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A fast and accurate method for the determination of total and soluble fluorine in toothpaste using high-resolution graphite furnace molecular absorption spectrometry and its comparison with established techniques

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ABSTRACT

A fast and reliable method has been developed for the determination of total and soluble fluorine in toothpaste, important quality control parameters in dentifrices. The method is based on the molecular absorption of gallium mono-fluoride, GaF, using a commercially available high-resolution continuum source atomic absorption spectrometer. Transversely heated platform tubes with zirconium as permanent chemical modifier were used throughout. Before each sample injection, a palladium and zirconium modifier solution and a gallium reagent were deposited onto the graphite platform and thermally pretreated to transform them into their active forms. The samples were only diluted and introduced directly into the graphite tube together with additional gallium reagent. Under these conditions the fluoride was stable up to a pyrolysis temperature of 550 °C, and the optimum vaporization (molecule formation) temperature was 1550 °C. The GaF molecular absorption was measured at 211.248 nm, and the limits of detection and quantification were 5.2 pg and 17 pg, respectively, corresponding to a limit of quantification of about 30 $\mu g\,g^{-1}$ (ppm) F in the original toothpaste. The proposed method was used for the determination of total and soluble fluorine content in toothpaste samples from different manufactures. The samples contained different ionic fluoride species and sodium monofluorophosphate (MFP) with covalently bonded fluorine. The results for total fluorine were compared with those obtained with a modified conventional headspace gas chromatographic procedure. Accuracy and precision of the two procedures were comparable, but the proposed procedure was much less labor-intensive, and about five times faster than the latter one.

1. Introduction

The benefits of fluorides in the prevention of dental caries have been discussed controversially for many years, but are generally accepted nowadays. Small quantities of fluorides are added to water, food and especially to toothpastes and dental gels [1–5]. Toothpastes are multi-component mixtures of different inorganic and organic components. They contain polishing, sweetening, and foaming agents, flavors, detergents, preservatives and different active ingredients. Among them are plaque inhibiting, antiphlogistic agents and fluorides as effective anti-caries agents. Today more than 95% of toothpastes contain fluorides for caries prevention. An effective toothpaste formula requires that fluoride is available to the enamel microenvironment in a bio-active form. Among the fluorides used are stannous fluoride (SnF_2), sodium fluoride (NaF), sodium monofluorophosphate (Na_2PO_3F), and amine fluoride, which have different chemical and biological properties. SnF_2 was the first fluoride used in toothpastes in the USA since the end of the 1960s [6]. SnF_2 and NaF are highly soluble in water and supply the bio-active fluoride. Amine fluorides provide an effective bacterial protection reducing the bacteria-producing plaque; they dissociate in water to fluoride anions and an organic cation.

It has been shown, however, that the abrasives used in toothpaste can have a dramatic influence on the stability of available fluoride in the toothpaste formulas [7]. With aluminum and calcium containing abrasives the loss of added fluorides from NaF is about 60–90% after one-week storage at room temperature. Sodium bicarbonate and sodium metaphosphate are less harmful, but also inactivate about 20–25% of the added fluoride within nine months of storage. These stability problems due to a change of water soluble fluoride forms to insoluble ones are accompanied by

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the undesired loss of the therapeutic effect of the toothpaste. For that reason, especially for calcium carbonate-based toothpastes, Na_2PO_3F (MFP) is added as a fluoride reservoir. MFP is water soluble and dissociates to sodium cations and the monofluorophosphate anion PO_3F^{2-} where the fluorine is more covalently bonded. During storage MFP can hydrolyze to the phosphate ion and release free fluoride ions.

Since the difference between toxic and therapeutic concentrations of fluoride is rather small, an accurate and fast method for the determination of ionic, dissolved and total fluoride in dentifrice is essential for quality control of active ingredients, for assessment of storage properties of the toothpaste, and for compliance with the specifications of health requirements for daily fluoride intake. Firstly, the formulator and the manufacturer are interested in the total fluoride to control the quantity introduced in a given formula. Secondly, the caries prevention efficiency depends on the bio-active free fluoride available in the toothpaste suspension, which requires the determination of ionic fluoride (free or bio-active) and the determination of soluble fluoride (ionic F + Na_2PO_3F). König and Walldorf [8,9] and Hattab [10] gave corresponding suggestions for fluoride determination in toothpastes using conventional methods.

The main sources of ionic fluoride, which is released from the toothpaste in water at neutral pH, are NaF, SnF_2 and amine fluorides, compounds that are dissociating completely in the toothpaste suspension. MFP releases about 6% of ionic fluoride because of spontaneously hydrolysis [11]; however, this process depends on time, temperature, Na_2PO_3F concentration and on the content of other dentifrice components. A variety of methods have been described in the literature for the determination of fluoride in dentifrice, such as spectrophotometric methods [12] and capillary electrophoresis [13,14]. The predominating methods for fluoride determination in toothpaste, etc., however, are ion chromatography (IC) and potentiometric methods, such as the use of fluoride ion selective electrodes (F-ISE) [15,16]. The F-ISE is sensitive, easy to use, and cost effective with a working range from 10^{-5} to 10^{-1} mol l^{-1} F. However, for a successful application of ISE the pH of the sample has to be between 5.2 and 5.5 to avoid interference from OH^- and conversion of F^- to HF and HF_2, the standards and samples must have the same ionic strength and interference of ions that form complexes or precipitates with fluoride have to be avoided [15]. Whereas the first two conditions could be accomplished by adding total ionic strength adjustment buffer (TISAB) solution to a neutral toothpaste suspension, the last one is almost impossible to implement because the main components of toothpastes consist of Si, Al, Ca and water-soluble organic compounds that form complexes and/or precipitates with fluoride [17].

Ion chromatography is also widely used [18–20] providing the possibility of determining several ions in the same sample, however, with the disadvantage of higher purchasing cost and a lower sampling rate compared to ISE. In addition, IC analysis requires particle-free samples making a filtration through a 0.15–0.45-μm filter mandatory. Another problem is that the fluoride ion is weakly retained on common columns and is eluted very close to the "dips" with many eluents; matrix components, such as bicarbonate can cause early baseline disturbance and a very large system peak. The use of suppressed conductivity detection is recommended for fluoride determination by IC.

The soluble fluoride is referred to as the sum of ionic and the fluoride of hydrolyzed PO_3F^{2-} from MFP in the supernatant of the toothpaste slurry. Concentrated HCl is added to reduce the pH to 2–3 and the samples are heated to hydrolyze the fluoride compounds; HF is liberated and vessels have to be closed immediately to avoid analyte loss. In order to comply with ISE working conditions the solution has to be neutralized, resulting in very high salt content [7]. There are also methods described for the simultaneous determination of fluoride and PO_3F^{2-} based on IC. The elution time of this element is strongly pH-dependent. Too low pH will result in a co-elution of phosphate and PO_3F^{2-} and too high pH will increase the retention time and hence reduce sampling frequency [19]. Although gradient IC would be the method of choice for such complex sample matrix, most IC users have isocratic systems. Michalski and Mathews [20] described a method using suppressed isocratic IC for toothpaste analysis.

The analytical procedure for the determination of total fluoride with ISE is based on an acid hexamethyldisiloxane (HMDS) diffusion method first described by Traves [21]. The method is based on the liberation of fluoride with concentrated $HClO_4$ and the reaction with HMDS to trimethylfluorosilane (TMFS). After the diffusion process, which takes 7 h at room temperature, the volatile TMFS is trapped in alkaline solution, neutralized and buffered for fluoride determination with ISE. The problems of this procedure are the high salt content after hydrolysis, which can cause interference and errors, and the long time that is required for sample preparation. An alternative is the application of gas chromatography (GC) [22–24], which is becoming the official method for total fluoride determination described in the Official Journal of the European Communities [25]. This method is based on a derivatization with trimethylethylchlorosilane (TECS) in the presence of hydrochloric acid and subsequent extraction with xylene in the presence of cyclohexane as an internal standard. A disadvantage of this method is that MFP has to be hydrolyzed before measurement. In contrast to ionic fluoride these methods for determination of soluble and total fluoride are more complex and time consuming, less accurate and prone to systematic errors due to analyte loss.

A completely different approach for the determination of ionic and covalently bond fluoride in toothpastes was proposed by Gomez et al. [26] in 1992. The method was based on the formation of the gaseous molecule AlF in a nitrous oxide–acetylene flame and the measurement of its absorption at 227.45 nm using the radiation emitted from a Pt hallow cathode lamp. Basic investigations about the possibility to determine non-metals, such as fluorine, using the absorption bands of their diatomic molecules formed in flames and furnaces had already been carried out in the late 1970s and early 1980s by Tsunoda et al. [27] and Dittrich et al. [28–30]. In all these studies conventional atomic absorption spectrometers equipped with hollow cathode lamps and/or a deuterium lamp were used. The relatively low resolution of these spectrometers and the limited background correction possibilities, however, resulted in a variety of problems, so that this approach has never found any wider application [30].

This situation has changed dramatically with the introduction of commercially available equipment for high-resolution continuum source atomic absorption spectrometry (HR-CS AAS) [31], which is equally applicable for the measurement of molecular absorption spectrometry (MAS) with high resolution and simultaneous background correction [32]. Examples for exploring HR-CS MAS for fluorine determination in flames and graphite furnaces were published by Huang et al. [33] and Heitmann et al. [34], respectively. The method for fluorine determination using the molecular absorption of the gaseous gallium monofluoride GaF formed in a graphite furnace was recently optimized by our group, and a limit of detection of 5.2 pg F was reported [35].

The goal of the present paper was to investigate the applicability of the optimized method for the rapid and accurate determination of total and soluble fluorine in toothpaste samples of different origin. Both, total and dissolved fluorine were determined analyzing the toothpaste slurry directly and after centrifugation, respectively. The results were compared with those obtained by ISE and by a modified GC method.

Table 1
Temperature program for platform coating with zirconium carbide using three injections of 50 μl each of 1.0 g l^{-1} Zr; argon gas flow rate of 2 l min^{-1} in all stages.

Step no.	Temperature/°C	Ramp/°C s^{-1}	Hold time/s
1	80	7	2
2	95	3	40
3	350	50	20
4	1100	300	15
5	2400	1500	4

2. Experimental

2.1. Molecular absorption spectrometry of GaF

2.1.1. Instrumentation

A Model contrAA 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena, Jena, Germany), equipped with a transversely heated graphite tube atomizer and a flame atomizer in two separate sample compartments, was used for all measurements. The instrument uses a 300-W xenon short-arc lamp, operating in a hot-spot mode, as continuous radiation source, a high-resolution double monochromator consisting of a prism pre- and an echelle grating monochromator, providing a spectral bandwidth per pixel of about 1.5 pm at 200 nm, and a linear charge-coupled device (CCD) array detector with 588 pixels, 200 of which are used analytically, displaying the spectral vicinity of the analytical line at high resolution [36].

All measurements were carried out at the 211.248-nm GaF molecular absorption 'line' using the graphite furnace (GF) technique and pyrolytically coated graphite tubes with integrated PIN platform (Analytik Jena Part No. 407-A81.025). The platforms were coated with zirconium carbide as a permanent modifier, injecting three portions of 50 μl each of a 1 g l^{-1} Zr solution and executing the temperature program shown in Table 1 after each injection. Peak volume selected absorbance (PVSA) [37], i.e., the summed integrated absorbance of seven pixels ($A_{\sum 7,int}$) has been used throughout for signal evaluation, as it resulted in the best sensitivity and signal-to-noise ratio.

2.1.2. Reagents and solutions

High purity water (conductance <0.055 μS cm^{-1}) obtained from an Elix 3 reverse osmosis system (Millipore, Bedford, MA, USA) and further purification with a Seralpur Delta ion exchanger (Seral, Ransbach-Baumbach, Germany) was used throughout. The fluorine standard has been prepared by dilution of a 1.00 g l^{-1} F stock solution (sodium fluoride in water, Merck, Darmstadt, Germany). The Pd, and Zr modifier solutions were also made by dilution with water starting from commercially available standard solutions, 10 g l^{-1} Pd dissolved in 15% (v/v) HNO_3 (Merck), and 1 g l^{-1} Zr dissolved in 2 M HCl (Merck), respectively. The Ga reagent 10 g l^{-1} Ga was prepared by dissolution of Ga(III) nitrate hydrate (Sigma–Aldrich Chemie, Steinheim, Germany) in water. If any sample dilution was necessary pure water has been used as diluent. The argon gas of 99.999% purity (5.0) was provided by Riessner Gase (Lichtenfels, Germany).

Table 3
Volume and concentration of the modifier solutions and the Ga reagent used for thermal pretreatment and injection together with the sample.

Solution	Concentration	Volume (μl)	Temperature program
Pd+Zr in HNO_3	0.1% *m*/*v* Pd, 20 mg l^{-1} Zr	5	Stages 1–5
Ga in H_2O	10 g l^{-1} Ga	10	Stages 1–5
Sample or standard	Variable	20	Stages 6–11
Ga in H_2O	10 g l^{-1} Ga	2	Stages 6–11
$NH_4H_2PO_4$ in H_2O	0.1% *m*/*v* $NH_4H_2PO_4$	4	Stages 6–11

2.1.3. Procedure

After coating the PIN platform with 150 μg Zr as permanent modifier using the temperature program given in Table 1, the tube was conditioned by measuring alternately a blank solution and a 20 μg l^{-1} F standard. At the beginning a considerable GaF molecular absorption signal of up to A_{int} = 1 s appeared, probably due to a contamination of the Zr standard or from fluorine retained in the graphite of a new tube. After a few heating cycles the blank signal became stable, normally around A_{int} = 0.03–0.04 s, and the tube was ready for measurement.

The temperature program used for fluorine determination in toothpaste is shown in Table 2, and the solutions, their volume and concentration used for the determinations are given in Table 3; the blank solution contained all reagents given in Table 3 with the sample or standard replaced by water. The conditions have only been modified slightly compared to those published previously [35]. First the palladium and zirconium mixed modifier and the gallium solution required for molecule formation were injected sequentially by the autosampler and the first four stages of the temperature program in Table 2 were executed. The goal of this pretreatment was to reduce the gallium to the metal to make it more effective for GaF molecule formation and to eliminate the acid content of the Pd/Zr-solution to avoid analyte losses in the form of HF in the drying and pyrolysis stages. After that a cooling stage (step 5) was introduced before the injection of the sample and the other modifier solutions, and the rest of the temperature program of Table 2 was executed, using a pyrolysis and vaporization temperature of 550 °C and 1550 °C, respectively. For an automatic performance of the pretreatment (steps 1–4), the cooling (step 5) and the analyte measurement (steps 6–11), software version ASpect CS 1.5.3 or higher has to be installed in the contrAA 700.

Another change in comparison to the previously optimized conditions was the use of $NH_4H_2PO_4$ as an additional modifier. The reason for that was to create a similar matrix for all samples, including those that did not contain MFP. The relatively weak PO molecular absorption bands that appeared due to the presence of the phosphate modifier [34] were almost completely separated in

Table 2
Graphite furnace temperature program for modifier pretreatment and GaF molecule formation and vaporization; for details see Section 2.1.3.

Step no.	Stage	Temperature/°C	Ramp/°C s^{-1}	Hold/s	Gas flow rate/l min^{-1}
1	Drying	90	7	2	2.0
2	Drying	110	3	5	2.0
3	Drying	350	300	10	2.0
4	Pretreatment	1100	500	10	2.0
5	Cool down	90			2.0
6	Drying	90	0	2	2.0
7	Drying	350	300	20	2.0
8	Pyrolysis	550	500	10	2.0
9	Auto zero	550	0	5	Stop
10	Vaporization	1550	1500	7	Stop
11	Cleaning	2400	500	4	2.0

Table 4
Fluoride species in the investigated toothpastes and their specified or assumed fluoride concentration.

Toothpaste		Components	Concentration/ ppm F
Colgate Total	70%	Sodium fluoride	1015[a]
	100%		1450[a]
	130%		1885[a]
Colgate Max	70%	MFP	1015[a]
	100%		1450[a]
	130%		1885[a]
Eurodent		Sodium fluoride	1450
Elmex		Amine fluoride (olafluor)	1400
Signal		Sodium fluoride + MFP	1450
Amin Med		Sodium fluoride	400
		Amine fluoride	800

[a] Assumed content; for details see Section 2.1.4.

time from the GaF absorption and could be eliminated easily using a correction spectrum and a least-squares algorithm that is available in the software of the contrAA 700.

2.1.4. Samples and sample preparation

Two toothpaste samples that were prepared by the manufacturer particularly for this investigation and four commercially available toothpastes that were purchased at a local supermarket have been investigated in this work. The two specially prepared toothpastes, Colgate Total and Colgate Max, were provided by the manufacturer in three forms, containing different fluorine concentrations. The standard concentration is referred to as 100%, and the same samples were also prepared with a 30% lower (70%) and 30% higher (130%) fluorine content. Although the manufacturer did not inform the fluoride content in these samples, it might be assumed that the 'standard concentration of 100%' is the same that is found in all commercially available toothpastes of this manufacturer, i.e., 1450 ppm. These samples were stored for 1 year before measurement of total and dissolved fluoride in order to investigate the influence of this parameter on the fluoride content. The different fluoride components of the purchased and the specially prepared toothpastes and the stated or assumed concentrations are listed in Table 4.

For the determination of total fluorine, about 10–20 mg of toothpaste sample was weighed accurately into polypropylene sample cups (Sarstedt) and filled up to 50 ml with de-ionized water. To increase dissolution of soluble matrix constituents the sample cups were placed in an ultrasonic bath for about 5 min. The toothpaste suspensions were diluted by a factor of 10 with de-ionized water without filtration and without any pH adjustment, and the fluorine content determined by HR-CS GF MAS.

For the determination of soluble fluorine, the samples were centrifuged for 5 min with 2700 rpm/rcf and the supernatant was used for further investigations.

2.2. Gas chromatography

2.2.1. Instrumentation

All determinations were carried out using a Model GC–MS 2010 Plus gas chromatograph (Shimadzu Kyoto, Japan), equipped with split/splitless injection port. A capillary column RTX-5MS (Restec, USA), with 30 m × 0.25 mm ID and a 0.25 μm thick stationary phase was used throughout. Analyses were carried out in scan mode (46–100 u) and electron ionization (70 eV). The chromatographic conditions were: injector temperature 280 °C, interface 200 °C and the oven temperature program of the column was: 35 °C (2 min) and a ramp of 20 °C min^{-1} to 200 °C. Helium gas (White Martins, São Paulo, Brazil) was used as carrier gas with a flow rate of 0.80 ml min^{-1}.

A headspace extraction has been used instead of a liquid-phase extraction with xylene, as recommended in the EC method [25]. The solid-phase micro extraction (SPME) device and the carboxen/polydimethylsiloxane (CAR/PDMS) fibers (75 μm film thickness) were from Supelco (Bellefonte, PA, USA). Before initial use, the fiber was preconditioned in the GC injection port at 300 °C for 2 h.

2.2.2. Reagents and solutions

The reagents used were dichlorodimethylsilane 99+% (Acros Organics, NJ, USA), sodium fluoride suprapur (Merck, Darmstadt, Germany), Hydrochloric acid 37% (Sigma–Aldrich, Steinheim, Germany). The sodium fluoride was dried at 100 °C for 2 h prior to the preparation of standard solutions. A NaF stock solution with 0.50 g l^{-1} F was prepared by dissolving sodium fluoride in deionized water.

2.2.3. Sample preparation

About 25 mg of toothpaste was weighed accurately into a 15 ml teflon tube; 10 ml of deionized water, 0.3 ml of hydrochloric acid and 30 μl of dichlorodimethylsilane were added, and the tube was tightly closed. The sample was left for 10 min of reaction time, where-upon the fiber was placed in the headspace for additional 10 min of extraction time. During the reaction and extraction, the samples were continuously agitated with a magnetic stir bar. Both stages of the procedure were conducted at a room temperature.

2.3. Ion selective electrode

2.3.1. Instrumentation

For ISE measurements a fluoride-selective electrode type F500 (WTW, Weilheim, Germany, No. 106284) was used.

2.3.2. Reagents and solutions

The TISAB solution was prepared from 300 g trisodium citrate dihydrate, 60 g sodium chloride and 22 g Titriplex IV (1,2-cyclohexylenedinitrilotetraacetic acid monohydrate) dissolved in warm deionized water and filled up to 1 l.

2.3.3. Sample preparation

Because of the relatively low sensitivity of the ISE measurement, the sample weight was increased to about 1 g; otherwise the sample preparation described in Section 2.1.4 for dissolved fluoride was followed. After centrifugation the total ionic strength of the measurement solutions was adjusted by adding TISAB solution; the pH of the resulting solution was 5.8.

3. Results and discussion

3.1. Molecular absorption spectrometry

3.1.1. Figures of merit

The calibration was performed in the range of 10–50 μg l^{-1} F with a sample volume of 20 μl, i.e., an absolute mass of 0.20–1.00 ng F using the temperature program given in Table 2 and the modifier and molecule-forming solutions given in Table 3. The calibration curve is displayed in Fig. 1, each of the five equidistant points was measured with three replicates, and the relative standard deviation (RSD) was between 1.0% and 4.5%. The limit of detection (LOD) of the method was calculated on the basis of three times the standard deviation of 11 repetitive blank measurements (using all the reagents listed in Table 3 and replacing the sample with de-ionized water), divided by the slope of the calibration curve. The LOD calculated in this way for 20 μl sample volume was 0.26 μg l^{-1} F and 5.2 pg F absolute, respectively. The limit of quantification (LOQ), defined as 10 times the standard deviation of 11 repetitive blank

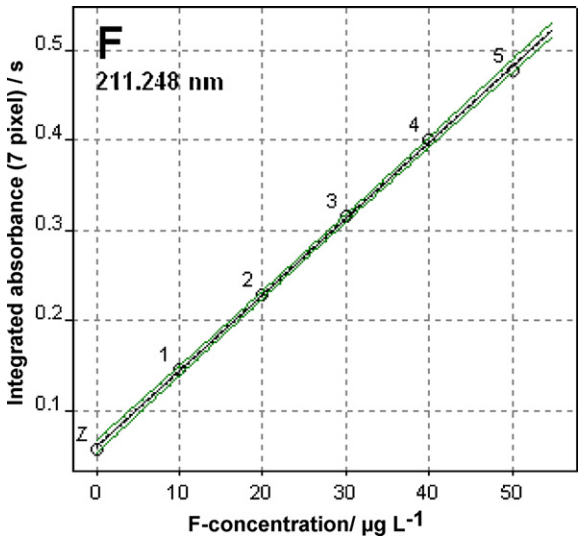

Fig. 1. Calibration curve for F using GaF molecular absorption at 211.248 nm; 10–50 $\mu g\,l^{-1}$ F, injection volume 20 µl; pyrolysis temperature 550 °C, vaporization temperature 1550 °C.

measurements was 0.87 $\mu g\,l^{-1}$ F and 17 pg F absolute, respectively. This corresponds to a LOQ of about 30 $\mu g\,g^{-1}$ (ppm) of F in the original toothpaste using the procedure described here, i.e., 15 mg of toothpaste diluted to a final volume of 500 ml. This LOQ perfectly serves the purpose of the current investigation, but could obviously be improved by at least one order of magnitude using less dilution. Two quality control (QC) standards containing 20 $\mu g\,l^{-1}$ and 40 $\mu g\,l^{-1}$ F, respectively, were routinely analyzed together with the toothpaste samples. The linear range extends from the LOQ (<1 $\mu g\,l^{-1}$) to at least 50 $\mu g\ l^{-1}$ F with a linear regression equation of $A_{int} = 0.00844c + 0.060$.

3.1.2. Analysis of toothpaste samples

The main interest of this investigation was to develop a simple method for the determination of total and dissolved fluoride in toothpaste independent of its chemical form (ionic or covalent). Among the fluorides that are typically used in toothpastes, i.e., NaF. Amine fluoride and MFP, the first one has been used for calibration, the second one decomposes readily at elevated temperatures, and in order to simulate the presence of MFP, $NH_4H_2PO_4$ was added to all solutions as an additional modifier; no significant differences in the sensitivity for the three compounds have been observed under these conditions. Interference due to the main matrix constituents, for example due to the formation of a competitive molecule such as CaF, could also be largely excluded due to the high sample dilution used in this work and the excess of the molecule-forming reagent gallium.

In order to investigate the influence of the storage time of the samples on these values the toothpaste samples Colgate Total and Colgate Max that have been spiked by the manufacturer with different fluoride concentrations, and were stored for 1 year before analysis. In addition, four commercially available samples, which were assumed to be relatively fresh, have been analyzed for their total and dissolved fluoride content. The results obtained by GaF molecular absorption spectrometry are shown in Table 5. All results are in good agreement with the values stated by the manufacturers. Both of the internal quality control standards measured after the toothpaste samples are also in good compliance with the expected concentrations, demonstrating calibration stability after the analysis of real samples with matrix. The results in Table 5 also demonstrate the independence of the developed method for total fluorine determination on the kind of fluoride species even after a very long storage time.

Most of the values for dissolved fluoride are within about ±5% of the values specified for total fluoride with no clear tendency with respect to any of the fluoride compounds added or in relation to the storage time. However, when a *t*-test was applied, comparing the values obtained with MAS for total F and dissolved F, five of the toothpaste samples, Colgate Total 100%, Colgate Total 130%, Colgate Max 130%, Signal and Amin Med exhibited a significantly lower value for dissolved fluoride on a 95% confidence level, but again, there was no correlation to a specific fluorine compound.

3.2. Established techniques

3.2.1. Gas chromatography

The linearity of the method has been studied for the calibration range of 0.75–5.0 $mg\,L^{-1}$ F. The LOD and LOQ, based on the parameters of the analytical curve, were determined as 0.25 $mg\,l^{-1}$ and 0.80 $mg\,l^{-1}$, respectively. This corresponds to a LOQ in the toothpaste of about 320 $\mu g\,g^{-1}$, based on a 25 mg sample mass. The results showed a good correlation coefficient (*R*) of 0.998, and the repeatability of the entire procedure, including sample pretreatment, SPME and determination was between 1% and 3% RSD, based on three repetitive determinations. The results of the determination of total fluoride in the toothpaste samples using GC–MS are summarized in Table 6, column 2.

3.2.2. Ion selective electrode

The LOQ for the F-ISE given by the manufacturer is 0.08 $mg\,l^{-1}$ and the precision of the results was assumed to be about 15%. Using an F-ISE for the determination of fluoride, by definition, only ionic

Table 5
Total and dissolved fluoride concentration found in the investigated toothpaste samples using HR-CS MAS; all concentrations in $\mu g\,g^{-1}$ F (ppm).

Toothpaste	Stated or assumed concentration	Total F concentration found[a]	RSD[b]/%	Dissolved F concentration found[a]	RSD[b]/%
Colgate Total 70%	1015	1000 ± 29	0.6	997 ± 30	0.9
Colgate Total 100%	1450	1520 ± 28	2.7	1330 ± 34	1.5
Colgate Total 130%	1885	1960 ± ±28	0.6	1850 ± 24	2.9
Colgate Max 70%	1015	1030 ± 30	2.8	1010 ± 32	2.9
Colgate Max 100%	1450	1530 ± 28	1.8	1510 ± 23	1.3
Colgate Max 130%	1885	1930 ± 25	2.1	1840 ± 22	2.2
Eurodent	1450	1430 ± 21	0.8	1510 ± 23	0.9
Elmex	1400	1390 ± 25	1.4	1420 ± 25	0.4
Signal	1450	1490 ± 26	2.7	1430 ± 17	1.0
Amin Med	1200	1180 ± 20	4.5	1130 ± 20	1.3
QC standard	20 $\mu g\,l^{-1}$	19.8 ± 0.63	3.7		
QC standard	40 $\mu g\,l^{-1}$	39.1 ± 0.59	0.6		

[a] Average ± confidence interval with $P = 0.95$.
[b] Relative standard deviation of three replicates.

Table 6
Comparison of values found for total fluoride using HR-CS GF MAS and GC–MS, of dissolved fluoride using HR-CS GF MAS and of ionic (bio-active) fluoride using F-ISE.

Toothpaste	Components	Found concentration/ppm F					
		Total F by MAS[a]	Total F by GC–MS[a]	Dissolved F by MAS		Ionic F by ISE	
	Column	1	2	3	% of MAS_{total}	4	% of $MAS_{dissolved}$
Colgate Total 70%	NaF	1000 ± 29	1030 ± 30	997 ± 30	100	990 ± 149	99
Colgate Total 100%	NaF	1520 ± 28	1480 ± 20	1330 ± 34	92	1390 ± 209	104
Colgate Total 130%	NaF	1960 ± 28	1930 ± 30	1850 ± 24	94	1850 ± 278	100
Colgate Max 70%	MFP	1030 ± 30	1040 ± 30	1010 ± 32	98	627 ± 94	62
Colgate Max 100%	MFP	1530 ± 28	1480 ± 30	1510 ± 23	98	770 ± 116	51
Colgate Max 130%	MFP	1930 ± 31	1990 ± 10	1840 ± 22	94	1020 ± 153	55
Eurodent	NaF	1430 ± 21	1400 ± 10	1510 ± 23	104	1190 ± 179	78
Elmex	Amine fluoride	1390 ± 25	1380 ± 10	1420 ± 25	102	1270 ± 191	89
Signal	NaF, MFP	1490 ± 26	1470 ± 6	1430 ± 17	97	376 ± 56	26
Amin Med	NaF, Amine fluoride	1180 ± 20	1360 ± 40	1130 ± 20	95	814 ± 122	72

[a] Average ± confidence interval with $P = 0.95$.

fluoride will be measured; this, however, is another important figure to test the quality of a toothpaste. Table 6, column 4 shows the results obtained for the bio-active quantity of fluoride. The data will be discussed in the following section in comparison with the results obtained with the other techniques.

3.3. Comparison of the results

3.3.1. Total fluoride content

The results obtained for the total fluoride content using HR-CS GF MAS and GC–MS, which are shown in Table 6, columns 1 and 2, are statistically not different for eight of the ten samples investigated, based on a *t*-test at a 95% confidence level; however, the results obtained with GC–MS are significantly higher for Colgate Max 130 and Amin Med. The determinations have been repeated, but the results were the same; no explanation could be given for the difference. Nevertheless, accuracy and precision of the two procedures are comparable, which means that both techniques can be used for the determination of total fluoride in toothpaste. Obviously, HR-CS GF MAS would be much more sensitive if less diluted samples would be used; however, this aspect is not of importance for this application, as the fluoride content in toothpastes is relatively high.

Other important aspects for the comparison of two methods, besides accuracy and precision, are the time and the amount of work that are involved in sample preparation and the final determination. In the case of the GC–MS procedure, the reaction time (10 min) and extraction time (10 min) have to be controlled precisely, and both, reaction and extraction have to be carried out immediately prior to the introduction into the chromatograph. The chromatogram takes about another 10 min, which means that a single determination by GC–MS takes slightly more than 30 min. Obviously, the preparation of the next sample can be carried out while the chromatogram is under way, making the whole process significantly faster. Nevertheless, the determination of fluoride in 10 samples in triplicate, as it has been done in this work, including establishing a calibration curve actually took three full working days. An autosampler would obviously facilitate this kind of determination and shorten the total analysis time.

In the case of the HR-CS GF MAS procedure, sample preparation consists of a dilution of the toothpaste with water and 5 min in an ultrasonic bath; obviously, all samples can be prepared and treated at the same time, independent when they will be analyzed. The coating and conditioning of the graphite tube (see Section 2.1.3) takes about 20 min, and this tube can then be used for hundreds of determinations without any further treatment. The calibration with one blank and five standards, all measured in triplicate, takes about 1 h. The temperature program shown in Table 2 takes about 3 min including sample injection and cool down, hence, a triplicate determination of fluorine takes about 10 min. This means that the whole procedure of coating and conditioning of the tube, calibration and analysis of 10 samples takes about 3 h – and most of the operations can be carried out using an autosampler. For a greater number of samples the procedure becomes even more economic, as coating, conditioning and calibration do not have to be repeated. This means that the proposed procedure, depending on the number of samples that have to be analyzed, is at least a factor of five faster than the GC–MS procedure used for comparison.

3.3.2. Dissolved and ionic fluoride

The results obtained with HR-CS GF MAS for dissolved fluoride after centrifugation and with F-ISE for ionic fluoride are compiled in Table 6, columns 3 and 4. As already discussed in Section 3.1.2, the values obtained for dissolved fluoride using MAS, although five of them are statistically different from the values found for total fluoride, based on a *t*-test on a 95% confidence interval, the differences are not really very great. Hence, neither the storage time nor the form in which fluoride was added had any significant effect on the part of fluoride that is dissolved in water, at least not for the samples investigated in this work.

This is in contrast to the ionic, i.e., bio-active fluoride determined by ISE. These values can be close to 100% of total fluoride even after 1 year of storage in the case of sodium fluoride as the added component, and they may drop to values between 50% and 60% when MFP is the active component. However, no rule could be established based on the present investigations, as values between 26% and 89% have been found for the bio-active fluoride in fresh toothpastes, and no relation with the composition of the toothpaste could be established. Obviously, the determination of bio-active fluoride cannot be carried out using HR-CS GF MAS, and hence remains the domain of ISE, which is fast and economic, although it is not without interference.

4. Conclusion

The results obtained for the fluoride content in toothpaste using the proposed HR-CS GF MAS procedure are in good agreement with the content stated by the manufacturers, and also with most of the results obtained with an independent GC–MS method. Both methods appear to be comparable with respect of accuracy and precision; however, the latter one is much more labor-intensive, and it requires an about five times longer time period to complete the same number of determinations. The proposed method does not require any significant sample preparation and can be easily automated. No difference in sensitivity has been found for the various fluoride compounds that are typically added in toothpaste formulations. The proposed method can also be used to determine dissolved fluoride; however, it cannot be used for the determination of bio-

active, ionic fluoride, which remains the domain of ion-selective electrodes. Because of the lack of fast and reliable alternative methods the determination of fluorine in different matrices is currently mainly limited to the determination of the ionic fluoride. Applying HR-CS GF MAS, covalently bound fluorine compounds could be determined as well, and the applicability of the proposed method might well be expanded to other sample types, such as urine, blood, foods and pharmaceutical products.

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