



Total Kjeldahl Nitrogen (TKN), USEPA by Flow Injection Analysis (FIA)

(Cartridge Part #A002054)

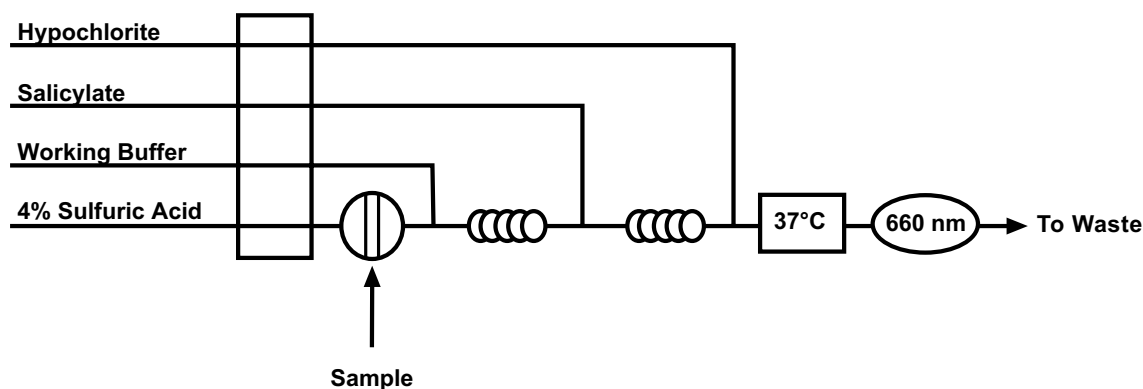
1.0 Scope and Application

- 1.1 This method is used for the determination of Total Kjeldahl Nitrogen (TKN) in drinking water, surface water, saline water, and domestic and industrial wastes according to USEPA Method 351.2 (Reference 15.4).
- 1.2 During digestion, amino acids, proteins, peptides, and other nitrogen compounds of biological origin are converted to ammonium sulfate. Nitrogenous compounds of some industrial wastes, such as amines, nitro compounds, hydrazones, oximes, semicarbazones, and some tertiary amines, may not be converted.
- 1.3 The Method Detection Limit (MDL) of this method is 0.013 mg/L TKN. The applicable range of the method is 0.05–20 mg/L TKN. The range may be extended to analyze higher concentrations by sample dilution.

2.0 Summary of Method

- 2.1 The sample is digested prior to analysis in the presence of sulfuric acid, potassium sulfate, and a mercury catalyst at a final temperature of 380°C. Free ammonia and organic nitrogen compounds are converted to ammonium sulfate under these conditions.
- 2.2 The ammonium reacts with salicylate and hypochlorite in a buffered alkaline solution in the presence of sodium nitroferricyanide (pH 12.8–13) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm (Reference 15.4).
- 2.3 The quality of the analysis is assured through reproducible calibration and testing of the Flow Injection Analysis (FIA) system.

- 2.4 A general flow diagram of the FIA system is shown below (see Section 17.0 for a detailed flow diagram).



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, “Glossary of Definitions and Purposes.”

4.0 Interferences

- 4.1 Precipitation of calcium and magnesium hydroxides is eliminated by potassium sodium tartrate in the working buffer.
- 4.2 Filter or centrifuge turbid digestates prior to the analysis.
- 4.3 Digestates with background absorbances at the analytical wavelength may interfere with the analysis.

5.0 Safety

- 5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.
- 5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Ammonium Sulfate, $(\text{NH}_4)_2\text{SO}_4$ (FW 132.15)

5.3.2 Chloroform, CHCl_3 (FW 119.38)

- 5.3.3 Potassium Sodium Tartrate Tetrahydrate, $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (FW 282.23)
- 5.3.4 Potassium Sulfate, K_2SO_4 (FW 174.26)
- 5.3.5 Red Mercuric Oxide, HgO (FW 216.61)
- 5.3.6 Sodium Hydroxide, NaOH (FW 40.00)
- 5.3.7 Sodium Hypochlorite, 5.25% available chlorine (household bleach), NaOCl (FW 74.44)
- 5.3.8 Sodium Nitroferricyanide Dihydrate, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ (FW 297.95)
- 5.3.9 Sodium Phosphate Dibasic Anhydrous, Na_2HPO_4 (FW 141.96)
- 5.3.10 Sodium Salicylate, $\text{NaC}_7\text{H}_5\text{O}_3$ (FW 98.08)
- 5.3.11 Sulfuric Acid, concentrated, H_2SO_4 (FW 98.08)
- 5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.
- 5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.
- 5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

- 6.1 Flow Injection Analysis (FIA) System (OI Analytical Flow Solution® 3000) consisting of the following:
 - 6.1.1 120-Place Autosampler
 - 6.1.2 Extended Range (ER) Photometric Detector with 5-mm path length flowcell and 660-nm optical filter
 - 6.1.3 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software
 - 6.1.4 TKN, USEPA Cartridge (Part #A002054)
- 6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.
- 6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

7.1 Raw Materials

- 7.1.1 Ammonium Sulfate, $(\text{NH}_4)_2\text{SO}_4$ (FW 132.15)
- 7.1.2 Brij®-35, 30% w/v (Part #A21-0110-33)
- 7.1.3 Chloroform, CHCl_3 (FW 119.38)
- 7.1.4 Deionized Water (ASTM Type I or II)
- 7.1.5 Potassium Sodium Tartrate Tetrahydrate, $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (FW 282.23)
- 7.1.6 Potassium Sulfate, K_2SO_4 (FW 174.26)
- 7.1.7 Red Mercuric Oxide, HgO (FW 216.61)
- 7.1.8 Sodium Hydroxide, NaOH (FW 40.00)
- 7.1.9 Sodium Hypochlorite, 5.25% available chlorine (household bleach), NaOCl (FW 74.44)
- 7.1.10 Sodium Nitroferricyanide Dihydrate, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$ (FW 297.95)
- 7.1.11 Sodium Phosphate Dibasic Anhydrous, Na_2HPO_4 (FW 141.96)
- 7.1.12 Sodium Salicylate, $\text{NaC}_7\text{H}_5\text{O}_3$ (FW 98.08)
- 7.1.13 Sulfuric Acid, concentrated, H_2SO_4 (FW 98.08)
- 7.1.14 Teflon® or glass boiling stones

7.2 Reagent Preparation

Note: For best results, filter and degas all reagents prior to use.

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge distilled/deionized water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

7.2.1.2 After preparing the degassed reagent water, store the reagent water in a tightly sealed container to protect it from reabsorption of atmospheric gases. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Digestion Reagents

7.2.2.1 1:4 Sulfuric Acid (50 mL)

7.2.2.1.1 While stirring, carefully add 10 mL of concentrated sulfuric acid to 40 mL of reagent water (Section 7.2.1).

Warning: Mixing sulfuric acid with water releases a great amount of heat. Take appropriate precautions.

7.2.2.2 Mercuric Sulfate Solution (100 mL)

7.2.2.2.1 Dissolve 8 g of red mercuric oxide in 50 mL of 1:4 sulfuric acid (Section 7.2.2.1).

7.2.2.2.2 Dilute to 100 mL with reagent water in a volumetric flask. Mix well.

Warning: Mercury compounds are highly toxic. Take appropriate precautions.

7.2.2.3 Digestion Solution (1 L)

7.2.2.3.1 While stirring, carefully add 200 mL of concentrated sulfuric acid to approximately 700 mL of reagent water.

7.2.2.3.2 Add 133 g of potassium sulfate and stir until dissolved.

7.2.2.3.3 Add 25 mL of mercuric sulfate solution (Section 7.2.2.2). Cool the solution to room temperature.

7.2.2.3.4 Quantitatively transfer the solution to a 1-L volumetric flask. Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing sulfuric acid with water releases a great amount of heat. Take appropriate precautions.

Warning: Mercury compounds are highly toxic. Take appropriate precautions.

7.2.3 Colorimetric Analysis Reagents

7.2.3.1 Start-up Solution (500 mL)

7.2.3.1.1 Add 1 mL of Brij-35 to approximately 400 mL of reagent water in a 500-mL volumetric flask.

7.2.3.1.2 Dilute to 500 mL with reagent water and mix well.

7.2.3.2 10 N Sodium Hydroxide (250 mL)

7.2.3.2.1 While stirring, carefully add 100 g of sodium hydroxide to approximately 175 mL of reagent water in a 250-mL volumetric flask.

7.2.3.2.2 Cool the solution to room temperature. Dilute to 250 mL with reagent water and mix well.

Warning: Mixing sodium hydroxide with water releases a great amount of heat. Take appropriate precautions.

Note: Store tightly capped in a plastic container. Prepare this solution monthly.

7.2.3.3 Stock Sodium Phosphate Buffer (1 L)

7.2.3.3.1 Dissolve 134 g of sodium phosphate dibasic anhydrous in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.2.3.3.2 Add 50 mL of 10 N sodium hydroxide (Section 7.2.3.2).

7.2.3.3.3 Dilute to 1,000 mL with reagent water and mix well.

7.2.3.4 Stock Potassium Sodium Tartrate Solution (1 L)

7.2.3.4.1 Dissolve 200 g of potassium sodium tartrate tetrahydrate in approximately 800 mL of reagent water in a 1-L volumetric flask.

7.2.3.4.2 Dilute to 1,000 mL with reagent water and mix well.

7.2.3.5 Working Buffer (1 L)

7.2.3.5.1 Mix 200 mL of stock sodium phosphate buffer (Section 7.2.3.3) with 200 mL of reagent water in a 1-L volumetric flask.

7.2.3.5.2 While stirring, add 250 mL of stock potassium sodium tartrate solution (Section 7.2.3.4). Continue stirring and slowly add 60 mL of 10 N sodium hydroxide (Section 7.2.3.2).

7.2.3.5.3 Dilute to 1,000 mL with reagent water and mix well.

7.2.3.5.4 Filter the solution through a 0.45- μ m filter. Add 0.5 mL of Brij-35 and mix gently to prevent foaming.

Note: Prepare this solution daily.

7.2.3.6 Salicylate/Nitroferricyanide Solution (500 mL)

7.2.3.6.1 Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroferricyanide dihydrate in approximately 300 mL of reagent water in a 500-mL volumetric flask.

7.2.3.6.2 Dilute to 500 mL with reagent water and mix well.

Note: Store in an amber bottle.

7.2.3.7 Sodium Hypochlorite Solution (200 mL)

7.2.3.7.1 Add 12 mL of sodium hypochlorite solution (5.25% available chlorine) to approximately 180 mL of reagent water in a 200-mL volumetric flask.

7.2.3.7.2 Dilute to 200 mL with reagent water and mix well.

Note: Store in an amber bottle. Prepare this solution daily.

7.2.3.8 Carrier/Sampler Wash Solution, 4% Sulfuric Acid (2 L)

7.2.3.8.1 While stirring, carefully add 80 mL of concentrated sulfuric acid to approximately 1,600 mL of reagent water in a 2-L volumetric flask.

7.2.3.8.2 Cool the solution to room temperature. Dilute to 2,000 mL with reagent water and mix well.

Warning: Mixing sulfuric acid with water releases a great amount of heat. Take appropriate precautions.

7.2.3.9 1 N Sodium Hydroxide Solution (500 mL)

7.2.3.9.1 Add 50 mL of 10 N sodium hydroxide (Section 7.2.3.2) to approximately 400 mL of reagent water in a 500-mL volumetric flask.

7.2.3.9.2 Dilute to 500 mL with reagent water and mix well.

7.3 Calibrant Preparation

7.3.1 Stock 1,000 mg/L Ammonia Nitrogen Solution (1 L)

7.3.1.1 Dissolve 4.717 g of ammonium sulfate (dried at 110°C) in approximately 900 mL of reagent water in a 1-L volumetric flask.

7.3.1.2 Dilute to 1,000 mL with reagent water. Preserve the solution by adding 2 drops of chloroform per liter.

Note: Store the solution at 4°C.

7.3.2 Intermediate Calibrant 20 mg/L Ammonia Nitrogen Solution (100 mL)

7.3.2.1 Use a volumetric pipet to add 2 mL of stock 1,000 mg/L ammonia nitrogen solution (Section 7.3.1) to approximately 80 mL of reagent water in a 100-mL volumetric flask.

7.3.1.2 Dilute to 100 mL with reagent water and mix well.

Note: Prepare this solution daily.

7.3.3 Working Calibrants

7.3.3.1 Add the designated volumes of stock or intermediate calibrant (see Equation 1) to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of reagent water.

7.3.3.2 Dilute each solution to the mark with reagent water and mix well.

EQUATION 1

$$C_1 V_1 = C_2 V_2$$

Where:

C_1 = Concentration (in mg/L) of stock solution (or calibrant)

V_1 = Volume (in L) of stock solution (or calibrant) to be used

C_2 = Desired concentration (in mg/L) of working calibrant to be prepared

V_2 = Final volume (in L) of working calibrant to be prepared

By solving this equation for the volume of stock solution to be used (V_1), the following equation is obtained:

$$V_1 = \frac{C_2 V_2}{C_1}$$

Since the desired concentration (C_2), the final volume (V_2), and the concentration of the stock solution (C_1) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_1) is easily calculated.

7.3.3.3 Calibrants covering the entire range of this analysis can be prepared from the following table.

| Final Concentration (mg/L) | Vol. of Inter. Cal. (μL) | Conc. of Inter. Cal. (mg/L) | Final Volume (mL) |
|----------------------------|--------------------------|-----------------------------|-------------------|
| 0.05 | 250 | 20 | 100 |
| 0.10 | 500 | 20 | 100 |

| Final Concentration (mg/L) | Vol. of Stock Cal. (μL) | Conc. of Stock Cal. (mg/L) | Final Volume (mL) |
|----------------------------|-------------------------|----------------------------|-------------------|
| 0.50 | 50 | 1,000 | 100 |
| 2.0 | 200 | 1,000 | 100 |
| 4.0 | 400 | 1,000 | 100 |
| 8.0 | 800 | 1,000 | 100 |
| 16.0 | 1,600 | 1,000 | 100 |
| 20.0 | 2,000 | 1,000 | 100 |

8.0 Sample Collection, Preservation and Storage

- 8.1 Samples should be collected in plastic or glass bottles that have been thoroughly cleaned and rinsed with reagent water (Section 7.2.1).
- 8.2 The volume of sample collected should be sufficient to ensure that a representative sample is obtained, replicate analysis is possible, and waste disposal is minimized.
- 8.3 Samples should be analyzed as soon as possible to reduce loss of analyte.
- 8.4 Samples may be preserved with 2 mL of concentrated sulfuric acid per liter of sample and refrigerated at 4°C.
- 8.5 Holding time for preserved, undigested samples is 28 days from the time of collection (Reference 15.5).

9.0 Quality Control

- 9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.2). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (MS/MSDs) as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.
 - 9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.
 - 9.1.2 In recognition of advances that are occurring in analytical technology and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.
 - 9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.3. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.
 - 9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification.

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

- a) calibration (Section 10.4)
- b) calibration verification (Section 9.5)
- c) initial precision and recovery (Section 9.2.2)
- d) analysis of blanks (Section 9.4)
- e) ongoing precision and recovery (Section 9.6)
- f) matrix spike and matrix spike duplicate (Section 9.3)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

- a) sample numbers and other identifiers
- b) analysis dates and times
- c) analysis sequence/run chronology
- d) sample weight or volume
- e) sample volume prior to each cleanup step, if applicable
- f) sample volume after each cleanup step, if applicable
- g) final sample volume prior to injection
- h) injection volume
- i) dilution data, differentiating between dilution of a sample or modified sample
- j) instrument and operating conditions
- k) other operating conditions
- l) detector

- m) printer tapes, disks, and other recording of raw data
 - n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported
- 9.1.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.
- 9.1.4 Analyses of laboratory reagent blanks (LRBs) are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of an LRB are described in Section 9.4.
- 9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical system is in control. This procedure is described in Section 9.6.
- 9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.
- 9.1.7 Accompanying QC for the determination of TKN is required per analytical batch. An analytical batch is a set of samples analyzed at the same time to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory reagent blank (LRB, Section 9.4), a laboratory control sample (LCS, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.
- 9.2 Initial Demonstration of Laboratory Capability
- 9.2.1 Method Detection Limit (MDL)—To establish the ability to detect TKN at low levels, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (Reference 15.1) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.3 must be achieved prior to practice of this method.
- 9.2.2 Initial Precision and Recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:
- 9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.
 - 9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery (\bar{x}) and the standard deviation of the percent recovery (s) for TKN. Use Equation 2 for the calculation of the standard deviation of the percent recovery (s).

EQUATION 2

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n-1}}$$

Where:

s = Standard deviation

n = Number of samples

x = Percent recovery in each sample

9.2.2.3 Compare s and x with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of s exceeds the precision limit or the value of x falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before the analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—The laboratory shall spike, in duplicate, a minimum of 10% of all samples (one sample in duplicate in each batch of 10 samples) from a given sampling site.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of TKN in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at one to five times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of TKN in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of TKN.

9.3.2.1 If necessary, prepare a stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of TKN (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution (Section 9.3.2.1) and analyze these aliquots to determine the concentration after spiking.

9.3.3 Calculate the percent recovery of TKN in each aliquot using Equation 3.

EQUATION 3

$$P = \frac{A - B}{T} \times 100$$

Where:

P = Percent recovery

A = Measured concentration of TKN after spiking (Section 9.3.2.2)

B = Measured background concentration of TKN (Section 9.3.2)

T = True concentration of the spike

9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch must be reanalyzed.

9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 4.

EQUATION 4

$$RPD = \left[\frac{|D_1 - D_2|}{(D_1 + D_2)/2} \right] \times 100$$

Where:

RPD = Relative percent difference

*D*₁ = Concentration of TKN in the spiked sample

*D*₂ = Concentration of TKN in the spiked duplicate sample

- 9.3.7 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be reanalyzed.
- 9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_a) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P_a - 2s_p$ to $P_a + 2s_p$. For example, if $P_a = 90\%$ and $s_p = 10\%$ for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each five to 10 new accuracy measurements).
- 9.4 Laboratory Reagent Blanks (LRB)—Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.
- 9.4.1 Analyze an LRB initially (i.e., with the tests in Section 9.2) and with each analytical batch. The LRB must be subjected to the exact same procedural steps as a sample.
- 9.4.2 If TKN is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB shows no evidence of contamination.
- 9.5 Calibration Verification—Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the midrange calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).
- 9.6 Laboratory Control Sample (LCS)—To demonstrate that the analytical system is in control and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:
- 9.6.1 Analyze an LCS with each analytical batch according to the procedure in Section 10.0.
- 9.6.2 If the precision and recovery for the LCS are within the acceptance criteria specified in Section 13.0, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.
- 9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for TKN by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85–105%.

- 9.7 Reference Sample—To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institute of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Configuration and Start-up

10.1 Instrument Configuration

- 10.1.1 Configure the OI Analytical Flow Solution 3000 Analyzer according to the Operator's Manual and verify that each module is properly powered on.
- 10.1.2 Verify that the TKN, USEPA Cartridge (Part #A002054) is configured as illustrated in the flow diagram shown in Section 17.0.
- 10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

- 10.2.1 Connect the reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.3.1). Start the pump, allowing the start-up solution to flow through the entire system.
- 10.2.2 Verify that the flowcell of each detector is purged of all bubbles and the flow is stable and free from surging before proceeding.

10.3 Baseline Verification

- 10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.6) for help on creating a Method.
- 10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.6).
- 10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

- 10.4.1 Prepare a series of at least three working calibrants using the stock solutions (Section 7.3) according to Equation 1, covering the desired analysis range.
- 10.4.2 Place the calibrants in the autosampler in order of decreasing concentration. Each calibrant should be analyzed according to the analytical procedures in Section 11.0. A calibration curve will be calculated by the WinFLOW software.
- 10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding “true” concentration.
- 10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.5). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Digestion

11.1.1 Blanks, Calibrants, Samples, and QC Samples

Note: The final diluted volume after digestion will be 25 mL.

11.1.1.1 For blanks, add 25 mL of reagent water to the tube.

11.1.1.2 For calibrants, use a volumetric pipet to add the appropriate amount of stock or intermediate calibrant (Section 7.3) to each tube. Completely rinse the material down the side of the tube with reagent water to an approximate volume of 25 mL. Mix well with a vortex mixer.

11.1.1.3 For samples, use a volumetric pipet to add 25 mL to the tube and mix well with a vortex mixer. If samples are known to be out of range, smaller volumes can be used and the result multiplied by the appropriate dilution factor.

11.1.1.3.1 For example, if 10 mL of sample is digested, multiply the result by 2.5 after digestion and analysis. If 5 mL of sample is digested, multiply the result by 5 after digestion and analysis.

11.1.1.4 For QC samples, prepare the samples according to its instructions and add 25 mL to the tube. Mix well with a vortex mixer.

11.1.2 Use a volumetric pipet to add 5 mL of digestion solution (Section 7.2.2.3) to each tube and mix well with a vortex mixer.

Note: Measure the digestion solution accurately to ensure uniform acid content in each tube.

11.1.3 Add 4–6 Teflon or glass boiling stones to each tube.

Note: Too many boiling stones will cause the sample to boil over.

11.1.4 Place the tubes in the block digester and set the temperature to 160°C. Allow the volume of each tube to be reduced to about 5 mL (approximately one hour).

Note: The evaporation step reduces the volume of liquid in the tube before the temperature is increased for digestion.

11.1.5 Increase the temperature to 380°C for 2½ hours. When the digestion is complete, the final volume will be about 2–3 mL. The liquid will be clear or straw-colored.

Note: If any amount of liquid boils out of the tube during the digestion, the result will be in error.

Note: If an exhaust system or scrubber is placed on top of the tubes during the digestion, care must be taken not to draw significant amount of fumes during the high temperature stage. Variable acid concentrations may result. An exhaust system is not necessary if the digestion apparatus is placed in a properly operating fume hood.

11.1.6 Remove the tubes from the block to cool. Do not allow the digests to crystallize while cooling. To prevent crystallization, add a few milliliters of reagent water to the digests after cooling the tubes for several minutes.

Caution: If the water is added too soon and the acid is still hot, the contents of the tube may react vigorously and some material may be lost from the tube.

11.1.7 Dilute the digestate to 25 mL with reagent water. The digests must be accurately diluted to a known volume in a calibrated container in order to ensure consistent acid content in the final solutions. If the contents of the tubes are removed to calibrated containers during this step, ensure that all of the material is transferred.

11.2 Analysis

Note: Pay special attention to Sections 11.2.2 and 11.2.7 to avoid precipitation of the salicylate/nitroferricyanide solution.

11.2.1 Set up the cartridge as shown in Section 17.0. Begin pump flow with the start-up solution (Section 7.2.3.1). Once the heater unit has reached 37°C, verify a stable baseline (Section 10.3).

11.2.2 Leave the salicylate line in reagent water while pumping all other reagents for at least 5 minutes. Then put the salicylate line into its reagent bottle and pump the salicylate/nitroferricyanide solution through the system.

11.2.3 If precipitate forms after addition of the salicylate/nitroferricyanide solution, it was added too soon or the pH of the stream was too low.

- 11.2.3.1 Immediately stop the pump and disconnect the manifold from the debubbler.
- 11.2.3.2 Place all reagent lines in 1 N sodium hydroxide (Section 7.2.3.9).
- 11.2.3.3 Turn on the pump and flush the system for at least 10 minutes or until all of the precipitate has been removed from the system.
- 11.2.3.4 Rinse with reagent water for 5 minutes. Return the reagent lines to their bottles, except the salicylate. Repeat Section 11.2.2.
- 11.2.4 When the system has stabilized, allow to pump at least 10–15 minutes and verify there are no bubbles in the flowcell. Obtain a stable baseline at 660 nm and autozero the baseline before beginning the analysis.
- 11.2.5 Load the sampler tray with calibrants, blanks, samples, and QC samples.

Note: The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.
- 11.2.6 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the “Fast Forward” button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the Sample Table.
- 11.2.7 When analysis is complete, remove the salicylate line from its reagent bottle and place it in reagent water. Pump the other reagents for at least 5 minutes. Then place the other lines in reagent water and pump for at least 10–15 minutes. Stop the pump, release the tension on all pump tubes, and power off the system.

11.3 Operating Notes

- 11.3.1 Variations in acid concentration can cause problems in the analysis of the digests. Titrate several aliquots from different tubes (after diluting to volume) to determine that a consistent acid content is being achieved.

Note: The final acid concentration of some samples (e.g., those containing high organic content, color, substantial amounts of carbonates or bicarbonates, etc.) may not be 4%.
 - 11.3.1.1 If the acid content determined by titration is consistent but different from the carrier/sampler wash solution (Section 7.2.3.8), remake the carrier/sampler wash solution to the same concentration.
 - 11.3.1.2 Raise or lower the amount of sodium hydroxide in the working buffer proportionately.
- 11.3.2 Problems can be isolated to either the cartridge or the digestion by preparing and analyzing undigested calibrants in carrier/sampler wash solution.

11.3.3 If a stable baseline on reagents cannot be achieved, investigate the following:

11.3.3.1 Filter the working reagents. Make sure the reagent containers are free from dust.

11.3.3.2 Disconnect the debubbler from the flowcell by removing the transmission tubing from the debubbler. Use a syringe fitted with a union to flush the flow cell with start-up solution (Section 7.2.3.1) followed by reagent water. Reconnect the debubbler.

11.3.3.3 Remove reagents from the cartridge and flush start-up solution through the cartridge using a syringe. Pump start-up solution through the lines until a stable baseline is achieved.

11.3.3.4 Clean the cartridge with 1 N hydrochloric acid. Remove the salicylate line to reagent water for 5 minutes before placing the reagent lines in the 1 N hydrochloric acid to prevent precipitation in the cartridge.

11.3.4 If poor peak shape or carryover is observed, investigate the following:

11.3.4.1 Check that the proper sample and wash times are set in the method.

11.3.4.2 Verify that the flow through the cartridge is smooth and consistent. Check for loose fittings.

11.3.4.3 Remake all reagents, including the stock reagents. Sodium hypochlorite solution must be fresh.

11.3.5 If high background or blank values are observed, carefully evaluate the source of water, the cleaning procedure for the digestion glassware, and the laboratory environment.

11.3.6 If poor sensitivity or nonlinearity is observed, investigate the following:

11.3.6.1 Check for excessive pump tube wear or a flow restriction. Replace pump tubes and verify that all lines are pumping freely.

11.3.6.2 Verify that the heater unit is on and the temperature is not too low.

11.3.6.3 Remake all reagents, including stock reagents. Sodium hypochlorite solution must be fresh.

11.3.6.4 The acid content of the digestates does not match that of the carrier/sampler wash (see Section 11.3.1).

11.3.7 The pH of the stream from the flowcell should be 13 before addition of the salicylate/nitroferriicyanide solution.

12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

13.0 Method Performance

| | |
|-------------------------------|-----------------|
| Range: | 0.05–20 mg/L |
| Throughput: | 55 samples/hour |
| Precision: | |
| 2.0 mg/L | <1% RSD |
| 16.0 mg/L | <1% RSD |
| Method Detection Limit (MDL): | 0.013 mg/L |

14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult Section 13.6 of *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 15.3).

15.0 References

15.1 *Code of Federal Regulations*, Part 136, Title 40, Appendix B, 1994.

15.2 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*; EPA-600/4-79-019; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1979.

15.3 *Less is Better: Laboratory Chemical Management for Waste Reduction*. Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036.

15.4 Nitrogen, Kjeldahl, Total (Colorimetric, Semi-Automated, Block Digester, AAII). *Methods for Chemical Analysis of Water and Wastewater*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 351.2.

15.5 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii.

15.6 WinFLOW Software and Operator's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

16.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

| | |
|----|--------------------------|
| °C | degrees Celsius |
| % | percent |
| ± | plus or minus |
| ≥ | greater than or equal to |
| ≤ | less than or equal to |

16.1.2 Alphabetical characters

| | |
|------|---------------------|
| g | gram |
| L | liter |
| mg | milligram |
| mg/L | milligram per liter |
| µg | microgram |
| µg/L | microgram per liter |
| mL | milliliter |
| ppm | parts per million |
| ppb | parts per billion |
| M | molar solution |
| N | normal solution |

16.2 Definitions

16.2.1 Initial Precision and Recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

16.2.2 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

16.2.3 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix that is treated like a sample, including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

- 16.2.4 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. The MS/MSD is analyzed like a sample. Its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot, and the measured values in the MS/MSD must be corrected for the background concentration.
- 16.2.5 Method Detection Limit (MDL)—The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 16.2.6 Minimum Level (ML)—The level at which the entire analytical system will give a recognizable signal and acceptable calibration point, taking into account method-specific sample and injection volumes.
- 16.2.7 Ongoing Precision and Recovery (OPR)—See Section 16.2.2, “Laboratory Control Sample.”

17.0 Figures

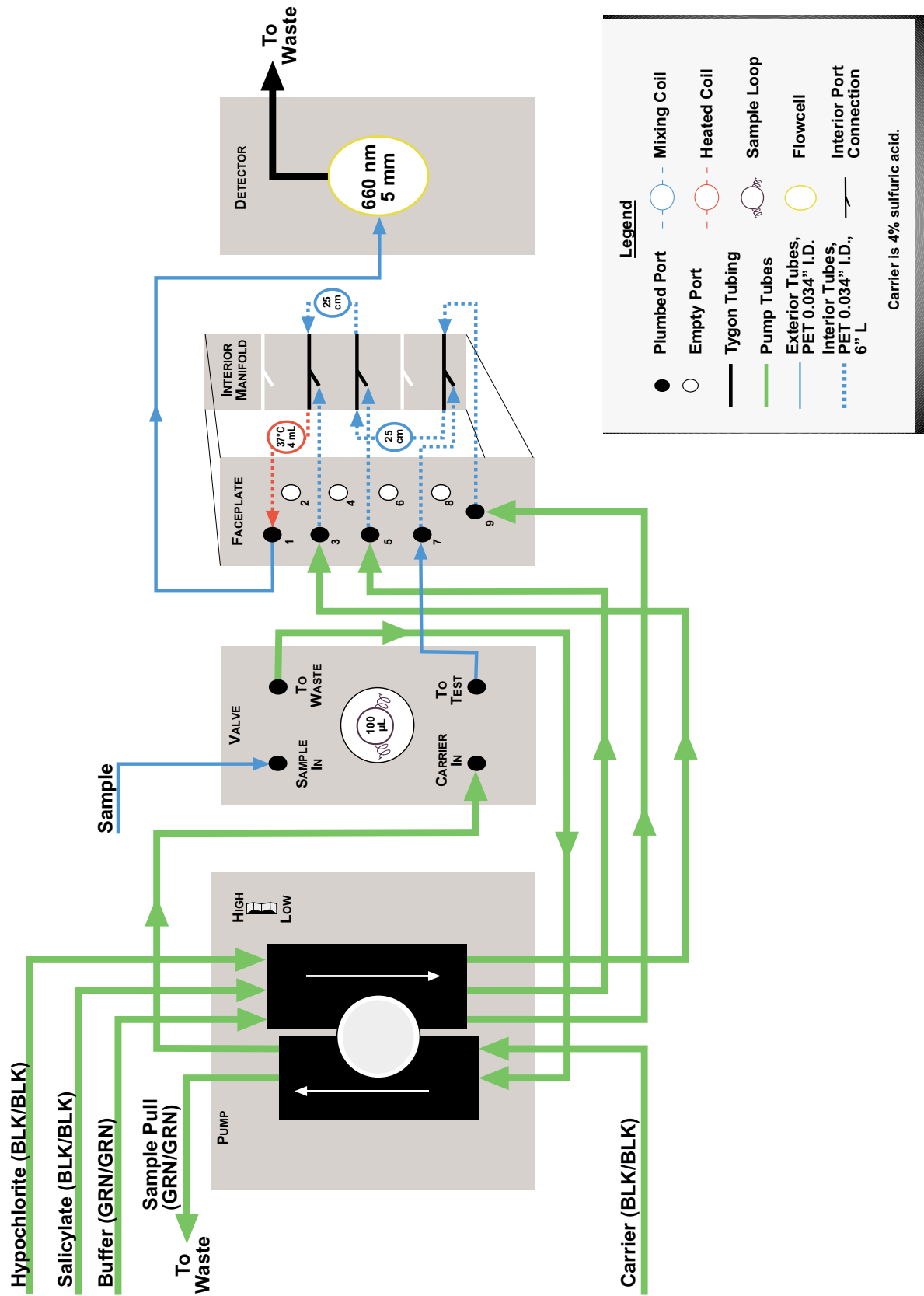


Figure 1. Detailed Flow Diagram for TKN, USEPA by FIA on a Flow Solution 3000, Cartridge Part #A002054

Results were obtained under optimal operating conditions. Actual results may vary depending on sample introduction, cleanliness of sample containers, reagent purity, operator skill, and maintenance of instruments.

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