JOURNAL OF FOOD AND DRUG ANALYSIS 21 (2013) 384–389

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Original Article

Determination of fluorides in pharmaceutical products for oral hygiene

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ARTICLE INFO

Article history:
Received 23 January 2013
Received in revised form
19 April 2013
Accepted 9 July 2013
Available online 11 September 2013

Keywords:
Fluorides
Mouthwash
Potentiometry
Sample preparation
Toothpastes

ABSTRACT

Fluorides are common ingredients in pharmaceutical products for oral hygiene due to their recognized effect in the prevention of tooth decay. In dental products, fluorides can be added in several different forms, such as sodium fluoride, sodium monofluorophosphate, tin fluoride, or in the form of different amines. This work describes potentiometric determination of fluorides in the samples of toothpastes and mouthwash. The method was optimized for the particular analytical purpose; namely, for the analysis of toothpastes and mouthwash by applying different sample preparation protocols depending on the fluoride source. Good recovery (93–103%) confirmed the correctness of the sample preparation procedures. Calculated limit of detection and limit of quantification for the optimized method were 1×10^{-3} mg/L and 2.8×10^{-3} mg/L fluoride, respectively. In the minority of the analyzed samples, calculated contents agreed well with the certified values, whereas the samples of mouthwash demonstrated better agreement.

1. Introduction

Pharmaceutical products for oral hygiene encompass several different formulations including toothpastes and mouthwash. Important ingredients of toothpastes are abrasives for mechanical cleaning, detergents, water, sweeteners, moisturizers, and flavors. These products might contain vitamins or therapeutic agents such as antiseptic or antibiotic compounds. Fluorides are common ingredients in pharmaceutical products for oral hygiene due to their recognized effect in the prevention of tooth decay [1,2]. Fluoride prevents early dental caries by several mechanisms. It reduces bacterial metabolism, especially glycolysis, thus reducing acid production and hence demineralization [3]. Fluoride also helps control decay by enhancing remineralization and altering the tooth structure, making the surface less soluble [4].

Tooth enamel is chiefly composed of crystals of a calcium phosphate mineral called hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. After a meal, acidity in the oral cavity decreases due to acid production. Produced acids further react with the hydroxyapatite from the tooth enamel, thus damaging its structure:

$$Ca_5(PO_4)_3OH_{(s)} + H^+_{(aq)} \leftrightarrow Ca_5(PO_4)^+_{3\,(aq)} + H_2O_{(l)}$$

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1021-9498
http://dx.doi.org/10.1016/j.jfda.2013.08.006

In the presence of fluorides in saliva or plaque fluid, fluoride substitutes the hydroxyl ions to form hydroxyfluorapatite [$Ca_{10}(PO_4)_6(OH)F$] or fluorapatite [$Ca_{10}(PO_4)_6F_2$]; both of which are less soluble under acidic conditions than hydroxyapatite. This mechanism prevents the loss of mineral ions, thus providing additional protection to mineral crystallites by laying fluoride-rich outer layers onto the apatite crystallites. Fluorapatite is characterized by its better resistance to acids in comparison to hydroxyapatite, building a more resistant structure in the tooth enamel. The critical pH for a mineral composed of a fluoride-rich apatite is significantly lower than that for hydroxyapatite.

Fluorides may be added to toothpastes in several different forms, such as sodium fluoride (NaF), sodium monofluorophosphate (Na_2FPO_3), tin fluoride (SnF_2), or in the form of different amines [5,6].

In order to prevent demineralization of the tooth enamel, the concentration of fluoride must be at least 1 mg/kg. In addition to being dependent on the content, the efficiency of fluoride sources in the prevention of tooth decay is highly influenced by the salt form, which in return, may be more or less efficiently incorporated in the tooth enamel structure. In most pharmaceutical products either NaF or Na_2FPO_3 is used as a source of fluoride ions. According to some investigations it was estimated that toothpastes containing NaF are more efficient when compared to those in which the fluoride is added in the form of Na_2FPO_3 [7]. The main mechanism by which these differences may be explained is linked to different quantities of the liberated fluoride during dissociation of the salts. Namely, in aqueous solutions NaF is completely dissociated, whereas Na_2FPO_3 during its dissociation releases about 6% of the F^- [8,9]:

$$NaF \rightarrow Na^+ + F^-$$

$$Na_2FPO_3 \rightarrow 2Na^+ + FPO_3^{2-}$$

$$FPO_3^{2-} + OH^- \rightarrow F^- + HPO_4^{2-}$$

The monofluorophosphate hydrolysis rate is low at neutral pH but can be significantly accelerated in acidic medium and temperatures above room temperature [8].

The differences in the efficiency of F^- and FPO_3^{2-} may further be explained by different diffusion rates, which are important in the process of the incorporation in the tooth enamel structure. The sole chemical reaction is rapid, whereas the diffusion rate is a limiting factor of the process. Fluoride ions are much more mobile in comparison to FPO_3^-, rapidly diffusing through saliva and reaching a target spot within 1 minute or even less [10]. In conclusion, for the same salt concentration, the efficiency of NaF can be considered better [7,11], due to higher concentrations of the formed fluoride ions in the dissociation process, which in return, generate a strong concentration gradient.

In toothpaste formulations, different polishing additives are normally added, and calcium carbonate is particularly popular. This salt can negatively reflect the stability of fluoride salts, reducing their concentration:

$$2F^- + CaCO_{3(s)} \leftrightarrow CaF_{2(s)} + CO_3^{2-}$$

The formed product (CaF_2) is poorly dissociated in the aqueous solutions, reducing the concentration of free fluorides [12].

When Na_2FPO_3 is used as a fluoride source, fluoride in the form of FPO_3^{2-} ions is practically protected from such a reaction. In addition, the lower toxicity of Na_2FPO_3 allows it to be used as a fluoride source in pharmaceutical formulations for oral hygiene.

Depending on various factors, for example, concentration, frequency of use, and age of the user, fluoride can be detrimental or beneficial [13]. The difference between toxic and therapeutic concentrations of fluoride is narrow, therefore, an accurate and fast method for the determination of total fluoride in dentifrice is important in quality control and for the assessment of compliance with the recommendations for daily fluoride intake. Several techniques have been used to determine water-soluble fluoride species in toothpastes: fluoride ion-selective electrode (ISE) electroanalysis [14–17], flow injection analysis [18,19], gas chromatography [13], ion chromatography [16], capillary electrophoresis [8], and flame atomic absorption spectrometry [20].

Potentiometry with ISE, the most widely used method for the determination of fluoride, is simple to perform and has good precision and sensitivity. Fluoride-specific electrodes are commercially available and are sensitive over a wide temperature range (0–50°C). The method, however, detects only free fluoride ions in solution.

Fluoride-selective electrodes are responsive to fluoride ions, however, hydroxide ions to some extent participate in the potential settings as well. Adjustment of pH with buffer is necessary because fluoride and hydroxide ions have the same valency and similar ion radius, so hydroxide ions can interfere in fluoride determination. Total ionic strength adjustment buffers (TISABs) are important reagents in the determination of fluoride using ISEs. TISAB solution in fluoride determination serves to reduce interferences originating from hydroxide ions by adjusting the pH, and to prevent complex formation between H^+ and F^- in acidic solutions. Solution is also efficient in reducing the interference from polyvalent cations, such as Al(III), Fe(III), and Mg(II), which are able to complex or precipitate with fluoride reducing the free fluoride concentration in the solution [21]. In potentiometric measurements, the potential is dependent on activity rather than concentration. Ionic strength of all solutions should be kept constant, to assure proportionality of the activity and concentration. Besides providing a constant pH, TISABs regulate the ionic strength of the samples and standard solutions.

The goal of the present study was to investigate the applicability of the optimized potentiometric method by using ISE for rapid and accurate determination of total fluoride in toothpaste and mouthwash samples of different origin. The emphasis was on defining the procedure for sample preparation.

2. Materials and methods

2.1. *Instrumentation*

The combined fluoride-selective electrode “Jenway” was used as a sensor for fluoride determination (Fig. 1).

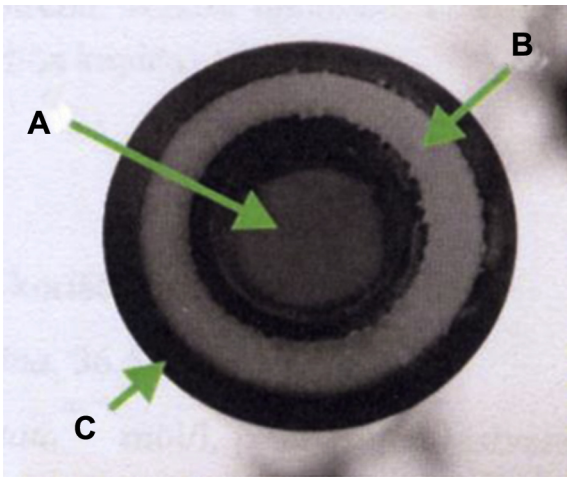

Fig. 1 – Fluoride-selective electrode. (A) Membrane; (B) body of the electrode; and (C) annular space with reference electrode.

In fluoride-selective electrodes, the active membrane was made of a LaF_3 monocrystal, doped with a small amount of europium(II) fluoride to lower its electrical resistance and to facilitate ionic charge transport. Fluoride ions bound to this crystalline surface according to:

$$LaF_{3(s)} + F^-_{(aq)} \leftrightarrow LaF^-_{4(s)}$$

The LaF_3 crystal, sealed into the end of a plastic tube, was in contact with the measuring solution. Internal electrolyte contained 0.1 M NaCl and 0.1 M NaF. The activity of fluoride ions in the inner solution controlled the potential on the inner surface of the crystal.

The electrode was connected to a read-out device HANNA potentiometer (USA). A magnetic stirrer (MM-510; Tehtnica, Železniki, Slovenia) was used to agitate solutions during the measurements. The usual laboratory glassware was used in the experiments. In the potentiometric measurements, polyethylene process vessels were used. All laboratory accessories were washed with nitric acid–water mixture (1:1, v/v), distilled, and triply distilled water.

2.2. Standards and reagent solutions

For all measurements, triply distilled water was used in order to reduce fluoride content to a minimum. A 1 g/L fluoride stock solution was prepared by dissolving an appropriate amount of NaF (Merck, Darmstadt, Germany, pro-analysis) in triply distilled water. Prior to dissolution, NaF was dried at 110°C for 2 hours.

TISAB was used in potentiometric measurements. There are several different formulations to make TISAB solutions. The preparation procedure of TISAB solution used in the present study was as follows: 58 g NaCl (Merck, Darmstadt, Germany, pro-analysis), 0.3 g sodium citrate (Merck, Darmstadt, Germany, pro-analysis), and 57 mL acetic acid (Merck, Darmstadt, Germany, pro-analysis) were dissolved in 500 mL triply distilled water. After dissolution, the pH was adjusted to 5.0–5.5 with 5 M NaOH (Merck, Darmstadt, Germany, pro-analysis) and the volume was adjusted to 1 L with triply distilled water. TISAB solution was stored in a plastic bottle until use. Recommended volume ratio between TISAB and test solutions was 1:1 (v/v). TISAB was used for the preparation of samples and standard free fluoride solutions. Fluoride calibration solutions in the range of 0.5–100 mg/L were prepared by serial dilution of the stock solution in triply distilled water. Finally, equal volumes (15 mL) of each calibration solution and TISAB solution were mixed and used in potentiometric measurements.

2.3. Samples

Fluorides were determined in six samples of commercial toothpastes and five samples of commercial mouthwash (Table 1). The samples were collected randomly from retail outlets in Novi Sad, Serbia. Collected samples encompassed domestic and foreign products including the most readily available and common ones. The intention of sampling strategy was to cover different products with different fluoride sources. Available products were fortified with one of two distinct fluoride sources as shown in Table 1.

2.4. Sample preparation

Sample preparation depended on the sample type, sample matrix, and the form of the fluoride ion, that is, in its free or complexed form.

Samples of toothpastes (1 g) were dissolved in triply distilled water and were quantitatively transferred to a 100-mL volumetric flask that was filled to the mark. To speed up the dissolution, the sample was heated for 10 minutes in a water bath prior to filling to the mark. Upon cooling, 10 drops of propanol (Kemika, Zagreb, Croatia) were added to reduce foaming. For samples in which Na_2FPO_3 was specified as the fluoride source, prior to filling to the mark, 4 mL 6 M HCl was added to release fluoride ions completely.

Samples of mouthwash were prepared by transferring 10 mL of a liquid sample to a 100-mL volumetric flask, adding 10 drops of propanol, and filling to the mark with triply distilled water.

Table 1 – Samples of toothpaste and mouthwash.

Sample no.	Sample	Fluoride source
1	Toothpaste	NaF
2	Toothpaste	NaF
3	Toothpaste	Na_2FPO_3
4	Toothpaste	Na_2FPO_3
5	Toothpaste	Na_2FPO_3
6	Toothpaste	NaF
7	Mouthwash	NaF
8	Mouthwash	NaF
9	Mouthwash	NaF
10	Mouthwash	NaF
11	Mouthwash	NaF

2.5. Potentiometric measurements

Prior to analysis, 15 mL of standards or prepared samples were transferred to polyethylene process vessels and 15 mL TISAB solution was added. A magnetic stirrer was used to facilitate the convective mass transfer during measurements, and the potentiometer was set to read the voltage. After thorough rinsing and drying with a paper tissue, the electrode was immersed in the solutions. Solutions were stirred mechanically for 2 minutes and then the potential was read. Measurements were repeated three times.

The analyte concentration was measured against a calibration curve that was prepared as described earlier. The recovery assays were performed by adding a known amount of the same salt, as specified by the manufacturer, to the sample prior to its preparation. Thus, for Samples 3–5, Na_2FPO_3 was added, whereas NaF was used for the rest of the samples. The percentage recovery values were calculated by comparing concentrations obtained from the spiked samples with actual added concentrations of fluoride, as follows:

$$\text{Recovery}(\%) = \left(C_{\text{Spiked Sample}} - C_{\text{Sample}}\right)/C_{\text{Add}} \times 100$$

$C_{\text{Spiked Sample}}$ represents mg/kg of determined fluoride content in spiked sample, C_{Sample} represents mg/kg of fluoride actually present in the sample, and C_{Add} represents mg/kg of fluoride added to the sample. Recovery test was performed for each toothpaste and mouthwash sample in three replicates.

3. Results and discussion

3.1. Determination of the equilibration time

In potentiometric measurements, the error is highest at the pre-equilibrium phase, that is, in the first moments of the electrode/solution contact. Over time the error diminishes and reaches a constant value. When the system is equilibrated, that is, when the equilibrium between the particular ion and a membrane potential is reached, the error is minimal. Equilibration time differs among the systems and depends on numerous factors. Prior to defining optimal experimental parameters, it was necessary to define the equilibrium time for the systems used in this particular investigation. Equilibration time was defined for different fluoride concentrations, 0.05 mg/L, 0.25 mg/L, 1 mg/L, 2.50 mg/L, and 5 mg/L. Potential was monitored in regular time intervals of 1 minute, 2 minutes, 3 minutes, and 5 minutes for each fluoride concentration and each measurement was repeated three times. It took longer in a solution of lower fluoride concentrations to reach potential equilibration, so in solutions containing 1 mg/L fluoride it needed 3 minutes for the potential to reach a standstill value (Fig. 2). Furthermore, the reproducibility was markedly better. In Fig. 2, the intervals around the values represent 2 standard deviations (SD) of the signal. In solutions containing >1 mg/L fluoride, the potential could be read after 1 minute when it reached a stable value. An equilibration time of 2 minutes was chosen for further experiments because it provided a relatively accurate determination over a wide concentration range. For general application, an equilibration time of 2 minutes allows time-efficient and accurate measurement of a broad concentration range, and it was thus adopted for practical measurements. Namely, according to specified contents in real samples of toothpastes and mouthwash, <0.05 mg/L fluoride could not be expected. Furthermore, the accepted equilibration time provided an acceptable duration of analysis.

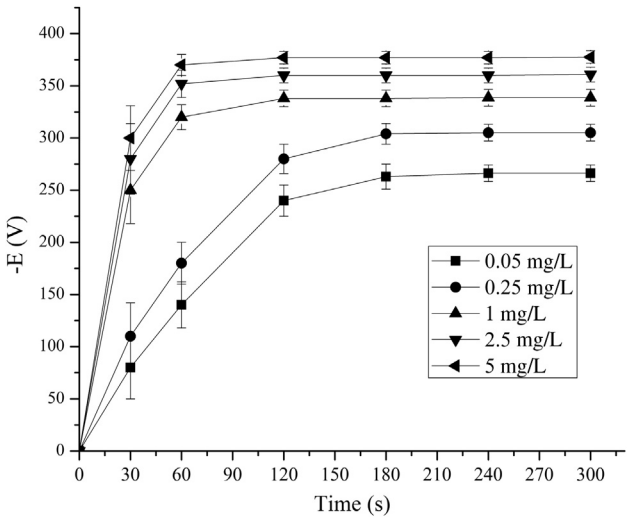

Fig. 2 – Equilibration times for different fluoride concentrations.

3.2. Linearity

When quantification is in question, it is of utmost importance to define the linear range. For the system used, the linearity was verified for a wide concentration range from 1 mg/L to 1000 mg/L of fluoride. Experimental results demonstrated that the dependence of the potential versus logC was correlated well with the assumed linear dependence ($-E = 338.7 + 55.6$ logC; $r = 0.9996$) over the whole examined concentration range. Furthermore, the slope of 55.6 mV/logC was in good agreement with the theoretical Nerstian slope for monovalent cations. A similar value of intercept (343.8 V) was reported in the literature [17], but the proposed method offered better agreement with the theoretical Nerstian slope.

3.3. Precision

Precision of fluoride determination was defined in terms of repeatability (intra-day assay) and reproducibility (inter-day assay). Repeatability was defined on the basis of seven replicate analyses of solutions containing 1 mg/L, 5 mg/L, and 50 mg/L fluoride under optimal experimental conditions in a single day. Reproducibility was defined by analyzing fluoride solutions every day in triplicate for three consecutive days. The results are summarized in Table 2. For all fluoride contents, calculated relative standard deviation (RSD) was <1% for both intra- and inter-day precision assays, indicating high precision of the method.

Table 2 – Intra- and inter-day precision of determination for different fluoride concentrations.

Fluoride content (mg/L)	Repeatability (intra-day precision)	Reproducibility (inter-day precision)
	RSD (%)	RSD (%)
1	0.46	0.75
5	0.02	0.04
50	0.0001	0.0002

RSD = relative standard deviation.

3.4. LOD

In defining LOD, multiple criteria can be applied. The sensitivity of a certain analytical method is determined by the lowest analyte concentration that can be reliably determined in statistically acceptable limits. This practically means that a signal detected in blank tests should be increased by its reproducibility in order to take into account the variation in the measurement results. This reproducibility component is normally expressed via 3 SD.

In this work the criterion $\bar{x} \pm 3$ SD was used to calculate the LOD, where $\bar{x}$ represented the mean of five blank measurements. The signal in the blanks increased by 3 SD was interpolated in the dependence −E versus logC, defined for low content range, in order to transform potential units into content units. Using such a statistical approach, a remarkable LOD of 1×10^{-3} mg/L of fluoride was calculated. In comparison to other previously reported potentiometric methods, which reported LOD of 10×10^{-3} mg/L [17] and 6.5×10^{-3} mg/L [22], there was evidently better sensitivity of the proposed method. In terms of sensitivity, the defined method demonstrated superiority with respect to flow injection analysis, which is able reliably to detect 38×10^{-3} mg/L [18], as well as over capillary electrophoresis (LOD = 0.17 mg/L) [8].

Limit of quantification was calculated on the basis of $x \pm 10$ SD and was 2.8×10^{-3} mg/L fluoride.

3.5. Accuracy

Taking into account that commercial products are fortified usually with NaF or Na_2FPO_3, accuracy was verified by spiking a non-fluoride product with these two fluoride salts at three different concentration levels. Non-fluoride toothpaste was spiked with 100 mg/kg, 450 mg/kg, and 1450 mg/kg of Na_2FPO_3 and NaF, respectively. Spiked samples were analyzed according to the defined method in triplicate. Good values of mean recovery (96–98%) confirmed the accuracy of the proposed method, as well as the correctness of the suggested sample preparation method for both fluoride sources.

3.6. Fluoride determination in samples

Taking into account fluoride content in samples specified by the manufacturers, calibration curves were defined for a broad concentration range, from 0.5 mg/L to 100 mg/L fluoride. Calibration was repeated three times, yielding a linear dependence ($r = 0.9999$) with good reproducibility, expressed via RSD for the intercept and the slope, 0.04% and 0.019%, respectively. Calculated contents, on the basis of three replicates, are presented in Table 3. According to the determined contents, an equilibration time of 2 minutes was sufficient, because the lowest content of fluoride in the working solution contained 5.95 mg/L fluoride (Sample 10; taking into consideration twofold dilution with TISAB), for which even 1 minute would allow measurement at the equilibration point.

Table 3 – Fluoride contents in the pharmaceutical products for oral hygiene.

Sample	Certified fluoride content	Calculated fluoride content
Toothpaste samples	(mg/kg)	(mg/kg)
1	1450[a]	1479 ± 20[b] (101)[c]
2	1450	987 ± 17 (96)
3	1450	896 ± 9 (98)
4	1450	1100 ± 17 (94)
5	1450	906 ± 10 (98)
6	1450	1345 ± 13 (103)
Mouthwash samples	(mg/L)	(mg/L)
7	1450	1305 ± 11 (99)
8	226	246 ± 5 (97)
9	226	226 ± 4 (101)
10	112	119 ± 3 (99)
11	450	443 ± 5 (103)

[a] The manufacturer did not specify the measurement uncertainty.
[b] Data are presented as mean ± 2 SD, $n = 3$.
[c] Results of the recovery test (%).

Good recovery yields (94–103%), besides confirming the accuracy of the proposed method, also confirmed the correctness of the sample preparation procedure. Discrepancies in specified and calculated contents were observed independently of the fluoride sources. In general, better agreement between the calculated and specified contents was observed in the samples of mouthwash. Samples 1 and 8–11 were relatively in concordance with the specified fluoride contents. Recovery tests were good for all samples, indicating reliable sample preparation, therefore, observations made in this study concluded that more strict control of fluoride content in pharmaceutical products for oral hygiene may be proposed, taking into consideration the toxicity and the specified benefits of fluorides.

One of the advantages of the proposed method was that it adapted sample pretreatment approaches depending on the fluoride source in the dentifrice. In another study that recognized the importance of sample preparation, acidic hydrolysis and diffusion through hexamethyldisiloxane was used to speciate ionic and soluble (ionic plus monofluorophosphate) fluoride species [14]. The authors reported inaccuracy in determination and suggested interchangeable use of direct hydrolysis of fluoride sources and hexamethyldisiloxane diffusion. In addition to marked sensitivity of the proposed method (LOD = 1×10^{-3} mg/L), a linear response with a characteristic Nerstian slope was observed for a broader concentration range (1–1000 mg/L) than for other similar previous studies [18].

In the present work, a potentiometric method for fluoride determination in pharmaceutical products for oral hygiene

was developed, particularly focusing on sample preparation due to different possible sources of fluoride. Analyzed samples encompassed toothpastes and mouthwash. In addition to differences in sample matrix, different fluoride salts were added to the samples as a source of fluoride, namely Na_2FPO_3 or NaF. For both types of sample, the procedure for sample preparation was rapid and simple, but for the samples with Na_2FPO_3, an additional step of acid hydrolysis should have been applied. In aqueous solutions, Na_2FPO_3 dissociates to FPO_3^{2-} ions, which does not produce a signal at the ion-selective membrane, so acid hydrolysis was necessary to liberate fluoride ions from FPO_3^{2-} ions. Calculated sensitivity of the developed method for fluoride determination was 1×10^{-3} mg/L of fluoride. The defined procedures for sample preparation were shown to be reliable, which was indicated by good recovery of the spiked non-fluoride samples (96–98%) as well as analyzed fluoride-containing samples (94–103%). Among 11 analyzed samples, in general, the samples of mouthwash exhibited better agreement with the specified fluoride contents. In a minority of the analyzed samples, calculated contents agreed well with the specified values. Taking into consideration the toxicity and the specified benefits of fluorides, more strict control of fluoride content in pharmaceutical products for oral hygiene is proposed.

Acknowledgments

This work was financially supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia through a national project (project number TR 31014).

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