SHIMADZU

Application News

High Performance Liquid Chromatography SSI LC 004

Anion Analysis of Ophthalmic Irrigation Solutions with the Shimadzu Prominence IC System

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Introduction

Ophthalmic solutions suitable for eye irrigation, eye strain relief, contact lens storage and tear replacement abound in the marketplace. While some of these serve as convenient agents for administering medicines in minute quantity, many are formulated to act as cleaning agents and may contain only dissociated ions like chloride or sulfate and organic preservatives such as benzalkonium chloride. The physiologic pH of blood and tears is approximately 7.4, but a pH range of 6.5 to 8.5 will prevent damage to the cornea. This is accomplished using buffers. To prevent eye tissue damage and maximize comfort, solutions are made to be iso-osmotic (equal osmotic pressure) with tears. The common term for this is isotonic. A 0.9% NaCl solution in pure water is said to be isotonic with tears. In this application note we will aid in the quality control of such ophthalmic solutions for their content of common anions.

Conductivity detection has long been used to determine aqueous ions separated by ion exchange. Although the combination of ion exchange separation and conductivity detection is a form of HPLC, it is known as ion chromatography (IC). In its simplest form, an IC is an HPLC system equipped with an appropriate separation column and conductivity detector. Because of the inherently high conductivity of IC mobile phases, a suppressor is commonly placed between the analytical column and the detector to reduce mobile phase background conductivity and increase the analyte signal to noise ratio. Suppressed IC results in 10x greater sensitivity for anionic species when compared to non-suppressed IC.

In suppressed IC, the suppressor is a post-column reactor, frequently a strong cation exchange resin which chemically alters the column effluent and lowers the conductivity of the mobile phase. At the same time, a heightening of the conductivity of anions like chloride and sulfate occurs. With such a system, detection limits for the common anions are at the ppb level, making it suitable for the determination of anionic species in EPA-regulated drinking water or ground water supplies. The Shimadzu Prominence HPLC system, when equipped with the SeQuant IC package, yields such anion analysis results reliably and accurately. For purposes of this application note, EPA Method 300.1 parameters will serve as a guideline for the quantitation of the common anions.

SeQuant SAMS™/CARS™ system is comprised of a suppressor column (strong cation exchange resin initially in the Resin-H+ form) and chemical regeneration system. Ion exchange reactions of the suppressor with mobile phase and analyte are as follows:

$$\text{Resin-H}^+ + \text{NaHCO}_3 \leftrightarrows \text{Resin-Na}^+ + \text{H}_2\text{CO}_3$$
$$\text{Resin-H}^+ + \text{NaCl} \leftrightarrows \text{Resin-Na}^+ + \text{HCl}$$

This chemical exchange gradually depletes the resin as a protic source and it must be regenerated. This is done automatically by the CARS™ regenerant (a high-molecular weight sulfonic acid solution) which flows continually while IC analysis is being performed.

Sample Preparation

The standard test mixture of seven anions was purchased from Alltech Associates, Inc. and used without modification. Over-the-counter ophthalmic irrigation solutions were purchased from local retailers and samples were diluted 50:1 without further modification. All injection volumes were 20µL.

Analytical Conditions (Suppressed IC)

Mobile Phase:	3.2mM sodium carbonate / 1.0mM sodium bicarbonate
Column(s):	Shodex IC SI-50 4E, 4.0 x 250mm, 5µm (polyvinylalcohol gel column)
Injection Volume:	20µL
Flow Rate:	0.7mL/min.
Column Temp:	40 °C
Detector Settings:	Gain = 0.1 (100µS/V); Cell Temp = 45 °C; Response = 1

Results

Under the mobile phase conditions described above, baseline drift was less than 2nS/min. (Method 300.1 specifies drift ≤ 5nS/min.). Standards were prepared in triplicate over 5 calibration levels by dilution of stock solution (50ppm chloride):

Anion	Level 1	Level 2	Level 3	Level 4	Level 5(ppm)	R^2
Fluoride	25.0	2.50	0.500	0.250	0.050	0.9994
Chloride	50.0	5.00	1.00	0.500	0.100	0.9994
Nitrite	50.0	5.00	1.00	0.500	0.100	0.9999
Bromide	50.0	5.00	1.00	0.500	0.100	0.9998
Nitrate	50.0	5.00	1.00	0.500	0.100	0.9997
Phosphate	50.0	5.00	1.00	0.500	0.100	0.9998
Sulfate	50.0	5.00	1.00	0.500	0.100	0.9998

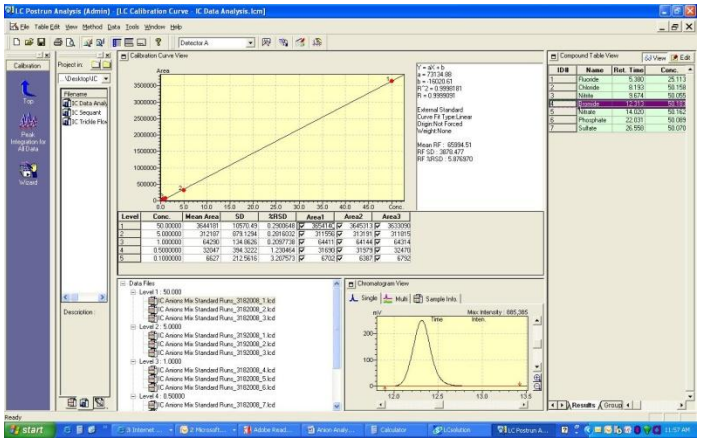
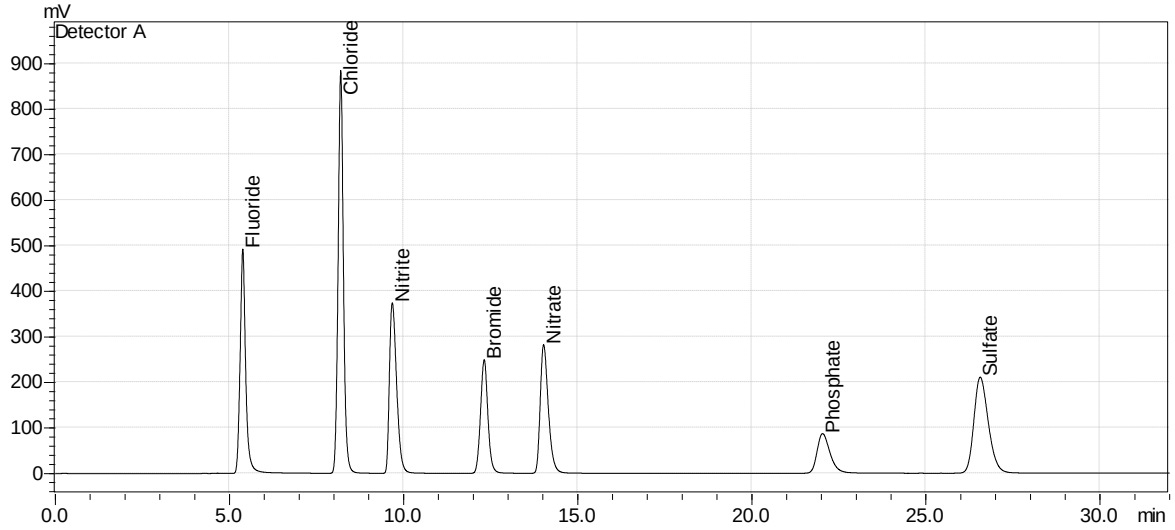
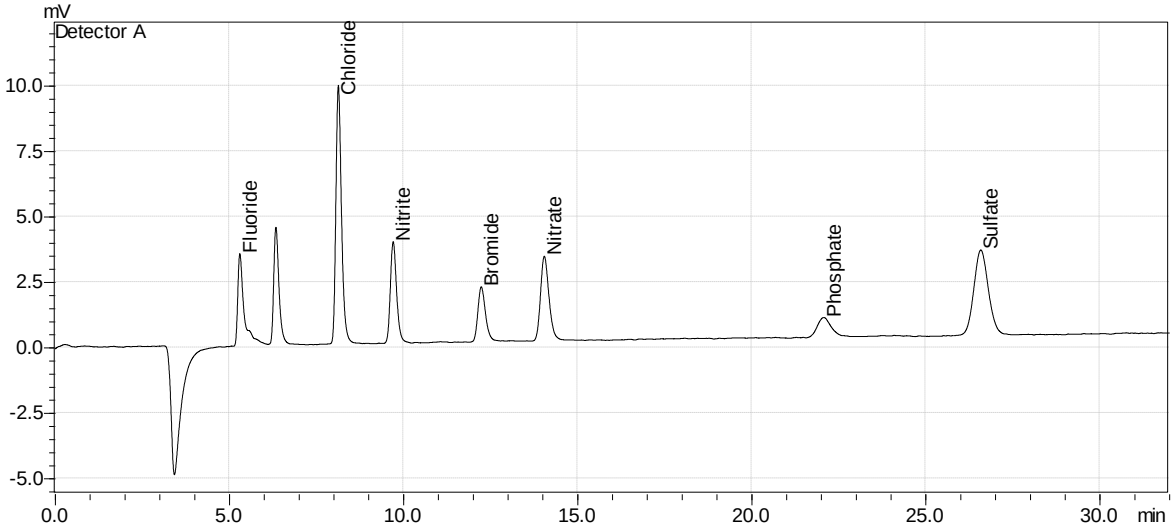


Figure 1: LCsolution calibration screen for bromide standard curve

Figure 2: Method 300.1 Anions Standard Mixture

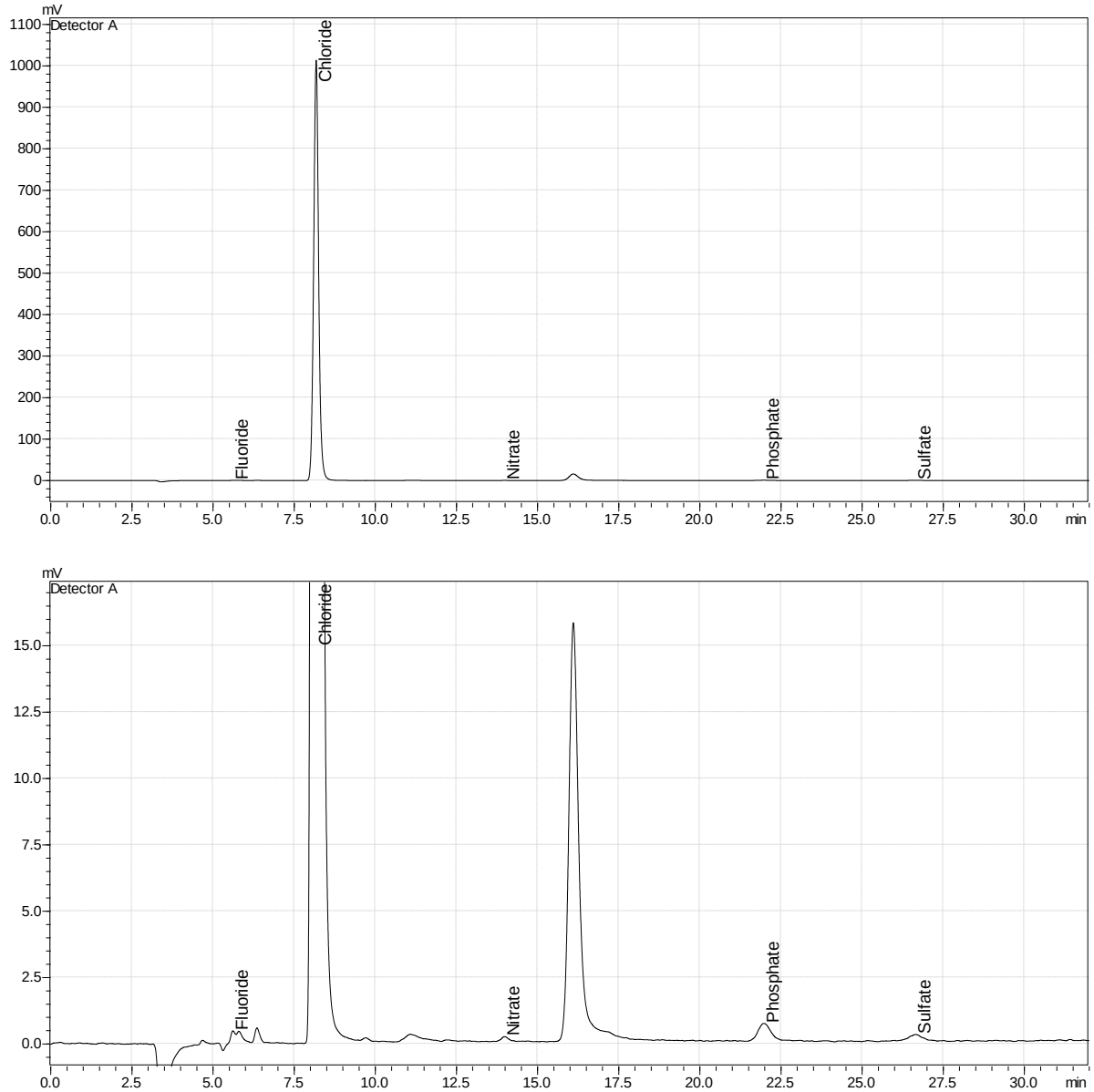


20µL injection
Fluoride 25ppm; Chloride 50ppm; Nitrite 50ppm; Bromide 50ppm; Nitrate 50ppm; Phosphate 50ppm; Sulfate 50ppm



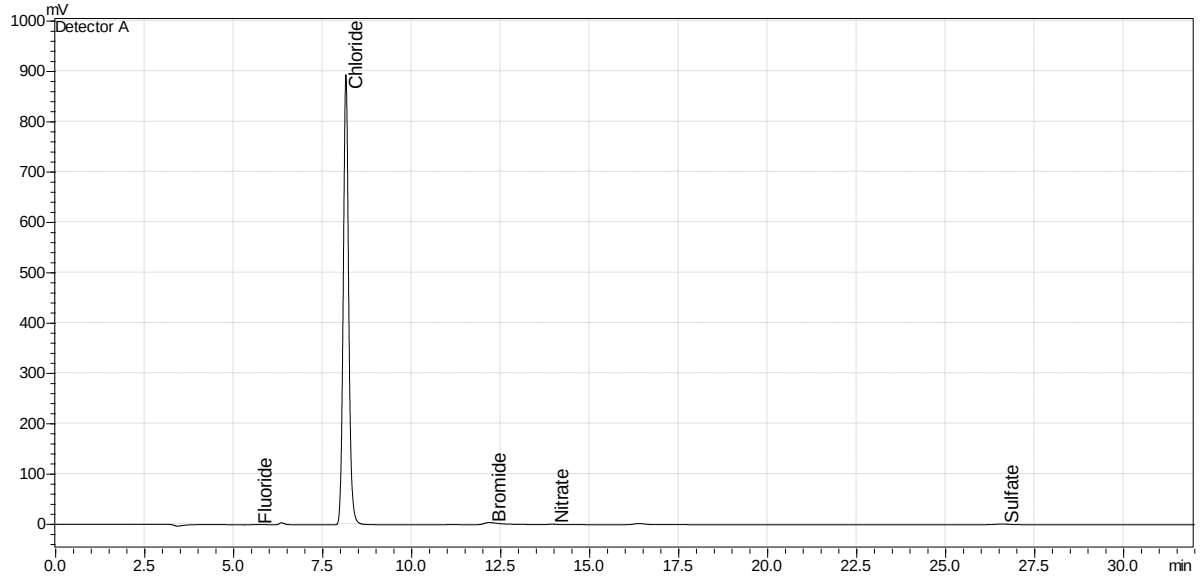
20µL injection
Fluoride 250ppb; Chloride 500ppb; Nitrite 500ppb; Bromide 500ppb; Nitrate 500ppb; Phosphate 500ppb; Sulfate 500ppb

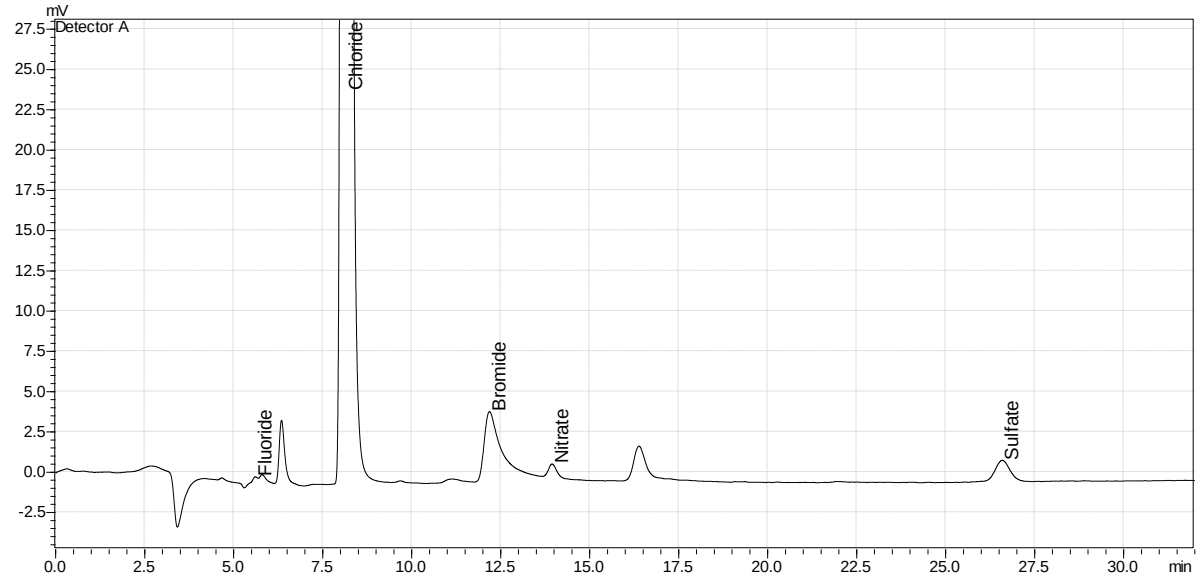
Figure 3
Eye Wash 1 (50:1 dilution)
Label Claim: hydroxylalkyl phosphonate, polyoxypropylene, EDTA, boric acid, sodium chloride, polyaminopropyl biguanide



Analyte	RT	Estimated Conc. (ppm)	Corrected Conc. (ppm)
Fluoride	--	0.000	0.000
Chloride	8.176	57.47	2874
Nitrite	--	0.000	0.000
Bromide	--	0.000	0.000
Nitrate	13.973	0.124	6.200
Phosphate	21.976	0.603	30.15
Sulfate	26.644	0.107	5.350

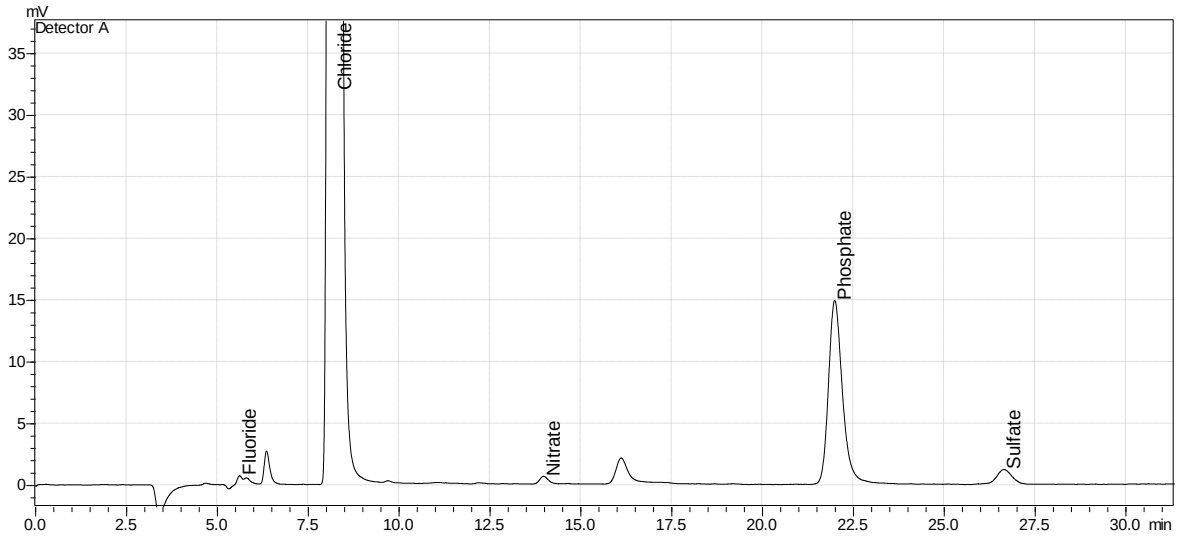
Eye Wash 2 (50:1 dilution)
Label Claim: Sodium chloride, sodium borate, boric acid





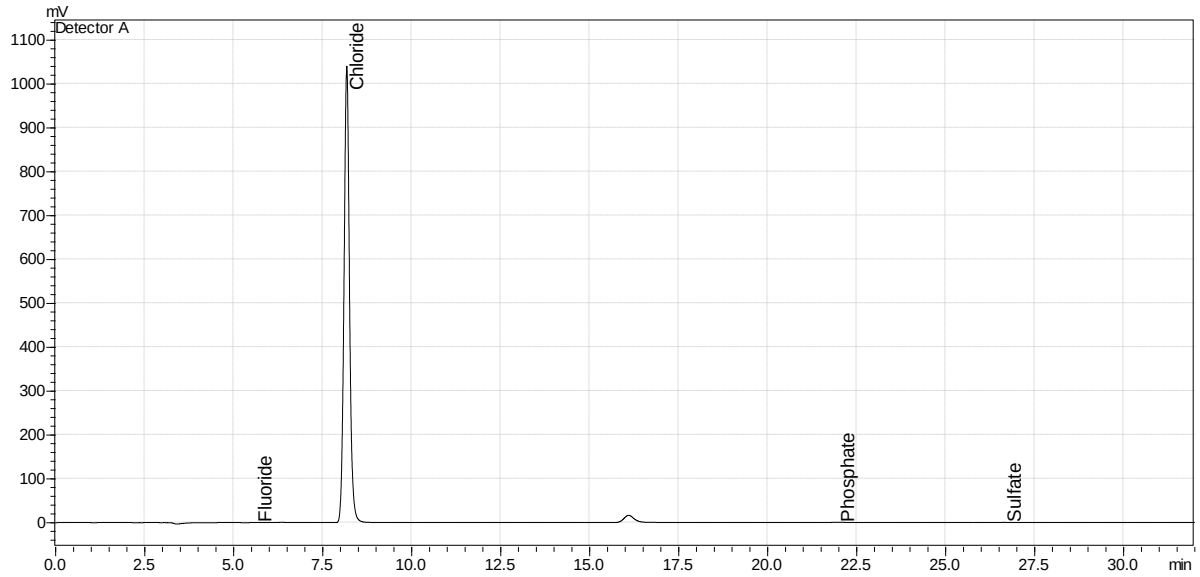
Analyte	RT	Estimated Conc. (ppm)	Corrected Conc. (ppm)
Fluoride	5.607	0.044	2.200
Chloride	8.152	50.51	2526
Nitrite	--	0.000	0.000
Bromide	12.185	2.403	120.2
Nitrate	13.943	0.316	15.80
Phosphate	--	0.000	0.000
Sulfate	26.583	0.176	8.800

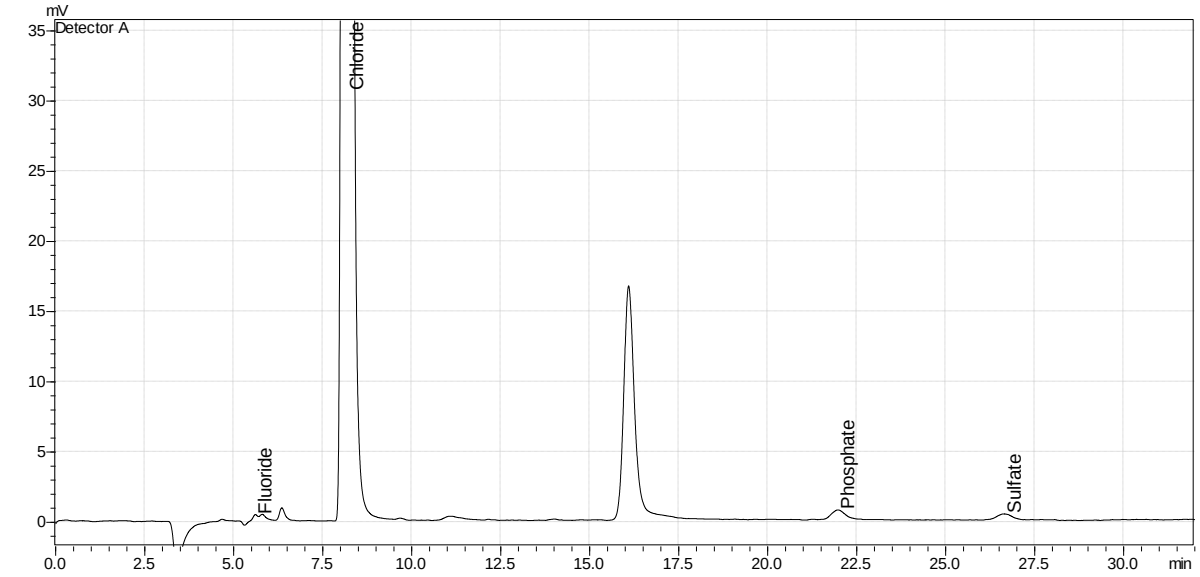
Eye Wash 3 (50:1 dilution)
Label Claim: Buffering soln., polyhexamethylene biguanide, sodium phosphate



Analyte	**RT**	**Estimated Conc. (ppm)**	**Corrected Conc. (ppm)**
Fluoride	--	0.000	0.000
Chloride	8.234	90.99	4550
Nitrite	--	0.000	0.000
Bromide	--	0.000	0.000
Nitrate	13.974	0.206	10.30
Phosphate	21.983	8.990	449.5
Sulfate	26.637	0.139	9.500

Eye Wash 4 (50:1 dilution)
Label Claim: Polyoxamine, sodium hydranate, polyaminopropyl biguanide, boric acid, EDTA, sodium borate, sodium chloride





Analyte	RT	Estimated Conc. (ppm)	Corrected Conc. (ppm)
Fluoride	5.611	0.060	3.000
Chloride	8.175	59.30	2965
Nitrite	--	0.000	0.000
Bromide	--	0.000	0.000
Nitrate	--	0.000	0.000
Phosphate	21.974	0.623	31.15
Sulfate	26.655	0.045	2.250

System Configuration

Part #	Description
220-91398-20	CBM-20A System Controller
228-45019-32	DGU-20A5 On-Line Degasser
228-45000-32	LC-20AD Pump
220-91397-02	SIL-20A Autosampler
228-45010-32	CTO-20AC Column Oven
228-45054-32	CDD-10Avp Conductivity Detector
220-97205-44	Windows XP Pro Data Station
220-91449-05	LCsolution 1.23 software
221-99999-G1	SeQuant (SAMS™/CARS™) Anion Suppressor & Regeneration Station

References

1. General guide to pharmaceutical formulations, Univ. of North Carolina School of Pharmacy, Chapel Hill, NC 2006.
2. Raaidah Saari-Nordhaus and James M. Anderson Jr., 'Recent Advances in Ion Chromatography Suppressor Improve Anion Separation and Detection', J. of Chromatogr. A, 956 (2002) 15.
3. Derick R. Douglas, Raaidah Saari-Nordhaus, Philippe Despres, and James M. Anderson, Jr., New Suppressor Technology Improves Trace Level Anion Analysis with Carbonate/Bicarbonate Mobile Phases', J. of Chromatogr. A, 956 (2002) 47.
4. Rakesh Bose, Raaidah Saari-Nordhaus, and James M. Anderson Jr., 'Solving Complex Anion Separation with carbonate/Bicarbonate Gradient', J. of Chromatogr. A, 956 (2002) 71.

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