Dental Journal
Majalah Kedokteran Gigi
Volume 46, Number 3, September 2013

Research Report

Determination of fluoride content in toothpaste using spectrophotometry

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ABSTRACT

Background: *Intake excessive fluoride in children's teeth are generally marked with white and brown patches. Excessive fluoride of more than 4.0 mg/L can cause a person suffering from poisoning, fragility of the bones (osteoporosis), liver and kidney damage. Knowledge about the spectrophotometry for determination method of fluoride content in commercially available toothpaste is very few.* ***Purpose:*** *The purposes of study were to examine the suitable method for fluoride extraction and to determine out the accuracy, precision, linearity, and stability of the measurement method of fluoride content in toothpaste.* ***Methods:*** *The suitable F extraction method was determined by the comparison among 3 methods of extraction; e.g. the dried samples were immersed in (1) distilled water, (2) 96% HCl, and (3) 96% HNO_3; and the validation methods of measurement were the maximum wavelength, standart curve, accuracy test, precision test, and stability test.* ***Results:*** *Result showed that the fluoride extraction by using the concentrated HNO_3 was found to have the highest levels of fluoride, followed by hydrochloric acid dissolution (HCl) and distilled water, while the method of validation showed that SPADNS revealed the acceptable accuracy. Precision has the RSD ≤ 2.00%. Furthermore the stability test result showed that the measurement of fluoride less than 2 hours was still reliable.* ***Conclusion:*** *The study suggested that the best result of fluoride extraction from toothpaste could be gained by using concentrate HNO_3, and the spectrophotometer (UV-Vis Mini Shimadzu U-1240) and SPADNS have the acceptable accuracy.*

Key words: *Spectrophotometry, fluoride content, toothpaste*

ABSTRAK

Latar Belakang: *Pemasukan fluoride yang berlebihan pada gigi anak ditandai dengan bercak putih dan coklat. Fluoride lebih dari 4.0 mg / L dapat menyebabkan seseorang menderita keracunan , kerapuhan tulang (osteoporosis), kerusakan hati dan ginjal. Pengetahuan tentang spektrofotometri untuk metode penentuan kadar fluoride dalam pasta gigi yang tersedia secara komersial sangat sedikit .* ***Tujuan:*** *Penelitian ini bertujuan meneliti metode yang tepat untuk mengektrak kandungan fluoride dan mengukur akurasi, presisi, linearitas dan stabilitas pengukuran kandungan fluoride pada pasta gigi.* ***Metode:*** *Metode ekstrak yang tepat ditentukan dengan membandingkan 3 metode, yaitu dengan perendamam sampel kering dalam (1) air destilasi, (2) HCl 96%, dan (3) HNO_3 96%; dan validasi metode yang memperhitungkan panjang gelombang, kurva standar, tes akurasi, presisi, dan stabilitas.* ***Hasil:*** *Hasil menunjukkan bahwa metode ekstrasi fluoride pada pasta gigi dengan menggunakan metode HNO_3 didapatkan level fluoride tertinggi, diikuti dengan metode HCl dan air destilasi. Hasil validasi metoda menunjukkan bahwa penggunaan SPADNS akurasinya dapat diterima. Presisi mempunyai RSD ≤2,00%. Pada tes stabilitas didapatkan hasil bahwa pengukuran kadar fluorida dalam waktu*

tidak lebih dari 2 jam masih dapat dilakukan. ***Simpulan:*** *Penelitian ini menunjukkan bahwa hasil terbaik ekstraksi fluoride dari pasta gigi dapat diperoleh dengan menggunakan HNO_3, dan spectrophotometer (UV-Vis Mini Shimadzu U-1240) dan SPADNS memiliki pengukuran yang akurat.*

Kata kunci: *Metode spektrofotometrik, kandungan fluorida, pasta gigi*

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INTRODUCTION

The use of toothpaste is a part of tooth brushing which is needed for healthy teeth. Toothpaste makes the teeth cleaner and reduce oral microorganism.[1] Fluoride in toothpaste is one of the substances that needed for healthy teeth. The mechanisms of fluoride in dental caries prevention are; reducing the enamel solubility caused by acid, lowering the enamel surface permeability and inhibiting the fermentation of carbohydrates by microorganisms of the oral cavity.[2] The needs of fluoride is between 0.7 to 0.9 mg/L (parts per million).[3] Therefore, despite the growing controversy, the provision of fluoride in toothpaste should not be exaggerated. This is due to the excess fluoride (fluorosis) can cause cells die and the teeth become brittle.

The degree of dental fluorosis depends on the amount of fluoride exposure up to age of 8 to10 years old. The fact that an adult shows no signs of dental fluorosis does not mean that his or her fluoride intake is within the safety limit.[4] Excessive fluoride in children's teeth are generally marked with white and brown patches. Excessive fluoride of more than 4.0 mg/L can cause a person suffering from poisoning, the fragility of the bones (osteoporosis), liver and kidney damage.[3-7]

To ensure accuracy of fluoride content measurement in toothpaste, the validation of method need to be done. Validation of the method according to United States Pharmacopeia (USP) in Martono[8] is aimed to determine that the analysis method is accurate, specific, reproducible, and hold in the range of analytes to be analyzed. Some parameters according to the USP are accuracy, precision, linearity, and stability.

Accuracy is the closeness between the measured values, which acceptable to the convention, the true value, or value of referrals. Accuracy is measured as the amount of analyte recovered in a measurement by performing spiking on a sample. Accuracy can be obtained by comparing the results of measurements with standard reference materials. International Conference on Harmonisation (ICH) recommends the collection of data from nine times the assay with 3 different concentrations (e.g. 3 concentrations with 3 times replication). Data are reported as percent recovery (% recovery).

Precision is a measurement of repeatability analysis method, and is usually expressed as relative standard deviation of statistically different samples. There are three levels of precision, i.e. repeatability, between the precision (intermediate precision), and reproducibility. The precision are include: Standard Deviation, Relative Standard Deviation (RSD) or Coefficient of Variation (CV), and the range of beliefs. Data for precision test is often collected as part of other studies related to precision, linearity or accuracy. Usually 6 to 15 replications are done for a single sample of each concentration. In testing, the value of RSD is 1-2% for the active compounds in large quantities, while for compounds with a little amount, RSD ranged between 1-5%.[8-10]

Linearity is the ability to obtain test result which is proportional to the concentration of analyte in a given range. Linearity is measurement of how well the calibration curve method connects between the response (y) with concentration (x). Linearity can be measured by performing a single measurement at different concentrations. The data obtained is processed by the method of least squares, and then determined the value of the slope (slope), intercept, and correlation coefficient (r).[10]

To obtain the reproducible and reliable test results, the sample, reagents and raw materials used should be stable at a certain time. The stability of all solutions and reagents is very important, either in relation to temperature or in respect of time. If the solution is not stable at room temperature, the temperature should be decreased 2-8°C to increase the stability of samples and standards.

The purposes of study were to examine the suitable method for the fluoride extraction and to determine the accuracy, precision, linierity, and stability of the method concerning to the measurement of fluoride content in toothpaste.

MATERIALS AND METHODS

Determination of maximum wavelength was done by mixing 5 mL of distilled water with 1 mL of SPADNS reagent, then performed using a scanning spectrophotometer (UV-VIS Mini Shimadzu U-1240). Measurements were taken at region of 350-700 nm wavelength. Ten series of fluoride standard solutions levels are: 0.2 mg/L; 0.4 mg/L; 0.6 mg/L; 0.8 mg/L; 1 mg/L; 1.2 mg/L; 1.4 mg/L; 1.6 mg/L; 1.8 mg/L and 2 mg/L respectively (each made in a volume of 10 ml) was prepared for configuration of standard curve. From each level taken 5 mL of solution

was then added 1mL of reagent SPADNS into each test tube, stirred until homogeneous and then incubated for 5 min at room temperature. The absorbance of each solution was measured with a spectrophotometer (UV-VIS Mini Shimadzu U-1240) at a wavelength of 550 nm (maximum wavelength after scanning).[8,10]

Accuracy and precision was obtained with standard solutions of fluoride levels of 0.4 mg/L, 1 mg/L and 1.8 mg/L respectively which is made from a standard solution of fluoride of 2 mg/L. From each level taken 5 mL of solution was then added 1 mL of reagent SPADNS into each test tube, stirred until homogeneous and then incubated for 5 min at room temperature. The absorbance of each solution was measured with a spectrophotometer (Shimadzu UV-VIS Mini U-1240) at a wavelength of 550 nm (maximum wavelength after scanning). Assay performed nine times, including three kinds of levels, each of 3 replications.[8,10]

Stability of reagent SPADNS was done by comparing volume of reagent at 0.5 mL and 1 mL within 5 mL of standard fluoride solution in three different levels of 0.4 mg/L, 1 mg/L, and 1.8 mg/L respectively and then measured with a spectrophotometer (UV-VIS Mini Shimadzu U-1240) at a wavelength of 550 nm. SPADNS reagent stability is determined by mixing 5 ml of distilled water with 1 mL of reagent SPADNS. Determination of wavelength and absorbance of the mixture was done by using a spectrophotometer (UV-VIS Mini Shimadzu U-1240). Measurements were made every 5 minutes in a span of 120 minutes and on the wavelength region of 350-700 nm.[11]

Six adults toothpastes and 3 children toothpastes which commercially available, were dried in order to make comparison between the method used in the comparison of Fluoride extraction by putting the samples in an oven at ±105^{o} C temperature until constant mass was obtained while cooling in the desicator. Furthermore as a first step, the optimization method performed by a comparison of three methods of sample dissolution.[12]

There were three methods used, in method 1**:** the dried samples (1 g) was immersed in 50 mL of distilled water for 24 hours in a porcelain dish. The mixture was filtered and put in a 100 mL volumetric flask, then add distilled water until the calibration line (tera-line fulfillment to be done by washing the residue). Prior to the measurement of fluoride levels, first centrifuge solution at a speed of 3000 rpm for 10 minutes.

Method 2: the dried samples (1 g) was included in a porcelain dish and immersed in 5 mL of concentrated HCl (96%) for 24 hours in the fumehood. After that, heated ± 2 hours at a temperature of 50ºC using a hot plate in the fumehood. Once heated, add another 5 mL of concentrated HCl and heating was continued until no white gas formed. The solution was then cooled, then filtered and put in a 100 mL volumetric flask, then add distilled water until the calibration line.

Method 3: the dried samples (1 g) was included in a porcelain dish and immersed in 5 mL of concentrated HNO_3 (96%) for 24 hours in the fumehood. After that, heated ± 2 hours at a temperature of 50ºC using a hot plate in the fumehood. Once heated, add another 5 mL of concentrated HNO_3 and a few drops of H_2O_2. Heating is continued until no more gas to form nitrogen oxides and the resulting brown solution nodes. The solution was cooled, then filtered and the filtrate was added in 100 ml flask, then add distilled water until the calibration line.

Table 1. The comparative analysis of fluoride method

Sample code	Fluoride level (mg/L)								
	Method 1			Method 2			Method 3		
	1	2	3	1	2	3	1	2	3
X	0.85	0.853	0.85	0.803	0.80	0.80	0.91	0.913	0.91
Y	0.713	0.71	0.716	0.807	0.807	0.81	0.857	0.85	0.85
Z	0.81	0.81	0.813	0.87	0.87	0.87	0.907	0.903	0.893

Table 2. The accuracy of fluoride standard solution based on the measurement of absorbance at λ 550 nm

Concentration of fluoride standard solution (mg/L)	A_{550} (x 100)			Average A_{550}	Measurable levels of fluoride (mg/L)	Recovery (%)	Average of recovery (%)
	I	II	III				
0.4	181.9	181.9	181.9	181.9	0.4286	107.15	99.84
1.0	181.2	181.2	181.2	181.2	0.8286	82.86	
1.8	179.2	179.2	179.2	179.2	1.9714	109.52	

Table 3. Repeatability of fluoride standard solution based on the measurement of absorbance at λ 550 nm

standard levels of fluoride (mg/L)	repeatation	A550 (x 100)	measurable levels of fluoride (mg/L)
0.4	1	181.9	0.4286
	2	181.9	0.4286
	3	181.9	0.4286
	average	181.9	0.4286
	SD	0	0
	RSD (%)	0	0
1	1	181.2	0.8286
	2	181.2	0.8286
	3	181.2	0.8286
	average	181.2	0.8286
	SD	0	0
	RSD (%)	0	0
1.8	1	179.2	1.9714
	2	179.2	1.9714
	3	179.2	1.9714
	average	179.2	1.9714
	SD	0	0
	RSD (%)	0	0

Table 4. Stability of SPADNS reagent

t (minutes)	λ (nm)	absorbance	t (minutes)	λ (nm)	absorbance
0	549	1.843	65	548	1.830
5	549	1.834	70	549	1.827
10	547	1.837	75	549	1.827
15	548	1.840	80	548	1.836
20	551	1.839	85	550	1.832
25	549	1.842	90	550	1.839
30	548	1.832	95	548	1.828
35	548	1.831	100	550	1.830
40	550	1.836	105	548	1.836
45	549	1.832	110	549	1.833
50	550	1.836	115	548	1.834
55	547	1.825	120	550	1.837
60	548	1.838			

RESULTS

The comparison of three extraction methods of samples could be seen in Table 1. Accuracy was expressed as percent recovery of analyte added.[13] (Table 2). Precision was determined by measuring the spread of individual results from the average, if the procedure was applied repeatedly in samples which were taken from a homogeneous mixture.[13] (Table 3). The stability of the volume of reagents was very important thing (Table 4).

DISCCUSION

All three methods of sample extraction were compared, and the result showed extraction by using concentrated HNO_3 was found to have the highest levels of fluoride, followed by hydrochloric acid dissolution (HCl) and distilled water. This result is supported by Oyewale[12] which states that the use of HNO_3 extraction gave the optimum extraction for all inorganic parameters or components of all different toothpastes.

The component of toothpastes are combined of calcium carbonate and phosphates, which have low solubility in water. Acidic condition is often required to release some of components into water soluble form. This may partly account for the acidic nature of some of the toothpaste samples. Furthermore Oyewale[12] stated that the fluoride content is all essentially available even in aqueous medium since it is often incorporated in toothpastes as sodium salts, which are generally soluble in water. Based on this experiment, the F extraction from toothpaste can be determined using concentrated HNO_3.

An important point related to validation method is selection of the maximum wavelength which in turn will be used for the manufacture of standard curves. The results showed that the wavelength of maximum absorption was at a wavelength of 550 nm. Validation method was done using reagents SPADNS and an instrument of spectrophotometer (UV-VIS Mini Shimadzu U-1240). SPADNS reagent is a mixture of solution SPADNS [Sodium 2 - (Para-sulfophenylazo) -1, 8-dihydroxy-3, 6-naphtalene disulfonate] with a solution of zirconyl chloride octahydrate, $ZrOCl_2.8H_2O$, in acidic conditions.[13,14]

Determination of fluoride was done by using spectrophotometry method and it based on metal displacement from a colored complex or the formation of a mixed-ligand complex, Zr(IV)-F-SPADNS. Fluoride addition will bleach SPADNS-Zirconyl chloride and degrade the red colored complex. The degree of bleaching was determined with a spectrophotometer, and the concentration of fluoride ions was assessed by comparison with standard solutions. The color loss was measured at wavelength on maximum absorbtion of mixed-ligand complex.[14] The reaction is as follows :

$$\underset{\text{(red)}}{\text{Zr-SPADNS}} + 6F^- \xrightarrow{H^+} \text{SPADNS} + \underset{\text{(colorless)}}{ZrF_6^{2-}} + nH_2O$$

This is consistent with the theory SPADNS method that the use of wavelength will be in the region of 550-580 nm wavelength. In addition, the use of 550 nm wavelength on a spectrophotometer supported by theory which stated that the red light located on the complementary wavelength region of 490-560 nm.[14] The red color observed (as seen by the eye) is the color of the reagent SPADNS. As an indication of the content of fluoride, the red color of the reagent SPADNS will be degraded, and if the higher fluoride content, the red color will fade (degradation increases) so that the smaller absorbance.[15] The results of absorbance measurements of fluoride standard solution at various levels in the manufacturing of standard curves. The results of the linear regression relationship is created from "y = bx + a", where "y" is the response (absorbance), "b" is the slope and "a" is the intercept. Equation of the regression line (content vs absorbance) and then "y = - 1.75 x + 182.6" with r = 1. Standard curve was made to give a perfect correlation coefficient (r = 1).

To examine the accuracy of the developed methods from the experiments, standard addition method was carried out, then the percentage recovery was calcutated.[16] Accuracy can be determined by the assay minimum of 9 times, covering a certain range e.g. 3 different levels, each of 3 replications. The average percent recovery were in the range of 98-102% [10] as seen in Table 2. The study showed that the spectrophotometer instrument (UV-VIS Mini Shimadzu U-1240) and methods developed SPADNS have acceptable accuracy.[8,14]

The test precision was conducted on three kinds of different levels with 3 repetitions for each target compound. The low Relative Standard Deviation (RSD) values indicates precision of the method. In majority, the determinations were below 2%, indicating high degree of agreement (repeatability) between experimental values.[17] Determination of repeatability can be done with a minimum of 6 times determination levels of 100%, or 9 times in the range of levels with 3 different levels, each repeated a number of 3 times. According to Ermer and Miller [10] it may be accepted if the repeatability RSD Ý 2.00% of the test. The repeatability of fluoride standard solution based on the measurement of absorbance at λ 550 nm, the three standard levels of fluoride (mg/L) and measurable levels of fluoride (mg/L) gave the RSD of 0%. This shows that the repeatability with RSD Ý 2.00% of the test is acceptable.[17]

The stability of all solutions and reagents is very important, whether in relation to temperature or in respect of time.[8] Comparison the stability of SPADNS reagent volume based on absorbance at λ 550 nm showed that the absorbance was stable, when the volume reached SPADNS reagent was added to the standard solution of 1 mL. In contrast the use of 0.5 mL of reagent gave absorbance measurement results of an unstable (fluctuating). The possibility of this result was because the amount of SPADNS reagent still not enough to indicate the amount of fluoride (levels) contained in the standard solution. Therefore, the determination of fluoride levels in this study was carried out by applying the ratio of the volume of the sample with a SPADNS reagent by 5:1 (v: v). The results showed that up to 120 minutes, a mixture of sample and SPADNS reagent continued to show stable and unaffected by environmental factors, especially from the air. This is according to US Environmental Protection Agency (USEPA)[19] which also states that during 2 hours of color formed from the sample and SPADNS reagent will remain stable. This means that measuring the levels of fluoride in the range of no more than 2 hours is still reliable.

The study suggested that the best result of fluoride extraction from toothpaste could be gained by using concentrate HNO_3, and the validation test results showed that the spectrophotometer (UV-Vis Mini Shimadzu U-1240) and SPADNS have the acceptable accuracy. Precision has RSD Ý 2.00%. The stability test results revealed that the measurement of fluoride in the range of less than 2 hours is still reliable.

REFERENCES

1. Andriewongso. Pasta gigi. (on line) http://www.andriewongso.com/awartikel-1984-Tahukah_Anda-Pasta_Gigi. 2008. Accessed March 20, 2009.
2. Arnold W, Dorow A, Langenhorst S, Gintner Z, Bánóczy J, Gaengler P. Effect of fluoride toothpastes on enamel demineralization. BMC Oral Health 2006; 6: 8.
3. Meenakshi, Maheshwari RC. Fluoride in drinking water and its removal. J Hazard Mater 2006; 137(1): 456-63.
4. Oxford Instruments Molecular Biotools Limited. Determination of fluoride content in toothpaste**.** USA: http://www.oxford-instruments.com/OxfordInstruments/media/industrial-analysis/magnetic-resonance-pdfs/Determination-of-Fluoride-Content-in-Toothpaste.pdf 2008. p. 1-2.
5. Darmawan L. Cara cepat membuat gigi sehat dan cantik dengan dental cosmetics. Jakarta: Gramedia Pustaka Utama; 2007. p.144.
6. Moghaddam AA, Fijani E. Distribution of fluoride in groundwater of Maku area, northwest of Iran. Environmental Geology 2008; 56(2): 281-7.
7. Msonda KWM, Masamba WRL, Fabiano E. A study of fluoride groundwater occurrence in Nathenje, Lilongwe, Malawi. Physics and Chemistry of the Earth 2007; 32: 1178-84.
8. Martono Y. Validasi metoda kromatografi cair kinerja tinggi isokratik untuk penetapan kadar asam galat, kafein dan epigalokatekin galat pada berbagai produk teh celup. Thesis. Yogyakarta: Program Studi Ilmu Farmasi, Fakultas Farmasi Universitas Gadjah Mada; 2009.
9. Gujarathi SC, Shah AR, Jagdale SC, Datar PA, Choudari VP, Bhanudas SK. Spectrophotometric simultaneous determination of Aspirin and Ticlopidine in combined tablet dosage form by first order derivative spectroscopy, AUC and ratio derivative spectrophotometric methods. Int J Pharm Sci Review and Research 2010; 3(1): 115-9.
10. Ermer J, Miller JH. Method validation in pharmaceutical analysis. A guide to best practice. Weinheim: WILEY-VCH Verlag GmBH & Co.KgaA; 2005. p. 4-22.
11. Gandjar IG, Rohman A. Kimia farmasi analisis. Yogyakarta: Pustaka Pelajar; 2007.
12. Oyewale AO. Estimation of the essential inorganic consituents of commercial toothpaste. Scientific & Industrial Research 2005; 64: 101-7.
13. Harmita. Petunjuk pelaksanaan validasi metoda dan cara perhitungannya. Majalah Ilmu Kefarmasian 2004; 1(3): 117-35.
14. Battaleb-Looie S, Moore F. A study of fluoride groundwater occurrence in Posht-e-Kooh-e-Dashtestan, South of Iran. World Applied Sci J 2010; 8(11): 1317-21.
15. HACH Company. 2006. Fluoride for water and seawater SPADNS, SPADNS 2 and ion-selective electrode methods. (on line) http://www.hach.com/fmmimghach?/CODE%3AEX_FLUORIDE15801/1. Accessed September 15, 2013.
16. Sharma MC, Sharma S. Validated simultaneous spectrophotometric estimation of Paroxetine HCl bulk and tablet dosage form using ferric chloride. J Opteoelectronics and Biomedical Materials 2010; 2(4): 185-9.
17. Garcia PL, Santoro MIRM, Singh AK, Kedor-Hackmann ERM. Determination of optimum wavelength and derivative order in spectrophotometry for quantitation of Hydroquinone in creams. Brazilian J Pharm Sci 2007; 43: 397- 404.
18. Sharma R, Pervez S. Study of dental fluorosis in subjects related to a phosphatic fertlizer plant environment in Chhattisgarh State. Scientific & Industrial Research 2004; 63: 985-88.
19. US Environmental Protection Agency. Method 13A - Determination of total fluoride emissions from stationary sources. SPADNS ZIRCONIUM LAKE METHOD. (on line) http://www.epa.gov/ttn/emc/promgate/m-13a. Accessed September 16,2013.