



Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility
VH-303, Florida International University, Miami, FL 33199
305-348-3095, 305-348-4096 fax,
<https://environment.fiu.edu/research/facilities-labs/cache-nutrient-analysis-core/index.html>

Method Summary of
CACHÉ-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 **$\text{NO}_2\text{-N}$**

Nitrogen, Ammonia/Ammonium as **$\text{NH}_3/\text{NH}_4\text{-N}$**

Soluble Reactive Phosphorus as **SRP**

Prepared by
Center for Aquatic Chemistry and Environment - Nutrient Analysis Core Facility
(CACHÉ-NACF)

1) Summary of Method

- a) Analysis for inorganic filtered nutrients, ammonia/ammonium as N ($\text{NH}_3/\text{NH}_4\text{-N}$), nitrite as N ($\text{NO}_2\text{-N}$), 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 $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$, EPA 349.0 for $\text{NH}_3/\text{NH}_4\text{-N}$ 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 nitroferrocyanide 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-phosphomolybdate 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 $\text{NO}_2\text{-N}$ holding times. $\text{NO}_2\text{-N}$ 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 °C with a holding time of 28 days from sample collection time. An aliquot of these samples are neutralized prior to $\text{NH}_3/\text{NH}_4\text{-N}$ analysis. $\text{NO}_2\text{-N}$ 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