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High performance liquid chromatography–tandem mass spectrometric determination of rupatadine in human plasma and its pharmacokinetics

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ABSTRACT

A simple, rapid, sensitive and selective liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method was developed and validated for the quantification of rupatadine in human plasma using estazolam as internal standard (IS). Following liquid–liquid extraction, the analytes were separated using a mobile phase of methanol–ammonium acetate (pH 2.2; 5 mM) (50:50, v/v) on a reverse phase C_{18} column and analyzed by a triple-quadrupole mass spectrometer in the positive ion and multiple reaction monitoring (MRM) mode, m/z 416 → 309 for rupatadine and m/z 295 → 267 for the IS. The assay exhibited a linear dynamic range of 0.1–100 ng/ml for rupatadine in human plasma. The lower limit of quantification (LLOQ) was 0.1 ng/ml with a relative standard deviation of less than 20%. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The validated LC–MS/MS method has been successfully applied to study the pharmacokinetics of rupatadine in healthy volunteers.

1. Introduction

Rupatadine (Fig. 1a) is a novel compound with potent dual antihistamine and platelet-activating factor antagonist activities and no sedative effects [1]. It has therapeutic efficacies towards allergic inflammatory conditions such as seasonal allergic rhinitis (SAR), perennial allergic rhinitis (PAR) and chronic idiopathic urticaria (CIU) at the recommended dosage of 10 mg rupatadine for adults and adolescents in Europe [2–4].

Following oral administration to humans, rupatadine is rapidly absorbed and subjected to considerable first-pass metabolism. Rupatadine is extensively metabolized in the liver, mainly by cytochrome P450 3A4 [5]. The major metabolites were identified as desloratadine (UR-12790, resulting from the *N*-dealkylation of the piperidine nitrogen) and 3-hydroxydesloratadine (UR-12788, formed by hydroxylation at the position 3 of the tricyclic ring system of desloratadine) [6].

Several analytical methods are available for the determination of rupatadine levels. Solans et al. [6] and Barbanoj et al. [7] developed methods for the simultaneous determination of rupatadine and its two main metabolites using a liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method that had a lower limit of quantification (LLOQ) of 0.2 ng/ml. In their methods, they both used enzymatic hydrolysis followed by a liquid–liquid extraction, which was expensive and complex. Wen et al. [8] reported 0.05 ng/ml as the limit of quantification of rupatadine using HPLC–MS/MS. However, the retention time of rupatadine (2.32 min) was so short that may cause matrix effect owing to the persistence of endogenous ingredients in plasma. In this paper, we describe a rapid and sensitive LC–MS/MS method for quantification of rupatdaine in human plasma employing liquid–liquid extraction without enzymatic hydrolysis. Although the simultaneous determination of main active metabolites was missing, the method developed is useful for the pharmacokinetic profiling of the parent drug.

2. Experimental

2.1. Chemicals and reagents

Rupatadine reference standard was supplied by Jiangsu Yangzijiang Pharmaceutical Factory (Jiangsu, P.R. China). Estazolam reference standard (internal standard, IS) was obtained from Hubei Pharmaceutical Factory (Hubei, P.R. China). HPLC grade methanol was purchased from VWR International Company (Darmstadt, Germany). Cyclohexane and diethyl ether were obtained from Nanjing Chemical Reagent No.1 Factory. Other chemicals were all of analytical grade. Water was distillated twice before use.

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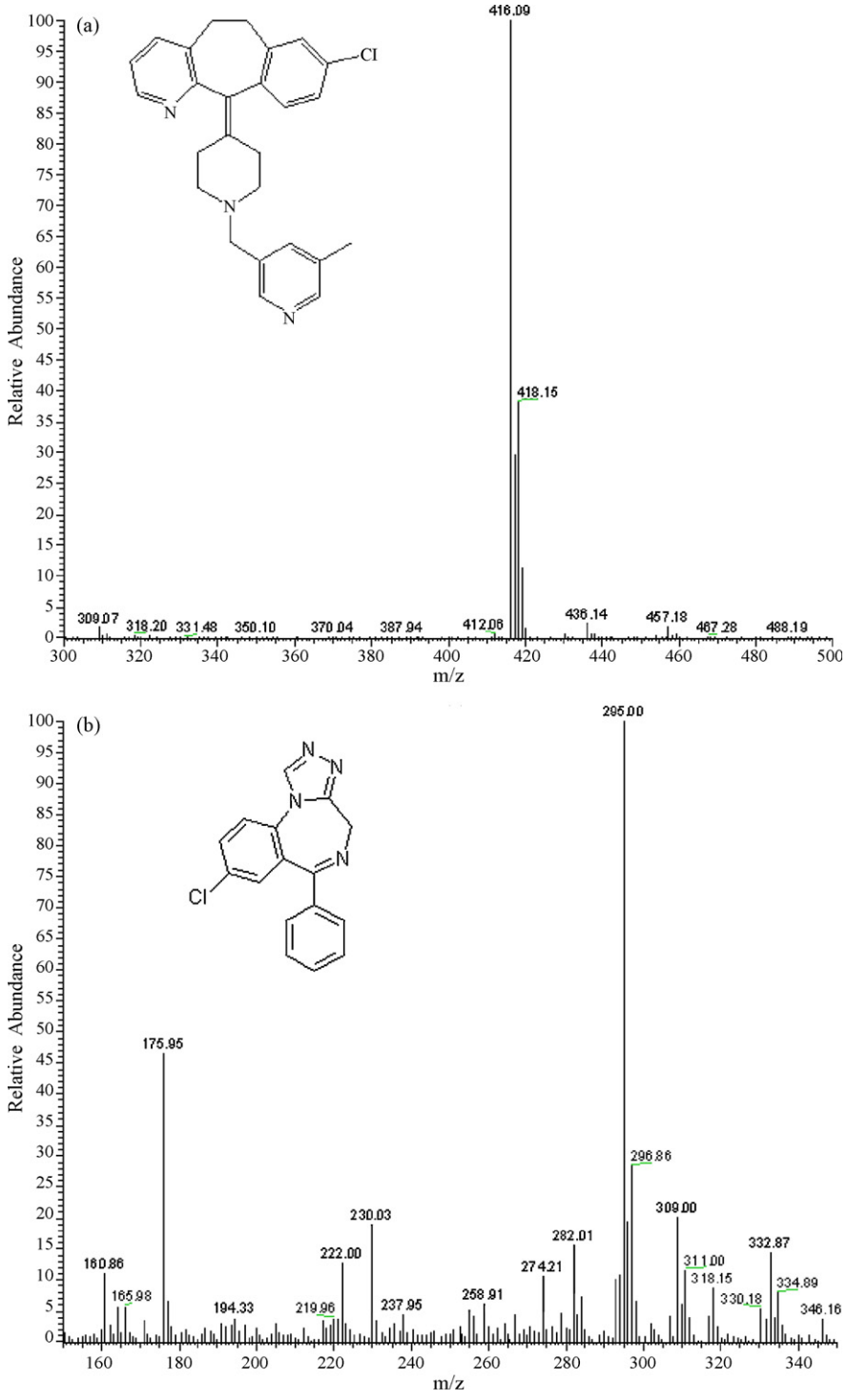

Fig. 1. Full scan ESI (+) precursor ion mass spectra of rupatadine (a) and the IS (b) and their chemical structures.

2.2. Preparation of stock and working solutions

The stock solution of rupatadine was prepared in methanol–water (20:80, v/v) and the stock solution of estazolam was prepared in methanol. Both of them were 1.0 mg/ml and were stored at 4 °C.

Working solutions of rupatadine were prepared daily in methanol–water (20:80, v/v) by appropriate dilution at 0.01, 0.1, 1, 10, 100 μg/ml.

The stock solution of estazolam was further diluted with methanol to prepare the working internal standard solution containing 100 ng/ml of estazolam.

2.3. LC–MS/MS instrument and conditions

Liquid chromatographic separation and mass spectrometric detection were performed using the Finnigan™ TSQ Quantum Discovery MAX™ LC–MS/MS system consisting of a Finnigan™

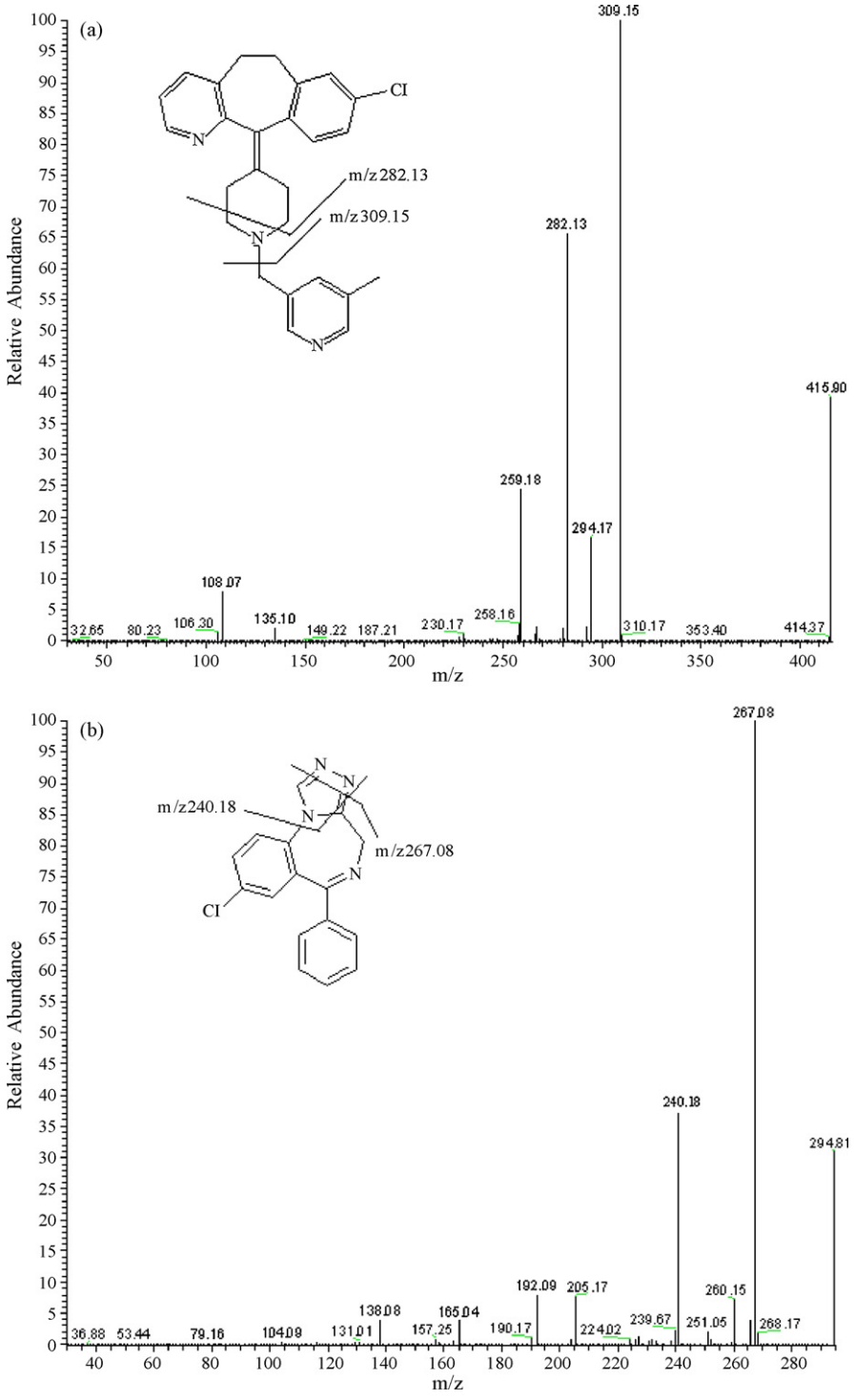

Fig. 2. Full scan ESI (+) product ion mass spectra of rupatadine (a) and the IS (b) and their proposed fragmentation patterns.

Surveyor LC pump, a Finnigan™ Surveyor auto-sampler and combined with a triple-quadrupole TSQ Quantum mass spectrometer (Thermo Electron Corporation). The chromatographic separation was on a Shimadzu Shim-pack VP-ODS C_{18} (150 mm × 2.0 mm, 5 μm) analytical column at 40 °C. The isocratic mobile phase composition was methanol–ammonium acetate (pH 2.2; 0.005 M) (50:50, v/v), which was pumped at a flow rate of 0.2 ml/min.

The tandem MS system is equipped with an ESI source, and run with the Xcalibur 2.0 software (Thermo Electron Corporation). The mass spectrometer was operated in the positive ion and MRM (multiple reaction monitoring) mode with precursor to product qualifier transition m/z 416 → 309 for rupatadine and m/z 295 → 265 for estazolam. Spray voltage was optimized at 5000 V, transfer capillary temperature at 320 °C, sheath gas and auxiliary gas (nitrogen) pressure at 29 and 5 arbitrary unites (set by the TSQ software, Thermo Electron Corporation), respectively. Argon was used as collision gas at a pressure of 1.5 m Torr and collision energy was 18 and 22 V for rupatadine and Estazolam, respectively. The scan width for

Table 1
The results of five calibration curves for determining rupatadine in human plasma

Concentration added (ng/ml)	Assay	0.1	0.3	0.5	1	2	5	10	50	100
	1	0.10	0.29	0.55	1.10	2.00	4.90	10.20	50.29	96.52
Concentration	2	0.12	0.33	0.57	0.94	2.04	4.62	9.69	51.55	98.00
found	3	0.09	0.28	0.49	0.89	1.79	4.95	9.44	47.77	101.32
(ng/ml)	4	0.08	0.26	0.50	1.01	1.92	4.99	10.75	49.22	104.13
	5	0.11	0.30	0.48	1.04	2.28	4.75	9.81	52.92	107.57
Mean (ng/ml)		0.10	0.29	0.52	1.00	2.01	4.84	9.98	50.35	101.51
S.D. (ng/ml)		0.02	0.03	0.04	0.08	0.18	0.15	0.51	2.00	4.50
Accuracy (%)		100.00	97.33	103.60	99.60	100.30	96.84	99.78	100.70	101.51

MRM was 0.01 *m*/*z* and scan time was 0.3 s. The peak width settings (FWHM) for both Q1 and Q3 were 0.7 *m*/*z*.

2.4. Sample preparation

The plasma samples were prepared by liquid–liquid extraction. The working internal standard solution (30 μl × 100 ng/ml) and 100 μl 1 M NaOH and 5 ml cyclohexane–diethyl ether (50:50, v/v) were added into a 1 ml aliquot of the collected plasma sample from a human volunteer and then was vortexed for 3 min. After centrifuging at 3000 rpm for 10 min, the upper organic phase was transferred to another 10 ml centrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas in water bath at 40 °C. The dried extract was redissolved in 200 μl mobile phase. Then an aliquot of 10 μl was injected into the LC–MS/MS system.

2.5. Standard curves

Calibration curves were prepared on 5 different days by spiking blank plasma with proper volume of one of the working solutions to produce the standard curve points equivalent to 0.1, 0.3, 0.5, 1, 2, 5, 10, 50 and 100 ng/ml of rupatadine. The following assay procedures were the same as described above. In each run, a blank plasma sample (processed without the IS) was analyzed to confirm absence of interferences but not used to construct the calibration function. The analysis of a sample without analyte but with IS showed no endogenous interference at the retention positions of rupatadine.

2.6. Preparation of quality control samples

Quality control (QC) samples were prepared daily by spiking blank plasma with proper volume of one of the working solution mentioned above to produce a final concentration equivalent to 0.3 ng/ml (low level), 5 ng/ml (middle level) and 100 ng/ml (high level) of rupatadine. The following procedures were the same as described above.

2.7. Method validation

2.7.1. Assay specificity

Blank plasma samples of healthy human, which was used to test specificity of the method, was obtained from six different sources.

2.7.2. Matrix effect

The potential matrix effect on the ionization of the analytes was evaluated by comparing the peak area of analytes resolved in blank sample (the final solution of blank plasma after extraction and reconstitution) to that of standard solutions at the same concentration. Three different concentration levels of rupatadine (0.3, 5 and 100 ng/ml) were evaluated by analyzing five samples at each set. The matrix effect of IS (3 ng/ml in plasma) was evaluated by the same method.

2.7.3. Linearity

The five calibration curves were generated by using the ratios of the analyte peak area to the IS peak area versus concentration and were fitted to the equation $y = bx + a$ by weighted least-squares linearity regression. Each back-calculated concentration should meet the following acceptable criteria: no more than 20% deviation at LLOQ and no more than 15% deviation above LLOQ.

The limit of detection (LOD) and the LLOQ were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively.

2.7.4. Precision and accuracy

The intra-batch precision and accuracy was determined by analyzing five sets of spiked plasma samples of rupatadine at each QC level (0.3, 5 and 100 ng/ml) in a batch. The inter-batch precision and accuracy was determined by analyzing five sets of spiked plasma samples of rupatadine at each QC level (0.3, 5 and 100 ng/ml) in three consecutive batches. The concentration of each sample was calculated using standard curve prepared and analyzed on the same day. Accuracy was assessed by calculating the percentage deviation from the theoretical concentration. Precision was determined by calculating the relative standard deviation for inter- and intra-run replicates.

2.7.5. Extraction recovery

The absolute recovery of rupatadine through the extraction procedures was determined at three concentrations (0.3, 5 and 100 ng/ml). A known amount of rupatadine was added to blank human plasma prior to extraction as described in the Section 2.4 and then the IS (estazolam) was added after extraction to eliminate bias introduced by sample processing ($n = 5$). As standards, firstly blank samples were extracted and evaporated to

Table 2
The precision and accuracy of the method for determining rupatadine in human plasma

Concentration added (ng/ml)	Intra-batch ($n = 5$)			Inter-batch ($n = 15$)		
	Concentration found (mean ± S.D., ng/ml)	Accuracy (%)	Precision (%)	Concentration found (mean ± S.D., ng/ml)	Accuracy (%)	Precision (%)
0.3	0.30 ± 0.04	100.14	11.8	0.29 ± 0.02	97.22	9.8
5	5.06 ± 0.40	101.17	7.1	5.16 ± 0.30	103.18	5.8
100	100.50 ± 8.95	100.50	8.9	99.53 ± 7.08	99.53	7.1

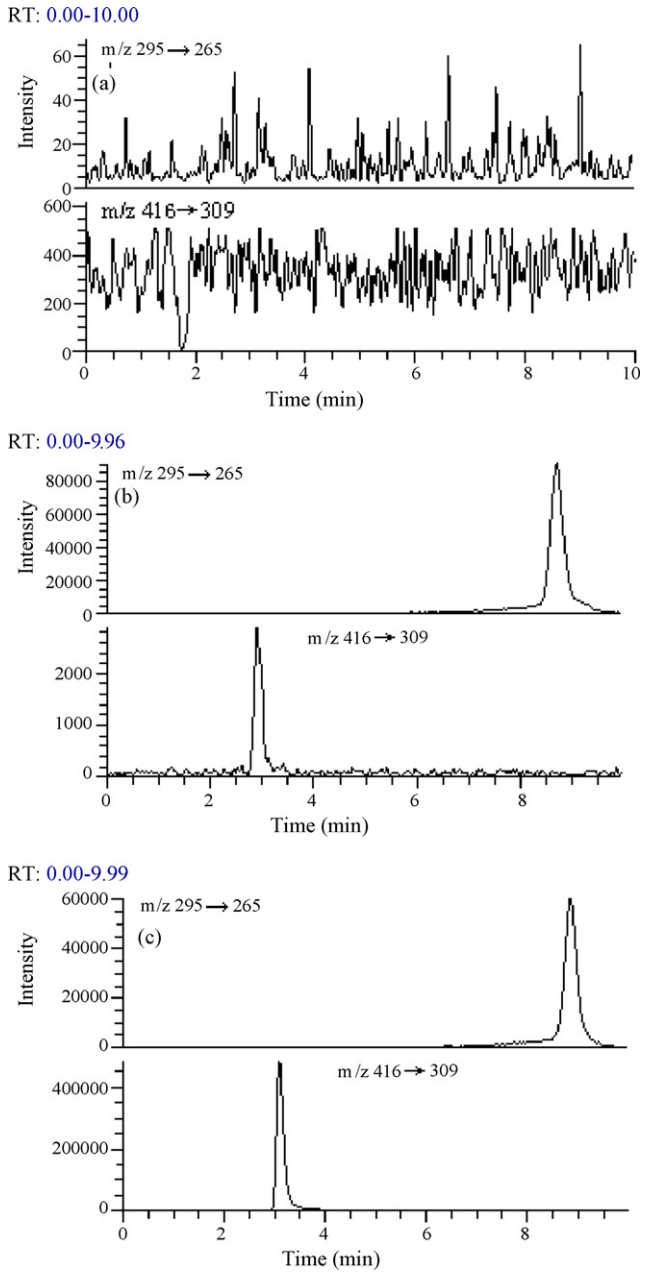

Fig. 3. Representative SRM chromatograms for rupatadine and the IS resulting from analysis of (a) blank plasma (drug and IS free); (b) 0.1 ng/ml (LLOQ) of rupatadine in human plasma spiked with the IS and (c) a plasma sample obtained at 0.33 h from a subject after a single oral dose (40 mg) of rupatadine and the sample concentration was determined to be 23 ng/ml for rupatadine.

dryness, the same concentration levels of rupatadine and IS were added to the residues and evaporated to dryness, then the residue was dissolved in 200 μl of mobile phase prior to analysis ($n = 2$). The extraction recovery was calculated by comparing the peak area ratio of rupatadine/estazolam of extracted samples to the peak area ratio of rupatadine/estazolam standards.

2.7.6. Stability

The short-term stability of rupatadine was assessed by determining QC plasma samples kept at room temperature for 24 h, which exceeded the routine preparation time of samples. The long-term stability was evaluated by determining QC plasma samples kept at low temperature (−20 °C) for 7 days. The post-preparative stability was measured by determining QC samples kept under the auto-sampler conditions (15 °C) for 24 h. The freeze and thaw stability was tested by analyzing QC plasma samples undergoing three freeze (−20 °C)–thaw (room temperature) cycles on consecutive days. The stock solution stability of rupatadine and the IS were evaluated by analyzing the working solutions (concentration in three QC levels for rupatadine and 3 ng/ml for the IS) kept at room temperature for 24 h and at low temperature (−4 °C) for 7 days, respectively. All these stability tests were performed using five replicates of QC samples.

2.7.7. Pharmacokinetic study

The above validated method was successfully applied to a pharmacokinetic study of rupatadine tablets in healthy Chinese volunteers. The study was approved by Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College. After assessment of medical history, physical examination, electrocardiogram and standard laboratory biochemical examination (blood cell count, biochemical profile and urine analysis), twelve healthy volunteers (6 males and 6 females) were selected to participate in the single-dose and the multiple-dose experiment. The age of the 12 healthy volunteers ranged from 25 to 40 years and the weight ranged from 50 to 68 kg. All volunteers gave written informed consent to participate in the study. The volunteers were asked to stop taking any medicines at least 2 weeks before the study.

The study was a single-dose, open-label, randomized, complete three-way crossover study. Every subject was administrated the following doses: 1 tablet (10 mg), 2 tablets (20 mg), 4 tablets (40 mg) of rupatadine in the first, second and third period, respectively. Then serial blood samples (3.5 ml) were collected from vein at 0, 0.17, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 36 and 48 h. The multiple-dose study started after the single-dose study with a week washout. In the multiple-dose study, the same 12 volunteers received an oral administration of 10 mg of rupatadine once daily (at 7:00 h) until the morning of the 7th day. Venous blood (3.5 ml) was drawn at the 5th Day (96 h) and 6th Day (120 h) morning before administration to make sure the steady state has appeared. In 7th Day (144 h) drawn blood again following the pre-specified time points after the last dose. Venous blood samples were obtained at 96, 120, 144, 144.17, 144.25, 144.33, 144.5, 144.75, 145, 1 45.5, 146,

Table 3
The stability of rupatadine in human plasma under tested conditions

	Accuracy (mean ± S.D.%)		
	0.3 (ng/ml)[a]	5 (ng/ml)[a]	100 (ng/ml)[a]
Short-term stability (24 h, room temperature)	101.32 ± 0.50	99.15 ± 1.80	95.08 ± 0.42
Long-term stability (7 days, −20 °C)	99.46 ± 0.01	87.32 ± 0.07	95.87 ± 7.38
Post-preparative stability (24 h, 15 °C)	97.13 ± 0.04	111.20 ± 0.02	108.57 ± 0.76
Freeze and thaw stability (3 cycles, −20 °C-room temperature)	99.86 ± 0.07	89.02 ± 0.13	90.87 ± 2.38

[a] Concentration added (ng/ml).

Table 4
Pharmacokinetic parameters of 12 healthy volunteers after oral administration of rupatadine in single- and multiple-dose study

Parameters	Single-dose study			Multiple-dose study
	10 mg	20 mg	40 mg	10 mg
$T_{1/2}$ (h)	13.47 ± 2.46	13.30 ± 2.60	12.85 ± 3.15	13.83 ± 4.98
T_{max} (h)	0.60 ± 0.24	0.67 ± 0.20	0.84 ± 0.36	0.57 ± 0.14
C_{max} (ng/ml)	6.06 ± 4.11	12.29 ± 5.75	30.19 ± 15.94	5.59 ± 3.34
AUC_{0-48}(ng*h/ml)	33.17 ± 19.57	51.99 ± 26.95	119.67 ± 46.67	33.12 ± 17.82
$AUC_{0-\infty}$(ng*h/ml)	35.46 ± 19.76	54.78 ± 27.39	124.47 ± 46.74	35.96 ± 17.86
MRT (h)	17.67 ± 3.48	13.77 ± 2.86	11.65 ± 3.39	18.97 ± 4.49
Ka (1/h)	5.95 ± 5.21	5.17 ± 4.10	6.47 ± 10.35	5.39 ± 3.27
C_{min} (ng/ml)	–	–	–	0.35 ± 0.20
C_{av} (ng/ml)	–	–	–	1.06 ± 0.68
AUC_{ss} (ng*h/ml)	–	–	–	25.49 ± 16.30
DF	–	–	–	5.09 ± 1.84

148, 152, 156, 168, 180 and 192 h post-dose. Each plasma sample was immediately separated by centrifugation at 3000 rpm for 5 min to separate the plasma fractions and stored frozen at −20 °C until analysis.

The pharmacokinetic parameters were calculated by Program Package (Drug and Statistics ver 2.0) software. Maximum plasma concentration after a single-dose (C_{max}), maximum plasma concentration observed at steady-state ($C_{max,ss}$), minimum plasma concentration observed at steady-state ($C_{min,ss}$), time corresponding to C_{max} (t_{max}), and time corresponding to $C_{max,ss}$ ($t_{max,ss}$) were determined from the plasma concentration–time curves. The elimination rate constant (k_e) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($t_{1/2}$) was calculated as $0.693/k_e$. The area under the plasma concentration–time curve (AUC) from time zero to the last data point (AUC_{0-t}) after the single-dose study was estimated by the linear trapezoidal rule, while the AUC from time zero to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/k_e$, where C_t was the last measurable concentration. The AUC_{ss} was the steady-state AUC after the multiple-dose administration, and was also calculated by the linear trapezoidal rule. The mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$. The steady-state average plasma concentration (C_{av}) was calculated as AUC_{ss}/τ, where τ represented the dosing interval, while the steady-state average plasma concentration (DF) was calculated as $(C_{max} - C_{min})/C_{av} \times 100\%$.

Statistical comparisons of the pharmacokinetic parameters (MRT, $t_{1/2}$ and C_{max}) of rupatadine were assessed utilizing the *t*-test on the ln-transformed data. A nonparametric test (Wilcoxon signed rank test) was applied to assess the effect of the conditions of administration on t_{max} values. A value of $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Selection of IS

Estazolam was selected as IS because its retention data, ionization and extraction efficiency were found to be appropriate among compounds we tried. The structures of rupatadine and estazolam are shown in Fig. 1.

3.2. Sample preparation

Liquid–liquid extraction was necessary and important because this technique could not only purify but also concentrate the sample. Diethyl ether, ethyl acetate, cyclohexane–diethyl ether (50:50, v/v) and *n*-hexane-isopropanol (95:5, v/v) were attempted and cyclohexane–diethyl ether (50:50, v/v) was finally adopted because it showed high extraction efficiency, and the extracted endogenous compounds did not interfere with the determination of rupatadine and IS.

3.3. LC–MS/MS conditions

The LC–MS/MS method for the determination of rupatadine in human plasma was investigated. Tandem mass spectrum analysis was carried out by electrospray ionization. Both positive and negative ion full scan were done to check the optimum response of rupatadine and estazolam, there was no signal response for rupata-

Table 5
Kinetic parameters about single- and multiple-dose study in male and female volunteers

Parameters	Single-dose study						Multiple-dose study	
	10 mg		20 mg		40 mg		10 mg	
	Male	Female	Male	Female	Male	Female	Male	Female
$T_{1/2}$ (h)	12.93 ± 2.58	14.01 ± 2.43	12.81 ± 3.50	13.80 ± 1.44	12.35 ± 2.24	13.35 ± 4.04	15.55 ± 5.94	12.12 ± 3.50
T_{max} (h)	0.50 ± 0.21	0.71 ± 0.25	0.58 ± 0.13	0.75 ± 0.22	0.68 ± 0.23	1.00 ± 0.42	0.60 ± 0.18	0.54 ± 0.10
C_{max} (ng/ml)	7.07 ± 4.74	5.04 ± 3.51	12.37 ± 5.93	12.21 ± 6.14	34.33 ± 16.51	26.06 ± 15.67	6.27 ± 3.83	4.91 ± 2.96
AUC_{0-48} (ng*h/ml)	36.85 ± 24.80	29.49 ± 13.97	53.53 ± 37.15	50.45 ± 14.56	121.79 ± 39.20	117.55 ± 56.95	38.66 ± 23.11	27.57 ± 9.11
$AUC_{0-\infty}$ (ng*h/ml)	39.09 ± 25.01	31.83 ± 14.21	56.19 ± 37.78	53.38 ± 14.77	126.06 ± 39.75	122.88 ± 56.74	42.31 ± 22.77	29.62 ± 9.31
MRT (h)	17.00 ± 4.39	18.34 ± 2.50	14.07 ± 2.70	13.48 ± 3.24	11.63 ± 2.29	11.66 ± 4.47	19.79 ± 5.69	18.16 ± 3.21
Ka (1/h)	8.63 ± 6.11	3.27 ± 2.24	7.08 ± 5.16	3.26 ± 1.28	3.56 ± 1.53	9.39 ± 14.59	–	–
C_{min} (ng/ml)	–	–	–	–	–	–	0.42 ± 0.23	0.29 ± 0.17
C_{av} (ng/ml)	–	–	–	–	–	–	1.25 ± 0.90	0.87 ± 0.34
AUC_{ss} (ng*h/ml)	–	–	–	–	–	–	30.10 ± 21.59	20.89 ± 8.23
DF	–	–	–	–	–	–	4.94 ± 1.48	5.24 ± 2.29

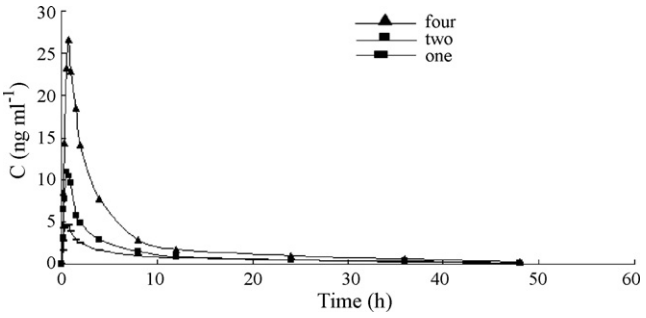

Fig. 4. Mean drug plasma concentration–time curves of rupatadine in 12 volunteers after oral administration of rupatadine in single-dose study.1 tablet: 10 mg; 2 tablets: 20 mg; 4 tablets: 40 mg.

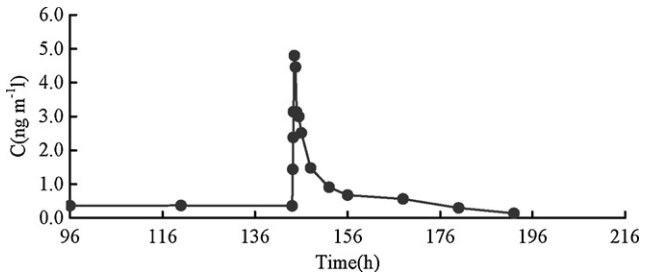

Fig. 5. Mean drug plasma concentration–time curve of rupatadine in 12 volunteers after oral administration of 10 mg of rupatadine in multiple-dose study.

dine in negative ions and the response of positive ions was stronger than negative ions for estazolam, so positive ion mode was chosen. In the precursor ion full scan spectra, the most abundant ions were protonated quasimolecular ion $[M+H]^+$ with m/z 416 for rupatadine and m/z 295 for IS. The product ion scan spectra were also investigated for using MRM mode; the most prominent product ions were m/z 309 for rupatadine and m/z 267 for the IS, respectively. Other main mass spectrometry parameters, such as spray voltage, capillary temperature, sheath gas and auxiliary gas pressure, source CID, collision gas pressure and collision energy, were also optimized by continuous infusion of a standard solution of rupatadine (1 μg/ml) and the IS (1 μg/ml) with a TSQ Quantum electronically controlled integrated syringe and the TSQ Quantum Tune program. Finally, the transition ions of m/z 416 → 309 for rupatadine, m/z 295 → 267 for the IS were set as detecting ions for obtaining maximum sensitivity. The positive ion ESI mass spectrum and the MS/MS product ion spectrum of these compounds are shown in Figs. 1 and 2.

3.4. Method validation

3.4.1. Assay specificity

No visible interferences were observed. Fig. 3a indicated no endogenous peaks at the retention positions of rupatadine and IS.

3.4.2. Matrix effect

All the ratios of the peak area of analytes resolved in blank sample (the final solution of blank plasma after extraction and reconstitution) to that of standard solutions at the same concentration were between 85% and 115%, which means that no significant matrix effect for rupatadine and the IS was implied in the method.

3.4.3. Linearity and LLOQ

The method exhibited excellent linear response over the selected concentration range of 0.1–100 ng/ml by weighted ($1/x$) least-squares linear regression analysis. The mean standard curve was typically described by the equation: $y = 0.221x - 0.0027$, $r = 0.9997$, where y corresponds to the peak area ratio of rupatadine to the IS and x refers to the concentration of rupatadine added to plasma. Results of five representative standard curves for LC–MS/MS determination of rupatadine are given in Table 1.

The lower limit of quantification for rupatadine proved to be 0.1 ng/ml (Fig. 3b), and the lower limit of detection was 0.05 ng/ml.

3.4.4. Precision and accuracy

Data for intra-batch and inter-batch precision and accuracy of the method for rupatadine are presented in Table 2. The precision deviation values for intra-batch and inter-batch are all within 15% of the relative standard deviation (R.S.D.) at each QC level. The accuracy deviation values for intra-batch and inter-batch are all within (100 ± 15)% of the actual values at each QC level. The results revealed good precision and accuracy.

3.4.5. Extraction recovery

The data of extraction efficiency measured for rupatadine and the IS in human plasma was consistent, precise and reproducible. The mean absolute extraction recovery of rupatadine at each QC level (0.3, 5 and 100 ng/ml) was (80.2 ± 5.8)%, (82.1 ± 5.9)% and (87.4 ± 4.7)%, respectively.

3.4.6. Stability studies

Table 3 summarizes the results of the short-term stability, long-term stability, post-preparative stability and freeze and thaw stability of rupatadine. The data showed reliable stability behavior of rupatadine under the condition tested.

3.4.7. Pharmacokinetic study

3.4.7.1. Single-dose study. A representative chromatogram of a plasma sample obtained at 0.33 h from a subject who received a single oral dose (40 mg) of rupatadine is shown in Fig. 3c. The mean plasma concentration–time curves of rupatadine following single oral doses of 10, 20 and 40 mg in 12 healthy volunteers are showed, respectively in Fig. 4. Kinetic parameters were listed in Table 4.

In our study, the C_{max} and AUC_{0-t} increased linearly with the increase from 10 to 40 mg. No significant differences were found in $t_{1/2}$, T_{max} and MRT ($P > 0.05$) between doses. The finding of dose proportionality suggests that processes responsible for the absorption and disposition of rupatadine are not saturated over the dose range of 10–40 mg.

3.4.7.2. Multiple-dose study. Mean plasma concentration–time data obtained at steady state after multiple oral doses of rupatadine in the same 12 healthy volunteers are depicted in Fig. 5. Kinetic parameters were listed in Table 4. The pharmacokinetic parameters, such as T_{max}, MRT, C_{max} ($P > 0.05$) obtained at steady state had no significant differences to those obtained after a single-dose of 10 mg.

3.4.7.3. Gender effect. Kinetic parameters about single- and multiple-dose study in male and female volunteers are listed in Table 5, respectively. The t-test result of C_{max} and AUC_{0-t} found no significant differences between the male and female groups.

4. Conclusion

In summary, a method is described for the quantification of rupatadine in human plasma by LC–MS/MS in positive ionization mode using multiple reaction monitoring. This method has shown

acceptable precision and adequate sensitivity for the quantification of rupatadine at the range of 0.1–100 ng/ml in human plasma samples obtained from pharmacokinetic, bioavailability or bioequivalence studies.

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