

**STANDARD OPERATING PROCEDURE**  
**For**  
**Determination of Total Phosphorus Using the**  
**Automated Ascorbic Acid Reduction Method by Standard Methods**  
**SM 4500-P B(5), F (SM 23<sup>rd</sup> Edition)**  
  
**Determination of Total Nitrogen Using the Automated**  
**Cadmium Reduction Method by Standard Methods Modified**  
**SM 4500-N C, SM 4500 NO<sub>3</sub>-N F (SM 23<sup>rd</sup> Edition)**

SOP #: SM 4500-P B(5), F  
Modified SM 4500-N C,  
SM 4500 NO<sub>3</sub>-N F

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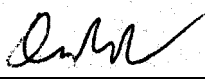
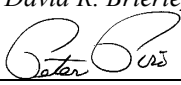
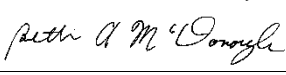

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## LIST OF REVISIONS

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**METHOD # SM 4500-P B(5), F**  
**METHOD # SM 4500-N C, SM 4500-NO<sub>3</sub>-N F**

**TITLE:** Phosphorus by Persulfate Digestion/Automated Ascorbic Acid Method  
Nitrogen by Persulfate Digestion/Automated Cadmium Reduction Method

**ANALYTES:** Total Phosphorus and Total Nitrogen

**INSTRUMENTATION:** Skalar Segmented Flow Analyzer

**1.0 SCOPE AND APPLICATION**

- 1.1 This method is applicable to acid-preserved surface waters and ground waters. Our laboratory's analytical range for Total Nitrogen (TN) is from 0.075 to 1.3 mg-N/L and for Total Phosphorus (TP) is from 0.0020 to 0.13 mg-P/L. The range may be extended with sample dilution or by using a higher curve.
- 1.2 These methods, with the above analytical ranges, are not appropriate for wastewaters, which may have concentrations two orders of magnitude higher than the calibration ranges. Wastewaters may also pose contamination problems for the instrument.
- 1.3 The methods may be run in combination or individually.
- 1.4 In natural waters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state: nitrate, nitrite, ammonia, and organic nitrogen. All these forms of nitrogen, as well as N<sub>2</sub>, are bio-chemically inter-convertible and are components of the nitrogen cycle. Phosphorus occurs almost solely as phosphates (-PO<sub>4</sub>). Forms of phosphate include orthophosphates, condensed phosphates, and organically bound phosphates.
- 1.5 Condensed phosphates are major constituents of commercial cleaning preparations. Orthophosphates present in fertilizers are carried into surface waters with storm runoff. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by human/animal wastes and food residues and may be formed from orthophosphates in biological treatment processes or by receiving water biota.
- 1.6 Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of freshwaters. Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.
- 1.7 Phosphates are classified analytically (based on analytical procedure) into three different types: reactive phosphorus, acid-hydrolyzable phosphorus, and organically bound phosphorus.
  - 1.7.1 Reactive phosphorus: Respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample. Reactive is largely a measure of orthophosphate, however, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure.
  - 1.7.2 Acid-hydrolyzable phosphorus: Acid hydrolysis at boiling water temperature converts condensed phosphates to dissolved orthophosphate.
  - 1.7.3 Organically bound phosphorus: Phosphate fractions that are converted to orthophosphate only by oxidative destruction of the organic matter present.



- 1.8 Analytically, the alkaline UV persulfate digestion collectively measures organic nitrogen, ammonia, nitrate and nitrite and is referred to as Total Nitrogen (TN). All nitrogen-containing compounds are oxidized to and determined as nitrate. The Total Phosphorus (TP) UV persulfate digestion measures the sum of all naturally occurring orthophosphates (reactive) and digested condensed and organic phosphates converted to orthophosphates.

## 2.0 SUMMARY OF METHOD

- 2.1 For total phosphorus, the sample is mixed with a potassium peroxodisulfate solution. The organic phosphates are broken down by means of UV radiation. Sulfuric acid is added to the sample stream and the solution is heated to 110°C. Complex inorganic phosphates are digested to orthophosphate. Sodium hydroxide is added to neutralize the solution. Ammonium heptamolybdate, catalyzed by antimony (III) oxide tartrate, reacts in an acidic medium with diluted solutions of phosphate to form a phospho-molybdic acid complex. This complex is reduced into a blue complex by ascorbic acid and measured colorimetrically at 880 nm.
- 2.2 For total nitrogen, the sample is mixed with a potassium peroxodisulfate/sodium hydroxide solution. This solution is mixed and brought into a UV digester and then heated to 110°C. After dialysis, the nitrate is reduced to nitrite by a cadmium copper reductor. The nitrite is determined colorimetrically with a sulfanilamide/NED color reagent at 540 nm.

## 3.0 DEFINITIONS

- 3.1 Dissolved Phosphorus: Sample filtered through a 0.45-µm membrane filter.
- 3.2 Reactive Phosphate: The fraction of phosphate (mostly orthophosphate) that will react with color reagents without any preliminary treatment.
- 3.3 Drift Standard: A standard run periodically to adjust for baseline and response shift; it is followed by a Wash.
- 3.4 Condensed Phosphates: Pyro-, meta-, and other polyphosphates
- 3.5 Calibration Blank: A volume of reagent water fortified with the same matrix as the calibration standards but without the analyte. The calibration blank is a zero standard and is used to calibrate the flow analyzer.
- 3.6 Calibration Standard (CAL): A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.7 Continuing Calibration Verification (CCV) or Instrument Performance Check (IPC) Solution: A solution of method analytes used to evaluate the performance of the instrument system with respect to a defined set of method criteria after calibration. Consists of calibration points (typically midrange) periodically reanalyzed within the test cycle to verify that the initial calibration is applicable throughout the sample run. A CCV is required after every tenth sample and at the end of the analytical run.
- 3.8 Initial Calibration Blank (ICB): A volume of reagent water fortified with the same matrix as the calibration standards but without the analyte. An ICB is analyzed after calibration.
- 3.9 Initial Calibration Verification (ICV): A solution of method analytes, at or near the calibration midpoint, used to evaluate the performance of the instrument system with respect to a defined set of method criteria immediately after calibration. An IPC is required after calibration.



- 3.10 Instrument Detection Limit (IDL): The concentration equivalent to the analyte signal, which is equal to three times the standard deviation of a series of ten replicate measurements of the calibration blank signal at the same wavelength.
- 3.11 Laboratory Duplicates (Sample Duplicate): Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analysis of the sample and the sample duplicate indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.12 Laboratory Fortified Blank (LFB): An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
- 3.13 Laboratory Fortified Sample Matrix (LFM): An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations for the purpose of determining LFM recoveries.
- 3.14 Laboratory Reagent Blank (LRB): An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.
- 3.15 Linear Calibration Range (LCR): The concentration range over which the instrument response to an analyte is linear.
- 3.16 Method Detection Limit (MDL): The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.17 Minimum Reporting Limit (MRL): The lowest amount of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy under stated analytical conditions. This defined concentration can be no lower than the concentration of the MRL check standard for that analyte and can only be used if acceptable quality control criteria for the analyte at this concentration are met.
- 3.18 MRL Check Standard: Low-level standard with concentration generally 3 to 5 times the MDL value. The standard is analyzed at the beginning of each analytical run before the samples are run.
- 3.19 Quality Control Sample (QCS): A solution of method analytes of known concentrations, which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory and instrument performance with externally prepared test materials.
- 3.20 Safety Data Sheet (SDS): Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
- 3.21 Stock Standard Solution (SSS): A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.



- 3.22 Tracer: An aliquot of the highest calibration standard used by the software to establish analyte elution time.
- 3.23 Wash: An aliquot of acid blank that follows a Drift standard; used to adjust for baseline and response shift.
- 3.24 Wash Ignore: An aliquot of acid blank used after samples that may have a high concentration of analytes and/or contaminants.
- 3.25 UCL: Upper Control Limit = Mean concentration (X) + 3 standard deviation (SD)
- UWL: Upper Warning Limit = X + 2 SD
- LCL: Lower Control Limit = X - 3 SD
- LWL: Lower Warning Limit = X - 2 SD

#### **4.0 INTERFERENCES**

- 4.1 Over acidification of samples can result in low recovery of inorganic and organic nitrogen. Samples must be preserved with 2 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> per 500 mL of sample. High organic carbon concentrations greater than 150 mg/L interfere by depleting persulfate during the digestion.
- 4.2 Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate. Concentrations as low as 0.1 mg As/L interfere with the phosphate determination.
- 4.3 Hexavalent chromium and nitrite interfere with phosphorus to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L.
- 4.4 Chloride concentrations up to 1,000 mg/L do not interfere. Seawater digestions are tolerated up to 10,000 mg/L provided that calibrants are matrix matched. Higher chloride concentrations interfere by depleting persulfate levels needed to oxidize inorganic and organic nitrogen species to nitrate. Resulting active chlorine species can also interfere in the colorimetric reactions used to determine TN and TP.
- 4.5 Barium, lead, and silver can interfere by forming insoluble phosphates, but their concentrations in natural water samples are usually lower than the interference threshold.

#### **5.0 SAFETY**

- 5.1 Protective clothing, gloves and glasses must be worn when working with sulfuric acid and other toxic and/or corrosive chemicals. Refer to Safety Data Sheets (SDS) for toxicity and hazard levels.
- 5.2 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations.

#### **6.0 EQUIPMENT AND SUPPLIES**

- 6.1 Skalar San+ automated segmented-flow analyzer, controller, and autosampler.
- 6.2 Skalar FlowAccess software, Version 3.3.4 (Windows 10)





- 6.3 Autosampler tubes: Greiner Bio-One Polystyrene 12 mL, 16 mm x 100 mm
- 6.4 Peristaltic pump tubing, assorted: See Skalar methodology.
- 6.5 Volumetric glassware and/or calibrated pipettors: Labware that is used for total phosphorus must be acid-washed before first use. Volumetric flasks and Class A pipets must also be acid-washed after each use.
- 6.6 Personal Protective Equipment (PPE): Lab coats, gloves, safety glasses, etc.
- 6.7 Polished ASTM Type I reagent water with low ionic and total organic content.
- 6.8 Tubing and air stone to helium-sparge reagent water.
- 6.9 NIST-calibrated electronic-digital thermometer gun for ensuring standards and samples are at room temperature.

## 7.0 REAGENTS AND STANDARDS

- 7.1 Sulfuric Acid, 9.4 N: Fill a 2-L volumetric flask with approximately 1 L of reagent water, add a Teflon stir bar and place the flask in an ice water bath on a stir plate; turn on the stir plate. Using a 500-mL volumetric flask and 10-mL graduated cylinder, carefully transfer 508.4 mL of trace metal grade sulfuric acid to the 2-L flask. Rinse the 500-mL flask and graduated cylinder several times with reagent water and add the rinses to the 2-L flask. Add more reagent water until the liquid level is just below the 2-L mark. After cooling, bring the solution up to 2 L. Store the solution in a polyethylene bottle.
- 7.2 TN Oxidizing Reagent: First dissolve 25 grams of sodium hydroxide in 800 mL of reagent water. When dissolved, add 49 grams of **low-nitrogen** potassium peroxodisulfate (such as SIGMA Puriss grade) and 38 grams of di-sodium tetraborate. Mix and fill up to 1 L with reagent water. Solution is stable for 1 week in a polyethylene bottle. Swirl the solution before starting the application. This reagent contains precipitation; keep tube end approximately 2 cm above container bottom.
- 7.3 TN 0.4-M Hydrochloric Acid Solution: Dilute 40 mL of concentrated hydrochloric acid in reagent water. Mix and fill up to 1 L with reagent water. Solution is stable for 1 month.
- 7.4 TN Buffer Solution (Under Stream): Dissolve 35 grams of imidazole (weigh in fume hood or wear an N95 mask) in approximately 800 mL of reagent water. Adjust the pH to  $8.2 \pm 0.1$  with hydrochloric acid. Dissolve 5 grams of di-sodium EDTA and then dilute to 1 L with reagent water. Add 1.0 mL of Brij 35 (30%). Solution is stable for 1 week at 4°C.
- 7.5 TN Buffer Solution (Upper Stream): Dissolve 50 grams of ammonium chloride in approximately 800 mL of reagent water. Adjust the pH to  $8.2 \pm 0.1$  with ammonium hydroxide. Dilute to 1 L with reagent water, add 1.0 mL of Brij 35 and mix. Solution is stable for 1 week at 4°C.
- 7.6 TN Color Reagent: Dilute 100 mL of concentrated hydrochloric acid in reagent water. Add 10 grams of sulfanilamide and 0.5 grams of NED, dissolve and then dilute to 1 L. This is the same color reagent for nitrate/nitrite. Solution is stable in amber for 2 weeks, store in the dark.
- 7.7 Sampler Rinsing Liquid/Acid Blank: Add 8 mL of 9.4-N  $\text{H}_2\text{SO}_4$  to reagent water and dilute to 2 L. Prepare daily. This solution is also used for the ammonia, nitrate+nitrite, and chloride tests.



- 7.8 TN ~2.5-M Sulfuric Acid Air Scrubber Solution: Dilute 7 mL of concentrated sulfuric acid in reagent water. Mix, cool and then dilute to 50 mL with reagent water. Solution is stable for 1 month.
- 7.9 TP Oxidizing Solution: Dissolve 5 grams of potassium peroxodisulfate in 900 mL of reagent water. Adjust the pH to 1.1-1.2 with concentrated sulfuric acid and then dilute to 1 L. Solution is stable in a polyethylene bottle for 1 week.
- 7.10 TP Sulfuric Acid Solution: In a chilled water bath, add 135 mL of sulfuric acid to reagent water. Allow to cool to room temperature and then bring the final volume to 1 L with reagent water. Add 2 mL of FFD6. Solution is stable in a polyethylene bottle for 1 week.
- 7.11 TP Sodium Hydroxide Solution: In a chilled water bath, dissolve 105 grams of sodium hydroxide in reagent water, cool and bring the final volume to 1 L. Solution is stable in a polyethylene bottle for 2 weeks.
- 7.12 TP Ascorbic Acid Solution: Dissolve 5.5 grams of ascorbic acid in reagent water, add 30 mL of acetone and dilute to 500 mL with reagent water. Solution is stable for 3 days at 4°C.
- 7.13 TP Ammonium Heptamolybdate Solution: Dissolve 0.225 g of potassium antimony oxide tartrate in reagent water. While stirring, first add 30 mL of sulfuric acid and dissolve 6 grams of ammonium heptamolybdate. Dilute to 1 L with reagent water. Solution is stable in a polyethylene bottle for 1 week. After remaining solution is disposed, the polyethylene container should be rinsed at least three times with reagent water, once with hypochlorite cleaning solution (see below), and at least six times with reagent water.
- 7.14 Stock TN Standard - 1000 mg/L as NO<sub>3</sub>-N: Quantitatively add 1.8045 g of potassium nitrate (KNO<sub>3</sub>) and 1.0 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> to a 250.0-mL volumetric flask. Dissolve and dilute to volume with reagent water. Solution is stable for 3 months at 4°C.
- 7.15 Stock TP Standard - 100 mg/L as PO<sub>4</sub>-P: Quantitatively add 0.1098 g of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) and 1.0 mL 9.4-N H<sub>2</sub>SO<sub>4</sub> to a 250.0-mL volumetric flask. Dissolve and dilute to volume with reagent water. Solution is stable for 3 months at 4°C.
- 7.16 Auto-Diluter TN/TP Stock Standard - 10/1.0 mg/L as NO<sub>3</sub>-N/PO<sub>4</sub>-P: Add 10.0 mL of stock KH<sub>2</sub>PO<sub>4</sub> and 10.0 mL of stock KNO<sub>3</sub> to a 1-L volumetric flask. Dilute to volume with acid blank or add 3.92 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> and dilute to volume with reagent water. Solution is stable for 1 month at 4°C.
- 7.17 Laboratory-Fortified Matrix (LFM) Spike Standard - 15/1.5 mg/L NO<sub>3</sub>-N/PO<sub>4</sub>-P: Add 15.0 mL of stock KH<sub>2</sub>PO<sub>4</sub> and 15.0 mL of stock KNO<sub>3</sub> to a 1-L volumetric flask. Dilute to volume with acid blank or add 3.88 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> and dilute to volume with reagent water. Solution is stable for 1 month at 4°C.
- 7.18 TN/TP Calibration Standards:
- 7.18.1 Standard # 9 (1.3/0.13 mg/L TN/TP): Auto-Diluter Stock diluted 7.69X (manual equivalent 13.0 mL/100 mL)
- 7.18.2 Standard # 8 (1.0/0.10 mg/L TN/TP): Auto-Diluter Stock diluted 10X (manual equivalent 5.0 mL/50 mL)
- 7.18.3 Standard # 9 (1.3/0.13 mg/L TN/TP): Auto-Diluter Stock diluted 7.69X (manual equivalent 13.0 mL/100 mL)



- 7.18.4 Standard # 8 (1.0/0.10 mg/L TN/TP): Auto-Diluter Stock diluted 10X (manual equivalent 5.0 mL/50 mL)
- 7.18.5 Standard # 7 (0.75/0.075 mg/L TN/TP): Auto-Diluter Stock diluted 13.3X (manual equivalent 15.0 mL/200 mL)
- 7.18.6 Standard # 6 (0.50/0.050 mg/L TN/TP): Auto-Diluter Stock diluted 20X (manual equivalent 5.0 mL/100 mL)
- 7.18.7 Standard # 5 (0.25/0.025 mg/L TN/TP): Auto-Diluter Stock diluted 40X (manual equivalent 5.0 mL/200 mL)
- 7.18.8 Standard # 4 (0.10/0.010mg/L TN/TP): Auto-Diluter Stock diluted 100X (manual equivalent 2.0 mL/200 mL)
- 7.18.9 Standard # 3 (0.075/0.0075mg/L TN/TP): Auto-Diluter Stock diluted 133X (manual equivalent 7.5 mL/1 L)
- 7.18.10 Standard # 2 (0.050/0.0050mg/L TN/TP): Auto-Diluter Stock diluted 200X (manual equivalent 5.0 mL/1 L)
- 7.18.11 Standard # 1 (0.00/0.00 mg/L TN/TP): Referred to as the Acid Calibration Blank, prepared as 4.0 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub>/1.00 L of reagent water.
- Manually prepared standards are diluted with acid blank. Calibration standards are prepared daily.
- 7.19 Quality Control Standard (QCS) – Glycine-N Standard (100 mg/L N): Quantitatively add 0.2680 g of glycine to a 500.0-mL volumetric flask. Dissolve and dilute to volume with reagent water. Solution is stable for 3 months at 4°C.
- 7.20 Stock TP QCS and Recovery Check Standard - 100 mg/L Pyridoxal: Quantitatively add 0.8561 g of pyridoxal 5-phosphate hydrate to a 1-L volumetric flask. Dissolve and dilute to volume in flask with reagent water. Solution is stable for 3 months at 4°C. Store solid pyridoxal phosphate between 2-8°C; stable for at least 1 year – monitor for degradation.
- 7.21 Manual LFM Sample Preparation: 2.0 mL of LFM spike std and 48.0 mL of sample into a 50.0-mL volumetric flask. The spike amount is 0.6250 mg/L for TN and 0.06250 mg/L for TP with a 1.0417 dilution.
- 7.22 Method Reporting Limit (MRL) Standards:
- 7.22.1 TN: Same as Calibrator # 3 prepared by the auto-diluter (0.075 mg/L TN).
- 7.22.2 TP: Add 2.0 mL of the Auto-Diluter Stock and 3.99 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> to a 1-L volumetric flask. Dilute to volume with reagent water (0.0020 mg/L TP).
- 7.23 Laboratory Fortified Blanks (LFB) Standard (0.25/0.025 mg/L TN/TP): Same as Calibrator # 4 and prepared by the auto-diluter.
- 7.24 Quality Control Sample (QCS) Standard (1.00/0.10 mg/L Glycine-N/ Pyridoxal-phosphate-P): Add 10 mL of 100-mg/L stock glycine-N, 1.0 mL of the 100-mg/L stock pyridoxal phosphate-P standard and 4.0 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> to a 1-L volumetric flask. Dilute to volume with reagent water.



- 7.25 UV Digester Recovery Check Standard (0.50/0.050 TN/Pyridoxal 5-Phosphate): 5.0 mL of 100-mg/L glycine, 0.50 mL of 100-mg/L pyridoxal 5-phosphate and 4.0 mL of 9.4-N H<sub>2</sub>SO<sub>4</sub> to a 1-L volumetric flask. Dilute to volume with reagent water.
- 7.26 Carryover Check Standard (2.5/0.25 mg/L TN/TP): Auto-Diluter Stock standard, diluted 4X by the instrument, followed by an acid blank. This solution is to check if carryover can be expected into the next sample. If the next field sample in a run was preceded by a high concentration sample, up to the carryover check standard concentration, it does not have to be repeated if no carryover occurs in the blank. However, the analyst has the option to repeat subsequent samples if the previous sample was above the curve, especially if there is suspicion that the matrix impacts peak symmetry. This is not used to extend the calibration range.
- 7.27 Hypochlorite Cleaning Solution: Dilute 160 mL of 12.5% sodium hypochlorite solution to 2 L with reagent water. This solution is also used for nitrate/nitrite and ammonia analyses.
- 7.28 0.1-N Hydrochloric Acid Cleaning Solution: Dilute 20 mL of trace metal-grade hydrochloric acid to 2 L with reagent water.
- 7.29 Phosphorus Detector Cleaning Solution # 1: Dilute ~0.15 mL FFD6 to 100 mL with reagent water. Do not use Brij, as it contains phosphorus.
- 7.30 Phosphorus Detector Cleaning Solution # 2: HPLC-grade methanol.
- 7.31 Phosphorus Detector Cleaning Solution # 3: Dilute 20 mL of trace metal-grade hydrochloric acid to 100 mL with reagent water.
- 7.32 Phosphorus Detector Storage Solution: Dilute 100 mL of HPLC-grade methanol to 500 mL with reagent water.
- 7.33 2-M Hydrochloric Acid Digester Cleaning Solution: Dilute 100 mL of trace metal-grade hydrochloric acid to 500 mL with reagent water.
- 7.34 2-M Sodium Hydroxide Digester Cleaning Solution: Dilute 40 g of sodium hydroxide to 500 mL with reagent water.
- 7.35 Ethanol, 96%
- 7.36 TP Glassware Cleaning Solution: Fill a carboy with 12.383 L of reagent water and add 650 mL of trace metal-grade hydrochloric acid.

## **8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE**

- 8.1 Sample collection techniques appropriate to the sample type must be practiced.
- 8.2 If dissolved analytes are to be measured, a sample aliquot must be filtered prior to acidification.
- 8.3 Samples must be preserved to pH < 2 with 9.4-N sulfuric acid.
- 8.4 Samples must be refrigerated at 4 ± 2°C.
- 8.5 Preserved/refrigerated samples must be analyzed within 28 days of collection.



## 9.0 QUALITY CONTROL

- 9.1 An LRB and an LFB are analyzed with every batch of up to 20 samples. The LRB must be below the reporting limit for total phosphorus (very low reporting limit) and below 50% of the reporting limit for total nitrogen. The LFB must be within 10% of true value.
- 9.2 Duplicates and LFM's are analyzed at a frequency of 10%. Duplicate RPDs and LFM recoveries should be within 20%.
- 9.3 A QCS is analyzed with every batch. It must be within 10% of true value.

## 10.0 CALIBRATION AND STANDARDIZATION

- 10.1 The instrument is calibrated on each day of use with at least three calibration standards for linear curves plus a calibration blank and uses the analysis procedure below.
- 10.2 Both calibration curves are linear and must have correlation coefficients ( $r$ )  $\geq 0.995$ .
- 10.3 Back-calculated values of calibration standards must be within 10% of the true values.

## 11.0 PROCEDURE

- 11.1 Remove standards, reagents and samples from refrigerators and allow them to equilibrate to room temperature; a warm water bath and electronic-digital thermometer gun can be used to accelerate the process for the standards and reagents.
- 11.2 If necessary, empty the instrument hazardous waste container into the drum in the hazardous waste room. Empty the instrument acidified water waste into a sink connected to the building pH neutralization tank.
- 11.3 Manually switch the outflow from the autosampler to the TN-TP splitter. Insert the TN flow cell and the 540-nm filter. Move the air tubes slightly to avoid pinching by the bars. Clamp down the platens on the autosampler and both pump units and turn them on; set the flow to high on the pump units for at least ten minutes to flush them. Make sure reagent water reservoirs are full. Turn on the analytical balance.
- 11.4 Rinse the reagent water carboy at least three times with polished reagent water and fill; sparge with helium for ten minutes.
- 11.5 Calibrate the analytical balance and 500- $\mu$ L pipettor. Other pipettors are also calibrated if they are expected to be needed for dilutions.
- 11.6 Turn on the backpressure pumps for TN and TP.
- 11.7 Partially fill the autosampler rinse and acid blank flasks with sparged water and acidify with 9.4-N sulfuric acid. Bring the flasks up to volume. Partially fill the quality control flasks (TP MRL Standard, UV Digester Recovery Check Standard, and QCS) with reagent water and acidify.
- 11.8 Start the flow for the autosampler acid blank then turn off the autosampler and turn it back on again to rinse the syringe with acid blank. Start the flow for the TP reagents, then turn on the ultraviolet lamp and heater (110°C). The flow for TP should remain on high, but the flow for TN can be set to normal to conserve water. Place the TP waste tube into the hazardous waste container.



- 11.9 Once a consistent bubble pattern is attained for TP, hold the power button until the off light is on and turn off the heater and ultraviolet lamp; the backpressure pump should remain on.
- 11.10 Perform the TP detector cleaning procedure (below), then turn on the heater and ultraviolet lamp; set the flow to high.
- 11.11 Once a consistent bubble pattern for TP is regained, set the flow to normal.
- 11.12 Turn on the Skalar control unit and allow it to initialize. Turn on the computer and start the Skalar FlowAccess software. Log in and select the "Unfiltered" system. Run the TP baseline. If a smooth and level baseline is not achieved, repeat the detector cleaning process with the flow, ultraviolet lamp and heater off; check the baseline again.
- 11.13 When a good TP baseline is achieved, start the flow for the TN reagents and turn on the ultraviolet lamp and heater (110°C) under high flow. Move the TN waste tube to the hazardous waste container. Once a consistent bubble pattern is achieved, turn on flow to the cadmium column and let it flush on high flow for at least one minute.
- 11.14 Set the TN flow to normal and run baselines for both analytes; meanwhile, insert pre-cleaned tubes for the drift and carryover standards and place the uncovered beaker of auto diluter stock into position; make sure the volume of the standard in the beaker is approximately 150 mL. Tubes are cleaned by filling with glassware cleaner, followed by at least three rinses with reagent water. The tubes are stored upside-down to dry.
- 11.15 Create the table for the run, using an existing table as a template. A Tracer starts the table, followed by a Drift and Wash. Next are the calibration standards, followed by a Wash-Ignore, a Drift and a Wash. Initial quality control samples follow. Every ten samples are followed by a Wash-Ignore, Drift, and a Wash, Continuing Calibration Verification Standard, Continuing Calibration Blank, Drift and a Wash. Print the table as a reference for filling the sample racks.
- 11.16 Turn off baseline acquisition and start autosampler preparation of the drift and carryover standards, click Control, then click on the autosampler image, then Pre-dilution Utility. The drift concentration should be 80% of the highest concentration in the calibration curve.
- 11.17 Once the drift and carryover standards are prepared, turn the pump units on high then turn the baselines on. Small carryover peaks from the standard preparation should appear in approximately 9-10 minutes for TN and 12-13 minutes for TP on high flow; meanwhile, complete preparation of the quality control standards in the flasks (TP MRL Standard, UV Digester Recovery Check Standard, and QCS).
- 11.18 Set the flows to normal.
- 11.19 Place the calibration standard tubes into position and start analysis. Turn on the auto shutdown feature, if needed.
- 11.20 Rinse the pre-dilution blank tubes with acid blank and fill one tube with acid blank; place the full tube in autosampler position A1 and the empty tube in position A2.
- 11.21 Fill the remaining sample tubes with unfiltered samples and place them into position in the autosampler rack(s).
- 11.22 During analysis, replenish the reagent water reservoirs.





- 11.23 Monitor the run for out-of-range samples. Dilutions of 2X (5 mL to 10 mL) and 5X (2 mL to 10 mL) may be prepared in pre-cleaned tubes with acid blank and acid-washed Class A glass pipets or calibrated pipettors. Higher dilution factors can be made with acid-washed volumetric flasks. Every ten samples are bracketed with continuing quality control, as mentioned above.
- 11.24 When the run is completed, turn off the flow to the TN cadmium column. Turn off the heaters and ultraviolet lamps for both analytes. Dip each reagent line into a 500-mL beaker of reagent water and place into the reagent water reservoir; turn the flow to high for both TN and TP.
- 11.25 Move the autosampler lines to the reagent water reservoir. Turn the autosampler off and on again to flush the syringe with reagent water.
- 11.26 Approximately 15 minutes after turning off the heaters, turn off both backflow pumps. Move the waste lines to the acidified water container. Set the flows to normal and rinse for at least 15 minutes more.
- 11.27 With the platen clamped down and the air tubes under the bars, use a syringe to flush the TP detector with approximately 3 mL of storage solution to limit bacterial growth.
- 11.28 Turn off the computer, controller, autosampler and pump units. Lift the platens on the autosampler and pump units.

## **12.0 DATA ANALYSIS AND CALCULATIONS**

- 12.1 The Skalar FlowAccess software performs peak identification and calculations, using peak heights and the calibration curves.
- 12.2 Unused calibrators may be deselected for each analyte; unused calibrators are noted on the chromatograms and result tables.
- 12.3 Peak heights may be adjusted manually; charting showing unadjusted and marked adjusted heights are printed.
- 12.4 Calibration curves, charting and result tables are printed and included in the run folders, along with reagent and standard preparation sheets.

## **13.0 MAINTENANCE**

- 13.1 Peristaltic pump tubing should be replaced monthly, or less often with reduced usage.
- 13.2 Platens on the pump units and autosampler must not be clamped down when not in use.
- 13.3 If the instrument is expected to go unused for more than several days, release the platens after use and wait at least 15 minutes for depressurization. Lift the pump bars and remove the air tubing from underneath them. Return the pump bars to the down position. This keeps the air tubing from being pinched and allows the pump bar springs to be in a more relaxed position.
- 13.4 The instrument's plastic tubing is replaced at least annually as part of a service contract with the manufacturer. More frequent tubing replacement may be needed if highly contaminated samples, such as wastewaters, are analyzed.
- 13.5 Weekly digester cleaning procedure:



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- 13.5.1 Make sure platens and air pump bars are clamped down.
  - 13.5.2 Turn black pressure regulators (with red dots): horizontal for TP, vertical for TN.
  - 13.5.3 Place small Kimwipes under the injection points.
  - 13.5.4 Connect an empty syringe and withdraw ~20 mL of air and water from each injection point to avoid overflow.
  - 13.5.5 Connect a syringe with ~20 mL of 2-M hydrochloric acid and flush. Wait 5 minutes.
  - 13.5.6 Use syringe to withdraw the hydrochloric acid and discard.
  - 13.5.7 Connect a syringe with ~ 20 mL of 2-M sodium hydroxide and flush. Wait 5 minutes.
  - 13.5.8 Use syringe to withdraw the sodium hydroxide and discard.
  - 13.5.9 Connect a syringe with ~20 mL of ethanol and flush. Wait five minutes.
  - 13.5.10 Use a syringe to withdraw the ethanol.
  - 13.5.11 Disconnect the syringe and rinse the system with reagent water for 30 minutes.
  - 13.5.12 Turn black pressure regulators (with red dots): back to vertical for TP, horizontal for TN.
  - 13.5.13 Unclamp the platens and air pump bars; shut the units off.
  - 13.6 Weekly cleaning procedure:
    - 13.6.1 Perform the weekly digester cleaning procedure above.
    - 13.6.2 Place both sets of reagent tubes in flasks with 0.1-M hydrochloric acid (HCl); place the three active autosampler tubes in another flask of 0.1-M HCl.
    - 13.6.3 Rinse the system for 45 minutes; longer if heavy contamination is suspected.
    - 13.6.4 Rinse the three sets of tubes by dipping them in 500-mL beakers of reagent water and place them into their respective reservoirs of reagent water.
    - 13.6.5 Rinse the system with reagent water for at least 30 minutes.
  - 13.7 Daily phosphorus detector cleaning procedure:
    - 13.7.1 Remove the lower detector tubing so the black connection remains on the upper detector tubing.
    - 13.7.2 Flush the detector with 3 mL of reagent water, using a syringe. Once air bubbles are flushed out, use a back-and-forth motion on the syringe, finish flushing.
    - 13.7.3 Flush the detector with 2 mL of cleaning solution (# 1), again with a back-and-forth motion.
    - 13.7.4 Rinse the syringe with reagent water and flush the detector with 2 mL of HPLC methanol (# 2), also with a back-and-forth motion.





13.7.5 Rinse the syringe with reagent water and flush the detector with 2 mL of 2-M HCl solution (# 3), using a back-and-forth motion.

13.7.6 Rinse the syringe with reagent water and flush the detector with 3 mL of reagent water, with a back-and-forth motion.

13.7.7 After flushing, reconnect the detector tubes.

## 14.0 METHOD PERFORMANCE

14.1 See the Initial Demonstration of Capability (IDC) for accuracy and precision data, along with results from the yearly Method Detection Limit (MDL) study, for method performance. All data are kept on file by the Quality Assurance Manager. Precision and accuracy quality control charts are generated automatically by the LIMS and reviewed monthly.

## 15.0 POLLUTION PREVENTION

15.1 The quantity of chemicals purchased should be based on expected usage during its shelf life.

15.2 Actual reagent preparation volumes should reflect anticipated usage during its shelf life.

## 16.0 WASTE MANAGEMENT

16.1 WES laboratory waste management practices comply with all applicable federal, state, and local rules and regulations.

## 17.0 REFERENCES

17.1 American Public Health Association (APHA). 2012. *Standard Methods for the Examination of Water and Wastewater*, 23rd Edition. APHA, American Public Health Association, and Water Environment Federation, Washington, D.C.

17.2 Total UV Digestible Nitrogen (Skalar Method). Catnr. 475-426 (+p6). Issue 092115/MH/99300143. Skalar Analytical B.V. Breda, The Netherlands.

17.3 Total UV Digestible Phosphate/Ortho Phosphate/Total Phosphate (Skalar Method). Catnr. 503-505w/r (+5290). Issue 092115/MH/99300143. Skalar Analytical B.V. Breda, The Netherlands.

17.4 Segmented Flow Analyzer: SA3000/5000 Chemistry Unit User Manual. 2013. Publication No: 0101063D.US Skalar Analytical B.V. Breda, The Netherlands.

17.5 FlowAccess V3 User Manual. 2013. Publication No: 0101092I.US. Skalar Analytical B.V. Breda, The Netherlands.



## 18.0 TABLES

**TABLE 1. Quality Control Tests and Acceptance Limits**

Accuracy			Precision		
QC Test	Acceptance Limits (% Recovery)	Frequency	QC Test	Acceptance Limits (RPD <sup>a</sup> )	Freq.
Laboratory Fortified Blank (LFB)	90 - 110	With every batch of ≤ 20 samples	Duplicates	≤ 20	10%
Laboratory Fortified Matrix (LFM)	80 - 120	10%			
Quality Control Standard (QCS)	90 - 110	With every batch of ≤ 20 samples			
Laboratory Reagent Blank (LRB)	> - MRL and < MRL (TP) > - ½ MRL and < ½ MRL (TN)	With every batch of ≤ 20 samples			
Initial Calibration Blank (ICB)	> - MRL and < MRL (TP) > - ½ MRL and < ½ MRL (TN)	After calibration			
Initial Calibration Verification (ICV)	90 - 110	After calibration			
Continuing Calibration Blank (CCB)	> - MRL and < MRL (TP) > - ½ MRL and < ½ MRL (TN)	After every 10 samples and at the end of the analytical run			
Continuing Calibration Verification (CCV)	90 - 110	After every 10 samples and at the end of the analytical run			
UV Digester Recovery Check Standard	≥ 90%	With every batch			
Minimum Reporting Limit (MRL) Standards	50 - 150	With every batch			

<sup>a</sup> RPD = relative percent difference among duplicates.



**TABLE 2. EXAMPLE ANALYSIS TABLE**

Position	Type	Identity	Comments	Weight	Ext. Dilution Factor	Pre-dil Factor	Pre-dil Position
ST8	T	Tracer		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	
B1	D	Drift		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	
ST25	S1	Blank Standard		1.0000	1.0000	1	
ST1	S4	0.050/0.0050 Standard		1.0000	1.0000	1	
ST2	S5	0.075/0.0075 Standard		1.0000	1.0000	1	
ST3	S6	0.10/0.010 Standard		1.0000	1.0000	1	
ST4	S7	0.25/0.025 Standard		1.0000	1.0000	1	
ST5	S8	0.50/0.050 Standard		1.0000	1.0000	1	
ST6	S9	0.75/0.075 Standard		1.0000	1.0000	1	
ST7	S10	1.0/0.10 Standard		1.0000	1.0000	1	
ST8	S13	1.3/0. Standard		1.0000	1.0000	1	
WT	WI	WashIgnore		1.0000	1.0000	1	
B2	D	Drift		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	
A1	U	Pre-dilution Blank		1.0000	1.0000	20	A2
B7	U	Carryover Standard		1.0000	1.0000	1	
A3	U	Carryover Blank		1.0000	1.0000	1	
A4	U	QCS 1.0/0.10		1.0000	1.0000	1	
A5	U	MRL TP 0.0020		1.0000	1.0000	1	
ST2	U	MRL TN 0.075		1.0000	1.0000	1	
ST5	U	ICV 0.50/0.050		1.0000	1.0000	1	
A6	U	ICB		1.0000	1.0000	1	
A7	U	LRB		1.0000	1.0000	1	
A8	U	0.50/0.050 Glycine/Pyridox		1.0000	1.0000	1	
ST4	U	LFB 0.25/0.025		1.0000	1.0000	1	
ST7	U	CCV 1.0/0.10		1.0000	1.0000	1	
A9	U	CCB		1.0000	1.0000	1	
B3	D	Drift		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	
A10	U	2000792		1.0000	1.0000	1	
A11	U	2000939		1.0000	1.0000	1	
A12	U	DUP 2000792		1.0000	1.0000	1	
A13	U	LFM 2000792		1.0000	1.0420	1	
A14	U	2001227 PT2:200mL		1.0000	100.0000	1	
A15	U	2001227 QC5:100mL		1.0000	20.0000	1	
A16	U	2001001		1.0000	1.0000	1	
A17	U	2001002		1.0000	1.0000	1	
A18	U	2001003		1.0000	1.0000	1	
WT	WI	WashIgnore		1.0000	1.0000	1	
B4	D	Drift		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	
ST6	U	CCV 0.75/0.075		1.0000	1.0000	1	
A19	U	CCB		1.0000	1.0000	1	
A20	U	2001227 PT2:100mL		1.0000	50.0000	1	
A21	U	2001227 PT5:100mL		1.0000	20.0000	1	
A22	U	2001227 PT5:50mL		1.0000	10.0000	1	
WT	WI	WashIgnore		1.0000	1.0000	1	
B5	D	Drift		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	
ST7	U	CCV 1.0/0.10		1.0000	1.0000	1	
A23	U	CCB		1.0000	1.0000	1	
B6	D	Drift		1.0000	1.0000	1	
WT	W	Wash		1.0000	1.0000	1	