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Determination of loratadine and its active metabolite in human plasma by high-performance liquid chromatography with mass spectrometry detection

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Abstract

A new sensitive and selective liquid chromatography coupled with mass spectrometry (LC/MS/MS) method for quantification of loratadine (LOR) and its active metabolite descarboethoxyloratadine (DSL) in human plasma was validated. After addition of the internal standard, metoclopramide, the human plasma samples (0.3 ml) were precipitated using acetonitrile (0.75 ml) and the centrifuged supernatants were partially evaporated under nitrogen at 37 °C at approximately 0.3 ml volume. The LOR, DSL and internal standard were separated on a reversed phase column (Zorbax SB-C18, 100 mm × 3.0 mm i.d., 3.5 μm) under isocratic conditions using a mobile phase of an 8:92 (v/v) mixture of acetonitrile and 0.4% (v/v) formic acid in water. The flow rate was 1 ml/min and the column temperature 45 °C. The detection of LOR, DSL and internal standard was in MRM mode using an ion trap mass spectrometer with electrospray positive ionisation. The ion transitions were monitored as follows: 383 → 337 for LOR, 311 → (259 + 294 + 282) for DSL and 300 → 226.8 for internal standard. Calibration curves were generated over the range of 0.52–52.3 ng/ml for both LOR and DSL with values for coefficient of determination greater than 0.994 by using a weighted (1/*y*) quadratic regression. The lower limits of quantification were established at 0.52 ng/ml LOR and DSL, respectively, with an accuracy and precision less than 20%. Both analytes demonstrated good short-term, long-term, post-preparative and freeze-thaw stability. Besides its simplicity, the sample treatment allows obtaining a very good recovery of both analytes, around 100%. The validated LC/MS/MS method has been applied to a pharmacokinetic study of loratadine tablets on healthy volunteers.

Keywords: Loratadine; Bioequivalence; Pharmacokinetics; LC–MS/MS

1. Introduction

4-(8-Chloro-5,6-dihydro-11*H*-benzo[5,6]-cycloheptal[1,2-*b*]-pyridin-11-ylidine)-1-piperidinecarboxylic acid ethyl ester (loratadine) is a long acting tricyclic antihistamine with selective peripheral histamine H_1-receptor antagonist activity that is used for relief of symptoms of seasonal allergies and skin rash (Fig. 1). Among the second-generation antihistamines, loratadine is free from sedation at recommended dosages. Following an oral administration of 10 mg tablet, loratadine (LOR) is rapidly absorbed and reaches peak concentration (T_{max}) at 1.3 h. For its major active metabolite, descarboethoxyloratadine, the T_{max} is 2.5 h [1]. Descarboethoxyloratadine or desloratadine (DSL) would be expected to produce results similar to LOR and other nonsedating antihistamines. The elimination half-life of LOR is 8–14 h, and that of DSL 17–24 h. An oral dose of loratadine (20 mg) leads to maximum plasma concentrations of only 11 and 10 ng/ml for LOR and DSL, respectively [2].

In view of these facts, the analytical method for LOR and DSL determination in human plasma has to be very sensitive and in the case of a large number of samples, the development of a chromatographic method suitable for this kind of analysis must take into account not only a sensitive procedure, but also a fast one, and as much as possible a simple sample preparation. An HPLC–MS or GC–MS method offers the solution from these points of view, in many cases.

Loratadine and desloratadine in plasma were studied by GC [3,4] or HPLC methods with UV [5] or fluorescence detec-

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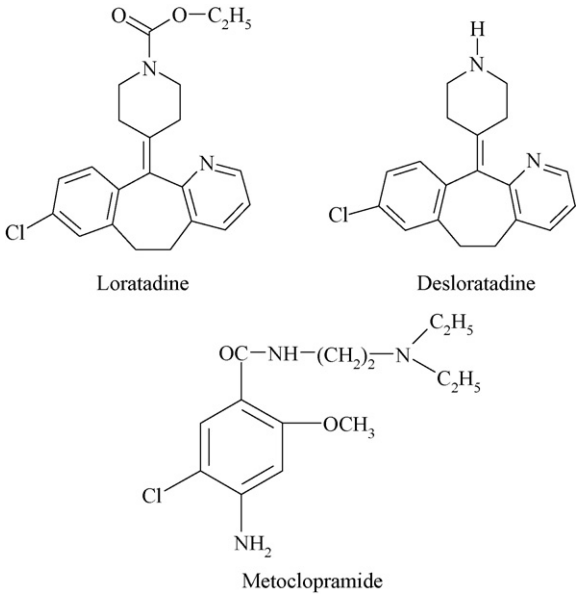

Fig. 1. Chemical structures of loratadine, desloratadine and metoclopramide (internal standard).

tion [6–9], with a sufficient lower limit of quantification for the purpose of study, by applying liquid–liquid or solid phase extraction. HPLC with MS detection was extensively used in the past years for the sensitive quantification of LOR and DSL [10–18], with a very low limit of quantification obtained mainly after liquid–liquid extraction of analytes.

Taking into account these facts, the aim of the present study was to develop a fast HPLC/MS/MS method able to quantify loratadine and desloratadine in human plasma after oral administration of a therapeutic dose of loratadine after a simple step of extraction. Finally, the developed and validated method was applied for bioequivalence investigation of two medicinal products containing 10 mg loratadine.

2. Experimental

2.1. Reagents

Loratadine and desloratadine were reference standards from Morepen Lab. Limited, India. Metoclopramide hydrochloride (MTC) (Fig. 1) was the internal standard (European Pharmacopoeia standard). Acetonitrile, methanol and formic acid were Merck products (Merck KgaA, Darmstadt, Germany). Distilled, deionised water was produced by a Direct Q-5 Millipore (Millipore SA, Molsheim, France) water system. The human blank plasma was supplied by the Local Bleeding Centre Cluj-Napoca, Romania.

2.2. Standard solutions

Two stock solutions of loratadine and desloratadine, respectively, with concentration of 2.5 mg/ml were prepared by dissolving appropriate quantities of reference substances (weighed on an Analytical Plus balance from Ohaus, USA) in 10 ml methanol. Two working solutions were then obtained for each substance by diluting specific volumes of stock solution with plasma. Then these were used to spike different volumes of plasma blank, providing finally eight plasma standards with the concentrations ranged between 0.52 and 52.3 ng/ml, equally for loratadine and desloratadine. Accuracy and precision of the method was verified using plasma standards with concentrations of 0.52, 1.68, 10.47 and 20.94 ng/ml loratadine and desloratadine, respectively. Quality control samples (QC) of 1.68, 10.47 and 20.94 ng/ml analytes were used during clinical samples analysis. The internal standard solution was prepared by sequential dilution of a stock solution of metoclopramide in acetonitrile (1 mg/ml) to reach a concentration of 3.65 ng/ml. This solution was used for precipitation of plasma proteins.

2.3. Chromatographic and mass spectrometry systems and conditions

The HPLC system was an 1100 series model (Agilent Technologies) consisted of a binary pump, an in-line degasser, an autosampler, a column thermostat and an Ion Trap VL mass spectrometer detector (Brucker Daltonics GmbH, Germany). Chromatograms were processed using QuantAnalysis software. The detection of LOR, DSL and internal standard was in MRM mode using an ion trap mass spectrometer with electrospray positive ionisation. The ion transitions were monitored as follows: $383 \rightarrow 337$ for LOR, $311 \rightarrow (259 + 294 + 282)$ for DSL and $300 \rightarrow 226.8$ for internal standard. Chromatographic separation was performed at 45 °C on a Zorbax SB-C18 100 mm × 3 mm, 3.5 μm column (Agilent Technologies), protected by an in-line filter.

2.4. Mobile phase

The mobile phase consisted of a mixture of water containing 0.4% formic acid and acetonitrile (92:8, v/v), each component being degassed, before elution, for 10 min in an Elma Transsonic 700/H (Singen, Germany) ultrasonic bath. The pump delivered the mobile phase at 1 ml/min.

2.5. Sample preparation

Standard and test plasma samples were prepared as follows in order to be chromatographically analyzed. In a test tube of 1.5 ml, 0.3 ml plasma and 0.75 ml acetonitrile containing internal standard (3.65 ng/ml metoclopramide as base) were added. The tube was vortex-mixed for 10 s (Vortex Genie 2, Scientific Industries) and then centrifuged for 6 min at 6000 rpm (204 Sigma centrifuge, Osterode am Harz, Germany). The supernatant was transferred in a glass centrifuge tube and evaporated at 37 °C under nitrogen to approximate 0.3 ml. The final solution was transferred to an autosampler vial and 10 μl were injected into the HPLC system.

2.6. Validation

As a first step of method validation [19–21], specificity was verified using six different plasma blanks obtained from

healthy human volunteers who had not previously taken any medication.

The concentration of analytes was determined automatically by the instrument data system using the internal standard method. Calibration was performed using singlicate calibration standards on five different occasions. The calibration curve model was determined by the least squares analysis. The applied calibration model was $y = c + bx + ax^2$, weight $1/y\,(1/y)$ quadratic response, where y, area ratio and x, concentration ratio. Distribution of the residuals (%difference of the back-calculated concentration from the nominal concentration) was investigated. The calibration model was accepted, if the residuals were within ±20% at the lower limit of quantification (LLOQ) and within ±15% at all other calibration levels and at least two-third of the standards meet this criterion, including highest and lowest calibration levels.

The lower limit of quantification was established as the lowest calibration standard with an accuracy and precision less than 20%.

The within- and between-run precision (expressed as coefficient of variation, CV%) and accuracy (expressed as relative difference between obtained and theoretical concentration, bias%) of the assay procedure were determined by analysis on the same day of five different samples at each of the lower (1.68 ng/ml), medium (10.47 ng/ml) and higher (20.94 ng/ml) levels of the considered concentration range and one different sample of each on five different occasions, respectively. The selected concentration values are relevant in practice taking in account to the fact that the reported maximum concentration levels of either loratadine or its metabolite are not greater that 8–10 ng/ml at usual oral doses (20 mg loratadine) [1,2].

The relative recoveries at each of the previously three levels of concentration and limit of quantification were measured by comparing the response, relative to the internal standard, of the treated plasma standards with the response of standards in solvent with the same concentration of analytes and internal standard as the plasma sample.

The stability of the analytes in human plasma was investigated in three ways, in order to characterize each operation during the process of bioequivalence studies: room-temperature stability (RTS), post-preparative stability (PPS) in the autosampler, freeze-thaw stability (FTS) and long-term stability (LTS) below −20 °C. For all stability studies, plasma standards at low (1.68 ng/ml both LOR and DSL) and high concentrations (20.94 ng/ml both LOR and DSL) were used. Four plasma standards at each of the two levels were prepared and let at room temperature 4 h before processing (RTS study). Other four pairs were prepared, immediately processed and stored in the autosampler (25 °C) of the HPLC system (PPS study). The samples were injected after 10 h, the expected longest storage time of the samples in autosampler before injection. For the freeze-thaw stability, aliquots at the same low and high concentrations were prepared. These samples were subjected to three cycles of freeze-thaw operations in 3 consecutive days. After the third cycle, the samples were analyzed against calibration curve of the day. The mean concentration calculated for the samples subjected to the cycles and the nominal ones were compared. For long-term stability, in the first validation day, there were injected and analyzed four samples at each of low and high concentrations, and values were calculated against calibration curve of the day. Other two sets with the same plasma concentrations were stored in freezer below −20 °C and analyzed together with calibration samples after 5 months. The values were calculated against calibration curve of the day and the mean values for the stored samples and nominal concentrations were compared. The requirement for stable analytes was that the difference between mean concentrations of the tested samples in various conditions and nominal concentrations had to be in ±15% range.

2.7. Clinical application and in-study validation

The validated method was applied in a bioequivalence study of two dosage forms—tablets containing 10 mg loratadine. The collecting times were 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48 h after oral administration of 40 mg loratadine. The accuracy and precision of the validated method was monitored to ensure that it continued to perform satisfactorily during analysis of volunteer samples. To achieve this objective, a number of QC samples prepared in duplicate at three concentration levels were analyzed in each assay run and the results compared with the corresponding calibration curve. At least 67% (four out of six) of the QC samples should be within 15% of their respective nominal values; 33% of the QC samples (not all replicates at the same concentration) can be outside ±15% of the nominal value.

3. Results and discussions

No significant interference at the retention time of LOR (7 min) and DSL (3.2 min) was observed in different plasma blank samples chromatograms (Figs. 2 and 3) due to the specificity of selected signals (Fig. 4). It is well known that an advantage of the ion trap over the triple quadrupole is the sensibility in scan mode. This allows adding multiple fragments from an MS spectrum in order to improve the overall signal. In the case of desloratadine, the sum of ions from MS spectrum (*m*/*z* 259, 294, 282) was chosen for quantification because the detection is about 40% more sensitive that the case based only on ion *m*/*z* 259.

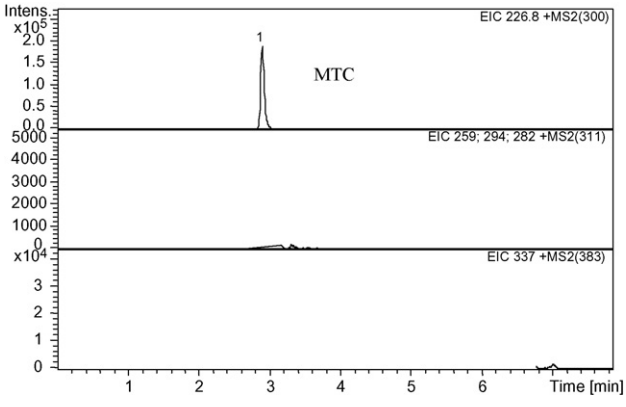

Fig. 2. Chromatograms of a plasma blank containing metoclopramide (MTC, internal standard).

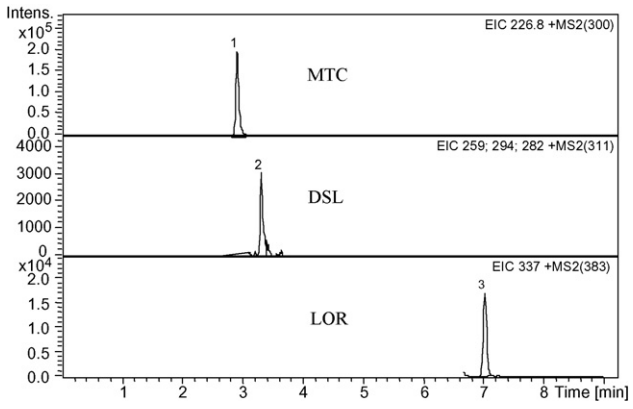

Fig. 3. Chromatograms of the LLOQ plasma standard with 0.52 ng/ml desloratadine (DSL) and 0.52 ng/ml loratadine (LOR), metoclopramide (MTC) being the internal standard.

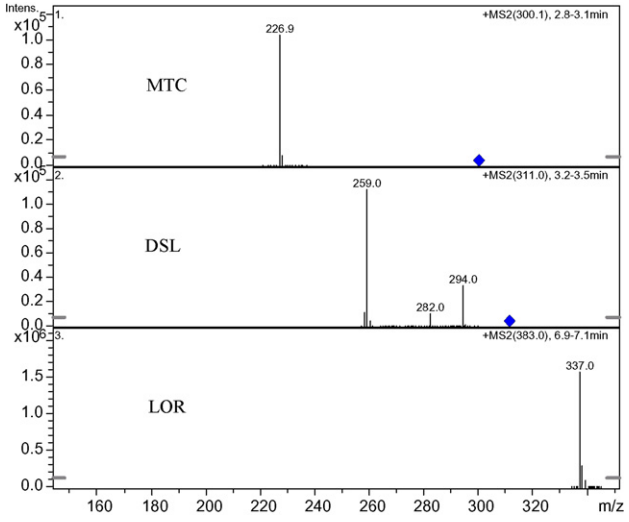

Fig. 4. Product ion mass spectra of the internal standard metoclopramide (MTC), *m/z* 300.1, desloratadine (DSL), *m/z* 311.0 and loratadine (LOR), *m/z* 383.0.

The applied calibration curves models proved to be accurate over the concentration range 0.52–52.3 ng/ml, with a correlation coefficient greater than 0.998, for both analytes. The residuals had no tendency of variation with concentration.

The method had within- and between-run accuracy and precision (Tables 1 and 2), in agreement to international regulations regarding bioanalytical methods validation [19–21]. The lower limit of quantification was established at 0.52 ng/ml LOR and DSL, respectively, with accuracy and precision less than 20% (Tables 1 and 2).

Table 2
Between-run precision and accuracy for loratadine and desloratadine ($n = 5$)

$c_{nominal}$ (ng/ml)	Mean c_{found} (ng/ml) (±S.D.)		CV%		Bias%	
	LOR	DSL	LOR	DSL	LOR	DSL
0.52	0.52 (0.10)	0.53 (0.03)	19.23	5.66	0.00	1.92
1.68	1.69 (0.19)	1.73 (0.09)	11.24	5.20	0.59	2.98
10.47	10.50 (0.70)	10.60 (0.32)	6.67	3.02	0.29	1.24
20.94	20.88 (0.61)	21.33 (1.22)	2.92	5.72	−0.28	1.86

The recovery was consistent and reproducible, either for LOR and DSL (Table 1).

The analytes proved their stability under various conditions (Table 3), the bias% of found concentration being less than 15%, the maximum accepted value for method's accuracy.

The validated method was verified during analysis of clinical samples from a bioequivalence study of two medicines containing 10 mg loratadine. The method continued to perform in terms of accuracy, in each analytical run not more than two out of six QC samples being outside of ±15% nominal value, but not all two at the same concentration. Figs. 5 and 6 show typical chromatograms of the plasma sample from a volunteer and concentration profiles of LOR and DSL obtained after oral administration of 40 mg loratadine, respectively.

In comparison with previously published HPLC–MS methods, the sensitivity of the proposed method (LLOQ of 0.52 ng/ml) is not better as those in which liquid–liquid [10–13,15,16,18] or solid-phase extraction [14,16,17] was applied (Table 4). But the main advantage is the simple sample preparation by protein precipitation, followed by a partial solvent evaporation, without significant matrix effect in comparison with Naidong et al. results [16] obtained by applying this sample pretreatment. Besides its simplicity, that sample treatment allows obtaining a very good recovery of both analytes

Table 3
Results of the stability studies ($n = 4$)

	RTS		PPS		FTS		LTS	
$c_{nominal}$ (ng/ml)	1.68	20.94	1.68	20.94	1.68	20.94	1.68	20.94
Bias%, LOR	2.61	−5.43	−2.61	−2.24	7.66	0.14	7.43	−2.79
Bias%, DSL	−9.05	−1.12	−13.82	−5.54	−0.51	−8.12	−2.00	−8.78

RTS, room temperature stability (22 °C, 4 h); PPS, post-preparative stability (22 °C, 10 h); FTS, freeze-thaw stability (three freeze-thaw cycles); LTS, long-term stability (−20 °C, 5 months).

Table 1
Within-run precision, accuracy and recovery for loratadine and desloratadine ($n = 5$)

$c_{nominal}$ (ng/ml)	Mean c_{found} (ng/ml) (±S.D.)		CV%		Bias%		Recovery% (±S.D.)	
	LOR	DSL	LOR	DSL	LOR	DSL	LOR	DSL
0.52	0.52 (0.10)	0.54 (0.05)	19.23	9.26	0.00	3.85	116.6 (18.0)	113.5 (10.0)
1.68	1.46 (0.20)	1.64 (0.17)	13.69	10.37	−13.09	−2.38	90.3 (13.2)	107.4 (10.8)
10.47	9.46 (0.75)	9.88 (0.73)	7.93	7.39	−9.65	−5.64	97.8 (7.4)	107.2 (7.5)
20.94	20.13 (2.07)	20.41 (2.43)	10.28	11.91	−3.87	−2.53	102.2 (14.9)	107.0 (11.5)

Table 4
Analytical characteristics of reported HPLC methods with MS detection for the determination of loratadine and desloratadine in plasma

References	Column	Mobile phase constituents and flow (ml/min)	Pretreatment/ extraction[a]	LOQ (ng/ml)	Run time (min)	Observation
Sun et al. [10]	C18	Acetonitrile–water–acetic acid, gradient mode, 0.2 ml/min	LL	0.05 LOR	11	DSL not determined
Salem et al. [11]	Phenyl	Acetonitrile–water–formic acid, 0.2 ml/min	LL	0.1 LOR	?	DSL not determined
Chen et al. [12]	C18	Acetonitrile–ammonium acetate, gradient mode, 0.2 ml/min	LL	0.2 LOR	?	DSL not determined
Naidong et al. [13]	Silica	Acetonitrile–water–trifluor-acetic acid, 0.5 ml/min	LL	0.01 LOR, 0.025 DSL	3	
Yang et al. [14]	C8	Acetonitrile–ammonium acetate, 0.25 ml/min	SPE	0.025 DSL	6	3-Hidroxy-DSL determined
Zhang et al. [15]	C8	Acetonitrile–water–formic acid, 0.5 ml/min	LL	0.2 LOR, 0.2 DSL	?	
Naidong et al. [16]	Silica	Acetonitrile–water–formic acid, gradient mode, 0.5 ml/min	LL, SPE, PP	0.2 LOR	4	DSL not determined
Yang et al. [17]	?	?	SPE	1.0 LOR, 1.0 DSL	?	Rat, rabbit, mouse plasma
Sutherland et al. [18]	C18	Acetonitrile–water–formic acid, gradient mode, 0.3 ml/min	LL	0.1 LOR, 0.1 DSL	7	

[a] LL, liquid–liquid extraction; SPE, solid-phase extraction; PP, protein precipitation.

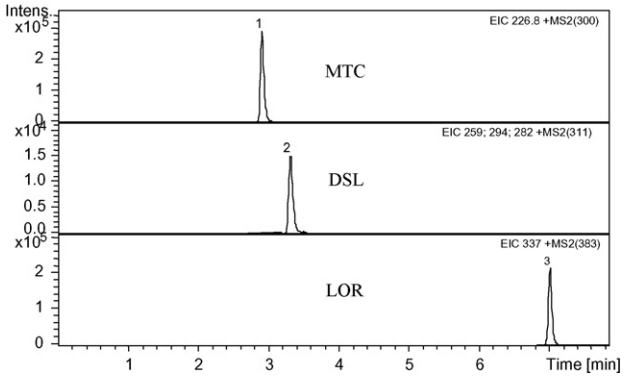

Fig. 5. The plasma sample chromatograms obtained from a healthy volunteer at 1.5 h after oral administration of 40 mg loratadine. Concentrations are 1.95 ng/ml desloratadine (DSL) and 3.01 ng/ml loratadine (LOR), metoclopramide (MTC) being the internal standard.

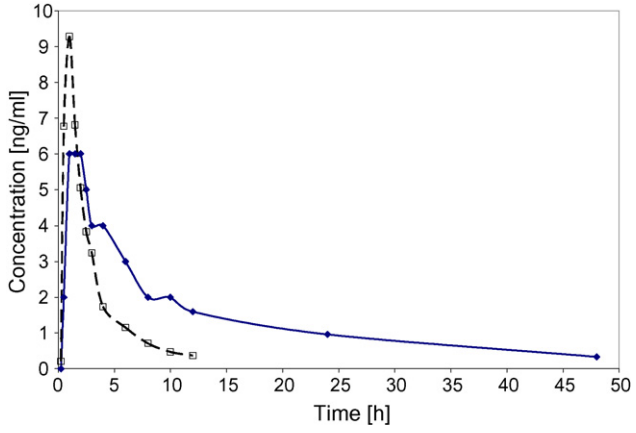

Fig. 6. Concentration profile of loratadine (□) and desloratadine (♦) after oral administration of 40 mg loratadine.

and, as far as we are aware, this is the first reported method for simultaneous analysis of LOR and DSL in human plasma after protein precipitation and partial evaporation of extract.

4. Conclusions

The proposed method provides accuracy and precision for quantitative determination of loratadine and desloratadine in human plasma after oral administration of 40 mg loratadine. The simple sample preparation by protein precipitation and selected signals for monitoring allowed a specific and efficient analysis of a large number of plasma samples, making the method more productive and thus more cost effective.

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