - \frac{}{S_{Depth}}\right)\right] \times 2 = \text{pycnocline threshold}$$

$S_{Conductivity}$ = Surface Conductivity $B_{Conductivity}$ = Bottom Conductivity
B_{Depth} = Bottom Depth in meters S_{Depth} = Surface Depth in meters

Upper Pycnocline: From the surface down, the shallowest depth where change in conductivity ≥ the Pycnocline Threshold

Lower Pycnocline: From the bottom up, the deepest depth where the change in conductivity ≥ the Pycnocline Threshold

No Pycnocline if hreshold < 500 µmho/cm, or no depth interval > threshold value.

If a pycnocline is present, samples are collected at 1 m above the upper limit (AP) and 1 m below the lower limit (BP) of the identified pycnocline depth(s). If a pycnocline is not present or the threshold was not reached, samples are collected at the nearest 1/3 and 2/3 the depth of the water column (at physicochemical profiling depths).

11.5 The Chief Scientist ensures all samples are collected, and checks recorded data and calculations for completeness, legibility, and verification that all data appears to be valid. Any measurements in question will be repeated for verification. Once complete the equipment is retrieved and the boat can proceed to the next station.

12. SAMPLE PROCESSING

The samples should be filtered immediately after collection. If not possible, store samples in a refrigerator or on ice. Storage temperature is documented on the CBP monitoring form. PC/PN, nutrient and chlorophyll samples must be filtered by the next day, TSS/PP samples within 7 days.

12.1 SUBSAMPLE ALIQUOTS

Samples and subsamples are not discarded until all processing is complete.

Prior to collection a 2L HDPE bottle labeled with the Project ID, Sample ID, and Sample log number with the -00 extension is labeled for each of the sample layers required (S, AP or 1/3, BP or 2/3, and B).

A set of 1L HDPE bottles for PC/PN filtration and 1L brown HDPE bottles for Chla filtration are labeled S, AP, BP, and B for use at each station. These bottles are rinsed with reagent water after use and are reused. If subsequent sample processing can not be performed prior to the next station, additional labeling of the subsamples must occur. *NOTE: The -00 aliquot can also be used for PC/PN filtration.*

12.1.1 Verify the bottle labels correspond to the carboy or go-flow.

12.1.2 Completely rinse each bottle 3 times with the sample, discarding the rinsate.

12.1.3 Fill each sample bottle, leaving about an inch of head space.

12.1.4 As soon as possible, mix the magnesium carbonate suspension and add 1 mL per liter of sample to the 1L brown bottle collected for chlorophyll. Invert the sample to mix.

12.2 FILTRATION AND NUTRIENT WATER COLLECTION

Refer to the following SOPs for labware and filter preparation requirements:

- Standard Operating Procedure for Total Suspended Solids Dried at 103-105°C
- Standard Operating Procedure for Chlorophyll: Spectrophotometric Method
- Standard Operating Procedure for Particulate Carbon and Nitrogen in Water and Seawater Using Flash Combustion/Chromatographic Separation and a Thermal Conductivity Detector
- Standard Operating Procedure for Particulate Phosphorous in Seawater and Brackish Water using the LACHAT QuickChem 8500 Series 2 FIA+

12.2.1 Filter for TSS/PPO_4 and nutrient water collection

The sample collected in the 2L bottle is used for concentration of total suspended solids (TSS) and particulate phosphate (PPO_4), as well as collection of nutrient water**.**

12.2.1.1 Filter for TSS:
Using forceps, transfer a pre-rinsed and pre-weighed 4.7 cm Whatman® GF/F glass fiber filter, wrinkled side up, to the filtration tower.
NOTE: Each filter for TSS must be pre-weighed and therefore pre-labeled with a unique identifier for future reference. Filters are prepped for each anticipated sample as well as a number of extra filters for use if needed.

12.2.1.2 Turn the vacuum on, moisten the filter with reagent water into the waste flask. Move the filtration tower to collect the filtrate for nutrient analysis.

12.2.1.3 Mix the sample thoroughly by inversion, immediately rinse the graduated cylinder twice with the sample, discarding the rinsate.

12.2.1.4 Measure the volume to be filtered into the rinsed graduated cylinder. *NOTE: A maximum volume of 500mL per filter is used for the CBMP. A gauge as to how much to filter: once the sample is measured, if the bottom of the graduated cylinder can not be seen, it probably will not finish in the alloted time.*

12.2.1.5 Filter the sample using ≤20 in. Hg vacuum pressure for no longer than 10 minutes. The vacuum pressure and time is to avoid cell damage. Record the volume and time filtered on the benchsheet under "TSS (-07)". *NOTE: If filtration exceeds 10 minutes: discard filter and remaining sample (collected filtrate may be saved), rinse filtration tower two times with reagent water into the waste flask, complete steps 12.2.1.1- 12.2.1.5 using a lesser sample volume. Record the new volume and time on the total suspended solids/particulate phosphate data benchsheet, initial and date the change. If the sample cannot be refiltered, note the time it took to filter on the filtration sheet.*

12.2.1.6 When complete, move the filtration tower to collect in the waste flask.

12.2.1.7 Completely rinse the graduated cylinder with reagent water, pour into the filtration tower. Repeat.

12.2.1.8 Rinse the tower and filter 2 more times with approximately 20 mL of reagent water from a squirt bottle.

12.2.1.9 Using forceps and avoiding the filtrate, fold the filter in half and place back in the filter holder labeled with the Project ID, sample ID, and sample log number. *NOTE: If an extra filter was used, record the sample ID and log number on the label.*

12.2.1.10 Transfer to a freezer as soon as possible. If a freezer is not available, store filter holders on ice, protected from water.

12.2.1.11 Completely rinse the empty filtration tower one time with reagent water before filtering the next sample.

12.2.1.12 Filter for PP:
Repeat steps 12.2.1.1 - 12.2.1.11 for each sample using an unweighed filter prepped for particulate phosphate analysis. Record the volume filtered on the benchsheet under "PP(-04)".

12.2.1.13 Duplicates:
Filter one randomly chosen sample for every 10 to 20 samples, following steps 12.2.1.1 - 12.2.1.11 for both TSS and PP, record the time and volume filtered on the benchsheet. *NOTE: PP dup info is recorded in the "PP DUP(-04)" column, TSS dups have a separate line due to pre-weighing.*

12.2.1.14 Nutrient water

The water collected in the filtration flask is divided as follows:
1 HDPE bottle, split code -01 (for NO3, NH4)
1 HDPE bottle, split code -02 (for OPO4, NO2)
1 HDPE bottle, split code -08 (for TDN, TDP)
1 HDPE bottle, split code -09 (for Si)
1 muffled glass bottle, split code -10 (for DOC, S at biology stations only)

If more than one trap flask was used for a single sample, combine the filtrate (see section 13.4 for exception).

- Rinse each sample bottle three times with the filtrate, discarding rinsate.
- Fill each sample bottle, leaving about an inch of head space.
- Put the -09 bottles on ice, packed to the neck of the bottle.
- Freeze all other bottles. If no freezer available, pack on ice to the neck.
- Discard any remaining filtrate, rinse the filtration flask three times with reagent water before filtering the next sample.

12.2.2 Filter for Chla/Pheo:

The sample collected in the 1L brown bottle is used for the concentration of chlorophyll.

12.2.2.1 Using forceps,transfer a 4.25 cm Whatman®GF/F glass fiber filter, wrinkled side up, to the filtration tower.

12.2.2.2 If not already added, add 1 mL of 1% $MgCO_3$ to the sample. Mix the sample thoroughly by inversion, immediately rinse the graduated cylinder twice with the sample, discarding the rinsate.

12.2.2.3 Measure the volume to be filtered into the rinsed graduated cylinder.
NOTE: A maximum volume of 500mL per filter is used for the CBMP. A gauge as to how much to filter: once the sample is measured, if the bottom of the graduated cylinder can not be seen, it probably will not finish in the alloted time.

12.2.2.4 Filter the sample using ≤10 in. Hg vacuum pressure for no longer than 5 minutes. The vacuum pressure and time is to avoid cell damage. Record the volume and time filtered on the benchsheet. *NOTE: If filtration exceeds 5 minutes: discard filter and remaining sample, rinse the filtration tower three times with reagent water, complete steps 12.2.2.1 through 12.2.2.4 using a lesser sample volume. Record the new volume and time on the benchsheet, initial and date the change. If the sample cannot be refiltered, note the time it took to filter on the filtration sheet.*

12.2.2.5 Remove the vacuum pressure as the sample leaves the filter. Avoiding the filtrate, fold the filter and wrap in a piece of aluminum foil labeled with the Project ID, Sample ID, and sample log number.

12.2.2.6 Place in a freezer or store on ice protected from water.

12.2.2.7 Completely rinse the filtration tower and graduated cylinder three times with reagent water before filtering the next sample.

12.2.2.8 Duplicates: Filter one randomly chosen sample for every 10 to 20 samples, following steps 12.2.2.1 - 12.2.2.7.

12.2.3 Filter for PC/PN:

The sample collected in the 1L HPDE bottle is used for the concentration of particulate carbon and nitrogen.

12.2.3.1 Using forceps, transfer a muffled 25mm Whatman® GF/F glass fiber filter, with the wrinkled side up, to the filtration tower.

12.2.3.2 Mix the sample thoroughly by inversion, immediately rinse the graduated cylinder twice with the sample, discarding the rinsate.

12.2.3.3 Measure the volume of sample to be filtered into the rinsed graduated cylinder. *NOTE: A maximum volume of 250mL per filter is used for the CBMP. A gauge as to how much to filter: once the sample is measured, if the bottom of the graduated cylinder can not be seen, it probably will not finish in the alloted time.*

12.2.3.4 Filter the sample using ≤10 in. Hg vacuum pressure for no longer than 10 minutes. The vacuum pressure and time is to avoid cell damage. Record the volume and time filtered on the benchsheet. *NOTE: If filtration exceeds 10 minutes: discard filter and remaining sample, rinse the filtration tower three times with reagent water, complete steps 12.2.3.1 through 12.2.3.4 using a lesser sample volume. Record the new volume and time on the benchsheet, initial and date the change. If the sample cannot be refiltered, note the time it took to filter on the filtration sheet.*

12.2.3.5 Using forceps and avoiding the filtrate, fold the filter and place in a muffled glass vial labeled with the Project ID, Sample ID, and sample log number.

12.2.3.6 Place in a freezer or store on ice protected from water.

12.2.3.7 Rinse the graduated cylinder and filtration tower three times with reagent water.

12.2.3.8 Duplicates and Triplicates:
Filter each sample in duplicate plus one randomly chosen sample filtered in triplicate for every 10 to 20, following steps 12.2.3.1 - 12.2.3.7.

13. OTHER SAMPLES COLLECTED

13.1 GRABS

Chlorophyll samples collected from the fluorometer for underway fluorometry calibration. One 1L brown HDPE bottle is used per cruise day, rinsed between uses. Samples are filtered following steps 12.2.2.1 - 12.2.2.7, duplicate grab samples are not collected. Typically 5 grab samples are collected per cruise day, with a minimum of 3 and collected at a range of voltage values from the fluorometer (if possible).

13.2 FIELD REPLICATE (co-located sampling, 2 at surface and 2 at bottom)

Collected at station CB7.4N. The sample carboys or go-flows are filled. Note bottle number if go-flows are used. For carboys: the "S" will be used as S-1, "AP" as S-2, "B" as B-1, and "BP" as B-2. These are treated as completely separate samples. This is to measure spatial variability, if any.

13.3 FIELD BLANKS

Near the end of each sampling day a field blank is processed for all parameters with the exeption of DOC, with one being collected per cruise (see below). In order for the necessary information to be properly and validly obtained, no special treatment or handling such as extra rinsing of the filtration apparatus may occur. Filter and portion nutrient water and DOC as described in section 12. Field Blanks are not duplicated.

- TSS/PPO4 and nutrient water: filter 1L of reagent water through a filter prepped for TSS analysis. No separate PP filter is collected. One field blank per month is collected for DOC on a day biology stations are collected.
- Chla: Rinse the grab bottle as normal and fill with reagent water, add 1mL of $MgCO_3$, filter 500mL.
- PC/PN: filter 250mL of reagent water

13.4 FIELD SPLITS

Two field split samples are collected from the nutrient water, chosen at random on different cruise days. Sample processing is as described in section 12 except the nutrient water collection flasks are not combined and 2 sets of sample bottles are collected. The parameters collected for the field split are NO_{23}, NH_4, OPO_4, NO_2, TDP, Si, and TDN. *NOTE: Only one field split per month is collected for DOC.*

14. LITERATURE CITED

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USEPA. 1983. Methods for Chemical Analysis of Water and Wastes. Method 330.5. U.S. Environmental Protection Agency. Cincinnati, OH. 521 pp.

APHA. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition. Method 10200 H. Chlorophyll. American Public Health Association. Washington, DC.

15. APPENDIX 1: STATIONS

Station Name	GPS NAD83	Location	typical depth	pycno cline	biology
CB5.4	37°48.00″ 76°10.50″	Deep Main Channel	~26m	X	
CB5.4W	37°48.798″ 76°17.706″	Mouth of Great Wicomico	~5m		
CB5.5	37°41.514″ 76°11.418″	Main Channel	~18m	X	
CB6.1	37°35.298″ 76°09.75″	Main Channel,Lower End off of Rappahannock River	~12m,	X	X
CB6.2	37°29.202″ 76°09.402″	Central Bay	~10m	X	
CB6.3	37°24.744″ 76° 09.468″	Central Bay Channel (Wolftrap)	~11m	X	
CB6.4	37°14.184″ 76°12.498″	Central Bay,Off York River mouth	~11m	X	X
CB7.1	37°41.01″ 75°59.40″	Eastern Shore Channel	~21m		
CB7.1N	37°46.506″ 75°58.494″	Tangier Sound Channel	~24m		
CB7.1S	37°34.872″ 76°03.498″	Eastern Shore Channel	~13m		
CB7.2	37°24.69″ 76°04.836″	Eastern Shore Channel	~21m		
CB7.2E	37°24.684″ 76°01.506″	Eastern Shore, Side Channel	~13m		
CB7.3	37°07.002″ 76°07.518″	Mainstem York Spit Channel	~13m	X	

CB7.3E	37°13.71″ 76°03.252″	Lower Eastern shore Channel South End, off old Plantation Fl	~17m,		X
CB7.4	36°59.73″ 76°01.248″	Baltimore Channel at Bay Bridge, Bay Mouth	~14m	X	X
CB7.4N	37°03.732″ 75°58.998″	North Channel at Bay Bridge, Bay Mouth	~13m		
CB8.1	36°59.712″ 76°10.698″	Lower Bay between James River mouth and Thimble Shoals Channel	~9m		
CB8.1E	36°56.832″ 76°02.112″	Thimble Shoals Channel at Bay Bridge, Bay Mouth	~19m		
EE3.4	37°54.498″ 75°47.502″	Pocomoke Sound	~4m		
EE3.5	37°47.784″ 75°50.682″	Pocomoke Sound Channel	~20m		
LE3.6	37°35.814″ 76°17.118″	Mouth of Rappahannock	~9m,		X
LE3.7	37°31.842″ 76°18.426″	Mouth of Piankatank	~7m		
LE5.5-W	36°59.928″ 76°18.81″	Mouth of James River	~7m,		X
WE4.1	37° 18.702″ 76° 20.802″	Mobjack Bay	~7m		
WE4.2	37°14.502″ 76°23.202″	Mouth of York River	~13m		X
WE4.3	37°10.602″ 76°22.398″	Mouth of Poquoson River	~4m		
WE4.4	37°06.60″ 76°17.598″	Mouth of Black River	~4m		

16. Appendix 2: EXAMPLE OF A COMPLETED FIELD DATA SHEET

CHESAPEAKE BAY MONITORING PROGRAM

STATION: **CB6.1**	R/V: FAY SLOVER	DATE: 6/2/14
	CRUISE NO.: CBP626	TIME: 1640
FIELD CHIEF: H.WRIGHT	STATION DEPTH: 12 m	LAT. 37°35.228′ N
	SECCHI DEPTH: 2.1 m	LONG. 076°09.747′ W

WEATHER DATA - CIRCLE APPROPRIATE WEATHER CODES

CLOUD COVER	PRECIP. TYPE	WIND SPEED	SEA STATE
0 – CLEAR (0-10%)	10 - NONE	0 0 – 1 KNOTS	0 CALM
1 - PARTIALLY CLOUDY: 10-50%	11 - DRIZZLE	1 2 – 10 KNOTS	1 < 1 FT.
2 - PARTIALLY CLOUDY: 50-90%	12 - RAIN	2 11 – 20 KNOTS	2 < 2 FT.
3 - OVERCAST: >90%	13 - RAIN HEAVY	3 21 – 30 KNOTS	3 < 3 FT.
4 - FOGGY	14 - SQUALLY	4 31 – 40 KNOTS	4 < 4 FT.
5 - HAZY	15 - FROZEN PRECIP.	5 > 40 KNOTS	5 > 4 FT.
6 - CLOUD (NO PERCENTAGE)	16 - RAIN SNOW		
☑ CHECK IF PHYTOPLANKTON IS COLLECTED.		WIND DIRECTION S	TIDAL STAGE: H L [E] E

Pycnocline and Biology Calculation

$$\left(\frac{21350 - 35659}{\underset{S_{Conductivity}}{} \quad \underset{B_{Conductivity}}{}} \right) \times 2 = 2862$$

$$\frac{11 - 1}{B_{Depth} \quad S_{Depth}}$$

$S_{Conductivity}$ = Surface Conductivity
$B_{Conductivity}$ = Bottom Conductivity
B_{Depth} = Bottom Depth from back in meters
S_{Depth} = Surface Depth in meters

DEFINITIONS:

Upper Pycnocline: The shallowest depth where change in conductivity ≥ the Pycnocline Threshold

Lower Pycnocline: The deepest depth where the change in conductivity ≥ the Pycnocline Threshold

No Pycnocline if Pycnocline Threshold < 500 μmho/cm, or no depth interval > threshold value.
Note: If no Pycnocline collect ⅓, ⅔.

Notes: _____

Initials:

Chief Scientist: _____

Supervisor: _____

GENERAL INFORMATION	
STATION: CB6.1	SAMPLING DATE: 6/2/14
COLLECTED BY: HW	

ENVIRONMENTAL DATA						
DEPTH (M)	TEMPERATURE °C	SALINITY	SP. CONDUCT. (µMHO/CM)	DO (PPM)	pH	COMMENTS
1	21.40	12.83	21350	8.68	8.25	
2	21.44	12.83	21348	8.77	8.25	
3	21.38	12.88	21416	8.77	8.25	
4	20.90	13.81	22836	8.68	8.22	
5	20.74	14.25	23500	8.46	8.18	
6	20.88	14.67	24148	8.11	8.14	
7	21.00	15.95	26049	7.05	8.08	
8 AP	20.61	16.18	26366	5.99	7.94	BIOLOGY
9	19.97	18.54	29896	3.56	7.66	PYC
10	20.21	20.08	32083	4.42	7.77	PYC
11 BP	20.18	22.53	35650	4.45	7.76	BIOLOGY
12						
13						
14						
15						
17						
19						
21						
23						
25						
27						
29						
31						
33						
35						

17. Appendix 3: CBP MONITORING FIELD SUMMARY SHEET

CBP MONITORING FIELD SUMMARY

CBP_____ Date: _____

Weather: _____ Day: 1 2 3 4 5

Field Chief/Crew:_____

Station	Arrive	Depart	Comments

Calibration date/time:_____

Post calibration date/time: _____

Post Calibration Check Values			
Conductivity	S.V.	58.6mmhos/cm	
	I.V.		mmhos/cm
pH	S.V.	7.00	10.00
	I.V.	_____	_____
D.O.	S.V.	_____	mg/L
	I.V.	_____	mg/L

Sample storage			
		Temperature	
	Thermometer ID#	AM	PM
cooler:	_____	_____°C	_____°C
freezer:	_____	_____°C	_____°C

Instruments used/Depth check			
UW sensor ID#: _____		YSI: _____	
SA sensor ID#: _____		1m boat = _____	m YSI

D.O check		
time	_____	
temperature	_____	°C
barometric pressure	_____	mmHg
D.O. S.V.	_____	mg/L
I.V.	_____	mg/L
within 0.3mg/L?	Y N	

S.V. = standard value, I.V. = instrument value

NOTES

Appendix 3:

SOP Particulate Carbon/Particulate Nitrogen

STANDARD OPERATING PROCEDURE
FOR
PARTICULATE CARBON AND NITROGEN IN WATER AND SEAWATER USING FLASH COMBUSTION/CHROMATOGRAPHIC SEPARATION AND A THERMAL CONDUCTIVITY DETECTOR

REVISED BY: Suzanne Doughten and Kevin Minga

FOR: Water Quality Laboratory
College of Sciences
Old Dominion University
Norfolk, Virginia 23529-0456

DOCUMENT FILE LOCATION: Water Quality Laboratory

EFFECTIVE DATE: April 16, 2018

TERMINATION DATE:

APPROVED BY:

John R. Donat, Ph.D. Date
Director, WQL

Suzanne C. Doughten. Date
WQL Supervisor

DISCLAIMER: This SOP applies to the analysis of marine and estuarine water samples in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the lower Chesapeake Bay. This SOP may not be applicable to any other studies.

TABLE OF CONTENTS

1. LOCATION

This analytical procedure will be used by Water Quality Laboratory staff located at 4211 Colley Avenue , Norfolk, Virginia. The analysis procedure will be performed in the carbon/nitrogen section of the laboratory. Sample collection is performed in the field. Filtration may be performed in the laboratory or the field.

2. PURPOSE

This procedure is designed to measure particulate carbon and nitrogen in water samples as an indication of water quality.

The intended data user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program (CBMP). These data will be used to assess whether the multijurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of the data, and its inclusion in computer models of the Chesapeake Bay. Trend analyses are also performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and it's tributaries.

3. APPLICABILITY

This procedure is applicable to the analysis of particulate carbon and nitrogen in fresh, estuarine and coastal water samples. The procedure assumes that by using flash combustion and chemical reduction, organic and inorganic carbon and nitrogen in the sample is converted to carbon dioxide and elemental nitrogen. The concentration of carbon dioxide and nitrogen is measured using a CE Instruments Flash EA 1112 gas chromatograph equipped with a thermal conductivity detector. The results are reported in mg of C and mg of N per liter of water.

4. OVERVIEW OF METHOD

This SOP is based on the procedures developed by instrument manufacturers Carlo Erba and ThermoQuest for quantifying total carbon and nitrogen and EPA Method 440.0.

Samples are collected using a submersible pump which is fitted with a polyethylene hose or go-flow bottles. Particulate carbon (PC) and particulate nitrogen (PN) in water samples are concentrated onto a precombusted 25mm Whatman® GF/F glass fiber filter by vacuum filtration at ≤10 Hg vacuum pressure. These samples are either frozen or dried then stored in a desiccator until analysis. NOTE: This applies for samples collected for the CBMP, other projects may have different sampling and filtration procedures.

Samples are analyzed using a ThermoQuest Italia S.p.A Flash EA 1112 Elemental Analyzer® . Particulate carbon and nitrogen are converted to a gaseous state by flash combustion. A series of catalytic and reducing reactors convert carbon and nitrogen to carbon dioxide and elemental nitrogen which are detected and quantified using a thermal conductivity detector. Particulate carbon and nitrogen concentrations are reported as mg particulate carbon and mg particulate nitrogen per liter of water. Software used is CE Elantech, Inc. Eager Xperience version 1.3.

5. DEFINITIONS AND ABBREVIATIONS

PC: Particulate Carbon
PN: Particulate Nitrogen
L: Liter(s)
mL: Milliliter(s)
mg: Milligram(s)
HDPE: High-density polyethylene
CRM: Certified Reference Material
CBP: Chesapeake Bay Program
CBMP: Chesapeake Bay Monitoring Program
USEPA: United States Environmental Protection Agency
QAPjP: Quality Assurance Project Plan
Reagent Water: Resistivity > 10 megohm-cm
Bypass: A run/analysis that contains no sample or cup. It is an empty run.
Blank: A run/analysis that contains a cup only.
Method Blank: A combusted filter analyzed in a cup. Weight is set to 25.

invert: In reference to homogenizing a sample by inverting, one inversion is: starting with the cap of the sample bottle pointing straight up, turn the sample bottle completely upside down so the cap is pointing straight down, then turn the sample bottle upright until the cap is pointing straight up again.

6. SAFETY EQUIPMENT AND PROCEDURES

Safety Equipment:

1. **Lab Coat with the addition of apron when handling strong acids and bases, i.e. SULFURIC ACID**
2. **Protective eye wear: Goggles when handling liquids, glasses are approved for handling solids only**
3. **Nitrile gloves (PVC is not sufficient for acetone and acids)**

7. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100 , pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

The copper used in this analysis must be disposed of properly. The copper is placed in an appropriate waste container provided by Old Dominion University's Environmental Health and Safety Office (EHSO), and EHSO collects the waste container and dispose of it. To reduce the amount of copper waste accumulated, the unreduced copper from a reduction reactor is reused.

8. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

Solid copper waste is collected in plastic container(s) provided by EHSO. The waste container(s) are labeled according to procedures provided by EHSO**.** When the container(s) are full the waste is collected by EHSO staff for disposal.

9. METHOD PERFORMANCE

Using samples analyzed in 2007, the average difference between 106 duplicate estuarine samples with a concentration below 0.8 mg/L and a mean concentration of 0.571 mg/L particulate carbon was 0.0270 mg/L. The standard deviation of the difference was 0.0239.

Using samples analyzed in 2007, the average difference between 106 duplicate estuarine samples with a concentration between 0.8 and 1.5 mg/L and a mean concentration of 1.121 mg/L particulate carbon was 0.0389 mg/L. The standard deviation of the difference was 0.0345.

Using samples analyzed in 2007, the average difference between 42 duplicate estuarine samples with a concentration above 1.5 mg/L and a mean concentration of 2.328 mg/L particulate carbon was 0.0971 mg/L. The standard deviation of the difference was 0.0861.

Using samples analyzed in 2007, the average difference between 61duplicate estuarine samples with a concentration below 0.1 mg/L and a mean concentration of 0.0776 mg/L particulate nitrogen was 0.0040 mg/L. The standard deviation of the difference was 0.0035.

Using samples analyzed in 2007, the average difference between 128 duplicate estuarine samples with a concentration between 0.1 and 0.2 mg/L and a mean concentration of 0.1439 mg/L particulate nitrogen was 0.0057 mg/L. The standard deviation of the difference was 0.0051.

Using samples analyzed in 2007, the average difference between 67 duplicate estuarine samples with a concentration above 0.2 mg/L and a mean concentration of 0.2925 mg/L particulate nitrogen was 0.0127 mg/L. The standard deviation of the difference was 0.0112.

Thermo Electron Corporation Soil Reference Material NC Lot Number 0704 was analyzed with a true value of 2.003 % Carbon and 0.193 % Nitrogen. In 2007 26 were run for nitrogen with an average value of 0.211 mg/L, which is a 110% recovery. In 2007 35 were run for carbon with an average value of 2.016 mg/L, which is a 101% recovery.

10. LABWARE

NOTE: In this SOP where labware numbers are specified, the amounts listed in this SOP are for routine Chesapeake Bay Monitoring Program sampling. Different cruises and/or projects may require different amounts and types of labware.

10.1 Labware Needed:

For sample collection and concentration:

4 -1L HPDE bottles labeled for PC/PN
2 to 4- Filtration units for PC/PN filtration
2 for each sample plus 1 extra for every 10 to 20 samples- Labeled and muffled vials with Teflon lined caps for PC/PN
approximately 250 - Precombusted 25 mm Whatman® GF/F filters or equivalent
1- stainless steel forceps for handling PC/PN filters
1- 250 mL plastic graduated cylinder

10.2 Labware cleaning:

Carbon contamination is almost impossible to eliminate, but this procedure should reduce background contamination to below detection limits. If contamination occurs, labware cleaning is highly suspect.

10.2.1 Small sample vials:

Place vials in muffle pan and muffle at 550±50°C muffle oven for 4 hours. **NOTE: If vials appear to contain any filter, remove before muffling.**

When cool, place sample vials in a container labeled **Clean PC/PN sample vials** or cap vials and store in PC/PN section.

10.2.2 Labware and vial caps:

Scrub with a brush (if able) using dilute Liquinox® and rinse with tap water. Rinse 2 times with 4N HCL. Rinse 6 times with reagent water.

Dry on a clean drying rack labeled **for clean labware only** completely covered. When dry, store with caps on, if applicable. When caps aren't applicable, cover items with aluminum foil.

11. CHEMICALS USED AND REAGENT PREPARATION

11.1 Safety Equipment

Safety Equipment:

1. **Lab Coat with the addition of apron when handling strong acids and bases, i.e. SULFURIC ACID.**
2. **Protective eye wear: Goggles when handling liquids, glasses are approved for handling solids only**
3. **Nitrile gloves (PVC not sufficient for acetone and acids)**

11.2 **Chemicals Used:**

Aspartic Acid certified as a standard and reference material
Certified or Standard Reference Material - a commercially available soil sample with known carbon and nitrogen amounts
A second source standard can also be used to check calibration (example: Atropine)
Oxidation Catalyst
Quartz Wool
High Quality Copper
Magnesium Perchlorate or equivalent drying agent. (Starting in June 2016 CE Elantech is replacing the magnesium perchlorate with Aquatak)
Solvent (such as methanol, acetone, etc) for cleaning

11.3 Wipe down the counter top or hood with reagent water before using reagents.

11.4 **Reagents needed for the analysis:**

NOTE: Refer to Safety Precautions and use care when preparing reagents.

Aspartic Acid is used as the standard for the calibration curve. Store the Aspartic Acid in the desiccator until needed.

Certified Reference Soil Material or second source standard (CRM). Follow directions supplied with CRM. Store the CRM in the desiccator until needed.

Other reagents are used to pack reactors or columns, and their use is documented in section 14.5.

12. QUALITY ASSURANCE AND QUALITY CONTROL

It is especially important to maintain the carbon/nitrogen analyzer in peak operating condition. Data quality is also defined by an ongoing assessment of calibration accuracy and analytical precision, as follows:

12.1 Verifying the accuracy of an initial calibration by processing and analyzing 3 certified reference material (CRM) samples each time the instrument is calibrated. At least two out of three CRM's must be in control to validate the calibration.

12.2 Analyzing a calibration check standard with a combusted filter after the initial calibration and CRM samples are analyzed.

12.3 Processing and analyzing a calibration check standard each day before the start of any analysis, after every 10 to 20 analytical results for samples (more frequently when quality control indicators indicate the need), and at the end of each analysis.

12.4 Analyze one duplicate sample after every 10 to 20 analytical runs to assess precision.

12.5 Analyze a method blank after every 10 to 20 analytical runs.

12.6 Performing maintenance and preventative maintenance according to the schedule in section 21 of this SOP or whenever there is a need for improved data quality.

12.7 The instrument is serviced by qualified service engineers whenever the correction of problems is beyond the capability of laboratory or Old Dominion University staff.

13. SAMPLE COLLECTION, CONCENTRATION, AND PRESERVATION

13.1 Filter Preparation:

13.1.1 Place 25mm Whatman® GF/F glass fiber filters in a muffle pan lined with aluminum foil.

13.1.2 Place the filter pan in the muffle oven for at least 15 minutes at 550°C ±50°C.

13.1.3 Remove the pan from the muffle oven, and cover with aluminum foil until cool.

13.1.4 Wrap the muffled filters in aluminum foil or some type of closed case, and store until use.

13.2 **Collection:**

NOTE: The procedures listed are for the typical CBMP cruise. Other projects may have different requirements for labware, filtration, sample containers and storage.

Labware needed:
4- 1 L HDPE sample bottles labeled for PC/PN

See section 10.2 for appropriate cleaning procedures.

13.2.1 Rinse the entire inside surface of the 1L HDPE sample bottle three times with sample, discarding the rinse.

13.2.2 Fill the sample bottle, leaving approximately one inch of air space in the sample bottle and cap tightly.

13.2.3 The samples should be filtered immediately after collection. If this is not possible, refrigerate samples or store samples by packing the sample bottles in ice up to the level of the top of the sample but below the bottom of the sample bottle cap, and filter by the next day. The sample can be taken out of a different size bottle, as long as it is HDPE and there is sufficient water to filter the correct amount. The PC/PN often are taken from the HDPE bottles used to collect the nutrient samples. Storage temperature must be measured using a thermometer which has been calibrated against an NIST-traceable thermometer within the last year. Storage temperature is documented on the CBP monitoring form.

13.3 **Concentration:**

Labware needed:

2 to 4 - Filtration units for PC/PN filtration
2 for each sample plus 1 extra for every 10 to 20 samples- Labeled and muffled vials with teflon lined caps for PC/PN
approximately 250 -Precombusted 25 mm Whatman® GF/F filters or equivalent
1 - stainless steel forceps for handling PC/PN filters
1 - 250 mL plastic graduated cylinder

See section 10.2 for the appropriate labware cleaning procedures.

13.3.1 Using forceps, gripping the filter edge only, transfer a muffled 25mm Whatman® GF/F glass fiber filter to the fitted base of the filter holder. Ensure that the grid is in place on the bottom of the filter holder, then screw the top of the holder onto the bottom of the holder. Maintain the forceps on a clean surface when not in use to protect from contamination.

13.3.2 Mix the sample thoroughly by inverting (see definitions) the sample bottle several times until well-mixed. Immediately rinse the 250 mL graduated cylinder three times with the sample to be concentrated. Pour the **maximum volume of sample that can be filtered in 10 minutes, up to 250 mLs,** into the pre-rinsed 250 mL graduated cylinder.

13.3.3 Record the log number, the sample ID and the volume filtered in the appropriate spaces on the bench sheet

13.3.4 Pour the sample from the graduated cylinder into a filter tower containing a muffled 25mm glass fiber filter.

13.3.5 The filtration tower is attached to a vacuum pump set to $\leq$10 Hg.

13.3.6 Using stainless steel forceps, remove the filter from the filter tower without touching the area containing sample.

13.3.7 Fold the filter in quarters with the sample facing inside, being sure not to touch or disturb the filtrate.

13.3.8 Place each filter into individually labeled, muffled glass vials with Teflon-lined

screw caps.

13.3.9 Place vials in a container and place in the freezer or pack on ice.

13.3.10 Rinse the graduated cylinder and the filtration tower a minimum of 2 times with reagent water before filtering the next sample.

13.3.11 After each station is completed rinse the forceps with reagent water.

14. PRE-ANALYTICAL PROCEDURES

14.1 **Sample Preparation:**

14.1.1 Use the Sample collection data sheets to determine the log numbers and/or cruise ID of the samples that will be analyzed.

14.1.2 Remove the samples from the freezer and log out in the chain-of-custody log.

14.1.3 Inspect samples to ensure that they have been properly stored and do not appear torn.

14.1.4 Remove the caps so they can dry. If samples are stored in containers other than glass vials, then those containers may be used for drying or the sample may be transferred to glass vials.

14.1.5 Dry the vials at 50°C± 2°C at least overnight.

14.1.6 Remove the vials from the oven, recap the vials tightly, and place in a desiccator until ready for analysis. Record this in the chain-of-custody log.

14.2 **Wrapping Filters in Tin Catalyst Cups:**

14.2.1 Clean one pair of stainless steel forceps using a Kimwipe or equivalent. Use a solvent such as methanol or acetone to clean. Never use acid on metal.

14.2.2 Using the stainless steel forceps, place a clean tin cup on a clean surface.

14.2.3 Using the stainless steel forceps, remove a glass fiber filter (sample) from the glass vial, place in the tin cup and pinch close with the steel forceps.

14.2.4 Store in a desiccator in the C/N section until analysis. If analyzing sample immediately after wrapping go to section 15.5.

14.3 **Preparing Standards:**

Always handle tin cups with clean stainless steel forceps. Wipe clean the forceps, the sealing device and a stainless steel spatula with Kimwipes or equivalent and a solvent (such as methanol or acetone, etc.). Always work on a clean aluminum foil-covered surface whenever handling standards or samples.

14.3.1 The Mettler® XP6 micro-balance is used to weigh the standards.

14.3.2 Tare the balance.

14.3.3 Next weigh 2 weights, one high and one low, that is expected to bracket the range of the standard that will be weighed. Record these readings in the micro-balance calibration check logbook. This must be done once every day before standards or samples are weighed.

14.4 **Standards:**

14.4.1 Aspartic Acid is the standard. The following guidelines for standard range are to bracket routine samples analyzed in this laboratory. Other projects or samples outside of the expected range may require a different standard curve and possibly a different chemical standard. Consult with the Laboratory Supervisor if this occurs.

The standards shall weigh approximately 0.06 up to a range of 1.3 to 1.7 mg.

Aspartic Acid is stored in a desiccator.

For one calibration curve, weigh out 6 standards (1 each of these approximate weights: 0.06 mg, 0.3 mg, 0.5 mg, 0.8 mg, 1.0 mg and a high standard in the range of 1.3 to 1.7) following steps 14.4.2 through 14.4.5 below.

14.4.2 Place the cup on the balance and tare.

14.4.3 Remove from balance and on a clean surface using a clean stainless steel spatula, place Aspartic Acid into the cup

14.4.4 Place the open cup with standard on the microbalance pan, weigh (add more

standard if necessary to achieve desired wieght), and record the weight in the PC/PN standards logbook and on data sheet. Remove from balance and seal cup. If preweighed standard is not used that day store in a desiccator.

14.4.5 Repeat steps 14.4.2 through 14.4.4 for each standard weight.

Note: CRM preparation is documented in the PC/PN Standards Logbook. Manufacturer instructions are followed.

14.4.6 Weigh out 3 CRM samples . Weigh out an amount that will give counts in the mid-point of the calibration curve. Using steps 14.4.2 through 14.4.4, to run as QC samples to validate the calibration.

14.4.7 Aspartic Acid calibration check standards are prepared at approximately mid range of the curve (0.5 mg- 0.9 mg), weighed according to steps 14.4.2 through 14.4.4

NOTE: Before opening up the reactors, changing the crucible sleeve, adjusting/tightening fitting nuts or the autosampler or changing the drying column the system must be depressurized. Shut off gas by selecting ashes removal under the tools menu and wait 2 minutes before proceeding. Once finished then restore gas flows and perform a Leak Test (see section 15.2 of this SOP).

14.5 **Renewal of Reactors (Columns):**

14.5.1 **Reduction Reactor (column)** : Install a reduction reactor with unreduced copper after approximately 1000 samples (sooner if analytical results indicate exhaustion of the reagents) according to section 14.5.4.

14.5.2 **Combustion Reactor (column)** : Remove and replace or clean the crucible sleeve approximately every 80 to 100 drops according to section 14.5.9. Repack the combustion reactor with new reagents at least every 1000 analyses (sooner if analytical results indicate exhaustion of the reagents) according to section 14.5.5.

14.5.3 **To clean out a reduction reactor:**

14.5.3.1 Due to the frequency of the reduction reactor breaking when trying to clean it, these reactors are not reused. A new reduction reactor is used each time.

14.5.3.2 The copper used in the old column must be disposed of as hazardous waste. Copper is placed in a waste container provided by ODU's Environmental Health and Safety Office. For handling of reduced copper see sections 7 and 8.

14.5.4 **To pack a reduction reactor:**

14.5 4.1 Always wear disposable gloves.

14.5.4.2 Place approximately 50 mm of quartz wool in the base of the reactor. The reactor has a beveled bottom with a flat top.

14.5.4.3 Refer to the ThermoQuest Operating Manual for Flash EA 1112 page 76, figure 5-22 for illustration of proper reduction reactor packing.
Pour copper into the reactor. It takes approximately 150 grams of copper**.** Do not pack the copper down. Gently tap bottom of reactor on a soft surface to allow the copper to settle, this will reduce air pockets. Record the Standard/Chemical Log number of the copper used to fill the reactor in the C/N Maintenance Log.

14.5.4.4 Place approximately 50mm of quartz wool on top of the copper (pack the quartz wool down slightly).

14.5.4.5 Wearing disposable gloves, wipe the reactor well with Kimwipes or equivalent to remove all fingerprints. **(Any fingerprints on the reactor will cause the** reactor **to break at operating temperatures.)**

14.5.4.6 Always use new o-rings when changing the reduction reactor. Leak check the reactor before bringing up to temperature.

14.5.5 **Combustion Reactor**

Reagents must be totally changed after 1000 samples. Deterioration of the standard curve correlation coefficient is an indication that the reagents need to be changed in the combustion reactor.

14.5.5.1 **To clean out a combustion reactor:**

14.5.5.2 Always wear disposable gloves.

14.5.5.3 Using stainless steel forceps, remove the quartz wool from the top of the reactor

.

14.5.5.4 Use a metal rod, with a serrated hollow cylinder on the end, to loosen the oxidation catalyst.

14.5.5.5 The reagents can be disposed of in the regular garbage.

14.5.6 **To repack a combustion reactor:**

Refer to the ThermoQuest Operating Manual for Flash EA 1112 page 76, figure 5-22 for illustration of proper combustion reactor packing. Always wear disposable gloves.
Record the Standard/Chemical Log number of the oxidation catalyst used to fill the reactor in the C/N Maintenance Logbook.
Reuse the same steel combustion reactor or a new steel one. The combustion reactor has holes in the top and a slight beveled bottom.
Note: Use a marked tool to help measure the amount of quartz wool and Oxidation Catalyst that is put in the reactor. Fill bottom of reactor with approximately 50mm of quartz wool. Pour the oxidation catalyst into a beaker and spoon into the reactor so have an equal mix of pellets and wires. It takes approximately 80 grams of oxidation catalyst to fill the reactor with 130mm of reagent. Place approximately 10mm of quartz wool on the top of the oxidation catalyst. Place a crucible sleeve with a small amount of quartz wool in the bottom in the combustion reactor. Always use new o-rings when changing the combustion reactor. Leak check the reactor before bringing up to temperature.

Note: Before turning the furnaces on, remove the cap from the reduction reactor. This allows moisture that is in the reagents to vent out and not contaminate the GC column. Once the instrument is up to temperature all of the moisture will be removed, and the reduction reactor can be capped.

Note: Once the instrument is at temperature, always analyze 10 conditioning standards after replacing the combustion column. Replace drying column after running conditioning standards.

14.5.7 The crucible sleeve is changed or cleaned at approximately 80 to 100 samples to remove used cups according to section 14.5.9 of this SOP.

14.5.8 **To clean out and repack a drying column. This should be changed after approximately 150 samples. If instrument is at operating conditions, insure gases have been off for 2 minutes to depressurize system before changing column:**

14.5.8.1 Wearing disposable gloves, remove the quartz wool from the ends of the column.

14.5.8.2 Empty the drying reagentout of the column. This can be placed in the regular trash. If the drying reagent is hardened inside the glass column, the column can be rinsed with reagent water and thoroughly dried using compressed air, oven drying or leaving to air dry.

14.5.8.3 Place quartz wool in one end of the glass column and fill the column with the drying reagent. Place quartz wool in the other end of the column so that it is sealed off at both ends with quartz wool. Inspect ring seals and replace if necessary.Always repack a drying column whenever the combustion reactor is repacked. This column should be changed after approximately 150 samples or sooner if needed.

14.5.8.4 Record the Standard/Chemical Log number of the drying reagent, used to fill the column, in the C/N Maintenance Logbook.

14.5.9 **To replace a crucible sleeve (must be done at approximately 80 to 100 drops). If instrument is at operating conditions, insure gases have been off for 2 minutes to depressurize system before changing column:**

14.5.9.1 Place about 5mm of packed quartz wool into the bottom of a clean crucible sleeve. The quartz wool should be just enough to trap the sample cups and the ash after combustion. The smallest amount of quartz wool should be used to optimize combustion of the cups. Scrape the outside of the crucible with a file to remove excess build up if necessary. Check to make sure crucible is not bent/warped too much from excessive heat. **Do not force crucible into the combustion reactor, it**

should just slide in rather easily.

14.5.9.2 Hand loosen the fitting on top of the combustion reactor.

14.5.9.3 Carefully lift the autosampler up and set to one side. **Be careful not to bend the helium lines connected to the autosampler too much. Excessive stress could cause leaks.**

14.5.9.4 Working quickly to minimize exposure to atmosphere, grasp the top of the old **HOT** crucible sleeve by inserting the crucible extractor tool ends into the holes in side of the crucible sleeve. Remove the sleeve from the combustion reactor by lifting straight up. Place the sleeve immediately in tray that can withstand high temperatures.

14.5.9.5 Grasp the top of the new or cleaned crucible sleeve with the crucible extractor tool and slide it carefully into the top of the combustion reactor.

14.5.9.6 Inspect the O-rings on the top of the combustion reactor fitting and replace at this time if needed.

14.5.9.7 Set the autosampler gently back in place.

14.5.9.8 Tighten the fitting on top of the combustion reactor by hand.

14.5.9.9 After inserting a new crucible sleeve, perform a leak check (see section 15.2 of this SOP).

NOTE: Let temperatures return to operating before performing leak check. If leak check is done while instrument is coming up to temperature from replacing crucible sleeve, the instrument frequently goes into Safety Cut Off mode. If it goes into Safety Cut Off, turn power off to instrument and then back on to reset.

Then analyze a blank cup to burn off contamination. If the chromatograms do not show contamination, analyze an Aspartic Acid standard as an unknown. If contamination is present, analyze another blank until no contamination is observed, then an Aspartic Acid standard as an unknown.

15. ANALYTICAL PROCEDURES

15.1 **Instrument Start Up:**

Cold Start up:

15.1.1 Switch on the instrument by lifting the lever located at the back of the instrument. After powering on, the indicating LEDs **Power on** and **Stand By** on the synoptic panel light up.

15.1.2 Turn on the Helium and Oxygen gases at the tanks by turning the knob counterclockwise. Helium should be set at 250 kpa and oxygen should be set at 300 kpa. Note on the gas usage log how much (psi) gas is in each tank.

Note: The instrument is controlled by Eager software. Use the help menu on the software for instructions. For purposes of this SOP Eager software has already been installed.

15.1.3 Switch the computer on.

15.1.4 Find the program file and open the Eager program. Select Analyzer #1. A little gray screen will appear for Eager start up, click on **Ok**. The main menu of Eager is the starting point to enter all menu and relevant functions.

15.1.5 If instrument already has the method loaded go to step 15.1.6. If not then in the main menu, select **File** and then the option **Load System Defined Method.** The file name of the loaded method is displayed in the grid *Filename of the method in use* of the main menu. **EA method filename** NC system.mth is the name of the file.

15.1.6 In the menu select **Edit** and then the option **Edit Elemental Analyzer Parameters**. A window appears where the analyzer operating parameters are displayed. Set left furnace temperature to 950^0 C, the right furnace to 840^0 C and the oven to 50^0 C. Then click on the **Flow/Timing** tab to set the gas flows. Set the carrier (Helium) to 140 ml/min, Oxygen to 250 ml/min and the reference to 100 ml/min. Cycle (Run Time) = 340 sec, Sampling Delay = 12 sec, Oxygen Inject End = 5 sec. Then click the **Detector** tab to set the filament to **On** and the Gain to 10. Press **Send** and then **Ok** to transfer the operating parameters to the instrument.

15.1.7 The analyzer is now working. The furnaces are heating, Helium and Oxygen are flowing and the **LEDs** furnace, Oven and TCD lights up on the synoptic panel. Perform a leak test according to section 15.2 before the furnaces heat up, because maintenance is easier to perform at room temperature.

15.1.8 After about 50 minutes the instrument furnaces reach the temperature settings and the **LED Ready** on the synoptic panel lights up. The instrument is ready to run analyses. Before running any samples or standards, after a cold start up or standby start up, a bypass sample needs to be analyzed.

15.2 **Leak Test**

15.2.1 The leak test must be performed any time a component of the pneumatic circuit is replaced. A leak test is done to check that the reactors, drying column and gas chromatographic columns have been properly installed or reinstalled.

15.2.2 In the main menu select **View** and the option **View Elemental Analyzer Status** or just click on the icon. A status window will appear, then click on **Special Functions** and press **Leak Test.** Press **Start** to begin the operation. Perform the autozero.

15.2.3 After 300-360 seconds (Leak test time), the *Carrier Flow* and the *Reference Flow* must be within 0-10 ml/min. Higher flow values indicate that the system is not leak free.

Warning: Leaks in the system are generally due to incorrect closure of the reactors and filter locking nuts. Leaks are also due to o-ring failure on the reactors or columns, or a cracked column. Rarely, leaks may be due to the autosampler. See Section 20 for steps to troubleshoot leaks if present.

15.2.4 To terminate the leak test and restore the operating flow values, press **Stop** and **Done.**

15.3 **Detector Signal Level**

To adjust the level of the TCD detector signal select **View** from the main menu and then the option **View Elemental Analyzer Status.** Select the option **Detector** then press the button **Auto-adjust Level at 1000 uV.** At the end of the operation, the value 1000 is set representing the analysis starting point.

15.4 **Component Table**

The Component Table consists of the names of the elements to be analyzed (Carbon and Nitrogen), retention times and a window with a range (in seconds) for each component. Retention time for Carbon should be approximately 210, for Nitrogen approximately 101. The window forCarbon should be 40 to 50 and for Nitrogen 20 to 30.

15.5 **ANALYSIS OF CALIBRATION STANDARDS AND GLASS FIBER FILTER SAMPLES:**

Note: The curve preparation listed is sufficient for samples typically analyzed for the Chesapeake Bay Program. For non-typical samples or other projects a different standard curve may be used as necessary. Consult the Lab Supervisor to determine proper curve concentration. Information on the standards are documented in the PC/PN Standards Logbook.

Perform these steps at the beginning of each day of analysis.(15.5.1 through 15.5.6)

15.5.1 Fill in gas usage log.

15.5.2 Perform leak test following step 15.2.

15.5.3 Check autosampler by making sure the plastic lid is placed on the autosampler with the groove side up. This will prevent air from mixing with the sample.

15.5.4 Set the autosampler tray with the space to be used first aligned with the window on the front of the autosampler.

15.5.5 From the Eager main menu select **File**, then **Save Method**. This saves the method that is currently in memory. Chesapeake Bay Program cruises should be saved in a directory such as **C:\CBP,** the filename should be **CBPXXX.mth.**. If more than one file needs to be made for a Chesapeake Bay Program cruise, use an extension such as A after the cruise #. A new file can be loaded into memory or the current file can be renamed and saved as a new file.
Note: Be sure to save after loading system method and before beginning analysis or data files will save in the system method folder and lead to issues with omitted data in the summary file. If it happens, can be resolved by moving files to the CBP folder and recalculating.

15.5.6 From the main menu select the **Edit Sample Table** icon and the sample table will appear. If this is a new file, click on **clear sample table**. New information can be added to the table. The sample table is divided into six categories: **ID, sample name, filename, type, standard name** and **weight.**

ID = Run number

Sample name:

Method Blank= cup with a combusted filter
Blank= empty cup
bypass=no sample
condstd = aspartic acid in a cup but weight is unkown
Cal Std= Aspartic Acid standard used to calibrate instrument and the standard number
Aspartic Acid + Filter = Aspartic Acid standard plus a combusted filter and the standard number
CRM=Certified Reference Material and the standard number
STD = In run standard and the standard number
LOG NUMBER AND STATION ID=sample
LOG NUMBER AND STATION ID with a D or T on end=sample duplicate or triplicate

F**ilename:** File name chromotagram is saved under. An example is CBPXXXNUMBER. The number is usually the run number.

Type: unknown is for all samples except the following:
Blank=blank cup
Bypass=no sample and conditioning standard
Standard=calibration standard

Standard: This is only used when a calibration standard is analyzed. The type of calibration standard used (aspartic acid) is selected.

Weight: The weight of the sample or standard. **Note: if volume filtered is used for the weight, that number must be divided by 10. (e.g. for 250ml, 25 would be entered for weight.)**

The following instructions are for analyzing a new calibration curve. If using a calibration curve from a previous date go to step 15.5.20.

15.5.7 Following section 14.4 of this SOP, weigh out at least 5 calibration standards and 3 CRM's for the calibration curve.

15.5.8 In the first position on the autosampler put an unknown quantity of Aspartic Acid (wrapped in a tin cup) to be designated as a conditioning standard, followed by 2 blank empty cups in the 2nd and 3rd positions, followed by the 5 or more calibration standards (standards can be analyzed in any order) in the next 5 or more positions.

15.5.9 To fill out the sample table information correctly; the conditioning standard is **condstd** for sample name and bypass for type, no weight is necessary. The blank is **Blank** for sample name and blank for type, no weight is necessary. Standards are **Calstdxxxx** for sample name (where **xxxx** is the standard # from the PC/PN standards logbook). Fill in the appropriate weight for each standard. Type is **standard, Aspartic Acid** is the standard name. **Note: Make sure the correct true values for Aspartic Acid appear in the box at the bottom left any time that standard is used. (e.g. Nitrogen-10.52 % and Carbon 36.09 % for Aspartic Acid)**

15.5.10 CRM's are CRM **-xxxx** for the sample name **(xxxx** is the standard # from the PC/PN standards logbook**)**.

15.5.11 Once all the information is entered into the sample table, click on the **X** at the top right corner to exit the sample table. A window will appear to ask if the sample table is to be modified/updated- click **OK.**

15.5.12 On the main menu click on the green arrow to start the analysis. A window will appear, click start and the analysis begins.

15.5.13 Once the curve has completely run, determine if an acceptable standard curve has been generated. The minimum acceptable correlation coefficient to continue analysis is 0.9980 for both Carbon and Nitrogen. If the standard curve is in control, skip to step15.5.18.

15.5.14 If the correlation coefficient is out of control, click on **View** (from the main menu) and click on **View calibration curve**. Click on **Component** and view both the Carbon and Nitrogen curves.

15.5.15 View the Nitrogen calibration linear regression and determine if the high standard is the worst-fit standard. Repeat for Carbon.

15.5.16 It is acceptable to have a four-point calibration curve if the high standard is the one that is thrown out. However, samples must be within the curve range of the highest point accepted. A correlation coefficient of at least 0.9980 must be achieved for both Carbon and Nitrogen for the calibration to be considered good.

15.5.17 If an acceptable calibration curve can not be achieved by throwing out the high standard, then run another calibration curve.

15.5.18 Once an acceptable calibration curve is achieved then run 3 **CRM's.**

15.5.19 Determine if at least 2 of 3 CRM's are in control. See step 17.2 to determine if CRM is in control. If those are out of control, see Troubleshooting in section 20 then try 3 more CRM's. If those are still out of control then try a different CRM and/or redry the CRM. If the CRM still does not work, analyze a new calibration curve. If the CRM's are in control, proceed with sample analysis.

15.5.20 Before proceeding with sample analysis run a calibration check standard-Aspartic Acid with a combusted filter (which is a mid-point of the curve) to validate the curve and then a method blank. The sample name for a calibration check standard with the filter is Aspartic Acid + Filter **xxxx**, where **xxxx** is the standard # from the PC/PN standards logbook. *** Always run std xxxx as an unknown for sample type.*** This will take the place of the calibration check standard whenever a new calibration curve is analyzed. If the same calibration curve is being used but it is a new day, start the analysis with a calibration check standard.

The **calibration check** standard must be within +/- 10% of the true value as calculated in step 17.3.

15.5.21 Analyze a method blank sample (a cup which contains a combusted filter). The weight is recorded as 25. See section 17.5 to see if the method blank is in control.

15.5.22 Analyze 10 to 20 samples, 1 duplicate, a method blank and a **calibration check standard** per analytical batch.

15.5.23 Samples, duplicates, field blanks, CRM's, a method blank and calibration check standards are **always** analyzed as unknown sample types.

15.5.24 The last samples analyzed in any series of analyses must be a duplicate or triplicate, a method blank and a standard in order to demonstrate that the instrument was still in

calibration at the end of the analytical run.

15.6 **Saving Data**

Once all samples have been analyzed the data needs to be saved and checked. From the main menu, select the **Summarize Results** icon and wait for the summarized data to appear. Select File. Select export to Text file. Save in the directory that the method file was saved in with the same name (15.5.5). To save dataclick on *save as* and save to Flash drive E: or other removable storage device. Repeat these steps saving file as Excel. The Excel file is printed and used to check data.

16. COMPLETING AND ANNOTATING DATA SHEETS AND CHROMATOGRAMS

16.1 Complete the header information as indicated on the data sheet (e.g. project, date and analyst).

16.2 An example of a data sheet is: Condition standard in 1, spaces 2-3 on the data sheet are calibration blanks, spaces 4- 9 on the data sheet are calibration standards in order of ascending weight, and spaces 10-12 are CRM samples. These numbers may change depending on what is analyzed.

16.3 Complete the information indicated on the data sheet for the calibration curve. Generate a curve (see section 15.5 of this SOP) and write the calibration coefficients for carbon and nitrogen in the comments column(see step 17.1 of this SOP for acceptable correlation coefficients).

16.4 Once an acceptable curve for both carbon and nitrogen have been obtained, complete the information indicated on the data sheet for 3 CRM standards (generally samples10-12). Write the value generated by the software when the CRM sample is run as an unknown in the comments column. If at least 2 out of the 3 CRM samples are in control (see step 17.2 of this SOP), analysis may continue.

16.5 Analyze a known weight of aspartic acid with a combusted filter (Aspartic Acid + Filter). Follow step 17.3 to see if this check standard is acceptable. Analyze a method blank sample. See section 17.5 to see if the method blank is in control. If these are in control analysis may continue.

16.6 Complete the information for 10 to 20 samples and one duplicate, one method blank and a calibration check standard in the next spaces on the data sheet.

16.7 Indicate if the difference between duplicate measurements is ≤ the MDL (MDLs are calculated each year) or relative percent difference (RPD) or coefficient of variance (CV) using step 17.4 of this SOP and record in the space provided on the data sheet.

16.8 Record the values for the calibration check standard in the space provided on the data sheet.

16.9 If all data quality assessment criteria are met (see section 17 of this SOP), no further annotation is required, and the information for the next 10 to 20 samples and one duplicate and a calibration check standard may be recorded in the next spaces on the data sheet.

16.10 If duplicate values are out of control, circle the sample index numbers of the preceding 10 to 20 samples which must be rerun. Sample analysis cannot be continued until an acceptable replicate is analyzed. When the rerun is completed, put the following annotation in the margin to indicate the sample index of the duplicate sample: see Drop xx, where xx is the sample index of the duplicate. If the duplicate was run in a new file, put the following annotation in the margin: see Drop xx, File=xxxxxxx, where xxxxxxx is the name of the new file.

16.11 If the calibration check standard is out of control, indicate this by placing an x next to the out of control value(s), and circle the sample index numbers of the preceding 10 to 20 samples which must be rerun. Annotate locations of the reruns using step 16.10 (above). Sample analysis cannot be continued until an acceptable calibration check standard is analyzed.

16.12 If the method blank is out of control, analyze another method blank immediately to see if there was contamination. If the second method blank is in control continue with the analysis. If the second method blank is out of control indicate this by placing an x next to the out of control value(s), and circle the sample index numbers of the preceding 10 to 20 samples which must be rerun. Annotate locations of the reruns using step 16.10 (above). Sample analysis cannot be continued until an acceptable method blank is analyzed.

17. DATA QUALITY ASSESSMENT

All data must meet all of the data quality assessment criteria specified in the following steps. If any data do not meet all of the specified criteria, the applicable part of the analysis is out-of-control and the data must be forwarded to the laboratory supervisor and quality assurance officer as soon as possible.

17.1 Evaluate the calibration correlation coefficient r for both carbon and nitrogen and proceed accordingly:

$r \geq 0.9980$	Acceptable
$r < 0.9980$	Stop analysis. Troubleshooting is required.

17.2 Assess whether the analytical results for at least 2 out of the 3 CRM samples validates the calibration. The result should be ± 10% of the True Value. If those are out of control, see Troubleshooting in section 20 then try 3 more CRM's. If those are still out of control then try a different CRM and/or redry the CRM. If the CRM still does not work, analyze a new calibration curve.

17.3 Assess whether the calibration error, as defined by the analytical result for each calibration check standard is within the range of the true value ± 10% when calculated as follows:

$$\% \text{ Recovery} = (\text{True value} \div \text{Actual value}) \times 100$$

17.4 If the difference between duplicate measurements is ≤ the MDL (MDLs are calculated each year), the duplicate is in control. If either measurement is > than the MDL, assess whether the precision for each duplicated sample is in-control, as follows:

17.4.1 The relative percent difference (RPD) must be ≤30%. The calculation is as follows:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \; X \; 100$$

D_1	=	Measured concentration of duplicate 1,
D_2	=	Measured concentration of duplicate 2, and
$\|D_1-D_2\|$	=	Absolute difference between duplicates
$(D_1+D_2)/2$	=	Mean.

17.4.2 Alternatively the coefficient of variance (CV) can be calculated if sample is analyzed in triplicate to show it is ≤ 20%. The calculation is as follows:

$$CV = \frac{\sigma}{\bar{x}} \times 100$$

where,

σ	=	Standard deviation,
$\bar{x}$	=	mean

17.5 Assess whether the method blank check is in-control. The blank should be less than the average of the last 10 calculated MDL values (average MDL).

If the method blank is greater than the average MDL, the blank is determined to be contaminated. Immediately analyze another blank to see if this is an isolated problem to that one cup or filter. If the second method blank is less than the average MDL, the analysis can proceed. If the second method blank is greater than the average MDL, the cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

An exception for reanalysis of samples for an out of control method blank is for the field blanks. Field blank data may be kept if it is analyzed with an out of control method blank, but all other quality control samples are in control and the field blanks are below the average MDL.

17.6 If the data meet all of the data quality assessment criteria defined in steps 17.1 through17.5, the analytical results are in-control. If any of the data quality objectives are not met, the analysis is out-of-control and the data must be forwarded to the laboratory supervisor as soon as possible.

For analytical results obtained within the out-of-control period (since the previous occurrence of in-control QC measurements and until the next occurrence of in-control QC results) the samples should be reanalyzed.

NOTE: In situations where there is not enough sample to reanalyze, the client will be notified of the problem and how they want to proceed. This is determined on a case by case basis with the client and the WQL Supervisor. The data will be marked on the analytical data sheet and on correspondence with the client, if the client requests the data which is measured with out of control QC measurements.

For the Chesapeake Bay Program when replicate samples do not meet the criteria for precision and there is not another sample to analyze, the data for that sample may be submitted with a problem code of FF attached to the data, and the relevant out of control QC is reported in the WQ_QAQC table.

17.7 Ecological patterns:

Once the data meets all of the data quality assessment criteria, forward the data to the laboratory supervisor for assessing whether the data demonstrate ecologically explainable patterns.

18. METHOD DETECTION LIMIT

Method Detection Limit is defined as the minimum concentration that can be detected and determined to be statistically different from zero. The method for determining MDLs is based upon student t times the standard deviation of not less than seven replicate analyses of the same sample. This MDL will be highly dependent upon the instrument, reagents, SOPs, personnel and, most importantly, the matrix of the media being analyzed.

The method detection limit is determined once a year for this analysis, using a low level sample collected in the field. The results are reported in a Method Detection Limit table in the WQ_DATA database maintained by the Water Quality Laboratory.

19. INSTRUMENT SHUT-DOWN

19.1 **STANDBY SHUT DOWN:** This should be done if the instrument will not be used for a few days. If the instrument will not be used for more than one week it should be shut down.

19.1.1 When analysis is completed, the instrument can be put in *Stand-by*. In this condition, the temperatures of the Left and Right Furnaces are reduced by 50% verses the operating temperatures, and the Helium flows on both channels are brought to approximately 10mL/min.

19.1.2 The *Stand-by* function can be activated manually or automatically at the end of the analytical sequence.

19.2 **Manual Setting of the Stand-by Function**

19.2.1 In the main menu, select **Edit** and then the option **Edit Elemental Analyzer parameters** or just press the icon.

19.2.2 In the section *Other*, select the function **Set instrument to Stand-by** by clicking the proper box.

19.2.3 Press **Send** to send the command to the instrument and press **Ok**.

19.3 **Automatic Setting of the Stand-by Function**

In the main menu, select the green bubble (Start Sequence of Sampler) icon. In the section *Elemental analyzer conditions while start sequence is finished,* enable the function **Force to stand-by** by clicking the appropriate box. The analyzer will automatically go to the stand-by condition, when the last sample has been analyzed.

19.4 **COLD SHUT DOWN** This should be done if the instrument will not be used for one week or more.

19.4.1 The furnaces, gas flows, detector and oven can also be manually shut off. Click on **Edit** and then **Edit Elemental Analyzer parameters** or just click on the instrument icon and the *EA 1112 Method N.C.eam* page will appear. Click on the *Temperature* tab and uncheck the left and right furnace boxes and the oven box. This will shut off the oven and furnaces. Check the Set instrument to Stand by. Then click on the *Detector*

tab and turn the filament off. Press **Send** to send the commands to the instrument, then press **Ok.** Do not save the method.

19.4.2 Once the instrument is at a low enough temperature to not crack the columns (approximately 200°C or lower), shut off the gases by clicking on the *Flow/timing* tab and unchecking the Carrier, Oxygen and Reference boxes.

19.4.3 Turn off the power to the instrument and turn off the gases.

19.5 ***Auto-Ready and Auto-Start Functions***
These are timed functions, which can be programmed to minimize dead times.

19.5.1 **Auto-Ready Function**

To pass from the **Stand-by** condition to the **Ready** status, operate as follows:

19.5.1.1 In the main menu, select the menu **View** and the option **View Elemental Analyzer status**, or press the relevant icon.

19.5.1.2 Select the menu **Auto-Ready.** In the section *Control*, set the desired date and time of activation of the auto-ready function.

19.5.1.3 Press **Activate** and then **Ok** to confirm. The LED Wake-up on the synoptic panel lights up.

19.5.2 **Auto-Start Function**

If Auto-Ready is to be followed by Auto-Start, programming an analytical sequence, do the following:

19.5.2.1 In the main menu, select the green arrow icon. Enable the function **Enable time programmed sequence start** by clicking the appropriate box.

19.5.2.2 In the section *Starting time* press **Now.** In the text box *Start/date/time* enter the date and time of the function activation.

CAUTION: The function activation should be programmed with a delay of at least 60 minutes versus the time programmed for the Auto-Ready Function to allow the analyzer to reach a good thermal equilibrium.

20. TROUBLESHOOTING

Troubleshoot leak in PC/PN analyzer:

Before taking off parts, turn off gases and wait 2 minutes to depressurize the system.

1) Take off autosampler and put cap on left furnace. Connect line 2 to cap. Do leak check. If no leak, than leak is in autosampler. If still leak go to step 2.

2) Get brass fitting. Cap off line 2 where goes to machine. Carrier gas goes to zero without leak test here. If not zero there is a leak. If no leak, than everything is good from back of machine to before the furnaces. Go to step 3. If leak, then leak is the regulator in back of machine or lines

3) Put port 2 back on right furnace cap. Use brass fitting and cap port 3 on right reactor. This tells if furnaces are OK. If no leak, then go to step 4. If leak than it is in the fittings of either top or bottom of either furnace, or a cracked column. If think it is the column place an empty column in combustion column place and if no leak it is the column. Can also do in reduction column.

4) Reconnect line to port 3. Use brass fitting and plug off port 4 on top of instrument. If leak, than the problem is to the right of the furnaces. Most likely is o-rings in drying column. Next change lines if that doesn't fix it.

SYMPTOM	DIAGNOSIS	REMEDY
1) High Nitrogen Blank	A. Presence of leak	A. Check that Helium and Oxygen lines are sealed and eliminate possible leak.
	B. Oxygen line or cylinder contaminated	B. Purge for a few minutes. Replace the contaminated cylinder.
	C. Autosampler not purged	C. Check that the Oxygen flow is correct.
	D. Contamination in tin cups.	D. Run bypass with no cup. If no high blank, replace cups.
2) High constant Nitrogen blank in several sequential analyses	A. Oxygen cylinder contaminated.	A. Replace the Oxygen cylinder.
	B. Presence of leak in the autosampler system.	B. Identify leaks and remove them.
3) Carbon peak tailing or split.	A. Too many ashes inside the reactor.	A. Check ashes and replace crucible sleeve.
	B. The sample analyzed was too large.	B. Weigh a lower amount of sample.

SYMPTOM	DIAGNOSIS	REMEDY
4) Bad separation between Nitrogen and Carbon peaks.	A. High Nitrogen blank value. B. Copper exhausted.	A. Check the Nitrogen blank value. May need to repeat analysis. B. Replace copper.
5) Peak between Nitrogen and Carbon peaks.	A. Oxygen line contaminated. B. Inadequate Oxygen purity.	A. Purge the oxygen line. B. Use Oxygen with adequate purity.

SYMPTOM	DIAGNOSIS	REMEDY
6) High Carbon blank.	A. Oxygen line contaminated.	A. Purge the oxygen line.
	B. Inadequate Oxygen purity.	B. Use Oxygen with adequate purity
	C. Contamination of tin cups.	C. Run bypass with no cup. If no high blank, replace cups.
	D. Failure in combustion column.	D. Replace crucible sleeve. If this does not work than reanalyze calibration curve. If this does not work then replace chemicals in combustion reactor. .
7) Increasing Nitrogen blank values.	A. Copper exhausted	A. Replace the copper in the reduction column.
8) Retention times very delayed with respect to the normal chromatogram.	A. Presence of leaks in the pneumatic circuit.	A. Perform leak check.
	B. Presence of obstructions in the pneumatic circuit.	B. Find the obstruction and remove it from the pneumatic circuit.

SYMPTOM	DIAGNOSIS	REMEDY
9) Peak broadening on chromatograph	A. Presence of dead volume in combustion reactor. B. Incomplete combustion	A. Replace crucible sleeve. B. Check flash combustion. Check for carry-over from high sample.
10) Deformed peak on chromatogram.	A. Presence of dead volume in combustion reactor.	A. Replace crucible sleeve.
11) Deterioration of standard curve correlation coefficient with time.	A. Combustion reactor reagents failing.	A. Replace crucible sleeve. If this does not work then replace chemicals in combustion reactor..
12) Baseline drift on chromatogram.	A. Presence of leaks. B. Flow variations. C. Incomplete combustion.	A. Perform leak check. B. Check temperatures and flow rates. Clean out gas lines. C. Check flash combustion. Check for carry-over from high sample.
13) Large peak on chromatogram.	A. Low carrier flow.	A. Check carrier flow (helium).

SYMPTOM	DIAGNOSIS	REMEDY
14) No signal on chromatogram.	A. Detector off.	A. Turn on detector.
	B. Filament broken or problem with circuit boards.	B. Contact service technician.
	C. Low carrier flow.	C. Check for blockage in flow lines.
15) "Ghost peaks" on chromatogram.	A. Presence of leaks	A. Check for leaks. Perform leak test.
	B. Drying column exhausted.	B. Replace packing in column.

SYMPTOM	DIAGNOSIS	REMEDY
16) Carbon value of standard out of control	A. Contamination of tin cups.	A. Use new cups.
	B. Standard weighed incorrectly.	B. Run new standard.
	C. Leak check and check flow rates.	C. Fix leak if one exists. Fix obstruction in line if flow is incorrect
	D. Failure in combustion column.	D. Replace crucible sleeve. If this does not work than reanalyze calibration curve. If this does not work then replace chemicals in combustion reactor.

SYMPTOM	DIAGNOSIS	REMEDY
18) Nitrogen value of standard out of control	A. Contamination of tin cups.	A. Use new cups.
	B. Standard weighed incorrectly.	B. Run new standard.
	C. Leak check and check flow rates.	C. Fix leak if one exists. Fix obstruction in line if flow is incorrect.
	D. Failure in reduction column.	D. Reanalyze calibration curve. If this does not work then replace chemicals in reduction reactor.
19) Baseline drift	A. Presence of leaks.	A. Check for leaks.
	B. Loss of flow.	B. Check flow rates..
	C. Poor combustion in combustion reactor..	C. Replace crucible sleeve.

SYMPTOM	DIAGNOSIS	REMEDY
20) Variability of check standards (high and low recoveries)	A. Gas leaks	A. Perform leak check
	B. Build up of cups in crucible sleeve.	B. Replace crucible sleeves.
	C. Debris in gas lines.	C. Clean out debris from gas lines. Especially the Teflon lines running to and from the drying column.
	D. Reagents in combustion reactor are packed down.	D. Check reagents in combustion column and replace if they are packed down.
21) High blank cups after replacing combustion column.	A. Contamination in combustion column.	A. Run 10 conditioning standards for burn off column.
22. High Nitrogen peak when running samples.	A. Autosampler top upside down or not seated properly	A. Position autosampler top correct way.
23. Deformed peak	A. Dead volume in combustion column.	A. Replace crucible sleeve.

SYMPTOM	DIAGNOSIS	REMEDY
24) Baseline starts at correct position then drops to zero between 150-220 seconds.	A. Helium and oxygen flow are interrupted. B. Combustion reactor failure. C. Contamination in helium or oxygen	A. Make sure gases are turned on. Check for blockages in gas lines. B. Replace reagents in combustion column. C. Stop oxygen inject and run a cycle. If baseline returns to proper position the oxygen is contaminated. Replace gas cylinders.
25) Excessive Helium loss after changing tank.	Gas line where attached to instrument is leaking	Reconnect ferrule to instrument.
26) Autosampler not moving smoothly or consistently	A. Autosampler piston needs to be cleaned.	A. See p. 278-282 of ThermoQuest Operating Manual.

21. MAINTENANCE

From the main menu go to **VIEW** and select View Maintenance. This page keeps track of the number of runs since maintenance was performed on the crucible sleeve (**Left 1)**, combustion reactor**(Left 2)**, reduction reactor **(Right)**, and the drying column **(Ads. Filter 1)**. Each time maintenance is performed the *maintenance page* should be updated: Select **Edit** then select *reset maintenance* then *reset* and then **Ok.**

21.1 **Regular Preventative Maintenance**:

Replace crucible sleeve every 80-100 drops.

When the Combustion and Reduction Reactors (columns) are changed (approximately 1000 drops):

- Replace bottom o-rings (seals) on the reactor coupling union
- Replace top o-rings on reactors
- Inspect reduction reactor for cracks before reusing
- Perform leak check whenever system is opened.

Repack drying column as needed or approximately every 150 drops.

21.2 Maintenance as needed:

- Replace ring seals on ends of drying column
- Change auto-sampler piston. Refer to instrument manual for instructions.
- Change helium and oxygen tanks when they contain below 500 psi.

  Close valve on top of tank. Remove regulator. Remove tank cap and place regulator on new tank. For the helium tank the line is purged. This is not necessary for the oxygen tank.

Purging gas line: Remove helium line where it enters the back of the instrument. Turn on gas and purge approximately one minute. This removes moisture from line. Connect line back on instrument.

22. LITERATURE CITED

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Appendix 4:

SOP CTD Parameters

STANDARD OPERATING PROCEDURE FOR THE YSI EXO2 WATER QUALITY MONITOR

REVISED BY: Heather Wright

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Norfolk, Virginia 23529-0456

DOCUMENT FILE LOCATION: Water Quality Laboratory

EFFECTIVE DATE: February 15, 2015

TERMINATION DATE:

APPROVED BY:

John R. Donat, Ph.D. Date
Director, WQL

Suzanne C. Doughten Date
Water Quality Laboratory Supervisor

DISCLAIMER: This SOP applies to the analysis of marine and estuarine water samples in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the lower Chesapeake Bay. This SOP may not be applicable to any other studies.

TABLE OF CONTENTS

1. LOCATION

This data collection procedure will be used by Water Quality Laboratory staff located at 4211 Colley Ave., Norfolk, Virginia. This procedure will be utilized in the cruise preparation area as well as in the field.

2. PURPOSE

This procedure is designed as a method of data collection and currently includes: pH, Salinity, Chlorophyll, Dissolved Oxygen, Depth, Temperature, and Specific Conductivity.Sensors for other parameters are available from the manufacturer.

The intended data user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program. These data will be used to assess whether the multi-jurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of the data and its inclusion in computer models of the Chesapeake Bay. Trend analyses are performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and its tributaries.

3. APPLICABILITY

The following procedures are applicable to the collection of data in fresh, estuarine, and marine environments. Sondes can be used for profiling and long term deployment.

4. OVERVIEW OF METHOD

The methods contained within this SOP are based on methods by Yellow Springs Instruments in the EXO User Manual: Advanced Water Quality Monitoring Platform. The methods include the use of the YSI EXO2 sonde and handheld display. This SOP describes currently used parameters, refer to the user manual for full range of operating parameters and reporting units available.

Data is controlled by, collected, and stored in the handheld, which is connected to a YSI EXO2 sonde. Stored data can be viewed on the handheld and downloaded to a PC using KOR software (currently version 1.0.9). *NOTE: Updates to software and firmware may be done as available.*

5. DEFINITIONS AND ABBREVIATIONS

mg:	Milligram(s)
µL:	Microgram(s)
mL:	Milliliter
L:	Liter(s)
ppt:	Part(s) per thousand
m:	Meter(s)
mmHg:	Millimeters of mercury
mS/cm:	Milli-Siemens per centimeter
µS/cm:	Micro-Siemens per centimeter
CBMP:	Chesapeake Bay Monitoring Program
USEPA:	United States Environmental Protection Agency
reagent water:	Resistivity>10 megohm-cm
D.O.:	Dissolved Oxygen
ODO:	Optical Dissolved Oxygen
T:	Temperature
C/T:	Conductivity/Temperature
HCl:	Hydrochloric Acid

6. SAFETY EQUIPMENT AND PROCEDURES

- Lab coat when handling strong acids and bases (i.e. HCl, H_2SO_4, and NaOH)
- Protective eye wear: Goggles for liquids; glasses for solids
- Nitrile gloves

7. LABWARE CLEANING

Volumetric flasks, graduated cylinders, other general labware and sample bottles:

Inspect labware for residue, breaks, or defects. Scrub with a bottle brush (if able) and a 1% dilute Liquinox® solution. Thoroughly rinse with tap water. Rinse twice with 4N HCl then 6 times with fresh reagent water, ensuring the entire inside surfaces are rinsed each time. Inspect again for residue, breaks, or defects. If none evident, dry on a clean drying rack (labeled "for clean labware only"), completely covering any open labware with paper towels.

When dry, store volumetric flasks, vials, and sample bottles with caps on. Seal the tops of filtration flasks, graduated cylinders, Erlenmeyer flasks, and the tops and bottoms of filtration towers with parafilm or aluminum foil. Store in the appropriate cabinet.

8. CHEMICALS USED AND REAGENT PREPARATION

8.1 Labware Needed - Use only Class A glassware.
500mL volumetric flask
1L volumetric flask
2L volumetric flask

8.2 Chemicals used: *NOTE: Must be analytical grade or of a higher purity except as noted.*
Potassium Chloride (KCl)
Rhodamine B ($C_{28}H_{31}ClN_2O_3$), non-reagent grade

8.3 Information recorded on each reagent bottle label:
- Reagent number
- Reagent identification (name and concentration)
- Date prepared
- Prepared by (initials)
- Expiration date (N/A if there is no holding time)
- Storage requirements (i.e. room temperature, dark, etc.)

8.4 Reagents
- Clean counter top and balance area with reagent water prior to making reagents.
- Containers are reused, rinse 3 times with reagent water and fresh reagent prior to filling.
- Use only fresh reagent water, pre-rinse volumetrics 3 times prior to use.
- Log each reagent into the Chemical/Reagent Logbook.

NOTE: Refer to section 6 and use care when preparing reagents.
Rhodamine B standards only made for the chlorophyll sensor2 point calibration.

8.4.1 Potassium Chloride Standard, 58600 µS/cm Conductivity Standard
In a 2 L volumetric flask, dissolve 74.557 g Potassium Chloride in reagent water. Dilute to volume, mix well by inversion. Expiration N/A.

8.4.2 Stock Rhodamine B Solution, 100mg/L
In a 500 mL volumetric flask, dissolve 0.05 g Rhodamine B in reagent water. Dilute to volume, mix well by inversion. Store refrigerated in a dark glass bottle. Expiration N/A.

8.4.3 Working Rhodamine B Standard, 0.5mg/L
In a 1 L volumetric flask, dilute 5 mL 100mg/L Stock Rhodamine B to volume in reagent water. Mix well by inversion. Expiration: 24 hours

9. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personal should try to minimize the evaporation, spilling, and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

10. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

11. INSTRUMENT AND ACCESSORIES

- YSI EXO2 Sonde
- YSI EXO Handheld
- Calibration cup
- Sonde guard
- Port plugs
- Field case
- Field cable
- Maintenance kit
- KOR software
- Rechargeable battery and charger for handheld
- Handheld battery compartment cover and 4 "C" batteries
- 4 "D" batteries for sonde
- Conductivity/Temperature sensor
- pH or pH/ORP sensor
- Optical DO sensor
- Total Algae sensor
- USB signal output adaptor

12. QUALITY CONTROL

12.1 DO and depth values are checked each morning of a cruise. If not within project specifications, the sonde is either recalibrated or replaced.

12.2 Assess if the post cruise calibration checks are within specifications. A correction factor may be applied. *NOTE: Correction factors are not applied to CBMP data, acceptance criteria has been established.*

12.3 Forward data and post cruise calibration check record to the laboratory supervisor within a day of cruise completion to verify calibration accuracy and assess if the data demonstrate ecologically explainable patterns.

13. SOFTWARE OVERVIEW

Refer to User Manual section 4.1 for further software information. The KOR software on the desktop and handheld are generally the same.

Icons and associated parameters of the KOR software are as follows:


RUN - live data, run settings, capture data


CALIBRATE - calibration of sensors


DEPLOY - used for long term deployment


SITES - manage sites, located within RUN menu on handheld.


DATA - file transfers, view/export files, file settings, calibration worksheets


OPTIONS - smart QC, application preferences, sonde settings, software and firmware updates, unit options, default calibration settings


CONNECTIONS - connect to sonde, identify installed senors, bluetooth settings


HELP - support for KOR


STATUS ICON - sonde connected


STATUS ICON - sonde not connected


STATUS ICON - logging


STATUS ICON - not logging

14. SONDE CALIBRATION

Calibrate sensors prior to each cruise, generally within 72 hours. Calibrations are stored within each sensor; multiple sensors of the same type may be calibrated on a single sonde.
NOTE: A C/T sensor must be present when calibrating D.O. and pH.

Calibration may be completed on a computer or handheld; the sonde must be connected by cable or bluetooth. Instructions refer to the computer software but the handheld is similar. Default information may be defined under *Options, Calibration*, and the individual parameters. Record calibration on an EXO2 Sonde Calibration Record sheet (section 20). Once calibrations are complete, refer to section 18 for storage requirements until use.

Rinse the sensors, guard, and cup by adding calibration solution to the cup, attach to the sonde and shake well. Repeat twice more then add sufficient solution for calibration. Rinse three times with reagent water before using another solution, and when calibration is complete. *NOTE: References to fill lines for calibration solutions are without the guard in the cup.*

If a calibration fails, repeat. If it fails again, troubleshoot with any applicable maintenance procedures and try again. Replace sensors at defined intervals or sooner if necessary.

Following each calibration save the record as a pdf file and transfer to the ODU network

1. view the calibration record
2. click *Print*
3. choose *CUTEpdf* printer, click *print*
4. save file as *Parameter#* (ie. DO1, DO2, etc.) in a folder for the cruise (ie. CBP638)

Name parameters as specified below:

- *DO* (Dissolved Oxygen)
- *Cond* (Specific Conductivity)
- *Chl RFU* (Chlorophyll RFU)
- *Chl ug/L* (Chlorophyll µg/L)
- *pH*
- *depth*

14.1 Dissolved Oxygen

14.1.1 Prepare calibration environment - water saturated air

- Dry the sensors and guard, ensure the thermistor and DO membrane are moisture free.
- Put a small amount of tap water in the calibration cup, cap the sonde and leave vented (do not tighten). Allow to sit ≥10 minutes. *NOTE: Hold the cup on the sonde when lifting.*

14.1.2 In the Calibrate menu:

- select *ODO*
- select *ODO %*
- enter the barometric pressure in mmHg (from handheld)
- choose *1 point calibration* and *air-saturated* (no other information needed)
- hit *Start Cal*
- once *Stable Data* is achieved, click *Apply*
- Click *Complete* if everything looks ok, the calibration report will display. *NOTE: "ODO gain" should be ~ 1.*
- Print pdf file

14.1.3 Prepare calibration verification environment - air saturated water:

- put a sufficient amount of tap water in a container
- bubble with aquarium bubble stones attached to outflow of vacuum pump for ~ 1 hour.

14.1.4 Verify calibration:

- open the dashboard, record D.O.
- record the standard D.O. value from standard saturation tables
- verify the reading is within 0.2mg/L of calibrated value (generally within 0.1mg/L)

14.2 Specific Conductivity

NOTE: Sensor also holds thermistor, which there is no calibration for but may be verified against a NIST thermometer as needed.

14.2.1 Fill KCl standard to 2nd line on calibration cup, install sonde, tap to remove bubbles.

14.3.2 In the Calibrate menu:

- select *Conductivity*
- select *SpCond µS/cm*
- enter standard information
  - std value = 58600
  - type = KCl and reagent number
  - manufacturer = technician's initials and date made
  - lot # = blank
- hit *Start Cal*
- once *Stable Data* is achieved, click *Apply*
- Click *Complete* if everything looks ok, the calibration report will display. *NOTE: cell constant should be ~ 5.*
- Print pdf file

14.3 pH - *NOTE: slow response time may indicate end of sensor life (can try to recondition)*

14.3.1 Fill pH 7 buffer to 1st line of calibration cup, install sonde, tap to remove bubbles.

14.3.2 In the Calibrate menu:
- select *pH/ORP*
- select *pH*
- enter standard information for a 2 point calibration
  - std value =7.00 and 10.00
  - type = pH (7 or 10) buffer and standard #
  - enter manufacturer and lot
- hit *Start Cal*
- once *Stable Data* is achieved, click *Apply*
- fill pH 10 buffer to 1st line on calibration cup when prompted
- hit *Start Cal*
- once *Stable Data* is achieved, click *Apply*
- Click *Complete* if everything looks ok, the calibration report will display.
  NOTE: Slope should be ~ 180, replace when it approaches 160.
- Print pdf file

14.3.3 Verify calibration
- fill pH 7 buffer to 1st line on calibration cup
- open the dashboard, record pH
- verify reading is within 0.2 of pH 7.0

14.4 Total Algae (Chlorophyll/BGA)
When grab samples are used for calibration of data (ie, the CBMP), a 1 point zero calibration is used. If a project necessitates a 2 point calibration, Rhodamine B may be used. Enter the standard information if applicable. Fill standard to 1st line when prompted continue.

14.4.1 Fill reagent water to 1st line of calibration cup, install sonde, tap to remove bubbles.

14.4.2 In the Calibrate menu:
- select *BGA-PE/Chlor*
- select *Chlorophyll RFU*
- enter standard information for a 1 point calibration
  - std value = 0.00
  - type = reagent water
  - manufacturer and lot # = blank
- hit *Start Cal*
- once *Stable Data* is achieved, click *Apply*
- Click *Complete* if everything looks ok, the calibration report will display.
- Print pdf file
- repeat for *Chlorophyll µg/L*

14.5 Depth

Place sonde in a stable, vertical position - no calibration cup is needed

In the Calibrate menu:

- select *Port D - Depth*
- select *Depth m*
- enter standard information for a 1 point calibration
  - std value = 0.00
  - type, manufacturer, and lot # = blank
- hit *Start Cal*
- once *Stable Data* is achieved, click *Apply*
- Click *Complete* if everything looks ok, the calibration report will display.
- Print pdf file

15. ONBOARD PREPARATION

15.1 Mount YSI for deployment *NOTE: Do not use metal clamps. If not using the rosette, attach to an array used to deploy the sonde without causing strain on the field cable.*

- Remove sensor storage caps
- Attach calibration cup with a small amount of tap or sample water
- Attach the field cable to the sonde and datalogger, secure the strain relief connection
- Secure the sonde to the rosette with duck tape where depth sensors are approximately level
- Secure the cable to the frame

15.2 Name a file : On the handheld, choose *Run*, then *run settings* - enter the cruise name in the *File Prefix* box as CBP### (ie. CBP638), the filename should be *station*.

15.3 Daily D.O. check (can be done prior to mounting for deployment)

- prepare water saturated air environment (see 14.1.1)
- follow steps outlined in 14.1.4 (requirement for field use is within 0.3mg/L)
- record data on the CBP Field Monitoring Sheet

16. DATA COLLECTION

The entire cruise will be saved to one file, named based on the first station collected.

16.1 Connect the handheld to the sonde.

16.2 From the dashboard, select *Collect Data.*
- press *6* - scroll to select the current station
- press *3* to log one point of data - sets the file for the cruise.
- select *done*, the dashboard will load

16.3 Press *1* to log the data when the sonde is at the desired depth and data is stable. *Data Captured* will appear in the banner. *NOTE: there is a small lag time before you can capture another point, hitting the button twice quickly will only log once.*

16.4 At each subsequent station:
- select *Collect Data* from the dashboard
- press *6* - scroll to select the current station
- select *done*, the dashboard will load. (Changes current station but logs to same file.)
- repeat step 16.3

17. POST CRUISE CALIBRATION CHECK

Only for sondes used in the field, completed as soon as possible following cruise completion. May be completed using the computer or handheld, the sonde must be connected by cable or bluetooth. Recorded on back side of EXO2 Sonde Calibration Record sheet. File in appropriate notebook once complete (used or not used).

Rinse the sensors, guard, and cup by adding calibration solution to the cup, attach to the sonde and shake well. Repeat twice more then add sufficient solution for calibration. Rinse three times with reagent water before using another solution, and when complete.
NOTE: References to fill lines for calibration solutions are without the guard in the cup.

If a calibration check fails, repeat procedure. If the check fails again, refer to section 18 and perform maintenance of the sensor.

17.1 Dissolved Oxygen

17.1.1 Prepare environment - can use either

Water saturated air:

- Dry the sensors and guard, ensure the thermistor and DO membrane are moisture free.
- Put a small amount of tap water in the calibration cup, cap the sonde and leave vented (do not tighten). Allow to sit ≥10-15 minutes. *NOTE: Hold the cup on the sonde when lifting.*

Air saturated water:

- put a sufficient amount of tap water in a container
- bubble with aquarium bubble stones attached to outflow of vacuum pump for ~ 1 hour.

17.1.2 Check calibration:

- open the dashboard, record D.O. value
- record the standard D.O. value from saturation tables
- verify reading is ± 0.2mg/L of standard value

17.2 Specific Conductance

- Fill KCl standard to 2nd line of calibration cup, install sonde, tap to remove bubbles.
- open the dashboard, record the Specific Conductance (µS/cm) value
- verify reading is ±1500 µS/cm of 58600 µS/cm

17.3 pH

- Fill pH 7 buffer to 1st line of calibration cup, install sonde, tap to remove bubbles.
- open the dashboard, record pH value
- verify the reading is ±0.2mg/L of pH 7.0 (or pH 10)
- Repeat for pH 10 buffer.

17.4 Total Algae (Chlorophyll/BGA)

- Fill reagent water to 1st line of calibration cup, install sonde, tap to remove bubbles.
- open the dashboard, record Chlorophyll µg/L and RFU values

17.5 Depth

- Place sonde in a stable, vertical position - no calibration cup is needed
- open the dashboard
- record the depth value
- verify the reading is ±0.1m of 0m

18. MAINTENANCE AND STORAGE (User Manual section 6)

Place in "long term storage" (section 18.8) if instrument will not be used in about a month.
Use Krytox (YSI) or 3M Silicone grease for o-rings and connections, do not grease metal.
Locking nuts for sensors can be replaced (User Manual pg.133).

Notify WQL Supervisor if any equipment is found to be damaged or in poor condition.

18.1 Software and Firmware

- Check exowater.com for software and firmware updates for sonde, sensors, and handheld
- Refer to User Manual sections 6.7, 6.10, and 6.11 for further instructions

18.2 Sonde

- Inspect for damage and rinse with reagent water after each cruise
- Use port plugs to protect ports when not in use
- Inspect top connections, remove debris with compressed air and wipe clean
- Clean as needed:
  - fill calibration cup 3/4 with tap water, insert guard
  - spray Simple Green in top
  - scrub with brush
  - with sensors attached: spray with Simple Green, scrub sensors and bulkhead
- Replace batteries as needed and keep spare available.
  - when compartment accessed: inspect and/or replace o-rings, grease prior to reinstallation
- Inspect ports, clear with compressed air, grease plugs and sensors prior to installation

18.3 Field Cable

- Inspect for damage after each cruise. *NOTE: Can be inspected on cruise.*
- Inspect connections for debris, remove with compressed air and wipe clean

18.4 Handheld

- Clean with wet cloth as needed
- Power down fully when not in use
- During cruises - have spare batteries available, the rechargeable battery pack charger, and the handheld back cover for battery use.

18.5 Depth Sensor

- Periodically rinse ports using a syringe and tap or reagent water.
- Can be stored dry or in a moist environment.

18.6 D.O. sensor

- rinse with tap or reagent water after use
- Inspect cap for scratches in black paint, may still function correctly, replace if necessary
- store in a moist environment, use tap water (do NOT use reagent water)
  - cap with moist sponge or calibration cup attached with small amount of water
- replace membrane cap every 1-2 years, refer to User Manual section 6.17
- Rehydrate: (when in dry air >8 hours)
  - soak in tap water for ~ 24 hours
  - calibrate, store in a moist environment

18.5 Conductivity/Temperature sensor

- Rinse with tap or reagent water after use
- Clean ports with wet brush (tap or reagent water), brushing 15-20 times per port
  - Use mild soap solution if deposits are observed
  - If necessary, soak in dilute white vinegar for 5-10 min to remove deposits
- Does not need to be stored moist

18.6 pH sensor

- rinse with tap or reagent water after use
- store in a moist environment, use tap water (do NOT use reagent water)
  - cap with moistened sponge or
  - calibration cup attached to sonde with small amount of water
- replace module as needed, usually 12-18 months, refer to User Manual section 6.21
- Clean when deposits appear on glass or response time slows noticeably:
  - Do NOT scrub
  - soak in soapy water - 10-15 minutes
  - rinse
  - if condition has not improved, soak in 1M HCl or white vinegar - 30-60 minutes
  - rinse, soak in tap water - 1 hour, stirring occasionally
  - rinse, test response time
  - if condition has not improved, soak in 1:1 tap water and bleach - 60 minutes
  - rinse, soak in tap water - at least 1 hour
  - rinse, test response time
- Recondition sensor: (recommended quarterly)
  - soak in 1M HCl - 5 minutes
  - rinse
  - soak in 1:1 tap water and bleach - 30 minutes
  - rinse
  - soak in tap water - 1hour
- Rehydrate: (if allowed to dry)
  - soak in 2M KCl - overnight (74.6g KCl dissolved in 500mL reagent water)
  - soaking in tap water or pH buffer 4 may work

18.7 Total Algae sensor

- Rinse with tap or reagent water after use
- Inspect for defects and wipe clean with a lint free cloth as needed
- does not need to be stored moist

18.8 Long Term Storage

- Remove batteries from sonde and handheld
- C/T and Total Algae sensors:
  - can store wet on sonde, dry on or off sonde
  - If removed, cap both ends to prevent damage
- D.O. sensor:
  - Can remove from sonde, cap port end if done
  - submerge sensor end in tap water or cap with moist sponge
- pH sensor:
  - Remove from sonde, cap port end
  - Store sensor end in pH 4 buffer or 1M KCl
- Protect any open ports on sonde with port plugs

19. DOWNLOAD DATA

- Turn on handheld, connect to computer with USB cable once powered up.
- Open KOR software on the computer, wait for software to recognize handheld
- In *Options* (gears):
  - choose *sync with handheld*
  - In the *File Sync* section, choose *Data Files*

  NOTE: Units of enabled parameters may be change. Select Options, then Units; select desired units and choose Apply. Currently selected units can now be viewed and exported.
- In *Data* (file folder):
  - choose *View/Export*
  - select the desired file to view
  - choose the *Export Data* button (2nd to the right of the file name), data will export to excel
  - choose *File, Save as*, name file CBP### where ### is the cruise number
- Transfer to the network → K:\sci\sci cbp water quality lab\FIELD_YSI\YSI_DATA\
- Refer to *Database Instructions* binder, alter and save file as .csv (do not import to database)

20. LITERATURE CITED

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1100 p 1-44. American Public Health Association. Washington, DC.

EXO User Manual, item #603789REF, Revision D, April 2014, YSI Incorporated, Yellow Springs, Ohio 45387.

Oxygen Solubility Table. USGS website. http:/water.usgs.gov/cgi-bin/dotables

21. CALIBRATION DATA SHEET EXAMPLE

EXO2 Sonde Calibration

Calibration date: _____ time: _____ tech: _____

for Cruise: _____ Sonde: _____

Dissolved Oxygen Calibration (circle one): air saturated water water saturated air

calibration complete & QC score good? (Y/N): _____ ODO gain: _____ (~ 1 is normal)

calibration check environment (circle one): air saturated water water saturated air

Barometric Pressure (mmHg): _____ Temperature: _____ °C

Standard Oxygen Solubility: _____ mg/L Sonde Solubility Reading: _____ mg/L

Within 0.1mg/L? (Y/N): _____

pH Calibration: pH 7 buffer log#: _____ pH 10 buffer log#: _____

calibration complete & QC score good? (Y/N): _____

pH slope: _____ mV (~ 180 is normal, replace when close to 160)

pH calibration check, pH 7.00 buffer reading: _____

Conductivity Calibration

KCl standard: _____ Date Made: _____ Conductivity: 58600 µS/cm Molarity: 0.5M

calibration complete & QC score good? (Y/N): _____

Sonde Conductivity Reading: _____ µS/cm

Total Algae Calibration

RFU calibration complete & QC score good? (Y/N): _____ sonde check reading: _____

µg/L calibration complete & QC score good? (Y/N): _____ sonde check reading: _____

Depth Calibration

calibration complete & QC score good? (Y/N): _____

0m standard - sonde reading: _____ m

Transfer calibration files to the network --> Data\EXO Calibrations\CBP### where ### = cruise number

files transferred to network: _____

Post Cruise Calibration Check

Check date: _____ time: _____ tech: _____

Cruise: _____ Sonde: _____

Dissolved Oxygen

Barometric Pressure (mmHg): _____ Temperature: _____ °C

Standard Oxygen Solubility: _____ mg/L Sonde Solubility Reading: _____ mg/L

Within 0.2mg/L? (Y/N): _____

Conductivity

KCl standard: _____ Date Made: _____ Conductivity: 58600 µS/cm Molarity: 0.5M

Sonde Conductivity Reading: _____ µS/cm

Within 1500µS/cm? (Y/N): _____

pH

pH 7 buffer log#: _____ pH 10 buffer log#: _____

Sonde reading: _____ Sonde reading: _____

Within 0.2 units? (Y/N): _____ Within 0.2 units? (Y/N): _____

Chlorophyll

Zero point sonde reading: _____ RFU, _____ mg/L

Depth

Sonde depth reading: _____ m

Within 0.1m? (Y/N): _____

Appendix 5:

SOP In Vivo Fluorescence Measurement

STANDARD OPERATING PROCEDURE FOR THE MEASUREMENT OF *IN VIVO* FLUOROMETRY IN WATER AND SEAWATER USING A MODEL 10-AU TURNER FLUOROMETER

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APPROVED BY:

John C. Donat, Ph.D. Date
Director, WQL

Suzanne C. Doughten Date
Supervisor, WQL

DISCLAIMER: This SOP applies to the analysis of marine and estuarine water samples in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay and Elizabeth River. This SOP may not be applicable to any other studies.

TABLE OF CONTENTS

1. LOCATION

This analytical procedure will be used by Water Quality Laboratory staff located at 4211 Colley Avenue, Norfolk, Virginia. The procedure will be performed in the field preparation section and in the field for *in situ* monitoring.

2. PURPOSE

This procedure is designed to use a fluorometric technique to estimate chlorophyll *a* in estuarine and coastal waters as an indicator of water quality and to provide estimates of chlorophyll *a* concentrations throughout the study area for comparison to remote sensing data.

The intended user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program (CBMP). These data will be used to assess the effectiveness of the multi jurisdictional action plan for restoring the water quality of the Chesapeake Bay. This assessment will include various analyses of the data such as transectional data compilations and comparison with photosynthetic pigment data collected using discrete grab samples at selected locations. These data may also be used for ground-truthing of remote sensing (i.e. satellite image) data. These data may also be used by state and federal government officials to implement management actions designed to help improve the quality of the water in the Chesapeake Bay.

3. APPLICABILITY

This procedure is applicable to the continuous *in vivo* measurement of chlorophyll *a* in estuarine and coastal waters. It can also be used for monitoring chlorophyll *a* in fresh water. The salinity ranges encountered in the Chesapeake Bay are 0-36 parts per thousand.

The procedure assumes that spectrophotometric chlorophyll *a* data obtained from grab samples for calibration purposes are accurate and that fluorescence of chlorophyll *a* using the excitation and emission wavelengths specified in the procedure is proportional to the pigment concentration of the grab samples. Chlorophyll *a* concentration is reported in units of µg/L.

A method detection limit is only applicable to the chlorophyll grab samples, refer to the current Chlorophyll: Spectrophotometric Method SOP for further information.

4. OVERVIEW OF METHOD

The procedure described in this SOP is based on the fluorescence of chlorophyll molecules at specific wavelengths of light. At these wavelengths, the fluorescence of chlorophyll is proportional to ambient chlorophyll concentration. Ambient water is pumped through a flow-through cuvette fitted in the fluorometer to provide *in vivo* measurement of chlorophyll concentrations. Fluorometry data are collected using an internal data logger that captures data every 30 seconds. The position (latitude and longitude) for each discrete sample or for continuous flow through measurements are determined by matching time stamps to the Global Positioning System (GPS) data.

The data collected from the fluorometer are calibrated using spectrophotometry. Discrete water samples are collected from the exhaust of the fluorometer flow-through system and analyzed using spectrophotometric analysis for chlorophyll *a* concentration. A regression equation is created with these values and used to interpolate chlorophyll *a* concentrations from fluorometric measurements.

To calculate µg/L chla: (FL value) - y-intercept/slope = Chl_F

Samples for fluorometry calibration are collected in brown high density polyethylene (HDPE) sample bottles and buffered using 1 mL of saturated (1%) magnesium carbonate per 1000 mL of sample and filtered onboard.

NOTE: If filtration immediately following sampling is not possible, store in a refrigerator or on ice and filter by the following day. Chesapeake Bay Program recommends chlorophyll samples be filtered within 4 hours of collection.

The procedures for sample collection, filtration, handling, preservation, analysis, quality control requirements, and acceptance criteria are described in the current Chesapeake Bay Program Cruise Deployment SOP and Chlorophyll: Spectrophotometric Method SOP.

Any defect to the cuvette or air in the water flow may interfere in the voltage readings.

There are no method performance data for the in vivo portion of the analysis. Refer to the current Chlorophyll: Spectrophotometric Method SOP for method performance data compiled from the filter analysis portion of the procedure.

5. DEFINITIONS AND ABBREVIATIONS

CBMP:	Chesapeake Bay Monitoring Program
chla:	Chlorophyll *a*
g:	Gram
HCl:	Hydrochloric Acid
HDPE:	High-density polyethylene
L:	Liter
m:	Meter
mg:	Miligram
mg/L:	Miligram per liter
mL:	Mililiter
$MgCO_3$:	Magnesium Carbonate
µG/L:	Micrograms per liter
USEPA:	United States Environmental Protection Agency

6. EQUIPMENT

6.1 Safety Equipment

- A lab coat, used when handling strong acids and bases.
- Protective eye wear: Goggles when handling liquids, glasses for handling solids only.
- Nitrile gloves

6.2 Method Equipment *NOTE: Includes items not always necessary, refer to the current Chlorophyll: Spectrophotometric Method SOP for filtration equipment.*

- Fluorometer with cuvette, light source, appropriate wavelength filters, and power cord.
- Intake hosing
- Exhaust hosing
- $MgCO_3$ suspension
- Sampling pump
- 1L brown HDPE sample bottles
- Dropper bottle with 1mL designation
- 2-way hose connector(s)

7. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100, pollution prevention involves waste minimization. There are several ways a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personal should try to minimize the evaporation, spilling, and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

8. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

9. LABWARE AND REAGENTS

9.1 Cleaning volumetric flasks, filtration towers, graduated cylinders, and sample bottles:
Inspect labware for residue, breaks, or defects. Clean labware by scrubbing with a bottle brush (if able) and dilute 1% Liquinox® soap. Thoroughly rinse with tap water, rinse twice with 4N HCl (refer to note below), and 6 times with fresh reagent water, ensuring the entire inside surfaces are rinsed each time. Inspect again for residue, breaks, or defects. If none evident, dry on a clean drying rack (labeled "for clean labware only"), completely covered with clean paper towels.

NOTE: Acid destroys chlorophyll and is not to be used on any labware dedicated to the chlorophyll analysis, i.e. brown 1L HDPE sample bottles, centrifuge tubes.

When dry, store volumetric flasks, vials, and sample bottles with the caps on. Seal the tops of filtration flasks, graduated cylinders, Erlenmeyer flasks, and the tops and bottoms of filtration towers with parafilm. Store in the appropriate cabinet.

9.2 Chemicals Used - must be analytical grade or of a higher purity except as noted.
Magnesium carbonate ($MgCO_3$)

9.3 Reagent Preparation *NOTE: There are no standards for use with the fluorometer, grab samples are taken on a daily basis to develop calibration curves.*

Reagent containers are used only for the intended reagent and are reused. Prior to fresh reagent being added it is rinsed 3 times with reagent water and 3 times with the fresh reagent.

All reagents are documented in the working standard/reagent logbook. Each is assigned a unique number and the following information is recorded: reagent name, reagent number, date prepared, prepared by, date expires, identity (including chemical number) and quantity of each component, and final volume.

The following information must be recorded on each reagent bottle: reagent (name) and concentration, reagent log number, analysis (which requires this reagent), date prepared, prepared by (initials), expiration date (entry is N/A if there is no holding time), storage requirements (e.g. room temperature, 4°C, dark, etc.).

NOTE: Wipe down the counter top, exhaust hood, and balance with reagent water before preparing reagents. The quantity of reagent made may be adjusted proportionally as needed.

9.3.1 Magnesium Carbonate suspension: Add 1.0g of finely powered magnesium carbonate to 100 mL of fresh reagent water, measured using a graduated cylinder, in a clean reagent bottle. Shake immediately before use.

10. INSTRUMENT MAINTENANCE

Removal and cleaning of the continuous flow cuvette is an in-laboratory procedure only. Refer to the diagram on page A7-13 in the Field Fluorometer User's Manual. Refer to the User Manual for any needed maintenance not described here.

10.1 Preventive Maintenance

- Flush cuvette with reagent water following each cruise.
- Inspect the cuvette monthly (without removing)
- Remove cuvette and clean as needed, clean and lube screw threads at this time.
- Ensure o-rings are in good condition and well lubed.
- Ensure moisture does not collect in the sample compartment, use desiccant packets as needed.
- Keep the instrument case free of salt residue

10.2 Inspection Procedure - Ensure the power is off and water is drained.

- Remove the sample compartment cover. *NOTE: The gasket is attached to the cover.*
- Inspect the sample compartment for any liquid, remove if present.
- Inspect the cuvette for noticeable damage or debris, clean or replace as needed.

10.3 Cleaning Procedure - Ensure the power is off, water is drained, and any hosing is removed from the fittings. Wear gloves when handling the cuvette.

- Remove the sample compartment cover. *NOTE: The gasket is attached to the cover.*
- Remove the fitting screws, carefully rotate and pull to remove the fittings. *NOTE: The cuvette may come out with either.*
- Inspect the sample compartment for any liquid, remove if present.
- Clean the cuvette with 1% dilute liquinox, rinse with reagent water, and dry with kimwipes. *NOTE: Do NOT use anything abrasive.*
- Clean the intake and exhaust fittings with cotton swabs and reagent water, inspect the o-rings and replace if necessary. Lube prior to reinstallation. *NOTE: do not use acids.*
- Position the cuvette securely onto either fitting, ensure it is smudge free.
- Install the fittings and seat the cuvette within the sample compartment; replace screws, lube if needed. *CAUTION: Excess force can easily break the cuvette.*
- Install cover

10.4 Check for leaks by forcing water through the intake fitting. If a leak occurs: remove the cuvette and dry the sample compartment prior to reseating the cuvette, test again.

10.5 Lamp Replacement - *NOTE: If "Lamp is Off/Bad" message is displayed, replace lamp even if it still emits light. Do not look at the lamp on with out eye protection. Wear gloves.*

- With power off, remove sample compartment cover.
- Remove old lamp: grip the black bars, rotate to align the prongs with the opening and lift.
- Install new lamp: grip the black bars, align the prongs with the opening, lower and rotate.

11. ONBOARD PREPARATION

Refer to the current Chesapeake Bay Program Cruise Deployment SOP for procedure to collect samples in the field. Refer to the current Chlorophyll: Spectrophotometric Method SOP for filtration procedures.

On the R/V Fay Slover the fluorometer is placed on the top of the survival coat storage area with the instrument panel facing in to the lab. If another research vessel is used, position it for easy access both for sampling and reading the instrument panel. Connect the power cable to the fluorometer and outlet.

11.1 Hose Arrangement - *NOTE: Ensure connections are as water tight as possible.*

11.1.1 On the R/V Fay Slover:

- There are two faucets to the left of the fluorometer. Attach a hose from the intake fitting to the faucet on the right (underway system), use hose clamps to secure.
- Attach a hose to the exhaust fitting, the other end in the sink for discharge and grab collection.

11.1.2 On another research vessel:

NOTE: Prevent back pressure, ensure an exhaust route is open prior to changing flow direction.
Horizontal profile only: as described above, attach the underway system to the intake fitting and collect grab samples from the exhaust fitting. Vertical profile, see below: (Also refer to Sec.13)

- Connect a 2-way hose connector to the intake fitting.
- Connect the sampling pump to one of those connections.
- Connect another 2-way hose connector to the other connection.
- Connect the underway system to one of these connections, an exhaust hose on the other.
- Connect a hose to the exhaust fitting at the top of the fluorometer.
- To collect the horizontal profile:
  Turn off the sampling pump valve.
  Turn on the underway valve to direct the flow through the sample compartment.
  Turn off the exhaust valve for the underway system.
- To collect the vertical profile:
  Turn on the exhaust valve for the underway system.
  Turn off the underway valve, directing the flow to the exhaust from of the cuvette.
  Turn on the valve from the sampling pump to the sample compartment..

11.2 Internal Data Logger

- Turn on and allow to warm up for 10 minutes, the HOME screen will be displayed.
- Press <ENT> to access the Main Menu.
- Press <5> to choose the Internal Data Logger menu.
- Press <3> to choose Status.
- Press <ENT> to toggle between "stop" and "logging."
- Press <ESC> 3 times to get back to the HOME screen. "Logging data" should be displayed on the screen and raw data will appear.

12. PROFILE CODES

The following codes are recorded on the field sheet and entered into the fluorometry data table after the cruise.

1 - start Vertical Fluorometry profile (0.5m)
3 - start Horizontal Fluorometry profile
4 - end Horizontal Fluorometry profile
5 - Horizontal grab
6 - Vertical grab
7 - each depth during the Vertical profile (1m, 2m, 3m, 6m, 9m, ...)
NOTE: "7" is not recorded if a vertical grab is taken and 6 has been recorded.

13. VERTICAL PROFILE SAMPLING - collected on station, typically with a chlorophyll sensor on the sonde. The following procedure is for use with a sampling pump collection system.

- Direct the underway flow to exhaust, the sampling pump flow through the fluorometer.
- Deploy the sampling array then begin pumping water. *NOTE: Prior to recording data at any depth allow sufficient time for the water to reach the sampling compartment.*
- Record the event number, time, and depth on the fluorometry field sheet (see section 14) at 0.5m, 1m, 2m, 3m, and at every 3 meters thereafter up to and including the bottom. Record "7" on the field sheet. fluorometer keypad to mark these events. *NOTE: If recording the profile from the bottom to the surface: record the bottom, then at each multiple of 3 on the way up, ensuring 1m and 2m are collected.*
- Vertical Grab Samples - collected at the surface, bottom, above pycnocline or 1/3, and below pycnocline or 2/3.
  - Rinse and fill a 1L amber HPDE bottle from the exhaust hose of the fluorometer.
  - Record "6" on the field sheet
  - Add 1 mL of magnesium carbonate suspension to the bottle.

14. HORIZONTAL PROFILE SAMPLING - collected between stations

Collected only if the research vessel is able to collect GPS data underway.

- Direct the underway system flow through the fluorometer.
  NOTE: Ensure the sample pump is off prior to changing the water flow.
- Record "3" and the time on the field sheet as the boat departs the station to begin profile.
- Record "4" and the time on the field sheet as the boat arrives on station to end profile.

14.1 Horizontal Grab Samples - Collect an average of 5 horizontal grab samples per collection date for a total of 15 per typical mainstem cruise. An effort should be made to collect them over a range of voltage values observed.

- Rinse and fill a 1L amber HPDE bottle from the exhaust hose of the fluorometer.
- Record "5" and the time on the field sheet.
- Add 1 mL of magnesium carbonate suspension to the bottle.

15. POST-CRUISE

15.1 Shut down internal data logger

- Press <ENT> to access the Main Menu.
- Press <5> to choose the Internal Datalogger menu.
- Press <3> to choose Status.
- Press <ENT> to toggle from "Logging" to "Stop".
- Turn off the power to the fluorometer.

15.2 Breaking down on the research vessel

- Remove the hoses from the intake and exhaust fittings.
- Unplug the power cord from the outlet.
- Remove the power cord from the fluorometer and replace the protective cap.
- Flush the sample compartment with reagent water.

15.3 Download the Fluorometry Data

- Attach the fluorometer to computer, power the fluorometer on.
- On the computer:
  1. Open *Fluorometry Download* (desktop icon).
  2. Click *Download and Convert Data from Instrument to File*.
- On the fluorometer:
  - Press *ENT* to exit the HOME screen.
  - Press *5* to choose Internal Data Logger.
  - Press *4* to access the downloading sequence.
  - Press *8* five times to start downloading.
- On the computer:
  - Name the file as *CBP###.BIN*, where ### = cruise number. (An ASCII file is also saved.)
  - Close the window to exit the program.

15.4 Maintenance : See section 10.1 of this SOP.

15.5 Storage: The fluorometer is stored in the cruise prep area until the next cruise.

15.6 Refer to current Chlorophyll: Spectrophotometric Method SOP for procedure to analyze the chlorophyll grab samples

16. FLUOROMETRY FIELD DATA SHEET example

NOTE: Fluorometry is typically recorded on the same sheet as the light data.

CBP Mainstem Light Attenuation and Fluorometry Field Data Sheet

Cruise #:CBP_____ Date:_____

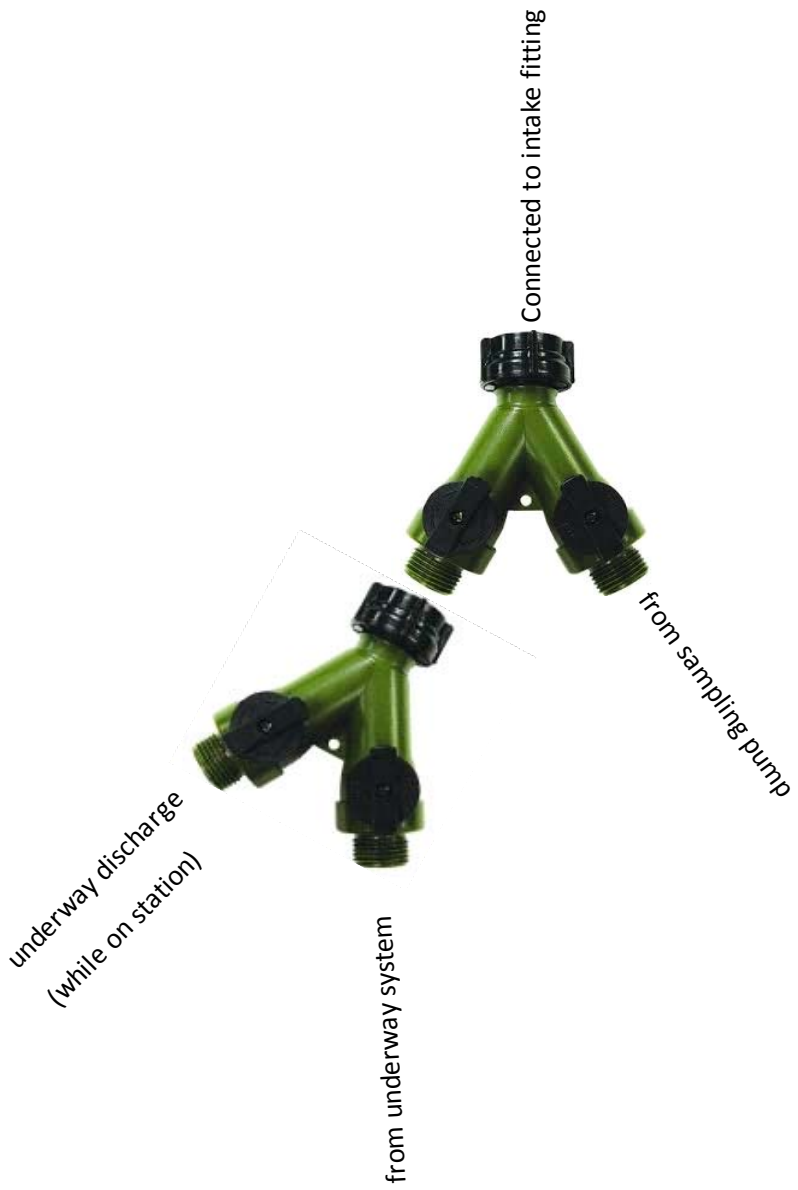
Grab	Event	Time	Station	Depth (m)	Light (µmol/sec/m²)		Comments
					Air (Ch. 1)	Water Up (Ch. 2)	

17. CHLOROPHYLL FIELD DATA SHEET example

CRUISE_____ CRUISE DATE _____
FILTERED BY _____ DATE FILTERED _____

LOG NUMBER	SAMPLE ID	Filtered		Extracted	Path Length	Analyzed		664/ 665 RATIO
		Time	Vol (L)	Date/ Initials		Date	By	

18. Figure 1: Hose schematic when using a submersible pump and collecting horizontal.



19. LITERATURE CITED

APHA. 1989. Standard Methods for the Examination of Water and Wastewater. 17th Edition. Method 10200 H. Chlorophyll. American Public Health Association. Washington, DC.

ASTM. 1979. Standard Practices for Measurement of Chlorophyll Content of Algae in Surface Waters. Method D3731-79. American Society for Testing and Materials. Philadelphia, PA.

Model 10-AU-005 Field Fluorometer User's Manual. October 1993. Order Number 998-5313.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1100 p 1-44. American Public Health Association. Washington, DC.

Appendix 6:

SOP Light Attenuation

STANDARD OPERATING PROCEDURE FOR MEASUREMENT OF LIGHT ATTENUATION IN SEAWATER USING INCIDENT RADIATION SENSORS AND A DATALOGGER

REVISED BY: Lori Ann Tew

FOR: Water Quality Laboratory
College of Sciences
Old Dominion University
Norfolk, Virginia 23529-0456

DOCUMENT FILE LOCATION: Water Quality Laboratory

EFFECTIVE DATE: July 1, 2015

TERMINATION DATE:

APPROVED BY:

John R. Donat, Ph.D. Date
Director, WQL

Suzanne C. Doughten Date
Water Quality Laboratory Supervisor

DISCLAIMER: This SOP applies to the physicochemical profiling of marine and estuarine water in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for the lower Chesapeake Bay and . This SOP may not be applicable to any other studies. **This SOP is for use with the LI-1500 Light Sensor Logger. If using the LI-1000 DataLogger, refer to SOP LIGHT-6.**

TABLE OF CONTENTS

1. LOCATION

This analytical procedure will be used by Water Quality Laboratory staff located at 4211 Colley Avenue, Norfolk, Virginia. The procedure will be performed in the cruise preparation section and in the field.

2. PURPOSE

This procedure is designed to measure photosynthetically active radiation between 400 and 700 nm, and how these light levels are attenuated by depth.

The intended data user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program (CBP). These data will be used to assess whether the multi-jurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of the data and its inclusion in computer models of the Chesapeake Bay. Trend analyses may also be performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and its tributaries.

3. APPLICABILITY

This procedure is applicable to the measurement of photosynthetically active radiation in fresh, estuarine, and coastal water samples. The amount of incident radiation are measured in units of $\mu mol\ s^{-1}\ m^{-2}$.

4. OVERVIEW OF METHOD

A 2 Π underwater radiation sensor, pointed upward, is lowered into the water column. A 2 Π surface sensor is mounted on the research vessel in a location unobstructed by shadows. The sensors are connected to a datalogger by cables. The data from the surface sensor are used as a reference measurement for changing levels of incident radiation on the surface of the water. By measuring light values at different depths and normalizing it to the radiation at the surface, the attenuation coefficient can be computed for the water column. This attenuation is used in the same manner as the secchi depth as a measure of the amount of radiation which penetrates the water column to support growth of phytoplankton and submerged aquatic vegetation.

Shadows interfere in obtaining a true measurement, therefore should be avoided.

There is no method detection limit for this procedure.

Neither reagents nor standards are required for this method and no continuing calibrations are performed. Sensors are returned for calibration every two years.

Neither samples nor waste is collected; pollution prevention methods are not needed.

No chemicals are used in this procedure; safety equipment is not necessary.

5. METHOD PERFORMANCE SPECIFICATIONS

The following are taken from the LI-COR light sensor logger instruction manual. There are 3 current inputs using BNC connectors. This procedure uses 2 sealed BNC current channels.

Current Accuracy: ± 0.3% of full scale reading (25°C)
Frequency Rejection: > 70 dB at 50 or 60 Hz
Range Selection: Auto range or Fixed range (selectable)

Current ranges	Resolution
0 - 0.250 µA (micro-amps)	0.0305 nA (nano-amps)
0 - 2.50 µA	0.1525 nA
0 - 25 µA	1.525 nA
0 - 250 µA	15.25 nA

Averaging: Only available in Standard Modes Sampling (see Table B-3 in instruction manual)
Display: Real time data in Monitor Mode
Real Time Clock: Year, Month, Day, Hour, Minute, Seconds
Accuracy ± 3 min per month

6. DEFINITIONS AND ABBREVIATIONS

°C	degrees Celsius
BNC	Bayonet Neill-Concelman (type of connector)
CBP	Chesapeake Bay Program
CTD	conductivity, temperature, depth sensor
nm	nanometer
PAR	photosynthetically active radiation
s or sec	Second(s)
SA	Surface Air
T	Temperature

USEPA	United States Environmental Protection Agency
UU	Underwater Up
WQL	Water Quality Laboratory
2 Π or 2 pi	type of radiation sensor
μmol s^{-1} m^{-2}	lumens, a measurement of light intensity, = μE s^{-1} m^{-2}

7. EQUIPMENT

LI-1500 Light Sensor Logger
Underwater Quantum Sensor (currently LI-192)
Terrestrial Quantum Sensor (currently LI-190R)
Cable for use with underwater sensor
Data transfer cable

8. MAINTENANCE

8.1 Clean the sensors after each use with dilute Liquinox® and water.

NOTE: Vinegar can be used to remove hard water deposits from the diffusor element.

CAUTION: Do not use organic solvents, abrasives, or strong detergents to clean the diffusor element on the sensors.

8.2 Return the sensors to the manufacturer (LI-COR) for recalibration every 2 years.

8.3 Check sensor operation each cruise by confirming a positive reading when in light and a near zero reading when covered.

NOTE: A negative reading in lit conditions indicates the polarity of the sensor is reversed (it is plugged into the cable backwards).

NOTE: A very positive or negative reading under dark indicates a possible short in the cable or some other electrical short.

9. ON BOARD PREPARATION

NOTE: PAR is determined from a sun lit position during daylight hours. Both surface and underwater sensors must be away from the shadow of the vessel.

9.1 Secure the surface sensor at an unobstructed high point. Connect the cable to the channel 1 BNC connection of the datalogger.

9.2 Connect the underwater sensor to the data cable and secure it to the sampling array pointing upward Connect the cable to the channel 3 BNC connection of the datalogger.

NOTE: If different channel inputs are used, ensure all the correct settings match the sensors in the datalogger.

The remaining set up is completed on the datalogger.

9.3 Power on the datalogger.

9.4 *CBPLIGHT will be highlighted. Press **OK**. The Monitor Mode screen will be displayed.
Display for CBPLIGHT
YYY/MM/DD HH:MM:SS
BATTERY: #.##V ###%
CONFIG: CBPLIGHT
STATUS: Not Logging (will say *Manual Log* when ready to record)
1SA: 0.000 (values will appear after 10 seconds)
3UU: 0.000

NOTE: Channel 1 is designated as SA (surface air) and channel 3 as UU (underwater up).

9.5 Verify the configuration file.
Press **MENU**, select *Configurations* and press **OK** twice.
Settings for CBPLIGHT
- Active: Yes
- Inputs: 2
- Samp Rate: 1 Hz
- Range: Auto
- Outputs: 2
- Routine: Manual

NOTE: If a new configuration file is required, see Section 3 of the instruction manual.

9.6 Verify the sensors to be used. Select *Inputs: 2* and press the right arrow key. If the serial numbers do not match, highlight the input and press the right arrow key. Select the correct sensor from the list and press **OK**.

NOTE: If more than two years have passed since the date of the sensor's last calibration date, a "**Sensor out of Calibration**" message will appear.

9.7 Verify the sensor information. Press **MENU**, highlight *Sensors* and press **OK**. Highlight the desired sensor serial number and press the right arrow key. If the multiplier value(s) and calibration date are correct, press **EXIT** back to the Monitor Mode screen.

NOTE: If a new sensor needs to be added, select < Add New Sensor> *, press* **OK**. *Select the model number and enter all pertinent information (serial number, multiplier(s), and calibration date).The multiplier is the calibration coefficient for each sensor and is specified on the calibration documentation for each sensor.*

9.8 Verify the averaging values. Press **MENU**, select *Configurations*, press **OK** twice. Select *Outputs: 2* and press the right arrow key. Select *1SA* and press **OK**. *Averaging* should be set to *10 sec* and *Min/Max* should read *No*. Press **EXIT**, and repeat with *3UU*. The settings should be the same.

NOTE: Averaging *is the number of seconds that the datalogger will calculate a running average. The CBP uses a 10 second average.*

NOTE: Steps 9.9 and 9.10 can be done at a later time.

9.9 Ensure both channels 1SA and 3UU are displaying positive values in the sunlight.

9.10 Cover both the surface and underwater sensors, creating a dark environment, ensure both channels 1SA and 3UU are displaying values close to zero.

9.11 Verify the time and date are correct. If it needs to be synched with the underway system on the boat, select **MENU** > *Console Settings* > *Set Time* and press **OK**. Use the left and right arrows to switch between fields and the up and down arrows to change the numbers. Press **OK** and **EXIT** back to the Monitor Mode screen.

9.12 Turn datalogger off until needed.

10. DATA COLLECTION

While collecting data keep in mind:

- Underwater eadings should display a decreasing trend descending through the water column.
- The SA value should always be higher than the UU value. (The angle of sunlight may also affect this if taking readings close to sunrise or sunset)
- The readings should not be negative. If they are, the sensor or cable may need replaced. If the readings are negative when in the air, the polarity of the sensor may be reversed (plugged in backwards).
- Wait at least 10 seconds for the readings to stabilize prior to recording measurements. The displayed numbers will constantly adjust but should be relatively stable.
- Note on the field datasheet if sensor(s) are in shade and it cannot be avoided; retake reading if able.

Readings are taken at 0.5m, 1m, 2m and 3m, and then at intervals based on the secchi for the station. Readings may be taken ascending or descending, to a depth where light values for channel 3UU are < 1% of the 0.5m value, or bottom is reached.

Secchi	Depth Intervals
0.0m - 0.3m	0.25m
0.4m - 0.6m	0.5m
0.7m - 1.9m	1.0m
2.0m - 2.9m	2.0m
3.0m+	3.0m

NOTE: Ensure 0.75m is collected if a 0.25m interval is required.

10.1 Position the sampling array at the desired depth in the water column and turn on the datalogger. When ******CBPLIGHT* is highlighted, press **OK**.

10.2 From the Monitor Mode screen, press the **START**│**STOP** key. Select *New File* (enter a name). Enter CBP###, where ### is the bay cruise number for the month. Press **OK**, and *Status* will now read *Manual Log*.

NOTE: For using an Existing File*, select* CBP### > Append To Data*.* ***Never*** *overwrite data.*

10.3 Record SA and UU values on the datasheet (along with the station name and depth); press

LOG to make an electronic recording.

10.4 Repeat step 10.3 for each depth needed.

NOTE: Turn the datalogger off between stations to conserve the battery.

11. DATA DOWNLOAD

11.1 Connect the datalogger to the computer with the USB cable. It will appear as a mass storage drive named **LI-1500**.

11.2 On the computer: Open the **DATA** folder in the **LI-1500** drive, locate the desired logging file, and copy it to the **LIGHT** folder on the network.

12. CALCULATIONS

The attenuation coefficient equation:

$$k_d = \frac{\ln(\text{light value at surface air}) - \ln(\text{light underwater up value at depth})}{\text{depth}}$$

13. DATA QUALITY ASSESSMENT

Measurements of accuracy, precision, and calibration checks are not practical for this analysis.

After the data have been downloaded, forward the data to the laboratory supervisor for assessing whether there are any outliers, and whether the data demonstrate explainable patterns.

14. LITERATURE CITED

LI-COR, 2014. LI-1500 Light Sensor Logger Instruction Manual, LI-COR®, Inc. Lincoln, NB. Version 2, October 2014. Publication No. 984-14406.

Appendix 7:

SOP Dissolved Organic Carbon

STANDARD OPERATING PROCEDURE FOR TOTAL AND DISSOLVED ORGANIC CARBON IN WATER AND SEAWATER USING COMBUSTIVE/NON-DISPERSIVE INFRARED GAS ANALYSIS

REVISED BY: Suzanne C. Doughten

FOR:

Water Quality Laboratory

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Norfolk, Virginia 23529-0456

DOCUMENT FILE LOCATION: Water Quality Laboratory

EFFECTIVE DATE: November 15, 2016

TERMINATION DATE:

APPROVED BY:

John R. Donat, Ph.D. Date
Water Quality Laboratory Director

Suzanne C. Doughten Date
Water Quality Laboratory Supervisor

DISCLAIMER: This SOP applies to analysis of marine and estuarine water samples in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

TABLE OF CONTENTS

1. LOCATION

This procedure will be used by Water Quality Laboratory (WQL) staff located at 4211 Colley Ave., Norfolk, Virginia. The procedure will be performed in the Water Quality Laboratory.

2. PURPOSE

This procedure is designed to measure the amount of total and/or dissolved organic carbon in ambient water samples. The dissolved organic carbon (DOC) measurement is an important component of the living resources collection for the USEPA Chesapeake Bay Monitoring Program (CBMP)

The intended data user groups are the subcommittees and workgroups of the CBP. These data will be used to assess whether the multi-jurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of the data, and its inclusion in computer models of the Chesapeake Bay. Trend analyses are also performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and its tributaries.

3. APPLICABILITY

This analytic procedure is designed to measure the amount of total and/or dissolved nonpurgeable organic carbon in ambient water samples from estuarine and near-coastal areas. It can also be used to analyze fresh water samples. The salinity ranges normally encountered in this laboratory are 0-36 parts per thousand. The total and/or dissolved organic carbon is reported in units of mg/L. This procedure is applicable to determining total and dissolved nonpurgeable organic carbon in water in the range from 0.5 to 1000 mg/L.

4. OVERVIEW

The method utilized is based on EPA method 415.1, combustion infrared method and SM 5310 B 19th Edition. The Standard Operating Procedure Method 2532 Carbon - July 16, 1999 - Total Organic and Dissolved Organic Nonpurgeable, Commonwealth of Virginia, Department of General Services, Division of Consolidated Laboratory Services was utilized to help set up this method.

The instrument used for this analysis is a Shimadzu® Total Organic Carbon Analyzer Model TOC-LCSH installed on June 10, 2016. The internal software is Shimadzu® TOC-Control version 1.04.

Samples collected by the WQL for the Chesapeake Bay Program are collected using either a Teflon go-flow bottle or a submersible pump which is fitted with a polyethylene hose. Water is filtered through a pre-rinsed 4.7 cm Whatman® GF/F (or equivalent) glass fiber filter by vacuum filtration at ≤20 in. Hg. The filtrate is collected in a pre-combusted glass bottle with a Teflon lined top or a Teflon bottle. Samples are frozen until analysis. Samples collected for different projects and by clients may have different protocols. To determine total organic carbon (TOC) content the sample is not filtered.

The filtered or unfiltered water sample is injected into a heated catalyzed reaction zone in which the carbonaceous matter is converted to carbon dioxide. The carbon dioxide flows through a detector, and peak area of the sample is displayed. The peak area is converted into TOC or DOC mg/L by utilization of the stored calibration curves. The instrument can store several calibration curves which can cover different ranges if necessary. If the calibration curve is exceeded the sample will be diluted into range.

The carbonates and bicarbonates will interfere with the determination of total and dissolved organic carbon. This interference is eliminated by acidification to a pH < 2 and sparging prior to analysis.

5. METHOD PERFORMANCE

Individual samples are injected a minimum of three times, and the standard deviation must be ≤ 0.200 or the CV must be ≤ 2.00. If this criteria is not met, then up to two additional samples are injected to try to meet the criteria.

Eight analyses of the RT Corporation® WP Demand PE 1130-20ML from June 2016 to August 2016 produced an average value of 3.698 mg/L The true value for this QC is 3.63 mg/L. This is an average recovery of 102%

Eleven analyses of blank samples from June 2016 to August 2016 produced an average value of 0.039 mg/L with a standard deviation of 0.0585 and a range of -0.053 to 0.17 mg/L.

Five analyses of 2.5 mg/L standard from June 2016 to August 2016 produced an average value of 2.503 mg/L. This is an average recovery of 100% with a standard deviation of 0.0542 and a range of 2.428 to 2.578 mg/L.

Eleven analyses of 5.0 mg/L standard from June 2016 to August 2016 produced an average value of 5.040 mg/L. This is an average recovery of 101% with a standard deviation of 0.0960 and a range of 4.884 to 5.195 mg/L.

6. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100 , pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personal should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

7. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

8. SAFETY EQUIPMENT AND PROCEDURES

8.1 A Lab Coat is to be used when handling strong acids and bases, i.e. HYDROCHLORIC ACID, SULFURIC ACID, SODIUM HYDROXIDE.

8.2 Protective eye wear: Goggles when handling liquids; glasses are approved for handling solids only.

8.3 Nitrile gloves

8.4 Furnace gloves: Gloves resistant to high temperatures must be used when the furnace heated to 550°C is used.

9. DEFINITIONS AND ABBREVIATIONS

DOC:	Dissolved Organic Carbon
TOC:	Total Organic Carbon
Reagent water:	Resistivity > 10 megohm-cm
L:	Liter(s)
mL:	Milliliter(s)
g:	Gram(s)
mg:	Milligram(s)
mg/L:	Milligram(s) per liter
N:	Normality of the chemical solution (g/L)
M:	Molarity of the chemical solution (mol/L)
CBP:	Chesapeake Bay Program
CBMP:	Chesapeake Bay Monitoring Program
USEPA:	United States Environmental Protection Agency

Refrigerator temperature: 0 to 6°C
Freezer temperature: -18°C or below

10. SAMPLES

10.1 Use the data sheets, master logbook, and/or chain-of-custody documents to determine the log numbers of the samples to be analyzed.

10.2 Remove samples from storage. Log out samples in the chain-of-custody logbook.

10.3 Inspect samples for breaks in the bottles or other problems.

10.4 If it is necessary to thaw frozen samples place them in a sink containing warm water.

11. LABWARE CLEANING

11.1 All labware except volumetric pipettes and sample tubes:

Inspect labware for breaks, defects, discoloration or residues. Clean labware by scrubbing with a bottle brush and dilute Liquinox® soap. Thoroughly rinse with tap water. Rinse twice with 4N hydrochloric acid (HCL), then 6 times with fresh reagent water, ensuring that the entire inside surfaces are rinsed each time. Dry on a clean drying rack (labeled **"for clean labware only**"). If discoloration or residue persists after cleaning, then discard labware.

Applicable glassware is combusted at 550 ± 50° C for 4 hours.
Note: Only glassware used exclusively for carbon analysis is combusted. Example: Filtration flasks and volumetric pipettes are not combusted. No contamination has been found in the field blank samples.

When dry, store volumetric flasks, vials and sample bottles with the caps on. Seal the tops of filtration flasks, graduated cylinders, and Erlenmeyer flasks, and the tops and bottoms of filtration towers. If labware has been combusted and no caps are available, seal with aluminum foil. Store in the appropriate cabinet.

11.2 Volumetric pipettes:

Check the label on the pipette soaking cylinder to verify that the soapy water has been changed within the last 7 days. If not, empty the soaking cylinder, rinse with tap water, then fill with fresh soapy water using Liquinox® soap and tap water. Replace the label on the soaking cylinder with a new label indicating the date the soapy water was changed and your initials.

Soak pipettes, with tips pointing up and completely submerged, overnight. Remove from soaking container and inspect for breaks, defects, discoloration or residues. Thoroughly rinse with tap water. Rinse twice with 4N hydrochloric (HCL) acid, then rinse with freshreagent water, twirling the pipettes to ensure that all of the inside surfaces are rinsed each time. Dry upside down and covered on a clean pipette rack.

11.3 Sample tubes:

After use, rinse tubes out with Liquinox® soap and tap water. Either acid rinse the tubes or place the tubes in the muffle furnace and combust at 550 ± 50° C for 4 hours. Both procedures can also be done, with muffling being the preferred method over only acid cleaning if both methods cannot be done. When cool, store covered with caps on.

12. REAGENTS

12.1 Chemicals Used:

Potassium hydrogen phtaltae ($C_8H_5O_4K$)
Hydrochloric Acid (HCl)
Soda Lime
Shimadzu® Platinum Catalyst
Shimadzu® Halogen scrubber
Quartz Wool
Sulfuric Acid
Phosphoric Acid (H_3O_4P)

12.2 On each reagent bottle the following information must be recorded:

Reagent Identification:
Date Prepared:
Prepared by:
Expiration date:
Storage Requirements: (ie. ambient temperature,refrigerated, in dark, etc.)
Reagent Number

12.3 Preparation of Reagents needed for analysis:

NOTE: Quantities of each reagent prepared may by adjusted (ie. halved or doubled) as necessary.

12.3.1 Stock standard (1000 mg carbon/L): Measure 2.1254 grams of potassium hydrogen phthalate. Dilute to volume in a one liter glass-stoppered volumetric flask with reagent water. Acidify to pH <2 by adding 0.5 mLs of sulfuric acid (H_2SO_4). Store in refrigerator. Holding time is at least one year.

12.3.2 Certified Reference Material (CRM): Follow manufacturer's instructions, then create a dilution within range of the calibration curve.

12.3.3 Matrix Water: reagent grade water.

12.3.4 2.4 N HCl: *NOTE: Prepare in an exhaust hood using gloves and eye protection.*
Measure 20 mL of Hydrochloric Acid (HCl) using a graduated cylinder and carefully pour into approximately 50 mL of fresh reagent water in a 100 mL volumetric flask. Mix, then dilute to volume with fresh reagent water.
Store in a glass stoppered glass reagent container at ambient temperature. Shelf life N/A.

12.3.5 IC Reagent: *NOTE: Prepare in an exhaust hood using gloves and eye protection.*
Measure 50 mL of Phosphoric Acid (H_3O_4P) using a graduated cylinder and carefully pour into approximately 100 mL of fresh reagent water in a 250 mL volumetric flask. Mix, then dilute to volume with fresh reagent water.
Store in a glass stoppered glass reagent container at ambient temperature. Shelf life N/A.

12.3.6 0.05 M HCl: *NOTE: Prepare in an exhaust hood using gloves and eye protection.*
Measure 5 mL of Hydrochloric Acid (HCl) using a graduated cylinder and carefully pour into approximately 50 mL of fresh reagent water in a 100 mL volumetric flask. Mix, then dilute to volume with fresh reagent water.
Store in a glass stoppered glass reagent container at ambient temperature. Shelf life N/A

13. PREPARING CALIBRATION STANDARDS AND MATRIX SPIKES

13.1 Matrix water, stock standard and CRM stock must be at ambient temperature .

13.2 10 mg C/L: Pipet 1.0 mL of carbon stock standard into a 100 mL glass-stoppered volumetric flask, and dilute to volume with reagent water.

13.3 NOTE: The calibration curve and spike preparation listed here is for most samples encountered in the Chesapeake Bay and analyzed in this laboratory. For other samples/projects a different calibration curve may need to be utilized. Consult with Laboratory Supervisor to determine proper curve, and document what standards were used and how they were prepared.

The instrument prepares the calibration curve from the 10 mg C/L standard. The recommended concentrations used are from 1-8 mg C/L. The recommended standards are 8.0, 5.0, 4.0. 2.5, 1.0 and 0.

13.4 Preparing spikes:

13.4.1 Prepare one matrix spike from a randomly-chosen sample for every 10 to 20 samples which will be analyzed.

13.4.2 Rinse a 25 mL glass-stoppered volumetric flask three times with the sample that will be spiked.

13.4.3 Add 0.1 mL of 1000 mg C/L stock to the rinsed 25 mL glass-stoppered volumetric flask.

13.4.4 Dilute to volume with the sample. The final concentration of the matrix spike is 4.0 mg C/L.

13.4.5 At the end of each day store the standards in a refrigerator for use up to 7 days if instrument maintains calibration.

14. PRE-OPERATION SET UP

14.1 Turn on instrument. Turn on power button on side of instrument. Wait approximately 30 seconds and turn on power on front of instrument.

14.2 Turn gas (ultra pure air) cylinder and regulator on. Record amount of gas in cylinder each day instrument is used.

14.3 Turn on computer and monitor. Open the TOC-Control software. Enter username. Open a new or existing table and click CONNECT to establish a link to the instrument.

NOTE: Under help there is an on-line manual that can be referred to during analysis.

14.4 Select INSTRUMENT. Select H/W SETTINGS. Select TOC tab. Choose 680 under furnace (if not already selected). This turns the furnace on.

14.5 Before starting analysis the following should be done each day the instrument is used:

Change the water in the rinse container and sample line. Use fresh reagent water each day.

Check the water in the dehumidifier drain vessel. It should be right below the outlet tube. If low level there is a leak. Need to troubleshoot. If bubbling there is a leak in the instrument. Need to troubleshoot.

Check the acid is bubbling in the B-type halogen scrubber. If the acid level is too low, add 0.05 mL HCL to scrubber. Change the stainless mesh if it has degraded

Check halogen scrubber is <4/5 black. Change if necessary.

Check water level in the humidifier. Add reagent water if below or close to lowest line.

Check 2.4 N HCL in the acid container on the side for sparging. Add acid if necessary.

Check IC solution level (diluted phosphoric acid). This is necessary for instrument operation even if IC is not being analyzed. Add IC reagent if necessary.

14.6 If starting instrument from cold shut down, run zero check to insure syringe fills correctly. Run this a minimum of once a month. This is found under INSTRUMENT MAINTENANCE.

14.7 After furnace warms up the READY light will light up. The TOC-L will have a green light when ready.

14.8 Analyze a blank before running the calibration curve and samples to check the instrument operation. The blank must be under 20 mV. In the WQL the blank generally has an area under 2 mV.

15. PLACING STANDARDS AND SAMPLES ON TOC INSTRUMENT

15.1 For the standard curve the sample line is placed directly in the volumetric flask that is used for the dilutions. Cover the flask with aluminum foil.

For samples use combusted glass tubes for analysis. Rinse the tubes three times with the sample before dispensing the sample.

15.2 Cover the sample tube with a cap or aluminum foil if not analyzing immediately.

15.3 A replicate, spiked sample, standard blank and calibration check standard must be analyzed after every 20 analytical results. Exceptions are when clients do not provide enough sample and do not require spikes and/or replicates. Some projects may require more QC. Always end the analysis with a replicate or spiked sample, standard blank and calibration check standard.

15.4 The following is for a typical CBP analysis for DOC. Some of the analysis parameters may need to be changed depending on the samples analyzed. (Example. The standard curve may need to be different or the sparge time increased.)

Select CALIBRATION CURVE in upper left box tab.

Select NEW. Select NEXT.

Page 2:
NORMAL. Check used dilution from Standard Solution. Select NEXT.

Page 3:
Select Analysis: NPOC
Default Sample Name and Sample ID: Leave blank
Unclick ZERO SHIFT.
Click MULTIPLE INJECTIONS.
Calculation Method: Linear Regression
File Name: StdCurveDATE.cal (Date:MMDDYYYY)

Page 4:
Units: mg/L
No. of injections: 3/5
No. of washes: 3
SD Max: 0.200
CV Max: 2.00
Sparge gas flow rate: 80
Sparge times: 5:00 min (this can change for different sample types)

Page 5:
Add point
Standard Solution Conc. 10.0 (Change to zero for Blank)
Cal Point: Refer to standards in section 13.3. Enter highest to lowest.
Other fields will be entered automatically by software.

Page 6:
Use default setting.
Select Finish.

Place sample line in Standard Solution.

In the sample table INSERT the CALIBRATION CURVE just made in the first line of the table. Press START to analyze the calibration curve. Select Single Measurement. Select Start.
NOTE: If all the standards are coming from same source and a blank is not analyzed, select BATCH and it will automatically advance to the next standard.

After each standard select NEXT then START to start next standard.

NOTE: If hit REPEAT it will reanalyze the standard concentration using the sparged sample in the syringe.

Move sample line to reagent water when analyze zero.

15.5 In the sample table choose INSERT the SAMPLE.

Click on calibration curve and select current calibration curve file. Click OPEN.

Page 1:
Click Edit Parameters manually. Click Next.

Page 2:
Default Sample Name: Station ID and depth or standard concentration.

Default Sample id: Log number or enter BLANK

Manual Dilution: 1.000 if not diluted or enter dilution.

No. of determinations: 1 (enter more if want to sample more than once)

Click Next

Page 3:
Confirm correct calibration curve. Click Next.

Page 4:
units: mg/L

Expected conc range: High Standard

No. of injections: 3/5

SD Max: 0.200

CV Max: 2.00

No. of washes: 3

Auto dilution: 1 (or add dilution if needed)

Sparge gas flow: 80

Sparge time: 5 minutes (different sample types may require different sparge times)

Acid Addition: 2%

Click multiple injections. (If this is not clicked will sparge for each injection)
Page 5:
Use default settings.

Page 6:
Pharmaceutical water testing: NONE

Click Finish.

15.6 Complete Sample Table.

16. STARTING THE ANALYSIS

16.1 Press **start** to start the instrument.

If analyzing standard curve select BATCH if all out of the same stock standard. Then select START.

If analyzing multiple lines out of the same sample vessel, select BATCH. Then select START.

If analyzing a sample and have to move the line for the next sample, select SINGLE MEASUREMENT. Then select START.

16.2 Check the calibration correlation coefficient of the calibration curve (See step 17.1 for DQA assessment). Select Preview. Select sample report. Calibration curve coefficient is displayed on this report. Alternatively, highlight calibration curve line in sample table. Right click measurement settings. Select Graph tab.

16.3 If calibration curve is acceptable, analyze the CRM.

16.4 Verify that the measured concentration of the CRM is acceptable (See step 17.2 for DQA assessment).

16.5 If the answer to steps 16.4 and 16.5 is yes, then the analysis is in control and may continue.

16.6 Next analyze a calibration check standard.

16.7 Verify that the measured concentration of the calibration check standard is acceptable (See step 17.3 for DQA assessment).

16.8 Next analyze a blank sample.

16.9 Verify that the measured concentration of the blank is acceptable (See step 17.6 for DQA assessment).

16.10 Samples are now able to be analyzed.

16.11 When the last sample has been analyzed, the instrument will stop.

16.12 If the last sampled analyzed is a BLANK, nothing else needs to be done at the end of the day. This has rinsed the machine.

If the last sample of the day is NOT a BLANK, rinse the sample line. The syringe is automatically rinsed at the end of the samples. Place the sample line in reagent water. Select INSTRUMENT, then MAINTENANCE, then WASHING. The line is OFFLINE. Press start. This rinses the sample line.

16.13 DATA REPORT

Refer to Database Instructions notebook on how to report the data.

17. DATA QUALITY ASSESSMENT

All data must meet all of the data quality assessment criteria specified in the following steps. In step 17.6 correction action is outlined if the criteria in steps 17.1 to 17.5 are not met.

17.1 Evaluate the calibration correlation coefficient and proceed accordingly:

$r \geq 0.9990$	Acceptable
$r = 0.9980 - 0.9989$	Acceptable but troubleshooting is required
$r < 0.9980$	Stop analysis. Troubleshooting is required

17.2 Assess whether the analytical result for the SRM sample confirms the calibration when calculated as follows:

$$\%\ Recovery = \frac{AMC}{CRM}\ x\ 100\%$$

Where,

AMC = Average measured concentration of the CRM sample

CRM = Concentration of the CRM sample

The analytical result must fall within the range of 90-110%.

17.3 Assess whether the calibration error, as defined by the analytical result for each calibration check standard is within the range of the true value ± 90-110%.

Calibration check standards are diluted automatically by the instrument so they are prepared the same as the calibration curve. The dilution factor is automatically calculated so the result displayed matches the standard the dilution was prepared from.

17.4 Assess whether the percent recovery for each spiked sample:

Is within 80 to 120% when calculated as:

$$MSR = \frac{SSR - SR}{SA x DF} \; x \; 100$$

where,

SSR	=	Measured concentration of spiked sample,
SR	=	Measured concentration of the unspiked sample
SA	=	Concentration of the spike.
DF	=	Dilution factor

17.5 Assess whether the precision for each duplicated sample is in-control, as follows for the coefficient of variation (CV) is ≤20%:

The coefficient of variation (CV) is defined as:

$$\frac{SD}{MEAN} * 100$$

where standard deviation (SD) is defined as:

$$SD = \sqrt{\sum_{i=1}^{n} \frac{(y_i - \bar{y})^2}{n-1}}$$

where MEAN is defined as:

$$\frac{(D_1 + D_2)}{2} = MEAN$$

The CV does not need to be calculated if $|D_1 - D_2|$ is less than or equal to the PQL or lowest standard.

17.6 Assess whether the method blank check is in-control. The blank should be less than or equal to the PQL. The PQL is normally the lowest standard.

Note: For a curve range of 1.0 mg/L to 8.0 mg/L, 0.5 mg/L is used as PQL for the blank.

If the method blank is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

An exception for reanalysis of samples for an out of control method blank is for the field blanks. Field blank data may be kept if it is analyzed with an out of control method blank, but all other quality control samples are in control and the field blanks are below the PQL.

17.7 If the data meet all of the data quality assessment criteria defined in steps 17.1 through 17.6, the analytical results are in-control. If any of the data quality objectives are not met, the analysis is out-of-control.

All analytical results obtained within the out-of-control period (since the previous occurrence of in-control QC measurements and until the next occurrence of in-control QC results) should be reanalyzed.

An exception is for out-of-control spike recovery. Replicate the spike and the sample, if all results agree than the out-of control spike recovery is due to matrix interferences and is not considered a problem with the analysis. The samples will be kept and it is documented on the analysis.

NOTE: In situations where there is not enough sample to reanalyze, the client will be notified of the problem and how they want to proceed. This is determined on a case by case basis with the client and the WQL Supervisor. The data will be marked on the analytical data sheet and on correspondence with the client, if the client requests the data which is measured with out of control QC measurements.

17.8 Ecological patterns:

Once the data meets all of the data quality assessment criteria, forward the data to the laboratory supervisor for assessing whether the data demonstrate ecologically explainable patterns.

18. METHOD DETECTION LIMIT

Method Detection Limit is defined as the minimum concentration that can be detected and determined to be statistically different from zero. The method for determining MDLs is based upon student t times the standard deviation of not less than seven replicate analyses of the same sample. This MDL will be highly dependent upon the instrument, reagents, SOPs, personnel and, most importantly, the matrix of the media being analyzed.

The method detection limit is determined once a year for this analysis, using a low level sample collected in the field or a standard made 1 to 5 times the current MDL. The results are reported in a Method Detection Limit table in the WQ_DATA database maintained by the Water Quality Laboratory.

19. INSTRUMENT SHUT DOWN

19.1 The instrument will stop after the last sample is analyzed

19.2 If a BLANK was the last sample analyzed, no more rinsing is needed. If a sample was analyzed last, wash the sample line before shutting instrument down.

19.3 Press the SHUTDOWN button. Press OK.

19.4 After approximately 30 minutes, the main power switch can be turned off on the instrument. This is the power switch on the side of the TOC-L.

19.5 Turn off the gas supply.

20. PREVENTATIVE MAINTENANCE

20.1 Prior to use:

Refer to section 14.5 for daily maintenance procedures.

20.2 To look for leaks:

If bubbling in the humidifier, leaks are before the humidifier.

Pinch the tubes that connect to the CO_2 absorber. If it bubbles in the dehumidifier drain vessel than there are leaks in the back of the instrument. Note: if notice bubbling in dehumidifier drain vessel when sampling, also suspect leaks in back of instrument (ie. filter clogged, halogen scrubber leak, etc.).

20.3 As needed:

Regeneration of TC catalyst. Place sample line in the 2.4 N HCL. Select INSTRUMENT. Select MAINTENANCE. Select REGENERATION OF TC CATALYST. This is in the TOC-L manual Section 4.2.1 on pages 236 and 237.

20.4 Monthly:

Zero point detection.

20.5 Annually:

Replace carbon dioxide absorber located behind the analyzer.

20.6 As needed:

20.6.1 If the calibration correlation coefficient exhibits a decreasing trend, or if precision decreases (standard deviation or coefficient of variation exhibits an increasing trend), wash the catalyst This is in the TOC-L manual Section 4.2.2 on pages 237 through 240.

20.6.2 If washing the catalyst does not correct the problem, than replace the catalyst. This is in the TOC-L manual Section 4.2.2 on pages 237 through 240 if using a regular combustion column.

For Chesapeake Bay samples a combustion tube for high salt samples is used. Refer to the Shimadzu TOC-L Series Combustion Tube for High Salt Samples Instruction Manual. Refer to Chapter 2 pages 2-13 with instructions on how to fill the column, and how to install the column on the instrument. In Figure 2.3 it shows a cooling unit, but the high salt combustion tube was too large to use the box. The tube is coiled on the bottom of the instrument.

21. TROUBLESHOOTING

See section 4.6 pages 261 to 276 of the Instruction Manual for Shimadzu® TOC-L for flow charts of error messages and how

In addition there is a program provided by Shimadzu that is entitled TOC-L Virtual Advisor that can be utilized for troubleshooting.If dehumidifier drain vessel overflows check the waste vessel that the line is not under the water level. This will cause back pressure.

If zeros are high and contamination of the sample container or water is eliminated, check the halogen scrubber. This may need to be replaced.

22. LITERATURE CITED

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1100 p 1-44. American Public Health Association. Washington, DC.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Method 5310B, High Temperature Combustion Method. p 5-17m American Public Health Association. Washington, DC.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1030 C. p 1-9. American Public Health Association. Washington, DC.

TOC-L CSH/CSN User's Manual. Shimadzu Corporation. September 2014.

Shimadzu TOC-L Series Combustion Tube for High Salt Samples Instruction Manual. Shimadzu Corporation. November 2012.

Appendix 8:

SOP Total and Fixed Suspended Solids

STANDARD OPERATING PROCEDURE FOR TOTAL SUSPENDED SOLIDS DRIED AT 103-105ºC AND FIXED SUSPENDED SOLIDS IGNITED AT 550ºC

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DOCUMENT FILE LOCATION: Water Quality Laboratory

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APPROVED BY:

John R. Donat, Ph.D. Date
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Suzanne C. Doughten Date
Water Quality Laboratory Supervisor

DISCLAIMER: This SOP applies to the analysis of marine and estuarine water samples in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to other studies.

TABLE OF CONTENTS

1. LOCATION

This analytical procedure will be used by Water Quality Laboratory staff at 4211 Colley Ave., Norfolk, Virginia. The procedure will be performed in the field or in the field preparation section of the laboratory.

2. PURPOSE

This procedure is designed to measure total and fixed suspended solids in water samples as an indicator of water quality. Suspended solids, along with algae, can significantly reduce light penetration, impacting survival of Submerged Aquatic Vegetation and disrupting light-dependent daily water column migrations of zooplankton. Elevated concentrations of suspended solids can also affect feeding rates of organisms like oysters and clams, which filter their food from overlying waters (Scope of Work for Chesapeake Bay Monitoring Program 1/1/2002-6/30/2002). The amount of sediment that is present as fixed suspended solids is thought to represent the inorganic solids which are a result of sediment runoff. The amount of sediment that is present as volatile suspended solids (which can be derived from subtracting the fixed suspended solids from the total suspended solids) is thought to represent the organic solids that indicate algae as the source of the solids.

The intended data user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program (CBMP). These data will be used to assess whether the multijurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of data, and its inclusion in computer models of the Chesapeake Bay. Trend analyses are also performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and its tributaries.

3. APPLICABILITY

This procedure is designed to measure the total suspended solids (TSS) and fixed suspended solids (FSS) in fresh, estuarine, and coastal water samples. The results are measured and reported in units of mg of TSS/L and mg of FSS/L of water.

This SOP does not meet the minimum requirements for obtaining TSS data in studies mandating compliance with 40CFR136 (USEPA, 1983).

4. OVERVIEW OF METHOD

This SOP is based on a modification of Standard Method 2540 D and E (APHA, 1989) for the analysis of estuarine and coastal water samples. Samples are collected according to the current CHESAPEAKE BAY PROGRAM CRUISE DEPLOYMENT SOP. *NOTE: Samples collected by other sources or projects may have different methods.*

Samples are filtered immediately, refrigerated or packed on ice until filtration.

A well-mixed sample is filtered through a pre-rinsed, pre-weighed and pre-combusted Whatman® GF/F glass fiber filter. The residue retained on the filter is dried to a constant weight at 103 to 105 ºC. The increase in weight of the filter is due to the filtered material and represents the total suspended solids. The filter is then ignited at 550±50°C. The amount of suspended material retained after ignition represents fixed suspended solids. If the suspended material clogs the filter and prolongs filtration time to more than 10 minutes, a lesser sample volume is filtered.

5. DEFINITIONS AND ABBREVIATIONS

*u*L: Microliter(s)
mL: Milliliter(s)
L: Liter(s)
*u*g: Microgram(s)
mg: Milligram(s)
g: Gram(s)
psi: Pounds per square inch
M: Molarity of the chemical solution (mol/L)
N: Normality of the chemical solution (g/L)
HDPE: High-density polyethylene
CBMP: Chesapeake Bay Monitoring Program
USEPA: United States Environmental Protection Agency
APHA: American Public Health Association
CRM: Certified Reference Material
Refrigerate: 0-6°C
Frozen: -18°C or below
reagent water: Resistivity > 10 megohm-cm
invert: In reference to homogenizing a sample by inverting, one inversion is: starting with the cap of the sample bottle pointing straight up, turn the sample bottle completely upside down so the cap is pointing straight down, then turn the sample bottle upright until the cap is pointing straight up again.

6. SAFETY EQUIPMENT

Lab coat
Furnace gloves
Nitrile gloves

A lab coat and Nitrile gloves should be worn if samples are suspected to contain contaminants. Gloves resistant to high temperatures must be used when the furnace heated to 550°C is used.

7. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

8. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

9. METHOD PERFORMANCE

A 0.1 gram class 1 weight standard is weighed before each time the balance is used. For 100 weighings of this standard the average weight obtained was 0.09999 grams. The range in weights was from 0.09993 to 0.10005.

The following applies to Total Suspended Solids (TSS) samples:

Using samples analyzed in 2007, the average difference between 118 duplicate saline samples with a concentration below 10 mg/L and a mean concentration of 6.6 mg/L was 0.799 mg/L. The standard deviation of the difference was 0.708.

Using samples analyzed in 2007, the average difference between 47 duplicate saline samples with a concentration between 10 and 20 mg/L and a mean concentration of 13.31 mg/L was 1.032 mg/L. The standard deviation of the difference was 0.915.

Using samples analyzed in 2007, the average difference between 14 duplicate saline samples with a concentration above 20 mg/L and a mean concentration of 33.38 mg/L was 1.647 mg/L. The standard deviation of the difference was 1.46.

The following applies to Fixed Suspended Solids (FSS) samples:

Using samples analyzed in 2007, the average difference between 141 duplicate saline samples with a concentration below 10 mg/L and a mean concentration of 4.63 mg/L was 0.740 mg/L. The standard deviation of the difference was 0.656.

Using samples analyzed in 2007, the average difference between 26 duplicate saline samples with a concentration between 10 and 20 mg/L and a mean concentration of 13.00 mg/L was 0.989 mg/L. The standard deviation of the difference was 0.877.

Using samples analyzed in 2007, the average difference between 8 duplicate saline samples with a concentration above 20 mg/L and a mean concentration of 35.8 mg/L was 2.324 mg/L. The standard deviation of the difference was 2.06.

10. LABWARE

10.1 Labware needed:

The labware needed noted in this SOP is for a typical Chesapeake Bay sampling. Other projects or sample numbers may change the amount or type of labware needed. Specific quantities of labware are located in each section.

10.2 Labware cleaning

Clean by scrubbing with a bottle brush (if able) and dilute Liquinox® soap. Then thoroughly rinse with tap water. Rinse twice with 4N hydrochloric acid (HCl), then 6 times with fresh reagent water. Dry on a clean drying rack labeled for clean labware only. When dry, store with caps on, if applicable. When caps aren't applicable, seal items with parafilm M or aluminum foil (or equivalent).

11. QUALITY ASSURANCE AND QUALITY CONTROL

It is especially important to maintain the balance in peak operating condition. Data quality is also defined by an ongoing assessment of calibration accuracy and analytical precision, as follows:

11.1 Processing and analyzing a sample in duplicate a minimum of every 10 samples.

11.2 Performing a calibration check using two class 1weights to bracket expected weight range each day the balance is used.

11.3 Monthly checking of the analytical balance’s accuracy by weighing a range of class 1 weights.

11.4 Processing and analyzing a standard reference material (CRM) or CBP blind audit sample at least semi-annually.

NOTE: CRM samples are prepared according to manufacturer’s instructions and documented in the Working Standard/Reagent Logbook.

11.5 Class 1 weights are re-certified annually.

11.6 The balances are serviced annually by a qualified service engineer.

12. CALIBRATION STANDARDS

A 100mg class 1 weight is used to verify that the calibration is linear and to measure accuracy of the balance.

13. EQUIPMENT

Perform weighings using the Sartorius® series MC1, model RC 210 S (or equivalent) analytical balance. Software used is Labtronic® Collect/W v 2.00 4-ch.

Oven capable of maintaining temperatures of 103 to 105°C.

Laboratory Furnace capable of maintaining temperatures of 550±50°C.

14. PRE-ANALYTICAL PROCEDURE

The labware needed is for a typical Chesapeake Bay sampling. Other projects or sample numbers may change the labware needed.

14.1 Labware Needed:

1- forceps
1- 2000 mL or 4000 mL filtration flask
1- filtration tower
1- filtration manifold
1- vacuum pump with hoses, stoppers and side arms

See section 10.2 for the appropriate cleaning procedures.

14.2 Preparation of Glass-Fiber filter:

NOTE: If only sampling TSS skip steps 14.2.6 and 14.2.7.

14.2.1 Using forceps, gripping only the filter edge, transfer a 4.7 cm Whatman® GF/F glass fiber filter (or equivalent) with the wrinkled side up onto the base of a filtration tower. Replace the top of the filtration tower onto the base.

14.2.2 Apply ≤20 in Hg vacuum and rinse the filter with 3 successive portions of fresh reagent water that are approximately 20mLs.

14.2.3 Continue suction to remove all traces of water.

14.2.4 Remove filter from filtration apparatus and transfer to an aluminum weighing pan. Refer to tracking sheet in Section 21 and record Date, Time, and Done by on the TSS tracking

sheet under "filter prep".

14.2.5 Dry the filters in an oven at 103 to 105ºC for at least 1 hour. Record Date, Time, Done by, and Oven Temperature on the TSS tracking sheet under "in oven".

14.2.6 Place the filters in a furnace preheated to 550±50° C for at least 15 minutes.

14.2.7 Remove the filters from the furnace and allow the filters to cool so they can be handled.

14.2.8 Transfer the filters to the desiccator. Verify that the desiccant in desiccator is blue. If it's not, place the desiccant in oven at 103 to 105ºC until blue. Cool the filters in a desiccator to ambient temperature (at least 1 hour). Record Date, Time, Done by, and Oven Temperature on the TSS tracking sheet under "out of oven".

14.2.9 Use the Sartorius® series MC1, model RC 210 S analytical balance linked with a computer with Labtronic® Collect/W v 2.00 4-ch. to weigh the filters. Record Date, Time, and Done by on the TSS tracking sheet under "pad weights".

Note: If this balance unavailable an equivalent analytical balance may be used. The instructions in steps 14.2.10 to 14.2.26 are specific for the software and balance from step 14.2.9.

14.2.10 Turn on computer and monitor. Ensure the balance computer cord is plugged into port A.

14.2.11 At the sign in screen enter the username and password.

14.2.12 Check the workstation only button on the sign in screen

14.2.13 Open Collect /W Jr.

14.2.14 Go to instrument, open and choose Sartorius® Balance

14.2.15 At the instrument Data Destination window, the application should read Microsoft Excel®. Click OK.

14.2.16 Return to the Collect /W window. Go to configure, instrument and choose Sartorius® Balance.

14.2.17 Locate the template needed (ie. CBP, ER or a split sample) in C:\TSS.

14.2.18 In the template, fill in the first log number of the samples. The template will automatically update the other log numbers.

14.2.19 Go to File. Use Save As and name the file. For the Chesapeake Bay cruises name the files as BAYXXX, where XXX stands for the cruise number. For the split samples use the name of the split sample, the month and the year (ie.. MCBMMYY).

14.2.20 Open the balance door.

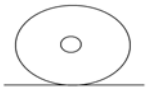
14.2.21 Clean off the balance pan with a brush.

14.2.22 Close the balance door. Tare the balance by pressing the long bar labeled T.

14.2.23 After the balance is stabilized, open the balance door and place a 100 mg weight on the balance pan.

14.2.24 Move the cursor on the computer to the cell for the standard weight.

14.2.25 Press the print key on the balance. This button is on the bottom right. It is a circle with a line under it and another circle inside of it.



14.2.26 The weight will appear on the Excel® spreadsheet when the balance has stabilized.

14.2.27 If the standard weight is not ± 0.0005g. of it's true weight, repeat Steps 14.2.20 to 14.2.26. If there still is not an accurate result, refer to the balance manual for calibration procedures.

DO NOT PROCEED WITH WEIGHING FILTERS UNTIL THE BALANCE IS DEMONSTRATED TO BE WITHIN CALIBRATION

14.2.28 The filters can now be weighed. Open the balance door.

14.2.29 Open the desiccator, remove a weigh pan with filter, and close the desiccator.

14.2.30 Place a filter on balance. *Note: Never touch a filter with fingers, only use forceps or by inverting weigh pan.*

14.2.31 Close the balance door.

14.2.32 Move the cursor on the computer to the cell for the sample to be weighed and the weight

to be recorded.

14.2.33 Press the print key on the balance.

14.2.34 The weight will appear on the Excel® spreadsheet when the balance has stabilized.

14.2.35 Record the cruise number or project name, sample ID, and log number on the bottom of the weigh pan.

14.2.36 Open balance door. Remove the filter using a pair of forceps without touching the filter, and place filter in a filter holder that is labeled with the appropriate cruise number, log number, and sample ID.

14.2.37 After all of the samples are weighed, save the file on the computer. In addition, save it to the ODU Server Network .

14.2.38 Check the tare of the balance after approximately every 20 samples weighed.

14.2.39 Repeat steps 14.2.5 and 14.2.8 through 14.2.38 for 10% of the samples to verify pad weight. Each filter must weigh ± 0.0005g. of the first weight. If the filters do not meet this criteria, repeat the cycle of drying and weighing until two successive weights are within ± 0.0005g for all of the samples.

15. SAMPLE COLLECTION, FILTRATION AND PRESERVATION

15.1 Collection

The labware needed is for a typical Chesapeake Bay sampling. Other projects or sample numbers may change the labware needed.

15.1.1 Labware needed:

1- 2000 mL HDPE sample bottle for each sample
See section 10.2 for the appropriate labware cleaning procedures.

15.1.2 Collect the samples according to the current CHESAPEAKE BAY PROGRAM CRUISE DEPLOYMENT SOP.

15.1.3 Rinse the entire inside surface of the 2000mL sample bottle three times with sample, discarding the rinsate.

15.1.4 Fill the sample bottle with sample, leaving approximately one inch of air space.

15.1.5 Samples should be filtered immediately after collection. If this is not possible, store samples by refrigerating or packing the sample bottles in ice up to the level of the top of the sample but below the bottom of the sample bottle cap for no more than 7 days.

NOTE: These holding times and sample collection apply to the Chesapeake Bay Program. Other clients may have different requirements.

15.2 Filtration

The labware needed is for a typical Chesapeake Bay sampling. Other projects or sample numbers may change the labware needed.

15.2.1 Labware needed:

4- filtration towers
4- 2000mL filtration flasks
1- forceps
4- 500mL graduated cylinders (a smaller or larger volume may be needed for specific samples)
1- filter holders (or weigh pans can be used if in laboratory) for each sample
1 extra-filter holders (or weigh pans can be used if in laboratory) for every 10 samples for duplicates
1- filter holder (or weigh pans can be used if in laboratory) for each scheduled sample date for field blank

See section 10.2 for the appropriate labware cleaning procedures.

15.2.2 Using forceps, gripping only the filter edge, transfer a pre-rinsed and pre-weighed 4.7 cm Whatman® GF/F glass fiber filter (or equivalent) with the wrinkled side up onto the base of a filtration tower. Replace the top of the filtration tower onto the base. Make sure the filtration tower is on the back row of the TSS/PPO_4 manifold.

15.2.3 Moisten the filter with Type I reagent water. Move the filtration tower from the back row to the front to collect the filtrate in a 2L filtration flask if the filtrate is needed for other analyses.

15.2.4 Mix the sample thoroughly by inverting (see definitions) the sample bottle several times until well-mixed. Immediately rinse the graduated cylinder twice with the sample to be filtered, discarding the rinsate.

15.2.5 Immediately pour a pre-determined volume of sample into the rinsed graduated cylinder. *Record the filtration volume in the appropriate space on the total suspended solids data sheet.*

NOTE: 500 mL is the maximum sample volume filtered in the field.

15.2.6 Filter the sample water through the filter using ≤20 in. Hg vacuum. To avoid cell damage during filtration, do not exceed this vacuum, and limit filtration duration to 10 minutes or less. If it takes longer than 10 minutes to filter, discard the filter and remaining sample. Rinse following step 15.2.12, then complete steps 15.2.2 through 15.2.6 using a lesser sample volume. Make sure to record the new volume on the total suspended solids/particulate phosphate data sheet and initial and date the change.

NOTE: If not enough sample to filter another TSS sample, record how long it took to filter and notify the Chief Scientist and/or Laboratory Supervisor.

15.2.7 When sample filtration is complete, move the filter and filtration tower to the back row of the manifold.

15.2.8 Apply vacuum of ≤20 in. Hg. Completely rinse all inside surfaces of the graduated cylinder with reagent water. Pour the reagent water into the filtration tower.

15.2.9 Rinse the graduated cylinder again, following step 15.2.8.

15.2.10 Rinse around filtration tower with approximately 20mL of reagent water, allowing it to pass through the filter.

15.2.11 Using forceps, being careful not to touch or disturb the filtrate, fold the filter in half. Remove the filter from the filtration tower and place it in a filter holder which is labeled with the Project ID, sample ID and sample log number. Place the cover on the filter holder.

15.2.12 Completely rinse the empty filtration tower at least one time with reagent water before filtering the next sample.

15.2.13 Repeat steps 15.2.2 through 15.2.12 for each sample. Record Date and Done by on the TSS tracking sheet under "filtered by".

NOTE: Filter one randomly chosen sample in duplicate for 10% of the samples collected following steps 15.2.2 through 15.2.12.

NOTE: At a time that is near the end of the Chesapeake Bay cruise, filter one "field blank" for each sampling date. Follow steps 15.2.2 through 15.2.12, except one liter of reagent water *is filtered instead of sample water. Other projects may not require field blanks, or they may require them at different frequencies.*

15.2.14 After filtration the filters are placed in a 103 to 105 °C oven or frozen. Filters dried overnight are considered stable, and can be shipped without ice. If an oven or freezer is unavailable, the samples may be kept on ice until they arrive in the laboratory. If samples have been dried in an oven before arriving, they will need to be redried according to this SOP before they are weighed.

16. SAMPLE ANALYSIS

NOTE: If only sampling TSS skip steps 16.1.6 through 16.1.14.

16.1.1 Dry the filter for at least 1 hour at 103 to 105°C in an oven. Record Date, Time, Done by, and Oven temperature on the TSS tracking sheet under "Samples in Oven".
NOTE: If removing samples from freezer note in chain of custody logbook.

16.1.2 Verify that the desiccant in the desiccator is blue. If not place desiccant in oven at 103 to 105°C until blue. Cool filters in a desiccator to ambient temperature (at least 1 hour). Record Date, Time, Done By, and Oven temperature on the TSS tracking sheet under " Out of Oven".

16.1.3 After the samples have been in the desiccator at least one hour, weigh the samples according to Steps 14.2.20 to 14.2.34, except the saved file will be opened instead of a template. Return the filter to the weigh pan after weighing. On the tracking sheet, record Date, Time and Done By under "Dry Weight**"**.

16.1.4 Repeat steps 16.1.1 through 16.1.3 for 10% of the samples to verify TSS dry weight. Filter must weigh ± 0.0005g. of the first weight. If the filters do not meet this criteria, repeat the cycle of drying and weighing until two successive weights are within ± 0.0005g for all of the samples.

16.1.5 Calculate the data using the Total Suspended Solids Database Instructions or calculate by hand using the instructions in step 17.1.

16.1.6 After the TSS data has been approved by the Laboratory Supervisor, the FSS analysis can be conducted.

16.1.7 Place the filters in a furnace preheated to 550±50°C for at least 20 minutes. If Particulate Phosphate (PPO4) analysis is also to be performed on these filters, leave the filters in the furnace for at least 1 ½ hours. Record Date, Time, Done By, and Oven Temperature on the tracking sheet under "**Samples Muffled**".

16.1.8 Remove the filters from furnace and allow them to cool so they can be handled.

16.1.9 Transfer the filters to a desiccator. Verify that the desiccant in the desiccator is blue. If not, place the desiccant in an oven at 103 to 105 °C until blue. Cool the filters in a desiccator to ambient temperature (at least 1 hour). Record Date, Time, Done by, and oven temperature on the TSS tracking sheet under "Out of Oven".

16.1.10 After the samples have been in the desiccator at least one hour, weigh the samples according to Steps 14.2.20 to 14.2.34, except the saved file will be opened instead of a template. Return the filter to the weigh pan after weighing. On the tracking sheet record Date, Time and Done by under "FSS Weights".

16.1.12 For samples which were muffled for 1.5 hours, repeat steps 16.1.1 through 16.1.3 for 10% of the samples to verify FSS dry weight. Filters must weigh ± 0.0005g of the first weight. If filters do not meet this criteria, repeat cycle of muffling in a furnace at 550±50°C and weighing until two successive weights are within ± 0.0005g for all of the samples.

For samples which were muffled for approximately 20 to 30 minutes, repeat steps 16.1.7 through 16.1.9 for 10% of the samples to verify FSS dry weight. Filter must weigh ± 0.0005g. of the first weight. If filters do not meet this criteria, repeat cycle of muffling in furnace at 550±50°C and weighing until two successive weights are within ± 0.0005g for all of the samples.

16.1.13 Calculate the data using the Fixed Suspended Solids Database Instructions or calculate by hand using instructions in step 17.2.

16.1.14 After the FSS data has been approved by the laboratory supervisor, the PPO_4 analysis can be conducted if appropriate.

17. CALCULATION OF ANALYTICAL RESULTS

17.1 Calculate total suspended solids (TSS) using the following equation:

$$mg\ TSS/L = \frac{(A-B) \times 1000}{sample\ volume,\ L}$$

where:

A = weight of filter + dried residue in grams; and
B = weight of filter in grams

NOTE: See database instructions under Total Suspended Solids for instructions on importing and calculating the data.

17.2 Calculate fixed suspended solids (FSS) using the following equation:

$$mg\ FSS/L = \frac{(C-B) \times 1000}{sample\ volume,\ L}$$

where:

C = weight of filter + dried residue in grams after ignition at 550°C; and
B = weight of filter in grams

NOTE: See database instructions under Total and Fixed Suspended Solids for instructions on importing and calculating the data.

17.3 Calculate volatile suspended solids (VSS) using the following equation:

$$mg\ VSS/L = mgTSS/L - mgFSS/L$$

18. DATA QUALITY ASSESSMENT

All data must meet all of the data quality assessment criteria specified in the following steps. If any data do not meet all of the specified criteria, the applicable part of the analysis is out-of-control and the data must be forwarded to the laboratory supervisor and quality assurance officer as soon as possible.

18.1 Assess whether the analytical result for the CRM sample when analyzed is within the 90 to 110 % confidence limits supplied with the CRM sample.
NOTE: If the purchased CRM's acceptability limits are wider they will be followed.

18.2 Assess whether the calibration error, as defined by the analytical result for the calibration check using a class S weight is within the range of ± 0.0005 grams.

18.3 Assess whether the precision for each set of replicated samples is in-control, as follows:

The coefficient of variation (CV) must be less than or equal to 20% when calculated as:

$$CV = \frac{sd}{\bar{x}}$$

where: CV = Coefficient of Variation;
sd = Standard Deviation; and
$\bar{x}$ = Mean Concentration.

18.4 Assess whether the field blanks are in-control. The blank should be less than the average of the last 10 calculated MDL values. If the field blank is greater than the average MDL, the blank is determined to be contaminated. If the field blanks are out of control, the data will be qualified.

18.5 If the data meet all of the data quality assessment criteria defined in steps 18.1 through 18.4, the analytical results are in-control. If any of the data quality objectives are not met, the analysis is out-of-control and the data must be forwarded to the laboratory supervisor and quality assurance officer as soon as possible.

If there is a problem with obtaining 20% CV between the two or three replicates of a sample, institute the following troubleshooting steps during sample filtration:

1) Increase the amount of reagent water used to rinse the filter and sample, in case salt residue is not being completely rinsed.

2) Increase the number of times the sample is inverted before filtration.

18.6 Ecological patterns:

Once the data meets all of the data quality assessment criteria, forward the data to the laboratory supervisor for assessing whether the data demonstrate ecologically explainable patterns.

19. METHOD DETECTION LIMIT

Method Detection Limit is defined as the minimum concentration that can be detected and determined to be statistically different from zero. The method for determining MDLs is based upon student t times the standard deviation of not less than seven replicate analyses of the same sample. This MDL will be highly dependent upon the instrument, reagents, SOPs, personnel and, most importantly, the matrix of the media being analyzed.

The method detection limit is determined once a year for this analysis, using a low level sample collected in the field. The results are reported in a Method Detection Limit table in the WQ_DATA database maintained by the Water Quality Laboratory.

20. LITERATURE CITED

APHA. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition. Method 209 C. American Public Health Association, Washington, DC.

APHA. 1989 Standard Methods for the Examination of Water and Wastewater. 17th Edition. Method 2540 D and E. American Public Health Association, Washington, DC.

USEPA. 1983. Methods for Chemical Analysis of Water and Wastes. Method 330.5. U.S. Environmental Protection Agency. Cincinnati, OH. 521 pp.

USEPA. 1983. Guidelines Establishing Procedures for the Analysis of Pollutants. Title 40 Code of Federal Regulations, Part 136, as amended.

Scope of Work for Chesapeake Bay Monitoring Program 1/1/2002-6/30/2003.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1100 p 1-44. American Public Health Association. Washington, DC.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1030 C. p 1-9. American Public Health Association. Washington, DC.

21. TRACKING SHEET FOR THE ANALYSIS OF TOTAL SUSPENDED SOLIDS AND FIXED SUSPENDED SOLIDS FOR THE CHESAPEAKE BAY AND ELIZABETH RIVER CRUISES

For other projects tracking sheet may be modified to meet the requirements of that project.

Cruise:_____ Sampling Date(s):_____

	Date	Time	Done By	Oven Temp. AM	Oven Temp. PM
Filter Prep					
In Oven (104°C ≥1hr.)					
Muffle (550±50°C≥15min.)					
Out of Oven (≥1hr.)					
Pad Weights					
Copy file to disks (2)					
10% in Oven (104°C ≥ 1hr.)					
Out of Oven (≥1hr.)					
2nd Pad Weights					
Transfer pad weight data to network					
Filtered By					
Samples in Oven (104°C ≥1hr.)					
Out of Oven (≥1hr.)					
Dry Weight					
10% in Oven (104°C ≥1hr.)					
Out of Oven (≥1hr.)					
2nd Dry Weight					
Tss approved					
Samples Muffled (550±50°C ≥1.5hr.)					
Out of Oven (≥1hr.)					
FSS Weight					
10% in Oven (104°C ≥1hr.)					
Out of Oven (≥1hr.)					
2nd FSS Weight					

22. EXAMPLE OF FILTRATION SHEET FOR THE ANALYSIS OF TOTAL SUSPENDED SOLIDS FOR THE CHESAPEAKE BAY

For other projects filtration sheet may be modified as long as they contain the log number, sample ID and filtration volume.

Total Suspended Solids/Particulate Phosphate

Cruise # _____ Sampling Date_____

LOG NUMBER	SAMPLE ID	TSS -(07) VOL (L)	PP (-04) VOL (L)	PP DUP (-04) VOL (L)	TIME FILTERED
	EE 3.4 S				
	EE 3.4 B				
	EE 3.5 S				
	EE 3.5 B				
	CB 5.4 W S				
	CB 5.4 W B				
	CB 5.4 S				
	CB 5.4				
	CB 5.4				
	CB 5.4 B				
	CB 5.5 S				
	CB 5.5				
	CB 5.5				
	CB 5.5 B				
	CB 6.1 S				
	CB 6.1				
	CB 6.1				
	CB 6.1 B				

Appendix 9:

SOP Chlorophyll and Phaeophytin

Standard Operating Procedure
For
Chlorophyll: Spectrophotometric Method

Revised by: Kevin L. Minga

For: Water Quality Laboratory
College of Sciences
Old Dominion University
Norfolk, VA 23508

Document File
Location: Water Quality Laboratory

Effective as of: April 15, 2014

Approved by: _____

John R. Donat, Ph.D. Date
Director, WQL

_____

Suzanne Doughten Date
Supervisor, WQL

DISCLAIMER: This SOP applies to the analysis of marine and estuarine water samples of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay and Elizabeth River. This SOP may not be applicable to any other studies.

TABLE OF CONTENTS

1. LOCATION

This analytical procedure will be used by the Water Quality Laboratory located at 4211 Colley Ave. Norfolk, Virginia.

2. PURPOSE

This procedure is designed to spectrophotometrically estimate the concentration of photosynthetic pigments in ambient water samples as an indicator of water quality.

The intended data user groups are the subcommittees and workgroups of the USEPA Chesapeake Bay Monitoring Program. These data will be used to assess whether the multi jurisdictional action plan for restoring the water quality of the Chesapeake Bay is effective. This assessment is performed, in part, by analysis of the data and its inclusion in computer models of the Chesapeake Bay. Trend analyses are also performed on the data to assess whether Chesapeake Bay restoration goals are being met. These data may also be used by State and Federal government officials to pass legislation designed to help improve the quality of the water in the Chesapeake Bay and its tributaries.

3. APPLICABILITY

This procedure is applicable to the analysis of photosynthetic pigments in fresh, estuarine, and coastal water samples. The procedure assumes that the spectrophotometric light absorbance of chlorophylls *a*, *b*, and *c*; and phaeophytin *a* at the wavelengths specified in this procedure is proportional to pigment concentration after correcting for extract solvent volume, sample volume, cuvette pathlength, and light absorbance due to extract turbidity and other pigments. The results are measured and reported as µg of pigment/L of water.

4. OVERVIEW OF METHOD

This SOP is based on ASTM Method D3731-79 (ASTM, 1979), which is substantially comparable to Standard Method 10200 H (APHA, 1995).

Samples are collected according to the current CHESAPEAKE BAY PROGRAM CRUISE DEPLOYMENT SOP. Photosynthetic organisms in water samples are concentrated onto a 4.25cm diameter Whatman® GF/F (or equivalent) glass fiber filter by vacuum filtration at no more than 10in.Hg. Photosynthetic pigments are extracted from planktonic algae concentrates by mechanical maceration in 90% aqueous acetone. The resulting extract is steeped overnight and refrigerated in the dark to complete the extraction of pigments from the cells of phytoplankton species that are more difficult to macerate (e.g. bluegreens).

NOTE: Samples collected by other sources or for different projects may have different methods.

A Shimadzu 2401-PC dual beam spectrophotometer with a path length range of 1-10cm is used, with software UVProbe Ver 2.10 produced by Shimadzu Corporation. The absorbencies of extracts are measured at 750nm, 664nm, 647nm, and 630nm before acidification and at 750nm and 665nm after acidification. Pigment concentrations are reported as µg of pigment/L of water.

5. DEFINITIONS AND ABBREVIATIONS

mL: Milliliter(s)
L: Liter(s)
µg: Microgram(s)
g: Gram(s)
N: Normality of the chemical solution
HDPE: High-density polyethylene
CBP: Chesapeake Bay Program
CBMP: Chesapeake Bay Monitoring Program
APHA: American Public Health Association
ASTM: American Society of Testing and Materials
QAPjP: Quality Assurance Project Plan
NIST: National Institute of Standards and Technology
CRM: Certified Reference Material
SRM: Standard Reference Material
CBP: Chesapeake Bay Program
frozen: -18°C or below.
refrigerated: 0 to 6°C
invert: In reference to homogenizing a sample by inverting, one inversion is: starting with the cap of the sample bottle pointing straight up, turn the sample bottle completely upside down so the cap is pointing straight down, then turn the sample bottle upright until the cap is pointing straight up again.
Reagent water: Resistivity > 10 megohm-cm

6. METHOD PERFORMANCE

Using samples analyzed in 2007, the average difference between 56 duplicate estuarine samples with a concentration below 5 *u*g/L and a mean concentration of 3.27 *u*g/L chlorophyll *a* was 0.309 *u*g/L. The standard deviation of the difference was 0.274.

Using samples analyzed in 2007, the average difference between 79 duplicate estuarine samples with a concentration between 5-10 *u*g/L and a mean concentration of 7.48 *u*g/L chlorophyll *a* was 0.563 *u*g/L. The standard deviation of the difference was 0.499.

Using samples analyzed in 2007, the average difference between 41 duplicate estuarine samples with a concentration between 10-20 *u*g/L and a mean concentration of 13.69 *u*g/L chlorophyll *a* was 0.668 *u*g/L. The standard deviation of the difference was 0.592.

Using samples analyzed in 2007, the average difference between 18 duplicate estuarine samples with a concentration above 20 *u*g/L and a mean concentration of 28.20 *u*g/L chlorophyll *a* was 2.982 *u*g/L. The standard deviation of the difference was 2.643.

25 separate measurements using NIST certified transmittance density values for SRM 930e set number 2077 have been completed on monthly intervals for January 2006 to January 2008. There are 3 separate filters with 3 different transmittance densities at each of 5 wavelengths. The wavelengths measured are 440.0 nm, 465.0 nm, 546.1 nm, 590.0 nm and 635.0 nm. The mean difference for the absorbance values obtained versus the absorbance values expected were less than 0.002 units at all of the wavelengths, with all of the wavelengths having a slight positive bias compared to the expected. The average percent difference varied from 100.1 to 100.3 %.

7. SAFETY EQUIPMENT

1. Lab Coat shall be worn during entire procedure, with the addition of apron when handling strong acids, i.e. HYDROCHLORIC ACID
2. Protective eye wear: Goggles when handling liquids, glasses are approved for handling solids only
3. Nitrile gloves for handling acetone and acids, PVC gloves for remainder of procedure

Preparation of reagents and dilutions of acids should be conducted under a chemical fume hood

8. POLLUTION PREVENTION

As stated in Standard Methods for the Examination of Water and Wastewater Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

For Chlorophyll *a* analyses, the purity of the acetone required makes recycling not possible. 90% acetone in which holding time has expired can be reused in the dishwashing laboratory to remove ink marks from glassware and to rinse the chlorophyll tubes.

The acetone waste is collected in an appropriate waste container provided by Old Dominion University's Environmental Health and Safety Office (EHSO), and EHSO collects the waste containers and disposes of them.

9. WASTE MANAGEMENT

Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office (EHSO).

Acetone waste is collected in metal container(s) provided by EHSO. The waste container is labeled according to procedures provided by EHSO**.** When the container(s) are full the waste is collected by EHSO staff for disposal.

10. LABWARE

The labware needed noted in this SOP is for a typical Chesapeake Bay sampling run. For other projects or sample numbers the amounts of labware and the volume of the labware may change.

10.1 Labware needed:

10.1.1 For sample collection and concentration:

- 4- 1000mL brown HDPE sample bottle per sampling day
- 1- Whatman® GF/F filter for every sample
- 1- Whatman® GF/F filter for every 10 to 20 samples (for duplicates)
- 1- 300mL filtration towers
- 1- 4000 mL trap flask
- 2- 500mL graduated cylinders
- 3- Filtration manifold

1- Forceps
1- vacuum pump with hosing, stoppers, and side arms

10.1.2 For sample extraction:

1- 10mL graduated centrifuge tube with Teflon-lined screw cap for each sample
1- TFE-fluorocarbon-to-glass pestle
1 or 2- macro pipette or repipettor
1- forceps

10.1.3 For reagent preparation: (Use only Class A volumetric labware)
Labware needed assumes that all reagents need to be prepared and bottles for each reagent are available.

3- 50mL volumetric flasks
1- 100mL graduated cylinder
1- 500mL volumetric flask
1- 250mL volumetric flask
1- 200mL volumetric flask

10.2 Labware cleaning:

10.2.1 This is for general labware. If labware is dedicated for chlorophyll use 10.2.2. Volumetric flasks, sample bottles with caps, filtration towers, and graduated cylinders:

Clean by scrubbing with a bottlebrush (if able) and dilute Liquinox® soap. Thoroughly rinse with tap water, rinse twice with 4N HCL, and 6 times with reagent water. Dry on a clean drying rack (labeled "for clean labware only") covered. When dry, store centrifuge tubes, volumetric flasks, and sample bottles with the caps on. Cover the top and bottom of filtration towers and graduated cylinders (top only) with parafilm or aluminum foil.

10.2.2 Centrifuge tubes with caps and TFE-fluorocarbon-to-glass pestle and any labware dedicated to just chlorophyll analysis including the brown sample bottles:

Clean by scrubbing with a bottlebrush (if able) and dilute Liquinox® soap. Thoroughly rinse with tap water, then 6 times with fresh reagent water. Dry on a clean drying rack (labeled "for clean labware only") covered.

11. CHEMICALS USED AND REAGENT PREPARATION

11.1 Labware needed: (Use only Class A volumetric labware unless noted otherwise)

Labware needed assumes that all reagents need to be prepared and bottles for each reagent are available.

3- 50mL volumetric flasks
1- 100mL graduated cylinder
1- 500mL volumetric flask
1- 250mL volumetric flask
1- 200mL volumetric flask

See section 10.2 for appropriate labware cleaning procedures.

11.2 Chemicals Used: All chemicals must be analytical grade or of a higher purity except as noted.

Hydrochloric acid (HCl), reagent grade
Magnesium carbonate ($MgCO_3$)
Acetone (CH_3COCH_3), reagent grade

11.3 Reagents and stock solutions needed for the analysis:

NOTE: Refer to Safety Precautions and use care when preparing reagents.

Reagent containers are used only for the intended reagent.

Wipe down the countertop, exhaust hood, and/or balance and balance table with reagent water before each use.

The following information must be recorded on each reagent bottle label:

Reagent identification (name) and concentration
Date prepared
Prepared by (initials)
Expiration Date
Storage requirements (e.g. ambient temperature, refrigerated, dark, etc.)
Reagent number

All reagents are documented in the working standard/reagent logbook. Each reagent is assigned a unique number and the following information is recorded: reagent name, reagent number, date prepared, date expires, quantities of each component (as well as each component's chemical number), and final volume.

NOTE: Quantities of each reagent prepared may be adjusted (ie., halved or doubled) as necessary.

11.3.1 1N HCL: Fill a 500mL and a 50mL class A volumetric flask to volume with reagent water and pour into the reagent container. Fill a 50mL class A volumetric flask to volume with concentrated hydrochloric acid and add to the reagent container, mix by inversion. For the analysis, use a smaller reagent container to dispense from to avoid the possible contamination of the stock solution. Cap and store both containers at ambient temperature. Shelf life N/A.

NOTE: Before filling smaller reagent container used for analysis, rinse three times with reagent water and three times with stock solution

11.3.2 Magnesium carbonate suspension: Measure 100mL of reagent water using a graduated cylinder. Weigh 1g of finely powered magnesium carbonate. Add each to the reagent container and shake. Shake immediately before each use. Shelf life N/A.

11.3.3 Aqueous acetone, 90%: Fill a 250mL and a 200mL class A volumetric flask with reagent grade acetone. Fill a 50mL class A volumetric flask with reagent water. Pour acetone and water into a reagent bottle and mix by inversion. Shelf life is two days.

NOTE: The volumetric relationship of acetone to water must be strictly maintained to prevent shifts in the absorption peaks.

NOTE: This reagent should be made as close to the time of use as possible, and must be used within 2 days of preparation for extractions and zeroing of the spectrophotometer.

12. QUALITY CONTROL

It is especially important to maintain the spectrophotometer in peak operating condition. This should be confirmed by the laboratory supervisor using the following guidelines:

12.1 Measuring the absorbance of a CRM for chlorophyll, if available.

12.2 Processing and analyzing a duplicate sample for every 10 to 20 samples.

12.3 Verifying the accuracy and stability of absorbance measurements monthly using NIST SRM filters.

13. SAMPLE COLLECTION, PRESERVATION AND CONCENTRATION

13.1 Collection and Preservation:

13.1.1 Labware needed:

1- 1000mL brown HDPE sample bottle for each sample

See section10.2 for the appropriate labware cleaning procedures.

13.1.2 Collection of sample and duplicates:

Collect samples according to the current CHESAPEAKE BAY PROGRAM CRUISE DEPLOYMENT SOP.

Collect and process one duplicate for every 10 to 20 samples by following steps 13.2.2 through 13.2.8 with sample from the same 1000mL brown HDPE sample bottle that the original sample was taken from.

13.1.3 Preservation of Samples: Mix the magnesium carbonate to suspension and immediately add 1mL per liter of sample. Invert the sample to mix.

Samples should be concentrated onto filters immediately after collection (section 13.2). If this is not possible, store samples for no longer than 24 hours by refrigerating or packing the sample bottles in ice up to the bottom of the cap.

NOTE: These holding times, preservation and sample collection apply to the Chesapeake Bay Program. Other clients may have different requirements.

13.2 Concentration

13.2.1 Labware needed:

1- Whatman® GF/F filter for every sample and one extra
1- Whatman® GF/F filter for every 10 to 20 samples (for duplicates)
1- 500mL graduated cylinders
1- 4000 mL trap flask
1- 300mL filtration tower
1- Filtration manifold
1- Forceps
1- Vacuum pump with hosing, stoppers, and side arms

See section 10.2 for the appropriate labware cleaning procedures.

13.2.2 Using forceps, gripping only the filter edge, transfer a 4.25 cm Whatman® GF/F glass fiber filter (or equivalent) with the wrinkled side facing up, onto the base of a filtration tower. Replace the top of the filtration tower onto the base.

13.2.3 Mix the sample thoroughly by inverting the sample bottle several times. Immediately rinse the graduated cylinder twice with the sample to be concentrated.

13.2.4 Immediately pour a pre-determined volume of sample into the rinsed graduated cylinder. If sample not immediately poured, mix the sample again by inverting the sample bottle several times.

NOTE: Record the filtered volume on the data sheet. 500 mL is the maximum sample volume filtered in the field.

13.2.5 Concentrate the plankton by vacuum filtration using $\leq$10in.Hg vacuum pressure. To avoid cell damage during filtration, do not exceed this vacuum pressure and limit filtration duration to 5 minutes or less. If it takes longer than 5 minutes to filter the selected sample volume, discard filter and remaining sample, rinse the filtration apparatus (see step 13.2.8), then complete steps 13.2.2 through 13.2.5 using a lesser sample volume. Record this new sample volume on the data sheet.

NOTE: If not enough sample to filter another sample, record how long it took to filter and notify the Chief Scientist and/or Laboratory Supervisor.

NOTE: **Do not suck the filter dry.**

13.2.6 Fold the filter in half twice using forceps being careful not to disturb the filtrate. Lightly blot the filter with a Kimwipe^{R} to remove excess moisture if necessary before placing the folded filter on a labeled rectangle of aluminum foil and seal tightly.

13.2.7 Freeze the filter as soon as possible . If freezing immediately is not possible, refrigerate or store in a water-tight container packed in ice and freeze as soon as possible. If applicable, storage temperature must be measured and documented using a thermometer which has been calibrated against an NIST-traceable thermometer within the last year.

13.2.8 Before processing another sample, completely rinse the empty filtration tower three times with reagent water before seating a new filter, and rinse the entire inside surface of a 500mL graduated cylinder two times with fresh reagent water.
Repeat steps 13.2.2 through 13.2.8 for each sample.

NOTE: Filter one randomly chosen sample in duplicate for every 10 to 20 samples following steps 13.2.2 through 13.2.8.

13.3 Holding Times:

The analysis must be completed within 28 days of collection.

NOTE: These holding times apply to the Chesapeake Bay Program. Other clients may have different requirements.

14. SAMPLE EXTRACTION AND CENTRIFUGATION

14.1 Labware needed:

1- 10mL graduated centrifuge tube with screw cap for each sample
1- TFE-fluorocarbon-to-glass pestle
1 or 2- macro pipette or repipettor
1- forceps
1- centrifuge

See section 10.2 for the appropriate labware cleaning procedures.

14.2 Pre-extraction Procedure:

14.2.1 Use the data sheets, master logbook, and/or chain-of-custody documents to determine the log numbers of the samples that will be extracted, their location, and validate they are within holding times. Notify Laboratory Supervisor if they are not within holding time. If Laboratory Supervisor not immediately available, continue with analysis but note on all applicable documentation that samples were out of holding time and notify Laboratory Supervisor as soon as possible.

14.2.2 Remove samples from the freezer and inspect them to ensure that they have been properly stored. If any questions on sample condition notify Laboratory Supervisor as soon as possible and make a note on all applicable documentation. Place the samples on ice in a covered cooler. Log the samples out in the chain-of-custody logbook.

14.3 Extraction:

Wipe down the countertop or exhaust hood with reagent water before extracting samples.

NOTE: Pigment samples must be maintained in the dark and on ice as much as possible throughout the entire extraction procedure. Remove one sample, extract it according to the steps in this section, and place it immediately in a test tube rack in the cooler before proceeding with the next sample.

14.3.1 Determine the number of samples to be extracted and rinse the same number of 10mL centrifuge tubes and Teflon-lined screw caps twice with 90% acetone solution. Place the tubes up side down in a test tube rack and caps face down on a clean paper towel until needed.

14.3.2 Remove the folded sample filter from the aluminum foil packet with forceps and place in a rinsed centrifuge tube.

14.3.3 Using a micropipette or repipettor add 3mL of fresh 90% aqueous acetone to the centrifuge tube containing the sample filter.

14.3.4 Disrupt the algal cells in the plankton concentrate by grinding with a TFE-fluorocarbon-to-glass pestle and thoroughly macerate the glass fiber filter and plankton.

14.3.5 Add 7mL of fresh 90% aqueous acetone to the centrifuge tube. Ensure that all filter particles are in the solvent.

14.3.6 Cap the centrifuge tube tightly, transfer the label from the aluminum to the tube, and place in a refrigerator to steep overnight. To ensure the samples are exposed to minimum light conditions, wrap the test tube rack in aluminum foil or duct tape and cover the top of the samples with aluminum foil. Document storage in the chain-of-custody.

NOTE: The minimum amount of time required for the samples to steep before being centrifuged is two hours.

14.3.7 Document the extraction on the appropriate data sheet by filling in the "Extracted By" and the "Date Chlorophyll Extracted" spaces provided

14.3.8 The next morning remove samples from the refrigerator. Centrifuge samples according to steps in section 14.4. If sample extracts cannot be analyzed at this time, they may be stored frozen. Document transfer in the chain-of-custody logbook.

14.4 Centrifugation:

14.4.1 Turn on the centrifuge and adjust the temperature so the unit cools to 4°C. If analyzing the samples the same day as centrifugation, adjust the temperature to 24ºC so the samples will come to ambient temperature before analysis.

14.4.2 When placing extracts into centrifuge, balance the load by placing centrifuge tubes containing equal volumes of extract in opposite buckets.

14.4.3 Centrifuge the samples for 5 minutes at 2300 rpm. Shake each centrifuge tube to ensure no filter particulates are stuck in or around the cap area. Centrifuge the samples for 15 minutes at 2300 rpm.

14.4.4 If not analyzing the same day as centrifugation, the centrifuged samples may be frozen and stored in the dark until analysis. Document storage in the chain-of-custody logbook.

15. EQUIPMENT AND PRE-ANALYTICAL PROCEDURE

15.1 Warming up:

15.1.1 Turn on the Shimadzu 2401-PC spectrophotometer and the computer.

15.1.2 Open the UV-Probe software.

15.1.3 Choose the Photometric method by clicking *Window*, then *Photometric*.

15.1.4 Click *Connect*. The spectrophotometer will go through a self-check process that takes about 5 minutes. After completion click *OK* and wait 30 minutes for warm up.

NOTE: Document instrument usage in logbook.

15.2 Create a Method: (can be completed while spectrophotometer is warming up)

Click *Edit*, then *Method*. The Photometric Method Wizard will open.

15.2.1 Wavelengths page:

NOTE: The wavelengths shown here are for routine Chesapeake Bay and Elizabeth River samples. Other wavelengths may be entered if they are required for a specific project.

Wavelength Type = Point
Column Name = adjusts with wavelength, change only if needed
Wavelength – type the following wavelengths in order, hitting *Add* after each one
630, 647, 664, 665, 750

NOTE: If analyzing chlorophyll grabs for fluorometry calibration, only enter wavelengths 664, 665, and 750.

Click *Next* once all wavelengths have appeared on the Entries table.

15.2.2 Calibration Page:
Type = Raw Data
Once "raw data" is chosen the remaining choices disappear, hit *Next*

15.2.3 Measurement Parameters (Sample) Page: (leave defaults)
Data Acquired By = instrument
Sample Repetitions = 1

15.2.4 File Properties Page:
Filename = C:\Program Files\Shimadzu\Data\chl\RunFiles\cruisefolder\cruiseID.unk
ie. C:\Program Files\Shimadzu\Data\chl\RunFiles\bay\C431.unk
Click *Finish*, the Photometric Method Page will open.
Close the Method to return to the Photometric Page.

16. MEASURING ABSORBENCIES USING SPECTROPHOTOMETER

NOTE: Equilabrate samples to ambient temperature before reading absorbencies.

16.1 Auto Zero: with nothing in the sample compartment click *Auto Zero*. Document this action in the logbook. This only needs to be done once a day after the spectrophotometer has warmed up.

16.2 Establish the baseline:

16.2.1 Fill 2 cuvettes with fresh 90% aqueous acetone. Clean the outside of the cuvettes with a Kimwipe^{R} or similar cloth until no smudges are observed. Ensure there are no air bubbles in the acetone, and the acetone is homogeneous.

NOTE: Samples are analyzed using 5 cm cuvettes when possible.

16.2.2 Place one cuvette in the reference cuvette holder (back) and cover with the white cap to help minimize evaporation, and place the other cuvette in the sample cuvette holder (front) in the spectrophotometer and close the lid.

NOTE: Place sample cuvette in same direction when analyzing samples.

16.2.3 Click *Baseline*. (Ensure that the range of the wavelengths used for the baseline encompasses the wavelengths used in the analysis.)

16.2.4 Click *Start* and run the baseline.

If the 750nm reading is >0.002 check the cuvettes for any smudges, floating particles, or air bubbles. If any are present, remedy the problem and repeat steps 16.2.2 and 16.2.3. If nothing is apparent, discard the acetone solution and begin at step 16.2 .

If the 750nm reading is ≤0.002 continue to the next step.

NOTE: The baseline should be checked periodically when numerous samples are being run. (ie. after every batch from the centrifuge)

16.2.5 Once the baseline is established leave the reference cuvette in place and discard the contents of the sample cuvette.

16.2.6 Indicate that a baseline has been established in the *Comments* column of the sample data sheet (ie. Est. baseline prior) and document this action in the logbook.

16.3 Run a Sample:

16.3.1 Rinse the sample cuvette 2-3 times with 90% aqueous acetone, making sure to completely rinse the windows. Remove excess acetone as much as possible.

16.3.2 Fill the cuvette with sample to be analyzed (about 6mLs).

16.3.3 Clean the outside of the cuvette with a Kimwipe^{R} until no smudges are observed.

16.3.4 Place the cuvette in the sample holder of the spectrophotometer and close the lid.

16.3.5 Click on the sample table in the *Sample ID* column. Type the sample information as:
Wlognumber.split code if applicable station ID (DUP if applicable)
ie. W23456.05 EE3.4S or W23456.05 EE3.4S DUP

16.3.6 Click on the *λ Go to WL* button. Enter 750 and click Ok. If the 750nm reading is >0.007 check the cuvettes for any smudges, floating particles, or air bubbles. If any are present, remedy the problem.

If the 750nm reading is ≤0.007 continue to the next step.

NOTE: Floating particles may be removed by filtering the sample with a syringe and a 0.2 µm or 0.45 µm syringe filter. Indicate that the sample was filtered in the *Comments* column (ie. Filtered).

NOTE: **If the 664 value is >1 analyze the sample using the 1 cm cuvette.**

16.3.7 Click out of the *Sample ID* column, this will release the *Read Unk*. button. Press *Read Unk*. (unknown). The readings will appear in the wavelength columns and "unknown" will appear in the *Type* column.

16.3.8 Open the lid and add 5 drops of 1N HCl to the sample cuvette with a disposable glass pasture pipette. Carefully mix the acid into the sample and close the lid. **The sample must be read between 1 and 2 minutes after acid addition.**

16.3.9 Click on the sample table in the *Sample ID* column. Type the sample information as:
wlognumber.split code station ID (DUP if applicable)
ie. W23456.05 EE3.4 S A or W23456.05 EE3.4 S DUP A

16.3.10 One minute after acidification (but no more than 2) press *Read Unk*. to take the phaeophytin absorbance readings. Again, the readings will appear in the wavelength columns and "unknown" will appear in the *Type* column.

If the 750nm reading is >0.007 check the cuvette for any smudges, floating particles, or air bubbles. If any are present, remedy the problem. Reread the phaeophytin value before the 2 minutes are up after acidification.

16.3.11 Calculate the corrected 664/665 ratio as follows and record on the data sheet.

ratio = (664-750) / (665a-750a)

where:

664 = the absorbance at 664nm before acidification
750 = the absorbance at 750nm before acidification
665a = the absorbance at 665nm after acidification
750a = the absorbance at 750nm after acidification

NOTE: This ratio should be between 1.0 and 1.7. If it is not between this number clean the cuvette and remix the acid, and reread the phaeophytin value before the 2 minutes are up after acidification. If the ratio does not improve, make a note that read phaeophytin twice. There is not enough sample to reanalyze. In natural estuarine samples with low

chlorophyll *a* and low phaeophytin *a* it is common to encounter samples with a ratio > 1.7 due to the low absorbances.

16.3.12 Remove the sample cuvette from the spectrophotometer and discard the sample.

16.3.13 Begin at step 16.3.1 again for all remaining samples. Following the final sample, thoroughly rinse both cuvettes with 90% aqueous acetone and allow to completely dry before storing.

NOTE: Remember to periodically run a new baseline.

NOTE: If the Auto Zero button is ever pushed when the cuvettes are in the sample compartment immediately repeat steps 16.1 to 16.2.6.

16.4 Save the data: Once all samples have been analyzed (and periodically during run) save the sample table data by selecting File, Save As and in the following locations:

C:\Program Files\Shimadzu\Data\chl\RunFiles\cruisefolder\cruiseID.unk
ie. C:\Program Files\Shimadzu\Data\chl\RunFiles\bay\C431.unk

C:\Program Files\Shimadzu\UVProbe\Data\Chl\TextFiles\cruise folder\cruiseID.txt
ie. C:\Program Files\Shimadzu\UVProbe\Data\Chl\TextFiles\ bay\C431.txt

NOTE: Columns *Type* and *EX* must be hidden before saving as a text file (.txt) in order for the data to be properly imported into the database.

NOTE: Document instrument usage in logbook.

NOTE: Transfer run files (.unk) to appropriate folder on the ODU Server Network. upon completion of each cruise.

17. CALCULATION OF ANALYTICAL RESULTS

Unless otherwise instructed by the lab supervisor, raw chlorophyll/pigment data are reported as absorbance units and are not calculated by the analyst by hand. In the event that it is necessary to calculate chlorophyll and phaeophytin data, the data are calculated using the equations in the following steps.

All absorbencies used in the calculations are corrected by subtracting the appropriate 750nm absorbance value (the absorbance value at 750 nm before acidification is subtracted from all absorbencies which were measured before acidification; and the absorbance value at 750nm after acidification is subtracted from the absorbance value at 665nm, which is measured after acidification).

17.1 Chlorophyll a corrected for Phaeophytin a:

$$\text{Chlorophyll a } u\text{g/L} = \frac{[26.7(\text{abs664nm} - \text{abs665nm})]\ \text{extract V in mL}}{(\text{sample V in L})(\text{cell pathlength})}$$

where:

abs 664nm	=	the 664nm absorbance minus the 750nm absorbance before acidification
abs 665nm	=	the 665nm absorbance minus the 750nm absorbance after acidification
extract V	=	90% aqueous acetone extract volume, in milliliters, recorded on the data sheet (usually 10mL)
sample vol.	=	filtered sample volume, in liters, recorded on the data sheet
cell pathlength	=	length of cell used in the spectrophotometer in cm

17.2 Phaeophytin a corrected for Chlorophyll a:

$$\text{Phaeophytin a } u\text{g/L} = \frac{26.7[1.7(\text{abs665nm}) - \text{abs664nm}] \text{ extract V in mL}}{(\text{sample V in L})(\text{cell pathlength})}$$

where:

abs 664nm	=	the 664nm absorbance minus the 750nm absorbance before acidification
abs 665nm	=	the 665nm absorbance minus the 750nm absorbance after acidification
extract V	=	90% aqueous acetone extract volume, in milliliters, recorded on the data sheet (usually 10mL)
sample vol.	=	filtered sample volume, in liters, recorded on the data sheet
cell pathlength	=	length of cell used in the spectrophotometer in cm

17.3 Chlorophyll b:

$$\text{Chlorophyll b } u\text{g/L} = \frac{[21.03(\text{abs647nm}) - 5.43(\text{abs664nm}) - 2.66(\text{abs630nm})] \text{ extract V in mL}}{(\text{sample V in L})(\text{cell pathlength})}$$

where:

abs 647nm	=	the 647nm absorbance minus the 750nm absorbance before acidification
abs 664nm	=	the 664nm absorbance minus the 750nm absorbance before acidification
abs 630nm	=	the 630nm absorbance minus the 750nm absorbance before acidification
extract V	=	90% aqueous acetone extract volume, in milliliters, recorded on the data sheet (usually 10mL)
sample vol.	=	filtered sample volume, in liters, recorded on the data sheet
cell pathlength	=	length of cell used in the spectrophotometer in cm

17.4 Chlorophyll c:

$$\text{Chlorophyll c } \mu\text{g/L} = \frac{[24.52(\text{abs630nm}) - 1.67(\text{abs664nm}) - 7.6(\text{abs647nm})]\ \text{extract V in mL}}{(\text{sample V in L})(\text{cell pathlength})}$$

where:

abs 630nm	=	the 630nm absorbance minus the 750nm absorbance before acidification
abs 647nm	=	the 647nm absorbance minus the 750nm absorbance before acidification
abs 664nm	=	the 664nm absorbance minus the 750nm absorbance before acidification
extract V	=	90% aqueous acetone extract volume, in milliliters, recorded on the data sheet (usually 10mL)
sample vol.	=	filtered sample volume, in liters, recorded on the data sheet
cell pathlength	=	length of cell used in the spectrophotometer in cm

17.5 Carotenoids: (Strickland and Parsons, 1972)

NOTE: in order to calculate carotenoids the wavelengths of 480nm and 510nm must be collected. These are currently not collected for chlorophyll for the routine Chesapeake Bay and Elizabeth River samples.

$$\text{Carotenoids } \mu\text{g/L} = \frac{7.6[\text{abs480nm} - 1.49(\text{abs510nm})]\ \text{extract V in mL}}{(\text{sample V in L})(\text{cell pathlength})}$$

where:

abs 480nm	=	the 480nm absorbance minus the 750nm absorbance before acidification
abs 510nm	=	the 510nm absorbance minus the 750nm absorbance before acidification
extract V	=	90% aqueous acetone extract volume, in milliliters, recorded on the data sheet (usually 10mL)
sample vol.	=	filtered sample volume, in liters, recorded on the data sheet
cell pathlength	=	length of cell used in the spectrophotometer in cm

17.6 CRM:
Currently there is no CRM available for the spectrophotometric chlorophyll analysis. Analyze a purchased chlorophyll standard monthly. These standards do not have a true value, one must be obtained. After a sufficient number of aliquots of the standard are analyzed (minimum of 5 analyzed on two different days), a true value can be obtained. When a new standard is purchased, if possible, analyze the new standard with the old standard to help determine the true value. Control charts will be utilized to establish acceptance limits.

NOTE: See database instructions under Chlorophyll for instructions on importing and calculating the data.

18. DATA QUALITY ASSESSMENT

All data must meet all of the data quality assessment criteria specified in the following steps. If any data do not meet all of the specified criteria, the analysis is out-of-control and the data must be forwarded to the laboratory manager and quality assurance officer as soon as possible to determine whether the data must be rejected.

18.1 Assess whether the analytical results for the chlorophyll standard are within confidence limits established through control chart tracking.

18.2 Assess whether the absorbance results for each of the NIST SRM filters is within the tolerances of the spectrophotometer (0.004 absorbance units). This is done once a month.

18.3 Assess whether the precision for each set of duplicated samples is in-control, as follows:

If the samples are ≤to the current calculated MDL, the replicates are considered in-control. If they do not meet this criteria, than the CV is calculated according to the method below:

The coefficient of variation (CV) must be less than or equal to 20% when calculated as:

$$CV = \frac{sd}{\bar{x}}$$

where: CV = Coefficient of Variation;
sd = Standard Deviation; and
x = Mean Concentration.

18.4 Assess whether the field blanks are in-control. The blank should be less than the average of the last 10 calculated MDL values. If the field blank is greater than the average MDL, the blank is determined to be contaminated. Another blank is not available to be analyzed. If the field blanks are out of control, the data will be qualified.

Note: If after calculating data more than 10% of the chlorophyll *a* data is not meeting the requirements for replicates, the technicians filtering the samples in the field should be notified. They can increase the amount of inversions they are doing in the field to see if this helps precision.

19. METHOD DETECTION LIMIT

Method Detection Limit is defined as the minimum concentration that can be detected and determined to be statistically different from zero. The method for determining MDLs is based upon student t times the standard deviation of not less than seven replicate analyses of the same sample. This MDL will be highly dependent upon the instrument, reagents, SOPs, personnel and, most importantly, the matrix of the media being analyzed.

The method detection limit is determined once a year for this analysis, using a low level sample collected in the field. The results are reported in a Method Detection Limit table in the WQ_DATA database maintained by the Water Quality Laboratory.

20. MAINTENANCE AND TROUBLESHOOTING

Refer to the Shimadzu 2401-PC manual and UV Probe Help index for maintenance and troubleshooting instructions as needed.

21. LITERATURE CITED

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Method 10200 H. Chlorophyll. American Public Health Association. Washington, DC.

ASTM. 1979. Standard Practices for Measurement of Chlorophyll Content of Algae in Surface Waters. Method D3731-79. American Society for Testing and Materials. Philadelphia, PA.

Strickland, J.D.H. and T.R. Parsons. 1972. A Practical Handbook of Seawater Analysis. Bulletin Fisheries of the Research Board of Canada. Ottawa, Canada.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1100 p 1-44. American Public Health Association. Washington, DC.

APHA. 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edition. Section 1030 C. p 1-9. American Public Health Association. Washington, DC.

APHA. 2012. Standard Methods for the Examination of Water and Wastewater. 22nd Edition. Section 10200 H. p 22-24. American Public Health Association. Washington, DC.

22. DATA SHEET

Note: A different data sheet is used if a project requires that the absorbance values are entered manually on a data sheet. This is used for routine Chesapeake Bay and Elizabeth River samples.

Chlorophyll

Cruise _____ Cruise Date _____

Filtered By _____ Date Filtered _____ $MgCO_3$#_____

Log Number	Sample ID	Filtered		Extracted	Path Length	Analyzed		664/665 Ratio
		Time	Vol. (L)	Date/ Initials		Date	By	

Figure: Data (bench) sheet for chlorophyll analysis.

Appendix 10:

SOP Silicate (Filtered)

STANDARD OPERATING PROCEDURE
for Silicate in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

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APPROVED BY

_____

John R. Donat, Ph.D. Date
Laboratory Director

_____

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of silicate in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.0234 to 2.337 mg Si/L (0.05 – 5.0 mg SiO_2/L). Higher concentrations can be determined by sample dilution. The method throughput is 40 injections per hour.

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

Dissolved silicate reacts with molybdate at 37°C in acidic conditions (pH 1.2) to form yellow β-molybdosilicic acid. This complex is then reduced by ascorbic acid to form molybdenum blue, which is measured at 660 nm. The absorbance is linearly proportional to the concentration of silicate in the sample. Interference from phosphate is reduced by the addition of oxalic acid.

In natural waters dissolved silicon is generally found in the form of silicate (Mullin and Riley, 1955). Particulate biogenic silica, which is not measured in this testing procedure, can also be found in the water column and represents the amount of silica present as diatom frustules (Hurley, Armstrong, Kenoyer and Bowser, 1985). Dissolved silicon, in the form of silicate, is readily available for biological uptake, and it will be found in extremely low amounts in surface waters when a diatom bloom in the spring is occurring (Brewer and Riley, 1966). This is due to the fact that diatoms use silicon in the formation of their shells. Therefore, it would follow that the availability of silica in water could affect the growth of diatoms. Scientists need to study the quantity of silicon in water in order to gain an understanding of diatom ecology. If a scientist is studying a system, such as a small lake, ground water may be a significant source of dissolved silicon (Hurley, et al, 1985). Physical oceanographers can use the concentration of silicon in water to trace the movement of water masses and determine mixing processes in bodies of water (Brewer and Riley, 1966). Silica in large amounts in water can be undesirable for many industrial applications. In the presence of magnesium, the dissolved forms of silica can form scales on equipment (APHA, 2012).

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

3.1.1 Freezer/Frozen: ≤ -18°C

3.1.2 Refrigerator/Refrigerated: 0 – 6°C

3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm

3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 g: Gram(s)

3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.6 L: Liter(s)

3.2.7 MDL: Method Detection Limit

3.2.8 mg: Milligram(s)

3.2.9 mg/L: Milligram(s) per Liter

3.2.10 mL: Milliliter(s)

3.2.11 NH_3/NH_4/NH4F: Ammonia

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 NO_3: Nitrate

3.2.14 NO_{23}/NO23F: Nitrate + Nitrite

3.2.15 NO_2/NO2F: Nitrite

3.2.16 OPO_4/PO4F: Orthophosphate

3.2.17 PO_4: Phosphate

3.2.18 ppm: Parts Per Million

3.2.19 PQL: Practical Quantitation Limit

3.2.20 QCS: Quality Control Sample

3.2.21 Si/SIF: Silicate

3.2.22 SOP: Standard Operating Procedure

3.2.23 SRM: Standard Reference Material

3.2.24 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without molybdate.

4.2 Phosphate causes interference but is reduced by the addition of oxalic acid. If the 7 cm reaction coil after the oxalic acid does not sufficiently reduce phosphate interference, a longer coil can be used.

4.3 Large amounts of iron or sulfides and tannins are interferences. The addition of disodium EDTA will eliminate the interference due to iron. The oxalic acid decreases interference from tannins.

4.4 Silicates can precipitate in acidic solutions, so ensure all labware is washed sufficiently to eliminate interference from acid.

4.5 Glassware should be avoided as much as possible as it contains silica. This can be accomplished by storing samples, standards and reagents in plastic and keeping any contact with glass to a minimum.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

- 5.4.1 Ammonium Molybdate
- 5.4.2 Oxalic Acid
- 5.4.3 Sodium Hydroxide
- 5.4.4 Sulfuric Acid

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

- 6.1.1 Sampler (Model ASX-520)
- 6.1.2 Auto dilutor (Model PDS-200)
- 6.1.3 Multichannel proportioning pump (Model RP-150)
- 6.1.4 Reaction unit or manifold (Model 31-114-27-2-A)
- 6.1.5 Heating unit (Part A85132)
- 6.1.6 Colorimetric detector (Part 85080)
- 6.1.7 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Class A volumetric pipettes (or pipettors) as required

6.5 Plastic volumetric flasks and graduated cylinders

6.6 All sample tubes and standard vials must be plastic.

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analysis, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Ammonium Molybdate Solution – Measure 20.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] and completely dissolve in approximately 400 mL Reagent H_2O in a 500 mL plastic volumetric flask. Make sure the molybdate is dissolved before adding the acid. Then add 8.0 mL concentrated sulfuric acid [H_2SO_4], dilute to the mark and mix by inversion.
Store in a plastic reagent container in the refrigerator.
Expiration is one month. Also, discard if precipitate or blue color is observed.

7.1.2 Oxalic Acid Solution – Dissolve 50.0 g oxalic acid [$HO_2CCO_2H \cdot 2H_2O$] in approximately 450 mL Reagent H_2O in a 500 mL plastic volumetric flask. Dilute to the mark and mix by inversion.
Store in a plastic reagent container at ambient temperature.
Expiration is N/A.

7.1.3 Ascorbic Acid Reducing Solution – In a 500 mL plastic volumetric flask, dissolve 20 g granular ascorbic acid [$C_6H_8O_6$] in approximately 450 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a plastic reagent container.
Expiration is one week. Also, discard if solution becomes yellow or precipitates.

7.1.4 Sodium Hydroxide – EDTA Rinse – Dissolve 65 g sodium hydroxide [NaOH] and 6 g tetrasodium ethylenediamine tetraacetic acid [Na_4EDTA] in 1.0 L Reagent H_2O. Use daily at the end of a run (~10 minutes, followed by Reagent H_2O rinse) or if the baseline begins to drift upwards.
Expiration is N/A.

7.2 PREPARATION OF STANDARDS

The following are purchased and used without further preparation unless specified.

Silica Stock Standard: 1 mL = 1 mg SiO_2, 1000 ± 5 ppm SiO_2 (467 ppm Si); Sodium Metasilicate Nonahydrate [$Na_2O_3Si \cdot 9H_2O$]. Traceable to NIST. Storage requirements and expiration date are on the label. (Ricca Chemical Company, or equivalent)

Silica Secondary Stock Standard: 1000 ± 6 mg SiO_2/L; Sodium meta-Silicate 9-Hydrate [$Na_2O_3Si \cdot 9H_2O$]. Traceable to NIST. Storage requirements and expiration date are on the label. (ERA, or equivalent)

NOTE: If the concentrations of the Stock Standards are the same, they can be interchanged as long as the same one is not used for both the calibration curve and the quality control sample.

Rinse all plastic volumetric flasks three times with Reagent H_2O prior to use.

7.2.1 Working Standard 23.37 mg Si/L (50.0 mg SiO_2/L) – In a 100 mL plastic volumetric flask, add 5.0 mL of Silicate Stock Standard. Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh on day of use.

7.2.2 Working Standard 2.337 mg Si/L (5.0 mg SiO_2/L) – In a 100 mL plastic volumetric flask, add 0.50 mL of Silicate Stock Standard. Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh on day of use.

7.2.3 Calibration curve – If making the calibration standards manually, dilute the specific volume of Working Standard 7.2.1 in a 100 mL plastic volumetric flask with Reagent H_2O. If using the autodilutor for the calibration standards, use Working Standard 7.2.2 and input the AutoDilutor Factor (ADF) values in the chart.

Working Standards (Prepared Daily)	Concentration mg Si/L	Manual	Autodilutor
		Standard 7.2.1 (23.37 mg Si/L) mL	ADF Value (Standard 7.2.2, 2.337 mg Si/L)
S1	2.337	10.0	--
S2	1.1685	5.0	2.00
S3	0.4674	2.0	5.00
S4	0.2337	1.00	10.00
S5	0.1169	0.50	20.00
S6	0.0584	0.25	40.00
S7	0.0234	0.10	100.00
S8	0.0000	--	--

7.2.4 Matrix Spikes – Add 0.50 mL of Working Standard 7.2.1 (23.37 mg Si/L) to a 25 mL plastic volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.4674 mg Si/L**. *NOTE: The spike concentration may be decreased/increased due to sample concentrations.*

7.2.5 Quality Control Sample 0.4674 mg Si/L – Add 0.10 mL of Silicate Secondary Stock Standard 1000 mg SiO_2/L to a 100 mL plastic volumetric flask. Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh day of use.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 12 for acceptance criteria.

9.2 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 12 for acceptance criteria.

9.3 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.4 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS)

containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.5 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.6 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. CALIBRATION AND STANDARIZATION

10.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.3). The minimum number of non-zero standards is four and a zero standard.

10.2 Calibrate the instrument as described in Section 11.

10.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 12.1 for acceptance criteria.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 12.2 for acceptance criteria.

11. PROCEDURE

11.1 PRE-ANALYTICAL PROCEDURE

11.1.1 Gather all Silicate reagents. Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

11.1.2 Fill a plastic vial with a sufficient volume of the Silicate Stock Standard. Use this portion to make the calibration curve.

11.1.3 Fill a plastic vial with a sufficient volume of Secondary Silicate Stock Standard. Use this portion to make the Quality Control Sample.

11.1.4 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

11.1.5 Remove samples from storage and log action in chain-of-custody.

11.1.6 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Allow the samples to come up to ambient temperature.

11.1.7 Rinse plastic sample tubes six times with Reagent H_2O.

11.2 CALIBRATION PROCEDURE

11.2.1 Prepare working standards and matrix spikes as described in Section 7.2. The calibration curve listed is for samples routinely analyzed in the laboratory. Different standards may be utilized for varying sample concentrations. The minimum number of non-zero standards is four, each sampled once. The zero standard is sampled in duplicate.

11.2.2 Set up manifold as shown in Section 17.2.

11.2.3 Verify/input data system parameters as shown in Section 17.1.

11.2.4 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

11.2.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

11.2.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.3 SYSTEM NOTES

11.3.1 For information on system startup/shutdown, maintenance and troubleshooting, refer to the Lachat Manual, Troubleshooting and Info Binder.

11.3.2 Allow the heating module to warm up to 37°C.

11.3.3 It is important to avoid using glassware as much as possible. This means that the preparation of standards and Reagent H_2O used to prepare the standards, carrier

and reagents should be done in plastic containers. Plastic autosampler cups must be used for both standards and samples.

11.3.4 A backpressure coil [200 cm x 0.5 mm (0.022") i.d. Teflon tubing] is used to prevent air bubble formation.

11.3.5 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.3.6 If the detection limit is greater than that specified in the method, the following outline should be followed.

- 11.3.6.1 Verify standards preparation procedures.
- 11.3.6.2 Verify that a 660 nm filter is being used.
- 11.3.6.3 Verify that the sample loop is completely filled by running dye.
- 11.3.6.4 Verify that the reagents are being added in the correct order.
- 11.3.6.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.
- 11.3.6.6 Prepare fresh reagents. Take extra care to be sure that all reagents are completely dissolved.

11.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

11.3.8 If a large number of bubbles are observed in the autodilutor water container, degas with helium for at least 15 minutes.

12. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 12.1 through 12.6, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 12.3 through 12.6 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

12.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.1.1 Acceptability of calibration correlation coefficient (r)

A.	r ≥ 0.9990	Acceptable
B.	r = 0.9980 – 0.9989	Acceptable but troubleshooting is required.
C.	r < 0.9980	Stop the analysis. Troubleshooting is required.

12.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

12.3 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

12.4 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

12.5 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA\times DF}\times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA\times DF}\times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

12.6 If the method blank is ≤ PQL (lowest non-zero standard) the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

12.7 Report results in mg Si/L.

13. METHOD PERFORMANCE

13.1 A Quality Control Sample prepared in Reagent H_2O (0.4674 mg Si/L) was analyzed on 17 separate analytical runs yielding the following results:

Silica Compound	Mean *mg Si/L*	Recovery of Si *%*	Standard Deviation *mg Si/L*	Relative Standard Deviation *%*
Sodium meta-Silicate 9-Hydrate	0.4582	98	0.0070	1.5

13.2 A standard prepared by the autodilutor at the reporting limit (0.0234 mg Si/L) was analyzed 10 times on four separate analytical runs. These gave a mean of 0.0235 mg Si/L, a standard deviation of 0.0010 mg Si/L, and a relative standard deviation of 4.5%.

13.3 A carry-over study was performed. The high standard (2.337 mg Si/L) was sampled twice followed by three samples of blanks. The average of the blanks was -0.0002 mg Si/L with a standard deviation of 0.0002 mg Si/L.

14. POLLUTION PREVENTION

14.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most

cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

15. WASTE MANAGEMENT

15.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

16. REFERENCES

16.1 U.S. Environmental Protection Agency, *Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Matrices, 2nd Ed.*, EPA/600/R-97/072, September 1997, Method 366.0.

16.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

16.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100 and 4500-SiO_2.

16.4 Lachat Instruments Method 31-114-27-2-A

16.5 Mullin, J.B. and Riley, J.P. 1955. The Colorimetric Determination of Silicate with Special Reference to Sea and Natural Waters. *Analytica Chimica Acta, 12*, 162 – 176.

16.6 Hurley, J.P., Armstrong, D.E., Kenoyer, G.J. and Bowser, C.J. 1985. Ground Water as a Silica Source for Diatom Production in a Precipitation-Dominated Lake. *Science, 227,* 1576 – 1578.

16.7 Brewer, P.G. and Riley, J.P. 1966. The Automatic Determination of Silicate-Silicon in Natural Waters with Special Reference to Sea Water. *Analytica Chimica Acta, 35,* 514 – 519.

17. TABLE, DIAGRAMS, AND FLOWCHARTS

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

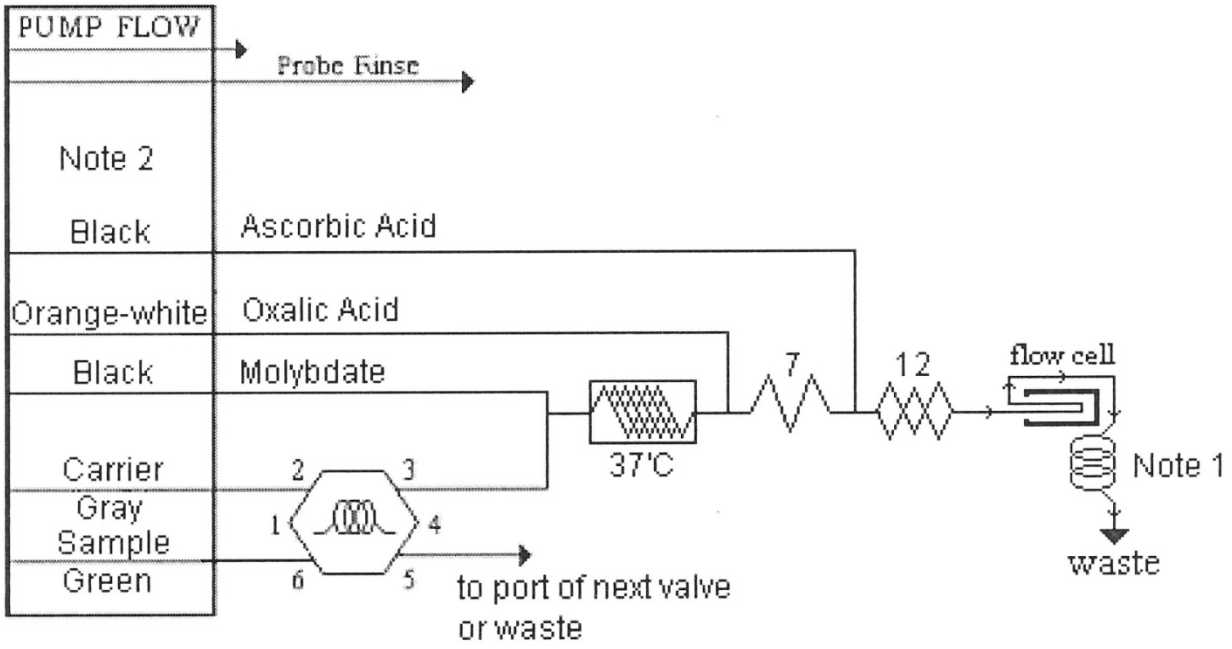
The timing values listed below were optimized using the universal dye. The brackish timing values were determined using artificial sea water as is and spiked at two low levels. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Timing Tab under Run Properties		Si
Run	Method Cycle Period (seconds)	90
	Sample Period (seconds)	50
	Min. Probe in Wash Period (seconds)	15
Channel 1	Load Period (seconds)	45
	Inject Period (seconds)	45
	Time to Valve (seconds)	20
SIF (Analyte)	Expected Inject to Peak Start (seconds)	21.5
	Expected Peak Base Width (seconds)	95.5
	Brackish Shutter Offset (seconds)	29.5
	Brackish Shutter Width (seconds)	20

Analyte Tab under Run Properties

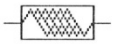
Concentration Units: mg Si/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: 1/X
QuikChem Method: 31-114-27-2-A
Chemistry: Brackish

17.2 SILICATE MANIFOLD DIAGRAM



Carrier: Reagent H_2O
Manifold tubing: 0.8 mm (0.032") i.d.
Sample Loop: 150 cm x 1.1 mm (0.042") i.d.
Interference Filter: 660 nm

7: 135 cm of tubing on a 7 cm coil support.
12: 255 cm of tubing on a 12 cm alternating coil support.

The  shows 175 cm of tubing wrapped around the heater block at the specified temperature. The flow cell is a 10 mm path length.

Note 1: 200 cm back pressure loop of 0.5 mm (0.022") i.d.
Note 2: Use 90 cm of 0.8 mm tubing with a Teflon PTA as line weight for all reagents in place of PVC and glass line weight transmission lines (only if not using reagent bottles with sipper caps). Each pump tube will require 2 PTA's and two collars for connections. (Glass can cause contamination by leaching silicate.)
Note 3: The sample loop should be cut at a 30 - 45° angle for the best fit.

Appendix 11:

SOP Total Dissolved Nitrogen

STANDARD OPERATING PROCEDURE
for Total Dissolved Nitrogen in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

Lori Ann Tew

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APPROVED BY

John R. Donat, Ph.D. Date
Laboratory Director

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of total dissolved nitrogen (TDN) in persulfate digests for seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.025 – 1.000 mg N/L. Higher concentrations can be determined by sample dilution. The method throughput is 38 injections per hour.

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

This method allows for the combined analysis of total dissolved nitrogen and total dissolved phosphorus from a single digestion. Nitrogen compounds require an alkaline medium for oxidation, whereas phosphorus compounds require an acidic environment for oxidation. The pH of the sample is alkaline in the beginning of the digestion. As the sodium hydroxide is consumed by the thermal decomposition of persulfate, the sample pH becomes acidic. This change in pH of the reaction allows for the oxidation of both nitrogen and phosphorus compounds (APHA, 2012).

Filtered water samples are digested with potassium persulfate in an alkaline environment to convert all forms of nitrogen containing compounds to the nitrate form, and the pH is adjusted with the addition of a boric acid buffer solution. The samples are analyzed by passage through a granular copperized cadmium column to reduce nitrate to nitrite. The nitrite (that already present plus the reduced nitrate) is then diazotized with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting magenta azo dye is colorimetrically measured at 520 nm.

Aqueous nitrogen compounds which are generally of greatest ecological interest are nitrate, nitrite, ammonia and organic nitrogen. These forms, in addition to nitrogen gas (N_2), are all part of the nitrogen cycle and can biochemically convert to one another. Nitrate is the most oxidized form of nitrogen, whereas organic nitrogen is the least oxidized (APHA, 2012). Total dissolved nitrogen (TDN) refers to the nitrogen present in

water after filtration. If the organic value is needed, inorganic fractions can be subtracted from the TDN value.

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

- 3.1.1 Freezer/Frozen: ≤ -18°C
- 3.1.2 Refrigerator/Refrigerated: 0 – 6°C
- 3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm
- 3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

- 3.2.1 CBP: Chesapeake Bay Program
- 3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard
- 3.2.3 CRM: Certified Reference Material
- 3.2.4 g: Gram(s)
- 3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard
- 3.2.6 L: Liter(s)
- 3.2.7 MDL: Method Detection Limit
- 3.2.8 mg: Milligram(s)
- 3.2.9 mg/L: Milligram(s) per Liter
- 3.2.10 mL: Milliliter(s)
- 3.2.11 NH_3/NH_4/NH4F: Ammonia
- 3.2.12 NIST: National Institute of Standards and Technology
- 3.2.13 NO_3: Nitrate
- 3.2.14 NO_{23}/NO23F: Nitrate + Nitrite
- 3.2.15 NO_2/NO2F: Nitrite
- 3.2.16 OPO_4/PO4F: Orthophosphate
- 3.2.17 PO_4: Phosphate
- 3.2.18 ppm: Parts Per Million
- 3.2.19 PQL: Practical Quantitation Limit
- 3.2.20 QCS: Quality Control Sample
- 3.2.21 Si/SIF: Silicate
- 3.2.22 SOP: Standard Operating Procedure
- 3.2.23 SRM: Standard Reference Material

3.2.24 TDN: Total Dissolved Nitrogen
3.2.25 TDP: Total Dissolved Phosphate
3.2.26 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation.

4.2 High concentrations of iron, copper or other metals can result in low results. The imidazole buffer reduces this interference.

4.3 Residual chlorine can interfere by oxidizing the reductor column.

4.4 High concentrations of sulfides, often present in anoxic water, rapidly deactivate cadmium columns by forming an insoluble layer of cadmium sulfide on the active metal surface.

4.5 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This can be eliminated by pre-extracting the sample with an organic solvent. This has not been seen as an issue with the typical samples analyzed by this lab.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

5.4.1 Cadmium granules
5.4.2 Chloroform

5.4.3 Hydrochloric Acid
5.4.4 Imidazole
5.4.5 Phosphoric Acid
5.4.6 Potassium Persulfate/Potassium Peroxodisulfate
5.4.7 Sodium Hydroxide
5.4.8 Sulfanilamide

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).
6.1.1 Sampler (Model ASX-520)
6.1.2 Auto dilutor (Model PDS-200)
6.1.3 Multichannel proportioning pump (Model RP-150)
6.1.4 Reaction unit or manifold (Model E31-107-04-4-B)
6.1.5 Colorimetric detector (Part 85080)
6.1.6 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Glassware – Class A volumetric flasks and pipettes (or pipettors) as required

6.5 Graduated cylinders

6.6 Digestion tubes – Threaded borosilicate glass tubes and polypropylene linerless screw caps, or equivalent

6.7 Autoclave – (GETINGE M/C 3522), or equivalent

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Imidazole Buffer, pH ~ 7.4 – *CAUTION: Prepare in fume hood as the addition of HCL will fume.* In a 1 L volumetric flask containing approximately 900 mL Reagent H_2O, add 6.8 g imidazole [$C_3H_4N_2$] and 2 mL concentrated hydrochloric acid [HCl]. Swirl to dissolve and dilute to mark with Reagent H_2O. Add two drops of 2% copper sulfate (Reagent 7.1.7) and invert to mix.
Expiration is one month.

7.1.2 Sulfanilamide Color Reagent – Add 100 mL 85% phosphoric acid [H_3PO_4] to a 1 L volumetric flask containing approximately 600 mL Reagent H_2O. Add 20.0 g sulfanilamide [$C_6H_8N_2O_2S$] and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride [NED – $C_{12}H_{14}N_2 \cdot 2HCl$] and stir to dissolve. Dilute to the mark and mix by inversion.
Store in a dark container.
Expiration is one month.

7.1.3 Oxidizing Reagent – Bring a 2 L volumetric flask to volume with Reagent H_2O. Using a portion of this water, add 40.2 g potassium persulfate or potassium peroxodisulfate [$K_2S_2O_8$, with ≤ 0.001% total nitrogen (N)] and 6.0 g sodium hydroxide [NaOH, low nitrogen (≤ 0.001% N)] and stir to dissolve. Transfer to a glass reagent container fit with a 5.0 mL bottle-top dispenser.
Use within two hours.

7.1.4 1 M Sodium Hydroxide – In a 1 L volumetric flask containing approximately 800 mL Reagent H_2O, add 40 g sodium hydroxide [NaOH, low nitrogen (≤ 0.001% N)] and stir to dissolve. Dilute to the mark and mix by inversion.
Store in a plastic container.
Expiration is N/A.

7.1.5 Boric Acid Buffer – Dissolve 61.8 g boric acid [H_3BO_3] approximately 600 mL of Reagent H_2O in a 1 L volumetric flask (the solution will be cloudy). Add 202 mL 1 M Sodium Hydroxide (Reagent 7.1.4) and stir to combine. Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is two months.

7.1.6 Carrier – Measure out 500 mL of Oxidizing Reagent (Reagent 7.1.3) and pour into a 2.5 L glass bottle. Add 1000 mL Reagent H_2O. Cover opening with foil and autoclave for 30 minutes at 105°C (120°C if also analyzing phosphorus) on the liquid cycle (See Section 10.3 for procedure). Before analysis, add 100 mL of Boric Acid Buffer (Reagent 7.1.5) and mix to combine.

7.1.7 2% Copper Sulfate – In a 100 mL volumetric flask containing approximately 80 mL Reagent H_2O, dissolve 2 g copper sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$]. Dilute to mark and mix by inversion.
Discard if a precipitate forms.
Expiration is N/A.

7.2 PREPARATION OF STANDARDS

Rinse all volumetric flasks with Reagent H_2O prior to use.

7.2.1 Nitrate Stock Standard, 100 mg NO_3-N/L – In a 1 L volumetric flask, dissolve 0.7218 g potassium nitrate [KNO_3] (dried overnight at 104 ± 2°C and stored in a dessicator) in approximately 800 mL Reagent H_2O. Add 2 mL chloroform [$CHCl_3$] (in an exhaust hood, using a macropipettor). Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is six months.

7.2.2 Nitrite Stock Standard, 250 mg NO_2-N/L – In a 1 L volumetric flask, dissolve 1.232 g sodium nitrite [$NaNO_2$] (dried overnight at 104 ± 2°C and stored in a dessicator) in approximately 800 mL Reagent H_2O. Dilute to volume and invert to mix. Add 2 mL chloroform [$CHCl_3$] (in an exhaust hood, using a macropipettor) and mix by inversion.
Store in a refrigerator.
Expiration is one month.

7.2.3 Glutamic Acid Stock Standard, 70.56 mg N/L – In a 500 mL volumetric flask, dissolve 0.3705 g glutamic acid [$C_5H_9NO_4$] in approximately 400 mL Reagent H_2O. Add 0.5 mL chloroform [$CHCl_3$] (in an exhaust hood, using a macropipettor). Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is ten months.

7.2.4 Working Standard 10.0 mg NO_3-N/L – In a 50 mL volumetric flask, add 5.0 mL of Nitrate Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Phosphate (2.0 mg PO_4-P/L)], add 0.10 mL of Phosphate Stock Standard to flask before diluting to mark. See specific SOP for details**.** Prepare fresh on day of use.

7.2.5 Calibration curve – Dilute the specific volume of Working Standard 7.2.4 in a 100 mL volumetric flask with Reagent H_2O.

Working Standards (Prepared Daily)	Concentration mg/L	Standard 7.2.4 (10.0 mg NO_3-N/L) mL
S1	1.000	10.0
S2	0.500	5.0
S3	0.200	2.0
S4	0.100	1.00
S5	0.025	0.25
S6	0.000	---

7.2.6 Matrix Spikes – Add 1.00 mL of Working Standard 7.2.4 (10.0 mg NO_3-N/L) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.4 mg NO_2-N/L** (**0.08 mg PO_4-P/L**). *NOTE: The spike concentration may be adjusted due to sample concentrations or sample volumes.*

7.2.7 Column Check Standard, 0.5 mg NO_2-N/L – Add 0.10 mL of Nitrite Stock Standard (7.2.2) to a 50 mL volumetric flask. Dilute to volume with Reagent H_2O. Prepare fresh day of use.

7.2.8 Glutamic Acid Standard, 0.3528 mg N/L – Add 0.50 mL of Glutamic Acid Stock Standard (7.2.3) to a 100 mL volumetric flask. Dilute to volume with Reagent H_2O. If making a combined standard [Glycerophosphoric Acid Standard (0.08 mg P/L)], add 1.00 mL of Working Glycerophosphoric Acid Standard (8.0 mg P/L) to flask before diluting to mark. See specific SOP for details. Prepare fresh day of use.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 13 for acceptance criteria.

9.2 Process and analyze a column check standard each time the analysis is performed to confirm the efficiency of the cadmium reduction. Refer to Section 13 for acceptance criteria.

9.3 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 13 for acceptance criteria.

9.4 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.5 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS)

containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.6 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.7 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. DISPENSING AND DIGESTION PROCEDURE

10.1 PRE-DISPENSING PROCEDURE

10.1.1 Fill a vial with a sufficient volume of the Nitrate Stock Standard (repeat for any standards of a combined analysis). Use this portion to make the calibration curve. Prepare working standards and matrix spikes as described in Section 7.2. The minimum number of non-zero standards is four and two zero standards.

10.1.2 Fill a vial with sufficient volume of the Nitrite Stock Standard. Use this portion to make the Column Check Standard.

10.1.3 Fill a vial with sufficient volume of the Glutamic Acid Stock Standard. Use this portion to make the Glutamic Acid Standard.

10.1.4 Remove a pre-dispensed CRM vial from the freezer and thaw. Record date of use in the Working Standards/Reagent Logbook. Dilute if necessary.

10.1.5 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

10.1.6 Remove samples from storage and log action in chain-of-custody.

10.1.7 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Thaw the samples.

10.1.8 Rinse digestion caps two times with 10% HCl and six times with Reagent H_2O. If contamination is suspected or becomes a problem, rinse the digestion tubes once with 10% HCl and six times with Reagent H_2O.

10.2 DISPENSING STANDARDS AND SAMPLES

10.2.1 Label a set of tubes for the standards as follows: S1, S2, S3, S4, S5, S6, CRM N, GA, and NO_2. Label enough tubes to ensure a sufficient volume for both the curve and in run check standards is available.

10.2.2 Label tubes with the log number of each sample, dup and spike to be dispensed. The last three numbers is usually sufficient, but if not, include the whole number.

10.2.3 Using a fixed volume pipette, dispense 10.0 mL of standard/sample into the appropriately labeled tube and cap. Change pipette tips between each standard/sample. The tubes may be frozen until a later date.

10.3 DIGESTION PROCEDURE

If previously frozen, remove a set of standards and samples from the freezer and thaw. The tubes must be digested within two hours of the preparation of the oxidizing reagent, and they will be autoclaved at 100-120°C for 30 minutes on the liquid cycle. Ensure there is ample room on the recording chart and the pen is in place. *Note: If digesting TDP set autoclave at 120°C.*

10.3.1 Using a bottle-top dispenser, add 5.0 mL of Oxidizing Reagent (7.1.3) to each tube and cap quickly and tightly to prevent volatilization. Invert to mix. *NOTE: Avoid aspiration of air bubbles when using the dispenser. If air bubbles are dispensed with the oxidizing reagent, the tube should be discarded and a new one prepared.*

10.3.2 Behind the metal door at the bottom of the autoclave, in the upper right hand corner of the boiler, turn the **CONTROL SWITCH** on.

10.3.3 On the control panel, press the **CONTROLS 'ON'** button. *NOTE: A light should turn on in the button. The EXHAUST and DOOR UNSEALED boxes should be lit.*

10.3.4 When the **EXHAUST** light turns off, press the **LIQUIDS** button (it should be lit).

10.3.5 Ensure the following settings are entered. If needed, adjust with the corresponding arrows.
SELECTED EXPOSURE TIME = 00:30
SELECTED EXHAUST TIME = 00:00
SELECTED TEMP = 105°C (acceptable range is 100 – 120°C)
NOTE: If also analyzing for phosphorus, set selected temp to 120°C.

10.3.6 When the standards and samples (or carrier reagent) are in place and the **READY** indicator lights, close the door and press the **START CYCLE** button. *NOTE: The READY indicator will go out and the CONDITIONING PHASE indicator will come on. When pressure and temperature are reached, the CONDITIONING PHASE indicator will turn off and the EXPOSURE PHASE indicator will come on.*

10.3.7 When the cycle is complete, the **COMPLETE** indicator will come on, and a buzzer will sound. The door will unseal automatically. Lower the door about an inch to allow residual steam to escape for about 15 minutes.

10.3.8 Remove the standards and samples and transfer to a refrigerator. If the carrier reagent was digested, remove it from the autoclave and transfer to the Lachat counter.

10.3.9 Once digestion is complete, record the date, parameter(s), and cruise(s) within the peak. Press the **CONTROLS 'OFF'** button and turn off the boiler **CONTROL SWITCH**.

11. CALIBRATION AND STANDARIZATION

11.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.3). The minimum number of standards is four and a zero standard. The standards should be digested at the same time as the samples being analyzed.

11.2 Calibrate the instrument as described in Section 12.

11.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 13.1 for acceptance criteria.

11.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 13.2 for acceptance criteria.

12. PROCEDURE

12.1 PRE-ANALYTICAL PROCEDURE

12.1.1 Gather all TDN reagents (and any reagents needed if running a combined analysis). Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

12.1.2 Remove samples and standards from the refrigerator.

12.1.3 Inspect samples and standards to ensure that they have been properly stored and that the sample containers have not been compromised. Allow the digestion tubes to come up to ambient temperature.

12.1.4 If there is precipitate in the bottom of the digestion tubes, invert to mix. Using a bottle top dispenser, add 1.0 mL of Boric Acid Buffer (7.1.5) to each tube, and mix well. *NOTE: If the precipitate is not homogenized prior to the addition of the Boric Acid Buffer, it will not dissolve.*

12.1.5 Rinse sample tubes/vials two times with 10% HCl and six times with Reagent H_2O. If Phosphorus is also analyzed, glass tubes must be used.

12.2 CALIBRATION PROCEDURE

12.2.1 Set up manifold as shown in Section 18.2.

12.2.2 Verify/input data system parameters as shown in Section 18.1.

12.2.3 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and ensure the buffer is through the manifold before putting the cadmium column on-line. Allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

12.2.4 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 18).

12.2.5 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

12.3 SYSTEM NOTES

12.3.1 For information on system maintenance and troubleshooting, refer to the Troubleshooting Guide in the System Operation Manual.

12.3.2 Do not switch the cadmium column in-line with the manifold until the imidazole buffer is in the system. Allow the carrier and buffer to rinse through the column for at least 10 minutes before starting analysis.

12.3.3 It is important to check column efficiency each time the column is replaced. Once the efficiency is known, a nitrite standard can be inserted in the sample

tray to verify that the column remains efficient (see Section 13 for acceptance criteria).

12.3.4 Poor correlation coefficients are sometimes the result of substandard column performance. If the standards are freshly prepared and the calibration fails consistently, replace the column.

12.3.5 The blank in this method will give a peak. If the blank peak is very negative (an inverse peak), the carrier is contaminated. If the blank peak is ≥ PQL (lowest non-zero standard), the blank is contaminated.

12.3.6 If the detection limit is greater than that specified in the method, the following outline should be followed.

- 12.3.6.1 Verify standards preparation procedures.
- 12.3.6.2 Verify that a 520 nm filter is being used.
- 12.3.6.3 Verify that the sample loop is completely filled by running dye.
- 12.3.6.4 Verify that the reagents are being added in the correct order.
- 12.3.6.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.
- 12.3.6.6 Prepare fresh reagents.

12.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

12.3.8 At the end of analysis, rinse the cadmium column with imidazole buffer and Reagent H_2O going through the carrier lines for ~10 minutes before taking the column off-line.

13. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 13.1 through 13.7, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 13.4 through 13.7 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

13.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

13.1.1 Acceptability of calibration correlation coefficient (r)

A.	r ≥ 0.9990	Acceptable
B.	r = 0.9980 – 0.9989	Acceptable but troubleshooting is required.
C.	r < 0.9980	Stop the analysis. Troubleshooting is required.

13.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

13.3 When the cadmium column is used, the percent column efficiency must be within 90 – 110 % when calculated as:

$$\%\ column\ eff. = \frac{MNO_3}{MNO_2} \times 100$$

Where, MNO_3 = Measured NO_3 standard concentration, MNO_2 = Measured NO_2 standard concentration. Both standard concentrations must be the same.

13.4 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

13.5 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

13.6 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA \times DF} \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA \times DF} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

13.7 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

13.8 Report results for total dissolved nitrogen in mg N/L.

14. METHOD PERFORMANCE

14.1 A Quality Control Sample prepared in Reagent H_2O was analyzed on 15 separate analytical runs yielding the following results:

RT-Corp. QC1051-2ML Lot 019822	*n*	Mean *mg NO_3-N/L*	Recovery of NO_3-N *%*	Standard Deviation *mg NO_3-N/L*	Relative Standard Deviation *%*
0.305 mg N/L	1	0.319	105	--	--
0.406 mg N/L	13	0.418	103	0.012	2.8
0.812 mg N/L	1	0.823	101	--	--

14.2 A standard prepared at the reporting limit (0.025 mg N/L) was analyzed 12 times on three separate analytical runs. These gave a mean of 0.023 mg N/L, a standard deviation of 0.005 mg N/L, and a relative standard deviation of 22%.

14.3 A carry-over study was performed. A high standard (1.0 mg N/L) was sampled twice followed by three samples of blanks. The average of the blanks was -0.008 mg N/L with a standard deviation of 0.017 mg N/L.

15. POLLUTION PREVENTION

15.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

15.2 The cadmium used in this analysis must be disposed of properly. The cadmium and any items contaminated by the cadmium are placed in an appropriate waste container provided by Old Dominion University's Environmental Health and Safety Office (EHSO). The EHSO collects the container and disposes of it.

16. WASTE MANAGEMENT

16.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

17. REFERENCES

17.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 353.2, Rev. 2.0.

17.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

17.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100, 4500-N and 4500-P.

17.4 Lachat Instruments Method 31-107-104-4-C

18. TABLE, DIAGRAMS AND FLOWCHARTS

18.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

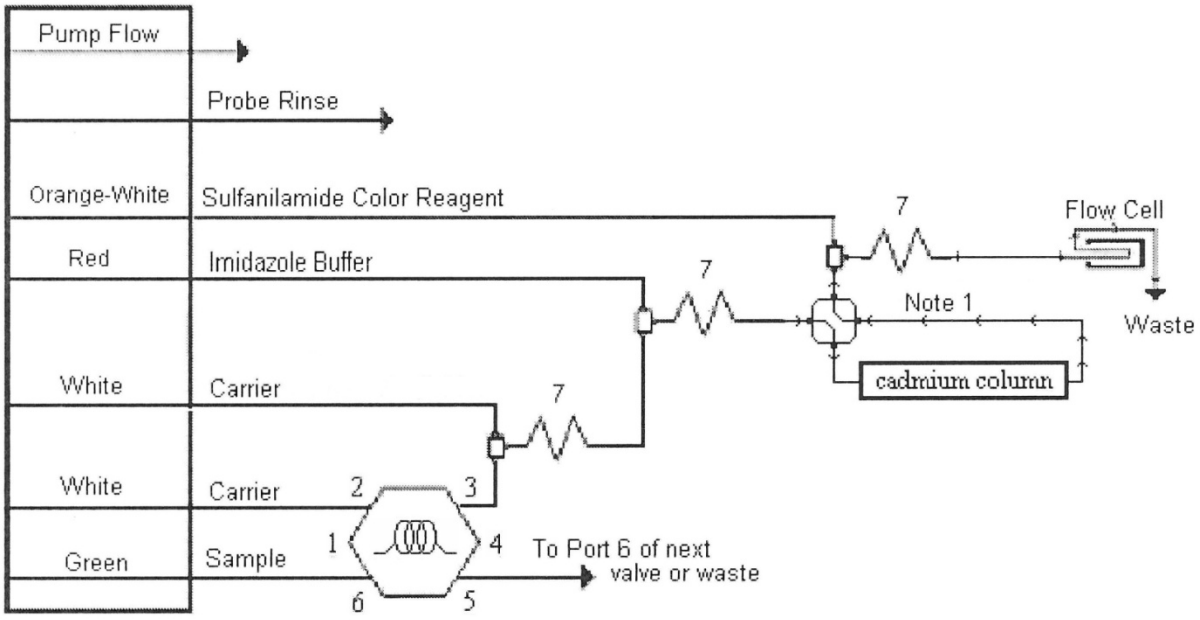
The timing values listed below were optimized using the universal dye. *Note: The times are approximate and may vary slightly with pump tubing wear*.

Time Tab under Run Properties		TDN	TDP/TDN
Run	Method Cycle Period (seconds)	45	95
	Sample Period (seconds)	13	27
	Min. Probe in Wash Period (seconds)	5	5
Channel 4	Load Period (seconds)	12	15
	Inject Period (seconds)	30	71
	Time to Valve (seconds)	26	33
TDN (Analyte)	Expected Inject to Peak Start (seconds)	15.5	15.5
	Expected Peak Base Width (seconds)	40.5	40.5

Analyte Tab under Run Properties

Concentration Units: mg N/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: 1/X
QuikChem Method: 31-107-104-4-C
Chemistry: Direct/Bipolar

18.2 TOTAL DISSOLVED NITROGEN MANIFOLD DIAGRAM



Carrier: Carrier (7.1.6)
Manifold tubing: 0.5 mm (0.022") i.d.
Sample Loop: 25 cm x 0.5 mm (0.022") i.d.
Interference Filter: 520 nm

7: 135 cm of tubing on a 7 cm coil support.

The flow cell is a 10 mm path length.

Note 1: This is a two state switching valve used to place the cadmium column in-line with the manifold. Do not switch the cadmium column in-line with the manifold until the imidazole buffer is in the system. Allow the carrier and buffer to rinse through the column for at least 10 minutes before starting analysis.

Appendix 12:

SOP Nitrate & Nitrite (Filtered)

STANDARD OPERATING PROCEDURE

for Nitrate + Nitrite in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

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APPROVED BY

John R. Donat, Ph.D. Laboratory Director	Date
Suzanne C. Doughten Quality Manager/Technical Director	Date

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of nitrate + nitrite in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.0025 – 0.50 mg N/L as NO_3+NO_2 (NO_{23}). Higher concentrations can be determined by sample dilution. The method throughput is 60 injections per hour.

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

Filtered water samples are analyzed by passage through a granular copperized cadmium column to reduce nitrate to nitrite. The nitrite (that already present plus the reduced nitrate) is then diazotized with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting magenta azo dye is colorimetrically measured at 520 nm. The concentration of nitrate can be calculated by subtracting nitrite.

Aqueous nitrogen compounds which are generally of greatest ecological interest are nitrate, nitrite, ammonia and organic nitrogen. These forms, in addition to nitrogen gas (N_2), are all part of the nitrogen cycle and can biochemically convert to one another. Nitrate is the most oxidized form of nitrogen. It is essential for many photosynthetic autotrophs and can be the growth-limiting nutrient in some cases (APHA, 2012).

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

3.1.1 Freezer/Frozen: ≤ -18°C

3.1.2 Refrigerator/Refrigerated: 0 – 6°C

3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm

3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 g: Gram(s)

3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.6 L: Liter(s)

3.2.7 MDL: Method Detection Limit

3.2.8 mg: Milligram(s)

3.2.9 mg/L: Milligram(s) per Liter

3.2.10 mL: Milliliter(s)

3.2.11 NH_3/NH_4/NH4F: Ammonia

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 NO_3: Nitrate

3.2.14 NO_{23}/NO23F: Nitrate + Nitrite

3.2.15 NO_2/NO2F: Nitrite

3.2.16 OPO_4/PO4F: Orthophosphate

3.2.17 PO_4: Phosphate

3.2.18 ppm: Parts Per Million

3.2.19 PQL: Practical Quantitation Limit

3.2.20 QCS: Quality Control Sample

3.2.21 Si/SIF: Silicate

3.2.22 SOP: Standard Operating Procedure

3.2.23 SRM: Standard Reference Material

3.2.24 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation.

4.2 High concentrations of iron, copper or other metals can cause low results. The imidazole buffer reduces this interference.

4.3 Residual chlorine can interfere by oxidizing the reductor column. This has not been an issue with the estuarine samples analyzed by this lab.

4.4 High concentrations of sulfides, often present in anoxic water, rapidly deactivate cadmium columns by forming an insoluble layer of cadmium sulfide on the active metal surface. These samples can be purged with an inert gas to remove the sulfides. This has not been an issue with the estuarine samples analyzed by this lab, but has been seen in some lake waters.

4.5 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This can be eliminated by pre-extracting the sample with an organic solvent. This has not been an issue with the estuarine samples analyzed by this lab.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

5.4.1 Cadmium granules

5.4.2 Chloroform

5.4.3 Hydrochloric Acid

5.4.4 Imidazole

5.4.5 Phosphoric Acid

5.4.6 Sulfanilamide

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).

6.1.1 Sampler (Model ASX-520)

6.1.2 Auto dilutor (Model PDS-200)

6.1.3 Multichannel proportioning pump (Model RP-150)

6.1.4 Reaction unit or manifold (Model 31-107-04-4-B)

6.1.5 Colorimetric detector (Part 85080)

6.1.6 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Glassware – Class A volumetric flasks and pipettes (or pipettors) as required

6.5 Graduated cylinders

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L. *NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.*

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Imidazole Buffer, pH ~ 7.4 – *CAUTION: Prepare in fume hood as the addition of HCL will fume.* In a 1 L volumetric flask containing approximately 900 mL Reagent H_2O, add 6.8 g imidazole [$C_3H_4N_2$] and 2 mL concentrated hydrochloric acid [HCl]. Swirl to dissolve and dilute to mark with Reagent H_2O. Add two drops of 2% copper sulfate (Reagent 7.1.3) and invert to mix.
Expiration is one month.

7.1.2 Sulfanilamide Color Reagent – Add 100 mL 85% phosphoric acid [H_3PO_4] to a 1 L volumetric flask containing approximately 600 mL Reagent H_2O. Add 20.0 g sulfanilamide [$C_6H_8N_2O_2S$] and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride [NED – $C_{12}H_{14}N_2 \cdot 2HCl$] and stir to dissolve. Dilute to mark with Reagent H_2O and mix by inversion.
Store in a dark container.
Expiration is one month.

7.1.3 2% Copper Sulfate – In a 100 mL volumetric flask containing approximately 80 mL Reagent H_2O, dissolve 2 g copper sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$]. Dilute to mark and mix by inversion.
Discard if a precipitate forms.
Expiration is N/A.

7.2 PREPARATION OF STANDARDS

Rinse all volumetric flasks with Reagent H_2O prior to use.

7.2.1 Nitrate Stock Standard, 100 mg NO_3-N/L – In a 1 L volumetric flask, dissolve 0.7218 g potassium nitrate [KNO_3] (dried overnight at 104 ± 2°C and stored in a dessicator) in approximately 800 mL Reagent H_2O. Add 2 mL chloroform [$CHCl_3$] (in an exhaust hood, using a macropipettor). Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is six months.

7.2.2 Nitrite Stock Standard, 250 mg NO_2-N/L – In a 1 L volumetric flask, dissolve 1.232 g sodium nitrite [$NaNO_2$] (dried overnight at 104 ± 2°C and stored in a dessicator) in approximately 800 mL Reagent H_2O. Dilute to volume and invert to mix. Add 2 mL chloroform [$CHCl_3$] (in an exhaust hood, using a macropipettor) and mix by inversion. Store in a refrigerator.
Expiration is one month.

7.2.3 Working Standard 10.0 mg NO_3-N/L – In a 100 mL volumetric flask, add 10.0 mL of Nitrate Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Ammonia (10.0 mg NH_3-N/L) and/or Phosphate (2.0 mg PO_4-P/L)], add 10.0 mL of Ammonia Stock Standard and/or 0.20 mL of Phosphate Stock Standard to flask before diluting to mark. See specific SOPs for details**.** Prepare fresh on day of use.

7.2.4 Working Standard 0.5 mg NO_3-N/L (with Ammonia and/or Phosphate)

7.2.4.1 Alone or with Ammonia (0.5 mg NH_3-N/L) – In a 100 mL volumetric flask, add 0.50 mL of Nitrate Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Ammonia (0.5 mg NH_3-N/L)], add 0.50 mL of Ammonia Stock Standard to flask before diluting to mark. See specific SOP for details**.** Prepare fresh on day of use.

7.2.4.2 With Phosphate (0.1 mg PO_4-P/L) and/or Ammonia (0.5 mg NH_3-N/L) – In a 100 mL volumetric flask, add 5.0 mL of Working Standard 10.0 mg NO_3-N/L (7.2.3). Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh on day of use.

7.2.5 Calibration curve – If making the calibration standards manually, dilute the specific volume of Working Standard 10 mg NO_3-N/L (7.2.3) in a 100 mL volumetric flask with Reagent H_2O. If using the autodilutor for the calibration standards, use Working Standard 0.5 mg NO_3-N/L (7.2.4) and input the AutoDilutor Factor (ADF) values in the chart.

Working Standards (Prepared Daily)	Concentration mg/L	Manual	Autodilutor
		Standard 7.2.3 (10.0 mg NO_3-N/L) mL	ADF Value (Standard 7.2.4, 0.5 mg NO_3-N/L)
S1	0.5000	5.0	--
S2	0.2000	2.0	2.50
S3	0.1000	1.00	5.00
S4	0.0500	0.50	10.00
S5	0.0100	0.10	50.00
S6	0.0050	1.00 (S1)*	100.00
S7	0.0025	0.50 (S1)*	200.00
S8	0.0000	--	--

*Use S1 instead of Standard 7.2.3.

7.2.6 Matrix Spikes – *NOTE: The spike concentration may be increased due to sample concentrations.*

7.2.6.1 Alone or with Ammonia – Add 2.0 mL of Working Standard 7.2.4 (0.5 mg NO_3-N/L) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.04 mg NO_3-N/L (0.04 mg NH_3-N/L).**

7.2.6.2 With Phosphate and/or Ammonia – Add 0.25 mL of Working Standard 7.2.3 (10.0 mg NO_3-N/L) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.1 mg NO_3-N/L** (**0.02 mg PO_4-P/L** and/or **0.1 mg NH_3-N/L**).

7.2.7 Working Standard 10.0 mg NO_2-N/L – Add 1.00 mL of Nitrite Stock Standard (7.2.2) to a 25 mL volumetric flask. Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh day of use.

7.2.8 Column Check Standard 0.05 mg NO_2-N/L – Add 0.50 mL of Working Standard 10.0 mg NO_2-N/L (7.2.7) to a 100 mL volumetric flask. Dilute to volume with Reagent H_2O. Prepare fresh day of use.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 12 for acceptance criteria.

9.2 Process and analyze a column check standard each time the analysis is performed to confirm the efficiency of the cadmium reduction. Refer to Section 12 for acceptance criteria.

9.3 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 12 for acceptance criteria.

9.4 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.5 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS) containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.6 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.7 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. CALIBRATION AND STANDARIZATION

10.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.5). The minimum number of standards is four and a zero standard.

10.2 Calibrate the instrument as described in Section 11.

10.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 12.1 for acceptance criteria.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 12.2 for acceptance criteria.

11. PROCEDURE

11.1 PRE-ANALYTICAL PROCEDURE

11.1.1 Gather all Nitrate reagents (and any reagents needed if running a combined analysis). Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

11.1.2 Fill a vial with a sufficient volume of the Nitrate Stock Standard (repeat for any standards of a combined analysis). Use this portion to make the calibration curve.

11.1.3 Fill a vial with sufficient volume of the Nitrite Stock Standard. Use this portion to make the Column Check Standard.

11.1.4 Remove a pre-dispensed CRM vial from the freezer and thaw. Record the date of use in the Working Standards/Reagent Logbook. Dilute if necessary.

11.1.5 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

11.1.6 Remove samples from storage and log action in chain-of-custody.

11.1.7 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Thaw the samples.

11.1.8 Rinse sample tubes two times with 10% HCl and six times with Reagent H_2O. If Phosphate is also analyzed, glass tubes must be used.

11.2 CALIBRATION PROCEDURE

11.2.1 Prepare working standards and matrix spikes as described in Section 7.2. The calibration curve listed is for samples routinely analyzed in the laboratory. Different standards may be utilized for varying sample concentrations. The minimum number of non-zero standards is four, each sampled once. The zero standard is sampled in duplicate.

11.2.2 Set up manifold as shown in Section 17.2.

11.2.3 Verify/input data system parameters as shown in Section 17.1.

11.2.4 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and ensure the buffer is through the manifold before putting the cadmium column on-line. Allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

11.2.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

11.2.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.3 SYSTEM NOTES

11.3.1 For information on system maintenance and troubleshooting, refer to the Troubleshooting Guide in the System Operation Manual.

11.3.2 Do not switch the cadmium column in-line with the manifold until the imidazole buffer is in the system.

11.3.3 It is important to check column efficiency each time the column is replaced. Once the efficiency is known, a nitrite standard can be inserted in the sample tray to verify that the column remains efficient (see Section 12 for acceptance criteria).

11.3.4 Poor correlation coefficients are sometimes the result of substandard column performance. If the standards are freshly prepared and the calibration fails consistently, replace the column.

11.3.5 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.3.6 If the detection limit is greater than that specified in the method, the following outline should be followed.

- 11.3.6.1 Verify standards preparation procedures.
- 11.3.6.2 Verify that a 520 nm filter is being used.
- 11.3.6.3 Verify that the sample loop is completely filled by running dye.
- 11.3.6.4 Verify that the reagents are being added in the correct order.
- 11.3.6.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.
- 11.3.6.6 Prepare fresh reagents.

11.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

11.3.8 If a large number of bubbles are observed in the autodilutor water container, degas with helium for at least 15 minutes.

12. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 12.1 through 12.7, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 12.4 through 12.7 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

12.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.1.1 Acceptability of calibration correlation coefficient (r)

A.	r ≥ 0.9990	Acceptable
B.	r = 0.9980 – 0.9989	Acceptable but troubleshooting is required.
C.	r < 0.9980	Stop the analysis. Troubleshooting is required.

12.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

12.3 When the cadmium column is used, the percent column efficiency must be within 90 – 110 % when calculated as:

$$\%\ column\ eff. = \frac{MNO_3}{MNO_2} \times 100$$

Where, MNO_3 = Measured NO_3 standard concentration, MNO_2 = Measured NO_2 standard concentration. Both standard concentrations must be the same.

12.4 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

12.5 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

12.6 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA \times DF} \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA \times DF} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

12.7 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

12.8 Report results in mg NO_{23}-N/L.

13. METHOD PERFORMANCE

13.1 A Quality Control Sample prepared in Reagent H_2O was analyzed on 23 separate analytical runs yielding the following results:

RT-Corp. QC1195-20ML	*n*	True Value *mg NO_3-N/L*	Mean *mg NO_3-N/L*	Recovery of NO_3-N *%*	Standard Deviation *mg NO_3-N/L*	Relative Standard Deviation *%*
Lot 017175	11	0.0625	0.0637	102	0.0026	4.0
Lot 017175	4	0.25	0.2568	103	0.0090	3.5
Lot 018782	10	0.0755	0.0776	103	0.0013	1.7
Lot 018782	3	0.151	0.1546	102	0.0016	1.0

13.2 A standard prepared by the autodilutor at the reporting limit (0.0025 mg NO_3-N/L) was analyzed 15 times on six separate analytical runs. These gave a mean of 0.0025 mg NO_3-N/L, a standard deviation of 0.0004 mg NO_3-N/L, and a relative standard deviation of 18%.

13.3 A carry-over study was performed. A high standard (0.5 mg NO_3-N/L) was sampled twice followed by three samples of blanks. The average of the blanks was 0.0001 mg NO_3-N/L with a standard deviation of 0.0004 mg NO_3-N/L.

14. POLLUTION PREVENTION

14.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

14.2 The cadmium used in this analysis must be disposed of properly. The cadmium and any items contaminated by the cadmium are placed in an appropriate waste container provided by Old Dominion University's Environmental Health and Safety Office (EHSO). The EHSO collects the container and disposes of it.

15. WASTE MANAGEMENT

15.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

16. REFERENCES

16.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 353.2, Rev. 2.0.

16.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

16.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100 and 4500-N.

16.4 Lachat Instruments Method 31-107-104-4-C

17. TABLE, DIAGRAMS AND FLOWCHARTS

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

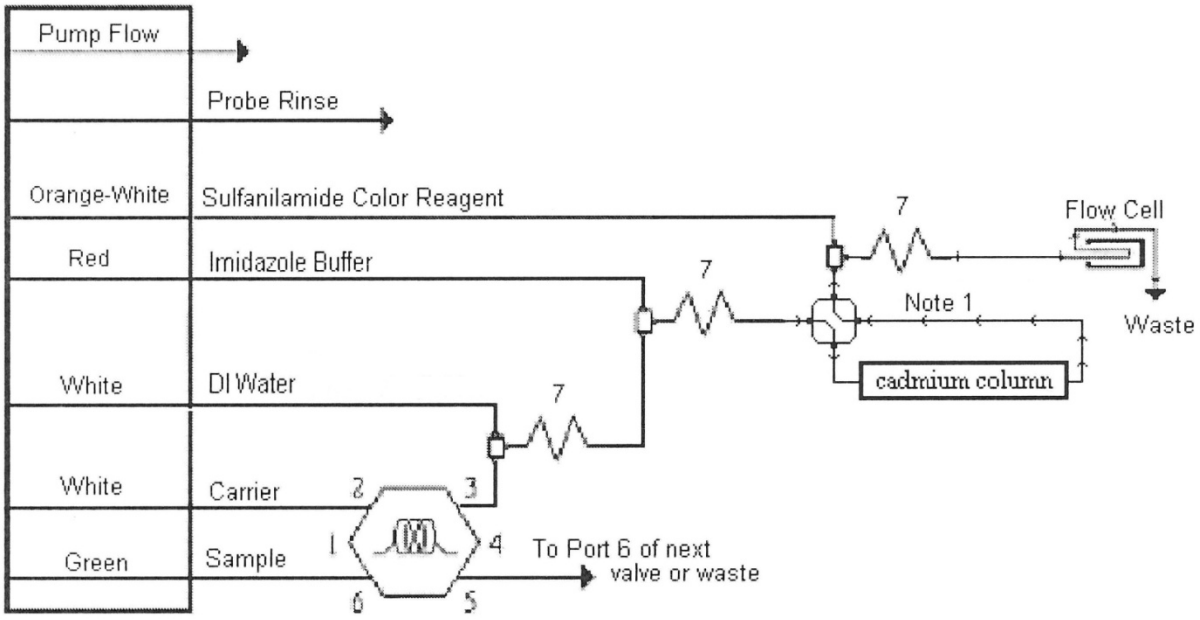
The timing values listed below were optimized using the universal dye. The brackish timing values were determined using artificial sea water as is and spiked at two low levels. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Time Tab under Run Properties		NO_3	NH_3/NO_3	$OPO_4/NH_3/NO_3$
Run	Method Cycle Period (seconds)	60	70	135
	Sample Period (seconds)	35	40	65
	Min. Probe in Wash Period (seconds)	5	20	59
Channel 4	Load Period (seconds)	25	25	30
	Inject Period (seconds)	30	30	30
	Time to Valve (seconds)	24	33	51
NO23F (Analyte)	Expected Inject to Peak Start (seconds)	19	19	13
	Expected Peak Base Width (seconds)	60.5	60.5	75.5
	Brackish Shutter Offset (seconds)	15.5	15.5	21
	Brackish Shutter Width (seconds)	24	24	21

Analyte Tab under Run Properties

Concentration Units: mg NO_3-N/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: 1/X
QuikChem Method: 31-107-104-4-C
Chemistry: Brackish

17.2 NITRATE/NITRITE MANIFOLD DIAGRAM



Carrier: Reagent H_2O
Manifold tubing: 0.5 mm (0.022") i.d.
Sample Loop: 130 cm x 0.8 mm (0.032") i.d.
Interference Filter: 520 nm

7: 135 cm of tubing on a 7 cm coil support.

The flow cell is a 10 mm path length.

Note 1: This is a two state switching valve used to place the cadmium column in-line with the manifold. Do not switch the cadmium column in-line with the manifold until the imidazole buffer is in the system.

Appendix 13:

SOP Nitrite (Filtered)

STANDARD OPERATING PROCEDURE
for Nitrite in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

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APPROVED BY

_____

John R. Donat, Ph.D. Date
Laboratory Director

_____

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of nitrite in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.0010 – 0.050 mg N/L as NO_2. Higher concentrations can be determined by sample dilution. The method throughput is 60 injections per hour. *NOTE: It was proven during comparison studies that the method is linear up to at least 0.20 mg N/L.*

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

Filtered water samples are analyzed for nitrite, which is diazotized with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting magenta azo dye is colorimetrically measured at 520 nm.

Aqueous nitrogen compounds which are generally of greatest ecological interest are nitrate, nitrite, ammonia and organic nitrogen. These forms, in addition to nitrogen gas (N_2), are all part of the nitrogen cycle and can biochemically convert to one another. Nitrite is an intermediate oxidation state of nitrogen, as a reduction of nitrate and oxidation of ammonia (APHA, 2012).

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

- 3.1.1 Freezer/Frozen: ≤ -18°C
- 3.1.2 Refrigerator/Refrigerated: 0 – 6°C
- 3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm
- 3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 g: Gram(s)

3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.6 L: Liter(s)

3.2.7 MDL: Method Detection Limit

3.2.8 mg: Milligram(s)

3.2.9 mg/L: Milligram(s) per Liter

3.2.10 mL: Milliliter(s)

3.2.11 NH_3/NH_4/NH4F: Ammonia

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 NO_3: Nitrate

3.2.14 NO_{23}/NO23F: Nitrate + Nitrite

3.2.15 NO_2/NO2F: Nitrite

3.2.16 OPO_4/PO4F: Orthophosphate

3.2.17 PO_4: Phosphate

3.2.18 ppm: Parts Per Million

3.2.19 PQL: Practical Quantitation Limit

3.2.20 QCS: Quality Control Sample

3.2.21 Si/SIF: Silicate

3.2.22 SOP: Standard Operating Procedure

3.2.23 SRM: Standard Reference Material

3.2.24 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation.

4.2 High concentrations of iron, copper or other metals can cause low results. The imidazole buffer reduces this interference.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

- 5.4.1 Chloroform
- 5.4.2 Hydrochloric Acid
- 5.4.3 Imidazole
- 5.4.4 Phosphoric Acid
- 5.4.5 Sulfanilamide

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).

- 6.1.1 Sampler (Model ASX-520)
- 6.1.2 Auto dilutor (Model PDS-200)
- 6.1.3 Multichannel proportioning pump (Model RP-150)
- 6.1.4 Reaction unit or manifold (Model 31-107-04-4-C)
- 6.1.5 Colorimetric detector (Part 85080)
- 6.1.6 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Glassware – Class A volumetric flasks and pipettes (or pipettors) as required

6.5 Graduated cylinders

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Imidazole Buffer, pH ~ 7.4 – *CAUTION: Prepare in fume hood as the addition of HCl will fume.* In a 1 L volumetric flask containing approximately 900 mL Reagent H_2O, add 6.8 g imidazole [$C_3H_4N_2$] and 2 mL concentrated hydrochloric acid [HCl]. Swirl to dissolve and dilute to mark with Reagent H_2O.
Expiration is one month.

7.1.2 Sulfanilamide Color Reagent – Add 100 mL 85% phosphoric acid [H_3PO_4] to a 1 L volumetric flask containing approximately 600 mL Reagent H_2O. Add 20.0 g sulfanilamide [$C_6H_8N_2O_2S$] and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride [$C_{12}H_{14}N_2 \cdot 2HCl$ or NED] and stir to dissolve. Dilute to mark with Reagent H_2O and mix by inversion. Add two drops of 2% Copper Sulfate (Reagent 7.1.3) and mix.
Store in a dark container.
Expiration is one month.

7.1.3 2% Copper Sulfate – In a 100 mL volumetric flask containing approximately 80 mL Reagent H_2O, dissolve 2 g copper sulfate pentahydrate [$CuSO_4 \cdot 5H_2O$]. Dilute to mark and mix by inversion.
Discard if precipitate forms.
Expiration is N/A.

7.2 PREPARATION OF STANDARDS

The following is purchased and used without further preparation unless specified.

Nitrogen Standard (as Nitrite): 1.00 mL = 1.00 mg N as NO_2^- (1000 ± 5 ppm N); Potassium Nitrite [KNO_2]. Certified traceable to NIST. Storage requirements and expiration date are on the label. (Ricca Chemical Company, or equivalent)

Rinse all volumetric flasks three times with Reagent H_2O prior to use.

7.2.1 Nitrite Stock Standard, 250 mg NO_2-N/L – In a 1 L volumetric flask, dissolve 1.232 g sodium nitrite [$NaNO_2$] (dried overnight at 104 ± 2°C and stored in a dessicator) in approximately 800 mL Reagent H_2O. Dilute to mark and invert to mix. Add 2 mL chloroform [$CHCl_3$] (in an exhaust hood, using a macropipettor) and mix by inversion. Store in a refrigerator.
Expiration is one month.

7.2.2 Working Standard 5.0 mg NO_2-N/L – In a 100 mL volumetric flask, add 2.0 mL of Nitrate Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard with Phosphate (10.0 mg PO_4-P/L), add 1.00 mL of Phosphate Stock Standard to flask before diluting to mark. See specific SOP for details**.** Prepare fresh on day of use.

7.2.3 Working Standard 0.5 mg NO_2-N/L – In a 100 mL volumetric flask, add 10.0 mL of Working Standard 5.0 mg NO_2-N/L (7.2.2). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard with Phosphate, the value is 1.0 mg PO_4-P/L. Prepare fresh on day of use.

7.2.4 Working Standard 0.05 mg NO_2-N/L – In a 100 mL volumetric flask, add 1.00 mL of Working Standard 5.0 mg NO_2-N/L (7.2.2). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard with Phosphate, the value is 0.1 mg PO_4-P/L. Prepare fresh on day of use.

7.2.5 Calibration curve – If making the calibration standards manually, dilute the specific volume of Working Standard 7.2.2 in a 100 mL volumetric flask with Reagent H_2O. If using the autodilutor for the calibration standards, use Working Standard 7.2.4 and input the AutoDilutor Factor (ADF) values in the chart.

Working Standards (Prepared Daily)	Concentration mg/L	Manual	Autodilutor
		Standard 7.2.2 (5.0 mg NO_2-N/L) mL	ADF Value (Standard 7.2.4, 0.05 mg NO_2-N/L)
S1	0.0500	1.00	--
S2	0.0250	0.50	2.00
S3	0.0125	0.25	4.00
S4	0.0050	0.10	10.00
S5	0.0025	5.0 (S1)*	20.00
S6	0.0010	2.0 (S1)*	50.00
S7	0.0000	--	--

*Use S1 instead of 7.2.2.

7.2.5 Matrix Spikes – Add 0.50 mL of Working Standard 0.5 mg NO_2-N/L (7.2.3) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.01 mg NO_2-N/L** (**0.02 mg PO_4-P/L**).

7.2.7 Working Secondary Standard 10.0 mg NO_2-N/L – Add 1.00 mL of Nitrogen Standard (as Nitrite) to a 100 mL volumetric flask. Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh day of use.

7.2.8 Quality Control Sample 0.02 mg NO_2-N/L – Add 0.20 mL of Working Secondary Standard 10.0 mg NO_2-N/L (7.2.7) to a 100 mL volumetric flask. Dilute to volume with Reagent H_2O. Prepare fresh day of use.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as the Quality Control Sample (QCS). Refer to Section 12 for acceptance criteria.

9.2 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 12 for acceptance criteria.

9.3 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.4 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS) containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.5 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.6 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. CALIBRATION AND STANDARIZATION

10.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.5). The minimum number of standards is four and a zero standard.

10.2 Calibrate the instrument as described in Section 11.

10.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 12.1 for acceptance criteria.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 12.2 for acceptance criteria.

11. PROCEDURE

11.1 PRE-ANALYTICAL PROCEDURE

11.1.1 Gather all Nitrite reagents (and any reagents needed if running a combined analysis). Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

11.1.2 Fill a vial with a sufficient volume of the Nitrite Stock Standard (repeat for any standards of a combined analysis). Use this portion to make the calibration curve.

11.1.3 Fill a vial with a sufficient volume of the Nitrogen Standard (as Nitrite). Use this portion to make the Quality Control Sample.

11.1.4 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

11.1.5 Remove samples from storage and log action in chain-of-custody.

11.1.6 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Thaw the samples.

11.1.7 Rinse sample tubes two times with 10% HCl and six times with Reagent H_2O. If Phosphate is also analyzed, glass tubes must be used.

11.2 CALIBRATION PROCEDURE

11.2.1 Prepare working standards and matrix spikes as described in Section 7.2. The calibration curve listed is for samples routinely analyzed in the laboratory. Different standards may be utilized for varying sample concentrations. The minimum number of non-zero standards is four, each sampled once. The zero standard is sampled in duplicate.

11.2.2 Set up manifold as shown in Section 17.2.

11.2.3 Verify/input data system parameters as shown in Section 17.1.

11.2.4 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

11.2.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

11.2.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.3 SYSTEM NOTES

11.3.1 For information on system maintenance and troubleshooting, refer to the Troubleshooting Guide in the System Operation Manual.

11.3.2 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.3.3 If the detection limit is greater than that specified in the method, the following outline should be followed.

11.3.3.1 Verify standards preparation procedures.

11.3.3.2 Verify that a 520 nm filter is being used.

11.3.3.3 Verify that the sample loop is completely filled by running dye.

11.3.3.4 Verify that the reagents are being added in the correct order.

11.3.3.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.

11.3.3.6 Prepare fresh reagents.

11.3.4 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

11.3.5 If a large number of bubbles are observed in the autodilutor water container, degas with helium for at least 15 minutes.

12. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 12.1 through 12.6, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 12.3 through 12.6 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

12.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.1.1 Acceptability of calibration correlation coefficient (r)

A.	$r \geq 0.9990$	Acceptable
B.	$r = 0.9980 – 0.9989$	Acceptable but troubleshooting is required.
C.	$r < 0.9980$	Stop the analysis. Troubleshooting is required.

12.2 Assess whether the analytical result for the QCS sample confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the

QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

12.3 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

12.4 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

12.5 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA \times DF} \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA \times DF} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

12.6 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

12.7 Report results in mg NO_2-N/L.

13. METHOD PERFORMANCE

13.1 A Quality Control Sample prepared in Reagent H_2O at two different concentrations was analyzed on 17 separate analytical runs yielding the following results:

Nitrite Concentration	*n*	Mean *mg NO_2-N/L*	Recovery of *NO_2-N %*	Standard Deviation *mg NO_2-N/L*	Relative Standard Deviation *%*
0.0200 mg NO_2-N/L	16	0.0202	101	0.0005	2.7
0.0800 mg NO_2-N/L	4	0.0805	101	0.0019	2.4

13.2 A standard prepared by the autodilutor at the reporting limit (0.0010 mg NO_2-N/L) was analyzed 10 times on three separate analytical runs. These gave a mean of 0.0009 mg NO_2-N/L, a standard deviation of 0.0004 mg NO_2-N/L, and a relative standard deviation of 43.0%.

13.3 A carry-over study was performed. The high standard (0.0500 mg NO_2-N/L) was sampled twice followed by three samples of blanks. The average of the blanks was 0.0000 mg NO_2-N/L with a standard deviation of 0.0002 mg NO_2-N/L.

14. POLLUTION PREVENTION

14.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

15. WASTE MANAGEMENT

15.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

16. REFERENCES

16.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 353.2, Rev. 2.0.

16.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

16.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100 and 4500-N.

16.4 Lachat Instruments Method 31-107-104-4-C

17. TABLE, DIAGRAMS AND FLOWCHARTS

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

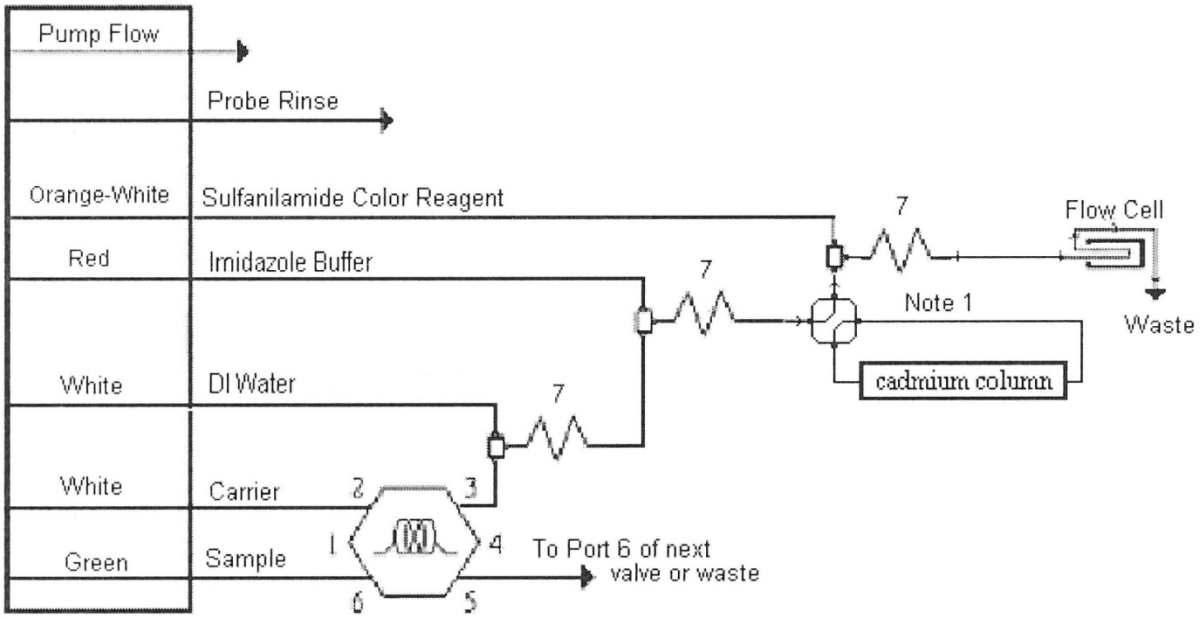
The timing values listed below were optimized using the universal dye. The brackish timing values were determined using artificial sea water as is and spiked at two low levels. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Time Tab under Run Properties		NO_2	OPO_4/NO_2
Run	Method Cycle Period (seconds)	60	135
	Sample Period (seconds)	35	50
	Min. Probe in Wash Period (seconds)	5	59
Channel 4	Load Period (seconds)	30	30
	Inject Period (seconds)	30	30
	Time to Valve (seconds)	26	42
NO2F (Analyte)	Expected Inject to Peak Start (seconds)	8	4
	Expected Peak Base Width (seconds)	95.5	135
	Brackish Shutter Offset (seconds)	21.5	33.5
	Brackish Shutter Width (seconds)	24	14.5

Analyte Tab under Run Properties

Concentration Units: mg NO_2-N/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: 1/X
QuikChem Method: 31-107-104-4-C
Chemistry: Brackish

17.2 NITRITE MANIFOLD DIAGRAM



Carrier: Reagent H_2O
Manifold tubing: 0.5 mm (0.022”) i.d.
Sample Loop: 100 cm x 1.1 mm (0.042”) i.d.
Interference Filter: 520 nm

7: 135 cm of tubing on a 7 cm coil support.

The flow cell is a 10 mm path length.

Note 1: This is a two state switching valve used to place the cadmium column in-line with the manifold. It is kept off-line for nitrite measurement.

Appendix 14:

SOP Ammonia (Filtered)

STANDARD OPERATING PROCEDURE
for Ammonia in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

Lori Ann Tew

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APPROVED BY

_____

John R. Donat, Ph.D. Date
Laboratory Director

_____

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of ammonia in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.0050 – 0.50 mg N/L as NH_4. Higher concentrations can be determined by sample dilution. The method throughput is 51 injections per hour.

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

Based on the Berthelot reaction, ammonia reacts with phenol and sodium hypochlorite in alkaline conditions. Indophenol blue is formed, and sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, which is directly proportional to the original concentration of ammonia in the sample.

Aqueous nitrogen compounds which are generally of greatest ecological interest are nitrate, nitrite, ammonia and organic nitrogen. These forms, in addition to nitrogen gas (N_2), are all part of the nitrogen cycle and can biochemically convert to one another (APHA, 2012). Ammonia is of interest biologically and chemically to oceanographers. It is usually the primary source of nitrogen for the phytoplankton in oligotrophic regions in the ocean. It constitutes more than half of the nitrogen excreted by marine animals (Degobbis, 1973). Ammonia is present in two forms in aqueous solutions: NH_4^+ (ionized) and NH_3 (non-ionized), and the concentration of total ammonia nitrogen (TAN) is the sum of NH_4^+ and NH_3 concentrations. The NH_3 form is toxic to fish, and the equilibrium of NH_4^+ and NH_3 is dependent on temperature and pH and to some extent salinity. Concentrations of NH_3 increase as temperature and pH increase but decrease as salinity increases (Bower and Bidwell, 1978). The United States Environmental Protection Agency (USEPA) recognized the toxicity of ammonia to aquatic life and originally published criteria recommendations in 1976. The National Ambient Water Quality Criteria were updated in 2013, and the acute and chronic criteria for ammonia in freshwater were set at 17 and 1.2 mg TAN/L, respectively (USEPA, 2013).

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

3.1.1 Freezer/Frozen: ≤ -18°C

3.1.2 Refrigerator/Refrigerated: 0 – 6°C

3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm

3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 g: Gram(s)

3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.6 L: Liter(s)

3.2.7 MDL: Method Detection Limit

3.2.8 mg: Milligram(s)

3.2.9 mg/L: Milligram(s) per Liter

3.2.10 mL: Milliliter(s)

3.2.11 NH_3/NH_4/NH4F: Ammonia

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 NO_3: Nitrate

3.2.14 NO_{23}/NO23F: Nitrate + Nitrite

3.2.15 NO_2/NO2F: Nitrite

3.2.16 OPO_4/PO4F: Orthophosphate

3.2.17 PO_4: Phosphate

3.2.18 ppm: Parts Per Million

3.2.19 PQL: Practical Quantitation Limit

3.2.20 QCS: Quality Control Sample

3.2.21 Si/SIF: Silicate

3.2.22 SOP: Standard Operating Procedure

3.2.23 SRM: Standard Reference Material

3.2.24 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation.

4.2 Calcium and magnesium ions may precipitate if present in sufficient concentrations. Tartrate is added to the sample in-line to prevent this problem.

4.3 If distillation is required, the sample is buffered at pH 9.5 with borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. This is not required for the typical samples analyzed in this lab.

4.4 Residual chlorine must be removed by pretreatment of the sample with sodium thiosulfate or other reagents before distillation. This is not required for the typical samples analyzed in this lab.

4.5 Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH dependent. Likewise, ensure that pH of standard ammonia solutions approximates that of the samples.

4.6 Ammonia is easily contaminated. If spike recoveries are consistently high, rinse the volumetric flasks three times with 10% HCl followed by six times with Reagent H_2O prior to use.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

5.4.1 Phenol

5.4.2 Sodium Hydroxide

5.4.3 Sodium Nitroprusside

5.4.4 Sulfuric Acid

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).

6.1.1 Sampler (Model ASX-520)

6.1.2 Auto dilutor (Model PDS-200)

6.1.3 Multichannel proportioning pump (Model RP-150)

6.1.4 Reaction unit or manifold (Model 31-107-06-1-F)

6.1.5 Heating unit (Part A85132)

6.1.6 Colorimetric detector (Part 85080)

6.1.7 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.1.8 PVC pump tubes must be used for this method

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Glassware – Class A volumetric flasks and pipets (or pipetters) as required

6.5 Graduated cylinders

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1.1 Buffer Chelating Agent – In a 1 L volumetric flask, dissolve 33.0 g potassium sodium tartrate tetrahydrate [$C_4H_4KNaO_6 \cdot 4H_2O$] and 24.0 g sodium citrate dehydrate [$HOC(COONa)(CH_2COONa)_2 \cdot H_2O$] in approximately 700 mL Reagent H_2O. Stir to mix and dilute to mark with Reagent H_2O. The initial pH after preparation should be around 8.2. Adjust the pH to 5.0 ± 0.1 with concentrated sulfuric acid [H_2SO_4] dropwise.
Expiration is one month.

7.1.2 Phenate Reagent – *CAUTION: Wear gloves. Phenol causes skin burns and is rapidly absorbed into the body through the skin. Prepare in fume hood.* Measure 41.5 g crystalline phenol [C_6H_5OH] in a tared glass beaker and dissolve in approximately 250 mL Reagent H_2O in a 500 mL volumetric flask. Add 16 g sodium hydroxide [NaOH]. Cool, dilute to mark and mix by inversion. *The color of this reagent darkens with age, increasing the baseline absorbance.*
Expiration is three days. Also, discard when reagent turns dark brown.

7.1.3 Sodium Hypochlorite – Dilute 50 mL sodium hypochlorite [5.65-6% NaOCl] to 250 mL with Reagent H_2O in a reagent container. Invert or shake to mix.
Expiration is one day.

7.1.4 Sodium Nitroprusside – In a 500 mL volumetric flask, dissolve 1.75 g sodium nitroprusside dihydrate [$Na_2Fe(CN)_5(NO) \cdot 2H_2O$] in approximately 400 mL Reagent H_2O. Dilute to mark and invert to mix.
Store in a refrigerator.
Expiration is two weeks.

7.2 PREPARATION OF STANDARDS

Rinse all volumetric flasks three times with Reagent H_2O prior to use.

7.2.1 Ammonia Stock Standard 100 mg NH_3-N/L – In a 1 L volumetric flask, dissolve 0.4717 g ammonium sulfate [$(NH_4)_2SO_4$] (dried overnight at 104 ± 2°C and stored in a desiccator) in approximately 800 mL Reagent H_2O. Add 1.0 mL concentrated sulfuric acid [H_2SO_4, 97%] with a macropipettor. Dilute to the mark and mix by inversion. Store in a refrigerator.
Expiration is six months.

7.2.2 Working Standard 10.0 mg NH_3-N/L – In a 100 mL volumetric flask, add 10.0 mL of Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Nitrate (10.0 mg NO_3-N/L) and/or Phosphate (2.0 mg PO_4-P/L)], add 10.0 mL of Nitrate Stock Standard and/or 0.20 mL of Phosphate Stock Standard to flask before diluting to mark. See specific SOPs for details**.** Prepare fresh on day of use.

7.2.3 Working Standard 0.5 mg NH_3-N/L (with Nitrate and/or Phosphate)

7.2.3.1 Alone or with Nitrate (0.5 mg NO_3-N/L) – In a 100 mL volumetric flask, add 0.50 mL of Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard with Nitrate (0.5 mg NO_3-N/L), add 0.50 mL of Nitrate Stock Standard to flask before diluting to mark. See specific SOP for details**.** Prepare fresh on day of use.

7.2.3.2 With Phosphate (0.1 mg PO_4-P/L) and/or Nitrate (0.5 mg NO_3-N/L) – In a 100 mL volumetric flask, add 5.0 mL of Working Standard 10.0 mg NH_3-N/L (7.2.2). Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh on day of use.

7.2.4 Calibration curve – If making the calibration standards manually, dilute the specific volume of Working Standard 7.2.2 in a 100 mL volumetric flask with Reagent H_2O. If using the autodilutor for the calibration standards, use Working Standard 7.2.3 and input the AutoDilutor Factor (ADF) values in the chart.

Working Standards (Prepared Daily)	Concentration mg/L	Manual	Autodilutor
		Standard 7.2.2 (10.0 mg NH_3-N/L) mL	ADF Value (Standard 7.2.3)
S1	0.5000	5.0	--
S2	0.2000	2.0	2.50
S3	0.1000	1.00	5.00
S4	0.0500	0.50	10.00
S5	0.0100	0.10	50.00
S6	0.0050	1.00 (S1)*	100.00
S8†	0.0000	--	--

* Use S1 instead of Standard 7.2.2.

† S7 is for NO_{23} only.

7.2.5 Matrix Spikes – *NOTE: The spike concentration may be increased due to sample concentrations.*

7.2.5.1 Alone or with Nitrate – Add 2.0 mL of Working Standard 7.2.3 (0.5 mg NH_3-N/L) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.04 mg NH_3-N/L (0.04 mg NO_3-N/L)**.

7.2.5.2 With Phosphate and/or Nitrate – Add 0.25 mL of Working Standard 7.2.2 (10.0 mg NH_3-N/L) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.1 mg NH_3-N/L** (**0.02 mg PO_4-P/L** and or **0.1 mg NO_3-N/L**).

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 12 for acceptance criteria.

9.2 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 12 for acceptance criteria.

9.3 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.4 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS) containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.5 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.6 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. CALIBRATION AND STANDARIZATION

10.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in Section 7.2.4). The minimum number of standards is four and a zero standard.

10.2 Calibrate the instrument as described in Section 11.

10.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 12.1 for acceptance criteria.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 12.2 for acceptance criteria.

11. PROCEDURE

11.1 PRE-ANALYTICAL PROCEDURE

11.1.1 Gather all Ammonia reagents (and any reagents needed if running a combined analysis). Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

11.1.2 Fill a vial with a sufficient volume of the Ammonia Stock Standard (repeat for any standards of a combined analysis). Use this portion to make the calibration curve.

11.1.3 Remove a pre-dispensed CRM vial from the freezer and thaw. Record date of use in the Working Standards/Reagent Logbook. Dilute if necessary.

11.1.4 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

11.1.5 Remove samples from storage and log action in chain-of-custody.

11.1.6 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Thaw the samples.

11.1.7 Rinse sample tubes three times with 10% HCl and six times with Reagent H_2O. If Phosphate is also analyzed, glass tubes must be used.

11.2 CALIBRATION PROCEDURE

11.2.1 Prepare working standards and matrix spikes as described in Section 7.2. The calibration curve listed is for samples routinely analyzed in the laboratory. Different standards may be utilized for varying sample concentrations. The minimum number of non-zero standards is four, each sampled once. The zero standard is sampled in duplicate.

11.2.2 Set up manifold as shown in Section 17.2.

11.2.3 Verify/input data system parameters as shown in Section 17.1.

11.2.4 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

11.2.5 Transfer the ammonia manifold waste line into a hazardous container (labeled according to the procedure provided by ODU EHSO) and cover the opening with laboratory film.

11.2.6 To reduce contamination by ambient ammonia, cover all of the sample and standard tubes with foil (Do not put laboratory film over the top of the tube, as the auto sampler probe will get clogged!). Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

11.2.7 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.3 SYSTEM NOTES

11.3.1 For information on system maintenance and troubleshooting, refer to the Maintenance and Troubleshooting Guide in the System Operation Manual.

11.3.2 Allow the heating module to warm up to 60°C.

11.3.3 A backpressure coil [200 cm x 0.5 mm (0.022") i.d. Teflon tubing] is used to prevent air bubble formation.

11.3.4 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.3.5 If the detection limit is greater than that specified in the method, the following outline should be followed.

- 11.3.5.1 Verify standards preparation procedures.
- 11.3.5.2 Verify that a 630 nm filter is being used.
- 11.3.5.3 Verify that the sample loop is completely filled by running dye.
- 11.3.5.4 Verify that the reagents are being added in the correct order.
- 11.3.5.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.
- 11.3.5.6 Prepare fresh reagents.

11.3.6 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure.

- 11.3.6.1 Place all reagent lines in Reagent H_2O and pump to clear reagents (2 to 5 minutes).
- 11.3.6.2 Place all reagent lines in 1 M HCl (1 volume concentrated HCl added to 11 volumes Reagent H_2O) and pump for several minutes.
- 11.3.6.3 Place all reagent lines in Reagent H_2O and pump until HCl is thoroughly washed out.
- 11.3.6.4 Resume pumping reagents.

11.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

11.3.8 Use consumer bleaches with caution. Proprietary additives may contribute to staining of tubing and data quality.

11.3.9 Add reagents in the order that they appear on the manifold to reduce staining.

11.3.10 If a large number of bubbles are observed in the autodilutor water container, degas with helium for at least 15 minutes.

12. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 12.1 through 12.6, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If any of data in Sections 12.3 through 12.6 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

12.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.1.1 Acceptability of calibration correlation coefficient (r)

A.	r ≥ 0.9990	Acceptable
B.	r = 0.9980 – 0.9989	Acceptable but troubleshooting is required.
C.	r < 0.9980	Stop the analysis. Troubleshooting is required.

12.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

12.3 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

12.4 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation.
NOTE: The RPD does not need to be calculated if this condition is satisfied.

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

12.5 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA \times DF} \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA \times DF} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

12.6 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

12.7 Report results in mg NH_3-N/L.

13. METHOD PERFORMANCE

13.1 A Quality Control Sample prepared in Reagent H_2O was analyzed on 13 separate analytical runs yielding the following results:

RT-Corp.	*n*	Mean *mg NH_3-N/L*	Recovery of *NH_3-N %*	Standard Deviation *mg NH_3-N/L*	Relative Standard Deviation *%*
QC1195-20ML Lot 017175	5	0.0504	101	0.0022	4.4
QC1195-20ML Lot 018782	8	0.0714	99	0.0035	4.9

13.2 A standard prepared by the autodilutor at the reporting limit (0.0050 mg NH_3-N/L) was analyzed 10 times on three separate analytical runs. These gave a mean of 0.0057 mg NH_3-N/L, a standard deviation of 0.0008 mg NH_3-N/L, and a relative standard deviation of 15%.

13.3 A carry-over study was performed. A high standard (0.5 mg NH_3-N/L) was sampled twice followed by three samples of blanks. The average of the blanks was -0.0006 mg NH_3-N/L with a standard deviation of 0.0005 mg NH_3-N/L.

14. POLLUTION PREVENTION

14.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

14.2 The phenol used in this analysis must be disposed of properly. The waste line from the ammonia manifold is separated from the other cartridge waste lines that do not contain hazardous reagents. The ammonia waste is placed in an appropriate waste container provided by Old Dominion University's Environmental Health and Safety Office (EHSO), and EHSO collects the waste container and disposes of it.

15. WASTE MANAGEMENT

15.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office (EHSO).

16. REFERENCES

16.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 350.1, Rev. 2.0.

16.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

16.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100 and 4500-N.

16.4 Lachat Instruments Method 31-107-106-1-I

16.5 U.S. Environmental Protection Agency, *Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater*, EPA-822-R-13-001, April 2013.

17. TABLE, DIAGRAMS AND FLOWCHARTS

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

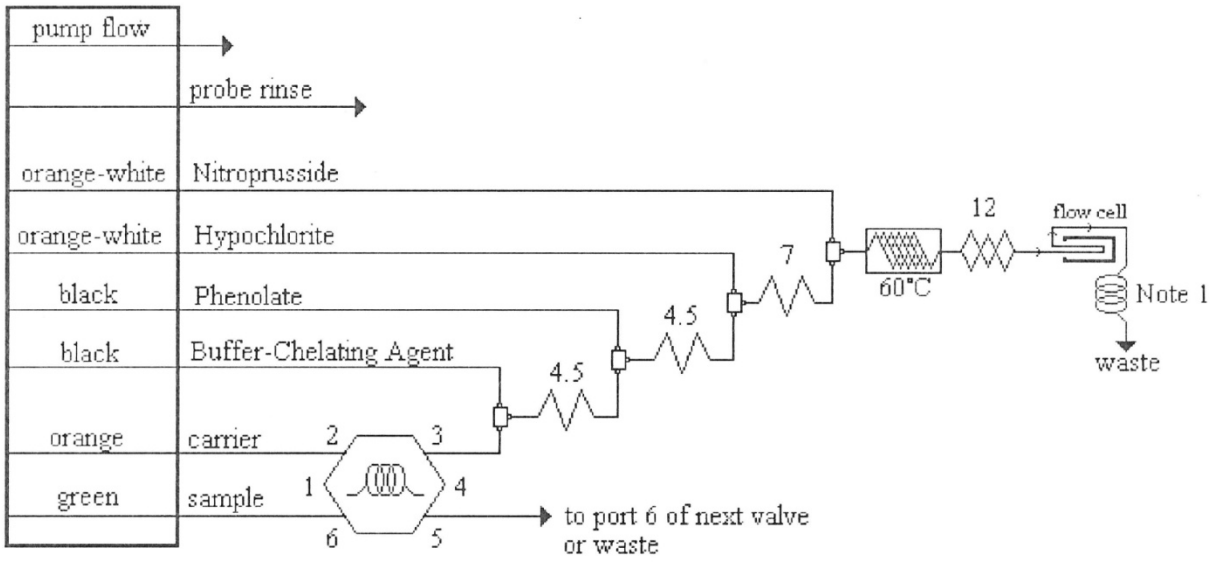
The timing values listed below were optimized using the universal dye. The brackish timing values were determined using artificial sea water as is and spiked at two low levels. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Time Tab under Run Properties		NH_3	NH_3/NO_3	$OPO_4/NH_3/NO_3$
Run	Method Cycle Period (seconds)	70	70	135
	Sample Period (seconds)	35	40	65
	Min. Probe in Wash Period (seconds)	20	20	59
Channel 3	Load Period (seconds)	25	25	30
	Inject Period (seconds)	40	40	50
	Time to Valve (seconds)	26	26	41
NH4F (Analyte)	Expected Inject to Peak Start (seconds)	58	58	45
	Expected Peak Base Width (seconds)	76.5	76.5	86
	Brackish Shutter Offset (seconds)	22	22	33.5
	Brackish Shutter Width (seconds)	21.5	21.5	21.5

Analyte Tab under Run Properties

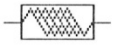
Concentration Units: mg NH_3-N/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: 1/X
QuikChem Method: 31-107-106-1-I
Chemistry: Brackish

17.2 AMMONIA MANIFOLD DIAGRAM



Carrier: Reagent H_2O
Manifold tubing: 0.5 mm (0.022") i.d.
Sample Loop: 100 cm x 1.1 mm (0.042") i.d.
Interference Filter: 630 nm

4.5: 70 cm of tubing on a 4.5 cm support coil.
7: 135 cm of tubing on a 7 cm coil support.
12: 255 cm of tubing on a 12 cm alternating coil support.

The  shows 650 cm of tubing wrapped around the heater block at the specified temperature. The flow cell is a 10 mm path length.

Note 1: 200 cm back pressure loop of 0.5 mm (0.022") i.d.
Note 2: The sample loop should be cut at a 30 - 45° angle for the best fit.
Note 3: PVC PUMP TUBES MUST BE USED FOR THIS METHOD.

Appendix 15:

SOP Particulate Phosphorus

STANDARD OPERATING PROCEDURE
for Particulate Phosphorus in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

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APPROVED BY

John R. Donat, Ph.D. Date
Laboratory Director

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of particulate phosphorus in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.200 – 3.00 mg P/L as PO_4. Higher concentrations can be determined by sample dilution. The method throughput is 45 injections per hour. *NOTE: It was proven during initial instrument setup that the method is linear up to at least 5.00 mg P/L.*

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

The samples are filtered through glass fiber filters, which are dried and muffled at 550°C for at least 1.5 hours. Then the residue is extracted in 1N HCl for 24 hours before analysis. The extracted orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate in an acid medium to form a complex. This complex is reduced with ascorbic acid to form an intensely blue-colored complex, which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Phosphorus is found in natural waters almost exclusively in the form of orthophosphate, condensed phosphates and organically bound phosphates. These can be found in soluble form or in a particulate form, composed of particles or detritus or in bodies of organic organisms (APHA, 2012). This method is used to analyze the amount of phosphorus in the particulate form found in the water.

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

3.1.1 Freezer/Frozen: ≤ -18°C

3.1.2 Refrigerator/Refrigerated: 0 – 6°C

3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm

3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 FSS: Fixed Suspended Solids

3.2.5 g: Gram(s)

3.2.6 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.7 L: Liter(s)

3.2.8 MDL: Method Detection Limit

3.2.9 mg: Milligram(s)

3.2.10 mg/L: Milligram(s) per Liter

3.2.11 mL: Milliliter(s)

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 OPO_4/PO4F: Orthophosphate

3.2.14 PO_4: Phosphate

3.2.15 PP: Particulate Phosphate

3.2.16 ppm: Parts Per Million

3.2.17 PQL: Practical Quantitation Limit

3.2.18 QCS: Quality Control Sample

3.2.19 Si/SIF: Silicate

3.2.20 SOP: Standard Operating Procedure

3.2.21 SRM: Standard Reference Material

3.2.22 TSS: Total Suspended Solids

3.2.23 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Arsenate can cause a positive interference in this analysis if present. This has not been a problem in seawater samples.

4.2 A high iron concentration can cause precipitation of and subsequent loss of phosphate from the dissolved phase. This is generally not encountered in seawater samples.

4.3 Silica forms a pale blue complex, which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

- 5.4.1 Ammonium Molybdate
- 5.4.2 Hydrochloric Acid
- 5.4.3 Sodium Hydroxide
- 5.4.4 Sulfuric Acid

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).

- 6.1.1 Sampler (Model ASX-520)
- 6.1.2 Auto dilutor (Model PDS-200)
- 6.1.3 Multichannel proportioning pump (Model RP-150)
- 6.1.4 Reaction unit or manifold (Model 31-115-01-4-B)
- 6.1.5 Heating unit (Part A85132)
- 6.1.6 Colorimetric detector (Part 85080)
- 6.1.7 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D, or equivalent)

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S, or equivalent)

6.4 Glassware – Class A volumetric flasks and pipettes (or pipettors) as required

6.5 Graduated cylinders

6.6 Screw top conical centrifuge tubes

6.7 All sample tubes and standard vials should be glass.

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Stock Ammonium Molybdate Solution – *CAUTION: Do not use any metal in preparation.* In a 1 L volumetric flask, dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a plastic container in a refrigerator.
Expiration is two months.

7.1.2 Stock Antimony Potassium Tartrate Solution – In a 1 L volumetric flask, dissolve 3.22 g antimony potassium tartrate [potassium antimonyl tartrate trihydrate ($C_8H_4O_{12}K_2Sb_2 \cdot 3H_2O$)] in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a dark bottle in a refrigerator.
Expiration is two months.

7.1.3 Molybdate Color Reagent – *CAUTION: Prepare in fume hood and solution will get hot!* In a 1 L volumetric flask, add 20 mL concentrated sulfuric acid [H_2SO_4, 97%] to approximately 500 mL Reagent H_2O. Swirl to mix. Add 213 mL Stock Ammonium Molybdate Solution (7.1.1) and 72 mL Stock Antimony Potassium Tartrate Solution (7.1.2). Dilute to the mark and mix by inversion.
Store at ambient temperature.
Expiration is one week or when the solution turns blue or precipitate is observed.

7.1.4 Ascorbic Acid Reducing Solution – In a 1 L volumetric flask, dissolve 60.0 g ascorbic acid [$C_6H_8O_6$] in approximately 700 mL Reagent H_2O. Add 1.0 g sodium dodecyl sulfate [SDS – $CH_3(CH_2)_{11}OSO_3Na$]. Dilute to the mark and mix by inversion. *NOTE: If reagent needs degassing, do so prior to the addition of SDS.*
Expiration is one week or if a yellow precipitate forms.

7.1.5 1N Hydrochloric Acid – *CAUTION: Prepare in fume hood.* In a 2 L volumetric flask, carefully add 166 mL concentrated hydrochloric acid [HCl] to approximately 1600 mL Reagent H_2O. Swirl to mix, dilute to mark and mix by inversion.
Store in a glass container at ambient temperature.

7.1.6 Sodium Hydroxide-EDTA Rinse – Dissolve 65 g sodium hydroxide [NaOH] and 6 g tetrasodium ethylenediamine tetraacetic acid [Na_4EDTA] in 1.0 L Reagent H_2O. Use daily at the end of a run (~10 minutes, followed by Reagent H_2O rinse) or if the baseline begins to drift upwards.
Expiration is one month.

7.2 PREPARATION OF STANDARDS

Rinse all volumetric flasks with Reagent H_2O prior to use. Rinse working standard volumetric flasks with 1N HCl prior to use.

7.2.1 Phosphate Stock Standard (1000 mg PO_4-P/L) – In a 1 L volumetric flask, dissolve 4.393 g of potassium phosphate monobasic [KH_2PO_4] (dried overnight at 104 ± 2°C and stored in a desiccator) in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is six months.

7.2.2 Working Standard 100 mg PO_4-P/L – In a 100 mL volumetric flask, add 10.0 mL of Stock Standard (7.2.1). Dilute to mark with 1N HCl and invert to mix. Prepare fresh on day of use.

7.2.3 Calibration curve – Dilute the specific volume of Working Standard listed in the table in a 100 mL volumetric flask with 1N HCl.

Working Standards (Prepared Daily)	Concentration mg/L	Standard 7.2.2 (100 mg PO_4-P/L) mL
S1	3.000	3.0
S2	2.000	2.0
S3	1.000	1.00
S4	0.500	0.50
S5	0.200	0.20
S6	0.000	--

7.2.4 Matrix Spikes – Using a macropipettor, pipette 3.0 mL of Standard S2 (2.00 mg PO_4-P/L) and 3.0 mL of sample into a sample tube. Mix using the pipettor or a transfer pipet. The final concentration of the matrix spike is **1.00 mg PO_4-P/L**. *NOTE: The spike concentration may be adjusted due to sample concentrations or sample volumes.*

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 FILTRATION PREPARATION

NOTE: If the filters are also used for TSS/FSS, follow the pre-analytical procedure in "Standard Operating Procedure for Total Suspended Solids Dried at 103–105°C and Fixed Suspended Solids Ignited at 550°C."

8.1.1 Using forceps to only grip the edge of the filter, transfer a 4.7 cm glass fiber filter (Whatman™ GF/F, or equivalent) with the wrinkled side up onto the base of a filtration tower. Replace the top of the filtration tower onto the base.

8.1.2 Apply ≤ 20 in. Hg vacuum pressure and rinse the filter with 3 successive portions of approximately 20 mL Reagent H_2O. Continue suction to remove all traces of water.

8.1.3 Remove filter from filtration apparatus and transfer to an aluminum weigh dish.

8.1.4 Repeat steps 8.1.1 through 8.1.3 for each filter.

8.1.5 Dry the filters in an oven set at 103-105°C for at least one hour.

8.2 SAMPLE COLLECTION

Follow Section 15 of the "Standard Operating Procedure for Total Suspended Solids Dried at 103–105°C and Fixed Suspended Solids Ignited at 550°C." *NOTE: Different projects may require different filters/procedures.*

8.3 PRESERVATION AND STORAGE

8.3.1 *NOTE: If using filters previously processed for TSS/FSS, skip to step 8.3.7.* Use the filtration sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

8.3.2 Remove the samples from storage and log action in chain-of-custody.

8.3.3 Inspect samples to ensure that they have been properly stored and are not torn or otherwise compromised.

8.3.4 Place filters in aluminum weigh dishes labeled with the sample ID, log number, and cruise ID and/or project ID.

8.3.5 Place the samples in a 104 ± 1°C oven and dry for at least one hour.

8.3.6 Muffle samples at 550 ± 50°C for at least 1.5 hours.

8.3.7 Transfer labels from the filter holders to screw top centrifuge tubes and place the muffled filters in the appropriately labeled tube. Samples can be stored covered prior to extraction.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 12 for acceptance criteria.

9.2 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 12 for acceptance criteria.

9.3 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.4 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS) containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.5 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.6 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. CALIBRATION AND STANDARIZATION

10.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.3). The minimum number of standards is four and a zero standard.

10.2 Calibrate the instrument as described in Section 11.

10.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 12.1 for acceptance criteria.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 12.2 for acceptance criteria.

11. PROCEDURE

11.1 PRE-ANALYTICAL PROCEDURE

11.1.1 Sample Extraction – Day before analysis

11.1.1.1 Using a 10 mL fixed volume bottle-top dispenser, add 10.0 mL of 1N HCl to centrifuge tube, ensuring the filter is completely submerged.

11.1.1.2 Allow sample to extract for 24 hours at ambient temperature, swirling two to three times. *NOTE: If unable to analyze the following day, remove the filter from the centrifuge tube and place the tubes in a refrigerator for up to 24 hours.*

11.1.2 Gather all Phosphate reagents. Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

11.1.3 Fill a vial with a sufficient volume of the Phosphate Stock Standard. Use this portion to make the calibration curve.

11.1.4 Remove a pre-dispensed CRM vial from the freezer and thaw. Record date of use in the Working Standards/Reagent Logbook. Dilute if necessary.

11.1.5 Rinse glass sample tubes two times with 10% HCl and six times with Reagent H_2O.

11.2 CALIBRATION PROCEDURE

11.2.1 Prepare working standards and matrix spikes as described in Section 7.2. The calibration curve listed is for samples routinely analyzed in the laboratory. Different standards may be utilized for varying sample concentrations. The minimum number of non-zero standards is four, each sampled once. The zero standard is sampled in duplicate.

11.2.2 Set up manifold as shown in Section 17.2.

11.2.3 Verify/input data system parameters as shown in Section 17.1.

11.2.4 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

11.2.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

11.2.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.3 SYSTEM NOTES

11.3.1 For information on system startup/shutdown, maintenance and troubleshooting, refer to the Lachat Manual, Troubleshooting and Info Binder.

11.3.2 Allow the heating module to warm up to 37°C.

11.3.3 A backpressure coil [200 cm x 0.5 mm (0.022”) i.d. Teflon tubing] is used to prevent air bubble formation.

11.3.4 Over time a blue film may accumulate on the walls of the flowcell and in the manifold tubing. This may be removed by pumping the manifold rinse solution (Reagent 7.1.6).

11.3.5 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.3.6 If the detection limit is greater than that specified in the method, the following outline should be followed.

11.3.6.1 Verify standards preparation procedures.

11.3.6.2 Verify that an 880 nm filter is being used.

11.3.6.3 Verify that the sample loop is completely filled by running dye.

11.3.6.4 Verify that the reagents are being added in the correct order.

11.3.6.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.

11.3.6.6 Prepare fresh reagents. Ensure the Stock Ammonium Molybdate Solution (7.1.1) is completely dissolved.

11.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

11.3.8 Add reagents in the order that they appear on the manifold to reduce staining.

12. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 12.1 through 12.6, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 12.3 through 12.6 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

12.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.1.1 Acceptability of calibration correlation coefficient (r)

A.	$r \geq 0.9990$	Acceptable
B.	$r = 0.9980 – 0.9989$	Acceptable but troubleshooting is required.
C.	$r < 0.9980$	Stop the analysis. Troubleshooting is required.

12.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

12.3 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

12.4 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

12.5 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = (SSR - 0.5SR) \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{(SR+2)0.5} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike.

12.6 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

12.7 For the final results use the following calculation:

$$result = \frac{(SR)(V_1)}{V_2}$$

Where, SR = measured concentration of sample, V_1 = volume of 1N HCl extract (L), V_2 = sample volume filtered through filter pad (L).
Report results in mg PO_4-P/L.

13. METHOD PERFORMANCE

13.1 A Quality Control Sample prepared in 1 N HCl (1.27 mg PO_4-P/L) was analyzed on 12 separate analytical runs yielding the following results:

RT-Corp.	Mean *mg PO_4-P/L*	Recovery of *PO_4-P %*	Standard Deviation *mg PO_4-P/L*	Relative Standard Deviation *%*
QC1195-20ML Lot 018782	1.2976	102	0.0389	3.0

13.2 A standard manually prepared at the reporting limit (0.20 mg PO_4-P/L) was analyzed eight times on two separate analytical runs. These gave a mean of 0.1928 mg PO_4-P/L, a standard deviation of 0.0018 mg PO_4-P/L, and a relative standard deviation of 1.0%.

13.3 A carry-over study was performed. A high standard (5.0 mg PO_4-P/L) was sampled twice followed by three samples of blanks. The average of the blanks was 0.0071 mg PO_4-P/L with a standard deviation of 0.0036 mg PO_4-P/L.

14. POLLUTION PREVENTION

14.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

15. WASTE MANAGEMENT

15.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

16. REFERENCES

16.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 365.1, Rev. 2.0.

16.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

16.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100 and 4500-P.

16.4 Lachat Instruments Method 31-115-01-4-B

17. TABLE, DIAGRAMS AND FLOWCHARTS

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

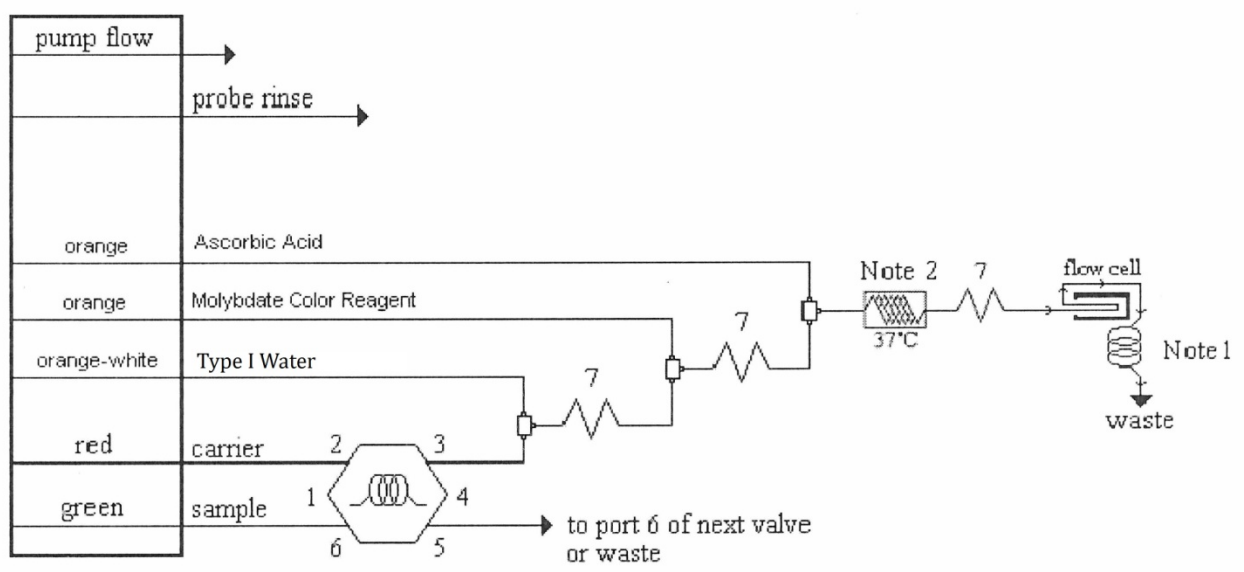
The timing values listed below were optimized using the universal dye. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Time Tab under Run Properties		PP
Run	Method Cycle Period (seconds)	80
	Sample Period (seconds)	15
	Min. Probe in Wash Period (seconds)	10
Channel 2	Load Period (seconds)	11
	Inject Period (seconds)	65
	Time to Valve (seconds)	23
PP (Analyte)	Expected Inject to Peak Start (seconds)	16.5
	Expected Peak Base Width (seconds)	76

Analyte Tab under Run Properties

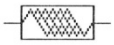
Concentration Units: mg PO_4-P/L
Calibration Fit Type: 1^{st} Order Polynomial
Force through Zero: No
Calibration Weighting: 1/x
QuikChem Method: 31-115-01-4-B
Chemistry: Direct/Bipolar

17.2 PARTICULATE PHOSPHATE MANIFOLD DIAGRAM



Carrier:	1 N HCl
Manifold tubing:	0.5 mm (0.022”) i.d.
Sample Loop:	50 cm x 0.8 mm (0.032”) i.d.
Interference Filter:	880 nm

7: 135 cm of tubing on a 7 cm coil support wrapped with 0.5 mm i.d. tubing.

The  shows 175 cm of tubing wrapped around the heater block at the specified temperature. The flow cell is a 10 mm path length.

Note 1: 200 cm back pressure loop of 0.5 mm (0.022”) i.d.
Note 2: 175 cm x 0.8 mm (0.032”) i.d. of tubing on the heater

Appendix 16:

SOP Total Dissolved Phosphorus

STANDARD OPERATING PROCEDURE
for Total Dissolved Phosphorus in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

Lori Ann Tew

Water Quality Laboratory
College of Sciences
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APPROVED BY

John R. Donat, Ph.D. Date
Laboratory Director

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of total dissolved phosphorus (TDP) in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.0050 – 0.2000 mg P/L. Higher concentrations can be determined by sample dilution. The method throughput is 38 injections per hour.

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

This method allows for the combined analysis of total dissolved nitrogen and total dissolved phosphorus from a single digestion. Nitrogen compounds require an alkaline medium for oxidation, whereas phosphorus compounds require an acidic environment for oxidation. The pH of the sample is alkaline in the beginning of the digestion. As the sodium hydroxide is consumed by the thermal decomposition of persulfate, the sample pH becomes acidic. This change in pH of the reaction allows for the oxidation of both nitrogen and phosphorus compounds (APHA, 2012).

Filtered water samples are digested with potassium persulfate in acidic conditions, converting phosphorus containing compounds to the orthophosphate form, and the pH is adjusted with the addition of a boric acid buffer solution. The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate in an acid medium to form a complex. This complex is reduced with ascorbic acid to form an intensely blue-colored complex, which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Phosphorus is found in natural waters almost exclusively in the form of orthophosphate, condensed phosphates and organically bound phosphates. These can be found in soluble form or in a particulate form, composed of particles or detritus or in bodies of organic organisms (APHA, 2012). This method measures the aqueous form of phosphorus.

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

3.1.1 Freezer/Frozen: ≤ -18°C

3.1.2 Refrigerator/Refrigerated: 0 – 6°C

3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm

3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 g: Gram(s)

3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.6 L: Liter(s)

3.2.7 MDL: Method Detection Limit

3.2.8 mg: Milligram(s)

3.2.9 mg/L: Milligram(s) per Liter

3.2.10 mL: Milliliter(s)

3.2.11 NH_3/NH_4/NH4F: Ammonia

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 NO_3: Nitrate

3.2.14 NO_{23}/NO23F: Nitrate + Nitrite

3.2.15 NO_2/NO2F: Nitrite

3.2.16 OPO_4/PO4F: Orthophosphate

3.2.17 PO_4: Phosphate

3.2.18 ppm: Parts Per Million

3.2.19 PQL: Practical Quantitation Limit

3.2.20 QCS: Quality Control Sample

3.2.21 Si/SIF: Silicate

3.2.22 SOP: Standard Operating Procedure

3.2.23 SRM: Standard Reference Material

3.2.24 TDN: Total Dissolved Nitrogen

3.2.25 TDP: Total Dissolved Phosphorus

3.2.26 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Sample turbidity may interfere but is removed by manual filtration.

4.2 A high iron concentration can cause precipitation of and subsequent loss of phosphate from the dissolved phase. This is generally not encountered in seawater samples.

4.3 Using ascorbic acid as the reductant, the color intensity is not influenced by variations in salinity.

4.4 Silica forms a pale blue complex, which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

4.5 Hydrogen sulfide effects can be treated by simple dilution since high sulfide concentrations are most often associated with high phosphate values.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

- 5.4.1 Ammonium Molybdate
- 5.4.2 Potassium Persulfate/Potassium Peroxodisulfate
- 5.4.3 Sodium Hydroxide
- 5.4.4 Sulfuric Acid

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).

- 6.1.1 Sampler (Model ASX-520)
- 6.1.2 Auto dilutor (Model PDS-200)
- 6.1.3 Multichannel proportioning pump (Model RP-150)
- 6.1.4 Reaction unit or manifold (Model 31-115-01-4-B)
- 6.1.5 Heating unit (Part A85132)
- 6.1.6 Colorimetric detector (Part 85080)
- 6.1.7 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Glassware – Class A volumetric flasks and pipettes (or pipettors) as required

6.5 Graduated cylinders

6.6 Digestion tubes – Threaded borosilicate glass tubes and polypropylene linerless screw caps, or equivalent

6.7 Autoclave – (GETINGE M/C 3522), or equivalent

6.8 All sample tubes and standard vials should be glass.

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Stock Ammonium Molybdate Solution – *CAUTION: Do not use any metal in preparation.* In a 1 L volumetric flask, dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a plastic container in a refrigerator.
Expiration is two months.

7.1.2 Stock Antimony Potassium Tartrate Solution – In a 1 L volumetric flask, dissolve 3.22 g antimony potassium tartrate [potassium antimonyl tartrate trihydrate ($C_8H_4O_{12}K_2Sb_2 \cdot 3H_2O$)] in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a dark bottle in a refrigerator.
Expiration is two months.

7.1.3 Molybdate Color Reagent – *CAUTION: Prepare in fume hood and solution will get hot!* In a 1 L volumetric flask, add 70 mL concentrated sulfuric acid [H_2SO_4, 97%] to approximately 500 mL Reagent H_2O. Swirl to mix. Add 213 mL Stock Ammonium Molybdate Solution (Reagent 7.1.1) and 72 mL Stock Antimony Potassium Tartrate Solution (Reagent 7.1.2). Dilute to the mark and mix by inversion.
Store at ambient temperature.
Expiration is one week or when the solution turns blue or precipitate is observed.

7.1.4 Ascorbic Acid Reducing Solution – In a 1 L volumetric flask, dissolve 60.0 g ascorbic acid [$C_6H_8O_6$] in approximately 700 mL Reagent H_2O. Add 1.0 g sodium dodecyl sulfate [SDS – $CH_3(CH_2)_{11}OSO_3Na$]. Dilute to the mark and mix by inversion. *NOTE: If reagent needs degassing, do so prior to the addition of SDS.*
Expiration is one week or if a yellow precipitate forms.

7.1.5 Oxidizing Reagent – Bring a 2 L volumetric flask to volume with Reagent H_2O. Using a portion of this water, add 40.2 g potassium persulfate or potassium peroxodisulfate [$K_2S_2O_8$, with ≤ 0.001% total nitrogen (N)] and 6.0 g sodium hydroxide [NaOH, low nitrogen (≤ 0.001% N)] and stir to dissolve. Transfer to a glass reagent container fit with a 5.0 mL bottle-top dispenser.
Use within two hours.

7.1.6 1 M Sodium Hydroxide – In a 1 L volumetric flask containing approximately 800 mL Reagent H_2O, add 40 g sodium hydroxide [NaOH, low nitrogen (≤ 0.001% N)] and stir to dissolve. Dilute to the mark and mix by inversion.
Store in a plastic container.
Expiration is N/A.

7.1.7 Boric Acid Buffer – Dissolve 61.8 g boric acid [H_3BO_3] approximately 600 mL of Reagent H_2O in a 1 L volumetric flask (the solution will be cloudy). Add 202 mL 1 M Sodium Hydroxide (Reagent 7.1.6) and stir to combine. Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is two months.

7.1.8 Carrier – Measure out 500 mL of Oxidizing Reagent (Reagent 7.1.5) and pour into a 2.5 L glass bottle. Add 1000 mL Reagent H_2O. Cover opening with foil and autoclave for 30 minutes at 120°C on the liquid cycle (See Section 10.3 for procedure). Before analysis, add 100 mL of Boric Acid Buffer (Reagent 7.1.7) and mix to combine.

7.1.9 Sodium Hydroxide – EDTA Rinse – Dissolve 65 g sodium hydroxide [NaOH] and 6 g tetrasodium ethylenediamine tetraacetic acid [Na_4EDTA] in 1.0 L Reagent H_2O. Use daily at the end of a run (~10 minutes, followed by Reagent H_2O rinse) or if the baseline begins to drift upwards.
Expiration is one month.

7.2 PREPARATION OF STANDARDS

Rinse all volumetric flasks with Reagent H_2O prior to use.

7.2.1 Phosphate Stock Standard, 1000 mg PO_4-P/L – In a 1 L volumetric flask, dissolve 4.393 g of potassium phosphate monobasic [KH_2PO_4] (dried overnight at 104 ± 2°C and stored in a desiccator) in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is six months.

7.2.2 Glycerophosphoric Acid Stock Standard, 400 mg P/L – In a 500 mL volumetric flask, dissolve 1.976 g of glycerophosphoric acid pentahydrate [$C_3H_7O_6PNa_2 \cdot 5H_2O$] in approximately 400 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is six months.

7.2.3 Working Standard 2.0 mg PO_4-P/L – In a 50 mL volumetric flask, add 0.10 mL of Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Nitrate (10.0 mg NO_3-N/L)], add 5.0 mL of Nitrate Stock Standard to flask before diluting to mark. See specific SOP for details**.** Prepare fresh on day of use.

7.2.4 Calibration curve – Dilute the specific volume of Working Standard 7.2.3 in a 100 mL volumetric flask with Reagent H_2O.

Working Standards (Prepared Daily)	Concentration mg/L	Standard 7.2.3 (2.0 mg PO_4-P/L) mL
S1	0.2000	10.0
S2	0.1000	5.0
S3	0.0400	2.0
S4	0.0200	1.00
S5	0.0050	0.25
S6	0.000	---

7.2.5 Matrix Spikes – Add 1.00 mL of Working Standard 7.2.3 (2.0 mg PO_4-P/L) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.08 mg PO_4-P/L** (**0.4 mg NO_2-N/L**). *NOTE: The spike concentration may be adjusted due to sample concentrations or sample volumes.*

7.2.6 Working Glycerophosphoric Acid Standard, 8.0 mg P/L – In a 100 mL volumetric flask, add 2.0 mL of Glycerophosphoric Acid Stock Standard (7.2.2). Dilute to mark with Reagent H_2O and invert to mix.

7.2.7 Glycerophosphoric Acid Standard, 0.08 mg P/L – In a 100 mL volumetric flask, add 1.00 mL of Working Glycerophosphoric Acid Standard (7.2.6). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Glutamic Acid Standard (0.3528 mg N/L)], add 0.50 mL of Glutamic Acid Stock Standard (70.56 mg N/L) to flask before diluting to mark. See specific SOP for details. Prepare fresh day of use.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 13 for acceptance criteria.

9.2 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 13 for acceptance criteria.

9.3 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.4 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS) containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.5 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.6 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. DISPENSING AND DIGESTION PROCEDURE

10.1 PRE-DISPENSING PROCEDURE

10.1.1 Fill a vial with a sufficient volume of the Phosphate Stock Standard (repeat for any standards of a combined analysis). Use this portion to make the calibration curve. Prepare working standards and matrix spikes as described in Section 7.2. The minimum number of non-zero standards is four and two zero standards.

10.1.2 Fill a vial with sufficient volume of the Glycerophosphoric Acid Stock Standard. Use this portion to make the Glycerophosphoric Acid Standard.

10.1.3 Remove a pre-dispensed CRM vial from the freezer and thaw. Record date of use in the Working Standards/Reagent Logbook. Dilute if necessary.

10.1.4 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

10.1.5 Remove samples from storage and log action in chain-of-custody.

10.1.6 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Thaw the samples.

10.1.7 Rinse digestion caps two times with 10% HCl and six times with Reagent H_2O. If contamination is suspected or becomes a problem, rinse the digestion tubes once with 10% HCl and six times with Reagent H_2O.

10.2 DISPENSING STANDARDS AND SAMPLES

10.2.1 Label a set of tubes for the standards as follows: S1, S2, S3, S4, S5, S6, CRM P, and GP. Label enough tubes to ensure a sufficient volume for both the curve and in run check standards is available.

10.2.2 Label tubes with the log number of each sample, dup and spike to be dispensed. The last three numbers is usually sufficient, but if not, include the whole number.

10.2.3 Using a fixed volume pipette, dispense 10.0 mL of standard/sample into the appropriately labeled tube and cap. Change pipette tips between each standard/sample. The tubes may be frozen until a later date.

10.3 DIGESTION PROCEDURE

If previously frozen, remove a set of standards and samples from the freezer and thaw. The tubes must be digested within two hours of the preparation of the oxidizing reagent, and they will be autoclaved at 120±2°C for 30 minutes on the liquid cycle. Ensure there is ample room on the recording chart and the pen is in place.

10.3.1 Using a bottle-top dispenser, add 5.0 mL of Oxidizing Reagent (7.1.5) to each tube and cap quickly and tightly to prevent volatilization. Invert to mix. *NOTE: Avoid aspiration of air bubbles when using the dispenser. If air bubbles are dispensed with the oxidizing reagent, the tube should be discarded and a new one prepared.*

10.3.2 Behind the metal door at the bottom of the autoclave, in the upper right hand corner of the boiler, turn the **CONTROL SWITCH** on.

10.3.3 On the control panel, press the **CONTROLS 'ON'** button. *NOTE: A light should turn on in the button. The EXHAUST and DOOR UNSEALED boxes should be lit.*

10.3.4 When the **EXHAUST** light turns off, press the **LIQUIDS** button (it should be lit).

10.3.5 Ensure the following settings are entered. If needed, adjust with the corresponding arrows.
SELECTED EXPOSURE TIME = 00:30
SELECTED EXHAUST TIME = 00:00
SELECTED TEMP = 120°C (acceptable range is 120±2°C)

10.3.6 When the standards and samples (or carrier reagent) are in place and the **READY** indicator lights, close the door and press the **START CYCLE** button. *NOTE: The READY indicator will go out and the CONDITIONING PHASE indicator will come on. When pressure and temperature are reached, the CONDITIONING PHASE indicator will turn off and the EXPOSURE PHASE indicator will come on.*

10.3.7 When the cycle is complete, the **COMPLETE** indicator will come on, and a buzzer will sound. The door will unseal automatically. Lower the door about an inch to allow residual steam to escape for about 15 minutes.

10.3.8 Remove the standards and samples and transfer to a refrigerator. If the carrier reagent was digested, remove it from the autoclave and transfer to the Lachat counter.

10.3.9 Once digestion is complete, record the date, parameter(s), and cruise(s) within the peak. Press the **CONTROLS 'OFF'** button and turn off the boiler **CONTROL SWITCH**.

11. CALIBRATION AND STANDARIZATION

11.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.3). The minimum number of standards is four and a zero standard. The standards should be digested at the same time as the samples being analyzed.

11.2 Calibrate the instrument as described in Section 12.

11.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 13.1 for acceptance criteria.

11.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 13.2 for acceptance criteria.

12. PROCEDURE

12.1 PRE-ANALYTICAL PROCEDURE

12.1.1 Gather all TDP reagents (and any reagents needed if running a combined analysis). Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

12.1.2 Remove samples and standards from the refrigerator.

12.1.3 Inspect samples and standards to ensure that they have been properly stored and that the sample containers have not been compromised. Allow the digestion tubes to come up to ambient temperature.

12.1.4 If there is precipitate in the bottom of the digestion tubes, invert to mix. Using a bottle top dispenser, add 1.0 mL of Boric Acid Buffer (Reagent 7.1.7) to each

tube, and mix well. *NOTE: If the precipitate is not homogenized prior to the addition of the Boric Acid Buffer, it will not dissolve.*

12.1.5 Rinse glass sample tubes/vials two times with 10% HCl and six times with Reagent H_2O.

12.2 CALIBRATION PROCEDURE

12.2.1 Set up manifold as shown in Section 18.2.

12.2.2 Verify/input data system parameters as shown in Section 18.1.

12.2.3 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

12.2.4 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 18).

12.2.5 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

12.3 SYSTEM NOTES

12.3.1 For information on system maintenance and troubleshooting, refer to the Troubleshooting Guide in the System Operation Manual.

12.3.2 Allow the heating module to warm up to 37°C.

12.3.3 A backpressure coil [200 cm x 0.5 mm (0.022”) i.d. Teflon tubing] is used to prevent air bubble formation.

12.3.4 Over time a blue film may accumulate on the walls of the flowcell and in the manifold tubing. This may be removed by pumping the manifold rinse solution (Reagent 7.1.9).

12.3.5 The blank in this method should not give a peak. If the blank peak is very negative (an inverse peak), the carrier is contaminated. If the blank peak is ≥ PQL (lowest non-zero standard), the blank is contaminated.

12.3.6 If the detection limit is greater than that specified in the method, the following outline should be followed.

12.3.6.1 Verify standards preparation procedures.

12.3.6.2 Verify that an 880 nm filter is being used.

12.3.6.3 Verify that the sample loop is completely filled by running dye.

12.3.6.4 Verify that the reagents are being added in the correct order.

12.3.6.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.

12.3.6.6 Prepare fresh reagents. Ensure the Stock Ammonium Molybdate Solution (7.1.1) is completely dissolved.

12.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

12.3.8 Add reagents in the order that they appear on the manifold to reduce staining.

12.3.9 If the samples are colored, this background can be subtracted. First calibrate in the normal manner. Next, replace the molybdate reagent with a solution containing 35 mL H_2SO_4/L. Finally, reanalyze the samples. The color interference concentration is then subtracted from the original determined concentration.

13. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 13.1 through 13.6, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 13.3 through 13.6 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

13.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

13.1.1 Acceptability of calibration correlation coefficient (r)

A.	$r \geq 0.9990$	Acceptable
B.	$r = 0.9980 – 0.9989$	Acceptable but troubleshooting is required.
C.	$r < 0.9980$	Stop the analysis. Troubleshooting is required.

13.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

13.3 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

13.4 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

13.5 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA \times DF} \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA \times DF} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

13.6 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated.

The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

13.7 Report results for total dissolved phosphorus in mg P/L.

14. METHOD PERFORMANCE

14.1 A Quality Control Sample prepared in Reagent H_2O was analyzed on 19 separate analytical runs yielding the following results:

RT-Corp. QC1051-2ML Lot 019822	*n*	Mean *mg P/L*	Recovery of P *%*	Standard Deviation *mg P/L*	Relative Standard Deviation *%*
0.0748 mg P/L	15	0.0729	97	0.0028	3.8
0.1122 mg P/L	1	0.1161	103	--	--
0.1496 mg P/L	3	0.1481	99	0.0037	2.5

14.2 A standard prepared at the reporting limit (0.0050 mg P/L) was analyzed 11 times on three separate analytical runs. These gave a mean of 0.0042 mg P/L, a standard deviation of 0.0017 mg P/L, and a relative standard deviation of 39.2%.

14.3 A carry-over study was performed. A high standard (0.2000 mg P/L) was sampled twice followed by three samples of blanks. The average of the blanks was -0.0014 mg P/L with a standard deviation of 0.0002 mg P/L.

15. POLLUTION PREVENTION

15.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

16. WASTE MANAGEMENT

16.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

17. REFERENCES

17.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 365.1, Rev. 2.0.

17.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

17.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100, and 4500-P.

17.4 Lachat Instruments Method 31-115-01-4-B

18. TABLE, DIAGRAMS AND FLOWCHARTS

18.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

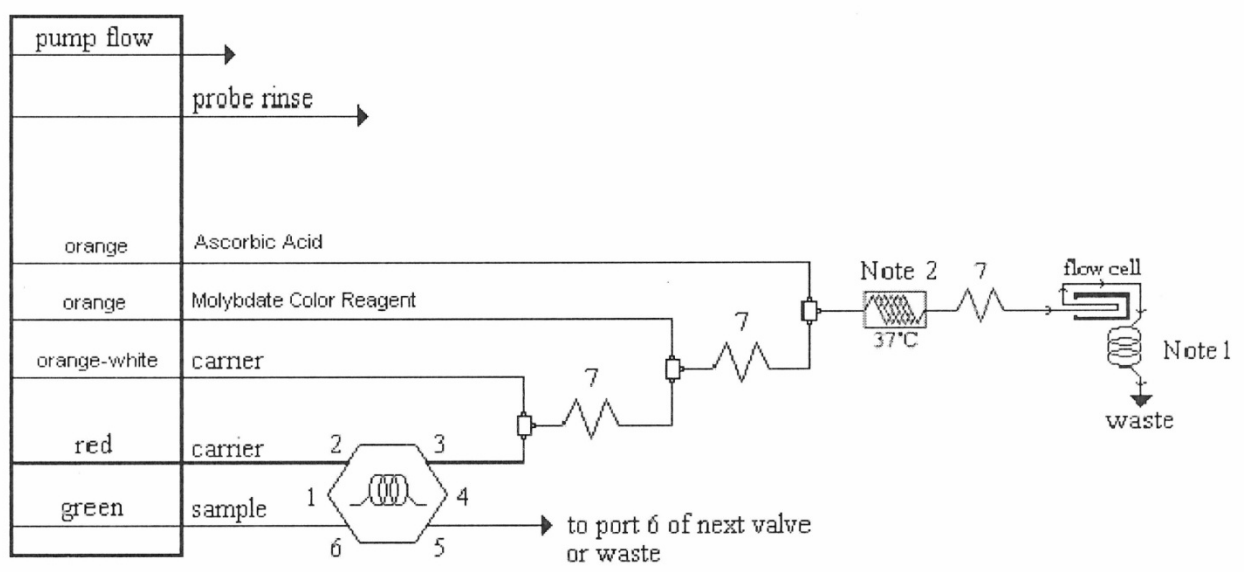
The timing values listed below were optimized using the universal dye. The brackish timing values were determined using artificial sea water as is and spiked at two low levels. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Time Tab under Run Properties		TDP	TDP/TDN
Run	Method Cycle Period (seconds)	95	95
	Sample Period (seconds)	27	27
	Min. Probe in Wash Period (seconds)	5	5
Channel 2	Load Period (seconds)	24	24
	Inject Period (seconds)	71	71
	Time to Valve (seconds)	23	23
TDP (Analyte)	Expected Inject to Peak Start (seconds)	14	14
	Expected Peak Base Width (seconds)	83	83
	Brackish Shutter Offset (seconds)	22	13.6
	Brackish Shutter Width (seconds)	20	17.3

Analyte Tab under Run Properties

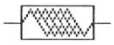
Concentration Units: mg P/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: 1/x
QuikChem Method: 31-115-01-4-B
Chemistry: Brackish

18.2 TOTAL DISSOLVED PHOSPHORUS MANIFOLD DIAGRAM



Carrier: Carrier (7.1.8)
Manifold tubing: 0.5 mm (0.022") i.d.
Sample Loop: 150 cm x 0.8 mm (0.032") i.d.
Interference Filter: 880 nm

7: 135 cm of tubing on a 7 cm coil support wrapped with 0.5 mm i.d. tubing.

The  shows 175 cm of tubing wrapped around the heater block at the specified temperature. The flow cell is a 10 mm path length.

Note 1: 200 cm back pressure loop of 0.5 mm (0.022") i.d.
Note 2: 175 cm x 0.8 mm (0.032") i.d. of tubing on the heater

Appendix 17:

SOP Dissolved Orthophosphate

STANDARD OPERATING PROCEDURE
for Orthophosphate in Seawater and Brackish water using the LACHAT QuikChem 8500 Series 2 FIA+

Effective Date: July 1, 2014

Written by

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APPROVED BY

John R. Donat, Ph.D. Date
Laboratory Director

Suzanne C. Doughten Date
Quality Manager/Technical Director

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of orthophosphate in seawater and brackish water but is also applicable to non-saline sample matrices.

1.2 The applicable range is 0.0010 – 0.1000 mg P/L as PO_4. Higher concentrations can be determined by sample dilution. The method throughput is 26 injections per hour.

1.3 This SOP applies to the analysis of fresh, estuarine and coastal waters in support of the USEPA Chesapeake Bay Water Quality Monitoring Program for samples collected in the Chesapeake Bay. This SOP may not be applicable to any other studies.

1.4 This SOP is to be used by all personnel conducting this analysis at the Water Quality Laboratory located in Norfolk, VA.

2. SUMMARY OF METHOD

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate in an acid medium to form a complex. This complex is reduced with ascorbic acid to form an intensely blue-colored complex, which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Phosphorus is found in natural waters almost exclusively in the form of orthophosphate, condensed phosphates and organically bound phosphates. These can be found in soluble form or in a particulate form, composed of particles or detritus or in bodies of organic organisms. Phosphate enters waterways by a variety of processes. Condensed phosphates are found in many cleaning products. Orthophosphates are found in many fertilizers. Organic phosphates are generally added to waterways through sewage, such as body wastes and food residues (APHA, 2012).

Phosphorus is essential to the growth of many organisms, and it can be a growth-limiting nutrient. When a system is lacking phosphate, the addition of it can stimulate production. When too much phosphate is added, blooms and other problems can arise. In this method the soluble form is measured. Dissolved orthophosphate is used to refer to the inorganic phosphorus present in water after filtration.

3. DEFINITIONS

3.1 The definitions used in this SOP can be found in the Quality Manual for Old Dominion University Water Quality Laboratory, Section 3. Some of the ones specific to this SOP

3.1.1 Freezer/Frozen: ≤ -18°C

3.1.2 Refrigerator/Refrigerated: 0 – 6°C

3.1.3 Reagent H_2O: Resistivity > 10 MΩ-cm

3.1.4 Brackish: 0.5 – 30‰ salinity

3.2 Some of the acronyms encountered in this SOP

3.2.1 CBP: Chesapeake Bay Program

3.2.2 CCV: Continuing Calibration Verification; also known as calibration check standard

3.2.3 CRM: Certified Reference Material

3.2.4 g: Gram(s)

3.2.5 ICV: Initial Calibration Verification; also known as calibration check standard

3.2.6 L: Liter(s)

3.2.7 MDL: Method Detection Limit

3.2.8 mg: Milligram(s)

3.2.9 mg/L: Milligram(s) per Liter

3.2.10 mL: Milliliter(s)

3.2.11 NH_3/NH_4/NH4F: Ammonia

3.2.12 NIST: National Institute of Standards and Technology

3.2.13 NO_3: Nitrate

3.2.14 NO_{23}/NO23F: Nitrate + Nitrite

3.2.15 NO_2/NO2F: Nitrite

3.2.16 OPO_4/PO4F: Orthophosphate

3.2.17 PO_4: Phosphate

3.2.18 ppm: Parts Per Million

3.2.19 PQL: Practical Quantitation Limit

3.2.20 QCS: Quality Control Sample

3.2.21 Si/SIF: Silicate

3.2.22 SOP: Standard Operating Procedure

3.2.23 SRM: Standard Reference Material

3.2.24 USEPA: United States Environmental Protection Agency

4. INTERFERENCES

4.1 Sample turbidity may interfere but is removed by manual filtration.

4.2 A high iron concentration can cause precipitation of and subsequent loss of phosphate from the dissolved phase. This is generally not encountered in seawater samples.

4.3 Using ascorbic acid as the reductant, the color intensity is not influenced by variations in salinity.

4.4 Silica forms a pale blue complex, which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 5 mg SiO_2/L would be required to produce a 0.14 µg P/L positive error in orthophosphate.

4.5 Hydrogen sulfide effects can be treated by simple dilution since high sulfide concentrations are most often associated with high phosphate values.

4.6 Sample color which absorbs light at 880 nm can interfere with the analysis. See Section 11.3.10 for correction procedure.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

5.2 All personnel working in the laboratory are required to wear lab coats, nitrile gloves and protective eye wear when handling strong acids and/or bases. Goggles are to be used when handling liquids; glasses are approved for handling solids only.

5.3 A reference file of material safety data sheets (MSDS) is available to all personnel involved in these analyses.

5.4 The following chemicals have the potential to be highly toxic or hazardous; for detailed explanation consult the MSDS.

- 5.4.1 Ammonium Molybdate
- 5.4.2 Sodium Hydroxide
- 5.4.3 Sulfuric Acid

6. EQUIPMENT AND SUPPLIES

6.1 Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios (LACHAT QuikChem® 8500 Series 2).

- 6.1.1 Sampler (Model ASX-520)
- 6.1.2 Auto dilutor (Model PDS-200)
- 6.1.3 Multichannel proportioning pump (Model RP-150)
- 6.1.4 Reaction unit or manifold (Model 31-115-01-1-I)
- 6.1.5 Heating unit (Part A85132)
- 6.1.6 Colorimetric detector (Part 85080)
- 6.1.7 Data system (Windows 7 PC, Omnion 3.0 software, Version 3.0.224)

6.2 Analytical balance, capable of weighing 0.1 mg – (OHAUS GA200D), or equivalent

6.3 Top-loading balance, capable of weighing 0.01 g – (SARTORIUS LC 2200 S), or equivalent

6.4 Glassware – Class A volumetric flasks and pipettes (or pipettors) as required

6.5 Graduated cylinders

6.6 All sample tubes and standard vials should be glass.

7. REAGENTS AND STANDARDS

Use Reagent water for all solutions. All chemicals must be analytical reagent grade or of a higher purity, except as noted. Preparation methods and concentrations are documented in the Working Standard/Reagent Logbook. See the current Quality Manual for Old Dominion University Water Quality Laboratory, Section 20 and Appendix L.

NOTE: Quantities of each reagent/standard prepared may be adjusted (i.e., halved or doubled) as necessary.

7.1 PREPARATION OF REAGENTS

Each reagent has a dedicated container. Prior to use, it is rinsed three times with Reagent water and three times with fresh reagent. The following information is recorded on the container: Reagent identification, pertinent analyses, date prepared, prepared by (initials), storage requirements, expiration date, and reagent number.

7.1.1 Stock Ammonium Molybdate Solution – *CAUTION: Do not use any metal in preparation.* In a 1 L volumetric flask, dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a plastic container in a refrigerator.
Expiration is two months.

7.1.2 Stock Antimony Potassium Tartrate Solution – In a 1 L volumetric flask, dissolve 3.22 g antimony potassium tartrate [potassium antimonyl tartrate trihydrate ($C_8H_4O_{12}K_2Sb_2 \cdot 3H_2O$)] in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a dark bottle in a refrigerator.
Expiration is two months.

7.1.3 Molybdate Color Reagent – *CAUTION: Prepare in fume hood and solution will get hot!* In a 1 L volumetric flask, add 70 mL concentrated sulfuric acid [H_2SO_4, 97%] to approximately 500 mL Reagent H_2O. Swirl to mix. Add 213 mL Stock Ammonium Molybdate Solution (Reagent 7.1.1) and 72 mL Stock Antimony Potassium Tartrate Solution (Reagent 7.1.2). Dilute to the mark and mix by inversion.
Store at ambient temperature.
Expiration is one week or when the solution turns blue or precipitate is observed.

7.1.4 Ascorbic Acid Reducing Solution – In a 1 L volumetric flask, dissolve 60.0 g ascorbic acid [$C_6H_8O_6$] in approximately 700 mL Reagent H_2O. Add 1.0 g sodium dodecyl sulfate [SDS – $CH_3(CH_2)_{11}OSO_3Na$]. Dilute to the mark and mix by inversion. *NOTE: If reagent needs degassing, do so prior to the addition of SDS.*
Expiration is one week or if a yellow precipitate forms.

7.1.5 Sodium Hydroxide – EDTA Rinse – Dissolve 65 g sodium hydroxide [NaOH] and 6 g tetrasodium ethylenediamine tetraacetic acid [Na_4EDTA] in 1.0 L Reagent H_2O. Use daily at the end of a run (~10 minutes, followed by Reagent H_2O rinse) or if the baseline begins to drift upwards.
Expiration is one month.

7.2 PREPARATION OF STANDARDS

Rinse all volumetric flasks with Reagent H_2O prior to use.

7.2.1 Phosphate Stock Standard, 1000 mg PO_4-P/L – In a 1 L volumetric flask, dissolve 4.393 g of potassium phosphate monobasic [KH_2PO_4] (dried overnight at 104 ± 2°C and stored in a desiccator) in approximately 800 mL Reagent H_2O. Dilute to the mark and mix by inversion.
Store in a refrigerator.
Expiration is six months.

7.2.2 Working Standard 10.0 mg PO_4-P/L (with Nitrite) – In a 100 mL volumetric flask, add 1.00 mL of Phosphate Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard with Nitrite (5.0 mg NO_2-N/L), add 2.0 mL of Nitrite Stock Standard to flask before diluting to mark. See specific SOP for details. Prepare fresh on day of use.

7.2.3 Working Standard 1.0 mg PO_4-P/L (with Nitrite) – In a 100 mL volumetric flask, add 10.0 mL of Working Standard 10.0 mg PO_4-P/L (7.2.2). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard with Nitrite, the value is 0.5 mg NO_2-N/L. Prepare fresh on day of use.

7.2.4 Working Standard 2.0 mg PO_4-P/L (with Ammonia and/or Nitrate) – In a 100 mL volumetric flask, add 0.20 mL of Phosphate Stock Standard (7.2.1). Dilute to mark with Reagent H_2O and invert to mix. If making a combined standard [Ammonia (10.0 mg NH_3-N/L)and/or Nitrate (10.0 mg NO_3-N/L)], add 10.0 mL of appropriate Stock Standard before diluting to mark. See specific SOPs for details. Prepare fresh on day of use.

7.2.5 Working Standard 0.1 mg PO_4-P/L (with Nitrite or Ammonia and/or Nitrate)

7.2.5.1 Alone or with Nitrite (0.05 mg NO_2-N/L) – In a 100 mL volumetric flask, add 1.00 mL of Working Standard 10.0 mg PO_4-P/L (7.2.2). Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh on day of use.

7.2.5.2 With Ammonia (0.5 mg NH_3-N/L) and/or Nitrate (0.5 mg NO_3-N/L) - In a 100 mL volumetric flask, add 5.0 mL of Working Standard 2.0 mg PO_4-P/L (7.2.4). Dilute to mark with Reagent H_2O and invert to mix. Prepare fresh on day of use.

7.2.6 Calibration curve – If making the calibration standards manually, dilute the specific volume of Working Standard listed in the table in a 100 mL volumetric flask with Reagent H_2O. If using the autodilutor for the calibration standards, use the Working Standard listed in the table and input the AutoDilutor Factor (ADF) values in the chart.

7.2.6.1 Alone or with Nitrite

Working Standards (Prepared Daily)	Concentration mg/L	Manual	Autodilutor
		Standard 7.2.2 (10.0 mg PO_4-P/L) mL	ADF Value (Standard 7.2.5, 0.10 mg PO_4-P/L)
S1	0.1000	1.00	--
S2	0.0500	0.50	2.00
S3	0.0250	0.25	4.00
S4	0.0100	0.10	10.00
S5	0.0050	5.0 (S1)*	20.00
S6	0.0020	2.0 (S1)*	50.00
S7	0.0000	--	--

*Use S1 instead of Standard 7.2.3.

7.2.6.2 With Ammonia and/or Nitrate

Working Standards (Prepared Daily)	Concentration mg/L	Manual	Autodilutor
		Standard 7.2.4 (2.0 mg PO_4-P/L) mL	ADF Value (Standard 7.2.5, 0.10 mg PO_4-P/L)
S1	0.1000	5.0	--
S2	0.0400	2.0	2.50
S3	0.0200	1.00	5.00
S4	0.0100	0.50	10.00
S5	0.0020	0.10	50.00
S8†	0.0000	--	--

†S6 and S7 are only for Nitrate.

7.2.7 Matrix Spikes - *NOTE: The spike concentration may be increased due to sample concentrations.*

7.2.7.1 Alone or with Nitrite – Add 0.50 mL of Working Standard 1.0 mg PO_4-P/L (7.2.3) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.02 mg PO_4-P/L** (**0.01 mg NO_2-N/L**).

7.2.7.2 With Ammonia and/or Nitrate – Add 0.25 mL of Working Standard 2.0 mg PO_4-P/L (7.2.4) to a 25 mL volumetric flask rinsed with sample. Dilute to volume with sample. The final concentration of the matrix spike is **0.02 mg PO_4-P/L** (**0.1 mg NH_3-N/L** and/or **0.1 mg NO_3-N/L**).

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

Refer to the current Quality Manual for Old Dominion University Water Quality Laboratory (Section 21 and Appendix O) and the SOP for Chesapeake Bay Program Cruise Deployment for collection, preservation and storage requirements for Chesapeake Bay Long Term Monitoring samples. Samples collected by other sources may have different methods.

9. QUALITY CONTROL

Perform an ongoing assessment of calibration accuracy, analytical accuracy, and analytical precision by doing the following:

9.1 Process and analyze a Certified Reference Material (CRM) when one is commercially available or a second source check standard each time the analysis is performed to confirm the accuracy of the instrument calibration. This is also known as a Quality Control Sample (QCS). Refer to Section 12 for acceptance criteria.

9.2 Process and analyze a duplicate sample, spiked sample, method blank, and calibration check standard (CCV) before and after every 10 to 20 samples with a minimum of one each per 20 samples. See Section 12 for acceptance criteria.

9.3 The MDL is established once a year for this analysis, using a low level sample collected in the field. To determine the MDL value, take a minimum of seven replicate aliquots of a low concentration sample and process through the entire analytical method. The aliquots are analyzed over a minimum of two separate days. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.143 for seven replicates], S = standard deviation of the replicate analyses.

9.4 The Limit of Quantitation (LOQ) is verified once a year for this analysis. The lowest non-zero standard is considered the LOQ. A Quality Control Sample (QCS) containing the analyte of concern is analyzed at 1 to 2 times the LOQ, and the result must be within ±LOQ of the expected result for it to be considered valid.

9.5 Perform all preventative maintenance as scheduled in Lachat Manuals, Troubleshooting and Info Binder.

9.6 A factory-trained service engineer performs any maintenance or repairs beyond the capability of laboratory and university staff.

10. CALIBRATION AND STANDARIZATION

10.1 Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (typical range in Section 7.2.6). The minimum number of standards is four and a zero standard.

10.2 Calibrate the instrument as described in Section 11.

10.3 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. See Section 12.1 for acceptance criteria.

10.4 After the calibration has been established, it must be verified by the analysis of a suitable Quality Control Sample (QCS). This is either a CRM or a second source standard. If the measurement exceeds ± 10% of the established QCS value, analyze a new QCS. If it still exceeds acceptable criteria, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. See Section 12.2 for acceptance criteria.

11. PROCEDURE

11.1 PRE-ANALYTICAL PROCEDURE

11.1.1 Gather all Phosphate reagents (and any reagents needed if running a combined analysis). Prepare any reagent out of holding time or of insufficient volume for analysis (see Section 7.1).

11.1.2 Fill a vial with a sufficient volume of the Phosphate Stock Standard (repeat for any standards of a combined analysis). Use this portion to make the calibration curve.

11.1.3 Remove a pre-dispensed CRM vial from the freezer and thaw. Record date of use in the Working Standards/Reagent Logbook. Dilute if necessary.

11.1.4 Use the data sheets, master sample logbook, and/or chain-of-custody to determine the log numbers and/or cruise ID of the samples to be analyzed.

11.1.5 Remove samples from storage and log action in chain-of-custody.

11.1.6 Inspect samples to ensure that they have been properly stored and that the sample containers have not been compromised. Thaw the samples.

11.1.7 Rinse glass sample tubes two times with 10% HCl and six times with Reagent H_2O.

11.2 CALIBRATION PROCEDURE

11.2.1 Prepare working standards and matrix spikes as described in Section 7.2. The calibration curve listed is for samples routinely analyzed in the laboratory. Different standards may be utilized for varying sample concentrations. The minimum number of non-zero standards is four, each sampled once. The zero standard is sampled in duplicate.

11.2.2 Set up manifold as shown in Section 17.2.

11.2.3 Verify/input data system parameters as shown in Section 17.1.

11.2.4 Pump Reagent H_2O through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved. Cover all reagent containers with laboratory film (not required if using a reagent container with a sipper cap).

11.2.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

11.2.6 Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.3 SYSTEM NOTES

11.3.1 For information on system startup/shutdown, maintenance and troubleshooting, refer to the Lachat Manual, Troubleshooting and Info Binder.

11.3.2 Allow the heating module to warm up to 50°C.

11.3.3 A backpressure coil [300 cm x 0.5 mm (0.022") i.d. Teflon tubing] is used to prevent air bubble formation.

11.3.4 Over time a blue film may accumulate on the walls of the flowcell and in the manifold tubing. This may be removed by pumping the manifold rinse solution (Reagent 7.1.5).

11.3.5 The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.3.6 If the detection limit is greater than that specified in the method, the following outline should be followed.

- 11.3.6.1 Verify standards preparation procedures.
- 11.3.6.2 Verify that an 880 nm filter is being used.
- 11.3.6.3 Verify that the sample loop is completely filled by running dye.
- 11.3.6.4 Verify that the reagents are being added in the correct order.
- 11.3.6.5 Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.
- 11.3.6.6 Prepare fresh reagents. Ensure the Stock Ammonium Molybdate Solution (7.1.1) is completely dissolved.

11.3.7 To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater – as is and spiked at one or two low levels – can be used to set timing parameters.

11.3.8 Add reagents in the order that they appear on the manifold to reduce staining.

11.3.9 If a large number of bubbles are observed in the autodilutor water container, degas with helium for at least 15 minutes.

11.3.10 If the samples are colored, this background can be subtracted. First calibrate in the normal manner. Next, replace the molybdate reagent with a solution containing 35 mL H_2SO_4/L. Finally, reanalyze the samples. The color interference concentration is then subtracted from the original determined concentration.

12. DATA ANALYSIS AND CALCULATIONS

If the data meet all criteria defined in Sections 12.1 through 12.6, the analytical results are in control. If any criteria are not met, the analysis is out of control and the data must be forwarded to the laboratory supervisor as soon as possible if it has not already been reanalyzed during the analysis. If the any of data in Sections 12.3 through 12.6 are out of control, the associated samples must be reanalyzed (samples run since last in control QC sample to next in control QC sample).

12.1 Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.1.1 Acceptability of calibration correlation coefficient (r)

A.	r ≥ 0.9990	Acceptable
B.	r = 0.9980 – 0.9989	Acceptable but troubleshooting is required.
C.	r < 0.9980	Stop the analysis. Troubleshooting is required.

12.2 Assess whether the analytical result for the Quality Control Sample (QCS) confirms the calibration. If the determined concentration is not within ± 10% of the stated value, reanalyze the sample in another cup. If it is still not within ± 10%, remake the QCS and analyze. If that fails, use a new vial and remake. If the new vial fails, assume the calibration is out of control and recalibrate.

12.3 In run calibration check standards must meet one of the following criteria:

Calibration error is within PQL of the true value: $|MCS - CCS| \leq PQL$ or

Percent error is 90 – 110% of true value: $\%\ Error = \frac{MCS}{CCS} \times 100$

Where, MCS = measured concentration of the calibration check standard, CCS = true value of calibration check standard, PQL = true value of lowest non-zero standard used in curve.

12.4 If the difference between replicates is ≤ PQL (lowest non-zero standard), the replicates are in control. If they do not meet this requirement then the relative percent difference must be calculated as shown in the following equation. *NOTE: The RPD does not need to be calculated if this condition is satisfied.*

A duplicate sample must have a Relative Percent Difference (RPD) equal to or less than 20% when calculated as:

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

Where, D_1 = measured concentration of duplicate 1, D_2 = measured concentration of duplicate 2.

12.5 The measured spike recovery (MSR) for each spiked sample must be within 80 – 120% when calculated by the following two equations:

$$\%MSR = \frac{SSR-SR}{SA \times DF} \times 100 \quad \text{and} \quad \%MSR = \frac{SSR}{SR+SA \times DF} \times 100$$

Where, SSR = measured concentration of spiked sample, SR = measured concentration of unspiked sample, SA = concentration of spike, DF = dilution factor. *NOTE: When the spike has been diluted in the cup, DF = 1.*

12.6 If the method blank is ≤ PQL (lowest non-zero standard), the blank is considered in control. If it is greater than PQL, the blank is determined to be contaminated. The cause must be investigated and measures taken to minimize or eliminate the problem. All associated samples are reanalyzed.

12.7 Report results in mg PO_4-P/L.

13. METHOD PERFORMANCE

13.1 A Quality Control Sample prepared in Reagent H_2O (0.0254 mg PO_4-P/L) was analyzed on 12 separate analytical runs yielding the following results:

RT-Corp.	Mean *mg PO_4-P/L*	Recovery of PO_4-P *%*	Standard Deviation *mg PO_4-P/L*	Relative Standard Deviation *%*
QC1195-20ML Lot 018782	0.0248	98	0.0006	2.5

13.2 A standard manually prepared at the reporting limit (0.0020 mg PO_4-P/L) was analyzed 12 times on three separate analytical runs. These gave a mean of 0.0020 mg PO_4-P/L, a standard deviation of 0.0003 mg PO_4-P/L, and a relative standard deviation of 16.2%.

13.3 A carry-over study was performed. A high standard (0.1 mg PO_4-P/L) was sampled twice followed by three samples of blanks. The average of the blanks was 0.0001 mg PO_4-P/L with a standard deviation of 0.0000 mg PO_4-P/L.

14. POLLUTION PREVENTION

14.1 As stated in *Standard Methods for the Examination of Water and Wastewater* Section 1100, pollution prevention involves waste minimization. There are several ways that a laboratory can minimize waste, with the easiest and most cost effective to be purchasing the smallest volume of chemical needed so that large volumes of chemicals are not discarded due to expiration dates. Where possible, methods that use less hazardous chemicals should be selected. Waste streams of hazardous versus non-hazardous chemicals should be segregated as much as possible. In addition, laboratory personnel should try to minimize the evaporation, spilling and leaks of hazardous chemicals and/or waste. Recycling and reclamation is not recommended as a viable option by Standard Methods for water laboratories, except for organic solvents.

15. WASTE MANAGEMENT

15.1 Old Dominion University's Hazardous Waste Program is managed by Old Dominion University's Environmental Health and Safety Office.

16. REFERENCES

16.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/4-79-020, August 1993, Method 365.1, Rev. 2.0.

16.2 Quality Manual for Old Dominion University Water Quality Laboratory, Rev. 1, January 2012.

16.3 American Public Health Association. 2012. *Standard Methods for the Examination of Water and Wastewater, 22nd Ed.* Sections 1030, 1100 and 4500-P.

16.4 Lachat Instruments Method 31-115-01-1-I

17. TABLE, DIAGRAMS AND FLOWCHARTS

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8500 SERIES 2

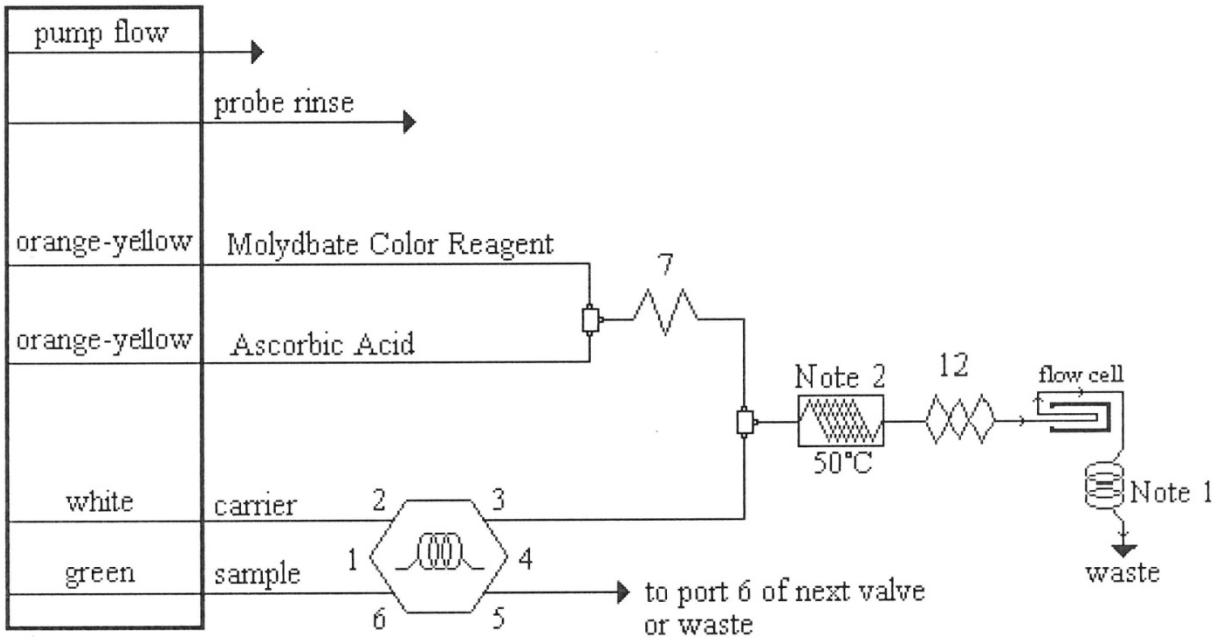
The timing values listed below were optimized using the universal dye. The brackish timing values were determined using artificial sea water as is and spiked at two low levels. *Note: The times are approximate and may vary slightly with pump tubing wear.*

Time Tab under Run Properties		OPO_4	OPO_4/NO_2	$OPO_4/NH_3/NO_3$
Run	Method Cycle Period (seconds)	135	135	135
	Sample Period (seconds)	50	50	65
	Min. Probe in Wash Period (seconds)	59	59	59
Channel 2	Load Period (seconds)	35	35	35
	Inject Period (seconds)	100	100	100
	Time to Valve (seconds)	23	23	23
PO4F (Analyte)	Expected Inject to Peak Start (seconds)	20	20	20
	Expected Peak Base Width (seconds)	132.5	132.5	132.5
	Brackish Shutter Offset (seconds)	27	27	26.3
	Brackish Shutter Width (seconds)	53.5	53.5	53.5

Analyte Tab under Run Properties

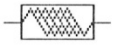
Concentration Units: mg PO_4-P/L
Calibration Fit Type: 1st Order Polynomial
Force through Zero: No
Calibration Weighting: None
QuikChem Method: 31-115-01-1-I
Chemistry: Brackish

17.2 ORTHOPHOSPHATE MANIFOLD DIAGRAM



Carrier: Reagent H_2O
Manifold tubing: 0.5 mm (0.022") i.d.
Sample Loop: 300 cm x 0.8 mm (0.032") i.d.
Interference Filter: 880 nm

7: 135 cm of tubing on a 7 cm coil support wrapped with 0.5 mm i.d. tubing.
12: 255 cm of tubing on a 12 cm alternating coil support wrapped with 0.5 mm i.d. tubing.

The  shows 175 cm of tubing wrapped around the heater block at the specified temperature. The flow cell is a 10 mm path length.

Note 1: 300 cm back pressure loop of 0.5 mm (0.022") i.d.
Note 2: 175 cm x 0.8 mm (0.032") i.d. of tubing on the heater

Appendix 18:

Virginia Mainstem Monitoring Program Log of Significant Changes

Changes ODU has made in methods and in the program

CHANGE	DATE	PARAMETERS EFFECTED
Sampled bi-monthly cruises from March to October and monthly cruises from November to February.	June 1984	None. Start of program at ODU.
Started sampling above and below pycnocline when a pycnocline was detected at designated stations.	March 26, 1985	All nutrients
ODU used a YSI oxygen meter in conjunction with a RS-5 salinometer from 1984 to April, 1986. Depth was calculated with meter markings on the cable. Switched to a Hydrolab Surveyor II from May, 1986 to February 1997. This has built in depth meter.	May 1986	salinity, DO, Specific conductivity, pH, depth, WTEMP
Started using < 15 in Hg for filtration vacuum pressure	BAY 59 May 1987	chlorophyll and phaeophytin
Started sampling at 1/3 and 2/3 of the water column when a pycnocline was not detected at designated stations.	October 1987	All nutrients
Dropped one March cruise due to budgetary constraints.	1988	All parameters
Started analyzing PC/PN, PP and TDN. Dropped the following parameters: TP, TOC, TKN and DKN. CBP decision.	1988	Phosphate, nitrogen and carbon
Dropped one October cruise due to budgetary constraints.	1989	All parameters
Started collected the Elizabeth River cruise. There are 6 stations as follows: ELI2, EBE1, WBE1, LAF1, SBE2 and SBE5.	February 1989	Started ER cruise
Stopped acidifying TDP, NH4F and NO23F samples. Freeze samples instead.	February 1990	TDP/NH4F/NO23F
Started rinsing sample bottles with filtered nutrient before adding sample to bottle.	August 1990	Nutrients
Station LAF1 dropped from Elizabeth River monitoring cruise	August 1990	ER cruise

Started collecting fluorometry data	December 1990	Fluorometry/ chlorophyll
Started using co-mounted system (sampling T) so profile data and sampling pump on same system and samples collected at same time as data. Prior to this the samples were collected in one area of the boat and the profile was done in another.	August 21, 1991 Bay 145	All
Samples for PC/PN filtered with vacuum filtration ≤15 in Hg instead of pressure filtration (were filtered using a syringe prior to this). Change implemented at request of AMQAW.	January 1992	Particulate carbon and nitrogen.
Chlorophyll samples filtered on Whatman® GF/F filters. Prior to this used Whatman® GF/C filters. Change implemented at request of AMQAW	January 1992	Chlorophyll *a* and phaeophytin
Starting collecting light data with Li-Cor sensors	January 1993	Light/KD
Tributary enhancement project. Added two stations near LE5.5 called LE5.5A and LE5.5B. Added parameters biogenic silica and particulate inorganic phosphate. This was only in effect for this one year.	January through December 1994	Biogenic silica and particulate inorganic phosophate
ODU started sampling entire VA mainstem. Prior to this ODU sampled and analyzed samples for 8 stations in lower Bay and VIMS sampled and analyzed rest of BAY for WQ.	January 1996	ALL. ODU's original 8 stations: LE5.5, CB8.1, CB8.1E, CB7.4, CB7.4N, CB7.3E, CB7.3, CB6.4.
Changed to 14 cruises per year from 18. Bi-monthly sampling July and August with monthly sampling the remainder of the year.	January 1996	All parameters.
Dropped DOC. CBP decision.	January 1996	Carbon
Switch from Scientific Instruments Corporation® autoanalyzer to Skalar® autoanalyzer	January 1996	NO23F, TDN, PP and SIF
Change station name from LE5.5 to LE5.5-W, because station switched to 0.6 miles west of where originally collected	September 1996	All parameters for station LE5.5

Switched from a Hydrolab Surveyor II to a YSI 6000 sonde.	March 1997	salinity, DO, Specific conductivity, pH, depth, WTEMP
Switch to Turner® digital flurometer from Turner® analog fluorometer	April 1997	Fluorometry
Switch to less NaOH in the oxidizing reagent	May 1997	TDN
Switch from manual method to analyze orthophosphorus to automated method on the Skalar® autoanlyzer. For orthophosphorus switched to using saline Type I reagent water and inverse predictions. Previously used filtered seawater from cruise analyzing and standard additions.	May 1997	PO4F
Switch from Scientific Instruments Corporation® autoanalyzer to Skalar® autoanalyzer	May 1997	NH4F
Discontinue using dichromic acid bath for cleaning labware	May 1997	PC, PN, PP, TDP and PO4F
Discontinue cleaning tin cups	May 1997	PC and PN
Switch from manual method to analyze total dissolved phosphorus and nitrite to automated method on the Skalar® autoanlyzer. For total dissolved phosphorus also switched to using saline Type I reagent water and inverse predictions. Previously used filtered seawater from cruise analyzing and standard additions.	July 1997	TDP and NO2F
Switched to Unicam® UV1 spectrophotometer for chlorophyll analysis. Perkin Elmer spectrophotometer using previously was no longer operational.	January 1998	CHLA and PHEO
Added two stations to the Elizabeth River monitoring cruise: SBA1 And SBD4	January 1998	ER cruise
Switch from 25 mL oxygen loop to 10 mL oxygen loop on Carlo Erba® N/A 1500	April 1998	PC and PN

Stopped collecting Winkler DO samples for every depth a sample is collected on the Bay and the ER. Only collect one in morning and one in afternoon to check probe (more if necessary due to not matching).	October 1998	Winkler DO
Added two stations to the Elizabeth River monitoring cruise: SBD1 and SBC1	October 1998	ER cruise
Switch to 2cm cell, filtration pressure <10 in Hg and do not filter to dryness	January 1999	CHLA and PHEO
Discontinue collecting light data with sensor that points up.	April 1999	EPARD_Z
Switch to grinding in centrifuge tube and adding known volume of acetone	April 1999	CHLA and PHEO
Stop collecting absorbances for 480 and 510 for the chlorophyll analysis at the request of the CBP and Rick Hoffman of Virginia DEQ.	July 1999	Carotenoids
Switch to taking mainstem chlorophyll samples from fluorometer instead of carboy used for nutrients. Change implemented by AMQAW to standardize fluorometry methods. Will use these samples in fluorometry calibrations curves.	January 2000	CHLA, PHEO and fluorometry
Instead of field blanks for every 10 samples, doing one field blank per day.	January 2000	none
ODU started using a CE Instruments Flash EA 1112 Elemantal Analyzer. A method comparison study between this new instrument and the Carlo Erba NA 1500 was completed (the instrument used prior to May 2001). The results are reported in A Comparion of Two Instruments for the Determination of Particulate Carbon and Particulate Nitrogen Concentrations in Estuarine Water Samples. In addition, started using 25mm GF/F filters for filtration of PC/PN instead of 13mm GF/F filters. This is because new instrument can accept larger filters. In past the largest volume that could be filtered was 50mL, now up to 250 mL can be filtered. New filtration towers employed.	May 2001	PC/PN

Discontinue using R/ Miss Jana, retired by ODU	December 2001	All parameters
Start using R/V Holton	January 2002	All parameters
Start analyzing FSS/VSS in addition to TSS at all stations in the Bay and ER. This is to help determine if the suspended solids are organic in nature (which would have a high VSS) or terrestrial as in run off (which would have a high FSS). To accomplish this change all filters for TSS/FSS/VSS are combusted at 550°C for 30 minutes in addition to rinsing them.	January 2002	All CBP and ER TSS.
Start using commercial vessel Sea Search, R/V Holton retried by ODU	June 2002	All parameters
Four stations on the Elizabeth River monitoring cruise were discontinued due to funding constraints in the state of Virginia. These were SBA1, SBD4, SBD1 and SBC1.	November 2002	ER cruise
Start using R/V Slover for CBP	January 2003	none
Start using R/V Slover for ER	March 2003	none
Switch from using Chloromine-T to aspartic acid as standard for PC/PN analyses	April 2003	PC/PN
Start using rosette bottles to collect samples instead of sampling pump	July 2003	none
Added two extra cruises to CBP mainstem monitoring to access dissolved oxygen. One cruise was added to June and one to September	2004	CBP mainstem cruise
Start using new procedure to collect Licor data. Will use secchi to determine depths to collect light data, and stop when underwater reading is <1% of reading at 0.5 meters.	June 2004	Light/KD
Start using a new sampler, integrator and software for the Skalar analysis. The new software is windows based versus dos based. The software package is called FlowAccess	June 2004	NO23F, NO2F, TDN, TDP, PP, PO4F, SIF and NH4F.

Start analyzing color dissolved organic matter (CDOM) at the surface for all ER and CBP stations once a month for July - October 2005 and April - June 2006. This is for the submerged aquatic vegetation (SAV) program.	July 2005	CDOM
Collect vertical fluorometry using Wet Star® fluorometric probe. Collect data every 1 meter. Chlorophyll samples now collected from go-flow where nutrient samples are collected.	November 2005	Vertical fluorometry
Stopped funding the two extra cruises to CBP mainstem monitoring to access dissolved oxygen. Only one cruise will be sampled in June and September. (NOTE: In 2006 due to boat problems could not sample Bay in Feb., so did 2 June cruises that year).	2006	CBP mainstem cruise
Switched to Shimadzu ® 2401PC spectrophotometer for chlorophyll analysis	January 2006	CHLA and PHEO
Stopped writing down wavelengths for chlorophyll analysis, this data is captured electronically. This change is due to new instrumentation. Oral permission given for this change by Mary Ellen Ley, QA officer CBP, and Rick Hoffman of Virginia DEQ	July 2006 BAY442 on and ER210 on	CHA/PHEO
Added back one extra cruise to CBP mainstem monitoring to access dissolved oxygen. One extra cruise was added to June.	2007	CBP mainstem cruise
Added DOC analysis to surface samples in CBP mainstem and Elizabeth River where phytoplankton is collected, to help with a phytoplankton index that was developed.	Jaunuary 2007	DOC Stations SBE5, CB6.4, CB6.1, CB7.3E, CB7.4, WE4.2, LE3.6 and LE5.5-W.
Stopped standardizing nitrite stock. Instead are buying a separate standard to confirm calibration accuracy.	July 1, 2007	NO2F/NO23F/TDN
Started using a 10 mL fixed volume macropipettor instead of a class A volumetric pipette to dispense TDN/TDP samples, spikes and duplicates.	July 1, 2007	TDN/TDP
Started using a 10 mL fixed volume bottle top dispenser instead of a class A volumetric pipette to extract PP samples and duplicates.	July 1, 2007	PP

Stopped collecting winkler dissolved oxygen samples on cruise. Will check DO calibration against a chart.	January, 2008	DO
Stopped funding the extra June cruise to CBP mainstem monitoring to access dissolved oxygen. Only one cruise will be sampled in June.	January, 2008	All parameters
At end of day on the R/V, will fill nutrient filtration flasks with reagent water and let it sit overnight. Use new graduated cylinders each day. Instituted this at request of AMQAW.	January, 2008	Nutrients.
Do not apply correction factor to physiochemical profile data if it is out of range during post-calibration. It is noted that it was out of range, and the data is either submitted with a problem code or it is not submitted at all. Instituted this at request of AMQAW.	January, 2008	salinity, DO, Specific conductivity, pH, depth, WTEMP
Switched from YSI 6000 sonde to YSI 6600 sonde with an optical DO probe.	April, 2008	salinity, DO, Specific conductivity, pH, depth, WTEMP
Collecting 2 field splits on the Chesapeake Bay Monitoring cruise in addition to the 2 replicate splits collected. Instituted this at request of AMQAW.	May, 2008	NO23F, NH4F, PO4F, NO2F, TDN, TDP, SIF
Collecting 1 field split on the Elizabeth River Monitoring cruise. Instituted this at request of AMQAW.	May, 2008	NO23F, NH4F, PO4F, TDN, TDP
Collecting 1 field split on the Elizabeth River or Chesapeake Bay Monitoring cruise for the DOC analysis. Instituted this at request of AMQAW.	May, 2008	DOC
Pycnocline calculation changed. Subtract bottom where collected sampled by surface and divide by that. Previously divided by depth of the station. Instituted this at request of AMQAW.	May, 2008	NO23F, NH4F, PO4F, TDN, TDP, TSS, FSS, CHLA, PHEO, PC, PN, PP
Collect equipment blank once a year that also goes through filtration equipment. Instituted this at request of AMQAW	May, 2008	NO23F, NH4F, PO4F, TDN, TDP, TSS, FSS, CHLA, PHEO, PC, PN, PP

Eliminate sodium potassium tartrate from buffer solution. If discover sodium potassium tartrate needs to be added back in will try to find a non contaminated source of the chemical.	July, 2008	NH4F
For CBP split samples (PMS-10 and MCB4.4) were processing and analyzing many of the parameters in replicate so do not lose sample. Since have to analyze all samples like routine samples for VA Chapter 45 certification, will stop this practice. Exception is AMQAW requires one duplicate and one spike (if applicable) for each date the split is analyzed, so will have additional QC on these samples. Also, if MCB4.4 is analyzed all in one day instead of separate dates, will continue to dup and spike one for B-1 and B-2 and then again for B-3 and B-4.	August 2009	All parameters analyzed for split samples
Filtration pressure $\leq$10 in Hg . Most have been filtered at this pressure since chla changed to this in January 1999, but SOP allowed $\leq$15 in Hg	October 2009	PC/PN
Stop sampling Elizabeth River cruise due to VA state budget cuts. Stations that were currently sampled by ODU: ELI2, WBE1, SBE2, SBE5 and EBE1.	January 2010	All parameters for ER
Switched cruise regimen to bi-monthly sampling in June and August, and monthly sampling all other months. Still 14 cruises per year.	January 2010	All parameters
All MDLs will be calculated by multiplying the standard deviation of at least 7 replicates by the Students' t value. Prior to this the CBP procedure of multiplying the standard deviation by 3 was used. Instituted this at request of AMQAW.	January 2011	All nutrient parameters
Muffle furnace Lindberg/Blue M Model #51828 broke on January 13, 2011. Replaced on January 27, 2011 with Lindberg/Blue M Model # BF51828C-1.	January 2011	FSS/VSS, PC/PN, PP
Old Dominion University Water Quality Laboratory is a Certified Laboratory by the Commonwealth of Virginia Department of General Services Division of Consolidated Laboratory Services under the requirements of 1-VAC 30-45. VA Laboratory ID # 450012	Interim Approval: June 15, 2010 Approval Effective: February 3, 2012	NH4F/NO2F/PP/SIF/ PC/PN/CHLA/DOC/ TSS/FSS/VSS/TDN/ NO23F/PO4F/TDP

Started reporting the Water Quality data in CIMS with a problem code of G for values between the MDL and the PQL	January 2012	All nutrient parameters
Added a second cruise to month of July only sampling the physicochemical profile to assess the low dissolved oxygen in the Chesapeake Bay in the summer months. No nutrient samples are collected on this cruise.	July 2012	DO/WTEMP/ SALINITY/ SPCOND/PH/ DEPTH/SECCHI
Will start analyzing MDLs on multiple analysis dates instead of all on the same date. Recommended by AMQAW.	January 2013	All nutrient parameters
Reuse chlorophyll bottles each day on the CBP mainstem cruises. Prior to this a different chlorophyll bottle was used for each sample. Always reused chlorophyll bottles for the fluorometery grab samples.	May 2013	CHLA/ PHEO
Due to budget cuts second cruise of the month during the summer only sampling the physicochemical profile to assess the low dissolved oxygen in the Chesapeake Bay in the summer months. No nutrient samples are collected on these cruises. Will collect horizontal fluorometery if boat used supports it.	August 2013 and June 2014	All nutrient parameters
Due to budget cuts eliminated one winter cruise, with preference being January. If unable to sample December cruise will sample in January.	August 2013	All paramters

For the Flash EA 1112 made the following changes to the instrument: Updated software from Eager 300 to Eager Experience. Port 4 was originally vented back into the sampler, but now is vented to the atmosphere. This will eliminate possible contamination. The combustion furnace is set at 950°C, it was at 900°C. The reduction furnace is set at 840°C, it was at 680°C. The chromatography column was removed from the oven, this is to give greater separation between the carbon and nitrogen peaks. The internal oxygen and helium flow controllers were tested, and the oxygen was set to the correct specifications. Some maintenance procedures were updated to new specifications. The technician also cleaned the instrument, and changed lines and connections. The SOP has been updated to reflect these changes. A calibration curve was analyzed with second source standards analyzed. Four filters were analyzed that were previously run under the old instrument conditions, and the results were comparable.	November 2013 CBP611 on	PC/PN
Due to budget changes, the January cruise was added back in for January 2015. In addition the nutrients were added back in for the June and August cruises for August 2014 and June 2015.	April 2014	All parameters
Switch from Skalar® continuous flow analyzer to Lachat® Flow Injection Analyzer.	July 2014 BAY628	SIF, NO2F, NO23F, NH4F, TDN, TDP, PP and PO4F.
Change autoclave temperature from 105°C to 120°C for the digestion of total dissolved phosphate samples. If total dissolved nitrogen samples are digested with phosphate then they are digested at the higher temperature also.	July 2014 BAY628	TDN/TDP
Following Good Laboratory Practice Conference discovered should label all documents that are copies with the word “copy”	August 2014	
Switched from YSI 6600 sonde to YSI EXO sonde.	November 2014 BAY637	salinity, DO, Specific conductivity, pH, depth, WTEMP

TDN low standard and reporting limit has been changed from 0.025 mg/L to 0.05 mg/L. MDL has been around or above 0.025 mg/L and no samples analyzed in the past several years have been <0.05mg/L	April 2015 BAY646	TDN
Switched to a new Li-Cor data logger LI-1500. Data since started collecting light data was LI-1000. This data logger will still be used as a back up.	July 20, 2015 BAY653	LIGHT/KD
Started tracking lot numbers of filters used for filtering samples.	March 2016 BAY668	All particulate and dissolved parameters.
On Lachat switched manifold used for total dissolved phosphate analysis. Use same manifold that is used for orthophosphate analysis. Regression curves and quality control samples have better results, and the total dissolved phosphate samples are higher than the orthophosphate analysis.	August 2016 BAY 678 on	TDP/PO4F
Start submitting null value with "V" problem code for light data that is excluded from the data	September 2016 BAY 681	LIGHT/ KD
Switched from TOC-5000 to TOC-L	October 2016 BAY 683	DOC
For chlorophyll samples the length of filtration time allowable was changed from 5 minutes to 10 minutes.	January 2017 CBP 689	CHLA/ PHEO
For chlorophyll samples the 1% $MgCO_3$ is now only added to freshwater samles.	February 2017 CBP 690	CHLA/ PHEO
Change dishwashing procedure to 10% HCL from 4N HCL. Have been using 10% HCL for Lachat analysis with no issues. Lower concentration of acid is less toxic and a cost saving.	September 2017	PC/PN, DOC, TSS/FSS

Water Quality Laboratory Changes

May 1994- Laboratory moved from Applied Marine Research Laboratory building on 45th Street in Norfolk near ODU to Nauticus in downtown Norfolk. Nauticus is a maritime museum. A special area with glass windows for viewing was built to accommodate the WQL.

March, 1997- The Water Quality Laboratory Manager Position is eliminated. Mr. Steven Sokolowski, who has been the head of WQL since 1984, is no longer employed by WQL. Ms. Suzanne C. Doughten, as Supervisor of Water Quality Laboratory, is now in charge of day to day field and laboratory operations.

January 2000- Laboratory moves from Nauticus in downtown Norfolk to 4211 Colley Ave. near ODU. Building owned by ODURF, laboratory section previously housed a laboratory from ODU's Chemistry Department.

May 1, 2000- Water Quality Laboratory of Old Dominion University was transferred from the Applied Marine Research Laboratory to the Chemistry and Biochemistry Department of Old Dominion University. Dr. John R. Donat assumed the position of Director of the Water Quality Laboratory, replacing Dr. Alan W. Messing.