

WHITEPAPER

Analytical and Measuring Instruments

Introducing a New ASTM Method for
the Determination of Total Nitrogen,
and TKN by Calculation, in Water
Samples



■ Introduction

The USEPA Clean Water Act (CWA) requires national approval for all methods used for CWA compliance.¹ There are approved methods for the determination of inorganic nitrogen ($\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NH}_3\text{-N}$), and for organic nitrogen (TKN – $\text{NH}_3\text{-N}$)². There are no EPA approved methods for the determination of total nitrogen although it is a required parameter in many USEPA permits, including monitoring of nutrient pollution for ambient water criteria.³ Because Part 136.3 Table 1b methods are required, laboratories have no recourse but to measure total nitrogen as the sum of TKN, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ ⁴ or as Simplified TKN (s-TKN™)⁵.

■ History and Explanation of the TKN Method

In 1883, Johan Kjeldahl published “A new method for the determination of N in organic substances”⁶ that became one of the greatest achievements in science up to that time. The new TKN method was faster, applicable to solids and liquids, and the data compared favorably to the previous methods. The TKN method remains virtually unchanged today. The TKN method obtains satisfactory results with almost all nitrogen compounds; however, recovery is incomplete with cyano-compounds, nitro-compounds, and certain alkaloids. Even with the addition of chemicals, such as salicylic acid, that aid in the recovery of samples with nitrate, the recovery of TKN in samples with nitrate ($\text{NO}_3\text{-N}$) is usually low.

The TKN method presents problems to the modern high-throughput environmental laboratory attempting to analyze large numbers of samples for trace concentrations of total nitrogen. The TKN method requires a preliminary manual digestion with concentrated sulfuric acid, a metal catalyst, and potassium sulfate. Mercury is the best catalyst but many laboratories choose to use copper sulfate instead. The mercury catalyst results in a clear digest solution while the copper catalyst results in a green-colored solution. The classical TKN procedure distills ammonia nitrogen (the product of the digestion) separating the analyte from the matrix prior to analytical determination by titration or colorimetry. In a rapid analysis scheme, the added distillation step is very time-consuming and severely limits laboratory throughput.

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Miniaturized methods for the determination of TKN by semi-automated block digestion followed by continuous flow colorimetric methods have been developed⁷. These continuous flow (CFA) methods omit the distillation step, speeding the analysis. However, CFA methods suffer from difficulties that result from the color of the sample digest absorbing light at the analytical wavelength. Improper matrix matching causes both positive and negative deflections of the baseline due to differences in refractive index between the sample solution and the carrier solution. In addition, excess acid in the digestion solution causes reagents to precipitate with the CFA chemistry cartridge.

The Technicon Instruments Corporation, the manufacturer that developed the first continuous flow analyzer, developed a continuous digestion and analysis system that automatically digested and analyzed TKN.⁸ This apparatus/analyzer produced very good comparison data with manual TKN. However, EPA never approved the fully automated TKN method for compliance reporting and the Technicon TKN digester is no longer available commercially.

¹ <https://www.epw.senate.gov/water.pdf>, Secs 301(a), 304 (h), and 501(a), accessed February 10, 2017

² Code of Federal Regulations, Protection of the Environment, Title 40, Chapter 1, Subchapter D, Part 136.3

³ <https://www.epa.gov/sites/production/files/documents/rivers1.pdf>, accessed February 10, 2017

⁴ <http://www.asaanalytics.com/total-nitrogen.php>, accessed February 10, 2017

⁵ <http://www.hach.com/quick-search-quick-search.jsa?keywords=simplified+TKN+epa>, accessed February 10, 2017

⁶ Kjeldahl, J., A new method for the determination of N in organic substances, *Zeitschrift fur Analytische Chemie*, 22 (1): 366-383

⁷ https://www.epa.gov/sites/production/files/2015-08/documents/method_351-2_1993.pdf, accessed February 10, 2017

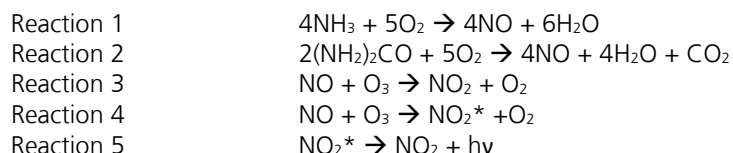
⁸ Ferrari Andres, Nitrogen Determination by a Continuous Digestion and Analysis System, *Annals of the New York Academy of Science*, 792

The United States Geological Survey National Water Quality Laboratory (USGS NWQL) in Denver, Colorado reported that TKN data should not be reported below about 0.2 mg/L.⁹ Ambient water criteria established for the State of Florida recommends maximum total nitrogen concentrations ranging from 0.24 mg/L – 1 mg/L depending on water type and location.¹⁰ Environmental scientists need a method that measures total nitrogen directly, has a low enough detection limit for ambient water quality monitoring, a large dynamic range allowing analysis of clean and polluted samples in one batch, and does not experience a high degree of carryover (contamination) from sample to sample.

■ ASTM D8083-16: A New Method for Total Nitrogen and TKN

This new method couples the Shimadzu TOC-L (Figure 1) High Temperature Catalytic Oxidation (or Combustion) Total Organic Carbon (TOC) analyzer, such as described in ASTM D7573¹¹ or Standard Methods 5310B¹² with the Shimadzu TNM chemiluminescent nitrogen detector. The combustion temperature is 720 °C. Zero carbon air carrier gas provides oxygen to support combustion and to the ozone generator of the nitrogen detector. The sample stream passes through a thermoelectric cooler immediately after exiting the combustion tube.

Nitrogen containing compounds in the sample introduced into the combustion tube convert to nitrogen monoxide (reactions 1 and 2). Nitrogen gas in the carrier gas (air) does not interfere. The carrier gas containing the nitrogen monoxide (NO) passes through a thermoelectric cooler. The cooled and dehumidified gas then enters the chemiluminescence analyzer where the NO reacts with ozone (O₃) and converts to a combination of nitrous oxide (NO₂) and excited nitrous oxide (NO₂^{*}) (reactions 3 and 4). As the NO₂^{*} returns to the ground state it emits radiation, which is measured photo-electrically (reaction 5). The detector signal generates a peak that is proportional to the nitrogen concentration in the sample.



The chemical reactions shown above do not portray the actual mechanism for combustion; they merely indicate the initial reactants and the final products. In most cases, reactions involve a sequence of steps that depend on temperature, pressure, and other factors. For oxidation to occur, the reactants must collide and have sufficient energy to cause the reaction to occur.

Catalytic combustion is the oxidation of combustibles on a catalytic surface without a flame. Catalytic combustion lowers the required temperature needed by providing a surface on which collisions happen. The catalyst is a substance that promotes the reaction but is not one of the reactants or a final product. In other words, the catalyst provides an alternative way for the reaction to occur and makes it possible for the reaction to occur at lower temperatures than if there was no catalyst.

■ Description of the Method

The Shimadzu TOC-L with TN module converts all nitrogen compounds to NO at 720 °C. The instrument uses an auto-sampler to automatically add a small amount of acid to ~50 micro-liter aliquot of sample and inject it onto a platinum catalyst inside the heated combustion chamber. After reaction with ozone, the quantitation is by chemiluminescence. A 1000 mg/L nitrogen stock standard is prepared from ammonium sulfate and potassium



Figure 1: Shimadzu TOC-L with TN Module

⁹ http://pubs.usgs.gov/sir/2012/5281/sir12_5281.pdf, accessed February 10, 2017

¹⁰ <http://cfpub.epa.gov/wqsets/nnc-development/>, accessed February 10, 2017

¹¹ ASTM D7573-09 Standard Test Method for Total Carbon and Organic Carbon in Water by High Temperature Catalytic Combustion and Infrared Detection, ASTM International, West Conshohocken, PA, 2009,

¹² <http://www.standardmethods.org/store/ProductView.cfm?ProductID=38>, accessed February 10, 2017

nitrate. The instrument automatically calibrates from a single 10 mg/L N solution to establish a multiple point calibration curve from 0.2 – 10 mg/L N. The instrument automatically dilutes (or injects less sample aliquot) off-scale peaks, enabling quantitation up to 500 mg/L. Concentrations higher than 500 mg/L N are diluted manually. Total analysis time, per injection, is 2 – 5 minutes. The Method Detection Limit (MDL) is 0.05 mg/L N.

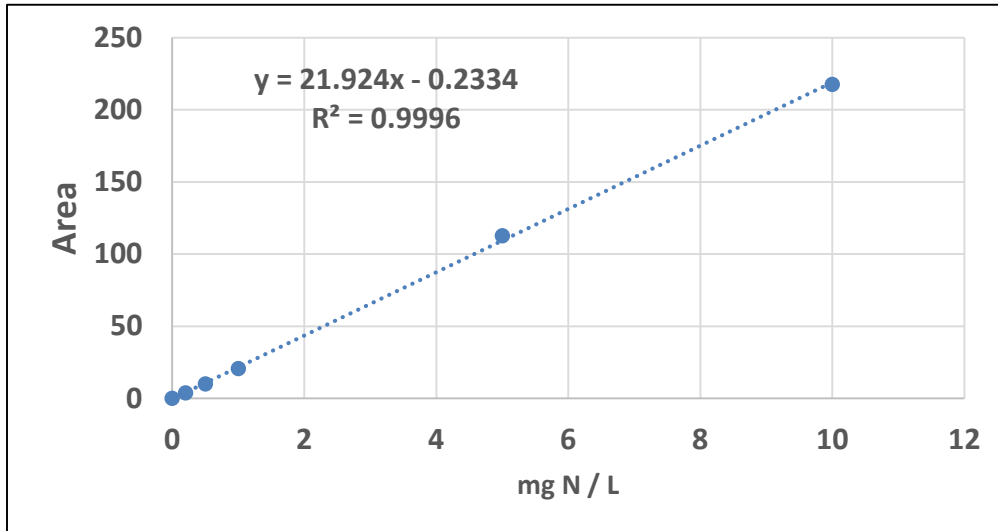


Figure 2: Calibration of Total Nitrogen by ASTM D8083-16

A calibration curve (Figure 2) was prepared using the mixed (NO₃-N + NH₄-N) calibrant and a series of inorganic and organic nitrogen compounds at 100 mg/L N analyzed for recovery (Figure 3). All compound recoveries were well within 90 – 110%.

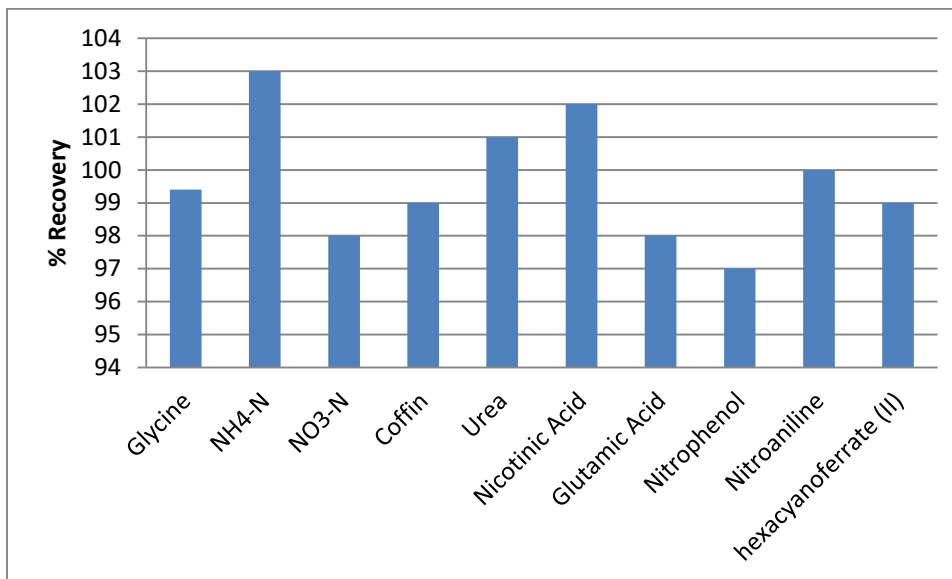


Figure 3: Recoveries of Various Nitrogen Compounds

■ Recommended Furnace Temperature and Simultaneous TOC

Furnace temperature was tested beginning at 680 °C and ramping upwards comparing the response of nitrate nitrogen and ammonia nitrogen. Between 720 °C and 730 °C, the responses were within 4 % of each other (Figure 4) so TN requires a furnace temperature of 720 °C. Other manufacturers use higher temperatures. The lower 720 °C temperature extends the life of the catalyst and combustion tube. For instance, when combusted at

975°C, salts melt and form a hard crust, but when combusted at 730 °C, salts remain friable¹³. As shown in Figure 3, the 720°C temperature is sufficient to quantitatively recover all nitrogen as NO. In addition, the operating temperature between 700 – 750 °C avoids the melting of Sodium Chloride (NaCl has a melting point of 801 °C) and at the same time minimizes loss of NO. Below 700 °C the loss of NO increases rapidly¹⁴.

ASTM D8083-16 includes in Appendix X1 two lab data for total organic carbon (TOC) analyzed at the 720 °C combustion temperature. Recoveries ranged from 100% at the higher concentrations to 119% at lower concentrations, demonstrating that you can analyze TOC and TN simultaneously with this new ASTM method.

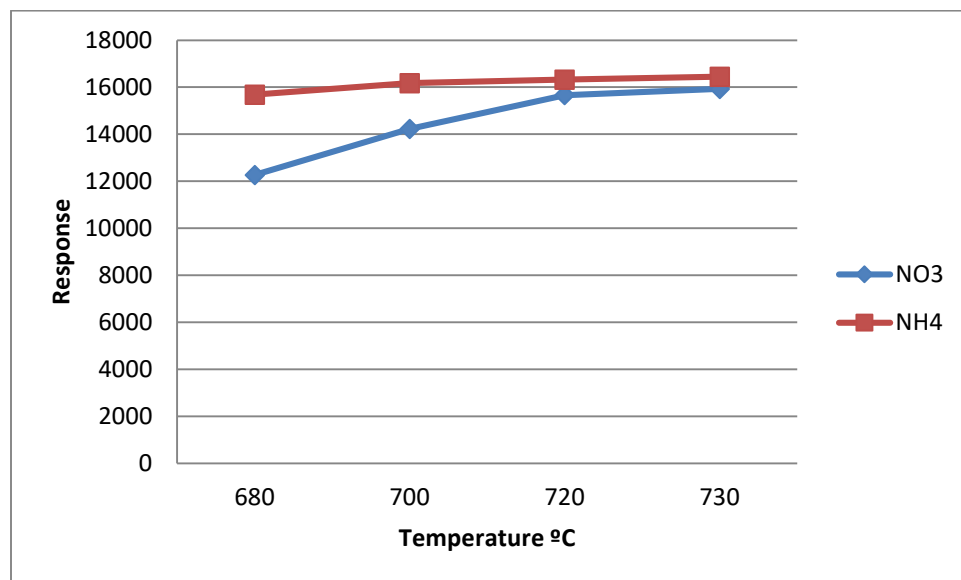


Figure 4: Nitrogen response with increasing temperature

■ Sample Preservation and Storage

Samples are collected in 40-milliliter vials containing 0.5 milliliters of 1 M H₂SO₄ and then checked with pH paper to verify the pH ≤ 2. If the pH is not less than 2, add more H₂SO₄. Refrigerate the samples at ≤ 6°C and analyze within 28 days. The 40-milliliter vials fit directly on the auto-sampler. Use larger sample containers and transfer to auto-sampler vials if necessary.

■ Interferences

Samples containing more than 500 mg/L suspended solids (TSS) could suffer poor precision. Homogenize samples prior to analysis and use the optional stirrer. The optional magnetic stirrer agitates the sample in the vials to prevent the settling of suspended solids. Magnetic stirrers at the measurement position and subsequent measurement position thoroughly agitate the samples prior to measurement. For very turbid samples, use the Oct-L 8 port sampler (Figure 5), placing the sample container with stirring magnet inserted directly on a bench-top magnetic stirrer.

Make sure to preserve samples with acid. Preservation with acid prevents negative bias from organic carbon (Figure 6).



Figure 5: TOC-L with Oct-L 8-Port Sampler

¹³ Gordon, Donald C., and Sutcliff, William H. Jr; *A New Dry Combustion Method for the Simultaneous Determination of Total Organic Carbon and Nitrogen in Seawater*, Marine Chemistry 1 (1973), 231-244

¹⁴ Hansel Dennis A., *Results and observations from the measurement of DOC and DON in seawater using a high temperature catalytic oxidation technique*, Marine Chemistry, 41 (1993), 195-202

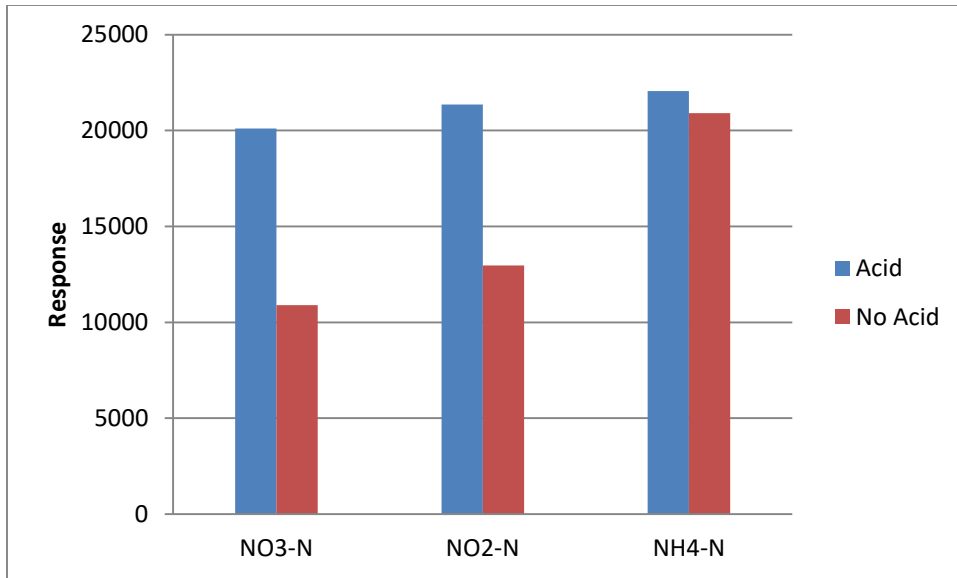


Figure 6: Mitigation of carbon interference on nitrogen response by acidification

With a 720 °C combustion temperature, and standards prepared from an equal concentration of nitrate and ammonia nitrogen, recoveries fall within 90 – 110%. Dissolved salts up to a conductivity of over 50,000 µmhos/cm do not interfere (Figure 7).

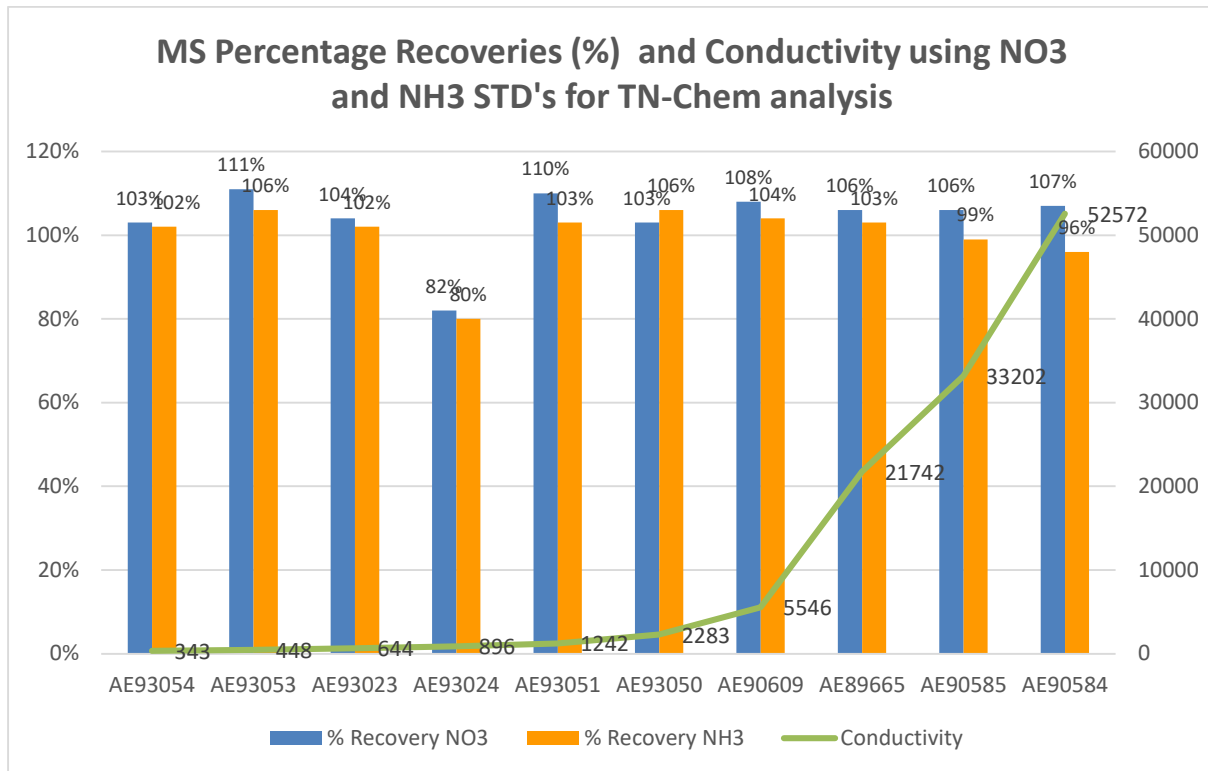


Figure 7: Recovery of TN matrix spikes in samples with increasing conductivity (TDS)

■ Comparison of ASTM D8083-16 TN with Existing EPA Methods

The City of Tacoma Washington obtained/validated an Alternative Test Procedure (ATP) for the “determination of Total Nitrogen by combustion and chemiluminescence” using a Shimadzu HTCC analyzer¹. This ATP is essentially equivalent to the new ASTM D8083-16 method. A comparison of the data (Figure 8) found the EPA calculated TN and the TN by the proposed method to be statistically the same.

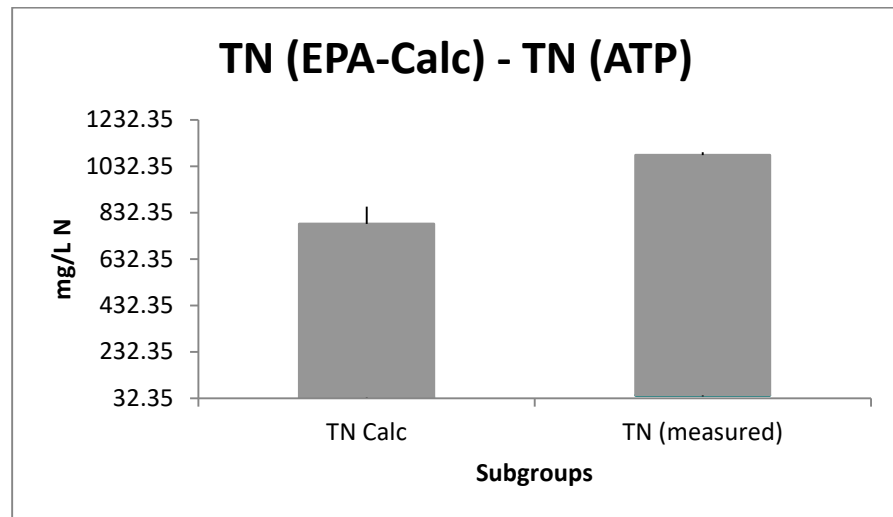


Figure 8: Comparison of EPA calculated TN with proposed method (WWTP)

In these samples ammonia nitrogen (a component of the TKN test) twice exceeded TKN. Since ammonia nitrogen is a part of TKN, the result can be equal to but never higher than TKN. A low TKN caused the EPA-calculated TN values to be lower than the NH₃-N. Measuring TN using the ATP corrected the problem (Table 1).

Table 1: City of Takoma TN data in mg/L N

TKN	NOx-N	TN (EPA-calc)	NH ₃ -N	TN (ATP)
858	0.027	858	853	1078
790	0.077	790	910	1083
776	0.211	776	922	1093
35.4	0.565	36.0	19.8	40.5
34.8	0.482	35.2	31.2	44.0
33.4	0.625	34.0	22.9	44.3
35.6	0.701	36.3	32.5	46.5

According to the ATP, the Total Dissolved Solids (TDS) of the samples with low TKN exceeds 10,000 mg/L. High TDS is a known interference with TKN¹⁵ causing the loss of nitrogen during the digestion. Scientists and wastewater treatment plant operators are often interested in organic nitrogen. CFR 40 Part 136.3 Table 1b defines organic nitrogen as TKN – (NH₃-N). The Takoma ATP and ASTM D8083-16 define organic nitrogen as TN – (NOx-N) – (NH₃-N). For the samples with NH₃-N higher than TKN, the EPA-calculated organic nitrogen is significantly less than zero. We evaluated the EPA calculated and the ASTM D8083-16 calculated organic nitrogen results (Table 2) and found them statistically different (Figure 9). These organic nitrogen results indicate that, for these samples, the ASTM D8083-16 method (and the equivalent ATP) is superior for the determination of organic nitrogen.

Table 2: Comparison of EPA organic nitrogen and HTCC organic nitrogen (mg/L)

EPA Organic Nitrogen	HTCC Organic Nitrogen
5	225
-120	173
-146	171
15.6	20.7
3.6	12.8
10.5	21.4
3.1	14

¹⁵ Kirk; Kjeldahl Method for Total Nitrogen; Anal. Chem. Volume 22, No. 2, 1950; pp 354-358

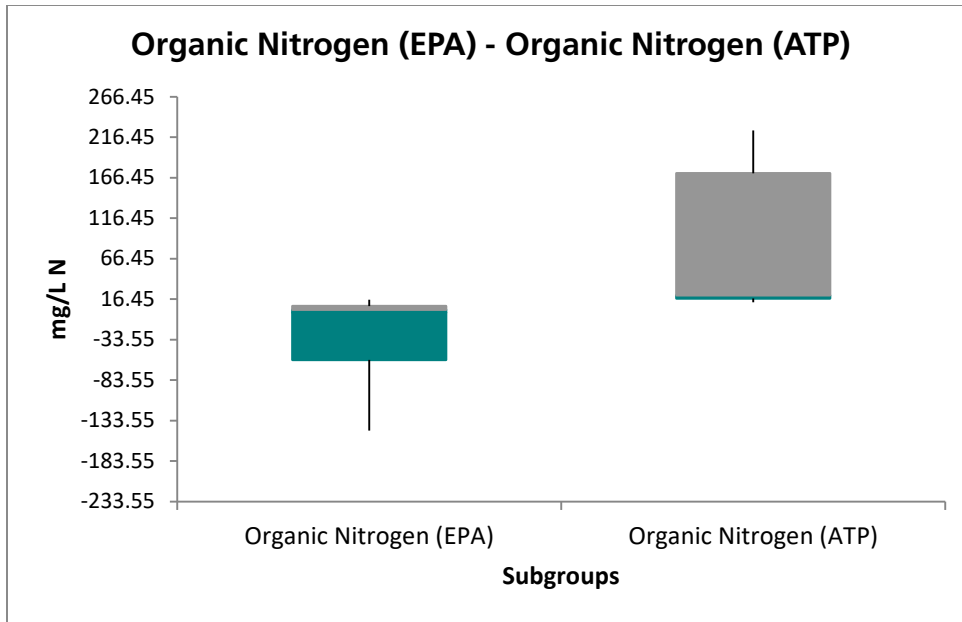


Figure 9: Statistical Analysis of Organic Nitrogen by the EPA calculation and the HTCC calculation

Standard Methods for the Examination of Water and Wastewater Part 4000 is also conducting testing on Total Nitrogen by the High Temperature Catalytic Combustion (HTCC) with Chemiluminescence Detection technique. The Standard methods committee compared TN (EPA-calculated) and TN (HTCC) on 52 effluent samples. We conducted a T test and found data for the 52 samples (one outlier) are statistically the same (Figure 10).

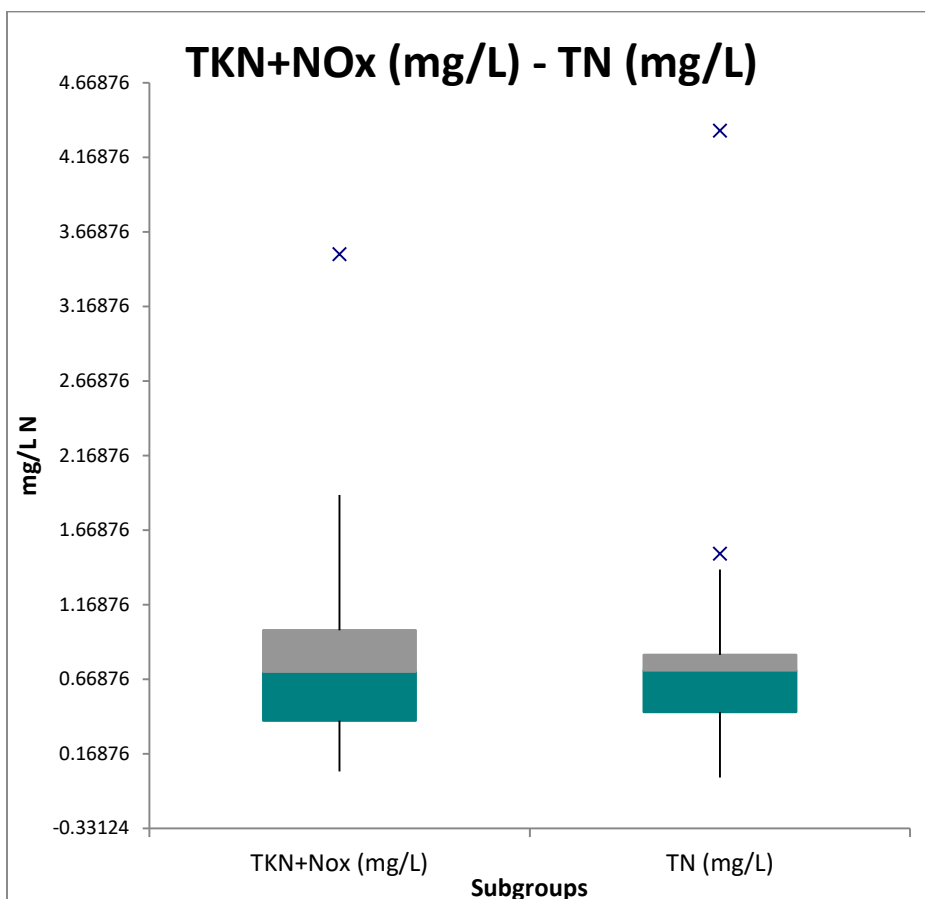


Figure 10: Statistical Analysis of 52 TN samples by the EPA calculation and the HTCC methods

■ Inter-laboratory Study Data

The ASTM D19.06 task group validating ASTM D8083-16 conducted an inter-laboratory study in accordance with ASTM Practice D2777 – 13¹⁶ with 8 operators in 7 laboratories using reproducible synthetic matrices, POTW matrices, and a pulp and paper effluent. Laboratories received each matrix, with the exception of a Laboratory Control Sample (LCS), as blind samples. The inter-laboratory study included three Youden pairs plotted in Figure 11. The tight grouping of pairs 1, 2 and 3 demonstrate good agreement of results between labs. Each laboratory analyzed the LCS shown in Table 3. Table 4 lists the statistical summary for all the inter-laboratory study results.

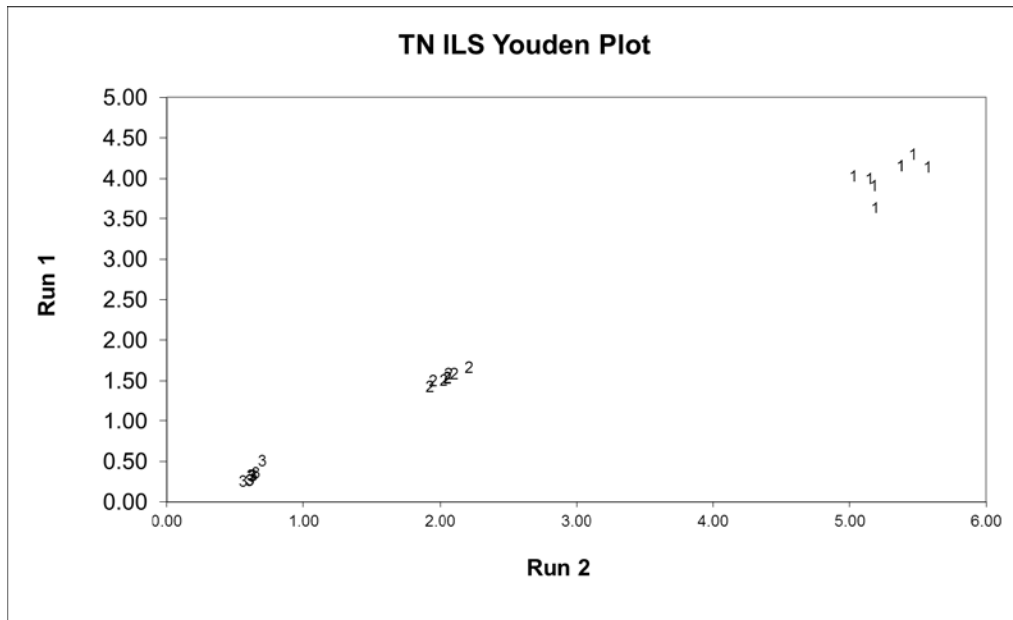


Figure 11: ASTM D8083-16 Youden Pairs

Table 3: Statistical Summary for the Laboratory Control Sample (LCS)

Number of useable values	32
True concentration (mg/L)	3.92
Mean Recovery (mg/L)	3.90
% Recovery	99.4
Overall Relative Standard Deviation (%)	5.7

¹⁶ ASTM D2777-13 Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water, ASTM International, West Conshohocken, PA, 2013.

Table 4: Summary of lab results reported as TN in mg/L

Matrix	Source of N	Known N (mg/L)	Found N (mg/L)	% Recovery	% RSD (multiple operator)	% RSD (single operator)
3 grams Instant Ocean® ¹⁷ per Liter (3000 mg TDS/L)	Glycine	5.00	5.15	103	3.56	1.49
		4.00	4.04	101	4.89	0.93
0.5 grams Instant Ocean® per liter (500 mg TDS/L)	Nicotinic Acid	2.00	1.93	97	4.71	1.20
		1.61	1.54	96	4.75	1.06
ERA ¹⁸ Ready-to-Use WasteWatR™ (CAT # 741 or equivalent)	Glycine	0.514	0.496	97	6.03	2.81
		0.313	0.303	97	12.2	4.56
ERA Ready-to-Use WasteWatR™ (CAT #505 or equivalent)	Mix of NH ₃ -N and NO ₃ -N	10.0	9.70	97	7.94	1.99
ERA Solids WP (CAT # 241 or equivalent) (contains TSS)	NH ₃ -N	30.0	28.5	95	9.50	2.98
WWTP influent (~300 ppm TSS)	unknown		29.6		7.74	2.71
WWPP effluent (~15 ppm TSS)	unknown		4.41		11.5	6.80
Pulp and Paper effluent (~500 ppm TSS)	unknown		9.30		31.0	3.42
WWTP aeration basin (~5000 ppm TSS)	unknown		339		37.9	6.76

Precision for the synthetic samples was, as expected, very good. These synthetic samples contained known, reproducible, matrices to enable laboratories to use them in their own internal validations. The “real-world” matrices (Figure 12) contained unknown nitrogen sources and high concentrations of Total Suspended Solids (TSS). As expected, precision suffers with higher TSS. Single operator precision is about three times less than the multiple operator precision for most matrices. The larger difference in single operator precision and multiple operator precision for the samples with the higher TSS indicates between lab sample variability.

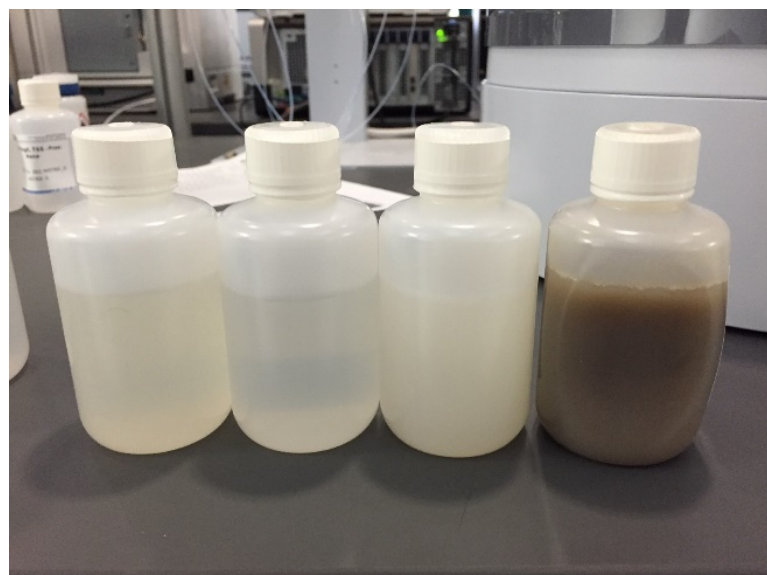


Figure 12: “Real World” samples from the ASTM D8083-16 Inter-laboratory Study

¹⁷ <http://www.instantocean.com/>

¹⁸ <http://www.eraqc.com/>

■ Conclusion

ASTM D8083-16 is a new method for the determination of total nitrogen, and TKN by calculation, in water samples. The method compares favorably with the current EPA approved calculated total nitrogen in samples without interferences. ASTM D8083-16 saves laboratories time, uses less reagent and smaller sample volumes, and overcomes several known issues with current TKN and TN methods.



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