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Determination of Fluoride in Acidulated Phosphate Topical Solutions Using Reagent-Free Ion Chromatography

INTRODUCTION

Dental caries (i.e., tooth decay), a widespread chronic disease, is formed through a complex interaction between acid-producing bacteria and fermentable carbohydrates. Although it is one of the most common and preventable childhood diseases, people remain susceptible throughout their lifetime.¹ Further evidence has also suggested that the oral pathogens involved in the formation of dental caries may contribute to other systemic diseases, such as heart disease and diabetes.² During the last several decades, preventive measures have helped to reduce the incidence of dental caries in industrialized countries. The most prominent measure is the exposure to fluoride, from various sources, such as drinking water, food, and toothpaste.³ There is significant evidence that fluoride reduces the incidence of dental caries and can slow or reverse the progress of carious lesions (i.e. prevents cavities). The widespread use of fluoride in the United States has been a major factor in the decline in the prevalence and severity of dental caries. The success of water fluoridation in preventing and controlling dental caries has led to the development of many fluoride-containing products, such as toothpaste, mouth rinse, supplements, and professionally-applied gels, foams, and varnishes.⁴

In the 1960s, acidulated phosphate fluoride (APF) was introduced in the form of a solution or gel. Professionally applied APF contains up to 1.23% fluoride (12,300 ppm fluoride) in the form of sodium fluoride at pH 3.0.⁵ This solution was developed based on studies designed to accelerate enamel fluorapatite formation, discourage calcium fluoride formation, prevent demineralization, and increase fluoride uptake at the surface. Earlier studies have shown that by reducing the pH of the solution to <4.0 there is a significant increase in the uptake of fluoride into the enamel, however, acidic solutions prepared from hydrochloric or acetic acid produced decalcification of the enamel surfaces. Therefore, phosphoric acid was chosen as the acidulent because it did not cause demineralization.² In the 1980s, the United States Food and Drug Administration (US FDA) permitted the sale of over-the-counter 0.05% sodium fluoride (226 ppm fluoride) or APF mouth rinses for daily use.⁶ The US FDA describes these treatment rinses as "...an aqueous solution of acidulated phosphate fluoride derived from sodium fluoride acidulated with a mixture of sodium phosphate, monobasic, and phosphoric acid to a level of 0.1 M phosphate ion and a pH of 3.0 to 4.5 and which yields an effective fluoride ion concentration of 0.02%."⁷ The determination of fluoride in APF is critical to ensure that the labeled amount of fluoride is within specifications for quality control.

The US Pharmacopeia (USP) describes an assay for determining fluoride in an acidulated phosphate topical solution using ion chromatography (IC) with suppressed conductivity detection.⁸ This method uses a combination of 1.4 mM sodium carbonate with 1 mM sodium hydroxide as the mobile phase to elute fluoride from the column. It is well known that carbon dioxide readily dissolves in dilute hydroxide solutions to form carbonate. A change in the mobile phase carbonate concentration can lead to poor retention stability of the target analyte and result in poor method performance. Here, we describe a simpler approach for determining fluoride in APF using a Reagent-Free™ IC system with eluent generation (RFIC-EG™). This approach eliminates the need to manually prepare eluents and thereby increases the automation, ease-of-use, and reproducibility between analysts and laboratories. Using the IonPac® AS18 column with electrolytically generated potassium hydroxide eluent enabled the separation of fluoride from the inactive ingredients, phosphate and sorbate, in less than 15 min. Using this method, the authors demonstrate linearity, limits of detection (LOD), limits of quantitation (LOQ), precision, and recovery of fluoride in a commercially available APF topical solution.

EQUIPMENT

Dionex ICS-3000 Reagent-Free Ion Chromatography (RFIC) system consisting of^a:
SP Single Pump or DP Dual Pump module
EG Eluent Generator module
DC Detector/Chromatography module (single or dual temperature zone configuration)

AS Autosampler

EluGen EGC II KOH cartridge (P/N 058900)

Continuously-Regenerated Anion Trap Column, CR-ATC (P/N 060477)

Chromeleon® 6.8 Chromatography Workstation

Polystyrene injection vials with caps and septa, 10 mL (P/N 055058)

^aAny Dionex RFIC-EG system can be used for this application.

REAGENTS AND STANDARDS

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

Sodium Fluoride (Fisher Scientific, S-299)

Sample

Sodium fluoride and acidulated phosphate topical solution (active ingredient = 0.44 mg/mL NaF or 0.044%)

CONDITIONS

Columns: IonPac AS18 Analytical,
2 × 250 mm (P/N 060553)

IonPac AG18 Guard,
2 × 50 mm (P/N 060555)

Eluent: 10 mM KOH from 0–5 min,
step to 45 mM at 5 min,
45 mM from 5–15 min*

Eluent Source: EGC II KOH with CR-ATC

Flow Rate: 0.25 mL/min

Temperature: 30 °C (column compartment)
30 °C (detector compartment)

Inj. Volume: 5 μL

Detection: Suppressed conductivity, ASRS 300
(2 mm), Autosuppression recycle mode,
power setting: 28 mA

Background

Conductivity: <0.50 μS

Noise: ~2-3 nS

System

Backpressure: ~2300 psi

*The column was equilibrated for 5 min at 10 mM KOH prior to each injection.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent Solution

Generate the potassium hydroxide eluent online by pumping deionized water through the EGC II KOH cartridge. Chromeleon software will track the amount of KOH used and calculate the remaining lifetime of the cartridge.

Alternatively, prepare 100 mM NaOH by pipetting 8.0 g of 50% (w/w) aqueous NaOH from the middle portion of the reagent bottle into a 1 L volumetric flask containing approximately 500 mL degassed deionized water. Dispense the NaOH below the surface of the water

to avoid introducing carbon dioxide from the air into the eluent. Bring to volume, mix thoroughly, and degas by sparging with helium or sonicating under vacuum for 10 min. Transfer to a plastic reservoir and maintain an inert helium atmosphere of 3–5 psi in the reservoir. Dilute the NaOH with deionized water to achieve the appropriate eluent concentration listed in the conditions section. When using manually prepared NaOH eluent, the ICS-3000 gradient pump should be used. (Note: Carbon dioxide readily dissolves in dilute hydroxide solutions to form carbonate. Carbonate contamination can lead to poor retention time reproducibility of the target analytes, resulting in performance that may be inferior to that achieved using an eluent generator.)

Stock Standard Solutions

All fluoride standard solutions described here were prepared as sodium fluoride (NaF) according to the USP method.⁸ Prepare a 2 mg/mL stock standard solution by dissolving 0.2 g of NaF in 100 mL of deionized water. Store the stock solution in a high-density polyethylene or polypropylene bottle at 4 °C.

Primary Dilution Standard

Prepare a 110 µg/mL NaF solution by adding 1.10 mL of the 2 mg/mL stock standard to a 20 mL scintillation vial, then bring to volume with deionized water.

Calibration Standards

Sodium fluoride calibration standards were prepared at 0.25, 0.50, 1.1, 3.0, and 5.0 µg/mL NaF by adding the appropriate volumes of the primary dilution standard to separate 100 mL volumetric flasks.

SAMPLE PREPARATION

The label of the APF topical solution reported 440 µg/mL NaF as active ingredient. This solution was diluted to a final concentration of 1.10 µg/mL NaF according to the procedure described in the USP method. To accomplish this, transfer 10 mL of the solution to a 1000 mL volumetric flask and bring to volume with deionized water (dilution = 1:100). Next, perform a second dilution by transferring 25 mL from the 1000 mL volumetric flask to a 100 mL volumetric flask and bring to volume with deionized water (total dilution = 1:400).

RESULTS AND DISCUSSION

In Application Note 156, the authors described determination of fluoride and monofluorophosphate (MFP) using the IonPac AS18 column with electrolytically generated potassium hydroxide eluent and suppressed conductivity detection.⁹ That method successfully demonstrated the separation of the active ingredients, fluoride and monofluorophosphate (MFP), and anionic inactive ingredients in the matrix. The IonPac AS18 column is ideal for the determination of fluoride because it provides excellent separation of the analyte from the void volume and organic acids, such as acetate and formate, which may be present in the sample or water source.

Here, we describe the determination of fluoride in a commercially available APF topical solution using the IonPac AS18 column with RFIC-EG. APF is a sodium fluoride solution that has been acidulated between pH 3.0 and 4.5 with an effective fluoride concentration of 0.02%, or has been acidulated to pH 3.5 with a fluoride concentration of 0.01%.⁷ The anionic inactive ingredients include phosphoric acid and sodium phosphate to buffer the solution, and potassium sorbate as a preservative. The lower solution pH and acidulated formula enables more calcium fluoride formation on the tooth surface to reduce or prevent dental caries. The US FDA is responsible for approving commercially available fluoride products marketed in the U.S. and for setting labeling standards for these products, which include toothpaste and mouth rinse.⁴ The USP has published appropriate test methods to assure that the fluoride concentration on the label is within FDA specifications. The USP monograph of APF states that the fluoride concentration cannot contain less than 90% and not more than 110% of the labeled amount. Therefore, it is critical to develop a simple, direct, and accurate test method for determining fluoride in APF to establish if the product meets the limits in the USP monograph.

To determine the linearity of the method, calibration standards were injected in duplicate at five concentration levels in the range of 0.25–5.0 µg/mL NaF (0.11–2.3 ppm F). A plot of peak area versus concentration produced a correlation coefficient (r^2) value of 0.9999 using a least squares regression fit. The USP compendial method for validation <1225> recommends a signal-to-noise (S/N) ratio of 10 for the determination of the limit of quantitation (LOQ).¹⁰ The baseline noise was determined by measuring the peak-to-peak noise in a representative

one-minute segment of the baseline where no peaks elute. Typical baseline noise for this method using the ASRS 300 suppressor in the recycle mode is ~2-3 nS/min. The LOQ for NaF was determined to be 11.2 ng/mL (5.1 ppb F). The LOD for NaF was estimated to be 3.4 ng/mL (S/N = 3).

The performance of the method was evaluated in terms of the precision from replicate injections of a standard or sample and the recovery from spiked APF solutions with known concentrations of NaF added to the sample. The relative standard deviations (RSDs) of the retention time and peak areas were calculated from 10 replicate injections of a standard solution prepared at a concentration 1.10 $\mu\text{g/mL}$ NaF. The calculated retention time and peak areas RSDs for replicate injections of the NaF standard were 0.04% and 0.41%, respectively.

The method was used to assay four independently prepared sample solutions prepared at a known concentration of 1.10 $\mu\text{g/mL}$ (based on the label claim) from a single APF product over four consecutive days. The average NaF concentration detected in the APF topical solution was $0.043 \pm 0.001\%$ (0.019% fluoride). This value is well within the 90–110% USP specifications of the labeled amount of 0.044% NaF. Figure 1 shows an overlay of chromatograms from four consecutive days using the IonPac AS18 with electrolytically generated potassium hydroxide eluent. As shown, fluoride is well resolved from the anionic inactive ingredients in the APF topical solution in less than 15 min. The intraday retention time and peak area precisions (i.e., a sequence of consecutive injections, n=10) were $\leq 0.07\%$ and $\leq 0.29\%$, respectively. The between-day retention time and peak area precisions over four consecutive days (i.e., day-to-day, n = 40) were 0.04% and 1.7%, respectively.

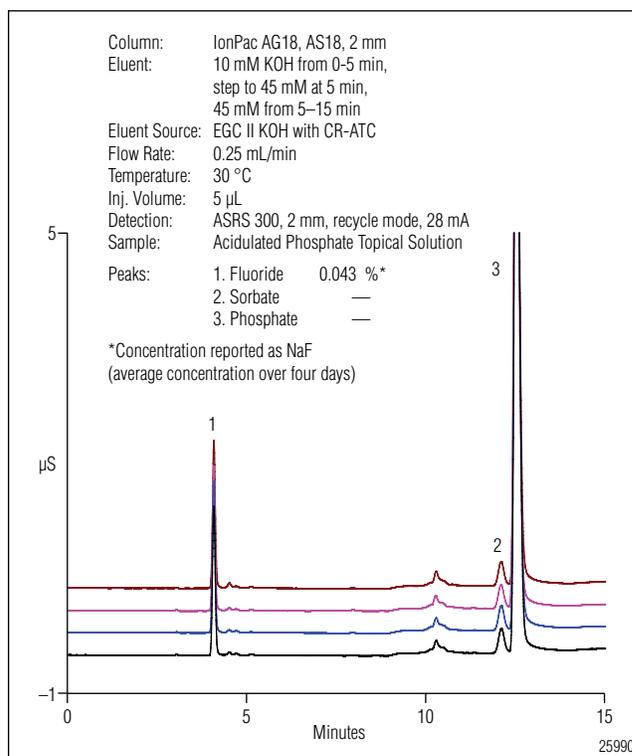


Figure 1. Overlay of chromatograms of fluoride in acidulated phosphate topical solution analyzed over four consecutive days.

Table 1 summarizes the amount of NaF determined in the independently prepared APF sample solutions and the retention time and peak area precisions.

The accuracy of the method was evaluated by spiking a known concentration of NaF in the sample over four consecutive days and calculating the recoveries based on the difference in response between the unspiked and spiked sample. Each independently prepared sample solution was spiked with approximately 1.10 $\mu\text{g/mL}$ NaF, which produced average recoveries in the range of 99.4–100.6%, confirming method accuracy (Table 2).

Table 1. Summary of NaF Determined in Independently-Prepared Solutions of Acidulated Phosphate Topical Solution Over Four Consecutive Days

Day	Analyte	n	Label Concentration (%)	Experimental Concentration (%)	Retention Time RSD	Peak Area RSD
1	NaF	10	0.044	0.043	0.05	0.17
2	NaF	10	0.044	0.044	0.03	0.26
3	NaF	10	0.044	0.042	0.07	0.21
4	NaF	10	0.044	0.043	0.03	0.29

Table 2. Recovery of NaF in Independently-Prepared Solutions of Acidulated Phosphate Topical Solution Over Four Consecutive Days

Day	Analyte	n	Average Amount Found ^a (µg/mL)	Amount Added (µg/mL)	Recovery (%)
1	NaF	6	1.07	1.10	100.4 ± 0.2
2	NaF	6	1.09	1.10	100.6 ± 0.2
3	NaF	6	1.04	1.09	100.6 ± 0.2
4	NaF	6	1.07	1.10	99.4 ± 0.2

^aAverage amount found represents the NaF concentration in the 1:400 diluted sample.

CONCLUSION

This method demonstrates a simple, accurate IC method using electrolytically generated potassium hydroxide eluent for the determination of fluoride in an acidulated phosphate topical solution. The method enabled the separation of fluoride and the anionic inactive ingredients, phosphate and sorbate, in less than 15 min with good retention time and peak area precisions. The results were within 0.001% of the label NaF concentration with recoveries nearly 100% over four consecutive days, demonstrating the high accuracy obtained by this method. The use of an eluent generator to deliver a precise potassium hydroxide eluent concentration requires only a source of deionized water and therefore enhances the method's ease-of-use by allowing the analyst to rapidly increase the eluent concentration to elute more strongly retained compounds, such as phosphate. In addition, the method precision is significantly improved by generating the eluent on-line compared to manual eluent preparation.

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LPN 2096 PDF 09/16
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