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Rapid determination of loratadine in small volume plasma samples by high-performance liquid chromatography with fluorescence detection

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Abstract

A simple and rapid high-performance liquid chromatographic method with fluorescence detection was developed for the determination of loratadine in small volume plasma samples. Liquid–liquid extraction of loratadine and diazepam (as internal standard) from plasma samples was performed with *n*-butyl alcohol/*n*-hexane (2:98, v/v) in alkaline condition followed by back-extraction into diluted perchloric acid. Chromatography was carried out using a C_8 column (250 × 4.6 mm, 5 μm) under isocratic elution with acetonitrile-20 mM sodium dihydrogen phosphate-triethylamine (43:57:0.02, v/v), pH 2.4. Analyses were run at a flow-rate of 1.0 ml/min at room temperature. The method was specific and sensitive with a quantitation limit of 0.62 ng/ml and a detection limit of 0.2 ng/ml at a signal-to-noise ratio of 3:1. The mean absolute recovery of loratadine from plasma was 84%, while the intra-and inter-day coefficient of variation and percent error values of the assay method were all less than 9.7%. Linearity was assessed in the range of 0.62–20 ng/ml in plasma with a correlation coefficient of greater than 0.999. The method has been used to analyze several hundred human plasma samples for bioavailability studies.

Keywords: Loratadine

1. Introduction

Loratadine, ethyl 4-(8-chloro-5,6-dihydro-11H-benzo [5,6]-cyclohepta[1,2-b]pyridin-11-ylidine)-1-piperidinecarboxylate (Fig. 1A), is rapidly effective and long-lasting tricyclic antihistamine with selective peripheral histamine H_1-receptor antagonist activity [1].

Several methods, such as gas chromatography-mass spectrometry [2,3], and high-performance liquid chromatography (HPLC) with mass spectrometry [4,5], ultraviolet (UV) [6], or fluorescence detection [7,8] have been described for the determination of loratadine in human plasma. Generally, these published methods are tedious and time-consuming, or need mass spectrometer which is expensive and not readily available in most clinical research laboratories. Therefore, a new simple and rapid method for measurement of plasma loratadine concentrations is still desirable.

This paper describes a rapid and simple HPLC method for the determination of loratadine in small volume of human plasma samples. This method was used for bioavailability studies of loratadine.

2. Experimental

2.1. Reagents

Loratadine (purity 99.72%) was supplied by Farmhispania (Spain). Diazepam (internal standard, Fig. 1B) was obtained from sigma (USA). Analytical grade triethylamine, phosphoric acid, perchloric acid and HPLC grade methanol were purchased from E. Merck (Darmstadt, Germany). Analytical grade *n*-butyl alcohol (butan-1-ol) was from BDH Chemicals (UK). HPLC grade acetonitrile was purchased from CARLO ERBA Reagenti (Italy). All other reagents were analytical grade.

2.2. Instrumentation

The analyses were performed on a Shimadzu chromatographic system (Kyoto, Japan) equipped with an LC-6A sol-

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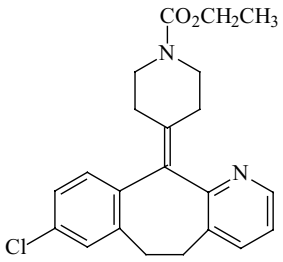
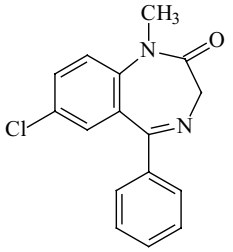


(A) Loratadine



(B) Diazepam

Fig. 1. Structures of loratadine (A) and diazepam (B, internal standard).

vent delivery pump, RF-10Axl fluorescence detector, and C-R8A integrator at attenuation 3. The fluorescence detection was performed at an excitation and emission wavelengths of 290 and 460 nm, respectively. The other parameters for detector were as follows: Gain = 2, Sens = 2 (medium), SPC Type = 2, Response = 2. The samples were applied by a Rheodyne 7725 loop injector with an effective volume of 100 μl. A Shimpack CLC-C_8 (250 × 4.6 mm i.d.; 5 μm particle size) with a guard column Shimpack C_8 (10 × 4 mm, 5 μm) were used for the chromatographic separation. The mobile phase comprised of acetonitrile-20 mM sodium dihydrogen phosphate- triethylamine (43:57:0.02, v/v), adjusted to pH 2.4 with concentrated phosphoric acid and 4 M sodium hydroxide. Analysis were run at flow rate of 1 ml/min at room temperature and the samples were quantified using loratadine to internal standard peak height ratio.

2.3. Standard solutions

Standard solutions of loratadine and diazepam were prepared by dissolving 5 mg of them in 50 ml of methanol and stored at −20 °C. The internal satandard stock solution was diluted in methanol to produce a final concentration of 50 μg/ml. Working solutions for loratadine were prepared daily in methanol producing 1000, 500, 250, 125, 62.5, 31.25 ng/ml.

2.4. Calibration curves

Plasma standards for calibration curves were prepared by spiking different samples of 1 ml drug-free plasma each with 20 μl of one of the above-mentioned loratadine working standard to produce 20, 10, 5, 2.5, 1.25, 0.62 ng/ml. They were shaked for 2 min, and then stored at least 15 min at room temperature before use. In each run, a plasma blank sample was also analyzed.

2.5. Extraction procedure

A 250 μl volume of plasma was transferred to a 1.5 ml polypropylene microcentrifuge tube. The internal standard (20 μl, equal to 1 μg diazepam) was added and vortex-mixed for 10 s. Extraction was performed by adding 40 μl 4 M NaOH and 1 ml of *n*-butyl alcohol/*n*-hexane (2:98, v/v) to the tube and shaking for 2 min. After centrifugation at 11 300 g for 3 min, the whole organic layer was separated and transferred into another tube. Then, 100 μl of 2.5% perchloric acid was added. The mixture was vortex-mixed and centrifuged (2 and 3 min, respectively). The aqueous phase was separated and transferred to another tube, and an 80 μl volume was injected into the chromatograph.

2.6. Assay validation

The specificity of the method was checked by comparing the chromatograms obtained from the samples containing loratadine and internal standards with those obtained from blank samples. Besides calibration standards, additional standards were prepared for the determination of accuracy and precision ($n = 3$) of the method. The absolute recovery ($n = 3$) was estimated by comparing peak heights of direct injection of drug solution made in 2.5% perchloric acid with corresponding loratadine peak of standard plasma concentrations after extraction.

2.7. Application

The assay was used for a comparative bioavailibility study of two tablets preparations containing 10 mg loratadine (Clarityn, Schering-Plough, Herts and a generic loratadine preparation from Arya pharmaceutical Co., Tehran, Iran).

Fourteen healthy volunteers participated in the study. The study was conducted using a two-way crossover design, as a single dose, randomized trial. The two formulations were administrated on two treatment days, separated by a washout period of 7 days, to fasted subjects who received a single oral dose (20 mg, two tablets) of one of the study medications. Food and drinks were not allowed until 3 h after ingestion of the tablet. Multiple blood samples (2.5 ml) were collected before and 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 8 h post dosing. The plasma was immediately separated by centrifugation and frozen at −20 °C until analysis.

3. Results

Representative chromatograms of drug-free plasma, plasma spiked with loratadine and a volunteer sample collected after oral dosing with loratadine are shown in Fig. 2.

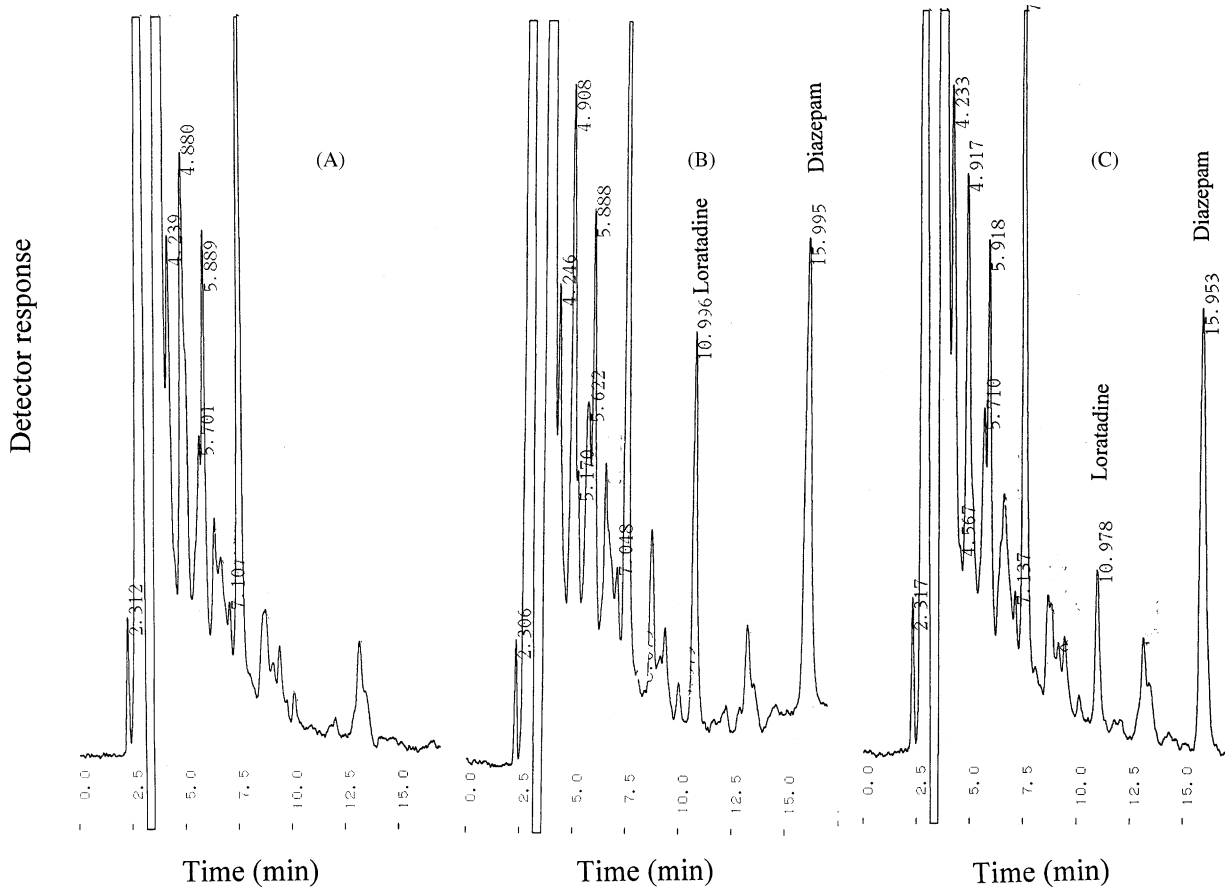


Fig. 2. Chromatogram of: (A) a blank plasma, (B) plasma spiked with 10 ng/ml loratadine, (C) a volunteer sample after oral administration of 20 mg of loratadine. The plasma loratadine concentration was determined to be 4.14 ng/ml.

The retention times for loratadine and the internal standard (diazepam) were 11 and 16 min, respectively. No interfering peaks from the endogenous plasma components were observed at the retention time of loratadine or internal standard. The calibration curves were linear over the concentration range of 0.62–20 ng/ml in human plasma, with a correlation coefficient greater than 0.999. Plasma concentrations above 20 ng/ml were diluted to meet the calibration range, while percent of variation was less than 5% for loratadine plasma standard of 40 ng/ml. The limit of quantitation was 0.62 ng/ml and the limit of detection was about 0.2 ng/ml at a signal to noise ratio of 3:1. The mean absolute recovery of the loratadine using the present extraction procedure was approximately 84% (Table 1). The mean absolute recovery of the internal standard was 87%. The results of the method intra- and inter-day accuracy and precision are presented in Table 1 and are all less than 9.7%.

The method showed good stability and performance for a long time and was used successfully for a comparative bioavailibility study. The plasma loratadine profiles for volunteers after taking the test or reference products are shown in Fig. 3.

4. Discussion

HPLC has proved to be the method of choice for the most of bioavailability studies. Mass spectrometry may offer better sensitivity than the conventional ultraviolet or fluorescence detectors, however, it is quite expensive and more

Table 1
The accuracy, intra- and inter-day precision and recovery data for the measurement of loratadine in human plasma ($n = 3$)

Nominal concentration (ng/ml)	Recovery (%)	Intra-day			Inter-day		
		Mean ± S.D.	Precision (%)	Accuracy (%)	Mean ± S.D.	Precision (%)	Accuracy (%)
0.62	88 ± 8.9	0.64 ± 0.062	9.7	3.2	0.60 ± 0.055	9.1	−3.2
1.25	82 ± 6.6	1.30 ± 0.099	7.6	4.0	1.35 ± 0.073	5.4	8
5	84 ± 5.8	5.26 ± 0.365	6.9	5.2	4.78 ± 0.369	7.7	−4.4
20	84 ± 2.1	20.1 ± 1.03	5.1	0.5	19.82 ± 0.782	3.9	−0.9

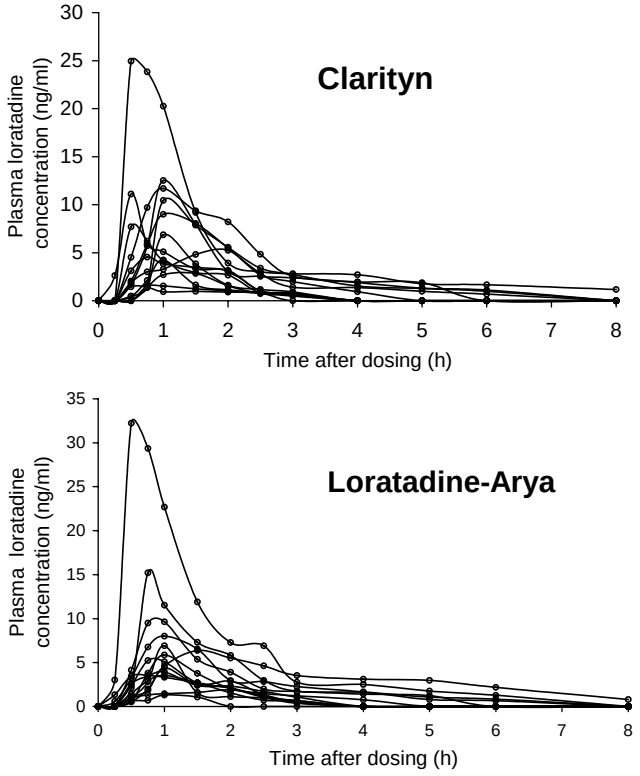


Fig. 3. Plasma concentration–time profiles of loratadine in 14 healthy volunteers following a 20 mg oral dose of Clarityn and a generic loratadine in a crossover study.

complicated. Loratadine has good fluorescence, especially in acidic mobile phases and fluorescence detection was found to be more sensitive and selective than UV, and therefore used in this work.

The most difficult aspect of loratadine assay by the published methods [2–8], is the sample preparation procedure. The need for at least 1 ml of plasma in previous methods [2–8] necessitates the use of high amounts of extraction solvent or double extraction [8]. Most of these methods also need to use glass tubes, which can not be centrifuged safely at high speed and therefore some of them freeze plasma for complete separation of extraction solvent. High-boiling point solvents such as toluene [4,8] and octane [5] are not easy to evaporate and increase the risk of loratadine loss during evaporation. Finally, total time needed for sample preparation is so long in some cases [6,8]. Knowing these limitations, we tried to find a suitable extraction method for loratadine from plasma.

Loratadine is not easily extracted from plasma using common extraction solvents. We checked several different extraction solvents. While loratadine in alkaline condition was rapidly eliminated from an aqueous phase into solvents, such as toluene, *n*-hexane, mixtures of *n*-butyl alcohol in *n*-hexane (as checked by direct injection of aqueous phase), it showed poor recovery in polypropylene tubes, when loratadine plasma standards were extracted with these solvents and the extracts was evaporated and injected after reconstitution. Re-extraction into phosphoric acid which has been used in an early work [7] was not successful in our experience, as other investigators have not repeated it. Instead of phosphoric acid, we used dilute perchloric acid that was able to effectively re-extract loratadine from *n*-hexane or low percents (lower than 5%) of *n*-butyl alcohol in *n*-hexane. Diluted perchloric acid was not able to extract loratadine from toluene or high percents (more than 20%) of *n*-butyl alcohol in *n*-hexane. Although loratadine from water standards could be extracted effectively into *n*-hexane and back-extracted into perchloric acid, it showed poor recovery when plasma standards of loratadine were examined. Addition of *n*-butyl alcohol into *n*-hexane produced good recovery for loratadine. The percent of *n*-butyl alcohol in *n*-hexane should be kept under minimum levels (less than 5%), because higher amounts decrease the recovery and also increase the interferences.

The pH of the mobile phase had great influence on the loratadine retention time and fluorescence. The retention time of loratadine was increased by decreasing acetonitrile volume in the mobile phase without loss of sensitivity, while the pH of the mobile phase was kept below 3. It is an important factor for obtaining an empty region in the chromatogram for loratadine and the internal standard peak. Unlike loratadine, the pH of the mobile phase had minor effects on the retention time of diazepam.

Although small volume of plasma was used in the present work, the limit of quantification was close to HPLC methods with ultraviolet [6], or fluorescence detection [7,8].

5. Conclusions

The present method provides a simple, rapid and precise means of assaying loratadine human plasma using liquid–liquid extraction followed by back extraction into diluted acid. The method allows the use of 1.5 ml polypropylene tubes which are much easier for working than glass tubes.The method is time-saving and economical, and is suitable for pharmacokinetic, bioavailability or bioequivalence studies.

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