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Application of bromate-bromide mixture as a green brominating agent for the determination of fexofenadine hydrochloride in pharmaceutical dosage form

Abstract

The present study describes one titrimetric and two spectrophotometric methods for the determination of fexofenadine hydrochloride (FFH) in bulk drug and in tablets based on bromination of FFH by bromine generated *in situ* by the action of acid on bromate-bromide mixture. In titrimetry, FFH is treated with a known excess amount of bromate-bromide mixture in acid medium followed by the determination of unreacted bromine iodometrically (method A). In spectrophotometry, the residual bromine is determined by its reaction with excess iodide and the liberated iodine (I_3^-) is either measured at 360nm (method B) or iodine reacted with starch followed by the measurement of the blue colored starch-iodide complex at 570nm (method C). Titrimetry allows the determination over the range of 4.5-30.0mg FFH whereas in spectrophotometry, Beer's law is obeyed in the concentration ranges of 2-20.0 and 0.6-6.0µg mL^{-1} FFH for method B and method C, respectively. The molar absorptivities are calculated to be $2.2x10^4$ and $5.3x10^4$ L $mol^{-1}cm^{-1}$ for method B and method C, respectively, and the corresponding Sandell sensitivity values are 0.0238 and 0.0101µg cm^{-2}. The limits of detection and quantification are also reported for both the spectrophotometric methods. The proposed methods were applied successfully to the determination of FFH in raw material and commercial tablets. Statistical comparison of the results was performed using Student's t-test and F-ratio at 95% confidence level and there was no significant difference between the official and proposed methods with regard to accuracy and precision. Further, the validity of the proposed methods was confirmed by recovery studies *via* standard addition technique.

Keywords: fexofenadine, bromate-bromide, pharmaceuticals, titrimetry, spectrophotometry; assay

Volume 7 Issue 1 - 2018

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Received: December 15, 2017 | **Published:** January 12, 2018

Abbreviations: FFH, fexofenadine hydrochloride; HPLC, high performance liquid chromatographic; USP, united states pharmacopeia; CAT, Chloramine-T

Introduction

Fexofenadine hydrochloride (FFH), chemically known as (±)-4-1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl-butyl]-a,a-dimethyl benzene aceticacid hydrochloride.[1] is a active metabolite of terfenadine and is a second-generation histamine H_1-receptor antagonist in piperidine-class drugs. These drugs are used in the treatment of seasonal allergic rhinitis, chronic idiopathic urticaria etc. FFH is a H_1-receptor antagonist that blocks peripheral histamine H_1-receptors selectively. It does not cause sedation or other central nervous system effects because it does not cross the blood–brain barrier (Figure 1).[2]

FFH is official in United States Pharmacopeia (USP),[3] which describes a high performance liquid chromatographic (HPLC) method for its assay. Literature survey revealed the availability of few methods for the assay of FFH in pharmaceuticals. Quantification of FFH has been achieved by high performance liquid chromatography (HPLC),[4-13] spectroflurimetry,[14] capillary electrophoresis,[15] cyclic voltammetry [16] and UV-spectrophotometry.[17–19] Direct potentiometric and potentiometric titrations methods employing polymeric membrane sensors were developed by Abbas et al.[20] the titrimetric method involved potentiometric titration of the drug with phosphomolybdic acid and membrane sensors were used for end point detection.[20] Some of these methods have sufficient sensitivity to determine lower concentrations of the drug. However, these methods involve several manipulation steps which are not simple for routine analysis of pharmaceutical formulations and require sophisticated instruments. To the best of our knowledge, no visual titrimetirc method has ever been reported for FFH. Visual titrimetry and visible spectrophotometry may serve as an useful alternatives to many of the aforesaid sophisticated techniques because of their cost-effectiveness, ease of operation, sensitivity, fair accuracy and precision and wide applicability.

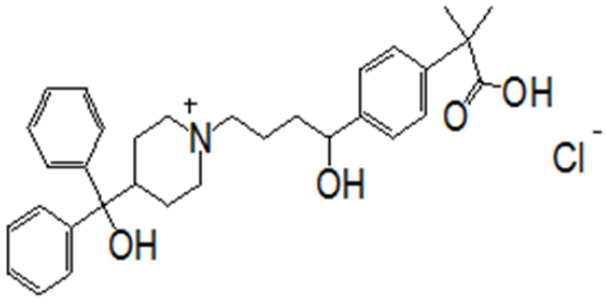

Figure 1 Structure of FFH.

Narayana et al.[21] developed two methods for the determination of FFH in bulk drug and in its tablet form. In their procedures, FFH was treated with a measured excess of chloramine-T (CAT), and the

unreacted oxidant was reacted with malachite green or xylene cyanol FF, and the change in absorbance was measured at 615 or 612nm. Apart from this, quite a few extractive spectrophotometric methods based on ion-pair formation reaction of FFH with dyes have also been reported. Suresh et al.[22] have reported a method based on the formation of chloroform-soluble ion-associate complex by the with bromothymol blue at pH 2.6 followed by absorbance measurement at 412 nm. Beer's law is obeyed over the concentration range 10-50µgmL^{-1} FFH. The drug has also been determined spectrophotometrically based on ion-pair complex formation with chromotrope 2R[23] at pH 5.0 followed by extraction into methylene chloride and measurement at 512nm. Alaa et al.[24] devised extractive spectrophotometric methods for the estimation of FFH based on ion- pair reaction employing some acidic dyes *viz*, bromothymol blue, bromophenol blue, bromocresol green and bromocresol purple. Based on similar reaction, Srinivas et al.[25] have also developed another method in which chloroform-soluble ion-associate complex formed by the interaction of drug with safranin-O or methylene blue at pH 9.8 were measured at 520 or 650nm. Another method based on the formation of charge transfer complex between FFH and 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) in non-aqueous medium. The method was applicable over a concentration range of 10-50µgmL^{-1}.[26] Soad et al.[27] developed a method based on oxidation of the drug with alkaline potassium permanganate where a green color peaking at 607nm is produced. Beer's law was obeyed in the range 0.25-1.12µgmL^{-1}. Another spectrophotometric determination of some antihistaminic and skeletal muscle relaxant drugs through ion-pair formation with xylene cyanol and orange G was also found in the literature.[28]

The visible spectrophotometric methods currently available suffer from one or the other disadvantage such as critical dependence on pH, poor sensitivity, labor-intensive, tedious and time-consuming liquid-liquid extraction step, use of large amount of organic solvents as indicated in Table 1. Extraction methods in general are prone to loss of analyte leading to erroneous results. The scientific references found in the CAS and SCI database, relating to green analytical chemistry or environmental-friendly analytical methods have been growing significantly in recent years and the recent development of new analytical methods with good characteristics such as selectivity and sensitivity are not sufficient; modern analytical methods need to be green.[29] Hence, the aim of this study was to develop three methods for the determination of FFH based on bromination reaction of FFH by an eco-friendly and green brominating agent (i.e. bromine-generated *in situ* by the action of the acid on bromate-bromide mixture), and potassium iodide and starch as auxiliary reagents. The reaction conditions were thoroughly studied, and under optimum conditions, the procedures provide highly sensitive and selective assays for FFH in commercial dosage forms. The proposed methods offer the advantage of simplicity, speed, accuracy and precision besides being free from interference from common tablet excipients.

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 5mM $KBrO_3$ and 10-fold molar excess of KBr was prepared by dissolving accurately weighed 0.209g of potassium bromate (S. D. Fine- Chem. Ltd., Mumbai, India) and 1.488g of potassium bromide (Merck, Mumbai, India) in water and diluting to volume in a 250mL calibrated flask, and directly used in the titrimetric method. Another stock standard solution of $KBrO_3$-KBr equivalent to 300µgmL^{-1} $KBrO_3$ was prepared by dissolving 30mg of $KBrO_3$ and 300mg KBr in a 100mL calibrated flask and this was diluted appropriately with water to get working concentrations equivalent to 30 and 15µgmL^{-1} 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 5g potassium iodide with water in a 100mL calibrated flask. This solution was prepared a fresh daily. A 2% solution was prepared separately for spectrophotometric work.

Starch solution

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

Hydrochloric acid

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

Sodium acetate

A 3M 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 FFH

Pharmaceutical grade fexofenadine hydrochloride certified to be 99.89% pure was received from Sanofi Aventis Pharma India, Mumbai, India, as gift and was used as received. A stock standard solution equivalent to 3.0mgmL^{-1} of FFH was prepared by dissolving accurately weighed 750mg of pure drug in 1:2 (acetic acid: water) and diluted to mark in a 250mL calibrated flask with the same solvent. The solution (3mgmL^{-1} FFH) was used in titrimetric work and diluted appropriately with water to get the working concentrations of 40 and 12µg mL^{-1} FFH for use in spectrophotometric method B and method C, respectively. The pharmaceutical preparations Allegra-180 and Allegra-120 (both from Aventis Pharma Ltd, Ankleshwar, India) were purchased from commercial sources in the local market and subjected to analysis.

Recommended procedures

Method A (titrimetry)

Different volumes (1.5-10mL) of standard FFH (3mgmL^{-1}) solution were measured accurately, transferred into a 100mL iodine flask and the total volume was made to 10mL with water. The solution was acidified by adding 5mL of 2M HCl followed by the addition of 10mL of bromate-bromide mixture (5 mM in $KBrO_3$) using a pipette. The content was mixed well and the flask was kept aside for 10min with occasional swirling. Then, 5mL of 5% (w/v) potassium iodide

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was added to the flask and the liberated iodine was titrated with 0.03M sodium thiosulphate to a starch end point. A blank titration was performed under the same conditions taking 10mL of acetic acid: water (1:2) mixture. The drug content in the measured aliquot was calculated from the following equation:

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

where B is volume of the titrant in the absence of the drug, S is volume of the 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 FFH).

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

Varying aliquots (0.5-5.0mL) of standard FFH solution (40µg mL^{-1}) were accurately transferred into a series of 10mL calibrated flasks and the total volume was adjusted to 5.0mL with water. OnemL of 2M HCl was added to each flask followed by the addition of 1mL bromate–bromide mixture solution (30µg mL^{-1} in $KBrO_3$). The content was mixed well and let stand for 10min with occasional shaking. Then, 1.0mL of 3M sodium acetate solution was added to each flask followed by 1mL 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 360nm after 5min against the water.

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

Into a series of 10mL calibrated flasks, different aliquots (0.5-5.0mL) of standard FFH (12µg mL^{-1}) solution were transferred using a micro burette. The total volume in each flask was brought to 5mL by adding required quantity of water. The solution was acidified by adding 1mL of 2M HCl, and 1mL of bromate–bromide (15µg mL^{-1} in $KBrO_3$) solution was then added to each flask. The flasks were kept aside for 10 min with periodic shaking; 1mL of 2% potassium iodide was added and the content was mixed well. After 5 min, 1mL 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 570 nm against water blank after 5min.

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 180mg or 120mg of FFH were weighed and finely powdered. An amount of the powder equivalent to 300mg of FFH was accurately weighed and transferred to a 100mL calibrated flask, 60mL of acetic acid: water (1:2) mixture was added and the content was shaken thoroughly for about 20min. The volume was diluted to the mark with same solvent, mixed well and filtered using Whatman No. 42 filter paper. The first 10mL portion of the filtrate was discarded and a suitable aliquot of the filtrate was assayed by titrimetric procedure. The same tablet extract (3mg mL^{-1} FFH) was appropriately diluted with water to get 40 and 12µg mL^{-1} with respect to FFH for the assay by the spectrophotometric method B and method C, respectively.

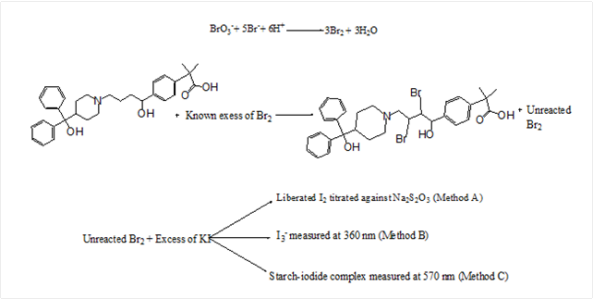
Procedure for the analysis of placebo blank and synthetic mixture

A placebo blank containing starch (50mg), acacia (45mg), hydroxyl cellulose (60mg), sodium citrate (70mg)lactose (20mg), talc (60mg), acacia (30mg)magnesium stearate (55mg) and sodium alginate (60mg) was prepared, and 50mg of the placebo blank was extracted with 1:2 (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 150mg of FFH to about 40mg 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 50mL flask. The synthetic mixture solution was analysed by titrimetry and then appropriately diluted with water to get 40.0 and 12.0mg mL^{-1} FFH solutions, and appropriate aliquots were subjected to analysis by method B and method C, separately.

Results and discussion

Bromate-bromide mixture in acid medium behaves as an equivalent solution of bromine and has been used for the assay of several organic pharmaceutical compounds.[30–33] From the preliminary experiments, FFH was found to undergo bromination reaction. The present study describes one titrimetric and two spectrophotometric procedures for the determination of FFH using bromine generated *in situ* as a green brominating agent and are based on the bromination reaction of FFH with a known excess of bromate-bromide mixture in acid medium through eletrophilic 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 FFH followed by determination of the residual bromine after the reaction between the drug and bromine is judged to be complete. In titrimetry, the reaction which was found to follow a 1:2 (FFH: $KBrO_3$) 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 360nm or reacted with starch and resulting blue colored chromogen of starch-iodine complex was measured at 570nm. In all methods, the amount of reacted bromate (*in situ* bromine) corresponded to the amount of FFH which formed the basis of the assay. The possible reaction pathways are proposed and illustrated in Scheme 1.



Scheme 1 Tentative reaction pathway for proposed methods.

Method development

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

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Titrimetry

The proposed titrimetric procedure is based on bromination reaction between FFH and bromine generated *in situ.* A 4.5-30mg of FFH 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 (5mL of 2 M HCl in a total volume of 25mL), the reaction was completedwithin 10 min. At lower acid concentration (≤3.0mL of 2M HCl) the reaction stoichiometry was slightly less than 2 and at higher acid concentration (≥7.0mL of 2 M HCl) the reaction stoichiometry was slightly higher than 2. Also the reaction stoichiometry was unaffected when (1.3-2.6) M HCl was maintained. Hence, 5mL of 2M HCl in a total volume of 25mL (2.0M overall) was used. Hence, 5mL of 2 M HCl was found adequate to be used in the titrimetric study. The reaction was found to be complete in 10min and contact time up to 20min had no effect on the stoichiometry or the results. Ten milliliters volume of 5mM $KBrO_3$-50mM KBr was found adequate for a quantitative bromination of FFH 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 360 in method B or iodine is reacted with starch and resulting blue colored chromogen of starch-iodide complex is measured at 570nm for method C (Figure 2).

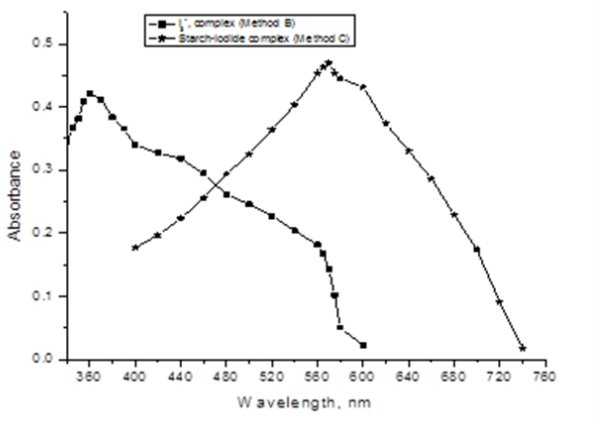

Figure 2 Absorption spectra: 12µg ml^{-1} FFH, tri-iodate ion (Method B): 3.6µg ml^{-1} FFH, starch-iodide complex (Method C).

Optimization of experimental variables

Selection of the solvent: FFH is insoluble in many solvents like water, chloroform, dichloromethane, toluene, ethyl acetate and acetone. Even though FFH 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 FFH, and the minimum ratio to get a stable solution was 1: 2 (acetic acid: water) for 3.0mgmL^{-1}

Effect of acid concentration: The reaction between FFH 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 FFH 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 0.08-0.5M (0.25mL -2mL 2M HCl) acid concentration was found sufficient for the instantaneous reaction between unreacted bromate-bromide and potassium iodide. Therefore, 1mL of 2M HCl in a total volume of 8mL (0.3M) was used in both methods.

Reaction time and color stability: The effect of time on the reaction between FFH 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 (5.0-90.0min) and the results showed that the reaction was complete within 10min in both the methods. The yellow tri-iodide ion in method B was stable upto 45min where as the absorbance of the blue colored starch-iodine complex chromogen in method C remained stable for at least 1 hr.

Role of sodium acetate: The liberation of iodine 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 1mL of 3M sodium acetate in a total volume of 10mL was found optimum.

Method validation

Linearity, detection and quantification limits: Under the optimum conditions a linear relation was obtained between absorbance and concentration of FFH in the ranges given in Table 1. The calibration graph in each instance is described by the equation:

$$Y = a + bX$$

(Where Y = absorbance, a = intercept, b = slope and X = concentration in µg mL^{-1}). The correlation coefficient, intercept and slope for the calibration data are summarized in Table 1. Sensitivity parameters such as apparent molar absorptivity and sandell sensitivity values, the limits of detection (LOD) and quantification (LOQ) are calculated as per the current ICH guidelines.[34] and compiled in Table 2. LOD and LOQ were calculated according to the same guidelines using the following formulae:

$$LOD = \frac{3.3 \times \sigma}{S} \quad \& \quad LOQ = \frac{10 \times \sigma}{S}$$

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

Accuracy and precision: The precision and accuracy of the proposed methods were studied by repeating the experiment seven times within the day to determine the repeatability (intra-day precision) and five times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed for three levels of analyte. The results of this study are summarized in Table 3. The percentage relative standard deviation (RSD, %) values were ≤77% (intra-day) and ≤2.38% (inter-day) indicating good precision of the methods. Accuracy was evaluated as percentage relative error (RE, %) between the measured mean concentrations and taken concentrations for FFH. The value of RE (%) was ≤2.89% demonstrate the high accuracy of the proposed methods.

Robustness and ruggedness: The robustness of the methods was evaluated by making small incremental changes in the volume of reagent and contact time, and the effect of these changes was studied on the absorbance of the formed colored product. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as RSD (≤64%).

Table 1 Comparison of the performance characteristics of the proposed methods with the existing visible spectrophotometric methods

Sl. No.	Reagent/s Used	Methodology	lmax(nm)	Linear Range (µg/ml)(ε = L/mol/cm)	Remarks	Ref
1	a)Chloramine -T-malachite green	Unrected chloramine T measured	615	0.2-4	Require standardization, unstable	20
	b)Chloramine-T- Xylene cyanol FF		612	0.6-4		
2	Bromothymol blue	Extractable ion-pair complex measured	412	10-50	Require extraction, less sensitive, pH dependent	21
3	Chromotrope 2R	Methylene chloride extractable ion-pair complex measured	512	30-120	Less sensitive, required close pH control and involved extraction step	22
4	a)bromothymol blue	Chloroform extractable 1:1ion-pair complex was measured	409	0.5-9.0	Required close pH control and involved tedious time consuming extraction steps	23
	b)bromophenol blue		411	1.0-6.0		
	c)bromocresol green		414	1.0-8.0		
	d)bromocresol purple		411	0.5-6.0		
5	a) Safranin-O	Chloroform extractable ion-pair complex was measured	520	10-50	Required close pH control and involved extraction steps, time consuming	24
	b)Methylene blue		650	10-50		
6	NBD-Cl	Charge-transfer complex measured	946	10-50	Requires heating, non-aqueous solvents and less sensitive	25
	Bromate-bromide mixture:					
	a) Iodide	Yellow colored tri-iodate ion measured	360	2.0-20.0 (ε =2.2×104)	Simple, sensitive and no heating step, no standardization. No use of organic solvent. Use of a green brominating reagent.	This Work
	b) Starch-iodide	Blue colored starch-iodide complex measure	570	0.6 -6.0 (ε =5.3×104)		

Table 2 Regression and analytical parameters

Parameter	Method A	Method B
l_{max}, nm	360	570
Beer's law limits, µg ml^{-1}	2-20.0	0.6-6.0
Molar absorptivity (ε) l mol^{-1} cm^{-1}	2.2×10^4	5.3×10^4
Sandell sensitivity[a], µg cm^{-2}	0.0238	0.0101
Limit of detection (LOD), µg ml^{-1}	0.2	0. 08
Limit of quantification (LOQ), µg ml^{-1}	0.6	0.25
Regression equation, Y[b]		
Intercept, (a)	0.9301	0.806
Slope, (b)	-0.0436	-0.1057
Correlation coefficient (r)	-0.9973	-0.9951
Standard deviation of intercept (S_a)	0.0061	0.0896
Standard deviation of slope (S_b)	0.0005	0.0265

*Limit of determination as the weight in µg per ml of solution, which corresponds to an absorbance of A = 0.001 measured in a cuvette of cross-sectional area 1cm^2 and l = 1cm. ** , where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in µg ml^{-1}.

Table 3 Intra-day and inter-day precision and accuracy evaluation. Mean value of five determinations; b. Relative standard deviation (%); c. Relative error (%)

Method	FFH* Taken mg/µg ml^{-1}	Intra-Day (n = 5)			Inter-Day (n = 5)		
		FFH found[a]	%RSD[b]	%RE[c]	FFH found[a]	%RSD[b]	%RE[c]
A (Titrimetry)	9	8.89	1.77	1.21	8.82	1.96	2.62
	18	17.66	1.06	1.79	17.56	1.84	2.41
	27	26.36	1.58	2.34	26.29	2.28	2.64
B (Spectrophotometry)	8	8.05	1.56	0.73	8.06	1.47	0.75
	12	12.07	1.15	0.63	12.14	2.16	1.16
	16	15.78	1.08	1.34	15.7	2.27	1.89
C (Spectrophotometry)	2.4	2.44	1.29	1.85	2.47	1.85	2.89
	3.6	3.68	1.14	2.33	3.7	1.81	2.71
	4.8	4.84	0.68	1.91	4.86	2.38	1.28

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Method ruggedness was demonstrated by having the analysis done by four analysts, and also by a single analyst performing the analysis on four different burettes/cuvettes in the same laboratory. Intermediate precision values (RSD, %) in both instances were in the range 0.64-2.94% indicating acceptable ruggedness. These results are presented in Table 4.

Selectivity: The placebo blank and synthetic mixture when subjected to analysis by the proposed methods did not reveal interference by the placebo components. The analysis of the synthetic mixture yielded percentage recovery values of FFH obtained from this study were in the range from 97.63 to 102.47. This unequivocally demonstrated the non-interference of the inactive ingredients in the assay of FFH. Further, the slopes of the calibration plots prepared from the synthetic mixture solutions were about the same as those prepared from pure drug solutions.

Application: The proposed methods were applied to the quantification of FFH in commercial tablets. The results presented in Table 5 showed that the methods are successful to the determination of FFH in pharmaceutical formulations without any detectable interference from the excipients present in the tablets. The reference method describes the measurement of ethanolic solution of FFH at 220nm.[19] When the results were statistically compared with those of the reference method by applying the Student's t-test for accuracy and F-test for precision, the calculated Student's t- value and F-value at 95% confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively. Hence, no significant difference exists between the proposed methods and the reference method with respect to accuracy and precision.

Recovery study: To assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. To a fixed and known amount of FFH in tablet powder (pre-analyzed), pure FFH was added at three concentration levels (50, 100 and 150% of the level present in the tablet) and the total was measured by the proposed methods. The determination with each concentration was repeated three times. In all the cases, the recovery percentage values ranged between 97.65 and 102.4 with relative standard deviation in the range 1.02-2.08 %. The results of this study presented in Table 6 indicated that the various excipients present in the formulations did not interfere in the assay.

Table 4 Method robustness and ruggedness study (%, RSD).*mg in titrimetry (Method A), µg ml^{-1} in spectrophotometry (Method B and C).

Method	Nominal Amount concentration mg/µg ml-1*	Robustness (%RSD)		Ruggedness (%,RSD)	
		Reaction times[a] (n=3)	Volume of acid[b]	Different analysts (n=4)	Different Burettes/ Cuvettes[c] (n=3)
	9	2.64	1.28	0.64	1.72
A	18	1.76	1.39	1.12	2.54
	27	2.14	2.37	0.72	2.37
	8	2.25	2.31	1.76	2.86
B	12	2.44	1.84	2.12	2.14
	16	2.58	2.13	1.42	2.52
	2.4	1.85	2.05	1.17	2.76
C	3.6	2.16	1.42	1.28	2.94
	4.8	2.08	2.37	1.46	2.82

[a]The reaction time was 8, 10 and 12min all the three methods,

[b]Volume of 2 M HCl was 4,5 and 6mL in method A, and 0.8. 1.0 and 1.2mL in method B and method C,

[c]Burettes in titimetry, cuvettes in spectrophotometry.

Table 5 Results of assay of tablets by the proposed methods and statistical evaluation.

Tablet Brand Name[b]	Label Claim*	Found[a] (Percent of Label Claim ±SD)			
		Reference method	Proposed methods		
			Method A	Method B	Method C
Allegra	180	99.36±1.65	97.98± 0.86 **t =**2.61 **F=**3.68	98.78± 1.42 **t =**2.80 **F =**1.35	98.62± 1.38 **t =**2.90 **F =**1.42
Allegra	120	100.4±1.86	100.8±1.27 **t**=2.09 **F**=2.14	100.65 ±1.58 **t =**2.79 **F**=1.38	99.16±1.22 **t =**2.94 **F =**2.32

*mg/tablet in tablets.

[a]Mean value of five determinations,

[b]Aventis Pharma Ltd., India.

The value of t and F (tabulated) at 95 % confidence level and for four degrees of freedom are 2.77 and 6.39, respectively.

Table 6 Recovery study via standard addition method

Method	Tablet Studied	FFH in Tablet µg ml^{-1}	Pure FFH Added µg ml^{-1}	Total Found µg ml^{-1}	Pure FFH* Percent±SD
A Titrimetry,	Allegra-180	8.82	4.5	13.08	98.24±1.98
		8.82	9	17.6	98.78 ±1.25
		8.82	13.5	21.79	97.65 ±2.08
B Spectrophotometry	Allegra-180	5.92	3	8.86	98.12±1.16
		5.92	6	11.87	99.26±1.86
		5.92	9	1.27	101.1±1.27

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Table Continued...

Method	Tablet Studied	FFH in Tablet µg ml^{-1}	Pure FFH Added µg ml^{-1}	Total Found µg ml^{-1}	Pure FFH* Percent±SD
		1.78	0.9	2.66	98.81±1.76
C Spectrophotometry	Allegra-180	1.78	1.8	7.96	102.4±1.02
		1.78	2.7	4.52	101.7±1.03

*Mean value of three determinations.

Conclusion

The present paper describes one titrimetric and two spectrophotometric methods for the determination of Fexofenadine hydrochloride in bulk drug and in its tablets and validated as per the current ICH guidelines. The 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 FFH in authentic samples. The titrimetric method which incidentally the first ever proposed for FFH, is much simpler method and it is applicable over a semi micro range (4.5-30mg FFH), yet provides very accurate and precise results. Unlike most of the existing spectrophotometric methods, the proposed spectrophotometric procedures are sensitive, simple, use eco-friendly chemicals, free from organic solvents and unwelcome steps such as heating or extraction and also from critical pH conditions. The spectrophotometric method C is more sensitive than the spectrophotometric method B as can be seen from the molar absorptivity values of both methods. The proposed methods rely on the inexpensive techniques and have the advantages of simplicity, cost-effectiveness and easily accessible technique in under-developed and developing countries. These advantages coupled with good accuracy and precision make the proposed methods highly suitable for routine use in laboratories as a part of industrial quality control.

Acknowledgments

None.

Conflicts of interest

The author declares no conflicts of interest.

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