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Simultaneous determination of fluoride, chloride, sulfate, phosphate, monofluorophosphate, glycerophosphate, sorbate, and saccharin in gargles by ion chromatography[*]

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Abstract: Simple, reliable and sensitive analytical methods to determine anticariogenic agents, preservatives, and artificial sweeteners contained in commercial gargles are necessary for evaluating their effectiveness, safety, and quality. An ion chromatography (IC) method has been described to analyze simultaneously eight anions including fluoride, chloride, sulfate, phosphate, monofluorophosphate, glycerophosphate (anticariogenic agents), sorbate (a preservative), and saccharin (an artificial sweetener) in gargles. In this IC system, we applied a mobile phased gradient elution with KOH, separation by IonPac AS18 columns, and suppressed conductivity detection. Optimized analytical conditions were further evaluated for accuracy. The relative standard deviations (RSDs) of the inter-day's retention time and peak area of all species were less than 0.938% and 8.731%, respectively, while RSDs of 5-day retention time and peak area were less than 1.265% and 8.934%, respectively. The correlation coefficients for targeted analytes ranged from 0.999 7 to 1.000 0. The spiked recoveries for the anions were 90%~102.5%. We concluded that the method can be applied for comprehensive evaluation of commercial gargles.

Key words: Ion chromatography, Anions, Gargles, Monofluorophosphate, Sorbate, Saccharin
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INTRODUCTION

There is a broad consensus in the research community that fluoridated species provide important anticariogenic benefits, and that the two most widely used anticariostatic agents are sodium fluoride (NaF) and sodium monofluorophosphate (SMFP). Apart from its positive cariostatic properties, fluoride, however, is a hazardous chemical. An acute intake of a large dose or chronic ingestion of lower doses of fluoride ions can result in a variety of side effects, including acute gastric and kidney disturbances, dental and skeletal fluorosis or even death (Stookey *et al.*, 1993).

Phosphorus-containing agents represent the greatest number of potential non-fluoride anticariostatic agents. Organic phosphates such as glycerophosphate (GP) and phytate have shown cariostatic activity. Calcium has also been of interest in anticaries. Across all attributes but metallic, calcium glycerophosphate and gluconate are less intense than calcium chloride (Forward, 1994). Calcium glycerophosphate has been increasingly commonly added to gargles, as it plays a critical role in preventing tooth decay. Besides, artificial sweeteners, such as saccha-

rin, and preservatives, such as sorbate, are commonly used in dentifrices as well as in food, beverage, confectionary, and pharmaceutical industries to maintain quality, characteristics, and safety of products (Reid *et al.*, 2007).

Several techniques have been used to analyze the water soluble species such as iodine and iodide in gargles. They include a flow injection (FI) system based on the chemiluminescence (Fujiwara *et al.*, 2000), micellar electrokinetic capillary chromatography for synthetic food dyes (Jaworska *et al.*, 2005), voltammetric at a film mercury electrodes for chlorhexidine (Wang and Tsai, 2001), and RP-HPLC for tetracaini (Huang *et al.*, 2006). In these methods, the species identified are limited. The anticariogenic agents including fluoride, monofluorophosphate, and glycerophosphate have been determined by ion chromatography (IC) in basic investigations of anticaries and quality control of toothpaste (Chen *et al.*, 2006); a method for simultaneous determination of preservatives and artificial sweeteners as well as anticaries has not been reported yet.

In this study, we developed a simple, reliable, and sensitive gradient IC method to spontaneously analyze the anticaries-related fluoride, monofluorophosphate, and glycerophosphate, sorbate as a preservative, and saccharin as an artificial sweetener in various commercial gargles.

MATERIALS AND METHODS

Chromatographic system

In the study, Dionex ICS 2000 Ion Chromatograph equipped with an isocratic pump, EG50 eluent generator, and Dionex ASRS-Ultra (2 mm) were obtained from Dionex (Sunnyvale, CA, USA). 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 (Dionex, Sunnyvale, CA, USA). An ASRS-Ultra (2 mm) suppressor was utilized in the recycle mode. Data were acquired by using Chromeleon software installed on a Dell P-IV computer.

An eluent generator was used to generate KOH gradient concentrations. For all analyses in this report, the gradient profile consisted of the following: 0.5~2.0 mmol/L from 0 to 5 min, 2~4.0 mmol/L from 5 to 12

min, 4~12 mmol/L from 12 to 20 min (curve=9), 20~28 mmol/L from 20 to 28 min, 28~50 mmol/L from 28 to 33 min, 50 mmol/L from 33 to 38 min, and 0.5 mmol/L from 38 to 43 min. Eluent flow rates were set at 0.25 ml/min with an injection volume of 25 μl.

Reagents and samples

The sodium monofluorophosphate was purchased from Fluka Chemical Corp. (Milwaukee, WI, USA) and had an assay value of 98.3% by the USP assay procedure. The calcium glycerophosphate (Fluka) had an assay value of 104% by the USP assay procedure (by titration with 1 mol/L HCl calculated on dry substance). Deionized water at 18.3 MΩ resistivity was used throughout. The samples were from various commercially available gargles or a tooth-cleaning bar (cotton swab bar), which were encoded as Gargles A, B, D, and E, and Cotton swab bar C.

Standard sample preparation

Working standard solutions were prepared daily by serial dilution of stock solution of each standard anion to 1000 mg/L.

Gargle sample preparation

Approximately 1.0 ml of a gargle sample was measured and all the liquid in cotton swab bar was weighted into a 100 ml volumetric flask and then diluted to the volume with deionized water, respectively. Then, the above solutions were diluted ten fold with deionized water and they filtered through a 0.45 μm membrane filter before sample injection.

RESULTS

Separation of standard mixture sample

We first generated the standard mixture sample and tested it for its chromatogram. Fig.1 illustrates the retention times for the studied compounds under the selected experimental conditions: fluoride (4.05 min), chloride (7.06 min), sorbate (13.11 min), glycerophosphate (18.30 min), carbonate (19.90 min), monofluorophosphate (21.52 min), sulfate (22.35 min), phosphate (24.96 min), and saccharin (33.03 min). The results show all the anions contained in the standard mixture sample were well separated by chromatograph, except for monofluorophosphate and

sulfate, which only had 0.43 min difference of retention time between each other.
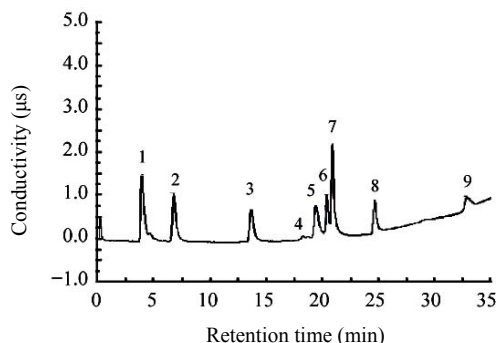
Fig.1 Chromatogram of standard anions
Peaks (mg/L): 1. Fluoride (0.1); 2. Chloride (0.1); 3. Sorbate (1.0); 4. Glycerophosphate (0.1); 5. Carbonate; 6. Monofluorophosphate (0.2); 7. Sulfate (0.1); 8. Phosphate (0.8); 9. Saccharin (0.5)

Table 1 shows relative standard deviations (RSDs) of the retention time and peak, linearity, and detection limits of standard anions. In brief, the RSDs of the retention time and peak area within one day were less than 0.938% and 8.731%, respectively, while the RSDs of 5-day retention time and peak area were less than 1.265% and 8.934%, respectively. The calibration curves were evaluated by plotting peak area against the concentration of anions, with the

linear calibration curves obtained within each concentration range. The correlation coefficients for anions ranged from 0.9990 to 1.0000. 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).

Gargle samples analysis

We then analyzed fluoride, chloride, sorbate, monofluorophosphate, glycerophosphate, sulfate, phosphate, and saccharin in gargle and cotton swab samples using the proposed method. The presence of these compounds was confirmed by comparing their retention times with those of the standard mixture sample. Each sample was analyzed after being diluted 1000 times and filtrated through a 0.45 μm membrane filter. Figs.2a and 2b display the chromatogram of Gargles A and E, respectively, while Table 2 summarizes the determination and reproducibility of anions (n=5). The recovery of the testing was evaluated by analyzing the spiked gargle samples, ranging between 90% and 102.5%. The concentrations of anions in 4 different gargle samples and 1 cotton swab sample are listed in Table 3.

Table 1 RSDs, linearity and detection limits of standard anions

Anion	RSD[a] (%)		Linear equations[b]	Linear range of anion (mg/L)	Coefficient of correlation (r^2)	Detection limit (S/N=3) (μg/L)
	Retention time	Peak area				
F^-	0.485	3.250	$Y=2.9652X+0.3824$	0.1~10	0.9997	0.12
Cl^-	0.938	5.003	$Y=2.0806X+0.0732$	0.1~10	1.0000	0.20
Sobic acid	0.737	1.397	$Y=2.3211X+0.0195$	0.1~10	1.0000	0.30
GP	0.903	8.731	$Y=0.1677X-0.0136$	0.1~10	0.9997	2.10
MFP	0.183	0.679	$Y=0.4522X+0.1684$	0.2~20	0.9997	0.40
SO_4^{2-}	0.844	6.174	$Y=1.0545X+0.0421$	0.1~10	0.9999	0.25
PO_4^{3-}	0.436	3.998	$Y=1.3797X-0.4006$	0.2~20	0.9990	0.52
Saccharin	0.252	6.015	$Y=0.1970X-0.0290$	0.5~50	0.9999	4.90

[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 species in a gargle sample (1000 times diluted)**

Anions	F^-	Cl^-	Sorbate	GP	MFP	SO_4^{2-}	PO_4^{3-}	Saccharin
Concentration (mg/L)	0.07	0.01	0.38	<DL[b]	0.03	0.02	0.01	6.31
RSD (%) (n=5)	1.63	4.32	1.45	8.93	1.21	6.53	4.04	0.81
Spiked (mg/L)	0.10	0.01	0.40	2.00	0.03	0.02	0.10	5.00
Found (mg/L)	0.17	0.18	0.79	1.95	0.06	0.36	0.10	11.30
Recovery (%)	98.0	90.0	102.5	97.5	96.6	90.0	98.2	99.8
RSD (%) (n=7)	1.82	4.78	3.46	8.08	1.37	6.56	4.26	1.16

[b]Detection limit

Table 3 Determination of species in gargle (1000 times diluted)

	Contents (mg/L)							
	F^-	Cl^-	Sorbate	GP	MFP	SO_4^{2-}	PO_4^{3-}	Saccharin
A	0.07	0.01	0.38	<DL[a]	0.03	0.02	0.01	6.31
B	0.15	0.07	0.19	0.05	0.05	0.06	0.08	2.55
C	0.01	7.00	<DL[a]	<DL[a]	0.04	0.01	<DL[a]	0.56
D	0.01	0.19	<DL[a]	<DL[a]	0.04	0.003	0.002	0.005
E	0.35	0.34	<DL[a]	<DL[a]	0.03	0.002	0.010	0.011

C: Liquid in cotton swab; [a]Detection limit
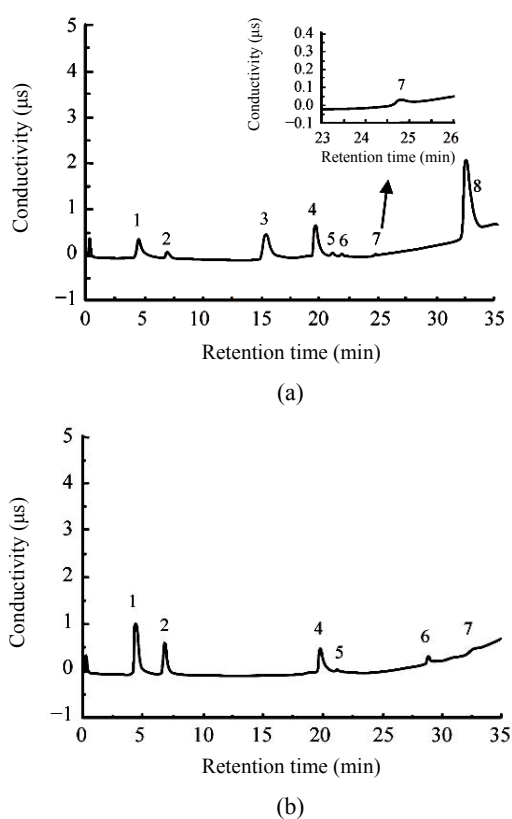
Fig.2 Chromatogram of Gargles (a) A and (b) E
1. Fluoride; 2. Chloride; 3. Sorbate; 4. Carbonate; 5. Monofluorophosphate; 6. Phosphate; 7. Saccharin

DISCUSSION

Since its introduction in 1975, IC has become a routine analytical method for the determination of inorganic ions, especially anions present in various matrices. At the early stage, due to the weak binding of fluoride to the ion-exchangers commonly used in IC, fluoride was generally eluted rapidly from the column and found to be very close to the "injection" peak. With the invention of high capacity columns such as IonPac AS18, the problem has been resolved.

Meanwhile, since the common anions, such as monofluorophosphate, glycerophosphate, sorbate, saccharin, and some organic acids, are strongly retained in anion exchange resin, the gradient elution generated by EG50 KOH is used.

In comparison with conventional carbonate-based chemistry, hydroxide eluent in EG50 KOH system can be suppressed in water, exhibiting very low conductivity because of its extremely low ionisation levels. The suppression of hydroxide in water offers several benefits over carbonate-based chemistry. The most obvious one is that the high power of gradient IC becomes possible, which results in a low background conductivity, delivering a very stable baseline (i.e. very low noise). This, in turn, offerers an increased signal-to-noise ratio, yielding a sensitivity of cotton swab parts per trillion (ng/L) and extremely high reproducibility.

In this study, the selection of ICS-2000 system and IonPac AS18 high capacity anion column allowed significant reduction of the interference problems caused by water dip, and a more sensitive detection of anions because of the low background conductivity (ca. 0.3 μs/cm) in the system (Liu et al., 2004). The KOH eluent generator generated predetermined concentrations of hydroxide through programmed current values. By using the electrolysis of water, the eluent can be automatically generated with high precision and accuracy from deionised water. Also, using the electrolysis of water, the acid (hydrogen ion) for the suppression of hydroxide eluent back to water can be done automatically with high precision and accuracy. Thus, using the principles of computer-controlled electrolysis, IC can be performed without the need for manually prepared eluent and regenerant reagents. Accordingly, reagent free ion chromatography (RFIC) offers simplicity of operation along with increased sensitivity and reproducibility, simplifying the use of gradient chroma-

tography.

Another advantage of the system is gradient ion chromatography with generated KOH efluent, which can simultaneously separate and determine both weak and strong retention anions, including anticaries such as fluoride, monofluorophosphate, glycerophosphate, preservatives such as sorbate, and sweetener such as saccharin, etc. These agents are necessary for basic investigations of anticaries and quality control of gargles.

CONCLUSION

In summary, we have developed a simple and effective protocol for analyzing various anions of interest in gargles. The method produces a clear separation of anions tested by ion chromatography, and the testing results are reliable. Compared with other current techniques, the proposed method quantifies the anticariogenic anions, preservatives, and additives in gargles simultaneously, offering a comprehensive quality control of gargles.

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