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Quantification of meclizine in human plasma by high performance liquid chromatography–mass spectrometry

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ABSTRACT

Purpose: Meclizine is an antihistamine and has been widely used for prophylactic treatment of motion sickness. To facilitate its pharmacokinetic study in human subjects, a high performance liquid chromatography–mass spectrometric method employing positive electrospray ionization was developed for the determination of meclizine concentration in human plasma.
Methods: Meclizine together with the internal standard (flunarizine) was extracted from 0.1 ml of human plasma by protein precipitation using acetonitrile. The chromatography was performed using a Zorbax SB-C18 column (150 × 2.1 mm, 5 μm, Agilent) with the mobile phase consisting of acetonitrile and 0.2% formic acid containing 2 mM amino acetate. Multiple reaction monitoring was used for quantification. The validation of the method including sensitivity, linearity, reproducibility and stability was examined.
Results: The lower limit of quantification (LLOQ) of the developed assay method for meclizine was 0.5 ng/ml and the linear calibration curve was acquired with $R^2 > 0.99$ between 0.5 and 200 ng/ml. The intra-day and inter-day variation of the current assay was evaluated with the coefficient of variations (CVs%) within 12.92% at LLOQ and 7.15% for other quality control samples, whereas the mean accuracy ranged from 99.2% to 102.7%. The samples were stable under the storage conditions at least for a month.
Conclusion: The present method provides a robust, fast and sensitive analytical tool for meclizine in human plasma and has been successfully applied to a clinical pharmacokinetic study in 20 subjects.

1. Introduction

Meclizine is an antihistamine which possesses marked protective activity against nebulized histamine and lethal doses of intravenously injected histamine in guinea pigs (Prod Info ANTIVERT®) [1,2]. It has a marked effect in blocking the vasodepressor response to histamine, but only a slight blocking action against acetylcholine. In one anecdotal report, serum levels of 10 ng/ml were reported after 12 h following an oral dose of 75 mg [3], and the elimination half-life of the parent compound was 6 h (USPID, 1994). Although metabolic study of meclizine in rat has been investigated long time ago [4], there was no clinical pharmacokinetic study of meclizine published. For detection of meclizine in human plasma, very few analytical methods were reported. A gas chromatography–mass spectrometry method was developed in 1977, but it was not well validated and the sensitivity was low and not suitable for the clinical pharmacokinetic study [5]. Other assay methods, such as HPLC–UV and capillary zone electrophoresis, were also developed and validated to detect meclizine in pharmaceutical preparation as well as in bio-samples, but the sensitivity is too low with LLOQ more than 500 ng/ml [6–8]. To facilitate clinical pharmacokinetic study of meclizine, a sensitive and specific high performance liquid chromatography–mass spectrometric method was developed and validated in terms of the accuracy, precision, reproducibility, and recovery as well. This method has also been successfully applied to a clinical pharmacokinetic study in 20 healthy subjects.

2. Materials and methods

2.1. Materials

The assay standards of meclizine and flunarizine were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) with purity of better than 99%. The analytical grade formic acid, amino acetate, and HPLC-grade methanol and acetonitrile were got from Fisher Scientific (Pittsburgh, PA, USA). The deionized water was prepared using a Barnstead Nanopure Diamond™ water purification system (APS Water Services Corporation, Van Nuys, CA, USA) and used throughout the study. Unless specified elsewhere, all reagents were used without further purification.

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The LC/MS/MS system consisted of an API 3200 LC/MS/MS system (Applied Biosystems, Foster City, CA, USA) and two Shimadzu LC-20AD Prominence Liquid Chromatograph pumps equipped with an SIL-20A Prominence autosampler (Shimadzu, Columbia, MD, USA). Chromatography was carried out using a Zorbax SB C_{18} column (150 × 2.1 mm, 5 μm, Zorbax, Agilent, Santa Clara, CA, USA) which was proceeded with a SB-C_{18} Guard Cartridges (12.5 × 2.1 mm, Zorbax, Agilent, Santa Clara, CA, USA).

2.2. Sample preparation

The stock solutions (1 mg/ml) of meclizine and flunarizine (internal standard, IS) were prepared by dissolving appropriate amount of corresponding compounds in methanol. All the stock solutions were stored at −20 °C and were stable for at least 6 months. The working solution of meclizine was prepared by diluting the stock solution with 50% methanol to yield eight concentrations over a range of 5–2000 ng/ml (5, 10, 100, 200, 1000, 2000 ng/ml for calibration samples and 5, 50, 800 ng/ml for quality control samples). The working solution (1 μg/ml) of flunarizine was also prepared by diluting the stock solution using 50% methanol.

The standard calibration samples were prepared by spiking 100 μl of the blank human plasma with 10 μl of working solutions mentioned above to yield six different concentrations, i.e. 0.5, 1, 10, 20, 100, 200 ng/ml. Quality control (QC) samples were prepared by adding 10 μl of QC working solutions to 100 μl of blank plasma. For the clinical plasma samples, 10 μl of 50% methanol was added instead of the working solutions.

An aliquot of 10 μl of flurnarizine working solution was added to all these samples. After mixing, 300 μl acetonitrile containing 2 mM ammonia acetate and 0.2% formic acid was added. The samples were then mixed using a vortex mixer for 1 min followed by centrifuging at 10,000 rpm for 6 min. The upper clear solution was transferred into a clean auto-sampler vial, and 10 μl was injected into HPLC–MS/MS system for analysis.

2.3. Liquid chromatography and mass spectrometry

A linear gradient elution was carried out using a mobile phase containing acetonitrile (A) and 0.2% formic acid containing 2 mM ammonia acetate (B) with the flow rate of 0.35 ml/min. The gradient began with 30% eluent A and 70% eluent B for 3 min and was changed linearly to 20% eluent B in 2 min and remained at 20% eluent B for 3 min. The gradient was then changed back to 30% eluent A in 0.5 min and kept at this percentage for 2 min. The temperatures of analytical column and autosampler were both set at room temperature.

All the liquid chromatographic eluent was then introduced into the ESI source. Typical mass spectrometric conditions were: gas 1, nitrogen (30 psi); gas 2, nitrogen (40 psi); ion spray voltage, 5500 V; ion source temperature, 550 °C; curtain gas, nitrogen (25 psi). Multiple reaction monitoring (MRM) scanning in positive ionization mode was used to monitor the transition of m/z 391–201 for meclizine and 405–203 for flurnarizine.

2.4. Validation of the assay method

The validity of the assay method was assessed according to FDA guidance [9], with regard to the linearity, sensitivity, precision, accuracy, recovery, and stability. The QC samples at three concentrations (low, medium and high) were utilized and analyzed for these tests. For construction of the standard calibration curve, blank plasma meclizine samples were spiked in triplicates at concentrations of 0.5, 1, 10, 20, 100, 200 ng/ml. The intra- and inter-day precision and accuracy of the method were evaluated using QC samples at concentrations of 0.5, 5 and 80 ng/ml.

2.5. Linearity and sensitivity

Calibration standards were prepared and analyzed in triplicate samples at each concentration. Calibration curve was constructed using the analyte/IS peak area ratio versus the analyte's nominal concentration, and fitted by linear least-squares regression analysis with weighting factor of 1, $1/x$ or $1/x^2$ (x is the value of the nominal concentration). Sensitivity of the method was evaluated in terms of the lower limit of quantification (LLOQ). LLOQ was determined based on the two criteria: (1) the analyte response at the LLOQ should be at least 5 times the response compared to blank response and (2) analyte peak (response) should be identifiable, discrete, and reproducible with a precision within 20% and accuracy of 80–120%.

2.6. Precision and accuracy

QC samples of five replicates were analyzed on the same day to determine the intra-day precision and accuracy, while on separate 3 days triplicate QC samples were analyzed to determine the inter-day precision and accuracy. Precision was calculated as the relative standard deviation (RSD), whereas accuracy was assessed as the percentage to the nominal concentration (%). The intra- and inter-day precision was set at <20% for QC samples at LLOQ concentration or 15% for other QC samples from nominal concentration.

2.7. Recovery

Extraction recovery of meclizine and flunarizine were assessed by comparing the peak areas of the extracted QC samples to the unextracted standard solutions containing equivalent amount of the analytes. Briefly, 300 μl acetonitrile containing 2 mM ammonia acetate and 0.2% formic acid was added to 100 μl of blank plasma and vortexed for 1 min. Afterwards, 10 μl of meclizine or flunarizine QC working solutions with 10 μl methanol were added and mixed well (post-extracted QC samples). After centrifuging for 5 min, the samples were analyzed. The peak area, representing 100% recovery, was compared with that from the extracted QC samples.

2.8. Matrix effect

To determine the matrix effect, six different blank plasma samples were utilized to prepare the QC samples at both low and high concentrations and used for assessing the lot-to-lot matrix effect. The samples were analyzed in triplicate and the relative standard deviation (RSD) of the peak area ratio of each analyte versus IS among the six lots was calculated as an indicator of the inter-lot matrix variation.

Since the endogenous substance may have the potential ion suppressing or enhancing effect on meclizine, the absolute matrix effect was also evaluated [10]. The QC samples at both low and high concentrations were prepared using deionized water instead of plasma. The peak areas were compared between the extracted samples with the post-extracted QC samples mentioned above.

2.9. Stability

The stability of meclizine in terms of storage stability, freeze/thaw stability, injector stability and handling stability was evaluated during the sample collection and handling, after long-term and short-term storage, freeze and thaw cycles, and after the analytical process. For storage stability, the QC samples were prepared and stored at −80 °C for 30 days. The samples were then processed and analyzed together with the freshly prepared

samples. For freeze–thaw stability assessment, QC samples were exposed to three freeze (−80 °C) and thaw (23 °C) for three cycles and then analyzed along with the freshly prepared samples. For injector stability, the prepared samples in the auto-sampler were evaluated by re-analyzing the samples after being placed in the auto-sampler for 12 h at room temperature. For the stability during the handling process, the QC samples were prepared and kept at room temperature for 4 h and then analyzed along with the freshly prepared samples. All these stability tests were performed on 3 QC concentrations with triplicate samples. The percent deviation in concentration was used as an indicator of stability. The analyte was considered to be stable when the percent deviation was within ±15% of the nominal concentration.

2.10. Application to the pharmacokinetic study

The assay was applied to a clinical pharmacokinetic study of the meclizine carried out in 20 healthy subjects of different ethnic populations including Chinese, Indian, American Caucasian, and Middle East population. Each subject received 25 mg of meclizine (Motion Sickness II, Bath No. R758, expire date July-2009, CVS pharmacy) along with approximately 240 ml of water after fasting for at least 12 h. The multiple blood samples were collected into the heparinized tubes at 0, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720 and 1440 min post-dose. After being centrifuged at 4000 rpm at 4 °C for 15 min, the supernatant was removed and stored at −80 °C until assay. The pharmacokinetic including AUC_{0-24}, T_{max}, C_{max}, $T_{1/2}$ was obtained from the concentration time curve or calculated using non-compartmental method. The study protocol and statement of informed consent were approved by the Institutional Review Board, Western University of Health Sciences. All volunteers were fully advised of the nature, purpose, procedures and possible risks of this study by a member of the study team. An acknowledgement of the receipt of this information and the participant's freely-tendered offer to volunteer were obtained by signing the informed consent form before participating in the study. The subjects were all non-smokers, 18–40 years old and within 25% of ideal weight. They were all in good health based on medical history, physical examination, ECG evaluation and routine laboratory tests including blood and urine analysis. A negative pregnancy test and not lactating were required for females. They were not allowed to consume caffeinated beverages, alcohol during each treatment period (24 h long).

3. Results and discussion

3.1. Mass spectrometry and chromatography

The full Q1 scan of meclizine and flunarizine was acquired in positive ion mode by infusing the standard solutions at concentration of 1 μg/ml in 0.2% formic acid into ESI source. The product ion mass spectra of these two compounds are shown in Fig. 1. The most abundant product ion of each analyte was selected from MRM monitoring, and the MS/MS conditions were optimized to maximize the response of each of precursor/product transition (see Table 1).

3.2. Chromatography and specificity

Representative chromatograms of blank, the QC samples of meclizine at concentrations of 0.5, 5, 80 ng/ml and flunarizine at

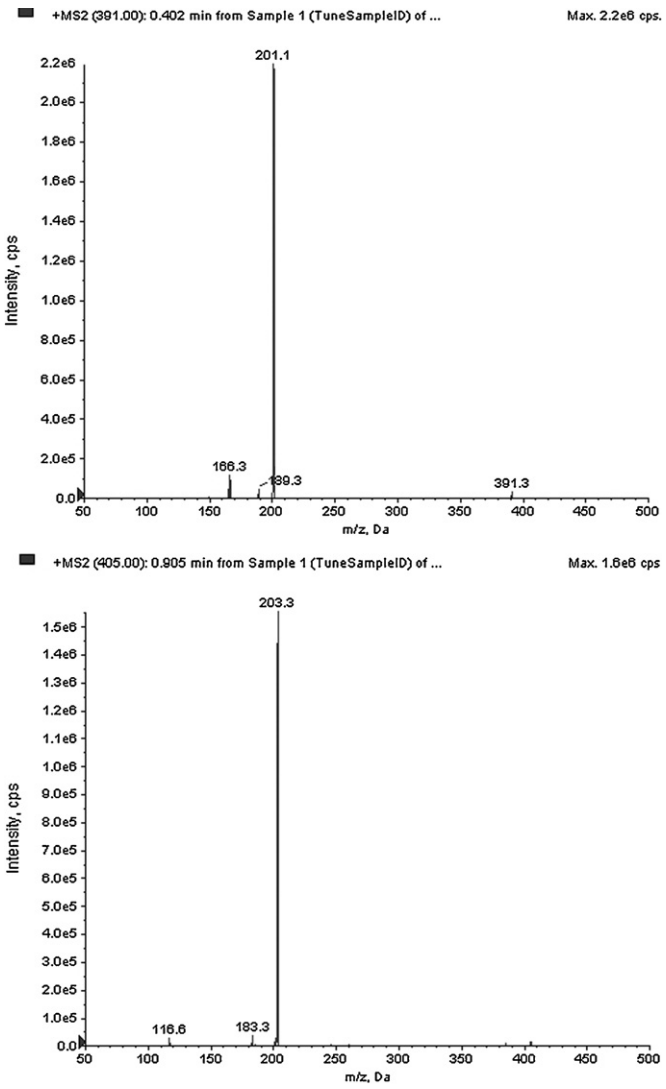

Fig. 1. Product ion mass spectra of (a) meclizine and (b) flunarizine.

200 ng/ml are shown in Fig. 2(a)–(e). Fig. 2(f) shows the representative chromatogram of the plasma samples obtained from a study subject at 2 h following an oral 25 mg of meclizine tablet. The typical retention time was 8.7 min for meclizine or 8.3 min for flunarizine. These results suggested that meclizine at high and low concentrations can be clearly detected. No interfering peaks at the retention time of meclizine and the IS were observed for all the six plasma samples from different subjects, which suggested there was a lack of interference observed (from the endogenous components in plasma) at the corresponding peaks of meclizine and flunarizine.

3.3. Linearity, sensitivity, precision and accuracy

The calibration curve of meclizine was linear over the concentration range of 0.5–200 ng/ml. The correlation coefficient

Table 1
MS conditions for meclizine and flunarizine (IS).

Compounds	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	DP (V)	EP (V)	CE (V)	CEP (V)	CXP (V)
Meclizine	391.3 $[M+H]^+$	201.2	41	8	16	23	4
Flunarizine	405.1 $[M+H]^+$	203.2	41	5	18	25	4

DP, declustering potential; EP, entrance potential; CEP, collision cell entrance potential; CE, collision energy; CXP, collision cell exit potential.

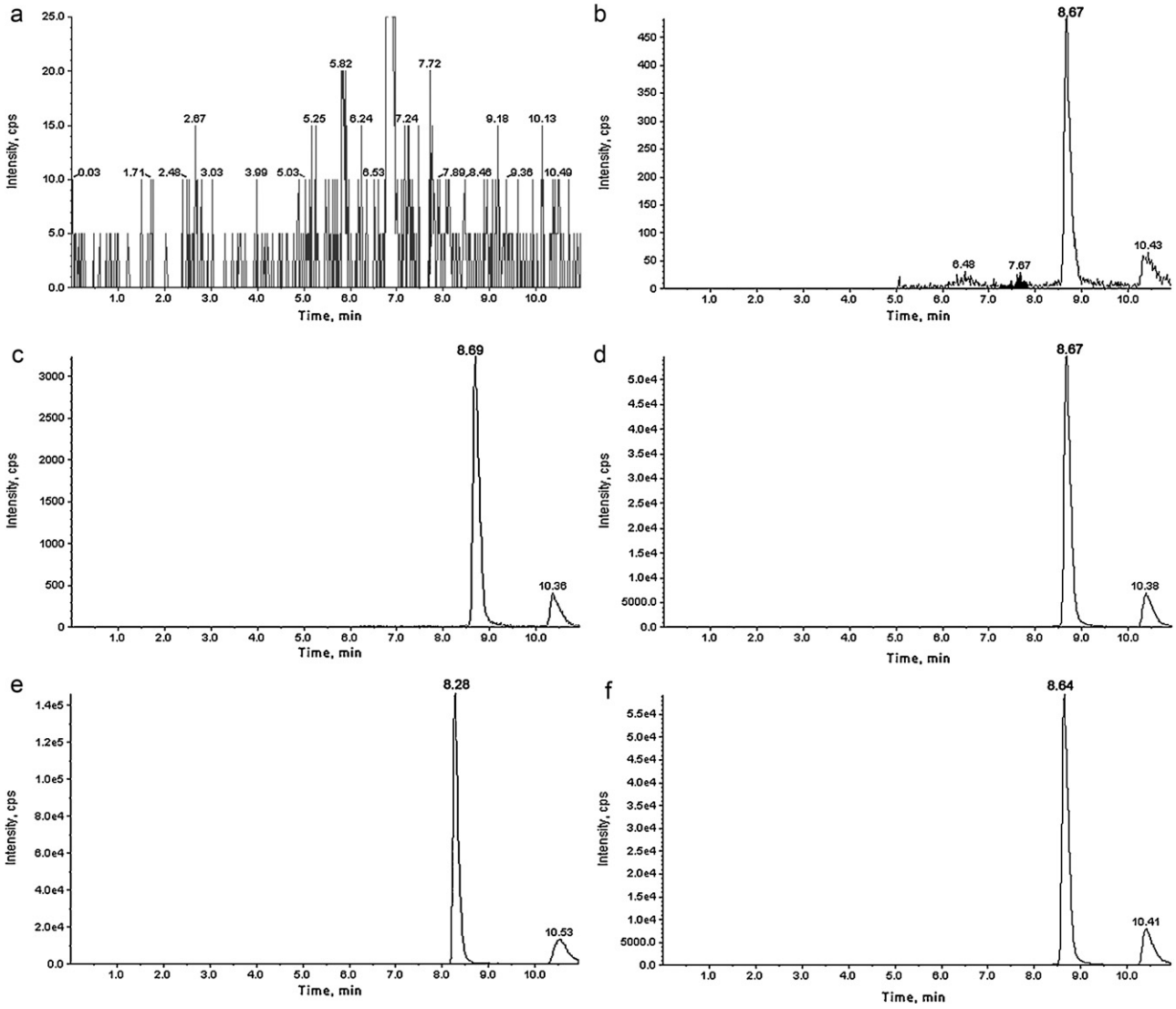

Fig. 2. MRM chromatogram of (a) blank plasma, (b) 0.5 ng/ml meclizine, (c) 5 ng/ml meclizine, (d) 80 ng/ml meclizine, (e) 200 ng/ml flunarizine (IS) and (f) real plasma sample obtained from a subject at 2 h post dosing (concentration determined was 80.3 ng/ml).

(r^2) was greater than 0.99 with different runs. The best-fit line of the calibration curve was obtained by using a weighting factor of $1/x^2$. The mean linear regression equations ($n = 3$) were: $Y = 0.009276\ (\pm 0.000931) \times -0.00089\ (\pm 0.001383)$, ($r = 0.998 \pm 0.001$). The LLOD and LLOQ were 0.1 ng/ml and 0.5 ng/ml respectively.

The precision and accuracy of the assay method are summarized in Table 2. For QC samples at concentrations of 0.5, 5, and 80 ng/ml, the intra-day coefficient of variations (CVs) ranged from 3.0 to 12.9%, while the inter-day coefficient of variations ranged from 5.1 to 8.3%. The accuracy, presented as percent deviation from the nominal concentrations, ranged from 98.2 to 103.0%. These results suggested that this method is accurate, precise and reproducible for detecting meclizine in human plasma.

Table 2
Precision and accuracy for determination of meclizine in human plasma.

Nominal conc. (ng/ml)	Intra-day ($n = 5$)		Inter-day ($n = 3$)	
	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
0.5	102.4	12.9	101.3	8.3
5	99.5	3.0	103.0	6.2
80	104.0	3.0	98.2	5.1

3.4. Recovery and matrix effect

This method yielded a recovery of 86.8–113.7% for QC samples which suggested that recovery rate was consistent over the calibration ranges. In this study, the presence of plasma enhanced the ion signal by average of 159% and these results were consistent in all the six human plasma. These results suggested that the endogenous substance in plasma can enhance the ion intensity of meclizine and thus increase the sensitivity (see Table 3).

3.5. Stability

The meclizine was found to be stable under the storage conditions (−80 °C) for at least 30 days and room temperature for 6 h

Table 3
Recovery of meclizine and flunarizine in plasma.

	Conc. (ng/ml)	% recovery ($n = 3$)	% matrix effect ($n = 3$)
Meclizine	0.5	113.7 ± 8.3	156.7 ± 18.7
	5	86.8 ± 8.6	173.2 ± 12.4
	80	104.1 ± 18.3	146.7 ± 9.4
Flunarizine	100	101.6 ± 13.0	123.5 ± 14.5

Table 4
Stability of meclizine under different conditions.

Time	Nominal conc. (ng/ml)	Measured conc. (ng/ml)			
		Sample #1	Sample #2	Sample #3	MeanSD
0 h	0.5	0.49	0.56	0.51	0.520.04
	5	4.85	4.58	4.51	4.640.18
	80	85.73	82.84	83.95	84.171.46
12 h in autosampler at room temperature	0.5	0.43	0.53	0.49	0.490.05
	5	4.80	4.68	4.61	4.700.09
	80	88.50	83.86	86.10	86.152.32
4 h at room temperature	0.5	0.49	0.43	0.53	0.480.05
	5	4.32	4.68	4.20	4.400.25
	80	93.09	90.75	92.12	91.991.17
24 h at −80 °C then exposed to 3 freeze and thaw cycles	0.5	0.47	0.50	0.40	0.460.05
	5	4.85	5.02	5.35	5.070.25
	80	89.35	85.94	89.51	88.272.02
1 month	0.5	0.47	0.42	0.51	0.470.05
	5	5.30	4.47	4.14	4.640.60
	80	77.16	81.75	95.06	84.669.30

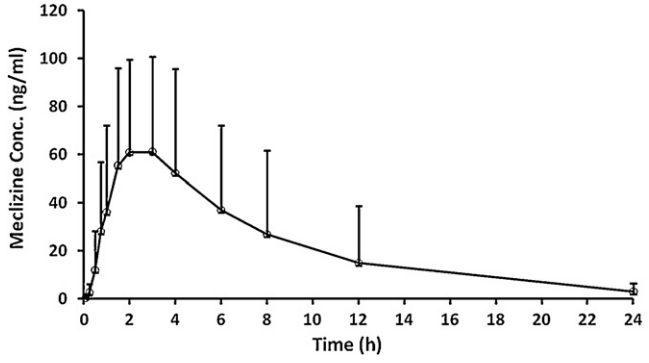

Fig. 3. Mean plasma concentration–time course in 20 subjects following an oral dose of 25 mg of meclizine tablet (Motion Sickness II®).

which is long enough to cover the whole study duration. Upon three freeze/thaw cycles, almost no difference was observed in the peak areas of QC samples in comparison to the freshly prepared samples. No significant degradation of meclizine and IS was observed when the extracted samples were kept in the autosampler at room temperature from 12 h (see Table 4).

Table 5
Mean pharmacokinetic parameters of meclizine in 20 subjects following an oral dose of 25 mg meclizine tablet (Motion Sickness II).

Parameters	Mean	Standard deviation
T_{max} (h)	3.1	1.4
C_{max} (h)	80.1	51.9
AUC_{0-24}(ng h/ml)	544.3	511.6
$T_{1/2}$ (h)	5.2	0.8

C_{max}, peak drug concentration, obtained directly from the original concentration–time data; T_{max}, time to peak drug concentration, obtained directly from the original concentration–time data; AUC_{0-24}, area under the concentration–time curve from time zero to the last sampling time 24 h, calculated using log linear trapezoidal rule; $T_{1/2}$, terminal elimination half-life, calculated as $0.693/\lambda z$.

3.6. Pharmacokinetic results

The mean plasma concentration–time course from 20 subjects is shown in Fig. 3 after receiving an oral dose of Motion Sickness II tablet (containing 25 mg of meclizine). The corresponding pharmacokinetic parameters of meclizine are summarized in Table 5.

4. Conclusion

The present LC–MS/MS assay method provides a robust, fast and sensitive analytical tool for meclizine in human plasma and has been successfully applied to a clinical pharmacokinetic study in 20 subjects.

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