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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF FEXOFENADINE IN PHARMACEUTICAL DOSAGE FORM BY USING LEVOCETIRIZINE AS AN INTERNAL STANDARD

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ABSTRACT

A simple, accurate and precise RP-HPLC method was developed and validated for determination of Fexofenadine in pharmaceutical dosage form by using Levocetirizine as an Internal standard (IS). The separation was achieved by Cap Cell Pack C18 column (250 × 4.5 mm, 5µ) column using acetonitrile: water (50:50 % v/v) as eluent at a flow rate of 1 mL/min, detection was carried out at 224 nm. The retention times for Fexofenadine and IS were found at 4.79 and 6.22 mins, respectively. Linearity was observed over the concentration ranging from 50-175 µg/mL and it was found to be linear with y = 0.011x + 0.168 (r^2 = 0.997). The precision of the method was demonstrated with % RSD values of < 2% while the % recovery was found in between 101.3-101.5%. Interference of the any additive components of formulations was not observed. Based on results obtained the proposed method was found to be accurate, specific and precise and could be applied to quantitative analysis of Fexofenadine by using Levocetrizine as IS.

KEY WORDS

Fexofenadine, Internal standard, Levocetirizine, RP-HPLC and recovery.

INTRODUCTION

Fexofenadine (FEX) is an anti-histamine drug which is used in the treatment of allergyic symptoms such as hay fever, nasal congestion and urticaria[1]. It is a second-generation anti-histamine and acts as peripheral H1-blocker. The molecular name of FEX is (±)-4-[1-Hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidin-yl]-butyl]-α, α-dimethylbenzene acetic acid (Fig 1). It is less permeable to blood brain barrier and causes less sedation when compared to first generation anti-histamines. It is usually taken orally in the form of tablet or suspension form, after oral administration it reaches maximum plasma concentration within few hours. It is usually taken after a high fat meal so that concentration of drug will reduce based upon the dosage forms, 60-70 % of drug was distributed in plasma and albumin. It metabolises in the liver and eliminated through feaces.

In literature, various analytical methods such as Spectroscopic[1-7], HPLC [8-18], HPTLC [19] and LCMS [20] were reported for quantification of FEX either in individual or in combined dosage forms both in formulations and biological matrices. In our study an attempt was made to develop a more precise and simpler RP-HPLC method based on use of Internal Standard (IS). In this study, Levocetrizine (Fig 1) an anti-histamine drug which exhibits structural similarities to that of FEX, hence it was considered as an IS. The method developed was found to be more appropriate in estimation of FEX in tablets dosage forms and also it was validated in terms of various analytical parameters as per ICH guidelines.

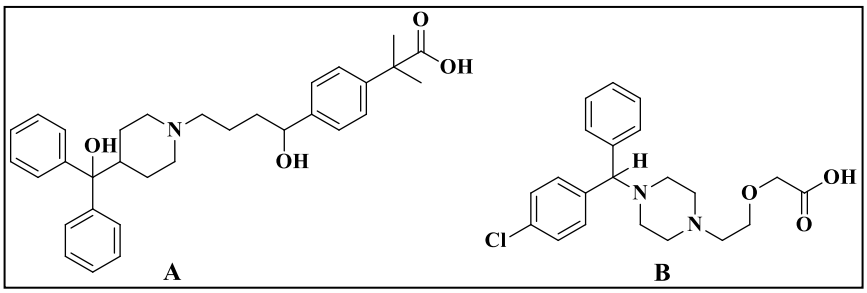

Fig 1: Structure of FEX (A) and Levocetrizine (IS, B).

MATERIALS AND METHODS

Instruments used:

Cyberlab HPLC (LC 100) accomplished with Cap Cell Pack C18 column, quantitative HPLC was performed on an isocratic mode with 20 µL injection sample loop. The output signal was monitored and integrated using Cyberlab LC 100 software. Double Beam UV-Visible Spectrophotometer (Lab India 3000^{+}) was used for detection of wavelength for HPLC analysis.

Chemicals and reagents:

The FEX and IS were procured as gift samples from Granules India Ltd, Hyderabad, India. The HPLC grade acetonitrile and water were purchased from Merck Life Sciences, Mumbai, India. Other reagents and solvents were analytical grade, purchased from SD Fine Chemicals Ltd, Mumbai, India.

Preparation of mobile phase:

A combination of acetonitrile and water (50:50 % v/v) was prepared and degassed with ultra-sonication for about 15 min. The resultant solution was filtered through 0.45 µ membrane filter.

Preparation of standard stock solution:

Transferred accurately 100 mg of FEX into 100 mL volumetric flask, containing 50 mL of diluent. The contents were dissolved and made up to the mark to get concentration of 1000 µg/mL. From this solution 10 mL was pipette out into another 100 mL volumetric flask containing 50 mL of diluent and sonicated to dissolve the contents. To this an equal volume of IS (50 µg/mL) is added and made up the volume, filtered the solution through 0.45 µ membrane filter.

Preparation of sample solution:

Weighed accurately twenty tablets of Allerga (FEX) and the powder equivalent to 100 mg of drug transferred in to 100 mL volumetric flask and added 50 mL of diluent. The solution was sonicated to dissolve and made up the volume to the mark with diluent. Transferred 10 mL of the above solution into another flask and added 50 mL of diluent. To this an equal quantity of IS (50 µg/mL) was added, sonicated to dissolve the contents and made up the volume to 100 mL (100 µg/mL). The resulted solution was filtered through 0.45 µ membrane filter.

Optimized chromatographic conditions:

Several trails were done for optimization of the RP-HPLC method by changing different chromatographic conditions such as mobile phase ratio, flow rate and pH. The optimization of chromatographic conditions was achieved in acetonitrile: water (50: 50 % v/v) as mobile phase at flow rate of 1 mL/min (Table 1).

Table 1: Optimized chromatographic conditions

Parameters	Chromatographic conditions
Column	Cap Cell Pack C18 (250×4.6 mm, 5 µ) column
Elution mode	Isocratic
Mobile phase	Acetonitrile: water (50:50 % v/v)
Flow rate	1.0 mL/min
Detection wavelength	224 nm
Injection volume	20 µL
Run time	10 min
Temperature	28 °C

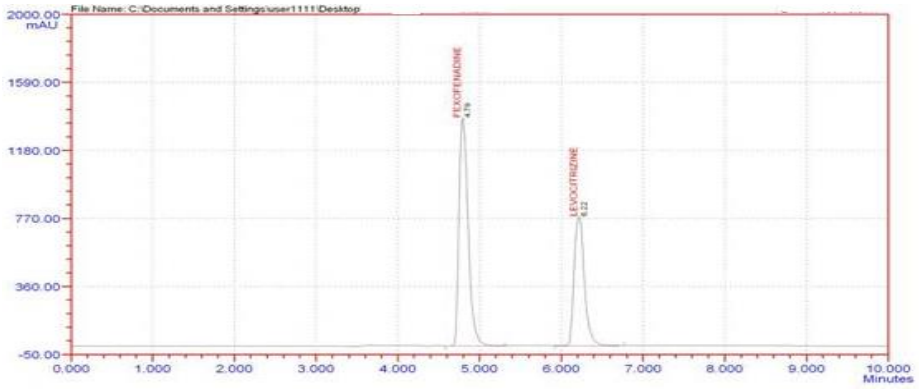

Fig 2: HPLC Chromatogram of FEX and IS in acetonitrile: water (50:50 % v/v).

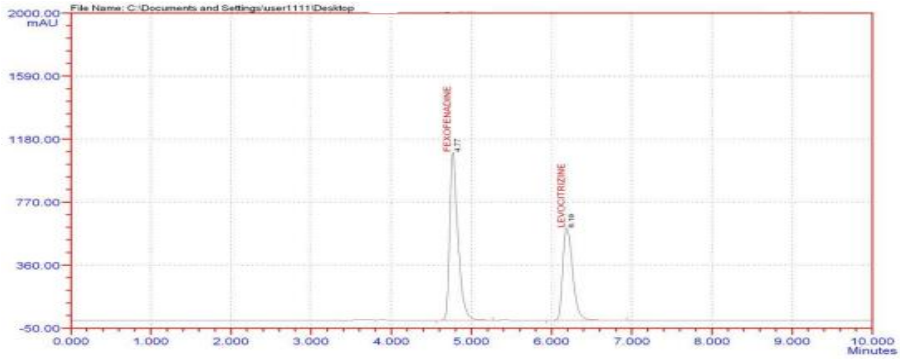

Fig 3: Chromatogram for sample (FEX) and IS in Acetonitrile: water (50:50 % v/v).

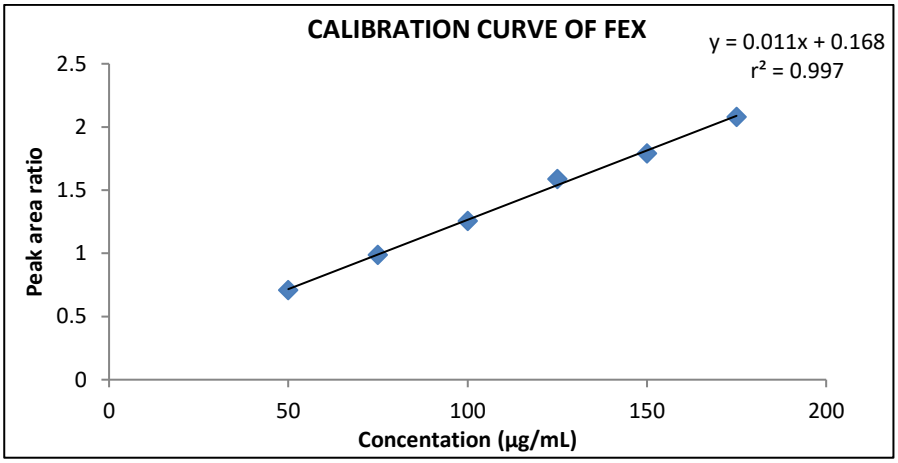

Fig 4: Calibration curve of FEX (concentration v/s peak area ratio of FEX/ IS).

Method validation

The optimized chromatographic method was validated for different parameters like system suitability, specificity, linearity, accuracy, precision, robustness, LOD and LOQ as per ICH guidelines[20, 21].

System suitability:

It was carried out for the assessment of system suitability of the equipment for the analysis. The test was carried out by injecting six replicate injections of standard solution. The results were validated for theoretical plates (N), tailing factor, % RSD and peak height.

Linearity:

A series of standard drug solutions were prepared in the concentration ranging from 50-175 µg/mL of FEX with 50 µg/mL of IS in each and injected into the chromatographic system to demonstrate linearity studies by using single plot. A calibration curve was plotted on peak area ratio of drug to IS v/s concentration (µg/mL) of FEX.

Accuracy:

Accuracy of the method was evaluated at 3 different concentration levels (at 50, 100, 150 % levels) by addition of known amounts of standard to the analyte

sample. For each concentration level, 3 sets were prepared and each injected three times and the results were recorded. The accuracy was expressed as percentage (%) analyte recovered by the proposed method.

Precision:

Precision was determined as repeatability; system precision was carried out by using the standard solution and method precision was carried out by using sample solution. The solutions were prepared as per the optimized method and injected into the chromatographic system for six times and % RSD was calculated by using peak area ratio.

Robustness:

Robustness study was conducted to determine the deliberate variations in the optimized chromatographic conditions like flow rate (1 ± 0.2 mL/min) and wavelength (224 ± 2 nm). The test was carried out by using the standard solutions.

Limit of detection (LOD) and limit of quantification (LOQ):

The LOD and LOQ were determined by using signal to noise ratio and were calculated by using 3.3σ/s and 10σ/s respectively, where 'σ' is the standard deviation of peak area ratio and 's' is the slope of calibration curve.

RESULTS AND DISCUSSION

Selection of UV detection wavelength:

The standard solutions of FEX and IS were prepared in the concentration of 100 µg/mL in different diluents (solvent systems). The resulted solution was scanned in the UV region (200-400 nm). After multiple scanning the overlain spectrum for FEX and IS was obtained from appropriate spectrum. Based on the peak absorption maxima of analyte (FEX) and IS, the detection wavelength was observed at 224 nm. It was also repeated by taking pharmaceutical dosage form for both FEX and IS.

Assay:

The sample solution was prepared and injected into the chromatographic system, the drug content in the tablet was quantified by using regression equation and the results were given in Table 2.

Table 2: Assay results for optimized method

Formulation	Label claim amount (mg)	% Amount found
Allerga	120	101.2%

Method validation

System suitability:

The working standard solution of FEX and IS were injected into HPLC system for five times. The system suitability parameters were evaluated from standard chromatograms by observing retention time, number of theoretical plates (N), tailing factor, peak area and peak height, the data was expressed in Table 3**.**

Table 3: System suitability parameters

Parameters	FEX	IS
Peak area (mV)	78673.2	54823.2
Retention time (min)	4.79	6.22
Tailing Factor	1.58	1.45
Theoretical plate number (N)	8413	10036
Height	75840	40521

Specificity:

The solutions of standard and sample were prepared as per the test method and injected into the chromatographic system. The chromatograms of standard and sample were found to be identical with same retention times.

Linearity:

The standard calibration curve was found to be linear over the concentration ranging from 50-175 µg/mL. The calibration curve was plotted for peak area ratio v/s concentration of FEX. The regression equation of calibration curve obtained was y = 0.011x + 0.168 and correlation coefficient (r^2) was 0.997. The linearity data

was showed in Table 4, whereas calibration curve graph was shown in Fig 4.

Table 4: Linearity data of FEX

S. No.	Concentration (µg/mL)		Peak area ratio (Drug/IS)	Linear regression equation
	FEX	IS		
1	50	50	0.715	
2	75	50	0.987	y = 0.011x+ 0.168
3	100	50	1.256	r^2 = 0.997
4	125	50	1.589	
5	150	50	1.791	
6	175	50	2.081	

Accuracy:

The accuracy was carried out at three concentration levels (50 %, 100 %, and 150 %). The % recovery obtained was found to be within the limits of 98-102 %. The results obtained were shown in Table 5.

Table 5: Accuracy data of FEX

S. No.	Spiked level	Sample area (n=3)	Sample height (n=3)	% recovery
1	50 %	78697.2	39789	101.3 %
2	100 %	157394.4	79578	101.5%
3	150 %	235091.6	119367	101.3%

Precision:

The results obtained from method precision showed in optimum range. The %RSD obtained was found within the acceptance limits (<2%). The results of system precision and method precision were shown in Table 6.

Table 6: Method precision of FEX

Injection no.	Retention time (mins)	Peak area ratio (drug/IS)
1	4.7	1.862
2	4.7	1.873
3	4.7	1.806
4	4.7	1.823
5	4.7	1.864
6	4.7	1.887
Mean	4.7	1.862
S. D	-	1.873
% RSD	-	1.806

S.D = Standard Deviation; **% RSD** = Percent Relative Standard Deviation

Robustness:

The optimized conditions were altered deliberately for evaluation of robustness of the method. The deviations obtained by deliberate changes were present within the limit the data was presented in table 7.

Table 7: Robustness data of FEX

Parameter	Chromatographic conditions	Peak area ratio 1 (Drug/IS)	Peak area ratio 2 (Drug/IS)	Mean peak area ratio	SD	%RSD
Flow rate (mL/min)	0.8 (low)	1.987	1.976	1.981	0.008	0.403
	1.0 (medium)	1.752	1.768	1.760	0.004	0.227
	1.2 (high)	1.623	1.652	1.637	0.002	0.122
Wavelength (nm)	222 (low)	2.064	2.052	2.069	0.010	0.338
	224 (medium)	1.986	1.952	1.969	0.014	0.711
	226 (high)	1.624	1.653	1.638	0.017	1.037

LOD and LOQ:

The LOD and LOQ of FEX was determined by using signal to noise ratio and they are calculated by using standard deviation of response (σ) and slope (s) of calibration curve. The LOD and LOQ were found to be 0.27 µg/mL and 0.84 µg/mL, respectively.

CONCLUSION

In present work a new RP-HPLC method has been developed for estimation of FEX by using Levocetrizine as an IS, in which a well resolved peak was observed for IS from that of analyte. A RP Cap Cell Pack C18 column (250 ×4.6 mm, 5µ) with mobile phase consisting of acetonitrile and water (50:50 % v/v) was used. The flow rate was 1 mL/min and the elution was monitored at 224 nm using UV detector. The peaks of standard (FEX) and IS were eluted at 4.79 and 6.22 mins, respectively. The developed method was found to be satisfactory with good precision, linearity and accuracy. It was validated according to ICH guidelines and all the results found to be within the specified limits. Hence the proposed RP-HPLC method was found to be simple, accurate, economical, and rapid can be applied for routine analysis in quality control of FEX in its pharmaceutical dosage forms.

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