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APPLICATION NOTE 72926

Sodium monofluorophosphate monograph modernization using ion chromatography

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Keywords
Dionex IonPac AS18 column, suppressed conductivity detection, pharmaceutical, USP monograph, drug substance, assay, impurity limit, toothpaste

Goal

To validate the IC methods for the assay of sodium monofluorophophate and an impurity (fluoride) in the proposed United States Pharmacopeia Sodium Monofluorophophate monograph

Introduction

Sodium monofluorophosphate (MFP, CAS 10163-15-2) is an active pharmaceutical ingredient (API) approved by the United States Food and Drug Administration (FDA) for use as an anti-caries agent in toothpastes and mouth rinses.[1,2] MFP produces fluoride, which blocks glycolysis[3] and exchanges with the hydroxyl group of the hydroxyapatite crystal of enamel.[4] As a result, oral bacteria produce reduced amounts of acidic compounds, and enamel hydroxyapatite, partially converted into fluorapatite, becomes more insoluble. MFP can hydrolyze to free fluoride and phosphate during storage.[5] Therefore, it is important to determine fluoride and MFP in MFP solid and in toothpastes to evaluate API and drug product quality and stability.

The United States Pharmacopeia (USP) has embarked on a global initiative to modernize many of the existing monographs across all compendia. As part of USP modernization effort, an ion chromatography (IC) method has been proposed to replace existing titration-based assays in the Sodium Monofluorophosphate monograph.[6]

This application note validates the IC method in the USP Sodium Monofluorophosphate monograph following the guidelines outlined in

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USP General Chapter <1225>, Validation of Compendial Methods (Figure 1).[7-9] We used a Thermo Scientific™ Dionex™ ICS-5000+ ion chromatography system with a Thermo Scientific™ Dionex™ IonPac™ AS18 anion-exchange column and a Thermo Scientific™ Dionex™ ADRS 600 (2 mm) Anion Electrolytically Regenerated Suppressor for suppressed conductivity detection. We also used this method to determine sodium monofluorophosphate in a commercial toothpaste.

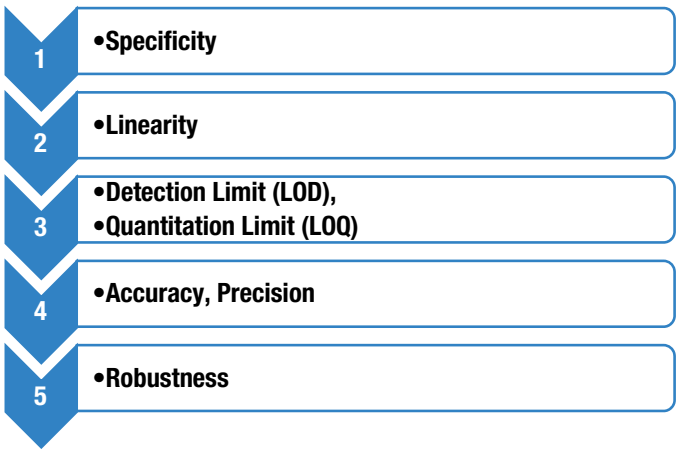

Figure 1. IC method validation

Experimental

Equipment

- A Thermo Scientific™ Dionex™ ICS-5000+ Ion Chromatography (RFIC™) system* was used in this work, which includes:
  - Dionex ICS-5000+ SP/DP Pump module
  - Dionex ICS-5000+ EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-5000+ DC Detector/Chromatography module with conductivity detector and Dual Temperature Zones.
- Thermo Scientific™ Dionex™ AS-AP Autosampler, with 250 µL syringe (P/N 074306), 1.2 mL buffer line assembly (P/N 074989), 2.5 µL injection loop
- Thermo Scientific™ Chromeleon™ 7.2 Chromatography Workstation

* This method can be run on any system supporting an electrolytic suppressor or any Thermo Scientific Dionex IC system using a chemically regenerated suppressor. Please note that this method was not tested with a chemically regenerated suppressor.

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Fluoride Dentifrice Reference standard, Sodium monofluorophosphate (1500 ppm) Silica dentifrice (USP, Cat 1277423, Lot PTG 07-30 containing 1.2% sodium monofluorophosphate)
- Sodium Monofluorophosphate (95%) (Sigma-Aldrich, 34443-500G)
- Sodium Fluoride (Baker, 3688-05)
- Sodium Acetate (Fluka, 71183)
- Sodium Sulfate (Sigma, 71959)

Sample

Over-the-counter toothpaste (0.76% Sodium MFP, purchased from a local store)

Conditions

Table 1. Chromatography conditions

Columns:	Dionex IonPac AS18 Analytical, 2 × 250 mm (P/N 060553) Dionex IonPac AG18 Guard, 2 × 50 mm (P/N 060555)
Eluent:	*Time (min)* — *KOH (mM)* -5 — 15 0 — 15 20 — 15 30 — 30 35 — 60 45 — 60 45.1 — 15 50 — 15
Eluent Source:	Thermo Scientific™ Dionex™ EGC KOH cartridge with Thermo Scientific™ Dionex™ CR-ATC column
Flow Rate:	0.25 mL/min
Injection Volume:	2.5 µL in Push-Full mode
Column Temp.:	30 ˚C
Detector Temp.:	35 ˚C
Detection:	Suppressed conductivity, Dionex ADRS 600 (2 mm) Suppressor, recycle mode, 38 mA current
System Backpressure:	~2500 psi
Run Time:	55 min (includes 5 min equilibrium time)

Preparations of solutions and reagents

Note: Do not use glassware to prepare the solutions. Polymeric containers made of high-density polyethylene (HDPE) are recommended.

Stock standard solutions 1000 to 3000 µg/mL in water

Accurately weigh 100 to 300 mg of solid into a 100 mL polypropylene bottle and dissolve in 100 mL (100.00 g) of DI water. Keep at 4 ˚C for up to a month.

Standard solution for assay, 150 µg/mL in water

Mix 1.0 mL (1.0 g) of 3000 µg/mL of sodium monofluorophosphate stock standard solution and 19.0 mL (19.0 g) of DI water to make the standard solution for assay. Prepare fresh for each sequence.

Calibration standard for sodium monofluorophosphate linearity

To prepare calibration standard solutions of 0.2, 2, 10, 20, 50, 100, 150, and 300 µg/mL sodium monofluorophosphate, dilute the stock standard solution (3000 µg/mL) to the appropriate concentrations with DI water.

Calibration standard for linearity and detection limit of sodium fluoride

Mix 1.0 mL (1.0 g) of 1000 µg/mL of sodium fluoride stock standard solution and 99.0 mL (99.0 g) of DI water to make 10 µg/mL sodium fluoride. Further dilute the 10 µg/mL standard solution to 0.05, 0.2, 0.5,1, 2.5, and 5 µg/mL with DI water.

System suitability solution

Dilute the stock standard solutions with DI water to make the system suitability solution containing 4.0 µg/mL of sodium fluoride, 1.4 µg/mL of sodium acetate, 150 µg/mL of sodium monofluorophosphate, and 150 µg/mL of sodium sulfate. Keep stock standard solutions at 4 ˚C.

Sample preparation

Two samples were used: 1) Fluoride Dentifrice Reference standard from USP, which contained 1.2% of sodium monofluorophosphate according to its data sheet.[2]) An over-the-counter toothpaste, which contained 0.76% sodium monofluorophosphate.

Weigh the Dentifrice Reference standard or toothpaste into a container (200 to 500 mg in vial or beaker) and record the weight of the sample. Add DI water (1 mL DI water per ~10 mg sample) to dissolve the sample and record the water weight. Filter through a 0.2 µm syringe filter before analysis. If the sample concentration is too high, dilute with DI water until the concentration is about 150 µg/mL.

To remove hydrophobic substances, a toothpaste sample was treated with a Thermo Scientific™ Dionex™ OnGuard™ II RP cartridge. This was only used during method development and was later omitted.

Robustness study

Following the guidelines of USP General Chapter <621> Chromatography,[10] the robustness of this method was evaluated by examining the retention time (RT), peak asymmetry, and assay result of a toothpaste sample and system suitability standard after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent gradient concentration, column temperature). The same procedure was applied to two column sets from two different lots. The following variations were tested:

- Flow rate at 0.25 mL/min, 0.225 mL/min, and 0.275 mL/min
- Column temperature at 30 ˚C, 27 ˚C, and 33 ˚C
- Eluent concentrations according to the method (Table 1), 10% more concentrated, and 10% less concentrated

Table 2. Preparation of standard stock solutions

Compound	Weight to prepare 100 mL stock standard (mg)	Concentration (mg/L= µg/mL)
Sodium Fluoride (NaF)	100	1000
Sodium Acetate (NaOAc)	100	1000
Sodium Monofluorophosphate (Na_2PO_3F)*	300	3000
Sodium Sulfate (Na_2SO_4)	150	1500

*The 95% sodium monofluorophosphate from Aldrich was used to prepare the standard because pure USP reference standard is not available yet.

Results and discussion

Separation

In this study, a 2 mm Dionex IonPac AS18 column, instead of the 4 mm column described in the proposal, was used to save eluent and thus be more environmentally friendly. The column temperature was decreased to 30 °C from the originally proposed 40 °C as 30 °C is the recommended temperature for the Dionex IonPac AS18 column. Both modifications are allowed according to the guidelines of USP General Chapter <621> Chromatography, which allows column diameter to be adjusted by as much as ±50% and column temperature to be adjusted by as much as ±10 °C.[10]

Figure 2 shows chromatograms of system suitability and calibration standards. There are good separations of fluoride from acetate and monofluorophosphate from sulfate. The retention times of monofluorophosphate (24.68 min for 40 °C, 19.15 and 20.28 min for 30 °C) and fluoride (3.95 min for 40 °C, 3.86 and 3.89 min for 30 °C) are consistent with the proposed USP method, which states about 22 min and 3.6 min. Note that the higher temperature of the proposed method relative to the lower temperature proposed here causes a multivalent anion such as MFP to be more retained and thus have a longer retention time.

Table 3 displays the results of the system suitability standard containing 4.0 µg/mL sodium fluoride, 1.4 µg/mL sodium acetate, 150 µg/mL sodium monofluorophosphate, and 150 µg/mL sodium sulfate separated with two column temperatures and on two columns from different column lots. It was found that temperature has minimal impact on the resolution between fluoride and acetate and between monofluorophosphate and sulfate. The methods at both 30 °C and 40 °C gave good resolution values (1.72 to 2.29) and precise peak areas (relative standard deviation (RSD) for fluoride (<0.7%) and monofluorophosphate (<0.14%)). The IC method with a column temperature of 30 °C was tested with two column lots. Both results satisfied the suitability requirements: RSD of the fluoride peak area (0.4% and 0.08%) is not more than (NMT) 5%, RSD of monofluorophosphate peak area (0.13% and 0.06%) is NMT 2%, the resolutions between fluoride and acetate (1.88 and 1.94) and between monofluorophosphate and sulfate (1.95 and 2.04) are not less than (NLT) 1.5. Therefore, the IC method with column temperature at 30 °C was chosen for the impurity determination to assay sodium monofluorophosphate and was validated in this application note.

Columns:	Dionex IonPac AG18, 2 × 50 mm and Dionex IonPac AS18, 2 × 250 mm
Eluent:	KOH
Gradient:	0–20 min, 15 mM; 20–30 min, 15–30 mM; 30–35 min, 30–60 mM; 35–45 min, 60 mM; 45.1 min, 15 mM; 45.1–50 min, 15 mM
Eluent Source:	Dionex EGC-KOH Cartridge with Dionex CR-ATC and Dionex High Pressure Degasser
Flow Rate:	0.25 mL/min
Inj. Volume:	2.5 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ADRS 600 (2 mm) Suppressor, 35 °C, 38 mA, recycle mode
Standards*:	A: 150 µg/mL sodium monofluorophosphate B: Suitability standard containing 4.0 µg/mL of sodium fluoride, 1.4 µg/mL of sodium acetate, 150 µg/mL of sodium monofluorophosphate, and 150 µg/mL of sodium sulfate
Peaks:	1 Fluoride 2 Acetate 3 Monofluorophosphate 4 Sulfate

*Prepared from 95% pure sodium monofluorophosphate

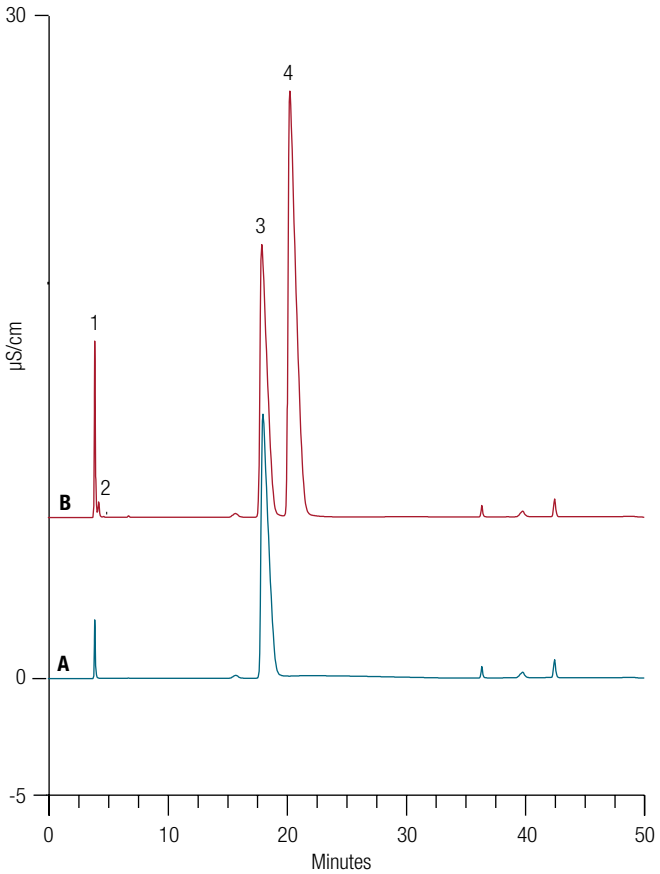

Figure 2. Chromatograms of system suitability and calibration standards

Table 3. Comparison of the suitability results at different column temperatures

Column Lot	Temp. (°C)	Compound	Concentration (µg/mL)	Ret. Time (min)	Relative Ret. Time	RSD	Peak area (µS*min)	RSD	Resol.	RSD
A	40	NaF	4*	3.95	0.18	0.15	0.769	0.05	2.29	0.41
		NaOAc	1.4	4.34	0.20	0.09	0.058	0.30		
		Na_2PO_3F	150	22.00	1.00	0.03	8.451	0.01	1.84	0.08
		Na_2SO_4	150	24.68	1.12	0.03	14.298	0.03		
B	40	NaF	4*	3.95	0.19	0.17	0.859	0.13	1.72	0.33
		NaOAc	1.4	4.306	0.21	0.12	0.101	2.03		
		Na_2PO_3F	150	20.8	1.00	0.00	8.644	0.21	1.77	0.05
		Na_2SO_4	150	23.276	1.12	0.01	14.655	0.20		
A	30	NaF	4*	3.89	0.22	0.17	0.824	0.08	1.94	0.04
		NaOAc	1.4	4.22	0.24	0.16	0.063	0.30		
		Na_2PO_3F	150	17.91	1.00	0.02	8.375	0.06	2.04	0.06
		Na_2SO_4	150	20.28	1.13	0.03	14.440	0.09		
B	30	NaF	4*	3.86	0.23	0.05	0.760	0.40	1.88	0.51
		NaOAc	1.4	4.17	0.25	0.00	0.041	0.69		
		Na_2PO_3F	150	16.94	1.00	0.02	8.322	0.13	1.95	0.07
		Na_2SO_4	150	19.15	1.13	0.03	14.532	0.17		

*Actual concentration was 5.9 µg/mL. 1.9 µg/mL of fluoride was the impurity in 150 µg/mL sodium monofluorophosphate.

Figure 3 shows chromatograms of 150 µg/mL of sodium monofluorophosphate, a toothpaste sample, and a dentifrice reference standard sample. It shows good separation of monofluorophosphate from the other peaks in the toothpaste product. Ten minutes of high concentration (60 mM) KOH is included in the IC method to elute an unknown compound eluting at about 42 min.

It was observed that the chromatograms were different for the first and second toothpaste sample injections with a broad peak at about 38 min in the second and all subsequent toothpaste injections. We believe this is caused by an unknown compound carrying over to the next injection. Figure 4 shows chromatograms of four successive injections, two from a toothpaste sample and two from DI water. After treating the sample with a reversed-phase SPE cartridge (Dionex OnGuard II RP cartridge), the unknown peak was absent. As this unknown compound does not interfere with either fluoride or monofluorophosphate and has no impact on their determinations in terms of retention time and peak area, the treatment was not used in this study.

Calibration of sodium monofluorophosphate

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and the USP General Chapter <1225> guidelines recommend a minimum of five concentrations to establish linearity in an assay. For a drug substance or finished product, the minimum specified range is from 80% to 120% of the test concentration for method accuracy validation testing.

Columns:	Dionex IonPac AG18, 2 × 50 mm and Dionex IonPac AS18, 2 × 250 mm
Eluent:	KOH
Gradient:	0–20 min, 15 mM; 20–30 min, 15–30 mM; 30–35 min, 30–60 mM; 35–45 min, 60 mM; 45.1 min, 15 mM; 45.1–50 min, 15 mM
Eluent Source:	Dionex EGC-KOH Cartridge with Dionex CR-ATC and Dionex High Pressure Degasser
Flow Rate:	0.25 mL/min
Inj. Volume:	2.5 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ADRS 600 (2 mm) Suppressor, 35 °C, 38 mA, recycle mode
Standards*:	A: 150 µg/mL sodium monofluorophosphate* B: Toothpaste sample C: USP Fluoride Dentifrice Reference Standard
Peaks:	1 Fluoride 2 Acetate 3 Monofluorophosphate 4 Sulfate

*Prepared from 95% pure sodium monofluorophosphate

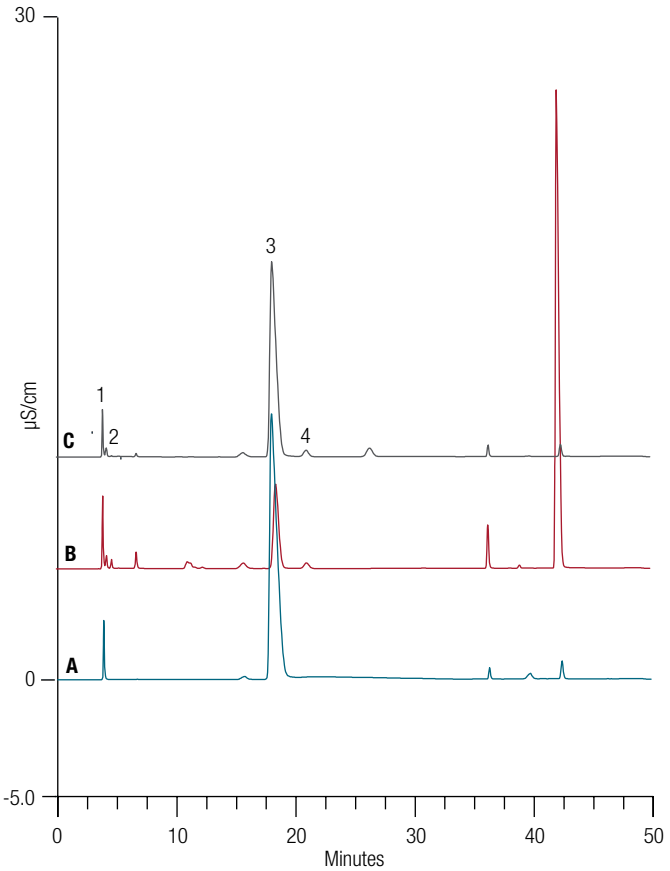

Figure 3. Chromatograms of a calibration standard and toothpaste products

Columns:	Dionex IonPac AG18, 2 × 50 mm and Dionex IonPac AS18, 2 × 250 mm
Eluent:	KOH
Gradient:	0–20 min, 15 mM; 20–30 min, 15–30 mM; 30–35 min, 30–60 mM; 35–45 min, 60 mM; 45.1 min, 15 mM; 45.1–50 min, 15 mM
Eluent Source:	Dionex EGC-KOH Cartridge with Dionex CR-ATC and Dionex High Pressure Degasser
Flow Rate:	0.25 mL/min
Inj. Volume:	2.5 µL
Column Temp.:	30 °C
Detection:	Suppressed conductivity, Dionex ADRS 600 (2 mm) Suppressor, 35 °C, 38 mA, recycle mode
Samples:	Top: S1: 1st injection of the toothpaste sample S2: 2nd injection of same toothpaste sample W1: DI water after the toothpaste sample W2: 2nd DI water Bottom: S1*: 1st injection of treated toothpaste sample S2*: 2nd injection of treated toothpaste sample W1: DI water after the toothpaste sample W2: 2nd DI water

*The toothpaste sample was treated by a Dionex OnGuard II RP cartridge to remove hydrophobic substances.

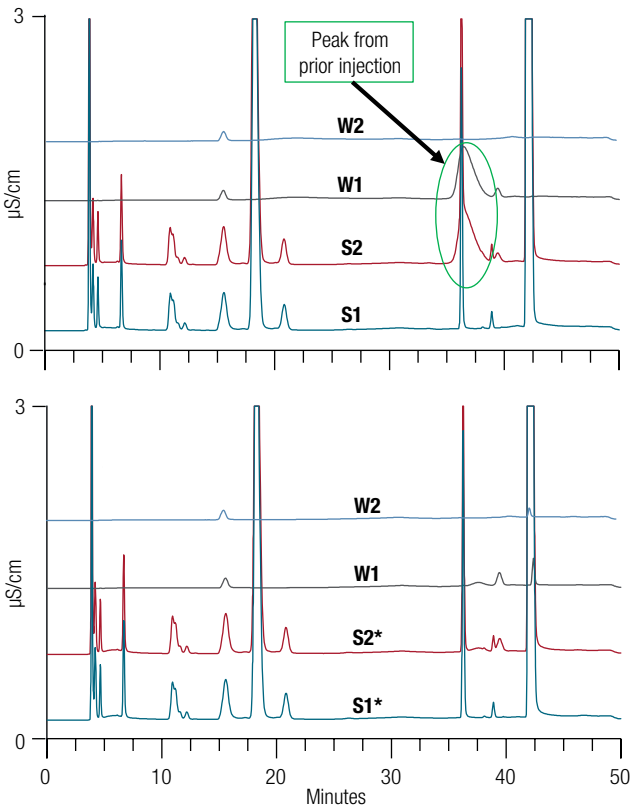

Figure 4. Chromatograms of four successive injections, two of a toothpaste sample and two of a DI water sample

In this study, sodium monofluorophosphate was calibrated at eight concentration levels ranging from 0.2 to 300 µg/mL. A linear relationship of peak area to concentration resulted in a coefficient of determination (r^2) of 0.998 (Table 4 and Figure 5). As the calibration is linear, using a one-point calibration at a 150 µg/mL sample concentration, as proposed in the respective USP monograph, is an acceptable method for assay.

Table 4. Comparison of calibration methods for sodium monofluorophosphate

Calibration Standards (µg/mL)	Calibration Type	r^2
0.2–300	Quadratic	1
0.2–300	Linear, through origin	0.998

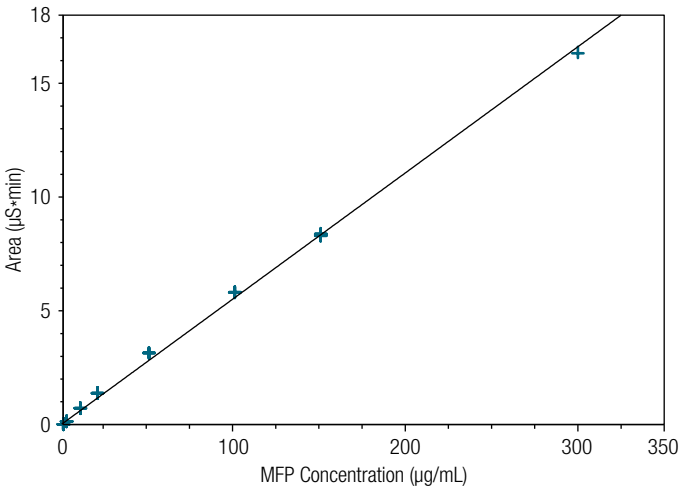

Figure 5. Calibration plot for sodium monofluorophosphate illustrating linearity

Calibration of fluoride, limit of detection (LOD), and limit of quantitation (LOQ)

According to the ICH and the USP guidelines, a minimum calibration range of 50% to 120% is required for the determination of an impurity with a minimum of five concentrations to establish its calibration curve. Sensitivity measurements (LOQ and LOD) are also required.

In this study, sodium fluoride was calibrated at seven concentration levels ranging from 0.05 to 10 µg/mL. The results yield a linear relationship of peak area to concentration (Table 5 and Figure 6) with the coefficient of determination (r^2) = 1.

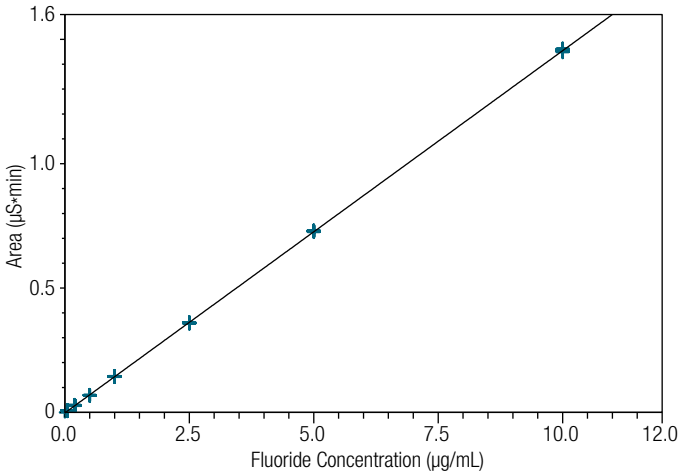

Figure 6. Calibration plot for fluoride illustrating linearity

The LOD and LOQ were determined by seven injections of 0.05 µg/mL sodium fluoride. The baseline noise was determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute but close to the peak of interest. The LOD and LOQ were determined for the concentration at the signal-to-noise ratio of 3 and 10, respectively (Table 5). The IC method is suited for the determination of fluoride impurities in toothpaste with an LOD of 0.008 µg/mL and an LOQ of 0.027 µg/mL.

Table 5. Calibration, limit of detection (LOD), and limit of quantitation data (LOQ) for fluoride

Analyte	Calibration Standards (µg/mL)*	Coefficient of Determination (r^2)	LOD (µg/mL)	LOQ (µg/mL)
Fluoride*	0.05–10	1.000	0.008	0.027

*Sodium fluoride salt

The acceptance criteria for impurity is NMT 1.2% of sodium fluoride in sodium monofluorophosphate. As the IC method is sensitive with linear calibration, a one-point calibration at 4 µg/mL sodium fluoride, as described in the proposed USP, is acceptable.

Sample analysis

The proposed USP monograph requires that sodium monofluorophosphate contains 91.7%–100.5% on the dried basis. However, the 100% pure USP sodium monofluorophosphate reference standard was not available at the time of this work (the USP was engaged in its validation). Therefore, a 95% pure sodium monofluorophosphate was used to prepare solutions for the determination of sodium monofluorophosphate in the toothpaste sample.

Using sodium fluoride calibration, the impurity (sodium fluoride) in sodium monofluorophosphate was determined. 1.9 µg/mL of sodium fluoride was found in 150 µg/mL sodium monofluorophosphate.

The percentage of fluoride in the portion of sodium monofluorophosphate taken is 0.6%, which passed the acceptance criteria of NMT 1.2% (Table 6).

Accuracy and precision

The method precision (Table 7) was evaluated by injecting the suitability standard. The method shows an intraday precision of 0.08% to 0.35% for sodium fluoride and 0.06% to 0.18% for sodium monofluorophosphate, and interday precision of 3.95% for sodium fluoride and 0.77% for sodium monofluorophosphate.

Method accuracy (Tables 8 and 9) was validated by comparing the measured sodium monofluorophosphate concentration with labeled relative amount of sodium monofluorophosphate in the USP Dentifrice Reference standard and in the toothpaste sample. The method shows a 99% accuracy for the USP Dentifrice Reference standard and 97% for the toothpaste.

Table 6. Sodium fluoride in sodium monofluorophosphate

	Fluoride (µg/mL)	RSD	Fluoride in Sodium Monofluorophosphate (%)
150 µg/mL Sodium monofluorophosphate	1.9	0.15	0.6

Table 7. Precision of the IC method for sodium monofluorophosphate assay and impurity determination

Analyte	Intraday Precision (%)*	Interday Precision (%) **
Sodium fluoride	0.1 to 0.4	4.0
Sodium monofluorophosphate	0.06 to 0.18	0.77

* Intraday precision range is calculated from n=3 injections.
** Interday precision is calculated from 3 days, n=3 injections each day.

Table 8. Sodium monofluorophosphate in the USP Dentifrice Reference standard with 1.2% sodium monofluorophosphate. Each sample was independently prepared. n=3 injections/sample. The five samples were prepared and analyzed over two days.

Sample	Measured Sodium Monofluorophosphate (%)	Accuracy (%)	Average Sodium Monofluorophosphate in Sample (%)	Average Accuracy (%)
1	1.19 ± 0.01	99	1.19 ± 0.04	99
2	1.20 ± 0.00	100		
3	1.14 ± 0.02	95		
4	1.24 ± 0.03	103		
5	1.20 ± 0.01	100		

Table 9. Sodium monofluorophosphate in toothpaste with 0.76% Sodium monofluorophosphate. Each sample was independently prepared. n=3 injections/sample. The six samples were prepared and analyzed over three days, two samples/day.

Sample	Measured Sodium Monofluorophosphate (%)	Accuracy (%)	Average Sodium Monofluorophosphate in Sample (%)	Average Accuracy (%)
1	0.76 ± 0.02	100	0.74 ± 0.2	97
2	0.75 ± 0.04	98		
3	0.73 ± 0.00	96		
4	0.73 ± 0.01	96		
5	0.73 ± 0.00	96		
6	0.71 ± 0.01	94		

Robustness

Assay robustness was evaluated by measuring the influence of small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature on the RT, peak asymmetry, and peak resolution). These tests were carried out on two column sets from two different lots. The peak asymmetry was measured following the USP standard. Table 10 summarizes the results for sodium monofluorophosphate. These results indicate the method was robust to both changes in chromatography parameters and column.

Table 10. Robustness of the IC-based assay for sodium monofluorophosphate (injected sample: system suitability standard)

Parameter		Column A					
		Ret. Time (min)		Asymmetry		Resolution	
		Average	% Diff	Average	% Diff	Average	% Diff
Flow Rate (mL/min)	0.225	15.98	-9	2.19	-1%	1.94	-1%
	0.25	17.58		2.22		1.97	
	0.275	19.39	10	2.25	2%	1.97	0%
Column Temp. (˚C)	27	16.34	-7	2.15	-3%	2.01	2%
	30	17.58		2.22		1.97	
	33	18.59	6	2.26	2%	1.90	-3%
Eluent Conc. (mM)	13.5/27/54	14.90	-15	2.11	-5%	1.89	-4%
	15/30/60	17.58		2.22		1.97	
	16.5/33/66	20.98	19	2.33	5%	1.99	1%
Parameter		**Column B**					
		Ret. Time (min)		Asymmetry		Resolution	
		Average	% Diff	Average	% Diff	Average	% Diff
Flow Rate (mL/min)	0.225	16.21	-4%	2.65	2%	1.96	1%
	0.25	16.93		2.60		1.95	
	0.275	19.66	16%	2.73	5%	1.97	1%
Column Temp. (˚C)	27	16.55	-2%	2.59	0%	2.01	3%
	30	16.93		2.60		1.95	
	33	18.83	11%	2.77	7%	1.90	-2%
Eluent Conc. (mM)	13.5/27/54	15.09	-11%	2.58	-1%	1.91	-2%
	15/30/60	16.93		2.60		1.95	
	16.5/33/66	21.29	26%	2.80	8%	2.00	3%

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Conclusion

This study evaluated the IC methods included in the proposed USP Sodium Monofluorophosphate monograph revision. The IC methods use a Thermo Scientific Dionex IonPac AS18 anion-exchange column and suppressed conductivity detection. The column temperature was changed from 40 ˚C to 30 ˚C to be consistent with the recommended column temperature and to positively impact column lifetime. Following the guidelines outlined in USP General Chapter <1225> (Validation of Compendial Methods) and the monograph instructions, the IC method with the 30 ˚C column temperature was validated. Deliberate variations in the IC method parameters (e.g., mobile phase concentration, column temperature) were also made to test robustness.

It was found that the IC method is linear over the established analytical range for both sodium monofluorophosphate (r^2 = 0.998, 0.2–300 µg/mL) and sodium fluoride (r^2 = 1, 0.05–10 µg/mL). The method is accurate (94–103%), precise (intraday precision from 0.06% to 0.18% and interday precision of 0.77%), and specific for sodium monofluorophosphate determination. The method is also sensitive (LOQ = 0.027 µg/mL), precise (intraday precision from 0.1% to 0.4% and interday precision of 4.0%), and specific for the determination of the impurity, sodium fluoride. The method is robust for both assay and impurity determination as deliberate IC method parameter variations had no significant impact on the parameters important to obtaining accurate results.

In conclusion, the IC method meets the guidelines outlined in USP General Chapter <1225> and can be used to replace existing titration-based assays in the USP Sodium Monofluorophosphate monograph.

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