Development and validation of a HPLC method for estimation of loratadine and its application to a pharmacokinetic study

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A simple, rapid, sensitive and accurate high-performance liquid chromatography method was developed and validated for the quantification of loratadine concentration in rabbit plasma using metoclopramide as an internal standard. Separation was performed on kromosil C18 column (250 × 4.6 mm 5 μm) using a mobile phase consisting of 0.1% perchloric acid : acetonitrile (55 : 45 v/v) at a flow rate of 1 ml/min. Validation of the method was performed in order to demonstrate its selectivity, linearity, precision, accuracy, ruggedness, recovery and matrix effect. The calibration curves of loratadine were linear over a concentration range of 5–1022 μg/ml. The with-in and between-day of coefficients of variation were <10%. The extraction recoveries of loratadine at the three levels of quality control samples were 99.961%, 99.767% and 99.938%. The method was rapid with a retention time of loratadine and the internal standard observed at 6.67 and 8.83 min respectively. The developed method was successfully applied for studying the pharmacokinetics of loratadine in rabbits.

Keywords: HPLC, internal standard, loratadine, metoclopramide.

LORATADINE is an H1 histamine antagonist which belongs to the second generation of antihistamines and is helpful in treating allergies. It is closely related to tricyclic antidepressants in its structure, such as imipramine and is distantly related to the atypical antipsychotic quetiapine[1]. Loratadine is free from sedation at the recommended doses among the second generation anti-histamines[2]. Its chemical name is 4-(8-chloro-5,6-dihydro-11 H-benzo [5,6] cyclohepta [1,2-b] pyridine-11-ylidene)-1-piperidinecarboxylic acid ethyl ester[3]. Loratadine is a long-acting second generation histamine antagonist that is structurally similar to cyproheptadine and azatadine[4]. Loratadine opposes free histamines and exhibits specific, selective peripheral H1 antagonistic activity. This hinders the action of endogenous histamine, which subsequently leads to short-term relief from the negative symptoms (e.g. nasal congestion, watery eyes) caused by the histamine[5].

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Loratadine is satisfactorily absorbed from the gastrointestinal tract and has quick first-pass hepatic metabolism when given orally. It is metabolized by isoenzymes of the cytochrome P450 system, including CYP3A4, CYP2D6, and to a lesser extent, several others. A literature survey carried out on the estimation of loratadine in rabbit plasma revealed only few analytical methods[6–8]. However, there was no standard method for loratadine estimation in rabbit plasma by high-performance liquid chromatography (HPLC). Hence, an attempt has been made to develop a new method for the estimation and validation of loratadine in rabbit plasma in accordance with the United States Food and Drug Administration (USFDA) guidelines.

Materials and method

Loratadine and metoclopramide pure drug (API) samples were obtained from DR. Reddy's Laboratories Ltd, Hyderabad, India. All the chemicals and solvents like distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen orthophosphate buffer, orthophosphoric acid were obtained from RANKEM-Mumbai, India.

Analytical method development – HPLC method

In order to estimate loratadine content in the plasma samples, HPLC method was developed using different mobile phases in different ratios: 0.1% orthophosphoric acid : acetonitrile (40 : 60); 0.01 N potassium dihydrogen phosphate : acetonitrile (55 : 45); 0.1% perchloric acid : acetonitrile (55 : 45). Different chromatographic columns were used (azilent C18 250 mm × 4.6 mm 5 μ, BDS C18 150 mm × 4.6 mm 5 μ, kromosil C18 250 mm × 4.6 mm 5 μ). Based on peak shape, retention time, tailing factor, the chromatographic conditions were optimized.

Preparation of loratadine stock and spiking solutions

The stock solution of loratadine was prepared by dissolving the drug in diluents (water : acetonitrile; 50 : 50) to produce 1 mg/ml.

From this stock solution, 0.012, 0.029, 0.094, 0.534, 1.150, 1.412, 1.860 and 2.350 ml were pipetted and transferred to eight individual 10 ml volumetric flasks and the volume was made up to the mark with diluent (water : acetonitrile; 50 : 50) to produce 1.20, 2.90, 9.40, 53.40, 115, 141.20, 186 and 235.0 µg/ml concentrations. Calibration standards and quality control (QC) samples were prepared by spiking blank plasma with working stock dilutions of analytes to produce 0.005, 0.013, 0.041, 0.232, 0.500, 0.614, 0.809 and 1.022 µg/ml concentrations.

Preparation of internal standard (IS) solution

10 mg of metoclopramide was taken in 10 ml volumetric flask and the volume was made with diluent (water : acetonitrile 50 : 50). From this stock solution, 0.1 ml of solution was taken into 10 ml volumetric flask and made up the volume with the same diluent to produce 10 µg/ml solution.

Buffer preparation (0.1% perchloric acid)

1 ml of perchloric acid was taken in 1000 ml volumetric flask and the solution was made up using HPLC grade water to produce 1000 ml.

Extraction procedure: 250 µl of plasma, 50 µl of IS and 10 µl of loratadine spiking solutions were taken into a centrifugal tube and 2 ml of acetonitrile was added. The solution was kept in a cyclomixer for 15 sec and vortexed for 2 min followed by centrifugation for 3 min at 3200 rpm. After centrifugation, the supernatant liquid was collected and 50 µl was injected directly into HPLC column.

Method validation: The optimized chromatographic method was completely validated according to the procedures described in USFDA guidelines for the validation of analytical methods and stability testing of the new drug respectively. The method was validated for different parameters such as system suitability, sensitivity, linearity, precision, accuracy, ruggedness, recovery, matrix effect, etc.

Sensitivity: Sensitivity was checked by preparing six lower limit of quantitation (LLOQ) samples independent of calibration curve standards.

Linearity: Calibration curves were obtained daily for three days using standards containing eight different concentrations. Curves were constructed by calculating the ratios of peak-area of loratadine to that of IS. For the preparation of calibration standards, working solutions of loratadine (50 µl) and IS (50 µl) were added to blank plasma (500 µl) to obtain final concentrations of 0.005, 0.013, 0.041, 0.232, 0.500, 0.614, 0.809 and 1.022 µg/ml.

Precision and accuracy studies: The accuracy and precision evaluation were assessed by repeated analysis of rabbit K_2EDTA plasma samples containing different concentrations of loratadine on separate occasions. A single run consisted of a calibration curve, six replicates of LLOQ, low-quality control (LQC), medium quality control (MQC) and high-quality control (HQC) samples.

Ruggedness: Ruggedness was performed by different analyses. The run consisted of a calibration curve and a total of six spiked samples; six replicates of each of LLOQ, LQC, MQC and HQC samples.

Recovery: Recovery of loratadine was evaluated by comparing mean analyte responses of six extracted samples of LQC, MQC and HQC samples to mean analyte response of six replicates injection of an unextracted quality control sample.

Matrix effect: Eighteen blank matrix samples from six different lots of matrices were processed. The reconstituted blank samples were spiked with HQC and LQC level (from each lot one blank, one HQC and one LQC sample) and compared against corresponding HQC and LQC samples injected in six replicates.

Pharmacokinetic studies

Healthy rabbits (New Zealand albino) of either sex weighing 2–3 kg were selected and housed with CPCSEA guidelines[9]. The animals were fasted overnight prior to treatment but had free access to water. None of the subjects received any other drug for at least two weeks

Table 1. Composition of loratadine test formulation (oral disintegrating tablets)

Compound	Quantity (mg)
Granules (containing loratadine 10 mg)	40
Sodium starch glycolate	18
Mannitol	130
Aspartame	2
Magnesium stearate	5
Talc	5
Total weight	200

Table 2. Optimized chromatographic conditions

Name	Parameters
Column	Kromasil C18 column (250 × 4.6 mm, 5µ)
Mobile phase composition	0.1% Perchloric acid buffer : acetonitrile (55 : 45)
Flow rate	1 ml/min
Injection volume	50 µl
Run time	12 min
Detection wavelength	280 nm
Column temperature	30°C
Diluent	Water : acetonitrile (50 : 50)

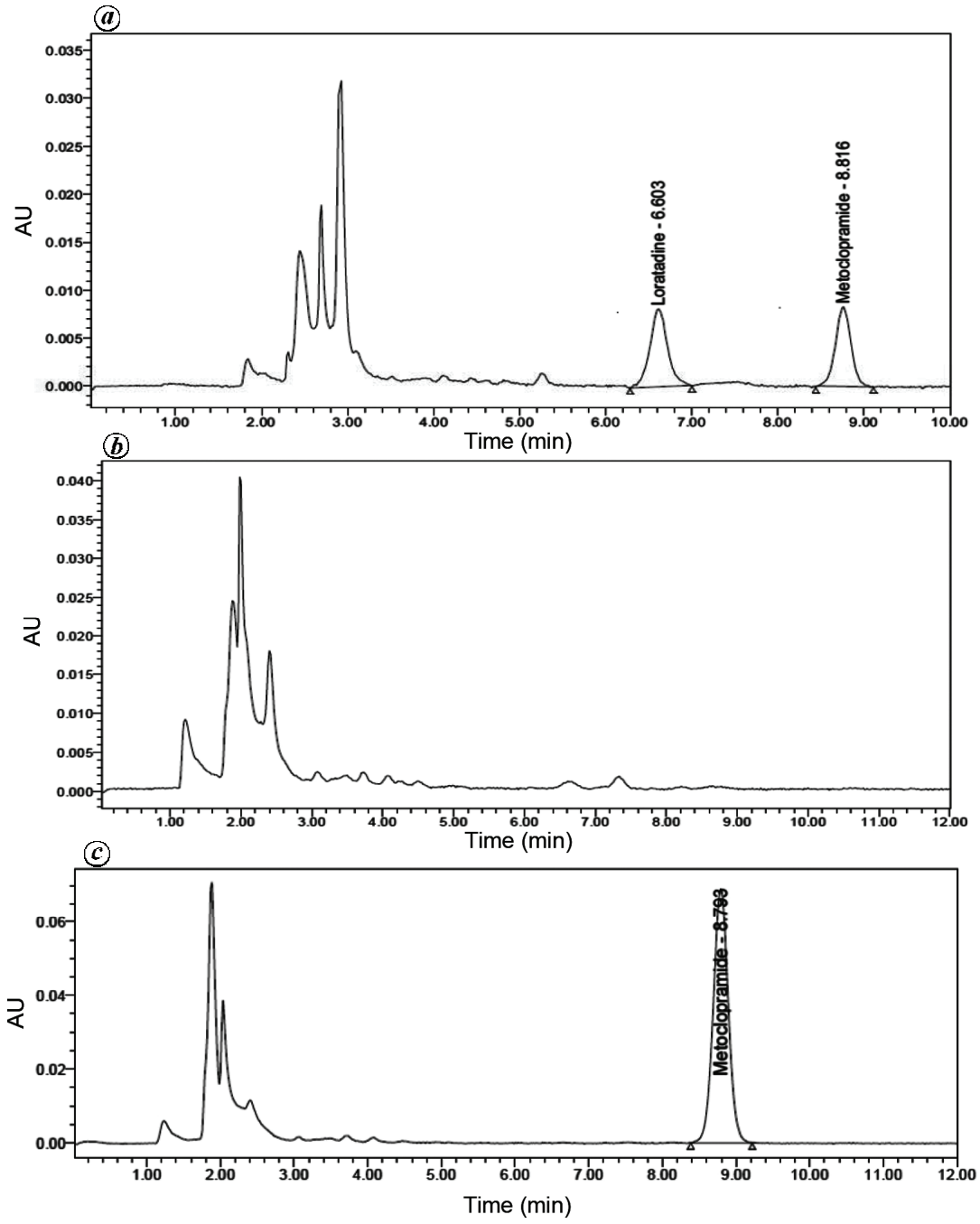

Figure 1. ***a***, Optimized chromatogram of loratadine and ISTD. ***b***, Blank chromatogram. ***c***, ISTD chromatogram.

before the commencement of the study and no other drug was given during the entire study[10,11].

Experimental design: Animals were separated into three experimental groups and each group consisted of three animals ($n = 3$). The test formulation of the batch was compared with reference/marketed formulation by the following treatment schedule under fasting condition. Group I – (Normal control) – received placebo; Group II – (Positive control) – reference formulation; Group III – (Sample) – loratadine test formulation.

Drug administration and sampling procedure

The test and reference formulations were administered via oral gauge at a dose of 0.164 mg loratadine (Table 1). Based on the conversion factor of adult dose to rabbit dose, the dose was calculated as shown below[12,13].

HED (mg/kg) = animal dose (mg/kg) × animal *Km* factor/human *Km* factor.

HED = human equivalent dose; animal *Km* factor = 12 and human *Km* factor = 37.

Table 3. Validated parameters of loratadine by HPLC method in plasma

	Analytical run ID	Spiked concentration (µg/ml)	Mean ± SD concentration (µg/ml)	%RSD	%CV
Accuracy and precision	LLOQ	0.005	0.00498 ± 0.00004	0.80	0.80712
	LQC	0.041	0.041 ± 0.001	1.21	1.249
	MQC	0.500	0.4988 ± 0.002	0.38	0.3891
	HQC	0.809	0.808167 ± 0.001	0.121	0.121657
Ruggedness	LLOQ	0.005	0.0049733 ± 0.00004	0.80	0.80712
	LQC	0.041	0.0411 ± 0.000126	0.306	1.249
	MQC	0.500	0.498833 ± 0.002041	0.409	0.3891
	HQC	0.809	0.808333 ± 0.0008	0.101	0.121657
Recovery	LQC	0.041	0.041 ± 0.0003	0.804	0.810
	MQC	0.500	0.499 ± 0.002	0.400	0.321
	HQC	0.809	0.809 ± 0.001	0.123	0.103
Matrix effect	LQC	0.041	0.041 ± 0.001	2.439	2.1815
	HQC	0.809	0.809 ± 0.001	0.123	0.101

Table 4. Data for calibration curve

Concentration (µg/ml)	Area response ratio
0.005	0.0077
0.013	0.0145
0.041	0.0225
0.232	0.0733
0.500	0.1453
0.614	0.1754
0.809	0.2287
1.022	0.2883

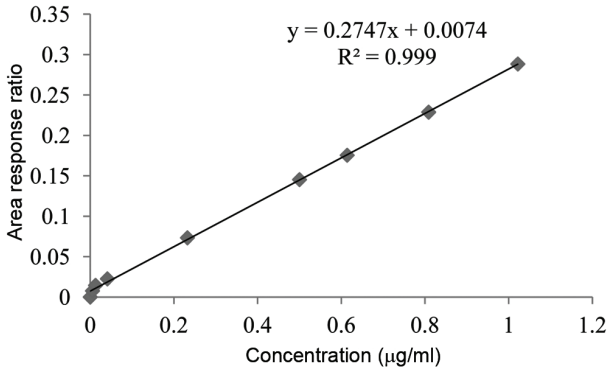

Figure 2. Calibration curve of loratadine in plasma.

Table 5. Plasma concentration values of loratadine formulations

	Concentration (ng/ml)	
Time (h)	Test formation	Reference formation
0	0	0
0.25	0.88 ± 0.03	0.82 ± 0.017
0.5	1.52 ± 0.06	1.23 ± 0.200
1	9.36 ± 0.20	6.5 ± 0.321
1.5	11.84 ± 0.10	9.5 ± 0.752
2	8.29 ± 0.10	6.8 ± 0.395
2.5	6.86 ± 0.152	5.03 ± 0.351
3	5.06 ± 0.350	3.25 ± 0.208
4	3.93 ± 0.360	2.1 ± 0.404
6	2.41 ± 0.264	1.6 ± 0.256
8	1.26 ± 0.050	1.243 ± 0.262
10	1.04 ± 0.090	1.106 ± 0.27
12	0.96 ± 0.10	0.8 ± 0.236
24	0.82 ± 0.12	0.79 ± 0.22

The protocol was approved by the institutional animal ethical committee at Malla Reddy Institute of Pharmaceutical Sciences (1662/PO/Re/S/12/CPCSEA), Andhra Pradesh, India. The experiments were conducted as per CPCSEA guidelines.

The blood samples (each about 2 ml) were drawn at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h after administration of formulation from the marginal ear vein of the rabbits held in the wooden box[14]. The collected blood samples were immediately centrifuged at 5000 rpm in an ultra cooling centrifuge for 10 min at 4°C. The supernatant plasma sample was separated and stored in a clean screw capped 5 ml polypropylene plasma tube at –20°C in a deep freezer, until further analysis[15].

Extraction of the drug from rabbit plasma

The stored plasma samples were processed at room temperature, 250 µl of plasma was added to 500 µl of acetonitrile to precipitate the proteins. The samples were vortexed on a vortex mixer for 15 min, followed by centrifugation at 10,000 rpm for 15 min. The respective samples were then injected into the HPLC column.

Pharmacokinetic analysis: The plasma concentrations were used to construct pharmacokinetic profiles by plotting drug concentration–time curves. To determine the

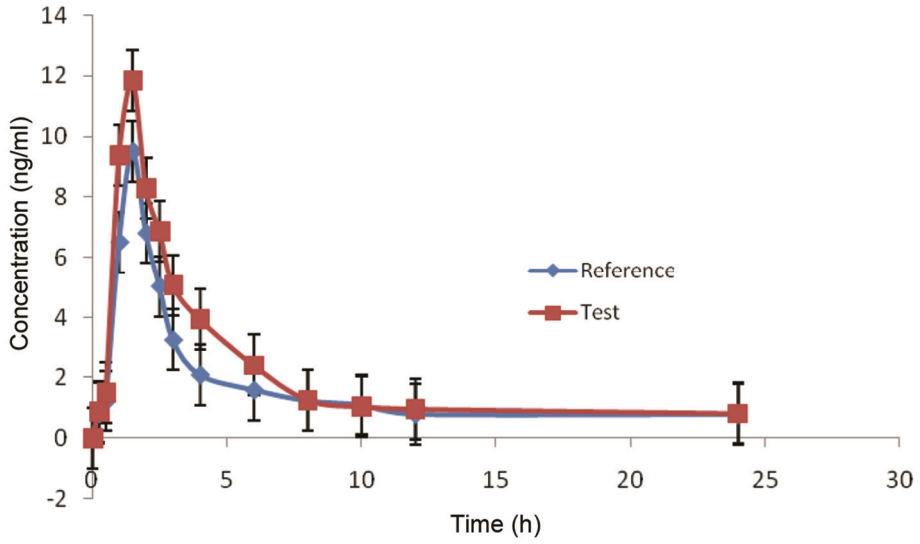

Figure 3. Plasma concentration–time profile.

Table 6. Pharmacokinetic parameters of loratadine formulations

Pharmacokinetic parameter	Test formulation	Reference formulation
C_{max} (ng/ml)	11.84 ± 0.296	9.5 ± 0.430
T_{max} (h)	1.5 ± 0.62	1.5 ± 0.64
AUC_{0-24}(mg h/l)	49.71 ± 1.01	38.4 ± 0.6
$AUC_{0-\infty}$ (mg h/l)	50.89 ± 1.52	39.53 ± 1.02
Kel (h^{-1})	0.11 ± 0.23	0.28 ± 0.56
$T_{1/2}$ (h)	6.47 ± 0.10	6.31 ± 0.31

pharmacokinetic parameters, all data obtained subsequently were fed into pharmacokinetic software 'WinNonlin®'. The pharmacokinetic parameters such as maximum plasma concentration (C_{max}), time to reach maximum concentration (T_{max}), area under the curve ($AUC_0 - t$) and ($AUC_{0-\infty}$), elimination rate constant (Kel) and biological half-life ($t_{1/2}$) were calculated.

Results and discussion

After estimating loratadine using different mobile phases with different ratios and by using different columns, the final optimized chromatographic conditions were developed as shown in Table 2 and the respective chromatograms by these conditions are given in Figure 1.

Method validation

System suitability: The retention times and area of loratadine and ISTD were measured. The per cent coefficients of variation (CV) of area ratio was found to be 1.34 and within the acceptable limits.

Sensitivity: Six LLOQ samples were prepared independent of calibration curve standards. The per cent CV of an area was found to be 1.14 and within the acceptable limits.

Accuracy and precision, ruggedness, recovery, matrix effect: The range of per cent CV for accuracy and precision was found to be from 0.12 to 1.24. The range of between-run nominal value percentage was found to be from 99.66 to 100.81. The range of within-run per cent CV for ruggedness was found to be from 0.1 to 0.8. Its nominal values percentage ranged from 99.73 to 100.24. The mean recovery values were 99.93%, 99.76% and 99.96% at HQC, MQC and LQC respectively. No outcome of quantitation for loratadine and IS was observed in the matrix effect. The results for accuracy and precision, ruggedness, recovery and matrix effect are given in Table 3.

Linearity and calibration curves: Calibration curves were found to be consistently accurate and precise in the range of 0.005 to1.022 µg/ml for loratadine. The regression coefficient (r) was equal to 0.999. Reverse-calculations were made from the calibration curves to determine loratadine concentration of each calibration standard. The corresponding data are presented in Table 4. A typical calibration curve is presented in Figure 2.

The plasma concentration–time profile values are given in Table 5 and the plasma concentration–time profile curve is given in Figure 3.

The pharmacokinetic parameters of loratadine test and reference formulations are shown in Table 6. AUC is an important parameter for comparative bioavailability studies. However, the other two parameters C_{max}, T_{max} are also important features and could affect the therapeutic behaviour of a drug. And hence were also considered in

this study. There was no difference in T_{max}, half-life ($t_{1/2}$) and elimination rate (Kel) of test and reference formulations. C_{max} and total bioavailability ($AUC_{0–24}$ and $AUC_{0–\infty}$) of test formulation were high compared to reference formulation. Thus the test formulation was successful in increasing bioavailability.

Conclusion

A simple, sensitive, accurate and precise HPLC method was developed and validated for the estimation of loratadine in rabbit plasma. The present method was successfully applied in the pharmacokinetic study of loratadine in rabbit plasma, in which all the pharmacokinetic parameters were determined. C_{max}, T_{max}, AUC, Kel, $t_{1/2}$ were measured. C_{max} and the total bioavailability ($AUC_{0–24}$ and $AUC_{0–\alpha}$) were high for test formulation compared to reference formulation. From the results of pharmacokinetic studies, it was thus concluded that the test formulation was better than reference formulation as it showed a greater extent of absorption.

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Received 29 August 2018; revised accepted 4 October 2018

doi: 10.18520/cs/v116/i2/243-248