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INVESTIGATION AND OPTIMIZATION OF TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF FLUNARIZINE DIHYDROCHLORIDE USING *IN SITU* BROMINE

KUDIGE NAGARAJ PRASHANTH, NAGARAJU SWAMY and KANAKAPURA BASAVAIAH*

Department of Chemistry, University of Mysore, Manasagangotri, Mysore 570006, Karnataka, India

Abstract: Three indirect methods for the assay of flunarizine dihydrochloride (FNH) in bulk drug and commercial formulation based on titrimetric and spectrophotometric techniques using bromate-bromide mixture are described. In titrimetry, a measured excess of bromate-bromide mixture is added to an acidified solution of FNH and the unreacted bromine is determined iodometrically (method A). Spectrophotometry involves the addition of a known excess of bromate-bromide mixture to FNH in acid medium followed by estimation of unreacted bromine by its reaction with excess iodide and the liberated iodine (I_3^-) is either measured at 370 nm (method B) or liberated iodine reacted with starch followed by the measurement of the blue colored starch-iodide complex at 575 nm (method C). Titrimetric method is applicable over the range 4.5-30.0 mg FNH (method A), and the reaction stoichiometry is found to be 1 : 2 (FNH : $KBrO_3$). The spectrophotometric methods are applicable over the concentration ranges 0.8-16.0 µg/mL and 0.4-8.0 µg/mL FNH for method B and method C, respectively. The molar absorptivities are calculated to be 2.83×10^4 and 4.96×10^4 L mol^{-1}cm^{-1} for method B and method C, respectively, and the corresponding Sandell sensitivity values are 0.0168 and 0.0096 µg cm^{-2}. The proposed methods have been applied successfully for the determination of FNH in pure form and in its dosage form and the results were compared with those of a literature method by applying the Student's t-test and F-test.

Keywords: flunarizine dihydrochloride, titrimetry, spectrophotometry, bromate-bromide mixture, pharmaceuticals

Flunarizine dihydrochloride (FNH) is one of the piperazine derivatives with antihistamine properties and calcium channel blocking activity and has been widely used for migraine prophylaxis (1). Calcium channel blocker FNH prevents calcium from entering certain cells in the brain and muscles. This, in turn, prevents blood vessels from dilating, which blocks part of the migraine process (2). FNH (Fig. 1) is chemically known as [trans-1-cinnamyl-4-(4,4-difluorobenzenhydryl) piperazine dihydrochloride] (3).

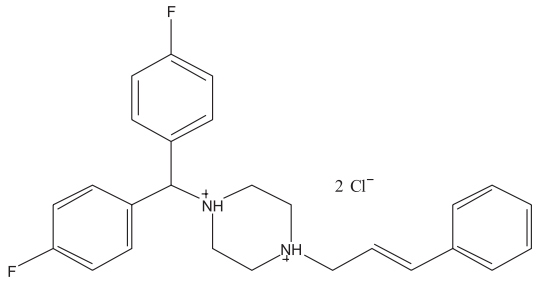


Figure 1. Structure of flunarizine dihydrochloride

FNH has official monographs in European Pharmacopeia (3) and British Pharmacopeia (4) which describe potentiometric titration for its assay using sodium hydroxide. Various other methods are also found in the literature such as high performance liquid chromatography (HPLC) (5, 6), high performance thin layer chromatography (HPTLC) (7), spectrofluorimetry (8) and UV-spectrophotometry (9–11).

Most of the reported methods are tedious, relatively less sensitive, require expensive instrumental setup and experienced personnel. In contrast to this, visible spectrophotometry is still considered to be a very convenient and economical technique because of its simplicity and speed, the inexpensive equipment needed and accuracy of results. Visible spectrophotometric methods based on diverse reaction chemistries have been proposed for the assay of FNH in pharmaceuticals. Adapa et al. (12) reported

* Corresponding author: e-mail: prashnisu@gmail.com; phone: +91-9902649708, fax: +91-8212516138

two methods based on the oxidation of drug with excess of NBS and estimating the unreacted NBS either with celestine blue (CB) or *p*-methyl aminophenol sulfate (PMAP)-sulfanilamide (SA). The same authors (13) reported two more methods for the assay of FNH. First method is based on the reaction of potassium permanganate with the olefinic double bond in FNH and estimating the unreacted permanganate with Fast Green FCF (FG FCF). Second method involves the treatment of the

Table 1. Comparison of the proposed and the existing visible spectrophotometric methods.

No.	Reagent used	Methodology	λ_{max} (nm)	Linear range, µg/mL and ε, L/mol cm	Remarks	Ref.
1.	NBS a) Celestine blue b) P-N-Me aminophenol sulfate-sulfanilamide	Unreacted NBS measured	-	-		12
2.	$KMnO_4$ a) Fast Green-FCF b) $NaIO_4$- MBTH	Unreacted $KMnO_4$ measured	620 620	1.0-5.0 (5.77×10^4) 4.0-24.0 (1.22×10^4)	Used expensive reagents.	13
3.	Iron(III) -o-phenanthroline mixture	Ferroin complex formed was measured	510	0.6-77.0		14
4.	Lead(II) and eosin	Ternary complex maesured	547.5	2.4-19.1 (3.2×10^4)	Time consuming and involve strict pH control, heating step.	15
5.	a) Carbol fuchsin b) Thorium nitrate-Thoron	Complexes formed were measured	285 555	10-200 50-300	Less sensitive and used expensive reagents.	16
6.	a) Molybdenum (V) thiocyanate b) Orange G c) Alizarin red S	Extractable ion-pair complexes were measured.	469-471 498-500 425-426		Involve extraction step, strict pH control and used organic solvents.	17
7.	Cobalt thiocyanate	Nitrobenzene soluble coordination complex was measured	620	NA	Involve extraction step and used organic solvents.	18
8.	a) Bromocresol green b) Bromocresol purple c) Bromophenol blue d) Bromothymol blue	Extractable ion-pair complexes were measured.	NA	NA	Involve extraction step, strict pH control and used organic solvents.	19
9.	a) Supracen violet-3B b) Tropacolin 000 c) Woolfast blue-BL	Chloroform soluble ion-association complexes measured	560 480 580	4.0-24.0 (1.27×10^4) 1.0-6.0 (2.50×10^4) 1.0-6.0 (4.21×10^4)	Involve extraction step, strict pH control and used organic solvents.	20
10.	Iodine	Charge-transfer complex measured.	355	8.0-13.0 ($\varepsilon = 4.40 \times 10^4$)	Used organic solvents.	21
11.	a) Picric acid b) DDQ	Charge-transfer complex measured.	402.8 460	12.0-65.0 30.0-175.0	Less sensitive and used organic solvents.	8
12.	Bromate-bromide mixture: a) Iodine b) Starch-iodine	Tri-iodide ion measured Starch-iodine complex measured	370 575	0.8-16.0 ($\varepsilon = 2.83 \times 10^4$) 0.4-8.0 ($\varepsilon = 4.96 \times 10^4$)	Simple, sensitive and no heating step. No use of organic solvent. Use of a green brominating reagent.	Proposed methods

DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, MBTH: 3-methyl-2-benzothiazolinone hydrazone hydrochloride, NA: Not available.

olefinic double bond in FNH with a Lemieux reagent ($KMnO_4$ and $NaIO_4$) and estimating the aldehyde formed with 3-methyl-2-benzothiazolinone hydrazone (MBTH). A method developed by El-Maamli (14) is based on the formation of tris (o-phenanthroline) iron(II) complex (Ferroin) upon reaction of FNH with iron(III) -o-phenanthroline mixture. Kelani et al. (15) reported a method based on the formation of ternary complex of FNH with eosin and lead(II). Two methods reported by Badalani et al. (16) describe the complexation reaction between FNH with either carbol fuchsin or thorium nitrate-thoron.

Besides, few extractive ion-pair methods have also been reported for FNH in pharmaceuticals. Elazazy et al. (17) reported three methods based on the formation of colored ion-pair complexes between the basic nitrogen of FNH and the inorganic complex; molybdenum (V) thiocyanate, Mo(V) (SCN) or the acid dyes; orange G (OR.G) and alizarin red S (ARS). In another method reported by Adapa et al. (18) colored coordination complex formed between FNH and cobalt-thiocyanate (CTC), which is extractable in nitrobenzene, was measured. Four methods reported by Zarapkar and Bapat (19) involve formation of ion-pair complexes between FNH and bromocresol green, bromocresol purple, bromophenol blue or bromothymol blue. Adapa et al. (20) reported three methods based on the formation of chloroform soluble ion-association complexes of FNH with supracen violet 3B, tropacolin 000 or woolfast blue BL. A method based on the molecular interaction between FNH and iodine, to form a charge-transfer complex was reported by El Walily et al. (21). Mohammad et al. (8) reported two methods based on the charge-transfer complexation reaction of drug either with picric acid or 2,3-dichloro-5,6-dicyano-*p*-benzoquinone.

Most of the above visible spectrophotometric methods suffer from one or other disadvantage such as poor sensitivity (8, 16), use of expensive reagents and organic solvents (8, 13, 16, 18-20), involve heating step (15), strict pH control (15, 17, 19, 20) etc., as indicated in Table 1.

The present investigation aims to develop simple, sensitive and cost-effective methods for the determination of FNH in pure form and in dosage form using titrimetric and spectrophotometric techniques. The methods utilize bromate-bromide mixture as brominating reagent, which has successfully been used for the sensitive spectrophotometric determination of many bioactive substances (22-28). The proposed methods have the advantages of speed, sensitivity and specificity besides being accurate and precise as depicted in Table 1, and can be adopted by the pharmaceutical laboratories for industrial quality control.

EXPERIMENTAL

Instrument

A Systronics model 106 digital spectrophotometer (Systronics, Ahmedabad, Gujarat, India) provided with 1 cm matched quartz cells was used for all absorbance measurements.

Reagents and materials

All reagents and chemicals used were of analytical or pharmaceutical grade and distilled water was used to prepare the solutions.

Bromate-bromide mixture

A stock standard solution of bromate-bromide mixture equivalent to 5 mM $KBrO_3$ and 10-fold molar excess of KBr was prepared by dissolving accurately weighed 0.209 g of potassium bromate (S.D. Fine-Chem. Ltd., Mumbai, India) and 1.488 g of potassium bromide (Merck, Mumbai, India) in water and diluting to volume in a 250 mL calibrated flask, and directly used in the titrimetric method. Another stock standard solution of $KBrO_3$-KBr equivalent to 300 µg/mL $KBrO_3$ was prepared by dissolving 30 mg of $KBrO_3$ and 300 mg KBr in a 100 mL calibrated flask and this was diluted appropriately with water to get working concentrations equivalent to 30 and 15 µg/mL in $KBrO_3$ for use in spectrophotometric method B and method C, respectively.

Potassium iodide

A 5% potassium iodide (Merck, Mumbai, India) solution was prepared by dissolving 5 g potassium iodide with water in a 100 mL calibrated flask. This solution was prepared afresh daily. A 2% solution was prepared separately for spectrophotometric work.

Starch solution

One gram of starch (LOBA Chemie Ltd., Mumbai, India) was made into paste with water and slowly poured with constant stirring into 100 mL boiling water, boiled for 5 min, cooled and used. This solution was prepared freshly every day.

Hydrochloric acid

Concentrated hydrochloric acid (Merck, Mumbai, India, Sp. gr. 1.18) was diluted appropriately with water to get 2 M HCl for use in all the methods.

Sodium acetate

A 3 M aqueous solution of sodium acetate was prepared by dissolving suitable quantity of sodium acetate trihydrate crystals (Merck, Mumbai, India) in water for use in method B.

Standard solution of FNH

Pharmaceutical grade flunarizine dihydrochloride certified to be 99.78% pure was received from Inga Pharmaceuticals, Mumbai, India, as gift sample and was used as received. A stock standard solution equivalent to 3.0 mg/mL of FNH was prepared by dissolving accurately weighed 750 mg of pure drug in 1 : 1 (v/v) (acetic acid : water) and diluted to mark in a 250 mL calibrated flask with the same solvent. The solution (3 mg/mL FNH) was used in titrimetric work and diluted appropriately with water to get the working concentrations of 40 and 20 µg/mL FNH for use in spectrophotometric method B and method C, respectively.

The pharmaceutical preparations Flunatrac-10 (Minova Life Sciences Ltd., Bangalore, India) and Flunarin-5 (FDC Ltd., Goa, India) were purchased from commercial sources in the local market and subjected to analysis.

Recommended procedures

Method A (Titrimetry)

Different volumes (1.5-10.0 mL) of standard FNH (3 mg/mL) solution were measured accurately, transferred into a 100 mL iodine flask and the total volume was made to 10 mL with acetic acid : water (1 : 1, v/v) mixture. The solution was acidified by adding 5 mL of 2 M HCl followed by the addition of 10 mL of bromate-bromide mixture (5 mM in $KBrO_3$) using a pipette. The content was mixed well and the flask was kept aside for 10 min with occasional swirling. Then, 5 mL of 5% (w/v) potassium iodide was added to the flask and the liberated iodine was titrated with 0.03 M sodium thiosulfate to a starch end point. A blank titration was performed under the same conditions taking 10 mL of acetic acid : water (1 : 1, v/v) mixture. The drug content in the measured aliquot was calculated from the following equation:

$$Amount\ (mg) = \frac{(B - S) \times Mol.\ wt. \times R\P}{n}$$

where B is the volume of titrant in the absence of the drug, S is the volume of titrant in the presence of the drug, Mol. wt. is relative molecular mass of the drug, R is molarity of bromate in the bromate-bromide mixture and n is the reaction stoichiometry (number of moles of bromate reacting with each mole of FNH).

Spectrophotometric method B (based on the measurement of tri-iodide ion)

Varying aliquots (0.2–4.0 mL) of standard FNH solution (40 µg/mL) were accurately transferred into a series of 10 mL calibrated flasks and the total volume was adjusted to 4.0 mL with acetic acid : water (1 : 1, v/v) mixture. One milliliter of 2 M HCl was added to each flask followed by the addition of 1 mL bromate–bromide mixture solution (30 µg/mL in $KBrO_3$). The content was mixed well and let stand for 10 min with occasional shaking. Then, 1.0 mL of 3 M sodium acetate solution was added to each flask followed by 1 mL of 2% potassium iodide. The volume was brought to the mark with water and the absorbance of the resulting tri-iodide ion was measured at 370 nm after 5 min against the water.

Spectrophotometric method C (based on the measurement of starch-iodine complex)

Into a series of 10 mL calibrated flasks, different aliquots (0.2-4.0 mL) of standard FNH (20 µg/mL) solution were transferred using a microburette. The total volume in each flask was brought to 4 mL by adding required quantity of acetic acid : water (1 : 1, v/v) mixture. The solution was acidified by adding 1 mL of 2 M HCl, and 1 mL of bromate–bromide (15 µg/mL in $KBrO_3$) solution was then added to each flask. The flasks were kept aside for 15 min with periodic shaking; 1 mL of 2% potassium iodide was added and the content was mixed well. After 5 min, 1 mL of 1% starch solution was added to each flask and the volume was made up to the mark with water and mixed well. The absorbance of the resulting blue chromogen was measured at 575 nm against water blank after 5 min. A standard graph was prepared by plotting absorbance against concentration and the unknown concentration was read from the graph or computed from the regression equation derived using Beer's law data.

Procedure for tablets

Twenty tablets each containing 5 mg or 10 mg of FNH were weighed and finely powdered. An amount of the powder equivalent to 300 mg of FNH was accurately weighed and transferred to a 100 mL calibrated flask, 60 mL of acetic acid : water (1 : 1, v/v) mixture was added and the content was shaken thoroughly for about 20 min. The volume was diluted to the mark with the same solvent, mixed well and filtered using Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and a suitable aliquot of the fil-

trate was assayed by titrimetric procedure. The same tablet extract (3 mg/mL FNH) was appropriately diluted with acetic acid : water (1 : 1, v/v) mixture to get 40 and 20 µg/mL with respect to FNH for the assay by the spectrophotometric method B and method C, respectively.

Procedure for the analysis of placebo blank and synthetic mixture

A placebo blank containing starch (10 mg), acacia (15 mg), hydroxyl cellulose (20 mg), sodium citrate (30 mg), lactose (10 mg), talc (60 mg), acacia (30 mg), magnesium stearate (25 mg) and sodium alginate (30 mg) was prepared, and 20 mg of the placebo blank was extracted with 1 : 1, v/v acetic acid : water mixture and the solution was made as described under "Procedure for the tablets" and then subjected to analysis.

A synthetic mixture was prepared by adding 150 mg of FNH to about 40 mg of the placebo blank prepared above, homogenized and the solution was prepared as done under "Procedure for the tablets". The filtrate was collected in a 50 mL flask. The synthetic mixture solution was analyzed by titrimetry and then appropriately diluted with water to get 40.0 and 20.0 µg/mL FNH solutions, and appropriate aliquots were subjected to analysis by method B and method C, separately.

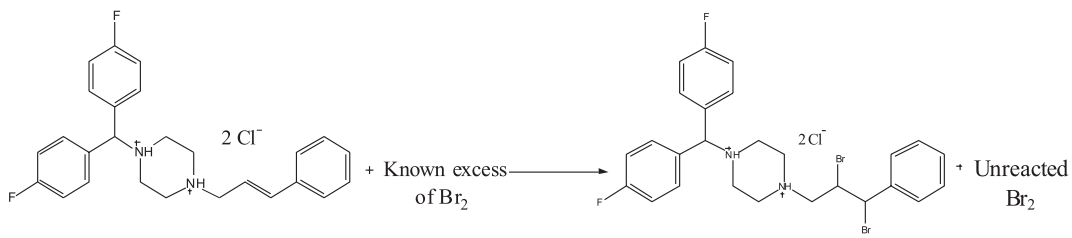
RESULTS AND DISCUSSION

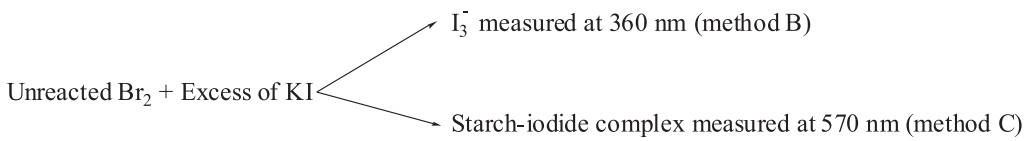
Bromate-bromide mixture in acid medium has been used for the assay of several organic pharmaceutical compounds (22-27) as it behaves as an equivalent solution of bromine. From the preliminary experiments, FNH was found to undergo bromination reaction. The present study describes one titrimetric and two spectrophotometric procedures for the determination of FNH using bromine generated *in situ* as a green brominating agent and are based on the bromination reaction of FNH with a known excess of bromate-bromide mixture in acid medium through electrophilic substitution reaction. The main advantages of this reagent are: replacement of the highly toxic and hazardous liquid bromine, no formation of hazardous byproducts and use of eco-friendly and easily available chemicals. The proposed methods entail the addition of a measured excess of bromate-bromide mixture in acid medium to FNH followed by determination of the residual bromine after the reaction between the drug and bromine is judged to be completed. In titrimetry, the reaction which was found to follow a 1 : 2 (FNH : $KBrO_3$) stoichiometry was followed by back titration of the unreacted bromine iodometrically, whereas in spectrophotometry, the amount of iodine liberated, by the reaction of unreacted bromine with potassium iodide, was either measured directly at 370 nm or reacted with starch and resulting blue colored chromogen of starch-iodine complex was measured at 575 nm.

In all methods, the amount of reacted bromate (*in situ* bromine) corresponded to the amount of FNH, which formed the basis of the assay. The possible reaction pathways are proposed and illustrated in Scheme 1.

Method development

The various experimental conditions providing accurate and precise results were carefully optimized.





Scheme 1. Proposed reaction pathway for the bromination of flunarizine dihydrochloride by bromate-bromide mixture

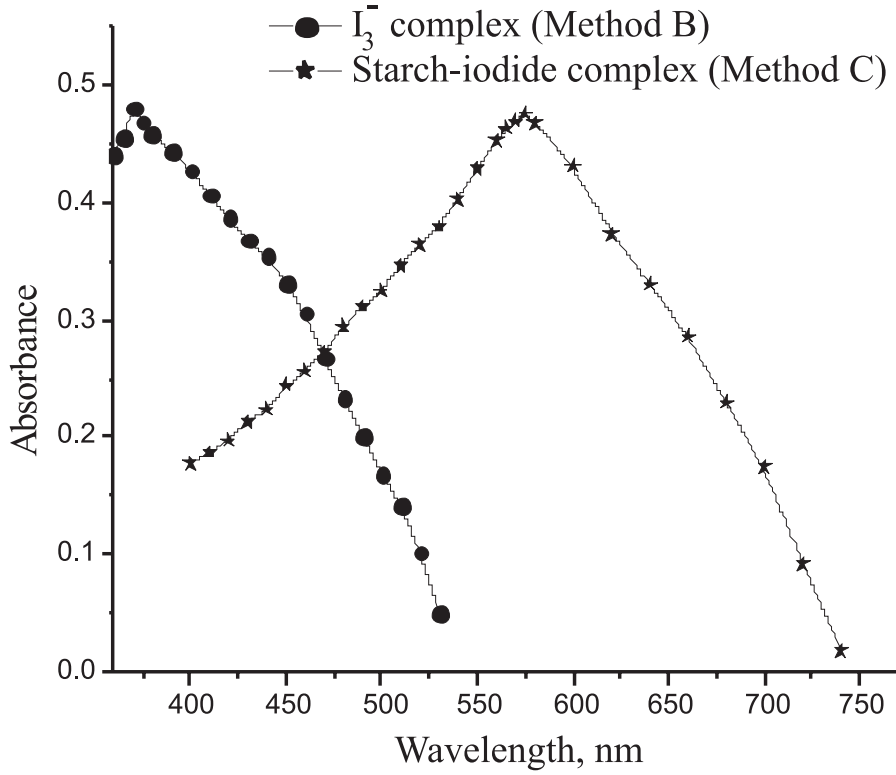


Figure 2: Absorption spectra: 8.0 µg/mL FNH, tri-iodate on (Method B): 4.0 µg/mL FNH, starch-iodide complex (Method C)

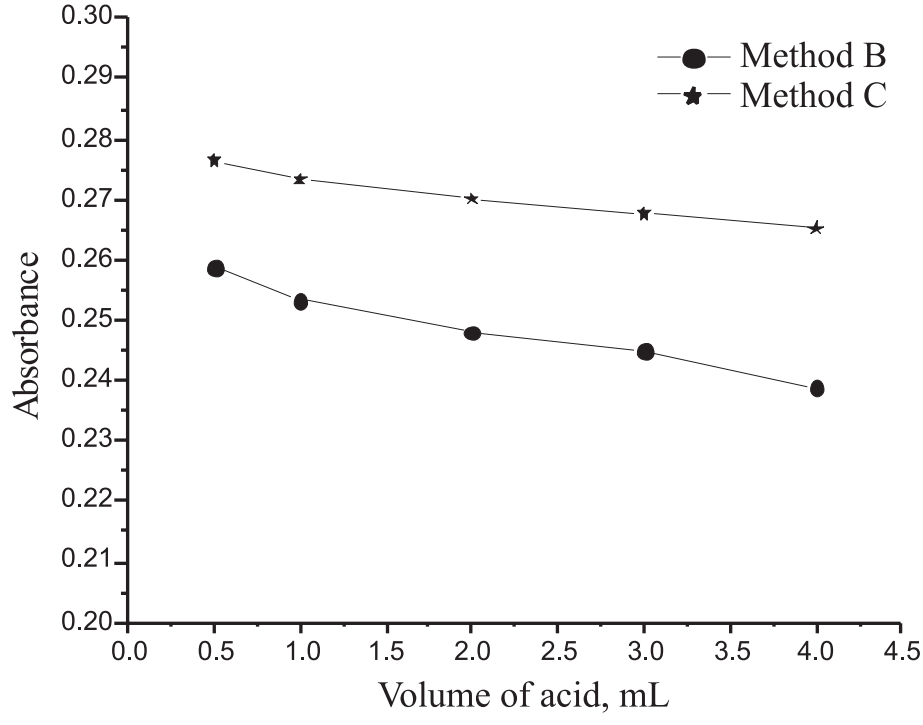


Figure 3. Effect acid concentration on 12 µg/mL FNH (Method B) and 6 µg/mL FNH (Method C)

Table 2. Regression and analytical parameters.

Parameter	Method B	Method C
λ_{max}, nm	370	575
Beer's law limits, µg/mL	0.8-16.0	0.4-8.0
Molar absorptivity (ε) L mol^{-1} cm^{-1}	2.83×10^4	4.96×10^4
Sandell sensitivity[a], µg cm^{-2}	0.0168	0.0096
Limit of detection (LOD), µg/mL	0.63	0. 28
Limit of quantification (LOQ), µg/mL	1.91	0.84
Regression equation, Y[b]		
Intercept, (a)	0.9269	0.8217
Slope, (b)	-0.0559	-0.0916
Correlation coefficient (r)	0.9997	0.9998
Standard deviation of intercept (S_a)	0.08015	0.08584
Standard deviation of slope (S_b)	0.00963	0.02063

[a]Limit of determination as the weight in µg/mL of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l = 1 cm. [b] Y = a + bX, where Y is the absorbance, X is concentration in µg/mL, a is intercept, and b is slope.

Table 3. Evaluation of intra-day and inter-day precision and accuracy.

Method	FNH* taken	Intra-day (n = 7)			Inter-day (n = 5)		
		FNH found[a]	% RSD[b]	% RE[c]	FNH found[a]	% RSD[b]	% RE[c]
A (Titrimetry)	9.00	9.06	1.13	0.67	9.10	1.71	1.08
	18.0	17.9	0.87	0.37	17.9	0.74	0.57
	27.0	27.2	0.48	0.81	27.3	0.57	0.95
B (Spectrophotometry)	4.00	3.95	1.64	1.20	3.94	1.95	1.40
	8.00	7.92	1.81	0.94	7.90	2.27	1.19
	12.0	12.2	0.80	1.28	12.3	1.20	2.25
C (Spectrophotometry)	2.00	2.02	1.89	1.15	2.03	2.30	1.35
	4.00	3.97	1.02	0.65	3.96	1.59	0.93
	6.00	6.10	0.69	1.69	6.11	0.84	1.80

*In method A, FNH taken/found are in mg and they are µg/mL in method B and C. [a]Mean value of five determinations; [b] Relative standard deviation (%); [c] Relative error (%).

Titrimetry

The proposed titrimetric procedure is based on bromination reaction between FNH and bromine generated *in situ*. A 4.5-30.0 mg of FNH were treated with known excess of bromate-bromide mixture in acid medium, and back titrating the unreacted bromine iodometrically after ensuring the completion of the reaction. Hydrochloric acid medium was found to be ideal and at optimum concentration (5 mL of 2 M HCl in a total volume of 25 mL), the reaction was completed within 10 min. At lower acid concentration (≤ 3.0 mL of 2 M HCl) the reaction stoichiometry was not stable and at higher acid concentration (≥ 4.0 mL of 2 M HCl) the reaction stoichiometry was remained constant. Hence, 5 mL of 2 M HCl in a total volume of 25 mL (1.0 M overall) was used. Hence, 5 mL of 2 M HCl was found adequate to be used in the titrimetric study. The reaction was found to be complete in 10 min and contact time up to 20 min had no effect on the stoichiometry or the results. Ten milliliters volume of 5 mM $KBrO_3$-50 mM KBr was found adequate for a

Table 4. Robustness and ruggedness.

Method A			Method B				Method C			
FNH studied mg	Robustness (RSD, %) Volume of HCl[a] (n = 3)	Ruggedness (RSD, %) Inter-burettes (n = 4)	FNH studied µg/mL	Robustness (RSD, %) Conditions altered		Ruggedness (RSD, %)	FNH studied µg/mL	Robustness (RSD, %) Conditions altered		Ruggedness (RSD, %)
				Volume of HCl[a] (n = 3)	Reaction time[b] (n = 3)	Inter-cuvettes (n = 3)		Volume of HCl[a] (n = 3)	Reaction time[b] (n = 3)	Inter-cuvettes (n = 3)
9.00	0.96	1.05	4.00	2.00	1.67	2.64	2.0	0.95	1.16	2.31
18.0	1.12	0.90	8.00	1.59	1.02	1.76	4.0	1.13	1.27	1.98
27.0	0.88	1.27	12.0	1.82	1.45	2.03	6.0	1.42	1.60	2.29

[a]In method A, volumes of 2M HCl varied were 5 ± 1 mL, in method B and C, 2M HCl (1 mL ± 0.1 mL); [b]In method B, the reaction time employed was 10 ± 1 min and in method C, 15 ± 1min.

quantitative bromination of FNH in the range investigated.

Spectrophotometry

In spectrophotometric methods, the amount of iodine liberated, by the reaction of unreacted bromine with potassium iodide, was either measured directly at 370 in method B or iodine is reacted with starch and resulting blue colored chromogen of starch-iodide complex is measured at 575 nm for method C (Fig. 2).

Optimization of experimental variables

Selection of the solvent

FNH is insoluble in many solvents like water, chloroform, dichloromethane, toluene, ethyl acetate and acetone. Even though FNH is soluble in methanol and ethanol, these solvents suppress the liberation of iodine, thus could not be used. Among the tested solvents, acetic acid was found to be an ideal solvent for the preparation of the standard solution of FNH, and the minimum ratio to get a stable solution was 1: 1, v/v (acetic acid : water) for 3.0 mg/mL FNH.

Effect of acid concentration

The reaction between FNH and bromate-bromide was performed in different acid media. Better results were obtained in hydrochloric acid medium. The effect of acid concentration on the reaction between FNH and bromate-bromide was studied by varying the concentration of HCl keeping the concentrations of bromate-bromide and drug fixed. The reaction was found to be rapid yielding a constant absorbance with maximum sensitivity and stability when the HCl concentration was maintained in the range of 0.15–0.67 M (0.5 to 3.0 mL 2 M HCl). Therefore, 1 mL of 2M HCl in a total volume of 7 mL (0.29 M) was used in both methods (Fig. 3).

Reaction time and color stability

The effect of time on the reaction between FNH and bromate-bromide mixture in the presence of HCl was studied by keeping all other reaction conditions unchanged. The absorbance of the colored species was measured after different reaction times (5.0-30.0 min) and the results showed that the reaction was complete within 10 min in method B and 15 min in method C. The yellow tri-iodide ion in method B was stable up to 45 min whereas the absorbance of the blue colored starch-iodine complex chromogen in method C remained stable for at least 1 h.

Role of sodium acetate

The liberation of iodine in method B did not stop even after 30 min under the specified acidic

conditions, but on adding sodium acetate the reaction ceased immediately. The amount of sodium acetate required was optimized and 1 mL of 3 M sodium acetate in a total volume of 10 mL was found optimum. In method C, the reaction was ceased by optimum amount of starch (1 mL of 1% starch).

Method validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity

Over the range investigated (4.5-30.0 mg), fixed stoichiometry of 1 : 2 (FNH : $KBrO_3$) was obtained in titrimetry, which served as the basis for calculations. In spectrophotometry, under optimum conditions, a linear relation was obtained between absorbance and concentration of FNH in the range of 0.8-16.0 µg/mL (method B) and 0.4-8.0 µg/mL (method C). The calibration graph is described by the equation:

$$Y = a + b X$$

(where Y = absorbance, a = intercept, b = slope and X = concentration in µg/mL) obtained by the method of least squares. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. Sensitivity parameters such as apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines (28) and compiled in Table 2. The results attest to the sensitivity of the proposed methods. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulae:

$$LOD = 3.3\sigma/s \text{ and } LOQ = 10\sigma/s$$

where σ is the standard deviation of five reagent blank determinations, and s is the slope of the calibration curve.

Precision and accuracy

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of the FNH were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table 3. The low values of the relative standard deviation (% RSD) and percentage relative error (% RE) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\% \text{ RE} = [\frac{\text{found - taken}}{\text{taken}}] \times 100$$

The assay procedure was repeated seven times, and percentage relative standard deviation (% RSD) values were obtained within the same day to evaluate repeatability (intra-day precision), and over five different days to evaluate intermediate precision (inter-day precision).

Robustness and ruggedness

To evaluate the robustness of the methods, volume of HCl was slightly altered (5 ± 1 mL) with reference to optimum values in titrimetry. However, in spectrophotometry, the reaction time (after adding BB, time varied was 10 ± 1 min in method B and 15 ± 1 min in method C) and volume of 2 M HCl were slightly altered (1 ± 0.1 mL) in both B and C methods. To check the ruggedness, analysis was performed using four different burettes in method A and three different cuvettes in method B and C. The robustness and the ruggedness were checked at three different drug levels (9, 18, 27 mg in method A; 4, 8, 12 µg/mL in method B and 2, 4, 6 µg/mL in method C). The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness, was within the acceptable limits (0.88–2.64%) as shown in Table 4.

Table 5. Results of analysis of tablets by the proposed methods.

Tablet brand name	Label claim mg/tablet	Found (Percent of label claim ± SD)[a]			
		Reference method	Proposed methods		
			Method A	Method B	Method C
Flunarin-5	5	100.1 ± 0.46	101.0 ± 0.79 *t* = 2.20 *F*= 2.95	99.51 ± 1.02 *t* = 1.18 *F*= 4.92	101.3 ±0.86 *t* = 2.75 *F*= 3.50
Flunaract-10	10	99.86 ± 0.57	100.8 ± 0.67 *t* = 2.39 *F*= 1.38	99.27± 1.07 *t* = 1.09 *F* = 3.52	100.9± 0.98 *t* = 2.05 *F* = 2.96

[a]Mean value of five determinations. Tabulated t-value at the 95% confidence level is 2.78. Tabulated F-value at the 95% confidence level is 6.39.

Table 6. Results of recovery study by standard addition method.

Tablets studied	Method A			Method B			Method C			
	FNH in tablets mg/mL	Pure FNH added, mg/mL	Total found, mg/mL	Pure FNH recovered*, % ± SD	FNH in tablets µg/mL	Pure FNH added, µg/mL	Total found, µg/mL	Pure FNH recovered*, % ± SD	FNH in tablets µg/mL	Pure FNH added, µg/mL	Total found, µg/mL	Pure FNH recovered*, % ± SD
Flunarin- 5	9.09	4.5	13.51	98.22 ± 0.24	3.98	2.0	6.01	101.5 ± 1.51	2.03	1.0	3.05	101.9 ± 1.66
	9.09	9.0	18.26	101.9 ± 0.10	3.98	4.0	7.91	98.25 ± 1.18	2.03	2.0	4.07	102.1 ± 1.02
	9.09	13.5	22.87	102.1 ± 0.24	3.98	6.0	9.90	98.67 ± 0.81	2.03	3.0	5.06	101.0 ± 1.27
Flunaract-10	9.07	4.5	13.63	101.3 ± 0.21	3.97	2.0	6.02	102.5 ± 2.31	2.02	1.0	3.01	99.00 ± 1.68
	9.07	9.0	18.02	99.44 ± 0.29	3.97	4.0	8.03	101.5 ± 1.16	2.02	2.0	4.05	101.5 ± 0.67
	9.07	13.5	22.81	101.8 ± 0.31	3.97	6.0	10.08	101.8 ± 0.59	2.02	3.0	5.06	101.3 ± 0.91

*Mean value of three determinations.

Selectivity

The proposed methods were tested for selectivity by placebo blank and synthetic mixture analyses. A convenient aliquot of the placebo blank solution, prepared as described earlier, was subjected to analysis by titrimetry and spectrophotometry according to the recommended procedures. In all the cases, there was no interference by the inactive ingredients present in the placebo mixture.

A separate experiment was performed with the synthetic mixture. The analysis of synthetic mixture solution prepared above yielded percent recoveries which ranged from 98.4 to 102.9% with standard deviation of 0.95-1.23% in all the cases. The results of this study indicate that the inactive ingredients present in the synthetic mixture did not interfere in the assay. These results further demonstrate the accuracy, as well as the precision, of the proposed methods.

Application to pharmaceutical formulation

In order to evaluate the analytical applicability of the proposed methods to the quantification of FNH in commercial tablets, the results obtained by the proposed methods were compared to those of the reference method (6) by applying Student's *t*-test for accuracy and the F-test for precision. The reported reference method describes HPLC determination of FNH in tablets. The results (Table 5) show that the Student's *t*- and F- values at a 95% confidence level are lower than the tabulated values, thereby confirming good agreement between the results obtained by the proposed methods and the reference method, with respect to accuracy and precision.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure FNH at three concentration levels (50, 100 and 150% of that in tablet powder) and total quantity then determined by the proposed methods. In all cases, the added FNH recovery percentage values ranged from 98.2 to 102.5% with a standard deviation of 0.10-2.31% (Table 6), indicating good recovery and absence of interference from the co-formulated substance in the determination

CONCLUSION

The proposed methods use bromate-bromide mixture as a green brominating reagent instead of hazardous liquid bromine. The assay results demonstrated that it is possible to use bromate-bromide

mixture as an environmental friendly reagent and potassium iodide and starch as auxillary reagents for the indirect titrimetric and spectrophotometric determination of FNH in authentic samples. The proposed titrimetric method is a first report on the application of titrimetry for the assay of FNH. The titrimetric method is simple, precise and accurate compared to the other methods reported earlier. The proposed methods make use of simple reagent, which an ordinary analytical laboratory can afford and, unlike most currently available spectrophotometric methods, the present methods are free from unwelcome steps such as heating or extraction and also from critical pH conditions. The spectrophotometric methods are sensitive more than many of the previously reported methods for determination of FNH. The methods are also useful for their high tolerance limit for common excipients found in drug formulations. These merits, coupled with the use of simple and inexpensive instruments, allow recommendation of the use of these methods in routine quality control laboratories.

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