Journal of Pharmaceutical and Biomedical Analysis 49 (2009) 347–353

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Simultaneous determination of rupatadine and its metabolite desloratadine in human plasma by a sensitive LC–MS/MS method: Application to the pharmacokinetic study in healthy Chinese volunteers

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ARTICLE INFO

Article history:
Received 8 August 2008
Received in revised form
22 September 2008
Accepted 10 October 2008
Available online 1 November 2008

Keywords:
Rupatadine
Desloratadine
LC–MS/MS
Human plasma
Pharmacokinetics

ABSTRACT

A sensitive liquid chromatography/tandem mass spectrometry (LC–MS/MS) method was developed for simultaneous determination of rupatadine and its metabolite desloratadine in human plasma. After the addition of diphenhydramine, the internal standard (IS), plasma samples were extracted with a mixture of methyl *tert*-butyl ether and *n*-hexane (1:1, v/v). The analysis was performed on a Ultimate™ AQ-C18 (4.6 mm × 100 mm, 5 μm) column using a mobile phase consisting of a 80/20 mixture of methanol/water containing 0.0005% formic acid pumped at 0.3 ml min^{-1}. The analytes and the IS were detected in positive ionization mode and monitoring their precursor → product ion combinations of *m/z* 416 → 309, 311 → 259, and 256 → 167, respectively, in multiple reaction monitoring mode. The linear ranges of the assay were 0.1–50 and 0.1–20 ng ml^{-1} for rupatadine and desloratadine, respectively. The lower limits of reliable quantification for both rupatadine and desloratadine were 0.1 ng ml^{-1}, which offered high sensitivity and selectivity. The within- and between-run precision was less than 7.2%. The accuracy ranged from −9.2% to +6.4% and −7.2% to +7.2% for rupatadine and desloratadine in quality control samples at three levels, respectively. The method has been successfully applied to a pharmacokinetic study of rupatadine and its major metabolite after oral administration of 10, 20 and 40 mg rupatadine tablets to healthy Chinese volunteers.

1. Introduction

Rupatadine, 8-chloro-6,11-dihydro-11-[1-[(5-methyl-3-pyridinyl)methyl]-4-piperidinylidene]-5*H*-benzo[5,6]-cyclohepta[1,2-*b*]pyridine (Fig. 1) is a long acting dual antagonist of both histamine H1 and platelet-activating factor (PAF) receptors. Pharmacological experiments have demonstrated that rupatadine is as potent as or even more potent than second-generation antihistamines such as loratadine, terfenadine and cetirizine [1–3]. Rupatadine is rapidly absorbed when administered orally and it is subjected to considerable first-pass metabolism [4]. Rupatadine is extensively metabolized mainly by cytochrome P450 3A4 in the liver. The major metabolites include desloratadine and 3-hydroxydesloratadine, both associated with pharmacological activity. Desloratadine (resulting from the *N*-dealkylation of the piperidine nitrogen of rupatadine, Fig. 1) is proved to be a selective, potent, orally active, peripheral H1 receptor antagonist. Clinical studies have demonstrated that desloratadine (5 mg) effectively relieves the signs and symptoms of seasonal allergic rhinitis patients [5,6]. Desloratadine is present in the plasma at low concentrations due to metabolism to several hydroxylated metabolites including the active metabolite 3-hydroxydesloratadine [7]. Due to the resulting low concentrations of rupatadine and its metabolite desloratadine after an intake of therapeutic doses, it is necessary to develop a very sensitive assay for determination of rupatadine and its metabolites in biosamples.

Several analytical methods have been reported for determination of desloratadine in plasma, employing HPLC-coupled ultraviolet detection [8], and tandem mass spectrometric detection [9–11]. Tian et al. [12] developed a liquid chromatography/tandem mass spectrometry (LC–MS/MS) method for the determination rupatadine in human plasma with an overall HPLC runtime of 10 min per sample. Few published methods for the determination of rupatadine and its metabolite desloratadine in human plasma are

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doi:10.1016/j.jpba.2008.10.014

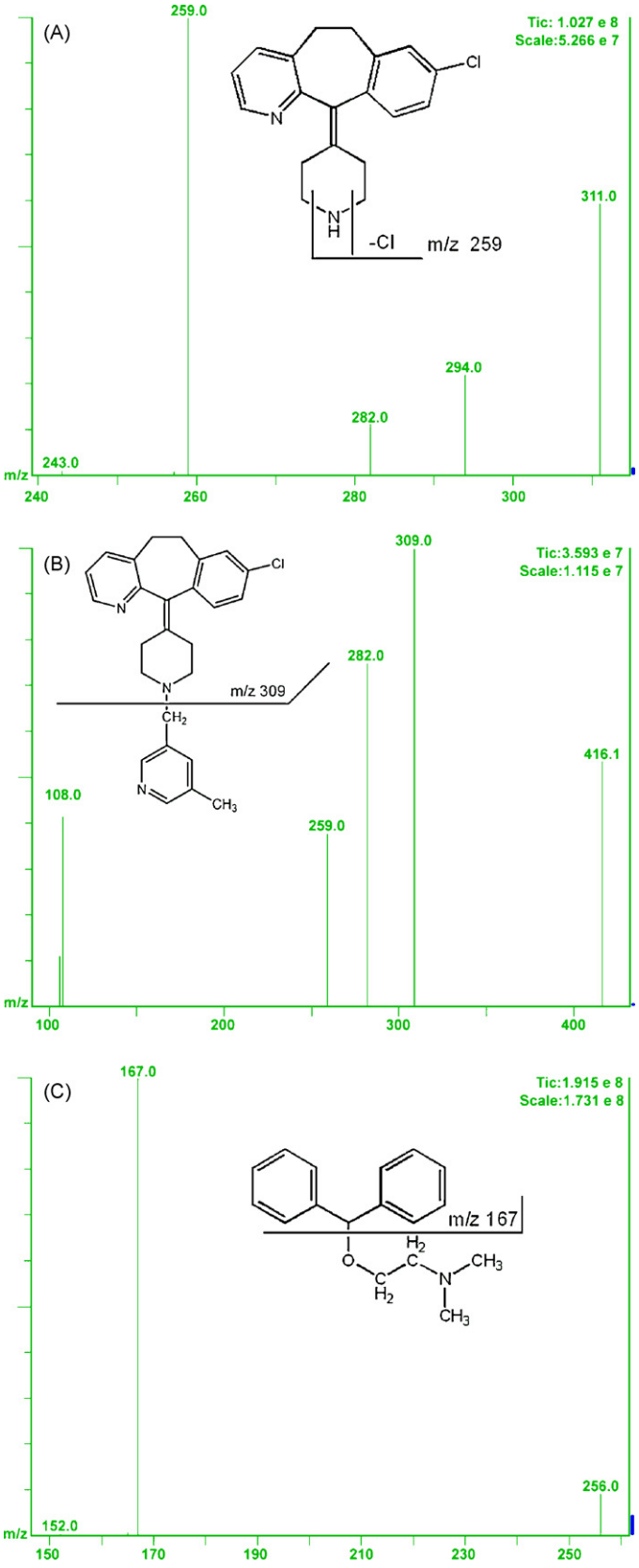

Fig. 1. Chemical structures and product ion spectra of $[M+H]^+$ of desloratadine (A), rupatadine (B) and the IS (C).

available. Solans et al. [13] investigated the effect of the concomitant intake of food on the oral bioavailability of rupatadine tablets, using a LC–MS/MS method for determination of rupatadine and its metabolites including desloratadine in human plasma. The method adopted time-consuming enzymatic hydrolysis (12 h) and gradient elution for LC analysis, with a lower limit of quantification (LLOQ) of 0.2 ng ml^{-1}.

The purpose of the present work was to develop and validate a sensitive, high-throughput liquid chromatography tandem mass spectrometric method for the simultaneous determination of rupatadine and desloratadine in human plasma using diphenhydramine as an internal standard (IS). In the present study, a one-step liquid–liquid extraction was used and an isocratic elution program was performed, which improved the throughput of the developed method without compromising the robustness and selectivity of the existing assay. The method was fully validated and applied to a pharmacokinetic study of rupatadine in healthy Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Rupatadine reference (99.0% purity), desloratadine reference (99.0% purity), diphenhydramine (IS, 99.0% purity) and tablet formulations of rupatadine 10 mg (Batch No. 061201) were supplied by Shanghai Shenjiu Medpharm Biotech Co. Ltd. (Shanghai, China). HPLC grade methanol was purchased from Merck KGaA (Darmstadt, Germany) and methyl *tert*-butyl ether and *n*-hexane of HPLC grade was purchased from Tedia (Fairfield, USA). Other chemicals were all of analytical grade. Ultrapure water was obtained by means of a Milli-Q apparatus from Millipore (Bedford, MA, USA). Human blank plasma (sodium heparin as an anticoagulant) was obtained from Shanghai Changhai Hospital (Shanghai, China).

2.2. LC–MS/MS instrumentation

A Varian LC-MS/MS system (Palo Alto, CA, USA) consisted of a ProStar 410 autosampler, two ProStar 210 liquid chromatographic pumps, and a 1200 L triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. The mass spectrometer and the data acquisition were fully controlled by Varian MS workstation Version 6.3 software.

2.3. Liquid chromatographic conditions

The liquid chromatographic separations were carried out on a Ultimate™ AQ-C18 column, 5 μm, 100 mm × 4.6 mm i.d. (Welch Materials, Inc., Ellicott, MD, USA) equipped with a security guard cartridge, 4.0 mm × 3.0 mm i.d. (Phenomenex, Macclesfield, Cheshire, UK). The mobile phase was composed of methanol–water (80:20, v/v) containing 0.0005% formic acid, at a flow rate of 0.3 ml min^{-1}. Before use, the mobile phase was filtered through a 0.45 μm nylon membrane filter. The column and the autosampler temperature were kept at room temperature of 25 °C and the injection volume was 10 μl. The total analysis time was 5.0 min per sample. The LC effluent at 1.8–4.0 min was sprayed into the mass spectrometer interface with a six-way valve and was discarded as waste at 0–1.8 and 4.0–5.0 min.

2.4. Mass spectrometer conditions

The mass spectrometer was operated in the positive ion mode. Infusion experiments were carried out to optimize the instrument parameters for maximal generation of protonated molecules

and capillary voltage was set to 36 V. Nitrogen gas set at 50 and 21 psi was used as nebulizing and drying gas, respectively. The API housing and drying gas was kept at 50 and 300 °C, respectively. Protonated analyte molecules were subjected to collision induced dissociation using argon as the collision gas at 1.8 mTorr to yield product ions. Automated MS/MS optimization for each analyte was accomplished using MS/MS Breakdown of Varian workstation and the collision energy ranged from 5 to 50 eV. MRM of the precursor–product ion transitions m/z 416 → 309 for rupatadine, m/z 311 → 259 for desloratadine and m/z 256 → 167 for the IS was monitored for quantification. The optimized collision energy was −14 eV for rupatadine, −16 eV for desloratadine and −10 eV for the IS. The detection voltage was set to 1600 V.

2.5. Standard solutions, calibration and quality control (QC) solutions

A stock solution of rupatadine of 1 mg ml^{-1} was prepared by dissolving the accurately weighed rupatadine in methanol. Working standard solutions at 2, 4, 10, 20, 40, 200 and 1000 ng ml^{-1} were prepared in methanol:water (1:1, v/v) from the stock solution. A stock solution of 1 mg ml^{-1} for desloratadine was also prepared in methanol. This solution was then serially diluted with methanol–water (1:1, v/v) to give a series of working standard solutions of 2, 4, 10, 20, 40, 100 and 400 ng ml^{-1}. A stock solution of 100 μg ml^{-1} for diphenhydramine was prepared in methanol and then was further diluted with methanol–water (1:1, v/v) to yield a working solution of 2 ng ml^{-1}.

Calibration standards were prepared by spiking 0.45 ml of human blank plasma with 25 μl of the working solutions of rupatadine and 25 μl of desloratadine. The resulting nominal plasma concentrations were 0.1, 0.2, 0.5, 1, 2, 10 and 50 ng ml^{-1} for rupatadine; 0.1, 0.2, 0.5, 1, 2, 5 and 20 ng ml^{-1} for desloratadine. For each validation and assay run, the calibration curve standards were prepared fresh from the working solutions.

QC samples were independently prepared at three-level concentrations of 0.2 ng ml^{-1} (low level), 2 ng ml^{-1} (medium level) and 40 ng ml^{-1} (high level) for rupatadine, 0.2, 2 and 16 ng ml^{-1} for desloratadine. The QC samples were stored at −20 °C and brought to room temperature before processed together with the clinical samples.

2.6. Sample preparation

Plasma samples were extracted employing a liquid–liquid extraction technique. To each tube containing 0.5 ml plasma, 50 μl of internal standard (2 ng ml^{-1}) was added and vortex mixed for 30 s. 5 ml methyl *tert*-butyl ether and *n*-hexane 1:1 (%, v/v) was then added and vortexed for 3 min. After centrifugation at 3500 × *g* for 10 min, 4 ml of the upper organic layer was transferred into a new tube and evaporated to dryness at 35 °C under a stream of nitrogen. The residue was reconstituted in 100 μl of mobile phase, followed by centrifugation at 10,000 × *g* for 10 min. A 10 μl aliquot of the supernatant was injected automatically into the LC–MS/MS system.

2.7. Method validation

2.7.1. Selectivity, calibration curve and LLOQ

The selectivity of the method was tested by screening five different batches of healthy human blank plasma. Each blank sample was tested for interferences in the MRM mode using the proposed extraction procedure and chromatographic/MS conditions.

A calibration curve ranging from 0.1 to 50 ng ml^{-1} for rupatadine or 0.1 to 20 ng ml^{-1} for desloratadine was used in each run by plotting the peak area ratios of the analyte to IS against the nominal standard curve concentrations. Least-squares linear regression was used for curve fitting with $1/x$ as the weighting factor.

The LLOQ of the assay was determined as the lowest concentration on the standard curve that could be quantitated with a precision of 20% and accuracy within ±20%.

2.7.2. Precision and accuracy

The within- and between-run accuracy and precision were evaluated by repeated analyses of QCs at three levels (low, medium and high) on three sequential runs in five replicates. Accuracy was assessed by calculating the percentage deviation from the theoretical concentration. Precision was determined by calculating the coefficient of variation for within- and between-run replicates. The criteria for acceptability of data induced accuracy within ±15% deviation from the nominal values and a precision within ±15% relative standard deviation (R.S.D.).

2.7.3. Matrix effect and extraction recovery

Five different batches of drug-free plasma from healthy volunteers were processed according to the described sample preparation. The absolute matrix effect of the plasma on ionization efficiency was assessed by comparing the peak areas of the analytes spiked in extracted blank plasma samples with that of the neat standards at corresponding concentrations. The relative matrix effects between five batches were measured by calculating the variability in the values. Three different concentration levels of rupatadine (0.2, 2 and 40 ng ml^{-1}) and desloratadine (0.2, 2 and 16 ng ml^{-1}) were evaluated by analyzing five samples at each level. The same evaluation was performed for the IS.

The extraction recoveries of rupatadine and desloratadine were determined by comparing the peak area of the QCs with the peak area of the corresponding standard solution spiked in extracted blank plasma. The recovery of IS was also determined similarly.

2.7.4. Stability

Short-term stability, long-term stability, autosampler stability and freeze–thaw cycles stability were assessed by analyzing three QC levels in five replicate. The QC samples were analyzed after storage at room temperature for 2 h, at −20 °C for 1 month, in the autosampler at room temperature for 8 h after liquid–liquid extraction and after three freeze–thaw cycles, which consisted of storage at −20 °C for a minimum of 12 h followed by thawing at room temperature. Deterioration of rupatadine or desloratadine was defined as greater than a 15% difference in the tested sample versus control at the sample nominal concentration.

2.8. Clinical pharmacokinetics design

The developed LC–MS/MS procedure was used to investigate the plasma profiles of rupatadine and its metabolite desloratadine following oral administration of rupatadine tablets at single doses of 10, 20 and 40 mg.

A clinical study was conducted on 30 Chinese volunteers (15 male and 15 female), aged from 20 to 28 years, who were judged to be in good health condition through medical history, physical examination and routing laboratory tests (hematology, blood biochemistry, and urine analysis). The volunteers were instructed to abstain from taking any medication including over the counter (OTC) drugs for at least 2 weeks prior to and during the study period and avoid any alcohol containing food and beverages 36 h prior to or during the course of the study. Informed consent was obtained from all the subjects after explaining the aim and risks of the study. The study protocol was approved by a local ethics committee. The subjects were divided randomly into three groups and received single

doses of 10, 20 and 40 mg of rupatadine under fasting conditions. Blood samples (2 ml) were collected into lithium heparinized tubes before administration (0 h) and at the time of 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, 36 and 48 h after oral administration. Plasma was separated by centrifugation (2000 × *g*) for 10 min at 4 °C and frozen at −20 °C until analysis.

3. Results and discussion

In the present study, LC–MS/MS was considered to be a preferred technique due to its sensitivity, speed and selectivity. In preliminary experiments the MS, chromatographic and extraction conditions were optimized for this purpose. And a full validation was performed in accordance with the recommendations published by FDA [14].

3.1. Chromatographic and mass spectrometry optimization

The chromatographic separation was performed on a Ultimate™ AQ-C18 column. The composition of mobile phase was optimized through several trials to achieve good resolution and retention for rupatadine, desloratadine and the IS. Initial mobile phase methanol–water (50:50, v/v) was used and with the increase of methanol content the retention for the analytes and the IS decreased. When the percentage of methanol in mobile phase reached up to 80%, the retention time for rupatadine, desloratadine and the IS was 12.1, 3.4 and 3.5 min. Further increase in methanol content could not shorten further the retention time of rupatadine. Thus several mobile phase additives were investigated, such as formic acid, ammonium formate, acetic acid and ammonium acetate, among which formic acid gave the best response and shortening of the retention time for the analytes, especially that of rupatadine. The retention time for rupatadine was shortened to 3 min when 0.0005% formic acid was used in the mobile phase. Therefore, a mixture of methanol–water (80:20, v/v) containing 0.0005% formic acid was adopted as the isocratic mobile phase. The LC effluent at 1.8–4.0 min was sprayed into the mass spectrometer interface with a six-way valve and was discarded as waste at 0–1.8 and 4.0–5.0 min, which reduced potential endogenous interference and also improved ionization efficiency.

The mass spectrum of rupatadine, desloratadine and the IS was acquired by mixing 5 μg ml^{-1} of each compound (20 μl min^{-1}) with mobile phase (200 μl min^{-1}) and infusing the mixture via a tee-union into the mass spectrometer. Each compound was run separately. At the optimum conditions described in Section 2.5, the electrospray ionization of rupatadine, desloratadine and the IS produced the abundant $[M+H]^+$ at mass-to-charge ratio (*m*/*z*) 416, 311 and 256, respectively. The corresponding $[M+H]^+$ ions from rupatadine, desloratadine and the IS were selected as the precursor ions and subsequently fragmented in MS/MS mode. Fig. 1 shows the product ion mass spectra of each compound. As can be seen in Fig. 1, there were predominant product ions at *m*/*z* 309 (the loss of $-CH_2C_5H_3NCH_3-$ from $[M+H]^+$ ion) for rupatadine, *m*/*z* 259 (the loss of –NH– and –Cl– from $[M+H]^+$ ion) for desloratadine, and *m*/*z* 167 (the loss of $-O(CH_2)_2N(CH3)_2$ from $[M+H]^+$ ion) for the IS, respectively.

3.2. Sample preparation and choice of IS

Liquid–liquid extraction was chosen because of its sufficient efficiency, specificity and lower experimental cost. Different organic extraction solvents were evaluated such as diethyl ether, methyl *tert*-butyl ether, diethyl ether/*n*-hexane (1:1, v/v) and methyl *tert*-butyl ether/*n*-hexane (1:1, v/v). The last of these was finally adopted because of its high extraction efficiency.

Internal standard was usually applied to obtain high accuracy when a mass spectrometer is used as the HPLC detector. In this study, diphenhydramine was adopted as the internal standard

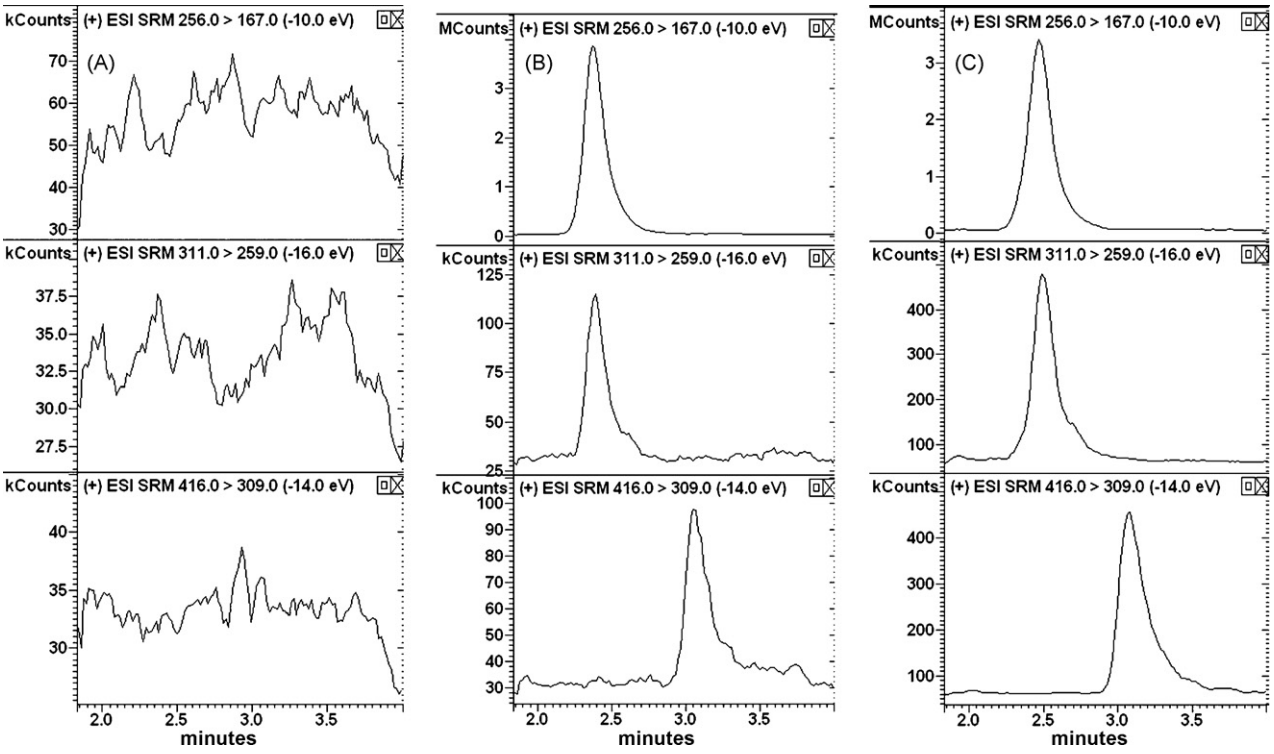

Fig. 2. Representative MRM chromatograms: (A) blank plasma sample; (B) blank plasma spiked with 0.1 ng ml^{-1} rupatadine, desloratadine and 2 ng ml^{-1} IS; (C) plasma sample collected from a subject 1.0 h after oral administration of 10 mg rupatadine (rupatadine: 0.99 ng ml^{-1}; desloratadine: 0.66 ng ml^{-1}).

Table 1
Accuracy and precision of rupatadine and desloratadine in spiked plasma samples.

Analyte	Rupatadine			Desloratadine		
Level	Low (0.2 ng ml^{-1})	Medium (2 ng ml^{-1})	High (40 ng ml^{-1})	Low (0.2 ng ml^{-1})	Medium (2 ng ml^{-1})	High (16 ng ml^{-1})
Between-run accuracy and precision (n = 15)						
Mean ± S.D. (ng ml^{-1})	0.194 ± 0.014	2.04 ± 0.10	39.65 ± 1.48	0.191 ± 0.012	2.11 ± 0.08	15.82 ± 0.68
%R.S.D.	7.2	5.0	3.7	6.1	3.8	4.2
%RE	−3.2	1.8	−0.9	−4.7	5.6	−1.2
Within-run accuracy and precision						
Run I (n = 5)						
Mean ± S.D. (ng ml^{-1})	0.203 ± 0.013	2.13 ± 0.10	39.86 ± 1.51	0.199 ± 0.013	2.14 ± 0.06	16.03 ± 0.70
%R.S.D.	6.2	4.6	3.8	6.5	2.7	4.4
%RE	1.4	6.4	−0.3	−0.4	7.2	0.2
Run II (n = 5)						
Mean ± S.D. (ng ml^{-1})	0.196 ± 0.014	1.96 ± 0.09	38.71 ± 1.57	0.187 ± 0.010	2.13 ± 0.11	15.56 ± 0.77
%R.S.D.	6.9	4.4	4.1	5.6	5.0	5.0
%RE	−1.8	−2.0	−3.2	−6.4	6.3	−2.7
Run III (n = 5)						
Mean ± S.D. (ng ml^{-1})	0.182 ± 0.009	2.02 ± 0.06	40.39 ± 1.28	0.186 ± 0.010	2.07 ± 0.07	15.85 ± 0.70
%R.S.D.	5.0	3.1	3.2	5.4	3.3	4.4
%RE	−9.2	1.0	1.0	−7.2	3.3	−0.9

because of its similarity of chromatographic behavior to those of the analytes and the minimal endogenous interferences in the MRM mode.

3.3. Method validation

3.3.1. Selectivity

The selectivity of the method was tested by comparing the chromatograms of five different batches of blank plasma and the spiked plasma. Fig. 2 shows the typical chromatograms of a blank plasma, blank plasma sample spiked with rupatadine, desloratadine at LLOQ and the IS, and test plasma sample obtained at 1 h after the oral dose of 10 mg rupatadine tablets to a volunteer. Under the optimized conditions the retention time of rupatadine, desloratadine and the IS was 3.1, 2.4 and 2.5 min, respectively. All blank plasma samples were found to be free of interferences with the compounds of interest.

3.3.2. Calibration curve and LLOQ

The assay was validated for rupatadine and desloratadine in human plasma in the concentration range of 0.1–50 and 0.1–20 ng ml^{-1}, respectively. Typical equations of calibration curves for rupatadine and desloratadine were $y = 0.1136x + 0.004310$ and $y = 0.1453x + 0.001156$, respectively, using a weighted $(1/x)$ linear regression. The correlation coefficients for the mean standard curve of five different lots of plasma were 0.9998 and 0.9995 for rupatadine and desloratadine, respectively. The difference between the nominal standard concentration and the back-calculated concentration from the weighted linear regression line varied from −17.1% to +12.2% for each point on the five calibration curves of rupatadine and −17.4% to +11.8% for that of desloratadine.

The LLOQ for rupatadine and desloratadine was proved to be 0.1 ng ml^{-1} with a precision of 15.0% and 10.9%, respectively, which was sufficient for pharmacokinetic study of rupatadine in human.

3.3.3. Precision and accuracy

For rupatadine and desloratadine, within- and between-run accuracy and precision were evaluated by the three levels of QCs on three sequential run in five replicates and the data were presented in Table 1. All QC levels for rupatadine and desloratadine had within- and between-run R.S.D. not greater than 7.2%. The deviation from the expected concentration, as a measurement of accuracy,

Table 2
Stability results of rupatadine and desloratadine in spiked plasma samples (n = 5).

Stability	Rupatadine			Desloratadine		
	Low (0.2 ng ml^{-1})	Medium (2 ng ml^{-1})	High (40 ng ml^{-1})	Low (0.2 ng ml^{-1})	Medium (2 ng ml^{-1})	High (16 ng ml^{-1})
Short-term stability (2 h at room temperature)						
Mean ± S.D. (ng ml^{-1})	0.193 ± 0.013	1.96 ± 0.11	38.44 ± 1.76	0.21 ± 0.012	2.17 ± 0.08	16.62 ± 0.69
%R.S.D.	6.6	5.5	4.6	5.8	3.0	4.2
%RE	−3.3	−2.0	−3.9	3.4	8.6	3.9
Long-term stability (1 month at −20 °C)						
Mean ± S.D. (ng ml^{-1})	0.193 ± 0.011	1.99 ± 0.10	38.28 ± 1.39	0.19 ± 0.009	2.15 ± 0.09	15.92 ± 0.62
%R.S.D.	5.6	4.9	3.6	5.0	4.0	3.9
%RE	−3.5	−0.1	−4.3	−4.9	7.6	−0.5
Autosampler stability (8 h at room temperature)						
Mean ± S.D. (ng ml^{-1})	0.21 ± 0.015	2.11 ± 0.13	41.04 ± 1.92	0.22 ± 0.013	2.08 ± 0.12	16.00 ± 0.69
%R.S.D.	7.0	6.0	4.7	5.0	5.7	4.3
%RE	5.6	5.6	2.6	8.0	3.8	0.1
Freeze–thaw stability (three cycles)						
Mean ± S.D. (ng ml^{-1})	0.21 ± 0.016	2.08 ± 0.15	40.31 ± 2.09	0.20 ± 0.017	1.98 ± 0.14	15.14 ± 0.80
%R.S.D.	7.5	7.2	5.2	8.2	7.2	5.3
%RE	7.0	4.1	0.8	2.7	−0.9	−5.4

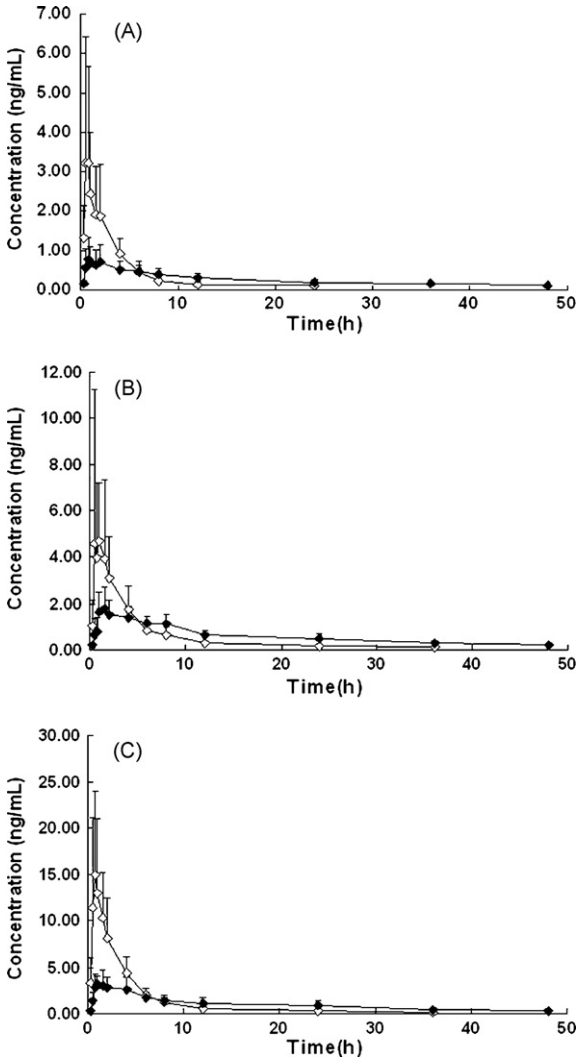

Fig. 3. Mean drug plasma concentration–time curves of rupatadine and desloratadine in 30 volunteers after single oral doses of 10 mg (A), 20 mg (B), and 40 mg (C) (◊: rupatadine; ♦: desloratadine).

ranged from −9.2% to +6.4% and −7.2% to +7.2% for rupatadine and desloratadine, respectively. These results were within the acceptance criteria and indicated that the method was accurate, reliable and reproducible.

3.3.4. Matrix effect and extraction recovery

The matrix effects are generally due to the influence of coeluting, undetected matrix components reducing or enhancing the ion intensity of the analytes and affect the reproducibility and accuracy of the assay. In this paper, the mean absolute matrix effect values (relative matrix effect values) obtained for rupatadine was 79.9 ± 5.3% (6.7%), 83.7 ± 4.6% (5.5%), and 81.2 ± 4.5% (5.4%) at concentrations of 0.2, 2.0, and 40 ng ml^{-1}, respectively (n = 5). The absolute matrix effect values (relative matrix effect values) for desloratadine were 74.2 ± 4.1% (5.5%), 77.1 ± 3.2% (4.1%), and 77.2 ± 3.9% (5.1%) at levels of 0.20, 2.0, and 16 ng ml^{-1}, respectively (n = 5), and the value for the IS was 87.1 ± 4.2% (4.8%) (n = 5). The results suggested ion suppression on quantification of rupatadine and desloratadine in plasma samples.

The extraction recovery of rupatadine was 78.4 ± 6.9%, 82.1 ± 4.7%, and 80.6 ± 4.9% at concentrations of 0.2, 2.0, and 40 ng ml^{-1}, respectively (n = 5). The extraction recoveries of desloratadine were 75.7 ± 5.6%, 80.8 ± 4.4%, and 75.3 ± 4.0% at concentrations of 0.2, 2.0, and 16 ng ml^{-1}, respectively (n = 5). The extraction recovery of the IS was 80.0 ± 5.6% (n = 5).

3.3.5. Stability

Although LC–MS/MS methods have demonstrated the capability of reducing the needs of sample clean up procedures because of its inherent selectivity and sensitivity, the duration of time required for sample processing is not short enough to complete instability issues. Therefore, analyte stability during sample transport, storage and preparation is a concern for the interpretation of the concentrations of drugs and their metabolites. In our study, QC plasma samples were used subject to short term (at room temperature for 2 h), long term (at 20 °C for 30 days), in the autosampler (at room temperature for 8 h) and three freeze–thaw (20 to +20 °C) cycles. The values obtained for present stability studies of rupatadine and desloratadine were summarized in Table 2. As can be seen from Table 2, there were no significant differences (R.S.D. 3.6–8.2%, RE −5.4 to +8.6%) in the assay concentrations at any QC level and no deterioration for rupatadine or desloratadine in plasma following the above conditions, thus indicating that rupatadine and desloratadine in plasma were stable.

3.4. Application to clinical pharmacokinetic study

The method described above has successfully been applied to analyze plasma samples obtained from 30 healthy volunteers who received single doses of 10, 20 and 40 mg rupatadine tablets. The mean plasma concentration–time profiles for rupatadine and its metabolite desloratadine after oral administration of rupatadine at three doses were presented in Fig. 3. Plasma concentrations of rupatadine were detectable up to 24, 36 and 36 h for 10, 20 and 40 mg doses, respectively. Desloratadine, the major metabolite, could be detected at all time points over the duration of 0.25–48 h at three doses. The procedure developed was sensitive enough to assure the quantitative analysis of rupatadine and desloratadine in plasma with acceptable accuracy over a period of 48 h. Pharmacokinetic parameters determined by non-compartment analysis method were listed in Table 3. The $AUC_{0-\infty}$ values of rupatadine were 11.2 ± 6.1, 19.3 ± 10.7 and 51.3 ± 24.7 ng h ml^{-1} for 10, 20 and 40 mg dose, respectively, which increased with the dose, showing apparent dose-dependent relationship (r = 0.9902). And the $AUC_{0-\infty}$ value of desloratadine also increased in a dose-related

Table 3
Pharmacokinetic parameters of rupatadine and desloratadine following oral administration of rupatadine tablets at different doses (mean ± S.D.).

Parameter	10 mg (n = 10)		20 mg (n = 10)		40 mg (n = 10)	
	Rupatadine	Desloratadine	Rupatadine	Desloratadine	Rupatadine	Desloratadine
C_{max} (ng ml^{-1})	3.9 ± 3.5	0.9 ± 0.5	7.2 ± 6.4	2.1 ± 0.9	18.0 ± 9.6	4.4 ± 1.5
T_{max} (h)	0.7 ± 0.2	1.1 ± 0.6	1.1 ± 0.5	1.7 ± 0.9	1.0 ± 0.3	1.5 ± 1.0
AUC_{0-t} (ng h ml^{-1})	10.4 ± 6.0	9.9 ± 3.9	18.3 ± 10.3	26.6 ± 8.3	49.7 ± 24.4	46.0 ± 18.4
$AUC_{0-\infty}$ (ng h ml^{-1})	11.2 ± 6.1	13.5 ± 4.9	19.3 ± 10.7	31.1 ± 10.8	51.3 ± 24.7	55.0 ± 20.8
$t_{1/2}$ (h)	4.6 ± 2.7	17.0 ± 5.2	3.9 ± 2.5	16.7 ± 6.0	5.6 ± 2.5	18.7 ± 7.1
MRT (h)	5.0 ± 1.8	23.5 ± 6.3	4.5 ± 2.3	21.6 ± 6.2	5.1 ± 1.3	24.8 ± 7.9

manner ($r = 0.9947$). The $t_{1/2}$ and MRT of rupatadine were much shorter than those of desloratadine at the corresponding dose.

4. Conclusion

A sensitive and selective LC–MS/MS method has been developed and validated for simultaneous determination of rupatadine and its active metabolite desloratadine in human plasma. The proposed method was very sensitive with the LLOQ 0.1 ng ml^{-1} for both rupatadine and desloratadine. Plasma samples were pretreated with one-step liquid–liquid extraction and analyzed under the isocratic LC condition. And the total analysis time was 5.0 min per sample, which made it an attractive procedure in high-throughput bioanalysis of rupatadine and desloratadine. The assay validation results were within the acceptable ranges for bio-analytical purposes. The method has been successfully applied to a pharmacokinetic study of rupatadine in healthy Chinese volunteers and demonstrated to be suitable for the analysis of rupatadine and its metabolite desloratadine in human plasma samples collected in pharmacokinetic studies.

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