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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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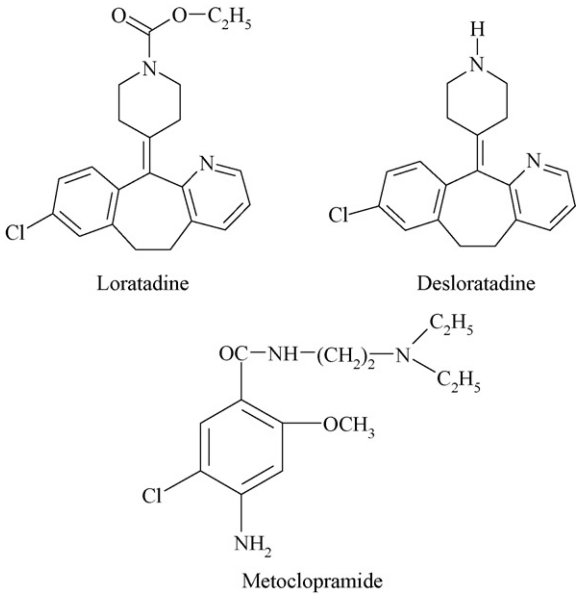


Fig. 1. Chemical structures of loratadine, desloratadine and metoclopramide (internal standard).

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