Designation: D8001 – 16

StandardTest 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.

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)
Nitrate-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	0.9999	1.3	4.2	19.5[E]	63[E]

[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:

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

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:

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

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

When using this test method:

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

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

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

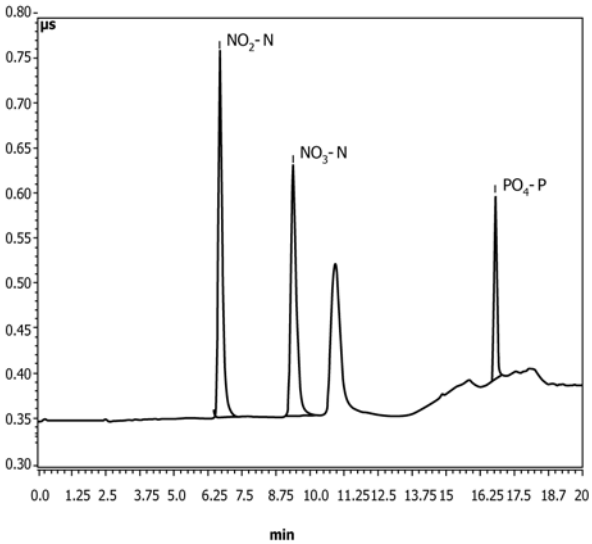
7.1 *Digestion Step*—Many techniques exist for heated digestion of water samples. Regardless of the instrumentation used, such as UV or microwave, the digestion must proceed long enough to consume all persulfate.

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.

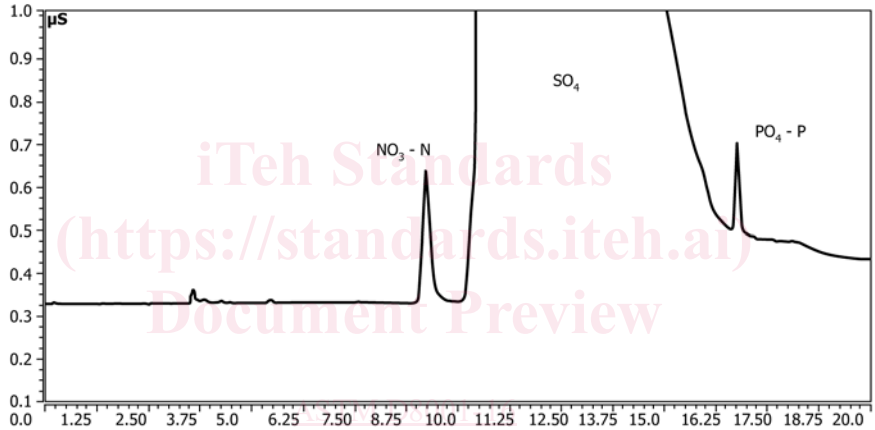
7.3 *Analytical balance,* capable of weighing up to 200 g accurately to ±0. 01 g.

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.



Column:		Dionex IonPac AG19, AS19, 2 mm	
Eluent:		Potassium Hydroxide by Electrolytic Eluent Generation	
Gradient:		20 mM 0-10 min, 20-50 mM 10-12 min, 50 mM 12-20 min	
Flow Rate:		0.30 mL/min	
Inj. VoL.:		5 µL	
Detection:		Electrolytic suppressed conductivity	
Peaks:	1.	Nitrite-N	100 µg/L
	2.	Nitrate-N	100
	3.	Phosphate-P	100

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.

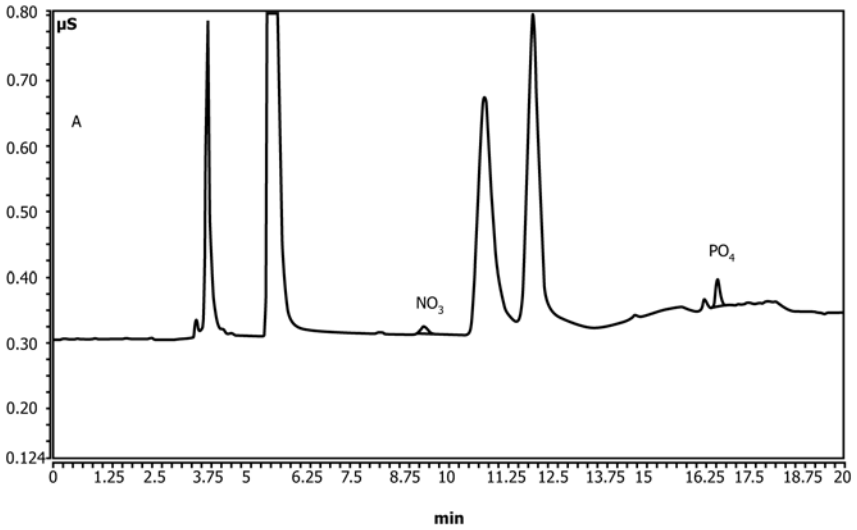
NOTE 1—*Sequential suppressor device*, when using carbonate based eluent, helps reducing background to achieve lower detection levels.

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.

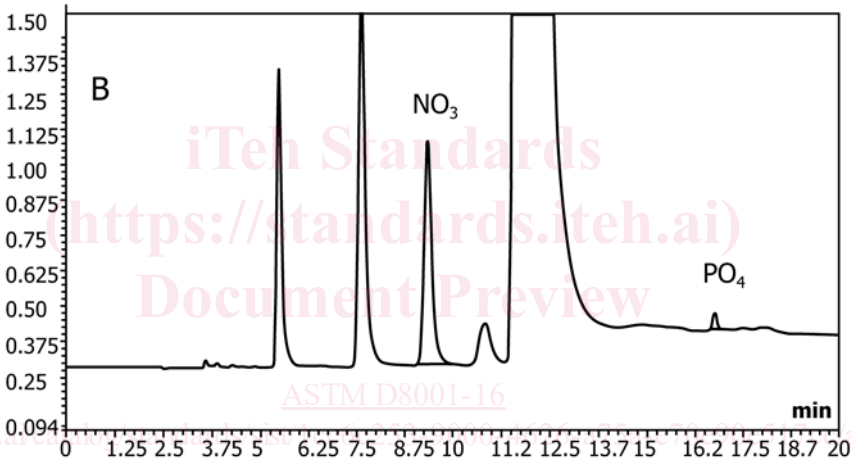
7.10 *Refrigerator,* capable of holding 6°C

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.

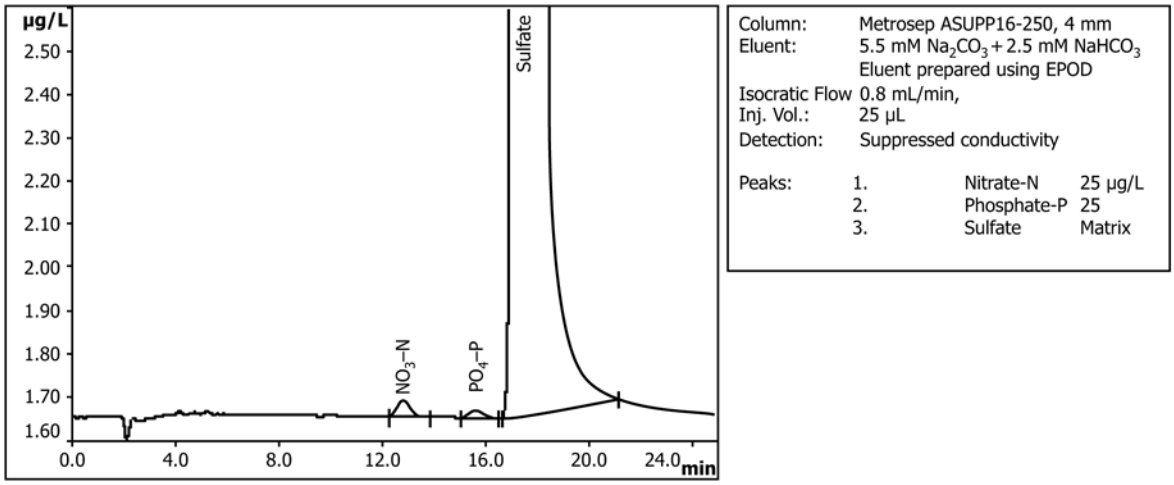
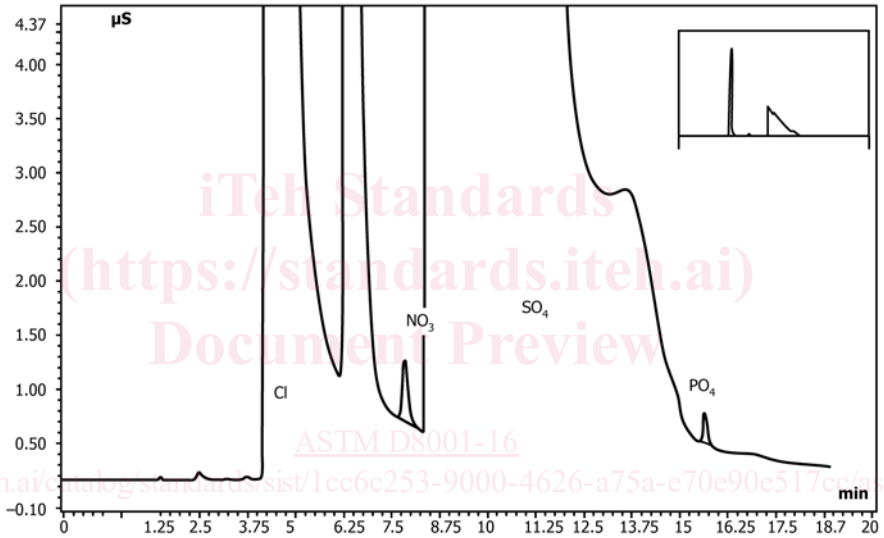


FIG. 5 Isocratic Separation of Nitrate (25 µg/L) and Phosphate (25 µg/L) from an Alkaline Persulfate Digested with Carbonate/ Bicarbonate Eluent



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

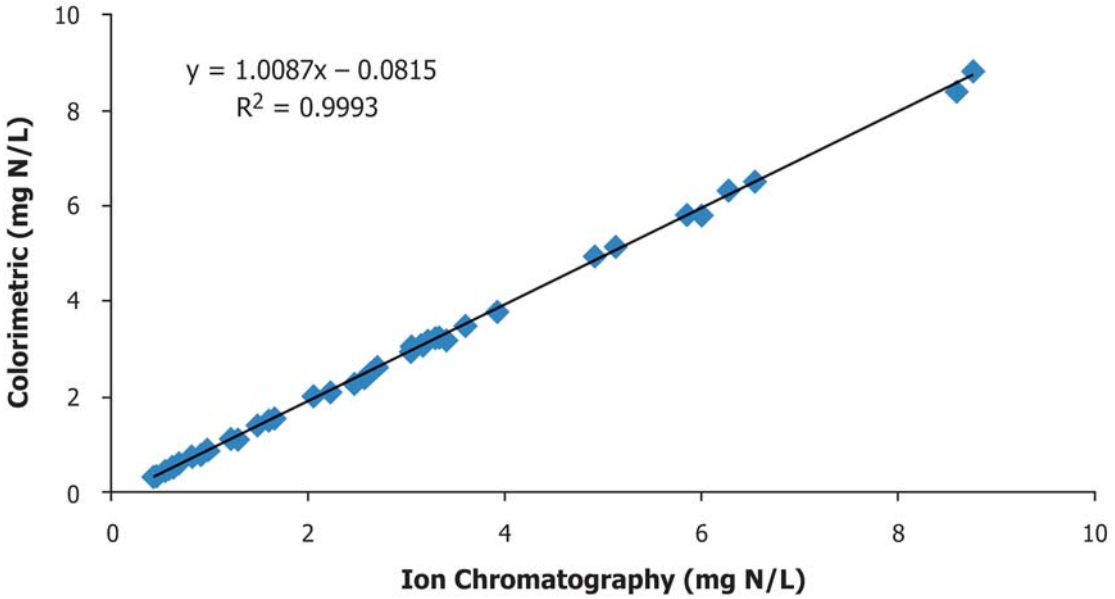


FIG. 7 Graph of Total Nitrate in River Water Samples from Alkaline Persulfate Digested Samples Using Colorimetric (Vanadomolydophosphoric Acid) and Ion Chromatography Determinative Steps

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