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Short communication

Simple, sensitive and rapid LC–ESI–MS method for the quantitation of lafutidine in human plasma—Application to pharmacokinetic studies

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Abstract

A sensitive and specific liquid chromatography electrospray ionization mass spectrometry (LC–ESI–MS) method has been developed and validated for the identification and quantification of lafutidine in human plasma. Lafutidine and internal standard were isolated from plasma samples by liquid–liquid extraction with diethyl ether. The chromatographic separation was accomplished on a stainless-steel column (C_{18} Shim-pack 5 μm 150 mm × 2.0 mm i.d. Shimadzu) at a flow rate of 0.2 ml/min by a gradient elution. Detection was performed on a single quadrupole mass spectrometer by selected ion monitoring (SIM) mode via electrospray ionization (ESI) source. The method was proved to be sensitive and specific by testing six different plasma batches. Linearity was established for the range of concentrations 1.0–400.0 ng/ml with a coefficient of determination (r) of 0.9998 and good back-calculated accuracy and precision. The intra- and inter-day precision (R.S.D.%) was lower than 10% and accuracy ranged from 85 to 115%. The lower limit of quantification was identifiable and reproducible at 0.5 ng/ml with 0.2 ml plasma. The proposed method enables the unambiguous identification and quantification of lafutidine for pharmacokinetic, bioavailability or bioequivalence studies.

Keyword: Lafutidine

1. Introduction

Lafutidine, ((±)-2-(furfurylsulfinyl)-*N*-[4-[4-(piperidinomethyl)-2-pyridyl]oxy-(*Z*)-2-butenyl] acetamide), is a novel antiulcer drug that exhibits long-lasting gastric antisecretory effects due to blockade of the histamine H2 receptor [1]. It has been reported that the gastroprotective activity of lafutidine was independent of antisecretory activity but partially or fully mediated by capsaicin-sensitive afferent neurons [2,3].

In order to fully evaluate the pharmacokinetics of lafutidine in human plasma to support phase I and II clinical studies, it was necessary to develop and validate an assay with appropriate sensitivity, selectivity, accuracy and precision. Potentially large number of samples in clinical studies needs a rapid and reliable assay. An ideal method should have simple sample preparation, fast on-column separation, and sensitive and specific detection. Liquid chromatography coupled with mass spectrometry (LC–MS) has become such an analytical tool which meets most of the above needs. In many cases, highly specific mass spectrometric detection, especially using tandem mass spectrometry (MS–MS) just requires minimum separation on column, but it is too expensive to use for the routine measurements in the preclinical laboratory [4].

Recently, Pan et al. described a method using HPLC and LC–MS for separation and identification of *cis* and *trans* isomers of 2-butene-l,4-diol and lafutidine in vitro [5]. In contrast, we report an LC–MS method for quantitative determining the lafutidine in biological samples with an excellent sensitivity for 2 μl injection volume on-column with a total run time of 7.5 min. This paper describes the development and validation of a highly sensitive LC–MS method for the quantitation of lafutidine in human plasma and shown the main pharmacokinetic parameters.

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2. Experimental

2.1. Chemicals and reagents

Lafutidine reference standard (99.7% purity) and ranolazine reference standard (99.3% purity) were identified and supplied by National Institute for the Control of Pharmaceutical and Biological Produces (Beijing, China). Methanol was chromatographic pure grade and purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Deionized water was distilled before using. Other reagents were used as received.

2.2. Instrumentation and operating conditions

Liquid chromatography was performed using a Shimadzu LC-10AD HPLC system consisting of an autosampler (SIL-HTc). The HPLC was coupled to a Shimadzu LCMS-2010A quadrupole mass spectrometer with an electrospray ionization (ESI) interface. Data acquisition and processing were accomplished using Shimadzu LCMS solution Software for LCMS-2010A system. Chromatographic separation was carried out with a stainless-steel column (C_{18} Shim-pack 5 μm 150 mm × 2.0 mm i.d. Shimadzu) column at 40 °C. Mobile phase A consisted of 1 μmol/l ammonium acetate and 0.04‰ triethylamine in water; mobile phase B was methanol. Each mobile phase was filtered through a 0.25 μm membrane and degassed under reduced pressure. Linear gradient elution was employed with a 7.5 min run time and its sequence was as follows: A–B (45:55) held for 1.5 min after injection, 20:80 at 3.0 min and held up to 4.0 min, and thereafter 45:55. Analyses were conducted at a flow rate of 0.2 ml/min. The ESI source was set at positive ionization mode. The $[M+H]^+$, m/z 432.15 for lafutidine and $[M+H]^+$, m/z 428.00 for ranolazine were selected as detecting ions, respectively. The quantification was performed via peak-area. The MS operating conditions were optimized as follows: drying gas 1.5 l/min, CDL temperature 250 °C, block temperature 200 °C, probe voltage +4.0 kV.

2.3. Preparation of stock solutions

The stock solutions of 1.0 mg/ml lafutidine and 1.0 μg/ml ranolazine (IS) were prepared after the correcting for purity and stored at 4 °C. The working solutions of lafutidine were prepared daily in methanol by appropriate dilution of the stock solution at 20 ng/ml, 0.2 μg/ml and 2.0 μg/ml.

2.4. Calibration curves

Typical calibration curves were constructed with six blank plasma samples spiked with appropriate amounts of the standard solutions. The calibration range was 1.0–400.0 ng/ml of lafutidine for plasma.

Each sample also contained 100.0 ng (20 μl × 1.0 μg/ml) of the internal standard. In each run, a plasma blank sample (no IS) was also analyzed Calibration curves were prepared by determining the best-fit of peak area ratios (peak area of analyte/peak area of internal standard) versus concentration, and fitted to the equation $R = bx + a$ by unweighted least-squares regression.

2.5. Extraction procedure

QC, calibration curve and clinical plasma samples were extracted employing a liquid–liquid extraction technique. To each tube containing 0.2 ml plasma, 100 ng (20 μl × 1.0 μg/ml) of internal standard, 100 μl of 0.1 M sodium hydroxide solution and 5 ml diethyl ether were added and then were vortexed for 2 min. Afterwards, samples were centrifuged for 10 min at 4000 rpm. The organic layer was evaporated under a stream of nitrogen at 40 °C. The residue was redissolved in 200 μl methanol. An aliquot of 2 μl was injected into the LC–MS system.

2.6. Method validation

The method validation assays were carried out according to the currently accepted US Food and Drug Administration (FDA) bioanalytical method validation guidance. The following parameters were considered. The specificity of the method was tested by screening six different batches of healthy human blank plasma. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/spectroscopic conditions and compared with those obtained with an aqueous solution of the analyte at a concentration near to the LLOQ.

The matrix effect on the ionization of analytes was evaluated by comparing the peak area of analytes resolved in blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in methanol. Three different concentration levels of lafutidine (2.0, 25.0 and 200.0 ng/ml) and 100 ng/ml of internal standard were evaluated by analyzing five samples at each level. The blank plasma used in this study was six different batches of healthy human blank plasma. If the ratio <85 or >115%, an exogenous matrix effect was implied.

Linearity was tested for the range of concentrations 1.0–400.0 ng/ml. For the determination of linearity, standard calibration curves of at least nine points (non-zero standards) were used. In addition, blank plasma samples were also analyzed to confirm absence of interferences but they were not used to construct the calibration function. Four out of nine non-zero standards including LLOQ and ULOQ were to meet the following acceptance criteria: no more than 15% deviation at LLOQ and no more than 10% deviation for standards above the LLOQ. The acceptance criteria for correlation coefficient was 0.998 or more, otherwise the calibration curve should be rejected. Five replicate analyses were done. The samples should been run from low to high.

The intra-day precision and accuracy of the assay was measured by analyzing five spiked samples of lafutidine at each QC level (2.0, 25.0 and 200.0 ng/ml). The inter-day precision and accuracy was determined over 3 days by analyzing 15 QC samples each day. The acceptable precision and accuracy deviation values should be within 15% of the actual values.

The extraction yield (absolute recovery) was determined by comparing the lafutidine/IS peak area ratios obtained following the outlined extraction procedure with those obtained from those that contained the same amount of lafutidine in extracted plasma but not be extracted after addition of drug. This procedure was repeated for the three different concentrations of lafutidine added, namely 2.0, 25.0 and 200.0 ng/ml. The recovery of IS was also determined.

For sensitivity determination, the lowest standard concentration in the calibration curve was considered as the lower limit of quantification (LLOQ), and was to meet the following criteria: LLOQ response should be 10 times that of noise and be identifiable, discrete and reproducible within the precision deviation of 20%. Samples at the concentration 0.5 ng/ml were investigated as the lower limit of quantification and the reproducibility and precision were also determined.

Short-term temperature stability. Stored plasma aliquots were thawed and kept at room temperature for a period of time exceeded that expected to be encountered during the routine sample preparation (around 6 h). Samples were analyzed as mentioned above.

Post-preparative stability. The autosampler stability was conducted reanalyzing extracted QC samples kept under the autosampler conditions (4 °C) for 24 h.

Freeze and thaw stability. QC plasma samples containing lafutidine were tested after three freeze (−20 °C) and thaw (room temperature) cycles. Long-term stability of lafutidine in human plasma was studied for a period of 10 days employing QC samples at three different levels. If after the stability study the analyte was found to be unstable at −20 °C, then it should be stored at −70 °C. The stability of lafutidine and internal standard working solutions were evaluated by testing their validity for 6 h at room temperature. Stability of working solutions was expressed as percentage recovery.

A calibration curve was generated to assay samples in each analytical run and was used to calculate the concentration of lafutidine in the unknown samples in the run. The calibration was analyzed in the middle of each run. In order to monitor the accuracy and precision of the analytical method a number of QC samples were prepared to ensure that method continues to perform satisfactorily. The QC samples in duplicate at three concentrations (2.0, 25.0 and 200.0 ng/ml) were prepared and were analyzed with processed test samples at intervals based on the total number of samples per batch.

3. Clinical study design

This was an open randomized, balanced study in 24 Chinese healthy volunteer with male and female in half. Each volunteer received in random order, single oral dose of 10.0 mg lafutidine test tablets. Blood samples (2 ml) for assay of plasma concentration of lafutidine were collected at the time of 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after oral administration of the medicals. They were put into lithium heparin tubes and immediately were centrifuged at 3000 × *g* for 10 min. The plasma obtained was frozen at −20 °C in coded polypropylene tubs until analysis.

4. Results and discussion

4.1. Selection of IS

It is necessary to use an IS to get high accuracy when a mass spectrometer is used as the HPLC detector. Ranolazine was adopted in the end because of its similarity of retention, ionization with the analyte and the less endogenous interferences at ranolazine $[M+H]^+$, *m/z* 428.00.

4.2. Sample preparation

Liquid–liquid extraction was necessary [6,7] and important because this technique can not only purify but also concentrate the sample. Ethyl acetate, trichlormethane and diethyl ether were all tested to do extraction and diethyl ether was finally adapted because of its high extraction efficiency. Sodium hydroxide (0.1 ml × 0.1 M) was added to the plasma in order to accelerate the drugs' dissociation from the plasma and reduce interference since most endogenous are of acidic nature.

4.3. Separation and specificity

The major ions observed from positive ion electrospray mass scan spectra for lafutidine were $[M+H]^+$, *m/z* 432.15; $[M+Na]^+$, *m/z* 454.20 and $[M+K]^+$, *m/z* 470.45. The ions of $[M+K]^+$, *m/z* 432.15 for lafutidine was selected for the SIM(+) due to their high stability and intensity.

The SIM(+) chromatograms extracted from plasma sample of a healthy volunteer who participated in a pharmacokinetics study conducted on 24 persons are depicted in Fig. 1. As shown, the retention times of lafutidine and the IS were 4.6 and 5.8 min, respectively.

The total HPLC–MS analysis time was 7.5 min per sample. No interferences of the analytes were observed because of the high selectivity of the SIM model. No ion suppression effects were observed under the developed sample preparation and chromatographic conditions. Fig. 2 shows an HPLC chromatogram for a blank plasma sample indicating no endogenous peaks at the retention positions of lafutidine or internal standard (ranolazine).

4.4. Method validation

The method exhibited a good linear response for the range of concentrations from 1.0 to 400.0 ng/ml. Respective regression equations were: $y = 0.231x - 0.003$ with a coefficient of 0.9998 for plasma.

Data for intra- and inter-day precision of the method for lafutidine as determined from the QC samples runs at the concentrations of 2.0, 25.0 and 200.0 ng/ml are presented in Table 1. The lower limit of quantitation for lafutidine was proved to be 0.50 ng/ml (LLOQ) and the lower limit of detection (LLOD) for lafutidine was 0.20 ng/ml. The extraction recoveries determined for lafutidine and IS were shown to be consistent, precise, reproducible and suitable to the bioanalysis.

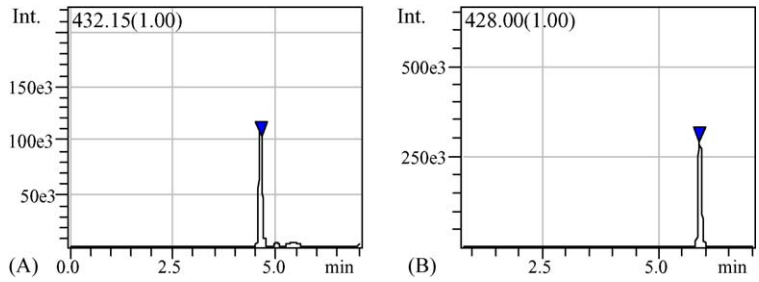

Fig. 1. The SIM(+) chromatogram for plasma sample of a healthy volunteer at 3 h after oral administration l0 mg lafutidine. Peaks were assigned with (▼). The retention times of lafutidine and the IS were 4.6 min (A) and 5.8 min (B), respectively.

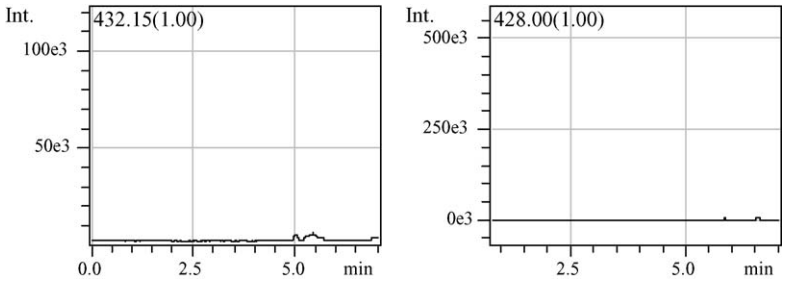

Fig. 2. The SIM(+) chromatogram for a blank plasma sample.

Table 1
The inter- and intra-day precision, accuracy of the method with determination of lafutidine ($n = 5$)

	Inter-day			Intra-day								
	2.0	25.0	200.0	1 day			2 days			3 days		
				2.5	25.0	200.0	2.5	25.0	200.0	2.5	25.0	200.0
Back-calculated concentration (ng/ml)	2.1	22.9	195.8	2.4	26.3	202.3	2.5	26.4	204.9	2.4	25.7	195.8
	1.8	24.0	204.9	2.3	25.6	194.7	2.5	25.6	202.7	2.3	25.0	200.8
	1.9	23.3	195.2	2.5	25.9	204.2	2.5	24.3	201.7	2.4	25.5	199.2
	2.1	24.0	200.1	2.5	25.4	200.0	2.5	24.4	206.7	2.4	25.0	207.3
	2.2	25.0	200.7	2.5	24.2	205.9	2.4	25.6	202.5	2.4	25.6	201.3
Mean	2.0	23.8	199.3	2.4	25.5	201.4	2.5	25.3	203.7	2.4	25.4	200.9
R.S.D.%	7.72	3.48	3.82	2.77	3.08	2.16	1.22	3.43	1.02	2.50	1.30	2.08
Mean accuracy%	99.77	95.34	99.67	97.79	101.90	100.71	99.02	101.03	101.86	95.62	101.49	100.44

2.0, 25.0 and 200.0 are added concentration in ng/ml.

4.5. Stability

Table 2 summarizes the stability data of the short-term, freeze and thaw, long-term as well as post-preparative test of lafutidine.

Short-term stability indicated reliable stability behavior under the experimental conditions of the regular runs. The results of freeze and thaw stability indicated that the analyte was stable in human plasma for three cycles of freeze and thaw, when stored at −20 °C and thawed to room temperature.

Table 2
Data showing stability of lafutidine in human plasma at different QC levels ($n = 5$)

	2.0 ng/ml		25.0 ng/ml		200.0 ng/ml	
	Recovery (mean ± S.D.) (%)	R.S.D. (%)	Recovery (mean ± S.D.) (%)	R.S.D. (%)	Recovery (mean ± S.D.) (%)	R.S.D. (%)
Short-term stability	94.08 ± 6.94	7.38	98.23 ± 4.05	4.12	96.63 ± 5.22	5.41
Freeze and thaw stability	90.03 ± 8.45	9.39	99.82 ± 3.98	3.99	92.08 ± 9.48	10.3
Long-term stability	87.58 ± 5.27	6.02	90.43 ± 2.39	2.64	88.51 ± 3.33	3.76
Post-preparative stability	98.91 ± 7.42	7.5	103.65 ± 11.26	10.86	94.94 ± 11.68	12.3

Table 3
Pharmacokinetic parameters of lafutidine in 24 volunteers (male and female in half) after oral administration

Gender	Parameter	C_{max} (ng/ml)	T_{max} (h)	$t_{1/2}$ (h)		V_c/F (l)	MRT (h)	Cl/F (l/h)	$AUC^{0-\tau}$ (ng h/ml)	$AUC^{0-\infty}$ (ng h/ml)
				α	β					
Male	Mean	151.55	1.6	1.43	4.29	25.45	5.74	12.70	919.57	938.73
	S.D.	54.49	0.4	0.18	0.53	25.35	0.72	5.73	429.59	443.13
Female	Mean	165.64	1.1	1.48	4.43	16.49	5.01	11.89	844.34	858.78
	S.D.	26.96	0.3	0.10	0.30	13.27	0.67	1.78	135.04	141.59

The post-preparative stability of QC samples shown that lafutidine was stable when kept at 4 °C in the autosampler for 24 h. The findings from long-term test indicate that storage of lafutidine's plasma samples at 20 °C is adequate when stored for 10 days and no stability-related problems would be expected during the samples routine analysis for the pharmacokinetic, bioavailability or bioequivalence studies.

The stability of working solutions was tested at room temperature. Based on the results obtained, these working solutions were stable within 6 h.

4.6. Results of pharmacokinetic study

The method was applied to analyze plasma samples obtained from 24 healthy volunteers which received a single dose of 10 mg lafutidine preparations each in the pharmacokinetics study. The procedure developed was sensitive enough to assure the quantitative analysis of lafutidine in plasma with acceptable accuracy over a period of 24 h after a single oral administration. Pharmacokinetic parameters are listed in Table 3. Except for T_{max}, no significant difference was found between male and female for the other parameters.

5. Conclusion

The proposed method of analysis provided a sensitive and specific assay for lafutidine determination in human plasma. Simple liquid–liquid extraction procedure and short run time can curtail test's time that is important for large sample batches. It was shown that this method is suitable for the analysis of lafutidine in human plasma samples collected for pharmacokinetic, bioavailability or bioequivalence studies in humans.

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