DIONEX

Application Note 116

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Quantification of Anions in Pharmaceuticals

INTRODUCTION

The United States Food and Drug Administration (U.S. FDA)[1–3] and regulatory agencies in other countries require that pharmaceutical products be tested for composition to verify their identity, strength, quality, and purity, with increased attention to inactive as well as active ingredients. Analytical techniques that can adequately test complex formulations composed of chromophoric and nonchromophoric ingredients are desirable. Nonchromophoric ingredients, many of which are ionic, cannot be detected by absorbance, but can be detected using suppressed conductivity. Suppressed conductivity is a powerful detection technique with a broad linear range and very low detection limits. Suppression lowers the background conductivity caused by the eluent and effectively increases the conductivity of the analyte.[4–5]

This Application Note describes the use of two anion-exchange columns with suppressed conductivity detection to analyze common anions in pharmaceutical formulations. Two oral, over-the-counter medications were selected as representative pharmaceutical products: a cough suppressant and a multisymptom cold/flu medication. These formulations contain complex mixtures of ingredients that are commonly found in other medications, many of which are ionic and nonchromophoric. Furthermore, these formulations also contain sugar alcohol, glycol, and carbohydrate ingredients that can be analyzed using the IonPac® ICE-AS1, CarboPac™ MA1, and CarboPac PA10 columns with electrochemical detection.[6]

In the methods outlined in this Note, the selectivities of the IonPac AS14 and AS11 columns for the analysis of anionic ingredients in pharmaceutical formulations were compared. The AS14 packing has a highly crosslinked core with an anion-exchange layer grafted to the surface. The anion-exchange layer is functionalized with alkyl quaternary ammonium functional groups and is grafted to crosslinked ethylvinylbenzene. This anion-exchange resin is selective for the more hydrophobic anions. The AS11 column packing has a pellicular structure consisting of an alkyl quaternary ammonium latex bonded to a crosslinked ethylvinylbenzene core. Another important difference between the two columns is that the AS14 column is designed for isocratic conditions, while the AS11 can be used with hydroxide gradients. Both columns are compatible with eluents containing organic solvents, which can be used to reduce undesirable secondary interactions some organic anions have with stationary phases. Expected detection limits, linearity, selectivity, accuracy, and precision are reported for the AS11 column.

EQUIPMENT

Dionex DX-500 system consisting of:

- GP40 Gradient Pump, with degas option
- CD20 Conductivity Detector or
  ED40 Electrochemical Detector
- LC30 or LC25 Chromatography Ovens or
  LC20 Chromatography Module
- AS3500 Autosampler

PeakNet Chromatography Workstation

REAGENTS AND STANDARDS

Reagents

Sodium carbonate, 0.5 M (Dionex P/N 37162)

Sodium bicarbonate, 0.5 M (Dionex P/N 37163)

Sodium hydroxide, 50% (w/w) (Fisher Scientific, P/N SS254-500)

Deionized water, 18 MΩ-cm resistance or higher (Prior to use, Dionex recommends testing the water for trace anions using the intended ion chromatography method.)

Standards

Five Anion Standard (fluoride, chloride, nitrate, phosphate, sulfate; Dionex P/N 37157)

Sodium acetate, anhydrous (Fluka Biochemika, P/N 71179)

Sodium bromide (Aldrich Chemical Co., P/N 31,050-6)

Sodium chloride (Fisher Scientific, P/N S271-500)

Sodium citrate, monohydrate (Fisher Scientific, P/N A104-500)

Sodium benzoate (Sigma Chemical Co., P/N B-3375)

Saccharin (Sodium salt; Sigma Chemical Co., P/N S-1002)

CONDITIONS

System 1

Column:	IonPac AS14 Analytical (P/N 46124), IonPac AG14 Guard (P/N 46134)
Eluent:	3.5 mM Sodium carbonate 0.8 mM Sodium bicarbonate
Flow Rate:	1.2 mL/min
Injection Vol.:	10 µL
Detection mode:	Suppressed conductivity, ASRS™, AutoSuppression™ recycle mode
Expected Background Conductivity:	15 µS
Expected System Operating Backpressure:	12.4 MPa (1800 psi)

System 2

Column:	IonPac AS11 Analytical (P/N 44076), IonPac AG11 Guard (P/N 44078), ATC-1 Anion Trap Column (P/N 37151)
Eluent:	Linear sodium hydroxide gradients: 0.5 mM Sodium hydroxide for 2.5 min, then 0.5 to 5 mM sodium hydroxide for 3.5 min, then 5 to 38 mM sodium hydroxide for 12 min, then 0.5 mM sodium hydroxide for 7 min.
Flow Rate:	2.0 mL/min
Injection Vol.:	10 µL
Detection mode:	Suppressed conductivity, ASRS, AutoSuppression recycle mode
Expected Background Conductivity:	0.5 µS
Expected System Operating Backpressure:	12.4 MPa (1800 psi)

PREPARATION OF SOLUTIONS AND REAGENTS

Sodium carbonate/bicarbonate eluent

3.5 mM Sodium carbonate/0.8 mM Sodium bicarbonate

Combine 1980 mL of deionized water with 14.0 mL of 0.5 N sodium carbonate and 3.2 mL of 0.5 N sodium bicarbonate. Degas for 20 minutes. Connect the eluent reservoir to the instrument and pressurize with helium.

Sodium hydroxide eluents

5 mM Sodium hydroxide

Degas 2000 mL of deionized water for 20 min and combine with 520 µL of 50% (w/w) sodium hydroxide.

100 mM Sodium hydroxide

Degas 1990 mL of deionized water for 20 min and combine with 10.4 mL of 50% (w/w) sodium hydroxide.

STOCK STANDARDS

Combine the Five Anion Standard (fluoride, chloride, nitrate, phosphate, sulfate; Dionex P/N 37157) with acetate, bromide, citrate, benzoate, and saccharin standards and purified water to yield stock concentrations of:

Fluoride	1.6 mg/L	Phosphate	12 mg/L
Acetate	9.8 mg/L	Chloride	2.4 mg/L
Nitrate	8.0 mg/L	Citrate	10 mg/L
Bromide	9.8 mg/L	Benzoate	10 mg/L
Sulfate	12.0 mg/L	Saccharin	100 mg/L

Dilute the stock solution with water to the desired concentrations.

For determinations of linear range, combine 10-g/L solutions of chloride, bromide, benzoate, citrate, and saccharin to make a 1.0-g/L solution of standard mix. Dilute with water to concentrations of 0.8, 0.6, 0.4, 0.2, 0.1, 0.08, 0.06, 0.04, 0.02, 0.01, 0.005, and 0.001 g/L. Dilute saccharin and citrate separately to evaluate these analytes without interference using concentrations of 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 g/L.

SAMPLE PREPARATION

Dilute viscous products with water on a weight per weight basis. A 10-fold (w/w) dilution was obtained by combining 1 gram of medication with 9 grams of water. The multisymptom cold/flu medication was further diluted to a 100-fold (w/w) final concentration. Determine the densities of the products by measuring the weights of known volumes. Calculate the final concentrations of the ingredients based on the densities of these medications. The ingredients of each medication are presented in Tables 1 and 2. The ingredients noted in bold-face type can be analyzed by anion-exchange columns with suppressed conductivity; other ingredients listed can be analyzed using CarboPac columns with electrochemical detection.[6]

Table 1 Cough suppressant

Dextromethorphan Hydrobromide	Active
Citric Acid	Inactive
FD&C Red 40	Inactive
Flavors	Inactive
Glycerin (glycerol)	Inactive
Propylene Glycol	Inactive
Saccharin Sodium	Inactive
Sodium Benzoate	Inactive
Sorbitol	Inactive
Water	Inactive

Table 2 Multisymptom cold/flu medication

Pseudoephedrine Hydrochloride	Active
Acetaminophen	Active
Dextromethorphan Hydrobromide	Active
Citric Acid	Inactive
FD&C Yellow #6	Inactive
Flavor	Inactive
Glycerin (glycerol)	Inactive
Polyethylene Glycol	Inactive
Propylene Glycol	Inactive
Purified Water	Inactive
Saccharin Sodium	Inactive
Sodium Citrate	Inactive
Sucrose	Inactive

Any purified water used for dilutions should be tested for trace anions by ion chromatography prior to use. Sample containers should be tested for residual anions prior to use by adding pure water, shaking or vortexing, and then testing the liquid. Plastic vials are usually lower in residual anions than glass. Prerinsing the vials with purified water can reduce artifacts.

DISCUSSION AND RESULTS

Selectivity

Figure 1 shows the separation of fluoride, acetate, chloride, nitrate, phosphate, and sulfate standards using a 3.5 mM sodium carbonate/0.8 mM sodium bicarbonate eluent with the IonPac AS14 column. The isocratic conditions eliminate any need to reequilibrate the column, thereby decreasing run times and increasing throughput. These anions can be analyzed within 14 minutes. Citrate, benzoate, and saccharin are not eluted by this method.

With the IonPac AS11 column, inorganic anions, acetate, citrate, benzoate, and saccharin are eluted using a sodium hydroxide gradient (see Figure 2). Although the gradient results in slightly longer run times, both organic and inorganic anions are effectively separated. Shorter run times are possible for either method by adjusting the eluent strength, but some resolution may be lost for fluoride and acetate.

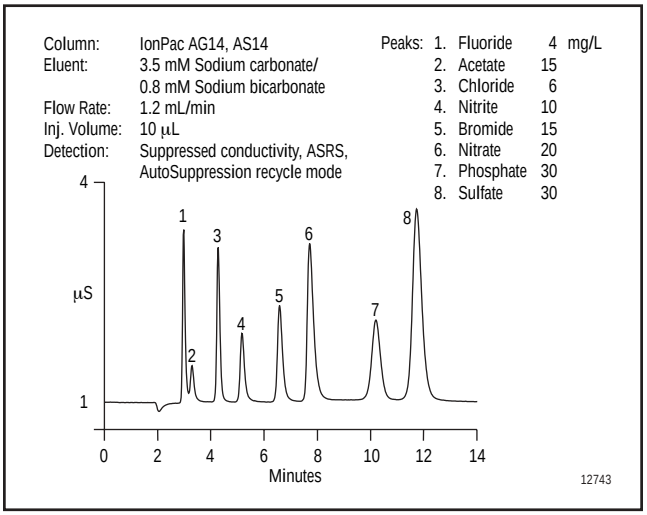

Figure 1 *Rapid separation of inorganic anions.*

Saccharin retention times significantly shorten above 1000 ng per injection, causing coelution with citrate. Saccharin retention depends on secondary interactions with the column. At high concentrations, saccharin exceeds the capacity of the column and may elute earlier

than at lower concentrations. When both saccharin and citrate coexist in a formulation, they both should be adjusted to below 100 mg/L for a 10-µL injection. Saccharin peaks tail, but the addition of organic solvents in the eluent (e.g., 10% acetonitrile) reduces tailing. Addition of acetonitrile alters the column selectivity, decreases peak area response, and increases background noise.

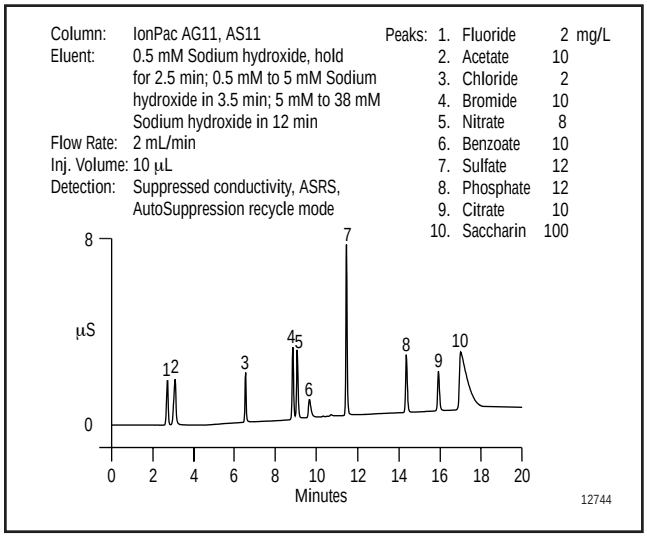

Figure 2 *Gradient separation of both inorganic and organic anions.*

Although not presented here, nearly a dozen over-the-counter medications have been analyzed using the IonPac AS14 and AS11 columns with suppressed conductivity. These medications include both solid and liquid formulations such as nasal and oral decongestants, astringents, antacids, enemas, sleep aids, analgesics, cleaning and disinfecting solutions, antihistamines, and allergy syrups. The known anionic ingredients in each formulation were separated from each other without any apparent interference. Trace levels of unlabeled ingredients were also detected as minor peaks and, when identified, measured at low concentrations.

Method Detection Limits

The method detection limits (MDL) for a 10-µL injection of common pharmaceutical anions with the AS11 column are described in Table 3. The MDL is defined as the minimum concentration required to produce a signal-to-noise ratio of 3. The MDL can be further decreased by increasing the injection volume above the 10 µL used in this Application Note or by using external water mode suppression.[7]

Table 3 Estimated lower detection limits

	System 2 (IonPac AS11 column)	
	ng	µg/L
Fluoride	0.3	30
Acetate	2	200
Chloride	0.5	50
Bromide	4	400
Nitrate	3	300
Sulfate	1	100
Phosphate	4	400
Citrate	4	400
Benzoate	7	700
Saccharin	20	2000

Linearity

Chloride, bromide, benzoate, citrate, and saccharin standards ranging from 1 to 1000 mg/L (10 ng to 10,000 ng) were injected (in triplicate) on the AS11 column (data not shown). The peak area response was found to be linear for chloride, bromide, citrate, and saccharin over this range ($r^2 \geq 0.999$). Benzoate was linear over the range of 1 to 200 mg/L (10 ng to 2,000 ng per injection; $r^2 = 0.999$). For the range of 1 to 1000 mg/L (10 ng to 10,000 ng), the correlation coefficient for benzoate was 0.995. Although linearity is good for citrate and saccharin when run independently, the two peaks coelute at concentrations above 100 mg/L (1000 ng). Therefore, linearity cannot be evaluated when both compounds are present at this concentration. For all analytes, linearity was demonstrated over at least two orders of magnitude.

Representative calibration curves for both the AS14 and the AS11 columns are presented in Figures 3 and 4.

Precision

The peak area and retention time RSDs using the AS11 column are presented in Table 4. Area precision is affected by concentration; RSD values increase as the concentrations approach the MDL.

Replicate, 10-µL injections (n = 10) of a 10-fold (w/w) dilution of cough suppressant yielded a bromide peak area RSD of 0.5% when measured at 30 µg/mL. A 100-fold (w/w) dilution of the multisymptom cold/flu medication resulted in a bromide RSD value of 1.0% when measured at 1 µg/mL for 10-µL injections; chloride was 0.3% at 3 µg/mL for 10-µL injections. The retention time RSD values ranged from 0.1% to 0.2% for these anions.

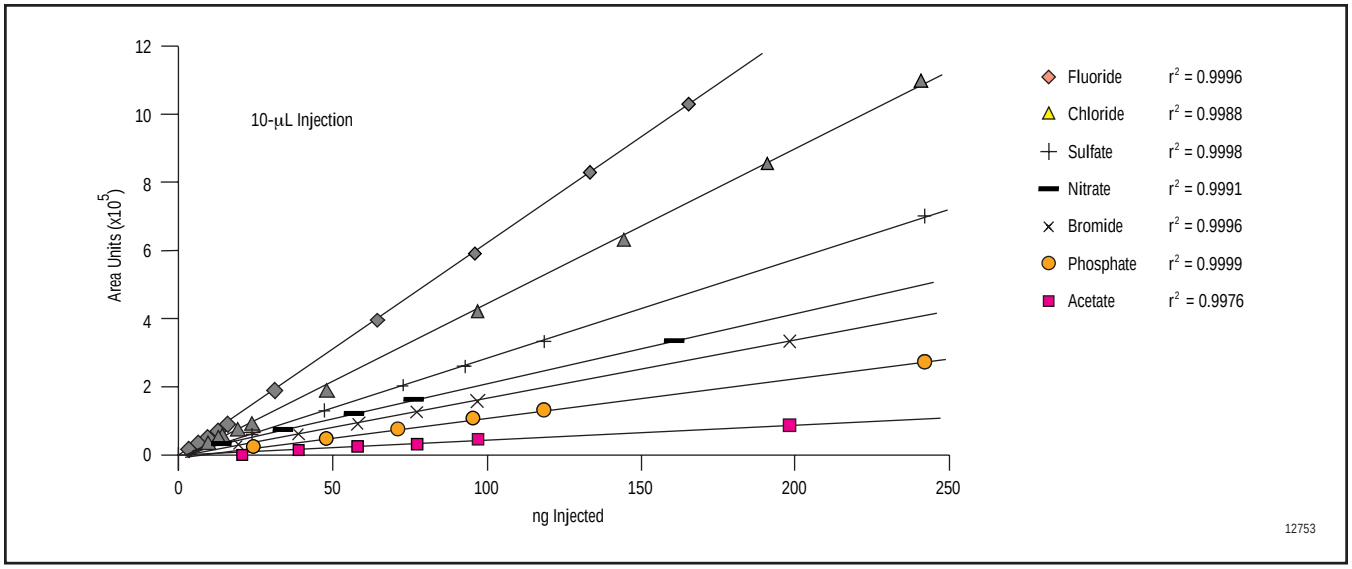

Figure 3 *Method linearity for the IonPac AS14 column (isocratic method).*

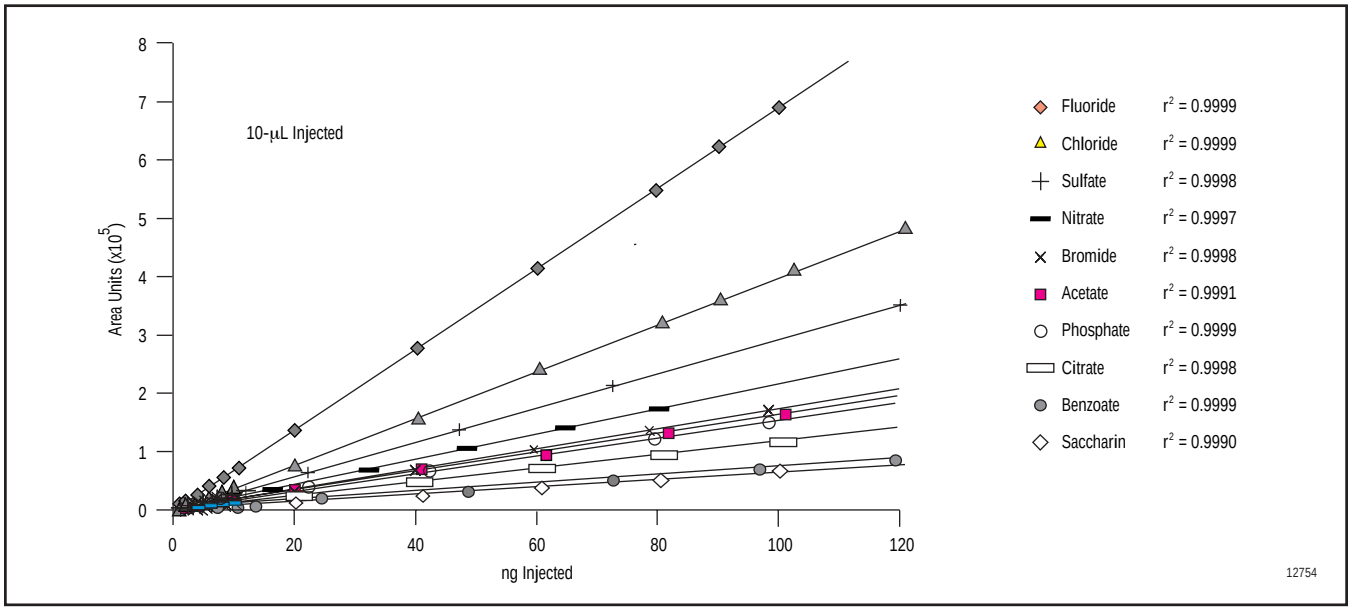

Figure 4 *Method linearity for the IonPac AS11 column (gradient method).*

Table 4 Peak area and retention time precision*

	RSD (%)	
	Area	Retention Time
Chloride	0.1	0.2
Bromide	0.1	0.1
Benzoate	0.2	0.1
Citrate	0.3	0.4
Saccharin	0.4	0.3

* 100-mg/L standards, 10 µL per injection (n = 15).

Table 5 Peak area and precision of pharmaceutical products*

Analyte	Measured Conc. (mg/L)	RSD (%)	
		Cough Suppressant	Cold/Flu Medication
Bromide	30	0.5	–
Bromide	1	–	1.0
Chloride	3	–	0.3

* 10-fold (w/w) dilutions of cough suppressant, 100-fold (w/w) dilutions of cold/flu medication, 10-µL injection (n = 10).

Table 6 Recovery of active ingredient counterions in medications		
	Dextromethorphan hydrobromide	Pseudoephedrine hydrochloride
Cough suppressant	98.9%	N/A
Multisymptom cold/flu formulation	97.3%	109%

These sample RSDs are summarized in Table 5 and are consistent with those found for standards analyzed at equivalent concentrations.

Recovery from Sample Matrix

Figures 5 and 6 show the separation of bromide from other ingredients in cough suppressant using the AS14 and AS11 columns, respectively. Bromide is the counterion in the active ingredient dextromethorphan hydrobromide. Figures 7 and 8 show the separation of both bromide and chloride from other ingredients in a multisymptom cold/flu formulation. Chloride is the counterion in pseudoephedrine hydrochloride. These anions are unique to these active ingredients in both medications; therefore, their quantification is an orthogonal method for the determinations of dextromethorphan and pseudoephedrine in these formulations.

The measured levels of these anions using the AS11 column are compared to the labeled values of these active ingredients in each formulation and expressed as a percent recovery (see Table 6). In all cases, the levels of bromide and chloride were within 10% of the label value. This orthogonal method for quantification of an active ingredient in a pharmaceutical can be a valuable troubleshooting tool. For example, during stability studies, samples can either change in concentration or components can decompose. Analysis of both the organic component and the inorganic counterion can serve to differentiate between these symptoms.

The dosage of inactive ingredients is not specified on the label of most medications. Therefore, it was not possible to evaluate the accuracy of the formulation for benzoate, citrate, and saccharin. However, these ingredients were quantified (see Figures 6 and 8).

Other Anions in Pharmaceutical Products

Besides the labeled content of the pharmaceutical products, other anionic ingredients may also be present. These may arise from trace levels of ingredients found in the raw materials used in manufacturing. Expanding the baseline of the above chromatograms (Figures 6B and 8B) reveals the presence of minor peaks. Some can be identified based on retention time, while others cannot. Sulfate (2 μg/mL) and phosphate (1 μg/mL) were measured in the cough suppressant. Five other minor peaks were observed but were not identified. Sulfate (13 μg/mL) was measured in the multisymptom cold/flu formulation, while three minor peaks were unidentified.

CONCLUSION

Using the IonPac AS11 column with suppressed conductivity detection, pharmaceutical formulations can be analyzed for both organic and inorganic anions. In the same injection, the IonPac AS11 resolves common inorganic anions such as fluoride, chloride, bromide, sulfate, nitrate, and phosphate, as well as common organic anions such as benzoate, sorbate, citrate, and saccharin. The IonPac AS14 resolves common inorganic anions, but is not suitable for common organic anions (except acetate). The AS14 column uses an isocratic eluent and is therefore faster for this analysis. Suppressed conductivity eliminates potential interferences from the nonionic ingredients in the formulation and provides a sensitive means to detect nonchromophoric analytes. Inorganic anions can be detected at the 30 to 400-μg/L levels, while organic anions can be detected at the 200 to 2000-μg/L levels. Both organic and inorganic anions were linear over more than two orders of magnitude. The recovery of chloride and bromide from pharmaceutical formulations was greater than 97% of the labeled concentrations for the active ingredients. Both methods are also well suited for evaluating trace levels of anionic contaminants.

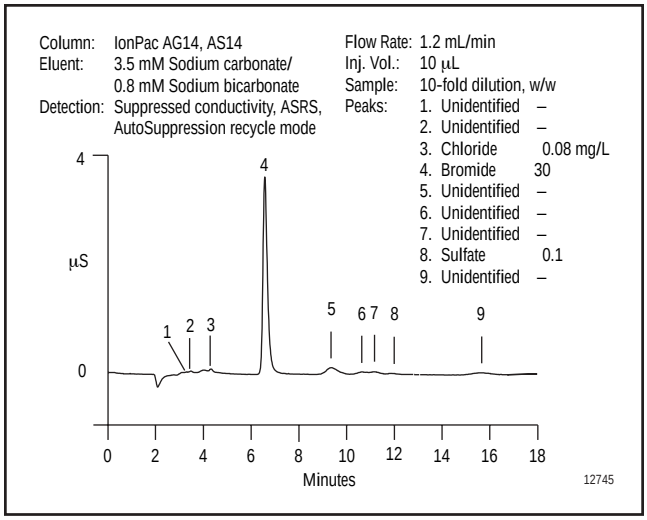

Figure 5 *Counterions in cough suppressant.*

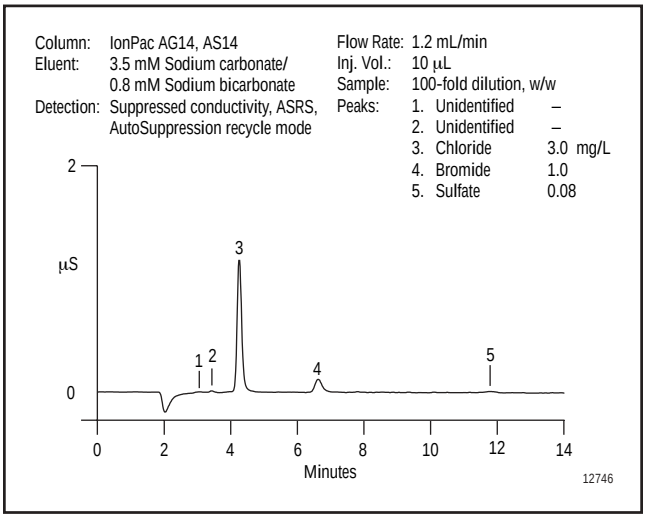

Figure 7 *Counterions in multisymptom cold/flu medication.*

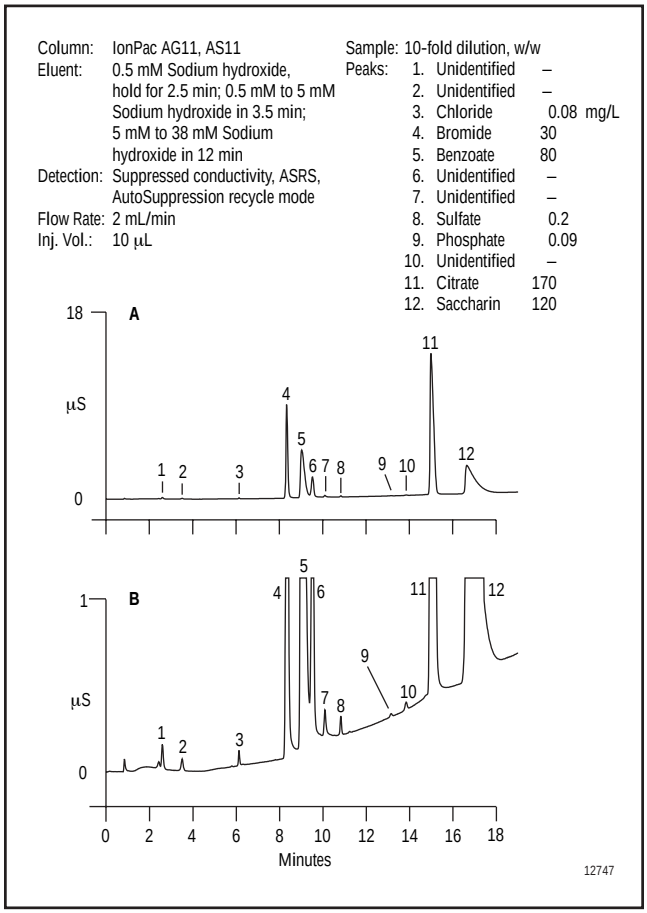

Figure 6 *Anionic ingredients in cough suppressant.*

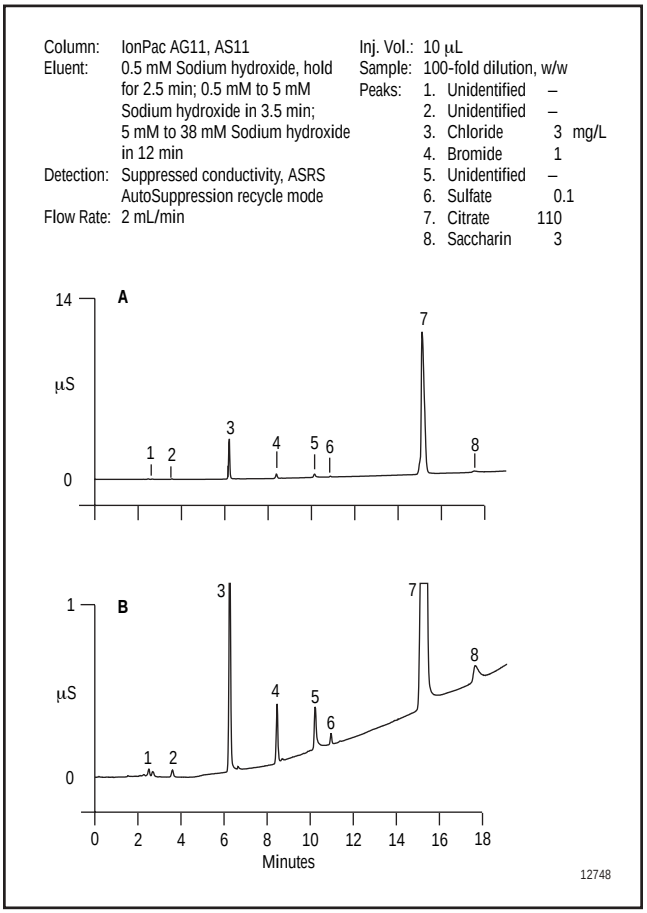

Figure 8 *Anionic ingredients in multisymptom cold/flu medication.*

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2. CFR Title 21, Foods and Drugs, Chapter 1, FDA, I Part 211.160, “General requirements.”
3. CFR Title 21, Foods and Drugs, Chapter 1, FDA, I Part 211.165, “Testing and release for distribution.”
4. Dionex Corporation, “Ion Chromatography in the Pharmaceutical Industry”, Application Note 106.
5. Rabin, S.; Stillian, J.R.; Barreto, V.; Friedman, K.; and Toofan, M. *J. Chromatogr.* **1993**, *640*, 97–109.
6. Dionex Corporation, “Quantification of Carbohydrates and Glycols in Pharmaceuticals”, Application Note 117.
7. Dionex Corporation, “Determination of Trifluoroacetic Acid (TFA) in Peptides”, Application Note 115.

LIST OF SUPPLIERS

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LPN 0924-01 11/02

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