Acta Pharmaceutica Turcica
XXXX (3) 99-102 (1998)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF LORATADINE IN HUMAN PLASMA USING FLUORESCENCE DETECTION

J. A. JILANI[1], N. M. NAJIB[1], M. S. SALEM[1], G. K. PILLAI[1], M. M. HASAN[2], F. M. ZGHOL[1], E. GANEM[1], I. JALAL[3]

[1]Faculty of Pharmacy Jordan University of Science and Technology Irbid-Jordan
[2]Faculty of Pharmacy and Medical Sciences Amman University
[3]Hikma Pharmaceuticals Amman-Jordan

A rapid and sensitive high-performance liquid chromatographic method has been developed for the determination of loratadine in human plasma using diazepam as the internal standard. The drugs were eluted from a 5/µm/ C-18 reversed-phase column with a mobile phase consisting of acetonitrile: 0.05 M ammonium phosphate buffer (42/58, v/v) adjusted to pH 3.0 with phosphoric acid and at a flow rate of 2.5 mL/min with fluorinetric detection at 290 nm for excitation and 460 nm for emission. Each analysis required no longer than 4 min. Quantitation was achieved by the measurement of peak-height ratio and the absolute recovery varied from 85.3 to 90.8%. Detection limits for loratadine in serum was 0.5 ng/mL. Intraday coefficients of variation (%CV) ranged from 3.42 to 5.56 and interday (%CV) from 3.75 to 6.74 at three different concentrations.

Keywords: *Loratadine; HPLC analysis; Fuorimetric detection; Assay validation; Application in pharmacokinetic studies*

Introduction

Loratadine (ethyl - 4 - (8 - chloro - 5, 6 - dihydro - 11 - H benzo-(5- 6) - cyclohepta-(1,2 -b)-pyridin-11-ylidine) piperidine-1 carboxylate) is a long-acting antihistamine devoid of central nervous system depressant and effects often associated with other antihistaminic agents. It is generally well tolerated and is effective in relieving symptoms associated with allergic diseases(1). The drug is rapidly and almost completely absorbed and metabolized to an active metabolite descarboethoxyloratadine. Plasma concentrations of loratadine reach a peak 1 hr after its administration, and then decrease in two phases with mean half lives of 1 and 10 hrs. The active metabolite appears rapidly in plasma, its maximal concentration is reached 2.4 hr after oral administrations of loratadine(2).

Plasma concentrations of loratadine and descarboethoxyloratadine have been determined in human plasma by radioimmuno assays, capillary column gas chromatography and high performance liquid chromatography (3-6).

This paper reports development of a rapid, sensitive, selective and reproducible HPLC assay using fluorescence detection for the determination of loratadine in human plasma. Compared to previously reported methods, the present method requires less sample manipulation, coupled with shorter retention time and use of a readily available internal standard. An application of the method for evaluation of pharmacokinetics of the drug in human is also described.

Materials and Methods

Apparatus

All analysis were performed using an HPLC system consisting of a Varian 2010 solvent delivery system, a fluorescence detector (Shimadzu model RF 530) and a Rheodyne injector fitted with a 100 µL loop. The chromatographic column was 25 cm Lichrospher 60 RP-select B (5 µm).

Reagents

Loratadine and diazepam were supplied by HIKMA Pharmaceuticals, Jordan. Cyclohexane and acetonitrile were of HPLC grade (Merck, Germany). All other chemicals and reagents used were of analytical grade. Loratadine tablets were supplied by HIKMA Pharmaceuticals, Jordan.

Standard solutions

Loratadine stock solution was prepared in 0.1% phosphoric acid so as to contain 0.1 µg/mL. Diazepam (internal standard for HPLC assay) was prepared in 0.1% phosphoric acid so as to contain 25 µg/mL.

Sample preparation

To 1.5 mL of human plasma was added 100 µL of internal standard solution (Diazepam, 25 µg/mL in 0.1% phosphoric acid). The mixture was extracted with 6 mL each of cyclohexane by vortex-mixing for

1 minute followed by centrifugation at 3000 r.p.m. for 5 minutes. The cyclohexane layer was transferred to a clean and dry test tube and evaporated to dryness with a stream of nitrogen at 60°C. The residue was reconstituted in 120 µL acetonitrile: 0.1% phosphoric acid (50:50) and was vortex-mixed for one minute. It was then transferred to a disposable polypropylene microcentrifuge tube and centrifuged at 12000 r.p.m. for 2 minutes to ensure that no particulate matter is injected on to the HPLC column. An appropriate aliquot was then introduced into the loop injector.

Calibration curve data

Loratadine stock solution (0.1 µg/mL) was used to spike serum sample (1.5 mL) to provide calibration standards of 2, 5, 10, 15 and 20 ng/mL. To each of these samples were added 100 µL of internal standard solution (25 µg/mL). The mixture was extracted and processed as described under sample preparation. Peak height ratios (Loratadine/internal standard) were measured and plotted against concentration of loratadine.

Chromatographic conditions

The mobile phase consisted of acetonitrile: ammonium phosphate, 0.05 M (42/58) adjusted to pH 3 by concentrated phosphoric acid. It was degassed daily by passing it through 0.45 µm membrane filter (Millipore, MA, U.S.A.) Flow rate of the mobile phase was 2.5 mL/min. Loratadine was monitored by a fluorescence detector set at an excitation wavelength of 290 nm and an emission wavelenght of 466 nm.

Recovery

In order to determine the absolute analytical recovery of loratadine from the plasma by this method, known concentrations of loratadine was mixed with drug-free plasma containing internal standard, extracted with cyclohexane as described and the peak height ratio (drug/internal standard) was measured. Six replicate analysis of three different concentrations (3, 8, 17 ng/mL)) were performed in aqueous and plasma medium and the peak ratios were compared (Table 1).

Table1. Analytical recovery of loratadine from plasma

Concentration*	Peak height ratio		
(ng/mL)	Aqueous	Serum	% recovery
3	0.207±0.0137	0.188±0.0183	90.8
8	0.625±0.0164	0.533±0.0137	85.3
17	1.327±0.0186	1.195±0.0152	90.1

*Six replicate analysis for each concentration

Linearity

Six calibration curves were constructed during the study period with concentrations ranging from 2 to 20 ng/mL. The peak-height ratios, the mean and standard deviation of the slope, intercept and correlation coefficient were calculated.

Table 2. Within-day and between-day precision of the HPLC assay of loratadine in plasma

Within-day			Between-day		
Added ng/mL	Measured ng/mL	Percentage +Bias	Added ng/mL	Measured ng/mL	Percentage +Bias
3.0			3.0		
Mean	3.23		Mean	3.167	
SD	0.179	7.67	SD	0.213	5.57
CV%	5.56		CV%	6.74	
8.0			8.0		
Mean	8.05		Mean	7.70	
SD	0.275	0.63	SD	0.289	-3.75
CV%	3.42		CV%	3.75	
17.0			17.0		
Mean	17.27		Mean	17.22	
SD	0.607	1.59	SD	0.803	1.29
CV%	3.52		CV%	4.66	

Mean values represent six different serum samples for each concentration

$$\%\text{Bias} = \frac{100\ (\text{measured conc.} - \text{added conc.})}{\text{added conc.}}$$

Precision

The within-day precision was evaluated by 6 replicate analysis of pooled serum samples containing loratadine at three different concentrations. The coefficient of variation ranged from 3.42-5.56%. The between-day precision was similarly evaluated on several days for two weeks. Coefficient of variation ranged from 3.75 to 6.74 (Table 2).

Application

A healthy human volunteer (age 24 yrs, weight 76 kg) participated in the study after informed consent. Two tablets of loratadine (2x10 mg) were administered following an overnight fast and blood samples (4 ml) were withdrawn through an indwelling cannula inserted in the forearm vein at 0 (pre-dose), 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 hours post-administration. Blood samples were centrifuged at 5000 r.p.m. for 5 minutes, plasma was separated and stored at -20°C until analysed by the HPLC method. No food or drinks were allowed for 3 hours following drug administration. The plasma samples were processed as described under sample preparation.

Results and Discussion

A representative chromatogram of blank plasma and plasma containing 10 ng/mL of loratadine and 2.5 µg/mL diazepam a readily availabe and suitable internal standard is shown in fig. 1. The mobile phase used for the analysis achieved optimum resolution of loratadine and diazepam (retention time of 2.70 and 4.1 minutes respectively) with no interference from plasma components. The lowest concentration of loratadine that could be detected under the conditions employed for the assay was 0.5 ng/mL plasma and the analytical recovery ranged from 85.3 to 90.8% (Table 1).

To determine the linearity of the assay, six calibration curves, each of five concentrations, were constructed during the study period. The peak height ratios, the mean and standard deviation of the slope and intercept were 0.0685±0.0034 and 0.0308±0.0019 respectively. The correlation coefficient was 0.9951±0.0012. The precision of the method was verified by evaluating the within-and between-day variations. The percent coefficient of variation ranged from 3.42 to 5.56 (within-day) and from 3.25 to 6.74 (between-day).

The applicability of the analytical procedure to pharmacokinetic studies in the human was evaluated by analysing plasma samples collected from a healthy male volunteer who received two tablets of loratadine (2x10 mg). The plasma level-time curve for a period of 12 hrs is shown in fig. 2. The peak plasma concentration

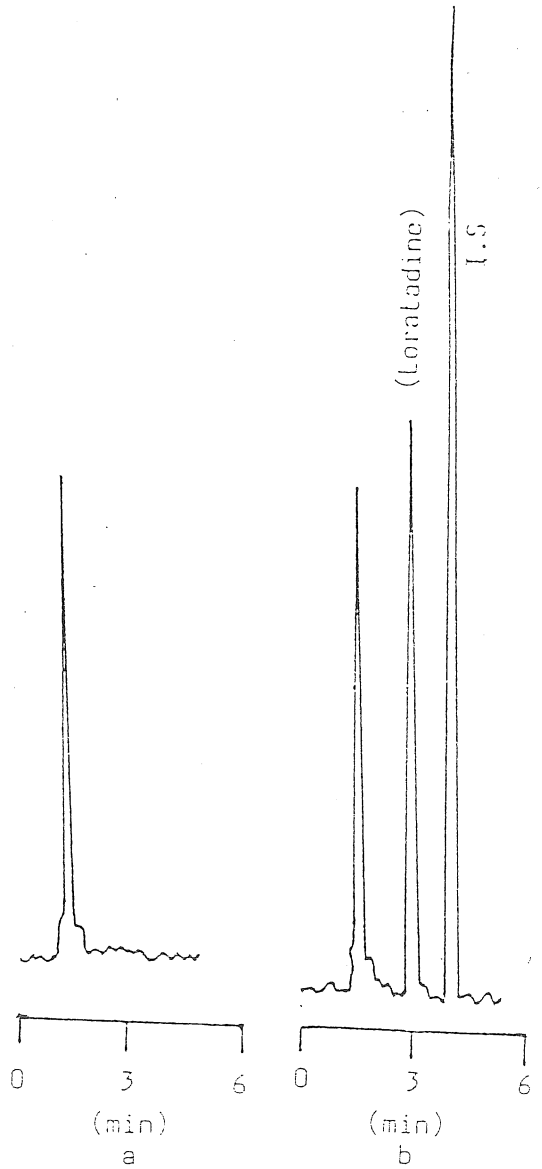

Fig.1. (a) Chromatogram of blank plasma
(b) Chromatogram of Loratadine (10 ng/mL) and Diazepam (2.5 µg/mL) in volunteers plasma

(C_{max}) of 25 ng/mL was reached in this volunteer at 1.0 hr post-oral dose (T_{max}) and the last measured concentration was 0.6

ng/mL at 12 hrs. The area under the plasma level-time curve, AUC_{0-12}, calculated by the trapezoid rule, was 45.6 ng.h/mL and the half life of elimination, calculated from the slope of the terminal elimination phase, was 2.8 hrs.

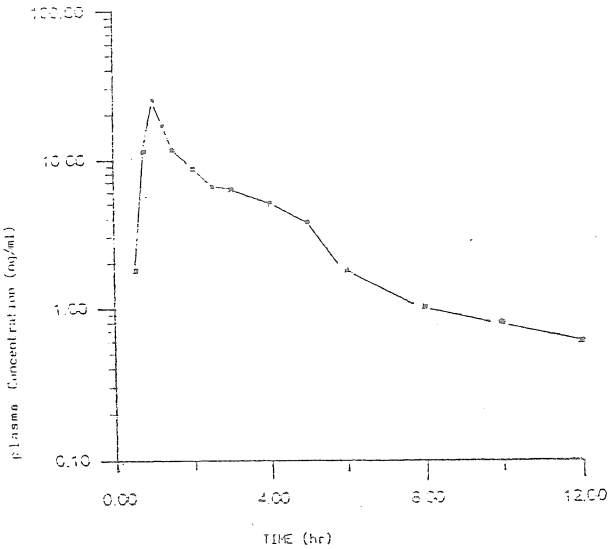

Fig. 2. Plasma concentration-time profile of Loratadine following oral administration of a 20 mg dose (2x10 mg tablet)

Conclusion

The HPLC method developed is relatively simple, rapid and possess the sensitivity and specificity to measure very low concentrations of loratadine that would be encountered in pharmacokinetic studies.

References

1. Villani, F.J. , Magatti, C.V., Vashi, D.B., Wong, J. and Popper, T.L. : Drug Res. 30, 1311 (1986)
2. Katchen, B., Cramer, J. and Chung, M.: Annals of Allergy 55, 293 (1985)
3. Radawanski, E., Hilbert synchowicz, J.S. and Zampaglione, N.: J. Clin. Pharmacol. 27, 530 (1987)
4. Wilson, J. : Drug Metab. Rev. 14, 619 (1983)
5. Zhong, D. and Blume, H.: Pharmazie 49, 736 (1994)
6. Jhonson, R., Christensen, J. and Lin, Chin-Chung: J. Chromatogr. 657, 125 (1994)

Accepted: 01.12.1997