Research Article

RP-HPLC METHOD DEVELOPMENT AND ITS VALIDATION FOR THE ESTIMATION OF FEXOFENADINE IN RAT PLASMA AND ITS APPLICATION FOR PHARMACOKINETIC STUDIESM.V. Kumudhavalli^{1*}, B. Jayakar², M. Kumar², P. Palanisamy²¹Department of Pharmaceutical Analysis, Vinayaka Missions College of Pharmacy, Vinayaka Missions University, Salem, Tamil Nadu, India²Vinayaka Missions College of Pharmacy, Vinayaka Missions University, Salem, Tamil Nadu, India

*Corresponding Author Email: palanisamy2907@gmail.com

Article Received on: 28/03/14 Revised on: 02/04/14 Approved for publication: 07/05/14

DOI: 10.7897/2230-8407.050587**ABSTRACT**

A simple, sensitive and rapid reverse phase performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of fexofenadine from small volumes of rat plasma. The Sample preparation was very simple and it involves protein precipitation method with ACN. From the results of all the validation parameters and applicability of the assay. The present method can be useful for pre-clinical pharmacokinetic studies of fexofenadine with desired precision and accuracy along with high-throughput. The developed analytical method is for fexofenadine in reverse phase chromatographic method utilizing C₁₈ column (250 mm × 4.6 mm, 5 μ). The peak was detected using a fluorescence detector set at Ex 220 nm and Em 280 nm. The mobile phase used was 1M triethylamine pH-4.0; Acetonitrile in the ratio of 65:35. The retention time of fexofenadine was found to be 8.25 minutes. The total chromatographic run time was 12.0 minutes. The flow rate of the mobile phase was 1.0 ml/min at room temperature. Method was found to be linear from 2 to 500 ng/ml for Fexofenadine ($r^2 \geq 0.9998$). The results indicate the bio-analytical method is linear, precise and accurate. The developed method was validated and found suitable for application in designing pharmacokinetic studies and simplified solvent system.

Keywords: Fexofenadine, RP-HPLC, Rat Plasma.**INTRODUCTION**

Fexofenadine (SP) (+)-2-[4-[1-hydroxy-4-[4-(hydroxydiphenyl methyl)piperidino]butyl[phenyl]-2-methyl propionic acid¹.

It is an antihistamine with selective peripheral H₁-receptor antagonist activity. It inhibits antigen-induced bronchospasm in sensitized guinea pigs and histamine release from peritoneal mast cells in rats. Absorption of Fexofenadine is rapid and complete, the bioavailability of fexofenadine capsule and tablet formulations are equivalent when administered in equal doses. The pharmacokinetics of fexofenadine is linear for oral doses up to 240 mg a day (120 mg twice a day). About 5 % of the total dose is metabolized approximately 0.5 to 1.5 % by cytochrome P450 3A4 isoenzyme metabolism and 3.5 % transformed to a methyl ester metabolite by intestinal micro flora. Renal and fecal excretion of Fexofenadine is 11 % and 80 % respectively²⁻⁵. HPLC uses a liquid mobile phase to transport the analyte (sample) through the column, which is packed with a stationary phase material. The mobile phase is continuously pumped at a fixed flow rate through the system and mixed (if required) by the pump. The injector is used to introduce a plug of sample into the mobile phase without having to stop the mobile phase flow, and without

introducing air into the system. The mixture of components is carried in a narrow band to the top of the column. Some compounds in the sample mixture will have a greater preference for the stationary phase than the mobile phase and will be retained in the column longer. Those components that are not retained as strongly are carried by the mobile phase down the column. The longer the column, the more opportunities for interaction with the stationary phase and there will be greater separation. As the components elute from the column they pass into a detector – where some physico-chemical property of the analyte produces a response from the detector. This response is amplified and plotted against time – giving rise to a ‘chromatogram’⁶⁻⁷. The main objective of this work was to develop and validate a RP-HPLC method for the quantification of fexofenadine in Rat plasma samples and its applicability to pharmacokinetic study. Different methods have been reported for detection and quantification of fexofenadine but they are expensive and time consuming. Objective of this method is to develop a sensitive and precise method which can overcome the above drawbacks and validate as per US FDA guidelines. Application of the developed method for analysis of plasma samples obtained from pharmacokinetic study in male SD rats.

MATERIALS AND METHODS**Chemicals**

Fexofenadine was gifted by Central Drug Research Institute, (Lucknow, India). Acetonitrile, triethylamine, methanol, ortho-phosphoric acid, potassium dihydrogen phosphate, ammonium acetate, plasma (blank) and tripple distil water were procured from Merck Laboratories.

Instrumentation and Chromatographic Condition

The HPLC method was performed using a Shimadzu LC-20 AT HPLC pump with a Rheodyne injector, Shimadzu RF 10-A XL fluorescence detector and Shimadzu Class-VP integrator. The developed analytical method is for fexofenadine in reverse phase chromatographic method utilizing C₁₈ column (250 mm × 4.6 mm, 5 μ). The peak was detected using a fluorescence detector set at Ex 220 nm and Em 280 nm. The mobile phase used was 1M triethylamine pH-4.0: Acetonitrile in the ratio of 65:35. The retention time of fexofenadine was found to be 8.25 minutes. The total chromatographic run time was 12.0 minutes.

Preparation of Standard Stock Solutions

A quantity of 2 mg of fexofenadine working standard was weighed and transferred to 10 mL volumetric Flask. It was dissolved in diluent (ACN: triethylamine pH4, 60:40). And required volume was made up with the same to achieve 1 μg/mL (1000 ng/mL) strength of the standard.

Preparation of Calibration Curve Standards

Various aliquots of stock solution (1 μg/mL = 1000 ng/mL) were diluted with the diluents (ACN: triethylamine, 60:40) to get different concentrations ranging from 2 – 500 ng/mL. This was done just prior to the preparation of spiked samples.

Preparation of Spiked Calibration Curve Standards

For preparation of calibration curve standard, first of all working stocks of different concentration ranging from 5000 ng/mL-20 ng/mL have to be prepared and then through parallel dilution spiked calibration curve standard can be prepared.

Extraction Trial

Step 1: Thawed samples were vortexed to ensure complete mixing of contents.

Step 2: 90 μL of above thawed blood sample were pipetted into respective RIA vials.

Step 3: 10 μL of above samples (Table 7) were pipetted into respective RIA vials.

Step 4: 900 μL of extracting solvent (ACN/MeOH) were added into respective RIA Vials.

Step 5: The above solution was vortex for 3 min

Step 6: The solution was centrifuged for 10 minutes at 13000 rpm at 28°C

Step 7: Separated 800 μL supernatant samples were transferred in respective labeled RIA vials.

Step 8: Separated 800 μL supernatant samples were dried in Heto MAXI dry plus (Denmark) for 2 hours at 50°C

Step 9: Reconstituted with 100 μL of mobile phase and vortexed.

Step 10: Loaded the processed sample into RP-HPLC.

Finalized Chromatographic Condition

Mobile Phase → ACN: 1M triethylamine-(pH- 4.0), 35:65 (%v/v)

Column Temp → Ambient

Flow rate → 1 mL/min

Injection volume → 20 μL

Run time → 20 minutes

Detector → Fluorescence detector (Ex: 220 nm, Em: 280 nm)

Column → LichroCART C18, (250 mm × 4.6 mm, 5 μm)

At lower concentration such as 2 ng/mL, the concentration is detectable and peak shape is also good. The method show Linearity from 2 ng/mL to 500 ng/mL

Preparation of Spiked Quality Control Samples

The quality control samples prepared using diluents were taken and spiked with plasma to achieve the predicted concentration range. Quality control samples concentration were fixed as per US FDA guidelines.

Reconstitution Solution

Reconstitution solution was prepared by mixing acetonitrile and triethylamine (1M, pH-4) in the ratio 35:65 %v/v.

RESULT AND DISCUSSION

HPLC Method Development

The chromatographic detection of fexofenadine was achieved on the Lichro CART C18 column (250 mm × 4.6 mm × 5 μm) using isocratic elution with 1M triethyl acetate buffer and acetonitrile as mobile phase (65:35). The flow rate of the mobile phase was 1.0 mL/min at room temperature. The retention time of fexofenadine was 8.2 minutes. The peak shape of the fexofenadine was found to be symmetrical.

Specificity and Selectivity

Procedure

Blank plasma, stock of fexofenadine in mobile phase and stock of fexofenadine spiked in plasma prepared and analyzed. Figure-1 shows a chromatogram for the control plasma (free of analytes) and Figure 8 shows chromatogram for analyte in mobile phase. Figure 9 shows the rat plasma spiked with fexofenadine. The retention time of fexofenadine was 8.25 minutes. The total chromatographic run time was 12.0 minutes.

Figure.1: Rat blank plasma

Figure 2: Analyte spiked in Mobile phase

Figure 3: Rat plasma sample spiked with Fexofenadine

There was no interference observed in the region of drug a Freeze Thaw injecting processed blank plasma sample obtained from rats. This indicated that the method was specific for the determination of drug in SD- rat plasma. The extraction procedure and chromatographic conditions yielded a clean chromatogram for the drug.

Recovery

Procedure

The percentage recovery of fexofenadine was determined by measuring the peak area of the prepared plasma quality control samples (HQC, MQC, LQC) with the peak area of the un-extracted aqueous standard containing the same concentrations of fexofenadine. The following formula is used to calculate recovery

$$\% \text{ Recovery} = \frac{\text{Mean peak area of extracted sample} \times 100}{\text{Mean peak area of aqueous sample}}$$

Table 1 Recovery study of lower quality control (LQC) sample

QC ID	Aqueous LQC Area	Spiked LQC Area	% Recovery
1	82396	77451	93.99
2	76853	69347	90.23
3	79256	65236	82.31
4	78643	64789	82.38
5	79462	66909	84.20
6	79468	64321	80.93
Mean			85.68
SD			5.23
%CV			6.10

Table 2: Recovery study of middle quality control (MQC) sample

QC ID	Aqueous MQC Area	Spiked LQC Area	% Recovery
1	1177814	1012920	86
2	1094673	976380	89.19
3	1285043	994550	77.39
4	1174694	1023769	87.15
5	1065387	987450	92.68
6	1378943	1014546	73.57
MEAN			84.33
SD			7.32
%CV			8.68

Table 3: Recovery study of higher quality control (HQC) sample

QC ID	Aqueous LQC Area	Spiked LQC Area	% Recovery
1	6347851	4903449	77.25
2	5985195	4903799	81.93
3	6189954	5395674	87.17
4	6410940	5434567	84.77
5	6190584	5075691	81.99
6	6073178	5689457	93.68
MEAN			84.46
SD			5.60
CV			6.63

Table 4: Percentage recovery for fexofenadine

	% Recovery	% CV
LQC	85.68 ± 5.23	6.10
MQC	84.33 ± 5.60	8.68
HQC	84.46 ± 5.60	6.63

Recovery of fexofenadine from spiked plasma samples (low, medium and high QC) was consistent and recovery from plasma was found to be more than 84 %. The % CV values are within the acceptable limits (< 15 %).

Analytical Standard Curve

Table 5: Accuracy data of calculated concentration of analytical samples for Fexofenadine in mobile phase

Sample ID	Concentration (ng/mL)	Area	Calculated Concentration	% Accuracy
AS1	2	39549	2.14	107.03
AS2	5	72602	4.47	89.4
AS3	10	140015	9.22	92.22
AS4	50	707712	49.24	98.47
AS5	100	1415424	99.12	99.12
AS6	200	2923213	205.40	102.7
AS7	500	7076045	498.12	99.62

The % accuracy of observed for back-calculated concentration for analytical standard curves was within 89.41 to 107.03.

Figure 4: Calibration curve for analytical standard of fexofenadine

Calibration Standard Curve

Table 6: Accuracy data of Calculated concentration of calibration standard of Fexofenadine in rat plasma

Sample ID	Concentration (ng/mL)	Area	Calculated Concentration	% Accuracy
CS-1	2	26594	2.37	118.56
CS-2	5	66985	5.34	106.8
CS-3	10	127700	9.80	98.02
CS-4	50	657850	48.77	97.53
CS-5	100	1339700	98.88049	98.88
CS-6	200	2749400	202.4892	101.24
CS-7	500	6788500	499.3509	99.87

The % accuracy of fexofenadine observed for back-calculated concentration for calibration curves was within 97.53-106.79 .

Linearity

Procedure

The calibration curves were constructed with seven concentrations including the LLOQ ranging from 2 to 500 ng/ml. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Acceptance criteria

The correlation coefficient should be ≥ 0.99 and regression line should be linear for given concentration (2 – 500 ng/ml).

Figure 5: Calibration curve for Fexofenadine

Table 7: Linearity of Fexofenadine

Concentration (ng/ml)	Observed Concentration	% Accuracy	% RE
2	2.37	118.56	-18.55
5	5.34	106.8	-6.80
10	9.8	98.02	1.98
50	48.77	97.53	2.47
100	98.88	98.88	1.12
200	202.49	101.25	-1.24
500	499.35	99.87	0.13
Slope	13606		
Intercept	-5668.3		

The values are in the acceptable limits and hence the method is linear in the range mentioned above.

Sensitivity

Procedure

Limits of detection (LOD) and quantitation (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that

provided a peak area. LOD and LOQ decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte by the method whereas LOQ is the minimum quantifiable concentration. On serial dilution and analysis by the method, LOQ was found to be 2 ng/ml for the analyte. Fexofenadine was having the LOD of 0.5 ng/ml.

Figure 6: Typical chromatogram of LOQ

Accuracy and Precision

Intraday Precision and Accuracy

Intraday precision

The intraday precision of fexofenadine was performed using LLOQ, LQC, MQC and HQC. Concentration of LOQC, LQC, MQC and HQC

Acceptance Criteria

The mean should be within 15 % of the actual value except at LLOQ, where it should not deviate by more than 20 %.

Procedure

Samples (n = 6) of each QC sample were analyzed. The % CV was calculated.

Figure 7: Typical chromatogram of HQC sample

Figure 8: Typical chromatogram of MQC sample

Figure 9: Typical chromatogram of LQC sample

Table 8: Intraday Precision and Accuracy

QC ID	LLOQ	LQC	MQC	HQC
Actual concentration (ng/ml)	2	6	75	400
1	2.15	6.12	74.86	360.8
2	2.20	5.51	72.17	387.73
3	2.08	5.21	73.51	396.98
4	2.13	5.18	75.66	399.84
5	2.39	5.33	72.99	373.46
6	2.38	5.14	74.98	418.57
Mean	2.23	5.42	74.03	389.56
SD	0.12	0.37	1.23	20.45
%CV	5.58	6.75	1.66	5.25
% Accuracy	111.28	90.25	98.70	97.39

The intra-day accuracy of fexofenadine of LOQQC was 111.28 %. For LQC, MQC, HQC it ranged from 90.25 % to 98.70 %. The intra-day precision of fexofenadine at LOQQC was 5.58 %. For LQC, MQC, HQC it ranged from 1.66 % to 6.75 %. The accuracy and precision was within accepted criteria.

Inter day accuracy

The total accuracy for fexofenadine was performed using LOQQC, LQC, MQC and HQC. Concentration of LOQQC, LQC, MQC, and HQC was shown in Table 15.

Acceptance Criteria

The mean value should be within 15 % of the actual value except at LLOQ, where it should not deviate by more than 20 %. The deviation of the mean from the true value serves as the measure of accuracy.

Procedure

Inter-Day Precision and Accuracy

Samples (n = 4) of each QC sample were analyzed on three different days. The % CV, % Accuracy was calculated.

Inter day precision

Inter day Precision for fexofenadine was performed using LOQQC, LQC, MQC and HQC. Concentration of LOQQC, LQC, MQC, and HQC was shown in Table 23, 24, 25.

Acceptance Criteria

The mean value should be within 15 % of the actual value except at LLOQ, where it should not deviate by more than 20 %. The deviation of the mean from the true value serves as the measure of accuracy.

Procedure

Samples (n = 4) of each QC sample were analyzed on three different days. The % CV was calculated and % accuracy were calculated.

Table 9: Day 1 Inter-day precision and accuracy

QC ID	LOQQC	LQC	MQC	HQC
Actual concentration (ng/ml)	2	6	75	400
1	2.24	5.79	75.59	407.28
2	2.29	5.35	73.49	399.8
3	2.25	5.04	73.73	402.94
4	2.32	4.97	77.15	410.5
Mean	2.26	5.04	75.74	405.13
SD	0.026	0.06	1.49	4.72
%CV	1.17	1.2	1.96	1.16
% Accuracy	113.03	88.14	100.99	101.28

Table 10: Day 2 Interday precision and accuracy

QC ID	LOQQC	LQC	MQC	HQC
Actual concentration (ng/ml)	2	6	75	400
1	2.45	5.32	75.2	402
2	2.3	5.04	73.84	400
3	2.24	5.19	73.74	393.3
4	2.3	5.0	76.01	402.93
Mean	2.3	5.14	74.7	399.79
SD	0.12	0.14	1.1	4.54
%CV	5.33	2.81	1.48	1.14
% Nominal	115.40	85.70	101.35	99.94

Table 11: Day 3 Interday precision and accuracy

QC ID	LOQQC	LQC	MQC	HQC
Actual concentration (ng/ml)	2	6	75	400
1	2.24	5.16	73.67	392
2	2.31	5.33	73.83	391
3	2.17	5.09	74.49	388
4	2.17	5.14	73.47	402
Mean	2.24	5.18	73.87	392
SD	0.07	0.11	0.44	5.78
%CV	3.16	2.04	0.6	1.47
% Accuracy	112.03	86.38	98.5	98.18

The inter-day accuracy of fexofenadine of LOQQC ranges from 112.03 % to 113.03 %. For LQC, MQC, HQC it ranged from 86.38 % to 115 %. The inter-day precision of fexofenadine at LOQQC was 5.58 %. For LQC, MQC, HQC it ranged from 1.66 % to 6.75 %. The inter-day accuracy and precision was within the accepted criteria.

Bench-Top Stability Procedure

Five-replicates of low and high concentration QC samples were removed from the freezer and stored unprocessed at room temperature for a period of 6 hours. Processed QC samples were evaluated against a freshly spiked calibration curve using chromatographic conditions. The bench top stability duration was calculated as the difference between the time QC samples were processed and the time the samples were removed from the freezer (in hours). QC concentrations were tabulated and the mean, standard deviation, % CV and % accuracy values were determined.

Table 12: Bench-Top Stability data (6 h) for compound Fexofenadine

Concentration	Stability	Mean conc. $\pm$ S.D.	CV (%)	Accuracy (%)
LQC (6 ng)	0 h	5.42 $\pm$ 0.37	6.75	90.25
	Bench-Top Stability (6 h)	5.16 $\pm$ 0.06	1.16	86.06
HQC(400 ng)	0 h	389.57 $\pm$ 20.45	5.25	97.39
	Bench-Top Stability (6 h)	371.42 $\pm$ 11.74	3.61	92.86

The mean percentage nominal of bench top stability samples calculated against freshly prepared samples at LOQ and HQC were 90.25 % and 97.39 % respectively. The mean percentage accuracy of stability samples when compared with

comparison samples at LQC and HQC were 80.06 % and 90.86 %, respectively demonstrating acceptable bench top stability for at least 26 hours at room temperature.

Table 13: Freeze and Thaw 2 Stability data for compound Fexofenadine

Concentration	Stability	Mean conc. $\pm$ S.D.	CV (%)	Accuracy (%)
LQC (6 ng)	0 h	5.42 $\pm$ 0.37	6.75	90.25
	Freeze and Thaw 2 Stability	5.51 $\pm$ 0.37	6.67	91.85
HQC(400 ng)	0 h	389.6 $\pm$ 20.42	5.25	97.39
	Freeze and Thaw 2 Stability	412.2 $\pm$ 20.94	5.08	103.05

Freeze and thaw stability Procedure

The freeze and thaw stability in matrix was assessed by analyzing five replicates of QC samples at low and high concentrations previously frozen and thawed over multiple cycles against freshly prepared calibration curve standards. Freeze and thaw cycle consists of first freezing at/or below -70°C for 24 hours and other cycles for a minimum of 12 hours followed by thawing unassisted at room temperature. The freeze and thaw stability was evaluated at the end of first and third cycle. The QC concentrations were tabulated and

the mean concentration, SD, % CV and % Accuracy values were determined at low and high QC levels.

Freeze thaw 2

Six aliquots at each of the low and high concentrations with plasma were stored at the storage temperature -80°C for 24 hours and thawed at room temperature. When completely thawed, the samples were refrozen for 12 to 24 hours under the same conditions, and then processed and analyzed.

Freeze thaw 3

Six aliquots at each of the low and high concentrations were stored at the storage temperature -80°C for 24 hours and

thawed at room temperature. The freeze-thaw cycle was repeated two more times, and then processed and analyzed.

Table 14: Freeze and Thaw 3 Stability data for fexofenadine

Concentration	Stability	Mean conc. $\pm$ S.D.	CV (%)	Accuracy (%)
LQC (6 ng)	0 h	5.42 $\pm$ 0.37	6.75	90.25
	Freeze and Thaw 3 Stability	5.6 $\pm$ 0.13	2.26	93.39
HQC (400 ng)	0 h	389.6 $\pm$ 20.42	5.25	97.39
	Freeze and Thaw 3 Stability	385.04	6.61	96.25

The mean percentage accuracy of freeze thaw 2 stability samples calculated against freshly prepared samples at LQC and HQC were 90.25 % and 97.39 % respectively. The mean percentage accuracy of stability samples when compared with comparison samples at LQC and HQC were 91.85 % and 103.05 %, respectively demonstrating acceptable two freeze thaw stability cycles. The mean percentage accuracy of freeze thaw 3 stability samples calculated against freshly prepared samples at LQC and HQC were 90.25 % and 97.39 % respectively. The mean percentage accuracy of stability samples when compared with comparison samples at LQC and HQC were 93.39 % and 96.25 %, respectively demonstrating acceptable three freeze thaw stability cycles.

Long-Term Stability Procedure

The storage time in a long-term stability evaluation should exceed the time between the date of first sample collection and the date of last sample analysis. Long-term stability should be determined by storing at least three aliquots of each

of the low and high concentrations under the same conditions as the study samples. The volume of samples should be sufficient for analysis on three separate occasions. The concentrations of all the stability samples should be compared to the mean of back-calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing.

Long Term Stability for 7 Days: Six aliquots at each of the low and high concentrations with plasma were stored at the storage temperature -80°C for 7 days and thawed at room temperature. Then processed and analyzed.

Long Term Stability for 30 Days: Six aliquots at each of the low and high concentrations with plasma were stored at the storage temperature -80°C for 30 days and thawed at room temperature. Then processed and analyzed. A Freeze Thaw analysis we observed that fexofenadine is stable for 30 days at all concentration.

Acceptance criteria

% Deviation should be < 15 %.

Table 15: Long term Stability (7 days) data for compound Fexofenadine

Concentration	Stability	Mean conc. $\pm$ S.D.	CV (%)	Accuracy (%)
LQC (6 ng)	0 h	5.42 $\pm$ 0.37	6.75	90.25
	Long term Stability (7 days)	5.97 $\pm$ 0.11	1.86	99.42
HQC (400 ng)	0 h	389.6 $\pm$ 20.42	5.25	97.39
	Long term Stability (7 days)	433.67 $\pm$ 11.34	2.61	108.42

Table 16: Long term Stability (30 days) data for compound fexofenadine

Concentration	Stability	Mean conc. $\pm$ S.D.	CV (%)	Accuracy (%)
LQC (6 ng)	0 h	5.42 $\pm$ 0.37	6.75	90.25
	Long term Stability (30 days)	6.23 $\pm$ 0.03	0.62	103.90
HQC (400 ng)	0 h	389.6 $\pm$ 20.42	5.25	97.39
	Long term Stability (30 days)	386.83 $\pm$ 19.36	5	96.71

The mean percentage accuracy of stability samples (7 days Long Term stability) calculated against freshly prepared samples at LQC and HQC were 90.25 % and 97.39 % respectively. The mean percentage accuracy of stability samples when compared with comparison samples at LQC and HQC were 91.85 % and 103.05 %, respectively demonstrating acceptable two freeze thaw stability cycle.

Pharmacokinetic Study**Animals**

The pharmacokinetic study of fexofenadine was carried out in young and healthy male rats (n = 5) weighing ~250 g. The animals were obtained from Laboratory Animal Services Division of CDRI, and housed in cages in standard laboratory conditions with a regular 12 h day-night cycle. The prescribed diet was given and water *ad libitum*. The animals were acclimatized for 2 days before carrying out the pharmacokinetic study and kept overnight in fasted condition. The study protocol was approved by local ethical committee of the institute.

Preparation of the Formulation

15 mg fexofenadine was taken in a mortar and pestle. 200 μ l of Tween 20 was added to it and then 0.25 % carboxy methyl cellulose solution was added drop by drop and the mixture was triturated until a suspension was obtained. The suspension was transferred to a 5 ml vial and the volume was made up to 3 ml by 0.25 % carboxy methyl cellulose suspension. The dose was calculated such that a 250 g rat received 500 μ l of the formulation which contained 2.5 mg of the drug. The dose was 10 mg/kg body weight.

Sampling Schedule

The pharmacokinetics of fexofenadine were investigated following a single dose of approximately 10 mg/kg fexofenadine (which corresponds to the clinical dose of 120 mg in humans) orally. Blood samples were withdrawn at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8 and 10 hour post dosing and collected in heparinised tubes. Plasma was separated by centrifugation at 14000 rpm for 5 minutes at 4°C within 1 h

of sampling and was stored at -80°C until analysis. The blood samples were withdrawn from the retro orbital plexus.

Sample Analysis

Sample analysis was carried out by using 100 μl plasma by the bio analytical method described in above session.

The rat plasma samples generated following administration of fexofenadine were analyzed by the newly developed and

validated method along with QC samples. All the QCs met the acceptance criteria (data not shown). The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the pharmacokinetics of fexofenadine in rats. Profiles of the mean plasma concentration versus time are shown in Figure.

Figure 16: The plasma concentration time profile of fexofenadine

Maximum concentration in plasma ($C_{\max}$ 154 $\pm$ 26.7 ng/mL) was achieved at 0.5 h ($t_{\max}$) which shows its faster absorption. All other pharmacokinetic parameters were as shown in Table.

Table18: Pharmacokinetic Parameters in male SD rats

Pharmacokinetic Parameters in male SD rats at 10mg/kg dose	
$T_{\max}$ (h)	0.5
$C_{\max}$ (ng/ml)	154 $\pm$ 26.7
$T_{1/2}$ (h)	1.81 $\pm$ 0.096
AUC ₀₋₁₀ (ng. h/ml)	476.5 $\pm$ 35.64
AUC _{0-∞} (ng. h/ml)	492.2 $\pm$ 12.59
MRT (h)	3.26 $\pm$ 0.16

CONCLUSION

Author have developed and validated a highly sensitive, specific, and reproducible and high-throughput RP-HPLC assay to quantify fexofenadine from small volumes of rat plasma. Sample preparation is very simple and it involves protein precipitation method with ACN. From the results of all the validation parameters and applicability of the assay, we can conclude that the present method can be useful for pre-clinical pharmacokinetic studies of fexofenadine with desired precision and accuracy along with high-throughput. The developed analytical method is for fexofenadine in reverse phase chromatographic method utilizing C_{18} column (250 mm $\times$ 4.6 mm, 5 μ). The peak was detected using a fluorescence detector set at Ex 220 nm and Em 280 nm. The mobile phase used was 1M triethylamine pH-4.0: Acetonitrile in the ratio of 65:35. The retention time of fexofenadine was found to be 8.25 minutes. The total chromatographic run time was 12.0 minutes. The flow rate of the mobile phase was 1.0 ml/min at room temperature. Method was found to be linear from 2 to 500 ng/ml for Fexofenadine ($r^2 \geq 0.9998$). Intra- and inter-day assay precision ranged from 1.66 to 6.75 and 1.66 to 6.75, respectively, and intra- and inter-day assay accuracy was between 90.25 to 98.70 % and 86.38 to 115 % respectively. The values of R.S.D are less than 15 %, indicating the accuracy and precision of the method. Stability of Fexofenadine in rat plasma was > 86.0 % in the battery of stability studies viz., bench-top, freeze/thaw cycles and 30 days storage in a freezer at -80°C . The assay was successfully applied to determine the pharmacokinetic parameters in

Sprague–Dawley rats after an oral administration of Fexofenadine at 10 mg/kg. As a result, the plasma half-life was 1.81 $\pm$ 0.096 h and the AUC (0– ∞) was 492.2 $\pm$ 12.59 ng/mL. The maximum plasma concentration ($C_{\max}$) 154 $\pm$ 26.7 ng/mL was achieved at 0.5 h ($t_{\max}$).

ACKNOWLEDGEMENTS

Authors are thankful to Prof (Dr.) B. Jaykar, Principal Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu, India and providing all the facilities for this research project.

REFERENCES

1. Merck Index, Published by Merck and Co. Inc. White house Station, NJ., 12th edition; 1996. p. 1062.
2. Goodman and Gilman's, Drug Therapy of Depression and Anxiety Disorders, 11th edition; 2006.
3. Tripathi DK. Anti Depressant Drugs, Essential of Medical Pharmacology,
4. Jaypee Brothers Medical Publishers Pvt. Ltd., New Delhi, Fifth edition; 2004. p. 413.
5. Drug bank.
6. Remington Psychopharmacologic Agents, The Science and Practice of Pharmacy, Published by Lipincott Williams and Wilkins, London; 21st edition, volume 2; 2006. p. 1521.
7. Beckett A and Stenlake *et al*, Practical Pharmaceutical Chemistry, first Indian edition IInd part, CBS Publishers and Distributors, New Delhi (India); 1997. p. 157.

Cite this article as:

M.V. Kumudhavalli, B. Jayakar, M. Kumar, P. Palanisamy. RP-HPLC method development and its validation for the estimation of Fexofenadine in rat plasma and its application for pharmacokinetic studies. Int. Res. J. Pharm. 2014; 5(5):418-426 <http://dx.doi.org/10.7897/2230-8407.050587>