Designation: D8001 – 16$^{\varepsilon 1}$

Standard Test Method for Determination of Total Nitrogen, Total Kjeldahl Nitrogen by Calculation, and Total Phosphorus in Water, Wastewater by Ion Chromatography[1]

This standard is issued under the fixed designation D8001; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Editorial changes were made throughout in September 2016.

1. Scope

1.1 This test method is applicable for the analysis total nitrogen (organic nitrogen + ammonia-N + nitrate-N + nitrite-N) as nitrate and total phosphorus as orthophosphate in unfiltered water samples by alkaline persulfate digestion followed by ion chromatography (IC).

1.2 Total Kjeldahl nitrogen (TKN) is determined by the calculation. To determine TKN subtract the nitrate-N and nitrite-N in a digested sample from a non-digested sample (see Section 4, Summary of Test Method).

1.3 The limit of detection (LOD), limit of quantitation (LOQ), and reporting range in Table 1 are based on the two-step process for this test method: digestion and analytical step. Because the digestion step requires a sample dilution, the LOD and LOQ are higher than undigested samples. The reporting range, LOD, and LOQ can be modified and perhaps improved depending on several factors (see Section 6, Interferences).

1.4 The method detection limits (MDL) are shown for reference. The digestion reagent contains background nitrate and results in higher detection limits. MDL will be shown after the interlaboratory study (ILS) is completed.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water
D4327 Test Method for Anions in Water by Suppressed Ion Chromatography
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
D5810 Guide for Spiking into Aqueous Samples
D6299 Practice for Applying Statistical Quality Assurance and Control Charting Techniques to Evaluate Analytical Measurement System Performance
D6792 Practice for Quality System in Petroleum Products and Lubricants Testing Laboratories

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *total Kjeldahl nitrogen (TKN), n*—the sum of organic nitrogen plus ammonia (NH_3).

3.2.2 *total nitrogen (TN), n*—the sum of all nitrate, nitrite, ammonia, and organic nitrogen, as N, in water or wastewater samples.

3.2.3 *total phosphorus (TP), n*—the sum of orthophosphates, polyphosphates, and organically bound phosphates, as P, in water or wastewater samples.

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

TABLE 1 Calibration, Linearity, Limits of Detection, and Quantitation from the Single Lab Validation Study

Analyte	Calibration Range (µg/L)	Linearity[A] (r^2)	System		Digested Sample	
			LOD[B] (µg/L)	LOQ[C] (µg/L)	LOD (µg/L)	LOQ (µg/L)
Nitrite-N	2.5–300	0.9999	0.76	2.5	—	—
Nitrate-N†	2.5–300	0.9999	1.0	3.4	171[D]	171
Phosphate-P	2.5–300	0.9998†	1.3	4.2	19.5[E]	63[E]

† Editorially corrected.

[A] Ten calibration levels, each injected in duplicate.
[B] LOD calculated as 3 × S/N.
[C] LOQ calculated as 10 × S/N.
[D]

$$\text{Nitrate MDL}_b = A + t_{(n-1,\,1-\alpha=0.99)} S_b$$

where:

A = the average method blank concentration,
$t_{(n-1,\,1-\alpha=0.99)}$ = the student's t-value for the single-tailed 99th percentile t statistic a standard deviation estimate with $n-1$ degrees of freedom, and
S_b = the sample standard deviation of the replicate blank analyses.

[E] Phosphate LOD/LOQ was calculated based on a dilution factor of 15× relative to the system concentrations.

4. Summary of Test Method

4.1 A water sample is digested with alkaline persulfate at a 2:1 ratio, the initial pH is >12. This sample is heated at 120°C for 60 min. Initial alkaline conditions oxidize dissolved/suspended nitrogen to nitrate. Over time the solution becomes acidic according to the following calculation:

$$S_2O_8^{2-} + H_2O \rightarrow 2HSO_4^- + \frac{1}{2}O_2 \quad (1)$$

The acidic conditions (pH ~2) result in the hydrolysis of dissolved/suspended phosphorus to orthophosphate.

4.2 The determinative step using IC is equivalent to Test Method D4327.

5. Significance and Use

5.1 This test method allows the simultaneous determination of total nitrogen and total phosphorous from one sample digestion step.

5.2 This test method measures oxidized ammonia and organic nitrogen (as nitrate) and soluble nitrate simultaneously. By subtracting the nitrate + nitrite value from a non-digested sample gives a TKN:

$$TN = TKN + (NO_3\text{-}N) + (NO_2\text{-}N) \quad (2)$$

$$TKN = NH_3\text{-}N + \text{Organic N} \quad (3)$$

When using this test method:

$$TKN = \text{Digested Sample} - \text{Non-Digested Sample} \quad (4)$$

$$TKN = TN - [NO_3\text{-}N + NO_2\text{-}N] \quad (5)$$

where:

TN = total nitrogen, and
TKN = total Kjeldahl nitrogen.

6. Interferences

6.1 Interferences can be caused by substances with similar ion chromatographic retention times, especially if they are in high concentration compared to the analyte of interest. Following digestion, samples contain high concentrations of sulfate that can cause column overloading and obscure nitrate and phosphate peaks. The use of columns with high capacity is required to overcome these limitations.

6.2 Samples high in chloride from brackish, seawater and brines may also result in column overloading. Chloride is also oxidized to chlorate during the digestion step, and thus contributes to depletion of the persulfate digestion reagent. These can either be diluted or pre-treated to remove excess chloride. Pretreatment using Ag^+ precipitation or the use of Cl^- removal cartridges are accepted for this test method. Dilution will increase the detection limits for total nitrogen and phosphate. The use of pretreatment cartridges may remove particulates if performed prior to the digestion step, giving a possible negative bias.

6.3 If very low µg/L concentrations are required, blank subtraction may be used provided the spike recoveries meet the methods detection limits. Approximately 92.5 µg/L nitrate were found in the potassium persulfate digestion chemical. This test method provides an MDL calculation where a peak is found in the blank samples/digestion reagent. (See Section 13.)

6.4 High levels of organic carbon concentrations greater than 800 mg/L of TOC, reducing agents, reduced forms of metals, etc. will consume the oxidative reagent that may limit oxidation of reduced nitrogen and phosphorous. (See Fig. 6 and Table 6.)

7. Instrumentation

7.1 *Digestion Step*—Many techniques exist for heated digestion of water samples. Regardless of the instrumentation used, such as UV or microwave, the digestion must proceed long enough to consume all persulfate.

7.1.1 *Autoclave or heating block or alternative,* capable of 120°C for 60 minutes.

7.2 *Digestion Tubes*—OD × L: 16 × 125 mm disposable glass tubes with screw caps.

7.3 *Analytical balance,* capable of weighing up to 200 g accurately to ±0. 01 g.

7.4 *Pipettes or Volumetric Transfer*—1- and 5-mL Class A volumetric pipettes or calibrated variable volume automatic pipettes fitted with disposable polypropylene tips.

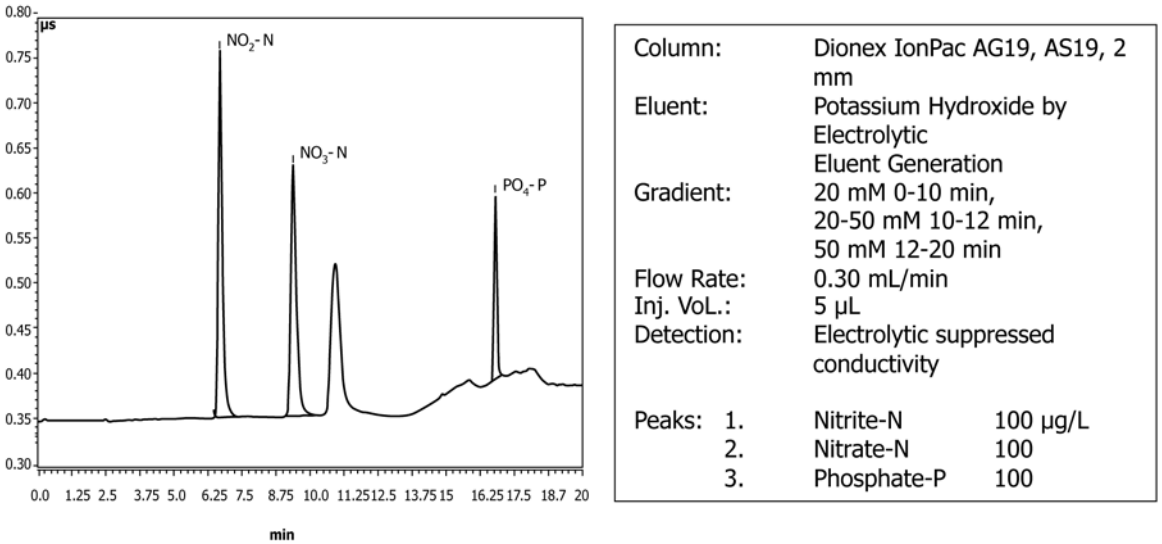
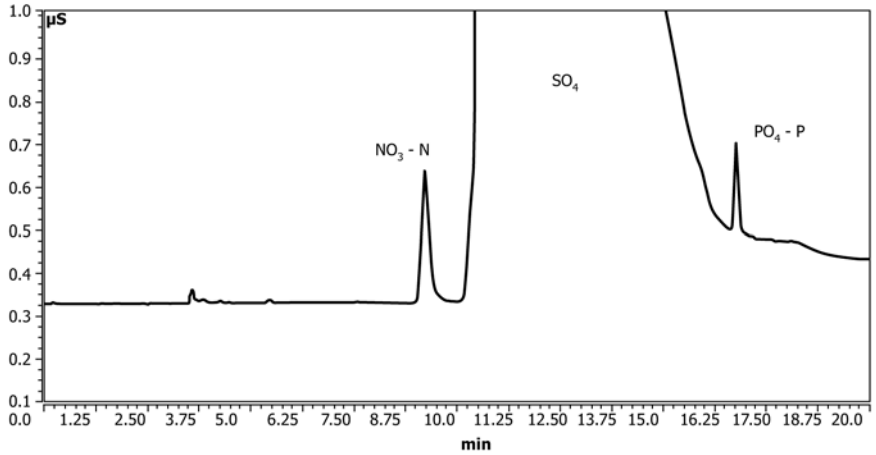


FIG. 1 Separation of Nitrite, Nitrate, and Phosphate Standards in Reagent Water by Ion Chromatography



NOTE 1—See Fig. 1 for chromatographic conditions.

FIG. 2 Separation of a Nitrate (101 µg/L) and Phosphate (105 µg/L) from an Alkaline Persulfate Digested Sample of Glycine and Glycerolphosphate

7.5 *Filter paper,* 0.45 µm, required for removing particulates from samples prior to injection into the ion chromatograph.

7.6 *Volumetric Flasks*—25-, 50-, 100-, and 1000-mL Class A volumetric flasks.

7.7 *Sample collection container,* standard HDPE plastic or glass 100-mL bottle with cap.

7.8 *Sonicator.*

7.9 *Ion Chromatograph*—Analytical system with all required accessories, columns, high-pressure dual piston pump, suppressor, and conductivity detector.

7.9.1 *Injection system,* capable of delivering 5 – 500 µL with a precision better than 1 %.

7.9.2 *Pumping system,* capable of delivering mobile phase flows between 0.1 and 5.0 mL/min with a precision better than 2 %. Due to the corrosive nature of the eluent, a PEEK (polyether ether ketone) pump head is recommended.

7.9.3 *Guard column,* for protection of the analytical column from strongly retained constituents.

7.9.4 *Anion exchange column,* capable of producing satisfactory analyte separation of anions.

7.9.5 *Anion suppressor device,* capable of using electrolytic or chemical suppression technology.

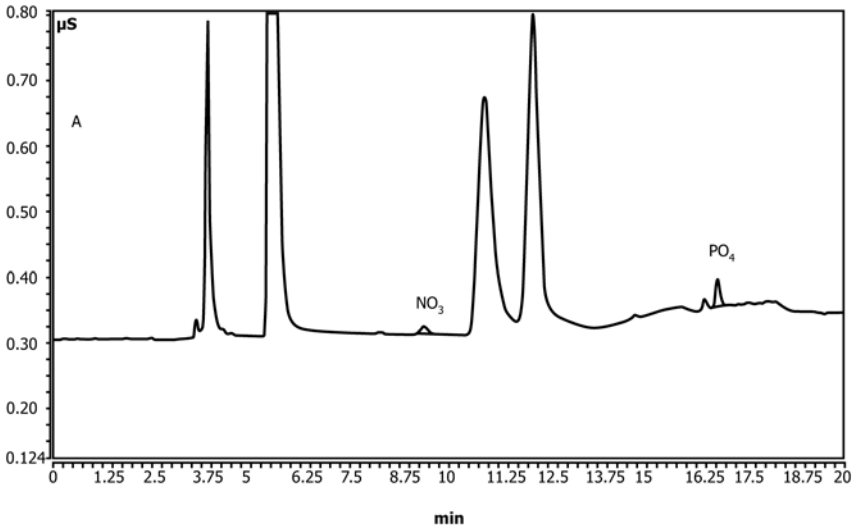
NOTE 1—*Sequential suppressor device*, when using carbonate based eluent, helps reducing background to achieve lower detection levels.

7.9.6 *Conductivity detector,* (low volume), temperature controlled to 0.01°C, capable of at least 0 to 3000 µS/cm or greater on a linear scale.

7.9.7 *Chromatography data system software,* capable of measuring peak areas or peak heights, retention times, and baseline correction capability.

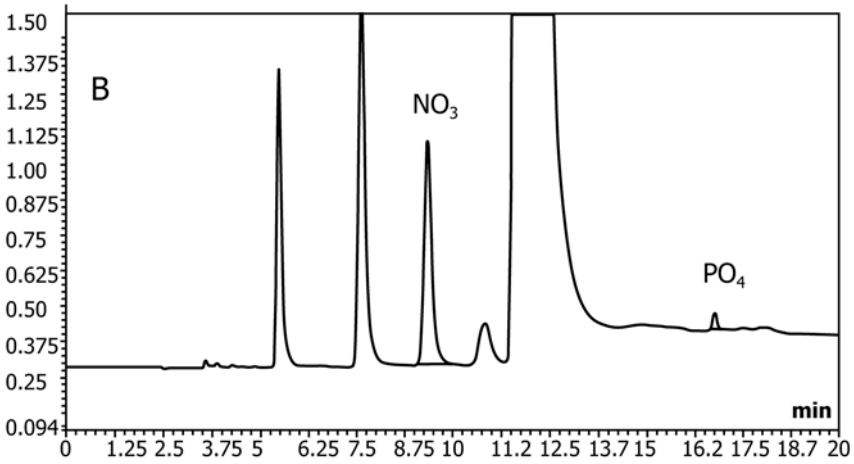
7.10 *Refrigerator,* capable of holding 6°C

7.11 *Borosilicate medicine bottle,* 100 mL.



NOTE 1—See Fig. 1 for chromatographic conditions.

FIG. 3 Separation of Anions, Including Nitrate (5 µg/L) and Phosphate (22 µg/L), from an Undigested, Sewage Sample



NOTE 1—See Fig. 1 for chromatographic conditions.

FIG. 4 Separation of Anions, Including Nitrate (262 µg/L) and Phosphate (30 µg/L) from an Alkaline Persulfate Digested, Raw Sewage Sample

8. Reagents

8.1 *Purity of Reagents*[3]—Reagent grade or higher purity chemicals and water shall be used for the preparation of all samples, standards and eluent solutions. See Specification D1193; type II water should be used.

8.2 *Sodium hydroxide, 1.5 M*—In a 100-mL volumetric flask add approximately 80 mL of filtered degassed deionized (DI) water. Add 8.0 mL of 50 % NaOH solution and swirl to mix. Fill to the mark with DI water, cap, and invert at least three times to mix. Transfer the solution to a polypropylene bottle in which it is stable for six months at 4°C.

8.3 *Alkaline Persulfate Digestion Reagent*—In a 50 mL volumetric flask add 40 mL of DI water. Add 5 mL of the 1.5-M stock NaOH solution followed by 2.0 g of potassium persulfate. Cap and sonicate for 10 min. Bring to mark with DI water, cap, and invert at least three times to mix. Do not heat. This solution should last at least three days if kept refrigerated. Excellent recoveries were achieved even with the formation of a precipitate after a few days as long as care is taken to not transfer any precipitate to the samples prior to digestion.

8.4 *IC Eluent Buffer Solution—Continuous Eluent Generation (optional),* to automatically prepare and purify the eluent used in the ion chromatography. Electrolytic eluent generation and auto-burette preparation of eluent by means of in-line dilution of a stock solution have been found satisfactory for this test method. Other continuous eluent generation devices may be used if the precision and accuracy of the method are not degraded.

[3] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the United States *Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

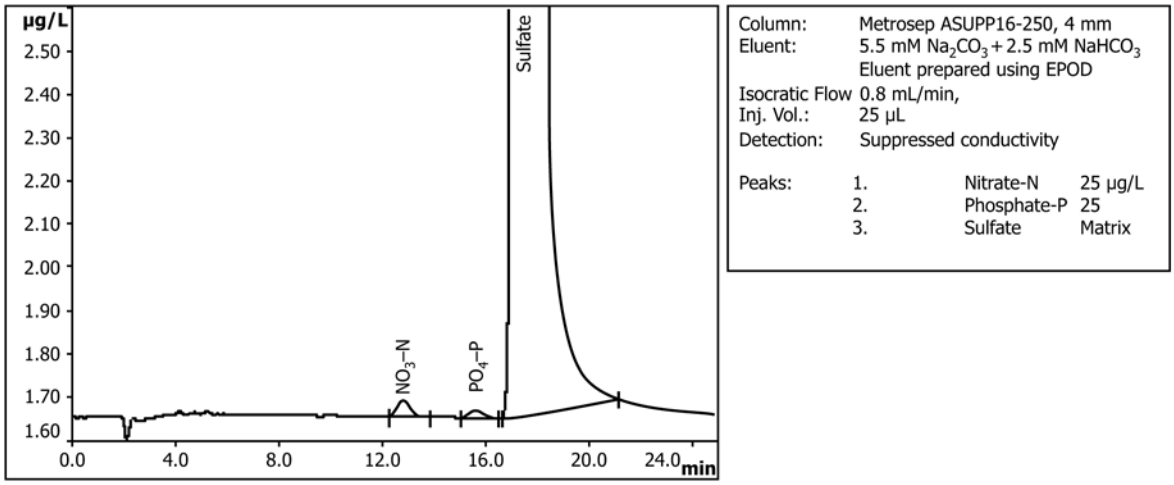
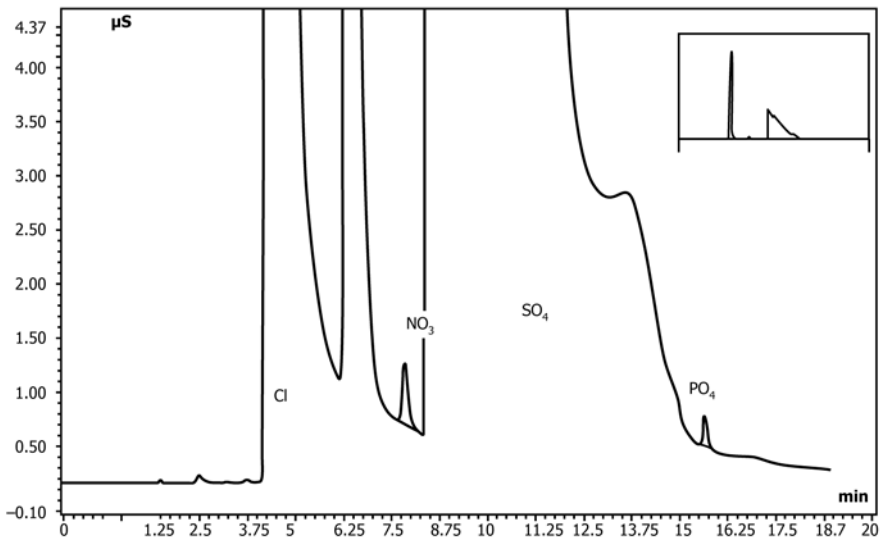


FIG. 5 Isocratic Separation of Nitrate (25 µg/L) and Phosphate (25 µg/L) from an Alkaline Persulfate Digested with Carbonate/ Bicarbonate Eluent



NOTE 1—See Fig. 1 for chromatographic conditions.

FIG. 6 Separation of Nitrate and Phosphate in the Presence of 1000 mg/L Chloride

8.5 *IC Eluent Suppression Anion Suppressor Device,* reduces the background conductivity of the eluent after separation by the anion separator column. Both chemical (sequential) and continuous electrolytic suppressors have been found satisfactory for this test method. Other anion suppressor devices may be used as long as the precision and accuracy of the method are not degraded.

8.6 *Suppressor Regeneration Solution (if needed)*—Prepare 0.5-M Sulfuric Acid Solution by adding 28 mL of concentrated sulfuric acid into 1 L of DI water. Sulfuric acid is not needed when using electrolytic eluent generation. (**Warning—** Solution will get hot, so use proper PPE while preparing this solution.) Alternatively, commercially available 0.5 M sulfuric acid may be used.

9. Preparation of Standard Solutions

9.1 Solutions used to calibrate the IC system:

9.1.1 *Potassium Nitrate Stock Calibrant Solution, 1 mL =1.0 mg-N*—Dissolve 0.72 g of potassium nitrate (KNO_3, FW = 101.1) in about 80 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock calibrant to a 100-mL borosilicate media bottle in which it is stable for 6 months at 4°C (see Note 2).

9.1.2 *Potassium Di-Hydrogen Phosphate Stock Calibrant Solution, 1 mL =1.0 mg-P*—Dissolve 0.44 g potassium dihydrogen phosphate (KH_2PO_4, FW = 136.09) in about 80 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion. Transfer the stock calibrant to a 100-mL borosilicate media bottle in which it is stable for 6 months at 4°C (see Note 3).

9.1.3 *Mixed IC Calibration Stock Solution 10 mg/L N and 10 mg/L P*—In a 100 mL volumetric flask add 1.0 mL of the

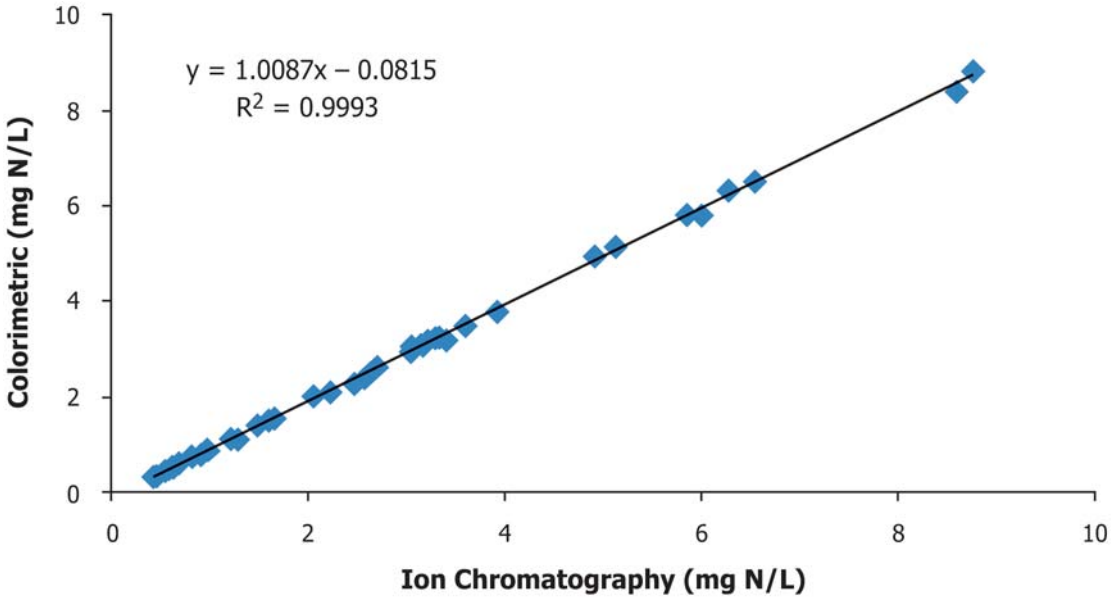


FIG. 7 Graph of Total Nitrate in River Water Samples from Alkaline Persulfate Digested Samples Using Colorimetric (Vanadomolydophosphoric Acid) and Ion Chromatography Determinative Steps

TABLE 2 Concentration Levels and Dilutions for Total N and Total P

Concentration Level	Amount of Mixed Calibration Solution (10 mg/L each P and N)	Final Volume (mL)	NO_3-N Final Concentration (µg/L)	PO_4-N Final Concentration (µg P/L)
1	0.025	100	2.5	2.5
2	0.05	100	5	5
3	0.1	100	10	10
4	0.25	100	25	25
5	0.5	100	50	50
6	1	100	100	100
7	2	100	200	200
8	3	100	300	300

TABLE 3 Spike Recoveries of Digestion Check Standards

Nitrogen Compounds	Expected Conc. (mg N/L)	Found Conc. (mg N/L)	Recovery
Nicotinic Acid	0.1289	0.1291	100.1
Urea	0.1335	0.1274	95.4
Glycine	0.0985	0.0941	95.6
Ammonium Chloride	0.1367	0.1273	93.1
Phosphorous Compounds	**Expected Conc. (mg N/L)**	**Found Conc. (mg N/L)**	**Recovery**
Glucose-1-Phosphate	0.1290	0.1253	97.1
Adenosine Triphosphate	0.1162	0.099	85.2
Phytic Acid	0.1232	0.1052	85.4
Glycerolphosphate	0.1077	0.107	99.3

stock N and 1.0 mL of the stock P solution. Fill to the mark with DI water, cap and mix thoroughly by manual inversion. Prepare this solution fresh each time calibration solutions are prepared.

NOTE 2—Alternatively, commercial stock calibration solutions can be used, provided that the solutions are traceable to primary stock solutions or certified reference materials, and are free from other analytes.

NOTE 3—In case of trace level of phosphorous contamination in reagents, it is highly recommended to matrix match the standards preparation in order to nullify the effect of phosphorous in reagent. (See Section 11 for additional information.)

9.1.4 *Working Calibration Solutions*—Use the amounts in Table 2 to prepare working calibration solutions.

9.2 *Digest-Check Stock Solutions—Total Nitrogen*—(it is recommended to use at least one of the digest check compounds):

9.2.1 *Glycine (1 mL = 1.0 mg-N)*—Dissolve 3.98 g glycine ($C_2H_5NO_2$-HCL, FW = 111.5) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark

TABLE 4 Total Nitrogen and Total Phosphate from a Domestic Wastewater Treatment Plant

Sample	Nitrogen		Phosphorous	
	Undigested (mg N/L)	Digested (mg N/L)	Undigested (mg N/L)	Digested (mg N/L)
Effluent 1	6.52	8.56	2.85	3.20
Primary Effluent 1	0.31	36.52	2.02	3.87
Filtered Effluent 2	11.02	13.01	0.23	0.43
Pre-Effluent 2	11.69	13.43	0.25	0.39
Raw Sewage 2	0.55	39.87	2.19	4.50

TABLE 5 Calculated Total Kjeldahl Nitrogen from Table 4 of Wastewater Samples Collected from a Domestic Wastewater Treatment Plant

Sample	Digested (mg N/L)	Undigested (mg N/L)	Calculated TKN
Effluent 1	8.5	6.52	2.03
Primary Effluent 1	36.52	0.31	36.22
Filtered Effluent 2	11.01	11.02	1.99
Pre-Effluent 2	13.43	11.69	1.75
Raw Sewage 2	39.87	0.55	39.32

TABLE 6 Recoveries of Nitrogen and Phosphorous Test Compounds in the Presence of Increasing Amounts of Chloride

Chloride Conc. (mg/L)	Test Compound	NO_3 or PO_4 Ret. Time (min)	Nominal Conc. (mg N or P/L)	% Rec.
560	Glycine-N	9.10	1.49	104.5
	Glycerolphosphate-P	16.8	1.63	96.2
997	Glycine-N	8.90	1.49	101.2
	Glycerolphosphate-P	16.7	1.63	94.0
1472	Glycine-N	8.70	1.49	87.5
	Glycerolphosphate-P	16.7	1.63	88.9

with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500-mL borosilicate media bottle in which it is stable for 6 months at 6°C

9.2.2 *Nicotinic Acid (1 mL = 1 mg-N)*—Dissolve 0.88 g nicotinic acid ($C_6H_5NO_2$, FW = 123.1) in about 50 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Store this solution at 4°C.

9.2.3 *Urea (1 mL = 1 mg-N)*—Dissolve 0.22 g urea (CH_4N_2O, FW = 60.06) in about 50 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Store this solution at 6°C.

9.2.4 *Ammonium Chloride (1 mL = 1.0 mg-N)*—Dissolve 0.38 g ammonium chloride (NH_4Cl, FW = 53.49) in about 50 mL of DI water in a 100 mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Store this solution at 6°C.

9.3 *Digest-Check Stock Solutions—Total Phosphorous* (it is recommended to use at least one of the digest check solutions):

9.3.1 *Glycerolphosphate Digest-Check Stock Solution (1 mL = 1.0 mg-P)*—Dissolve 4.94 g glycerolphosphate ($C_3H_7O_6PNa_2{\cdot}5H_2O$, FW = 306.1) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500-mL borosilicate media bottle in which it is stable for 6 months at 6°C.

9.3.2 *Adenosine Triphosphate (ATP) (1 mL = 0.1 mg-P)*—Dissolve 0.06 g ATP ($C_{10}H_{16}N_5O_{13}P_3$, FW = 551.15) in about 50 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. The final concentration of this solution was adjusted for the percent water content. Store this solution at 6°C.

9.3.2.1 *Sodium Pyrophosphate (1 mL = 0.1 mg-P)*—Alternative to ATP solution above if desired. Sodium pyrophosphate ($Na_4P_2O_7{\cdot}10H_2O$, MW = 446.06). Dissolve 0.07 g in about 50 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. The final concentration of this solution was adjusted for the percent water content. Store this solution at 6°C.

9.3.3 *Phytic Acid (1 mL = 0.1 mg-P):*—Dissolve 0.06 g phytic acid ($C_6H_{18}O_{24}P_6{\cdot}12Na{\cdot}xH_2O$, FW = 935.91, anhydrous basis) in about 50 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. The final concentration of this solution was adjusted for the percent water content. Store this solution at 6°C.

9.3.4 *Glucose-1-Phosphate: (1 mL = 0.1 mg-P)*—Dissolve 0.12 g glucose-1-phosphate ($C_6H_{11}K_2O_9P{\cdot}2H_2O$, FW = 372.3) in about 50 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Store this solution at 6°C.

9.3.5 *Glucose Digest-Check Stock Solution (1 mL = 1.25 mg-C)*—Weigh 1.6 g glucose in a 500 mL volumetric flask. Fill to the mark with DI water, cap, and mix thoroughly by manual inversion. Transfer the solution to a polypropylene bottle in which it is stable for six months at 6°C. This solution may be added as a carbon source to check for digestion interference.

9.3.6 *Mixed Digest-Check Solution (concentration 4 mg-N/L, 1.6 mg-P/L and 50 mg-C/L)*—Dispense 1 mL of glycine stock solution, 0.4 mL of the glycerolphosphate stock solution, and 10 mL of the glucose stock solution into a 250-mL volumetric flask that contains about 200 mL of DI water. Dilute the contents of the flask to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 250-mL borosilicate media bottle in which it is stable for one month at 6°C.

10. Sampling and Preservation

10.1 Collect samples in accordance with Test Method D4327.

10.2 Analyze the samples as soon as possible after collection. Preservation by refrigeration at 4°C is required for nitrite, nitrate, or phosphate if not analyzed within 24 hrs. Sample stability of refrigerated samples will vary but typically should be analyzed within 48 hrs.

10.3 Filter the digested samples through a prewashed 0.45-m filter prior to analysis to avoid fouling or clogging the resin of the columns.

11. Calibration

11.1 Set up the ion chromatograph according to the manufacturer's instructions. For details on the chromatography conditions see Fig. 1.

11.2 The retention time for each anion is determined by injecting a standard solution containing only the anion of interest and noting the time required for a peak to appear on the chromatogram. Retention times vary with operating conditions and are influenced by the concentration of ion(s) present. Prepare separate standard solutions in accordance with Table 1. Note the time in minutes for each peak to appear on the chromatogram.

11.3 Concentrations other than those listed in Table 1 may be used if they better approximate concentrations expected in the samples.

NOTE 4—If the concentrations of the sample ions of interest are known or estimated, the concentration of standard solutions prepared for instrument calibration may be varied to better approximate or bracket the concentration range of interest. Anions of no interest may be omitted.

NOTE 5—The mid-range combination anion standard may be used to verify resolution of all anions.

NOTE 6—Each analytical curve should be established using only one scale setting. Changing the scale setting may result in a slight change in the slope of the analytical curve.

11.4 The analytical calibration plot shall be verified daily or whenever samples are to be run, or component change or new eluent prior to the analysis of samples to verify the system resolution, calibration, and sensitivity as part of the quality verification process.

11.5 Inject a known volume of each calibration solution from Table 1 into the ion chromatograph, and measure the areas of the peaks corresponding to nitrate and phosphate.

11.6 Construct the calibration plots by plotting the peak area against the nitrate and phosphate standard concentrations. Use linear regression to determine the best straight-line calibration; the plots should each have a linear least squares correlation coefficient of 0.99 or greater. The response factor for each ion, Rf, is the slope of the calibration plot straight line, in mg/L (area count).

NOTE 7—If the plot of the peak area values against the ion concentrations is not linear (the correlation factor should be at least 0.99), the procedure should be checked for errors, and if necessary, the calibration should be repeated.

11.7 Other calibration methods may be used as long as they meet the statistical requirements for the method for MDL, spike recovery, etc.

12. Procedure

12.1 Alkaline persulfate digests are prepared by dispensing samples and alkaline persulfate digestion reagent into disposable glass tube with screw cap in the volume ratio of 2 to 1. For this test method, 4 mL of sample and 2 mL of digestion solution should be used.

12.2 Mix by inversion and place in a heating block for 60 minutes at 120°C. Samples are allowed to cool in the heating block prior to analysis.

12.3 Dilute samples with DI water prior to injection if the expected concentrations exceed the calibration range. For the data presented in Section 14, dilutions were 15×. Different dilutions can be used if the user desires lower detection limits as long as the equivalent results are achieved. Also, any dilution of sample must fall within the calibration range. If not, re-dilute or prepare a calibration range that fits within the dilution range.

12.4 Set up the ion chromatograph according to the manufacturer's instructions.

12.5 The detector ranges are variable. Normal background operating ranges are from 0 to 2 µS/cm when using hydroxide eluents.

12.6 Samples can be injected manually or using an autosampler.

13. Calculation

13.1 TN – N is measured after sample digestion as the molecular weight of N / molecular weight of nitrate.

13.2 TP – P is measured after sample digestion as the molecular weight of P / molecular weight of phosphate.

13.3 LOD is determined as three times the signal to noise from a blank and low calibration standard injections.

$$\mathrm{LOD} = 3 \times S/N \qquad (6)$$

where:

S/N = $(n = 7)$,

Signal = average signal from 7 lowest calibration std. injections, and
Noise = average noise from 7 blank injections.

13.4 *Minimum Detection Limit (MDL):*

$$MDL_s = t_{(n-1,\,1-\alpha=0.99)} S_s \quad (7)$$

where:

t = 3.14, and
S_s = standard standard deviation of the replicate (n = 7) spiked blank sample analyses.

The spiking level should be 2–10 times the estimated MDL. The estimated MDL should be determined using the mean plus 3 times the standard deviation of an n = 7 set of method blanks.

13.4.1 Minimum detection limit for method blanks (MDL_b) used where there is a blank contaminant. In this test method, nitrate is found in the digestion reagent.

$$MDL_b = \bar{X} + t_{(n-1,\,1-\alpha=0.99)} S_b \quad (8)$$

where:

MDL_b = the MDL based on method blanks,
$\bar{X}$ = mean of the method blank results,
$t_{(n-1,\,1-\alpha=0.99)}$ = the Student's t-value appropriate for the single tailed 99th percentile t statistic and a standard deviation estimate with $n - 1$ degrees of freedom, and
S_b = sample standard deviation of the replicate blank sample analyses, n = 7.

A blank subtraction is $MDL_s - MDL_b$.

13.5 LOQ is determined as 10 times the signal to noise from a blank injection and low calibration standard injections.

$$LOQ = 10 \times S/N \quad (9)$$

13.6 Determination of TKN is determined by subtracting the nitrate and nitrite values of an undigested sample from the nitrate value of a digested sample.

$$TKN = (\text{digested}) - (\text{nondigested}) \quad (10)$$

$$[TKN] = [TN] - [NO_3^- + NO_2^-] \quad (11)$$

13.7 Recoveries are used to determine if the check standards are properly digested, ensuring that the organic, bound and non-free nitrate and phosphate are accurately determined.

$$\text{Percent Recovery} = 100[A\,(V_s + V) - B\,V_s]/C\,V \quad (12)$$

where:

A = analyte concentration (mg/L) in spiked sample,
B = analyte concentration (mg/L) in unspiked sample,
C = concentration (mg/L) of analyte in spiking solution,
V_s = volume (mL) of sample used, and
V = volume (mL) of spiking solution added.

13.8 Percent difference is used to determine the difference between the results from separate techniques as shown in Table 7.

$$\text{Percent Difference} = (V1 - V2)/((V1 + V2)/2) \times 100 \quad (13)$$

where:

$V1$ = value obtained in technique 1, and
$V2$ = value obtained in technique 2.

14. Precision and Bias

14.1 This test method is based on a two-step process that includes sample preparation (digestion) followed by analysis using ion chromatography. The analytical step is based on Test Method D4327 and is expected to have similar performance.

14.2 The standard deviation resulting from digestion repeatability studies was less than 3 %.

14.3 Practice D2777 should be used for determination of precision and bias.

14.4 A full interlaboratory study has not been completed. Tables 3-6 show precision and bias results for an intralaboratory study.

15. Quality Assurance and Quality Control

15.1 Confirm the performance of the instrument or the test procedure by analyzing one or more quality check sample(s) after each calibration and on at least each day of use thereafter.

15.2 When quality control (QC)/quality assurance (QA) protocols are already established in the testing facility, these can be used when they confirm the reliability of the test result.

15.3 When there is no QC/QA protocol established in the testing facility, Appendix X1 can be used as the QC/QA system.

15.4 In order to verify that the digestion is working properly, a variety of digestion check standard suggestions are provided using nitrogen and phosphate within this test method.

TABLE 7 Comparison of Total Phosphate in River Water Samples from Alkaline Persulfate Digested Samples using Colorimetric (Vanadomolydophosphoric Acid) and Ion Chromatography Determinative Steps

TP (mg P/L) Colorimetric	TP (mg P/L) Ion Chromatography	% Diff.
0.124	0.128	3.17
0.154	0.162	5.06
0.29	0.362	21.0
0.449	0.662	38.0
0.108	0.101	6.69
0.128	0.133	3.83
0.133	0.143	7.24
0.102	0.11	7.54
0.131	0.126	3.89
0.158	0.157	0.63

Other standards can be used provided that they require a digestion procedure. Free nitrate and orthophosphate alone should not be used, but can be used to develop the linear range, system detection limits, etc.

15.5 In order to be certain that analytical values obtained using this test method are valid and accurate within the confidence limits of the test, the following QC procedures must be followed when running the test. For a general discussion of quality control and good laboratory practices, see Practice D5847 and Guide D3856.

15.6 *Calibration and Calibration Verification:*

15.6.1 Analyze the calibration standards daily prior to analysis to calibrate the instrument as described in Section 11.

15.6.2 Verify instrument calibration for each analytical batch of 10 samples by analyzing a mid-point standard. The recovery should be 80 to 120 % or else corrective actions should be taken.

15.7 *Initial Demonstration of Laboratory Capability:*

15.7.1 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability.

15.7.2 Analyze seven replicates of an independent reference solution containing between 2 to 25 µg/L the spike reference standards. The matrix of the solution should be equivalent to the solution used in the sample study. Each replicate must be taken through the complete analytical procedure.

15.8 *Laboratory Control Samples:*

15.8.1 To ensure that the test method is in control and to verify the quantitative value produced by the test method, analyze a laboratory control sample (LCS) with each batch of samples. It is preferred to use an independent reference material (IRM) within the concentration range of this test method. The observed test result must fall within the control limits specified by the outside source. See Guide D5810 for reference.

15.9 *Method Blank:*

15.9.1 Analyze a method blank with each batch of samples. A laboratory method blank can be prepared using distilled water. The method blank is used to verify the system is running optimally and used to distinguish between the system, the digestion reagent and the actual sample.

15.10 *Matrix Spike (MS):*

15.10.1 To check for interferences in the specific matrix being tested, perform an MS on at least one sample from each batch by spiking an aliquot of the sample with a known concentration of nitrogen and phosphorous. The spike must produce a concentration in the spiked sample 2 to 5 times the background concentration.

15.10.2 The recovery for the test compounds should be between 80 and 110 %. If the recovery is not within these limits, matrix interference may be present in the sample selected for spiking. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results should be qualified with an indication that they do not fall within the performance criteria of the test method.

15.11 *Duplicate:*

15.11.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch. If the concentration in the sample is below the LOQ, then a spiked sample may be used.

15.11.2 Calculate the standard deviation of the duplicate values and compare to the single operator precision from the collaborative study using an *F* test. Refer to 6.5.5 of Practice D5847 for information on applying the *F* test.

15.11.3 If the result exceeds the precision limit, the batch must be reanalyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the method.

15.12 The analyst is permitted certain options to improve the performance of this test method, provided that all performance specifications are met. These options include sample pretreatment to remove interferences. Any time such modifications are made, the initial demonstration of proficiency must be successfully repeated.

16. Keywords

16.1 alkaline persulfate digestion; ion chromatography; nitrate; ortho-phosphate; phosphate; total Kjeldahl nitrogen; total nitrogen; total phosphate

APPENDIX

(Nonmandatory Information)

X1. QUALITY CONTROL

X1.1 Confirm the performance of the instrument or the test procedure by analyzing a QC sample.

X1.2 Prior to monitoring the measurement process, the user of the test method needs to determine the average value and control limits of the QC sample (see Practices D6299 and D6792 and MNL 7[4]).

X1.3 Record the QC results and analyze by control charts or other statistically equivalent techniques to ascertain the statistical control status of the total testing process (see Practices D6299 and D6792 and MNL 7[4]). Any out-of-control data should trigger investigation for root cause(s). The results of this investigation may, but not necessarily, result in instrument re-calibration.

X1.4 In the absence of explicit requirements given in the test method, the frequency of QC testing is dependent on the criticality of the quality being measured, the demonstrated stability of the testing process, and customer requirements. Generally, a QC sample is analyzed each testing day with routine samples. The QC frequency should be increased if a large number of samples are routinely analyzed. However, when it is demonstrated that the testing is under statistical control, the QC testing frequency may be reduced. The QC sample precision should be checked against the ASTM test method precision to ensure data quality.

X1.5 It is recommended that, if possible, the type of QC sample that is regularly tested be representative of the material routinely analyzed. An ample supply of QC sample material should be available for the intended period of use, and must be homogeneous and stable under the anticipated storage conditions. See Practices D6299 and D6792 and MNL 7[4] for further guidance on QC and control charting techniques.

[4] *ASTM MNL 7*, Manual on Presentation of Data Control Chart Analysis, 6th ed., available from ASTM Headquarters.