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# Determination of Anions in Toothpaste by Ion Chromatography

## INTRODUCTION

The U.S. Food and Drug Administration has approved the use of three decay-preventing compounds in toothpaste: stannous fluoride, sodium fluoride, and sodium monofluorophosphate (MFP). All three compounds are derived from hydrofluoric acid that is produced by reacting sulfuric acid with fluor spar, a calcium fluoride-rich ore.<sup>1</sup> Regardless of the source, fluoride is well known to have a critical role in preventing tooth decay. However, fluoride can react with other ingredients in toothpaste to form insoluble compounds, and therefore be unavailable to fulfill its therapeutic function.<sup>2</sup> MFP can hydrolyze to free fluoride and phosphate ions during storage.<sup>3</sup> Therefore, the determination of fluoride and MFP in toothpastes is important to evaluate the quality and stability of these cavity-preventing compounds.

Several techniques have been used to determine fluoride, MFP, and other phosphates in dental care products, including gas chromatography (GC), ion-selective electrodes, and colorimetry.<sup>4-6</sup> These techniques measure the amount of MFP indirectly by either sample derivatization followed by GC analysis, or acid hydrolysis followed by a fluoride determination with a calorimetric or ion-selective electrode. These procedures are subject to interferences, have poor reproducibility, and are very labor intensive. Ion chromatography (IC) provides a convenient and simple method for the direct determination of fluoride and MFP in a single analysis. In this application note, we describe the use of electrolytic eluent generation combined with a high-capacity, hydroxide-selective, anion-exchange column—the IonPac® AS18—for the determination of fluoride, MFP, and other inactive anionic ingredients in different toothpastes.

## EQUIPMENT

A Dionex ICS-2000 Reagent-Free™ Ion Chromatography (RFIC) System was used in this work. The ICS-2000 is an integrated ion chromatograph and consists of:

- Eluent Generator
- Column Heater
- Pump Degas
- EluGen® EGC-KOH Cartridge  
(Dionex P/N 058900)

CR-ATC (Dionex P/N 060477)

AS50 Autosampler

Chromeleon® Chromatography Workstation

This application note is also applicable to other RFIC systems.

## REAGENTS AND STANDARDS

Deionized water, Type I reagent-grade, 18 MΩ-cm resistivity or better

Sodium and potassium salts, ACS reagent-grade or better, for preparing anion standards (VWR or other)

Fluoride standard 1000 mg/L, 100 mL  
(Dionex P/N 037158)

Chloride standard 1000 mg/L, 100 mL  
(Dionex P/N 037159)

Sulfate standard 1000 mg/L, 100 mL  
(Dionex P/N 037160)

Sodium fluorophosphate (MFP), 95%  
(Sigma-Aldrich P/N 33,444-3)

Potassium dihydrogenphosphate, 99%  
(Sigma-Aldrich P/N 22,130-9)

Sodium benzoate, 99%  
(Sigma-Aldrich P/N 10,916-9)

## CONDITIONS

### Method A

Columns: IonPac AS18 Analytical, 4 × 250 mm (Dionex P/N 060549)  
IonPac AG18 Guard, 4 × 50 mm (Dionex P/N 060551)

Eluent: 22–30 mM KOH from 7–8 min,  
30–40 mM from 12–13 min,  
40–60 mM from 16–20 min

Eluent Source: ICS-2000 with CR-ATC

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Injection: 25 µL

Detection: Suppressed conductivity,  
ASRS® ULTRA, 4 mm (Dionex P/N 053947)  
AutoSuppression® recycle mode  
200 mA current

System  
Backpressure: ~2500 psi  
Run Time: 30 min (4 min reequilibration)

### Method B

Columns: IonPac AS17 Analytical, 4 × 250 mm (Dionex P/N 055682)  
IonPac AG17 Guard, 4 × 50 mm (Dionex P/N 055684)

Eluent: 3 mM KOH from 0–5 min, 3–15 mM from 5–14 min, 15–40 mM from 14–18 min

Eluent Source: ICS-2000 with CR-ATC

Flow Rate: 1.5 mL/min

Temperature: 30 °C

Injection: 25 µL

Detection: Suppressed conductivity,  
ASRS ULTRA, 4 mm (Dionex P/N 053947)  
AutoSuppression recycle mode  
150 mA current

System  
Backpressure: ~2200 psi  
Run Time: 30 min (4 min reequilibration)

## PREPARATION OF SOLUTIONS AND REAGENTS

### Stock Standard Solutions

#### Stock Anion Standard Solutions (1000 mg/L)

For several of the analytes of interest, 1000-mg/L standard solutions are available from Dionex and other commercial sources. When commercial standards are not available, 1000-mg/L standards can be prepared by dissolving the appropriate amounts of the required analytes in 1000 mL of deionized (DI) water according to Table 1.

Table 1. Preparation of Stock Standard Solutions

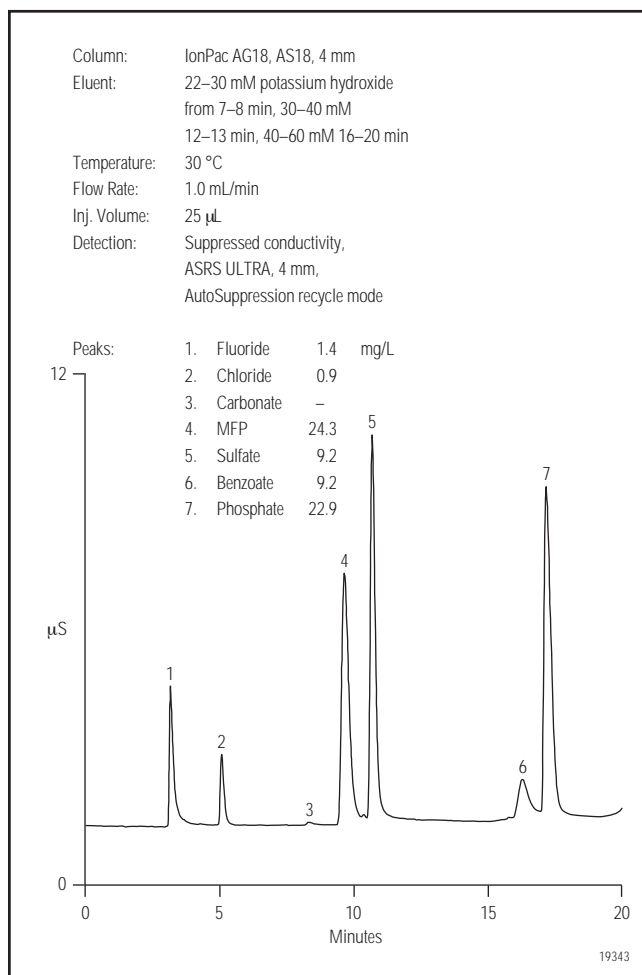
Anion	Compound	Amount (g)
Fluoride	Sodium fluoride (NaF)	2.210
Chloride	Sodium chloride (NaCl)	1.648
Fluorophosphate (MFP)	Sodium fluorophosphate (Na <sub>2</sub> PO <sub>4</sub> F)	1.469
Phosphate	Potassium phosphate, monobasic (KH <sub>2</sub> PO <sub>4</sub> )	1.433
Sulfate	Sodium sulfate (Na <sub>2</sub> SO <sub>4</sub> )	1.479
Benzoate	Sodium benzoate (C <sub>6</sub> H <sub>5</sub> CO <sub>2</sub> Na)	1.190

### Calibration Standards

Composite working standard solutions at lower analyte concentrations are prepared from the 1000-mg/L stock standard solutions. Six levels of calibration standards were used in this study to cover the expected concentrations found in different toothpaste formulations. However, separate calibration standards for fluoride were prepared because monofluorophosphate preparations contain some free fluoride.

### SAMPLE PREPARATION

Approximately 1 g of toothpaste was dissolved into 100 mL of DI water. This sample was placed on a vortex to fully disperse the toothpaste and then filtered through a 0.45 µm Supor® (PES) membrane filter.



**Figure 1.** Standard separation of anions in toothpaste on the IonPac AS18 column.

## RESULTS AND DISCUSSION

Sodium fluoride is the most common form of fluoride found in toothpaste. The choice of fluoride compound is determined by its compatibility with other ingredients.<sup>1</sup> MFP is the source of fluoride in some brands of toothpaste. The MFP breaks down to fluoride and phosphate, and all three analytes can be easily determined by IC.<sup>7</sup> In addition, pyrophosphate may be found in some toothpastes as a tartar control agent<sup>8</sup> and saccharin is often present as an artificial sweetener. Although, both the IonPac AS18 (Method A) and AS17 (Method B) were found to be ideal for the separation of the primary analytes in toothpaste (i.e., fluoride and MFP), each column has certain advantages. For

**Table 2. Calibration Ranges and Linearity for Anions in Toothpaste**

Analyte <sup>a</sup>	Concentration Range (mg/L)	Linearity (r <sup>2</sup> )
Fluoride	0.25–25	0.9998
Chloride	0.10–10	0.9999
MFP	1–100	0.9991
Sulfate	0.50–50	0.9998
Benzoate	0.25–25	0.9999
Phosphate	1–100	0.9997

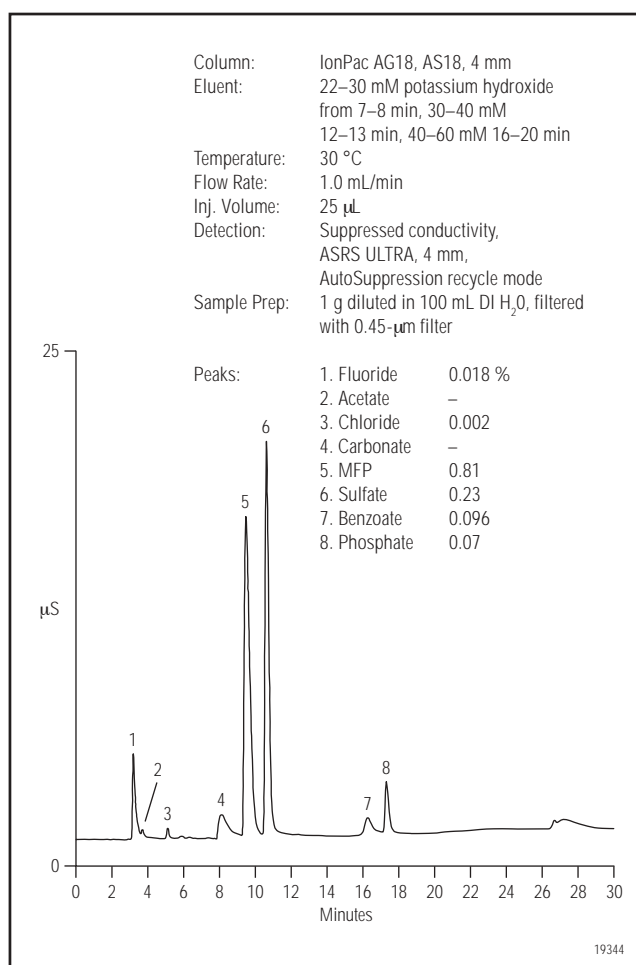
<sup>a</sup>Method A experimental conditions

example, the AS18 has a significantly higher capacity (285 µeq/column), due primarily to its macroporous substrate, in comparison to the AS17 (30 µeq/column). This higher capacity enables the AS18 to tolerate high-ionic-strength matrices and have a larger linear working range than the AS17. However, the separation of stronger retained analytes, such as saccharin, result in extraordinarily long retention times on the higher-capacity AS18 and are therefore best separated on the lower-capacity AS17 column. For polyphosphates with more than two phosphorus groups (i.e., triphosphate, trimetaphosphate, etc.) the most appropriate column is the IonPac AS11, as described in Application Notes 71 and 104.

Although either column can be used for the analysis of toothpaste, this application note primarily focuses on the AS18 column. Figure 1 shows a standard separation on the AS18 column of typical anions found in toothpaste, including fluoride, chloride, MFP, sulfate, benzoate, and phosphate. Table 2 shows the calibration ranges and correlation coefficients for these analytes. Correlation coefficients for the primary target analytes, fluoride and MFP, were 0.9998 and 0.9991, respectively. Three different toothpastes were analyzed for the presence of both active ingredients, fluoride and MFP, and for inactive anionic ingredients.

Two toothpastes (A and B) had MFP as their primary source of fluoride, whereas the other toothpaste (C) had sodium fluoride as its active ingredient. Table 3 shows the average concentrations found in these toothpastes, based on 10 replicate injections. The results showed a higher percentage of MFP in toothpastes A and B, whereas toothpaste C contained a greater amount of free fluoride, as expected from the product labels. Figures 2, 3, and 4 illustrate the application of this method for toothpastes A, B, and C, respectively. Although not quantified, a large amount of pyrophosphate was detected in toothpaste C, indicating it is a tartar protection toothpaste.

Table 4 shows the precisions based on retention time and peak area from the replicate analyses of the different toothpastes. Overall, retention time RSDs were <0.2% and the peak area precisions were <0.9%. The high retention time precision is a reflection of the precise eluent concentrations delivered by the ICS-2000.



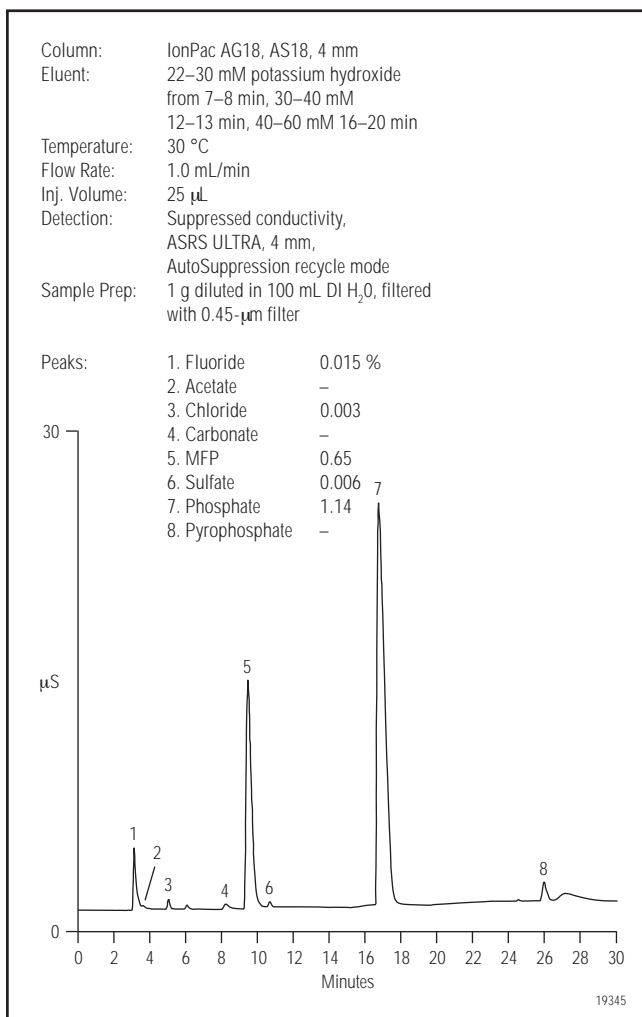
**Figure 2.** Determination of anions in toothpaste A with the IonPac AS18 column.

**Table 3. Anion Concentrations<sup>a</sup> Found in Toothpastes**

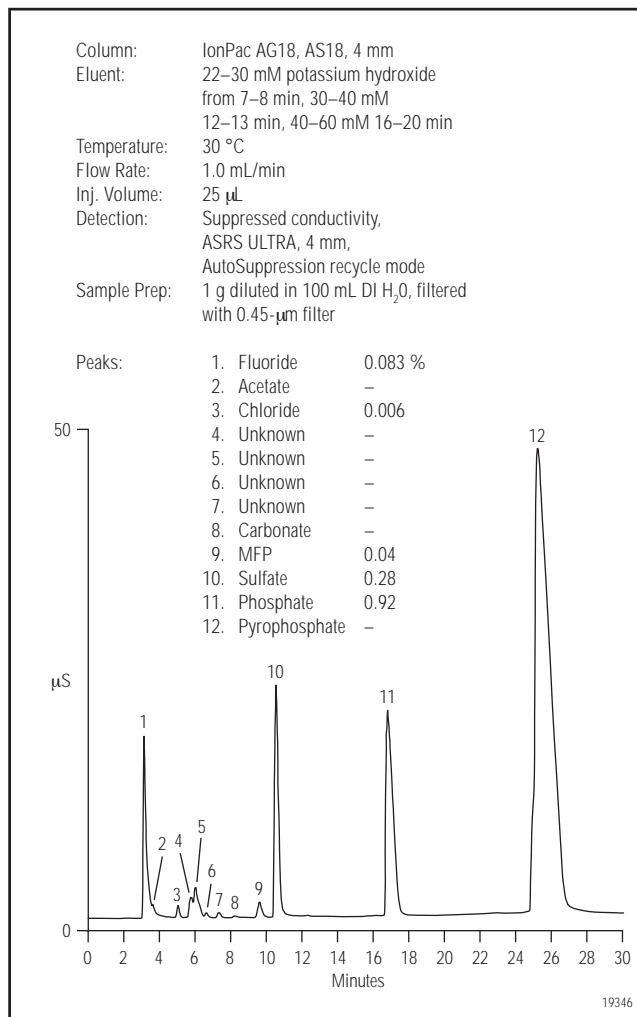
Toothpaste <sup>b</sup>	Fluoride (%)	Chloride (%)	MFP (%)	Sulfate (%)	Benzoate (%)	Phosphate (%)
A	0.018	0.002	0.81	0.230	0.096	0.07
B	0.015	0.003	0.65	0.006	–	1.14
C	0.083	0.006	0.04	0.280	–	0.92

<sup>a</sup>Average concentrations were calculated from ten replicate injections (n = 10)

<sup>b</sup>Method A experimental conditions



**Figure 3.** Determination of anions in toothpaste B with the IonPac AS18 column.



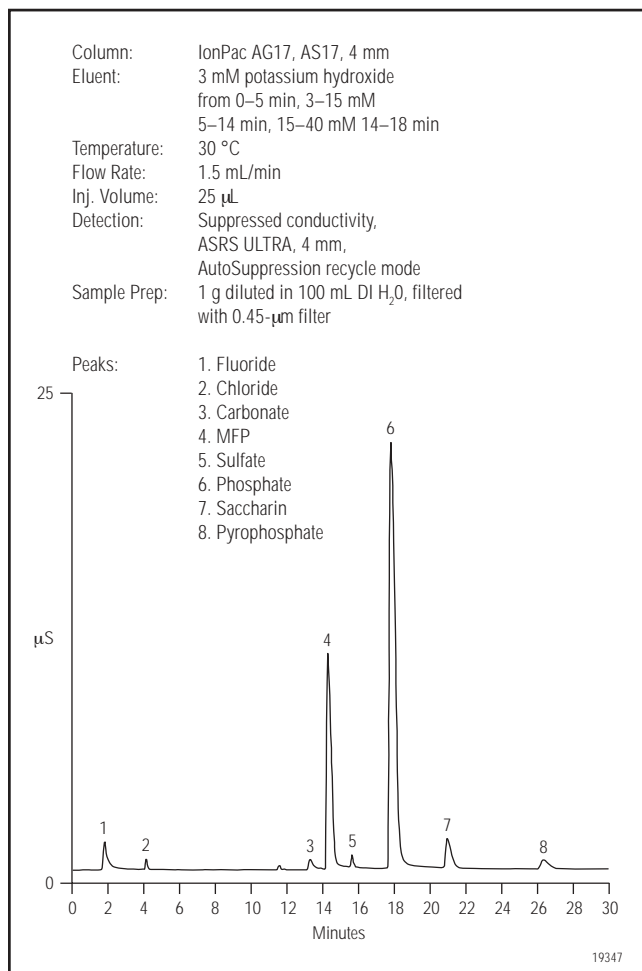
**Figure 4.** Determination of anions in toothpaste C with the IonPac AS18 column.

**Table 4. Retention Time and Peak Area Precision for Anions Found in Toothpaste**

Analyte <sup>a</sup>	Toothpaste A		Toothpaste B		Toothpaste C	
	RT RSD <sup>b</sup> %	Peak Area RSD %	RT RSD %	Peak Area RSD %	RT RSD %	Peak Area RSD %
Fluoride	0.08	0.40	0.07	0.89	0.03	0.41
Chloride	0.13	0.79	0.12	0.72	0.05	0.78
MFP	0.14	0.26	0.08	0.19	0.09	0.76
Sulfate	0.09	0.29	0.06	0.59	0.06	0.30
Benzoate	0.12	0.87	–	–	–	–
Phosphate	0.07	0.79	0.06	0.19	0.05	0.24

<sup>a</sup>Method A experimental conditions

<sup>b</sup>The relative standard deviations (RSDs) were calculated from ten replicate injections (n = 10)



**Figure 5.** Separation of anions in toothpaste B with the IonPac AS17 column.

As previously mentioned, the AS17 may be substituted for this application using the conditions listed under Method B. As shown in Figure 5, the selectivity of the AS17 allowed the separation of saccharin and pyrophosphate. The AS17 and AS18 had equivalent run times.

## CONCLUSION

This application note demonstrates that IC with electrolytic eluent generation provides a convenient and reliable method for the simultaneous determination of fluoride, MFP, and other anionic ingredients in various toothpastes. The results are comparable with values reported in the literature. The electrolytic eluent generator provides additional convenience and ease of use by allowing the user to rapidly increase—with high fidelity—the eluent concentration to elute more strongly retained components, such as pyrophosphate. In addition, precision is significantly improved by generating the eluent on-line compared to manual eluent preparation.

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