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Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Determination of Ammonium Plus Organic Nitrogen by a Kjeldahl Digestion Method and an Automated Photometric Finish that Includes Digest Cleanup by Gas Diffusion

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U.S. Department of the Interior
U.S. Geological Survey

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By Charles J. Patton and Earl P. Truitt

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CONVERSION FACTORS, ABBREVIATED WATER-QUALITY UNITS, AND OTHER ABBREVIATIONS

Multiply	By	To obtain
centimeter (cm)	3.94×10^{-1}	inch
gram (g)	3.53×10^{-2}	ounce, avoirdupois
liter (L)	2.64×10^{-1}	gallon
microliter (µL)	3.38×10^{-5}	ounce, fluid
micrometer (µm)	3.94×10^{-5}	inch
milligram (mg)	3.53×10^{-5}	ounce, avoirdupois
milliliter (mL)	3.38×10^{-2}	ounce, fluid
millimeter (mm)	3.94×10^{-2}	inch
nanometer (nm)	3.94×10^{-8}	inch

Degree Celsius (°C) may be converted to degree Fahrenheit (°F) by using the following equation:

$$°F = 9/5\ (°C) + 32.$$

Abbreviated water-quality units used in this report are as follows:

µg/L	microgram per liter
mg-N/L	milligram nitrogen per liter

Other abbreviations also used in this report:

A/D	analog-to-digital
ASTM	American Society for Testing and Materials
ATP	adenosine triphosphate
FW	formula weight
h	hour
Hz	hertz
ID	identification
LCL	lower control limit
M	molarity (moles/liter)
MDL	method detection level
N	normality (equivalents/liter)
NASQAN	National Stream Quality Accounting Network
NWQL	National Water Quality Laboratory
PC	personal computer
QC	quality control
RSD	relative standard deviation
s	second
sp gr	specific gravity
TKN	total Kjeldahl nitrogen
TP	total phosphorus
UCL	upper control limit
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
v/v	volume/volume
w/w	weight/weight
w/v	weight/volume
≈	nearly equal to
<	less than
≥	greater than or equal to
≤	less than or equal to
±	plus or minus

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ABSTRACT

The National Water Quality Laboratory (NWQL) determined ammonium plus organic nitrogen (Kjeldahl nitrogen) by using semi-automated, block digester methods for filtered and whole-water samples from 1986 until October 1, 1991. During that time, phosphorus was determined by a persulfate digestion method. In 1991, projected increases in demand for both tests by the U.S. Geological Survey's National Water-Quality Assessment Program led the NWQL to develop and validate methods for determining both analytes in a common digest.

This report describes a rapid and accurate method to determine Kjeldahl nitrogen. The batch, high-temperature (block digester), Hg (II)-catalyzed digestion step used in the new methods I-2515-91/4515-91 is similar to U.S. Geological Survey methods I-2552-85/4552-85 and U.S. Environmental Protection Agency method 351.2 except that sample and reagent volumes are halved. Prepared digests are desolvated at 220 degrees Celsius (°C) and digested at 370°C in separate block digesters set at these temperatures, rather than in a single, temperature-programmed block digester. This approach permits 40 calibrants, reference materials, and samples to be digested and resolvated in about an hour. Ammonium ions originally present in samples, along with those released during the digestion step, are determined photometrically by an automated, salicylate-hypochlorite Berthelot reaction procedure at a rate of 90 tests per hour. About 100 microliters of digest are required per determination. The upper concentration level is 10 milligrams per liter (mg/L) with a method detection level of 0.05 mg/L. Repeatability for a sample containing about 4.1 mg/L of Kjeldahl nitrogen in a high suspended-solids matrix is 3.1 percent. Between-day precision for the same sample is 4.8 percent.

A gas diffusion cell in the air-segmented continuous flow analyzer eliminates particulates and ions that otherwise would interfere in the photometric finish. A single-channel analyzer can process the resolvated digests from two pairs of block digesters each hour. Statistical analysis of paired data for about 1,500 samples determined by U.S. Geological Survey methods I-2552-85/4552-85 and I-2515-91/4515-91 during method validation revealed a median concentration difference between the former and the latter methods of about 0.1 mg-N/L. This result was expected because digestion blank concentrations (nearly equal to 0.1 mg/L) were not subtracted from concentrations reported by methods I-2552-85/4552-85. A 10-year record of National Water Quality Laboratory Kjeldahl nitrogen blind blank concentration data also supports a step-change decrease in Kjeldahl nitrogen concentrations of about 0.1 mg/L after methods I-2552-85/4552-85 were replaced by methods I-2515-91/4515-91 on October 1, 1991. Somewhat larger concentration differences between the two

methods were observed for a subset of about 350 samples with nitrate plus nitrite concentrations greater than 1 mg-N/L.

INTRODUCTION

From 1986 until October 1, 1991, the National Water Quality Laboratory (NWQL) determined ammonium plus organic nitrogen (Kjeldahl nitrogen) by using semi-automated, block digester methods I-2252-85 (filtered samples) and I-4551-85 (whole-water samples). These methods are similar to U.S. Environmental Protection Agency (USEPA) method 351.2 (U.S. Environmental Protection Agency, 1993). During that time, phosphorus was determined by a persulfate digestion method similar to USEPA method 365.1 (U.S. Environmental Protection Agency, 1983a). In 1991 projected increases in demand for both tests, brought on by the National Water-Quality Assessment program, led the NWQL to develop and validate methods for determining both analytes in a common digest. Details of the phosphorus method, which was developed and validated concurrently with the Kjeldahl nitrogen method described here, can be found in Patton and Truitt (1992).

Previous work suggested that streamlining the Kjeldahl digestion step improved data quality for these tests and also increased production capacity, decreased production costs, and lowered analysts' exposure to corrosive and toxic chemicals. Jirka and others (1976), for example, reported that digestion times for Kjeldahl nitrogen could be reduced from about 4 hours to 90 minutes by halving sample and reagent volumes prescribed in USEPA method 351.2. Later, Bowman and Delfino (1982) demonstrated that further reduction in digestion time could be achieved by replacing a single, temperature-programmed block digester with a pair of block digesters set at the desolvation (≈220°C) and digestion (370°C) temperatures. Methods I-2515-91/4515-91, which are described here, have been in routine use at the NWQL since October 1, 1991. They combine the Jirka and others (1976) and Bowman and Delfino (1982) digestion procedure improvements with a novel, highly robust automated colorimetric finish (salicylate-hypochlorite reaction) for separating ammonia in resolvated Kjeldahl digests from ionic and particulate interferents by in-line, gas diffusion across a microporous, polypropylene membrane. A batch of 40 calibrants, reference materials, and samples can be digested and made ready for colorimetric analysis in about 1 hour by using these methods.

Data in this report result from two distinct studies in 1991. The aim of the first study, conducted in April, was to assess the feasibility of using a common (Kjeldahl) digestion procedure for Kjeldahl nitrogen and phosphorus determinations and to document the performance of updated photometric finishes associated with these determinations. Analytical performance of the newly developed methods was documented, and quality-control (QC) guidelines for routine operation of the new methods were established. In many cases, data that appear in this report relating to analytical figures of merit—precision and accuracy of results, method detection levels, and blank concentrations—were collected during the April study. At that time, all samples received at the NWQL with test requests for Kjeldahl nitrogen and phosphorus also were analyzed for Kjeldahl nitrogen by USGS method numbers I-2515-91 (filtered samples), I-4515-91 (whole-water samples) and phosphorus by USGS method numbers I-2610-91 (filtered samples), I-4610-91 (whole-water samples).

At that time, these methods were in the initial stages of validation (Patton and Truitt, 1992). The resulting data set consists of about 400 pairs of Kjeldahl nitrogen concentrations that had been determined by USGS method numbers I-2552-85/4552-85 and I-2515-91/4515-91, which were used to assess relative performance of the two methods.

The second study, conducted in July, August, and September, was performed to address U.S. Geological Survey (USGS) concerns that the full range of water types, which are commonly sent to the NWQL for nutrient determinations, was not adequately represented in the April study. During these months, about 1,100 additional samples, selected by the USGS, were determined by both methods. Results of this comparison are included here, but typically QC and other analytical performance-related data that closely matched those from the April study are not.

This report describes USGS methods I-2515-91/4515-91 for determining Kjeldahl nitrogen developed for use at the NWQL. The method was implemented in the NWQL on October 1, 1991. All aspects of methods I-2515-91/4515-91 are described from sample preparation through calculation and reporting of results. Precision and accuracy data are included. Method I-2515-91/4515-91 supplements other methods of the USGS for determination of inorganic substances in water that are described by Fishman and Friedman (1989) and Fishman (1993).

Several individuals merit special thanks for assistance with various aspects of work reported here. Andrea M. Jirka (U.S. Environmental Protection Agency, Kansas City, Kan.), George T. Bowman (State Laboratory of Hygiene, Madison, Wis.), and Jack W. Kramer (Water Quality Laboratory, Heidelberg College, Tiffin, Ohio) are gratefully acknowledged for helpful discussions during the planning phases. Kramer also supplied high-particulate, agricultural run-off samples (Heidelberg sample) used as a control sample throughout this work. Dr. Ivan Sekerka (National Water Research Institute, Canada), Bertin Francoeur (National Laboratory for Environmental Testing, Canada), and Andrea Jirka served as colleague reviewers for a previous draft of this report.

The authors especially thank Jeffrey W. Pritt (U.S. Geological Survey), who performed statistical analysis on surface-water data included in this report. Assistance from several other individuals at the National Water Quality Laboratory, the Branch of Quality Systems, the Office of Water Quality, and the Branch of Systems Analysis also is gratefully acknowledged.

Former USGS chemist Earl P. Truitt (deceased) made substantial contributions to the development of this analytical method. The principal author gratefully acknowledges Earl's able assistance and analytical work upon which this report is based.

ANALYTICAL METHOD

Parameter and Codes:

Ammonium plus organic nitrogen, dissolved, I-2515-91 (mg/L as N): 00623 Ammonium plus organic nitrogen, total, I-4515-91 (mg/L as N): 00625

1. Application

This method is used to determine Kjeldahl nitrogen in water, drinking water, wastewater, brines, and water-suspended sediment. The suitability of this method for determination of Kjeldahl nitrogen in

bottom materials has not been investigated. The analytical range of this method is 0.1 to 10.0 mg/L of nitrogen.

2. Summary of Method

2.1 Organic nitrogen is converted to ammonium ions at a temperature of 370ºC in a reaction medium of sulfuric acid, potassium sulfate, and mercury (II). In principle, nitrate and nitrite are not reduced to ammonium ions under these conditions; USGS nomenclature refers to Kjeldahl nitrogen as ammonium plus organic nitrogen to emphasize this distinction. In practice, however, nitrate and nitrite might interfere positively or negatively (see section 3, Interferences).

2.2 The digestion procedure was adapted from the method of Jirka and others (1976) and Bowman and Delfino (1982), which is identical to USEPA method 351.2 (U.S. Environmental Protection Agency, 1983b), except that sample and reagent volumes are halved, as is the time required for digestion.

2.3 An air-segmented continuous flow analyzer is used to automate the photometric determination of ammonium ions in resolvated Kjeldahl digests by the salicylate analog (Reardon and others, 1966) of the Berthelot reaction (Patton and Crouch, 1977; Harfmann and Crouch, 1989). Resolvated Kjeldahl digests often contain suspended particulates (clays) and ions that interfere with the photometric finish. Both classes of interferents are eliminated by means of an on-line gas diffusion cell, which consists of a continuous-flow, parallel-plate dialyzer assembly that replaces the dialysis membrane with a hydrophobic, microporous, polypropylene membrane. Gases pass through the microporous polypropylene membrane; particles and ions do not. Before passage through the gas diffusion cell, ammonium ions in acidic resolvated Kjeldahl digests mix with the alkaline donor stream and are converted to ammonia—

$$NH_4^+{}_{(aq)} \text{ plus } {}^-OH \rightarrow NH_{3\,(g)}\uparrow + H_2O.$$

Inside the diffusion cell, gas-phase ammonia in the donor stream passes through the polypropylene membrane and is trapped in the interferent-free recipient stream (see fig. 1).

The authors determined ammonium in filtered (nominal pore size, 0.45μm) and unfiltered portions of highly turbid Heidelberg sample digests during the April experiments to demonstrate that the gas diffusion cell effectively removed suspended particles (clays) from resolvated Kjeldahl digests. (Samples were provided for this study by the Water Quality Laboratory at Heidelberg College in Tiffin, Ohio.) Equivalent ammonium concentrations in unfiltered and filtered sample digests are listed in table 1. Furthermore, the authors observed no surface fouling or mechanical failure of the porous polypropylene gas-diffusion membrane during the course of this work, and photometer flow-cell clogging was never a problem.

3. Interferences

3.1 As described in section 2.3, in-line digest cleanup by gas diffusion (Seifter and others, 1971) eliminates all particulate and potential ionic interference in the photometric finish. Thus, resolvated digests containing suspended particulates (clays) do not require filtration prior to analytical determinations. Likewise, colorimetric reagents do not contain complexing agents, such as citrate, tartrate, or EDTA, that are required to prevent precipitation of calcium (II) and magnesium (II) in the alkaline analytical stream of methods that lack a gas-diffusion cleanup step.

EXPLANATION

① Double injection fitting, Alpkem P/N 303-0107.

② 4.3-inch (11-centimeter) dialyzer assembly, Alpkem P/N 303-0803. Use a 45 inch-pound torque wrench, Bran-Leubbe P/N 178-5121-01, to tighten the dialyzer plates.

③ Gelman, Metricel® 0.1-micrometer pore size, 47-millimeter diameter polypropylene filter membrane. Never allow surfactants to contact this membrane, even during instrument start up or shutdown.

④ Reagent addition tee, Alpkem P/N 303-0100.

⑤ Thermally insulate the 0.034-inch (0.086 cm) inside diameter (i.d.) polyethylene tubing that connects the heat bath coil outlet to the flow cell by sheathing it with a piece of 0.10-inch (0.25 cm) i.d. polyvinyl chloride transmission tubing. Also stretch-wrap the flow cell barrel with several layers of thin foam plastic.

⑥ Collect this waste stream in a separate container to prevent precipitation of salicylic acid that would otherwise occur if were allowed to mix with acidic wastes from other analytical cartridges.

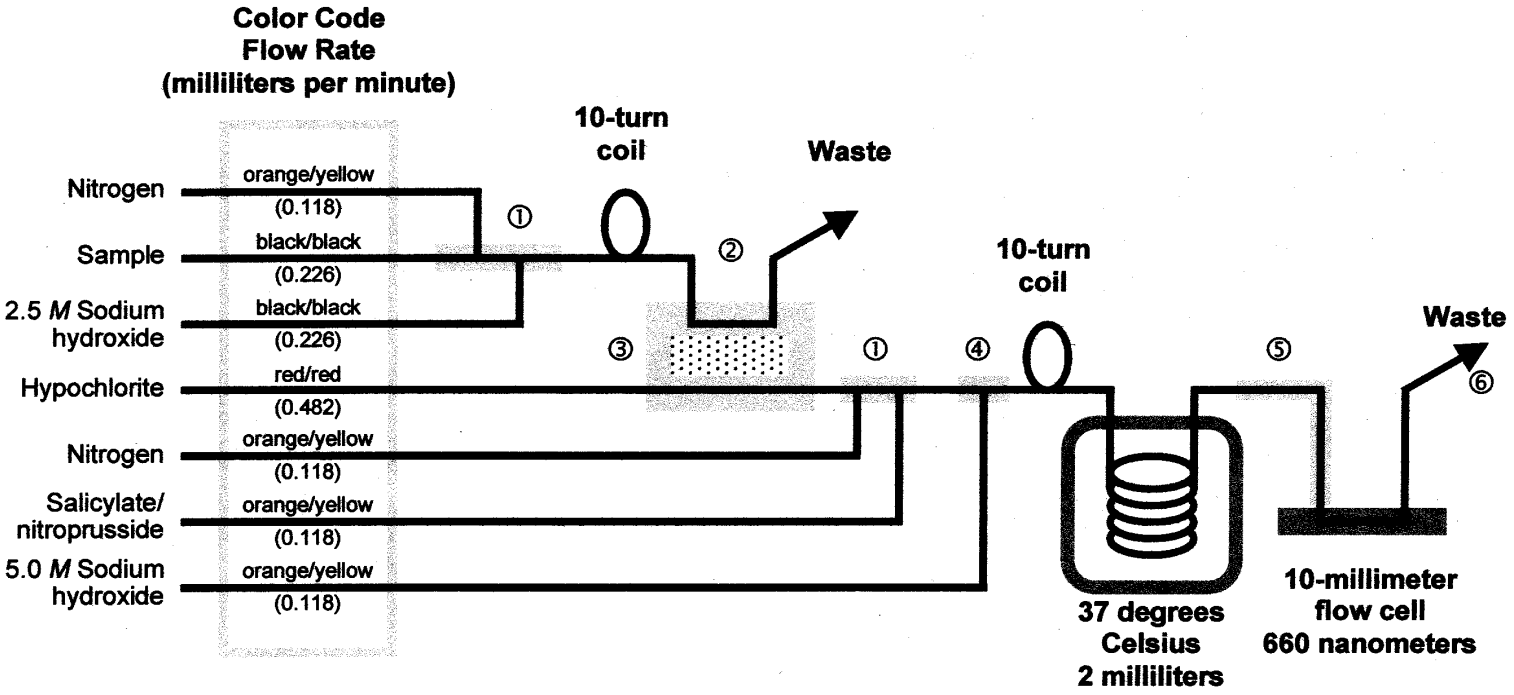

Figure 1. Analytical cartridge for colorimetric determination of Kjeldahl nitrogen in filtered samples (I-2515-91) and whole-water samples (I-4515-91) by U.S. Geological Survey methods.

Table 1. Kjeldahl nitrogen concentrations determined by methods I-2515-91/4515-91 for resolvated Heidelberg sample digests before and after passage through a 0.45-micrometer nylon syringe filter

[μm, micrometer; mg-N/L, milligram nitrogen per liter]

Julian date	Concentration (mg-N/L)		Julian date	Concentration (mg-N/L)	
	Unfiltered	Filtered		Unfiltered	Filtered
98	3.96	4.03	107	3.78	3.80
99	4.28	4.25	107	4.15	4.08
99	4.11	4.10	108	4.38	4.46
100	3.54	3.62	108	4.44	4.34
100	4.26	4.28	109	4.32	4.32
101	4.17	4.11	109	4.15	3.99
101	4.14	4.07	113	4.12	4.25
101	4.09	4.07	113	4.29	4.29
101	3.95	4.02	114	3.90	3.71
102	3.95	4.05	114	4.11	4.09
106	4.33	4.29	115	4.05	4.27
106	4.12	4.11	116	4.39	4.30
106	4.16	4.20	116	4.38	4.44
		Average concentration (mg-N/L):		**4.14**	**4.14**
		Standard deviation:		**0.20**	**0.20**
		Number of samples:		**26**	**26**
		Relative standard deviation (percent):		**4.88**	**4.83**

3.2 Once samples have been acidified, they are subject to contamination by ammonia in the laboratory atmosphere. The digestion process, therefore, must be performed in a hood that is located in an ammonia-free area of the laboratory. Other analytical or housekeeping procedures with potential to contribute ammonia vapor to the laboratory atmosphere may not be performed in or near this hood. Avoid delays between sample preparation, sample digestion, and digest analyses to minimize the risk of ammonia contamination.

3.3 Nitrate can exert both a positive and negative interference in Kjeldahl nitrogen determinations. As stated by the American Public Health Association (1992, p. 4–94):

> During [Kjeldahl] digestion, nitrate in excess of 10 mg/L can oxidize a portion of the ammonia released from the digested organic nitrogen, producing N_2O and resulting in a negative interference. When sufficient organic matter in a low state of oxidation is present, nitrate can be reduced to ammonia, resulting in a positive interference. The conditions under which significant interferences occur are not well defined and there is no proven way to eliminate the interference….

4. Instrumentation

4.1 A third-generation, air-segmented continuous flow analyzer (Alpkem RFA-300™) was used to automate photometric determination of ammonium ions in resolvated digests. Modules in this system include a 301 sampler, 302 peristaltic pump, 313 analytical cartridge base, 314 power module, 305A photometer, 311 recorder, and a data acquisition and processing system for use with

a personal computer (PC). Alternative procedures to automate the photometric finish by using flow injection analyzers or other second or third generation continuous flow analyzers also could be implemented (Patton and Wade, 1997).

4.2 Photometric data from the continuous flow analyzer were acquired and processed with a software package (Alpkem SoftPack™), operated on a PC equipped with a 12-bit, analog-to-digital (A/D) converter plug-in card. This A/D converter provided a resolution of 1 part in 4,095 (≈ 0.0012 volts when 5 volts is full scale), which is conservatively 20 times better than that afforded by a 10-inch (26-centimeter) strip-chart recorder. The A/D converter must be able to acquire data at frequencies in the range of 0.5 to 2 Hz—that is, 30 points/min to 120 points/min. As a general rule, data acquisition frequencies for air-segmented continuous flow analyzers should match the roller lift-off frequency of the peristaltic pump (Patton and Wade, 1997)—that is, 0.5 Hz for Technicon AutoAnalyzer II ™ and 1.5 Hz for Alpkem RFA-300 equipment. Most PC-based data acquisition and processing systems sold by vendors of continuous flow analyzers meet or exceed these specifications.

4.3 Operating characteristics for this equipment follow:

Analytical wavelength	660 nm
Flow cell path length	10 mm
Standard calibration control setting	≈1.4
Dialyzer[1]	4.3 in. (10.9 cm)
Segmentation frequency	1.5 Hz
Reaction coil volume	2 mL
Reaction coil temperature	37°C
Sample time	20 s
Wash time	20 s
Analysis rate	90/h, 1:1

[1]Microporous polypropylene membrane, 0.1 µm pore size (Gelman Metricel®)

5. Apparatus

5.1 Tecator Digestion System 40, Model 1016 block digesters or equivalent, which accommodate 40, 75-mL tubes, are used to desolvate and digest samples.

5.2 In this procedure, block digesters are operated in pairs. Prepared samples are desolvated in one block set at 220°C and immediately digested in another one set at 370°C. Time required for desolvation is 30 minutes and for digestion is 15 minutes.

6. Reagents

6.1 Digestion Reagents

CAUTION: Heat is produced when concentrated sulfuric acid is mixed with water. Wear protective eyeglasses, gloves, and clothing. Hot sulfuric acid solutions are hazardous.

NOTE: All references to deionized water shall be understood to mean ASTM Type I deionized water (American Society for Testing and Materials, 1995).

6.1.1 *Sulfuric acid, 3.6 M:* Cautiously add 200 mL of concentrated sulfuric acid [H_2SO_4, sp gr 1.84] to ≈700 mL of deionized water contained in a 1-L volumetric flask with constant mixing. Allow this solution to cool, dilute it to the mark with deionized water, and mix it well. Transfer this reagent to a plastic bottle where it is stable indefinitely at room temperature.

6.1.2 *Mercury (II) sulfate reagent:* Add 25 mL of 3.6 *M* sulfuric acid to 4.0 g of red mercury (II) oxide [HgO, FW = 216.59] contained in a 100-mL Griffin beaker. Place the beaker in an ultrasonic bath to speed dissolution. Use the resulting solution

immediately to prepare the digestion reagent as described in the next paragraph.

6.1.3 *Digestion reagent:* Add 268 g of potassium sulfate [K_2SO_4, FW = 174.27] to ≈1,300 mL of deionized water contained in a 2-L volumetric flask. Cautiously add 400 mL of concentrated sulfuric acid [H_2SO_4, sp gr = 1.84] with constant mixing, and then add the mercury (II) sulfate reagent. Stir the mixture magnetically or place the flask in an ultrasonic bath to speed dissolution. Allow this solution to cool, dilute it to the mark with deionized water, and mix it well. Transfer this reagent to a glass bottle or dispensing apparatus and store it at or above 20°C to prevent precipitation of potassium sulfate.

6.2 Colorimetric Reagents

6.2.1 *Sampler wash reservoir solution (≈1.3 N sulfuric acid + ≈0.3 M potassium sulfate):* Cautiously add 78 mL of concentrated sulfuric acid [H_2SO_4, sp gr 1.84] to ≈1,500 mL of deionized water contained in a 2-L volumetric flask with constant mixing. Add 54 g of potassium sulfate [K_2SO_4, FW = 174.27 g], and after it has dissolved, allow the solution to cool. Then dilute it to the mark with deionized water, and mix it well. Transfer this solution to plastic bottles where it is stable indefinitely at room temperature.

NOTE: This solution has hydronium and sulfate ion concentrations similar to those of resolvated digests. It can be used as the matrix for undigested ammonium ion calibrants.

6.2.2 *Sodium hydroxide, 5.0 M (alkaline recipient stream reagent):* Cautiously add 200 g of sodium hydroxide pellets [NaOH, FW = 40.00] to ≈700 mL of deionized water contained in a 1-L volumetric flask. Swirl the flask repeatedly to dissolve its contents. Allow the resulting solution to cool, dilute it to the mark with deionized water, and mix it well. Transfer this reagent to a plastic bottle where it is stable indefinitely at room temperature.

CAUTION: Heat is produced when sodium hydroxide pellets are mixed with water. Wear protective eyeglasses, gloves and clothing. Hot sodium hydroxide solutions are very hazardous.

6.2.3 *Sodium hydroxide, 2.5 M (alkaline donor stream reagent):* Add 50 mL of 5 *M* sodium hydroxide solution to 50 mL of deionized water contained in a 125-mL plastic bottle. Swirl the bottle to mix its contents. Prepare this reagent daily.

6.2.4 *Brij-35 surfactant (30 percent w/w):* Most commercially available solutions are satisfactory.

6.2.5 *Sodium bicarbonate buffer (0.1 M, pH ≈ 10.5):* Add 16.8 g of sodium bicarbonate ($NaHCO_3$, FW = 84.00), and 15 mL of 5 *M* sodium hydroxide solution to ≈ 1,500 mL deionized water contained in a 2-L volumetric flask. Shake the flask vigorously to dissolve its contents. Dilute the resulting solution to the mark with deionized water and mix it well. Transfer this reagent to plastic bottles where it is stable indefinitely at room temperature.

6.2.6 *Sodium hypochlorite reagent (STOCK, 5.25 percent w/v):* Most commercially available solutions of household chlorine bleach containing 5.25 percent available chlorine, Clorox®, for example, are satisfactory.

6.2.7 *Sodium hypochlorite reagent (WORKING):* Add 375 µL of stock sodium hypochlorite reagent to 250 mL of bicarbonate buffer reagent contained in a plastic bottle. Swirl the bottle to mix its contents. Prepare this reagent daily.

6.2.8 *Sodium salicylate/sodium nitroferricyanide reagent (STOCK):* Dissolve 150 g sodium salicylate [$C_7H_5O_3^-Na^+$, FW = 160.10] and 0.30 g sodium nitroferricyanide [$Na_2Fe(CN)_5NO \bullet 2H_2O$, FW = 297.95] in about 800 mL deionized water. Dilute the resulting solution to the mark with deionized water and mix it well. Store this reagent in an amber-colored glass bottle, where it is stable for several months at room temperature.

6.2.9 *Sodium salicylate/sodium nitroferricyanide reagent (WORKING):* Add 100 µL of Brij-35 surfactant per 100 mL of stock solution and mix it well.

7. Calibrants

7.1 *Primary calibrant, inorganic (1.000 mL = 2.50 mg-N):* Dissolve 4.7736 g of ammonium chloride [NH_4Cl, FW=53.49] previously dried at 110°C for ≈2 h and stored in a desiccator, in ≈400 mL of deionized water contained in a 500-mL volumetric flask. Dilute this solution to the mark with deionized water and mix it well. Transfer this calibrant to a plastic bottle and store it in a refrigerator where it is stable for several months.

7.2 *Primary calibrant, organic (1.000 mL = 2.50 mg-N):* Dissolve 9.9500 g of glycine hydrochloride [$C_2H_5NO_2 \bullet HCl$, FW = 111.5] in ≈400 mL of deionized water contained in a 500-mL volumetric flask. Dilute this solution to the mark with deionized water and mix it well. Transfer this calibrant to a plastic bottle and store it in a refrigerator where it is stable for several months.

7.3 *Spike solution, organic (1.000 mL = 0.1 mg-N):* Use an adjustable pipet (100 to 1,000 µL) to dispense 1,000 µL of organic nitrogen primary calibrant into ≈20 mL of deionized water contained in a 25-mL volumetric flask. Dilute the resulting solution to the mark with deionized water and mix it well. Transfer this solution to a 40-mL amber glass vial and store it in a refrigerator where it is stable for about a month.

NOTE: When Kjeldahl nitrogen and phosphorus are to be determined in the same digest, prepare mixed primary calibrant and spike solutions that contain both nitrogen and phosphorus.

7.4 *Working calibrants*: Use two adjustable pipets (ranges 10 to 100 µL and 100 to 1,000 µL) to dispense the volumes of primary calibrant, listed in table 2, and 1.000 mL of field preservative solution[1] (a solution containing 1.3 g $HgCl_2$ + 10.0 g NaCl in 100 mL of deionized water) into a series of 250-mL volumetric flasks that each contain ≈240 mL of deionized water (U.S. Geological Survey Office of Water Quality Technical Memorandum No. 94.16, 1996; U.S. Geological Survey Office of Water Quality Technical Memorandum No. 99.04, 1999). Rinse these flasks with a dilute solution of hydrochloric acid (≈5 percent v/v) and deionized water just prior to calibrant preparation. Dilute the contents of the flasks to the mark with deionized water, and shake them with repeated inversion to ensure thorough mixing. Transfer calibrants to plastic bottles and store them in a refrigerator when they are not in use. Prepare working calibrants as needed or biweekly, whichever comes first.[2]

[1]The USGS no longer amends nutrient samples with mercuric chloride as it did at the time this method was validated. The preparation of calibrants, blanks, and check standards in a matrix that closely matches that of samples being analyzed is necessary to ensure the accuracy of analytical results. Beginning in 1996, some nutrient samples received at the NWQL for analysis have been amended with sulfuric acid. Since January 1999, all whole-water nutrient samples sent to the NWQL require sulfuric acid amendment.

[2]Undigested calibrants, which are useful for assessing instrument function, can be prepared from the primary ammonium calibrant by using the sampler wash reservoir solution (see section 6.2.1) as the matrix.

Table 2. Calibrant preparation protocol

[µL, microliter; mg-N/L, milligrams of nitrogen per liter; mL, milliliter]

Calibrant identification	Primary calibrant volume (µL)	Field preservative volume (µL)	Nominal concentration[1] (mg-N/L)
C1	1,000	1,000	10.0
C2	500	1,000	5.0
C3	250	1,000	2.50
C4	125	1,000	1.25
C5	50	1,000	0.50
C6	10	1,000	0.10
(Blank)	0	1,000	0.00

[1]Based on a final volume of 250 mL.

8. Sample Preparation

8.1 Dispense calibrants, blanks (generally, ASTM type I deionized water), reference materials, and samples into digestion tubes as follows. Be sure the matrix of calibrants, blanks, and reference materials match that of samples amended with preservatives—sulfuric acid, for example—at collection sites. Note that three positions in each block are reserved for duplicate and spiked samples. To spike samples at a concentration of 1.0 mg-N/L, dispense 100 µL of the organic spike solution (see section 7.3) directly into appropriate tubes prior to digestion.

8.2 Rinse all glassware first with a dilute solution (≈5 percent v/v) of hydrochloric acid and then with deionized water before each use. This step is critical to achieve consistently low-concentration digestion blanks for both nitrogen and phosphorus.

8.3 Use an adjustable (5.0 to 10.0 mL) pipet to dispense 10.0-mL aliquots of calibrant, blank, reference, and sample solutions into digestion tubes. The suggested block protocol can be found in table 3. Vigorously shake sample containers and immediately aspirate aliquots to avoid sampling errors.

NOTE: When samples contain large quantities of suspended solids, continuous stirring during sample aspiration may provide the only means of obtaining representative aliquots.

8.4 Use an adjustable (2.0 to 100.0 µL) pipet to dispense 100 µL of the organic spike solution, equivalent to 1 mg-N/L when added to 10 mL of sample (see section 7.3), directly into samples designated as spikes.

8.5 Use an adjustable volume, syringe-based repetitive dispenser to add 2.00 mL of digestion reagent into each tube. Then add several acid-rinsed (≈5 percent HCl), Teflon™ boiling chips to each tube.

8.6 Desolvate prepared digests under a hood for 30 minutes in a block digester set at 220°C. At the end of this time, digest volumes should be less than 3 mL.

Table 3. Suggested block protocol for determination of Kjeldahl nitrogen by methods I-2515-91/4515-91

[ID, identification; mg-N/L, milligrams nitrogen per liter; SRWS, U.S. Geological Survey Standard Reference Water Sample]

Block position	Sample ID	Block position	Sample ID	Block position	Sample ID
1	CALIBRANT 1	15	SAMPLE	29	SAMPLE
2	CALIBRANT 2	16	ORG_CHK[1]	30	SAMPLE
3	CALIBRANT 3	17	SAMPLE	31	SAMPLE
4	CALIBRANT 4	18	SAMPLE	32	BLANK
5	CALIBRANT 5	19	SAMPLE	33	SAMPLE
6	CALIBRANT 6	20	SAMPLE	34	SAMPLE
7	BLANK	21	SAMPLE	35	SAMPLE
8	BLANK	22	SAMPLE	36	SAMPLE
9	SAMPLE	23	SAMPLE	37	SAMPLE
10	SAMPLE	24	SRWS	38	SPIKE[2]
11	SAMPLE	25	SAMPLE	39	DUPLICATE[2]
12	SAMPLE	26	SAMPLE	40	DUPLICATE[2]
13	SAMPLE	27	SAMPLE		
14	SAMPLE	28	SAMPLE		

[1]A solution of glycine (7.5 mg-N/L) is recommended.
[2]Samples for spiking and duplicate determinations should be chosen randomly.

8.7 Immediately transfer (CAUTION) desolvated digests to the block digester set at 370°C, and leave them there for 15 minutes. At the end of this time, digest volumes should be less than 0.5 mL and appear as a clear, syrupy bead at the bottom of each digestion tube. Crystallized digests indicate a problem in the acid to sulfate ratio of the digestion reagent.

8.8 Cautiously remove digestion tubes from the block digester and allow them to cool for about 10 minutes in the hood. With extreme caution, immediately dispense 10.0 mL of deionized water into each tube with vigorous agitation, by using an adjustable volume, syringe-based repetitive dispenser and a vortex mixer.

9. Instrument Performance

When a pair of block digesters is used as described in the Introduction, 40 calibrants, check standards, and samples can be digested and made ready for colorimetric analysis in about 1 hour. The air-segmented continuous flow analyzer used in this method can perform 90 ammonium determinations per hour with less than 1 percent interaction. Thus, a single channel analyzer can process the resolvated digests from two pairs of block digesters each hour.

10. Calibration

With a second-order polynomial least-squares curve-fitting algorithm ($y = a+bx+cx^2$, where y is the corrected peak height and x is the concentration), the correlation coefficient (r^2) of the calibration plot should be greater than 0.999. A typical calibration plot for Kjeldahl nitrogen calibrants in the concentration range of 0.1 to 10.0 mg-N/L is shown in figure 2.

11. Procedure and Data Evaluation

Set up the analytical cartridge of the continuous flow analyzer as shown in figure 1. Turn on electrical power to all

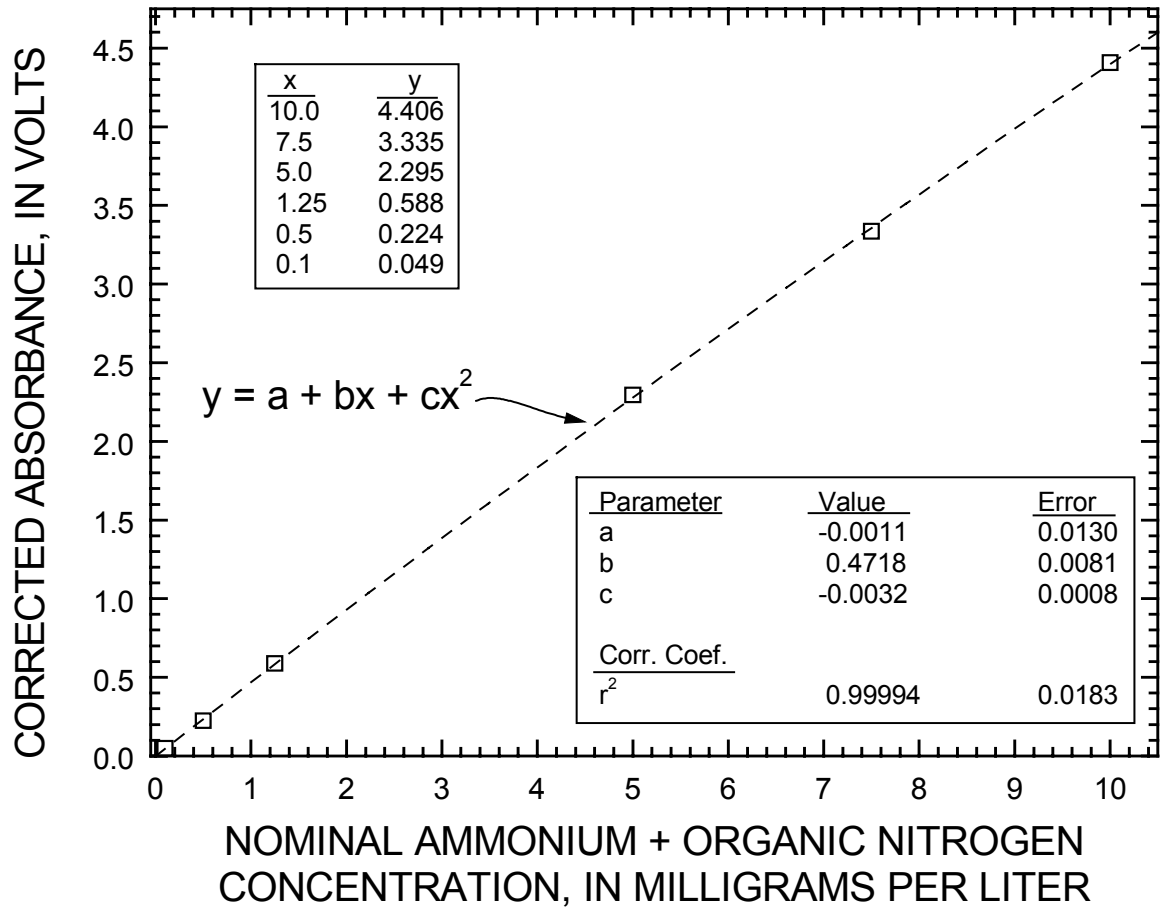

Figure 2. Typical calibration plot for determination of ammonium ions in Kjeldahl digests prepared by methods I-2515-91/4515-91. Corr. Coef = correlation coefficient.

system modules and put fresh sampler wash reservoir solution and reagents on-line. After about 10 minutes, verify that the output of sample and reference detectors is ≈5 volts. A suggested sampler tray protocol for automated determination of ammonium ions in resolvated digests is provided in table 4.

NOTE: To minimize errors that result from contaminated analyzer cups, rinse them several times with the solution they are to contain before placing them on the analyzer sampler tray.

NOTE: The full-scale absorbance range control (STD CAL) of the photometer should not require daily adjustment. Between-run/between-day variations in baseline-absorbance level and calibration curve slope of about ±5 percent are acceptable. Adjustment of the STD CAL control to compensate for larger variations in sensitivity or baseline (reagent blank) levels will only mask underlying problems, such as incipient light-source failure, partially clogged flow cells, or contaminated or improperly prepared reagents, any of which could compromise analytical results. Dwell time for the analyzer may increase by

Table 4. Suggested tray protocol for automated determination of ammonium ions in resolvated digests by methods I-2515-91/4515-91

[ID, identification]

Cup number	Sample ID	Cup number	Sample ID	Cup number	Sample ID
1	SYNC	15	SAMPLE	29	U_BLANK[1]
2	CALIBRANT 1	16	SAMPLE	30	SAMPLE
3	CALIBRANT 2	17	SRWS[3]	31	SAMPLE
4	CALIBRANT 3	18	SAMPLE	32	SAMPLE
5	CALIBRANT 4	19	SAMPLE	33	SAMPLE
6	CALIBRANT 5	20	SAMPLE	34	SAMPLE
7	CALIBRANT 6	21	SAMPLE	35	SAMPLE
8	U_BLANK[1]	22	SAMPLE	36	SAMPLE
9	BLANK	23	SAMPLE	37	SAMPLE
10	BLANK	24	SRWS[3]	38	SAMPLE
11	ORG_CHK[2]	25	SAMPLE	39	SAMPLE
12	SAMPLE	26	SAMPLE	40	SPIKE
13	SAMPLE	27	SAMPLE	41	DUPLICATE
14	SAMPLE	28	SAMPLE	42	DUPLICATE

[1]Undigested blank (sampler wash reservoir solution, see 6.2.1).
[2]Organic nitrogen check sample; see note 2 on table 3.
[3]USGS standard reference water sample.

a few seconds per day as pump tubes wear, but it need not be checked or updated more than once per week.

12. Calculations

12.1 Instrument calibration requires the preparation of a set of solutions (calibrants) in which the analyte concentration is known. These calibrants are digested along with samples and used to establish a calibration function that is estimated from a least-squares fit of nominal calibrant concentrations (x) in relation to peak absorbance (y). A second-order polynomial function ($y = a+bx+cx^2$) usually provides improved concentration estimates at the upper end of the calibration range than the more conventional linear model ($y = a+bx$). Accuracy is not lost when a second-order fit is used, even if the calibration function is strictly linear, because, in this case, the value estimated for the quadratic parameter c will approach zero.

12.2 Before the calibration function can be estimated, the baseline absorbance component of measured peak heights, including drift (continuous increase or decrease in the baseline absorbance during the course of an analysis), if present, needs to be removed. Baseline absorbance in continuous flow analysis is analogous to the reagent blank absorbance in batch analysis. Correction for baseline absorbance is an automatic function of most data acquisition and processing software sold by vendors of continuous flow analyzers.

NOTE: These correction algorithms are based on linear interpolation between initial and intermediate or final baseline measurements, and so they do not accurately correct for abrupt, step-changes in baseline absorbance that usually indicate partial blockage of the flow cell. It is prudent, therefore, to reestablish baseline absorbance at intervals of 20 samples or so.

12.3 After peaks are baseline corrected, they need to be digestion blank corrected.

This correction can be applied in several ways:

1. Subtract the baseline-corrected absorbance of the digestion blank—compute an average concentration if multiple digested blanks are included in each block—from the baseline-corrected absorbance of all calibrants, check standard, and samples in the block. Then estimate regression parameters (a, b, and c terms) for the calibration function by using a second-order polynomial least-squares algorithm. For second- and higher order calibration functions, use the Newton-Raphson successive approximations algorithm (Draper and Smith, 1966; Swartz, 1976, 1977, 1979) to convert corrected peak heights into concentrations.

2. Designate digestion blanks as a calibrant with a nominal concentration of zero. The calibration function estimated as described in section 12.3 then will have a positive y-intercept, with a magnitude that will approximate the baseline-corrected absorbance of the digestion blank. If this method is used, be sure that the curve-fitting algorithm does not force a zero y-intercept by including one or more "dummy" (0,0) points in the data set used for calibration.

3. Designate digested blanks as baseline correction samples. In this case initial, intermediate (if included), and final baselines are interpolated between digested blank peak maxima. Thus, baseline and digestion blank corrections are performed in a single operation.

NOTE: Digestion blank corrections for data in this report were performed by method 1. Note, however, that analytical results calculated by the other two methods should be equivalent, although in the authors' opinion the second correction method is most sound statistically. In any case, choose the digestion blank correction algorithm that is most easily implemented with the software package available. Regardless of the algorithm chosen, make sure that it is documented in the standard operating procedure (SOP) and that it is understood by analysts. This process also applies to algorithms used to convert corrected peak heights to concentration units. The SOP for this method must be updated whenever any changes in data acquisition and processing software or in calculation algorithms are implemented.

12.4 Most software packages provide a data base for entering appropriate dilution factors. Usually, these factors can be entered before or after analyses are performed. If dilution factors are entered, then reported concentrations will be compensated automatically for the extent of dilution. The dilution factor is the number by which a measured concentration must be multiplied to obtain the analyte concentration in the sample before dilution. For example, dilution factors of 2, 5, and 10 indicate that sample and diluent were combined in proportions of 1+1, 1+4, and 1+9, respectively.

13. Reporting Results

Report Kjeldahl nitrogen, dissolved (test ID = 00623), and total (test ID = 00625) concentrations as follows: 0.1 to 0.9 mg-N/L, one decimal; 1.0 mg-N/L and greater, two significant figures.

NOTE: The laboratory reporting level (LRL)—about twice the method detection level (MDL) as defined in section 14.1—for this method was reduced from 0.2 mg-N/L to 0.1 mg-N/L on November 10, 1997 (U.S. Geological Survey National Water Quality Laboratory Technical Memorandum 98.07, 1998).

14. Detection Levels, Precision, and Accuracy

14.1 A method detection level (MDL) of ≈0.05 mg-N/L was estimated for methods I-2515-91/4515-91 by using the protocol set forth in the U.S Environmental Protection Agency (1990). This MDL estimate was obtained with homogenous solutions; therefore, the MDL for particulate-laden samples might be somewhat higher because of the increased difficulty of obtaining a representative sample for analysis (see table 5).

14.2 Within-run precision (repeatability) for methods I-2515-91/4515-91 on the basis of 55 replicate determinations of the same high suspended-solids sample was 3.1 percent. The average concentration and standard deviation of these replicates was 4.1 ± 0.1 mg-N/L (see table 6).

Between-day precision based on 26 replicate determinations of the same sample between April 8 and April 26, 1991, was 4.8 percent. The average concentration and standard deviation of between-day replicates was 4.1 ± 0.2 mg-N/L (see table 7). Figure 3 shows duplicate data for 564 randomly selected samples determined for Kjeldahl nitrogen at the NWQL by method I-2515-91/4515-91 between the dates of 2/11/92 and 2/13/93. The top panel of this figure presents trial 1 concentra-tion in relation to trial 2 concentration as a scatter plot about the line of equal relation. The bottom panel shows a scatter plot of the percent difference between duplicates in relation to the trial 1 concentration. About 90 percent of the 300 samples with Kjeldahl nitrogen concentrations ≥ 0.5 mg-N/L (10 MDLs) agreed to within ± 25 percent.

14.3 Between-day accuracy for methods I-2515-91/4515-91, which is based on concentrations determined for reference materials provided by the USEPA and the USGS, is listed in table 8. In all cases, Kjeldahl nitrogen concentrations determined by this method were within published control limits for these reference materials.

14.4 The average recovery for 1.00 mg of glycine nitrogen added to 94 randomly selected samples analyzed during the July–August experiment was 1.04 ± 0.10 mg-N/L. Figure 4 shows that concentrations of Kjeldahl nitrogen in samples did not affect spike recovery.

14.5 When the 1,487 data pairs from April and July–August experiments were combined, the median difference between Kjeldahl nitrogen concentrations determined by the present (I-2515-91/4515-91) and old (I-2552-85/4552-85) methods was about 0.1 mg-N/L, which is highly significant ($p < 0.0001$) on the basis of a Wilcoxon signed rank test. Linear regression analysis of these data pairs shows a statistically significant ($p < 0.0001$), nonzero intercept, which also suggests a possible additive difference between the former and new methods. Table 9 lists mean and median Kjeldahl nitrogen concentration differences determined for filtered- and whole-water samples by the former and the new methods as well as the results of the regression of the former method in relation to the new methods. A concentration difference of this magnitude was expected because digestion blank concentrations (≈ 0.1 mg-N/L) were

Table 5. Data used to estimate the method detection level (MDL) for Kjeldahl nitrogen determination by methods I-2515-91/4515-91

[mg-N/L, milligrams nitrogen per liter. Measured concentrations pertain to eight replicate digestions of a 0.25 mg-N/L glycine calibrant that was prepared in deionized water that contained an appropriate volume of field preservative solution.]

Replicate number	Nominal concentration (mg-N/L)	Measured concentration (mg-N/L)
1	0.25	0.194
2	.25	.196
3	.25	.202
4	.25	.229
5	.25	.192
6	.25	.204
7	.25	.166
8	.25	.196
	Average concentration (mg-N/L):	**.197**
	Standard deviation (mg-N/L):	**.017**
	Number of samples:	**8**
	Degrees of freedom:	**7**
	One-sided *t* value (99 percent confidence):	**2.998**
	Method detection level (MDL):	**0.052**

Table 6. Repeatability (within-run precision, May 3, 1991) data for 55 replicate determinations of Kjeldahl nitrogen concentration in the Heidelberg sample by using methods I-2515-91/4515-91

[mg-N/L, milligrams nitrogen per liter]

Trial number	Concentration (mg-N/L)	Trial number	Concentration (mg-N/L)	Trial number	Concentration (mg-N/L)
1	3.97	20	4.12	38	4.13
2	4.36	21	4.29	39	4.06
3	4.16	22	4.29	40	4.25
4	4.19	23	4.09	41	3.94
5	4.36	24	4.20	42	4.06
6	4.07	25	4.25	43	4.22
7	4.21	26	3.99	44	3.93
8	4.37	27	4.13	45	4.11
9	4.04	28	4.20	46	4.23
10	4.21	29	3.88	47	3.99
11	4.26	30	4.03	48	4.18
12	4.09	31	4.15	49	4.21
13	4.33	32	3.94	50	3.97
14	4.22	33	3.98	51	4.04
15	4.15	34	4.11	52	4.19
16	4.34	35	3.89	53	3.90
17	4.11	36	4.06	54	4.11
18	4.14	37	4.22	55	4.14
19	4.24				
			Average concentration (mg/L):		**4.13**
			Standard deviation (mg/L):		**0.13**
			Relative standard deviation (percent):		**3.1**

Table 7. Between-day (April 8–26, 1991) precision of digested calibrants and the Heidelberg sample for Kjeldahl nitrogen determination by using methods I-2515-91/4515-91

[ID, identification; mg-N/L, milligrams nitrogen per liter; std. dev., standard deviation; RSD, relative standard deviation; ≈, nearly equal to; µm, micrometer]

Sample ID	Nominal concentration (mg-N/L)	Number of samples	Average concentration (mg-N/L)	Std. dev. (mg-N/L)	RSD (percent)
CALIBRANT 1	10.0	24	9.99	0.04	0.38
CALIBRANT 2	7.5	25	7.52	.08	1.10
CALIBRANT 3	5.0	23	4.98	.08	1.65
CALIBRANT 4	2.5	25	2.50	.06	2.30
CALIBRANT 5	1.25	25	1.27	.06	4.39
CALIBRANT 6	.5	25	.48	.06	3.51
Heidelberg[1]	[3] ≈ 4.1	26	4.14	.20	4.88
Heidelberg[2]	[3] ≈ 4.1	26	4.14	.20	4.83

[1]Diluted digest was shaken and poured into analyzer cup.
[2]Diluted digest was dispensed into analyzer cup through a 0.45-µm nylon syringe filter.
[3]National Water Quality Laboratory consensus concentration.

not subtracted from concentrations reported by the former methods. Indeed, a step change of about 0.1 mg-N/L in NWQL Kjeldahl nitrogen blind blank concentrations is evident after October 1, 1991, when the new methods became operational, as shown in figure 5. Complete details of the method comparison studies can be found in the Discussion of Results section that follows.

DISCUSSION OF RESULTS

Analytical Methods

Before beginning validation studies, the authors performed several experiments to characterize block digester and continuous flow analyzer performance. For example, to estimate the extent of temperature variation throughout the 40 positions within the block digester, a batch of 40 test solutions as described in section 8 was prepared and digested. The first eight tubes in row 1 of the block digester contained calibrants and blanks (see table 3 and fig. 6b). The remaining 32 tubes in rows 2 through 5 contained an aqueous solution of nicotinic acid with a nominal concentration of 7.5 mg-N/L. Nicotinic acid was chosen for this study because it is one of the most difficult compounds that contain organic nitrogen to digest by the Kjeldahl method (Bradstreet, 1965; Jirka and others, 1976; Bowman and Delfino, 1982). Digestion time was limited to 90 minutes at 370°C—less than the time required for 100-percent recovery of nicotinic acid. The authors hypothesized that recovery of nicotinic acid in each tube would indicate the relative temperature at each block position. That is, tubes with lower nicotinic acid recoveries would be correlated with cooler positions in the block. Results of this experiment are presented in figure 6a, which shows isopleths for nicotinic acid concentration found in each tube relative to its position in the block digester. This figure shows that recovery of nicotinic acid approached 100 percent only in tubes near the center of the block digester and decreased radially from the digester's center to its perimeter.

Next, the authors performed a set of experiments to assess how the temperature gradient detected within the block digester would affect method performance for

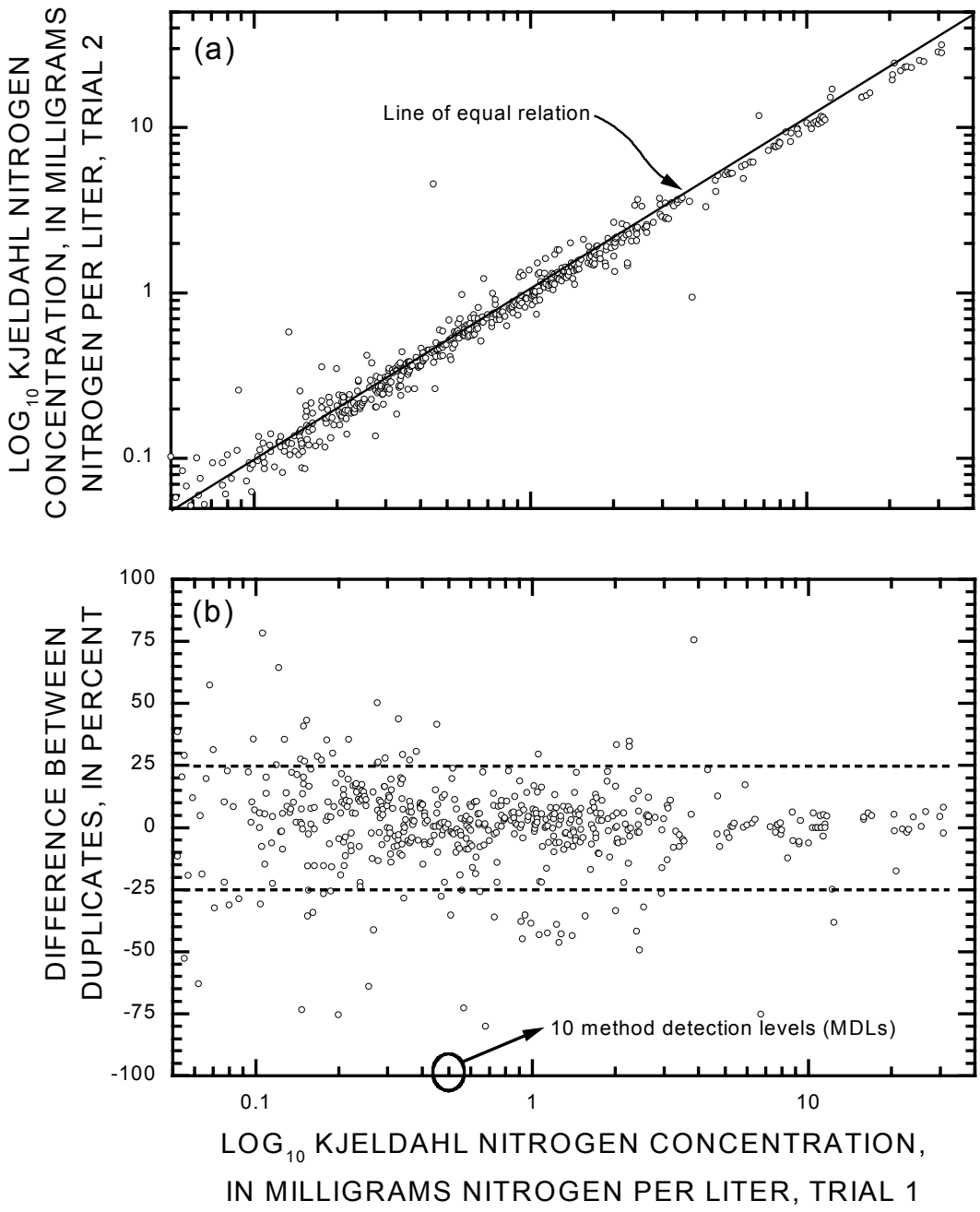

Figure 3. Duplicate data for 564 randomly selected samples determined at the National Water Quality Laboratory by methods I-2515-91/4515-91 between 2/11/92 and 2/13/93. About 90 percent of the 300 samples with Kjeldahl nitrogen concentrations ≥ 0.5 milligram nitrogen per liter (10 MDLs) agreed to within ± 25 percent. Note that several extreme outliers shown in graph (a) do not appear in graph (b), because the *y*-axis scale is limited to differences between trial 1 and trial 2 of plus or minus 100 percent.

Table 8. Between-day (April 8–26, 1991) accuracy of digested U.S. Environmental Protection Agency and U.S. Geological Survey reference samples for Kjeldahl nitrogen determination by using methods I-2515-91/4515-91

[ID, identification; mg-N/L, milligrams nitrogen per liter; ±, plus or minus; std. dev., standard deviation; RSD, relative standard deviation; LCL, lower control limit; UCL, upper control limit; µL, microliters; mL, milliliters; SRWS, standard reference water sample]

Sample ID	Nominal concentration (mg-N/L)	Number of samples	Average concentration (mg-N/L)	Std. dev. (mg-N/L)	RSD (percent)
USEPA LOW[1]	2.50 ± 0.34	13	2.40	0.19	8
(LCL - UCL)[2]	(1.82 – 3.18)				
USEPA[3]	4.95 ± 0.44	10	5.00	.20	4
(LCL - UCL)[2]	(4.07 – 5.83)				
SRWS N-28	0.26 ± 0.17	11	.15	.10	67
(LCL - UCL)	(-0.08 – 0.60)		[4] (0.03 – 0.35)		
SRWS N-29	1.21 ± 0.21	13	1.02	.09	8
(LCL - UCL)	(0.79 – 1.63)		[4] (0.88 – 1.17)		

[1]A solution containing 500 µL USEPA "Nutrient 2" and 400 µL National Water Quality Laboratory $HgCl_2$/NaCl preservative solution diluted to 100 mL with deionized water.

[2]Confidence interval of 95 percent.

[3]A 1,000 µL USEPA "Nutrient 2" solution and 400 µL National Water Quality Laboratory $HgCl_2$/NaCl preservative solution diluted to 100 mL with deionized water.

[4]Concentration range.

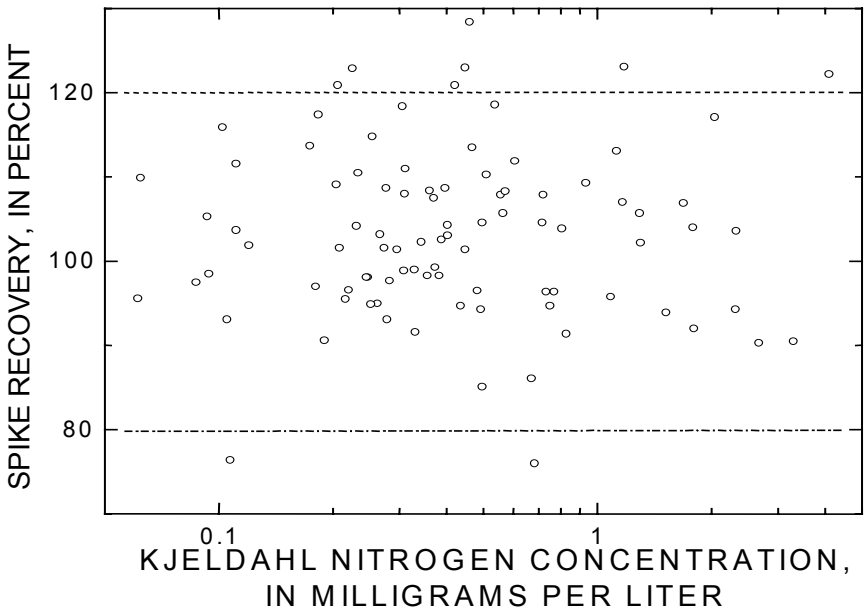

Figure 4. Spike recovery in relation to Kjeldahl nitrogen concentration determined by methods I-2515-91/4515-91 during the July–August, 1991 experiment. Number of samples = 94, nitrogen added (as glycine) = 1.0 milligram nitrogen per liter (mg-N/L), average nitrogen found = 1.04 ± 0.10 mg-N/L, and average percent recovery = 104 ± 10.

Table 9. Statistical data and regression analysis results for Kjeldahl nitrogen methods I-2515-91/4515-91 (new methods) and I-2552-85/4552-85 (former methods)

[Mean and median concentration differences, in milligrams nitrogen per liter, are the result of subtracting former-method concentrations from new-method concentrations. Slopes and *y*-intercepts are the result of using new-method concentrations as the independent (*x*) variable. Data for surface-water and ground-water samples are combined.]

Sample type	Number of samples	Median difference	Mean difference	Slope	*y*-intercept
Filtered	508	-0.074	-0.091	0.922	0.159
Whole-water	979	-0.115	-0.049	0.962	0.096

several real and synthetic samples that contain various forms of organic nitrogen. Percent recovery and precision of analytical results were used to evaluate method performance. In this second set of experiments, full blocks for each of three different test solutions were prepared and digested. As before (see section 8), the first eight tubes in row 1 of the block digester contained calibrants and blanks. The remaining 32 tubes in rows 2 through 5 contained an aqueous solution of nicotinic acid with a nominal concentration of 7.5 mg-N/L in batch one, an aqueous solution of adenosine triphosphate (ATP) with a nominal concentration of 1.0 mg-N/L in batch 2, and a high suspended-solids sample with a consensus concentration of 6.3 ± 0.5 mg-N/L in batch 3. Each batch was desolvated in a block digester set at 220°C for 30 minutes and then transferred to the high-temperature block digester set at 370°C. After 15 minutes, all eight tubes were removed from row 1, which contained calibrants and blanks, and tubes 1 through 4 from row 2. At 15-minute intervals thereafter, tubes 5 through 8 were removed from row 2, tubes 1 through 4 from row 3, tubes 5 through 8 from row 3, tubes 1 through 4 from row 4, tubes 5 through 8 from row 4, tubes 1 through 4 from row 5, and tubes 5 through 8 from row 5 (see fig. 6b). This procedure yielded 4-tube subsets of each test solution that had undergone digestion at 370°C for 15, 30, 45, 60, 75, 90, 105, and 120 minutes.

Results of these experiments are summarized in figure 7, where symbols plotted as a function of digestion time represent the average Kjeldahl nitrogen concentration measured in each 4-tube subset for each of the three test solutions. Error bars associated with the symbols represent ±1 standard deviation. As shown in figure 7, recovery of nitrogen from digested ATP solutions and high suspended-solids samples were ≥ 95 percent after 15 minutes and remained constant at longer digestion times. Standard deviations for these two test solutions fluctuated randomly as digestion time increased. Typically, however, shorter digestion times are desirable because digestion blank concentrations and variability tend to increase as digestion time increases. In contrast, but in keeping with the block digester temperature gradient experiment, digestion times greater than 90 minutes were required to approach nitrogen recoveries ≥ 95 percent in nicotinic acid solution digests. The authors interpret the increased precision of nicotinic acid concentrations observed at the longest digestion time as an indication that the effect of the block digester's temperature gradient on nicotinic acid recovery decreased substantially as digestion time approached 120 minutes.

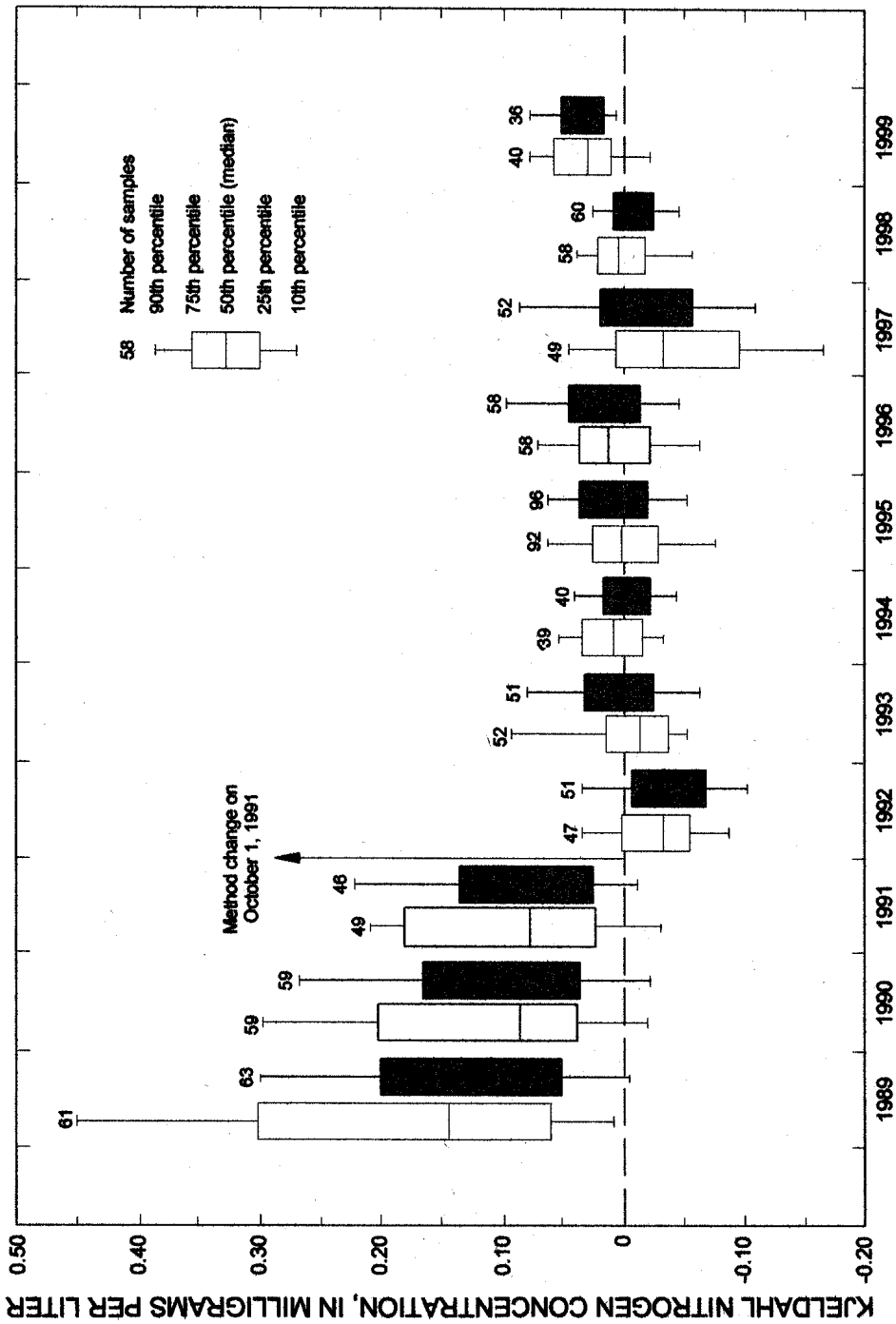

Figure 5. Concentration ranges for Kjeldahl nitrogen blind blank samples analyzed at the U.S. Geological Survey National Water Quality Laboratory from January 1989 through September 1999. Data from October through December 1994 do not exist, and therefore are not included in box plots for that year. Outliers, typically 3 to 5 per box plot, are not shown in this figure. White boxes and shaded boxes represent blind blanks submitted as filtered (parameter code 00623) and whole-water recoverable (parameter code 00625) samples, respectively. Note that regardless of designation, the sole constituent of blind blank samples is ASTM Type 1 deionized water.

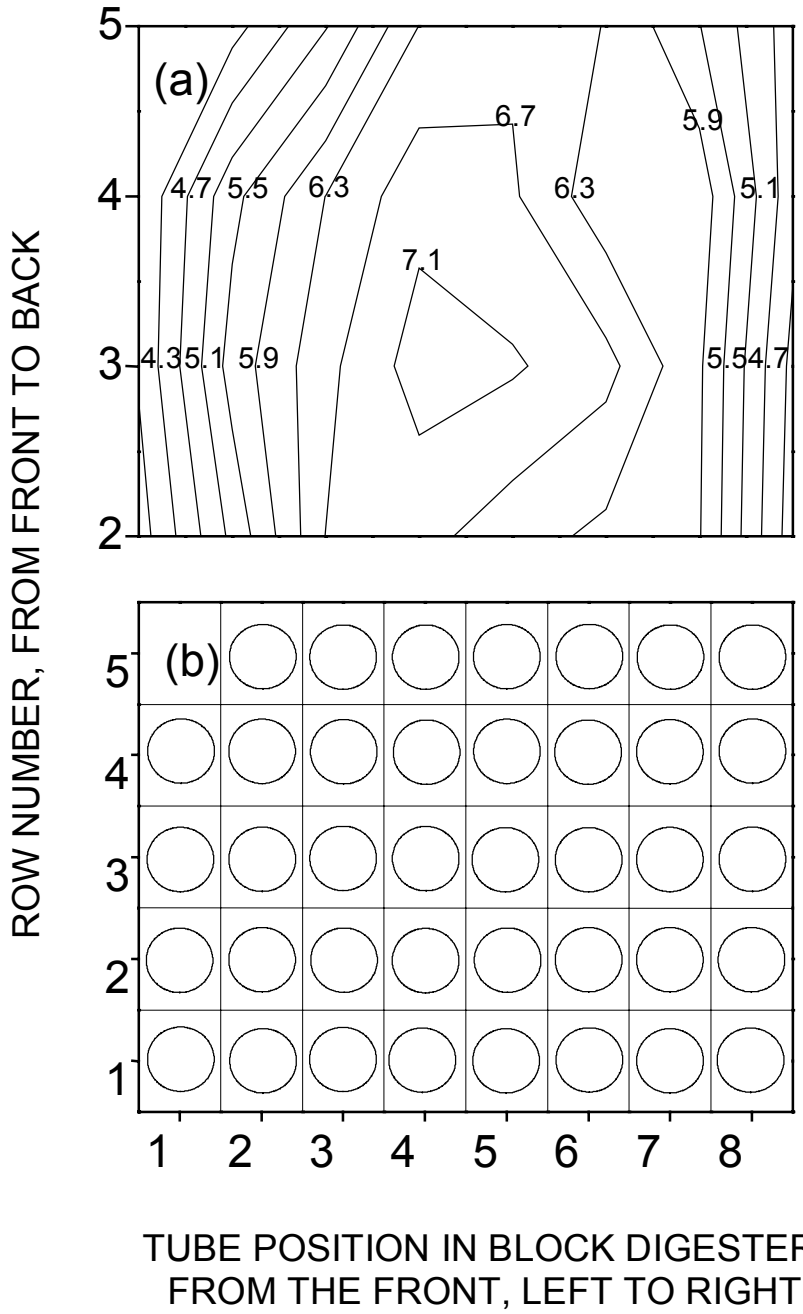

Figure 6. Isopleths of nicotinic acid concentration (a), in milligrams nitrogen per liter, found in each tube in relation to the position of each tube in the block digester (b).

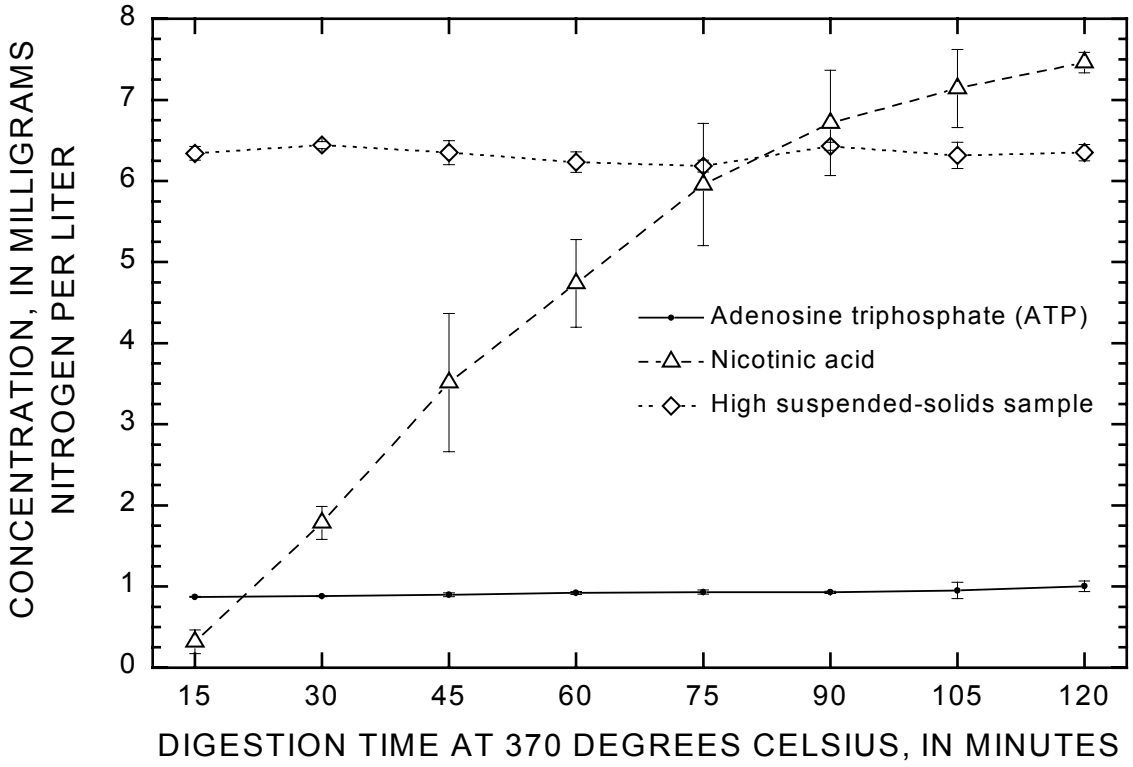

Figure 7. Kjeldahl nitrogen concentration recovered in digests of a high-suspended-solids sample, an aqueous solution of adenosine 5' triphosphate, and an aqueous solution of nicotinic acid by methods I-2515-91/4515-91 as a function of high-temperature digestion time. Error bars represent ±1 standard deviation for the average of four determinations of each sample type at specified digestion times.

On the basis of these results and statistical analysis of Kjeldahl nitrogen concentrations determined by USGS methods I-2552-85/4552-85 (370°C digestion time ≈90 minutes) and I-2515-91/4515-91 (370°C digestion time =15 minutes), the authors conclude as follows:

1. Digestion times of 15 minutes at 370°C are adequate for full recovery of organic nitrogen compounds in natural-water samples typically received for analyses at the NWQL, and

2. Longer digestion times required for quantitative recovery of nicotinic acid might contribute to over- or underestimation of Kjeldahl nitrogen concentrations in samples containing nitrate concentration ≥ 1 mg-N/L (see discussion that follows).

Statistical Analysis

The lack of precision in the former USGS methods (I-2552-85/4552-85) for several years preceding validation of the new methods (I-2515-91/4515-91) is indicated by NWQL blind blank data for the years 1989 through 1999 (see fig. 5). In an effort to mitigate errors arising from statistical comparison of methods with differing precision, the authors used medians and median-based, nonparametric statistics (Wilcoxon signed rank test) in the discussions that follow in preference to means and means-based, parametric paired *t*-tests. As listed in table 9, when filtered- and whole-water-sample data from the April and July–August experiments are combined, median Kjeldahl nitrogen concentrations determined by the new methods are about 0.1 mg-N/L less than those determined by the former methods. This was expected because Kjeldahl nitrogen concentrations reported for the former methods were not corrected for digestion blank concentrations, which are approximately equal to this difference. Median concentration differences between the two methods were comparable during the April and July–August experiments, as listed in table 10.

When data pairs from the April and July–August experiment were grouped into the categories *filtered* and *whole* surface-water samples and *filtered* and *whole* ground-water samples, similar trends were observed. Data for each of these four categories are shown as scatter plots in figure 8 and as box plots in figure 9. As shown in these figures, the deviations of plotted data from the line of equal relation, which represent the concentration difference between the new and former methods, was least for unfiltered surface-water samples from both studies. Additional trends in the data emerge when method median differences are calculated for the subset of samples in these four categories with nitrate plus nitrite concentrations either less than or equal to 1.0 mg-N/L or greater than 1.0 mg-N/L. The results of such an analysis are presented for data from the April experiment (table 11) and July–August experiments (table 12).

Inspection of tables 11 and 12 reveals that median concentration differences between the two methods in all four categories more closely matches the median digestion blank concentration in the subset of samples with nitrate plus nitrite concentrations less than or equal to 1 mg-N/L than for the entire population. Likewise, larger concentration differences than can be accounted for by the lack of blank correction in the former methods are observed for the subset of samples with nitrate plus nitrite concentrations greater than 1 mg-N/L. For a substantial number of samples in these subsets, the differences between Kjeldahl nitrogen concentrations determined by the former and new methods increases as nitrate plus nitrite concentrations increase. The authors hypothesize that this effect is caused by complex and poorly characterized interactions among nitrate, ammonium, and organic matter during Kjeldahl digestion as discussed in section 3.3. The authors further speculate that the much longer 370°C digestion time of the former methods in relation to the new methods—90 minutes rather than 15 minutes—intensifies these undesirable side reactions.

The concentration difference between the new and former methods was positive in a single case in the July–August experiment. This difference was observed in the subset of whole surface-water samples with nitrate plus nitrite concentrations greater than 1 mg-N/L (see table 12).

Table 10. Median difference between paired Kjeldahl nitrogen concentrations determined by methods I-2515-91/4515-91 and I-2552-85/4552-85

[Median differences are the result of subtracting methods I-2552-85/4552-85 concentrations from methods I-2515-91/4515-91 concentrations. Data for surface-water and ground-water samples are combined. mg–N/L, milligrams nitrogen per liter]

Data source	Number of samples	Median Kjeldahl nitrogen concentration (mg-N/L)		
		I-2515-91 I-4515-91	I-2552-85 I-4552-85	Median difference
April	411	0.495	0.566	-0.071
July–August	1,076	.422	.519	-.097
Both	1,487	.437	.531	-.094

Careful inspection of original data eliminated inadvertent transposal of data columns for the two methods during initial stages of data analysis as a possible explanation for this apparent anomaly. In a further effort to account for the anomaly, the ammonium concentrations were examined in the subset of samples with nitrate plus nitrite concentrations greater than 1 mg/L (see tables 11 and 12). As indicated in table 13, the average nitrate plus nitrite and ammonium concentrations in the July–August subset have higher than average ammonium concentrations and, in general, lower than average nitrate plus nitrite concentrations than the April subset. Furthermore, the authors found that 14 samples in the July–August subset (126 samples)—seven each from two USGS stations near minimally treated sewage discharge outfalls—were largely responsible for the observed positive concentration difference between the two methods (table 12). Average ammonium and nitrate plus nitrite concentrations were 11.1 and 2.2 mg-N/L for the seven samples from station 9419700 and 9.4 and 2.6 mg-N/L for the seven samples from station 9419753. Samples with Kjeldahl nitrogen and ammonium concentrations in this range would have required dilution before analysis. The authors, therefore, can only speculate whether dilution errors, the atypically high ammonium to nitrate ratio (table 13), other unknown factors, or some combination of all three are responsible for the positive concentration difference between the new and former methods in this data set.

This study provides evidence that nitrate plus nitrite concentrations greater than 1 mg-N/L might cause positive or negative interference in Kjeldahl nitrogen determinations. This information should serve as a caution to data users who calculate total nitrogen concentrations by summing Kjeldahl nitrogen and nitrate plus nitrite concentrations.

Data analysis in this section also provides insight into the difficulties of comparing Kjeldahl nitrogen (ammonium plus organic nitrogen) concentrations with total nitrogen concentrations determined by high-temperature combustion methods (U.S. Environmental Protection Agency, 1997) or alkaline persulfate digestion (D'Elia and others, 1977) methods as discussed in previous studies (Ameel and others, 1993; Kroon, 1993).

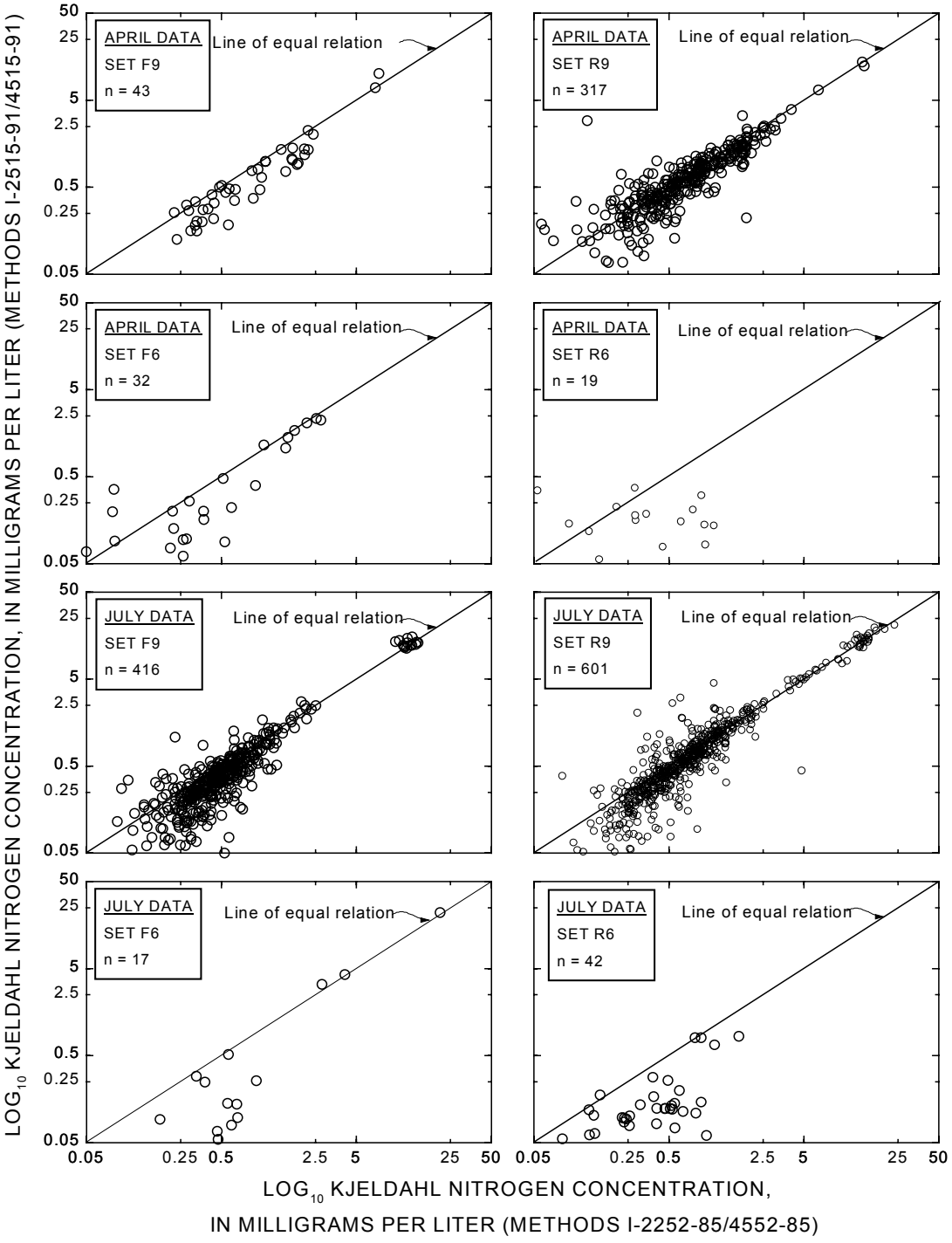

Figure 8. Relation between Kjeldahl nitrogen concentrations determined by methods I-2552-85/ 4552-85 and I-2515-91/4515-91 in the April and July–August experiments for each of the four water types. Sets F9, R9, F6, and R6 were composed of filtered and whole surface-water samples and filtered and whole ground-water samples, respectively. The letter *n* represents the *number of points* in each set.

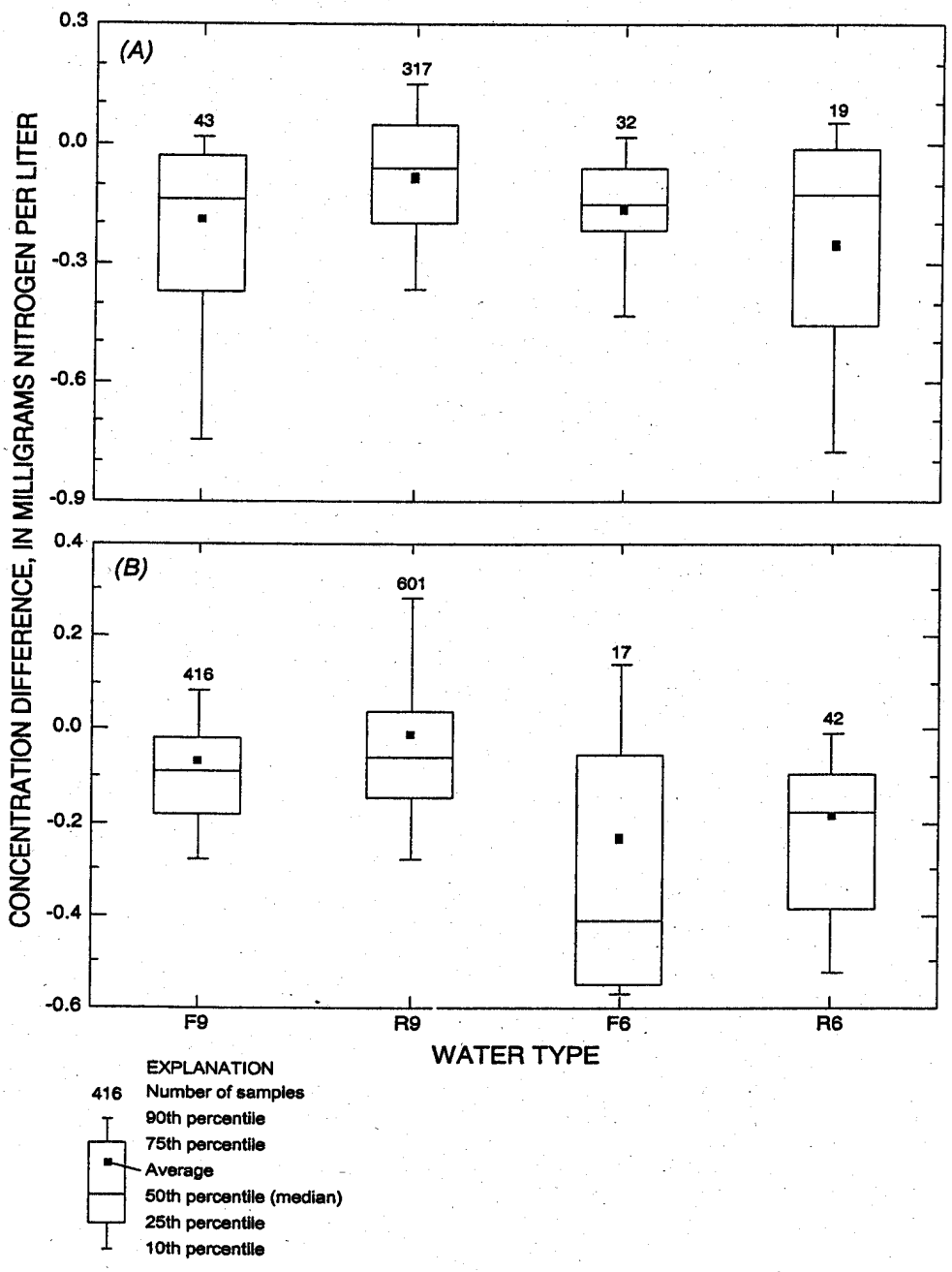

Figure 9. Concentration differences between Kjeldahl nitrogen determined by methods I-2515-91/4515-91 and I-2552-85/4552-85 in the April (A) and July–August (B) experiments for each of the four water types (F9, R9, F6, and R6). Points above and below the 90th and 10th percentile whiskers (3 to 5 per plot, typically) are not shown. The labels F9, R9, F6, and R6 stand for filtered and whole surface-water samples and filtered and whole ground-water samples, respectively.

Table 11. Effect of nitrate plus nitrite concentrations on median differences between Kjeldahl nitrogen concentrations determined by U.S. Geological Survey methods I-2515-91/4515-91 and I-2552-85/4552-85 (April data set)

[mg-N/L, milligrams nitrogen per liter; ≤, less than or equal to; >, greater than]

Water type	All samples			
	Number of samples	Median Kjeldahl nitrogen concentration (mg-N/L)		
		I-2515-91 I-4515-91	I-2552-85 I-4552-85	Difference
Filtered surface	43	0.475	0.636	-0.161
Whole surface	317	.561	.592	-.031
Filtered ground	32	.143	.325	-.182
Whole ground	19	.144	.281	-.137
		Samples with NO_3^- + NO_2^- concentrations (≤ 1 mg-N/L)		
Filtered surface	25	.375	.442	-.067
Whole surface	249	.459	.496	-.037
Filtered ground	20	.231	.33	-.099
Whole ground	10	.149	.207	-.058
		Samples with NO_3^- + NO_2^- concentrations (> 1 mg-N/L)		
Filtered surface	18	.843	1.281	-.438
Whole surface	68	1.217	1.48	-.263
Filtered ground	12	.075	.319	-.244
Whole ground	9	.144	.451	-.307

Table 12. Effect of nitrate plus nitrite concentrations on median differences between Kjeldahl nitrogen concentrations determined by U.S. Geological Survey methods I-2515-91/4515-91 and I-2552-85/4552-85 (July–August data set)

[mg-N/L, milligrams nitrogen per liter; ≤, less than or equal to; >, greater than]

Water type	All samples			
	Number of samples	Median Kjeldahl nitrogen concentration (mg-N/L)		
		I-2515-91 I-4515-91	I-2552-85 I-4552-85	Difference
Filtered surface	416	0.352	0.452	-0.100
Whole surface	601	.566	.616	-.050
Filtered ground	17	.138	.579	-.441
Whole ground	42	.109	.382	-.273
		Samples with NO_3^- + NO_2^- concentrations (≤ 1 mg-N/L)		
Filtered surface	341	.311	.413	-.102
Whole surface	475	.455	.53	-.075
Filtered ground	7	.272	.446	-.174
Whole ground	15	.064	.172	-.108
		Samples with NO_3^- + NO_2^- concentrations (> 1 mg-N/L)		
Filtered surface	75	.582	.713	-.131
Whole surface	126	1.123	1.017	+.106
Filtered ground	10	.073	.573	-.5
Whole ground	27	.122	.493	-.371

Table 13. Average concentrations of nitrate plus nitrite and ammonium for sample subsets with nitrate plus nitrite concentrations greater than 1 milligram nitrogen per liter

[mg-N/L, milligrams nitrogen per liter]

Water type	Average concentration (mg-N/L)			
	April		July – August	
	$NO_2^- + NO_3^-$	NH_4^+	$NO_2^- + NO_3^-$	NH_4^+
Filtered surface (F9)	11.1	0.12	2.5	2.1
Whole surface (R9)	6.0	.62	2.4	1.6
Filtered ground (F6)	5.0	.00	19.6	.04
Whole ground (R6)	8.3	.00	6.1	.06

CONCLUSIONS

U.S. Geological Survey (USGS) methods I-2515-91/4515-91 for determining Kjeldahl nitrogen in filtered and whole-water samples were implemented at the National Water Quality Laboratory (NWQL) on October 1, 1991. The methods, in which high-temperature block digestion is followed by automated, colorimetric ammonium determination that includes digest cleanup by continuous flow gas diffusion, have method detection levels of about 0.05 mg-N/L as estimated by using U.S Environmental Protection Agency protocols. Within-run precision (repeatability) and between-day precision for filtered and whole-water samples analyzed by methods I-2515-91/4515-91 are about 3 percent and 5 percent, respectively. Duplicate determinations for these methods typically agree to within 25 percent, and spike recoveries are typically 100 ± 20 percent. Data provided in this report demonstrate that digestion times of 15 minutes at 370°C are adequate for full recovery of organic nitrogen compounds in natural-water samples typically received for analyses at the NWQL. Furthermore, these data suggest that digestion times longer than 15 minutes might contribute to over- or underestimation of Kjeldahl nitrogen concentrations in samples with nitrate plus nitrite concentrations ≥ 1 mg-N/L.

Statistical analysis of paired data collected during method validation experiments and NWQL blind blank data are consistent with a step-change decrease of about 0.1 mg-N/L in Kjeldahl nitrogen concentrations reported by the NWQL beginning on October 1, 1991. On that date, USGS methods I-5225-85/4525-85 were replaced by USGS methods I-2515-91/4515-91. Generally, this concentration difference resulted from the lack of digestion blank correction in methods I-5225-85/4525-85.

This work documents the importance of considering factors, such as digestion time and whether digestion blank corrections have been applied, when Kjeldahl nitrogen data produced at different laboratories are compared. It also provides insight into the difficulties of comparing Kjeldahl nitrogen concentration with true total nitrogen concentrations. This work also indicates that nitrate plus nitrite concentrations greater than 1 mg-N/L might cause positive or negative interference in Kjeldahl nitrogen determinations. This information should serve as a caution to data users who either combine Kjeldahl nitrogen concentrations from different laboratories, or who calculate total nitrogen concentrations by summing Kjeldahl nitrogen and nitrate plus nitrite concentrations, or both.

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