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Simultaneous determination of loratadine and pseudoephedrine sulfate in human plasma by liquid chromatography–electrospray mass spectrometry for pharmacokinetic studies

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Abstract

To support the pharmacokinetic and bioavailability study of an extended-release loratadine (LOR)/pseudoephedrine sulfate (PES) tablet, a high performance liquid chromatographic-electrospray ionisation–mass spectrometric method (LC–MS) was developed for the simultaneous determination of LOR and PES in human plasma. Diazepam (DP) and phenylpropanolamine (PPA) were used as internal standards for LOR and PES, respectively. Analytes were extracted from alkalized human plasma by liquid/liquid extraction using ethyl ether. Chromatographic separation was performed on an ODS column at flow rate of 0.2 ml/min. The total chromatographic run time was 10.5 min with the retention time of 7.1 min and 6.2 min for LOR and DP, respectively, and 2.2 min for both of PES and PPA. The LOQ was 10 pg/ml and 50 pg/ml for LOR and PES, respectively. The method is accurate and precise enough for its intended purpose.

Keywords: Loratadine; Pseudoephedrine sulfate; Pharmacokinetics; LC–MS

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1. Introduction

Loratadine (LOR) and pseudoephedrine sulfate (PES) are two common components of many preparations used for the relief of symptoms associated with allergic rhinitis. LOR (4-(8-chloro-5,6-dihydro-11*H*-benzo[5,6]-cycloheptal[1,2-*b*]-pyridin-11-ylidine)-1-piperidinecarboxylic acid ethyl ester) 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. Among the second-generation antihistamines, LOR is free from sedation at recommended dosages [1]. PES (benzenemethanol,α-[1-(methylamino)ethyl]-,[*S*-(R^*,R^*)]sulfate) is the synthetic salt of one of the naturally occurring dextrorotatory diastereomers of ephedrine and is classified as an indirect and direct-acting sympathomimetic amine.

Extended-release LOR/PES twice- or once-daily tablet formulations have been marketed in the United States since 1993 and have been shown to be safe and effective for the relief of symptoms associated with allergic rhinitis. The twice-daily tablet consists of loratadine 5 mg in an immediate-release coating and pseudoephedrine sulfate 120 mg, of which 60 mg is in an immediate-release coating and 60 mg is in a barrier-protected core. Different formulations based on the same mechanism as the extended-release formulation have been developed recently. To study the bioavailability and bioequivalence of these formulations, both PES and LOR must be measured in plasma of participants. Various analytical procedures have been reported for separate quantitative determination of loratadine or pseudoephedrine sulfate in dosage forms and in biological fluids. These include high performance liquid chromatography with ultraviolet detection (HPLC-UV) for PES [2] and LOR [3,4], HPLC with fluorescence detection for LOR [5], capillary electrophoresis (CE) for LOR [6] and PES [7], and LC–MS

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for LOR [8,9]. Sutherland et al. [10] and Weng et al. [11] described different methods for the simultaneous detection of LOR and its metabolite descarboethoxy-loratadine (DCL) using liquid chromatography–tandem mass spectrometry (LC–MS/MS).

Due to the differences in the pK_a values and polarities, it is difficult and time consuming to chromatograph both LOR and PES in a single chromatographic run by isocratic mobile phase when biological samples are involved. Few researches have addressed the simultaneous detection of LOR and PES in plasma. Feyyaz et al. [12] described a method of simultaneous determination of PES, LOR and other compounds in formulations by first-derivative spectrophotometry and ratio-spectra derivative spectrophotometry, as well as HPLC to separate PES and dexbrompheniramine maleate. The method was only suitable for the analysis of drugs in formulations, and not applicable to pharmacokinetic studies due to endogenous interferences and low sensitivity. Since both LOR and PES have basic functional groups, they can be extracted from alkalized human plasma using liquid/liquid extraction. A simple one-step liquid/liquid extraction of LOR and PES from alkalized plasma was developed. With the selectivity provided by mass spectrometry, determination can be optimized by gradient elution. To support the bioequivalence study of extended-release LOR/PES tablet, we developed a sensitive method for simultaneous determination of LOR and PES in human plasma using a gradient elution mode.

2. Experimental

2.1. Chemicals and reagents

LOR (purity 99.90%), PES (purity 99.98%) DP (purity 99.80%), PPA (purity 99.70%), descarboethoxy-loratadine (DCL, purity 99.80%) were supplied by Jiangsu Institute for the Control of Pharmaceutical Products (Nanjing, China). HPLC grade acetonitrile (ACN) and methanol were obtained from Fisher Scientific (St. Louis, MO, USA). Milli-Q water was generated by passing distilled water through a Quantum EX ultrapure organex cartridge (Cat No. QTUM 000EX, Millipore S.A. 67120, Molshem, France). Blank plasma was obtained from the Blood Supply Center (Nanjing, China) and was stored in a freezer at −/20 °C. Sodium heparin used as an anticoagulant, was obtained from Huixing Biochemistry Co. Ltd. (Shanghai, China). Ethyl ether was obtained from Nanjing Chemical Reagent No. 1 Factory (Nanjing, China).

2.2. LC–mass spectrometry (LC–MS)

The LC–MS system consisted of a Shimadzu 10ADvp Pump, DGU-14AM degasser, SIL-HTC Autoinjector and LCMS2010A mass spectrometer with an electrospray ionization source (Shimadzu, Kyoto, Japan). A Shimadzu LCMass Solution Version 2.04 on a Windows 2000 operating system was used for data analysis.

The analysis was carried out on an ODS column (Shim-pack, 5 μm, 2.1 mm × 150 mm i.d., Shimadzu, Japan) equipped with an ODS guard column (Security Guard, Phenomenex, USA). Mobile phases of acetonitrile–water (35:65), containing 0.1% of glacial acetic acid (v/v) (A) and acetonitrile–water (80:20), containing 0.1% of glacial acetic acid (v/v) (B) were used. The column was equilibrated with mobile phase A before injection. After the injection was initiated, the mobile phase was changed immediately to mobile phase B. PES and internal standard PPA were eluted within 3 min and LOR and DP were eluted within 8 min. The column was equilibrated with mobile phase A for 3 min before the second injection was initiated. The total period for one sample was about 11 min (see Fig. 1).

2.3. Standard solutions and sample preparation

LOR, DCL, PES, DP (internal standard 1, IS-1) and PPA (internal standard 2, IS-2) stock solutions were prepared by weighing out the reference standards, which were dissolved in methanol. Intermediate stock solutions were prepared by diluting the stock solution with methanol. Standard solutions for preparing calibration curve and quality control (QC) samples were obtained by serial dilutions of intermediate stock solutions with water, except for DP which was diluted with methanol.

Volumes (1.0 ml) of QC and blank plasma samples were transferred into glass tubes. Twenty microliters of IS-1 solution (500 ng/ml of DP in methanol) and IS-2 solution (20 μg/ml of PPA in water) were added to all tubes except blank plasma samples. After 30 s vortex-mixing, 100 μl of 0.1 mol/l sodium hydroxide solution was added to each tube and samples were vortex-mixed for another 30 s. Ethyl ether (6 ml) was added to each tube and the samples were vortex-mixed for 3 min. The tubes were then centrifuged at 1200 × *g* for 10 min at room temperature using a Centrifuge (Model 0412-1, Shanghai Surgical Instruments Factory, Shanghai). The upper organic layer was transferred into a glass tube and the lower aqueous layer was discarded. The organic solvent was evaporated to dryness under nitrogen in thermostatic water bath at 45 °C. The samples were reconstituted with 200 μl of acetonitrile by vortex-mixing for 60 s. After further centrifuging at 21 000 × *g* for 10 min at 4 °C (Micromax RF, Thermo IEC, USA), the supernatant was transferred to 1.5 ml autosampler vial. A 5 μl volume of the supernatant was used for analysis.

2.4. Calibration standard samples and quality control (QC)

During validation and batch analysis of plasma samples, calibration standard samples were prepared freshly by adding aliquot of LOR and of PES calibration solutions into glass tubes containing 1.0 ml of blank plasma to obtain serial concentrations of 0.05/5.0, 0.10/10.0, 0.25/25.0, 0.50/50.0, 1.00/100.0, 2.50/250.0, 5.00/500.0, 10.00/1000.0 ng/ml for