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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 681–690

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Simultaneous determination of fexofenadine and its related compounds by HPLC ☆

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Received 24 November 2001; received in revised form 27 February 2002; accepted 17 March 2002

Abstract

A simple reversed phase liquid chromatographic (RPLC) method has been developed and subsequently validated for the determination of fexofenadine hydrochloride and its related compounds A and B. The method utilizes a C8 column for the separation and determination of meta-isomer (related compound B). The separation was achieved using an Eclipse XDB C8, 5μm, 4.6 × 150 mm column and a mobile phase comprising 1% triethylamine phosphate (pH 3.7), acetonitrile and methanol in the ratio 60:20:20 (v/v/v). 5-Methyl 2-nitrophenol has been used as internal standard for the purpose of quantitation of fexofenadine. The described method was linear over a range of 0.7–18.7 μg/ml for related compounds A and B and 60–750 μg/ml for assay of fexofenadine. The relative standard deviation ($n = 3$) was 0.5% for the drug and 3.4% for related compounds. The intermediate precision was 0.79% ($n = 9$) for assay and 5.16% ($n = 9$) for related impurities. The mean recovery of both the related compounds were in the range of 94–103%. Limits of detection (LOD) and quantification (LOQ) for the related compounds A and B were 0.18, 0.12 and 0.56, 0.48 μg/ml, respectively. The precision of the method was checked by *F*-test using a reported method as reference and the calculated value (1.35) was found to be less than the table value at 95% confidence levels. The obtained results confirm that the method is highly suitable for its intended purpose.

Keywords: Fexofenadine; RPLC; Related compound A; Related compound B; C8 column

1. Introduction

Fexofenadine is the active acid metabolite of terfenadine and possesses the beneficial antihistaminic effects of the parent compound, but is devoid of the arrhythmogenic side effects of the later. It is undergoing review by the FDA for the treatment of seasonal allergic rhinitis [1]. The molecular structure of fexofenadine hydrochloride, (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-alpha,alpha-dimethyl benzene acetic acid hydrochloride is shown in Fig. 1.

Only a few LC methods have been reported for the determination of fexofenadine [2–9]. Furthermore, two LC methods have been described in US pharmacopeial Forum Previews [9], in which one

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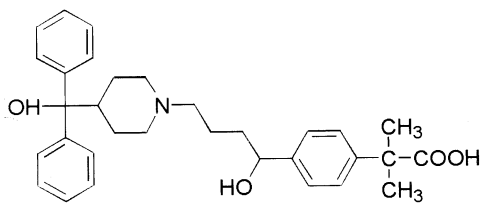
☆ DRF Publication number 180

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00181-4

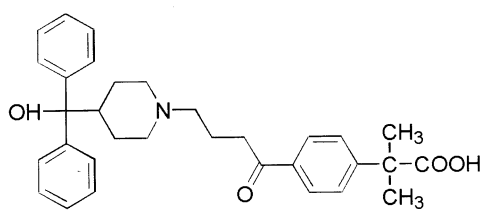
of the methods is used for the separation and determination of related compound-B (meta-isomer) using a expensive beta-cyclodextrin modified silica column (USP L45) and the other method for the determination of both fexofenadine and its related compound A using a phenyl bonded column (USP L11). No single method is reported for the simultaneous determination of fexofenadine and its related compounds A and B (Fig. 1).

The objective of this work was to develop an analytical LC procedure, which would serve as reliable and rapid method for the simultaneous determination of fexofenadine and its two impurities A and B.

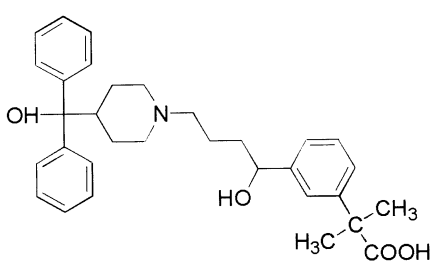
This manuscript describes the development and subsequent validation of isocratic reversed phase HPLC method using C8 column as stationary phase for the above determination. In the proposed LC method, the two impurities were well separated from fexofenadine and eluted before 25 min run time using 5-methyl 2-nitrophenol as internal standard. The precision of the described method for assay of fexofenadine has been checked in terms of *F*-test using a reported method as reference.



fexofenadine



related compound A (keto acid)



related compound B (meta-isomer)

Fig. 1. Structures of fexofenadine and its related compounds.

2. Experimental

2.1. Chemicals

Samples of fexofenadine hydrochloride and its related compounds A and B were received from Dr. Reddy's laboratories, Hyderabad, India. HPLC grade acetonitrile (99.7%, B. No. RK1 MF51011) was obtained from Merck, E-merck (India) Ltd., Mumbai, India. HPLC grade methanol (99.8%, B.No. R024M01) was purchased from Rankem, Ranbaxy, S.A.S. Nagar, India. Triethylamine (99.5%) was procured from Chemica, Fluka, Switzerland. Analytical reagent grade orthophosphoric acid (85%) was purchased from Qualigens Fine chemicals, Mumbai, India. Tablets of Altiva (120 mg of active drug) were purchased from the market. Capsules of Allegra (60 mg of active drug) were obtained through local pharmacy. 5-Methyl-2-nitrophenol was obtained from S.D. Fine chemicals, Mumbai, India. Milli-Q grade water was prepared by using Waters Milli-Q plus purification system, Millipore Corporation, USA.

2.2. Instrumentation

The LC system consisted of Waters 510 pump with 996 Photodiode array detector and a Rheodyne injector equipped with 10 μl sample loop was used for the chromatographic separation. The output signal was monitored and integrated using Millenium 2010 Chromatography Manager software (Waters).

2.3. Preparation of solutions

2.3.1. Mobile phase

Triethylamine (10 ml) was mixed and made upto the mark with Milli Q water in a 1-l volumetric flask. Six hundred ml of above aqueous triethylamine solution, adjusted to pH 3.7 with concentrated orthophospharic acid, was mixed with 200 ml of acetonitrile and 200 ml of methanol. The mixture was filtered and degassed through 0.45 μ Nylon filter using a Millipore vacuum pump.

2.3.2. Sample preparation

Sample solutions of fexofenadine and its related compounds were prepared using mobile phase as diluting solvent. The working concentration of fexofenadine for assay determination was 0.3 mg/ml and for related substances was 0.75 mg/ml. The concentration of the internal standard was maintained at 0.2 mg/ml in each solution of fexofenadine, used for validation studies.

2.3.3. Degradation of fexofenadine

For acid degradation, fexofenadine sample was refluxed with 0.1 N HCl at 60 ± 1 °C for 12 h and then neutralized by adjusting the pH to 7.0 with NaOH. The solution was further diluted to required concentration with mobile phase.

For basic degradation, fexofenadine sample was refluxed with 0.1 N NaOH at 60 ± 1 °C for 12 h and then neutralized the solution by adjusting pH to 7.0 with HCl. The solution was further diluted to required concentration with mobile phase.

For oxidative degradation, fexofenadine sample was refluxed with 3% H_2O_2 for 3 h and then diluted to required concentration with mobile phase.

For photo and thermal degradations, two separate solutions of pure fexofenadine (0.5 mg/ml in mobile phase) were prepared. One solution was exposed to ultraviolet light (254 nm) for 18 h, and the other was kept at 70 °C temperature for 12 h.

2.4. LC conditions

The LC conditions for the determination of fexofenadine and its related compounds were using UV detection set at a wavelength of 210 nm and an injection volume of 10 μl. The chromatographic separations were performed using Eclipse XDB C8, 5 μm, 150×4.6 mm column (Agilent Technologies) with a mobile phase flow rate of 1.2 ml/min and at ambient temperature (~ 22 °C).

3. Results and discussion

3.1. Method development

According to US pharmacopeial Previews [9], the separation between fexofenadine (*para*-form) and related compound B (*meta*-form) can be achieved using a chiral column, which is very expensive. However, this separation can be done using less-expensive reversed phase columns. In the method development stage different reversed phase columns, containing C18, C8, phenyl, NH_2 and CN as stationary phases, and mobile phase systems were employed to achieve the separation between these positional isomers. Initial conditions using sodium phosphate or acetate buffers at different pH values (ranging from 3 to 8) in mobile phase preparations didnot give any indication of separation on all the columns employed. After the introduction of triethylamine phosphate buffer at pH 3.5 in the mobile phase, a marginal separation was observed on a C18 stationary phase. Interestingly very good separation was achieved on a C8 stationary phase with the same mobile phase containing varying proportions of triethylamine phosphate buffer and organic solvents such as acetonitrile and methanol. Finally the mobile phase consisting of 1.0% triethylamine phosphate (pH 3.7), acetonitrile and methanol (60:20:20, v/v/v) at a flow rate of 1.2 ml/min was found to be an appropriate mobile phase allowing adequate separation of three compounds using a Eclipse XDB C8 150 mm column. The typical chromatogram of fexofenadine sample spiked with two impurities recorded using the proposed method is shown in Fig. 2a. In the present method the selectivity was found to be more than 1.14 with a resolution more than 2.7 for the separation of fexofenadine and its impurities. System suitability results of the developed method are presented in Table 1.

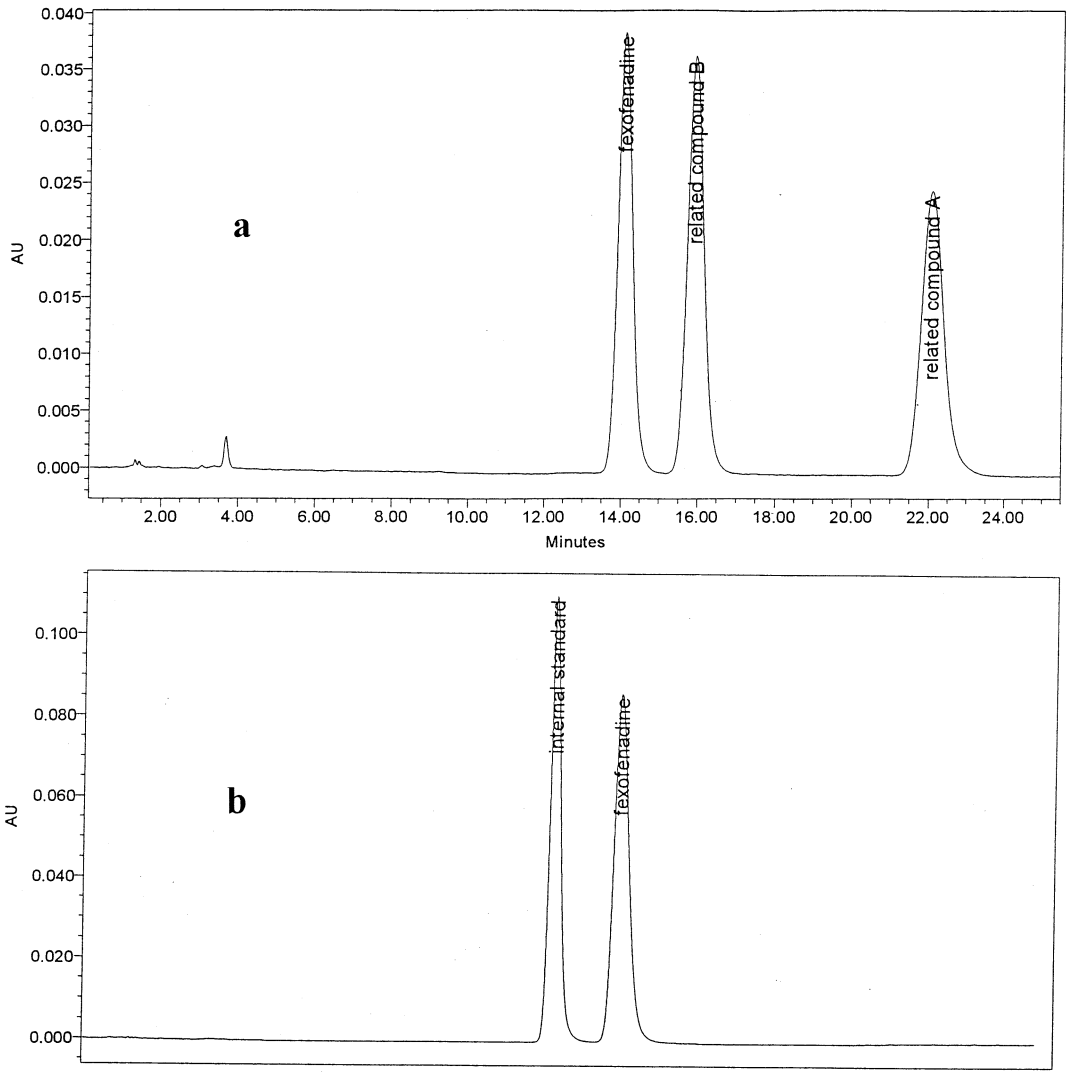


Fig. 2. Representative chromatograms: (a) separation of fexofenadine and its related compounds, (b) formulated fexofenadine (Allegra 60 mg) with internal standard (conditions as described in Section 2).

Table 1
System suitability results of the method

Compound	k^a	RRT[a]	α^a	R^a	N^a	T^a	R.S.D. (area precision)
Fexofenadine	13.06	–	–	–	6515	1.173	0.42
Related compound B	14.89	1.13	1.14	2.72	6658	1.173	0.38
Related compound A	21.05	1.56	1.41	6.71	6786	1.212	0.53

k, capacity factor; α, selectivity; R, resolution (USP); N, number of plates(USP); RRT, relative retention time; T, tailing factor (USP); dead volume (k_0), 1.0; conc., 50 μg/ml; R.S.D., relative standard deviation for area of six injections (instrument precision).
[a] $n = 3$.

Selectivity of the method was checked by using four different columns of different manufacturers, which are nearly equivalent to Eclipse XDB C8 column.

The columns used for study were: (1) Waters, Symmetry C8 150 mm, (2) Waters, Symmetry Shield RP8 150 mm, (3) Shimadzu Shimpak CLC C8 150 mm and (4) Waters, Novapak C8 150 mm. The separation between all the compounds was achieved on each of the columns. But the resolution between fexofenadine and related compound on Symmetry Shield RP8 and Novapack C8 columns were found to be only 1.2 and 1.5, respectively. On Symmetry C8 and Shimpack CLC C8 columns, the resolution was more than 3.0 with little bit longer retention times. These two columns can be used as an alternative to Eclipse XDB C8 column for the above determinations.

3.2. Quantification of related compounds

For the quantification of impurities, a High–Low chromatographic technique [10] was used. In this technique a concentrated (2.0 mg/ml) sample of fexofenadine was injected and the response of each impurity was recorded. A typical chromatogram of High–Low chromatography of fexofenadine is shown in Fig. 3. The impurities can be identified by matching the UV spectrum and the retention times with that of standards. Weight percentages of each impurity present in fexofenadine sample (in 2 mg/ml) were calculated using its peak response and relative response factors (RRFs). The RRFs of related compounds A and B with respect to fexofenadine were found to be 1.022 and 1.072, respectively, at the detection wavelength of 210 nm. RRF is the ratio of the peak response per unit concentration for the each impurity to the peak response per unit concentration for the reference compound (fexofenadine) under the given analytical conditions. The weight percentage of related compounds present in fexofenadine sample were calculated using its RRF values and peak responses [11]. The unknown impurities UK1 and UK2, eluted at retention times 10.4 and 18.9 min, respectively, were not characterized (Fig. 3). UV absorption spectra of these impurities were similar to that of fexofenadine. Therefore, the RRF for these unknowns was taken as equal to 1.0 and their weight percentages were calculated.

3.3. Method validation

The described method has been extensively validated for assay and related substances of fexofenadine using the following parameters. 5-Methyl 2-nitrophenol used as internal standard for the purpose of quantification of fexofenadine.

3.3.1. Specificity

Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components.

To demonstrate the specificity of the method, all the possible known impurities discussed above were added to pure fexofenadine sample and the mixture was analyzed for assay and the results were compared with pure sample results. Reproducibility was observed in both the cases (R.S.D. < 2.0)

Accelerated degradation studies were also performed to demonstrate the validity of the method. The samples, refluxed with 0.1 N NaOH or 0.1 N HCl or subjected to high temperature or exposed to UV light, did not give any degradation products. With oxidative degradation, fexofenadine mostly converted to an oxidative degradation product (eluted at 1.2 min) that was not characterized.

Photodiode array detection was used as evidence of the specificity of the method, and to evaluate the homogeneity of the peak. Chromatographic peak purity was determined using wavelength comparison (210 vs. 220 nm)[12]. The samples exposed to acidic, basic, oxidative, thermal, and UV stress conditions were subjected to photo-diode array analysis for peak purity of fexofenadine. The plot reports with flat tops in all instances showed that fexofenadine peak had no detectable impurity peaks embedded in and are free of co-eluting degradation compounds. From above results, it is clear that the proposed method can be used for determining the stability of fexofenadine as bulk and pharmaceutical formulations.

3.3.2. Linearity

Linearity was checked by preparing standard solutions at five different concentration levels ranging from 60 to 750 μg/ml. The linearity was also checked for 3 consecutive days for the solutions of same concentrations prepared from the stock solution (2.5 mg/ml). The precision for inter day linearity is below 1.6% R.S.D. The equation for calibration curve was $y = 3.72x + 0.025$ ($Sy/x = 0.003$, $Sb = 0.0055$ and $Sa = 0.0023$). The correlation coefficient was found to be more than 0.999, indicating good linearity.

3.3.3. Accuracy

The accuracy of the method for assay determination was checked at three concentration levels i.e. at 300, 450, and 600 μg/ml ($n = 3$) for 3 consecutive days. Solutions for the standard curves were prepared fresh every day. The percentage recoveries are tabulated in Table 2. Fur-

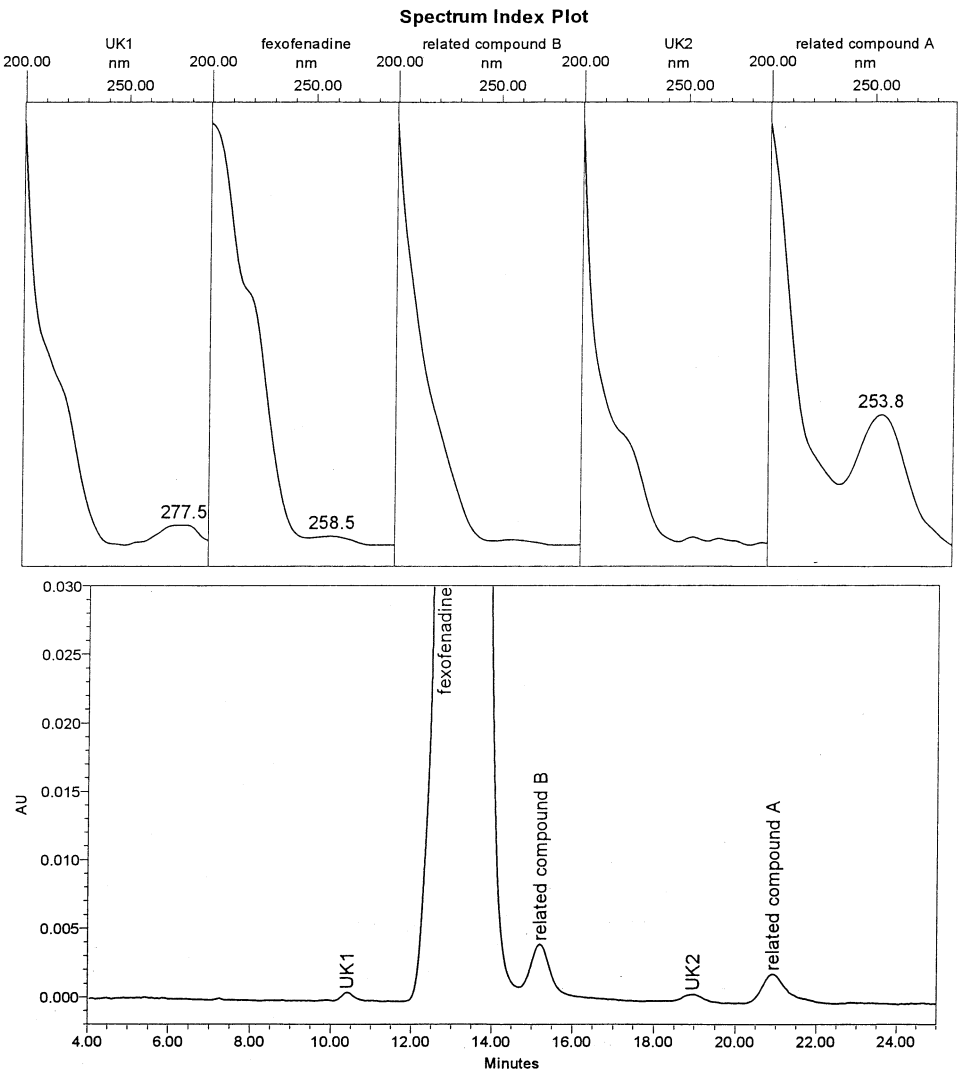


Fig. 3. High–low chromatogram of fexofenadine (2 mg/ml) with spectrum index plot using PDA detection (conditions as described in Section 2).

Table 2
Precision and accuracy in the assay determination of fexofenadine

Day of analysis	Taken (mg)	Recovery (mg) ($n = 3$) Mean ± C.V[a])	Percentage of recovery
Repeatability			
0 day	0.304	0.300 ± 0.50	98.68
	0.442	0.438 ± 0.45	99.10
	0.613	0.616 ± 0.34	100.4
1 day	0.304	0.302 ± 0.49	99.44
	0.442	0.440 ± 0.34	99.61
	0.613	0.617 ± 0.37	100.7
2 day	0.304	0.305 ± 0.39	100.23
	0.442	0.443 ± 0.33	100.29
	0.613	0.619 ± 0.37	101.03
Intermediate precision			
Inter day	0.304	0.302 ± 0.79	99.34
	0.442	0.442 ± 0.61	100.0
	0.613	0.617 ± 0.29	100.65

[a] Coefficient of variation.

Table 3
F-test for comparison of methods

	Assay results from proposed method ($n = 9$)	Assay results from reference method ($n = 9$)	F-test $= s_A^2/s_B^2$	Value from table ($F_{(8,8)}$)
Bulk drug	Mean: 99.93, S.D.:1.00	Mean: 100.0, S.D.: 1.04	1.08	4.43
Formulation (Allegra 60 mg)	Mean: 100.36, S.D.: 0.80	Mean: 100.52, S.D.: 0.93	1.35	4.43

ther, the accuracy of the method was checked by *F*-test using a reported method [9] as reference. The results are presented in Table 3. The calculated value of *F*-test is less than the table value at 95% confidence levels indicates that the developed method and reference methods not to differ significantly in terms of precision and accuracy.

Standard addition and recovery experiments were also conducted to determine the accuracy of the present method for the quantification of related compounds A and B. The range of addition levels of impurities to the parent compound was done at 0.15–0.75% of the concentration (0.75 mg/ml) of the fexofenadine. The recovery of each impurity was calculated from the slope and intercept of the calibration curve drawn in the concentration range 0.75–15 μg (0.1–2.5%) using its authentic standard [13]. The equation of calibration curve used for the recovery studies of related compound B was $y = 27472x + 7930$ and for the related compound A was $y = 25160x - 3410$ in the concentration range of 0.8–12.8 and 0.84–14.4 μg, respectively. The intercept values were found to be less than the 10% of area response produced by 2% concentration level [14]. The mean recovery of both the impurities were found to be in the range of 94–103%. The recovery data is presented in Table 4.

3.3.4. Precision

The precision of the method for the determination of assay and related compounds of fexofenadine were studied using the parameters viz. repeatability and intermediate precision. Repeatability is the intra-day variations in assay or in recoveries of related compounds obtained at different concentration levels, which are expressed in terms of R.S.D. values calculated from the data of each day for 3 days. R.S.D. values of assay and recoveries of related compounds were found to be

Table 4
Recovery of fexofenadine related compounds

Compound	Added (μg)	Recovered (μg) ($n = 3$) (Mean ± C.V[a])	Percentage of recovery
Repeatability			
Related compound B	1.12	1.06 ± 3.48	94.6
	2.48	2.53 ± 2.11	102.0
	5.17	5.32 ± 1.57	102.9
Related compound A	1.23	1.17 ± 3.0	95.1
	2.35	2.30 ± 2.35	97.8
	4.85	4.98 ± 1.51	102.6
Intermediate precision ($n = 9$)			
Related compound B	1.12	1.07 ± 4.90	95.5
	2.48	2.54 ± 3.35	102.4
	5.17	5.28 ± 1.89	102.1
Related Compound A	1.23	1.15 ± 5.16	93.4
	2.35	2.33 ± 2.82	99.1
	4.85	5.03 ± 2.01	103.7

[a] Coefficient of variation.

well below 0.5 (Table 2) and 3.48% (Table 4), respectively.

The intermediate precision is the inter-day variation at the same concentration levels, determined on successive days. The intermediate precision for assay and related compounds of fexofenadine were found to be 0.79 R.S.D. (Table 2) and 5.16% R.S.D. (Table 4), respectively.

3.3.5. Robustness

To determine the robustness of the method, experimental conditions were purposely altered and the effects on chromatographic characteristics were evaluated. The effect of change in buffer pH on retention (capacity factor k') of fexofenadine, and related compounds A and B is shown in Fig. 4. The retention of all the three compounds was increased with decrease of pH. The resolutions between fexofenadine and related compound B and related compounds A and B were varied from 2.7 to 2.8 and 6.3 to 8.7, respectively, with decrease of pH (4.0–3.0).

In another experiment, column temperature varied from 20 to 40 °C in steps of 5 °C each time. The increase in temperature resulted in decrease in retention of all the compounds. The resolution between fexofenadine and related compound B was found to be constant as 2.7 and the resolution between related compounds A and B

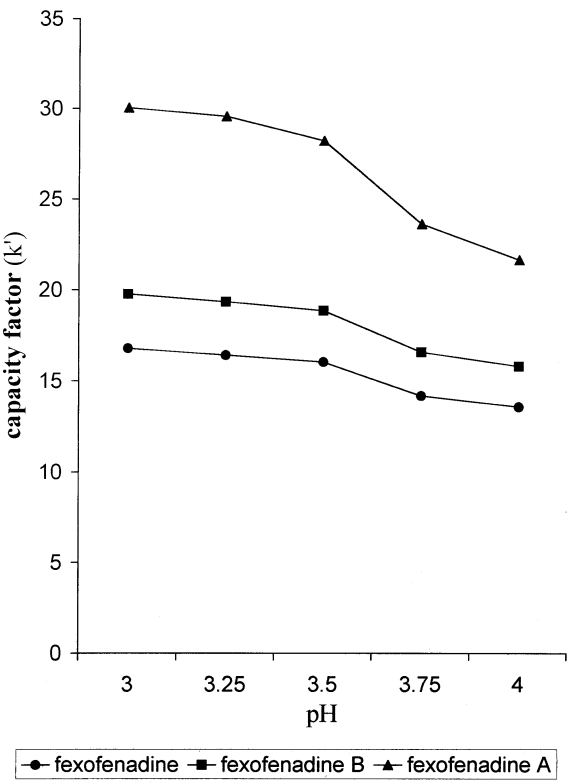


Fig. 4. Effect of change in buffer pH on retention of fexofenadine and its impurities.

Table 5
Assay determination of fexofenadine in formulations

Formulation	Amount taken (mg)	Amount recovered (mg) ($n = 3$)	Percentage of recovery
Allegra (60 mg)	0.322	0.318	98.7
	0.483	0.488	101.0
	0.644	0.649	100.7
Altiva (120 mg)	0.261	0.262	100.3
	0.392	0.396	101.0
	0.522	0.530	101.5

was varied from 7.7 to 5.8 with increase of temperature (20–40 °C). Effect of percent organic strength on retention was studied by varying the percentage of acetonitrile from -4 to $+4\%$ while other mobile phase components were held constant as stated in conditions. The increase of percentage of acetonitrile in the mobile has decreased the retention and resolution of all the compounds.

3.3.6. Limits of detection (LOD) and quantification (LOQ)

The detection sensitivity can be demonstrated by the LOD. A signal-to-noise ratio of approximately 2–3 is generally considered to be acceptable for estimating the detection limit, which is the lowest concentration that can be detected. The LOD of related compounds A and B by the proposed method were found to be 0.18 and 0.12 μg/ml, respectively. The quantitation limit is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. A typical signal-to-noise ratio is 10:1. The LOQ of related compounds A and B were found to be 0.56 and 0.48 μg/ml, respectively.

3.3.7. Stability

In routine testing in which many samples are prepared and analyzed every day, it is essential that solutions are stable enough to allow for delays such as instrument breakdowns or overnight analysis using auto samplers. The stability of the fexofenadine in solution containing the mobile phase and the internal standard was determined for the samples stored in refrigerator and at room temperature. The samples were checked after 3 successive days of storage and the data were compared with freshly prepared samples. In each case the R.S.D. values of assay were found to be below 2.0% R.S.D. This indicates that the fexofenadine is stable in the solution for at least 3 days and compatible with internal standard.

3.4. Assay determination of fexofenadine from formulation samples

Twenty capsules of Allegra (60 mg of active drug) and 20 Altiva (120 mg of active drug) tablets were extracted separately into methanol and centrifuged. The supernatant liquid was diluted with mobile phase to the required concentrations and analyzed. The results of the extracted samples from both the formulations at three different concentrations were ($99 \pm 1.5\%$) comparable with the claimed values (Table 5). A typical LC chromatogram shown in Fig. 2b indicates that the fexofenadine and the internal standard are well separated from the excipient peaks in the formulation sample (Allegra 60 mg).

4. Conclusion

An isocratic-reversed phase HPLC method has been developed and subsequently validated for the simultaneous determination of fexofenadine and its two impurities A and B using C8 column as stationary phase to avoid expensive chiral column and longer analysis time. The developed method was found to be selective, precise and stability indicating. The accuracy of the proposed method has been checked in terms of *F*-test using a reported method as reference. The obtained results confirm that the method is highly suitable for its intended purpose and is useful in the quality

control of bulk manufacturing and pharmaceutical formulations.

Acknowledgements

The authors wish to thank the management of Dr. Reddy's group for supporting this work. We would also like to thank the colleagues in separation science division of Analytical Research of Dr. Reddy's Research Foundation for their co-operation and encouragement in carrying out this work.

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