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Development and full validation of a sensitive quantitative assay for the determination of clemastine in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

A sensitive high-performance liquid chromatography–tandem mass spectrometry (LC/MS/MS) method was developed and validated for the determination of clemastine in human plasma. After having been extracted from plasma samples by ethyl acetate, clemastine and internal standard, diphenhydramine, were separated on a C_{18} column. Detection was performed on Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer by selected reaction monitoring (SRM) mode via electrospray ionization (ESI) source. The method was linear in the concentration range of 5.0–1000.0 pg/ml for clemastine. The intra- and inter-day precisions were within 13.4% and the deviations were between −1.1% and 5.6%. The fully validated LC/ESI-MS/MS method has been successfully applied to the preliminary pharmacokinetic study in healthy male Chinese volunteers.

Keywords: Clemastine; LC/MS/MS; Determination; Human plasma; Quantitative assay

1. Introduction

Clemastine hydrogen fumarate (Fig. 1) is an ethanolamine-derivative antihistamine used for the relief of symptoms associated with allergic rhinitis, seasonal rhinitis, urticaria, angioedema, and the common cold. Clemastine competes against histamine for the H_1 receptor binding sites. As an ethanolamine derivative, it also possesses anticholinergic activity, which resulting in a sedative effect. The oral therapeutic dose of clemastine is only 1 or 2 mg each day which in turn leads to very low plasma levels (C_{max} is around 0.6 ng/ml/1 mg dose) [1], so a sensitive analytical method is needed for its determination in plasma.

The lack of sufficiently sensitive analytical methods of clemastine therefore has hindered the extensive study of its pharmacokinetic properties for a long time. GC–ECD [2], GC–MS [3,4], LC–MS [5], RIA (radioimmunoassay) [1], GC–nitrogen–phosphorous detection [6], and LC–MS–MS [7,8] methods have been developed for the detection of clemastine in biological samples. An early GC–ECD method oxidized clemastine to chlorobenzophenone prior to analysis of plasma samples. The lowest concentration of clemastine that could be measured was 1 ng/ml. Meanwhile, the selectivity of the method is questionable since metabolites of clemastine are oxidized to the same compound [2]. A GC–MS method was developed later with a similar lower limit of quantification (LLOQ), 1 ng/ml, for the determination of clemastine in human plasma, which did not give sufficient sensitivity to enable the study of its pharmacokinetics [3]. Hasegawa et al. reported a method for simultaneous determination of ten antihistamine drugs in human plasma using pipette tip solid-phase extraction and GC–MS [4]. However, the LLOQ

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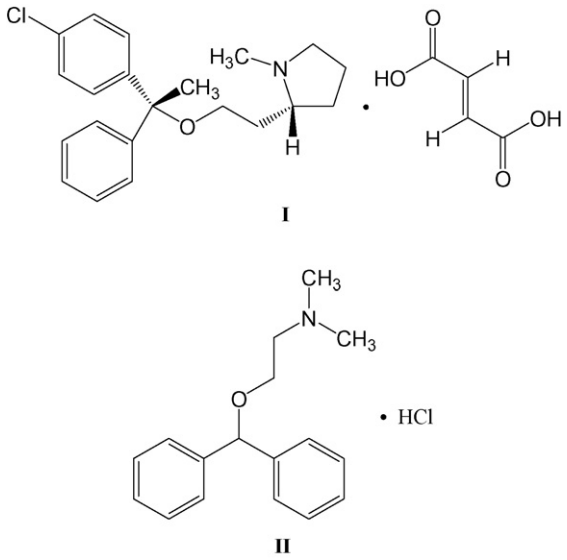

Fig. 1. Chemical structures of clemastine hydrogen fumarate (I) and diphenhydramine hydrochloride (II; internal standard).

for clemastine was only 10 ng/ml. Although LC–MS methods have been developed for the determination of clemastine in biological samples, these methods were neither validated nor used for the analysis of clemastine in plasma samples of pharmacokinetic studies [5]. Radioimmunoassay has been proven to be selective and sensitive enough to measure pharmacokinetics and bioavailability of clemastine in several studies with the minimum measurable concentration of 0.1 ng/ml [1]. Davydova et al. have also validated a same sensitive GC–nitrogen–phosphorous detection method with a LLOQ of 0.1 ng/ml [6]. Recently two LC–MS–MS methods have been developed by Gergov et al. and Horvath et al. with the LLOQ of 0.5 ng/ml and 0.01 ng/ml, respectively [7,8].

The present study describes a rapid and sensitive LC/MS/MS method using diphenhydramine (Fig. 1), which has been successfully used as internal standard in many LC/MS/MS methods for biomedical analysis [9–11], as IS for the determination of clemastine in human blood sample. The fully validated method can quantify clemastine between the concentration range of 5.0 and 1000.0 pg/ml. This concentration range corresponds to therapeutic levels and makes it possible to follow its elimination after a very low oral dosage administration of clemastine (compound tablets containing 1000 mg paracetamol, 60 mg pseudoephedrine and 0.67 mg clemastine hydrogen fumarate).

2. Experimental

2.1. Materials

Clemastine hydrogen fumarate (99.7% pure) and diphenhydramine hydrochloride (99.8% pure) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, acetonitrile and formic acid of HPLC grade were purchased from Tedia Company Inc. (Beijing, China). All other reagents were of analytical grade. Blank human plasma was obtained from the Changsha Blood Donor Service (Changsha, China). Ultra-pure water was obtained from a Milli Q-plus system (Billerica, MA, USA).

2.2. Instrumentation and operating conditions

Liquid chromatography was performed using a Surveyor MS Pump (Thermo Finnigan, USA) and a Surveyor Autosampler (Thermo Finnigan, USA). Chromatographic separation was achieved on an Aquasil-C_{18} (150 mm × 2.1 mm i.d., 5 μm; Thermo Finnigan, USA) with a 4.0 mm × 2.0 mm i.d. Security-Guard C_{18} (5 μm) guard column (Phenomenex, Torrance, CA, USA). The mobile phase, methanol–acetonitrile–1% formic acid (70:10:20 (v/v/v)), was delivered at a flow rate of 0.3 ml/min. The column temperature was maintained at 25 °C.

Mass spectrometric detection was performed on a Thermo Finnigan TSQ Quantum triple quadrupole mass spectrometer (San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. The ESI source was set at the positive ionization mode. Quantification was performed using selected reaction monitoring (SRM) mode of the transitions of m/z 344 → 215 for clemastine and m/z 256 → 167 for diphenhydramine (IS), respectively, with a scan time of 0.3 s per transition. The MS operating conditions were optimized as following: the spray voltage was 4000 V with a source collision-induced dissociation (CID) voltage of 12 V, the heated capillary were 350 °C. Nitrogen was used as the sheath gas (50 psi) and auxiliary gas (12 psi). Argon was used as the collision gas at a pressure of approximately 1.0 mtorr (1 torr = 133.3 Pa). The optimized collision energies for clemastine and IS were 15 and 10 eV, respectively. Data acquisition was performed by Xcalibur 1.3 software (Thermo Finnigan, USA). Peak integration and calibration were performed using LCQuan software (Thermo Finnigan, USA).

2.3. Preparation of stock solutions

The stock solutions of clemastine (400.0 μg/ml) and IS (400.0 μg/ml) were prepared in methanol and diluted to produce a 1.0 μg/ml stock solution of clemastine in methanol–water (50:50 (v/v)), which was serially diluted to give working solutions of 100.0, 200.0, 800.0, 2000.0, 4000.0, 10000.0, and 20000.0 pg/ml in methanol–water (50:50 (v/v)). IS working solution was also prepared by diluting the 400 μg/ml stock solution of diphenhydramine with methanol–water (50:50 (v/v)) to a concentration of 400 ng/ml. All stock solutions and working solutions were stored at 4 °C.

2.4. Calibration curves

Calibration curves were prepared by spiking 50 μl of the appropriate working solution to 1.0 ml of blank human plasma to produce the calibration curve points equivalent to 5.0, 10.0, 40.0, 100.0, 200.0, 500.0, and 1000.0 pg/ml of clemastine. Each sample also contained 20.0 ng/ml of IS (50 μl × 400 ng/ml). In each batch, a blank plasma sample (without IS) was also analyzed. Calibration curves were prepared by determining the best

fit of the peak area ratios (peak area of analyte/peak area of IS) versus concentration, and fitted to the equation $y = a + bx$ (y represented the ratios of clemastine peak area to that of IS and x represented the plasma concentrations of clemastine) by weighted ($1/x^2$) least squares linear regression.

2.5. Quality control samples

The quality control samples (QCs) used in the validation and during the pharmacokinetic study had been prepared in the same way as the calibration curves. The nominal plasma concentrations of QCs were 10.0 pg/ml (low level), 100.0 pg/ml (middle level), and 1000.0 pg/ml (high level) for clemastine with 20.0 ng/ml of the IS. The calibration curve and quality control plasma samples were extracted on each analytical batch along with the unknown samples.

2.6. Sample preparation

Clinical plasma samples, QC and calibration curve samples were extracted employing a liquid–liquid extraction method. To a 1.0 ml aliquot of plasma sample, 50 μl of internal standard (400.0 ng/ml diphenhydramine), 50 μl of methanol–water (50:50 (v/v)) and 200 μl of 1 M sodium hydroxide solution were added. The mixed samples were briefly mixed and 3 ml of ethyl acetate were added. The mixture was vortex-mixed for approximately 1 min, then shaken on a mechanical shaker for 15 min. After centrifugation at 3000 rpm for 10 min, the organic layer was transferred to another 10 ml centrifuge tube and evaporated to dryness under a gentle stream of air at 40 °C. The dry residue was reconstituted in 200 μl of the mobile phase, then vortex-mixed. A 20 μl aliquot of the final solution was injected onto the LC/MS/MS system.

2.7. Method validation

The method validation assays were carried out according to the currently accepted USA Food and Drug Administration (FDA) bioanalytical method validation guidance [12].

The specificity of the method was tested by analyzing blank plasma samples from six healthy individuals. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/mass spectroscopic conditions and was compared with those obtained from an aqueous solution of the analyte at a concentration near the LLOQ.

As described by Matuszewski et al. [13], the matrix effects (MEs) on the ionization of the analytes were evaluated by comparing the peak areas of the analytes resolved in the blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in the mobile phase. Three different concentration levels of clemastine (10.0, 100.0 and 1000.0 pg/ml) were evaluated by analyzing five samples at each level. The blank plasma used in this study was five different batches of healthy individual's blank plasma. If the ratio is less than 85% or more than 115%, an exogenous matrix effect is implied. The assessment of the relative MEs was made by a direct comparison of the analyte peak area values between different sources of human plasma. The relative ME was expressed as R.S.D.s (%).

Calibration curves of clemastine of seven concentrations ranged from 5.0 to 1000.0 pg/ml were extracted and assayed. Blank plasma samples were analyzed to confirm the absence of interference but were not used to construct the calibration function. The lower limit of quantification (LLOQ), defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, was evaluated by analyzing samples which were prepared in six replicates as following: 50 μl of the standard solution containing 100.0 pg/ml clemastine was spiked to 1.0 ml human blank plasma.

The precision of the assay was determined from the QC plasma samples by replicate analyses of three concentration levels of clemastine (10.0, 100.0 and 1000.0 pg/ml). Intra-day precision and accuracy were determined by repeated analyses of the group ($n = 6$) of standards on 1 day. Inter-day precision and accuracy were determined by repeated analyses on three consecutive days ($n = 6$ series per day). The concentration of each sample was determined using the calibration curve prepared and analyzed on the same day. Accuracy was described as relative error (RE) and precision was described as relative standard deviation (R.S.D.).

For the determination of extraction recovery, blank human plasma was processed according to the sample preparation procedure as described above. The organic layer was evaporated to dryness, and dry extracts were reconstituted in the mobile phase added appropriate standards at concentrations corresponding to the final concentration of the extracted plasma samples. These spike-after-extraction samples represented 100% recovery. The extraction recoveries of clemastine were determined by comparing the mean peak areas of six extracted low (10.0 pg/ml), medium (100.0 pg/ml) and high (1000.0 pg/ml) QC samples to mean peak areas of six spike-after-extract samples at the same concentrations. Recovery of IS was also evaluated by comparing the mean peak areas of six extracted medium QC samples to mean peak areas of six reference solutions spiked in extracted plasma samples of the same concentrations.

- *Freeze and thaw stability*: QC plasma samples at three concentration levels were stored at the storage temperature (−20 °C) for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycles were repeated twice, and the samples were analyzed after three freeze (−20 °C)–thaw (room temperature) cycles.
- *Short-term temperature stability*: QC plasma samples at three concentration levels were kept at room temperature for a period that exceeded the routine preparation time of the samples (around 4 h).
- *Post-freezing stability*: QC plasma samples at three concentration levels stored at low temperature (−20 °C) were studied after 45 days.
- *Post-treatment stability*: The autosampler stability was conducted by reanalyzing extracted QC samples kept under autosampler conditions (16 °C for 24 h).

- *Stock solutions and working solutions stability*: The stability of clemastine and the IS working solutions were evaluated at room temperature for 6 h and at 4 °C for 15 days.

Standard curves in each analytical batch were used to calculate the concentrations of clemastine in the unknown samples in the run. They were prepared along with the unknown samples in the same batch and analyzed in the middle of the run.

The QC samples of clemastine in triplicates at three concentrations (10.0, 100.0 and 1000.0 pg/ml) were prepared and were analyzed along with processed test samples at intervals in each batch.

3. Preliminary application

Two healthy male Chinese volunteers participated in this preliminary pharmacokinetic study. After overnight fasting, each volunteer received an oral dosage tablet (containing 1000 mg paracetamol, 60 mg pseudoephedrine and 0.67 mg clemastine hydrogen fumarate), which were taken with 200 ml water. Blood samples were collected before and 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0, and 72.0 h after dosing. Plasma was separated by centrifugation of the heparinized samples at 3000 rpm for 10 min and was stored at −20 °C until analysis. The volunteers were all adult male Chinese who were selected after completing a thorough medical, biochemical and physical examination. Informed consent was obtained from all subjects after explaining the aims and risks of the study. The study protocol was approved by the Human Investigation Ethical Committee at the School of Pharmaceutical Sciences at Central South University, Changsha, China.

Plasma-concentration data for each individual subject were analyzed by non-compartmental analysis using the TopFit 2.0 software package (Thomae GmbH, Germany). Maximum plasma concentration (C_{max}) and the time-to-maximum concentration (T_{max}) were estimated by visual inspection of semi-logarithmic plots of the concentration–time curves. The area under the curve (AUC_{0-t}) was calculated using the linear-trapezoidal rule, with extrapolation to infinity ($AUC_{0-\infty}$) from the last detectable concentration using the terminal elimination rate constant (k_e) calculated by linear regression of the final log-linear part of the drug concentration–time curve. Apparent elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = 0.693/k_e$.

4. Results and discussion

4.1. Selection of the mass spectrometric conditions

An LC/MS/MS method for the determination of clemastine in human plasma was investigated. Firstly, the possibility of using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) source under positive ion detection mode was evaluated during the early stage of assay development. Results showed that ESI could offer higher sensitivity for the analytes than APCI. Consequently, ESI was chosen as the source for further study.

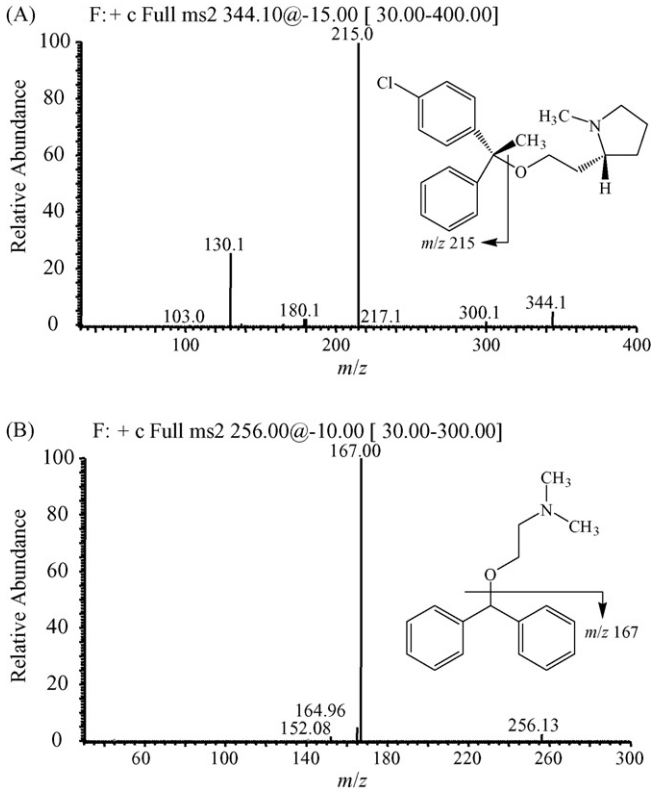

Fig. 2. Product ion mass spectra of $[M+H]^+$ ions of (A) clemastine and (B) diphenhydramine (internal standard).

In the mass spectra of clemastine and IS, clemastine and IS were formed predominantly protonated molecule $[M+H]^+$ at *m/z* 344 and *m/z* 256, respectively. The product ion spectra of clemastine and IS can be seen in Fig. 2. Fig. 2 showed that, after collision-induced dissociation, the major fragment ions observed in each product ion spectrum were at *m/z* 215 and 167 for clemastine and IS, respectively, so the SRM transitions *m/z* 344 → 215 and *m/z* 256 → 167 were selected to analysis clemastine and IS, respectively. To obtain maximum sensitivity of the SRM transition *m/z* 344 → 215 under the developed mobile phase conditions, some parameters such as spray voltage, capillary temperature, source CID, sheath gas (nitrogen) pressure, auxiliary gas (nitrogen) pressure, collision gas (argon) pressure, and collision energy were all optimized. After optimization, parameters were chosen as following: the spray voltage: 4000 V; the source CID voltage: 12 eV; the heated capillary temperature: 350 °C; the sheath gas (nitrogen): 50 psi; the auxiliary gas (nitrogen): 12 psi; the collision gas (argon) pressure: 1.2 mtorr (1 torr = 133.3 Pa); the collision energy: 15 eV for clemastine and 10 eV for IS. The other MS parameters were adopted from the recommended values for the instrument.

4.2. Selection of the liquid chromatographic conditions

The mobile phase consisted of 70% methanol provided low background noise and rapid separation. To achieve symmetrical peak shapes, 10% acetonitrile was added to the mobile phase. Under the present chromatographic conditions

(methanol–acetonitrile–1% formic acid = 70:10:20 (v/v/v)), the running time of each sample was only 4.0 min. The retention times were 3.2 and 2.2 min for clemastine and IS, respectively.

4.3. Selection of IS

It is necessary to use an IS to get high accuracy when a mass spectrometer is used as the HPLC detector. Using deuterated clemastine as an internal standard, Horvatha et al. had developed a HPLC–MS–MS method for the determination of clemastine in human plasma with an LLOQ of 10 pg/ml [8]. In this present paper, diphenhydramine was chosen as internal standard because of the similarity of its retention time, ionization and extraction efficiency with the clemastine, as well as the less endogenous interference at the transition *m/z* 256 → 167. At the same time, in many methods which were used to analysis drugs in biological fluids by LC–MS–MS [9–11], diphenhydramine was chosen as internal standard with acceptable repeatability.

4.4. Sample preparation

Liquid–liquid extraction was important because this technique cannot only purify but also concentrate the sample. Ethyl acetate, *n*-hexane–dichloromethane–isopropanol (300:150:15 (v/v/v)), diethyl ether–dichloromethane (3:2 (v/v)), and diethyl ether were all attempted. Meanwhile, three kinds of aqueous pH modifiers (500 μl of 0.5 M Na_2CO_3, 200 μl of 1.0 M NaOH, and 500 μl 10 mM phosphate buffer with pH 14) were evaluated. Ethyl acetate and 200 μl of 1.0 M NaOH were finally adopted because of its high extraction efficiency.

4.5. Matrix effects

The absolute MEs and the relative MEs (expressed as R.S.D.s, %) at QC concentrations of clemastine in five different sources of human plasma are presented in Table 1. For clemastine, the absolute MEs were measured to be between 92.7% and 96.7%. These observations indicated that endogenous substances slightly suppressed the ionization of clemastine under the present chromatographic and extraction conditions when the ESI interface was employed. Moreover, the absolute MEs observed were similar over the QC concentration (10.0, 100.0 and 1000.0 pg/ml), without showing any analyte concentration-dependence for different sources of human plasma. The variability of the R.S.D.s of absolute MEs at different concentrations of clemastine in five different sources of human plasma was acceptable, with R.S.D. values below 13.0% at the QC concentration of clemastine, indicating that the relative MEs for the analyte were minimal in this study. Meanwhile, the absolute and relative MEs values of IS at the concentration of 20.0 ng/ml in plasma were observed to be 95.1% and 10.3%, respectively.

Table 1
Matrix effects data for clemastine at 10.0, 100.0 and 1000.0 pg/ml in five different sources of human plasma ($n = 5$)

Nominal concentration (pg/ml)	Matrix effect (%) (mean ± S.D.)	R.S.D. (%)
10.0	92.7 ± 14.3	13.0
100.0	96.7 ± 6.9	6.7
1000.0	93.4 ± 9.3	9.6

4.6. Method validation

Assay specificity was assessed by comparing the chromatograms of six different sources of blank human plasma with the corresponding spiked plasma. Fig. 3 shows the typical chromatograms of a blank, a spiked plasma sample with clemastine (5.0 pg/ml, LLOQ) and IS (20.0 ng/ml), and a plasma sample from a healthy volunteer 24 h after an oral dosage tablets (containing 1000 mg paracetamol, 60 mg pseudoephedrine and

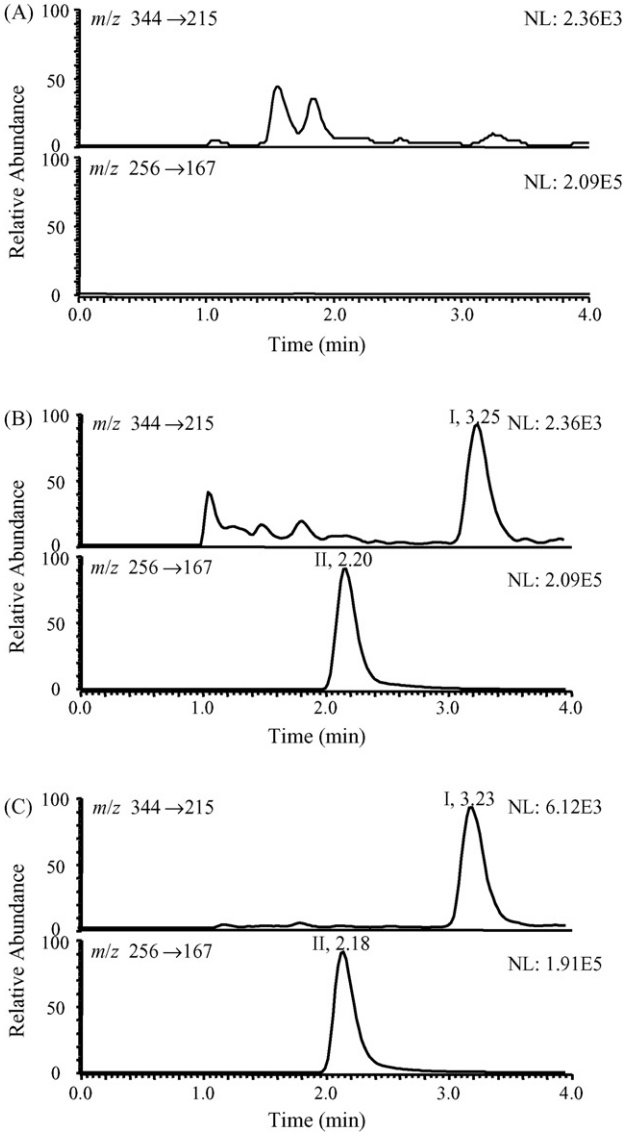

Fig. 3. Representative SRM chromatograms for clemastine in human plasma: (A) a blank plasma sample; (B) a blank plasma sample spiked with clemastine at the LLOQ of 5.0 pg/ml; (C) volunteer A plasma sample 24 h after an oral dosage tablets (containing 1000 mg paracetamol, 60 mg pseudoephedrine and 0.67 mg clemastine hydrogen fumarate). I: clemastine; II: diphenhydramine (internal standard).

Table 2
Accuracy and precision for clemastine at the plasma concentrations of LLOQ

Nominal plasma concentration (pg/ml)	Mean measured concentration (pg/ml)	Relative error (%)	Intra-day R.S.D. (%)
5.0	4.5	−6.2	12.8
	4.5		
	4.7		
	4.0		
	4.6		
	5.8		

Table 3
Intra- and inter-day precision and accuracy data for assays of clemastine in human plasma (3 days, six replicates per day)

Nominal plasma concentration (pg/ml)	Mean measured concentration (pg/ml)	Relative error (%)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
10.0	10.4	3.7	8.7	13.4
100.0	98.9	−1.1	7.1	8.6
1000.0	1055.8	5.6	7.4	3.4

0.67 mg clemastine hydrogen fumarate). As shown in Fig. 3, no significant peaks interfering with analyte were observed in the drug-free human plasma. The retention times for clemastine and IS were 3.2 and 2.2 min, respectively. Because of the high selectivity of the SRM mode, the determination of clemastine was not be interfered by paracetamol and pseudoephedrine, which were co-administration with clemastine.

The linear regressions of the peak area ratios versus concentrations were fitted over the range 5.0–1000.0 pg/ml for clemastine in human plasma. Typical equation of the calibration curve using weighted ($1/x^2$) least squares linear regression was as following: $y = 1.685 \times 10^{-3} + 3.771 \times 10^{-3}x$, $r^2 = 0.9943$, where y and x are defined as above.

The LLOQ was established at 5.0 pg/ml of clemastine, which was sufficient for the clinical pharmacokinetic studies following an oral administration of a compound tablets. The precision and accuracy values corresponding to LLOQ are shown in Table 2. Those values were below 20%.

Table 4
The extraction recovery for clemastine and diphenhydramine (internal standard) in human plasma ($n = 6$)

Compound	Nominal concentration (pg/ml)	Recovery (%) (mean ± S.D.)	R.S.D. (%)
Clemastine	10.0	65.7 ± 3.9	5.9
	100.0	63.9 ± 6.2	6.3
	1000.0	62.3 ± 5.5	8.8
Diphenhydramine (IS)	20000.0	77.5 ± 1.1	1.4

Precision and accuracy of the assay were determined by replicate analyses ($n = 6$) of QC samples at three concentrations, by performing the complete analytical runs on the same day and also on three consecutive days. The data from these QC samples were examined by a one-way analysis of variance (ANOVA). The intra-day and inter-day precisions were less than 13.4% for each QC level of clemastine. The bias, determined from QC samples, was within ±5.6% for each QC level. The results are summarized in Table 3.

The extraction recoveries determined for clemastine were shown to be consistent, precise and repeatable. Data are shown in Table 4. The extraction recovery of IS was 77.5 ± 1.1% at the plasma concentration of 20.0 ng/ml.

The stability of clemastine in human plasma was investigated under a variety of storage and process conditions such as the freeze and thaw stability, short-term temperature stability, post-freezing stability, and post-treatment stability. Table 5 summarizes these stability data. All the results showed good stability during these tests and there were no stability related problems during the routine analysis of samples for pharmacokinetic study. The stability of the stock solutions and working solutions were stable for 6 h at room temperature and stable for 15 days at 4 °C.

4.7. Preliminary application

This fully validated LC/ESI-MS/MS method was used to study the preliminary pharmacokinetic study of clemastine in

Table 5
Stability of clemastine under various storage conditions ($n = 3$)

Storage condition	Concentration (pg/ml)		S.D.	R.S.D. (%)	Relative error (%)
	Added	Found (mean)			
Three freeze-thaw cycles	10.0	9.9	0.5	4.6	−1.1
	100.0	106.7	0.9	0.9	6.7
	1000.0	1116.6	47.7	4.3	11.7
Post-freezing (−20 °C) for 45 days	10.0	9.8	1.2	12.6	−1.9
	100.0	96.2	4.7	4.9	−3.8
	1000.0	964.2	101.2	10.5	−3.6
Post-treatment for 24 h (16 °C)	10.0	10.6	1.5	14.2	6.4
	100.0	102.8	9.1	8.8	2.8
	1000.0	1064.2	101.2	9.5	6.4
Benchtop for 4 h (room temperature)	10.0	10.9	0.8	7.6	9.3
	100.0	97.6	5.0	5.1	−2.4
	1000.0	1102.0	73.4	6.7	10.2

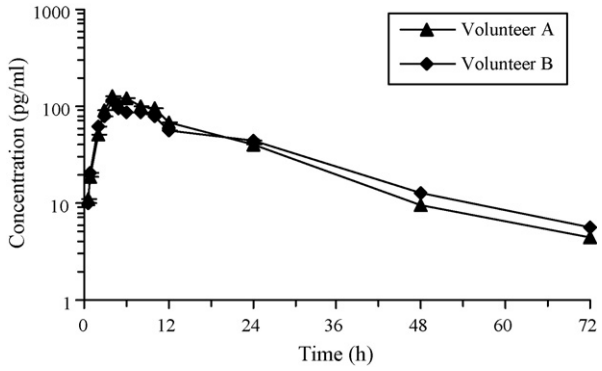

Fig. 4. Plasma concentration–time curves of clemastine after an oral dosage tablets (containing 1000 mg paracetamol, 60 mg pseudoephedrine and 0.67 mg clemastine hydrogen fumarate).

Table 6
The main pharmocokinetic parameters of clemastine after an oral dosage tablets (containing 1000 mg paracetamol, 60 mg pseudoephedrine and 0.67 mg clemastine hydrogen fumarate)

Parameter	Volunteer A	Volunteer B
C_{max} (pg/ml)	129.4	115.7
T_{max} (h)	4.0	4.0
$t_{1/2}$ (h)	14.8	18.0
k_e (1/h)	0.047	0.041
AUC_{0-t} (pg h/ml)	2419.8	2383.3
$AUC_{0-\infty}$ (pg h/ml)	2513.9	2518.4

human after an oral administration of a compound tablets (containing 1000 mg paracetamol, 60 mg pseudoephedrine and 0.67 mg clemastine hydrogen fumarate). Profile of the plasma concentration of clemastine versus time is shown in Fig. 4. The main pharmacokinetic parameters of clemastine are presented in Table 6.

5. Conclusions

An LC/ESI-MS/MS method was developed and fully validated for the determination of clemastine in human plasma. The sample pretreatment was a single-step liquid–liquid extraction procedure. Full validation following FDA guideline indicated that the developed method had high sensitivity with an LLOQ of 5.0 pg/ml, reliability, specificity and excellent efficiency with a total running time of 4.0 min per sample, which is important for large batches of samples. The sensitive, simple and rapid LC/MS/MS assay is suitable for pharmacokinetic, bioavailability or bioequivalence studies of clemastine in human subjects.

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