

o-Phosphate–P in Drinking, Saline and Surface Waters, and Domestic and Industrial Wastes

1 SCOPE

This method covers the determination of o-Phosphate–P in surface and saline waters, drinking waters and domestic and industrial waste waters. Samples which have been digested according to USEPA methods 365.1, 365.2 or 365.3 and have been neutralized for pH may also be analyzed using this method.

This method, equivalent to USEPA Method 365.1, Rev 2.0, is approved for the Clean Water Act for use in wastewater compliance monitoring under National Pollutant Discharge Elimination System (NPDES). For NPDES monitoring, this method is also equivalent to Standard Methods 4500-P-F (1999 forward) and USGS Method I-4601-85.

This method is also approved for the Safe Drinking Water Act for use in drinking water compliance monitoring under National Primary Drinking Water Regulations (NPDWR).

2 RANGE OF APPLICATION

Range	0.003 – 0.3 mg P/L
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3 METHOD DETECTION LIMIT

By EPA Procedure	MDL = 0.0007 mg P/L
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4 METHOD PRINCIPLE

Reaction with acidic molybdate in the presence of antimony forms an antimony phospho-molybdate complex. This complex is chemically reduced by ascorbic acid to an intensely blue complex: phosphomolybdenum blue. The absorbance of this complex is measured photometrically at 880nm

5 REFERENCES

Methods for the Determination of Inorganic Substances in Environmental Samples, USEPA 600/R 93/100, August 1993: Method 365.1, Rev. 2.0

Standard methods for the Examination of Water and Wastewater, APHA/AWWA/WEF, method 4500-P-F (1999 forward).

6 INTERFERENCES

Interferences can come from a variety of analytes including barium, lead, silver, silica, nitrite, sulfide, arsenate, hexavalent chromium, and arsenic; however the effects of these are usually negligible in natural waters. See Section 11.1, Chemistry Troubleshooting Tips for specifics.

Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in seawater, it does not interfere.

Filtration of samples for total or total hydrolysable phosphorus should only occur after digestion.

Bias from sample turbidity or color is correctable, using sample blanking feature of AQ software.

7 REAGENTS: CHEMICALS REQUIRED AND SAFETY INFORMATION

Consult the safety data sheets (SDS) for details on safe handling of chemicals.

<u>Chemical</u>	<u>Formula</u>	<u>C.A.S. No.</u>	<u>Safety Classification</u>
Ammonium molybdate tetrahydrate	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	12054-85-2	--
Antimony potassium tartrate trihydrate	$\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$	28300-74-5	GHS07, GHS09
Ascorbic acid	$\text{C}_6\text{H}_8\text{O}_6$	50-81-7	--
Potassium phosphate monobasic	KH_2PO_4	7778-77-0	--
Sulfuric acid, concentrated	H_2SO_4	7664-93-9	GHS05

GHS01: Danger – Explosive

GHS04: Warning – Compressed Gas

GHS07: Warning – Irritant

GHS02: Danger – Flammable

GHS05: Warning/Danger – Corrosive

GHS08: Danger – Health Hazard

GHS03: Danger – Oxidizing

GHS06: Danger – Toxic

GHS09: Warning/Danger – Aquatic Toxicity

Note: Persons using this method should be familiar with normal laboratory practice. This method does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with local regulatory conditions.

8 REAGENT SOLUTIONS

Use reagent-grade chemicals certified for analytical or general laboratory use. SEAL recommends using ACS Reagent Grade whenever possible. Reagents of technical or commodity grade must be validated by the user. Use high-purity reagent water, distilled or deionized, and free from organic contamination, therefore Grade 1 (ISO Standard 3696) or better than Type II (ASTM Standard D1193) waters are suitable.

8.1 AMMONIUM MOLYBDATE, 4% (w/v)

Ammonium molybdate tetrahydrate	4.0 g
DI Water	dilute to 100 mL

Dissolve 4.0 g ammonium molybdate tetrahydrate in approximately 80 mL deionized water. Stir until completely dissolved, may take up to 1 hour. Dilute to 100 mL and store in a plastic bottle.

Store this reagent in a plastic container for up to 3 weeks. Refrigerate.

8.2 SULFURIC ACID, 5 normal

Sulfuric acid, concentrated	70 mL
DI Water	dilute to 500 mL

Slowly add 70 mL sulfuric acid, concentrated to about 400 mL of deionized water. Caution, the container will become very warm. Cool to room temperature and dilute to 500 mL with deionized water.

8.3 ANTIMONY POTASSIUM TARTRATE, 3g/L

Antimony potassium tartrate trihydrate

1.5 g

DI water

dilute to 500 mL

Dissolve 1.5 g antimony potassium tartrate trihydrate in about 400 mL deionized water. Dilute to 500 mL and mix well. Store at 4°C in a dark bottle.

Refrigerate in a dark bottle. Prepare fresh monthly.

8.4 WORKING PHOSPHATE COLOR REAGENT

Sulfuric acid, 5 normal (above)

see table

Antimony potassium tartrate, 3 g/L (above)

10 mL

Ammonium molybdate, 4% w/v (above)

10 mL

DI water

dilute to 100 mL

To a 100 mL volumetric flask, add the volume of sulfuric acid, 5 normal according to the table:

Calibration diluent (Section 9.2), preservation level	None	0.5mL/L	1.0mL/L	1.5 mL/L	2.0mL/L
Sulfuric acid, 5 normal added to color reagent	30 mL	27 mL	24 mL	21 mL	18 mL

Then add 10 mL antimony potassium tartrate, 3 g/L to the sulfuric acid, 5 normal, and swirl to mix. Add 10 mL ammonium molybdate, 4% w/v. Swirl the contents, fill the flask to the mark with deionized water and mix well.

Store this reagent in a plastic container for up to 3 weeks. Discard if the reagent turns blue or becomes turbid.

8.5 WORKING ASCORBIC ACID, 5.0 g/L

Ascorbic acid, fine granular

0.50 g

DI water

dilute to 100 mL

Dissolve 0.50 g ascorbic acid, fine granular, in about 80 mL deionized water. Dilute to 100 mL and mix well.

Prepare fresh daily.

9 STANDARD SOLUTIONS

9.1 PHOSPHATE-P STOCK STANDARD SOLUTION (1000 mg X/L)

Potassium dihydrogen orthophosphate, anhydrous	4.394 g
Sulfuric acid, 5 normal (above)	up to 10 mL
DI water	dilute to 1 L

Dissolve 4.394 g potassium dihydrogen orthophosphate, anhydrous (dried at 105°C) in about 800 mL deionized water in a 1 liter volumetric flask. Up to 10 mL sulfuric acid, 5 normal, may be added for preservation. Dilute to the mark with deionized water.

Store at 4°C.

9.2 CALIBRATION DILUENT (for acid-preserved samples)

Sulfuric acid, 5 normal (above)	see table
DI water	dilute to 2 L

Use this solution as the diluent for automated and manual dilutions and also as the blank and diluent for auto-calibrations. **Note:** use of sulfuric acid for preservation of field samples is not allowed for compliance monitoring analysis of o-phosphate in drinking waters. See section 10 for details.

Choose the level of sulfuric acid closest to the level of acid added to field samples for preservation. Transfer the volume of sulfuric acid, 5 normal, shown in the table (± 0.25 mL) to a 2 liter bottle. Dilute to volume with deionized water.

Sulfuric acid, preservation level	None	0.5mL/L	1.0mL/L	1.5 mL/L	2.0mL/L
Sulfuric acid, 5 normal, volume added per 2 L	--	7 mL	14 mL	21 mL	29 mL

9.3 PHOSPHATE INTERMEDIATE STANDARD SOLUTION (10 mg P/L)

Phosphate Stock Standard (1000 mg P/L, above)	2.0 mL
Calibration diluent	dilute to 200 mL

Pipet 2.0 mL PHOSPHATE-P stock standard (1000 mg P/L) to a 200 mL volumetric flask. Fill to the mark with Calibration Diluent and invert to mix.

Store at 4°C

9.4 AUTO CALIBRATION STANDARD – PHOSPHATE-P (0.3 mg P/L)

PHOSPHATE Intermediate Standard (10 mg X/L, above)	15 mL
Calibration diluent	dilute to 500 mL

Transfer 15 mL PHOSPHATE-P intermediate standard (10 mg P/L) to a 500 mL volumetric flask. Fill to the mark with Calibration Diluent and invert to mix.

Prepare fresh weekly. Store at 4°C.

9.5 MANUAL CALIBRATION

These are suggested concentrations for manual calibration standards.

Concentration, mg P/L	0	0.003	0.015	0.03	0.075	0.15	0.225	0.3
Volume (mL) Auto Calibration Standard Solution diluted as indicated with Calibration Diluent	--	1.0	5.0	10	25	50	75	100
Final Volume, mL	100	100	100	100	100	100	100	100

Prepare weekly

10 SAMPLE PRESERVATION AND STORAGE

Samples may be collected into plastic or glass containers.

Samples to be reported for ortho-phosphate for EPA's drinking water compliance monitoring programs should NOT be filtered, digested, or undergo hydrolysis. The samples should be cooled to $\leq 6^{\circ}\text{C}$ and must be analyzed within 48 hours of collection. Do not use acid for preservation.

Samples to be reported for dissolved ortho-phosphate for EPA's wastewater compliance monitoring programs must be filtered immediately, then cooled to $\leq 6^{\circ}\text{C}$ and must be analyzed within 48 hours of collection. Do not use acid for preservation.

Samples to be reported for total-phosphorus for EPA's wastewater compliance monitoring programs must be preserved with sulfuric acid to a $\text{pH} < 2$, cooled to $\leq 6^{\circ}\text{C}$ and must be analyzed within 28 days. Digestion is required as described in USPEA 365.1, 365.2 or 365.3. Do not filter prior to digestion. After digestion, neutralize with sodium hydroxide as described in USEPA method 365.1 or 365.2. If pH neutralization is not desired, analyze the finished digests using SEAL method EPA-119-D instead. Call SEAL Analytical technical Support for assistance.

11 PROCEDURE

Prepare standards and reagents as described in Sections 8 and 9. The laboratory must determine standard concentrations that are most suitable to their needs for calibration within the range of application for this method. The concentrations listed within this method are only suggestions.

Any reagents having visible particulates should be filtered.

Pour calibration standards, quality control solutions and samples into sample cups and reagent wedges, according to the user-defined work list.

Before running the analysis batch, check the analyzer for sufficient reagent water, fresh reaction segments, correct location of reagent wedges and a waste collection bottle in place.'

After o-phosphate analyses are finished for the day, clean the cuvette to remove deposits. Fill a reagent wedge with SEAL Cuvette Cleaning Solution (p/n 5676) and place it in Position 1 of the reagent tray. Within SEAL discrete analyzer software, go to Maintenance—Cuvette Functions and select Extra Wash.

11.1 CHEMISTRY TROUBLESHOOTING TIPS

Glassware should be washed with 1:1 HCl rinsed with phosphate color reagent, and rinsed thoroughly with deionized water. If glassware is reserved solely for the determination of phosphorus, then treatment with 1:1 HCl and phosphate color reagent is only required occasionally. Commercial detergent should never be used.

As much as 50 mg Fe^{3+}/L , 10 mg Cu^{2+}/L , and 10 mg SiO_2/L can be tolerated. High silica concentrations cause positive interference and high iron concentrations can cause precipitation, which causes a subsequent loss of phosphorus. For silica concentrations of 20, 50, and 100 mg/L, phosphorus results will be elevated by 0.0005, 0.015, and 0.025 mg/L, respectively.

Hexavalent chromium and nitrite interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L, respectively.

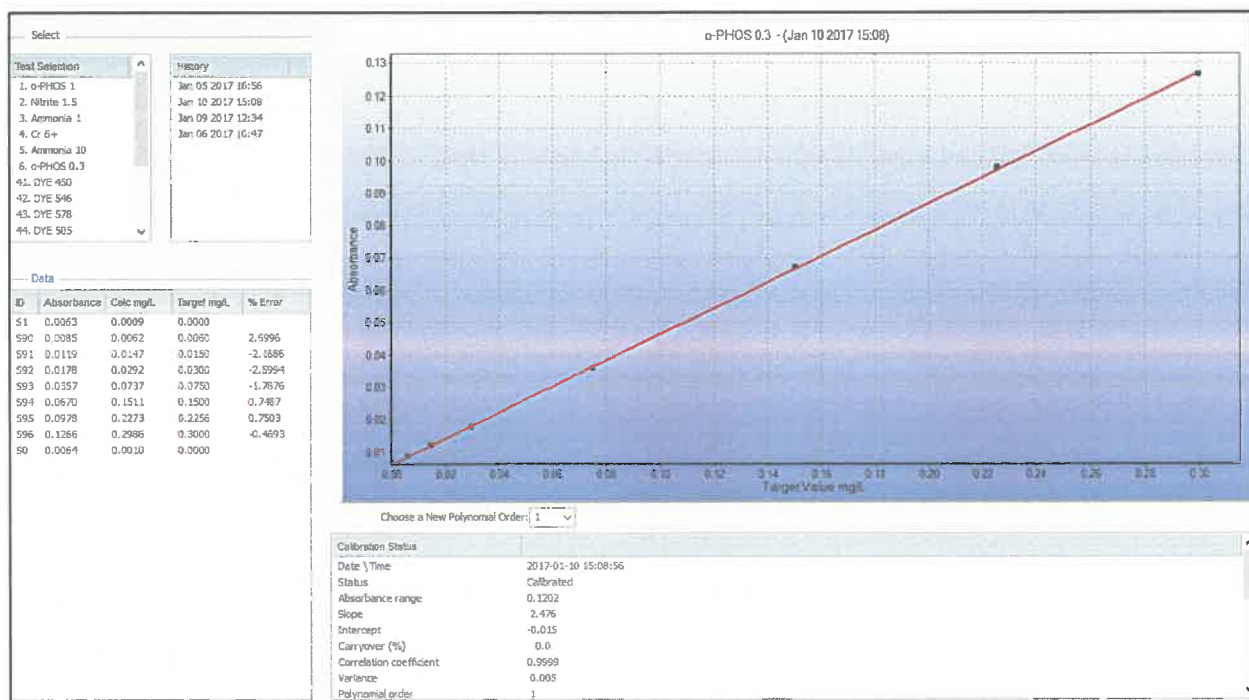
12 TEST PARAMETERS

PARAMETER	AQ300 SETTING
Test name	o-PHOS 0.3
Units	mg P/L
Decimals	4
Test type	End Point
Sample volume (µL)	500
Water volume (µL)	0
Number of mixes	2
Cuvette primes	4
Cuvette washes	2
Baseline on Wash	Ticked
Reaction time (seconds)	480
Wavelength (nm)	880
Polynomial order	1
Number of reagents	2
1. PO4 color reagent (µL)	55
2. Ascorbic acid (µL)	60

DEFINITIONS:

Test name:	Name which appears on the final report
Units:	Concentration units appearing against the reported result
Decimals:	Number of decimal places in the reported result
Test type:	The type of test the software will perform
Sample volume:	Volume (in microliters) of the sample used in the test
Water volume:	Volume (in microliters) of water from the water reservoir used in the test
Number of mixes:	Number of mixes of the sample + reagent in the reaction well
Cuvette primes:	Number of times the cuvette is flushed with reagent (no sample) prior to sampling
Cuvette washes:	Number of washes of the cuvette between aspirations of finished reaction liquid
Baseline on Wash:	Water baseline measured between aspirations to provide drift correction
Reaction time:	Time (in seconds) between addition of sample + reagent and measurement of the reaction mixture
Wavelength:	Interference filter wavelength to be used in the test
Polynomial order:	Polynomial order to be used when fitting the standard points to the standard graph (1 = linear)
Number of reagents:	Number of reagents defined for the test
Reagent volume:	Volume (in microliters) of the reagent used in the test

13 STANDARDIZATION



DEFINITIONS:

Abs. Range: Difference between the absorbance of the highest standard and the absorbance of the blank

Variance: Calculated as follows:

$$\text{Variance} = \frac{\sum(\text{Deviations, i.e., errors})^2}{\sum(\text{Observed values})^2} \times 100$$

Carryover (%): Calculated as follows:

$$\text{Carryover (\%)} = \frac{(A - B)}{C} \times 100\%$$

Where,

- A = absorbance of carryover blank (S0)
- B = absorbance of blank (S1)
- C = calibration absorbance range

14 PERFORMANCE VALIDATION DATA

14.1 DETECTION LIMIT STUDY

Replicates of 0.003 mg P/L nitrate standard in reagent water***

Concentration (mg P/L)				
	0.0029	0.0023	0.0027	0.0027
	0.0030	0.0023	0.0029	0.0026
	0.0033	0.0022	0.0029	0.0027
	0.0024	0.0018	0.0027	0.0020
	0.0021	0.0022	0.0025	0.0020
	0.0027	0.0021	0.0026	0.0022
	0.0027	0.0016	0.0029	0.0024
	0.0028	0.0018	0.0030	0.0025
	0.0029	0.0022	0.0027	0.0020
	0.0028	0.0021	0.0028	0.0029
	0.0027	0.0019	0.0031	0.0025
	0.0032	0.0023	0.0032	0.0032
	0.0032	0.0022	0.0028	0.0031
	0.0026	0.0022	0.0030	0.0026
	0.0029	0.0019	0.0028	0.0033
Statistics				
Mean	0.0028	0.0021	0.0028	0.0026
Std. Deviation	0.0003	0.0002	0.0002	0.0004
MDL*	0.0008	0.0006	0.0005	0.0011

14.2 PERFORMANCE STUDY

Replicates of 0.015 mg P/L nitrate standard in reagent water***

Concentration (mg P/L)				
	0.0126	0.0125	0.0132	0.0124
	0.0130	0.0128	0.0128	0.0131
	0.0135	0.0131	0.0131	0.0134
	0.0129	0.0126	0.0130	0.0133
	0.0127	0.0123	0.0130	0.0129
	0.0138	0.0123	0.0133	0.0132
	0.0134	0.0125	0.0129	0.0140
	0.0133	0.0125	0.0132	0.0139
	0.0128	0.0126	0.0129	0.0136
	0.0138	0.0126	0.0137	0.0138
Statistics				
Mean	0.0132	0.0126	0.0131	0.0134
Std. Deviation	0.0004	0.0002	0.0003	0.0005
% RSD**	3.4	1.9	2.0	3.7
% Recovery	87.9	83.9	87.4	89.1

14.3 PRECISION STUDY

Replicates of 0.15 mg P/L nitrite standard in water***

Concentration (mg P/L)				
Statistics				
Mean	0.1471	0.1464	0.1464	0.1477
% Recovery	98.0	97.6	97.6	98.4
Std. Deviation	0.0007	0.0011	0.0009	0.0009
% RSD**	0.5	0.8	0.6	0.6
Maximum	0.1481	0.1488	0.1481	0.1493
Minimum	0.1456	0.1450	0.1454	0.1458

Replicates of 0.3 mg P/L nitrite standard in water***

Concentration (mg P/L)				
Statistics				
Mean	0.2974	0.2957	0.2967	0.2998
% Recovery	99.1	98.6	98.9	99.6
Std. Deviation	0.0017	0.0024	0.0016	0.0014
% RSD**	0.6	0.8	0.6	0.5
Maximum	0.3003	0.2993	0.3006	0.3008
Minimum	0.2952	0.2906	0.2945	0.2957

* MDL is calculated by multiplying the Standard Deviation by the Student-t value for the number of replicates run.

** %RSD is calculated by dividing the Standard Deviation by the mean, then multiplying by 100.

*** To prepare these standards, refer to Section 9.

15 REVISIONS

January 31, 2017 Rev 0

Method created and support data acquired