Application note | 002345

Pharma

Determination of fluoride in sodium fluoride oral solution

Authors

Jingli Hu, Jeffrey Rohrer

Thermo Fisher Scientific,
Sunnyvale, CA, USA

Keywords

Dionex ICS-6000 HPIC system, Dionex IonPac AS22 column, Dionex ADRS 600 suppressor, ion chromatography, USP, mouth rinse, dental

Goal

To determine fluorine in sodium fluoride oral solution using the mobile phase from the United States Pharmacopeia (USP) Sodium Fluoride Oral Solution monograph

Introduction

Fluoride is the active ingredient for anticavity mouth rinse products. Other components can also be added to mouth rinse products, such as sodium benzoate, sodium chloride, and disodium phosphate. Mouthwash containing 0.02–0.05% sodium fluoride can be purchased over the counter and used daily to prevent cavities. Mouthwash manufacturers are required to determine the fluoride concentration in the final products. The determination of fluoride in oral solution using ion chromatography (IC) is described in the USP Sodium Fluoride Oral Solution monograph.[1]

The USP monograph method lists an L46 column set for the determination of fluoride in oral solution. The Thermo Scientific™ Dionex™ CarboPac™ PA1 column set belongs to this category and was reported to be the column used for this method.[2] An IC system was set up using a Dionex CarboPac PA1 column set with the same mobile phase and chromatographic conditions as the USP monograph. However, we found the retention

time of chloride is about 37 min, which is much longer than the expected time of 5 min reported in the monograph. The retention time of chloride remained around 37 min after extensive troubleshooting that included testing different column lots, mobile phase chemicals, and IC system. Therefore, after testing a few other Thermo Scientific™ Dionex™ IC columns, the Thermo Scientific™ Dionex™ IonPac™ AS22 column set was chosen in this study as an alternate column because it elutes chloride within 10 min while keeping the fluoride peak far away from the water dip (void volume).

In this application note, we developed a new method using the same mobile phase as the USP monograph method. The method was validated for separation, calibration range, accuracy, and precision. The fluoride amounts in three commercially available mouth rinse products were determined and compared with the labeled value.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC system* including:
  - Dionex ICS-6000 DP Pump module
  - Dionex ICS-6000 DC Detector/Chromatography module with Conductivity Detector
  - Dionex AS-AP Autosampler with sample tray cooling, 250 µL sample syringe (P/N 074306), 1,200 µL buffer loop (P/N 074998), and 10 mL vial trays (P/N 074938)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.9

*This method can be run on any Thermo Scientific™ Dionex™ IC system that supports electrolytic eluent suppression.

Consumables

- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor, 4 mm (P/N 088666)
- Fisherbrand™ Narrow-Mouth field sample bottles, high-density polyethylene (HDPE), 125 mL, 250 mL sizes for storage of standards and samples (Fisher Scientific P/N 02-895A, B)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ sterile disposable filter units (Fisher Scientific P/N 09-740-46)

Reagents and standards

- Deionized (DI) water, Type 1 reagent grade, 18 MΩ·cm resistivity or better
- Sodium fluoride standard (USP P/N 1614002)
- Sodium chloride standard (USP P/N 1613804)
- Sodium benzoate standard (Fisher Scientific P/N AAA1594630)
- Thermo Scientific™ Dionex™ Phosphate standard, 1,000 mg/L (P/N 303172)
- Sodium hydroxide solution (1 N) (Fisher Scientific P/N SS266-4)
- Sodium carbonate anhydrous (Fisher Scientific P/N S263-500)

Samples

Three anticavity fluoride-containing mouthwashes were purchased from a local store.

Chromatographic conditions

Parameter	Value
Columns	Dionex IonPac AG22 (4 × 50 mm) guard column (P/N 064139) Dionex IonPac AS22 (4 × 250 mm) analytical column (P/N 064141)
Eluent	150 mg/L of anhydrous sodium carbonate and 1.0 mL/L of 1 N sodium hydroxide in water (1.42 mmol/L Na_2CO_3, 1 mmol/L NaOH)
Flow rate	1.5 mL/min
Injection volume	20 µL
Column temperature	30 °C
Detection	Suppressed conductivity
Suppressor	Dionex ADRS 600 (4 mm) Suppressor, AutoSupression recycle mode, 15 mA current
Detection/Suppressor compartment temperature	25 °C
Cell temperature	35 °C
Background conductance	~10 µS/cm
System backpressure	~2,300 psi (100 psi = 689.5 kPa)
Noise	<2 nS/cm
Run time	10 min

Preparation of solutions and reagents

Anions stock standard solutions

Stock standard solutions (1,000 mg/L) were prepared by dissolving the appropriate analyte amounts in 100 mL of DI water, according to Table 1.

Table 1. Masses of compounds used to prepare 100 mL of 1,000 mg/L ion standards

Analyte	Compound	Amount (mg)
Fluoride	Sodium fluoride (NaF)	221.0
Chloride	Sodium chloride (NaCl)	164.9
Benzoate	Sodium benzoate (NaC_6H_5COO)	119.0

Fluoride calibration standard solution

Fluoride calibration standard solutions were prepared by diluting the 1,000 mg/L stock solution with DI water. Five levels of calibration standard (0.1, 0.25, 0.5, 0.75, and 1 mg/L) were used in this study to check if the fluoride concentration of the USP monograph (0.5 mg/L) is within the linearity range.

System suitability solution

The USP monograph states 1.0 µg/mL of USP Sodium Fluoride RS and 0.5 µg/mL of USP Sodium Chloride RS in water as the system suitability solution. This corresponds to fluoride 0.45 mg/L and chloride 0.30 mg/L. This system suitability solution mixture was prepared by diluting the fluoride and chloride stock solutions with DI water.

Four anions mixture solution

An anions mixture solution (fluoride 0.5 mg/L, chloride 0.5 mg/L, benzoate 10 mg/L, and phosphate 5 mg/L) was prepared to investigate the separation of fluoride from other common anions found in oral mouth rinse samples.

Eluent preparation

Anhydrous sodium carbonate (150 mg) and 1 mL of 1 N sodium hydroxide were added to a 1 L volumetric flask. The volumetric flask was filled approximately two-thirds full of DI water, and the solution was mixed well. Then, it was brought to 1 L with DI water. The solution was filtered through a Nalgene rapid-flow disposable filter unit.

Results and discussion

Separation

Dionex IonPac AS22 columns are designed for compliance monitoring of inorganic anions in accordance with U.S. EPA Methods 300.0 (A) and 300.1 (A). The selectivity of the Dionex IonPac AS22 column was optimized to retain fluoride well out of the water dip (a dip in the baseline at the column's void volume) while separating common anions with an isocratic mobile phase.[3]

Phosphate is listed as an ingredient in all three oral rinse products and benzoate is listed as an ingredient in sample #1. Figure 1 shows the separation of four anions (fluoride, chloride, benzoate, and phosphate) using a Dionex IonPac AS22 column with a run time of 40 min. Fluoride is well away from the water dip, and it is well separated from chloride. The USP monograph method is a fast analysis method that ends the separation immediately after chloride elution. Therefore, it is important to choose a run time that allows benzoate and phosphate from the first injection to elute in positions in subsequent injections that will not affect the integration of fluoride. In addition, the retention of phosphate can vary depending on the column lot and autosampler setting. The run time needs to be adjusted accordingly to make sure phosphate does not interfere with the fluoride integration. Figure 2 shows an overlay of injections of the four-standard mixture and two subsequent DI water blanks. It shows that benzoate will elute in the first injection and phosphate will elute at ~6.5 min of the third injection and does not interfere with fluoride integration.

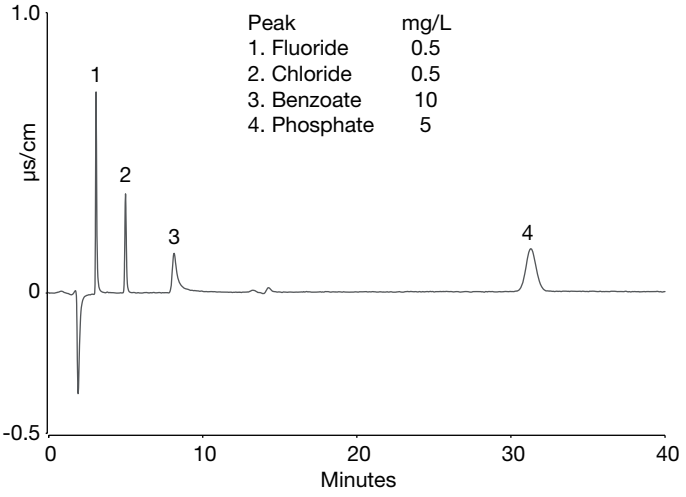

Figure 1. Separation of four anions (40 min run)

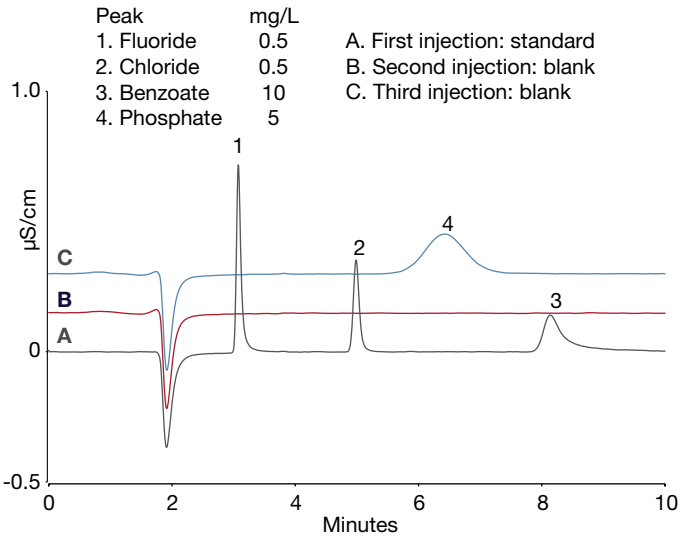

Figure 2. Overlay of anion mixture (10 min run) with two subsequent blank injections

The USP monograph uses a system suitability standard of 1.0 µg/mL of USP Sodium Fluoride RS and 0.5 µg/mL of USP Sodium Chloride RS in water to evaluate resolution, tailing factor, and relative standard deviation. The separation reported here passes USP specifications as shown in Table 2.

Table 2. System suitability. Fluoride is used for the tailing factor and % RSD calculations because it is the target of interest. The resolution is between the fluoride and chloride peaks.

Parameter	Required	Found
Resolution	NLT = 1.5	12.6
Tailing factor	NMT = 2.0	1.55
RSD %	NMT = 2	0.29

Method linearity

The USP monograph method calculates the percentage of fluoride in a sample using one calibration level of 1.1 µg/mL of sodium fluoride standard, which is equivalent to 0.5 mg/L fluoride. To ensure 0.5 mg/L fluoride is in the method's linear range, the linearity of fluoride was investigated in the concentration range of 0.1–1 mg/L (0.1, 0.25, 0.5, 0.75, 1). Figure 3 shows the calibration curve; the coefficient of determination (r^2) is 0.9999 using linear fitting, which suggests an excellent fit between the experimental data and the linear calibration model.

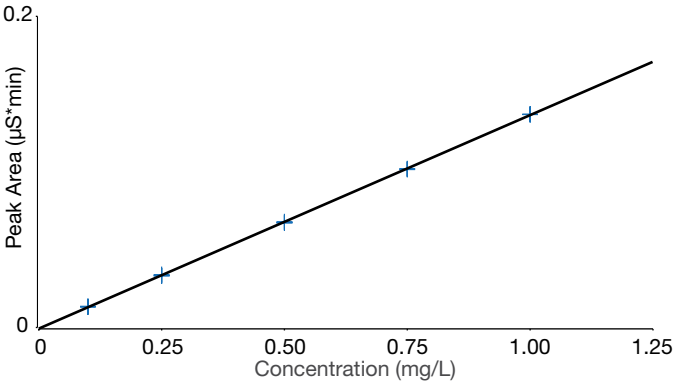

Figure 3. Fluoride calibration curve

Sample analysis

The USP monograph specifies a sample solution as follows: "Nominally 1.1 µg/mL of sodium fluoride from a portion of Oral Solution in water". This corresponds to a fluoride concentration of 0.5 mg/L. The sample was diluted to approximately 0.5 mg/L fluoride according to the active ingredients label (Table 3).

Figure 4 shows the chromatogram of three oral rinse samples with a run time of 40 min to prevent unwanted interferences due to the late eluting phosphate in case of non-autosampler configurations. Fluoride is always resolved from the water dip and well separated from chloride. Benzoate is found in sample #1, as expected from the product label. All three oral rinse products contain phosphate. Chloride is not found in sample #3.

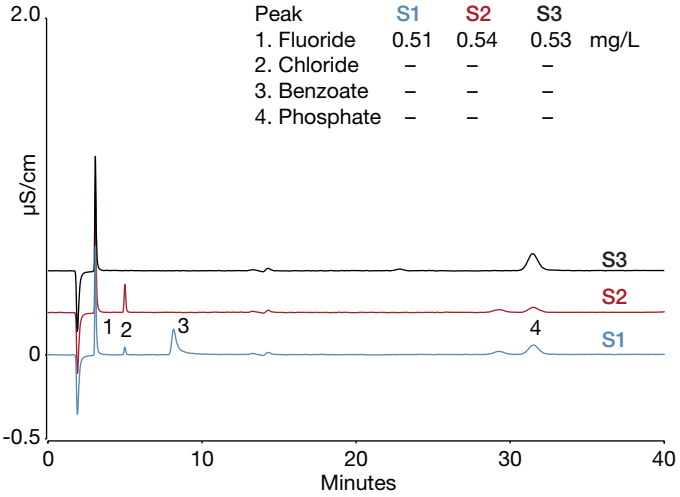

Figure 4. Fluoride determination of three oral rinse samples (40 min run)

Figure 5 shows the chromatogram of three oral rinse samples with a run time of 10 min. Phosphate has not eluted and is expected to elute in the chromatogram of the third injection. Figure 6 shows the phosphate from the first injection will not interfere with quantification of fluoride in the third injection.

Table 3. Preparation of samples to achieve approximately 0.5 mg/L fluoride

Sample	Sample description	Active ingredient (sodium fluoride) labeled %	Convert label to fluoride conc. (mg/L)	Dilution fold	Fluoride conc. (mg/L) after dilution
1	Alcohol free for children	0.05	226.2	450	0.503
2	Alcohol free for adults	0.02	90.5	180	0.503
3	With alcohol for adults	0.02	90.5	180	0.503

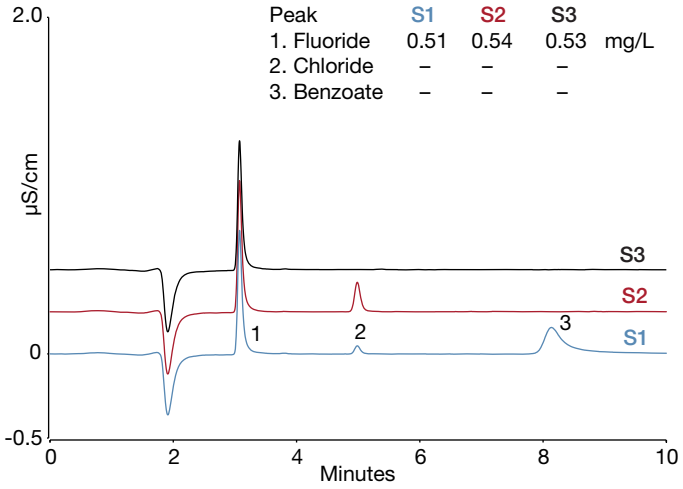

Figure 5. Fluoride determination of three oral rinse samples (10 min run)

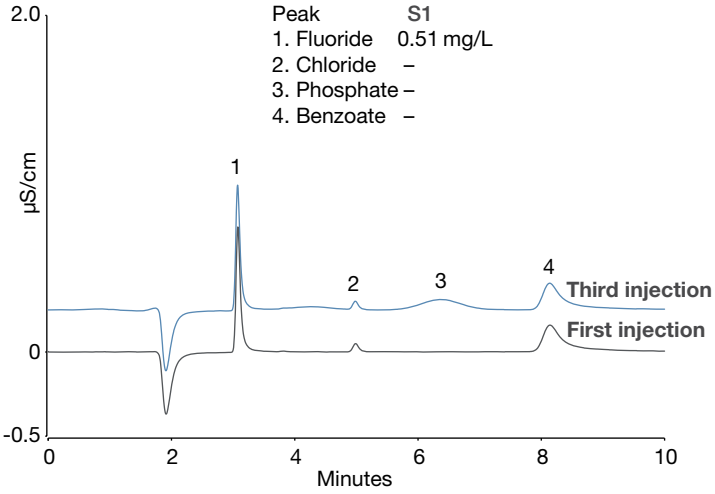

Figure 6. Fluoride determination of oral rinse sample #1 (10 min run, overlay first and third injections)

The USP monograph uses a one-point calibration to calculate the fluoride amount in samples as shown below.

$$\text{Result} = (R_u/R_s) \times (C_s/C_u) \times 100$$

R_u = peak response of fluoride from the sample solution

R_s = peak response of fluoride from the standard solution

C_s = concentration of USP sodium fluoride RS in the standard solution (µg/mL)

C_u = nominal concentration of sodium fluoride in the sample solution (µg/mL)

Table 4 summarizes the sample results using one-point calibration and a calibration curve, respectively. Results show that the fluoride concentrations calculated by the two methods are similar, suggesting that one-point calibration calculation is accurate for this analysis. All three samples pass the USP acceptance criteria of 90%–110%.

Method accuracy and precision

Method accuracy was evaluated by determining the recoveries of the target analytes spiked into the oral rinse sample at concentrations ranging 20% to 50% of the original amount. Table 5 summarizes the analyte recoveries for the three oral rinse samples. The analyte recoveries were all in the range of 90–110%.

Method precision was determined by triplicate injections of the 0.5 mg/L fluoride standard on three separate days. The calculated peak area precision varied 0.71% with retention time precision <0.1% for all target anions.

Table 4. Fluoride in oral rinse sample

Sample	Labeled value (mg/L)	Measured (one point method, mg/L)	Recovery (%)	Measured (Calibration curve method, mg/L)	Recovery (%)
1	226.2	229.3	101	228.4	101
2	90.5	96.8	107	96.3	106
3	90.5	95.5	105	95.0	105

Table 5. Recovery of fluoride spiked in oral rinse sample

Sample	Spike level 1 (~20%)			Spike level 2 (~50%)		
	Amount found (mg/L)	Amount added (mg/L)	Recovery (%)	Amount found (mg/L)	Amount added (mg/L)	Recovery (%)
1	0.51	0.1	105	0.51	0.25	103
2	0.54	0.1	101	0.54	0.25	105
3	0.53	0.1	103	0.53	0.25	103

Conclusion

This application modified the existing USP method for determining fluoride in oral rinse products with a modern, high-performance anion-exchange column optimized for using carbonate-based eluents, as suggested by the USP. The method demonstrates excellent accuracy and precision for fluoride in commercially available oral rinse sample, and, therefore, can be used as a quality control method by oral rinse manufacturers.

References

1. Sodium Fluoride Oral Solution, United States Pharmacopeia National Formulary USP43-NF38 (2023) 4072.
2. Sodium Fluoride Oral Solution (2017) Pharmacopeia Forum 43(1).
3. Thermo Scientific Dionex IonPac AS22 column manual. https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-065119-IC-IonPac-AS22-Fast-Man065119-EN.pdf

Learn more at **thermofisher.com/ic**