Analysis of the Data Impacts of Changing Total Phosphorus Methodology

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Introduction

Recently, the water quality data that Mote Marine Laboratory (MML) has been generating for Sarasota County since 1998 was examined to assist in the determination of numeric nutrient criteria. An apparent step trend in the values of total phosphorus was noted and investigated for its origin and influence on the overall data set.

The trend was clearly linked to method requirements that were imposed (and then subsequently revised) as part of the NELAP (National Environmental Laboratory Accreditation Program) requirements for MML to maintain our certification through the Florida Department of Health. The following is a summary and assessment of likely impacts on the data.

Analytical History

The history of total phosphorus methods used by MML for Sarasota County data is as follows:

February 1998-January 1999	Technicon 329-74W/B
February 1999-August 2008	EPA 365.4
September 2008-present	EPA 365.4, modified

All three methods are colorimetric autoanalyzer analyses that used samples with an identical digestion process.

NELAC Certification Requirements

The NELAC program began to affect Florida laboratories in the late 1990's when NELAC was established (1995), adopted their first standards (1997), and first recognized accrediting authorities. FDEP also transferred their oversight of environmental laboratories and Quality Assurance Plans to the Florida Department of Health, who had always performed on-site laboratory inspections. Auditor of MML in 1997 was extremely thorough and provided an interpretation of the coming NELAC inspections (2001) in which approved methods were to be a rigid application of only those listed in 40 CFR Part 136, and only conducted exactly as written. Subsequent agency mailings were similar in tone. For total phosphorus, the listed method applicable to MML and the County program was EPA 365.4. Prior to this time, MML had been using the manufacturer's method for total phosphorus (Technicon 329-74W/B, attached) which was applied to digested samples. The Technicon method was based on an antimony-molybdate complex and reduction with ascorbic acid.

In anticipation of NELAP certification and subsequent inspections, MML altered the method for total phosphorus to EPA 365.4 (attached) and applied this to Sarasota County samples in February 1999 through August 2008. This method was also based on an antimony-molybdate complex reduced with ascorbic acid. There were also different amounts of sulfuric acid, sodium chloride, potassium sulfate, and ammonium molybdate from the prior Technicon method. The same auditor as in 1997 performed MML's first inspection under the NELAP rules in 2001 and

was satisfied with MML's analytical systems and record-keeping. Unfortunately, while spike recoveries under the Technicon method had been generally satisfactory, recoveries under the EPA method were occasionally problematic; samples spiked prior to digestion (Figure 1) would sometimes exceed limits, but when the same samples were spiked post-digestion (per Quality Assurance Plan/Quality Plan protocols), recoveries fell within the specified limits (Figure 2). The higher recoveries were generally associated with the more saline samples but many saline samples demonstrated acceptable recoveries of pre-digestion spikes.

In November 2007, EPA issued a memorandum (attached) regarding the ability of laboratories to modify Part 136 methods without necessitating a review and specific approval by EPA. Specifically, now permitted were "minor changes in reagents used where the underlying reaction and principles remain virtually the same." Changes in pH, pH adjustment reagents, buffers, complexing reagents, etc. were all permissible, but changes were to be allowed only "if the modified method produces equivalent performance."

Analytical Impacts

MML learned of EPA's memorandum in the summer of 2008 and conducted a test series of analyses which analyzed Sarasota Bay samples from a range of salinities and sources (various bays, Myakka River) by both the EPA 365.4 and a modified 365.4 method. The modified method (attached) was again an antimony-molybdate method reduced with ascorbic acid, and again used different amounts of acid, sodium chloride, potassium sulfate, and ammonium molybdate. Differences between sample values tested by the two methods were generally less than two times the method detection limit of 0.05 mg/L. The required precision of duplicate analyses (of the same sample using the same method) is set at 13% relative standard deviation or *three* limits of detection, whichever is greater. A slight concentration dependence was present, with freshwater stations (high phosphorus concentrations) somewhat higher by the modified method, and saline stations (low concentrations) somewhat lower. Spike recoveries were much improved. Analytical protocol was changed to the modified method based on the superior spike recoveries and the difference between methods of 1-2 limits of detection for individual samples.

The improved spike recoveries for the modified method are shown in data analyzed since September 2008. Pre-digestion spikes (Figure 3) have few instances outside control limits, and the few required post-digestion spikes (Figure 4) were also acceptable. (Note that there were no longer any low salinity samples from the Myakka River in the sampling program.). Between the three methods, MML has higher confidence in the data generated with the Technicon and the modified EPA methods although all were produced with acceptable quality assurance procedures.

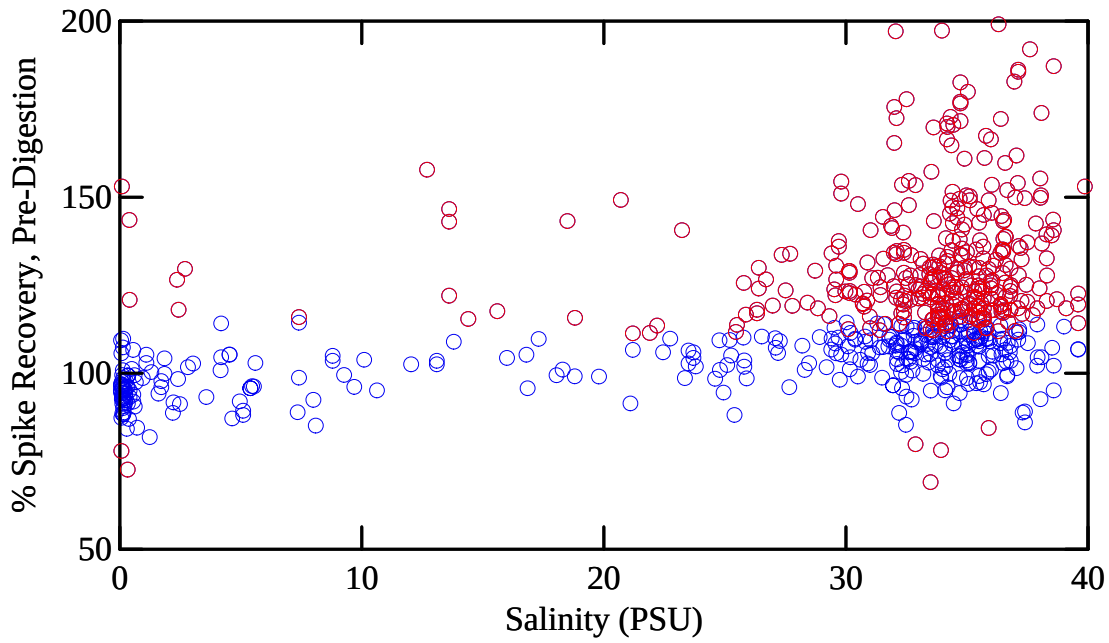

Figure 1. Pre-digestion spike recovery of total phosphorus analyses using EPA 365.4. Red indicates outside control limits, blue is acceptable or within warning limits.

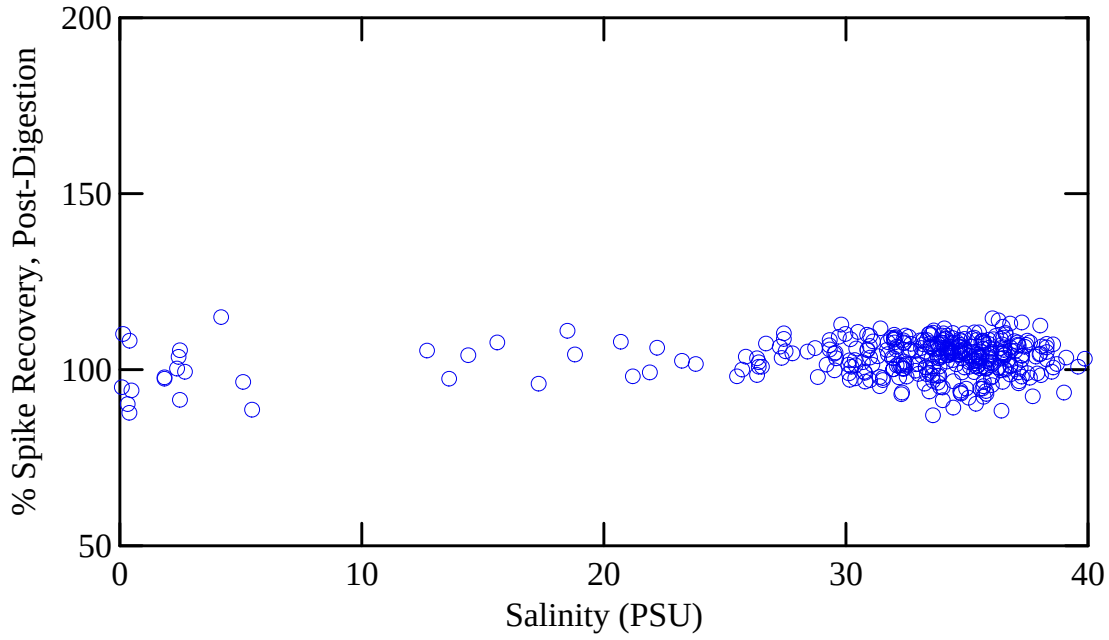

Figure 2. Post-digestion spike recovery of total phosphorus analyses using EPA 365.4. Blue is acceptable or within warning limits.

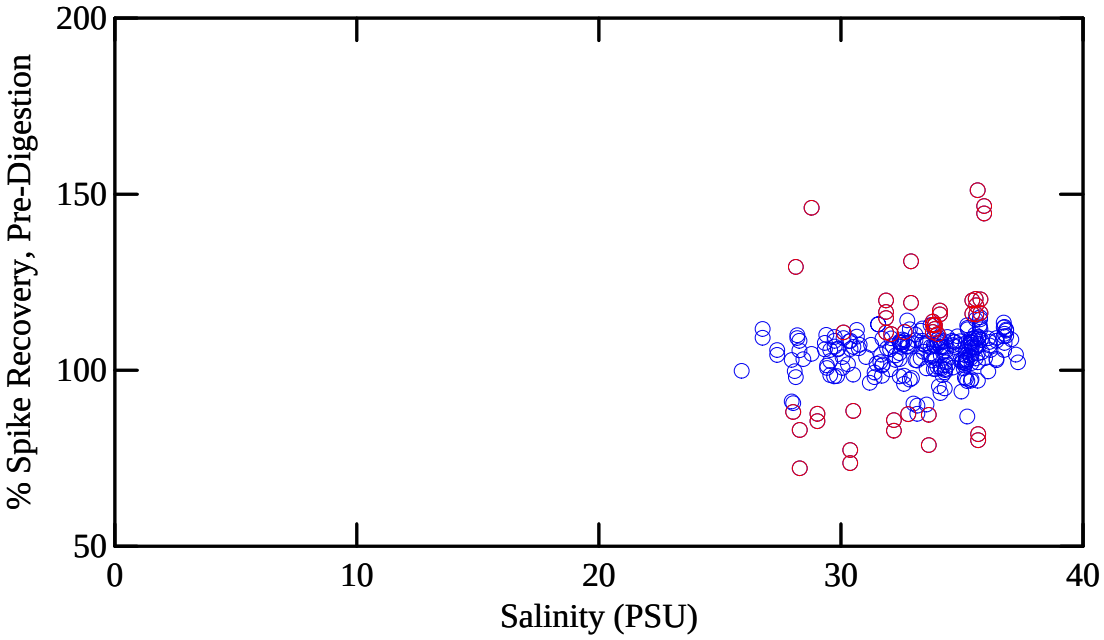

Figure 3. Pre-digestion spike recovery of total phosphorus analyses using modified EPA 365.4. Red indicates outside control limits, blue is acceptable or within warning limits.

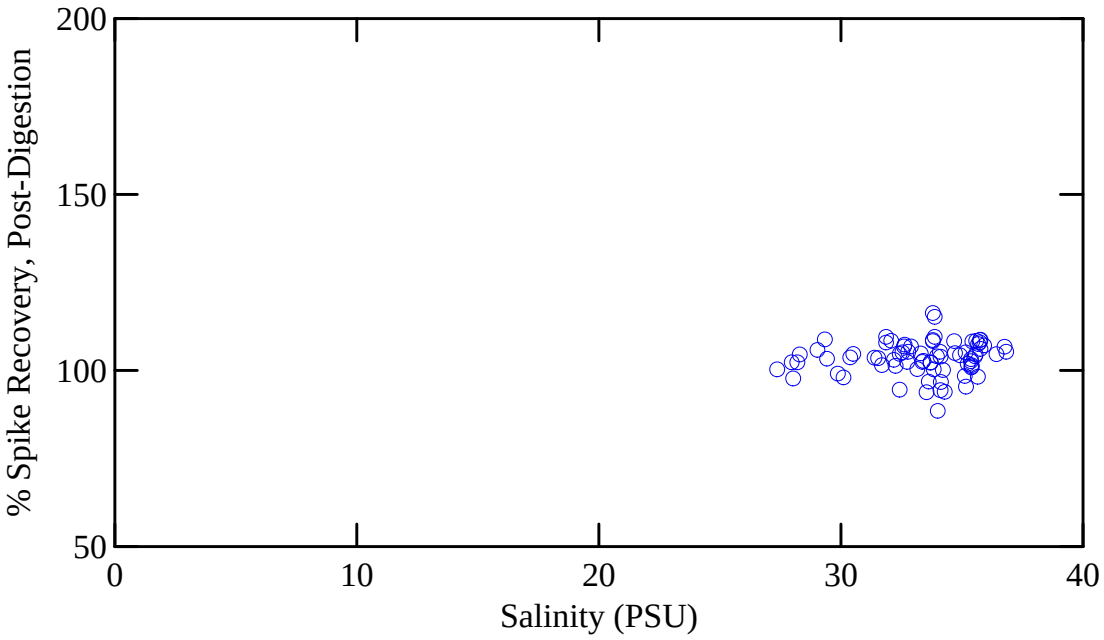

Figure 4. Post-digestion spike recovery of total phosphorus analyses using modified EPA 365.4. Blue is acceptable or within warning limits.

Data Trends

Unfortunately, the median total phosphorus levels in the saline bays (as determined by the un-modified EPA method) were between two to four times the limit of detection or between 0.10 and 0.20 mg/L (Figure 5). The resulting step trend of 0.05-0.10 mg/L can be seen in the Sarasota Bay (saline) samples both when the EPA method was adopted in early 1999 and when the modified EPA method began in late 2008 (Figure 6). The net change represented a large fraction of the values obtained using the unmodified value and was visually apparent. The Myakka River samples (Figure 7) show a possible decline with the change between the Technicon and EPA method in early 1999, but the change is a smaller fraction of the median sample value and harder to detect visually.

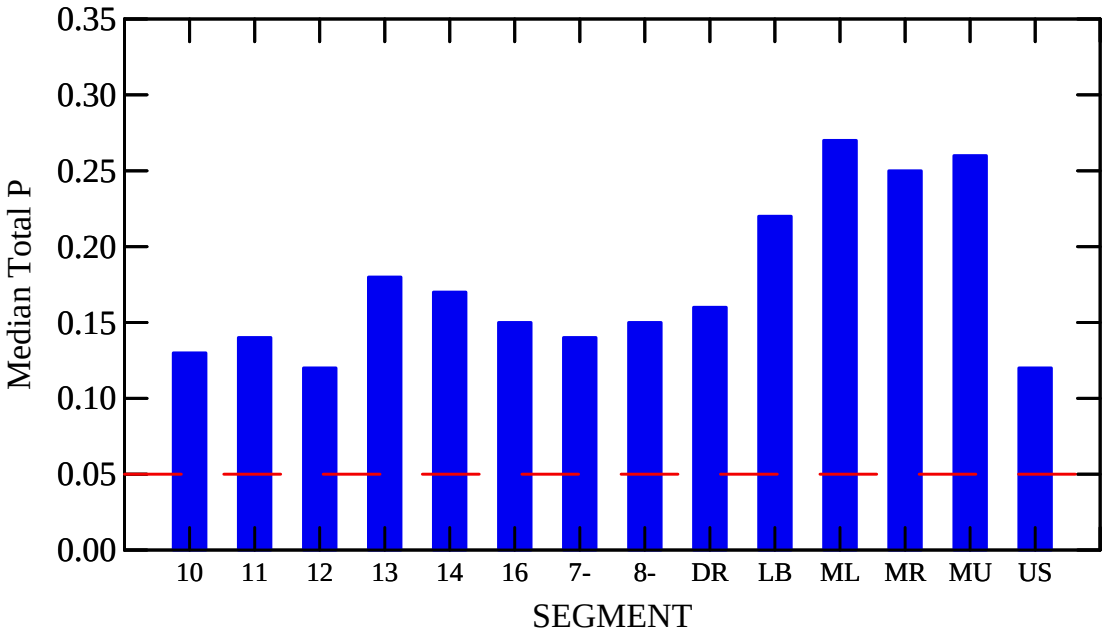

Figure 5. Median values of total phosphorus by segment using the EPA 365.4 method. Red dashed line is method detection limit of 0.05 mg/L.

Potential Causes

MML spent considerable time trying to identify the cause of the varying response between methods. The sodium chloride content of the analytical stream varied between methods. In the EPA method, when taking into consideration both the differing reagents and pump tube sizes, the analytical stream has approximately one-third of the amount of sodium chloride per liter, making the analysis perhaps more sensitive more sensitive to varying salt content in samples and to any possible salt effects on the chemistry of analysis. While salt in samples may contribute to the poor pre-digestion spike recoveries of the EPA method, however, the effect was not constant or predictable.

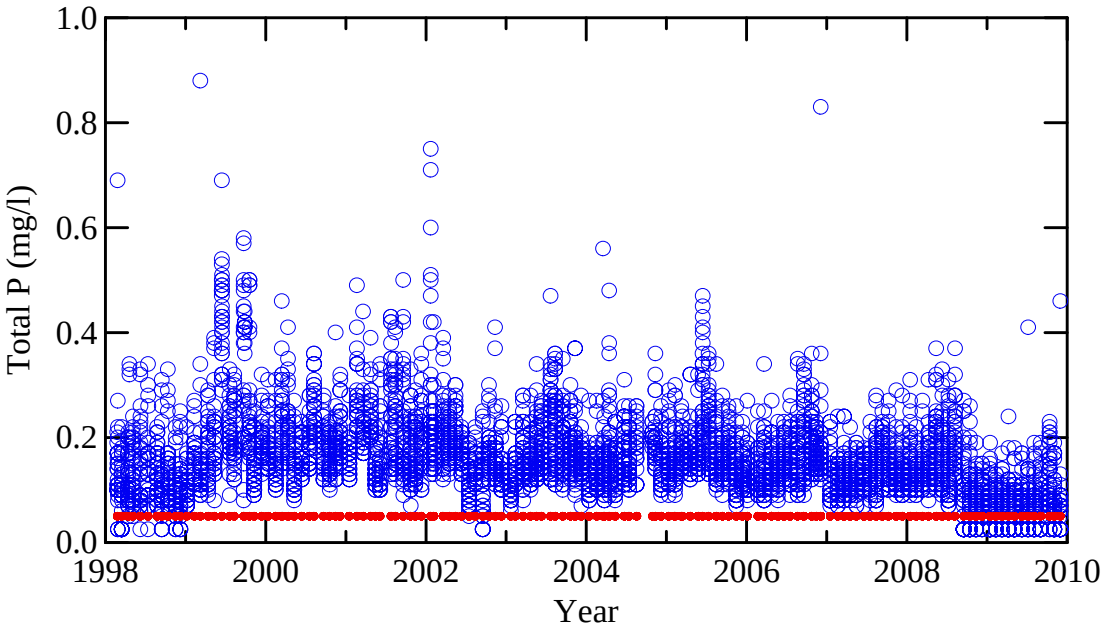

Figure 6. Total phosphorus results for Sarasota Bay samples using all three methods, 1998-2010. Red dashed line is method detection limit of 0.05 mg/L.

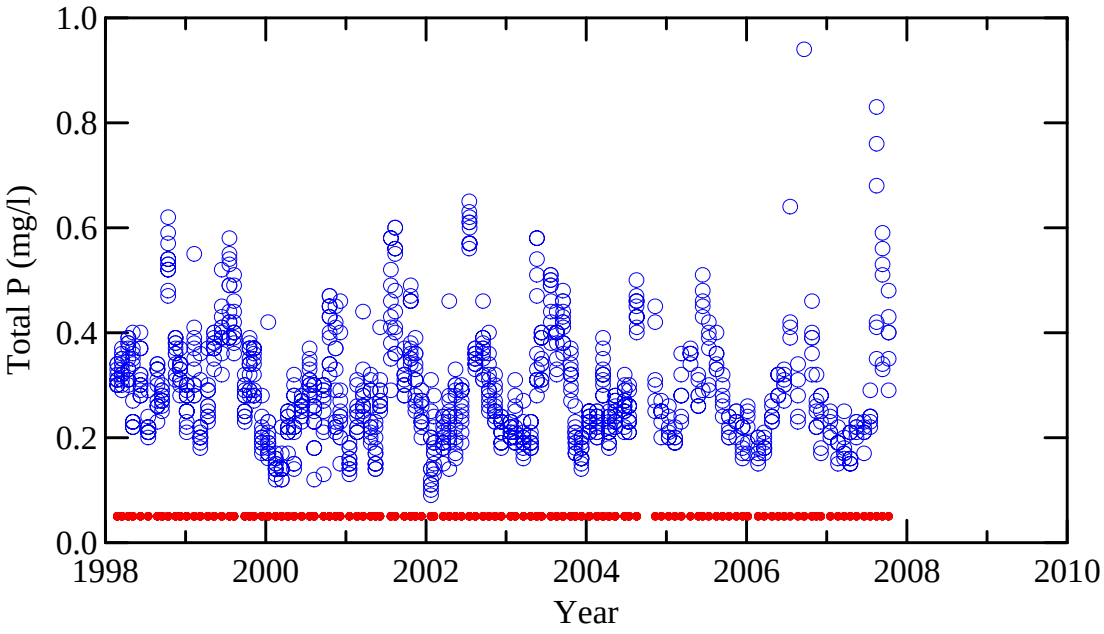

Figure 7. Total phosphorus results for Myakka River samples using two methods, 1998-2007. Red dashed line is method detection limit of 0.05 mg/L.

The un-modified EPA method suffered from an enhanced response factor in the presence of only *some* saline samples, as evidenced by both the slightly higher values for the tested saline samples and the excessive spike recoveries. Regression analyses of the recovery of predigested spikes for the un-modified EPA method as a function of other measured parameters (salinity, pH, DO, color, turbidity, TKN, chlorophyll, inorganic phosphorus, inorganic N species) indicated no other significant explanatory sample variable for enhanced spike recoveries other than salinity. While the relationship with salinity was highly significant ($p<0.001$), correlations were low ($r^2=0.179$) with a standard error of the estimate slightly over three method detection limits. There was no constant offset in the spiked samples that could be attributed to false absorption signals due to the optical mixing patterns due to density differences between the fresher sampler wash and the more saline samples.

A related explanation for the change in sensitivity between methods has to do with the pH of the sample and reagent stream. The development of the molybdenum blue color resulting from the reaction of phosphorus with reagents is very pH sensitive. The un-modified EPA method has approximately one third of the acid per liter as the modified method, making it much less acidic, and perhaps more sensitive to any buffering abilities that may vary between samples. Overall, however, the effect remains puzzling in that not all saline samples exhibited excessive spike recoveries with the un-modified EPA method.

Potential Corrections and Data Uses

The lack of a high correlation between pre-digestion spike recovery and salinity (Figure 1), however, makes the use of a regression-based correction factor to 'transform' data from the un-modified EPA method results to the modified EPA method difficult to support and apply. The noise associated with the salinity relationship would result in an uncertainty in the transformed data as large as or larger than the observed step trend between analytical methods. There are statistical methods to correct for a step trend due to method changes that should be employed in any future trend analyses of Sarasota Bay data.

For data uses such as setting nutrient criteria or evaluating against standards, a bias between methods may be present depending on the period of record employed. It should be emphasized, however, that the bias between methods is less than two limits of detection and that all data were generated in compliance with MML's NELAP certificate (2001-present) and in accordance with procedures identified within MML's FDEP/FDOH-approved Quality Manual (1998-present).

Appendix A
Technicon 329-74W/B

extra

Technicon™ AutoAnalyzer™ II

METHOD

Industrial Method No. 329-74W/B

Revised: November 1978

A/II/a/1

INDIVIDUAL/SIMULTANEOUS* DETERMINATION OF NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

RANGE: Nitrogen 0.024 - 1.2 mg/l; 1.5 - 75 mg/l
Phosphorus 0.024 - 1.2 mg/l; 1.5 - 75 mg/l

BD-40 (LOW LEVEL)

GENERAL DESCRIPTION

NITROGEN

The determination of nitrogen is based on a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffered alkaline medium at a pH of 12.8 - 13.0. The ammonia-salicylate complex is read at 660 nm.

PHOSPHORUS

The determination of phosphorus is based on the colorimetric method in which a blue color is formed by the reaction of ortho phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 660 nm.

The acid digest samples are prepared by digestion with the Technicon BD-40 Block Digestor. Refer to Manual No. TA4-0323-11 for sample preparation.

PERFORMANCE AT 40 SAMPLES PER HOUR

MANUALLY PREPARED STANDARDS

NITROGEN	1.5 - 75 mg/l	0.024 - 1.2 mg/l
Sensitivity	at 75 mg/l 1.00 absorbance units	at 1.2 mg/l 0.20 absorbance units
Coefficient of Variation	at 37.5 mg/l ±0.3%	at 0.60 mg/l ±0.8%
Detection Limit	1.5 mg/l	0.024 mg/l

PHOSPHORUS	1.5 - 75 mg/l	0.024 - 1.2 mg/l
Sensitivity	at 75 mg/l 1.00 absorbance units	at 1.2 mg/l 0.20 absorbance units
Coefficient of Variation	at 37.5 mg/l ±0.3%	at 0.60 mg/l ±0.4%
Detection Limit	1.5 mg/l	0.024 mg/l

* See Operating Note 11.

Bran + Luebbe, Inc. 1025 Busch Parkway Buffalo Grove, IL 60089-4516 • Phone (708) 520-0700 • Telefax (708) 520-0855

REAGENTS

Unless otherwise specified, all reagents should be of ACS quality or equivalent.

LIST OF RAW MATERIALS

Potassium Sulfate (K_2SO_4)
Sulfuric Acid, Concentrated (H_2SO_4)
Sodium Hydroxide Solution, 50% w/w (NaOH)
Sodium Potassium Tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$)
Methanol (CH_3OH)
Sodium Phosphate, dibasic, crystal ($Na_2HPO_4 \cdot 7H_2O$)
or
Sodium Phosphate, dibasic, anhydrous (Na_2HPO_4)
Sodium Salicylate ($Na_2C_7H_5O_3$)
Sodium Nitroprusside [$Na_2Fe(CN)_5NO \cdot 2H_2O$]
Sodium Hypochlorite Solution, 5.25%
Sodium Chloride (NaCl)
Ammonium Molybdate [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$]
Antimony Potassium Tartrate [$K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$]
Ascorbic Acid ($C_6H_8O_6$)
or
Araboascorbic Acid ($C_6H_8O_6$)
Triton X-100* (Technicon No. T21-0188)
Brij-35, 30% Solution ** (Technicon No. T21-0110)
Levor V ***

GENERAL REAGENTS

SAMPLER IV WASH RECEPTACLE SOLUTION

Potassium Sulfate	31.7	g
Sulfuric Acid, concentrated	48	ml
Deionized Distilled Water, q.s.	1000	ml

Preparation:

Dissolve 31.7 g of potassium sulfate in 800 ml of distilled water. Add 48 ml of concentrated sulfuric acid and dilute to one liter with deionized distilled water.

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* Trademark of Rohm & Haas Company

** Trademark of Atlas Chemical Industries

*** Trademark of Lever Brothers

TRITON X-100 SOLUTION, 50% in methanol

Triton X-100	50	ml
Methanol	50	ml

Preparation:
Add 50 ml of Triton X-100 to 50 ml of methanol and mix thoroughly.

SYSTEM WASH WATER SOLUTION
(For System Shut-Down and Start-Up Only)

Triton X-100 Solution	1.0	ml
Distilled Water	1000	ml

Preparation:
Add 1.0 ml of Triton X-100 solution to one liter of distilled water and mix thoroughly.

NITROGEN REAGENTS

STOCK SODIUM HYDROXIDE SOLUTION, 20%

Sodium Hydroxide Solution, 50% w/w	400	g
Distilled Water, q.s.	1000	ml

Preparation:
To 600 ml of distilled water, add 400 g of sodium hydroxide solution, 50% w/w. Cool to room temperature and dilute to one liter with distilled water.

STOCK SODIUM POTASSIUM TARTRATE SOLUTION, 20%

Sodium Potassium Tartrate	200	g
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 200 g of sodium potassium tartrate in about 600 ml of distilled water. Dilute to one liter with distilled water and mix thoroughly.

STOCK BUFFER SOLUTION, 0.5 M

Sodium Phosphate, Dibasic, crystal	134	g
-OR-		
[Sodium Phosphate, Dibasic, anhydrous	(70	g)
Sodium Hydroxide Solution, 50% w/w	40	g
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 134 g of sodium phosphate, dibasic, crystals (or 70 g of sodium phosphate, dibasic, anhydrous) in about 800 ml of distilled water. Add 40 g of sodium hydroxide solution, 50% w/w, dilute to one liter with distilled water and mix thoroughly.

WORKING BUFFER SOLUTION

Stock Buffer Solution, 0.5 M	200	ml
Stock Sodium Potassium Tartrate Solution, 20%	250	ml
Stock Sodium Hydroxide Solution, 20%	100	ml
Distilled Water, q.s.	1000	ml
Brij-35, 30% Solution	1.0	ml

Preparation:
Combine the reagents in the stated order: add 250 ml of stock sodium potassium tartrate solution, 20%, to 200 ml of stock buffer solution, 0.5 M, with swirling. Slowly, with swirling, add 100 ml of sodium hydroxide solution, 20%. Dilute to one liter with distilled water, add 1.0 ml of Brij-35, 30% solution (20 - 25 drops), and mix thoroughly.

SODIUM SALICYLATE/SODIUM NITROPRUSSIDE SOLUTION

Sodium Salicylate	150	g
Sodium Nitroprusside	0.30	g
Distilled Water, q.s.	1000	ml
Brij-35, 30% Solution	1.0	ml

Preparation:
Dissolve 150 g of sodium salicylate and 0.30 g of sodium nitroprusside in about 600 ml of distilled water. Filter through fast filter paper into a one liter volumetric flask and dilute to volume with distilled water. Add 1.0 ml of Brij-35 and mix thoroughly. Store in a light-resistant container.

SODIUM HYPOCHLORITE SOLUTION, 0.315%

Sodium Hypochlorite Solution,5.25%	6.0	ml
Distilled Water, q.s.	100	ml
Brij-35, 30% Solution	0.1	ml

Preparation:
Dilute 6.0 ml of sodium hypochlorite solution to 100 ml with distilled water. Add 0.1 ml (two drops) of Brij-35 and mix thoroughly. Prepare fresh daily. [Any commercial bleach solution (e.g. Clorox) containing 5.25% available chlorine is satisfactory.]

SALINE DILUENT (0.9% NaCl Solution with Brij-35/Nitrogen Channel Only)

Sodium Chloride	9.0	g
Distilled Water, q.s.	1000	ml
Brij-35	1.0	ml

Preparation:
Dissolve 9.0 g of sodium chloride in approximately 600 ml of distilled water. Dilute to one liter with distilled water. Add 1.0 ml of Brij-35 and mix thoroughly.

PHOSPHORUS REAGENTS

MOLYBDATE/ANTIMONY SOLUTION

Ammonium Molybdate	30.0	g
Antimony Potassium Tartrate	0.20	g
Levor V	1	ml
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 30.0 g of ammonium molybdate and 0.20 g of antimony potassium tartrate in 600 ml of distilled water, using heat if necessary. Dilute to one liter with distilled water, add 1 ml of Levor V and mix thoroughly. Transfer to a light-resistant container. This solution is stable for about one month.

ASCORBIC ACID SOLUTION, 6%

Ascorbic Acid	60	g
-OR-		
Araboascorbic Acid		
Levor V	1	ml
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 60.0 g of ascorbic or araboascorbic acid in 600 ml of distilled water and then dilute to one liter with distilled water. Add 1 ml of Levor V and mix thoroughly. Transfer to a light-resistant container. If kept tightly stoppered when not in use, this solution is stable for at least five days.

SALINE DILUENT, (2% NaCl solution with Levor V/ Phosphorus Channel Only)

Sodium Chloride (NaCl)	20	g
Levor V	0.5	ml
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 20 g of sodium chloride in about 600 ml of distilled water and then dilute to one liter with distilled water. Add 0.5 ml of Levor V and mix thoroughly.

ACID DILUENT

Sodium Chloride (NaCl)	20.0	g
Sulfuric Acid, 95 - 98% (H_2SO_4)	80	ml
Levor V	0.5	ml
Distilled Water, q.s.	1000	ml

Preparation:
Dissolve 20.0 g of sodium chloride in about 600 ml of distilled water. Add 80 ml of concentrated sulfuric acid, swirling constantly. Cool to room temperature and dilute to one liter with distilled water. Add 0.5 ml of Levor V and mix thoroughly. This reagent is stable for at least five days.

OPERATING NOTES

1. Start-Up
   a. Check the level of all reagents to ensure an adequate supply.
   b. Excluding the salicylate and molybdate/antimony lines, place all reagent lines in their respective containers. Connect the sample probe to the Sampler IV and start the proportioning pump.
   c. Flush the Sampler IV wash receptacle with about 25 ml of Sampler IV Wash Receptacle Solution.
   d. When reagents have been pumping for at least five minutes, place the salicylate and molybdate/antimony lines in their respective containers and allow the system to equilibrate for 10 minutes.

   NOTE: If a precipitate forms after the addition of salicylate, immediately stop the proportioning pump and flush the coils with water using a syringe. Precipitation of salicylic acid is caused by a low pH. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

   e. To prevent precipitation of salicylic acid in the waste tray (which can clog the tray outlet), keep the nitrogen flowcell pump tube and the nitrogen Colorimeter TO WASTE tube separate from all other lines or keep tap water flowing in the waste tray.

2. Shut-Down
   a. Remove the salicylate and molybdate/antimony lines from their containers and allow them to pump air. When the air bubbles enter the analytical system, place all reagent lines (excluding the Sampler IV Wash Receptacle Solution line) in the System Wash Water Solution.
   b. After 15 minutes, stop the proportioning pump and remove the platen.

3. Colorimeter
   If necessary, the system may be operated with the colorimeters in the DAMP 1 position.

4. Reagent Background Color
   a. Place all lines in the system wash water container and start the proportioning pump. After making the necessary adjustments on the colorimeters, set the SID CAL controls to 1.00 and adjust the water baselines to zero.
   b. Following the start-up procedure, place all reagent lines in their respective containers and allow the system to equilibrate.
   c. The reading of the reagents compared to distilled water should not be more than 35 units (0.35 absorbance) for the nitrogen channel and not more than 5 units (0.05 absorbance) for the phosphorus channel. If the absorbance of either channel is much higher than the above values, one or more of the reagents or the water used to make up the reagents is probably contaminated.

5. Concentration Ranges

   a. Nitrogen Channel -- Concentration ranges from 0.024 - 1.2 mg/l to 1.5 - 75 mg/l can be accommodated by changing the pump tube size of the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure 1 and flow diagram).

   b. Phosphorus Channel -- Concentration ranges from 0.024 - 1.2 mg/l to 1.5 - 75 mg/l can be accommodated by changing the pump tube size of the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure 2 and flow diagram).

   c. If samples at any time exceed 100% of scale, dilute with the blank solution to maintain the same sample matrix before reassaying.

   d. Two pump tubes of each size necessary to accommodate the ranges listed are included in the accessories and spares kit.

   e. For any one manifold configuration, an approximate five-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 100 or higher.

6. Saline Diluents

   Be certain that the saline diluent with Brij-35 is used only in the nitrogen system, as Brij-35 contains phosphorus. To avoid confusion, clearly label the saline diluent containing Brij-35 "for Nitrogen Channel Only".

7. Manifold Configurations

   a. Individual Determination of Nitrogen and Phosphorus

   When N or P is being determined individually, the PT fitting is omitted and the sample line is attached directly to the sample probe of the Sampler IV.

   b. Simultaneous Determination of Nitrogen and Phosphorus

   When N and P are being determined simultaneously, both initial sample lines are connected to a PT stream-splitter fitting which is in turn connected to the sample probe on the Sampler IV.

FIGURE 1: CONFIGURATIONS FOR THE NITROGEN CARTRIDGE

#	* SAMPLE LINE	SALINE LINE	RESAMPLE LINE	WORKING BUFFER LINE	APPROX. STD CAL	RANGE PPM N
1	.23(ORN/WHT)	.80(RED/RED)	.32(BLK/BLK)	.80(RED/RED)	7.00 1.00	.024-1.2 .09 -4.5
2	.10(ORN/GRN)	.80(RED/RED)	.16(ORN/YEL)	.80(RED/RED)	7.00 1.00	0.06-3.0 0.30-15.0
3	.10(ORN/GRN)	2.50(PUR/PUR)	.10(ORN/GRN)	.80(RED/RED	7.00 1.00	0.30-15.0 1.5 -75.0

FIGURE 2: CONFIGURATIONS FOR THE PHOSPHORUS CARTRIDGE

#	SAMPLE LINE	SALINE LINE	RESAMPLE LINE	WORKING DILUENT LINE	APPROX. STD CAL	RANGE PPM N P
1	*.42(ORN/ORN)	.80(RED/RED)	.42(ORN/ORN)	.60(WHT/WHT	7.30 1.30	.024-1.2 0.12-6.0
2	.32(BLK/BLK	.60(WHT/WHT	.23(ORN/WHT)	.60(WHT/WHT	7.30 1.00	0.06-3.0 0.30-15.0
3	.10(ORN/GRN)	.60(WHT/WHT)	.10(ORN/GRN)	.80(RED/RED)	7.30 1.00	0.30-15.0 1.5 -75

* See Operating Note #7.

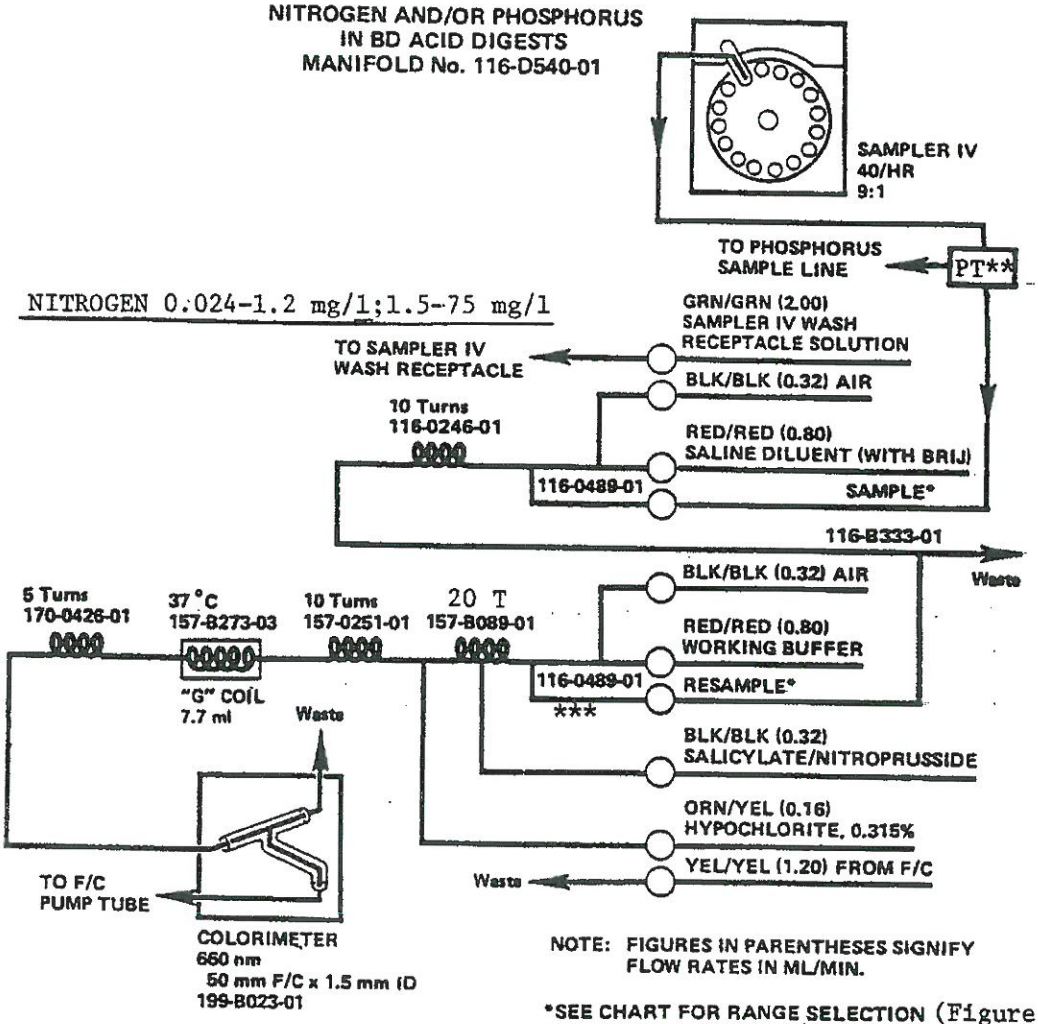
8. Sample Probe & PT Stream Splitter
Because stainless steel is susceptible to attack by sulfuric acid solutions, this method utilized a special Kel-F sample probe (Technicon No. 171-0745) and a special PT stream splitter with platinum nipples (Technicon No. 166-B331).

9. Phosphorus Channel (only)
   a. Cleansing Procedure
   Before initially operating the system, the following procedure should be performed to cleanse the system. Once a week, thereafter, this procedure should be repeated during system start-up.

   With the exception of the ascorbic acid and molybdate/antimony lines, place all phosphorus reagent lines into their respective containers. Start the proportioning pump and allow five minutes pumping time. Place both the ascorbic acid and molybdate/antimony lines in sodium hydroxide solution, 20% for five minutes, then into hydrogen peroxide, 50% for five minutes, then into distilled water. After five minutes follow the start-up procedure (Operating Note 1) and allow the system to equilibrate.
   b. Conditioning Procedure
   After the initial cleansing of the system is performed, condition the phosphorus channel as described below. Once this channel has been conditioned, there is no need to repeat the procedure; only the cleansing procedure need be performed once each week during shut-down.

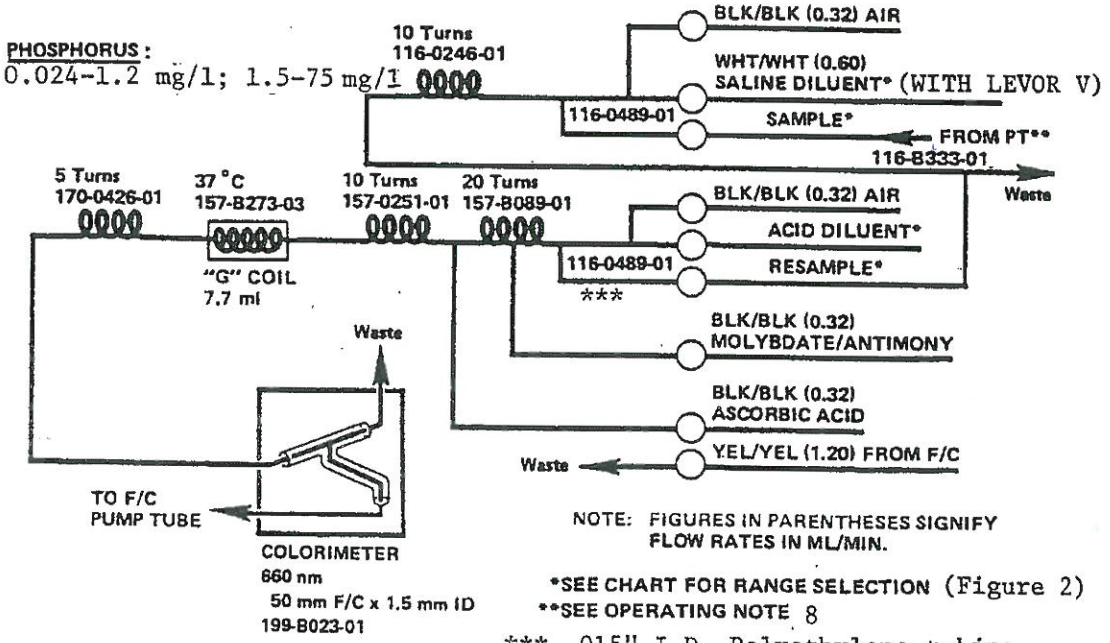
   Following the Start-Up procedure (Operating Note #1), place all reagent lines for phosphorus in their respective containers and allow the system to equilibrate. Place three sample cups containing midscale standard solution on the Sampler IV tray (with a stop-pin at the third cup) and start the sampler. Aspirate the set of standards three times, allowing five minutes of wash between each set. After the Recorder traces the last standard peak, wait ten minutes and adjust the baseline tracing to zero using the BASELINE control.

NITROGEN AND/OR PHOSPHORUS
IN BD ACID DIGESTS
MANIFOLD No. 116-D540-01



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

*SEE CHART FOR RANGE SELECTION (Figure 1)
**SEE OPERATING NOTE 8



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

*SEE CHART FOR RANGE SELECTION (Figure 2)
**SEE OPERATING NOTE 8

*** .015" I.D. Polyethylene tubing

Appendix B
EPA 365.4

PHOSPHORUS, TOTAL

Method 365.4 (Colorimetric, Automated, Block Digestor AA II)

STORET NO. 00665

1. Scope and Application
   1.1 This method covers the determination of total phosphorus in drinking water, surface water and domestic and industrial wastes. The applicable range of this method is 0.01 to 20 mg P/1.
2. Summary of Method
   2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 ml and placed on the AutoAnalyzer for phosphorus determination.
3. Sample Handling and Preservation
   3.1 Sample containers may be of plastic material, such as a cubitainer, or of Pyrex glass.
   3.2 If the analysis cannot be performed the day of collection, the sample should be preserved by the addition of 2 ml of conc. H_2SO_4 per liter and refrigeration at 4°C.
4. Apparatus
   4.1 Block Digestor BD–40
   4.2 Technicon Method No. 327–74W for Phosphorus
5. Reagents
   5.1 Mercuric sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 ml of 1:4 sulfuric acid (10 conc. H_2SO_4: 40 ml distilled water) and dilute to 100 ml with distilled water.
   5.2 Digestion solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 600 ml of distilled water and 200 ml of conc. H_2SO_4. Add 25 ml of mercuric sulfate solution (5.1) and dilute to 1 liter.
   5.3 Sulfuric acid solution (0.72 N): Add 10 ml of conc. sulfuric acid to 800 of distilled water, mix and dilute to 1 liter.
   5.4 Molybdate/antimony solution: Dissolve 8 g of ammonium molybdate and 0.2 g of antimony potassium tartrate in about 800 ml of distilled water and dilute to 1 liter.
   5.5 Ascorbic acid solution: Dissolve 60 g of ascorbic acid in about 600 ml of distilled water. Add 2 ml of acetone and dilute to 1 liter.
   5.6 Diluent water: Dissolve 40 g of NaCl in about 600 ml of distilled water and dilute to 1 liter.
   5.7 Sulfuric acid solution, 4%: Add 40 ml of conc. sulfuric acid to 800 ml of ammonia-free distilled water, cool and dilute to 1 liter.
6. Procedure

   Digestion

   6.1 To 20 or 25 ml of sample, add 5 ml of digestion solution and mix. (Use a vortex mixer).
   6.2 Add 4–8 Teflon boiling chips. Too many boiling chips will cause the sample to boil over.

Issued 1974

6.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digestor and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

6.4 Cool sample and dilute to 25 ml with distilled water. If TKN is determined the sample should be diluted with ammonia-free water.

Colorimetric Analysis

6.4.1 Check the level of all reagent containers to ensure an adequate supply.

6.4.2 Excluding the molybdate/antimony line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

6.4.3 Flush the Sampler IV wash receptacle with about 25 ml of 4% sulfuric acid (5.7).

6.4.4 When reagents have been pumping for at least five minutes, place the molybdate/antimony line in its container and allow the system to equilibrate.

6.4.5 After a stable baseline has been obtained, start the sampler.

7. Calculations

7.1 Prepare a standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with the standard curve.

8. Precision and Accuracy

8.1 In a single laboratory (EMSL) using sewage sample containing total P at levels of 0.23, 1.33, and 2.0, the precision was ±0.01, ±0.04, and ±0.06, respectively.

8.2 In a single laboratory (EMSL) using sewage samples of concentration 1.84 and 1.89, the recoveries were 95 and 98%, respectively.

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2. Gales, M.E. and Booth, R.L., "Evaluation of Organic Nitrogen Methods", EPA Office of Research and Monitoring, June, 1972.
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5. Gales, M.E., and Booth, R.L., "Evaluation of the Technicon Block Digestor System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-6001/4–78–015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

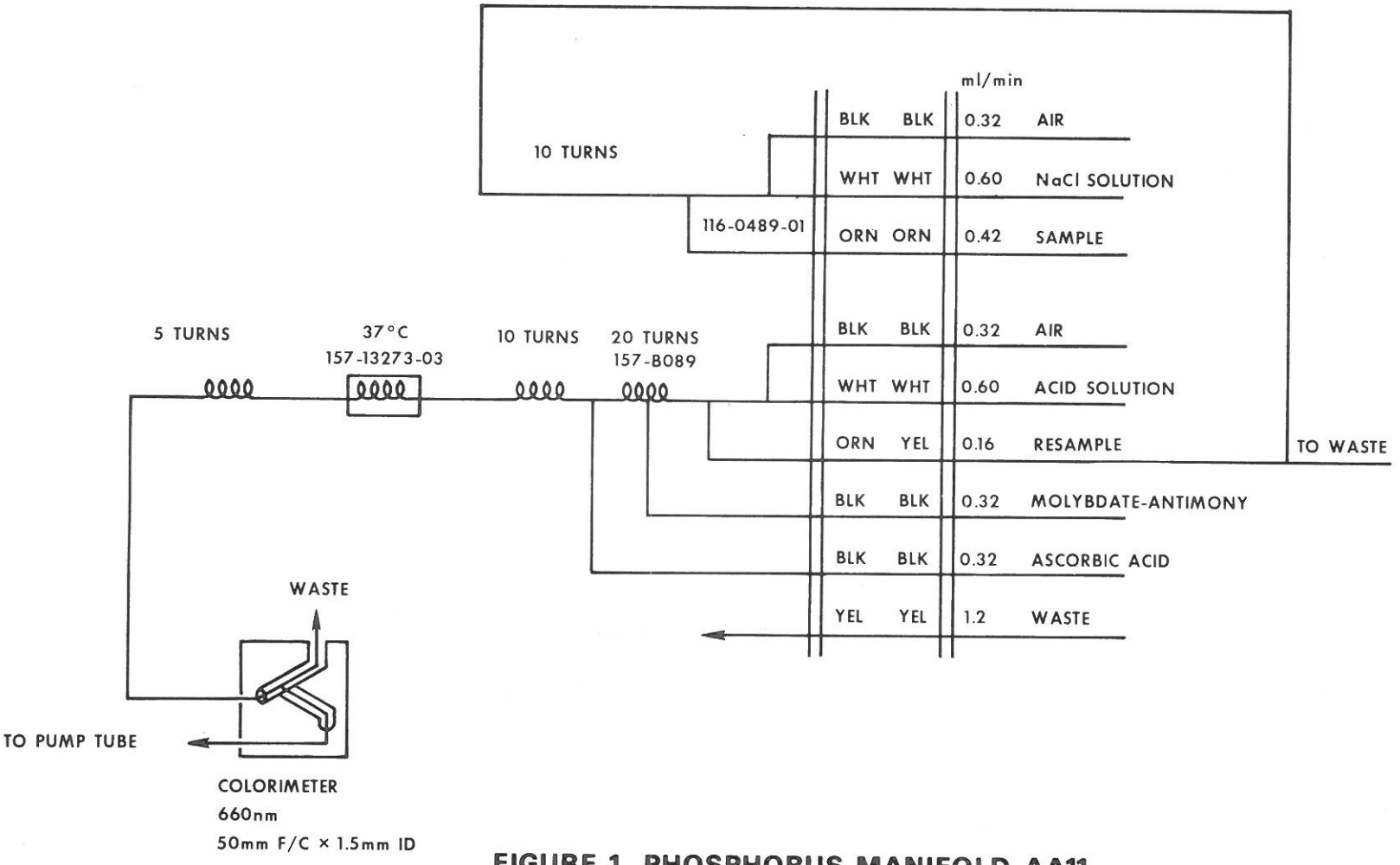


FIGURE 1. PHOSPHORUS MANIFOLD AA11

Appendix C
MML Modified EPA 365.4

STANDARD OPERATING PROCEDURE - TOTAL Phosphorus (Total P or ΣP)

Method Reference: Phosphorus, total, EPA 365.4 /600/4-79/020, March 1983, (colorimetric, automated, block digestor) MML modified according to Technicon method 329-74W/B.

Scope and application: EPA 365.4, 1.0

Applicable matrix or matrices: DW, SW, GW, EFF, SA

Summary of the test method: EPA 365.4, 2.0

Procedure: EPA 365.4, 6.0 AA3 Checklist

Digestion:

1. Document all digest information on the TKN/Total P Digest Log.
2. Pipette 20.0 mL of standard or sample into each digestion tube. Clearly label each tube with black Sharpie.
3. Add 5.0 mL of digest solution to each tube and vortex to mix. Tubes may be stored in the cold room for several days at this point.
4. Plug in Block Digestor and press Auto button to begin warm up and turn fume hood on. Thermostat needle will move to green shading and will drop to base when warmed up (about ½ hour). Make sure both upper and lower dials are turned on.
5. Set top timer (high temp) for at least 3 hours, set bottom timer (low temp) for at least one hour. (Use preset temperatures of 160°C for the low temperature and 380°C for high temperature)
6. Tubes should be at room temperature before digesting, if not place in hot water bath to warm to room temperature. Dry tubes and place in metal digester rack. Remove stoppers and place in bucket of Barnstead Reverse Osmosis water (BRO).
7. Add 5-8 micro Hengar granules[1] to each tube just before putting on digestor. (If added too soon the air will come out of the boiling chips and cause the liquid to boil over!)

NOTE 1: EPA 365.4, 6.2 requires Teflon boiling chips, but those were found to cause bumping and boil over of samples. This created a dangerous situation that was corrected by using Hengar granules.

8. Put tube rack on digestor after it is preheated and set timer for 1 hour. Check that tubes are at a boil and not boiling over after 10-15 minutes and put sides on rack.
9. Leave at low temp for 1 hour. After 1 hour, turn bottom knob off (low temp), leave at high temp for 90 min.
10. After 90 min, remove rack and place on counter in fume hood with hood on. Push the OFF button on the digester and unplug.

11. After tubes have cooled for a few minutes, begin reconstitution. Add 20mL of BRO water from repipettor bottle. Shake stoppers dry and place in tubes. If tubes are not to be reconstituted within a few minutes of digestion, place stoppers in tubes immediately and reconstitute before the end of the day. Vortex 3 times and place in plastic rack. If samples do not fully dissolve, they can be placed in a warm water bath and vortexed, before storing in the cold room or just prior to analysis.

If digesting a second rack....Leave digestor unplugged for about an hour. When plugged back in be sure that the OVER TEMP light is not on. Start the procedure from the beginning.

Instrument Setup:
Follow the AA3 checklist for general instructions. See Table 1 for specific analysis instructions.

Table 1: Total P specific analysis instructions

Filters	660 nm
Sampling rate	30/hr 6:1 sample:wash*
Heater temp.	37°C
Wetting agent	Aerosol 22

*This sampling rate was found to work the best by MML

Prior to analysis:
1. Turn on instrument, plug in heater, and place reagent lines in BRO with aerosol 22 (1:2) (1mL/L). Place the sampler wash line in BRO.
2. Place ascorbic acid and antimony/molybdate reagent lines in 5 N NaOH for 5 min.
3. Place ascorbic acid and antimony/molybdate reagent lines in 30% H_2O_2 for 5 min.
4. Place reagent lines in BRO with approximately 2 mL/L of aerosol 22 (1:2) for 10 min.
5. If running analysis immediately, see analysis instructions below. If not running immediately, remove reagent lines and sampler probe from water and allow the system to dry.

Analysis:
1. Insert all reagent lines in reagents except molybdate/antimony.
2. Allow to pump for 5 min. and then place the reagent line in molybdate/antimony. Continue analysis according to AA3 checklist.

Shutdown:
1. When removing reagent lines- remove molybdate/antimony solution 5 minutes prior to all other reagents.

2. Remove remaining reagent lines from reagents and place in BRO with aerosol 22 (1:2). Place sampler wash line in water.
3. Allow the system to wash for 10 minutes then remove the lines from water and allow to dry.
4. Unplug heater and turn off instrument.

Digestion Tube cleaning Procedure:

1. Remove the sample numbers from the tubes with acetone and a paper towel.
2. Remove neoprene stoppers and place in plastic beaker. Cover with DI water.
3. Pour remaining digested sample into the satellite hazardous waste container located in the lab. A swirling motion just before pouring suspends the boiling chips, which fall into the waste container along with the sample. If needed, use a funnel in the waste container. Rinse with small portion of RO to remove mercury from tubes.
4. Place each tube in the metal rack and secure the rack cover.
5. Rinse 3 times with RO water to be sure the tubes are rinsed of sample and boiling chips.
6. Wash with approx 10% liquinox solution with hot soapy water and a brush. Then rinse well with hot tap water, and at LEAST 3 times with RO water.
7. From a wash bottle containing 25% sulfuric acid, squeeze about 5-10 mL down along the sides of each of the tubes. Drain into an acid waste container using a rotary motion. Fill tubes with RO water and rinse at LEAST 3 times, until satisfied that there is no longer any acid residue in the tubes. You can make sure by testing with a narrow range pH strip in one of the tubes.
8. Invert the whole rack on paper towels and allow to drain a few minutes.
9. While the tubes are draining, rinse the stoppers with 25% sulfuric and copious amounts of RO water, and drain.
10. Right the rack of tubes, and stopper them as soon as possible to avoid contamination.

Definitions: SM 20 4500-P A, 2.0

Safety: MSDS, Standard laboratory safety equipment suggested (i.e. gloves, glasses, lab coat, and appropriate footwear)

Sample collection, preservation, shipment, and storage: EPA 365.4, 3.0, QA manual section 6.5

Equipment and Supplies: EPA 365.4, 4.0 Technicon Block digestor BD-40, 75 mL digestion tubes. Automated continuous flow analytical instrument is the AutoAnalyzer 3 by Bran+Luebbe. A flow cell of 10 mm path length and 1.0 mm internal diameter is used. 4.0 ml polystyrene autoanalyzer cups are used in the sampler tray.

Reagents and Standards: EPA 365.4, 5.0 with MML modifications from Technicon method 329-74W/B. Reagents were modified to improve method performance.

1. **1:5 sulfuric acid**: Add 10 mL concentrated sulfuric acid (H_2SO_4) (CASRN 7664-93-9) to 40 mL of BRO. Store in a glass bottle at room temperature. Expiration 1 year.

2. **Mercuric sulfate solution**: Dissolve 8.0 g red mercuric oxide (HgO) (CASRN 21908-53-2) in 50 mL of 1:5 sulfuric acid and dilute to 100 mL with BRO in a volumetric flask. Store at room temperature in a glass bottle. Expiration 5 years.

3. **Digestion solution**: Dissolve133 g of potassium sulfate (K_2SO_4) (CASRN 7778-80-5) in about 700 mL of BRO and 200 mL of concentrated sulfuric acid (H_2SO_4) (CASRN 7664-93-9). Cool, add 25 mL of mercuric sulfate solution and dilute to 1 L with BRO in a volumetric flask. Store at room temperature in a glass bottle. Expiration 5 years.

4. **Sampler wash**: Dissolve 31.7 g of potassium sulfate (K_2SO_4) (CASRN 7778-80-5) in about 800 mL of BRO. Add 48 mL of concentrated sulfuric acid (H_2SO_4) (CASRN 7664-93-9) and dilute to 1 L with BRO in a volumetric flask. Store in a plastic bottle at room temperature. Expiration 1 month.

5. **Molybdate/antimony solution**: Dissolve 15.0 g of ammonium molybdate 4-hydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) (CASRN 12054-85-2) and 0.10 g of antimony potassium tartrate ($C_8H_4K_2O_{12}Sb_2\cdot 3H_2O$) (CASRN 28300-74-5) in about 300 ml of BRO. Dilute to 500 mL with BRO in a volumetric flask. Pour into a dark bottle and add 1 mL of aerosol 22 (1:2) into the bottle and mix thoroughly. It is best to add the aerosol on the day of analysis. Store at room temperature. Expiration 1 month.

6. **Ascorbic acid solution**: Dissolve 30.0 g of ascorbic acid ($C_6H_8O_6$) (CASRN 50-81-7) into about 300 mL of BRO. Dilute to 500 mL with BRO in a volumetric flask. Pour into a dark bottle and add 0.5 mL of aerosol 22 (1:2) into the bottle and mix thoroughly. It is best to add the aerosol on the day of analysis. Store at 4 °C. Expiration 2 weeks.

7. **Saline diluent**: Dissolve 20 g of sodium chloride (NaCl) (CASRN 7647-14-5) into about 600 mL of BRO. Dilute to 1 L with BRO in a volumetric flask. Pour into a dark bottle and add 0.5 mL of aerosol/22 (1:2) into the bottle and mix thoroughly. Store at room temperature. Expiration 1 month.

8. **Acid diluent**: Dissolve 20.0 g of sodium chloride (NaCl) (CASRN 7647-14-5) in about 600 mL of BRO. Add 80 mL of concentrated sulfuric acid (H_2SO_4) (CASRN 7664-93-9), swirling constantly. Cool to room temperature

and dilute to 1 L with BRO in a volumetric flask. Pour into a dark bottle and add 0.5 mL of aerosol/22 (1:2) into the bottle and mix thoroughly. Store at room temperature. Expiration 1 week.

9. **Stock phosphate solution**: Dissolve 4.393 g of potassium dihydrogen phosphate (KH_2PO_4) (CASRN 7778-77-0) into about 600 mL of BRO. Dilute to 1 L with BRO in a volumetric flask. Concentration = 1000 mg/L as P. Store in a dark bottle at 4 °C. Expiration 6 months.

10. **Stock phosphate ICV solution**: Dissolve 4.393 g of potassium dihydrogen phosphate (KH_2PO_4) (CASRN 7778-77-0) (different lot number than stock standard) into about 600 mL of BRO. Dilute to 1 L with BRO in a volumetric flask. Concentration = 1000 mg/L as P. Store in a dark bottle at 4 °C. Expiration 6 months.

11. **QC sample**: Use an unexpired standard from a proficiency test study and dilute to the appropriate concentration such that it will fit in the range of the calibration curve.

Table 1: Preparation of intermediate and working standards for Total P. Combined NH_4N and PO_4P standards may be used when analyzing TKN and Total P together.

Standard	Initial Conc. mg/L	Initial Vol. mL	Final Vol. mL	Final Conc. mg/L
50 mg/L P	1000	5.0	100	50
ICV 50 mg/L P	1000	5.0	100	50
CAL 9	50	0.0	100	0
CAL 7	50	0.400	100	0.200
CAL 6	50	1.000	100	0.500
CAL 5	50	2.00	100	1.00
CAL 4	50	2.50	100	1.25
CAL 3	50	3.00	100	1.50
CAL 2	50	4.00	100	2.00
CAL 1	50	5.00	100	2.50
ICV	50	3.00	100	1.50

Interferences: SM 20 4500-P F, 1 b., EPA 365.1, 5.0

Calibration and Standardization: EPA 365.4, 7.0, AACE software

Method Detection Limit: 0.05 mg/L

Method Performance: EPA 365.4, 8.0

Quality Control: EPA 351.2, 9.0, QA manual
Duplicate precision 13%,

Spk. Recovery 90-110%,
ICV 90-110%,
CCV 90-110%

Calculations: EPA 365.4, 7.0, AACE software

Data assessment and acceptance criteria for quality control measures: Quality Manual 5.0, 11.0

Corrective actions for out-of-control data: Quality Manual 13.0

Contingencies for handling out-of-control or unacceptable data: Quality Manual 13.0

Pollution prevention: Any waste that has been in contact with digestion solution should be treated as hazardous and disposed of properly.

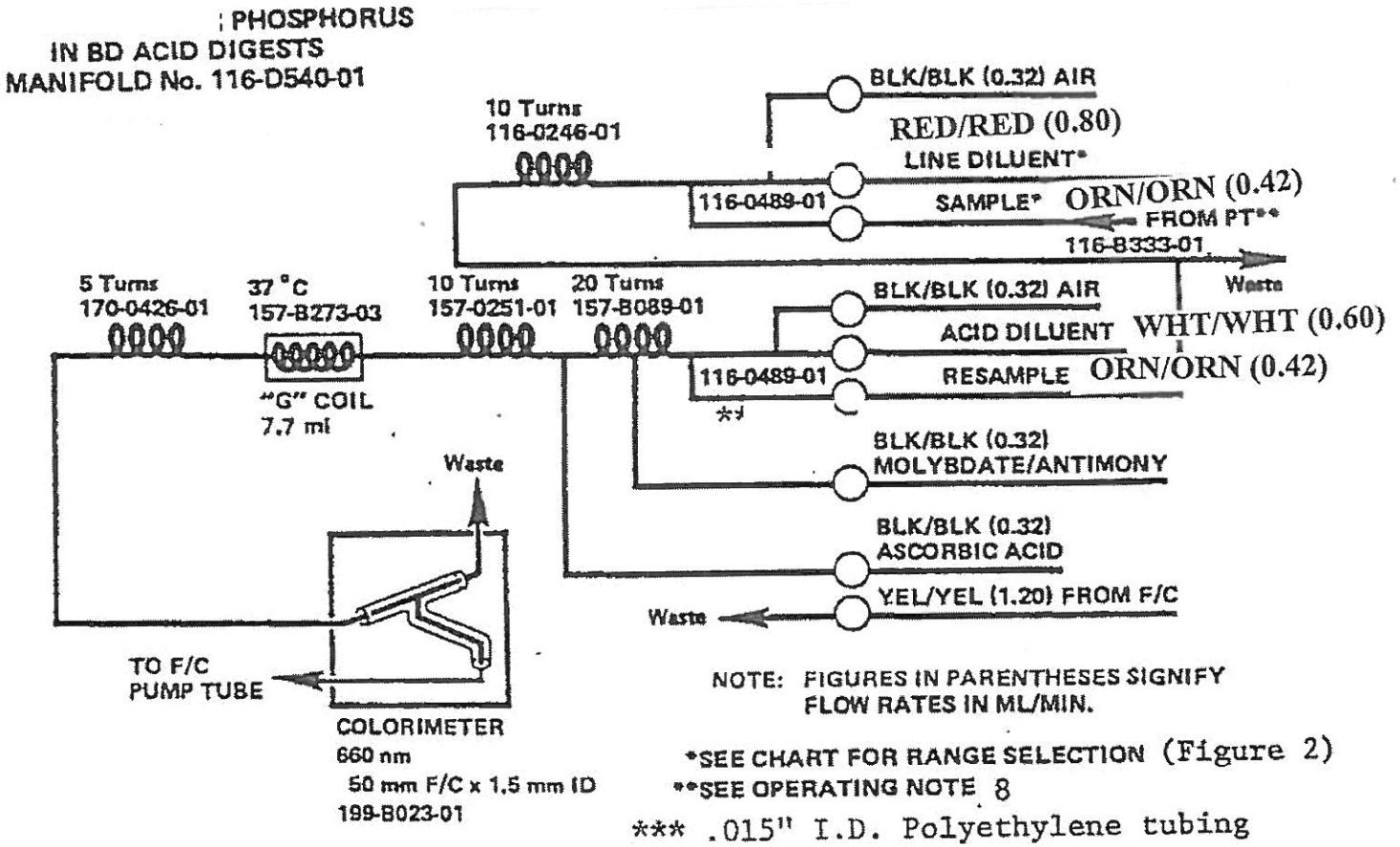
Waste Management: Quality Manual 8.2.3
Any sample or reagent waste that has not been in contact with mercury may be neutralized and disposed of down the drain. Waste with mercury should be collected in a properly labeled container and stored in the hazardous waste shed.

References: EPA 365.4, Bibliography
Technicon Industrial Method NO. 329-74W/B Revision Nov. 1978
SM20 4500-P B5,E

Tables, diagrams, flowcharts, validation data: EPA 365.4-3 modified by MML. Technicon Industrial Method NO. 329-74W/B Revision Nov. 1978 Manifold No. 116-D540-01

Revision History: EPA method in use since June 11, 1997.

Revision 1.0 Documented additional categories following initial NELAC inspection, May, 2001.
Revision 1.1 Standardized formatting, June, 2003.
Revision 1.2 Update QA limits, 10/1/2005.
Revision 1.3 Clarify 660 nm filters used for analysis.
Revision 1.4 Update standards/procedure notes and manifold diagram to improve the spike recovery of saline samples
Revision 1.5 Update procedures for AACE software
Revision 1.6 Update instrument for AutoAnalyzer 3 September, 2010



EPA method modified to enhance method performance. Diagram from Technicon method 329-74W/B. Saline diluent line changed from 0.60 mL/min to 0.80 mL/min and resample line changed from 0.16 mL/min to 0.42 mL/min.

Appendix D
EPA Memo, "Flexibility of CWA Methods"

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

SUBJECT: **Flexibility to Modify CWA Methods**

FROM: Richard Reding, Chief
Engineering & Analytical Support Branch, EAD, OST

OFFICE OF WATER

TO: Quality Assurance Managers
ATP Coordinators
NPDES Coordinators

DATE: November 20, 2007

The CWA methods team has conducted conference calls and other outreach with our regional and state colleagues about administering the CWA methods program. We have received many questions and suggestion about interpreting a user's current flexibility to modify a CWA, i.e. a Part 136 or 304(h), chemical analytical method without prior review or rulemaking by EPA. This flexibility is embodied in the quality assurance/control (QA/QC) section of many 1600-series methods, and more recently (March 12, 2007) in CWA regulations at 40 CFR 136.6. We appreciate your collaborative and cooperative approach in helping us implement this flexibility, and thereby get sound technical solutions to analytical problems in use more quickly than in the past. This memorandum is our current thinking about this flexibility. You may use it when auditing a laboratory, or fielding inquiries about allowed modifications to Part 136 chemical methods. As we gain experience with this flexibility we will update, as needed, via memoranda or updates to the Q&As at our CWA methods website http://www.epa.gov/waterscience/methods/.

You asked for examples of allowed flexibility. Lem Walker has prepared the following descriptions of developer and user responsibilities to document modifications they make to CWA methods. Developer responsibilities are germane to those, for example, who automate manual methods and often market these solutions for nationwide use for CWA compliance monitoring. User responsibilities are germane to those who modify existing CWA methods to solve matrix problems, or to speed or otherwise improve the analysis. Laboratories that modify Part 136 methods may be private, public or commercial and may conduct analyses for one or more clients or facilities.

Examples of Allowed Method Modification

In the past and often on a case-by-case basis, EAD wrote letters to co-regulators, developers, and others about modifications to Part 136 methods. If the underlying chemistry and determinative technique were essentially the same as the unmodified Part 136 method, we agreed that these modified methods were equivalent and acceptable alternatives. The current state is that those who develop or use a modification to an

approved (Part 136) method and document the modification as described at 136.6 will no longer receive or require a letter from us. The March 12th Methods Update Rule promulgated 136.6 which allows the regulated community more flexibility that includes:

- Changes between manual method, flow analyzer and discrete instrumentation
- Changes between automated and manual sample preparation such as digestions, distillations, and extractions; in-line sample prep is an acceptable form of automated sample preparation for CWA methods
- Changes in calibration range (provided that the modified range covers any relevant regulatory limit)
- Changes in equipment such as using similar equipment from a different vendor than that mentioned in the method
- Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter or modifying temperature program for a specific GC column, or sensible changes in reaction time and temperature as needed to achieve the chemical reactions defined in the unmodified CWA method
- Changes to chromatographic columns, including the use of capillary GC columns
- Changes in purge-and-trap sample volumes or operating conditions
- Adjusting sample sizes or changing extraction solvents to optimize method performance in meeting regulatory requirements (except for parameters that are defined by the method, such as oil and grease
- Minor changes in reagents used where the underlying reaction and principles remain virtually the same. Some examples are:

A. Changes in pH. A change in pH is allowed if the pH improves performance specifications. One example would be prevention of the formation of a precipitate as used by Rhine et al. Their article, "Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water" *(Soil Sci. Soc. Am. J. 62:473-480 (1198))* is attached. Another example is lowering the pH from 8.5 to 7.5 using an imidazole buffer for the nitrate nitrogen by cadmium reduction test.

B. Changes in pH Adjustment Reagents - Changes in compounds used to adjust pH are acceptable as long as they do not produce interference. For example, using HCL in place of H_3PO_4.

C. Changes in buffer reagents provided that the change does not produce an interference. The purpose of a buffer is to maintain or adjust the sample to a certain optimized pH. If one buffer is found to work better than another in a certain matrix, or is found to improve performance, or is at a different pH, the buffer is allowed.

D. Changes in complexing reagent provided that the change does not produce interferences. The ammonia paper cited in section A provides an example of using a different complex reagent (citrate) other than either reagent specified in the EPA method

(sodium potassium tartrate and EDTA) because it was found to be more effective and not interfere.

E. Changes in reactants provided that the change does not produce interference. The ammonia paper cited in section A gives an example and references other examples of changing the precursor to a final product that still results in the same reaction (Berthelot reaction and formation of indophenol).

F. Changes in the order of reagent addition provided that the change does not produce interference. Using the same reagents, but adding them in different order or preparing them in combined or separate solutions (so they can be added separately), is allowed provided reagent stability or method performance is improved.

The underlying philosophy of reagent modification should always include safety along with method performance. If equal or better performance can be obtained with an alternative reagent, then it is allowed.

NOTE: Changes in method parameters are not allowed, if such changes would alter the defined methodology (i.e. method principle) of the unmodified CWA method. For example, phenol method 420.1 or 420.4 defines phenolics as ferric iron oxidized compounds that react with 4-aminoantipyrine (4-AAP) at pH 10 after being distilled from acid solution. Because total phenolics represents a group of compounds that all react at different efficiencies with 4-AAP, changing test conditions likely would change the behavior of these different phenolic compounds.

Technologies allowed as alternatives under Part 136.6 include the following:

- discrete analyzers
- segmented flow analyzers
- flow injection analyzers
- micro distillation apparatus
- midi distillation apparatus
- prepackaged reagents
- colorimetric methods
- digital titrators and methods where the underlying chemistry used for the determination is similar as that used in the approved method
- ion chromatography
- TOC analyzers (oxidative method and detection)
- UV digestion

Changes are only allowed, if the modified method produces equivalent performance for the analyte(s) of interest, and the equivalent performance is documented.

EPA encourages regulatory authorities to allow flexibility in the spirit of method improvement. For example, because it is impossible to address all matrix interference in all wastewaters, it may be necessary to tailor a method modification to a specific matrix interference problem. The reason for allowing a method modification is to improve

method performance such as accuracy (e.g. recovery), lower detection limits, and better precision.

Evaluating Method Modifications

Regions and states periodically audit laboratories. When they do so, we recommend using the following items to evaluate the suitability of a modified Part 136 method:

Developer Responsibilities

- Provide the laboratory with a side-by side method comparison table
  The developer should provide to its customers an in-depth comparison of the modified method with the EPA approved method, and document the comparison in a two-column method comparison table. The two-column method comparison table shall include the number and title of each method, the latest revision date of the modified method and a detailed discussion of each of the 17 topics required by the standard EPA method format. Each topic should be discussed on a separate row in the method comparison table. The developer should highlight any differences between the modified method and EPA approved method. If the modified method is an automation of a previously approved manual method, any difference in kinetics and interferences should be presented and a comparison of final ratios of the concentrations of the reactants in the proposed and approved methods included.

- The developer should provide to their clients the modified method written in the standard EPA format: http://www.epa.gov/waterscience/methods

- Provide a copy of the data comparing the modified method performance to the approved method to demonstrate that the method is capable of yielding reliable data for compliance monitoring purposes. Test results from validation of a modified method are used to demonstrate that the modified method produces results are equivalent to results produced by the EPA-designated approved method. Equivalency is established by demonstrating that the modified method produces results meet or exceed the QC acceptance criteria of the EPA-designated approved method.

Verify that all items of the "Equivalency Checklist" are met:

Equivalency checklists:

1) Concentrations of calibration standards. Document the range of the concentrations of material used to establish the relationship between response of the measurement system and analyte concentration.

2) %RSD or correlation coefficient of calibration regression.

3) Performance range tested with units.

4) Sample(s) used in initial demonstration have the recommended preservative, where applicable.

5) Sample(s) used in initial demonstration met recommended holding times, where applicable.

6) Interferences.

7) Document the qualitative identification criteria used.

8) Performance evaluation studies performed for analytes of interest, where available.
Latest study sponsor or title
Latest study number.

9) Analysis of external reference material
Results of analyses on reference material from a source different from that used to prepare the calibration standards, if applicable.

10) Sources of external reference material, if applicable.

11) Surrogates used, if applicable.

12) Concentrations of surrogates, if applicable.

13) Recoveries of surrogates appropriate to the proposed use, if applicable.

14) Sample preparation.

15) Clean-up procedures.

16) Method blank result.

17) Matrix (reagent water, drinking water, effluent)
Matrix spikes.

18) Spiking system, appropriate to the method and application.

19) Spike concentrations (with units corresponding to the final sample concentration) and recoveries.

20) Source of spiking material.

21) Number of replicate spikes
Initial demonstration of capability.

22) Precision (analyte by analyte)
Duplicates.

23) Bias (analyte by analyte).

24) Detection limit (with units; analyte by analyte).

25) Confirmation of detection limit, if applicable.

26) Quantitation limit (with units; analyte by analyte)
Minimum level (ML), practical quantitation level (PQL) or limit of quantitation (LOQ).

27) Qualitative confirmation.

User Responsibilities

Although no comparative data between methodologies need to be provided to EPA prior to use, the user or laboratory should have a data package available for review that demonstrates proficiency by:

- making a detailed Standard Operating Procedure (SOP) available
- performing and documenting an initial demonstration of capability
    - Verify the modified method by analyzing and documenting 3-7 representative effluents (performed on different days of the week). The facility/lab is to show they can get the modified method to work and that it gets comparable results for their effluent.
- a demonstration of calibration linearity or use of a calibration curve
- periodic calibration verification
- an ongoing demonstration of performance (ongoing precision and recovery (OPR) and a blank with each sample batch)
- a demonstration of the method detection limit (MDL)
- matrix spike and matrix spike duplicate for each discharge the first time that the sample of the discharge is analyzed and at a frequency of 5% thereafter
- meeting the quality control (QC) specifications of the method
    - If the reference method does not provide sufficient QC specifications, the targets listed in the December 1996 Streamlining Guide *(applies only to CWA methods)* may be used (http://www.epa.gov/waterscience/methods/guide/flex.html).
- having the modified method manufacturer's supporting data available for review – when the manufacturer has developed the method modification.

6.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digestor and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

6.4 Cool sample and dilute to 25 ml with distilled water. If TKN is determined the sample should be diluted with ammonia-free water.

Colorimetric Analysis

6.4.1 Check the level of all reagent containers to ensure an adequate supply.

6.4.2 Excluding the molybdate/antimony line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

6.4.3 Flush the Sampler IV wash receptacle with about 25 ml of 4% sulfuric acid (5.7).

6.4.4 When reagents have been pumping for at least five minutes, place the molybdate/antimony line in its container and allow the system to equilibrate.

6.4.5 After a stable baseline has been obtained, start the sampler.

7. Calculations

7.1 Prepare a standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with the standard curve.

8. Precision and Accuracy

8.1 In a single laboratory (EMSL) using sewage sample containing total P at levels of 0.23, 1.33, and 2.0, the precision was ±0.01, ±0.04, and ±0.06, respectively.

8.2 In a single laboratory (EMSL) using sewage samples of concentration 1.84 and 1.89, the recoveries were 95 and 98%, respectively.

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5. Gales, M.E., and Booth, R.L., "Evaluation of the Technicon Block Digestor System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus", EPA-6001/4–78–015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.