4500-N$_{org}$ NITROGEN (ORGANIC)*

4500-N$_{org}$ A.   Introduction

1. Selection of Method

The kjeldahl methods (B and C) determine nitrogen in the trinegative state. They fail to account for nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone. "Kjeldahl nitrogen" is the sum of organic nitrogen and ammonia nitrogen.

The major factor that influences the selection of a macro- or semi-micro-kjeldahl method to determine organic nitrogen is its concentration. The macro-kjeldahl method is applicable for samples containing either low or high concentrations of organic nitrogen but requires a relatively large sample volume for low concentrations. In the semi-micro-kjeldahl method, which is applicable to samples containing high concentrations of organic nitrogen, the sample volume should be chosen to contain organic plus ammonia nitrogen in the range of 0.2 to 2 mg.

The block digestion method (D) is a micro method with an automated analysis step capable of measuring organic nitrogen as low as 0.1 mg/L when blanks are carefully controlled.

2. Storage of Samples

The most reliable results are obtained on fresh samples. If an immediate analysis is not possible, preserve samples for kjeldahl digestion by acidifying to pH 1.5 to 2.0 with concentrated H_2SO_4 and storing at 4°C. Do not use $HgCl_2$ because it will interfere with ammonia removal.

3. Interferences

a. Nitrate: 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 in conjunction with the kjeldahl methods described herein.

b. Inorganic salts and solids: The acid and salt content of the kjeldahl digestion reagent is intended to produce a digestion temperature of about 380°C. If the sample contains a very large quantity of salt or inorganic solids that dissolve during digestion, the temperature may rise above 400°C, at which point pyrolytic loss of nitrogen begins to occur. To prevent an excessive digestion temperature, add more H_2SO_4 to maintain the acid-salt balance. Not all salts cause precisely the same temperature rise, but adding 1 mL H_2SO_4/g salt in the sample gives reasonable results. Add the extra acid and the digestion reagent to both sample and reagent blank. Too much acid will lower the digestion temperature below 380°C and result in incomplete digestion and recovery. If necessary, add sodium hydroxide-sodium thiosulfate before the final distillation step to neutralize the excess acid.

Large amounts of salt or solids also may cause bumping during distillation. If this occurs, add more dilution water after digestion.

c. Organic matter: During kjeldahl digestion, H_2SO_4 oxidizes organic matter to CO_2 and H_2O. If a large amount of organic matter is present, a large amount of acid will be consumed, the ratio of salt to acid will increase, and the digestion temperature will increase. If enough organic matter is present, the temperature will rise above 400°C, resulting in pyrolytic loss of nitrogen. To prevent this, add to the digestion flask 10 mL conc H_2SO_4/3 g COD. Alternately, add 50 mL more digestion reagent/g COD. Additional sodium hydroxide-sodium thiosulfate reagent may be necessary to keep the distillation pH high. Because reagents may contain traces of ammonia, treat the reagent blank identically with the samples.

4. Use of a Catalyst

Mercury has been the catalyst of choice for kjeldahl digestion. Because of its toxicity and problems associated with legal disposal of mercury residues, a less toxic catalyst is recommended. Digestion of some samples may be complete or nearly complete without the use of a catalyst. Effective digestion results from the use of a reagent having a salt/acid ratio of 1 g/mL with copper as catalyst (B.3*a*), and specified temperature (B.2*a*) and time (B.4*c*). If a change is made in the reagent formula, report the change and indicate percentage recovery relative to the results for similar samples analyzed using the previous formula.

Before results are considered acceptable, determine nitrogen recovery from samples with known additions of nicotinic acid, to test completeness of digestion; and with ammonium chloride to test for loss of nitrogen.

4500-N$_{org}$ B.   Macro-Kjeldahl Method

1.   General Discussion

a. Principle: In the presence of H_2SO_4, potassium sulfate (K_2SO_4), and cupric sulfate ($CuSO_4$) catalyst, amino nitrogen of many organic materials is converted to ammonium. Free ammonia also is converted to ammonium. After addition of base, the ammonia is distilled from an alkaline medium and absorbed in boric or sulfuric acid. The ammonia may be determined colorimetrically, by ammonia-selective electrode, or by titration with a standard mineral acid.

b. Selection of ammonia measurement method: The sensitivity of colorimetric methods makes them particularly useful for determining organic nitrogen levels below 5 mg/L. The titrimetric and selective electrode methods of measuring ammonia in the distillate are suitable for determining a wide range of organic nitrogen concentrations. Selective electrode methods and automated colorimetric methods may be used for measurement of ammonia in digestate without distillation. Follow equipment manufacturer's instructions.

2. Apparatus

a. Digestion apparatus: Kjeldahl flasks with a total capacity of 800 mL yield the best results. Digest over a heating device adjusted so that 250 mL water at an initial temperature of 25°C can be heated to a rolling boil in approximately 5 min. For testing, preheat heaters for 10 min if gas-operated or 30 min if electric. A heating device meeting this specification should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: See Section 4500-NH$_3$.B.2*a*.

c. Apparatus for ammonia determination: See Section 4500-NH$_3$.C.2, D.2, F.2, or G.2.

3. Reagents

Prepare all reagents and dilutions in ammonia-free water.

All of the reagents listed for the determination of Nitrogen (Ammonia), Section 4500-NH$_3$.C.3, D.3, F.3, or G.3, are required, plus the following:

a. Digestion reagent: Dissolve 134 g K_2SO_4 and 7.3 g $CuSO_4$ in about 800 mL water. Carefully add 134 mL conc H_2SO_4. When it has cooled to room temperature, dilute the solution to 1 L with water. Mix well. Keep at a temperature close to 20°C to prevent crystallization.

b. Sodium hydroxide-sodium thiosulfate reagent: Dissolve 500 g NaOH and 25 g $Na_2S_2O_3 \cdot 5H_2O$ in water and dilute to 1 L.

c. Borate buffer solution: See Section 4500-NH$_3$.B.3*b*.

d. Sodium hydroxide, NaOH, 6*N*.

4. Procedure

a. Selection of sample volume and sample preparation: Place a measured volume of sample in an 800-mL kjeldahl flask. Select sample size from the following tabulation:

Organic Nitrogen in Sample mg/L	Sample Size mL
0–1	500
1–10	250
10–20	100
20–50	50.0
50–100	25.0

If necessary, dilute sample to 300 mL, neutralize to pH 7, and dechlorinate as described in Section 4500-NH$_3$.B.4*b*.

b. Ammonia removal: Add 25 mL borate buffer and then 6*N* NaOH until pH 9.5 is reached. Add a few glass beads or boiling chips such as Hengar Granules #12 and boil off 300 mL. If desired, distill this fraction and determine ammonia nitrogen. Alternately, if ammonia has been determined by the distillation method, use residue in distilling flask for organic nitrogen determination.

For sludge and sediment samples, weigh wet sample in a crucible or weighing bottle, transfer contents to a kjeldahl flask, and determine kjeldahl nitrogen. Follow a similar procedure for ammonia nitrogen and organic nitrogen determined by difference. Determinations of organic and kjeldahl nitrogen on dried sludge and sediment samples are not accurate because drying results in loss of ammonium salts. Measure dry weight of sample on a separate portion.

c. Digestion: Cool and add carefully 50 mL digestion reagent (or substitute 6.7 mL conc H_2SO_4, 6.7 g K_2SO_4, and 0.365 g $CuSO_4$) to distillation flask. Add a few glass beads and, after mixing, heat under a hood or with suitable ejection equipment to remove acid fumes. Boil briskly until the volume is greatly reduced (to about 25 to 50 mL) and copious white fumes are observed (fumes may be dark for samples high in organic matter). Then continue to digest for an additional 30 min. As digestion continues, colored or turbid samples will become transparent and pale green. After digestion, let cool, dilute to 300 mL with water, and mix. Tilt flask away from personnel and carefully add 50 mL sodium hydroxide-thiosulfate reagent to form an alkaline layer at flask bottom. Connect flask to a steamed-out distillation apparatus and swirl flask to insure complete mixing. The pH of the solution should exceed 11.0.

d. Distillation: Distill and collect 200 mL distillate. Use 50 mL indicating boric acid as absorbent solution when ammonia is to be determined by titration. Use 50 mL 0.04*N* H_2SO_4 solution as absorbent for manual phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Sections 4500-NH$_3$.C, D, F, and G, respectively.

f. Standards: Carry a reagent blank and standards through all steps of the procedure.

5. Calculation

See Section 4500-NH$_3$.C.5, D.5, F.5, or G.5.

6. Precision and Bias

Two analysts in one laboratory prepared reagent water solutions of nicotinic acid and digested them by the macro-kjeldahl method. Ammonia in the distillate was determined by titration. Results are summarized in Table 4500-N$_{org}$:I

7. Bibliography

KJELDAHL, J. 1883. A new method for the determination of nitrogen in organic matter. *Z. Anal. Chem.* 22:366.

PHELPS, E.B. 1905. The determination of organic nitrogen in sewage by the Kjeldahl process. *J. Infect. Dis.* (Suppl.) 1:225.

MCKENZIE, H.A. & H.S. WALLACE. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion conditions. *Aust. J. Chem.* 7:55.

MORGAN, G.B., J.B. LACKEY & F.W. GILCREAS. 1957. Quantitative determination of organic nitrogen in water, sewage, and industrial wastes. *Anal. Chem.* 29:833.

BOLTZ, D.F., ed. 1978. Colorimetric Determination of Nonmetals. Interscience Publishers, New York, N.Y.

JONES, M. & D. BRADSHAW. 1989. Copper: An alternative to mercury; more effective than zirconium in kjeldahl digestion of ecological materials. *Commun. Soil Sci. Plant Anal.* 20:1513.

TABLE 4500-N$_{org}$:I. PRECISION DATA FOR KJELDAHL NITROGEN METHOD BASED ON MEAN OF TRIPLICATE ANALYSES OF NICOTINIC ACID

Lab/ Analyst	Nicotinic Acid mg N/L	Recovery of N %	Standard Deviation mg/L	Relative Standard Deviation %
1/1	5	93.3	0.16	3.46
1/2	5	101	0.16	3.17
1/1	10	87.7	0.16	1.84
1/2	10	91.5	0.28	3.06
1/1	20	95.7	0.16	0.84
1/2	20	95.7	0.58	3.03
2/1	0.5	97.4	0.005	1.04
2/2	0.5	95.3	0.027	5.46
3/1	0.5	87.3	0.130	29.9
4/1	0.5	113	0.235	41.7
2/1	1.0	103	0.012	1.15
2/2	1.0	101	0.046	4.63
3/1	1.0	84.3	0.081	9.66
4/1	1.0	99.3	0.396	39.9
2/1	2.0	104	0	0
2/2	2.0	99.2	0.029	1.44
3/1	2.0	89.2	0.071	3.98
4/1	2.0	112	0.139	6.18

4500-N$_{org}$ C.   Semi-Micro-Kjeldahl Method

1. General Discussion

See Section 4500-N$_{org}$.B.1.

2. Apparatus

a. Digestion apparatus: Use kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus* equipped with heating elements to accommodate kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 375 to 385°C for effective digestion.

b. Distillation apparatus: Use an all-glass unit equipped with a steam-generating vessel containing an immersion heater† (Figure 4500-N$_{org}$:1).

c. pH meter.

d. Apparatus for ammonia determination: See Section 4500-NH$_3$.C.2, D.2, F.2, or G.2.

3. Reagents

All of the reagents listed for the determination of Nitrogen (Ammonia) (Section 4500-NH$_3$.B.3) and Nitrogen (Organic)

macro-kjeldahl (Section 4500-N$_{org}$.B.3) are required. Prepare all reagents and dilutions with ammonia-free water.

4. Procedure

a. Selection of sample volume: Determine the sample size from the following tabulation:

Organic Nitrogen in Sample mg/L	Sample Size mL
4–40	50
8–80	25
20–200	10
40–400	5

For sludge and sediment samples weigh a portion of wet sample containing between 0.2 and 2 mg organic nitrogen in a crucible or weighing bottle. Transfer sample quantitatively to a 100-mL beaker by diluting it and rinsing the weighing dish several times with small quantities of water. Make the transfer using as small a quantity of water as possible and do not exceed a total volume of 50 mL. Measure dry weight of sample on a separate portion.

b. Ammonia removal: Pipet 50 mL sample or an appropriate volume diluted to 50 mL with water into a 100-mL beaker. Add

---

* Rotary kjeldahl digestion unit, Kontes, Model 551000-0000, or equivalent.
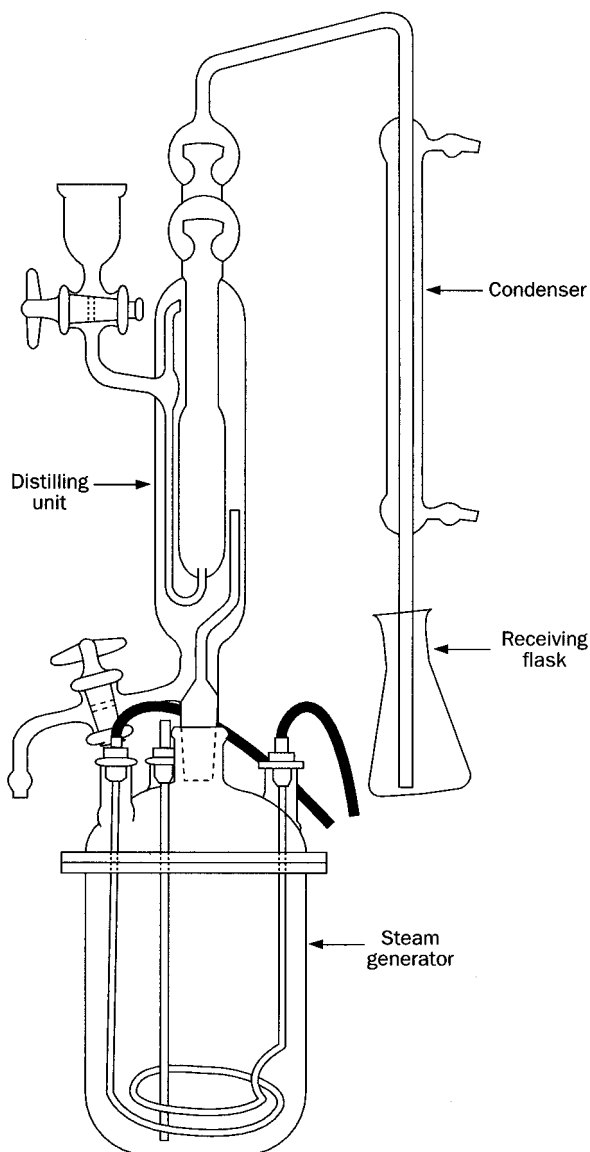† ASTM E-147 or equivalent.

Figure 4500-N$_{org}$:1. Micro-kjeldahl distillation apparatus.

3 mL borate buffer and adjust to pH 9.5 with 6N NaOH, using a pH meter. Quantitatively transfer sample to a 100-mL kjeldahl

flask and boil off 30 mL. Alternatively, if ammonia removal is not required, digest samples directly as described in ¶ *c* below. Distillation following this direct digestion yields kjeldahl nitrogen concentration rather than organic nitrogen.

c. Digestion: Carefully add 10 mL digestion reagent to kjeldahl flask containing sample. Add five or six glass beads (3- to 4-mm size) to prevent bumping during digestion. Set each heating unit on the micro-kjeldahl digestion apparatus to its medium setting and heat flasks under a hood or with suitable ejection equipment to remove fumes of SO$_3$. Continue to boil briskly until solution becomes transparent and pale green and copious fumes are observed. Then turn each heating unit up to its maximum setting and digest for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 mL. Add 10 mL sodium hydroxide-thiosulfate reagent and turn on steam.

d. Distillation: Control rate of steam generation to boil contents in distillation unit so that neither escape of steam from tip of condenser nor bubbling of contents in receiving flask occurs. Distill and collect 30 to 40 mL distillate below surface of 10 mL absorbent solution contained in a 125-mL erlenmeyer flask. Use indicating boric acid for a titrimetric finish. Use 10 mL 0.04N H$_2$SO$_4$ solution for collecting distillate for the phenate or electrode methods. Extend tip of condenser well below level of absorbent solution and do not let temperature in condenser rise above 29°C. Lower collected distillate free of contact with condenser tip and continue distillation during last 1 or 2 min to cleanse condenser.

e. Standards: Carry a reagent blank and standards through all steps of procedure and apply necessary correction to results.

f. Final ammonia measurement: Use the titration, ammonia-selective electrode, manual phenate, or automated phenate method, Sections 4500-NH$_3$.C, D, F, and G, respectively.

5. Calculation

See Section 4500-NH$_3$.C.5, D.5, F.5, or G.5.

6. Precision and Bias

No data on the precision and bias of the semi-micro-kjeldahl method are available.

7. Bibliography

See Section 4500-N$_{org}$.B.7.

4500-N$_{org}$ D. Block Digestion and Flow Injection Analysis

1. General Discussion

a. Principle: Samples of drinking, ground, and surface waters and of domestic and industrial wastes are digested in a block digestor with sulfuric acid and copper sulfate as a catalyst. The digestion recovers nitrogen components of biological origin,

such as amino acids, proteins, and peptides, as ammonia, but may not recover the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some refractory tertiary amines. Nitrate is not recovered. See Section 4500-N for a discussion of the various forms of nitrogen found in waters and wastewaters, Sections

4500-N$_{org}$.A and B for a discussion of kjeldahl nitrogen methods, and Section 4130, Flow Injection Analysis (FIA).

The digested sample is injected onto the FIA manifold where its pH is controlled by raising it to a known, basic pH by neutralization with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction that follows. The ammonia thus produced is heated with salicylate and hypochlorite to produce a blue color that is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The resulting peak's absorbance is measured at 660 nm. The peak area is proportional to the concentration of total kjeldahl nitrogen in the original sample.

b. Interferences: Remove large or fibrous particulates by filtering the sample through glass wool.

The main source of interference is ammonia. Ammonia is an airborne contaminant that is removed rapidly from ambient air by the digestion solution. Guard against ammonia contamination in reagents, water, glassware, and the sample preservation process. It is particularly important to prevent ammonia contamination in the sulfuric acid used for the digestion. Open sulfuric acid bottles away from laboratories in which ammonia or ammonium chloride have been used as reagents and store sulfuric acid away from such reagents. Ensure that the open ends of the block digestor's tubes can be covered to prevent ammonia from being scrubbed from the fume hood make-up air during the digestion.

If a sample consumes more than 10% of the sulfuric acid during digestion, the pH-dependent color reaction will show a matrix effect. The color reaction buffer will accommodate a range of 5.4% $\pm$ 0.4% H$_2$SO$_4$ (v/v) in the diluted digested sample. Sample matrices with a high concentration of carbohydrates or other organic material may consume more than 10% of the acid during digestion. If this effect is suspected, titrate digested sample with standardized sodium hydroxide to determine whether more than 10% of the sulfuric acid has been consumed during digestion. The block digestor also should have a means to prevent loss of sulfuric acid from the digestion tubes during the digestion period.

Also see Sections 4500-N$_{org}$.A and B.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

a. Block digestor capable of maintaining a temperature of 380°C for 2 h.

b. Digestion tubes capable of being heated to 380°C for 2 h and having a cover to prevent ammonia contamination and loss of sulfuric acid.

c. FIA injection valve with sample loop or equivalent.

d. Multichannel proportioning pump.

e. FIA manifold (Figure 4500-N$_{org}$:2) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-N$_{org}$:2. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

f. Absorbance detector, 660 nm, 10-nm bandpass.
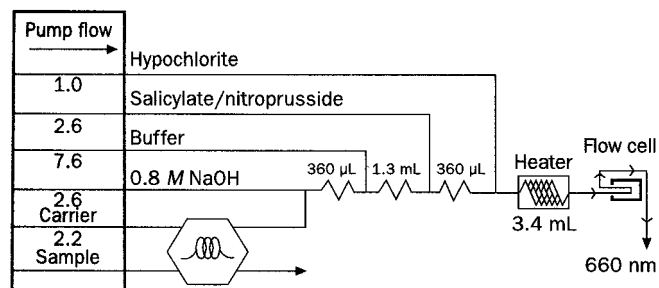
g. Injection valve control and data acquisition system.

Figure 4500-N$_{org}$:2. FIA total kjeldahl nitrogen manifold.

3. Reagents

Use reagent water ($>$10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Digestion solution: In a 1-L volumetric flask, dissolve 134.0 g potassium sulfate, K$_2$SO$_4$, and 7.3 g copper sulfate, CuSO$_4$, in 800 mL water. Then add slowly while swirling 134 mL conc sulfuric acid, H$_2$SO$_4$. Let cool, dilute to mark, and invert to mix.

b. Carrier and diluent: To a tared 1-L container, add 496 g digestion solution (¶ 3*a*) and 600 g water. Shake until dissolved.

c. Sodium hydroxide, NaOH, 0.8*M*: To a tared 1-L plastic container, add 32.0 g NaOH and 985.0 g water. Stir or shake until dissolved.

d. Buffer: To a tared 1-L container add 941 g water. Add and completely dissolve 35.0 g sodium phosphate dibasic heptahydrate, Na$_2$HPO$_4$ · 7H$_2$O. Add 20.0 g disodium EDTA (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add 50 g NaOH. Stir or shake until dissolved.

e. Salicylate/nitroprusside: To a tared 1-L dark container, add 150.0 g sodium salicylate (salicylic acid sodium salt), C$_6$H$_4$(OH)(COO)Na, 1.00 g sodium nitroprusside (sodium nitroferricyanide dihydrate), Na$_2$Fe(CN)$_5$NO · 2H$_2$O, and 908 g water. Stir or shake until dissolved. Prepare fresh monthly.

f. Hypochlorite: To a tared 250-mL container, add 16 g commercial 5.25% sodium hypochlorite bleach solution* and 234 g deionized water. Shake to mix.

g. Stock standard, 250 mg N/L: In a 1-L volumetric flask dissolve 0.9540 g ammonium chloride, NH$_4$Cl (dried for 2 h at 110°C), in about 800 mL water. Dilute to mark and invert to mix.

h. Standard ammonia solutions: Prepare ammonia standards in desired concentration range, using the stock standard (¶ 3*g*) and diluting with water.

i. Simulated digested standards: To prepare calibration standards without having to digest the standards prepared in ¶ 3*h*, proceed as follows:

Stock standard, 5.00 mg N/L: To a tared 250-mL container add about 5.0 g stock standard (250 mg N/L). Divide actual

---

* Regular Clorox, The Clorox Company, Pleasanton, CA, or equivalent.

weight of solution added by 0.02 and make up to this resulting total weight with diluent (¶ 3*b*). Shake to mix. Prepare working standards from this stock standard, diluting with the diluent (¶ 3*b*), not water.

4. Procedure

a. Digestion procedure: Carry both standards and samples through this procedure.

To a 75-mL block digestor tube add 25.0 mL sample or standard and then add 10 mL digestion solution (¶ 3*a*) and mix. Add four alundum granules to each tube for smooth boiling. Place tubes in preheated block digestor for 1 h at 200°C. After 1 h, increase block temperature to 380°C and continue to digest for 1 h at 380°C. Remove tubes from block and let cool for about 10 min. Dilute each to 25.0 mL with water and mix with vortex mixer. Cover tubes to prevent ammonia contamination.

b. FIA analysis: Set up a manifold equivalent to that in Figure 4500-N$_{org}$:2 and analyze digested standards and samples by method supplied by manufacturer or laboratory standard operating procedure. Follow quality control protocols described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus ammonia concentration. The calibration curve is linear.

6. Precision and Bias

a. Recovery and relative standard deviation: Table 4500-N$_{org}$:II gives results of single-laboratory studies.

b. MDL: A 130-µL sample loop was used in the method described above. Using a published MDL method,[1] analysts ran 21 replicates of a 0.1-mg N/L standard. These gave a mean of 0.103 mg N/L, a standard deviation of 0.014 mg N/L, and MDL of 0.034 mg N/L. A lower MDL may be obtained by increasing the sample loop volume and increasing the ratio of carrier flow rate to reagents flow rate.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 rev. 1.11 amended June 30, 1986. 49 CFR 43430.

TABLE 4500-N$_{org}$:II. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

Matrix	Sample/Blank Designation	Known Addition mg N/L	Recovery %	Relative Standard Deviation %
Wastewater treatment plant influent	Reference sample*	—	97	—
	Blank†	3.0	97	—
		6.0	99	—
	Site A‡§	0	—	3.3
		3.0	91	—
		6.0	95	—
	Site B‡§	0	—	3.6
		3.0	115	—
		6.0	93	—
	Site C‡§	0	—	5.1
		3.0	97	—
		6.0	107	—
Wastewater treatment plant effluent	Reference sample*	—	92	—
	Blank†	3.0	97	—
		6.0	100	—
	Site A‡‖	0	—	5.4
		3.0	94	—
		6.0	100	—
	Site B‡‖	0	—	4.1
		3.0	119	—
		6.0	81	—
	Site C‡‖	0	—	7.3
		3.0	93	—
		6.0	105	—
Landfill leachate	Reference sample*	—	96	—
	Blank†	3.0	101	—
		6.0	99	—
	Site A‡#	0	—	3.3
		3.0	95	—
		6.0	98	—
	Site B‡#	0	—	4.4
		3.0	134	—
		6.0	85	—
	Site C‡#	0	—	3.8
		3.0	98	—
		6.0	105	—

* U.S. EPA nutrient QC sample, 1.52 mg N/L.
† Determined in duplicate.
‡ Samples without known additions determined four times; samples with known additions determined in duplicate.
§ Sample dilutions: A - 5-fold; B - 10-fold; C - 5-fold. Typical relative difference between duplicates 3%.
‖ Sample dilutions: A - none; B - 2-fold; C - none. Typical relative difference between duplicates 1%.
Sample dilutions: A, B, and C - 25-fold. Typical relative difference between duplicates 4%.