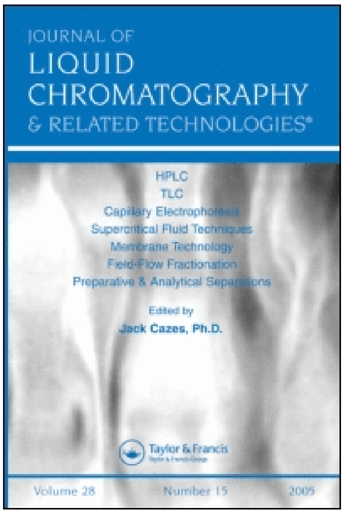

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DETERMINATION OF SOLUBLE FLUORIDE AND MONOFLUOROPHOSPHATE IN DENTAL CREAM USING GRADIENT ELUTION ION CHROMATOGRAPHY

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ABSTRACT

A single column, stability indicating, Ion Chromatographic method has been developed for the determination of Soluble Fluoride and Monofluorophosphate (MFP) in dental cream. The limits of detection (3x signal-to-noise) for Fluoride and MFP in dental cream are 5.0 ppm and 87 ppm (wt./wt.), respectively, using the sample preparation procedure described. These LODs correspond to operating concentrations of F and MFP of 0.05 ppm and 0.87 ppm (wt./vol), respectively. The method is linear for F$^-$ ($r = 0.9998$) over an operating range of 1.0 to 4.5 ppm (wt./vol) (40%-180% of 2.5 ppm target) and for MFP ($r = 0.9999$) over an operating range of 35.7 to 66.3 ppm (wt/vol) (70%-130% of 51.0 ppm target). Both anions (MFP and F) were found to have no interference with a placebo dentifrice and each passed resolution and tailing factor requirements of $R \geq 2.0$ and $T<1.5$. Quantitative recoveries were obtained for both species from placebos spiked with 70, 100, and 130% of target. Twenty replicate preparations and analyses of a homogenized commercial sample, conducted by two operators over the course of 4 days, showed agreement to within 5.5%RSD for F and 2.23%RSD for MFP.

INTRODUCTION

Many methods have been reported in the literature for the determination of different fluoride species.[1-10] For dental cream there are four fluoride species of interest: Ionic Fluoride, all fluoride that is soluble in the ionic form (F^-); Soluble Fluoride (Total Soluble Fluoride), all fluoride that is soluble including fluorine in the form of Monofluorophoshate (MFP, PO_3F^{2-}), Stannous Fluoride (SnF_2), and other fluoride compounds; Insoluble Fluoride, all fluoride in a sample that may be insoluble such as Calcium and other metal salts; Total Fluoride, all fluoride from the insoluble and soluble categories.[1-4]

The determination of Fluoride content in dental cream is important as Fluoride is an active ingredient regulated by government and private agencies.[11-12] Total Fluoride is used as a measure of highest allowable fluoride content and is based on potential toxicity. Many agencies require no more than 1500 ppm Total F in dental cream.[12] Ionic Fluoride and Soluble Fluoride are used as measures of bioavailability. Bioavailablity is a measure of the effective deliverable fluoride during use of the product. For Sodium Fluoride (NaF) dental cream, Ionic Fluoride is the measure of bioavailability. For dentifrice using Sodium Monofluorophosphate (Na_2PO_3F) as the active fluoride donor, Soluble Fluoride is the measure of bioavailability since fluoride will be present in two forms, the Ionic form (F^-) and as MFP (PO_3F^{2-}).

Ionic Fluoride as a species can be determined experimentally by a number of techniques such as Ion Selective Electrode (ISE),[1-4] Colorimetric techniques,[5] and Capillary Electrophoresis (CE).[6] Total Fluoride can be determined by use of Gas Chromatography (GC),[5, 10] and ISE using acid digestion of the sample.[1] Soluble Fluoride is determined by ISE using acid digestion of a sample aliquot, Colorimetric titration, or CE. Insoluble Fluoride as a species would be determined by calculation (Insoluble F = Total F - Soluble F). Ion Chromatography (IC) has also been used to determine Fluoride ions and other negatively charged fluoride containing compounds such as MFP.[8-9]

Determination of Soluble Fluoride in dental cream using Ion Chromatography has been reported using a single column system.[8] However, resolution between Fluoride and other low molecular weight ions in dental cream (such as phosphates and sulfates) or the system peak make quantitation difficult. This paper describes a validated method for simultaneous determination of Fluoride ion and Monofluorophosphate ion (Soluble Fluoride) using gradient elution IC with auto-suppression.

EXPERIMENTAL

Apparatus

Analyses were performed using the Dionex DX500 analytical module with self suppression using an ASRS-Ultra (Dionex, Sunnyvale, CA, USA). The module consisted of the GP40 gradient pump and CD20 conductivity detector (Dionex) and a Midas autosampler fitted with a 3µL fixed injection loop (Spark-Holland, 7800 AJ Emmen, Netherlands). Dionex AS15-2mm/AG15-2mm analytical and guard columns were used for the determination of Fluoride and Monofluorophosphate anions. A Dionex GM-4 gradient mixer and an ATC-2 mm Anion trap column was used post-pump to remove carbonate and other contaminate anions in dentifrice from the hydroxide eluent.

Chromatographic Conditions

The mobile phase consisted of A) 80 mN NaOH, prepared using 50/50 w/w NaOH solution (See Reagents Section) and DI/distilled water (See Reagents Section) and B) DI/Distilled Water. The pump flow rate was set to 0.32 mL/min. A gradient was used for elution (see Table 1 for gradient parameters, and Table 2 for detector and pump settings). Eluents were stored under helium gas. The detector was set at 20 µS full scale and the suppressor at 100mA. The injection volume was set on a fixed loop at 3 µL. Using these conditions, fluoride ion elutes at about 2.7 minutes and monofluorophosphate elutes at about 7.6 minutes.

Table 1

Programmed Gradient Profile

Time (Min.)	Program Curve #	% Eluant A (80 mN NaOH)	% Eluant B (Distilled Water)
0.00	---	40	60
0.01	5	40	60
9.50	5	90	10
12.50	5	90	10
12.51	5	40	60

Table 2

Instrument Parameters

Detection Mode	Conductivity, 100 mA
Detector Range	20 µS
Temp. Comp.	1.7
DS3 Setpoint	32°C
Pump	0.32 mL/min
Run Time	25 min
Injector	3 µL

Reagents

Sodium monofluorophosphate (Na_2PO_3F, SMFP) reagent, USP grade, was obtained from Ozark-Manhoning Co. Elf Atochem (Tulsa, OK, USA). Sodium Fluoride (NaF) was USP Grade (USP, Rockville, MD, USA). Deionized/distilled water was obtained from a Barnstead Meagpure MP-12A system, model number A42012 (Thermolyne, Debuque, IO, USA). 50%-50% w/w NaOH solution was used from J.T. Baker (Phillipsburgh, NJ, USA).

Standard Preparation

Fluoride standard stock solutions were prepared in duplicate from dried NaF at two different levels, 50 and 5 ppm fluoride anion. Monofluorophosphate standard stock solutions were also prepared in duplicate from Sodium Monofluorophosphate at the 800 and 200 ppm MFP levels.

Working Standard Preparation

Four calibration standard solutions were prepared, one for each set of standards (two high and two low). A 10 mL aliquot of high concentration (50ppm) fluoride anion and a 10 mL aliquot of high concentration (800ppm) MFP anion were added to a 100mL volumetric flask. This solution was duplicated with a second set of standards. Final concentrations were 5 ppm F^- and 80 ppm PO_3F^{2-}. The same procedure was carried out for the two sets of low concentration stock solutions. Final concentrations of the low working standard were 0.5 ppm F^- and 20 ppm PO_3F^{2-}. The MFP standards contain a significant amount of fluoride ion. The concentration of fluoride anion present in the monofluorophosphate raw material was measured previously. The final fluoride concentrations of each working standard was adjusted for this additional fluoride ion.

Sample Preparation

Approximately 10g of dentifrice was thoroughly dispersed into 30mL of DI/distilled water, quantitatively transferred and diluted to volume in a 100mL volumetric flask (100mg/mL). A portion of the 10% sample mixture was then centrifuged at 8,000 X G for 30 minutes to obtain a clear supernatant. This supernatant solution was further diluted 10% with DI/distilled water. The resultant 1% sample mixture (10mg/mL) was used for injection into the chromatographic system.

RESULTS AND DISCUSSION

The AS15 IC column shows good retention and resolution for fluoride and MFP which allowed a single run soluble fluoride method to be developed. Initial isocratic methods showed good separation and retention, but elution of the ortho-phosphate ion and other anionic species in dental cream was well over 25 minutes; thus, a gradient system of 32mN NaOH at time 0 to 72 mN NaOH at time 9.5 was used to speed the elution of the of phosphate and the other anions. The 72 mN concentration of NaOH was held for 3 minutes and the system switched back to initial conditions and allowed to return to baseline before re-injection. Table 1 shows the linear gradient parameters. As shown in Figure 1, a sample run time of 25 minutes gave enough time for all of the anions in the dental cream to elute and recycle back to initial conditions.

To validate the methodology, each anion, F and MFP, was analyzed separately for linearity, recovery, reproducibility, and specificity/selectivity. A five point calibration curve of both Fluoride and MFP anions ranging from 1.0 to 4.5 ppm and 36.4 to 67.0 ppm (equivalent to 0.76% (wt./wt.) Sodium Monofluorophosphate dentifrice prepared as specified), respectively, were generated. Each anion passed F-tests ($p = 0.5$) and regression lack of fit ($r = 0.9998$ for F and $r = 0.9999$ for MFP). Spiked placebo samples were prepared by adding the appropriate amounts of F and MFP anions and homogenized. Recoveries from the spiked placebos containing 100.0, 200.0, and 450.0 ppm F were 75.3, 88.1, and 96.0% respectively. The loss of fluoride anion was due to low levels of calcium in the dentifrice. Recoveries from the spiked placebos containing 700, 1000, 1300 ppm MFP as fluoride anion were 102.4,102.1, and 101.1 % respectively ($n = 3$ at each level). Reproducibility studies were performed on 20 samples for each anion. Five samples were analyzed by one operator on each of two days, and five samples were analyzed by a second operator on each of two days. The same instrument was used but the samples were quan-

Table 3

Reproducibility of Analyses for Homogenized Placebo Spiked with Fluoride and Monofluorophosphate Anions by Day and by Operator*

Day	Operator	Average (ppm F)	Std. Dev. (ppm F)
1	1	87.7	0.91
2.	1	87.7	0.27
3	2	76.8	0.57
4	2	84.6	0.42

Day	Operator	Average (ppm MFP)	Std. Dev. (ppm MFP)
1	1	97.7	0.10
2	1	96.6	0.66
3	2	100.4	0.55
4	2	102.0	0.20

* n = 5 for each data set.

titated vs. independent calibration curves on each day. The results for both fluoride and MFP anions are summarized in Table 3. Operator-to-operator and day-to-day reproducibility was acceptable. Calibration and quantitation was done using external standards and area counts. A set of calibration standards were run before each set of 10 samples. Subsequent standards are evaluated at ± 5% the original calibration standards for check purposes only. This criteria works well within internal specifications. The day-to-day and operator variability is due to temperature fluctuations throughout the chromatographic runs and previous calibration as discussed. The apparatus was not run in a climate controlled area nor was a column heater or temperature stabilizer used. The overall recoveries and RSDs were 84.2 ± 4.6%, 5.5%, respectively, for F and 99.2 ± 2.2%, 2.2%, respectively, for MFP. As referred previously, the lower than 100% values for fluoride are due to calcium fluoride precipitate. Recoveries for fluoride agreed with the previous findings for the same level (200 ppm F) in a spiked placebo. No interferences were observed for common anions including chloride, sulfate, phosphate, and bromide. Both fluoride and MFP passed resolution ($R \geq 2.0$) and tailing factor ($t < 1.5$) requirements (see Figure 1). Each analyte and the placebo was chromatographed to show the method

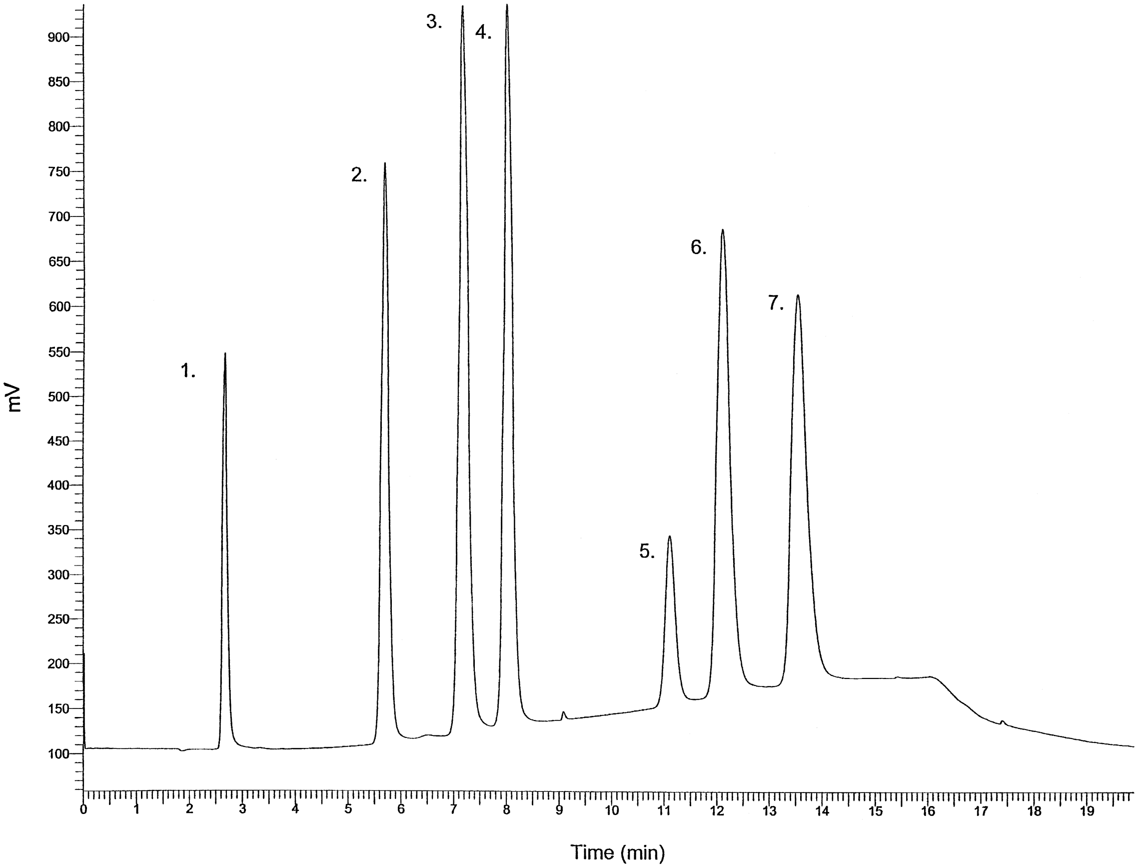

Figure 1. Chromatogram of common analytes. 1. Fluoride, 5 ppm. 2. Chloride, 15 ppm. 3. Monofluorophosphate, 80 ppm. 4. Sulfate, 25 ppm. 5. Nitrate, 30 ppm. 6. Bromide, 70 ppm. 7. Orthophosphate, 50 ppm. Chromatographic conditions are specified in the text.

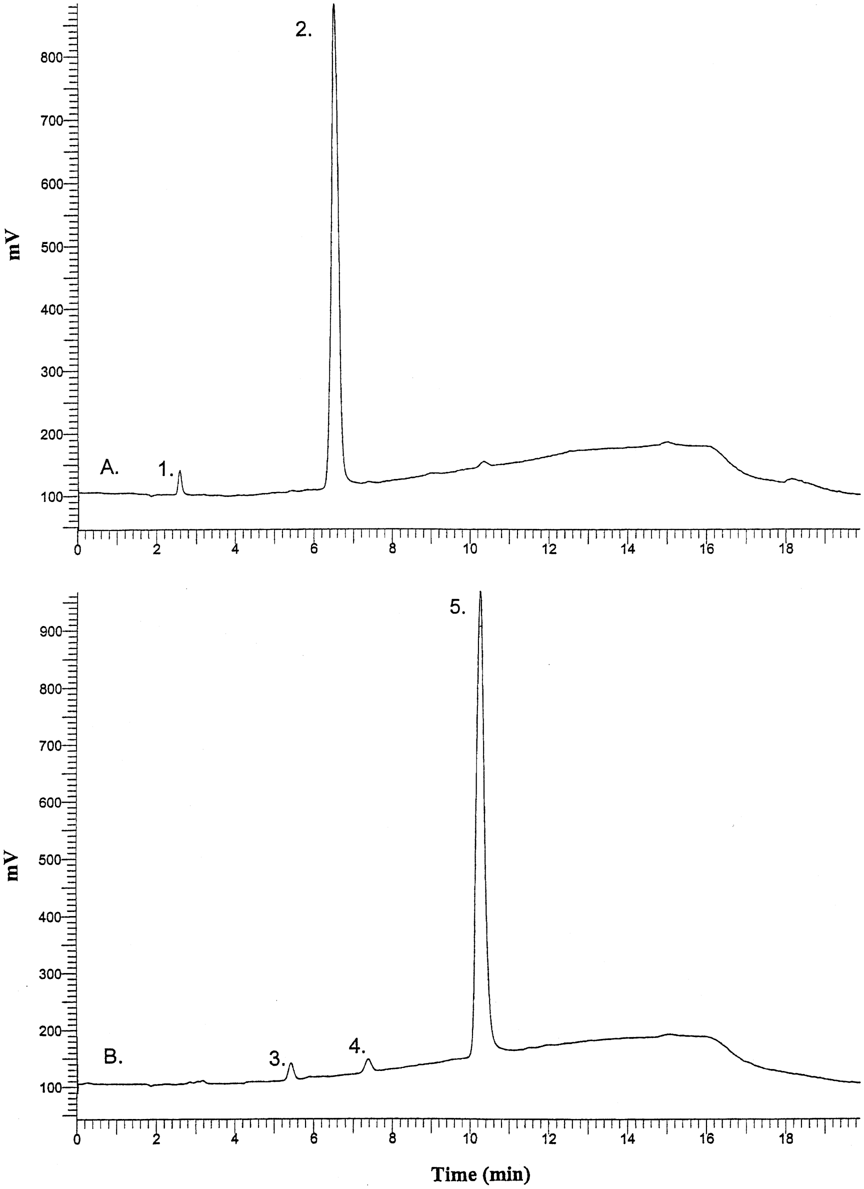

Figure 2. Chromatograms of A) MFP standard stock solution, 1. Fluoride. 2. MFP. and B) MFP dentifrice placebo, 3. Chloride, 4. Sulfate, 5. Orthophosphate.

was specific and selective for fluoride and MFP. No interferences were found (see Figure 2).

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