Asian Journal of Chemistry; Vol. 23, No. 3 (2011), 1088-1090

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

# RP-HPLC Estimation of Fexofenadine Hydrochloride in Rat Plasma

B. JEEVANA JYOTHI* and T. JYOTHI

Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswavidyalayam (Women's University), Tirupati-517 502, India

*Corresponding author: E-mail: jeevanajyothib@yahoo.com

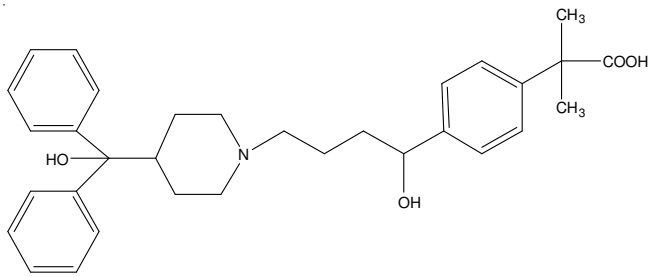
(*Received*: 19 March 2010; *Accepted*: 2 November 2010) AJC-9241

A validated reverse phase HPLC method was developed for the estimation fexofenadine hydrochloride in rat plasma to determine pharmacokinetic parameters such as peak plasma concentration ($C_{max}$), peak time ($t_{max}$), area under the curve ($AUC_{0-24}$) and biological half-life ($t_{1/2}$). The samples were chromatographed on a reverse phase column, Luna $C_{18}$ (250 mm × 4.6 mm, 5 µm). Detection of fexofenadine HCl was carried out at 220 nm using SPDMP 10A photodiode array detector. Mixture of acetonitrile and 0.5 % phosphate buffer (60:40, v/v) having the pH of 3.5 was used as mobile phase. The drug was extracted from rat plasma samples by liquid-liquid extraction using methanol as extraction solvent. Calibration curve was linear over the range of 0.05-2.00 µg/mL of fexofenadine hydrochloride. After oral administration of 10 mg/kg of rat weight of fexofenadine hydrochloride, the plasma concentration-time curve was best confirmed to two-compartment open model. The maximum concentration ($C_{max}$) 5.65 ± 2.10 µg/mL was obtained at time ($t_{max}$) of 1.00 ± 0.87 h. The mean area under the curve at 24 h, $AUC_{0\rightarrow24}$ was 57.09 ± 17.3 µg h/mL and at infinity time, $AUC_{24\rightarrow\infty}$ was 0.35 ± 14.8 µg h/mL. The biological half-life $t_{1/2}$ was 2.4 ± 1.65 h.

**Key Words: Fexofenadine hydrochloride, RP-HPLC, Citrizine.**

## INTRODUCTION

Fexofenadine hydrochloride (FXF HCl) is an antihistaminic drug and chemically, it is α-α-dimethyl-4-[1-hydroxy-4-{4-(hydroxyl diphenylmethyl)-1-piperidinyl}butyl]benzene acetic acid. It is used in the treatment of hay fever and similar allergy symptoms. It was developed as a successor of and alternative to terfenadine[1,2].




Structure of fexofenadine hydrochloride

**Fexofenadine hydrochloride:** Literature reveals that there are reports on liquid chromatographic determination of fexofenadine hydrochloride in biological fluids using HPLC-MS[3]. There were few methods for the quantitative determination of fexofenadine hydrochloride and its related compounds in bulk of pharma-ceutical dosage forms by HPLC method with UV detection[4,5] and by spectrophotometric method in combined pharmaceutical dosage forms[6]. HPLC method for estimation of fexofenadine hydrochloride during dissolution tests for containing capsules and coated tablets[7].

But so far no reports are available in literature on validated HPLC technique for estimation of fexofenadine hydrochloride in plasma samples, which is useful in clinical studies and in evaluation of pharmacokinetic parameters. Hence the objective of the present research work is to develop high pressure liquid chromatographic technique for estimation of fexofenadine hydrochloride in rat plasma to estimate pharmacokinetic parameters of fexofenadine hydrochloride.

## EXPERIMENTAL

Pure and certified sample of fexofenadine hydrochloride was gifted by M/s Karnataka Antibiotics and Pharmaceuticals Ltd., Bangalore, India. Acetonitrile (HPLC grade), water (HPLC grade) and methanol (HPLC grade) were purchased from Merck specialties Pvt. limited (Mumbai). All other chemicals and reagents were of analytical grade.

A gradient high pressure liquid chromatograph (Shimadzu HPLC with Sphinchrome Software series, Japan) with two CC-10 AT VP pumps was used for the analysis. The samples

were chromatographed on a reverse phase Luna $C_{18}$ (250 mm × 4.6 mm, 5 µm) column. Detection of fexofenadine hydrochloride was carried out at 220 nm using SPDMP 10A photodiode array detector. Different mobile phases were tested in order to find the sensitive conditions for the sharp peaks characteristic of the selected internal standard such as cetrizine (CBZ) and the drug, fexofenadine hydrochloride. Mixture of 0.5 % phosphate buffer (pH 3.5) and acetonitrile (60:40, v/v) was selected as optimum composition for mobile phase. The flow rate was maintained at 1 mL/min. The injector having the capacity of 0-250 µL, Rheodyne Hamilton type was used to inject the samples. The mobile phase was vacuum filtered through 0.45 µm millipore filter paper before use. Samples that are filtered through 0.45 µm millipore filter paper using sample filtration syringe system were used to inject in the system.

**Preparation of standard solution of fexofenadine hydrochloride:** The standard stock solution was prepared with mobile phase to obtain 10 µg/mL solution of fexofenadine hydrochloride. Working standard solutions equivalent to 100, 200, 300, 400 and 500 ng/mL of fexofenadine hydrochloride were prepared from stock solution.

**Preparation of internal standard solution:** Citrizine (IS), 10 mg was accurately weighed and dissolved in 100 mL of mobile phase. This solution was suitably diluted to obtain 50 ng/mL of citrizine.

**Extraction procedure:** Blood sample, 1 mL volume were collected from retro orbital plexus of rats into eppendorf tubes containing dipotassium ethylenediamine tetra acetic acid ($K_2$EDTA). The collected blood samples were coagulated by centrifugation at 3000 rpm for 20 min and plasma was separated. 100 µL of clear plasma was spiked with 25 µL of cetrizine, (IS, 50 ng/mL in mobile phase) and methanol (for denaturation and precipitation of plasma proteins) was added to make the volume up to 1 mL. The tubes were tightly capped, vortexed for 10 min and centrifuged for 20 min at 3000 rpm. Then the supernatant clear sample was filtered through 0.45 µm Millipore filter paper and then 100 µL of sample was injected into the system.

**Calibration curve:** Standard solutions containing 100, 200, 300, 400 and 500 ng/mL of fexofenadine hydrochloride were prepared in mobile phase. An aliquot of drug free plasma 100 µL was accurately measured into a stoppered centrifuge vials followed by the addition of 100 µL of 50 ng/mL solution of cetrizine (IS) along with the addition of 100 µL of each fexofenadine hydrochloride standard solution. The samples were processed as describe above. The quantification of chromatogram was performed using peak area ratios of the drug to internal standard and average value for 5 such determinations were calculated. A standard graph was plotted between the plasma concentrations of fexofenadine hydrochloride and the peak area ratio of fexofenadine hydrochloride to citrizine (IS) is shown in Fig. 1. The relevant chromatograms are presented in Fig. 2.

**Accuracy:** Accuracy of the present method was determined by recovery studies. The recovery studies were conducted by adding 1, 2 and 4 µg of fexofenadine hydrochloride to the preanalyzed plasma drug samples containing 500 ng of fexofenadine hydrochloride per 500 µL of plasma and subjecting

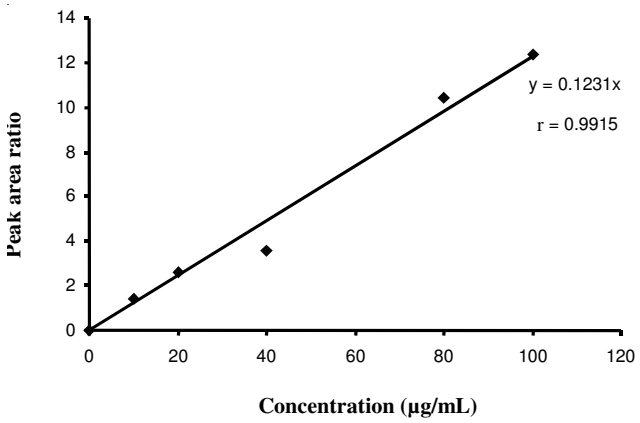



Fig. 1. Standard curve of fexofenadine hydrochloride in rat plasma

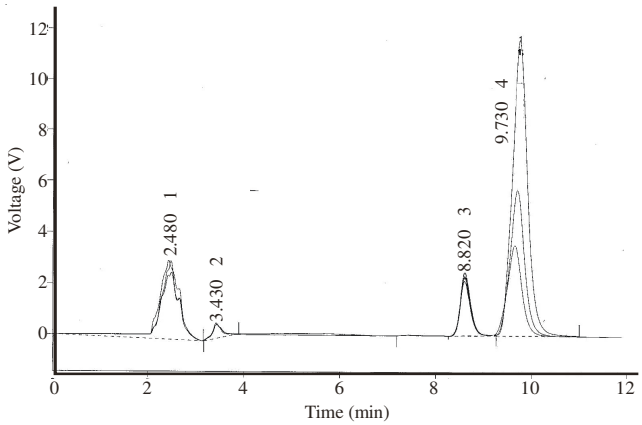



Fig. 2. HPLC chromatogram of blank plasma spiked with cetrizine and fexofenadine hydrochloride

them to present HPLC method of estimation. The accuracy was expressed in terms of present recovery of fexofenadine hydrochloride from the preanalyzed samples. The results are given in the Table-1.

TABLE-1
RECOVERY OF FEXOFENADINE HCl FROM PREANALYZED PLASMA SAMPLE CONTAINING 5 µg OF FEXOFENADINE HCl PER 500 µL PLASMA (n = 6)

| Amount of fexofenadine HCl added (µg) | Mean percent recovery (± SD) |
|---|---|
| 1 | 99.95 ± 2.65 |
| 2 | 99.89 ± 3.25 |
| 4 | 99.92 ± 2.28 |

**Method validation:** The intra-day and inter-day variation of the present HPLC method was estimated by subjecting plasma drug samples (100, 200 and 300 ng/mL) prepared on different five days of HPLC analysis for five different times. In each case the coefficient of variation in mean peak area ratios of the drug was calculated to find the precision of the present HPLC method. The results are given in the Table-2.

**Pharmacokinetic study:** Male wistar albino rats weighing 180-220 g were used in the study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animals were given standard rat pellets (Gold Mohor Ltd.) and drinking water *ad libitum*.

TABLE-2
PRECISION OF THE HPLC METHOD USED FOR THE ESTIMATION OF FLUTAMIDE IN RAT PLASMA (n = 6)

| Conc. of FXF (µg/mL) | Intra day | | Inter day | |
|---|---|---|---|---|
| | Mean peak area ratio | CV (%) | Mean peak area ratio | CV (%) |
| 1 | 2.03 | 0.98 | 2.14 | 1.22 |
| 2 | 4.02 | 0.96 | 3.98 | 1.06 |
| 3 | 6.25 | 1.02 | 5.98 | 1.24 |

A group of rats (containing 4 animals) were administered with fexofenadine hydrochloride powder (equivalent to10 mg/kg, p.o. in water containing 2 % w/v tragacanth). At the predetermined time intervals, blood samples (0.2-0.3 mL) were collected from retro orbital plexus of rats into eppendorf tubes containing dipotassium ethylenediamine tetraacetic acid ($K_2$EDTA). The collected blood samples were coagulated by centrifugation at 3000 rpm for 20 min and plasma was separated and stored in deep freezer until used. Plasma samples of fexofenadine hydrochloride were thawed after withdrawal from deep freezer. The plasma concentration of fexofenadine hydrochloride was estimated by HPLC present method.

## RESULTS AND DISCUSSION

**Linearity:** A good linear relationship was observed between the concentrations fexofenadine hydrochloride and the peak area ratios of fexofenadine hydrochloride to that of internal standard with a high correlation coefficient (r = 0.999) in the range of 0.05-2.00 µg/mL plasma. The regression of fexofenadine hydrochloride concentration over its peak area ratio was found to be y = 1253x - 0.9917 where 'y' is the peak area ratio and 'x' is the concentration of fexofenadine hydrochloride. The low coefficient of variation values in the peak area ratios indicated the reproducibility of the method.

**Accuracy:** The high recovery values closer to 100 % with less standard deviation as shown in the Table-2 indicated accuracy of the method.

**Method validation:** The low percent coefficient of variation values shown in the Table-2 of inter and intra day (precision) studies indicated that the present method is reproducible. Hence this method was used for the estimation of flutamide *in vivo* studies.

**Pharmacokinetic study:** Following oral administration of 10 mg/kg of rat weight of fexofenadine hydrochloride, the plasma concentration of fexofenadine hydrochloride was estimated by present developed HPLC technique and the data is given in Table-3. Plasma concentration-time curve was plotted and given in Fig. 3. The mean area under the curve after 24 h, $AUC_{0\rightarrow24}$ was 57.09 ± 17.3 µg h/mL and at infinity $AUC_{24\rightarrow\infty}$ was 0.35 ± 14.8 µg h/mL. Peak plasma concentration $C_{max}$ was 5.65 ± 2.1 µg/mL which appeared at peak time tmax of 1.00 ± 0.87 h. The half-life $t_{1/2}$ of the drug was 2.4 ± 1.65 h.

TABLE-3
MEAN PLASMA CONCENTRATION-TIME DATA AFTER ORAL ADMINISTRATION OF PURE FXD (n = 4)

| Time (h) | Rat 1 | Rat 2 | Rat 3 | Rat 4 | Avg. plasma conc (µg/mL) | CV* (%) |
|---|---|---|---|---|---|---|
| 0.25 | 2.1760 | 2.180 | 2.1790 | 2.175 | 2.1780 | 0.28 |
| 0.50 | 2.6012 | 2.601 | 2.6018 | 2.024 | 2.6016 | 0.41 |
| 0.75 | 3.9600 | 3.968 | 3.9620 | 3.966 | 3.9640 | 0.64 |
| 1.00 | 5.6000 | 5.680 | 5.6200 | 5.700 | 5.6500 | 0.79 |
| 2.00 | 4.8000 | 4.850 | 4.7300 | 4.780 | 4.7900 | 0.66 |
| 4.00 | 3.8300 | 3.900 | 3.8700 | 3.800 | 3.8500 | 0.57 |
| 6.00 | 3.0100 | 3.110 | 3.0400 | 3.080 | 3.0600 | 0.52 |
| 8.0 | 2.6200 | 2.720 | 2.7500 | 2.590 | 2.6700 | 0.48 |
| 12.00 | 2.0100 | 2.180 | 2.1500 | 2.040 | 2.0900 | 0.23 |
| 24.00 | 0.9100 | 0.970 | 0.8700 | 1.010 | 0.9400 | 0.95 |

*Coefficient of variance. No significant variation between the rat groups ($p < 0.05$).

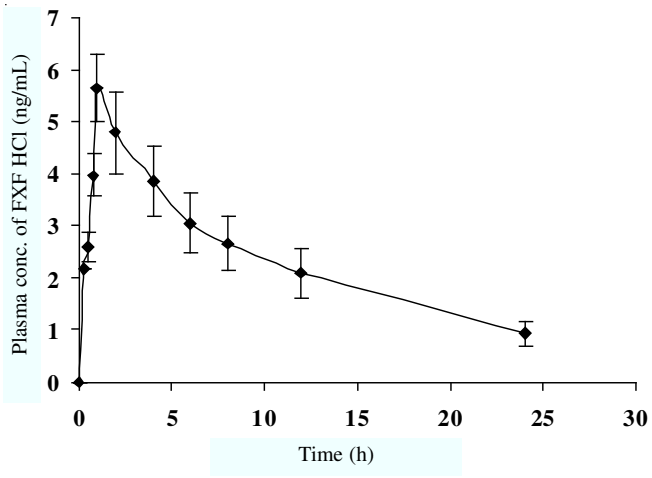



Fig. 3. Mean plasma concentration-time curve

## ACKNOWLEDGEMENTS

The authors thank M/s Karnataka Antibiotics & Pharmaceuticals Limited, Peenya, Bangalore, India, for providing a gift sample of fexofenadine hydrochloride.

## REFERENCES

1. K. Simpson and B. Jarvis, *Drugs*, **59**, 301 (2000).
2. S.G. Meeves and S. Appajosyulla, *J. Allergy Clin. Immunol*., **112**, S69 (2003).
3. U. Hofmann, M. Seiler, S. Drescher and M.F. Fromm, *J. Chromatogr. B*, **766**, 227 (2002).
4. T. Radhakrishna and G.O. Reddy, *J. Pharm. Biomed. Anal*., **29**, 681 (2002).
5. T.A. Walker and G.L. Schmitt, *J. Liq. Chromatogr. Rel. Tech*., **29**, 25 (2006).
6. H. Mahgoub, A.A. Gazy, F.A. EI-Yazbi, M.A. EI-Sayed and R.M. Youssef, *J. Pharm. Biomed. Anal.*, **31**, 801 (2003).
7. A.R. Breier, C.S. Paim, M. Steppe and E.E. Schapoval, *J. Pharm. Sci.*, **8**, 289 (2005).