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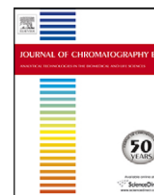

A single LC–tandem mass spectrometry method for the simultaneous determination of four H_2 antagonists in human plasma

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ABSTRACT

A sensitive and universal LC–MS/MS method for the simultaneous determination of famotidine, cimetidine, ranitidine and lafutidine in human plasma was presented. This is the first single LC–MS/MS method reported for the simultaneous analysis of these four H_2 antagonists in human plasma. Following liquid–liquid extraction with ethyl acetate, the separation was performed on an Agilent Zorbax SB-CN (150 mm × 2.1 mm I.D., 5 μm) column using a mobile phase consisted of methanol:20 mM ammonium acetate (55:45, v/v). The total run time was 7 min per sample. Quantification was performed by electrospray ionization–triple quadrupole mass spectrometry by selected reaction monitoring (SRM) detection in the positive mode. All calibration curves showed good linear regression ($r^2 > 0.99$) from 0.5 to 1000 ng/mL for famotidine and lafutidine, and 5–20,000 ng/mL for cimetidine and ranitidine. The method showed good precision and accuracy with overall intra- and inter-day variations of 1.37–9.29% and 3.51–9.40%, respectively. The assay was successfully applied to a bioequivalence study using ranitidine as the model compound.

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1. Introduction

Ranitidine, famotidine, cimetidine and lafutidine are all histamine (H_2) antagonists used in treatment of gastro-oesophageal reflux disease and gastric and duodenal ulceration.

A number of analytical methods have been reported for the determination of individual H_2 antagonists in human plasma since 1990 years, cimetidine [1–8], ranitidine [9–17], famotidine [18–25], and lafutidine [26–29]. Apart from some uncommonly used methods such as free capillary zone electrophoresis [4], micellar electrokinetic capillary chromatography [7], HPTLC [13], most reports utilized HPLC-UV method [1–7,9–16,18–23,26,27]. Although HPLC method is mature and relatively economical, limitations such as sensitivity, selectivity and throughput were often encountered during the method development. Only several studies employed LC–MS or LC–MS/MS method [8,17,24,25,28,29]. Xu et al. [8] published an HPLC–APCI-MS/MS method for the quantification of cimetidine. Zhang et al. [17] developed an LC–MS/MS method for the simultaneous determination of ranitidine and some other drugs in human plasma. These methods both showed broad linearity and high sensitivity. Campanero et al. [24] and Zhong et al. [25] reported LC–MS and LC–MS/MS methods, respectively, for the determination of famotidine in human plasma. Wu et al. [28], as well as Chen et al. [29] developed LC–MS methods for the determination of lafutidine. Sample preparation involved protein precipitation, liquid–liquid extraction (LLE), and solid-phase extraction (SPE). However, limitations of these LC–MS and LC–MS/MS methods include use of structure analogue as internal standard which is commercial unavailable [25]; utilization of large volumes of plasma, usually more than 200 μL [24,28]; long run time was needed because of gradient elution [>10 min] [8]; relatively high lower limit of quantification (LLOQ), and/or the relatively narrow dynamic range of the calibration curve [24,28]. Furthermore, these LC–MS and LC–MS/MS methods are only available for analyzing one of the four H_2 antagonists. Only a few of methods have been published for the simultaneous determination of some of these H_2 antagonists. Ashiru et al. reported an HPLC method for the simultaneous determination of cimetidine, famotidine, ranitidine and nizatidine in urine samples [30]. Zendelovska and Stafilov developed an HPLC-UV method for the simultaneous quantification of cimetidine and ranitidine in human plasma [31]. To date, there has been no report of a single LC–MS/MS method that is capable of simultaneously analyzing these four H_2 antagonists in biological samples such as human plasma. A sensitive method for the simultaneous determination of the four H_2 antagonists could be very meaningful and imperative. It is also very useful in a routine

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laboratory to save time and solvents. The initial of this study was to develop a single LC–MS/MS method for the simultaneous analysis of all these four H_2 antagonists in human plasma. The method was also applied to a bioequivalence study using ranitidine as model compound.

2. Experimental

2.1. Reagents and chemicals

The reference standards of famotidine and cimetidine were supplied by Longcheng Pharmaceutical Company (Chaoyang, P.R. China). Ranitidine hydrochloride was supplied by Jiangsu Lanjian Pharmaceutical Company (Jiangsu, P.R. China). Lafutidine was obtained from Chengdu Hengrui Pharmaceutical Company (Chengdu, P.R. China). Omeprazole (internal standard, IS) was obtained from Zhejiang Huayi Pharmaceutical Company (Zhejiang, P.R. China). The purities of reference standards are all >99.0%. HPLC grade methanol was purchased from VWR International Company (Darmstadt, Germany). Sodium hydroxide and ammonium acetate (analytical reagent) were purchased from Nanjing Chemical Reagent No. 1 Factory. Ethyl acetate (analytical reagent) was purchased from Nanjing Chemical Reagent Co. Ltd. Other chemicals were all of analytical grade. Water was distillated twice before use.

2.2. Instrumentation and chromatographic conditions

Liquid chromatographic separation and mass spectrometric detection were achieved on the Finnigan™ TSQ Quantum Discovery MAX™ LC–MS/MS system consisted of a Finnigan Surveyor LC pump, a Finnigan Surveyor auto-sampler, an on-line vacuum degasser and a triple quadrupole TSQ Quantum mass spectrometer (Thermo Electron Corporation).

Chromatographic separation of famotidine, cimetidine, ranitidine, lafutidine and the IS was performed on Agilent Zorbax SB-CN (150 mm × 2.1 mm I.D., 5 μm) column at 40 °C using methanol:20 mM ammonium acetate (55:45, v/v) as the mobile phase. Analysis was complete within 7 min with a flow rate of 0.2 mL/min.

Detection was performed using a Finnigan TSQ triple quadrupole mass spectrometer equipped with an ESI source using selected reaction monitoring (SRM) in positive ion mode. The parameter settings were as follows: spray voltage 4000 V, transfer capillary temperature 300 °C, sheath gas and auxiliary gas (nitrogen) pressure 17 and 15 arbitrary units, respectively. The optimized source CID was −15 V. Argon was used as the collision gas with a collision cell gas pressure of 1.5 mtorr (1 torr = 133.3 Pa). The optimized collision energy was 21 V for famotidine, 40 V for cimetidine, 14 V for ranitidine, 15 V for lafutidine, and 40 V for the IS. The mass spectrometer was set to monitor the transitions of the precursors to the product ions as follows: m/z 338.2 → 189.0 for famotidine, m/z 253.1 → 159.0 for cimetidine, m/z 315.1 → 176.1 for ranitidine, m/z 432.2 → 193.1 for lafutidine and m/z 346.1 → 136.1 for the IS. The scan width for SRM was 0.01 m/z and scan time was 0.2 s. The peak width settings (FWHM) for both Q1 and Q3 were 0.7 m/z.

2.3. Preparation of stock and working solutions and quality control samples

Primary stock solutions of analytes and the IS were prepared at 1 mg/mL in methanol and stored at 4 °C. Serial (working) dilutions of famotidine and lafutidine were prepared at the concentrations of 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, and 10,000 ng/mL. Serial (working) dilutions of cimetidine and ranitidine were prepared at the concentrations of 50, 100, 200, 500, 2000, 5000, 10,000, 20,000, 50,000, 100,000, and 200,000 ng/mL. Stock solution of the IS was prepared by dissolving omeprazole in methanol at a concentration of 1 mg/mL. The IS working solution was prepared by diluting the stock solution to 200 ng/mL.

Quality control (QC) samples were prepared daily by spiking blank plasma with proper volume of one of the working solution to produce a final concentration equivalent to 1, 100, and 800 ng/mL for famotidine and lafutidine, 10, 2000, and 16,000 ng/mL for cimetidine and ranitidine.

2.4. Sample preparation

Sample preparation was performed by liquid–liquid extraction. 100 μL aliquots of plasma samples were transferred to 10 mL centrifuge tubes, after addition of 100 μL of 2 M NaOH, 10 μL IS working solution and 3 mL ethyl acetate, the mixture was vortex-mixed for 3 min, then centrifuged at 4000 rpm for 10 min. The organic layer was transferred to another clean glass tube and evaporated under a steady stream of nitrogen to dryness in a water bath at 40 °C. The residue was reconstituted in 100 μL mobile phase and 10 μL injected into the LC–MS/MS system.

2.5. Bioanalytical method validation

The method was validated following the USFDA guidelines [32].

Selectivity was evaluated by analyzing the blank plasma samples from six different sources to ensure that no visible interferences were present at the retention time of famotidine, cimetidine, ranitidine, lafutidine, and the IS.

Linearity was determined at five replicates with concentration levels of 0.5, 1, 2, 5, 10, 20, 50 100, 200, 500, and 1000 ng/mL for famotidine and lafutidine, and 5, 10, 20, 50, 200, 500, 1000, 2000, 5000, 10,000, and 20,000 ng/mL for cimetidine and ranitidine. 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.

The limit of detection (LOD) and the LLOQ were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively. Each back-calculated concentration standard should meet the following acceptable criteria: no more than 20% deviation at LLOQ and no more than 15% deviation above LLOQ.

Intra-batch accuracy and precision were determined by analyzing five sets of spiked plasma samples at QC levels in a batch. Inter-batch accuracy and precision were performed by analyzing five sets of spiked plasma samples at QC levels on three consecutive days. The determined concentrations, which were obtained from a calibration curve prepared on the same day, were used to evaluate the method accuracy and precision. The accuracy was determined by the relative error (RE%), which was calculated by the equation: (mean of determined concentration − nominal concentration)/nominal concentration × 100%, and the precision was evaluated by the relative standard deviation (RSD%).

Stability was evaluated under different conditions that occurred during sample analysis. The short-term stability was evaluated by keeping QC samples at room temperature for 24 h. The long-term stability was assessed by QC plasma samples kept at low temperature (−20 °C) for 10 days. The post-preparative stability was measured by placing QC samples under the auto-sampler conditions (15 °C) for 24 h. The freeze and thaw stability was tested by analyzing QC samples undergoing three freeze (−20 °C)–thaw (room temperature) cycles on consecutive days. The working solutions and stock solutions of famotidine, cimetidine, ranitidine, lafutidine and the IS were also evaluated for stability at room temperature for 24 h and at 4 °C for 10 days, respectively. All QC samples were run in triplicate.

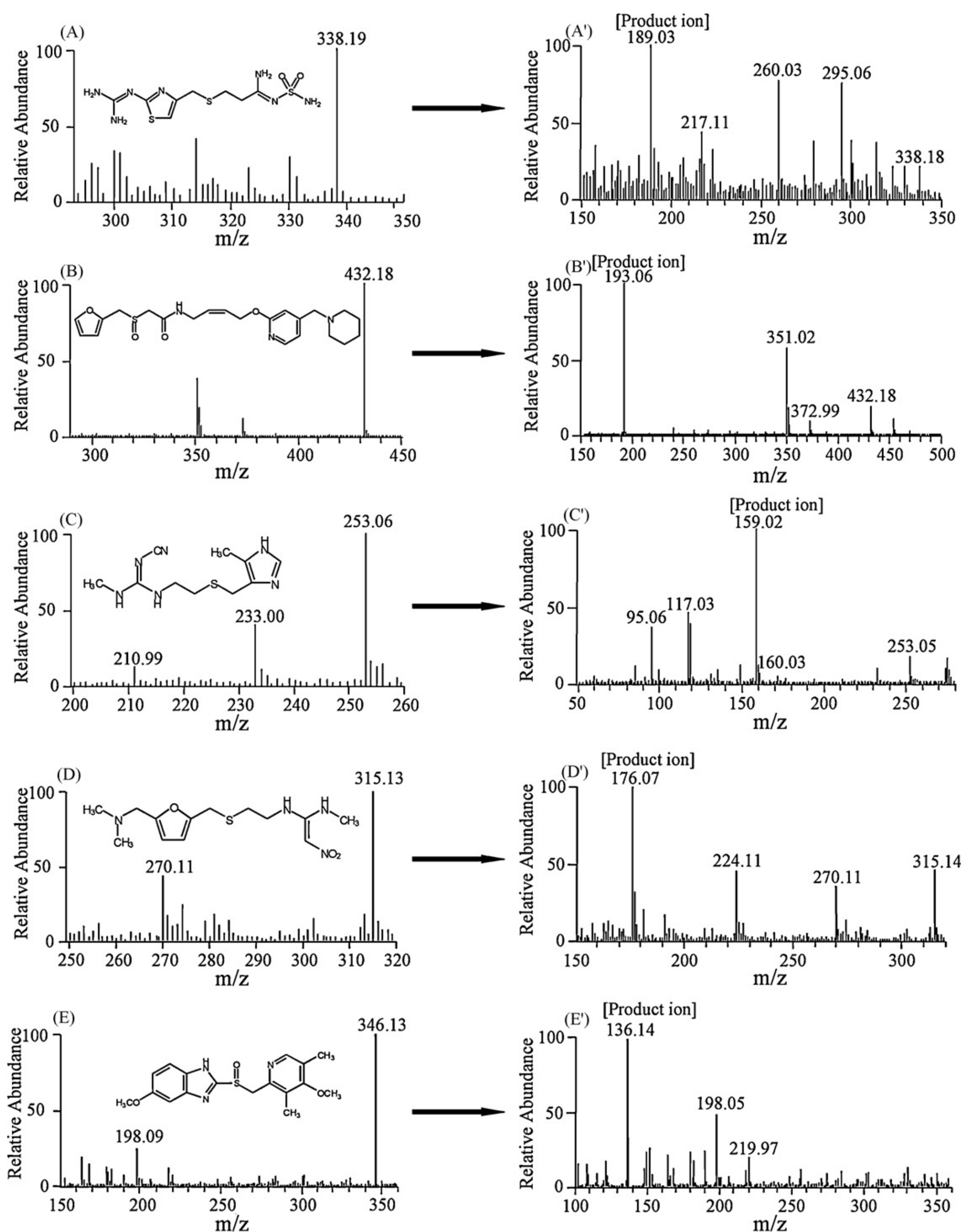

Fig. 1. The positive ion ESI-MS/MS spectra of famotidine (A), lafutidine (B), cimetidine (C), ranitidine (D), the IS (E) and their chemical structures.

Extraction recovery was evaluated by comparing the analyte–IS peak area ratios obtained from extracted samples to those from the samples containing the same amount of analytes which was added after the extraction step (on the extraction regents). The procedure was performed at three QC concentration levels for five replicates.

Matrix effects were thoroughly evaluated using blank plasma from different sources. A quantitative estimation of the matrix effects was obtained by comparing the peak area of the analytes dissolved in the supernatant of the processed blank plasma to that of standard solutions at the same concentration. Three QC concentration levels were evaluated by analyzing five samples at each set. The matrix effect of internal standard (20 ng/mL of omeprazole in plasma) was evaluated in the same way.

2.6. Application

The validated method was used to quantify ranitidine concentrations in a pharmacokinetic and bioequivalence study. After fasting overnight, six volunteers were administrated ranitidine hydrochloride capsules (containing 300 mg ranitidine) in the single dose study. Then serial blood samples were collected from the vein at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 15.0 h post-dose. Blood samples were put into lithium hep-

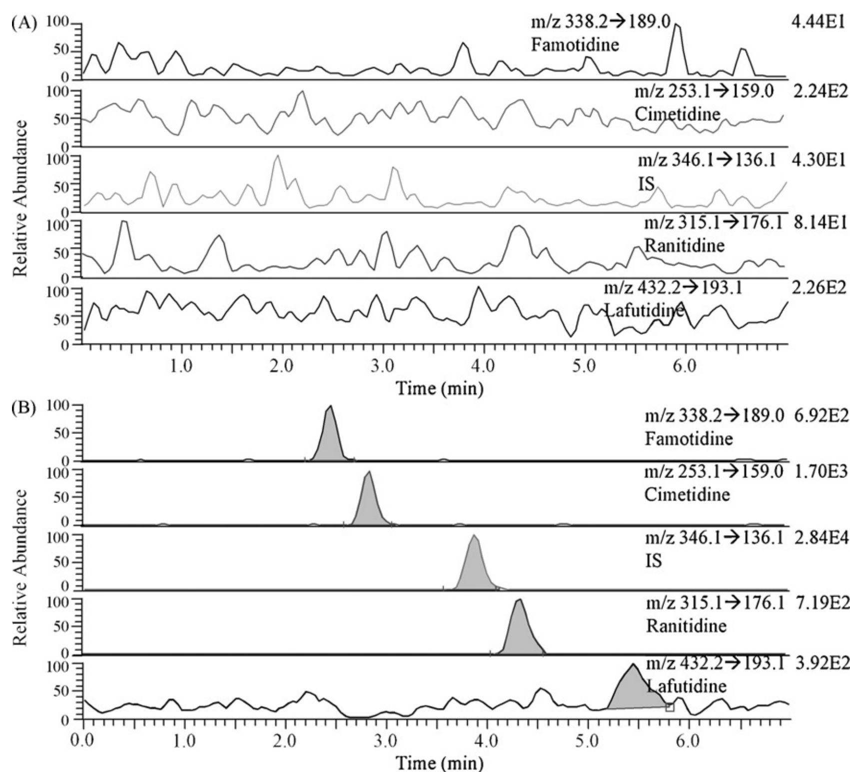

Fig. 2. Representative SRM chromatograms for famotidine, cimetidine, lafutidine, ranitidine and the IS resulting from analysis of (A) blank plasma (drugs and IS free) and (B) 0.5 ng/mL (LLOQ) of famotidine and lafutidine, 5 ng/mL (LLOQ) of cimetidine and ranitidine in human plasma spiked with the IS.

arin tubes and immediately centrifuged at 2000 × *g* for 10 min. The obtained plasma was frozen at −20 °C in coded polypropylene tubes until analysis.

3. Results and discussion

3.1. Method development

3.1.1. LC–MS/MS conditions

Since the four H_2 antagonists are all basic chemicals, the best signal was observed in positive ion electrospray ionization (ESI) mode. In the precursor ion full-scan spectra, the most abundant ions were protonated molecules $[M+H]^+$ at *m*/*z* 338.2 for famotidine, *m*/*z* 253.1 for cimetidine, *m*/*z* 315.1 for ranitidine, *m*/*z* 432.2 for lafutidine, and *m*/*z* 346.1 for the IS. The major fragment ions observed in each product-ion scan spectrum in SRM mode were at *m*/*z* 189.0 for famotidine, *m*/*z* 159.0 for cimetidine, *m*/*z* 176.1 for ranitidine, *m*/*z* 193.1 for lafutidine, and *m*/*z* 136.1 for the IS. Other main mass spectrometry parameters, such as spray voltage, capillary temperature, sheath gas and auxiliary gas pressure, scour CID, collision gas pressure and collision energy, were also optimized by continuous infusion of standard solutions (1 μg/mL) with a TSQ Quantum electronically controlled integrated syringe and the TSQ Quantum Tune program. Finally, the transition ions of *m*/*z* 338.2 → 189.0 for famotidine, 253.1 → 159.0 for cimetidine, 315.1 → 176.1 for ranitidine, 432.2 → 193.1 for lafutidine and 346.1 → 136.1 for the IS were set as detecting ions for obtaining maximum sensitivity. The positive ion ESI-MS/MS spectra of the compounds are shown in Fig. 1.

Because of high polarity and hydrophilicity of the H_2 antagonist, problems such as severe peak tailing were observed on C_{18}

Table 1
Precision and accuracy of the method for famotidine, lafutidine, cimetidine and ranitidine in human plasma.

Concentration added		Intra-batch (*n* = 5)			Inter-batch (*n* = 15)		
		Concentration found (mean ± SD, ng/mL)	Precision (RSD%)	Accuracy (RE%)	Concentration found (mean ± SD, ng/mL)	Precision (RSD%)	Accuracy (RE%)
Famotidine (ng/mL)	1.00	1.02 ± 0.0674	6.62	1.84	1.04 ± 0.0530	5.11	3.77
	100	91.8 ± 4.75	5.17	−8.22	96.9 ± 5.59	5.77	−3.12
	800	745 ± 39.0	5.23	−6.84	755 ± 26.6	3.51	−5.67
Lafutidine (ng/mL)	1.00	1.03 ± 0.0637	6.17	3.05	1.01 ± 0.0570	5.67	0.552
	100	107 ± 4.36	4.09	6.53	97.8 ± 7.92	8.10	−2.20
	800	801 ± 25.5	3.18	0.0864	775 ± 40.9	5.27	−3.18
Cimetidine (ng/mL)	10.0	9.85 ± 0.672	6.82	−1.47	9.50 ± 0.569	5.98	−4.96
	2,000	1,890 ± 176	9.29	−5.38	1,960 ± 146	7.42	−1.91
	16,000	14,400 ± 198	1.37	−9.83	14,900 ± 738	4.95	−6.86
Ranitidine (ng/mL)	10.0	9.37 ± 0.685	7.31	−6.33	9.87 ± 0.928	9.40	−1.32
	2,000	1,920 ± 151	7.81	−3.80	2020 ± 153	7.53	1.10
	16,000	16,300 ± 459	2.82	1.71	15,700 ± 1170	7.40	−1.87

Table 2
The stability of famotidine, lafutidine, cimetidine and ranitidine in human plasma under tested conditions[a] ($n = 3$).

	Mean ± SD					
	Famotidine			Lafutidine		
	1.00 ng/mL	100 ng/mL	800 ng/mL	1.00 ng/mL	100 ng/mL	800 ng/mL
Short-term stability (24 h, room temperature)	101 ± 5.81	90.3 ± 3.25	94.1 ± 5.24	97.4 ± 5.58	106 ± 4.24	95.9 ± 5.97
Long-term stability (10 days, −20 °C)	101 ± 4.65	95.8 ± 8.68	93.7 ± 5.25	96.4 ± 6.79	99.7 ± 7.45	102 ± 5.54
Post-preparative stability (24 h, 15 °C)	98.5 ± 5.27	88.6 ± 0.98	92.3 ± 3.39	97.1 ± 6.95	107 ± 4.22	99.3 ± 3.10
Freeze and thaw stability (3 cycles, −20 °C–room temperature)	104 ± 4.86	95.0 ± 7.46	94.3 ± 5.67	93.9 ± 4.85	104 ± 5.16	96.2 ± 5.31
	Mean ± SD					
	Cimetidine			Ranitidine		
	10.0 ng/mL	2000 ng/mL	16,000 ng/mL	10.0 ng/mL	2000 ng/mL	16,000 ng/mL
Short-term stability (24 h, room temperature)	95.9 ± 7.47	101 ± 7.18	90.6 ± 1.94	94.2 ± 8.85	103 ± 5.72	98.9 ± 3.65
Long-term stability (10 days, −20 °C)	92.5 ± 5.50	105 ± 6.25	94.0 ± 4.81	99.2 ± 6.28	106 ± 5.82	96.4 ± 5.40
Post-preparative stability (24 h, 15 °C)	101 ± 9.16	96.8 ± 9.37	90.6 ± 2.49	98.1 ± 10.61	107 ± 4.67	97.3 ± 5.59
Freeze and thaw stability (3 cycles, −20 °C–room temperature)	95.4 ± 6.95	100 ± 7.33	92.9 ± 4.73	97.2 ± 7.99	105 ± 5.40	104 ± 3.22

[a] Stability data were expressed as mean percentage of the analyte concentration determined at certain time point relative to that at time zero (nominal concentration).

column. While the elution on an SB-CN column permitted much better peak shape and separation. After evaluation of a variety of elution conditions, the separation, sensitivity, peak shapes and retention time were found to be satisfactory when an isocratic elution with a mobile phase consisted of methanol–water (containing 20 mM ammonium acetate) at the ratio of 55:45 (v/v) was used. All the compounds had retention time less than 6 min, and chromatographic run could be completed within 7 min.

3.1.2. Sample preparation

Liquid–liquid extraction and protein precipitation were both investigated to acquire precise, consistent and reproducible recovery. However, ion suppression, interferences from sample matrix, and poor recovery of cimetidine (<20%) were observed when protein precipitation was tried. Then liquid–liquid extraction involved several solvents including ethyl acetate, diethyl ether, *n*-hexane–isopropanol (95:5, v/v), and methylene chloride–ethyl acetate (20:80, v/v) was evaluated. Because of the differences of polarity and other qualities among the four H_2 antagonists, the extraction recovery of each analyte varied with the reagents, while ethyl acetate showed sufficient and reproducible recovery for all the four H_2 antagonists. The addition of sodium hydroxide solution (2 M) helped the dissociation of drugs from the plasma and reduced most of interferences result from acidic endogenous materials.

3.1.3. Selection of internal standard

A number of chemical compounds sharing some structural/chemical similarities with these H_2 antagonists were evaluated as potential internal standards. As a result, omeprazole was selected because of satisfactory chromatographic profile, stable signal intensity and reproducible extraction efficiency.

3.2. Method validation

3.2.1. Selectivity

No peaks from endogenous compounds were observed at the retention time of each analyte and IS in any of six blank plasma extracts evaluated. Typical chromatograms of drug free plasma and spiked plasma at the LLOQ of 0.5 ng/mL for famotidine and lafutidine, and 5 ng/mL for cimetidine and ranitidine are shown in Fig. 2A and B.

3.2.2. Linearity and LLOQ

Eleven-point calibration curve was found linear over the concentration range of 0.5–1000 ng/mL for famotidine and lafutidine 5–20,000 ng/mL for cimetidine and ranitidine. The best linearity fit for the calibration curve was achieved by weighted linear regression with a $1/\chi^2$ weighing factor, with a mean equation (curve coefficients ± SD) of $y = (9.10 \pm 0.260) \times 10^{-3}x + (1.98 \pm 0.137) \times 10^{-2}$ for famotidine, $y = (1.39 \pm 0.0274) \times 10^{-3}x + (3.68 \pm 0.113) \times 10^{-2}$ for cimetidine, $y = (9.13 \pm 0.325) \times 10^{-4}x + (4.93 \pm 0.313) \times 10^{-2}$ for ranitidine, $y = (9.87 \pm 0.285) \times 10^{-3}x + (1.03 \pm 0.110) \times 10^{-2}$ for lafutidine, where y is the peak area ratio of the analyte to the IS and x is the concentration of the analyte. The mean correlation coefficients of the calibration curves were all >0.99.

The lower limit of quantification proved to be 0.5 ng/mL for famotidine and lafutidine, 5 ng/mL for cimetidine and ranitidine (Fig. 2B), and the lower limit of detection 0.2 ng/mL for famotidine and lafutidine, 2 ng/mL for cimetidine and ranitidine.

3.2.3. Accuracy and precision

Data for the intra- and inter-batch precision and accuracy are presented in Table 1. The method was found to be highly accurate and precise. Intra-batch accuracy ranged from −9.83% to 6.53% and inter-batch accuracy ranged from −6.86% to 3.77% for the four compounds. Intra-batch precision was less than 9.29%, while inter-batch precision was less than 9.40% for each compound.

3.2.4. Stability

Stability of famotidine, lafutidine, cimetidine and ranitidine in human plasma under different conditions were evaluated. The detailed results are shown in Table 2. No significant degradation of any analytes in human plasma occurred after short-term storage for 24 h at room temperature, long-term storage for 10 days at

Table 3
Recoveries (%) of famotidine, lafutidine, cimetidine and ranitidine (mean ± SD).

Concentration (ng/mL)	n	Famotidine	Lafutidine
1.00	5	81.7 ± 2.07	86.9 ± 4.58
100	5	77.2 ± 3.96	86.4 ± 1.18
800	5	75.0 ± 3.94	84.2 ± 5.90
Total	15	78.0 ± 4.19	85.8 ± 3.98
Concentration (ng/mL)	n	Cimetidine	Ranitidine
10.0	5	73.1 ± 7.90	88.7 ± 4.50
2000	5	80.0 ± 3.73	90.5 ± 3.25
16,000	5	78.0 ± 1.80	85.4 ± 4.62
Total	15	77.1 ± 5.43	88.2 ± 4.22

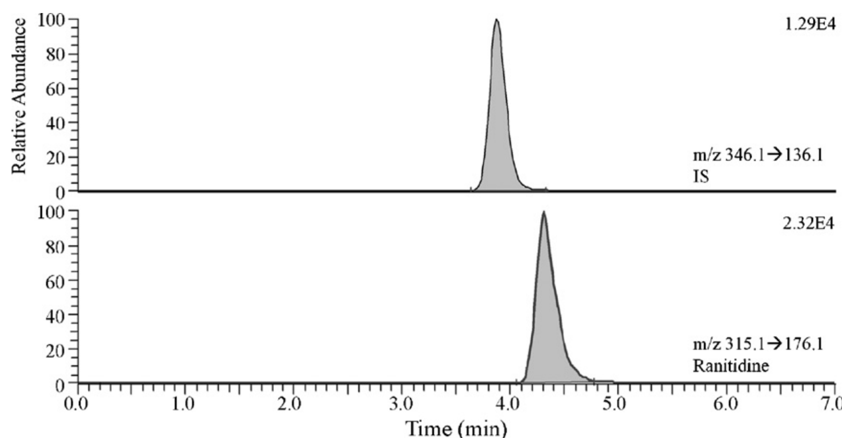

Fig. 3. A plasma sample obtained at 0.5 h from a subject after a single oral dose (300 mg) of ranitidine and the sample concentration was determined to be 93.37 ng/mL for ranitidine.

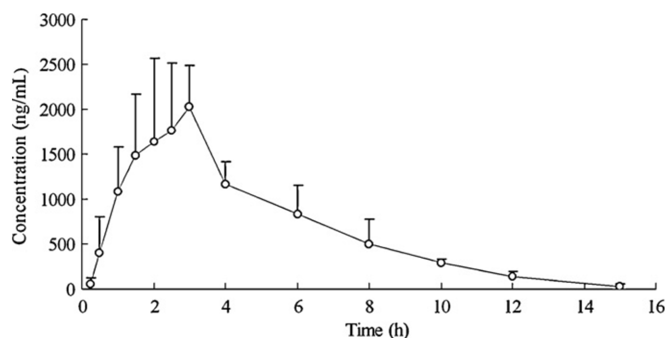

Fig. 4. Mean drug plasma concentration–time curve of ranitidine from six volunteers after oral administration. The vertical bars represent standard deviation.

−20 °C, three freeze and thaw cycles, or post-preparative storage for 24 h at 15 °C. The stock solutions and the working solutions of the four analytes and IS were stable for 10 days at 4 °C and 24 h at room temperature, respectively.

3.2.5. Extraction recovery

The extraction recovery determined for famotidine, cimetidine, ranitidine and lafutidine was shown to be consistent, precise and reproducible. The average recoveries of the four analytes from human plasma were 78.0%, 77.1%, 88.2%, and 85.8%. Data of extraction recoveries are presented in Table 3.

3.2.6. Matrix effect

The average matrix effect values were 96.2%, 100%, and 107% for lafutidine; 98.3%, 103%, and 98.7% for famotidine; 96.9%, 101%, and 105% for ranitidine; and 97.3%, 101%, and 98.4% for cimetidine at three QC concentration levels. The average matrix effect for the IS was 101%. These results indicated that the extracts had little or no detectable coeluting endogenous substances that could influence the ionization of the analytes and IS.

3.3. Application

The validated method was successfully applied to quantify ranitidine concentrations in a bioequivalence study. A representative chromatogram of a plasma sample obtained from a subject who received a single oral dose of ranitidine hydrochloride capsules is shown in Fig. 3. The mean plasma concentration–time profiles of six volunteers are represented in Fig. 4.

4. Conclusions

The paper presents a simple, rapid and sensitive HPLC–ESI-MS/MS method for the simultaneous determination of famotidine, cimetidine, ranitidine and lafutidine in human plasma for the first time. The method exhibited good linearity over a concentration range of 0.5–1000 ng/mL for famotidine and lafutidine, and 5–20,000 ng/mL for cimetidine and ranitidine, respectively. Isocratic elution and shorter run time (7 min) are helpful in carrying out analysis rapidly. The internal standard used for this method is more easily available. Utilization of small volume of plasma (100 μL) also allows the method to pediatric studies. The method has been successfully applied to the bioequivalence study of the ranitidine hydrochloride capsules in human volunteers.

References

[1] E. Jantratid, S. Prakongpan, J.P. Foley, J.B. Dressman, Biomed. Chromatogr. 21 (2007) 949.
[2] J. Hempenius, J. Wieling, J.P.G. Brakenhoff, F.A. Maris, J.H.G. Jonkman, J. Chromatogr. B 714 (1998) 361.
[3] T. Iqbal, C.S. Karyekar, M. Kinjo, G.C. Ngan, T.C. Dowling, J. Chromatogr. B 799 (2004) 337.
[4] J. Lukga, D. Josid, J. Chromatogr. B 667 (1995) 321.
[5] M.T. Kelly, D. McGuirk, F.J. Bloomfield, J. Chromatogr. B 668 (1995) 117.
[6] F.G. Russel, M.C. Creemers, Y. Tan, P.L. van Riel, F.W. Gribnau, J. Chromatogr. B: Biomed. Appl. 661 (1994) 173.
[7] H. Soini, T. Tsuda, M.V. Novotny, J. Chromatogr. A 559 (1991) 547.
[8] K.Y. Xu, V.K. Arora, A.K. Chaudhary, R.B. Cotton, I.A. Blair, Biomed. Chromatogr. 13 (1999) 455.
[9] C. López-Calull, L. García-Capdevila, C. Arroyo, J. Bonal, J. Chromatogr. B 693 (1997) 228.
[10] L.G. Hare, D.S. Mitchel, J.S. Millership, P.S. Collier, J.C. McElnay, M.D. Shields, D.J. Carson, R. Fairc, J. Chromatogr. B 806 (2004) 263.
[11] A. Ahmadiani, H. Amini, J. Chromatogr. B 751 (2001) 291.
[12] C.F. Pérez, H.J. Olguín, J.F. Pérez, A.T. López, I.L. Asseff, C.A. García, J. Chromatogr. B 795 (2003) 141.
[13] V.D. Mody, M.C. Satia, T.R. Gandhi, I.A. Modi, R.I. Modi, B.K. Chakravarthy, J. Chromatogr. B 676 (1996) 175.
[14] C.F. Wong, K.K. Peh, K.H. Yuen, J. Chromatogr. B 718 (1998) 205.
[15] T.G.D. Nascimento, E.D.J. Oliveira, R.O. Macêdo, J. Pharm. Biomed. Anal. 37 (2005) 777.
[16] D. Farthing, K.L.R. Brouwerb, I. Fakhry, D. Sica, J. Chromatogr. B 688 (1997) 350.
[17] Y. Zhang, N. Mehrotra, N.R. Budha, M.L. Christensen, B. Meibohm, Clin. Chim. Acta 398 (2008) 105.
[18] L. Zhong, K.C. Yek, J. Pharm. Biomed. Anal. 16 (1998) 1051.
[19] A. Zarghi, A. Shafaati, S.M. Foroutan, A. Khoddamc, J. Pharm. Biomed. Anal. 39 (2005) 677.
[20] S. Wanwimolruk, A.R. Zoest, S.Z. Wanwimolruk, C.T. Hung, J. Chromatogr. Biomed. Appl. 572 (1991) 227.
[21] A. Zarghi, H. Komeilizadeh, M. Amini, L. Kimiagar, Pharm. Pharmacol. Commun. 4 (1998) 77.
[22] T.C. Dowling, R.F. Frye, J. Chromatogr. B: Biomed. Sci. Appl. 732 (1999) 239.
[23] M.A. Campanero, I. Bueno, M.A. Arangoa, M. Escolar, E.G. Quetglas, A. Lopez-Ocariz, J.R. Azanza, J. Chromatogr. B: Biomed. Sci. Appl. 763 (2001) 21.

[24] M.A. Campanero, I. Bueno, M.A. Arangoa, M. Escolar, E.G. Quetglás, A. López-Ocáriz, J.R. Azanza, J. Chromatogr. B 763 (2001) 21.
[25] L. Zhong, R. Eisenhandler, K.C. Yeh, J. Mass Spectrom. 36 (2001) 736.
[26] H. Itoh, T. Naito, M. Takeyama, Biol. Pharm. Bull. 25 (2002) 379.
[27] K. Ikawa, T. Shimatani, S. Hayato, N. Morikawa, S. Tazuma, Biol. Pharm. Bull. 30 (2007) 1003.
[28] L.L. Wu, Z.J. Zhang, Y. Tian, W. Li, F.G. Xu, Y. Chen, H.L. Wei, J. Mass Spectrom. 40 (2005) 1637.
[29] W.D. Chen, Y. Liang, H. Li, Y. Xiong, X.D. Liu, G.J. Wang, L. Xie, J. Pharm. Biomed. Anal. 41 (2006) 256.
[30] D.A.I. Ashiru, R. Patel, A.W. Basit, J. Chromatogr. B 860 (2007) 235.
[31] D. Zendelovska, T. Stafilov, J. Pharm. Biomed. Anal. 33 (2003) 165.
[32] Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation, 2001.