

Determination of Total Nitrogen in Water

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Revision # 6	11/23/2015
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Document	Document Changes	Author (s)
SOP Rev. 11	Pg.3, Modifications made in Summary of Method (2): • Information of ANTEK Instrument was removed; • Information of Trace Elemental Xper-TN_b custom was added and combined with information of Elementar Trace S/N Cube; • The combustion temperature has been revised to 730 °C, which is the manufacturer-recommended setting for the Archie-Xpert-TN_b custom configuration. A comparison study was performed using the Elementar S/N Cube to evaluate results generated at 1050 °C versus 730 °C. Statistical analysis confirmed that the measurements produced at both temperatures were equivalent. Therefore, the operating temperature of the Elementar S/N Cube was reduced to 730 °C to prevent column cracking and breakage associated with extended operation at 1050 °C. Pg.4, MDL values were updated to current (released 08/01/25) in Table 1. Pg.4-5, Description of Matrix Spike (MS) and Matrix Spike Duplicate (MSD) (3.i) were updated to reflect the change of UMS number per analytical batch. Pg.6, Change “QC cover page” to “Quality control check” (3.j). The information of QC page was simplified, data validation through Element7 LIMS was added. Pg.6-7, Analytical balance and pipette information in Equipment and	YD IL MK

	Supplies (6) was referenced to CAChE-NACF SOP-009 and 010. Pg.7, Instrument Parameters of ANTEK (7.a) was replaced by those of Archie-Xpert-TN_b custom. Instrument parameters of Elementar Trace S/N Cube (7.b) was updated. Pg.7, Matrix Spike Sample Preparation (8.c) section was re-worded: the volumes in the spiking procedure (8.c.iii) were proportionally increased to make up enough total volume to prepare approximately 30 MS vials. Shelf life of UMS, MS and MSD was added to the section. Pg.8, Logbook of ANTEK was obsoleted, and logbook for Archie-Xpert-TN_b Custom was added to the working standard section (10.a) Pg.9-10, Combustion process (12.a) was updated to reflect the change of instrument; analytical batch sequence (12.b) was updated to reflect the change of method blank (MB) position in a sequence: MB would be run at the beginning and end of the run as well as after every 10 samples. The number of UMS per analytical batch was changed from two to one. Pg.11, Data Qualifiers (13.a.ix) were updated to reflect minor verbiage changes. Pg.11, Auto-analyzer Preventative Maintenance (14) was updated to reflect the change of instrument. Pg.11-12, Manuals of Trace Elementar S/N Cube and Archie-Xpert-TN_b Custom were added into the References (17); CAChE-NACF SOP-009 and 010 were added into the References (17). Pg.13, Example of QC page was removed. Pg.15, Instrument schematic diagram of ANTEK was replaced by instrument schematic diagram of Xpert-TN_b Custom (Figure 2)	
ADM_250528	TN ANTEK Decommission_Addition of Current Instruments	IL
SOP Rev.10	Pg. 3. In section 2, the purpose of the use of Oxygen gas instead of Argon/Oxygen was revised from “to promote complete <u>recovery</u> of the nitrogen in the water samples” to “promote complete <u>combustion and oxidation</u> of the nitrogen in the water samples”. Pg. 4. MDL values were updated in Table 1. Pg. 4 and Pg.5. The following typos were corrected: • Section 3.b: (Table 2 in 10.a) to (Table 2 in 10.c) • Section 3.h: QC standard (8.e) to QC Standard (8.e.ii)	IL YD MK

	Pg. 4. ADM_220711 was included in the Calibration section 3.c. “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. 5. ADM_221101B was included in section 3.h. Quality Control (Secondary Standard): “CACHÉ-NACF purchases certified reference material standards (CRM) or standards with NIST traceability certificates as the Secondary Standards or QC standard. 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”. 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.” $Precision(\%) = \left(\frac{Average\ Deviation}{Mean} \right) \times 100$ Pg. 6. Addition of new pipette (300 µl) and balance to Section 6. (Equipment and Supplies). Pg. 7. Insertion of dry air supply regulated to 10 psi for Nafion driers in ANTEK 9000 and Elementar Trace S/N Cube in section 6.g Pg. 7. Removal of Eppendorf pipette from section 6.h. Pg. 7. Update Pyrolysis Oxygen flow in section 7.a (ANTEK) from 220 ml/min to 100 ml/min. Pg. 8. Replacement of the Mixed QC Standards in sections 8.e.ii, for NIST traceable or CRM standard to be used as Secondary Standard (QC Standard) as a result of ADM_221101B	
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	Pg. 8. QC standard preparation was deleted from section 8.e.ii since the lab now uses the NIST traceable Secondary Standard (QC Standard) which is prepared according to the manufactures' specifications. Pg. 9. The following statement was added to Section 10 (Calibration and Standardization): "Any sample result that falls outside the Linear Dynamic Range (LDR, 13.a.iv) will be diluted and re-analyzed." Pg. 9. 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." $Uncertainty (\%) = \left(\frac{2 \times Standard\ Deviation}{Mean} \right) \times 100$ Pg. 10. 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. 10. The following reference section was corrected: Section 12.b: QC sample (8.e.iii) to QC sample (3.h) Pg. 11. Description for the Data Qualifier "J" in section 13.a.ix.3 was modified from "Estimated Value, qualifier has to be accompanied by detailed explanation. Value outside the established criteria" to "Value outside the established criteria". Pg.12. Addition of the 2016 NELAC Standard as a reference in Section 17.	
ADM_221101B	Quality Control Standard or Secondary Standard used for the analysis will be purchased from a NIST traceable provider and prepared according to the provider's instructions.	IL
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.3, MDL values were updated to currents ones (released 07/01/21)	IL YD MK

	in table 1 Pg.3, Additional sentence was added to clarify that the ANTEK instrument is only used as a backup. Pg.5, Percent recovery ranges for Matrix Spike were updated to 80-120% Pg.10, Additional sentence was added to clarify that the LDR is determined annually based on the quarterly collected data.	
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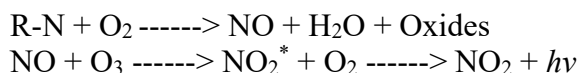
Method Summary of
CACHÉ-NACF SOP-006, Rev.11
Prepared for Battelle NEON Program

Determination of Total Nitrogen in Water

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

1) Summary of Method

- a) CACHÉ-NACF follows the ASTM D5176-91(2003) - “Standard Test Method for Total Chemically Bound Nitrogen in Water by Pyrolysis and Chemiluminescence Detection” (the procedure is a modification of the classical Dumas (1831) method of determining nitrogen by a combustion technique with the addition of chemiluminescence). The method involves converting all forms of nitrogen into nitric oxide (NO) upon combustion of a sample with oxygen at a temperature of 730°C. The NO is reacted with ozone (O₃) to form a metastable form of nitrogen dioxide (NO₂^{*}). As the metastable form of nitrogen dioxide decays, a quantum of light is emitted in an amount directly proportional to the amount of nitrogen in the sample. The chemiluminescent emission is detected by a photomultiplier tube (PMT) or a chemiluminescence detector (CLD).



Two instruments are available to perform TN analysis: (1) An Xpert-TNb analyzer (custom edition) from Trace Elemental, Inc. equipped with an Archie Autosampler and a built-in PMT detector; (2) a Trace S/N Cube from Elementar, Inc. equipped with a Horiba APNA-370 CLD detector. Both instruments are optimized and maintained according to their manufacturing recommendations, under the same chemistry and principles, except that Xpert uses Oxygen as the carrier gas while Trace S/N uses Argon/Oxygen mixture as the carrier gas. Total nitrogen is determined on unfiltered samples, while total dissolved nitrogen is determined on filtered samples.

2) Equipment and Supplies

- a) Trace Elemental, Inc. Xpert-TNb analyzer (custom edition)
b) Elementar, Inc. Trace S/N Cube Analyzer

3) Sample Storage

Sample bottles are stored in refrigerators at 2-6 °C. Samples have a holding time of 28 days from sample collection time. Storage of samples is in refrigerators designated for sample storage only.

4) Analysis

- a) Combustion of the sample and its reaction with ozone is completed by the Archie-Xpert-TNb Custom or the Elementar Trace S/N Cube. Both instruments are programmed to perform a triplicate analysis of each sample.
- a) Every analytical batch (20 samples) include:
- Calibration curve standards when needed, MB, ICV, QC, UMS and MS/MSD.
 - First set of 10 samples.
 - Samples are bracketed by CCVs and MBs. Each bracket includes no more than 10 samples. RBs are run every 10 samples to monitor baseline and intra-run calibration drifts.

- One replicate sample for every 25 samples.
- If running multiple batches of samples, a set of quality control samples including QC, UMS, MS/MSD are required for every 20 samples.

Appendix A. Text and Diagram of ANTEK removed from SOP Rev.10

2) Summary of Method

An ANTEK Instrument, Inc. Model 9000 Nitrogen Analyzer is used to determine TN of a 5 μ L injection from a prepared water sample. The instrument is run according to the Installation/Operation/Service Manual provided by ANTEK Instruments, Inc., except that Oxygen gas is used as a carrier gas instead of Argon/Oxygen to promote complete combustion and oxidation of the nitrogen in the water samples. Total nitrogen is determined on unfiltered samples, while total dissolved nitrogen is determined on filtered samples. An auto-sampler is used to inject the samples into the analyzer.

6) Equipment and Supplies

- c) ANTEK Instrument, Inc. Model 9000 Nitrogen Analyzer equipped with the following: A200S auto-sampler, Chemiluminescent Nitrogen Detector (CLD), Pyro Tech Furnace, ozone generator, vacuum pump and accessories.
- g) Dry air supply regulated to 10 psi for Nafion driers in ANTEK 9000 and Elementar Trace S/N Cube.
- i) Borosilicate glass sample vials (2.0 ml), with teflon/silicone septa seals for the ANTEK-9000 auto-analyzer

7) Instrument Parameters

a) ANTEK-9000

Pyrolysis Temperature:	1050°C
Pyrolysis Oxygen Flow:	100 ml/min
Oxygen to Ozone Flow:	25 ml/min
System Pressure:	0.5-0.75 psi
Vacuum:	25 in/Hg
PMT Voltage:	650-900
Gain:	X50 Hi
Sample Volume:	5 μ l + 3 μ l air

