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Determination of glycerophosphate and other anions in dentifrices by ion chromatography

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Abstract

Simple, reliable and sensitive analytical methods to determine the anions, such as fluoride, monofluorophaosphate, glycerophosphate related to anticaries are necessary for basic investigations of anticaries and quality control of dentifrices. A method for the simultaneous determination of organic acids, organic anions and inorganic anions in the sample of commercial toothpaste is proposed. Nine anions (fluoride, chloride, nitrite, nitrate, sulfate, phosphate, monofluorophaosphate, glycerophosphate and oxalic acid) were analyzed by means of ion chromatography using a gradient elution with KOH as mobile phase, IonPac AS18 as the separation column and suppressed conductivity detection. Optimized analytical conditions were further validated in terms of accuracy, precision and total uncertainty and the results showed the reliability of the IC method. The relative standard deviations (RSD) of the retention time and peak area of all species were less than 0.170 and 1.800%, respectively. The correlation coefficients for target analytes ranged from 0.9985 to 0.9996. The detection limit (signal to noise ratio of 3:1) of this method was at low ppb level (<15 ppb). The spiked recoveries for the anions were 96–103%. The method was applied to toothpaste without interferences.

Keywords: Ion chromatography; Fluoride; Monofluorophaosphate; Calcium glycerophosphate; Anions

1. Introduction

Sodium monoflurophosphate (sodium MFP) and sodium fluoride are two agents commonly added to toothpaste for caries prevention. Both of them were used for decades in the toothpaste production. Clinical research has proved that fluoride plays an important role in the prevention of caries, while MFP hydrolyzes spontaneously during the application of the product, producing fluoride and phosphate in situ. Apart from its positive cariostatic properties, fluoride is a hazardous substance. An acute intake of a large dose or chronic ingestion of lower doses of fluoride ions can result in a variety of side effects, such as acute gastric and kidney disturbances, dental and skeletal fluorosis or even death [2–4].

Calcium was of interest because of its use in nutritional fortification. Calcium glycerophosphate and gluconate were less intense than calcium chloride across all attributes but metallic [1]. Calcium glycerophosphate is an increasingly common practice that is added to the toothpaste, because it plays a critical role in preventing tooth decay.

Simple, reliable and sensitive analytical methods to determine the anions, such as fluoride, monofluorophaosphate and glycerophosphate that are related to anticaries are necessary for basic investigations of anticaries and quality control of dentifrices.

Several techniques have been used to analyze the water-soluble fluoride species in toothpaste: ion chromatography (IC) [2,3], fluoride ion-selective electrode electroanalysis [5], gas chromatography (GC) [6], capillary electrophoresis (CE) [7,8] and spectrophotometry [9]. Among these methods, ion selective electrode analysis is useful for analysis of fluoride in products; it would not be convenient to measure F^- and PO_3F^{2-} simultaneously in aged oral care formulations. GC has a disadvantage that MFP must be hydrolyzed first before the total fluoride is measured. The CE method suffers from a rather poor sampling rate. The method used to determinate glycerophosphate in the toothpaste has not been reported.

Since its introduction in 1975, ion chromatography (IC) has become a routine analytical method for the determination of inorganic ions, especially anions present in various matrices.

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With the development of new separation methods, novel stationary phases, a great variety of detectors, etc. the concept of IC was later successively widened and IC can applied in different area. [10,11].

Due to the weak binding of fluoride to the ion-exchangers commonly used in IC, fluoride was generally eluted rapidly from the column and is found very close to the "injection" peak. IC methods for direct determination of fluoride in toothpaste were limited. With the inventiveness of high capacity columns such as IonPac AS18, the problem has been resolved. Meanwhile, the common anions, monoflurophosphate, glycerophosphate and some of organic acids are strong retention in anion exchange resin. For simultaneous determination of all anionic species in toothpaste, the gradient eluent of KOH, which is generanted by EG50 KOH eluent generator, is necessary.

In this paper, Using AS18 column, six common anions, monoflurophosphate, glycerophosphate and organic acids can be determined simultaneity by gradient IC. The method developed was successfully applied to determination of anionic species in a sample of commercial toothpaste.

2. Experimental

2.1. Chromatographic system

All systems and components for ionic analysis were from Dionex (Sunnyvale, CA, USA). The hardware used for these analyses consisted of an ICS-2000 ion chromatograph equipped with an isocratic pump, a conductivity detector an EG50 eluent generator. Data were acquired by using Chromeleon software installed on a Dell P-IV computer.

The eluent generator was used to generate KOH gradient concentrations. For all analyses in this report, the gradient profile consisted of the following: 1.0–3.5 mM from 0 to 5 min, 4.0 mM from 5 to 12 min, 4 to 12 mM from 12 to 20 min (curve = 9), 12 to 28 mM from 20 to 28 min, 80 mM from 28 to 40 min.

Eluent flow rates were set at 0.25 ml min^{-1} with an injection volume of 25 μl.

For the analytical separation, an IonPac AG18 (50 mm × 2 mm) guard column and an IonPac AS18 (250 mm × 2 mm) analytical column were used. In addition, an ASRS-Ultra (2 mm) suppressor was utilized in the recycle mode.

2.2. Reagents and samples

All reagents were of analytical reagent grade unless otherwise specified. Aliquots of commercially available stock solutions (1000 mg l^{-1}) were used to prepare anion mixtures containing the ions to be investigated.

The sodium monofluorophosphate was obtained from Fluka and had an assay value of 98.3% by the USP assay procedure. The calcium glycerophosphate was purchased from Fluka (USA) and had an assay value of 104% by the USP assay procedure (by titration with 1N HCl caculated on dry substance). Deionized water at 18.3 MΩ resistivity was used throughout.

2.3. Sample preparation

Approximately 1.0 g of a toothpaste sample was weighed into 100 ml volumetric flask. The flask was filled with about 50 ml of deionized water, stand ultrasonic agitation for up to 30 min, then dilution to volume with deionized water. Then, the solution above was diluted five folds with deionized water. Sample solutions were filtered through a 0.45 μm membrane filter before sample injection.

3. Results and discussion

3.1. Method development

The selection of ICS-2000 system and IonPac AS18 high capacity anion column allowed a significant reduction of the interference problems caused by water dip, as well as higher detection sensitivity because of the low background conductivity [12]. There were so many analytes in the toothpaste that gradient elution was needed.

Advances in hardware, such as the EG50 eluent generator, which through programmed current values can generate hydroxide in predetermined concentrations, simplify the use of gradient chromatography. Only deionized water needs to supplied by the user, conductivity background are low, and gradients are more reproducible, human error or potential problems with the proportioning valve in the eluent pump not being factors.

The back ground conductivity of the KOH eluent generated by eluent generator after suppression was ca. 0.3 μs cm^{-1}. In addition, the low detection noise leads to an improvement in the detection sensitivity of anions. Another advantage of measuring under lower background conductivity conditions was to decrease the water dip volume on the chromatogram leading to a good separation of fluorine and water dip.

By using low concentration KOH from 1.0 to 3.5 mM in 5 min, the resolution for fluride between water dip ($\alpha = 7.02$) and between acetate ($\alpha = 1.60$) were remarkable improved.

Under the selected experimental conditions, the retention times and capacity factor for the studied compounds were as follows: fluoride (6.55 min, 0.50), chloride (11.16 min, 1.56), nitrite (13.67 min, 2.14), nitrate (22.80 min, 4.23), glycerophosphate (24.69 min, 4.66), monoflurophosphate (27.12 min, 5.22), sulfate (27.75 min, 5.36), oxalic acid (28.96 min, 5.64), and phosphate (33.43 min, 6.67).

3.2. Hydrolyzation of monoflurophosphate and glycerophosphate

The Hydrolyzation of monoflurophosphate and glycerophosphate (GP) was investigated in this experiment. The MFP breaks down to fluoride and phosphate. And the GP also hydrolyzes, producing glycerol and phosphate. In the standard samples of MFP and GP, the concentration of phosphate increased. The appearance of phosphate was in balance with the hydrolysis of MFP and GP. Figs. 1 and 2, respectively show the measured concentration of phosphate in a 0.10 mmol l^{-1} standard sample

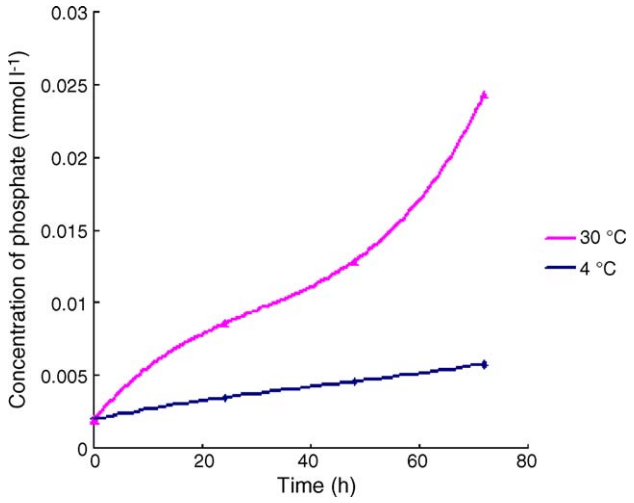

Fig. 1. The influence of placement time on the measured concentration of phosphate in 0.10 mmol l^{-1} monoflurophosphate standard solution.

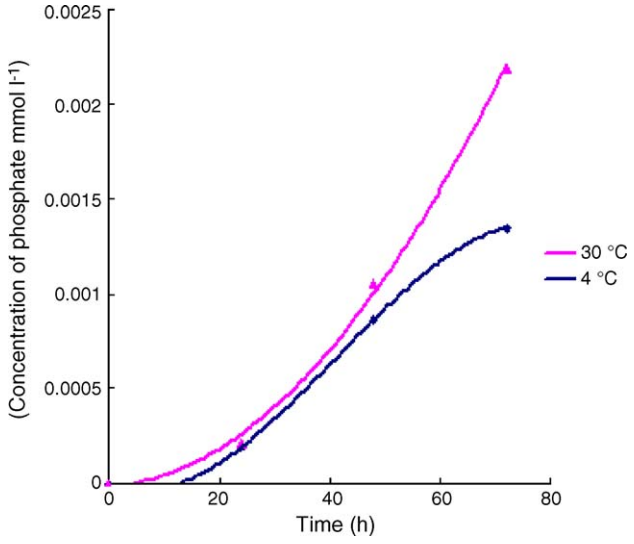

Fig. 2. The influence of placement time on the measured concentration of phosphate in 0.10 mmol l^{-1} glycerophosphate standard solution.

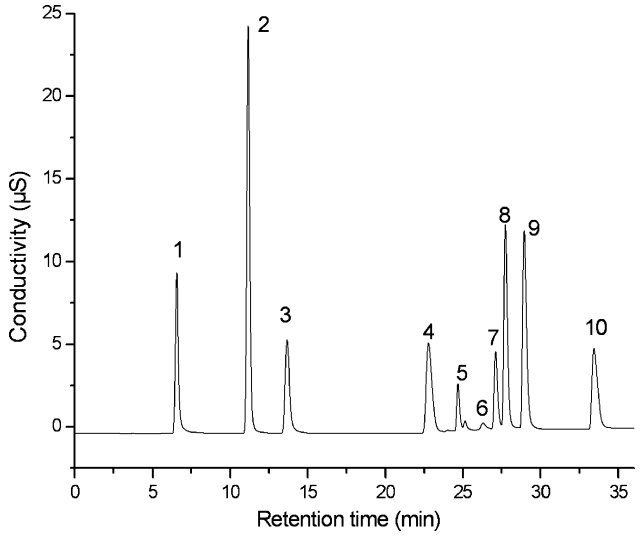

Fig. 3. Chromatogram of standard anions; peaks (mg l^{-1}): (1) fluoride(1); (2) chloride(4); (3) nitrite(2); (4) nitrate(3); (5) glycerophosphate(4); (6) carbonate; (7) monoflurophosphate(2); (8) oxalate(4); (9) sulfate(3); and (10) phosphate(4).

of MFP and GP at 4 °C and 30 °C. The solution of GP is much more stable than that of MFP. The hydrolysis of MFP was very slow at 4 °C, only 1.5% of MFP hydrolyzed after 24 h. At 30 °C, MFP hydrolyzes faster, 6.6% hydrolyzed in 24 h. However, temperature did not have great effect on the hydrolysis of GP. GP can keep stable at 4 °C or room temperature. Totally speaking, all the samples should be detected after preparation as soon as posssible in order to purue good accuracy of the results.

3.3. Method validation

According to Metha [13] requirements the selectivity of the proposed method is right because the peaks showed resolutions ≥1.5 for all the determined compounds. Fig. 3 presents ionic chromatograms showing the anion separation of a standard mixture sample.

The relative standard deviations (RSDs) of the retention time and peak area within one day were less than 0.17 and 1.80%, respectively. And interday RSDs of retention time and peak area (5 days) were less than 0.28 and 2.75%, respectively. The linearity and detection limits data are summarized in Table 1. The

Table 1
RSDs, linearity and detection limits data of standard anions

Anion	RT RSD[a] %	Peak area[a] RSD %	Linear equations[b]	Linear range of anion (mg l^{-1})	Coefficient of correlation (r^2)	Detection limit (S/N = 3, mg l^{-1})
Fluoride	0.16	1.78	$Y = 2.2813X - 0.0026$	0.05–20	0.9994	0.0010
Chloride	0.17	0.15	$Y = 1.4313X - 0.0110$	0.1–50	0.9998	0.0015
Nitrite	0.21	0.47	$Y = 0.9478X - 0.0119$	0.5–60	0.9995	0.0034
Nitrate	0.14	0.33	$Y = 0.8180X - 0.0210$	0.5–40	0.9989	0.0047
Glycerophosphate	0.14	1.68	$Y = 0.1482X - 0.0068$	0.5–40	0.9993	0.0133
Monoflurophosphate	0.11	1.45	$Y = 0.6157X - 0.0054$	0.5–50	0.9991	0.0040
Oxalic acid	0.15	1.26	$Y = 0.9218X - 0.0180$	0.1–30	0.9990	0.0022
Sulfate	0.15	0.64	$Y = 1.1318X - 0.0232$	0.5–50	0.9996	0.0031
Phosphate	0.08	1.44	$Y = 0.5172X - 0.0215$	0.5–50	0.9985	0.0077

[a] The RSDs were calculated from 10 replicate injections within one day.
[b] X: concentration of anions; Y: peak area.

Table 2
Concentrations and spiked recoveries of anions in a real sample (500 times diluted)

Anions	F^-	Cl^-	NO_2^-	NO_3^-	GP	MFP	SO_4^{2-}	Oxalate	PO_4^{3-}
Concentration (mg l^{-1})	0.14	0.11	<DL[b]	5.78	1.83	8.96	0.77	0.28	1.83
RSD (%, $n=5$)	2.00	0.22	–	0.59	1.91	2.01	1.00	0.50	0.91
Spiked (mg l^{-1})	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00
Found (mg l^{-1})	0.66	0.61	0.51	6.80	2.79	9.94	1.79	1.27	2.84
Recovery (%)[a]	103.4	99.7	102.0	102.1	96.0	97.6	102.2	98.5	101.5
RSD (%, $n=7$)	1.01	0.89	0.45	0.34	2.00	1.54	0.49	1.69	1.44

[a] Spiked recoveries were obtained by adding standard solution to the sample, and then determined.
[b] Detection limit.

Table 3
Determination of anions in toothpaste (500 times diluted)

Toothpaste	Contents (mg l^{-1})								
	F^-	Cl^-	NO_2^-	NO_3^-	GP	MFP	SO_4^{2-}	Oxalate	PO_4^{3-}
A	0.14	0.11	<DL[a]	5.78	1.83	8.96	0.77	0.28	1.83
B	0.12	0.15	<DL[a]	6.24	2.18	9.45	0.75	0.36	1.84
C	0.23	0.18	0.01	6.14	1.12	8.17	0.83	0.29	2.44

[a] Detection limit.

calibration curves were evaluated by plotting peak area against the concentration of anions. The linear calibration curves were obtained in each concentration range. The correlation coefficients for anions ranged from 0.9985 to 0.9996. As it can be seen a good linearity was obtained in all cases. The limit of detection (LOD) of each compound was defined as the detectable concentration of an anion giving a peak three-times as high as the background noise (S/N = 3). These values were rather low as the conductivity IC.

3.4. Glycerophosphate and other anions in toothpaste samples

Fluoride, chloride, nitrite, nitrate, monoflurophosphate, glycerophosphate, oxalic acid, sulfate and phosphate were determined in toothpaste samples using the proposed method (Table 2).

The presence of these compounds was confirmed by comparing their retention times with those of standard solutions. After ultrasonic agitation for 20 min, the concentrations of anions did not increase. So 30 min was chosen as the ultrasonic agitation time. Each sample was analyzed after dilution to 500 times and filtration through a 0.45 μm membrane filter. Table 1 summarized the determination and reproducibility of anions ($n=5$) in the real samples, and the chromatogram corresponding to one of the three samples in shown in Fig. 4.

The accuracy of the method was evaluated form recovery assays, preparing spiked samples of toothpaste. The obtained values ranged between 96 and 103%. Although the recoveries of glycerophosphate and monoflurophosphate are often reduced by hydrolysis in the solution, high recovery of glycerophosphate seems to be obtained in this analytical system within 5 h after dilution.

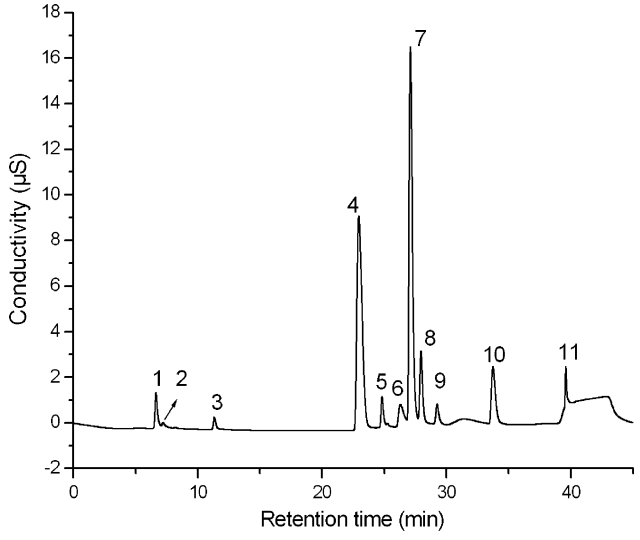

Fig. 4. Chromatogram of a real sample; peaks: (1) fluoride; (2) acetate; (3) chloride; (4) nitrate; (5) glycerophosphate; (6) carbonate; (7) monoflurophosphate; (8) oxalic acid; (9) sulfate; (10) phosphate; and (11) pyrophosphate.

Three different kinds of toothpaste were tested and all the contents data were summarized in Table 3. It seems this IC system would be suitable for practical use.

4. Conclusion

The method represents improvements over prior reported techniques to quantitate anions considered important in the management of dental caries. The calibration curves showed stability and matrix spike recoveries were quite acceptable. Contents in the commercial toothpaste were analyzed by means of ion chromatography using a gradient elution with KOH as mobile phase

and conductor detection. The Chromatographic peaks for all nine analytes were clearly separated. The method was applied to toothpaste without interferences.

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