Determination of Sodium Fluoride in Dental Mouth Wash and Oral Multivitamin Formula Using RP-HPLC Precolumn Derivatization Reaction

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***Abstract*-An accurate, selective and sensitive reversed phase-HPLC method has been developed and validated for selective determination of sodium fluoride in dental mouth wash and oral multivitamin formula. The method was based on a precolumn derivatization reaction of sodium fluoride with triphenyl silyl hydroxide in acidic conditions then the product (triphenyl silyl fluoride) was extracted into n-heptane. Limit of quantification and limit of detection for sodium fluoride were found to be 1.07 and 0.35 µg/ml; respectively. A Zorbax® eclipse HC-C18 column (250 mm x 4.6 mm x 5 µm) was used for separation with acetonitrile: water (75:25, v/v) as the mobile phase (flow rate 0.5 ml/min), temperature was adjusted at 40˚C with detection wavelength of 222nm. The retention time was found to be about 8.6 min. The method exhibited a wide linear range of response from 1-300µg/ml with a correlation coefficient of 0.9998 and percentage recovery of 99.50 ± 0.792. Recoveries in dosage forms were found to be 99.33± 0.818 and 98.76 ± 0.930 for dental mouth wash and oral multivitamin formula; respectively. The developed analytical method has been validated for selectivity, linearity, precision, accuracy, and robustness according to ICH guidelines. The developed method could be successfully employed for routine analysis of fluoride ion in pharmaceutical formulations as well as in a wide range of matrices due to high selectivity of the method.**

***Keywords*: fluoride, precolumn, derivatization, selective, RP-HPLC**

I. INTRODUCTION

Over the past few decades, tooth decay has been reduced dramatically. The key reason is fluoride. Fluoride decreases cavities in children and adults. It also aids to fix the first stages of teeth decay. Fluoride is like any other nutrient; it is safe and effective when used appropriately [1].

Fluoride is effective in avoiding and treating the first stages of dental caries (teeth decay). Researchers have shown that there are several ways through which fluoride achieves its decay-protective effects. It renders the tooth structure stronger, so teeth are less susceptible to acid attacks. Repeated acid attacks decompose the teeth, which causes cavities. Fluoride also acts to fix, or remineralize, areas in which acid attacks have already started. The remineralization effect of fluoride is important because it reverses the first stages of decay process as well as creating a tooth surface that is less susceptible to decay.

Fluoride is obtained in two forms: topical and systemic. Topical fluorides reinforce teeth present in the mouth rendering them more decay-resistant. Topical fluorides include toothpastes, mouth washes and applied fluoride therapies [1].

Systemic fluorides are those that are ingested into the body and become involved into forming teeth structures. Systemic fluorides can also give topical prophylaxis because fluoride is present in saliva, which continually washes the teeth. Systemic fluorides include water fluoridation or dietary fluoride supplements in the form of tablets, drops or lozenges[1].

It is well known that fluoridation of the water supply (1 ppm fluoride) during the period of teeth development leads to a great decrease in the incidence of dental caries. Hydroxyapatite is the main crystal for all calcified tissues in the human body. The fluoride ion reacts with the hydroxyapatite in the teeth to develop the more caries-resistant crystal, fluorapatite. The reaction may be expressed by the equation [2]

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \text{------} Ca_{10}(PO_4)6F_2 + 2OH^-$$

(Hydroxyapatite) (Fluorapatite)

Three stages of fluoride deposition in teeth enamel can be distinguished [2]:

Small quantities (reflecting the low levels of fluoride in tissue fluids) are incorporated into the enamel crystals while they are being formed. After enamel has been set down, fluoride deposition continues in the surface enamel. Diffusion of fluoride from the surface inward is apparently prevented. After eruption, the surface enamel takes fluoride from water, food, supplementary fluoride and smaller quantities from saliva.

The American Academy of Pediatrics recommends that children up to age 16, in regions, where drinking water contains less than optimal levels of fluoride, receive daily fluoride supplementation.

Multivitamin Fluoride drops supply fluoride in drop form for infants and young children 6 months to 6 years of age. Each 1.0 ml supplies sodium fluoride (0.25 mg fluoride) in addition to nine essential vitamins.

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The American Academy of Pediatrics [3] and the American Dental Association [4] currently recommend that infants and young children 6 months to 3 years of age, in regions where drinking water contains less than 0.3 ppm of fluoride, and children 3-6, in areas where the drinking water contains 0.3 through 0.6 ppm of fluoride, take 0.25 mg of supplemental fluoride daily which is supplied in a full dose (1 ml) of Multivitamin Fluoride (0.25 mg) drops.

Several methods have been reported in the literature for the determination of fluoride in pharmaceutical formulations and dental preparations, such as spectrophotometric methods [5] and capillary electrophoresis [6,7]. Among the methods for fluoride determination are potentiometric methods, such as fluoride ion selective electrode [8] and flow system [9].

Ion chromatography is also described [10,11,12] providing the possibility of determining several ions in the same sample. An alternative is the use of gas chromatography (GC) [13, 14], which is beneficial for total fluoride determination.

Considering common use of HPLC-UV instrumentation in analytical laboratories, we developed a RP-HPLC method for fluoride determination. The foundation of the method is a reaction originally developed for the determination of fluoride by GC, based on the derivatization of fluoride with hydroxysilane to give a corresponding fluorosilane.

$$R_3SiOH+H^++F^- \text{ ------- } R_3SiF+ H2O$$

Where R is the organic group.

Following the concept of the GC method, we have developed a RP-HPLC method with UV detection, using triphenyl silyl hydroxide as the derivatizing agent [15].

The goal of the present paper is to test the applicability of the optimized method for the selective and accurate determination of sodium fluoride in dental mouth wash and oral multivitamin formula. The results were compared with those obtained by ion selective electrode reported method [16].

II. MATERIALS AND METHODS

A. *Materials*

- *Pure Standards*

Sodium fluoride (99.1%) was obtained from Koch-Light Laboratories Ltd (Colnbrook Bucks, England). Dentiplus® mouth wash and Pediavit® oral drops were purchased from the market.

- *Chemicals and reagents*

All chemicals used in the experiment were of analytical purity grade. Solvents used: n-heptane, HPLC water and acetonitrile were obtained from Sigma-Aldrich (Steinheim, Germany). Silanes used in fluoride derivatization were the derivatizing agent – triphenyl silyl hydroxide (98%), obtained from Sigma-Aldrich (Steinheim, Germany).Mineral acid used in the derivatization procedure: perchloric acid (70%) was obtained from POCh.

B. *Instrumentation*

Waters Alliance e2695 HPLC binary pump (Waters Technology, Milford, MA) equipped with an in-line vacuum degassing auto-sampler with capacity for 120 vials with programmable temperature control, heated column compartment and photodiode array detector (model 2998). All components of the HPLC system were controllable through the Empower 3 chromatography data software. Zorbax® eclipse HC-C18 column (250 mm x 4.6 mm x 5 µm) was used for analytical separation. Sonicator (Crest ultrasonics, scotch road. Mercer, country airport, Trenton, NJ 08628). Vortex type 16700 was obtained from Thermolyne (Thermo).

C. *Procedures*

Optimized procedure of fluoride derivatization was as follows: 0.02, 0.4, 1.8, 3.5 and 6 ml of aqueous fluoride solution of 100 µg/ml (water in case of blank) was placed into a set of 15mL falcon test tubes, mixed with 6 ml of concentrated perchloric acid and 0.15 ml of 0.5 mmol of triphenyl silyl hydroxide. Then, 2 ml of n-heptane was added to the mixture and the tube was vigorously agitated using vortex for approximately 50 min at room temperature. Finally, the 2 ml n-heptane layer was aspirated and subjected to HPLC measurement.

Optimized HPLC method included the use of a Zorbax® eclipse HC-C18, and isocratic elution with acetonitrile/H2O (75:25, v/v) at 0.5 ml/min. All measurements were performed at 40˚C. Injection volumes of 10 µl and detection wavelength of 222 nm were used. Quantification was carried out using peak area.

Pharmaceutical formulations samples (80µg/ml) were prepared by transferring 711 µl and 640 µl of each of Dentiplus® mouth wash (0.225 mg/ml) and Pediavit® oral drops (0.25 mg/ml); respectively, into a 15 ml falcon tube, mixed with 6 ml of concentrated perchloric acid and 0.15 ml of 0.5 mmol of triphenyl silyl hydroxide. Then, 2 ml of n-heptane was added to the mixture and the tube was vigorously agitated using vortex for approximately 50 min at room temperature. Finally, the 2 ml n- heptane layer was aspirated and subjected to HPLC measurement.

III. RESULTS

A. *Optimization of chromatographic parameters*

Development of the RP-HPLC method for fluoride determination required selection or optimization of following parameters: (i) proper solvent for product of fluoride derivatization (triphenyl silyl fluoride), compatible with both derivatization procedure and the chromatographic conditions; (ii) mobile phase composition and elution program, allowing resolution of analyte (triphenyl silyl fluoride) from derivatizing reagent (triphenyl silyl fluoride) and any potential impurities; (iii) UV detection parameters

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and the injection volume, providing highest sensitivity and selectivity.

Water-immiscible solvent is beneficial considering it is an easy way to avoid high sample acidity required for the derivatization [15]. n-heptane was used as the sample solvent.

Satisfactory separation of triphenyl silyl fluoride was achieved in less than 10 min using isocratic elution. By applying the optimized procedure, a calibration curve in the concentration range 1.00–300.00 µg/ml of triphenyl silyl fluoride in n-heptane was constructed. The calibration was linear over the tested concentration range with correlation coefficient value of 0.9998.

In order to select a suitable mobile phase allowing separation of analyte (triphenyl silyl fluoride) from any potential impurities, several trials were done , taking in consideration, the system suitability parameters, retention time, tailing factor, number of theoretical plates and HETP and the most optimum mobile phase was selected.

Initially acetonitrile and water in different proportions were tried. Different flow rates and column temperatures were tried. The final method, achieving the best separation and the least run time, was optimized with the following conditions: The mobile phase was: acetonitrile: water (75:25, v/v). A Zorbax® eclipse HC-C18 (250 mm × 4.6 mm, 5µm particle size) was used for chromatographic separation. The flow rate was adjusted to 0.5 ml/min and the column oven was adjusted at 40°C. Isocratic elution is simpler than gradient elution in application. The injection volume was set to 10µl and the photodiode array detector was adjusted at 222 nm. The average retention time ± SD for 3 replicate injections was found to 8.6 ± 0.4.

B. Method Application

The next step after method optimization is its application to determine sodium fluoride in its pure form, in mouth wash and oral multivitamin formula. The method showed good linearity when the peak area was plotted against sodium fluoride concentration in the range of 1.00-300.00 µg/ml. The computed regression equation was found to be:

$$A = 45075C - 150776 \quad r = 0.9998$$

Where A is the peak area, C is the concentration in µg/ml and r is the correlation coefficient. Calibration curve parameters are given in Table 1.

Table 1: Analytical Parameters of the Proposed RP- HPLC Method for Determination of Naf In Pure Form:

Parameter	Value
Range (µg/ml)	1.00-300.00
Slope	45075
Intercept	-150776
Correlation coefficient	0.9998
Accuracy (mean ± S.D)	99.21± 0.419
Repeatability[a] (SD_r)	1.126
Intermediate precision [b] (SD_{int})	0.986
LOD	0.35
LOQ	1.07
Robustness	0.785

[a] *The intraday (n=9) average of three different concentrations (1,10,50 µg/ml) repeated three times within 1 day,* [b] *the inter-day (n=9) average of three different concentrations (1,10,50 µg/ml) repeated three times in 3 successive days.*

Also, results of recovery studies were found to be acceptable at all tested levels, proving the high accuracy of the proposed method, (Table 2, 3&4).

Table 2: Accuracy Results for the Determination of Naf in Pure Form by the Proposed Method:

Taken (µg/ml)	Found (µg/ml)	%Recovery*
5.00	4.95	99.00
10.00	9.99	99.90
30.00	29.72	99.07
50.00	49.63	99.26
200.00	197.61	98.81
Mean ± S.D	99.21± 0.419	

**Average of three determinations*

Table 3: Determination Of Naf In Dentiplus® Mouth Wash And Application Of Standard Addition Technique To The Assessment Of Naf In Dentiplus® Mouth Wash By The Proposed HPLC Method:

Dosage form	Taken (µg/ml)	Found (µg/ml)	%Recovery*	Pure added (µg/ml)	Pure found (µg/ml)	%Recovery*
Dentiplus® mouth wash	80.00	80.56	100.70	10.00	9.84	98.40
				20.00	19.93	99.65

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				50.00	49.97	99.94
	Mean ± S.D			99.33 ± 0.818		

*Average of three determinations

Table 4: Determination of NaF in Pediavit® oral drops and application of standard addition technique to the assessment of NaF in Pediavit® oral drops by the proposed HPLC method:

Dosage form	Taken (µg/ml)	Found (µg/ml)	%Recovery*	Pure added (µg/ml)	Pure found (µg/ml)	%Recovery*
Pediavit® oral drops	80.00	78.84	98.55	10.00	9.88	98.80
				20.00	19.62	98.10
				30.00	29.47	98.23
				40.00	40.14	100.35
				50.00	49.15	98.30
	Mean ± S.D			98.76 ± 0.930		

*Average of three determinations

The proposed method was applicable for the determination of NaF in Dentiplus® mouth wash and Pediavit® oral drops where it revealed good percentage recoveries (Table 3&4), as shown in Fig. 3&4.

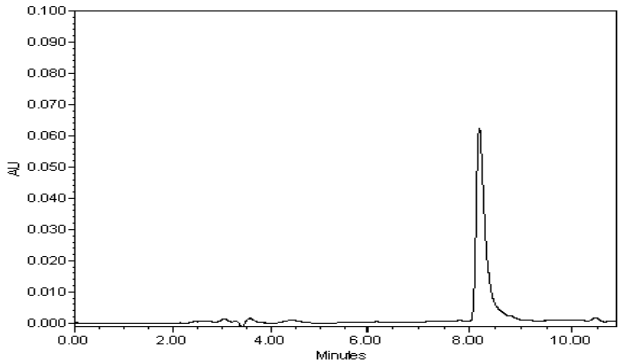

Figure 1: HPLC Chromatogram of Pediavit® oral drops (80µg/ml) at λ222 nm.

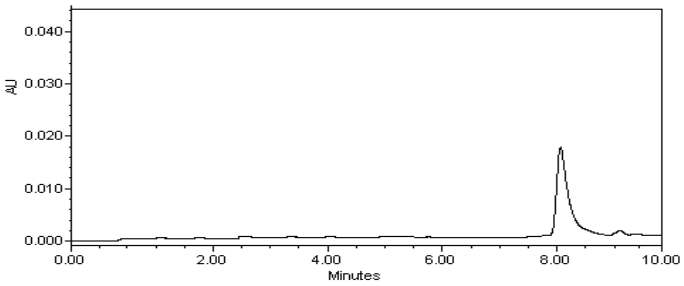

Figure 2: HPLC Chromatogram of Dentiplus® mouth wash (80µg/ml) at λ222 nm.

Results obtained by applying the proposed RP-HPLC method for the determination of sodium fluoride in bulk powder were statistically compared with those obtained by the reported ion-selective electrode method [16] using student's t-test and variance ratio F-test, revealing no significant difference between the proposed and reported methods regarding accuracy and precision,Table 5.

Table 5: Statistical comparison between the results obtained by applying the proposed method and the official method [16] for the analysis of NaF in pure form:

Parameter	The proposed method	Reported method [a]
Mean	99.21	100.55
SD	0.419	0.950
Variance	0.176	0.903
N	5	5
t (2.78) *	1.68	--
F(6.39)*	5.14	--

*The values between parentheses are the corresponding theortical values of **t** and **F** at the 95% confidence level.

[a] Ion selective electrode for fluoride [16].

C. *Method validation*

- *Linearity*

The linearity of the established method was estimated, and the linear regression data for the calibration curve of sodium fluoride showed good linearity (r=0.9998) over the concentration range of 1.00-300.00 µg/ml relative to peak area (Table 1)

- *Accuracy*

The accuracy was calculated as the % recovery of pure sodium fluoride and was found to be 99.21 as shown in **Error! Reference source not found.**. Moreover, when the developed method was applied for quantification of sodium fluoride in Dentiplus® mouth wash and Pediavit® oral drops, good mean % recovery was obtained and the results obtained are listed in Table 3&4.

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- *Precision*

The precision of the proposed method was checked by testing intraday (repeatability) and interday (intermediate precision) variations, and it was represented in terms of standard deviation. The obtained values of S.D. (Table 1) confirmed the high precision of the proposed method.

- *Limits of detection and quantification*

Limit of detection was found to be 0.35, whereas limit of quantification was found to be 1.07. This showed the adequate sensitivity of the proposed method (Table 1).

- *Selectivity of analytical method*

The selectivity of the proposed method was assessed by its ability to resolve (triphenyl silyl fluoride) from any potential impurities as shown in Fig. 1&2. The results revealed that the developed method was able to completely discriminate sodium fluoride from other components in dosage form, confirming the selectivity of the method. Moreover, acceptable results were obtained upon applying the method to dosage forms (Table 3&4) to assess the method's selectivity, confirming there is no interference between other components in dosage form.

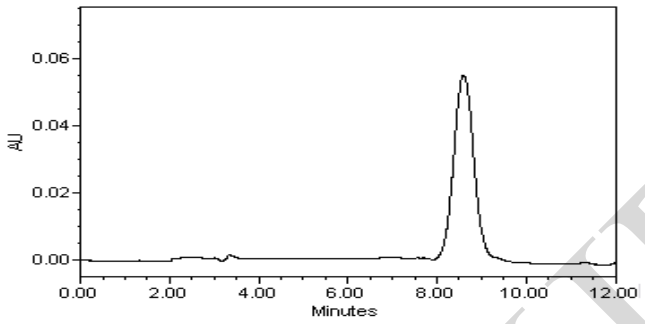

Fig. 3: HPLC Chromatogram showing triphenyl silyl fluoride 50.00µg/ml in pure form at λ222 nm

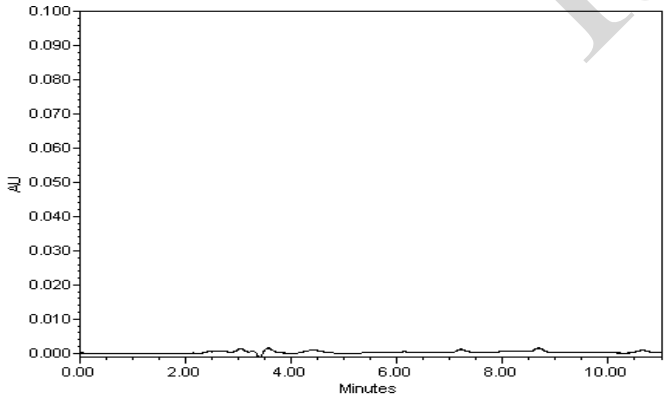

Fig. 4: HPLC Chromatogram of blank at λ222 nm

Table 6: System Suitability Testing Parameters of the Developed RP-HPLC Method:

Parameter	Value
Capacity factor (K')	3.50
Tailing factor (T)	1.00
Number of theoretical plates (N)	766.86
HETP*	0.33

* *Height equivalent to theoretical plate (in mm)*

- *Robustness*

The robustness was carried out by changing flow rate and reinjection of 50 µg/ml sample again. Standard deviation was calculated. The low value of S.D. (Table 1) indicates the robustness of the method.

- *System Suitability Test*

System suitability testing was done during method development and validation. The capacity factor was found to be within the range of 0.8 < k'> 10. Other parameters such as number of theoretical plates and height equivalent to theoretical plates were calculated, and their values were within the acceptable limits (Table 6).

IV. DISCUSSION

The proposed RP-HPLC method offered selective determination of sodium fluoride, and its application to dental mouth wash and oral multi-vitamin formula. The analytical method was developed and validated according to ICH guidelines. Analytical variable parameters such as selectivity, linearity, precision, accuracy (per cent recovery), and system suitability were tested using the above mentioned chromatographic conditions and instruments.

V. CONCLUSION

As a conclusion; the developed method offered distinct advantage in selectivity and sensitivity and could be easily used in quality control laboratory for the analysis of sodium fluoride in its bulk powder and dosage forms. The proposed method could be used for daily analysis of sodium fluoride in Dentiplus® mouth wash and Pediavit® oral drops. It is the first developed pre-column derivatization reaction RP-HPLC method for selective determination of sodium fluoride in dental mouth wash and oral multi-vitamin formula. The short chromatographic run time of only 10 minutes makes this method suitable for processing many samples in a limited time interval, which is very crucial in quality control and daily analysis of the drug.

REFERENCES

[1] http://www.ada.org/en/member-center/oral-health-topics/fluoride-supplements(Accessed on 16 November, 2016).
[2] Brudevoid F, McCann HG: Fluoride and caries control - Mechanism of action, in Nizel AE (ed): The Science of Nutrition and its Application in Clinical Dentistry. Philadelphia, WB Saunders Co, pp.331-347, 1966.
[3] American Academy of Pediatrics Committee on Nutrition: Fluoride supplementation, Pediatrics, vol. 77, pp.758-761, 1986.

IJRS

[4] American Dental Association Council on Dental Therapeutics: Accepted Dental Therapeutics, ed 38, Chicago, pp.321-326, 1979.
[5] R. Sandulescu, E. Florean, L. Roman, S. Mirel, R. Oprean, and P. Suciu, "Spectrophotometric determination of fluoride in dosage forms and dental preparations", Journal of Pharmaceutical and Biomedical Analysis, vol. 14, pp.951-958, 1996.
[6] T. Wang, and S.F.Y. Li, "Development of a capillary electrophoresis method with indirect photometric detection for the determination of anions related to anticaries", Journal of Chromatography A, vol. 781, pp.457-466, 1997.
[7] P. Wang, S.F.Y. Li, and H.K. Lee, "Simultaneous determination of monofluorophosphate and fluoride in toothpaste by capillary electrophoresis", Journal of Chromatography A, vol. 765, pp.353-359.
[8] T. Atin et al. "Deposition of fluoride on enamel surfaces released from varnishes is limited to vicinity of fluoridation site", Clin Oral Invest, vol. 11, pp.83-88.
[9] Y. Okabayashi, M. Hikawa, T. Nakagawa, H.Tanaka, and M. Chikuma, "Simple Flow System for the Rapid Pre-concentration and Potentiometric Determination of Fluoride Using a Micro-column and a Wall-jet Electrode", Analyst, vol. 114, pp.1267-1270, 1989.
[10] Y.C. Chen, M. Ye, H. Cui, F. Wu, Y. Zhu, and J.S. Fritz, "Determination of glycerophosphate and other anions in dentifrices by ion chromatography", Journal of Chromatography A, vol. 1118, pp.155-159, 2006.
[11] T.A. Biemer, N. Asral, and A. Sippy, "Ion chromatographic procedures for analysis of total fluoride content in dentifrices", Journal of Chromatography A, vol. 771, pp. 355 – 359, 1997.
[12] R. Michalski, and B. Mathews, "Simultaneous determination of fluoride and monofluorophosphate in toothpastes by suppressed ion chromatography", Central European Journal of Chemistry, vol. 4, pp.798 – 807, 2006.
[13] Luois, A.J. Wilkes, and J.M. Talbot, "Optimisation of total fluoride analysis by capillary gas chromatography Part I: silica based dental creams", Pharmaceutica Acta Helvetiae, vol. 71, pp.273 – 277, 1996.
[14] G. Wejnerowska, A. Karczmarek, and J. Gaca, "Determination of fluoride in toothpaste using headspace solid-phase microextraction and gas chromatography flame ionization detection', Journal of Chromatography A, vol. 1150, pp. 173 – 177, 2007.
[15] J. Musijowski, B. Szostek, M. Koc, and M. Trojanowicz, "Determination of fluoride as fluorosilane derivative using reversed-phase HPLC with UV detection for determination of total organic fluorine", Journal of Separation Science, vol. 33(17-18), pp.2636–2644, 2010.
[16] J. R. Marzilli et al. "Determination of fluoride in fluoride tablets and solutions by ion selective electrode: collaborative study", Journal Association of Official Analytical Chemists, vol. 67 (4), pp. 682-684, 1984.

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