FIU Center for Aquatic Chemistry and Environment
NSF Center of Research Excellence in Science and Technology

Center for Aquatic Chemistry and Environment
Nutrient Analysis Core Facility
Document Change Record

Determination of Filtered Nutrients in Water

Document ID	**CAChE-NACF SOP-004**
Issue Date	08/28/1998
Revision History	**Effective Date**
Revision # 7	11/23/2015
Revision # 8	01/12/2018
Revision # 9	07/01/2019
Revision # 10	05/01/2022
Revision # 11	09/18/2023

The following were editorial changes which have been incorporated the CACHE-NACF SOP-004.

Document Reviewed	**Document Changes**	**Author (s)**
SOP Rev.10	Pg. 3. MDL values were updated in Table 1. Pg. 4. ADM_220711 was included in the Calibration section 3.b. "As part of the calibration evaluation, calculation of the Percent Relative Error (%RE) will be performed for two of the analyses calibration levels (low and midpoint). The Relative Error, at both of these levels, shall meet the criteria of %RE low <20% and %RE mid < %10 and can be calculated using the following equation: $$RE(\%) = \left(\frac{|X'i - Xi|}{Xi}\right) \times 100$$ Where: Xi = True value for the calibration standard X'i = Measured concentration of the calibration standard. Pg. 4. ADM_221101B was included in section 3.h. Quality Control (Secondary Standard): "CAChE-NACF purchases certified reference material standards (CRM) or standards with NIST traceability certificates as the Secondary Standards or QC standards. These standards will be prepared according to the instructions provided by the company. Based on the results for each analysis, appropriate dilution factors will be determined so that the results fall within the middle range of the calibration for each analysis".	IL YD MK

Pg. 6. In order to assess method precision, the following statement was included in section 3.i.:
"For method performance evaluation during a long period of time (such as quarter or annual evaluation), precision can be calculated from the Average Deviation of the data set from a quality control standard (i.e. ICV, QC, MS, MSD, CCV) and the Mean of that standard collected during certain time period."

$$Precision\ (\%) = \left(\frac{Average\ Deviation}{Mean}\right) \times 100$$

Pgs. 7 and 8. Addition of new pipette (300 µl) and balance to Section 6. (Equipment and Supplies).

Pg. 12. The following statement was deleted from section 8.c.ii: "This reagent is used to acidify the samples automatically prior to being purged for 6 minutes."

Pg. 13, It was specified that "solid" phenol is added in the preparation of 20%phenol in section 8.e.i.3.

Pg. 14. Correction of DIW volume in section 8.e.iv.2, from 900 mL of DIW to 800 mL DIW. Warning for the addition of acid was reworded: "Use caution when making acid solutions. Always add acid to water. The mixing of acid in water may generate heat."

Pg. 14. Recipe of Sodium dodecyl sulfate (SDS) in section 8.e.iv.3 was updated. "30 g sodium dodecyl sulfate is added to 170 ml DIW" instead of 45 g in 255 ml of DIW.

Pg. 14. It was specified that the antimony potassium tartrate is a "trihydrate" in section 8.e.iv.4.

Pg. 14. It was specified that the ammonium molybdate is a "tetrahydrate" in section 8.e.iv.5., and that the filter used is a 0.8 µm glass fiber filter.

Pg. 14 to16. The following typos were corrected:

- Section 8.e.iv.7: (8.e.ix.3) to (8.e.iv.3)
- Section 8.e.iv.7: (8.e.ix.6) to (8.e.iv.6)
- Section 8.f.i.1: iso-propanol to 2-propanol
- Section 8.f.ii.1: iso-propanol to 2-propanol
- Section 8.f.iv.2: heptamolybdate to molybdate tetrahydrate
- Section 8.f.iv.2: tartrate to tartrate trihydrate

Pg. 14. The following reference sections were added to the preparation

	of Ammonium buffer in Section 8.f.i.2: • NaOH (8.c.i) and 3 N HCl (8.c.ii) Pg. 16. Replacement of the Mixed QC Standards in sections 8.g.ii, 8.g.iv., 8.g.vi. and 8.g.ix for NIST traceable or CRM standard to be used as Secondary Standard (QC Standard) as a result of ADM_221101B. Pg. 16. For the easiness of calculation, the amount of ammonium molybdate tetrahydrate in section 8.f.iv.2 was updated. Pg. 16. Recipe of Stock molybdate in section 8.f.iv.2 was doubled. Added "The resulting solution is filtered through a 0.8 µm glass fiber filter." Pg. 16. Diluent preparation procedure and shelf life were updated in section 8.f.iv.3. Pg. 17. The following statement was added to Section 10.b. (Calibration and Standardization): "Any sample result that falls outside the Linear Dynamic Range (LDR, 13.a.iv) will be diluted and re-analyzed." Pg. 20. In order to assess method uncertainty, the following statement was included in section 13.a.vi.: "For method performance evaluation during a long period of time (such as quarter or annual evaluation), uncertainty (such as quarter or annual evaluation) is calculated from 2 times of the Standard Deviation of the data set from a quality control standard (i.e., ICV, QC, CCV) and the Mean of that standard collected during certain time period." $$Uncertainty\ (\%) = \left(\frac{2 \text{ x } Standard\ Deviation}{Mean}\right) \times 100$$ Pg. 20. Linear Calibration range in section 13.a.iii was rephrased to "The analysis of the calibration curves is performed to ensure that the resulting curve is linear and within the acceptance criteria". Pg. 21. Description for the Data Qualifier "J" in section 13.a.ix.3 was modified form "Estimated Value, qualifier has to be accompanied by detailed explanation. Value outside the established criteria" to "Value outside the established criteria". Pg. 22. Addition of the 2016 NELAC Standard as a reference in Section 18.	
ADM_221101B	Quality Control Standard or Secondary Standard used for the analysis will be purchased from a NIST traceable provider and prepared	IL

	according to the provider's instructions.	
ADM_220711	Calculation of Percent Relative Error (%RE) for two of the analysis calibration's points	IL
SOP Rev.9	Pg.3, MDL definition is updated to "The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results". Pg.4, MDL values were updated to currents ones (released 07/01/21) in table 1. Pg.5, Percent recovery ranges for Matrix Spike were updated to 80-120%. Pg.7, New SEAL instrument (Serial # 5909A55890) was added to the equipment (section 6.e). Pg.7, 20 uL pipette was added to the list of equipment (section 6.g.). Pg.13, Additional sentence was added to clarify that the Walmart bleach brand is a substitute for the Clorox brand which causes interferences with the analysis. Pg.20, Additional sentence was added to clarify that the LDR is determined annually based on the quarterly collected data.	IL YD MK

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Method Summary of

CAChE-NACF SOP-004, Rev. 9

Prepared for Battelle NEON program

Determination of Filtered Nutrients in Water

Nitrogen, Nitrite (NO_2) + Nitrate (NO_3) as **N+N**

Nitrogen, Nitrite as **NO_2-N**

Nitrogen, Ammonia/Ammonium as **NH_3/NH_4-N**

Soluble Reactive Phosphorus as **SRP**

Prepared by

Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility

(CAChE-NACF)

1) Summary of Method

a) Analysis for inorganic filtered nutrients, ammonia/ammonium as N (NH_3/NH_4-N), nitrite as N (NO_2-N), nitrate and nitrite as N (N+N), and soluble reactive phosphorus as P (SRP), are simultaneously performed by wet chemical analysis using a four-channel Rapid Flow Analyzer based on standard procedures EPA 353.4 for NO_3-N and NO_2-N, EPA 349.0 for NH_3/NH_4-N and EPA 365.1 for SRP. The indophenol blue method for ammonia/ammonium is used. Ammonia/Ammonium reacts with alkaline phenol and hypochlorite to form indophenol. Sodium nitroferricyanide intensifies the blue color and it is detected photometrically at 640nm (Astoria) or 660nm (Seal Quattro39). Nitrite is determined as an azo dye formed by the reaction of nitrite with sulfanilamide and subsequent coupling with N-1-naphthylethylenediamine (NEDA) and detected photometrically at 540nm. Nitrate is determined by the quantitative reduction of nitrate to nitrite using an activated cadmium column, and then determination of nitrite as described above. The nitrite concentration before reduction is subtracted from the nitrite concentration after reduction to give nitrate concentration. Soluble reactive phosphorus is determined by reacting phosphate with molybdenum (IV) and antimony (III) in an acid medium to form an antimony-phospho-molybdate complex; this complex is reduced with ascorbic acid to form a blue colored complex and measured photometrically at 880nm

2) Sample Storage

a) Filtered Samples preserved frozen without acid: These samples should be analyzed within 48 hours of sample collection to accommodate for SRP and NO_2-N holding times. NO_2-N and SRP holding times are 48 hours from collection time without preservation or 48 hours from the time the samples are removed from the freezer. If they are going to be analyzed within 48 hours, then the samples can be stored in a refrigerator.

Per project or contract specific request, samples preserved by freezing are also acceptable. Prior to analyzing, the samples need to be thawed slowly by leaving them at room temperature, no heating allowed, until liquid and shaken well. All samples have a limit of 28 days from collection time to complete the full analysis.

b) Filtered Samples preserved with acid**:** Acidified filtered samples are kept in refrigerators at 2 to 6 ºC with a holding time of 28 days from sample collection time. An aliquot of these samples are neutralized prior to NH_3/NH_4-N analysis. NO_2-N and SRP will be analyzed within 48 hours of collection only if it is requested by the client, agreed under contract and samples are received the same day of the sampling event.

3) Analysis

Every analytical batch (20 samples) include:

- S6 as SYNC or primer to mark the start of analysis, two carryover (CO), reagent blanks (RB), calibration curve standards in **decreasing order of concentration**, MB, ICV, QC, UMS(D) and MS(D).
- First set of 10 samples
- Samples are bracketed by CCVs. Each bracket includes no more than 10 samples plus one analytical replicate at the end of each bracket. RB and CCVs are run every 10 samples to monitor baseline and intra-run calibration drifts.

- Second set of 10 samples
- If running multiple batches of samples, a set of quality control samples including MB, QC, UMS(D), MS(D) are required for every 20 samples