United States
Environmental Protection
Agency

Environment Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

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EPA

Test Method
Technical Addition to Methods for Chemical Analysis of Water and Wastes (EPA-600/4-79-020)

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The Determination of Inorganic Anions in Water by Ion Chromatography — Method 300.0

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1. Scope and Application

1.1 This method covers the determination of the following inorganic anions.

Analyte	Storet No. Total	Dissolved
Chloride	00940	—
Fluoride	00951	00950
Nitrate-N	00620	—
Nitrite-N	00615	—
Ortho-Phosphate-P	—	00671
Sulfate	00945	—

1.2 This is an ion chromatographic (IC) method applicable to the determination of the anions listed above in drinking water, surface water, and mixed domestic and industrial wastewater.

1.3 The Method Detection Limit (MDL, defined in Section 13) for the above analytes is listed in Table 1. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of ion chromatography and in the intrepretation of the resulting ion chromatogram. Each analyst must demonstrate the ability to generate acceptable results with this method, using the procedure described in Section 10.2.

1.5 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the addition of spike solutions covering the anions of interest. The spike procedure is described in Section 11.6.

2. Summary of Method

2.1 A small volume of sample, typically 2 to 3 mL, is introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, separator column, suppressor column, and conductivity detector.

3. Definitions

3.1 Stock standard solution — a concentrated solution containing a certified standard that is a method analyte. Stock standard solutions are used to prepare secondary standard solutions.

3.2 Calibration standards — a solution of analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare aqueous calibration solutions.

3.3 Quality control check sample — a solution containing known concentrations of analytes, prepared by a laboratory other than the laboratory performing the analysis. The analyzing laboratory uses this solution to demonstrate that it can

obtain acceptable identifications and measurements with a method.

3.4 Performance evaluation sample — a solution of method analytes distributed by the Quality Assurance Branch (QAB), Environmental Monitoring and Support Laboratory (EMSL-Cincinnati), USEPA, Cincinnati, Ohio, to multiple laboratories for analysis. A volume of the solution is added to a known volume of reagent water and analyzed with procedures used for samples. Results of analyses are used by the QAB to determine statistically the accuracy and precision that can be expected when a method is performed by a competent analyst. Analyte true values are unknown to the analyst.

3.5 Laboratory control standards — a solution of analytes prepared in the laboratory by adding appropriate volumes of the stock standard solutions to reagent water.

3.6 Laboratory duplicates — two aliquots of the same sample that are treated exactly the same throughout laboratory analytical procedures. Analyses of laboratory duplicates indicate precision associated with laboratory procedures but not the sample collection, preservation, or storage procedures.

3.7 Field duplicates — two samples taken at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of field duplicates indicate the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

4. Interferences

4.1 Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or spiking can be used to solve most interference problems.

4.2 The water dip or negative peak that elutes near and can interfere with the fluoride peak can be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (7.3 100X) to 100 mL of each standard and sample.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms.

4.4 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage to instrument columns and flow systems.

5. Safety

5.1 Normal, accepted laboratory safety practices should be followed during reagent preparation and instrument operation. No known carcinogenic materials are used in this method.

6. Apparatus and Materials

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

6.2 Ion chromatograph — Analytical system complete with ion chromatograph and all required accessories including syringes, analytical columns, compressed air, detector, and stripchart recorder. A data system is recommended for peak integration.

6.2.1 Anion guard column: 4 x 50 mm, Dionex P/N 030825, or equivalent.

6.2.2 Anion separator column: 4 x 250 mm, Dionex P/N 030827, or equivalent.

6.2.3 Anion suppressor column: fiber, Dionex P/N 35350, or equivalent.

6.2.4 Detector — Conductivity cell: approximately 6 μL volume, Dionex, or equivalent.

7. Reagents and Consumable Materials

7.1 Sample bottles: Glass or polyethylene of sufficient volume to allow replicate analyses of anions of interest.

7.2 Reagent water: Distilled or deionized water, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.3 Eluent solution: Sodium bicarbonate (CAS RN 144-55-8) 0.003 M, sodium carbonate (CAS RN 497-19-8) 0.0024M. Dissolve 1.0081 g sodium bicarbonate ($NaHCO_3$) and 1.0176 g of sodium carbonate (Na_2CO_3) in reagent water and dilute to 4 liters.

7.4 Regeneration solution (fiber suppressor): Sulfuric acid (CAS RN 7664-93-9) 0.025N. Dilute 2.8 mL conc. sulfuric acid (H_2SO_4) to 4 liters with reagent water.

7.5 Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased as certified solutions or prepared from ACS reagent grade materials (dried at 105°C for 30 min.) as listed below.

7.5.1 Chloride (CL^-) 1000 mg/L: Dissolve 1.6485 g sodium chloride (NaCL, CAS RN 7647-14-5) in reagent water and dilute to 1 liter.

7.5.2 Fluoride (F^-) 1000 mg/L: Dissolve 2.2100 g sodium fluoride (NaF, CAS RN 7681-49-4) in reagent water and dilute to 1 liter.

7.5.3 Nitrate (NO_3^--N) 1000 mg/L: Dissolve 6.0679 g sodium nitrate ($NaNO_3$, CAS RN 7631-99-4) in reagent water and dilute to 1 liter.

7.5.4 Nitrite (NO_2^--N) 1000 mg/L: Dissolve 4.9257 g sodium nitrite ($NaNO_2$, CAS RN 7632-00-0) in reagent water and dilute to 1 liter.

7.5.5 Phosphate ($PO_4^{\equiv}$-P) 1000 mg/L: Dissolve 4.3937 g potassium phosphate (KH_2PO_4, CAS RN 7778-77-0) in reagent water and dilute to 1 liter.

7.5.6 Sulfate ($SO_4^{=}$) 1000 mg/L: Dissolve 1.8141 g potassium sulfate (K_2SO_4, CAS RN 7778-80-5) in reagent water and dilute to 1 liter.

7.5.7 Stability of standards: Stock standards (7.5) are stable for at least one month when stored at 4°C. Dilute working standards should be prepared weekly, except those that contain nitrite and phosphate should be prepared fresh daily.

8. Sample Collection, Preservation and Storage

8.1 Samples should be collected in scrupulously clean glass or polyethylene bottles.

8.2 Sample preservation and holding times for the anions that can be determined by this method are as follows:

Analyte	Preservation	Holding Time
Chloride	None required	28 days
Fluoride	None required	28 days
Nitrate-N	Cool to 4°C	48 hours
Nitrite-N	Cool to 4°C	48 hours
O-Phosphate-P	Filter and cool to 4°C	48 hours
Sulfate	Cool to 4°C	28 days

8.3 The method of preservation and the holding time for samples analyzed by this method are determined by the anions of interest. In a given sample, the anion that requires the most preservation treatment and the shortest holding time will determine the preservation treatment and holding time for the total sample.

9. Calibration and Standardization

9.1 Establish ion chromatographic operating parameters equivalent to those indicated in Table 1.

9.2 For each analyte of interest, prepare calibration standards at a minimum of three concentration levels and a blank by adding accurately measured volumes of one or more stock standards (7.5) to a volumetric flask and diluting to volume with reagent water. If the working range exceeds the linear range of the system, a sufficient number of standards must be analyzed to allow an accurate calibration curve to be established. One of the standards should be representative of a concentration near, but above, the method detection limit if the system is operated on an applicable attenuator range. The other standards should correspond to the range of concentrations expected in the sample or should define the working range of the detector. Unless the attenuator range settings are proven to be linear, each setting must be calibrated individually.

9.3 Using injections of 0.1 to 1.0 mL (determined by injection loop volume) of each calibration standard, tabulate peak height or area responses against the concentration. The results are used to prepare a calibration curve for each analyte. During this procedure, retention times must be recorded. The retention time is inversely proportional to the concentration.

9.4 The working calibration curve must be verified on each working day, or whenever the anion eluent is changed, and after every 20 samples. If the response or retention time for any analyte varies from the expected values by more than ± 10%, the test must be repeated, using fresh calibration standards. If the results are still more than ± 10%, an entire new calibration curve must be prepared for that analyte.

9.5 Nonlinear response can result when the separator column capacity is exceeded (overloading). Maximum column loading (all anions) should not exceed about 400 ppm.

10. Quality Control

10.1 Each laboratory using this method should have a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability (10.2) and the analysis of spiked samples as a continuing check on performance. The laboratory should maintain performance records to define the quality of data that are generated.

10.1.1 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Section 10.2

10.1.2 The laboratory should spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. Field and laboratory duplicates should also be analyzed.

10.2 Before performing any analyses, the analyst should demonstrate the ability to generate acceptable accuracy and precision with this method, using a laboratory control standard.

10.2.1 Select a representative spike concentration for each analyte to be measured. Using stock standards, prepare a quality control check sample concentrate in reagent water 100 times more concentrated than the selected concentrations.

10.2.2 Using a pipet, add 1.00 mL of the check sample concentrate (10.2.1) to each of a minimum of four 100-mL aliquots of reagent water. Analyze the aliquots according to the procedure in Section 11.

10.2.3 Calculate the average percent recovery (R), and the standard deviation(s) of the percent recovery, for the results.

10.2.4 Using the appropriate data from Table 2, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 10.2.3. If the data are not comparable within control limits (10.3.1), review potential problem areas and repeat the test.

10.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration of analyte being measured.

10.3.1 Calculate upper and lower control limits for method performance as follows:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 10.2.3. The UCL and LCL can be used to construct control charts that are useful in observing trends in performance.

10.4 The laboratory should develop and maintain separate accuracy statements of laboratory performance for water and wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analyses of four aliquots of water or wastewater, as described in Section 10.2.2, followed by the calculation of R and s.

10.5 Before processing any samples, the analyst must demonstrate through the analysis of an aliquot of reagent water that all glassware and reagent interferences are under control. Each time there is a change in reagents, a laboratory reagent blank must be processed as a safeguard against laboratory contamination.

10.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak in the chromatogram, confirmatory techniques such as sample dilution and spiking, must be used. Whenever possible, the laboratory should perform analysis of quality control check samples and participate in relevant performance evaluation sample studies.

11. Procedure

11.1 Table 1 summarizes the recommended operating conditions for the ion chromatograph. Included in this table are estimated retention times that can be achieved by this method. Other columns, chromatographic conditions, or

detectors may be used if the requirements of Section 10.2 are met.

11.2 Check system calibration daily and, if required, recalibrate as described in Section 9.

11.3 Load and inject a fixed amount of well mixed sample. Flush injection loop thoroughly, using each new sample. Use the same size loop for standards and samples. Record the resulting peak size in area or peak height units. An automated constant volume injection system may also be used.

11.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.5 If the response for the peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze.

11.6 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, spike the sample with an appropriate amount of standard and reanalyze.

Note: Retention time is inversely proportional to concentration. Nitrate and sulfate exhibit the greatest amount of change, although all anions are affected to some degree. In some cases, this peak migration can produce poor resolution or misidentification.

12. Calculation

12.1 Prepare separate calibration curves for each anion of interest by plotting peak size in area, or peak height units of standards against concentration values. Compute sample concentration by comparing sample peak response with the standard curve.

12.2 Report results in mg/L.

13. Precision and Accuracy — Method Detection Limit

13.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations listed in Table 1 were obtained using reagent water.

13.2 Single-operator accuracy and precision for reagent, drinking and surface water, and mixed domestic and industrial wastewater are listed in Table 2.

14. References

14.1 Annual Book of ASTM Standards, Part 31 Water, proposed test method for "Anions in Water by Ion Chromatography," p. 1485-1492 (1982).

14.2 Standard Methods for the Examination of Water and Wastewater, Method 400Z, "Anions by Ion Chromatography" proposed for the 16th Edition of Standard Methods.

14.3 Dionex, IC 16 operation and maintenance manual, PN 30579, Dionex Corp., Sunnyvale, California 94086.

14.4 Method detection limit (MDL) as described in "Trace Analyses for Wastewater," J. Glaser, D. Foerst, G. McKee, S. Quave, W. Budde, *Environmental Science and Technology,* Vol. 15, Number 12, p. 1426, December 1981.

Table 1. *Chromatographic Conditions and Method Detection Limits in Reagent Water*

Analyte	Retention[1] Time (Min)	Relative Retention Time	Method[2] Detection Limit mg/L
Fluoride	1.2	1.0	0.005
Chloride	3.4	2.8	0.015
Nitrite-N	4.5	3.8	0.004
O-Phosphate-P	9.0	7.5	0.061
Nitrate-N	11.3	9.4	0.013
Sulfate	21.4	17.8	0.206

Standard Conditions:

Columns — As specified in 6.2 *Sample Loop — 100 µL*
Detector — As specified in 6.2 *Pump Volume — 2.30 mL/Min*
Eluent — As specified in 7.3

[1] *Concentrations of mixed standard (mg/L)*

Fluoride 3.0 *O-Phosphate-P 9.0*
Chloride 4.0 *Nitrate-N 30.0*
Nitrite-N 10.0 *Sulfate 50.0*

[2] *MDL calculated from data obtained using an attenuator setting of 1 µMHO full scale. Other settings would produce an MDL proportional to their value.*

Table 2. *Single-Operator Accuracy and Precision*

Analyte	Sample Type	Spike (mg/L)	Number of Replicates	Mean Recovery %	Standard Deviation (mg/L)
Chloride	RW	0.050	7	97.7	0.0047
	DW	10.0	7	98.2	0.289
	SW	1.0	7	105.0	0.139
	WW	7.5	7	82.7	0.445
Fluoride	RW	0.24	7	103.1	0.0009
	DW	9.3	7	87.7	0.075
	SW	0.50	7	74.0	0.0038
	WW	1.0	7	92.0	0.011
Nitrate-N	RW	0.10	7	100.9	0.0041
	DW	31.0	7	100.7	0.356
	SW	0.50	7	100.0	0.0058
	WW	4.0	7	94.3	0.058
Nitrite-N	RW	0.10	7	97.7	0.0014
	DW	19.6	7	103.3	0.150
	SW	0.51	7	88.2	0.0053
	WW	0.52	7	100.0	0.018
O-Phosphate-P	RW	0.50	7	100.4	0.019
	DW	45.7	7	102.5	0.386
	SW	0.51	7	94.1	0.020
	WW	4.0	7	97.3	0.04
Sulfate	RW	1.02	7	102.1	0.066
	DW	98.5	7	104.3	1.475
	SW	10.0	7	111.6	0.709
	WW	12.5	7	134.9	0.466

RW = Reagent Water *SW = Surface Water*
DW = Drinking Water *WW = Wastewater*

*USGPO:1984-759-102-862