

## Research Article

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# Extractive Spectrophotometric and Conductometric Methods for Determination of Fexofenadine Hydrochloride in Pharmaceutical Dosage Forms

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**Abstract**

Two simple and sensitive extractive spectrophotometric and conductometric methods have been developed for the determination of fexofenadine hydrochloride in bulk and in pharmaceutical preparations. The first method is based on the formation of colored chloroform extractable ion-association complexes (1:1) of fexofenadine with bromocresol purple (BCP) and bromophenol blue (BPB) dyes in aqueous acidic buffer pH 3.0. The extracted complex species were quantitatively measured at 411 and 415 nm for FEX-BCP and FEX-BPB, respectively (method I). The second method is based on the conductometric determination of 2.5-13.45 mg of fexofenadine by titration with sodium tetraphenylborate (TPB) in aqueous solution at 20°C (method II). All the reaction conditions for the proposed methods have been studied. Beer's law was obeyed in the FEX concentration ranges 1.1-47.8 and 1.2-45.0  $\mu\text{g mL}^{-1}$  with detection limit of 0.21 and 0.15  $\mu\text{g mL}^{-1}$  for FEX-BCP and FEX-BPB, respectively. The proposed methods were applied successfully for the determination of the FEX in pharmaceutical formulations, the RSD% values were found to be 0.38, 0.24 and 0.74% for extractive spectrophotometric and conductometric methods, respectively. The results obtained were compared statistically with those obtained by the official method and showed no significant differences regarding accuracy and precision.

**Keywords:** Fexofenadine hydrochloride; Spectrophotometry; Conductometry; Bromocresol purple; Bromophenol blue; Sodium tetraphenylborate and Pharmaceutical preparations

**Introduction**

Fexofenadine, ( $\pm$ )-4-[1-Hydroxy-4-[4-(hydroxyl diphenylmethyl)-1-piperidinyl]butyl]- $\alpha$ ,  $\alpha$ -dimethyl benzene acetic acid, an active metabolite of terfenadine, is a selective histamine  $H_1$ -receptor antagonist, and is clinically effective in the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria as a first-line therapeutic agent, such as loratadine and cetirizine [1]. Several methods have been described for the quantitative determination of fexofenadine hydrochloride in pharmaceutical dosage forms by HPLC with ultra violet detection [2-7] potentiometry [8] and capillary electrophoresis [9,10]. Various spectrophotometric methods have been reported for the determination of fexofenadine hydrochloride [5,11-18] from its individual and combined formulations with other active ingredients. Fexofenadine has been determined in human plasma by HPLC with UV detection [19], fluorescence detection [20] and tandem mass spectrometry detection [21-23].

In our studies, fexofenadine has basic cationic nitrogen reacts with anionic dye at a suitable pH, to form highly colored chloroform extractable ion pair complex. Therefore, fexofenadine is determined spectrophotometrically through formation of ion pair complex with bromocresol purple and bromophenol blue, as acidic dye. On the other hand, fexofenadine was found to reacts with sodium tetraphenylborate in aqueous solution. This reaction was studied for the new conductometric titration of the drug. Optimum conditions were established and both the methods were validated for linearity. The validated methods when applied to the determination of FEX in formulations yielded results which were in agreement with the label claim.

**Materials and Methods****Apparatus**

A Jasco V-530 UV-VIS spectrophotometer (Japan) with 1 cm quartz cells was used for all absorbance measurements under the following operating conditions: scan speed medium (400 nm/min), scan range 350–500 nm and slit width 2 nm. Spectra were automatically obtained by Jasco system software. A conductometer – pH meter Consort C830 (Belgium) equipped with conductivity cell (cell constant of 1.0) and combined glass pH electrode was used. The desired temperature was maintained with circulating water bath thermostat connected to a jacket around the analysis vessel.

**Chemicals**

All chemicals and reagents used throughout this work were of analytical-reagent grade and supplied by Merck and solutions were made with doubly distilled water. Fexofenadine hydrochloride (FEX) was obtained from Chem Pharma, India. The purity of FEX was found to be 99.86% according to BP [24]. Pharmaceutical preparations containing FEX were purchased from commercial sources in the local market.

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## Solutions

Stock solutions, 0.01 M of sodium tetraphenylborate,  $1 \times 10^{-3}$  M of bromocresol purple (BCP) and bromophenol blue (BPB) were prepared by dissolving the accurately weighed amounts of the pure solid in doubly distilled water. Buffer solutions (pH range from 1.2 to 4.0) were prepared by mixing specific volumes of KCl 0.2 M and HCl 0.2 M. absolute methanol was used. Stock solution, 1.0 mg mL<sup>-1</sup> of FEX was prepared in doubly distilled water, stored in dark bottles and kept in the refrigerator for not more than 10 days. Other concentrations of working solutions were then prepared by suitable dilution of the stock solution with water.

## General procedures

**Extractive Spectrophotometry:** Into a series of 50 mL separating funnels, 3 mL or 4.0 mL of buffer solution of pH 3.0 and 4.0 mL of BCP or BPB were placed. An appropriate volume of  $10^{-3}$  M FEX solution (0.020-0.90 mL for BCP; 0.022-0.84 mL for BPB) was added to each funnel and mixed well. The funnels were shaken vigorously with 10 mL chloroform for 2 min, then allowed to stand for clear separation of the two phases. The separated organic phase was transferred to a 50 mL beaker, dried over anhydrous sodium sulfate, and transferred to a 10 mL volumetric flask. Then the combined extract was made up to the mark with the solvent and mixed. The absorbance of the organic phase was measured at 411 and 415 nm for FEX-BCP and FEX-BPB complexes, respectively, against a reagent blank similarly prepared. The standard calibration plot was prepared to calculate the amount of the analyst drug in unknown samples. The color is stable for at least 24 hrs up to 30°C.

**Conductometry:** A volume containing 2.5-13.45 mg of FEX was transferred to a 25 mL volumetric flask and made up to the mark with water. The contents of the volumetric flask were transferred to a beaker and the conductivity cell was immersed in the sample solution. 0.01 M sodium tetraphenylborate solution was then added from a microburette with precision  $\pm 0.005$  mL and the conductance was measured subsequent to each addition of reagent solution and after thorough stirring. The conductance reading was taken 1 min after each addition of the reagent. A graph of conductivity (corrected values for dilution) versus volume of titrant added was constructed and the end-point was determined.

## Stoichiometric ratio

The molar ratio and continuous variation methods were applied to study the stoichiometric ratio of the ion pair formed. A  $1 \times 10^{-3}$  M standard solution of each drug and reagent were used. In the former method a constant volume of  $1 \times 10^{-3}$  M drug solution was employed and the reagent concentration was changed to obtain a ratio for the ion pair complex, while in the latter method, a series of solutions was kept at 2.0 mL. The reagent was mixed in various proportions and then diluted to volume in a 10 mL calibrated flask with chloroform. The absorbances of the resulting solutions were measured at  $\lambda_{\max}$  against reagent blanks treated similarly.

## Procedure for pharmaceutical dosage form samples

Twenty tablets or the contents of 20 capsules were weighed and finely powdered. An accurately weighed quantity of the powder equivalent to 100 mg of FEX was dissolved in a 100 mL of methanol and sonicated for 5 minutes and then filtered. The combined filtrate was evaporated to the dryness. The remaining portion of the solution was dissolving in a 100 mL volumetric flask to the volume with double

distilled water, and the resulting solution was used for analysis by the recommended procedures in the concentration ranges mentioned above.

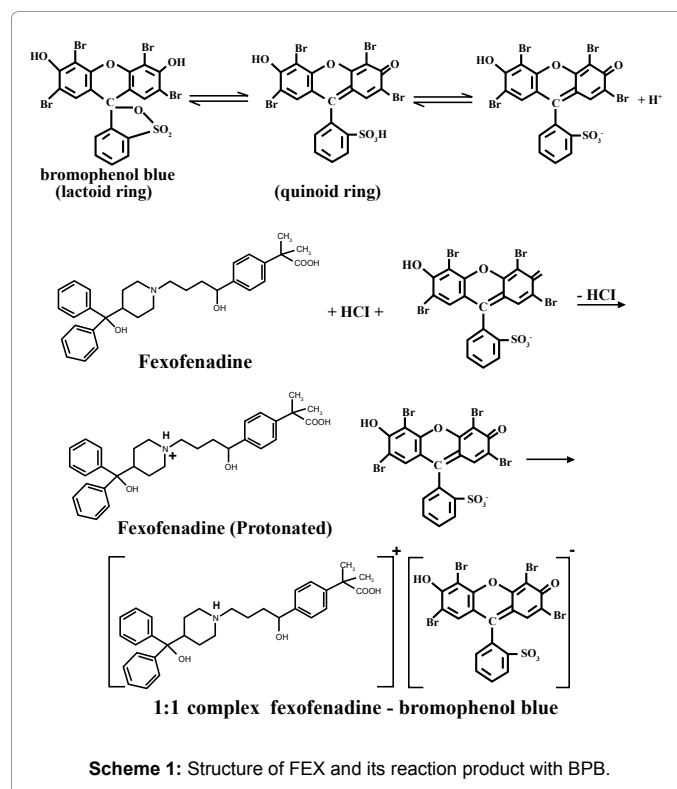
## Results and Discussion

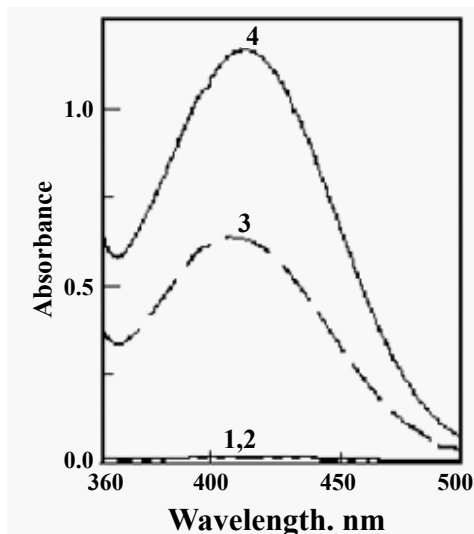
### Method I

Containing cationic nitrogen, the cited drug reacts with BPB to form a yellow ion-pair complex between the basic nitrogen of the drug in KCl-HCl buffer and BPB. Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic force of attraction. The complex is quantitatively extracted into chloroform. The probable mechanism of formation of the complex is shown in scheme 1.

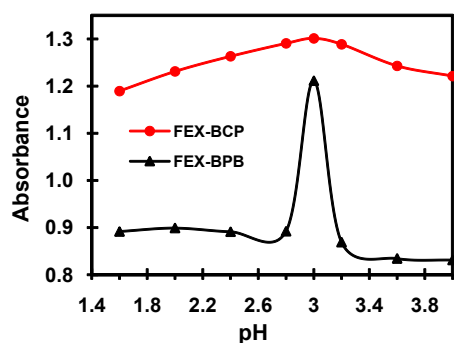
Absorption spectra of the yellow FEX-BCP and FEX-BPB ion-pair complexes extracted into chloroform with its  $\lambda_{\max}$  at 411 and 415 nm, respectively, are shown in Figure 1. The colorless blanks have practically negligible absorbance.

**Optimization of variables:** Optimum conditions necessary for rapid and quantitative formation of colored ion-pair complexes with maximum stability and sensitivity were established by a number of preliminary experiments. KCl-HCl buffer (Clark & Lubs buffer) was found to be suitable for both BCP and BPB methods. Chloroform was preferred to other solvents (carbon tetrachloride, dichloromethane and ether) for both methods for its selective and quantitative extraction. Optimum conditions were fixed by varying one parameter at a time while keeping other parameters constant and observing its effect on the absorbance at 411 and 415 nm for BCP and BPB, respectively. For BCP, effect of pH was studied (Figure 2) by extracting the colored complex species at different pH. Maximal absorbance was observed at the pH 3.0 using 3 mL of buffer (Figure 3). Similarly, for BPB, a maximal absorbance was observed at the pH 3.0 using 4 mL of buffer.

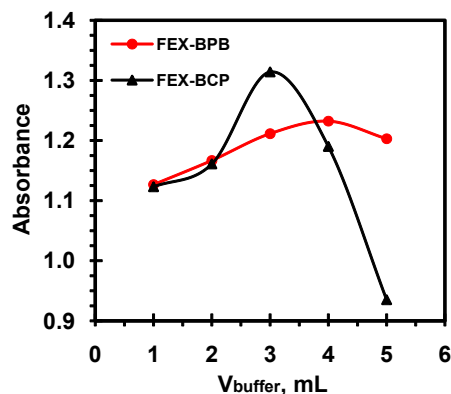




**Figure 1:** Absorption spectra of FEX - dye complexes extracted into 10 mL chloroform: (3) 0.25 mL of  $10^{-3}$  M FEX + 4 mL of  $10^{-3}$  M BPB + 4 mL of pH 3.0 buffer, (4) 0.52 mL of  $10^{-3}$  M FEX + 4 mL of  $10^{-3}$  M BCP + 3 mL of pH 3.0 buffer, against their respective blanks (1,2) vs. distilled water.



**Figure 2:** Effect of pH on the absorbance of FEX ( $28 \mu\text{g mL}^{-1}$ ) - dye complex.



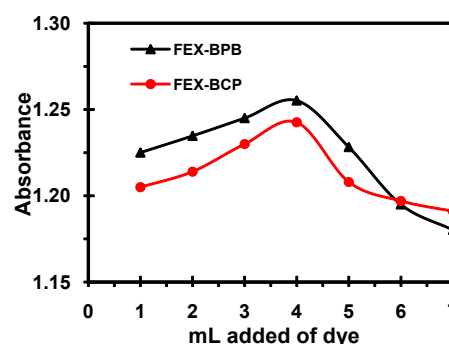
**Figure 3:** Effect of volume buffer on the absorbance of (●) FEX ( $28 \mu\text{g mL}^{-1}$ ) - BPB at pH 3.0 and 415 nm, (▲) FEX ( $28 \mu\text{g mL}^{-1}$ ) - BCP at pH 3.0 and 411 nm.

A volume of 4 mL of  $1 \times 10^{-3}$  M BCP or BPB was found to be optimal for complete complexation, since the absorbance was found to be maxima at the mentioned volumes. The effect of the reagent's concentration on the absorbance is shown in Figure 4.

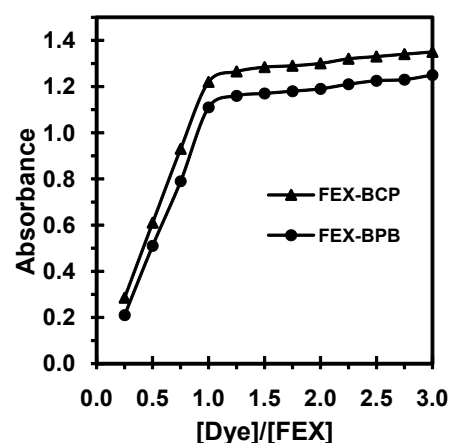
**Stoichiometric relationship:** The stoichiometric ratio of the drug to dye in each of the colored complexes was determined using the molar ratio method and Job's method of continuous variation [25]. It is apparent from the data that ion-pair complexes with drug to dye ratio 1:1 are formed (Figure 5 and 6).

The logarithmic stability constant of the formed complexes are calculated from the Harvey and Manning method [26] using the data of the molar ratio and continuous variation methods (Table 1).

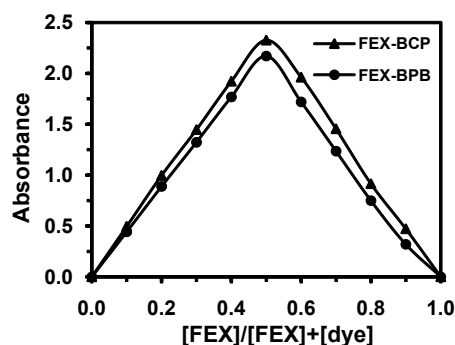
**Linearity and range:** The Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation and correlation coefficient determined for each method are given in Table 1. A linear relationship was found between the absorbance at  $\lambda_{\text{max}}$  and the concentration of the drug in the range 1.1-47.8 and 1.2-45.0  $\mu\text{g mL}^{-1}$  for BCP and BPB method, respectively, in the final measured volume of 10 mL. Regression analysis of the Beer's law plots at  $\lambda_{\text{max}}$  reveals a good correlation. The graphs show negligible intercept and are described by the regression equation,  $A = mC + b$  (where  $A$  is the absorbance of 1 cm layer,  $m$  is the slope,  $b$  is the intercept and  $C$  is the concentration of the measured solution in  $\mu\text{g mL}^{-1}$ ) obtained by the least-squares method [27]. The



**Figure 4:** Effect of reagent concentration on the formation of the yellow colored ion-pair complexes [FEX (▲) 28.6 and (●)  $27 \mu\text{g mL}^{-1}$ ].



**Figure 5:** Mole - ratio method of FEX-dye complexes ( $C_{\text{FEX}} = 5 \times 10^{-5}$  M).



**Figure 6:** Job's method of continuous variations of FEX-dye systems,  $C_{\text{FEX}} + C_{\text{dye}} = 2 \times 10^{-4}$  M.

Parameters	Extraction method with	
	BCP	BPB
$\lambda_{\text{max}}$ (nm)	411	415
pH	3.0	3.0
Logarithmic stability constant	6.64	6.70
Beer's law range ( $\mu\text{g mL}^{-1}$ )	1.1–47.8	1.2–45.0
Ringbom optimum concentration range ( $\mu\text{g mL}^{-1}$ )	3.5–25.0	2.2–28.0
Detection limit ( $\mu\text{g mL}^{-1}$ )	0.21	0.15
$\epsilon$ ( $\text{L mol}^{-1} \text{cm}^{-1}$ )	$2.75 \times 10^4$	$2.50 \times 10^4$
Sandell's sensitivity ( $\mu\text{g cm}^{-2}$ per 0.001 absorbance unit)	0.039	0.040
Regression equation <sup>a</sup>		
Slope (m)	0.0445	0.0439
Intercept (b)	0.0309	-0.0028
Correlation coefficient (r)	0.9999	0.9998
Recovery %	100.30 $\pm$ 0.38	100.16 $\pm$ 0.24

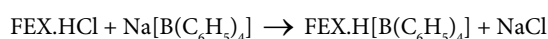
<sup>a</sup>With respect to  $A = mC + b$ , where C is the concentration ( $\mu\text{g mL}^{-1}$ ) and A is absorbance

**Table 1:** Statistical data of the regression equations for the determination of FEX with the proposed method I.

high molar absorptivities of the resulting colored complexes indicate the high sensitivity of the methods.

## Method II

Conductometric measurements can be used in quantitative titrations of ionic solutions in which the conductance of the solution varies before and after the equivalence point, so that two intersecting lines can be drawn to indicate the end-point. The shape of the titration curve depends on all the species present during the titration process and other factors such as viscosity, dielectric constant, solvation, ion-pair association and proton transfer. The results show an obvious inflection point in the conductance curve at drug-reagent molar ratio of 1:1 (FEX:TPB). The reaction may be represented by the equation:



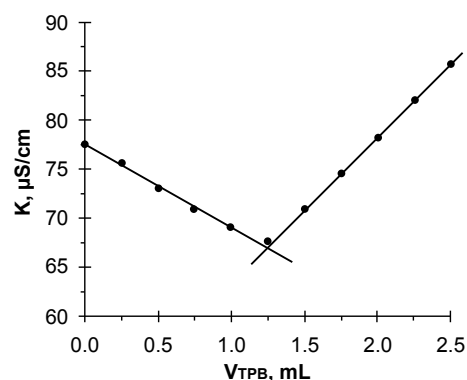
The optimum conditions for performing the titration in a quantitative manner were elucidated as described below.

Three different titrations were described: (i) aqueous drug solution with aqueous reagent solution; (ii) methanolic drug solution with methanolic reagent solution and (iii) ethanolic drug solution with ethanolic reagent solution. Preliminary experiments showed that procedure (i) was the most suitable for successful results, because

in procedures (ii) and (iii) precipitates were formed which caused some errors. The reagent concentration in each titration must be not less than ten times that of the drug solution in order to minimize the dilution effect on the conductivity throughout the titration. The optimum concentration of sodium tetraphenylborate was  $1 \times 10^{-2}$  moles  $\text{L}^{-1}$  to achieve a constant and highly stable conductance reading after one minute mixing. Concentration less than this led to unstable readings and more time was needed to obtain constant conductance values. On raising the temperature from  $20^\circ\text{C}$  to  $60^\circ\text{C}$ , a change in the shape of the titration curve was observed and so decreased the precision of the end-point. The relationship between the conductance values and the concentration of fexofenadine hydrochloride and sodium tetraphenylborate solutions was linear increasing in the range of 0.1–1.0 mmole  $\text{L}^{-1}$ . The conductance value of fexofenadine hydrochloride solution was greater than that for sodium tetraphenylborate solution at the same concentration with about three times.

Representative titration curve is shown in Figure 7. Two straight lines are obtained, intersecting at the end-point. The first branch gradually decreasing and the second sharply ascending. The decrease of conductance may be attributed to the formation of  $\text{FEX.H}[\text{B}(\text{C}_6\text{H}_5)_4]$  complex in the solution as a result of the reaction. After the end-point, the titration curve indicate a sharply increase of conductance. This may be due to the ionization of the reagent added.

In order to establish whether the proposed method exhibits any fixed or proportional bias, a simple linear regression [27] of observed drug concentration against the theoretical values (6 points) was calculated. Student's t-test (at 95% confidence level) was applied to the slope of the regression line (Table 2) and showed that it did not differ significantly from the ideal value of unity. Hence, it can be concluded that there are no systematic differences between the determined and true concentrations over the cited range. The standard deviation (SD) can be considered satisfactory, at least for the level of concentrations examined.



**Figure 7:** Conductometric titration curve of 25 mL of 0.5 mM FEX with 10 mM Na-TPB at  $20^\circ\text{C}$ .

Parameters	Na - tetraphenylborate
Optimum concentration range (mg/25ml)	2.50–13.45
Intercept of the regression line <sup>a</sup>	1.227
Slope of regression line	0.993
Student's t <sup>b</sup> (2.310) <sup>c</sup>	1.837
Range of error (%)	$\pm 0.74$

<sup>a</sup>Observed vs. theoretical. <sup>b</sup>Comparison with pharmacopoeial method [24]  
<sup>c</sup>Value in parenthesis is the theoretical t-value for five degrees of freedom

**Table 2:** Linear regression analysis for FEX using sodium tetraphenylborate.



## Validation of the methods

The validity of the methods for the analysis of FEX in its pure state and in its formulations was examined by analyzing the samples using the proposed procedures. The results obtained for pure drug are given in Table 3. The precision and accuracy of the methods were tested by analyzing six replicates of the drug. The low values of relative standard deviation (RSD) indicate good precision and reproducibility of the methods and the average percent recoveries obtained were quantitative, indicating good accuracy of the methods.

## Application to the pharmaceutical dosage forms

The proposed techniques were applied to the tablets and capsules. The ingredients in the tablets and capsules did not interfere in the experiments. The applicability of the proposed methods for the assay of fexofenadine hydrochloride in formulations was examined by analyzing various formulations and the results are tabulated in Table 4 were compared to the official non-aqueous titration method for fexofenadine [24] by means of *t*- and *F*-values at 95% confidence level. In all cases, the average results obtained by proposed methods and official method were statistically identical, as the difference between the average values had no significance at 95% confidence level. The low values of RSD show the results are reproducible. The proposed methods are simple, sensitive and reproducible and can be used for routine analysis of fexofenadine hydrochloride in pure form and in formulations. The commonly used additives such as starch, lactose, glucose, titanium dioxide, and magnesium stearate do not interfere with the assay procedures.

## Conclusion

The developed spectrophotometric and conductometric methods for the determination of fexofenadine hydrochloride are simple, accurate, precise and use simple reagents and apparatus. Therefore, this approach could be considered for the analysis of fexofenadine hydrochloride in the quality control laboratories. Methods are sufficiently sensitive to permit determination even down to 0.15 µg mL<sup>-1</sup>.

Method	µg mL <sup>-1</sup>		Relative error %	RSD %	Recovery %
	Taken	Found*			
BPB	1.20	1.19	-0.83	2.82	99.17
	5.00	5.01	0.20	1.61	100.20
	10.00	10.03	0.30	0.55	100.30
	20.00	20.06	0.30	0.50	100.30
	40.00	40.10	0.25	0.13	100.25
	45.00	45.16	0.35	0.11	100.36
BCP	1.10	1.08	-1.82	3.06	98.18
	5.00	5.02	0.40	1.64	100.40
	10.00	10.04	0.40	0.83	100.40
	20.00	20.00	0.00	0.61	100.00
	40.00	40.06	0.15	0.14	100.15
	47.00	47.10	0.21	0.12	100.21
Conductometry	100.0	99.80	-0.20	3.04	99.80
	200.0	200.20	0.10	1.18	100.10
	250.0	250.10	0.04	0.85	100.04
	300.0	300.07	0.02	0.81	100.02
	400.0	401.30	0.33	0.76	100.33
	500.0	502.40	0.48	0.61	100.48

\*Average of six determinations

**Table 3:** Determination of fexofenadine hydrochloride in pure forms by BPB, BCP and conductometric methods.

Drug	Label claim	% Found <sup>a</sup> ± SD			Official method
		Proposed methods			
		BPB	BCP	Conductometry	
Allergy stop	60 mg/ cap	100.80 ± 0.19 <i>t</i> = 1.99 <i>F</i> = 2.14	101.20 ± 0.21 <i>t</i> = 2.17 <i>F</i> = 2.61	101.04 ± 0.17 <i>t</i> = 1.91 <i>F</i> = 1.71	100.79 ± 0.13 <i>t</i> = 1.32
	120 mg/ tab	101.40 ± 0.20 <i>t</i> = 2.05 <i>F</i> = 1.78	100.80 ± 0.18 <i>t</i> = 1.75 <i>F</i> = 1.44	100.01 ± 0.19 <i>t</i> = 2.01 <i>F</i> = 1.60	101.05 ± 0.15 <i>t</i> = 1.28
	180 mg/ tab	99.88 ± 0.18 <i>t</i> = 1.68 <i>F</i> = 1.65	100.60 ± 0.20 <i>t</i> = 1.36 <i>F</i> = 2.04	99.91 ± 0.16 <i>t</i> = 1.73 <i>F</i> = 1.31	99.78 ± 0.14 <i>t</i> = 1.79
Fexodine	60 mg/ cap	99.76 ± 0.16 <i>t</i> = 2.20 <i>F</i> = 1.00	99.85 ± 0.19 <i>t</i> = 1.67 <i>F</i> = 1.41	100.95 ± 0.21 <i>t</i> = 1.82 <i>F</i> = 1.72	99.71 ± 0.16 <i>t</i> = 1.24
Fenadin	120 mg/ tab	101.32 ± 0.22 <i>t</i> = 1.91 <i>F</i> = 1.67	102.15 ± 0.21 <i>t</i> = 1.31 <i>F</i> = 1.52	100.22 ± 0.14 <i>t</i> = 1.07 <i>F</i> = 1.47	99.69 ± 0.17 <i>t</i> = 1.23
	180 mg/ tab	100.95 ± 0.20 <i>t</i> = 1.70 <i>F</i> = 1.23	101.08 ± 0.23 <i>t</i> = 1.12 <i>F</i> = 1.63	100.30 ± 0.32 <i>t</i> = 2.09 <i>F</i> = 3.16	100.52 ± 0.18 <i>t</i> = 1.81

\*Five independent analyses. At 95% confidence level *t*-value is 2.776 and *F*-value is 6.26

**Table 4:** Determination of FEX in different pharmaceutical formulations by the proposed and official methods.

The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients in the estimation. Moreover, the lower reagents consumption along with the short analytical run time leads to an environmentally friendly spectrophotometric procedure, which makes it especially suitable for routine quality control analysis work. The commonly used additives such as starch, lactose and magnesium stearate do not interfere with the assay procedures.

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