International Scholarly Research Network
ISRN Spectroscopy
Volume 2012, Article ID 648510, 10 pages
doi:10.5402/2012/648510

Research Article

Use of Alizarin Red S as an Ion-Pair Reagent for the Spectrophotometric Assay of Fexofenadine in Pharmaceuticals and in Spiked Human Urine

Madihalli S. Raghu, Kanakapura Basavaiah, Kudige N. Prashanth, and Kankapura B. Vinay

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

Correspondence should be addressed to Kanakapura Basavaiah, basavaiahk@yahoo.co.in

Received 19 October 2012; Accepted 19 November 2012

Academic Editors: C. Liang and I. Milošev

The present study describes two simple, rapid, selective, and cost-effective spectrophotometric methods for the determination of an antiallergic drug, fexofenadine hydrochloride (FFH), in bulk drug, tablets, and in spiked human urine. The first method (method A) is based on the formation of yellow-colored ion-pair complex between FFH and alizarin red S (AZS) in acid medium which was extracted into dichloromethane, and the absorbance was measured at 440 nm. The second method (method B) is based on the breaking of the yellow FFH–AZS ion-pair complex in alkaline medium followed by the measurement of the violet-colored free dye at 590 nm. Under the optimized conditions, Beer's law is obeyed over the concentration ranges of 0.4–12.0 and 0.2–3.5 μg mL^{-1} FFH for method A and method B, respectively, and the corresponding molar absorptivity values are 3.80×10^4 and 1.61×10^5 L mol^{-1} cm^{-1}. The Sandell's sensitivity, detection, and quantification limits are also reported. The proposed methods were successfully applied to the determination of FFH in pure drug and commercial tablets. The accuracy and reliability of the proposed methods were further established by recovery studies via standard addition technique.

1. Introduction

Fexofenadine hydrochloride (FFH), chemically known as (±)-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]-butyl]-a,a-dimethyl benzene acetic acid hydrochloride [1], is an 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, and so forth. FFH is an 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 [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], spectrofluorimetry [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.; 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. But, involve several manipulation steps which are not simple for routine analysis of pharmaceutical formulations besides requiring sophisticated instruments. Visible spectrophotometry may serve as an useful alternatives to many of the aforesaid

sophisticated techniques, because of its cost effectiveness, ease of operation, sensitivity, fair accuracy and precision, and wide applicability.

Narayana and Veena [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 612 nm. Apart from this, quite a few extractive spectrophotometric methods based on ion-pair formation reaction of FFH with dyes have been also reported. Kumar et al. [22] have reported a method based on the formation of chloroform-soluble ion-associate complex with bromothymol blue at pH 2.6 followed by absorbance measurement at 412 nm. Beer's law is obeyed over the concentration range of 10–50 μg mL^{-1} FFH. The drug has been also 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 512 nm. Beer's law is obeyed over the concentration range 30–120 μg mL^{-1} FFH. Alaa et al. [24], devised extractive spectrophotometric methods for the estimation of FFH based on ion-pair reaction employing some acidic dyes namely, 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 was measured at 520 or 650 nm. However, many of the previously reported methods suffered from one or more disadvantage such as poor sensitivity as indicated in Table 1.

Extractive spectrophotometric procedures have received considerable attention for quantitative determination of many organic compounds of pharmaceutical interest [26–30] employing many dyes including alizarin red S (AZS) [31–33]. This study was directed to develop two accurate, selective, precise, and inexpensive procedures for the determination of FFH in pharmaceuticals based on ion-pair complex formation using AZS as a reagent.

2. Experimental

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

2.2. Materials and Reagents. Chemicals used were of analytical reagent grade. Spectroscopic grade organic solvents and distilled water were used throughout the investigation. Pharmaceutical grade fexofenadine hydrochloride (99.89% pure) was gifted from Sanofi-Aventis Pharma India, Mumbai, India, and was used as received. 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.

Alizarin red S (S. d. Fine Chem., Mumbai, India): 0.05% (w/v) solution in water, hydrochloric acid (Merck, Mumbai, India, sp. gr. 1.18):0.1 M in water and potassium hydroxide (Merck, Mumbai, India):1.0% (w/v) in ethanol were prepared in the usual manner.

2.3. Standard Stock Solution. A stock standard solution of 100 μg mL^{-1} of FFH was prepared by dissolving accurately weighed 10 mg of pure drug in 5 mL of acetic acid and diluting to the mark with water in a 100 mL calibrated flask. The stock solution was diluted to 20 μg mL^{-1} with water and used in method A.

2.4. Recommended Procedures

2.4.1. Method A (Based on the Measurement of Ion-Pair Complex). Different aliquots (0.2, 0.50–6.0 mL) of a standard FFH (20 μg mL^{-1}) solution were accurately transferred into a series of 125 mL separating funnels, and the total volume was adjusted to 6.0 mL by adding adequate quantity of water. To each funnel 5 mL of 0.1 M HCl was added, followed by 5 mL of 0.05% AZS solution. The content was mixed well and after 10 min; the formed ion-pair complex was extracted with 10 mL of dichloromethane after shaking for 1 min. The two phases were allowed to separate, and the dichloromethane layer was dried over anhydrous sodium sulphate, and the absorbance of the yellow FFH-AZS ion-pair complex was measured at 440 nm against a reagent blank.

2.4.2. Method B (Based on the Measurement of the Free Form of AZS from the Broken Ion-Pair). Different aliquots of 0.2, 0.5, 1.0, 2.0, 3.0, and 3.5 mL FFH-AZS complex (5 μg mL^{-1} in FFH, prepared by following the procedure described in method A) equivalent to 0.2–3.5 μg mL^{-1} with respect to FFH, were transferred into a series of 5 mL standard flasks, and the total volume was brought to 3.5 mL by adding dichloromethane. To each flask, 1.5 mL of 1% ethanolic KOH was added, the content was mixed, and the absorbance of the violet colored species was measured at 590 nm against the reagent blank.

Standard graph was prepared by plotting the absorbance *versus* drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation derived using the absorbance-concentration data.

2.5. Procedure for Tablets. Twenty tablets each containing 180 and 120 mg of FFH were weighed, ground into a fine powder and mixed well. An accurately weighed quantity of finely ground tablet powder equivalent to 10 mg of FFH was accurately weighed into a 100 mL calibrated flask, and 5 mL of acetic acid, and 50 mL of water was added, and the flask was shaken for 20 min, and finally, made up to the mark with water. The flask was kept aside for 5 min and filtered using Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded, and a suitable aliquot of the filtrate (containing 100 μg mL^{-1} FFH) was diluted with water to get a working concentration of 20 μg mL^{-1} FFH and was used

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

Sl. no.	Reagent/s used	Methodology	λ_{max} (nm)	Linear range (μg/mL) (ε = L/mol/cm)	Remarks	Ref
1	(a) Chloramine-T-mala chite green	Unrected chloramine-T measured	615	0.2–4	Exact concentration of CAT and dye to be known	[21]
	(b) Chloramine-T-Xylene cyanol FF		612	0.6–4		
2	Bromothymol blue	Chloroform extractable ion-pair complex measured	412	10–50	Less sensitive, narrow linear dynamic range	[22]
3	Chromotrope 2R	Methylene chloride extractable ion-pair complex measured	512	30–120	Less sensitive, narrow linear dynamic range	[23]
4	(a) Bromothymol blue	Chloroform extractable 1 : 1 ion-pair complex was measured	409	0.5–9.0	Less sensitive	[24]
	(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	Less sensitive	[25]
	(b) Methylene blue		650	10–50		
6	Alizarin red S	Dichloromethane extractable ion-pair measured	440	0.4–12.0 ($\varepsilon = 3.8 \times 10^4$)	Simple, highly sensitive and wide linear range, economical, and no standardization.	This Work
	FFH-AZS ion pair broken with ethanolic KOH	breaking of the yellow FFH -AZS ion-pair complex and measurement of violet-colored dianionic form of the dye in alkaline medium	590	0.2–3.5 ($\varepsilon = 1.6 \times 10^5$)		

for the assay by method A. The ion-pair complex FFH-AZS from tablet powder was used for the assay by applying the procedure described under method B.

2.6. Procedure for the Analysis of Placebo Blank and Synthetic Mixture. A placebo blank containing starch (50 mg), acacia (45 mg), hydroxyl cellulose (60 mg), sodium citrate (70 mg) lactose (20 mg), talc (60 mg), acacia (30 mg) magnesium stearate (55 mg), and sodium alginate (60 mg) was prepared, and 20 mg of the placebo blank was extracted with 5 mL acetic acid and water, and the solution was made as described under "Procedure for the tablets" and then subjected to analysis.

A synthetic mixture was prepared by adding 10 mg of FFH to about 10 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 100 mL flask. The synthetic mixture solution was diluted with water to get 20.0 mg mL^{-1} FFH solutions, and appropriate aliquots were subjected to analysis by method A and method B.

2.7. Procedure for Spiked Human Urine. Five mL of 100 μg mL^{-1} FFH solution was taken in a 25 mL calibrated flask using a micro burette, the flask was made up to the mark with spiked human urine. The content was mixed well for two minutes, and 5.0 mL of this spiked urine sample was analyzed using the procedures described earlier in methods A and B.

3. Results and Discussion

3.1. Absorption Spectra. The reaction of FFH with AZS in an acidic medium to form a yellow ion-pair complex was investigated, and this complex was extracted into dichloromethane. The absorption spectrum (Figure 1) of the formed ion-pair complex was recorded at 370–520 nm against the reagent blank solution and exhibited a maximum absorption at 440 nm (method A). In method B, this FFH–AZS ion-pair complex was treated with ethanolic KOH to yield a chromogen, the dissociated form of AZS, which exhibited bathochromic shift to maximum absorbance of 590 nm (Figure 1).

3.2. Reaction Mechanism. The anionic dyes such as AZS form ion-pair complex with the nitrogenous drug in acid medium. Since the drug contains tertiary amino group which is protonated in acid medium. So, when the FFH is treated with AZS dye in acid medium, a yellow ion-pair complex extractable into dichloromethane is formed, and the possible reaction pathway for the formation of ion-pair complex (method A) is given in Scheme 1. Cañamares et al. [34] have shown that alizarin can exist in three different forms (neutral absorbing at 433 nm, monoionized absorbing at 526 nm, and deionized absorbing at 567 nm) based on different pHs 3.4, 10.0, and 12.8, respectively. Nemodruk and Karalova [35] have stated that alizarin red S has a yellow color in acid solutions (pH < 5) which becomes a violet on increasing the pH to about 11. They explain that this phenomenon

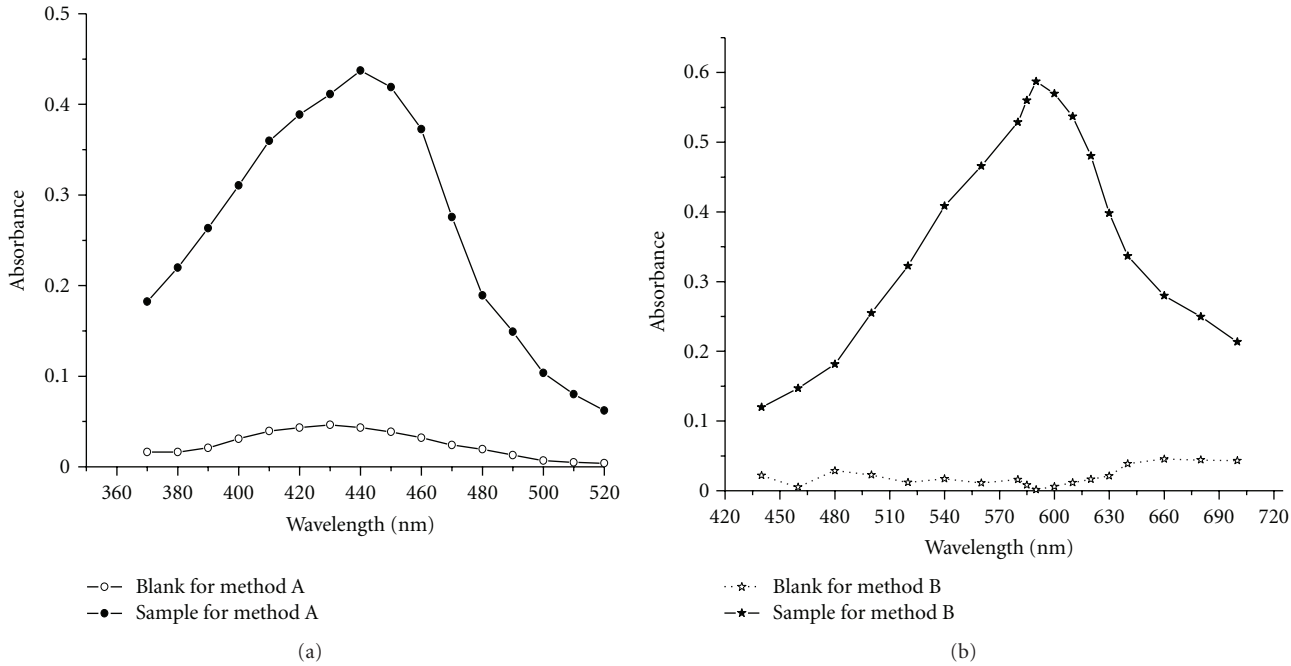
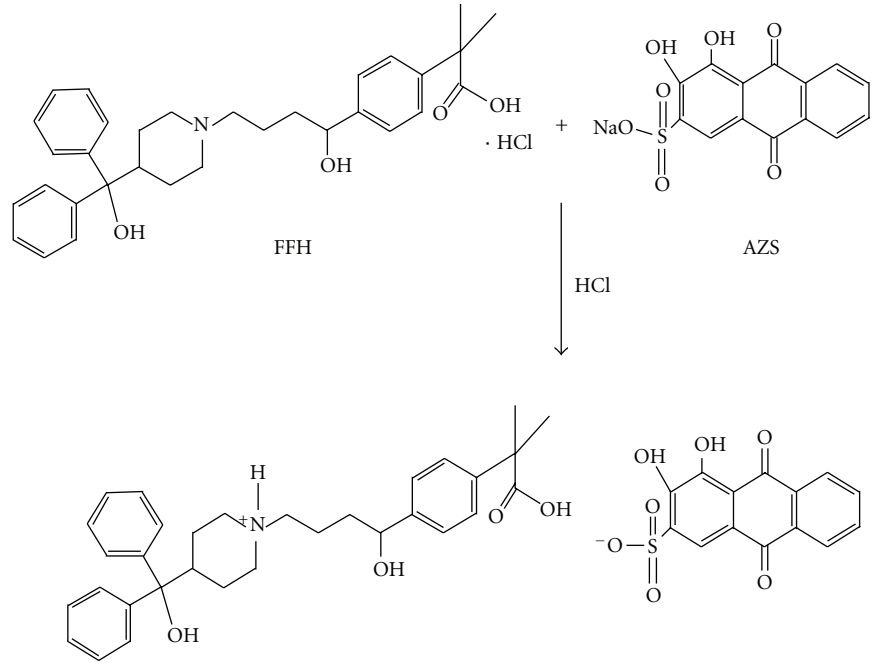
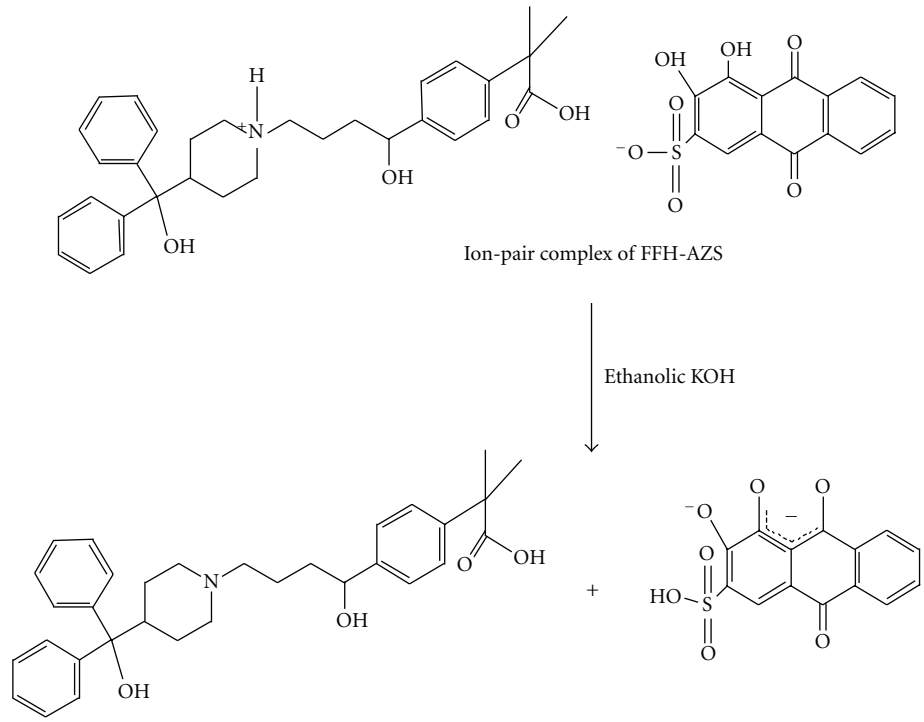


Figure 1: Absorption spectra of the ion-pair complex of FFH-AZS (6 μg mL^{-1} FFH) in method A and dianionic form of the dye (2 μg mL^{-1} FFH-AZS ion-pair) in method B.



Scheme 1: The proposed reaction pathway for ion-pair complex formation.

is due to the dissociation of the alizarin red S molecule at the two phenol groups and forming dianionic form of the dye (AZS2-). So, when the FFH-AZS ion-pair complex, formed in method A, is treated with alcoholic KOH, the ion-pair complex will break, and the yellow color will change to violet color due to the formation of dianionic form of the



Scheme 2: The proposed reaction pathway for the formation of dianionic form of AZS.

dye (AZS2-). The possible reaction pathway for method B is illustrated in Scheme 2.

3.3. Optimization of Reaction Variables. A number of preliminary experiments established optimum conditions necessary to achieve complete reaction formation, quantitative extraction of the ion-pair complex, complete breaking of the ion-pair complex, and highest sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at the respected wavelength.

3.4. Effect of pH on the Ion-Pair Complex Formation. The effect of pH of the aqueous phase was studied by extracting the colored complex in the presence of either hydrochloric acid or acidic buffers of pH 3.0-4.0. It was noticed that the maximum color intensity was observed using hydrochloric acid. Also, the formed ion-pair complex was found to be pH independent since no remarkable changes were observed while using different concentrations of HCl such as 0.1, 0.3, and 0.5 M. Further, 5.0 mL of 0.1 M HCl gave reproducible results, and it was fixed throughout the study.

3.5. Effect of Reagent Concentration. The effect of the AZS concentration in method A was studied by measuring the absorbance of solutions containing a fixed concentration of 6.0 μg mL^{-1} FFH and different amounts of the AZS at 440 nm. The study showed that 5.0 mL of 0.05% AZS solution was necessary as the difference between the absorbances of the blank and the measured species was the maximum (Figure 2). Similarly, the effect of alcoholic KOH concentration required to break the ion-pair complex and formation of the dianionic form of the dye in method B was studied by measuring the absorbance of solutions containing a fixed concentration of ion-pair complex and different volumes of 1.0% alcoholic KOH at 590 nm. The results showed that 1.5 mL of 1.0% (w/v) ethanolic KOH was sufficient to yield a maximum absorbance as can be seen in Figure 3.

3.6. Selection of the Extracting Solvent. Three organic solvents, *namely*, chloroform, dichloromethane and 1,2-dichloroethane were examined for quantitative extraction of the formed ion-pair complex. From the results shown in Table 2, dichloromethane was selected as extracting solvent due to its efficiency, the greater stability of the extracted ion-pair complex (>24 h), its high sensitivity, maximum absorbance of the measured species, and short time required for the separation of the two phases.

3.7. Effect of Time, Sequence of Addition, and Stability. The effect of contact time between FFH and AZS in the presence of 0.1 M HCl (method A) was studied in the time range of 0–25 min before extraction, and it was found that 10 min was the minimum time to achieve maximum absorbance at 440 nm. Shaking times of 0.5–3 min were studied, and

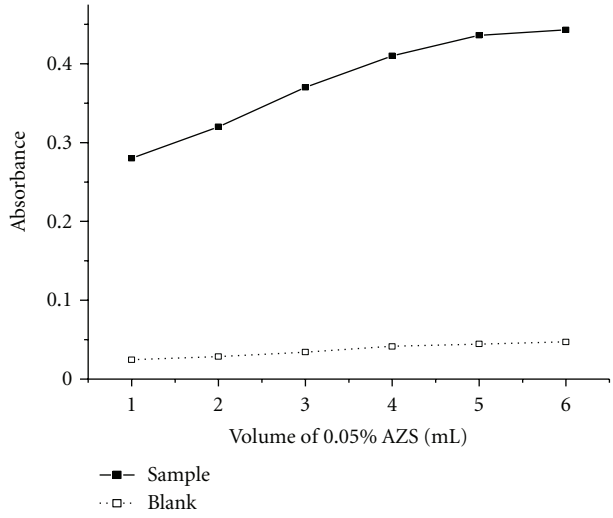


Figure 2: Effect of alizarin red S concentration on the color development (6 μg mL^{-1} FFH).

Table 2: Effect of the extracting solvent on absorbance of the formed ion-pair complex[a].

Solvent	A_{blank}	$A_{\text{ion-pair}}$
Chloroform	0.030	0.403
1,2-dichloroethane	0.039	0.368
Dichloromethane	0.028	0.435

[a] Concentration of FFH is 6 μg mL^{-1}.

the results showed that 1.0 min was sufficient to produce a constant absorbance. In method B, the effect of the time required to break the ion-pair complex was studied after the addition of ethanolic KOH to the ion-pair complex, and it was noticed that the breaking of the complex was instantaneous. There was no appreciable change in the absorbance of the measured species if the order of addition of the reactants was varied in method A. The absorbance of the yellow ion-pair complex remained stable for more than 24 h at room temperature (method A), and the absorbance of the violet color of the dianionic form of AZS (method B) was found to remain stable for about 2 hr.

3.8. Effect of the Volume of Aqueous Phase. The effect of volume of aqueous phase was studied by using different volumes of aqueous phase (including drug, AZS, and HCl) such as 15, 20, 25, 30, and 35 mL and extracting with 10 mL of dichloromethane. The use of 25 mL of aqueous phase was found to be sufficient to achieve maximum absorbance of measured species and minimum absorbance of reagent blank and hence an aqueous phase of 25 mL was fixed throughout.

3.9. Effect of Number of Extractions and Volume of Organic Solvent. The results showed that one extraction with 10 mL of organic solvent and a second extraction with identical quantity of dichloromethane yielded negligible absorbance.

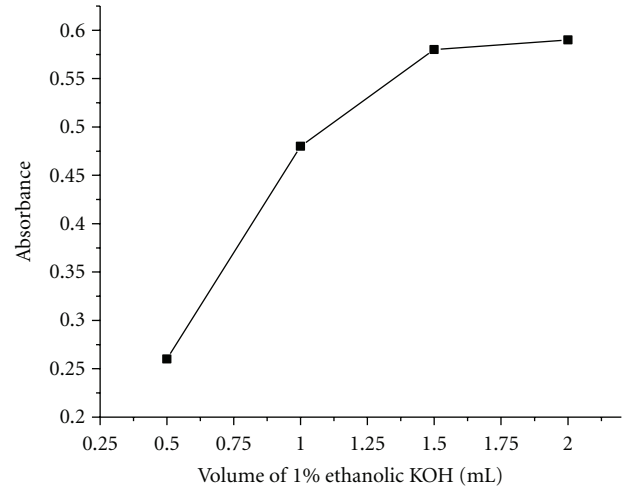


Figure 3: Effect of 1.0% ethanolic KOH (2 μg mL^{-1} FFH-AZS ion-pair).

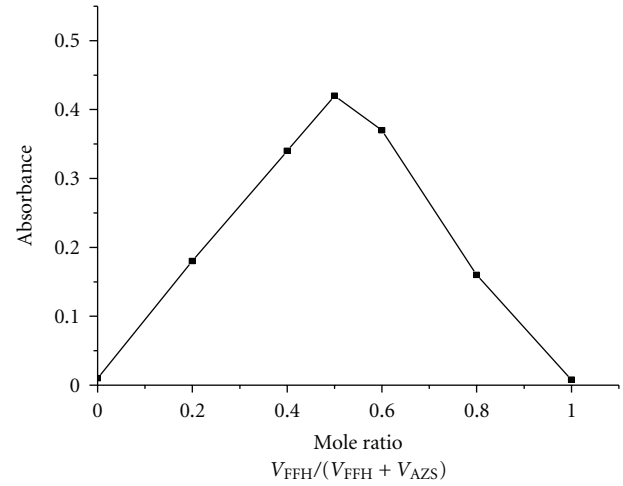


Figure 4: Job's continuous variations plot for FFH-AZS complex.

3.10. Composition of the Ion-Pair Complex. The composition of the ion-pair complex formed in method A between FFH and AZS was established by applying Job's method of continuous variations. In this method, 6.27×10^{-4} M solutions of FFH and AZS were used and mixed in varying volume ratios in such a way that the total volume of the drug and AZS was kept at 5 mL in the total volume of 15 mL of aqueous layer. The absorbance of extracted ion-pair in each instance was measured and plotted against the mole fraction of the drug (Figure 4). The plot reached a maximum value at a mole fraction of 0.5 which indicated that a 1 : 1 (FFH: AZS) ion-pair complex was formed through the electrostatic attraction between positive protonated FFH and AZS anion. The conditional stability constant (K_f) of the ion-pair complex was calculated [36] from the data of continuous variations method and found to be 4.53×10^5.

Table 3: Regression and Analytical Parameters.

Parameter	Method A	Method B
λ_{max}, nm	440	590
Beer's law limits (μg mL^{-1})	0.4–12.0	0.2–3.5
Molar absorptivity (l mol^{-1} cm^{-1})	3.8×10^4	1.6×10^5
Sandell sensitivity* (μg cm^{-2})	0.0142	0.0033
Limit of detection (μg mL^{-1})	0.23	0.04
Limit of quantification (μg mL^{-1})	0.68	0.12
Regression equation, Y**		
Intercept, (a)	0.0052	0.0236
Slope, (b)	0.0726	0.2714
Correlation coefficient (r)	0.9995	0.9995
Standard deviation of intercept (S_a)	0.1095	0.0358
Standard deviation of slope (S_b)	0.0151	0.0184

* 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 1 cm^2 and l = 1 cm. ** $Y = a + bX$, where Y is the absorbance, a is the intercept, b is the slope and X is the concentration in μg mL^{-1}.

3.11. Validation of the Proposed Methods. The proposed methods have been validated for linearity, sensitivity, selectivity, precision, accuracy, and recovery.

3.12. Analytical Parameters. A linear relation is found between absorbance and concentration of FFH within the Beer's law range given in Table 3. The calibration graphs are described by the equation:

$$Y = a + bX, \tag{1}$$

(where Y = absorbance, a = intercept, b = slope, and X = concentration in μg/mL) obtained by the method of least squares. Sensitivity parameters such as apparent molar absorptivity and Sandells sensitivity values and the limits of detection and quantification are calculated as per the current ICH guidelines [37] which are compiled in Table 3 that speaks of the excellent sensitivity of the proposed method. Limits of detection (LOD) and quantification (LOQ) were calculated from the following equations.

$$\text{LOD} = \frac{3.3 \times \sigma}{S}, \qquad \text{LOQ} = \frac{10 \times \sigma}{S}, \tag{2}$$

where σ is the standard deviation of "n" reagent blank determinations and S is the slope of the calibration curve.

3.13. Accuracy and Precision. In order to study the precision and accuracy of the proposed methods, three concentrations of pure FFH within the linearity range were analyzed, each determination being repeated seven times (intra-day precision) on the same day and one time each for five days (inter-day precision). The percentage relative standard deviation (%RSD) was ≤1.91% (intra-day) and ≤2.00% (inter-day). In addition, the accuracy of the proposed method was measured by calculating the percentage relative error (%RE), which varied between 0.86% and 2.85%. The results of this study compiled in Table 4 indicate the high accuracy and precision of the proposed methods.

3.14. Robustness and Ruggedness. The evaluation of the method robustness was done by slight alteration of some parameters, namely, volume of HCl and the contact time for method A or volume of alcoholic KOH for method B and performing the analysis under the optimized experimental conditions. The effect of these changes on the absorbance reading of the colored systems in both methods was studied and found to be negligible confirming the robustness of the proposed methods. Method ruggedness was expressed as %RSD of the same procedure applied by three analysts and also by a single analyst performing analysis on three different cuvettes. The results presented in Table 5 showed that no statistical differences between different analysts and instruments suggesting that the proposed methods were rugged.

3.15. Selectivity. In the present methods, interference by the excipients often used in pharmaceutical formulations as possible coactive substances was studied. Selectivity was evaluated by both placebo blank and synthetic mixture analyses. The placebo blank, consisting the composition as mentioned under "analysis of placebo blank" was prepared and analyzed as described under the recommended procedures. The resulting absorbance readings for the methods were same as the reagent blank, inferring no interference from the placebo. The selectivity of the methods was further confirmed by carrying out recovery study from synthetic mixture. The percent recoveries of FFH were 98.7 ± 1.47 and 101.3 ± 1.58, for method A and method B, respectively. This confirms the selectivity of the proposed methods in the presence of the commonly employed tablet excipients.

3.16. Application to Analysis of Tablets. The proposed methods were applied to the quantification of FFH in commercial tablets. The results presented in Table 6 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 the absorbance of ethanolic solution of FFH at 220 nm [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. The proposed methods were also applied to the determination of MBD in spiked human urine sample. The percent recoveries of FFH in spiked human urine were 103.2 ± 1.56 and 104.3 ± 1.58, for method A and method B, respectively.

3.17. Recovery Studies. To further ascertain the accuracy of the proposed methods, a standard addition technique was followed. A fixed amount of drug from preanalyzed tablet powder was taken and pure drug at three different levels (50%, 100%, and 150% of that in tablet powder) was added. The total was found by the proposed methods. The

Table 4: Evaluation of intraday and interday precision and accuracy.

Method	FFH taken (μg mL^{-1})	Intraday ($n = 5$)			Interday ($n = 5$)		
		FFH found[a] (μg mL^{-1})	%RSD[b]	%RE[c]	FFH found[a] (μg mL^{-1})	%RSD[b]	%RE[c]
A	4.00	4.08	1.26	2.11	4.10	1.06	2.65
	6.00	6.14	1.32	2.34	6.17	0.62	2.85
	8.00	8.15	1.91	1.77	8.18	0.59	2.34
B	1.00	1.01	1.52	1.20	1.02	2.00	2.04
	2.00	1.98	1.88	0.86	1.97	1.74	1.27
	3.00	2.95	0.87	1.62	2.93	0.91	2.05

[a]Mean value of five determinations; [b]relative standard deviation (%); [c]relative error (%).

Table 5: Robustness and ruggedness.

Method	FFH taken, μg mL^{-1}	Robustness		Ruggedness	
		Parameters interchanged			
		Volume of HCl/ethanolic KOH*	Reaction time**	Interanalysts (%RSD), ($n = 3$)	Interinstruments (%RSD), ($n = 3$)
A	4.00	1.84	2.24	1.96	1.84
	6.00	1.68	1.87	2.04	1.68
	8.00	2.02	2.32	2.18	2.02
B	1.00	1.12	—	1.78	1.97
	2.00	1.24		1.96	2.28
	3.00	1.58		2.29	2.56

*In method A, the volumes of 0.1 M HCl were 4.8, 5.0, and 5.2 mL, and in method B the volumes of ethanolic KOH added were 1.4, 1.5, and 1.6 mL. **In method A, the reaction times were 8, 10, and 12 min.

Table 6: Results of analysis of tablets by the proposed methods.

Tablet brand name	Label claim[b] mg/tablet	Found (Percent of label claim ± SD)[a]		
		Reference method	Proposed methods	
			Method A	Method B
Allegra-180	180	99.36 ± 1.65	98.28 ± 1.09	98.62 ± 1.26
			$t = 2.71$	$t = 2.63$
			$F = 2.30$	$F = 1.71$
Allegra-120	120	100.4 ± 1.86	100.9 ± 1.24	101.4 ± 0.94
			$t = 2.16$	$t = 2.13$
			$F = 2.25$	$F = 3.92$

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

Table 7: Results of recovery study by standard addition method.

Tablets studied	Method A				Method B			
	FFH in tablets, μg mL^{-1}	Pure FFH added, μg mL^{-1}	Total found, μg mL^{-1}	Pure FFH recovered*, Percent ± SD	FFH in tablets, μg mL^{-1}	Pure FFH added, μg mL^{-1}	Total found, μg mL^{-1}	Pure FFH recovered*, percent ± SD
Allegra-180	3.93	2.0	5.91	99.20 ± 0.93	1.00	0.5	1.51	102.8 ± 1.00
	3.93	4.0	7.87	98.56 ± 0.57	1.00	1.0	1.98	98.66 ± 0.89
	3.93	6.0	9.86	98.88 ± 0.90	1.00	1.5	2.52	101.4 ± 1.63
Allegra-120	3.95	2.0	5.97	101.2 ± 0.82	1.01	0.5	1.50	98.53 ± 1.99
	3.95	4.0	8.03	102.1 ± 1.53	1.01	1.0	2.00	99.23 ± 0.60
	3.95	6.0	9.89	99.10 ± 1.40	1.01	1.5	2.53	101.6 ± 2.53

Mean value of three determinations.

determination at each level was repeated three times and the percent of recovery of the added standard was calculated. Results of this study presented in Table 7 reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

4. Conclusions

The results of this study demonstrate that it is possible to use alizarin red S as an ion-pairing reagent for the spectrophotometric determination of FFH in bulk drug and pharmaceutical samples as well as in spiked human urine. The proposed methods are highly reliable owing to the stability of the ion-pair complex and the dianionic form of the AZS which are ultimately measured. The methods can be performed at room temperature and make use of cheaper and readily available reagent. Moreover, the procedures do not involve any critical reaction conditions and no pH adjust is required. As can be seen from the molar absorptivity values of both methods, the method B is more sensitive than method A. From the Student's t-test and F-test values, it is clear that the results obtained by the proposed methods are in a good agreement with those obtained by the established method and indicate a high accuracy and precision. Thus, the methods are useful for the quality control and routine analysis of FFH in pharmaceutical preparations since there is no interference was observed from the common excipients that might be found in commercial formulations.

Acknowledgments

The authors are thankful to Sanofi-Aventis Pharma., Mumbai, India, for providing pure FFH sample. Authors also thank the University of Mysore, Mysore, India, for providing facilities to carry out this work.

References

[1] The Merck, *The Merck Index, An Encyclopedia of Chemicals, Drugs, And Biologicals*, Merck & Company, Whitehouse Station, NJ, USA, 2001.

[2] C. Dollery, *Therapeutic Drugs*, Churchill Livingstone, London, UK, 2nd edition, 1999.

[3] "The United States Pharmacopoeia," 24th Revision, the 19th National Formulary, USP Convention, Rockville, Md, USA, 2000.

[4] A. R. Breier, C. S. Paim, M. Steppe, and E. E. S. Schapoval, "Development and validation of dissolution tests of fexofenadine hydrochloride capsules and coated tablets," *Journal of Pharmacy and Pharmaceutical Sciences*, vol. 8, no. 2, pp. 289–298, 2005.

[5] H. M. Maher, M. A. Sultan, and I. V. Olah, "Development of validated stability-indicating chromatographic method for the determination of fexofenadine hydrochloride and its related impurities in pharmaceutical tablets," *Chemistry Central Journal*, vol. 5, no. 1, article 76, 2011.

[6] T. Radhakrishna and G. Om Reddy, "Simultaneous determination of fexofenadine and its related compounds by HPLC," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 29, no. 4, pp. 681–690, 2002.

[7] F. Zafar, M. H. Shoaib, and R. I. Yousuf, "Development of Rp-HPLC method for fexofenadine determination in tablet formulations and development of dissolution method," *Pakistan Journal of Pharmacology*, vol. 28, pp. 43–49, 2011.

[8] B. R. Vaghela, A. M. Reddy, P. Venkatesh, and N. Kumar, "Identification and characterization of an oxidative degradation product of fexofenadine, development and validation of a stability-indicating RP-UPLC method for the estimation of process related impurities and degradation products of fexofenadine in pharmaceutical formulations," *Scientia Pharmaceutica*, vol. 80, no. 2, pp. 295–309, 2012.

[9] S. Karakus, I. Küçükgüzel, and S. G. Küçükgüzel, "Development and validation of a rapid RP-HPLC method for the determination of cetirizine or fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 46, no. 2, pp. 295–302, 2008.

[10] M. K. Sharaf El- Din, F. Irahim, M. I. Eid, and M. E. K. Wahb, "Validated stability indicating liquid chromatographic method for the determination of fexofenadine hydrochloride in presence of its degradation products application to tablets and content uniformity testing," *Journal of Pharmacy Research*, vol. 4, no. 7, pp. 2377–2380, 2011.

[11] D. C. Oliveira, A. Weich, and C. M. B. Rolim, "Simple and reliable HPLC analysis of fexofenadine hydrochloride in tablets and its application to dissolution studies," *Pharmazie*, vol. 62, no. 2, pp. 96–100, 2007.

[12] L. Cui, L. Qian, S. Hong-Wei, Y. Lun, S. Zhen-Peng, and L. Hong, "Determination of fexofenadine hydrochloride and its related substances by HPLC," *Chinese Journal of New Drugs*, no. 18, pp. 1511–1513, 2007.

[13] M. S. Arayne, N. Sultana, H. Shehnaz, and A. Haider, "RP-HPLC method for the quantitative determination of fexofenadine hydrochloride in coated tablets and human serum," *Medicinal Chemistry Research*, vol. 20, no. 1, pp. 55–61, 2011.

[14] Z. A. Alothman, N. Bukhari, S. Haider, S. M. Wabaidur, and A. A. Alwarthan, "Spectrofluorimetric determination of fexofenadine hydrochloride in pharmaceutical preparation using silver nanoparticles," *Arabian Journal of Chemistry*, vol. 3, no. 4, pp. 251–255, 2010.

[15] P. Mikuš, I. Valášková, and E. Havránek, "Determination of fexofenadine in tablets by capillary electrophoresis in free solution and in solution with cyclodextrins as analyte carriers," *Drug Development and Industrial Pharmaceuticals*, vol. 31, no. 8, pp. 795–801, 2005.

[16] A. Golcu, B. Dogan, and S. A. Ozkan, "Anodic voltammetric behavior and determination of antihistaminic agent: fexofenadine HCl," *Analytical Letters*, vol. 38, no. 12, pp. 1913–1931, 2005.

[17] İ. Kozan, I. M. Palabiyik, E. Karacan, and F. Onur, "Spectrophotometric and high performance liquid chromatographic determination of fexofenadine hydrochloride in pharmaceutical formulations," *Turkish Journal of Pharmaceutical Sciences*, vol. 5, no. 3, pp. 175–189, 2008.

[18] H. J. Vekaria, K. S. Muralikrishna, and G. F. Patel, "Development and validation of spectrophotometric method for estimation of fexofenadine hydrochloride and montelukast sodium in combined dosage form," *Inventi Impact: Pharm Analysis & Quality Assurance*, vol. 2011, Article ID Inventi:ppaqa/240/11, 2011.

[19] A. R. Breier, M. Steppe, and E. E. S. Schapoval, "Validation of UV spectrophotometric method for fexofenadine hydrochloride in pharmaceutical formulations and comparison with HPLC," *Analytical Letters*, vol. 40, no. 12, pp. 2329–2337, 2007.

[20] M. N. Abbas, A. A. A. Fattah, and E. Zahran, "A novel membrane sensor for histamine H1-receptor antagonist 'fexofenadine'," *Analytical Sciences*, vol. 20, no. 8, pp. 1137–1142, 2004.

[21] B. Narayana and K. Veena, "A new method for the spectrophotometric determination of fexofenadine hydrochloride," *Indian Journal of Chemical Technology*, vol. 17, no. 5, pp. 386–390, 2010.

[22] K. S. Kumar, V. Ravichandran, M. K. Mohan Maruga Raja, R. Thyagu, and A. Dharamsi, "Spectrophotometric determination of Fexofenadine hydrochloride," *Indian Journal of Pharmaceutical Sciences*, vol. 68, no. 6, pp. 841–842, 2006.

[23] S. S. Abd El-Hay, M. Y. El-Mammli, and A. A. Shalaby, "Spectrophotometric determination of clemastine hydrogen fumarate, fexofenadine HCl and moxepril HCl through ion-pair formation with chromatrope 2 R," *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 2, no. 1, pp. 497–509, 2011.

[24] S. A. Alaa, S. A. Ibrahim, and A. M. Hassan, "Utility of extracted colored ion—associate complexes formation reaction for the determination of fexofenadine hydrochloride in pure forms and in dosage forms," *Journal of Chemical Engineering & Process Technology*, vol. 1, article 101, 2010.

[25] L. D. Srinivas, P. Ravi kumar, and B. S. Sastry, "Ion association methods for the determination of fexofenadine in pharmaceutical preparations," *E-Journal of Chemistry*, vol. 2, no. 3, pp. 199–202, 2005.

[26] S. Ashour, M. F. Chehna, and R. Bayram, "Spectrophotometric determination of alfuzosin HCl in pharmaceutical formulations with some sulphonephthalein dyes," *International Journal of Biomedical Science*, vol. 2, no. 3, pp. 273–278, 2006.

[27] K. Harikrishna, B. S. Nagaralli, and J. Seetharamappa, "Extractive spectrophotometric determination of sildenafil citrate (viagra) in pure and pharmaceutical formulations," *Journal of Food and Drug Analysis*, vol. 16, no. 1, pp. 11–17, 2008.

[28] N. Rajendraprasad, K. Basavaiah, K. B. Vinay, and H. D. Revanasiddappa, "Sensitive and selective extractive spectrophotometric method for the determination of hydroxyzine dihydrochloride in pharmaceuticals," *Journal of the Mexican Chemical Society*, vol. 54, no. 4, pp. 233–239, 2010.

[29] N. Rajendraprasad, K. Basavaiah, and K. B. Vinay, "Extrtactive spectrophotometric determination of quetiapine fumarate in pharmaceuticals and human urine using calmagite as an ion-pair reagent," *Chemical Industry Chemical Engineering Qurterly*, vol. 17, no. 3, pp. 259–267, 2011.

[30] S. Mostafa, M. El-Sadek, and E. A. Alla, "Spectrophotometric determination of enrofloxacin and pefloxacin through ion-pair complex formation," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 28, no. 1, pp. 173–180, 2002.

[31] K. Basavaiah, SriLatha, and J. M. Swamy, "Spectrophotometric determination of ceterizine hydrochloride with Alizarin Red S," *Talanta*, vol. 50, no. 4, pp. 887–892, 1999.

[32] M. S. Kamel, B. N. Barsoum, and R. Sayed, "Spectrophotometric microdetermination of anthelmentic drug in pure form and pharmaceutical formulation by ion-pair complexation," *Journal of Applied Science Research*, vol. 4, no. 10, pp. 1242–1248, 2008.

[33] S. A. M. Abdulrahman and K. Basavaiah, "Use of alizarin red S as a chromogenic agent for the colorimetric determination of dothiepin hydrochloride in pharmaceutical formulations," *Journal of Saudi Chemical Society*. In press.

[34] M. V. Cañamares, P. Sevilla, S. Sanchez-Cortes, and J. V. Garcia-Ramos, "Surface-enhanced Raman scattering study of the interaction of red dye alizarin with ovalbumin," *Biopolymers*, vol. 82, no. 4, pp. 405–409, 2006.

[35] A. A. Nemodruk and Z. K. Karalova, *Analytical Chemistry of Boron*, Ann Arbor-Humphrey Science, London, UK, 1969.

[36] A. S. Amin, A. A. E. Gouda, R. El-Sheikh, and F. Zahran, "Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation," *Spectrochimica Acta A*, vol. 67, no. 5, pp. 1306–1312, 2007.

[37] "Validation of analytical procedures: text and methodology, Q2 (R 1), complementary guideline on methodology dated 06 November 1996," in *Proceedings of the International Conference on Harmonization of Technical Requirements Registration of Pharmaceuticals for Human Use, ICH Harmonized Guideline*, London, UK, November 2005.