Date: 01/03/06
Revision #: 1
Prepared by: PB

CDFG FISH AND WILDLIFE WATER POLLUTION CONTROL LABORATORY STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF INORGANIC ANIONS IN WATER BY ION CHROMATOGRAPHY - EPA 300.0

1.0 Scope and Application

1.1 This method is applicable to the determination of the following inorganic anions.

Bromide
Chloride
Fluoride
Nitrate-N
Nitrite-N
Ortho-Phosphate-P
Sulfate

1.2 The matrices applicable to this method include:drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, solids (after extraction 2.3), leachates (when no acetic acid is used 2.4).

1.3 The Single Laboratory Method Detection Limit (MDL, defined in Section 10) 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 The reporting limits are based on water quality objectives[a/] specified in National Pollution Discharge Elimination System (NPDES) permits by Regional Water Quality Control Boards. Water quality objectives in mg/L for the Hatchery Monitoring Program (based on the Hot Creek Fish Hatchery requirements) are as follows:

Nitrate (as N)	0.21
Phosphate (as P)	0.65
Sulfate	24.0

The current reporting limits for the ICS 1000 are listed in Table 1 on page 15.

Scope and Application (continued)

1.5 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation 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.

1.6 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of fortified sample matrix covering the anions of interest. The fortification procedure is described in Section 8.4.5.

2.0 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 device, and conductivity detector.

2.2 In order to use this method for solids an extraction procedure must be performed as described in section 8.1.2.

3.0 Sample Collection, Preservation, and Storage

3.1 Samples should be collected in scrupulously clean glass of polyethylene bottles.

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

Analyte	Preparation/Preservation	Holding Time
Bromide	Filter/None required	28 days
Chloride	Filter/None required	28 days
Fluoride	Filter/None required	28 days
Nitrate-N		
chlorinated	Filter/Cool to 4°C	28 days
nonchlorinated	Filter/conc. H_2SO_4-pH <2	14 days
Nitrite-N	Filter/Cool to 4°C	48 hours
O-Phosphate-P	Filter/Cool to 4°C	48 hours

Sulfate	Filter/Cool to 4°C	28 days

Sample Collection, Preservation, and Storage (continued)

3.3 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. It is recommended that all samples be cooled to 4°C and held no longer than 28 days.

4.0 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 fortification 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 usually 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 Any anion that is not retained by the column or only slightly retained will elute in the area of fluoride and interfere. Known coelution is caused by carbonate and other small organic anions. At concentrations of fluoride above 1.5 mg/L this interference may not be significant, however, it is the responsibility of the user to generate precision and accuracy information in each sample matrix.

4.5 The acetate anion elutes early during the chromatographic run. The retention times of the anions also seem to differ when large amounts of acetate are

Interferences (continued)

present. Therefore, this method is not recommended for leachates of solid when acetic acid is used for pH adjustment.

4.6 The quantitation of unretained peaks should be avoided, such as low molecular weight organic acids (formate, acetate, propionate, etc.) which are conductive and coelute with of near fluoride and would bias the fluoride quantitation in some drinking and most waste waters.

5.0 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.

6.0 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 auto-sampler, analytical column, guard columns, compressed helium gas, detectors and data system. The current system is the Dionex ICS-1000, S/n 04090303, purchased In October 2004.

6.2.1 Anion guard column: A protector of the separator or analytical column. If omitted from the system the retention times will be shorter. Usually packed with a substrate the same as that in the analytical column. Ag14-4mm guard column currently utilized (P/N 46134).

6.2.2 Anion separator (analytical) column: Separation is generated using a Dionex AS14A-4mm column.

Apparatus and Materials (continued)

6.2.3 Anion suppressor device: The Dionex ASRS-Ultra Anion Suppressor is used for this system (P/N 061561.

6.2.4 Detector – 35°C Heated Conductivity cell: 25 μL loop (P/N 057985).

6.2.5 Dionex Chromeleon DC36R051 Data Chromatography Software used to generate the data.

6.2.6 The Dionex AS-40 Autosampler.

7.0 Reagents and Standards

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**: AS 14A Eluent diluted 1:100 to make a final concentration of sodium carbonate, 8.0 mM and sodium bicarbonate, 1.0 mM.

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

7.4.1 **Bromide** (Br^-) 1000 mg/L: Dissolve 1.2876 g sodium bromide (NaBr) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

7.4.2 **Chloride** (Cl^-) 1000 mg/L: Dissolve 1.6485 g sodium chloride (NaCl) in reagent water and

Reagents and Standards (continued)

dilute to 1 L or use purchased ERA certified stock standard.

7.4.3 **Fluoride** (F^-) 1000 mg/L: Dissolve 2.2100 g fluoride (NaF) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

7.5.4 **Nitrate** (NO_3^--N) 1000 mg/L: Dissolve 6.0679 g sodium nitrate ($NaNO_3$) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

7.5.5 **Nitrite** (NO_2^--N) 1000 mg/L: Dissolve 4.9257 g sodium nitrite ($NaN0_2$) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

7.5.6 **Phosphate** (HPO_4^{2-}-P) 1000 mg/L: Dissolve 4.3937 g potassium phosphate, monobasic (KH_2PO_4) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

7.5.7 **Sulfate** (SO_4^{2-}) 1000 mg/L: Dissolve 1.8141 g potassium sulfate (K_2SO_4) in reagent water and dilute to 1 L or use purchased ERA certified stock standard.

Note: Stability of standards: Stock standards are stable for at least one month when stored at room temperature. Dilute working standards should be prepared at 28 day intervals, except those that contain nitrite and phosphate which should be prepared fresh daily.

8.0 Procedure

8.1 Sample Preparation

Procedure (continued)

8.1.1 **Water**: Clear water samples are already filtered through a 0.45 um filter prior to analysis. The samples are poured into the autosampler vials and the plug/filter is placed on the top of the vial and is forced into the vial using the tool supplied by Dionex. The plug filter is optional.

8.1.2 **Solid materials**: The following extraction should be used for solid materials. Add an amount of reagent water equal to ten times the weight of dry solid material taken as a sample. This slurry is mixed together for ten minutes using a magnetic stirring device. Filter the resulting slurry before injecting using a 0.45 μm membrane type filter. This can be the type that attaches directly to the end of the syringe. Care should be taken to show that good recovery and identification of peaks is obtained. A matrix spike should be performed.

8.2 Instrument Operating Conditions: Instrument operating conditions are controlled by the Data System and a copy of the instrument operating parameters is presented in the Dionex Operator's Manual.

8.3 Calibration

8.3.1 Ion chromatographic operating parameters should be established which are equivalent to parameters in the Manual.

8.3.2 For each analyte of interest, prepare calibration standards at six concentration levels consisting of a blank, and five standards ranging from the method detection limit to the established upper limit. Each attenuation range of the instrument used to

analyze a sample must be calibrated individually.

Calibration (continued)

8.3.3 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 also be recorded. These steps are done by the Data System which displays the peak height and area, the calibration curves and the linearity of the calibrations at the different concentrations.

8.3.4 The calibration curve must be verified on each working day, or whenever the anion eluent is changed, and after every 10-15 samples. A certified reference standard at a concentration near the mid-range of the curve must be used to verify the calibration curve. The standard must be purchased from a different vendor than the working standard. If the response or retention time for any analyte varies from the expected values by more than $\pm 20\%$, the test must be repeated, using fresh calibration standards. If the results are still more than $\pm 20\%$, a new calibration curve must be prepared for that analyte.

8.3.5 Non-linear response can result when the separator column capacity is exceeded (overloading). The response of the detector to the sample when diluted 1:1, and when not diluted, should be compared. If the calculated responses are the same, samples of this total anionic concentration need not be diluted.

8.4 Analysis

8.4.1 Table 2 summarizes the operating conditions stored under the file name AS14A in the data

system Program section. Included in the table are the estimated retention times of

Analysis (continued)

the anions of interest established under the conditions of this method.

8.4.2 Check system calibration daily, if required, recalibrate as described in Section 8.3.

8.4.3 Load standards, blanks, and samples by filling polyethylene autosampler vials and inserting cap/filter into the vial with the tool supplied by Dionex. Load autosampler holders with the ridges facing the front of the autosampler.

8.4.4 Begin analysis.

8.4.4.1. Turn gas on. Adjust helium to 20 psi.

8.4.4.2. Switch eluant degas module on and set pressure to 50 psi.

8.4.4.3. If the eluent is freshly prepared, prime the pump by opening the priming valve one-quarter to one-half turn counterclockwise. On the Chromeleon Control Panel click Pump Settings and then click **Eluent Flow Valve Open**. Place a 10 ml syringe in the hole of the priming valve and draw back about 3-4 syringes of eluent to eliminate air bubbles from the system.

8.4.4.4. After priming the lines thoroughly, close the priming valve. Close the eluent valve by clicking **Eluent Flow Valve Closed** on the control panel. Open the waste valve in the secondary pump head by turning the

knob one-quarter to one–half turn counterclockwise. Click **Prime on the Control Panel** and continue

Analysis (continued)

until no air bubbles are exiting the waste pump line. Press **Pump Off** and close the waste valve.

8.4.4.5. Press **startup AS14A** to begin the pump and **control acquisition on** to view the baseline. If the AS14A does not begin, make sure the connected boxes are checked in the ICS-1000_Panel_1.pan.

8.4.4.6. The pump setting and eluent flow valve icons should be green.

8.4.4.7. Create a schedule or call up an existing one and modify it in the sequence folder. The calibration curve may be saved as raw data to use it in the current schedule.

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

8.4.5 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, fortify 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 may produce poor resolution or identification.

8.5 Data Review and Reporting

8.5.1 After the standards have been analyzed review the calibration curves using the data system. The calibration curves for each anion should be reviewed for response and linearity ($r \geq 0.995$). If a problem is observed the run should be stopped and corrective action should be taken prior to restarting the run. The standards may need to be prepared and analyzed again.

8.5.2 For the blank, a response less than the MDL should be observed for all analytes. If there is a positive response for the blank the run should be stopped and corrective action should be taken prior to restarting the run. The blank should be prepared again and reanalyzed.

8.5.3 All results should be reported in mg/L.

8.5.4 Report NO_2^- as N
NO_3^- as N
HPO_4^{2-} as P

9.0 Quality Assurance/Quality Control and Acceptance Criteria

9.1 The minimum requirements of this program consist of an initial demonstration of laboratory capability (9.2) and the analysis of fortified samples as a continuing check on performance. The laboratory should maintain records to define and document the quality of data that are generated.

9.1.1 If any changes or modifications are made to this procedure due to improvements in technology the analyst is required to repeat the procedure in Section 9.2.

9.1.2 The laboratory should fortify and analyze a minimum of 5% of all samples to monitor continuing laboratory performance. A minimum of 5% of all samples should be run in

Quality Assurance (continued)

duplicate.

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

9.2.1 Select a representative check concentration for each analyte to be measured. Using stock standards, prepare a laboratory performance check sample concentrate in reagent water 100 times more concentrated than the selected concentrations. Alternatively, purchase a commercially available certified reference standard.

9.2.2 Using a pipet or calibrated autopipetor, add 1.00 mL of the check sample concentrate (9.2.1) to each of a minimum of four 100-mL aliquots of reagent water. Analyze the aliquots according to the procedure in Section 8.4.

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

9.2.4 Using the appropriate data from Table 1, determine the recovery and single operator precision expected for the method, and compare these results to the values calculated in Section 9.2.3. If the data are not comparable within the control limits, review potential problem areas and repeat the test.

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

9.4 The laboratory should develop and maintain separate accuracy statements of laboratory performance for each

Quality Assurance (continued)

matrix being analyzed by the laboratory. 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 9.2.2, followed by the calculation of R and s.

9.5 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.

9.6 Additional quality assurance practices have been adopted by the laboratory to be used with this method. The analysis of field duplicates when they are submitted 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 fortification, must be used. Whenever possible, the laboratory should perform analysis of quality control check samples and participate in relevant performance evaluation sample studies.

9.7 In order to verify that standards have been prepared correctly and have not degraded, a reference standard check should be performed using a standard of known concentration prepared by an independent source with each set of samples. Results must be within the listed acceptance limits of the control sample or ± 20% if no limit is listed.

9.8 With each batch of samples processed analyze a single laboratory fortified blank (LFB) or matrix spike containing each analyte of concern at a concentration at or near those used in Section 9.2. If more than 20 samples are run in a batch analyze one LFB or matrix spike for every 20 samples. Evaluate the accuracy by If acceptable data cannot be obtained, locate the

Quality Assurance (continued)

problem and correct it.

9.9 A replicate LFBs and matrix spikes should be analyzed to determine the precision of the laboratory measurements for every 5% of the sample batch. Add these results to the ongoing control charts to document data quality.

9.10 One sample should be analyzed in duplicate for every 20 samples analyzed or for each batch of samples and duplicate results should be within ± 20%. If the duplicate results do not fall within this range the duplicates should be reanalyzed.

10.0 Method Performance

10.1 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 waters.

10.2 Single operator accuracy and precision data are given for each anion in Table 1.

11.0 Reporting

11.1 All results are reported in mg/L with nitrate and nitrite reported as nitrogen (N) and ortho-phosphate reported as phosphorous (P).

11.2 The reporting limits (RL) are based on reporting requirements set by the regulatory agency. In the case of nitrate and nitrite, reporting requirements set by the Regional Water Quality Control Boards will be used. If there are no reporting requirements set, the reporting limits will be equal to the Practical Quantitation Limit (PQL) defined by the WPCL.

11.3 All results will be reported using three significant figures. If a target analyte is detected between the

Reporting (continued)

MDL and the RL, the result will be reported as detected not quantified (DNQ) and if the result is detected below the MDL the result will be reported as ND (not detected) and be followed by the MDL value.

12.0 References

1. U.S. Environmental Protection Agency, *The Determination of Inorganic Anions in Water by Ion Chromatography,* Method 300.0. August 1991. Environmental Monitoring and Systems Laboratory, Cincinnati, OH 45268.

2. Dionex Corporation, Document No. 031879, ICS -1000, *Ion Chromatography System Operator's Manual.*

Table 1: Method Detection Limits (MDL) and Reporting Limits (RL)

ANION	MDL	RL	Precision RL, (SD)	Accuracy RL, Mean % Recovery
Bromide	0.50	1.0	0.025	106
Chloride	0.2	0.35	0.022	95
Nitrate-N	0.1	0.20	0.00	95
Fluoride	0.01	0.125		
Ortho-P	0.1	0.25	0.01	97
Sulfate	0.5	0.70	0.078	108

Table 2: Operator Retention Times for AS14 Program:

ANION	Retention Times Minutes	Peak Area us/min	Peak Height us
Bromide	6.51	0.031	0.129
Chloride	4.474	0.0361	0.233
Nitrate-N	7.347	0.0439	0.167
Ortho-P	8.994	0.0741	0.196
Sulfate	10.900	0.0625	0.210

Section Approval: _____

QC Officer Approval: _____

Date: 01/03/06
Revision #: 1
Prepared by: PB

Final Approval: _____