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Short Communication

Development of a highly sensitive LC–MS/MS method for simultaneous determination of rupatadine and its two active metabolites in human plasma: Application to a clinical pharmacokinetic study

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ABSTRACT

An easy LC–ESI–MS/MS method was developed and validated for simultaneous determination of rupatadine (RT) and its two active metabolites, namely desloratadine (DT) and 3-hydroxydesloratadine (3-OH-DT), in human plasma. The chromatographic separation was carried out on a C_{18} column with gradient elution by using methanol and 10 mM ammonium acetate containing 0.1% (v/v) formic acid. The lower limit of quantification (LLOQ) was 0.05, 0.035 and 0.035 ng/mL for RT, DT and 3-OH-DT, respectively. The intra- and inter-day precision of analytes were within the range of 1.0–4.7% and 2.2–12.1%, respectively. The intra- and inter-day accuracy of analytes were within the range of −7.7% to 5.2% and −4.1% to 4.8%, respectively. The method was successfully applied to a pharmacokinetic study of RT and its two metabolite DT and 3-OH-DT in healthy volunteers following single (10, 20, 40 mg) and multiple (10 mg) oral doses of rupatadine fumarate tablets.

1. Introduction

Rupatadine (RT) is a non-sedating and selective histamine H1-receptor antagonist and platelet-activating factor inhibitor [1–3], which is safe and effective for the treatment of allergic rhinitis and chronic urticaria [3–7]. It is extensively metabolized by P450 3A4 [2,8], and the major metabolites of RT are desloratadine (DT) and 3-hydroxydesloratadine (3-OH-DT), which are active and contribute to the overall efficacy of the drug [4,9,10]. It is essential to evaluate the potential effect of the main metabolites and their back-conversion to the parent drug during analysis.

There is a growing demand for the simultaneous quantification of RT with metabolites for the pharmacokinetic and pharmacodynamic studies in human. However, few LC–MS/MS methods dealing with biological matrices have been reported. An LC–MS/MS method was developed for the determination of RT in human plasma, but it did not allow simultaneous determination of any metabolite [11]. Wen et al. [12] developed an LC–MS/MS method to determine RT and DT in human plasma. Two other reports from the same group [9,13] descried a LC–MS/MS method for the simultaneous determination of RT, DT and 3-OH-DT in human plasma, both of which focused on the effect of food or the co-administration of azithromycin on the pharmacokinetics and no chromatograms and validation details were presented. Besides, the high concentration of 0.2 M ammonium acetate employed in the mobile phases may have potential adverse effect on the mass spectrometer. LLOQ of the reported methods for the analyte was between 0.1 to 0.2 ng/mL [9,11–13], which could not meet the requirement in our clinical pharmacokinetic research.

To address this challenge, an LC–ESI–MS/MS method for the simultaneous determination of RT and its active metabolites DT and 3-OH-DT with greater sensitivity was developed and validated. The method was used in a human study following single and multiple oral doses of rupatadine fumarate tablets (RTFT). To the best

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of our knowledge, there is no report so far on the back-conversion of DT and 3-OH-DT to their parent drug, as well as the comparison of pharmacokinetic parameters of RT and its two metabolites between single and multiple oral doses of RTFT. More interestingly, the two metabolites were found to have different pharmacokinetic profiles in male and female subjects.

2. Experimental

2.1. Chemicals and reagents

Rupatadine fumarate tablets (RTFT) were supplied by Zhuhai Kinhoo Pharmaceutical Co., Ltd. (Guangdong, China). Rupatadine fumarate (RTF), desloratadine (DT) and the internal standard (IS, letrozole) were purchased from National Institutes for Food and Drug Control (Beijing, China). 3-Hydroxydesloratadine (3-OH-DT) was supplied by TLC PharmaChem, Inc (Mississauga, Canada). Methanol was HPLC grade (Merck KGaA, Darmstadt, Germany). All other reagents and solvents were analytical grade. Water was prepared with double distillation.

2.2. LC–MS/MS instruments and conditions

Liquid chromatography was performed on an Agilent 1260 Series liquid chromatography (Agilent Technologies, Palo Alto, CA, USA). The separation was carried out on a Hedera ODS-2 column (2.1 mm × 150 mm, 5 μm, Hanbon Sci&Tech, Huai'an, China) with a security Guard-C_{18} column (4 mm × 2.0 mm, 5 μm, Phenomenex, Torrance, CA, USA). The mobile phase was composed of 10 mM ammonium acetate containing 0.1% (v/v) formic acid (A) and methanol (B) with a gradient elution program of 60% B (0–3.0 min), from 60 to 100% of B (3.0–3.1 min), 100% of B (3.1–6.0 min), followed by the column equilibration for 5 min. Autosampler and column temperature were set at 8 °C and 38 °C, respectively. The flow eluate was directed to the mass spectrometer in the first 7 min and afterwards to the waste.

An AB Sciex API 4000 tandem mass spectrometer (Applied Biosystems, Toronto, Canada) equipped with a Turbo-V® ionspray source operation in the positive ESI mode was used for the detection. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of *m/z* 416.0 → 309.1, 311.0 → 259.1, 327.0 → 275.2 and 286.1 → 217.1 for RT, DT, 3-OH-DT and IS, respectively, The nebulizer gas (GS1) and heater gas (GS2) were set at 60 and 30 psi, respectively. The curtain gas (CUR) was kept at 10 psi and the collision gas (CAD) was 10 psi, with ion spray temperature and ionspray voltage kept at 400 °C and 5500 V. The system control and data analysis were performed by AB Sciex Analyst software (version 1.5.2).

2.3. Preparation of calibration standards and quality control (QC) samples

The stock solutions of RT, DT and 3-OH-DT prepared in methanol (1.00 mg/mL) were serially diluted with methanol to provide working standard solutions. The stock solution of IS in methanol (1.00 mg/mL) was diluted to 400 ng/mL as IS working solution.

A mixture of RT, DT and 3-OH-DT (each of 20 μL of the working standard solution) was added into a clean tube and evaporated to dryness under nitrogen stream at 35 °C. The residual was reconstituted with 1.0 mL drug-free human plasma to obtain calibration standards with the concentration of 0.050, 0.15, 0.50, 1.5, 5.0, 10, 20, and 35 ng/mL for RT, and 0.035, 0.10, 0.30, 0.60, 1.5, 3.0, 6.0 and 10 ng/mL for DT and 3-OH-DT. The QC samples were prepared at three concentration levels, namely low (0.10/0.080/0.080 ng/mL), medium (2.0/0.80/0.80 ng/mL), and high (30/8.0/8.0 ng/mL) for RT/DT/3-OH-DT. All the solutions were kept at −20 °C and brought to room temperature before use.

2.4. Sample preparation

All frozen samples were thawed and allowed to equilibrate at room temperature before analysis. An aliquot of 1.0 mL plasma was mixed with 20 μL IS solution (400 ng/mL), 1.0 mL saturated solution of sodium bicarbonate and 4.0 mL ethyl acetate. The mixture

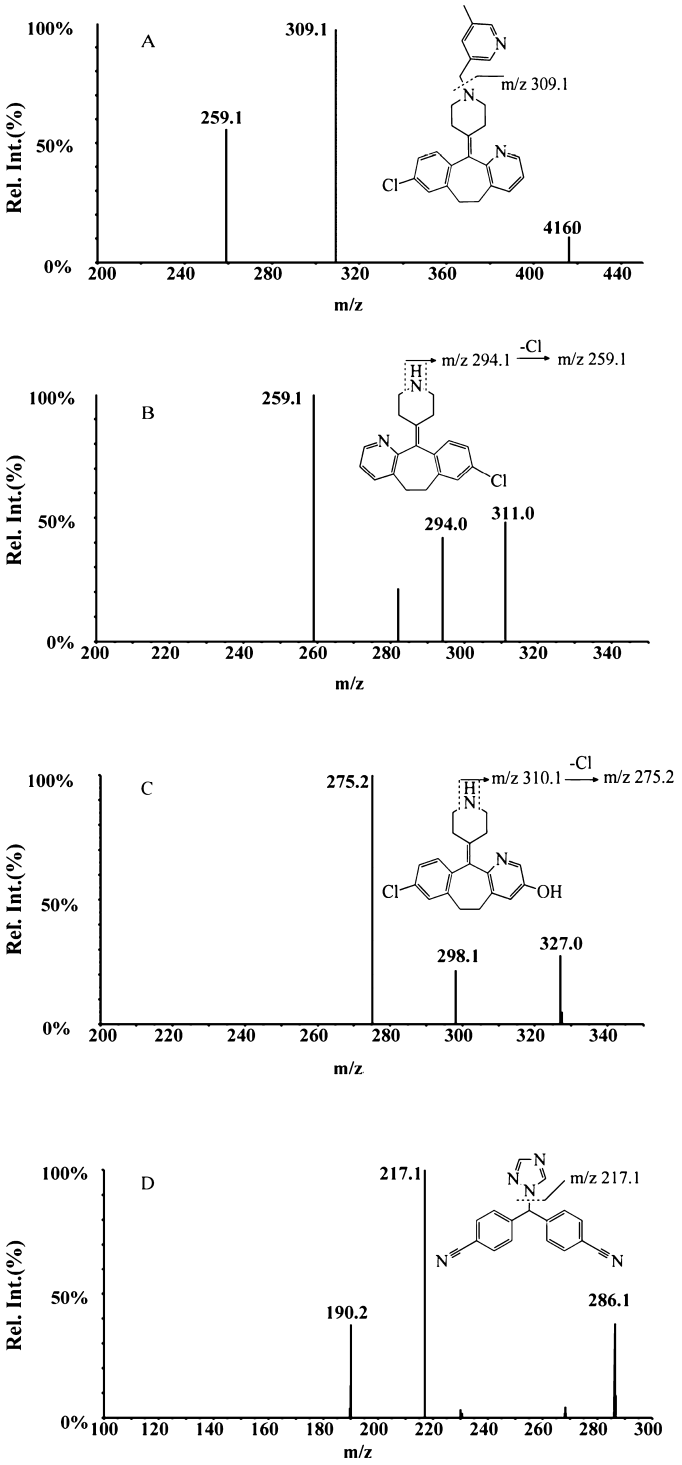

Fig. 1. Positive product ion mass spectra of $[M+H]^+$ of RT (A), DT (B), 3-OH-DT (C) and IS (D) and their proposed fragmentation patterns.