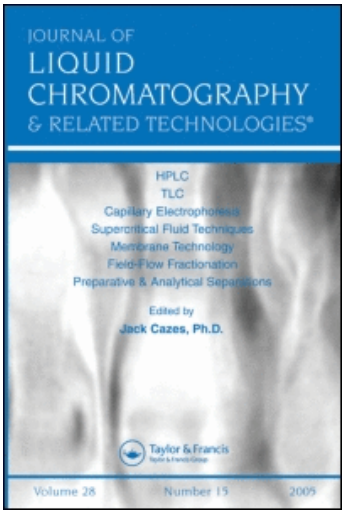

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DETERMINATION OF FLUORIDE IONS BY SINGLE COLUMN HIGH PRESSURE ANION CHROMATOGRAPHY IN DENTIFRICE PREPARATIONS AND BODY FLUIDS: SALIVA AND BLOOD SERUM

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DETERMINATION OF FLUORIDE IONS BY SINGLE COLUMN HIGH PRESSURE ANION CHROMATOGRAPHY IN DENTIFRICE PREPARATIONS AND BODY FLUIDS: SALIVA AND BLOOD SERUM

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ABSTRACT

Fluoride ions are commonly used as anticaries agents in dentifrice preparations. However, when the fluoride level exceeds the recommended limits some fluorosis may be noticed. This paper focuses on the single column ion chromatographic determination of fluoride using conductometric detection. Chromatography was achieved on a low capacity anion exchange column, Hamilton PRP-X100 150×4.1 mm, $10\,\mu$m, with a low conductivity mobile phase consisting of 4 mM p-hydroxybenzoic acid and 0.5 mM sodium benzoate (pH 9.0 adjusted with 1 N NaOH) at a flow rate 1.8 mL/min.

For the quantitative determination, peak height was used, while in some cases bromide can be used as internal standard (suggested

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concentration 15 mg/L). A rectilinear relationship was observed up to 30 mg/L. The limit of detection (S/N = 3) was 1 ng, while the limit of quantitation (LOQ) was 5 ng at 0.1 μS, when 50 μL of the samples were injected onto the analytical column.

The time of analysis is less than 3.5 min. The statistical evaluation of the method was examined performing within-day (n = 8) and inter-day calibration (n = 8) with RSD values ranging from 2.6 to 5.2 and from 7.1 to 11.7 respectively.

The applicability of the method was demonstrated in the analysis of sodium fluoride dentifrices: lozenges, toothpastes, mouth wash solutions, as well as in body fluids: saliva and blood serum. Recovery of fluoride ions in spiked body fluid samples ranged from 91.7 to 110.1%.

INTRODUCTION

Fluoride ions are commonly formulated into oral care products in the management of dental caries. Mass measures include topical fluorides such as fluoridated toothpastes, mouthwash solutions, and pharmaceutical formulations. Over 95% of widely used toothpastes contain fluoride compounds including sodium salt (NaF), sodium monofluorophosphate, stannous fluoride, and amine. Fluoridation of public water supplies has also been used as a caries control measure based on epidemiological studies. Concentration of fluoride up to 1.0 mg/L in drinking water has no harmful effect on health. In countries where drinking water is not fluoridated, pharmaceutical preparations like lozenges are recommended, especially for children, to prevent dental caries and to form healthy teeth. In places where fluoride content in water is more than 0.7 mg/L, no need for fluoride supplements is noticed. However, if the occurring fluoride concentration exceeds 10 mg/L water should be defluoridated.(1,2)

Cariostatic properties of various restorate materials (sealants, amalgams, zinc-phosphate cements) are enhanced by adding fluoride. In order to estimate the success of these measures, fluoride analysis is of great importance in several biological materials, among them body fluids, bone, teeth, and soft tissues.(3,4)

Existing analytical methods employ ion chromatography with conductometric or post-column spectrophotometric or fluorometric detection and fluoride ion specific electrode. However, the latter are difficult to be applied to low volume samples, such as blood serum or saliva.(3,5–9)

Ion chromatographic (IC) methods for fluoride determination present various problems. One of them is its low affinity towards strongly basic anion exchangers. By eluent suppressed ion chromatography, fluoride ions elute very close to the system void volume and their determination is difficult due to the interference from

water dip. Single Column Ion Chromatography (SCIC) overcomes the limitation of common eluent suppressed IC methods where fluorides are thus early eluted, or not efficiently resolved from the unretained components peaks.(10–13)

Fluoride was determined in a previously published work of the authors among other inorganic ions in drinking water.(14) The current study aimed the determination of fluoride concentration in dentifrices and body fluids using SCIC.

EXPERIMENTAL

Instrumentation

The ion chromatograph used consisted of an SSI model 222D Pump (SSI, State College PA, USA) and a model Wescan 315 conductometric detector (Alltech, Deerfield, IL, USA) maintained at $35 \pm 0.1^{\circ}$C.

A Rheodyne model 9125 six-port high pressure switching injection valve (Rheodyne, Cotati, CA, USA) was assembled with a 50-µL injection loop.

The separation column, Hamilton PRP-X100, 150×4.1 mm (Hamilton, Reno, NV, USA) was packed with spherical 10-µm polystyrene-divinylbenzene trimethylammonium anion exchanger (Hamilton, Reno, NV, USA). The capacity of the column was 0.19 ± 0.02 meq/g. Data acquisition was performed using a Hewlett-Packard integrator; model HP 3396 II (Hewlett-Packard, Avondale, PA, USA).

A Vac-Elut vacuum manifold column processor purchased from Analytichem International, a division of Varian (Harbor City, USA), was used for SPE. All evaporations were performed with a nine-port Reacti-Vap evaporator (Pierce, Rockford, IL, USA).

A glass vacuum-filtration apparatus obtained from Alltech Associates was employed for the filtration of mobile phase, using 0.2 µm membrane filters, obtained from Schleicher and Schuell (Dassel, Germany).

Degassing of solvents was achieved by sonication in a Transonic 460/H Ultrasonic bath (Elma, Germany) or by vigorous Helium sparging prior to use. A Glass-col, Terre Haute 47802 small vortexer and a Hermle centrifuge, model Z 230 (B. Hermle, Gosheim, Germany) were employed for the sample pre-treatment.

Samples, Chemicals, and Reagents

Fluoride sodium salt, sodium benzoate, and 4-hydroxy benzoic acid were supplied from Merck (Darmstadt, Germany). Methanol (HPLC-grade, Merck, Darmstadt, Germany) and Acetonitrile (Riedel de Haen, Germany) were used throughout analyses. Fluoride supplements, Apoflux, formulated as lozenges,

were supplied from SANTA Corp. (Acharnai, Greece). Two brands of toothpaste and one mouth wash solution were purchased from local stores. All samples contained fluoride solely in the form of sodium salt.

Preparation of Standard Solutions

A stock standard solution (1000 mg/L) of fluoride was prepared by dissolving an appropriate amount of analytical reagent grade sodium salt in de-ionised water. From this stock, working standards were prepared by sequential dilution. All standard solutions and samples were kept at 4°C before and after analysis in polyethylene bottles.

Stock solutions 20 mM of 4-hydroxybenzoic acid and sodium benzoate containing 2% v/v CH_3OH were used for daily preparation of dilute mixtures at the working concentration of 0.5 mM and 4.0 mM respectively.

The pH of the mobile phase was adjusted at 9.0 by adding 4 mL of 1 N sodium hydroxide solution per 1 liter of eluent. The sodium hydroxide solution was prepared by properly diluting the content of Fixanal ampoule (Riedel-de Haen, Hannover, Germany). The mobile phase was filtered through 0.2 μm membrane filters prior to use.

Sample Preparation

Lozenges

Three to five lozenges from three different initial fluoride concentrations (0.25, 0.5 and 1 mg) were weighed and the mean value was recorded. After finely powdering the lozenges in a porcelain mortar, a solution was prepared by dissolving the appropriate amount that corresponds to the average weight of one lozenge in 10 mL water. The insoluble excipients were removed by filtration through a 0.2 μm membrane filter and the filtrate was diluted to yield three different concentrations within the working range of the analytes: 5, 10, and 15 ng/μL F^-.

Mouth Wash Solutions

Solid Phase Extraction was used to remove interfering constituents. Abselut Nexus by Varian, Bond Elut C_8 (by Varian), Merck PR-18 (200 mg/3 mL), Merck PR-18 (sorbent 500 mg/3 mL), Adsorbex PR-18 (by Merck), were tested regarding their efficiency in retaining interference. Solutions were sucked

through the cartridge that was preconditioned with 2 mL methanol and 2 mL of water. The sample was further injected into the ion chromatograph.

Toothpaste

A portion of the toothpaste (1–2 g) accurately weighed was transferred into a glass beaker where deionized water was added. After mixing with a glass rod and magnetically stirring the content of the beaker for 30 min. in room temperature, the slurry solution was filtered through a 0.2 mm filter. The filtrate was diluted with water to final volume of 50 mL. Two dilute solutions were prepared from this stock solution to yield concentrations within the working range 10–15 ng/μL F^-.

Blood Serum

After protein precipitation with acetonitrile, vortex mixing for two minutes, centrifugation at 3500 rpm for 20 min, and solvent evaporation under nitrogen stream, aliquots of 100 μL of pooled human blood serum were spiked with 100 μL of fluoride standard solution, at concentration levels of 2.5, 5.0, and 10.0 ng/μL.

Saliva

In order to determine the fluoride concentration in saliva, a spiked blank sample was used to construct a calibration curve. Sample preparation involved filtration through a 0.2-μm membrane filter and a five-fold dilution.

RESULTS AND DISCUSSION

Chromatographic Conditions

Eluent systems examined for their efficiency, regarding peak shape and analysis time, as well as resolution from other inorganic anions were mixtures of 4-Hydroxybenzoic acid and sodium benzoate at various concentrations. The effect of methanol content in mobile phase as organic modifier was studied in different percentages (0–1% v/v). Table 1 summarizes the results obtained by the different eluent systems, while Table 2 tabulates the optimal chromatographic conditions. Table 3 shows the retention times of common inorganic ions separated with the developed method. Bromide anions can be used as internal standard (15 ng/μL). A typical chromatogram is illustrated in Figure 1.

Table 1. Eluent Systems Examined for Fluoride Determination by Single Column Ion Chromatography

Parameters	Value
p-Hydroxybenzoic acid	4 mM
Sodium benzoate	0.5 mM
pH	9.0
Flow rate	1.8 mL/min
Background conductivity	6.92 μ S/cm,
Pressure	875 psi
Detection	Conductivity
Sensitivity	0.1 μ S cm^{-1} FS

Calibration Data-Linearity Range

The calibration curve was constructed using standard solutions prepared by sequential dilution from the stock at concentrations: 0.1, 0.5, 1, 5, 8, 10, 15, 20, and 30 ng/μL.

Quantitation was based on linear regression analysis of peak height versus analyte concentration in ng/μL.

Regression equations were: $y = (0.0036 \pm 0.0002)x + (0.1464 \pm 0.0984)$ with $R = 0.9957$, where $x =$ ng and $y =$ peak height ratio of fluoride to internal standard bromide, and $y = (178.75 \pm 1.48)x + (-5949.5 \pm 978.4)$ with $R = 0.9999$, where $x =$ ng and $y =$ peak height of fluoride. Correlation coefficients indicate that the use of an internal standard is not necessary in the proposed method, taking into account that it prolongs the total analysis time as well.

The method is linear in the range 0.1–30 ng/μL. The minimum detectable concentration LOD was defined as a peak height that produces three times of baseline noise at at 0.1 μS and was found to be 1 ng. The LOQ was the lowest concentration of calibration standards with acceptable precision and accuracy and found to be 5 ng at 0.1 μS.

Method Validation: Accuracy and Precision

The precision of the method based on within-day repeatability was performed by replicate injections ($n = 8$) of three standard solutions covering different concentration levels: low, medium, and high where peak heights were measured. Statistical evaluation revealed relative standard deviations, at different values. Results are shown in Table 4.

The reproducibility (day-to-day variation) of the method was established using the same concentration range as above. A triplicate determination of each

Table 2. Chromatographic Parameters for Fluoride Determination by Single Column Ion Chromatography

p-Hydroxy-Benzoic Acid (mM)	Sodium Benzoate (mM)	CH_3OH (% v/v)	NaOH (mmol/L)	Flow Rate (mL/min)	Pressure (psi)	t_R (min)
1	1	0	0	0.8	600	3.3
1	1	8	0	0.8	700	3.714
1	1	5	4	0.8	650	3.69
1	1	5	2	0.8	675	4.51
3	4	5	4	0.8	650	3.837
1	1	10	2	1	900	4.49
1.5	1	5	2	1	525	2.768
1	1	8	4	1	850	4.8
0.5	1	8	4	1	850	2.69
1.5	1	5	4	1	775	3.78
4	0.5	0	2	1.8	875	3.336

Table 3. Retention Time of Other Inorganic Anions

Ions	Retention Time (min)
SO_4^{2-}	>10
CO_3^{2-}	3.90
Cl^-	4.36
PO_4^{3-}	>10
NO_2^-	5.14
NO_3^-	8.08
Br^-	5.233

concentration was conducted during routine operation of the system over a period of eight consecutive days. Reproducibility results are illustrated in Table 4.

Accuracy was determined by replicate analysis of three different levels (5.0, 10.0, and 20.0 ng/μL) and calculating the recoveries of actually found versus theoretical values.

Application to Dentifrices and Body Fluids

Lozenges

The developed method was applied to pharmaceuticals (lozenges) used for in situ dental fluorosis. Results are illustrated in Table 5. A typical chromatogram of fluoride in toothpaste is depicted in Figure 2a.

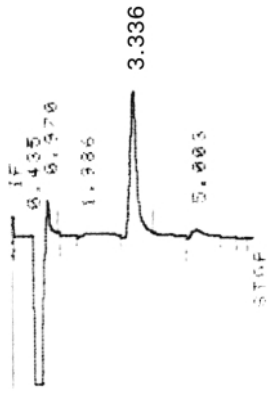

Figure 1. High performance liquid chromatogram of fluoride 3.336 min. Chromatographic conditions are described in text.

Table 4. Day-to-Day (Over a Period of 8 Consecutive Days) and Within-Day (n = 8) Precision and Accuracy Study for Fluoride Determination

	Within-Day			Inter-Day		
Added F^- (ng)	Found ± SD (ng)	RSD	Recovery of Theoretical Value %	Found ± SD (ng)	RSD	Recovery of Theoretical Value %
250	247.7 ± 10.6	4.3	99.1	255.0 ± 18.1	7.1	102.0
500	478.0 ± 25.1	5.2	95.6	533.2 ± 62.3	11.7	106.6
1000	946.9 ± 24.2	2.6	94.7	942.7 ± 91.0	9.6	94.3

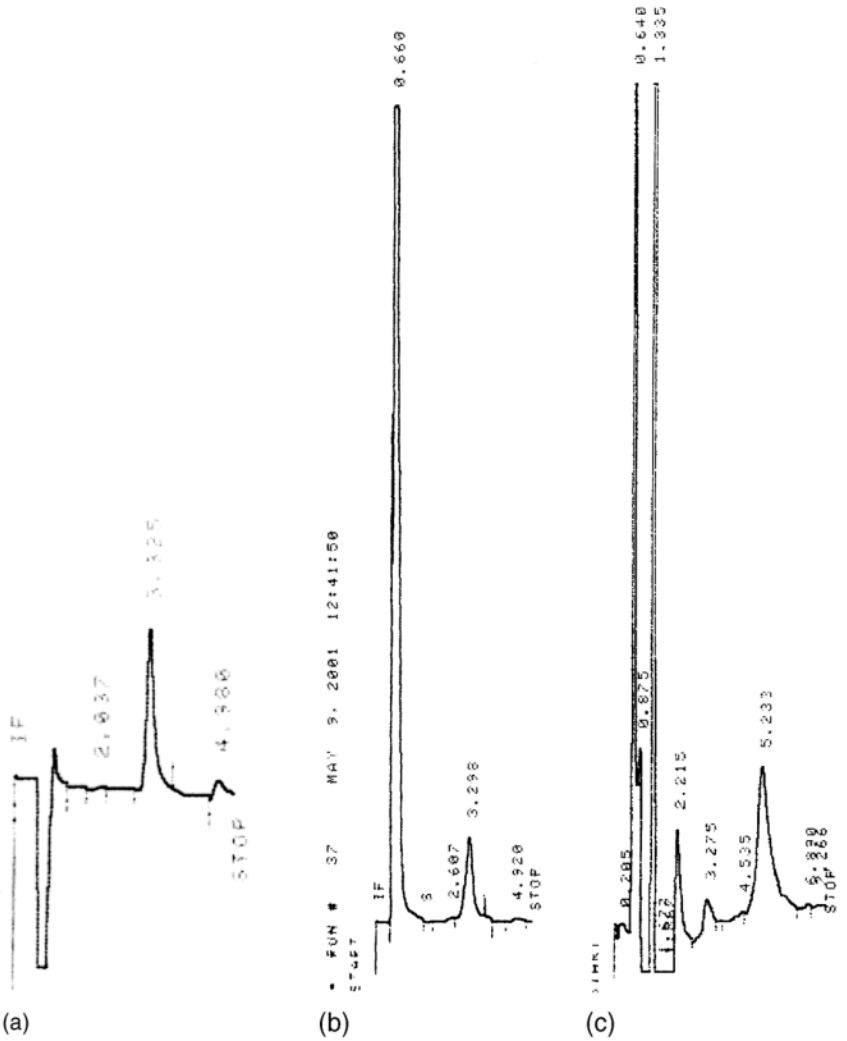

Figure 2. **a.** High performance liquid chromatogram of determination of fluoride (3.325 min) in pharmaceutical preparation (lozenges). **b.** High performance liquid chromatogram of determination of fluoride (3.298 min) in toothpaste. **c.** High performance liquid chromatogram of determination of fluoride (3.275 min) in mouthwash fluoride solution.

Table 5. Fluoride Determination in Pharmaceuticals (Apoflux)

Added F^- (ng)	Found F^- (ng)	RSD
	Apoflux 0.25 mg	
250	318.2 ± 28.3	8.9
500	542.6 ± 30.6	5.6
750	730.1 ± 43.9	6.0
	Apoflux 0.5 mg	
250	253.9 ± 2.3	0.9
500	489.6 ± 13.1	2.7
750	683.0 ± 43.9	6.4
	Apoflux 1.0 mg	
250	250.0 ± 8.5	3.4
500	460.0 ± 44.8	9.8
750	708.2 ± 48.0	6.8

Mouth Wash Solutions

The direct determination of fluoride in mouth wash solutions was not possible due to ingredients such as color, or taste/flavor improving additives causing interference. Therefore, solid phase extraction was applied to remove interfering constituents. Nexus, Varian Bond C_8, Merck PR-18 (200 mg/3 mL), Merck PR-18 (500 mg/3 mL), and Adsorbex PR-8 were tested regarding their efficiency in retaining interference. Comparative results indicating interference removal are presented in Table 6. Obviously, Adsorbex PR-18 provided better sample purification. A typical chromatogram of fluoride in toothpaste is depicted in Figure 2b.

Table 6. Interference Removal by SPE

Cartridge	Interference
Nexus	+ + + +
Varian Bond C_8	+ + +
Merck PR-18 (200 mg/3 mL)	+ +
Merck PR-18 (500 mg/3 mL)	+
Adsorbex PR-18	−

The calibration curve for quantitation of mouth wash solution was constructed by standard solutions extracted, following the above-described protocol was:

$$y = (167.5 \pm 5.5)x + (25.4 \pm 0.036), \quad R = 0.9957,$$

where $x =$ ng and $y =$ peak height of fluoride. Results are presented in Table 7.

Toothpaste

In order to apply the method under study to toothpastes, two commercially available products were analyzed. Two dilute solutions were prepared from this stock solution to yield concentrations within the working range 10–15 ng/μL F^-. A typical chromatogram of fluoride in toothpaste is depicted in Figure 2c. Results are presented in Table 7.

Table 7. Fluoride Determination in Commercial Products of Oral Care

Commercial Product	Labeled Amount F^-	Found $F^- \pm$ SD	RSD
Apoflux	0.25[a]	0.28 ± 0.03	10.7
Apoflux	0.5[a]	0.48 ± 0.03	6.2
Apoflux	1[a]	0.95 ± 0.04	4.2
Mouth Wash Solution	113.1[b]	110.8 ± 7.4	6.6
Toothpaste I	29.11[b]	31.66 ± 2.53	8.0
Toothpaste II	48.05[b]	49.5 ± 6.9	1.4

[a] (mg/Lozenge).
[b] (ng/μL).

Table 8. Recovery of Fluoride from Saliva and Human Blood Serum

Added Ng	Found ± SD ng	Recovery %
Saliva		
400	406.5 ± 25.0	101.6
600	587.1 ± 38.8	97.8
800	806.5 ± 19.7	100.8
Serum		
125	137.6 ± 6.6	110.1
250	229.3 ± 12.1	91.7
500	504.6 ± 24.3	101.0

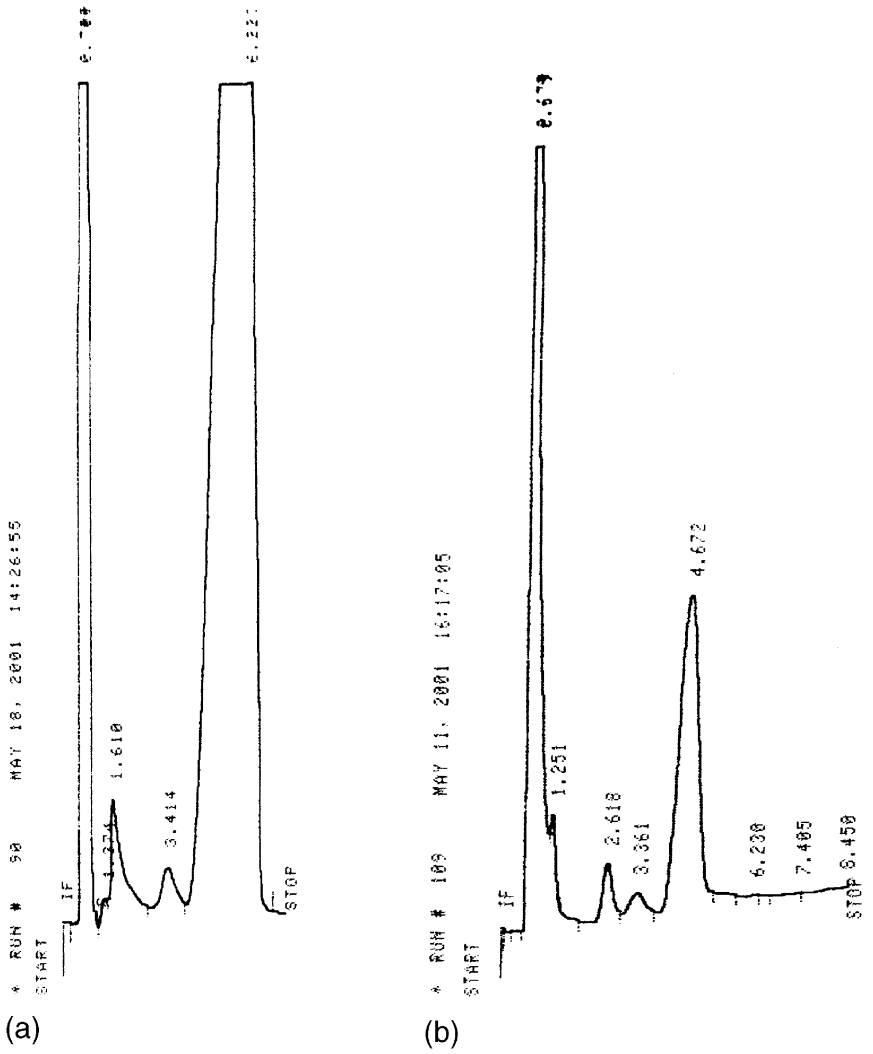

Figure 3. **a.** High performance liquid chromatogram of determination of fluoride 3.414 min in spiked human blood serum. **b.** High performance liquid chromatogram of determination of fluoride 3.361 min in saliva after mouthwashing with commercial fluoride solution.

Blood Serum

In order to determine the fluoride concentration in human serum, the calibration curve was constructed using the standard addition technique:

$$y = (108.5 \pm 0.99)x + (150 \pm 0.32) \quad R = 0.9958,$$

where $x =$ ng and $y =$ peak height of fluoride.

Absolute recovery was measured at the three concentration levels and calculated by comparing the concentrations for spiked serum samples with those for direct injection of pure compounds. Results are shown in Table 8. No interference from endogenous compounds was observed, as shown in Figure 3a.

Table 9. Fluoride Determination in Saliva After Mouth Washing with Commercial Solution

Time After Mouth Wash (min)	$F^- \pm$ SD (ng)	RSD
0	465.1 ± 57.2	12.3
15	436.6 ± 11.4	2.6
30	376.8 ± 11.8	3.1
60	358.6 ± 28.9	8.0

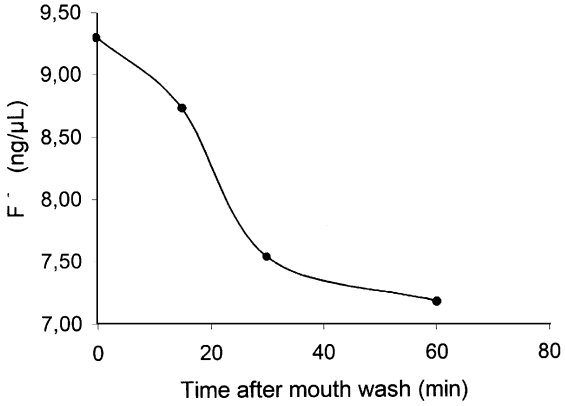

Figure 4. Saliva fluoride content in various time intervals after mouthwashing with commercial fluoride solution.

Saliva

In order to determine the fluoride concentration in saliva, a spiked blank sample was used to construct the calibration curve that is:

$$y = (180.932 \pm 10.136)x + (-31027 \pm 6303.335), \quad R = 0.9984.$$

Recovery results are tabulated in Table 8, while a typical chromatogram of fluoride determination in saliva is illustrated in Figure 3b.

Fluoride concentration was measured shortly after brushing teeth with Toothpaste I, and after mouth rinsing with commercial fluoride solution. Saliva samples taken in different time intervals, 15, 30, and 60 min after mouth washing were analyzed. Results are tabulated in Table 9. Fluoride concentration in saliva after different time intervals is depicted in Figure 4.

CONCLUSION

Single column ion chromatography can be used for the determination of fluoride in body fluids and commercial products of oral care. Eluent suppression is not necessary due to the low background conductivity of the eluent system. Simple sample preparation provides rapid determination of fluoride level in the examined samples. Retention time of less than 3.5 min provides short analysis time and efficient resolution from early-eluted peaks.

The developed method in this assay is characterized by selectivity and sensitivity, as well as accuracy and precision, both within day and day-to-day. It can be used in monitoring the effectiveness of fluoride dentifrices and the monitoring of fluoride level in serum.

REFERENCES

1. Hattab, F.N. Analytical Methods for the Determination of Various Forms in Toothpastes. J. Dental. **1989**, *17*, 77–83.
2. *Standard Methods* 1995.
3. Michigami, Y.; Kuroda, Y.; Ueka, K.; Yamamoto, Y. Determination of Urinary Fluoride by Ion Chromatography. Anal. Chim. Acta **1993**, *274* (2), 299–302.
4. Venkateswarlu, P. Determination of Fluorine in Biological Materials. A Review. Ad. Dent. Res. **1994**, *8*, 80–86.
5. Vogel, G.L.; Carrey, C.M.; Chow, L.C.; Ekstrand, J. Fluoride Analysis in Nanoliter- and Microliter-Size Fluid Samples. J. Dent. Res. **1990**, *69*, 522–528.

6. Barnett, N.W.; Handley, P.J.; Handley, H.W. Use of Zirconyl Xylenol Orange for the Post-Column Spectroscopic Detection of Fluoride in Ion Chromatography. Anal. Letters **1993**, *26* (11), 2525–2529.
7. Biemer, T.A.; Asral, N.; Sippy, A. Ion Chromatographic Procedures for Analysis of Total Fluoride Content in Dentifrices. J. Chromatogr. A **1997**, *771*, 355–359.
8. Moskvin, L.N.; Katruzov, A.N.; Nikitina, T.G. Ion Chromatographic Determination of Fluoride and Chloride Ions in High-Purity Water. J. Anal. Chem. **1998**, *53* (2), 173–177.
9. Montes Bayon, M.; Rodriguez Garcia, A.; Garcia, Alons; Sanz-Medel, A. Indirect Determination of Trace Amount of Fluoride in Natural Waters by Ion Chromatography: A Comparison of On-line Post-Column Fluorometry and ICP-MS Detectors. Analyst **1999**, *124* (1), 27–31.
10. Potter, J.; Hilliker, A.; Breen, G. Determination of Fluoride and Monofluorophosphate in Toothpastes by Ion Chromatography. J. Chromatogr. **1986**, *367*, 423–427.
11. Sheridan, R.C. Simultaneous Determination of Fluoride, Phosphate, and Sulfate in Wet-Process Phosphoric Acid by Ion Chromatography. J. Chromatogr. **1986**, *371*, 383–386.
12. Kumagai, H.; Tajima, I.; Sakai, T.; Inoue, Y. Evaluation of a Complexation Solid-Phase Extraction Method for Eliminating Interferences Caused by Metal-Ions in the Determination of Fluoride-Ion in Water. Anal. Sci. **1995**, *11* (6), 995–997.
13. Weiss, J.; Reinhard, S.; Pohl, C.; Saini, C.; Narayaran, L. Stationary Phases for the Determination of Fluoride and Other Inorganic Anions. J. Chromatogr. A **1995**, *706* (1–2), 81–92.
14. Papadoyannis, I.; Samanidou, V.; Zotou, A. Highly Selective Simultaneous Determination of Eight Inorganic Anions by Single Column High Pressure Anion Chromatography in Drinking Water. J. Liq. Chromatogr. & Rel. Technol. **1995**, *18* (7), 1383–1403.

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