

Ions In Physiological Fluids

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INTRODUCTION

Ion chromatography (IC) has gained wide acceptance over the last twenty years. Advances in column technology and detection schemes have led to ion chromatography applications in a wide variety of matrices and industries. One of the most important applications of IC in clinical chemistry is the analysis of physiological fluids such as urine, plasma, and serum both for inorganic and organic anions and cations.¹ Profiles of physiological fluids can aid in the investigation of certain diseases such as lactic acidosis, hyperoxaluria, and urinary stone disease. The analysis of physiological fluids requires a highly sensitive and selective analytical technique, since the matrix is complex and the concentrations of the ions of interest are often very low. While high-performance liquid chromatography has become an important technique for the determination of biological markers for the diagnosis of diseases, IC is being widely used for the analysis of ionic species (see Figure 1).

The separation mechanism of IC is based on an ion-exchange displacement process occurring between the sample ions and eluent ions with the ion-exchange functional groups bonded to the stationary phase. A typical solid phase consists of ethylvinylbenzene cross-linked with divinylbenzene and functionalized with ion-exchange groups. The separation of organic acids and anions illustrated in this application note was accomplished using a stationary phase that is functionalized with quaternary ammonium groups; whereas, a carboxylate group was used for exchange sites for the separation of cations. Effluent from the analytical column is passed through a suppressor that reduces the total background conductance of the eluent

and increases the electrical conductance of the analyte ions. When suppressed conductivity is used, significant improvements in sensitivity are obtained.

This application note outlines the analysis of physiological fluids using ion chromatography. Using this method, common inorganic ions such as chloride, nitrate, nitrite, phosphate, sulfate, sodium, potassium, magnesium, and calcium may be easily separated. This IC method requires minimum sample preparation, and the analytes can be separated without any significant interferences.

EQUIPMENT

Dionex DX 500 system consisting of:

GP40 Gradient Pump

CD20 Conductivity Detector

LC10 or LC20 Chromatography Module

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Sodium hydroxide (NaOH), 50% w/w
(Fisher Scientific)

Sulfuric acid, (H₂SO₄), reagent grade

Deionized water 17.8 MΩ-cm resistance or higher

Acetonitrile (CH₃CN), HPLC grade

PART I: ANION AND ORGANIC ACID ANALYSIS

CONDITIONS

Columns: IonPac® AS11 Analytical
IonPac AG11 Guard, and IonPac ATC-1

Eluents: E1: Deionized water
E2: 5.0 mM Sodium hydroxide
E3: 100 mM Sodium hydroxide

Gradient Program:

	Time (min)	E1 %	E2 %	E3 %	Comments
<i>Equilibration</i>	0.0	90	10	0	0.5 mM NaOH for 20 min
<i>Analysis</i>	20.0	90	10	0	0.5 mM NaOH, inject
	20.2	90	10	0	Inject Valve to "Load"
	22.5	90	10	0	0.5–5.0 mM NaOH in 3.5 min
	26.0	0	100	0	5.0–38.25 mM NaOH in 12 min
	38.0	0	65	35	

Flow Rate: 2.0 mL/min

Inj. Volume: 10 µL

Detection: Suppressed conductivity, ASRS,
AutoSuppression™ recycle mode

Expected

Background

Conductivity: 0.5 mM NaOH: <1 µS
35 mM NaOH: <3.5 µS

Expected

System

Operating

Backpressure: 11 MPa (1600 psi)

PREPARATION OF SOLUTIONS AND REAGENTS

100 mM Sodium Hydroxide

Weigh 992 g (992 mL) of deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Tare the bottle on the balance, and add 8.00 g (5.25 mL) of 50% sodium hydroxide directly to the bottle. Quickly transfer the eluent reservoir to the instrument, and pressurize it with helium.

5.0 mM Sodium Hydroxide

Weigh 1.00 kg (1.00 L) of deionized water into an eluent reservoir bottle. Degas the water for approximately 10 minutes. Tare the bottle on the balance, and add 0.40 g (0.26 mL) of 50% sodium hydroxide directly to the bottle. Quickly transfer the eluent reservoir to the instrument, and pressurize the bottle with helium.

STOCK STANDARDS

Prepare 1000 mg/L (1000 ppm) stock analyte standard solutions by dissolving the amount of each salt listed in Table 1 in deionized water and diluting it to 1.000 L.

SAMPLE PREPARATION

Urine

Dilute with deionized water (e.g., 1:25) as required and inject sample.

Plasma and Serum

Acetonitrile deproteinization procedure is used. Mix 0.2 mL of serum with an equal volume of acetonitrile, then centrifuge at 5000 x g for 7 minutes. Dilute 0.1 mL of the supernatant with 1.0 mL deionized water.

Table 1 Preparation of Stock Standard Solution

Analyte	Salt	Amount (g)
Fluoride	Sodium fluoride	2.210
Chloride	Sodium chloride	1.648
Acetate	Sodium acetate	1.389
Formate	Sodium formate	1.510
Bromate	Sodium bromate	1.179
Pyruvate	Pyruvic acid	1.000
Nitrate	Sodium nitrate	1.371
Isocitrate	Isocitric acid trisodium dihydrate	1.306
<i>cis</i> -Aconitate	<i>cis</i> -Aconitic acid	1.000
<i>trans</i> -Aconitate	<i>trans</i> -Aconitic acid	1.000
Selenite	Sodium selenite	1.362
Maleate	Maleic acid	1.000
Malonate	Malonic acid	1.000
Phthalate	Potassium hydrogen phthalate	1.238
Phosphate	Potassium dihydrogen phosphate	1.433
Citrate	Citric acid	1.000
Sulfate	Potassium sulfate	1.814
Oxalate	Sodium oxalate	1.522
Uric acid	Uric acid	1.000
Creatinine	Creatinine	1.000
Choline	Choline chloride	1.342
Sodium	Sodium chloride	2.542
Ammonium	Ammonium chloride	2.964
Potassium	Potassium chloride	1.906
Calcium	Calcium chloride hydrate	3.668
Magnesium	Magnesium chloride hexahydrate	8.365

DISCUSSION AND RESULTS

A range of inorganic and organic acid anions are of particular interest in clinical chemistry studies. Figure 2 demonstrates the extensive range of anions that may be separated and detected by IC. Elevated levels of many organic acids in physiological fluids can be used as indicators of diseases such as lactic acidosis, hyperoxaluria, and urinary stone disease. IC is frequently used for the routine determination of oxalate in urine as elevated oxalate levels are indicative of renal failure. This method is outlined in Application Note 36, "Determination of Oxalate in Urine by Ion Chromatography". The IC method is twice as fast and more reliable than the colorimetric method.

The method outlined in this application note is *not recommended* for determination of oxalate because the sodium hydroxide eluent decomposes urinary ascorbate to oxalate. Several studies have established the role of nitrate and nitrite in mammalian acute toxicosis.²⁻⁵ These studies have included the biotransformation of the nitrate and nitrite and resulting methemoglobinemia and anemic hypoxia. As illustrated in Figure 1, nitrite and nitrate can be easily monitored using this IC method.

IC may be used for complex matrices such as serum. Figure 2 shows the separation of inorganic anions and organic acid anions in a serum sample. Uric acid as urate is monitored in patients suspected to be suffering from hyperuricemia, a condition that may lead to gout. Uric acid is also monitored in patients under therapy for chronic myelogenous leukemia. The separation shown in Figure 3 represents an alternative to the HPLC method, offering comparable specificity and sensitivity.

Chloride can be monitored to detect a variety of electrolyte disturbances in blood, often paralleling changes in sodium concentration. Blood chloride levels increase in dehydration and decrease in overhydration, congestive heart failure, Addison's disease, and other disturbances of sodium-water balance. IC is a well established technique for chloride analysis. Figure 2 shows the separation of chloride in a complex serum matrix.

As ion exchange is used as the separation process for this method, ions of different valency will exhibit a broad range of retention behavior. Consequently, a wide range of eluent strengths need to be employed to achieve satisfactory separations. In this method, the IonPac AS11 is coupled with the Anion Self-Regenerating Suppressor (ASRS) to dramatically reduce eluent conductivity, thus allowing the use of a rapidly increasing hydroxide gradient for the separation of an extensive group of monovalent through trivalent

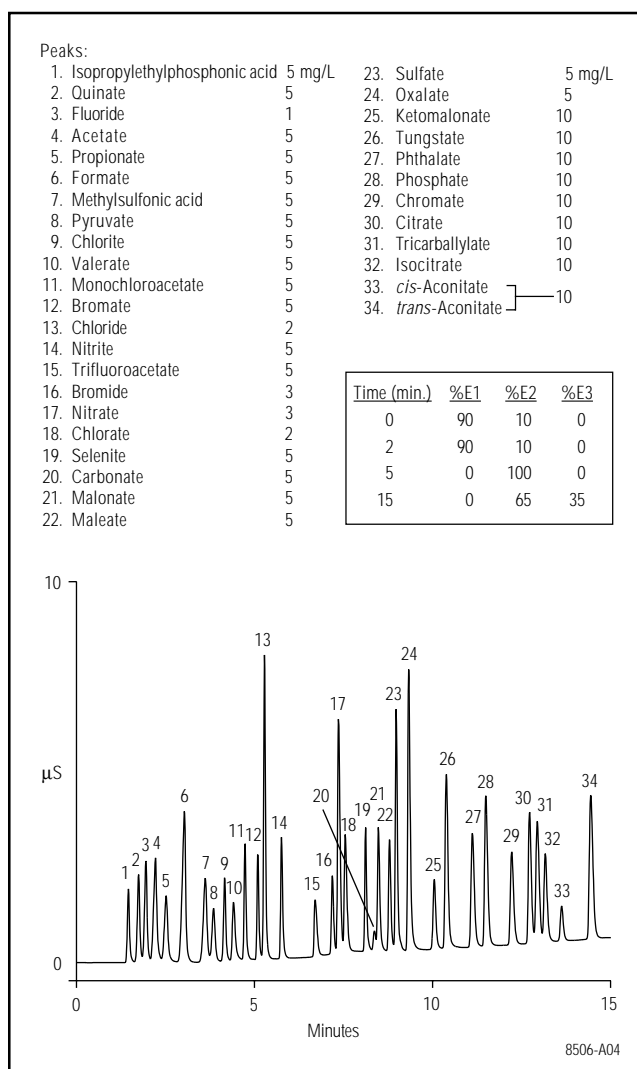


Figure 1 Inorganic and organic acid anions standards.

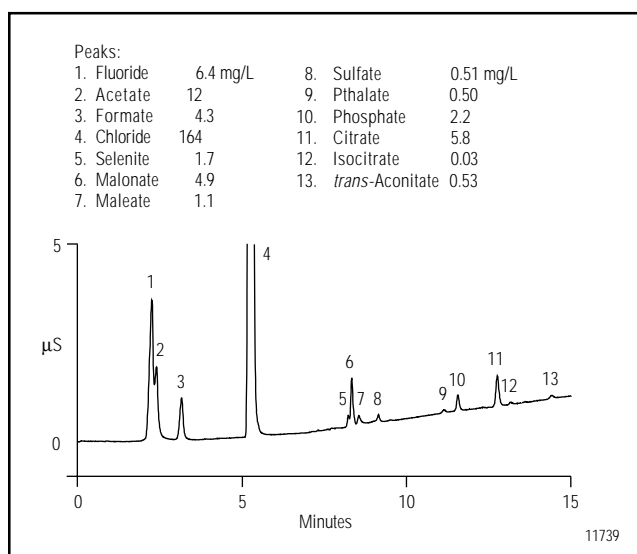


Figure 2 Separation of inorganic and organic acid anions in serum.

inorganic anions and organic acid anions from a single injection (see Figures 3 and 4). Suppression greatly enhances sensitivity by decreasing background eluent conductivity and increasing analyte conductivity. Figure 5 shows the mechanism of suppression. The ASRS removes the sodium ions (and other cations) from the eluent and replaces them with hydronium ions from the electrolysis of the water regenerant. These hydronium ions combine with the

hydroxide ions from the eluent to form water that is significantly less conductive than the sodium hydroxide eluent. Analyte conductivity is enhanced because the analyte anions associate with the more conductive hydronium ions.

The IonPac AS11 column selectivity and capacity coupled with suppressed conductivity make this method highly sensitive and selective. Tables 2 through 4 show recoveries for inorganic anions and organic acid anions in urine, plasma, and serum.

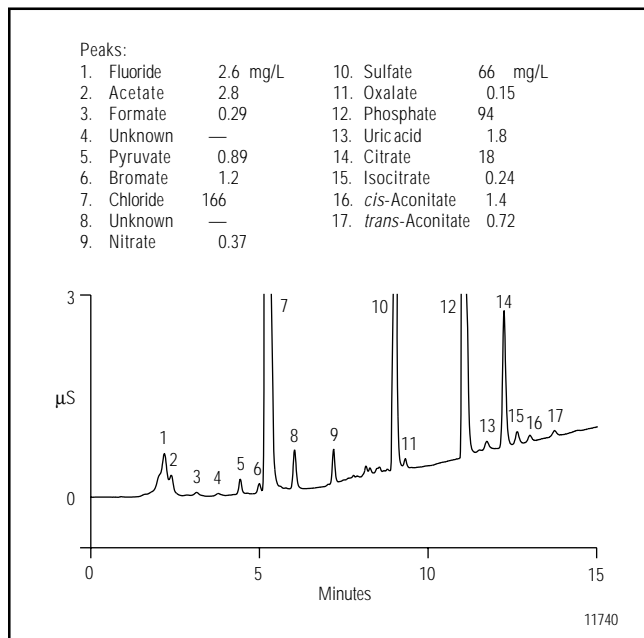


Figure 3 Determination of organic acid and inorganic anions in urine.

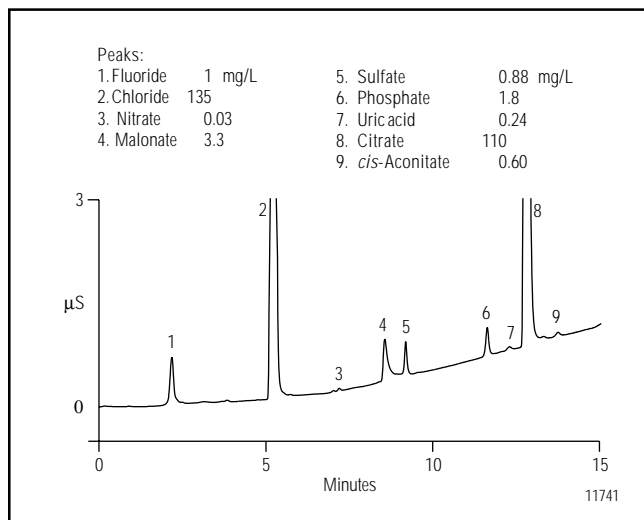


Figure 4 Separation of inorganic and organic acid anions in plasma.

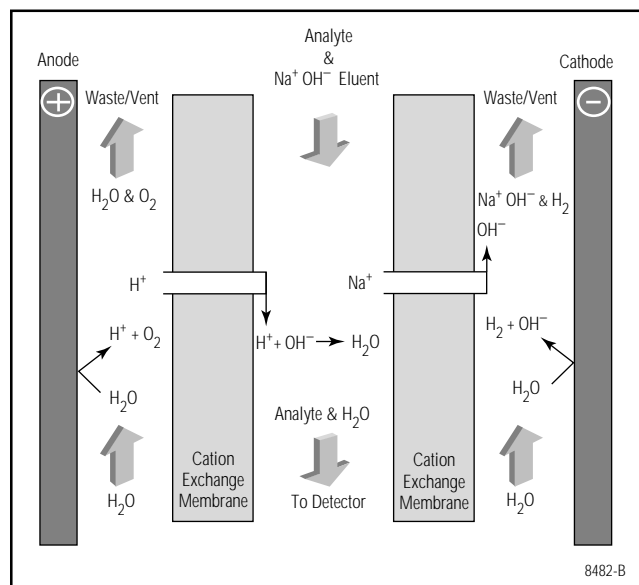


Figure 5 AutoSuppression with the Anion Self-Regenerating Suppressor (ASRS).

Table 2 Single operator precision and recovery for anions and organic acids in urine^a

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Fluoride	2.60	2.0	97	1
Acetate	2.67	2.0	98	4
Formate	0.30	1.0	95	4
Pyruvate	0.90	1.0	97	1
Bromate	1.22	2.0	98	2
Nitrate	0.37	1.0	100	1
Urate	2.5	2.0	92	1
Isocitrate	0.25	1.0	94	6
<i>cis</i> -Aconitate	1.4	2.0	97	9
<i>trans</i> -Aconitate	0.75	1.0	99	7

^a Precision and recovery data for urine sample diluted 1:25, 10- μ L sample volume injected, n = 7.

PART II: CATION ANALYSIS

CONDITIONS

Columns:	IonPac CS12A Analytical IonPac CG12A Guard
Eluent:	18 mN Sulfuric acid
Flow Rate:	1.0 mL/min
Injection Vol.:	25 µL
Detection:	Suppressed conductivity, CSRS, AutoSuppression recycle mode
Expected Background Conductivity:	<3 mS
Expected System Operating Backpressure:	8.3 MPa (1200 psi)

PREPARATION OF SOLUTIONS AND REAGENTS

1.0 N Sulfuric Acid Stock Solution

Calculate the amount (in grams) of concentrated sulfuric acid (H_2SO_4) needed to add to a 1-L volumetric flask by using the % H_2SO_4 composition stated on the label of the particular bottle of H_2SO_4 . For example, if the H_2SO_4 concentration is 98%, weigh out 50 g of concentrated H_2SO_4 . Carefully add this amount of H_2SO_4 to a 1-L volumetric flask containing about 500 mL of deionized water. Dilute to 1 L and mix thoroughly.

18 mN Sulfuric Acid

Pipet 18 mL of the 1.0 N H_2SO_4 into a 1-L volumetric flask. Dilute to 1 L using deionized water. Degas for approximately 10 minutes.

STOCK STANDARDS

Prepare 1000 mg/L (1000 ppm) stock analyte standard solutions by dissolving the amount of each salt listed in Table 1 in deionized water and diluting it to 1.000 L.

SAMPLE PREPARATION

Urine

No sample preparation is required; simply dilute with deionized water (e.g., 1:25) as desired and inject sample.

Plasma & Serum

Acetonitrile deproteinization procedure is used. Mix 0.2 mL of serum with an equal volume of acetonitrile, then centrifuge at 5000 x g for 7 minutes. Dilute 0.1 mL of the supernatant with 1.0 mL deionized water.

DISCUSSION AND RESULTS

The determination of alkali and alkaline earth metals is also of significant importance in clinical chemistry. Using the conditions provided in this application note, mono and divalent cations such as lithium, sodium, ammonium, potassium, magnesium, and calcium can be easily determined (see Figure 6). This IC method is more sensitive, specific, precise, and reliable when compared to the traditional analytical methods such as atomic absorption, flame emission, gas chromatography, and ion-selective electrodes. Cations routinely monitored in clinical chemistry include lithium, which is prescribed to control the manic

Table 3 Serum sample precision and recovery for anions and organic acids in serum^a

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Fluoride	6.30	10.0	98	2
Acetate	11.50	10.0	95	2
Formate	4.00	5.0	97	2
Selenite	1.40	2.0	92	5
Malate	4.50	10.0	94	5
Malonate	0.25	1.0	90	3
Sulfate	0.72	2.0	97	1
Pthalate	1.50	3.0	97	1
Phosphate	3.10	5.0	99	0.5
Citrate	7.50	10.0	101	0.3
Isocitrate	0.10	1.0	92	7
<i>trans</i> -Aconitate	1.70	5.0	103	1

^aPrecision and recovery data for serum sample, 10-µL sample volume injected.

Table 4 Precision and recovery for anions in plasma^b

Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Fluoride	1.02	2.0	101	0.3
Nitrate	0.10	2.0	98	0.5
Malonate	3.50	5.0	97	1
Sulfate	0.90	2.0	100	0.5
Phosphate	1.90	5.0	103	0.3
<i>cis</i> -Aconitate	0.60	2.0	95	2

^bPrecision and recovery data for plasma sample diluted 1:10, 10-µL sample injected, n = 7.

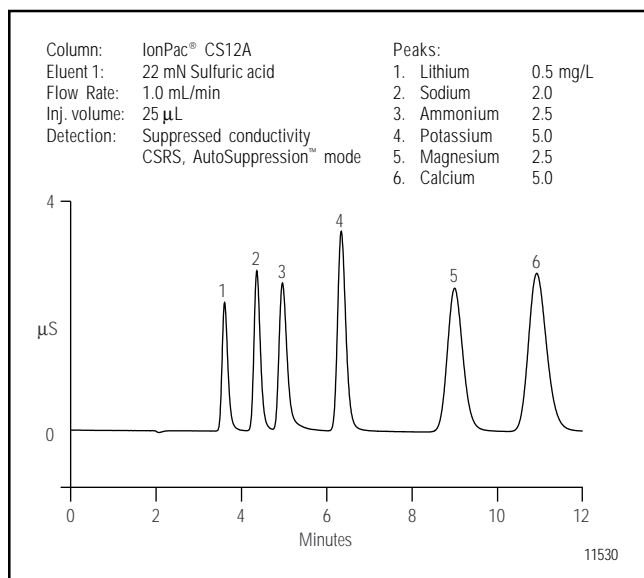


Figure 6 Determination of alkali and alkaline earth metals.

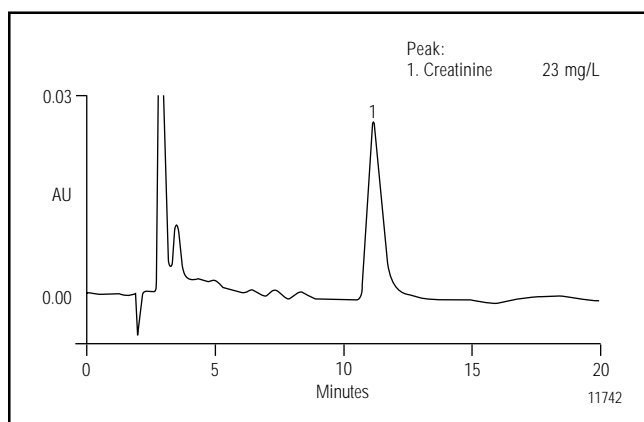


Figure 7 Determination of creatinine in urine.

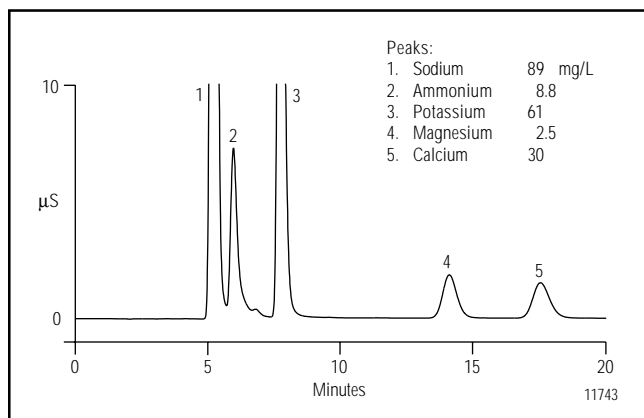


Figure 8 Separation of cations in urine.

phase of bipolar or manic depression. Ionized calcium measurement is frequently performed during major surgical procedures such as open heart operations, liver transplants, and other operations in which large volumes of blood anticoagulated with citrate are given. Decisions about providing calcium supplementation are based on ionized calcium measurements. Magnesium is another divalent cation that is frequently monitored in physiological fluids. Magnesium is monitored for hypomagnesemia and hypermagnesemia. Ammonia levels are useful in the diagnosis of Rye's syndrome because blood ammonia is often elevated before liver enzymes. Creatinine measurements are performed to estimate renal functions. Creatinine can be determined using this method with an absorbance detector set to 210 nm (see Figure 7).

The IonPac CS12A cation-exchange column used in this application provides fast, isocratic separations of lithium, sodium, ammonium, potassium, magnesium, and calcium in less than 12 minutes using a dilute sulfuric acid eluent (see Figure 6). However, in complex matrices such as physiological fluids where a high concentration of one analyte might obscure the neighboring peak, a more dilute eluent can be used. Figures 8 through 10 show the separation of cations with a more dilute eluent. The cation-exchange substrate is functionalized with carboxylic acid/phosphonic acid functional groups that permit the elution of mono and divalent cations using a dilute hydronium ion eluent such as sulfuric acid or methanesulfonic acid. The eluent is suppressed with a Cation Self-Regenerating Suppressor (CSRS) prior to conductivity detection.

The chemistry of the CSRS follows the same principles as the ASRS, except the hydroxide ions accomplish the suppression. In the CSRS, hydroxide ions generated in the cathode chamber move across the anion-exchange membrane and react with the hydronium ion in the eluent, thus forming water. Simultaneously, anions in the eluent enter the anode chamber and are removed by a continuous flow of water. Response is maximized by the association of analyte cations with the more conductive hydroxide ions (see Figure 11). As with the ASRS, the net result is a significant improvement in the signal-to-noise ratio.

Recoveries for the mono and divalent cations are listed in Tables 4 through 6 and they range from 95 to 110%. The method outlined in this application note offers the following advantages: high sensitivity (mg/L), wide linear dynamic range, high specificity, extreme ruggedness, ease of operation, and minimum sample preparation.

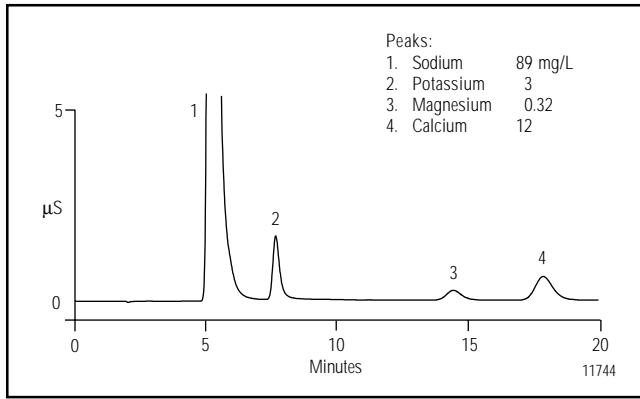


Figure 9 Determination of alkali and alkaline earth metals in plasma.

Table 5 Precision and recovery for cations in plasma ^a				
Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Sodium	85.00	50.0	105	1
Potassium	3.00	5.0	98	0.5
Magnesium	0.45	2.0	101	0.5
Calcium	12.15	10.0	98	0.5

^aPrecision and recovery data for a plasma sample diluted 1:25 with deionized water, 25- μ L sample volume injected, n = 7.

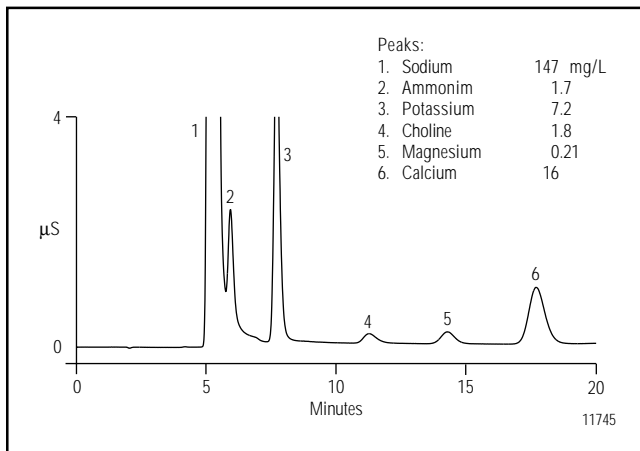


Figure 10 Determination of various cations and choline in serum.

Table 6 Precision and recovery for cations in serum ^b				
Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Sodium	150.00			1
Ammonium	1.65	5.0	95	0.3
Potassium	7.30	5.0	97	0.4
Choline	1.90	5.0	99	0.9
Magnesium	0.25	2.0	105	0.7
Calcium	15.25	10.0	97	0.5

^bPrecision and recovery data for a serum sample diluted 1:10, 25- μ L sample volume injected, n = 7.

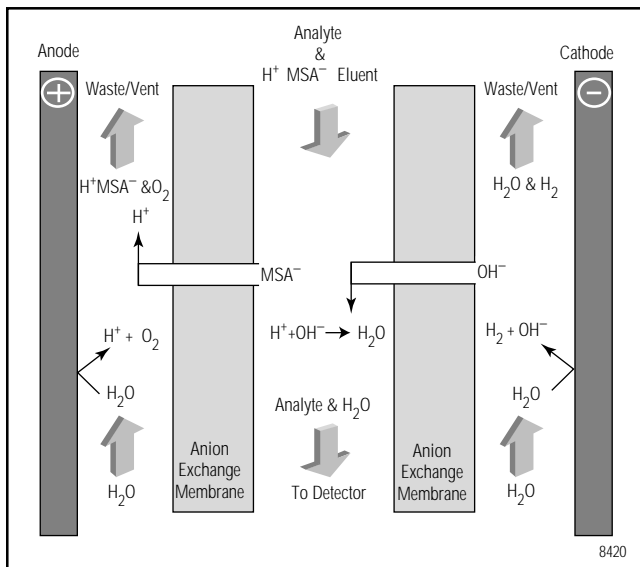


Figure 11 AutoSuppression with the Cation Self-Regenerating Suppressor (CSRS).

Table 7 Precision and recovery for cations in urine ^c				
Analyte	Amount Found (mg/L)	Amount Spiked (mg/L)	Mean Recovery (%)	RSD (%)
Sodium	89.00	50.0	110	2
Ammonium	8.80	10.0	95	0.9
Potassium	60.50	50.0	98	1
Magnesium	2.50	5.0	101	0.3
Calcium	29.50	50.0	97	0.8

^cPrecision and recovery data for a urine sample diluted 1:25, 25- μ L sample volume injected, n = 7.

PRECAUTIONS

Some physiological fluid samples may contain organic material capable of fouling an ion-exchange column; therefore, the use of a guard column is strongly recommended for this analysis. Generally, fouling of the ion-exchange resin is characterized by a gradual decrease in retention times of the analytes. If fouling occurs, the columns can be cleaned with a combination of solvent and salt. Refer to the appropriate column manual for a detailed description of the column cleanup procedure. Sodium hydroxide eluents should be stored under an inert gas at all times. Eluents should not be stored for more than a week. The expected background conductivity listed in the conditions sections should be used for troubleshooting carbonate contamination. A high conductivity background is generally associated with carbonate contamination.

REFERENCES

1. Ohsawa, K.; Yoshimura, Y.; Watanabe, S.; Tanaka, H.; Yokota, A.; Tamura, K. *Anal. Sci.*, **1986**, *2*, 165.
2. Lewis, D. *Biochem. J.* **1951**, *48*, 175–180.
3. Ishigami, K.; Inoue, K. *Res. Bull. Obihiro Univ.*, **1976**, *10*, 45–55.
4. Osweiler, G.D.; Carson, T.L.; Buck, W.B.; Van Gelder, G. *Clinical and Diagnostic Veterinary Toxicology, 3rd Ed.*, **1985**, *1A*, 460–467.
5. Bradley, W.H.; Eppson, H.E.; Beath, O.A. *J. Am. Vet. Med. Assoc.*, **1939**, *94*, 541–542.
6. Pesce, A.J., Kaplan, L.A. *Methods in Clinical Chemistry* **1987**, *5*, 27–87.

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