

METHOD 6500

DISSOLVED INORGANIC ANIONS IN AQUEOUS MATRICES BY CAPILLARY ION ELECTROPHORESIS

1.0 SCOPE AND APPLICATION

1.1 This test method is applicable for determination of the dissolved inorganic anions; fluoride, bromide, chloride, nitrite, nitrate, ortho-phosphate, and sulfate in aqueous matrices using capillary ion electrophoresis with indirect UV detection.

1.2 This test method is applicable to drinking water, wastewater and ground water for the analysis of inorganic anions in the concentration range of 0.1 to 50 mg/L, except for fluoride, which has a range of 0.1 to 25 mg/L. It is the user's responsibility to ensure the applicability of this test method for other anion concentration ranges and other aqueous sample matrices.

1.3 Capillary ion electrophoresis provides a simultaneous separation and determination of several inorganic anions using nanoliters of sample in a single injection. Only 500 μ L of sample is required to fill the analysis vial. Analysis time is less than 5 minutes.

2.0 SUMMARY OF METHOD

2.1 Capillary ion electrophoresis (Figs. 1 - 4) is a free zone electrophoretic technique optimized for the analysis of anions with molecular weights less than 200. The anions migrate and are separated according to their mobility in the electrolyte when an electrical field is applied through the open tubular fused silica capillary. The electrolyte's electroosmotic flow (EOF) modifier dynamically coats the inner wall of the capillary, changing the surface to a net positive charge. This reversal of wall charge reverses the natural EOF. The modified EOF in combination with a negative power supply augments the mobility of the analyte anions towards the anode and detector achieving rapid analysis times. Cations migrate in the opposite direction towards the cathode and are removed from the sample during analysis. Water and other neutral species move toward the detector at the same rate as the EOF. The neutral species migrate slower than the analyte anions and do not interfere with anion analysis (Figs. 2 and 3).

2.2 The sample is introduced into the capillary using hydrostatic sampling. The inlet of the capillary, containing electrolyte, is immersed in the sample and the sample raised 10 cm for 30 seconds where 36 nanoliter volumes are siphoned into the capillary. After sample loading, the capillary is immediately immersed back into the electrolyte. The voltage is applied initiating the separation process. Pressure injection may also be used as long as the performance specifications of this method are achievable.

2.3 Anion detection is based upon the principles of indirect UV detection. The UV absorbing electrolyte anion is displaced charge-for-charge by the separated analyte anion. The analyte anion zone has a net decrease in background absorbance. This decrease in UV absorbance is quantitatively proportional to analyte anion concentration (Fig. 4). Detector output polarity is reversed to provide positive mV response to the data system, and to make the negative absorbance peaks appear positive.

2.4 The analysis is complete once the last anion of interest is detected. The capillary is then vacuum purged by the system of any remaining sample, and replenished with fresh electrolyte. The system is now ready for the next analysis.

3.0 DEFINITIONS

3.1 Capillary Ion Electrophoresis: An electrophoretic technique in which an UV absorbing electrolyte is placed in a 75 μm fused silica capillary. Voltage is applied through the capillary causing electrolyte and anions to migrate towards the anode and through the capillary's UV detector window. Anions are separated based upon the anion's differential rates of migration in the electrical field which is directly related to the anion's equivalent ionic conductance. Anion detection and quantitation are based upon the principles of indirect UV detection.

3.2 Electrolyte: A combination of a UV absorbing salt and an electroosmotic flow modifier placed inside the capillary, used as a carrier for the analytes, and for anion detection and quantitation. The UV absorbing portion of the salt must be anionic and have an electrophoretic mobility similar to the analyte anions of interest.

3.3 Electroosmotic Flow (EOF): The direction and velocity of electrolyte solution flow within the capillary under an applied electrical potential (voltage); the velocity and direction of flow is determined by electrolyte chemistry, power supply polarity and applied voltage.

3.4 Electroosmotic Flow Modifier (OFM): A cationic amine in the electrolyte that dynamically coats the negatively charged silica wall reversing the direction of the electrolyte's natural electroosmotic flow and directing it towards the anode and detector. This modifier augments anion migration and enhances speed of analysis (Fig. 2).

3.5 Electrophoretic Mobility: The specific velocity of a charged analyte in the electrolyte under specific electroosmotic flow conditions. The mobility of an analyte is directly related to the analyte's equivalent ionic conductance and applied voltage, and is the primary mechanism of separation.

3.6 Electropherogram: A graphical presentation of UV detector response versus time of analysis; the x axis is the migration time which is used to qualitatively identify the anion, and the y axis is the UV response which can be converted to time corrected peak area for quantification.

3.7 Hydrostatic Sampling: A sample introduction technique in which the capillary with electrolyte is immersed in the sample, and both are elevated to a specific height, typically 10 cm, above the receiving electrolyte reservoir for a preset amount of time, typically less than 60 seconds. Nanoliters of sample are siphoned into the capillary by differential head pressure and gravity.

3.8 Indirect UV Detection: A form of UV detection in which the analyte displaces an equivalent net charge amount of the highly UV absorbing component of the electrolyte causing a net decrease in background absorbance. The magnitude of the decreased absorbance is directly proportional to analyte concentration. Detector output polarity is switched in order to obtain a positive mV response.

3.9 Migration Time: The time required for a specific analyte to migrate through the capillary to the detector. The migration time in capillary ion electrophoresis is analogous to retention time in chromatography.

3.10 Time Corrected Peak Area (normalized peak area): Peak area divided by migration time. CIE principles state that peak area is dependant on migration time, i.e. for same concentration of analyte, as migration time increases (decreases) peak area increases (decreases). Timed corrected peak area accounts for these changes.

3.11 Midpoint of Peak Width - CIE peaks are typically asymmetrical with the peak apex shifting with increasing concentration, and peak apex may not be indicative of true analyte migration time. Midpoint of peak width is the midpoint between the analyte peak's start and stop integration.

4.0 INTERFERENCES

4.1 The most difficult quantitation and possible comigration occurs when one anion is in significant excess to other anions in close proximity. For two closely adjacent peaks reliable quantitation can be achieved when the concentration differential is less than 100:1. As the resolution between two anion peaks increase so does the tolerated concentration differential.

4.2 Dissolved carbonate, as HCO_3^{-1} , is an anion present in all aqueous environmental samples, especially alkaline samples. Under the defined analysis conditions, carbonate at less than 1000:1 concentration differential to the anions will not interfere with the quantitation of the anions listed in Section 1.1.

4.3 Most monovalent organic acids and neutral organics commonly found in wastewater and groundwater migrate later in the electropherogram, after carbonate, and do not interfere with the anions listed in Section 1.1. Formate, a common organic acid found in environmental samples, migrates shortly after fluoride but before phosphate. At high formate concentrations the quantification of fluoride may be incorrectly identified. Include 5 mg/L formate into the mixed anion working solution to aid with fluoride identification and quantitation (Fig. 5).

4.4 Other inorganic or organic anions present in the sample will be separated and detected yielding an anionic profile of the sample. Other matrix anions commonly found in drinking water or wastewater do not interfere with the analysis of anions given in Section 1.1. However, unknown matrix anions may co-migrate or be a direct interferant with the analyte anions of interest.

4.5 Divalent organic acids usually found in wastewater migrate after phosphate. At concentrations greater than 10 mg/L, they may interfere with phosphate identification and quantitation.

4.6 Chlorate also migrates in the phosphate region but does not interfere with phosphate identification or quantitation at concentrations less than 3 mg/L. For chlorate concentrations greater than 3 mg/L, add 5 mg/L chlorate to the mixed anion working solution to aid in identification of phosphate and chlorate.

4.7 As the concentration of analyte increases the analyte peak shape becomes asymmetrical. If adjacent analyte peaks are not baseline resolved, the data system will drop a perpendicular line between them to the baseline. This causes a decrease in peak area for both analyte peaks and a low bias for analyte amounts. For optimal quantitation, ensure that adjacent peaks are fully resolved, if they are not, dilute the sample 1:1 with reagent water.

5.0 SAFETY

5.1 Refer to Chapter Three for additional guidance on safety protocols.

5.2 It is the responsibility of the user to prepare, handle, and dispose of electrolyte solutions in accordance with all applicable federal, state, and local regulations.

WARNING -- This capillary electrophoresis method uses high voltage as a means for separating the analyte anions, and can be hazardous if not used properly. Use only those instruments with the appropriate safety features.

6.0 EQUIPMENT AND SUPPLIES

6.1 Capillary Ion Electrophoresis System: Consists of the following components, as shown in Fig. 1, or equivalent.

6.1.1 High Voltage Power Supply: Capable of generating voltage potential between 0 and minus 30 kV relative to ground.

6.1.2 Covered Sample Carousel: To prevent environmental contamination of the samples during a multi-sample analysis.

6.1.3 Sample Introduction Mechanism: Capable of hydrostatic or pressure sampling techniques.

6.1.4 Capillary Purge Mechanism: To automatically purge the capillary after every analysis to eliminate any cross contamination from the previous sample matrix and to replenish the capillary with fresh electrolyte, or clean the capillary with other reagents, such as sodium hydroxide.

6.1.5 UV Detector: Capable of monitoring 254 nm with a time constant of 0.1 s.

6.1.6 Fused Silica Capillary: A 75 μm (inner diameter) x 375 μm (outer diameter) x 60 cm (length) having a polymer coating for flexibility, and a non-coated section to act as the cell window for UV detection.

6.1.7 Constant Temperature Compartment: To keep the samples, capillary and electrolytes at constant temperature.

6.2 Data System: Computer system capable of acquiring data at 20 points per second, ability to express migration time or relative migration time in minutes to 3 decimal places, use midpoint of the analyte peak width to determine the migration time of the analyte, use reference peaks and normalized migration time relative to the reference peak for qualitative identification, report time corrected peak area, and express results in concentration units.

6.3 Anion exchange cartridge, hydroxide form or equivalent.

6.4 Plastic syringes, 20 mL disposable.

6.5 Vacuum filtration apparatus using a 0.45 μm aqueous compatible filter.

7.0 REAGENTS AND STANDARDS

7.1 Purity of Reagents: Unless otherwise indicated, it is intended that all reagents shall conform to the reagent grade specification of the Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the performance or accuracy of the determination. Detection limits of this method are limited by the purity of the reagents.

7.2 Reagent Water: All references to water in this method refer to reagent water unless otherwise specified. Reagent water will be interference free. Refer to Chapter One for a definition of reagent water.

7.3 Individual Anion Solution, Stock Standard (1000 mg/L Anion): Individual stock solution may be purchased from an appropriate vendor or may be prepared in the laboratory. Recommend use of certified 1000 ppm stock standards.

NOTE: All weights given are for anhydrous or dried salts.

7.3.1 Bromide Solution, Standard: Dry approximately 0.5 g of sodium bromide (NaBr) for 6 hours at 150°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.128 g of the dry salt with water, and fill to mark with water.

7.3.2 Chloride Solution, Standard: Dry approximately 0.5 g of sodium chloride (NaCl) for 1 hour at 100°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.165 g of the dry salt with water, and fill to mark with water.

7.3.3 Fluoride Solution, Standard: Dry approximately 0.5 g of sodium fluoride (NaF) for 1 hour at 100°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.221 g of the dry salt with water, and fill to mark with water.

7.3.4 Formate Solution, Standard: Dissolve 0.151 g of sodium formate in a 100 mL volumetric flask with water, and make to volume.

7.3.5 Nitrate Solution, Standard: Dry approximately 0.5 g of sodium nitrate (NaNO₃) for 48 hours at 105°C and cool in a desiccator. In 100 mL volumetric flask dissolve 0.137 g of the dry salt with water, and fill to mark with water (1000 mg/L NO₃ = 225.8 mg/L N-NO₃).

7.3.6 Nitrite Solution, Standard: Dry approximately 0.5 g of sodium nitrite (NaNO₂) for 24 hours in a desiccator containing concentrated sulfuric acid. In 100 mL volumetric flask dissolve 0.150 g of the dry salt with water, and fill to mark with water. Store in a sterilized glass bottle. Refrigerate and prepare monthly. (1000 mg/L NO₂ = 304.3 mg/L N-NO₂)

NOTE: Nitrite is easily oxidized, especially in the presence of moisture. Use only fresh reagent.

NOTE: Prepare sterile bottles for storing nitrite solutions by heating for 1 hour at 170°C in an air oven.

7.3.7 Ortho-Phosphate Solution, Standard: In 100 mL volumetric flask dissolve 0.150 g of anhydrous dibasic sodium phosphate (Na_2HPO_4) with water, and fill to mark with water (1000 mg/L $\text{PO}_4 = 326.1$ mg/L P- PO_4).

7.3.8 Sulfate Solution, Standard: Dry approximately 0.5 g of sodium sulfate (Na_2SO_4) for 1 hour at 105°C and cool in a desiccator. In a 100 mL volumetric flask dissolve 0.148 g of the dry salt with water, and fill to mark with water.

7.4 Mixed Anion Solution, Working: Prepare a blank, and at least 3 different working standard concentrations for the analyte anion of interest within the desired range of analysis, typically between 0.1 and 50 mg/L. To a pre-rinsed 100 mL volumetric flask add an appropriate aliquot of individual anion stock standard solution (Section 7.3) and dilute with water. Add 5 mg/L formate to all standards.

NOTE: Use 0.1 mL of individual anion stock standard solution (Section 7.3) per 100 mL for 1 mg/L anion.

NOTE: Anions of no interest may be omitted.

NOTE: The mid-range mixed anion working solution (Section 7.4) may be used for the determination of migration times and resolution described in Section 10.1, and for quality control evaluation described in Section 9.0.

7.5 Electrolyte Reagents: Although any electrolyte meeting the performance criteria of this method may be used. This method has been validated using a chromate-based electrolyte.

7.5.1 Chromate Concentrate: (100 mM Chromate)- In a 1 L volumetric flask dissolve 23.41 g of sodium chromate tetrahydrate ($\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$) in 500 mL of water, and dilute to 1L with water. This concentrate may be stored in a capped glass or plastic container for up to 1 year.

7.5.2 Electroosmotic Flow Modifier Concentrate: (100 mM Tetradecyltrimethyl ammonium bromide, TTABr) - In a 100 mL volumetric flask dissolve 3.365 g of tetradecyltrimethyl ammonium bromide (TTABr) in 70 mL of water, and dilute to 100 mL with water.

NOTE: TTABr needs to be converted to the hydroxide form using the anion exchange cartridge. TTAOH is commercially available from Waters Corp. (sole source).

7.5.3 Buffer Solution: (100 mM CHES/1mM Calcium Gluconate) - In a 1 L volumetric flask dissolve 20.73 g of CHES (2-[N-Cyclohexylamino]-Ethane Sulfonic Acid) and 0.43 g of calcium gluconate in 500 mL of water, and dilute to 1 L with water. This concentrate may be stored in a capped glass or plastic container for up to one year.

7.5.4 Sodium Hydroxide Solution: (500 mM Sodium Hydroxide) - In a 100 mL volumetric flask dissolve 2 g of sodium hydroxide in 50 mL of water and dilute to 100 mL with water.

7.5.5 Electrolyte Solution, Working: (4.7 mM Chromate/4 mM TTAOH/10mM CHES/0.1mM Calcium Gluconate) -Wash the anion exchange cartridge in the hydroxide form using the 20 mL plastic syringe with 10 mL of 500 mM NaOH followed by 10 mL of water. Discard the washings. Slowly pass 4 mL of the 100 mM OFM Concentrate Solution through the cartridge into a 100 mL volumetric flask. Rinse the cartridge with 20 mL of water, adding the washing to the volumetric flask.

NOTE: The above procedure is used to convert the TTABr to TTAOH which is used in the electrolyte. If using commercially available 100 mM TTAOH, this step is not necessary.

Into the 100 mL volumetric flask add 4.7 mL of chromate concentrate solution and 10 mL buffer solution. Mix and dilute to 100 mL with water. The natural pH of the electrolyte should be 9.0 ± 0.1 . Filter and degass using the vacuum filtration apparatus. Store the remaining electrolyte in a capped glass or plastic container at ambient temperature. The electrolyte is stable for one year. This electrolyte is commercially available from Waters Corp.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample collection procedures should address the considerations described in Chapter Nine of this manual.

8.2 See the introductory material in Chapter Three, Inorganic Analytes, for information on sample handling and preservation.

8.3 Rinse sampling containers with the sample and discard to eliminate any contamination from the container, fill to overflowing, and cap to exclude air.

8.4 Analyze samples as soon as possible after collection. For nitrite, nitrate, and phosphate refrigerate the sample at 4°C after collection and warm to room temperature before dilution and analysis. Determine nitrite and nitrate within 48 hours.

8.5 Filter samples containing suspended solids through a pre-rinsed 0.45 µm aqueous compatible membrane filter before transferring the sample to the analysis vial.

8.6 If sample dilution is required, dilute with reagent water only.

9.0 QUALITY CONTROL

9.1 All quality control data should be maintained and available for easy reference or inspection.

9.2 For each batch of samples processed, method blanks must be carried throughout the entire sample preparation and analytical process according to the frequency described in Chapter One. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing blanks.

9.3 Matrix Spike/Matrix Spike Duplicates (MS/MSDs): MS/MSDs are intralaboratory split samples spiked with identical concentrations of target analytes. The spiking occurs prior to sample

preparation and analysis. An MS/MSD is used to document the bias and precision of a method in a given sample matrix. MS/MSDs are to be analyzed at the frequency of one per analytical batch as described in Chapter One. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols. Each laboratory should calculate its own acceptance criteria based on its historical data for each matrix type. Refer to Chapter One for guidance.

9.4 A laboratory control sample shall also be processed with each sample batch. Refer to Chapter One for more information.

9.5 Recalibrate after 15 analyses to account for any changes in migration time or response. Use the single mixed anion working solution (Sec. 7.4). Replace the new calibration results with the previous calibration results.

9.6 The new calibration curve is validated if the single point calibration response factor of new recalibration generated in Section 9.5 is $\pm 5\%$ of the previous calibration response factor, and if analyte migration time is $+ 5\%$ of previous migration time determined in Section 10.1.

9.7 If the calibration curve is not validated then discard the spent electrolyte and replace with fresh electrolyte. Calibrate as described in Section 10.1.

NOTE: Replace the electrolyte working solution in the instrument daily.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Determination of migration times - The migration time of an anion is dependent upon the electrolyte compositions, pH, capillary surface and length, applied voltage, the ionic strength of the sample, and temperature. For every fresh electrolyte determine the analyte migration time in minutes, to the third decimal place, of the mid-range mixed anion standard working solution (Section 7.4), using the analysis scheme described in (Section 11.0). Use mid-point of analyte peak width as the determinant of analyte migration time (Fig. 5 and Table 2).

NOTE: Analyte peak apex may be used as the migration time determinant, but potential analyte misidentification may result with asymmetrical shape at high analyte concentrations.

10.2 For each anion concentration (X-axis) plot time corrected peak area response (Y-axis). Determine the best linear calibration line through the data points, or use the linear regression calibration routine available in the data systems. Do not force the line through zero.

10.3 After verification of linear multiple calibration, a single point calibration can be used between 0.1 and 50 mg/L anion. This single point calibration solution can be used for subsequent recalibration.

11.0 PROCEDURE

11.1 Set up the capillary electrophoresis system according to the manufacturer's instructions. Fill the electrolyte reservoirs with fresh electrolyte. Transfer the blank, standard, or sample into a prerinsed plastic sample analysis vial and place in the covered sample carousel.

11.2 Program the system according to manufacturer's instructions using the following instrument settings as guidelines for analysis of standards, and samples.

11.2.1 Condition a new 75 μm i.d. x 375 μm o.d. x 60 cm capillary with 100 mM NaOH for 5 minutes followed by working chromate electrolyte solution A for 5 minutes.

NOTE: This conditioning step should be repeated weekly in order to regenerate the capillary surface for optimum reproducibility.

Program the system for at least a one minute purge of the capillary with electrolyte between each standard or sample. Using a 15 psi vacuum purge mechanism, one 60 cm capillary volume can be displaced in 30 seconds.

11.2.2 Program the system for the hydrostatic sampling technique for 30 seconds. Different sampling times may be used provided that samples and standards are analyzed identically. Approximately 1.2 nL of sample per second is siphoned into a 75 μm capillary.

11.2.3 Program the system for constant current 14 μA and a run time of 5 minutes; if an anionic profile of the sample is of interest set the time to 7 minutes. Using a capillary 60 cm in length, the field strength at 15 μV applied voltage is 250 V/cm.

11.2.4 Program the integrator or computer for data acquisition rate of 20 points per second with a run time designated in Section 11.2.3. Set up data processing method according to manufacturers instructions.

11.2.5 Monitor UV response at 254 nm. Since detector ranges are variable, the range setting required for analysis will depend on the concentration of anions in the sample and should be chosen accordingly.

11.2.6 The electropherogram of the working calibration standards (Section 7.4) should be similar to the inorganic anion electropherogram shown in Fig. 5.

11.3 Analyze all standards (Section 7.4) and samples as described in Section 11.2. Refer to Figs. 5-9 for representative anion standard, 0.1 mg/L anion standard, drinking water, and waste water (municipal and industrial).

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Relate the time corrected peak area for each sample anion with the calibration curve generated in Section 10.2 to determine mg/L concentration of anion. If the sample was diluted prior to analysis, then multiply mg/L anion by the dilution factor to obtain the original sample concentration.

$$\text{Original Sample mg/L Anion} = (A \times \text{SF})$$

where:

A = mg/L anion determined from the calibration curve

SF = scale or dilution factor

13.0 METHOD PERFORMANCE

13.1 Figures 6-12 display representative examples of electropherograms and linearity of calibration curves.

13.2 Tables 1-10 provide collaborative design, migration time reproducibility, comparison of CIE with other approved EPA methods, and interlaboratory reproducibility and precision for the capillary ion electrophoresis technique.

13.3 Table 11 is entitled "Capillary Ion Electrophoresis Anion Analysis Round Robin Using Chromate Electrolyte (mg/L)" and provides precision data in some common environmental matrices.

13.4 The following documents may provide additional information regarding this method and technique:

13.4.1 Romano, J., Krol, J, "Capillary Ion Electrophoresis, An Environmental Method for the Determination of Anions in Water", J. of Chromatography, Vol. 640, 1993, p. 403.

13.4.2 Romano, J., "Capillary Ion Analysis: A Method for Determining Ions in Water and Solid Waste Leachates", Amer. Lab., May 1993, p. 48.

13.4.3 Jones, W., "Method Development Approaches for Ion Electrophoresis", J. of Chromatography, Vol. 640, 1993, p. 387.

13.4.4 Jones, W., Jandik, P., "Various Approaches to Analysis of Difficult Sample Matrices for Anions using Capillary Electrophoresis", J. of Chromatography, Vol. 608, 1992, p. 385.

13.4.5 Bondoux, G., Jandik, P., Jones, W., "New Approaches to the Analysis of Low Level of Anions in Water", J. of Chromatography, Vol. 602, 1992, p. 79.

13.4.6 Jandik, P., Jones, W., Weston, A., Brown, P., "Electrophoretic Capillary Ion Analysis: Origins, Principles, and Applications", LC-GC, Vol. 9, Number 9, 1991, p. 634.

13.4.7 Romano, J., Jackson, P., "Optimization of Inorganic Capillary Electrophoresis for the Analysis of Anionic Solutes in Real Samples", J. of Chromatography, Vol. 546, 1991, p. 411.

13.4.8 Jandik, P., Jones, W., "Optimization of Detection Sensitivity in the Capillary Electrophoresis of Inorganic Anions", J. of Chromatography, Vol. 546, 1991, p. 431.

13.4.9 Jandik, P., Jones, W., "Controlled Changes of Selectivity in the Separation of Ions by Capillary Electrophoresis", J. of Chromatography, Vol. 546, 1991, p. 445.

13.4.10 Foret, R., et.al., "Indirect Photometric Detection in Capillary Zone Electrophoresis", J. of Chromatography, Vol. 470, 1989, p. 299.

13.4.11 Hjerte'n, S. et. al., "Carrier-free Zone Electrophoresis, Displacement Electrophoresis and Isoelectric Focusing in an Electrophoresis Apparatus", J. of Chromatography, Vol. 403, 1987, p. 47

13.4.12 Jandik, P., Bonn, G., "Capillary Electrophoresis of Small Molecules and Ions", VCH Publishers, 1993.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical management for Waste Reduction* available from the American Chemical Society, Department of Government Relations and Science Policy, 1155 16th Street, NW, Washington, DC, 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Section 14.2.

16.0 REFERENCES

1. Waters Chromatography, "Innovative Methods for Ion Analysis", Method N-601b, 1992.
2. Waters Chromatography, Validation Data for Method 6500, Millipore Corporation Waters Chromatography Division, Ion Analysis Group; Milford, Massachusetts.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain Table 1 through 11, Figures 1 through 12, and a flow diagram of method procedures.

TABLE 1

COLLABORATIVE DESIGN AS FOUR YOUTDEN PAIR SETS¹

Individual Youden Pair Standards, in mg/L

	1	2	3	4	5	6	7	8	
Analyte Anion	Cl	0.7	2.0	3.0	15.0	40.0	20.0	50.0	0.5
	Br	2.0	3.0	15.0	40.0	20.0	50.0	0.7	0.5
	NO₂	3.0	40.0	20.0	15.0	50.0	0.5	2.0	0.7
	SO₄	40.0	50.0	0.5	0.7	2.0	3.0	15.0	20.0
	NO₃	15.0	20.0	40.0	50.0	0.5	0.7	2.0	3.0
	F	2.0	0.7	0.5	3.0	10.0	7.0	20.0	25.0
	PO₄	50.0	40.0	20.0	0.5	3.0	2.0	0.7	15.0

Source: Reference 2

¹The collaborative design is intended to demonstrate performance between 0.1 and 50 mg/L anion, except for Fluoride between 0.1 and 25 mg/L. The concentrations among anions is varied as not to have any one standard at all low or all high anion concentrations.

TABLE 2

ANION MIGRATION TIME REPRODUCIBILITY FROM YODEN PAIR STANDARDS
USING CHROMATE ELECTROLYTE AND CONSTANT CURRENT

Analyte Mid-Point Migration Time, Ave of Triplicate Samplings

Youden Standards	Analyte	Cl	Br	NO ₂	SO ₄	NO ₃	F	PO ₄
	1	3.132	3.226	3.275	3.405	3.502	3.761	3.906
	2	3.147	3.239	3.298	3.431	3.517	3.779	3.931
	3	3.138	3.231	3.283	3.411	3.497	3.771	3.925
	4	3.158	3.244	3.307	3.434	3.510	3.781	3.963
	5	3.184	3.271	3.331	3.435	3.551	3.787	3.981
	6	3.171	3.260	3.312	3.418	3.537	3.776	3.964
	7	3.191	3.272	3.315	3.437	3.544	3.773	3.978
	8	3.152	3.248	3.294	3.418	3.526	3.739	3.954
Std Dev	0.021	0.015	0.018	0.012	0.20	0.03015	0.027	
%RSD	0.67%	0.46%	0.55%	0.36%	0.56%	0.40%	0.68%	

Ave Migration Time Std Dev = 0.018 min = 1.1 sec

Ave %RSD = 0.53%

TABLE 3

COMPARISON OF CAPILLARY ION ELECTROPHORESIS WITH CHROMATE
ELECTROLYTE AND APPROVED METHODS USING A PERFORMANCE EVALUATION
STANDARD

	Analyte	Cl	NO ₂	SO ₄	NO ₃	F	PO ₄
Performance Evaluation Standard¹	True Value in mg/L	43.00	1.77	37.20	15.37	2.69	6.29
Official Anion Methods Wet Chem & IC	Measured Mean ²	43.20	1.77	37.00	15.42	2.75	6.38
	Measured Std Dev	3.09	0.07	2.24	1.15	0.26	0.21
CIE Using Chromate Electrolyte³	Ave CIE n=18	42.51	1.78	37.34	14.06	2.63	6.34
	CIE/Mean CIE/True Value	0.984 0.989	1.006 1.006	1.009 1.003	0.911 0.945	0.956 0.978	0.994 1.008

Source: Reference 2

¹The performance evaluation standard was purchased from APG Laboratories and diluted 1:100 with Type I DI water.

²The measured result is the average from numerous laboratories using Approved Standard Methods and EPA wet chemistry and ion chromatography methods

³The CIE results were determined using the proposed EPA and ASTM method, and are the average from 4 laboratories using the Youden Pair Standards for quantitation.

TABLE 4

CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE
INTERLABORATORY REPRODUCIBILITY AND PRECISION¹

Analyte ²	Cl	NO ₂	SO ₄	NO ₃	F
Lab 1 n = 5	43.22 ± 0.22	1.58 ± 0.09	36.39 ± 0.33	14.57 ± 0.12	2.54 ± 0.10
Lab 2 n=5	43.68 ± 0.61	1.58 ± 0.08	37.01± 0.37	13.94 ± 0.09	2.69 ± 0.02
Lab 3 n=5	43.93 ± 0.39	1.60 ± 0.06	37.68 ± 0.24	15.05 ± 0.11	2.69 ± 0.03
Lab 4 n=3	42.51 ± 0.22	1.78 ± 0.06	37.34 ± 0.19	14.06 ± 0.07	2.69 ± 0.02
Average Mean ± Std Dev	43.34 ± 0.36	1.64 ± 0.07	37.11 ± 0.28	14.41 ± 0.10	2.64 ± 0.04
% RSD	0.83%	4.5%	0.77%	0.67%	1.61%

¹Results from 4 laboratories analyzing the performance evaluation standard using the Youden Pair Standards for quantitation. Results expressed as mg/L.

²Only 1 lab reported results for PO₄ as 6.34 ± 0.02 mg/L on triplicate samplings yielding an %RSD of 0.07%

TABLE 5

CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE KNOWN
 RECOVERY AND PRECISION USING PERFORMANCE EVALUATION STANDARD WITH
 DRINKING WATER

Analyte	Cl	NO ₂	SO ₄	NO ₃	F	PO ₄
Milford Drinking Water n=3, as ppm	24.27 ± 0.18	Not Detected	7.99 ± 0.07	0.36 ± 0.05	Not Detected	Not Detected
%RSD	0.73%		0.91%	13.3%		
Performance Evaluation Std¹	43.00	1.77	37.20	15.37	2.69	6.29
MDW + PES n=3, as ppm	66.57 ± 0.34	1.74 ± 0.03	45.19 ± 0.17	15.42 ± 0.12	2.62 ± 0.07	5.55 ± 0.31
%RSD	0.51	1.85	0.38	0.79	2.69	5.52
% Recovery	97.9%	98.3%	100.2%	98.1%	97.4%	88.2%

Source: Reference 2.

¹The performance evaluation standard was diluted 1:100 with Drinking Water.

TABLE 6

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS
WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF CHLORIDE

Data given as mg/L

Analyte	Sample #	Titration ¹	IC ²	CIE
Effluent	1	-- ³	149	147
	2	--	162	161
	3	--	153	152
	4	--	139	140
	5	--	111	110
	6	--	109	107
	7	--	3.6	3.5
Drinking Water	1	5.5	5.1	5.0
	2	5.5	5.0	4.9
	3	5.3	5.2	5.1
	4	5.5	5.1	5.1
	5	5.3	5.0	5.0
	6	5.3	4.9	4.9
	7	5.5	4.9	4.9
Landfill Leachate	1	0.1	<0.1	ND
	2	230	245	240

Source: Reference 2.

¹ Chloride determined using 4500 Cl C, Iodometric Method

² Chloride determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

³ A dash line indicates test not performed. ND indicates anion not detected

TABLE 7

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS
WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF FLUORIDE

Analyte	Sample #	Electrode ¹	IC ²	CIE
Effluent	1	1.7	1.2	1.5
	2	0.9	0.6	0.6
	3	0.8	0.5	0.6
	4	0.8	0.4	0.7
	5	0.9	0.5	0.8
	6	0.9	0.5	0.7
	7	<0.1	ND	<0.1
Drinking Water	1	1.2	0.9	0.9
	2	1.3	0.9	0.9
	3	1.3	0.9	0.9
	4	1.3	0.9	0.9
	5	1.3	0.9	0.9
	6	0.9	0.6	0.6
	7	1.3	0.9	0.9
Landfill Leachate	1	<0.2	ND	ND
	2	16	10.6	10.9

Source: Reference 2.

¹ Fluoride determined using 4500-F C, Ion Selective Electrode Method

² Fluoride determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

TABLE 8

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS
WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF SULFATE

Data given as mg/L

Analyte	Sample #	Turbidimetric ¹	IC ²	CIE
Effluent	1	98	87.5	98.0
	2	110	95.3	95.9
	3	130	118	115
	4	130	139	136
	5	110	113	110
	6	100	107	106
	7	6	5.6	5.8
Drinking Water	1	6	5.8	6.0
	2	6	5.8	6.0
	3	6	5.9	6.1
	4	6	5.9	6.1
	5	5	5.8	6.2
	6	4	3.0	3.4
	7	5	5.8	6.1
Landfill Leachate	1	<1	ND	ND
	2	190	211	201

Source: Reference 2.

¹ Sulfate determined using 4500 SO₄ E, Turbidimetric Method² Sulfate determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

TABLE 9

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS
WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF NITRITE + NITRATE³
Data given as mg/L

Analyte	Sample #	Cd Red'n ¹	IC ²	CIE
Effluent	1	0.3	ND	ND
	2	--	ND	ND
	3	--	ND	ND
	4	--	ND	0.5
	5	--	2.1	2.4
	6	2.4	1.9	2.2
	7	0.7	0.3	0.4
Drinking Water	1	0.6	0.3	0.4
	2	0.6	0.3	4.4
	3	0.4	0.3	4.4
	4	0.6	0.3	0.3
	5	0.6	0.3	0.4
	6	0.3	0.1	0.1
	7	0.5	0.3	0.4
Landfill Leachate	1	--	ND	ND
	2	--	ND	ND

Source: Reference 2.

¹ Total nitrite + nitrate determined using 4500-NO₃ F, Cadmium Reduction Method

² Nitrite + nitrate determined using 4110 C, Single Column Ion chromatography Using Direct Conductivity Detection

³ Each technique gave separate nitrite and nitrate values; because of their liability results were added for comparison purposes

TABLE 10

COMPARISON OF APPROVED METHOD AND CAPILLARY ION ELECTROPHORESIS
WITH CHROMATE ELECTROLYTE FOR THE DETERMINATION OF ORTHO-PHOSPHATE
Data given as mg/L

Analyte	Sample #	Ascorbic Acid ¹	IC ²	CIE
Effluent	1	3.4	ND	2.8
	2	4.9	ND	4.4
	3	4.7	ND	4.5
	4	5.3	ND	4.2
	5	3.0	ND	3.0
	6	2.9	ND	2.3
	7	<0.1	ND	<0.1
Drinking Water	1	<0.1	ND	ND
	2	<0.1	ND	ND
	3	--	ND	ND
	4	<0.1	ND	ND
	5	<0.1	ND	ND
	6	--	ND	ND
	7	--	ND	ND
Landfill Leachate	1	<0.1	ND	<0.1
	2	2.2	1.6	1.4

Source: Reference 2.

¹ Phosphate determined using 4500 PO₄ E, Ascorbic Acid Method

² Phosphate determined using 4110 C, Single Column Ion Chromatography Using Direct Conductivity Detection

TABLE 11

CAPILLARY ION ELECTROPHORESIS ANION ANALYSIS ROUND ROBIN¹
USING CHROMATE ELECTROLYTE (mg/L)

Sample	Chloride	Bromide	Nitrite	Sulfate	Nitrate	Fluoride	Phosphate
1. Bleachwaste	<0.046	<0.046	<0.072	0.30±0.37	<0.84	<0.020	<0.041
2. Creekwater	3.06±0.27	<0.046	<0.072	3.00±0.30	0.37±0.19	0.11±0.09	<0.061
3. Wastewater	24.6±0.62	<0.046	<0.072	2.02±0.56	<0.084	0.08±0.08	3.74±0.75
4. Wastewater	59.7±2.9	0.85±0.52	<0.072	109±4.4	44.9±1.6	0.988±0.21	4.94±1.32
5. Wastewater	63.8±2.0	0.68±0.52	<0.072	115±3.9	44.3±1.06	1.04±0.17	4.78±1.55
6. Wastewater	72.0±5.4	0.05±0.01	<0.072	144±11.8	5.38±2.57	0.57±0.21	1.18±1.01
7. Wastewater	139±10.0	<0.046	4.0±1.3	584±35	353±25.5	3.01±0.80	9.34±5.17
8. Wastewater	51.4±7.7	<0.046	<0.072	40.2±6.1	39.9±7.9	1.17±0.24	6.99±1.31
9. Wastewater	29.9±4.3	<0.046	2.14±1.35	217±19	13.9±4.9	1.33±0.28	9.95±5.04
10. Wastewater	766±44	<0.046	<0.072	489±46	12.9±6.9	<0.020	41.3±8.5
11. Surfacewater	3.71±0.39	<0.046	<0.072	2.70±0.39	0.23±0.20	0.11±0.097	<0.041
12. Wastewater	22.1±0.62	8.47±0.30	<0.072	133±4.4	<0.084	0.76±0.11	<0.041
13. Drinking Water	5.15±0.35	<0.046	<0.072	2.64±0.26	0.50±0.27	0.59±0.097	<0.041
14. Drinking Water	4.95±0.24	<0.046	<0.072	2.62±0.21	0.54±0.25	0.56±0.09	<0.041

Source: Reference 2.

¹ Five laboratory interlaboratory precision.

FIGURE 1

HARDWARE SCHEMATIC OF A CAPILLARY ION ELECTROPHORESIS SYSTEM

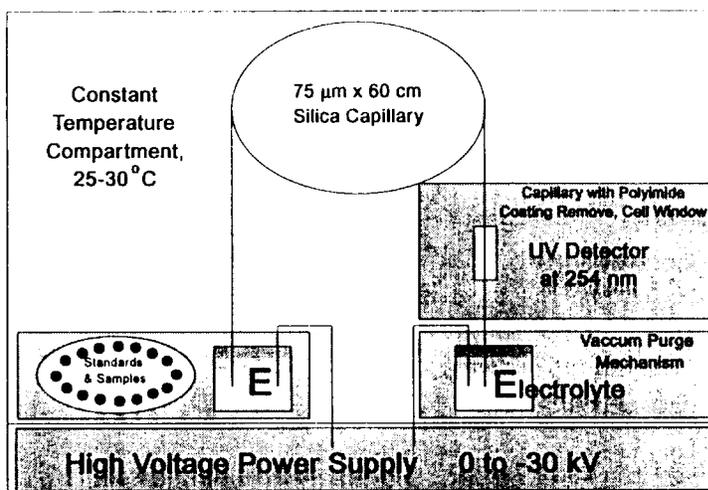


FIGURE 2

PICTORIAL DIAGRAM OF ANION MOBILITY AND ELECTROOSMATIC FLOW MODIFIER

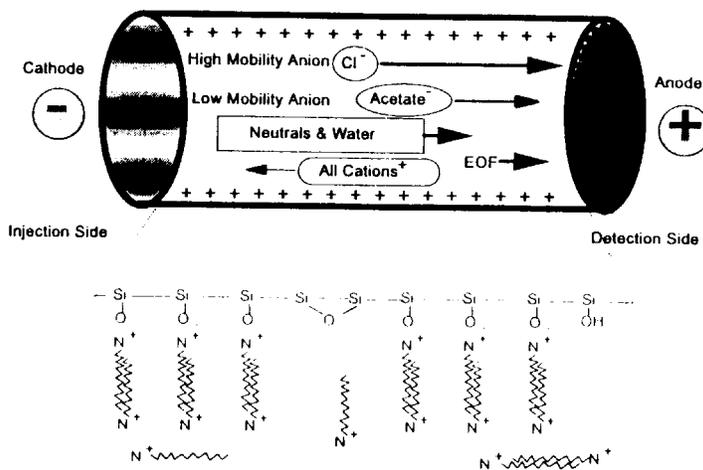


FIGURE 3

SELECTIVITY DIAGRAM OF ANION MOBILITY USING CAPILLARY ION ELECTROPHORESIS

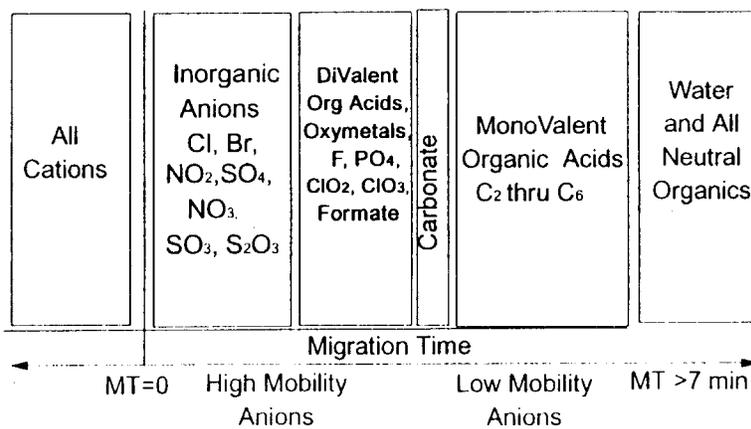


FIGURE 4

PICTORIAL INDIRECT UV

DIAGRAM OF DETECTION

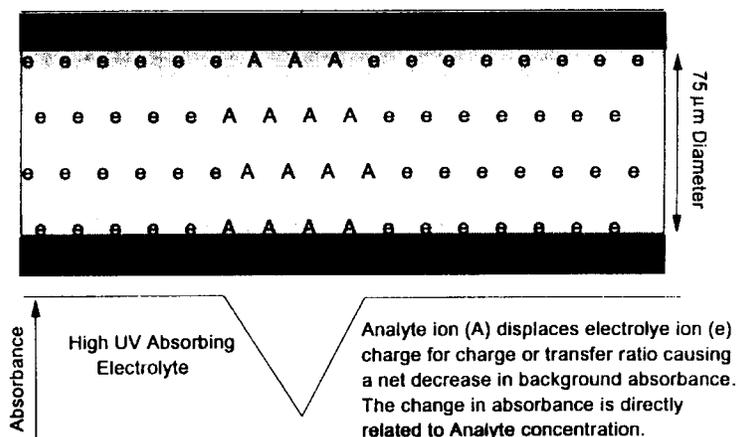
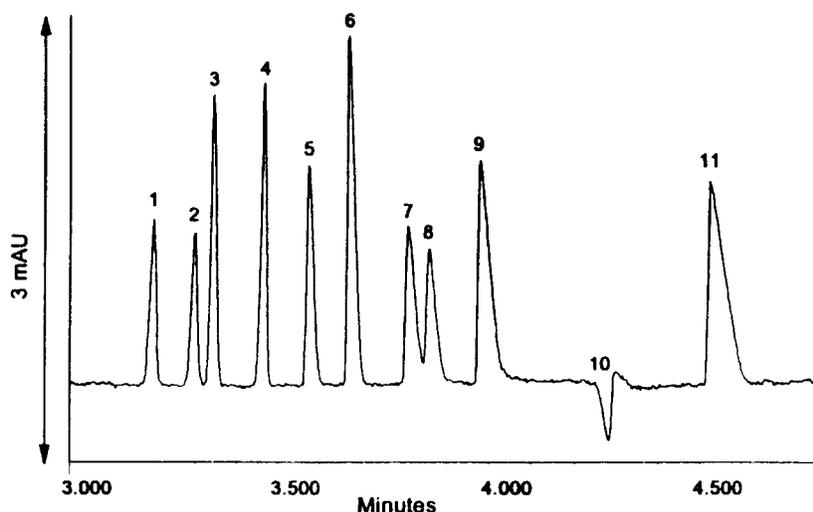


FIGURE 5

ELECTROPHEROGRAM OF THE INORGANIC ANIONS AND TYPICALLY FOUND ORGANIC ACIDS USING CAPILLARY ION ELECTROPHORESIS WITH CHROMATE ELECTROLYTE



Electrolyte: 4.7 mM Na₂CrO₄/4.0 mM TTAOH / 10 mM CHES / 0.1 mM Calcium Gluconate
 Capillary: 75 μm (id) x 375 μm x 60 cm (length), Uncoated Silica
 Voltage: 15 kV using a Negative Power Supply
 Current: 14 ± μA, Constant Current
 Sampling: Hydrostatic at 10 cm for 30 seconds
 Detection: Indirect UV using a Hg Lamp and 254 nm Filter

Anion	Conc. Mg/L	Migration Time in Minutes	Migration Time Ratio to Cl	Peak Area	Time Corrected Peak Area
1. Chloride	2.0	3.200	1.000	1204	376.04
2. Bromide	4.0	3.296	1.030	1147	348.05
3. Nitrite	4.0	3.343	1.045	2012	601.72
4. Sulfate	4.0	3.465	1.083	1948	562.05
5. Nitrate	4.0	3.583	1.120	1805	503.69
6. Oxalate	5.0	3.684	1.151	3102	842.14
7. Fluoride	1.0	3.823	1.195	1708	446.65
8. Formate	5.0	3.873	1.210	1420	366.61
9. O-Phosphate	4.0	4.004	1.251	2924	730.25
10. Carbonate	--	4.281	1.338	--	--
11. Acetate	5.0	4.560	1.425	3958	868.01

FIGURE 6

ELECTROPHEROGRAM OF 0.1 MG/L INORGANIC ANIONS
MINIMUM DETECTION LIMIT WITH CHROMATE ELECTROLYTE

Seven replicates of the 0.1 mg/L inorganic anion standard was used to calculate the minimum detection limits, as mg/L, using analytical protocol described in Standard Methods 1030 E.

Chloride = 0.046	Bromide = 0.090	Nitrite = 0.072	Sulfate = 0.032
Nitrate = 0.084	Fluoride = 0.020	phosphate = 0.041	

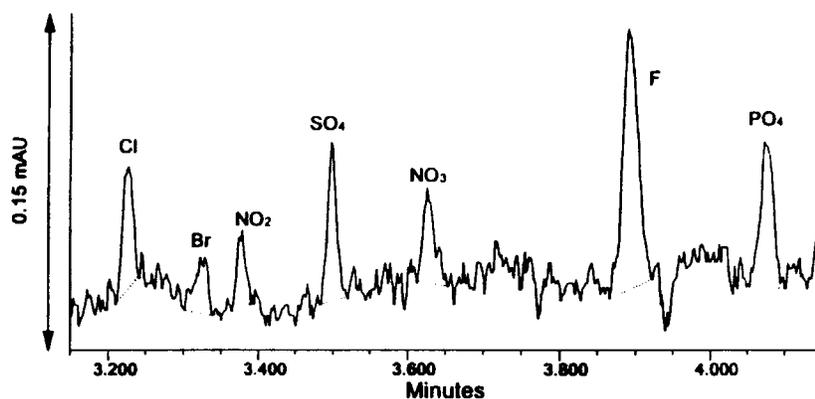


FIGURE 7

ELECTROPHEROGRAM OF TYPICAL DRINKING WATER
USING CHROMATE ELECTROLYTE

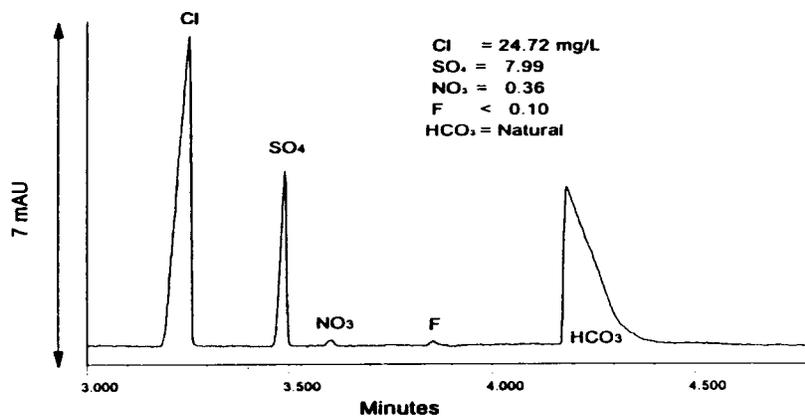


FIGURE 8

ELECTROPHEROGRAM OF TYPICAL MUNICIPAL WASTEWATER DISCHARGE
USING CHROMATE ELECTROLYTE

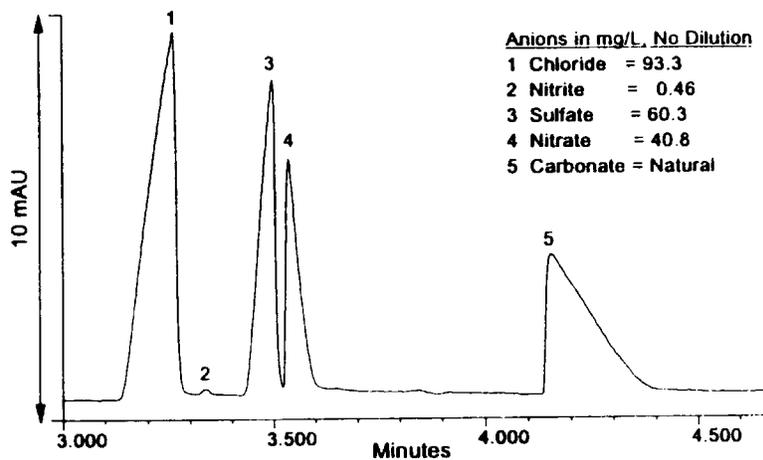


FIGURE 9

ELECTROPHEROGRAM OF TYPICAL INDUSTRIAL WASTEWATER DISCHARGE
USING CHROMATE ELECTROLYTE

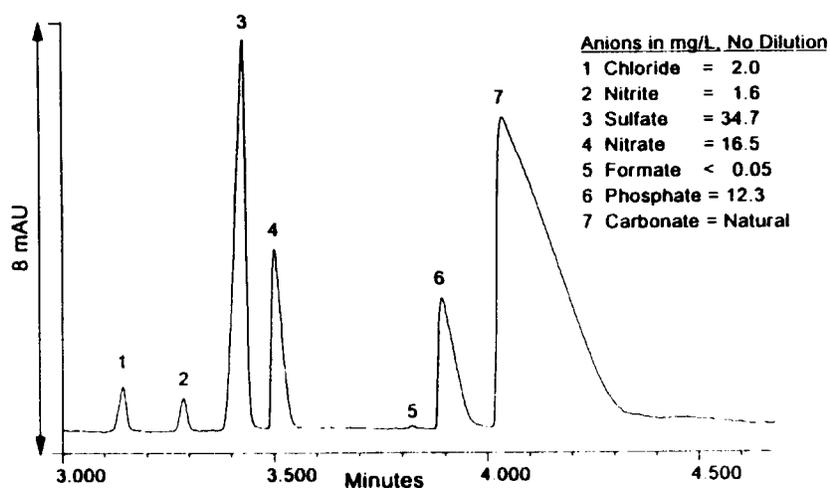


FIGURE 10

LINEARTY CALIBRATION CURVE FOR CHLORIDE, BROMIDE, AND SULFATE USING CHROMATE ELECTROLYTE

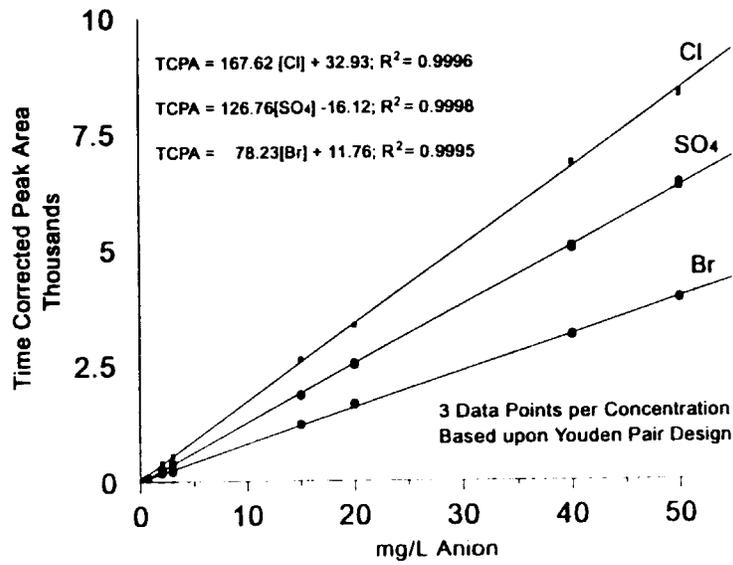


FIGURE 11

LINEARTY CALIBRATION CURVE FOR FLUORIDE AND O-PHOSPHATE USING CHROMATE ELECTROLYTE

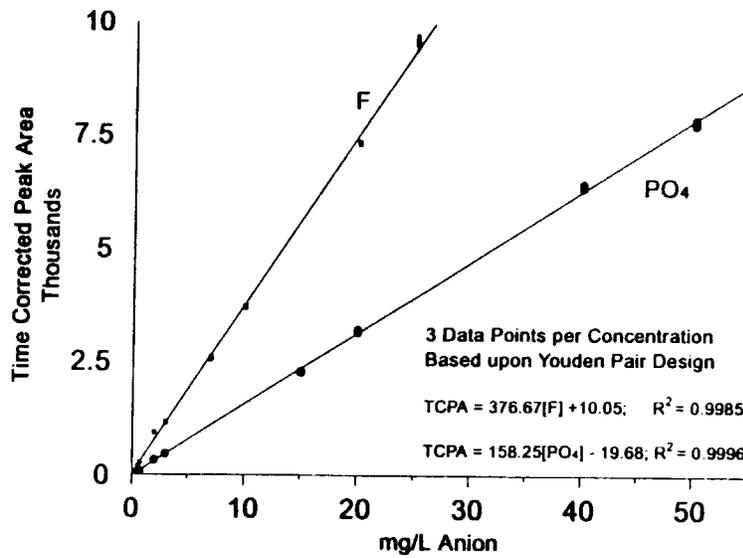
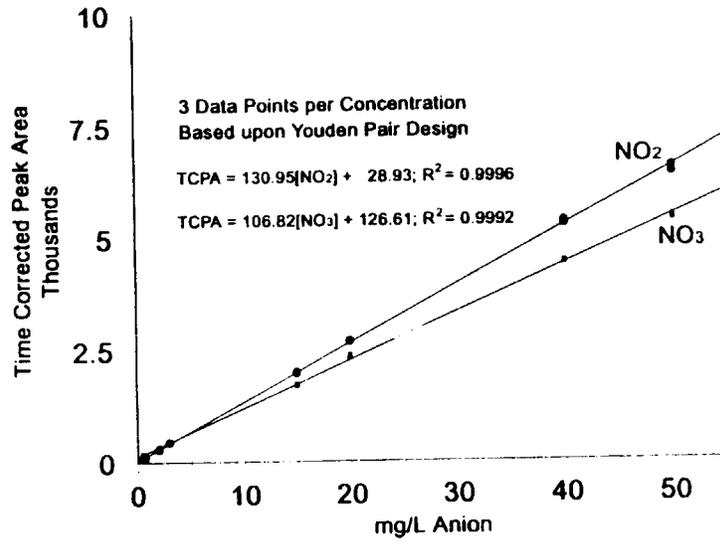


FIGURE 12

LINEARITY CALIBRATION CURVE FOR NITRITE AND NITRATE
USING CHROMATE ELECTROLYTE



METHOD 6500

DISSOLVED INORGANIC ANIONS IN AQUEOUS MATRICES
BY CAPILLARY ION ELECTROPHORESIS

