# DRINKING WATER METHOD:

# Drinking Water Orthophosphate for Thermo Scientific Gallery Discrete Analyzer

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| **Name of the method**: | Drinking Water Orthophosphate |
| **Reference**: | Standard Methods (SM) 4500-P E. Phosphorous /Ascorbic Acid Method [1]. EPA approved method under 40 C.F.R. § 141.23 (National Primary Drinking Water Regulations, NPDWR, based on SDWA, Safe Drinking Water Act). |
| **Intended use**: | This paper presents Drinking Water Orthophosphate method for Thermo Scientific™ Gallery™ discrete analyzer. The method was designed to follow the standard method SM 4500-P E. as closely as possible. |
| **Revision number:** | 5 |
| **Revision date (mm/dd/yyyy):** | 02/18/2016 (Editorial revision date 08/22/2017) |

## 1. Scope and Application

**1.1** This automated method covers the determination of orthophosphate (CAS: 14265-44-2) in drinking water with the Thermo Scientific Gallery discrete analyzer.

**1.2** The method is based on reaction that is specific for the orthophosphate ion.

**1.3** The applicable range for this method is from 0.0125 to 0.5 mg P/L. An extension of the range to 5 mg P/L is achieved when 1:10 automated dilution is used. The automated dilution feature must be confirmed with acceptable analysis of quality control samples by the user.

## 2. Summary of Method

**2.1** Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of phosphorous to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

**2.2** Only orthophosphate forms a blue color in the test. Polyphosphates (and some organic phosphate compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphate compounds may be converted to the orthophosphate form by persulfate digestion. These conversion methods are not covered in this method.

## 3. Definitions

**3.1** Units and symbols from the international metric system (SI) are used. Definitions, acronyms, and abbreviations are explained as they occur for the first time.

## 4. Interferences

**4.1** No interference is caused by copper, iron or silicate at concentrations many times greater than their reported concentration in seawater. However, high iron concentrations can cause precipitation of, and subsequent loss, of phosphorous.

**4.2** Arsenates react with the molybdate reagent to produce a blue color similar to that formed with orthophosphate. Thus, it should be considered when present in concentrations higher than orthophosphate. Concentrations as low as 0.1 mg/L can interfere with the orthophosphate determination.

**4.3** Hexavalent chromium and nitrite-ion can interfere with the orthophosphate determination yielding in approximately 3 % lower results at concentrations of 1 mg/L and 10 % to 15 % lower results at 10 mg/L concentrations.

**4.4** Sample turbidity is not to be removed by filtration prior to analysis for orthophosphate, unless dissolved reactive phosphorous is studied. Samples for total phosphorous may be filtered only after digestion. Sample color that absorbs in the photometric range used for analysis will also interfere. The sample blank is measured automatically in the application for sample color and turbidity correction.

## 5. Safety

**5.1** The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

**5.2** The following chemicals have the potential to be toxic or hazardous. Consult MSDS (Material Safety Data Sheet) for details.

**5.2.1** Sulfuric acid (in Phosphate R1) (CAS# 7664-93-9)

**5.2.2** Ammonium molybdate tetrahydrate (in Phosphate R1) (CAS# 12027-67-7)

**5.2.3** Antimony potassium tartrate (in Phosphate R1) (CAS# 28300-74-5)

**5.2.4** Ascorbic acid (in Phosphate R2) (CAS# 50-81-7)

**5.2.5** Potassium phosphate monobasic (CAS# 7778-77-0)

## 6. Equipment and Supplies

**6.1** Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

**6.2** Water purification system for producing suitable water for autoanalyzers. Refer to analyzer user manual.

**6.3** Thermo Scientific Gallery, automated photometric discrete analyzer. Later referred as Gallery.

**6.4** Filter, wavelength 880 nm.

**6.5** DECACELL™ Cuvettes for Gallery. DECACELL cuvettes must always be used with Gallery. Cuvettes are for single use only. Ordering code 986540.

**6.6** Washing solution 4.5 % hypochlorite solution, 4 × 20 mL, is used for daily analyzer cleansing. Ordering code 984030 (4 x 20 ml).

## 7. Reagents and Standards

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|---|---|
| Reagents for Gallery: | THERMO SCIENTIFIC ORDERING CODES |
| | 984366 Phosphate R1, 4 × 20 mL |
| | 984367 Phosphate R1, 20 × 20 mL |
| | 984368 Phosphate R2, 4 × 20 mL |
| Standards for Gallery: | THERMO SCIENTIFIC ORDERING CODES |
| | 984729 Phosphate (as P) Std, 1000 ppm, 500 mL |
| | 984726 Phosphate (as PO4) Std, 1000 ppm, 500 mL |

**7.1** Preparation of reagents and standard solutions needed for this method is described under. Also ready to use reagents and standard solutions are available for this method.

**7.2** Reagent water — Distilled or deionized water, free of the analyte of interest. Water stored in bottles should be substituted by fresh water after one week.

**7.3** Phosphate R1 — Prepared by adding 15 mL of ammonium molybdate solution (20 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ /500 mL water) to 50 mL of sulfuric acid solution (70 mL conc. $H_2SO_4$ /500 mL water) followed by 5 mL addition of antimony potassium tartrate solution (1.5 g $K(SbO)C_4H_4O_6\cdot \frac{1}{2}H_2O$ /500 mL water) to this mixture.

**7.4** Phosphate R2 — Ascorbic acid solution containing 1.76 g of ascorbic acid in 100 mL water. This solution is stable for 5 days when refrigerated between 2 -8 °C.

**7.5** Phosphate Standard (stock), 1000 mg P/L — 984729 Phosphate standard 1000 mg/L (as P), or other certified stock solution, is for calibrating the Gallery analyzer. For a self made standard (stock) solution, dissolve 0.4394 g of predried (105 °C for one hour) potassium phosphate monobasic ($KH_2PO_4$) in distilled water and dilute to 100 mL.

**7.6** Phosphate calibration solutions — Prepare an appropriate series of standards by diluting suitable volumes of Phosphate Standard (stock) with distilled water, or use automated Gallery calibration dilution feature.

**7.7** Quality Control Sample (QCS) — A second source standard from an external source, e.g. 984726 Phosphate (as PO4) Std, 1000 ppm (326.2 ppm as P). Dilute to appropriate concentration with distilled water. Do not use Phosphate calibration solutions as QCS-samples.

**7.8** Laboratory Fortified Blank (LFB) — A certified standard stock solution e.g. Phosphate (as PO4) Std, 1000 ppm (326.2 ppm as P) or a self made LFB stock solution is used for LFB samples. Dilute to appropriate concentration with distilled water. Do not use Phosphate calibration solutions as LFB-samples.

## 8. Sample Collection, Preservation, and Storage

**8.1** Sample containers may be of plastic material or of Pyrex glass. All bottles must be thoroughly cleaned and rinsed with distilled water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal. Do not store samples containing low concentrations of phosphorous in plastic bottles because orthophosphates may be adsorbed to the walls of plastic bottles.

**8.2** Samples should be analyzed as soon as possible, after collection, within 48 hours at maximum. Samples must be cooled and maintained at 4 °C.

## 9. Quality Control

**9.1** Quality control (QC) program — Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of laboratory reagent blanks, continuing calibration check standards, fortified blanks and fortified samples as duplicates as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data generated.

**9.2** The initial demonstration of capability (IDC) — IDC is used to characterize analyzer performance in an individual laboratory by determination of calibration curve and analysis of quality control samples (QCS) and laboratory performance by determination of method detection limit (MDL), minimum reporting level (MRL) and the initial precision and recovery (IPR) test.

**9.2.1** Calibration Curve — Calibration curve must be determined initially and reanalyzed at least every six months or whenever a significant change in analyzer response is observed or expected. The calibration curve must use a minimum of five standards and a blank to ensure that the resulting curve is fitted correctly. The Gallery analyzer fits the calibration first as a linear curve. The user can then fit the calibration to 2nd degree curve, if the fitting is better thereby. The correlation coefficient of the calibration curve should be equal to or greater than 0.995.

**9.2.2** Quality Control Sample (QCS) — When beginning the use of this method, whenever new standard materials are used, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable analyzer performance with the analyses of a secondary standard solution (QCS) from an external source. If the determined concentrations are not within ± 15 % or ± 20 % when the concentration is ≤ 2x MRL of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before proceeding with the initial determination of MDL or continuing with on-going analyses.

**9.2.3** Method Detection Limit (MDL) — An MDL should be established using reagent water (blank) fortified at a concentration of one to five times the estimated detection limit (MDLest). The estimate is calculated as three times the standard deviation of replicate measurements of the analyte in reagent water.

$$MDLest = 3 \times (SD_0)$$

where $SD_0$ = standard deviation of the replicate analyses (n=10) of reagent water.

Prepare a laboratory fortified blank sample (LFB) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit (recommended 1 – 5 x MDLest). Perform all calculations defined in the method and report the concentration values in the appropriate units. Each individual result must be within 70 – 130 % of the theoretical value. Calculate the MDL as follows:

$$MDL = (t) \times (SD)$$

where t = Student's t-value for a 99 % confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for 7 replicates]
SD = standard deviation of the replicate analyses

MDL should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or analyzer response.

**9.2.4** Minimum Reporting Level (MRL) Confirmation — The minimum concentration that can be reported by a laboratory as a quantified value for the method analyte in a sample following analysis. The MRL must be at or above the level of the lowest fortified calibrator, where it must meet the criteria set for MRL confirmation. It would also have to be considered that criteria for Laboratory Reagent Blank (LRB) must be met (LRB ≤ 1/3 x MRL).

Fortify and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the mean (*Mean*) and standard deviation (SD) for these replicates. Determine the Half Range for the Prediction Interval of Results ($HR_{PIR}$):

$$HR_{PIR} = 3.963 \text{ x } (SD)$$

Where SD = standard deviation
3.963 = constant value for seven replicates

Calculate the upper and lower limits for the Prediction Interval of Results (PIR = *Mean* +$HR_{PIR}$) from the results and confirm that the results meet the criteria. Accepted results confirm the MRL to be valid.

$$PIR\,Upper\,Limit = \frac{Mean + HR_{PIR}}{Fortified\,Concentration} \times 100$$

$$PIR\,Lower\,Limit = \frac{Mean - HR_{PIR}}{Fortified\,Concentration} \times 100$$

Criteria: The Upper PIR Limit must be ≤ 150 % Recovery.
The Lower PIR Limit must be ≥ 50 % Recovery

**9.2.5** Initial Precision and Recovery (IPR) — For initial precision and recovery test the laboratory should analyze four replicate volumes of reagent water spiked with the analyte of interest (LFB). Calculate accuracy as percent recovery and precision as relative standard deviation (% RSD) as shown under.

$$\%RSD = \frac{100}{\overline{X}} \times \sqrt{\sum_{i=1}^{n} \frac{(X_1 - \overline{X})^2}{n-1}}$$

where $\overline{X}$ = mean of replicate measurements
$X_1$ = measured value of the replicate
n = number of replicates

$$\text{Recovery}\,\% = \frac{C_s - C}{s} \times 100$$

where Cs = spiked sample concentration
C = sample background concentration
s = concentration equivalent of analyte added to sample

Criteria for % Recovery: ±15% (or 85 - 115 %), when c is > 2 x MRL and ±20% (or 80 - 120 %), when c is ≤ 2 x MRL
Criteria for % RSD: ±15%

**9.3** Assessing laboratory performance with ongoing QC includes the use of Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB), Laboratory Fortified Matrix (LFM) and Continuing Calibration Check (CCC) samples. Quality Control Samples (QCS) are analyzed periodically.

**9.3.1** Laboratory Reagent Blank (LRB) — The laboratory should analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment.

Criteria for LRB: Must be below 1/3 of MRL

**9.3.2** Laboratory Fortified Blank (LFB) — The laboratory should analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery as shown in 9.2.5. If the recovery of any analyte falls outside the required control limits, the source of the problem should be identified and resolved before continuing analyses. LFB analyses data is used to assess laboratory performance against the required control limits.

Criteria for % Recovery: ±15% (or 85 - 115 %), when c is > 2 x MRL and ±20% (or 80 - 120 %), when c is ≤ 2 x MRL

**9.3.3** Continuing Calibration Check (CCC) — For all determinations the laboratory should analyze the CCC (a mid-range check standard) and a calibration blank immediately following calibration, after every tenth sample and at the beginning and end of the sample run. Analysis of the CCC solution and calibration blank immediately following calibration must verify that the analyzer is within ± 10 % of calibration. Subsequent analyses of the CCC solution must verify the calibration is still within ± 10 %. This procedure is done automatically by the analyzer. **Note:** Application has manual result acceptance instead of automatic acceptance to ensure CCC protocol is followed.

If the calibration cannot be verified within the limits specified in the application, the analyzer gives a message of outlier result. In such case reanalyze the CCC solution by triggering the Ongoing QC procedure. If the second analysis of the CCC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the analyzer recalibrated. All samples following the last acceptable CCC solution must be reanalyzed, even if the resumed CCC is acceptable. The analysis data of the calibration blank and CCC solution must be kept on file with the sample analyses data.

Criteria for % Recovery: ±10% (or 90 -110 %)

**9.3.4** Laboratory Fortified Matrix Sample (LFM) (also termed Matrix Spike, MS and Matrix Spike Duplicate MSD) — The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank in section 9.3.2.

Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 85-115 %. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control, the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related. The Gallery analyzer has a standard addition feature for difficult sample matrixes.

$$\text{LFM Recovery \%, } R = \frac{(C_s \cdot f) - C}{s} \times 100$$

where $C_s$ = spiked sample concentration
f = spike dilution correction
C = sample background concentration
s = concentration equivalent of analyte added to sample

**Note:** If the added spike volume is less than 1% of the total LFM sample volume, the factor f can be excluded.

Criteria: LFM % Recovery: ±15%

The precision of the LFM determinations is assessed by measuring LFM samples as duplicates (LFMD also termed Matrix Spike Duplicate or MSD). Precision is then calculated as follows:

$$\%RPD = 100 \times \frac{LFM - LFMD}{½ \times (LFM + LFMD)}$$

where LFM = analyte concentration measured in LFM sample
LFMD = analyte concentration measured in LFM duplicate

Criteria: % RPD ±20%

**9.3.5** Quality Control Samples (QCS) — Standard solution samples (9.2.2), that must reach laboratory acceptance criteria are analyzed with each calibration, whenever new standard materials are used, on a quarterly basis or as required to meet data-quality needs in addition to the IDC measurements.

Criteria for % Recovery: ±15% (or 85 - 115 %), when c is > 2 x MRL and
±20% (or 80 - 120 %), when c is ≤ 2 x MRL

## 10. Calibration and Standardization

**10.1** Dilute Phosphate standard (stock) (7.5) with distilled water to get a suitable phosphate standard for calibration. Use the automated Gallery calibration dilution feature for a calibration curve. Alternatively, prepare a series of at least five standards, covering the desired range, and a blank, for a calibration curve.

**10.2** Process standards and blanks as described in Section 11.0 Procedure.

**10.3** The Gallery analyzer plots automatically the analyzer responses against standard concentrations. The user must accept this calibration curve before the analyzer starts to measure blanks and samples. The calibration correlation coefficient shall be equal to or greater than 0.995.

**10.4** After the calibration has been established, it must be verified by the analysis of a suitable control sample (QCS). If measurements exceed ± 15 % of the established LFB value, the analysis should be terminated and the analyzer recalibrated. Periodic reanalysis of the QCS is recommended quarterly at minimum. Ongoing QC is done automatically by the analyzer and it includes analyzing LRB, CCC and LFB samples with each batch of ten samples. In addition LFM samples are to be done in duplicates with every batch of ten samples.

## 11. Procedure

**11.1** Preparation before analysis — Add all required reagents, samples, other consumables and requests for tests following the analyzer instructions according to the manufacturer.

**11.2** The reagents and samples needed for the analysis are dispensed automatically according to the pre-defined application to single-use cuvettes, where all the reactions and measurements take place.

**11.3** Gallery Drinking Water Orthophosphate test flow — To 120 µL of sample or standard or blank 14 µL of Phosphate R1 is added and solution is mixed. After blank measurement, 6 µL of Phosphate R2 is added and solution is mixed. After 9 minutes incubation, the absorbance is measured at 880 nm. Suggested application is shown in Appendix A.

## 12. Data Analysis and Calculation

**12.1** The Gallery analyzer plots automatically the analyzer responses against standard concentrations to create a calibration curve. The analyzer computes sample concentration by comparing sample response with the standard curve.

**12.2** Results are reported in mg P/L.

## 13. Method Performance

**13.1** According to a validation study of the Gallery Drinking Water Orthophosphate method, single laboratory result for MDL was 0.00036 mg P/L and MRL was confirmed to be 0.0125 mg P/L. Initial precision was analyzed to be 0.1 - 1.0 % RSD and accuracy 102-103 % Recovery at concentrations 0.1 - 0.4 mg P/L.

**13.2** Precision for LFB samples was 0.2 – 1.1 % RSD and for LFM samples 0.1 – 0.7 % RSD corresponding to sample concentrations 0.1 – 0.4 mg P/L.

**13.3** Accuracy for LFB samples was 102 – 103 % Recovery and for LFM samples 92 – 98 % Recovery corresponding to sample concentrations 0.1 – 0.4 mg P/L.

**13.4** % Recovery for CCC was 100 – 105 % and LRB was always below 1/3 MRL.

**13.5** Method limits for LFB and LFM samples are 15 % RSD and 85 - 115 % Recovery and for CCC 90 - 110 % Recovery. LRB must be below 1/3 MRL.

## 14. Pollution Prevention

**14.1** The analyzer uses small amounts of reagents, which reduces the quantity of wastes significantly compared to manual methods or flow analyzers. The small packing size facilitates the use of reagents during their shelf lives and thus reduces disposal cost of unused materials.

## 15. Waste Management

**15.1** Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner.

**15.2** The containers for cuvette and liquid waste must be emptied and rinsed with water at the end of the day.

## 16. References


**1.** 4500-P E. Phosphorous/Ascorbic acid method. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association (APHA), 2012.

**2.** Code of Federal Regulations 40 § 141.23. Inorganic chemical sampling and analytical requirements.

**3.** Code of Federal Regulations 40 § 136, Appendix B — Definition and Procedure for the Determination of the Method Detection Limit – Revision 1.11.

**4.** 4020 Quality Assurance/Quality Control. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association (APHA), 2012.

**5.** 1020-B.4 Method Detection Level Determination and Application. Standard Methods for the Examination of Water and Wastewater, 22nd Edition, American Public Health Association (APHA), 2012.

# 17. Tables, diagrams, flowcharts and validation data

**17.1** Suggested test parameters and test flow for Gallery Drinking Water Orthophosphate method are presented in Appendix A.

**17.2** Method performance data

**17.2.1** Calibration and QCS-samples

Calibration curve was prepared between 0 - 0.5 mg P/L for DW o-PO4P application. Calibration was done with automatic dilution from working standard solution of 1.5 mg P/l and laboratory reagent water. Calibration was verified with QCS-samples.

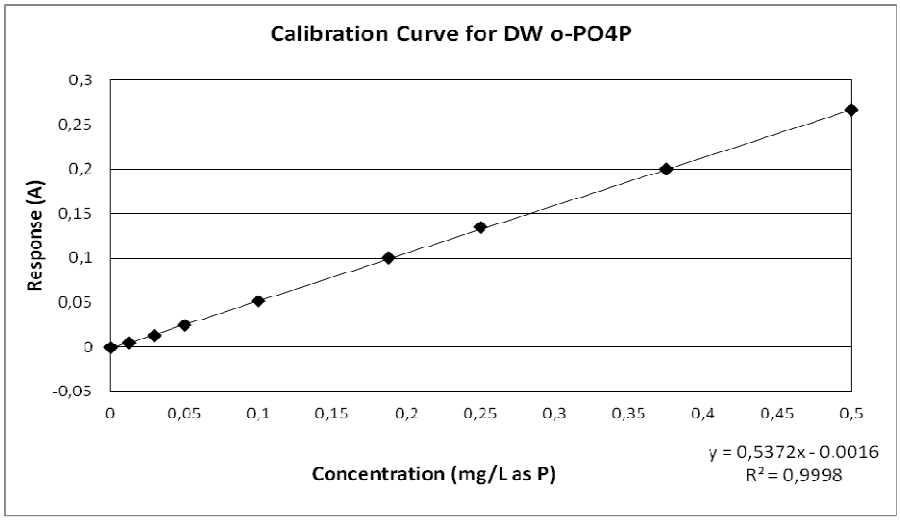



Figure 1. Calibration curve for DW o-PO4P application.

Table 1. Results for Phosphate QCS-samples after calibration.

| Orthophosphate as phosphorous | | |
|---|---|---|
| **QCS-sample** | **Duplicate % RSD** | **% Recovery** |
| PO4P 0.1mg/L | 0.13 % | 96 % |
| PO4P 0.4mg/L | 0.19 % | 103 % |

**17.2.2** Method Detection Limit (MDL)

MDL was established using reagent water (blank) fortified at a concentration approximately five times the estimated detection limit (MDLest). The estimate was calculated as three times the standard deviation of replicate measurements of the analyte in reagent water.

$$MDLest = 3 \times (SD_0)$$

where $SD_0$ = standard deviation of the replicate analyses (n=10) of reagent water.

Table 2. Values for determining MDLest for DW o-PO4P application.

| Sample | Results (mg/L) |
|---|---|
| LRB min. | 0.001800 |
| LRB max. | 0.002122 |
| Average | 0.00188 |
| n | 10 |
| std. deviation (SD) | 0.00013 |
| MDLest calculated | **0.000402** |
| **MDLest assigned** | **0.0022** |

**Note:** The MDLest had to be assigned higher than the calculated MDLest, because the highest individual LRB result of this test was as high as 0.002122 mg P/L, and criteria for LRB sample in this test is, that it should never be above the MDL est.

A laboratory fortified blank sample (LFB) at a concentration which was approximately five times the MDLest, was analyzed in seven replicates. Each individual result had to be within 70 – 130 % of the theoretical value. MDL was calculated as follows:

$$MDL = (t) \times (SD)$$

where t = Student's t-value for a 99 % confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for 7 replicates]

SD = standard deviation of the replicate analyses

Table 3. Values for determining MDL for DW o-PO4P application.

| Sample | Result (mg/L) | % Recovery |
|---|---|---|
| LFB 0.01 mg/L PO4P | 0.00793 | 79 % |
| LFB 0.01 mg/L PO4P | 0.00791 | 79 % |
| LFB 0.01 mg/L PO4P | 0.00795 | 80 % |
| LFB 0.01 mg/L PO4P | 0.00768 | 77 % |
| LFB 0.01 mg/L PO4P | 0.00796 | 80 % |
| LFB 0.01 mg/L PO4P | 0.00803 | 80 % |
| LFB 0.01 mg/L PO4P | 0.00797 | 80 % |
| Average | **0.007919** | **79 %** |
| n | **7** | |
| Std. deviation (SD) | **0.000114** | |
| % RSD | **4.5 %** | |
| **MDL** | **0.00036** | |

**17.2.3** Minimum Reporting Level (MRL)

Seven replicate LFBs were analyzed at the proposed MRL concentration.
The mean (*Mean*) and standard deviation (s) for these replicates were calculated. The Half Range for the Prediction Interval of Results ($HR_{PIR}$) was determined using the equation:

$$HR_{PIR} = 3.963 \times (SD)$$

Where SD = standard deviation
3.963 = constant value for seven replicates

The upper and lower limits for the Prediction Interval of Results (PIR = *Mean* +$HR_{PIR}$) were calculated from the results.

$$PIR\,Upper\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100$$

$$PIR\ Lower\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100$$

Criteria: The Upper PIR Limit must be ≤ 150 % Recovery.
The Lower PIR Limit must be ≥ 50 % Recovery

Table 4. Results for MRL confirmation for DW o-PO4P application.

| Sample | Result (mg P/L) | % Recovery |
|---|---|---|
| LFB 0.01 mg/L PO4P | 0.01009 | 101 % |
| LFB 0.01 mg/L PO4P | 0.00966 | 97 % |
| LFB 0.01 mg/L PO4P | 0.00957 | 96 % |
| LFB 0.01 mg/L PO4P | 0.00938 | 94 % |
| LFB 0.01 mg/L PO4P | 0.00989 | 99 % |
| LFB 0.01 mg/L PO4P | 0.00962 | 96 % |
| LFB 0.01 mg/L PO4P | 0.00961 | 96 % |
| average | 0.00969 | 97 % |
| n | 7 | |
| std. deviation (SD) | 0.000232 | |
| $HR_{PIR}$ | 0.000921 | |
| **Upper PIR Limit** | **106.1 %** | **≤ 150 %** |
| **Lower PIR Limit** | **87.7 %** | **≥ 50 %** |
| % RSD | 2.4 % | |

Calculated limits met the criteria and confirmed the MRL to be valid.

### 17.2.4 Initial Precision and Recovery (IPR)

Four replicate volumes of reagent water spiked with the analyte of interest (LFB) were analyzed at three different concentrations. % Recovery and % RSD were calculated. Results are shown in the following table.

Criteria for % Recovery: 85 % - 115 %, when c is > 2 x MRL and
80 % - 120 %, when c is ≤ 2 x MRL
Criteria for % RSD: ±15%

Table 5. Results for initial precision and recovery (IPR) for DW o-PO4P application.

| Sample | Result (mg P/L) | % Recovery |
|---|---|---|
| LFB 0.1 mg/L | 0.1027 | 103 % |
| LFB 0.1 mg/L | 0.1028 | 103 % |
| LFB 0.1 mg/L | 0.1027 | 103 % |
| LFB 0.1 mg/L | 0.1028 | 103 % |
| **Average** | **0.1027** | **103 %** |
| **Std.deviation** | **0.000074** | |
| **% RSD** | **0.1 %** | |
| **Acceptable** | **YES** | **YES** |
| LFB 0.3 mg/L PO4P | 0.3056 | 102 % |
| LFB 0.3 mg/L PO4P | 0.3020 | 101 % |
| LFB 0.3 mg/L PO4P | 0.3096 | 103 % |
| LFB 0.3 mg/L PO4P | 0.3051 | 102 % |
| **Average** | **0.3056** | **102 %** |
| **Std.deviation** | **0.0031** | |
| **% RSD** | **1.0 %** | |
| **Acceptable** | **YES** | **YES** |
| LFB 0.4 mg/L | 0.4074 | 102 % |
| LFB 0.4 mg/L | 0.4083 | 102 % |
| LFB 0.4 mg/L | 0.4088 | 102 % |
| LFB 0.4 mg/L | 0.4083 | 102 % |
| **Average** | **0.4082** | **102 %** |
| **Std.deviation** | **0.00059** | |
| **% RSD** | **0.1 %** | |
| **Acceptable** | **YES** | **YES** |

**17.2.5** Precision and accuracy from LFB samples

Precision (%RSD) and accuracy (% Recovery) were determined using LFB samples at 0.1, 0.3 and 0.4 mg/L concentrations. Results are shown in the following table.

Criteria for % Recovery: 85 - 115 %, when c is > 2 x MRL and
80 - 120 %, when c is ≤ 2 x MRL
Criteria for % RSD: ±15%

Table 6. Precision and accuracy data for DW o-PO4P application.

| **Sample:**<br>**Concentration:** | PO4P-LFB 0.1<br>0.1 mg P/L | PO4P-LFB 0.3<br>0.3 mg P/L | PO4P-LFB 0.4<br>0.4 mg P/L |
|---|---|---|---|
| **Average** | 0.103 | 0.305 | 0.409 |
| **n** | 10 | 10 | 10 |
| **Std.deviation** | 0.00021 | 0.00324 | 0.00077 |
| **% RSD** | 0.2 % | 1.1 % | 0.2 % |
| **Avg. % Recovery** | 103 % | 102 % | 102 % |
| **Acceptable** | **YES** | **YES** | **YES** |

**17.2.6** Precision and accuracy from LFM samples

Laboratory Fortified Matrix (LFM) samples were prepared by spiking three different tap water samples with Phosphate standard solution to gain spike concentrations of 0.1 and 0.4 mg P/L for each sample. The analyte concentrations were high enough to be detected above the original sample and were not less than four times the MDL. The added analyte concentrations were chosen to be the same as those used for LFB. Precision was estimated by analyzing four replicates and calculating the %RSD. Accuracy was estimated from LFM and corresponding unspiked samples by analyzing them and calculating the % Recovery using the following equation. Results for LFM precision and accuracy are shown in table 9.

$$\text{LFM Recovery \%, } R = \frac{(C_s \cdot f) - C}{s} \times 100$$

where $C_s$ = spiked sample concentration
f = spike dilution correction
C = sample background concentration
s = concentration equivalent of analyte added to sample

**Note:** Because the added spike volume was less than 1% of the total LFM sample volume (0.4 % and 0.12 % of total LFM sample volume), the factor f could be excluded.

Criteria for LFM % Recovery: 85 - 115 %, when c is > 2 x MRL and
80 - 120 %, when c is ≤ 2 x MRL

Criteria for LFM % RSD: ±15%

Table 7. Laboratory Fortified Matrix (LFM) sample results for DW o-PO4 application.

| Sample | Result avg. n = 3 (mg P/L) | Spike. mg P/L | % Recovery | % RSD. n =3 | Acceptable |
|---|---|---|---|---|---|
| TW-02 | 0.0021 | | | | |
| TW-02 +0.1 mg/L PO4P | 0.0946 | 0.10 | 92 % | 0.7 % | **YES** |
| TW-02 +0.4 mg/L PO4P | 0.3906 | 0.40 | 97 % | 0.1 % | **YES** |
| TW-03 | 0.0026 | | | | |
| TW-03 +0.1 mg/L PO4P | 0.0944 | 0.10 | 92 % | 0.3 % | **YES** |
| TW-03 +0.4 mg/L PO4P | 0.3943 | 0.40 | 98 % | 0.2 % | **YES** |
| TW-04 | 0.0028 | | | | |
| TW-04 +0.1mg/L PO4P | 0.0989 | 0.10 | 96 % | 0.7 % | **YES** |
| TW-04 +0.4mg/L PO4P | 0.3754 | 0.40 | 93 % | 0.3 % | **YES** |
| **Average:** | | | **95 %** | **0.4 %** | **YES** |

### **17.2.7** Continues Calibration Check (CCC)

Continues Calibration Check (CCC) solution was analyzed in intervals of every 10th sample, at the beginning and end of run. CCC sample concentration was from the mid calibration area – 0.25 mg P/L. CCC sample result summary is in table 10.

Criteria for CCC % Recovery: 90 – 110 %

Table 8. PO4P CCC result summary. Sample**:** PO4P CCC, concentration 0.25 mg/L.

| | Analyzer 1 | Analyzer 2 |
|---|---|---|
| **Average** | 0.254 | 0.256 |
| **% Recovery** | 102 % | 102 % |
| **n** | 20 | 6 |
| **Std.deviation** | 0.0033 | 0.0048 |
| **% RSD** | 1.29 % | 1.87 % |
| **Minimum result** | 0.249 | 0.252 |
| **Maximum result** | 0.261 | 0.261 |
| **Acceptable** | **YES** | **YES** |

**17.3** The validation study results showed the Drinking Water Orthophosphate method for Thermo Scientific Gallery discrete analyzer is equally effective in meeting the QC acceptance criteria given in the reference method.

Table 9. Performance of the Drinking Water Orthophosphate method for Thermo Scientific Gallery discrete analyzer compared to reference method acceptance criteria.

| **QC acceptance criteria for SM 4500-P E** | **Performance of DW o-PO4P for Gallery analyzer** |
|---|---|
| Minimum detectable concentration: 10 µg P/l | MDL calculated: 0.00036 mg P/L<br>MRL: 0.0125 mg P/L |
| Recovery IPR: NA (laboratory specific)<br>% Recovery CCC: 90 – 110 %<br>Recovery LFB: Mean ± 3 x SD (n ≥ 20)<br>Recovery LFM: NA (laboratory specific) | % Recovery IPR: 102 – 103%<br>% Recovery CCC: 100 – 105%<br>% Recovery LFB: 102 – 103%<br>% Recovery LFM: 92 – 98 % |
| % RSD IPR: NA (laboratory specific)<br>% RSD LFB: NA (laboratory specific)<br>% RPD LFM: NA (laboratory specific) | % RSD IPR: 0.1 – 1.0 %<br>% RSD LFB: 0.2 – 1.1 %<br>% RSD LFM: 0.1 – 0.7 % |
| Method Blank: ≤ ½ x MRL | Method Blank (LRB): ≤ 1/3 x MRL |

# APPENDIX A.

## DW o-PO4P application for the Gallery analyzer

thermo scientific

Test parameters

DW o-PO4P Version number 1.1



Gallery™ NPDWR application

Thermo Fisher Scientific Oy

Prior to change

**Date** 11.5.2017

**Time** 15:33:45

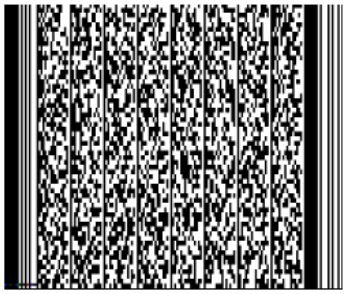
**User** Test designer

**Software version: 6.0.1**

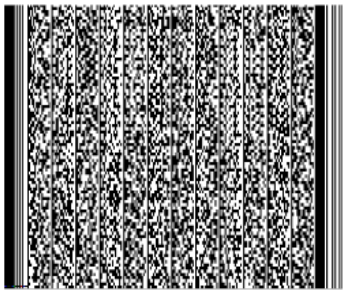
*Info*

| | |
|---|---|
| Tag | INT001 |
| Last time changed | 11.5.2017 15:29 |
| User name | Test designer |
| Full name | DW Orthophosphate |
| In use | Yes |
| Type | Photometric |
| Online name | |
| Acceptance | Manual |
| Result unit | mg P/L |
| Number of decimals | 3 |
| Correction factor | 1 |
| Correction bias | 0 |
| Sample type | |

**Barcode 1**



**Barcode 2**



*Flow*

Blank type Yes Primary dilution 1 + 0 Dispensed volume 140

# Test parameters

DW o-PO4P Version number 1 . 1

**Date** 11.5.2017
**Time** 15:33:45

**User** Test designer
**Software version: 6.0.1**

| Sample | Volume (µl) | Dispense with | Extra volume (µl) | Extra wash |
|---|---|---|---|---|
| | 120 | Extra | 60 | No |

| Incubate | Time (sec) | Actual time (sec) |
|---|---|---|
| | 18 | 18 |

| End-point blank | Blank resp. min.(A) | Blank resp. max.(A) |
|---|---|---|
| | * | * |

| Reagent | Reagent | Volume (µl) | Dispense with | Extra volume (µl) | Syringe speed | Replacing reagent |
|---|---|---|---|---|---|---|
| | Phosphate R1 | 14 | Extra | 20 | Normal | None |
| | Barcode ID | Alarm limit (ml) | Onboard stability (days) | | | |
| | A04 | 2,0 | 5 | | | |

| Incubate | Time (sec) | Actual time (sec) |
|---|---|---|
| | 120 | 117 |

| Reagent | Reagent | Volume (µl) | Dispense with | Extra volume (µl) | Syringe speed | Replacing reagent |
|---|---|---|---|---|---|---|
| | Phosphate R2 | 6 | Extra | 20 | Normal | None |
| | Barcode ID | Alarm limit (ml) | Onboard stability (days) | | | |
| | A05 | 2,0 | 5 | | | |

| Incubate | Time (sec) | Actual time (sec) |
|---|---|---|
| | 540 | 540 |

| End-point measurement | Main wavelength (nm) | Side wavelength (nm) | Residual net abs. (A) |
|---|---|---|---|
| | 880 | None | 0 |

# Test parameters

DW o-PO4P Version number 1 . 1

Gallery™ NPDWR application
Thermo Fisher Scientific Oy
Prior to change

**Date** 11.5.2017
**Time** 15:33:45

**User** Test designer
**Software version: 6.0.1**

*Dilution*

| | |
|---|---|
| Dilution with | Water |
| Primary dilution 1 + | 0 |

*Limits*

| | Measuring range (mg P/L Min | Max | Next dilution ratio (1+) Low | High |
|---|---|---|---|---|
| Primary dilution | * | 0,5000 | * | 9,0 |
| 2nd dilution | * | * | * | * |
| 3rd dilution | * | * | * | * |
| 4th dilution | * | * | * | * |

| | | | |
|---|---|---|---|
| Test limit | * | 5,0000 | mg P/L |
| Critical limit | * | * | mg P/L |
| Init. abs. | * | 2,5 | A |

*Calibration*

| | |
|---|---|
| Calibration type | 2nd order |
| Repeat time (days) | 1 |
| Points/calibrator | Duplicate |
| Acceptance | Manual |

| | |
|---|---|
| Abs. error (A) | * |
| Rel. error (%) | * |
| Factor limit min. | * |
| Factor limit max. | * |

| Nbr | Calibrator | Current lot | Concentration | Dilution 1 + |
|---|---|---|---|---|
| 1 | PO4P-0 | Default | 0,0000 | 0 |
| 2 | PO4P-cal | Default | 1,5000 | 119 |
| 3 | PO4P-cal | Default | 1,5000 | 49 |
| 4 | PO4P-cal | Default | 1,5000 | 29 |
| 5 | PO4P-cal | Default | 1,5000 | 14 |
| 6 | PO4P-cal | Default | 1,5000 | 7 |
| 7 | PO4P-cal | Default | 1,5000 | 5 |
| 8 | PO4P-cal | Default | 1,5000 | 3 |
| 9 | PO4P-cal | Default | 1,5000 | 2 |

Coeff. of det. min. 0,995

*QC*

thermo scientific

# Test parameters

DW o-PO4P Version number 1.1



Gallery™ NPDWR application
Thermo Fisher Scientific Oy
Prior to change

**Date** 11.5.2017
**Time** 15:33:44

**User** Test designer
**Software version: 6.0.1**

| | | | |
|---|---|---|---|
| Procedure | Ongoing QC | QC profile | PO4P |
| Interval type | Requests | In use | Yes |
| Requests | 10 | Acceptance | Manual |
| Time (hh:mm) | 0:00 | Trigger | Manual,Interval,Reagent lot change,Reagent vial change |

| | | | |
|---|---|---|---|
| Procedure | QCS | QC profile | PO4P QCS |
| Interval type | | In use | Yes |
| Requests | | Acceptance | Manual |
| Time (hh:mm) | 0:00 | Trigger | Manual,Calibration |

| Procedure | Control | Current Lot | Conc. | SD | Req. count |
|---|---|---|---|---|---|
| Ongoing QC | LRB | Default | 0,000 | 0,0021 | 1 |
| Ongoing QC | PO4P-CCC | Default | 0,250 | 0,0125 | 1 |
| Ongoing QC | PO4P-LFB 0.1 | Default | 0,100 | 0,0075 | 1 |
| Ongoing QC | PO4P-LFB 0.4 | Default | 0,400 | 0,0300 | 1 |
| QCS | PO4P-QCS 0.1 | Default | 0,100 | 0,0075 | 1 |
| QCS | PO4P-QCS 0.4 | Default | 0,400 | 0,0300 | 1 |

| Procedure | Nbr of controls | SD multiplier |
|---|---|---|
| Ongoing QC | 1 | 2 |
| QCS | 1 | 2 |