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Simultaneous determination of methylephedrine and noscapine in human plasma by liquid chromatography–tandem mass spectrometry

Lin Zhu, Xiaoyan Chen, Yifan Zhang, Hualing Yu, Dafang Zhong *

Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China

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Abstract

A selective and sensitive method has been developed and validated for simultaneous quantification of methylephedrine and noscapine in human plasma. Analytes were extracted from human plasma samples by liquid–liquid extraction, separated on a Diamonsil C_{18} column and detected by tandem mass spectrometer with an atmospheric pressure chemical ionization (APCI) interface. Diphenhydramine was used as the internal standard (I.S.). The method was found to be precise and accurate within the linear range 0.1–100 ng/ml for each analyte. The intra- and inter-day relative standard deviations (R.S.D.s) were below 5.2% for methylephedrine and 6.7% for noscapine. The inter-day relative error (RE) as determined from quality control samples (QCs) was less than 3.0% for each analyte. The assay was successfully employed in a pharmacokinetic study after an oral administration of a multicomponent formulation containing 20 mg DL-methylephedrine hydrochloride, 16 mg noscapine, 300 mg paracetamol and 1 mg of chlorpheniramine maleate.

Keywords: Methylephedrine; Noscapine

1. Introduction

Methylephedrine is a sympathominetic agent with similar action to ephedrine, but has less pressor activity and central nervous system effect [1]. Noscapine is an opium alkaloid which was widely used in pharmaceutical preparations for its antitussive effect [2]. Methylephedrine and noscapine are often given by mouth in combination preparations for the relief of cough and cold symptoms. Therefore, simultaneous quantification of these two drugs in human plasma is desired for pharmacokinetic studies.

Several methods have been described for the assay of methylephedrine in plasma samples, such as gas chromatography with flame-ionization detection [3] or nitrogen–phosphorus detection [4], reversed-phase high performance liquid chromatography (HPLC) with ultraviolet (UV) detection [1]. Recently, a liquid chromatographic tandem mass spectrometric (LC/MS/MS) method [5] was reported to determine methylephedrine in human plasma, which provided a lower limit of quantification (LLOQ) of 5 ng/ml. The plasma levels of noscapine have been determined by either normal-phase or reversed-phase HPLC with UV detection [6–10]. These methods required tedious sample preparations and a relatively long chromatographic run time (>7 min for one sample). The methods reported were mostly used in the pharmacokinetic studies following single oral doses of 100 mg or 200 mg noscapine [7,8], while the dose of noscapine in the multicomponent formulation was only 16 mg. Due to the low dose of noscapine in the multicomponent formulation, a more sensitive method is needed for determination of noscapine plasma levels. To date, no analytical methods have been reported for simultaneous determination of methylephedrine and noscapine in human plasma.

This paper presents a highly sensitive and selective LC/MS/MS method suitable for the simultaneous determination of methylephedrine and noscapine in human plasma. This assay was successfully applied to a pharmacokinetic study of multicomponent formulation containing methylephedrine and noscapine.

* Corresponding author. Tel.: +86 24 239 025 39; fax: +86 24 239 025 39.
E-mail address: zhongdf@china.com (D. Zhong).

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2. Experimental

2.1. Materials

DL-Methylephedrine hydrochloride, noscapine and diphenhydramine hydrochloride (internal standard, I.S.) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was of HPLC grade, and other chemicals used were of analytical grade. Blank (drug free) human plasma was obtained from Shenyang Blood Donor Service (China). Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Instrumentation

A Shimadzu LC-10ADvp pump (Kyoto, Japan) and a Agilent 1100 autosampler (Wilmington, DE, USA) were used for solvent and sample delivery. A Thermo-Finnigan TSQ (API II) triple quadrupole mass spectrometer (San Jose, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) source was used for mass analysis and detection. Data acquisition was performed with Xcalibur 1.1 software (Thermo-Finnigan). Peak integration and calibration were performed using LCQuan software (Thermo-Finnigan).

2.3. Chromatographic conditions

Chromatography was performed on a Diamonsil C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Dikma, Beijing, China) with a 4 mm × 3.0 mm i.d. SecurityGuard C_{18} (5 μm) guard column (Phenomenex, Torrance, CA, USA), using a mobile phase of methanol–water–formic acid (70:30:0.5, v/v/v), which was degassed by sonication before use. The flow-rate was set at 0.5 ml/min. The column temperature was maintained at room temperature (24 °C). The injected volume was 20 μl.

2.4. Mass spectrometric conditions

The mass spectrometer was operated in the positive mode. The tuning parameters were optimized for methylephedrine, noscapine and I.S. by infusing a solution containing 1 μg/ml of each analyte at a flow-rate of 10 μL/min into the mobile phase (0.5 ml/min) using a post-column "T" connection. The optimized temperatures of the vaporizer and heated capillary were 150 and 280 °C, respectively. The corona discharge current was set at 4.0 μA with a source collision induced dissociation (CID) voltage of 10 V. The sheath and auxiliary gases (nitrogen) were set at 80 psi and 3 l/min, respectively. Argon was used as the collision gas at a pressure of approximate 0.19 Pa. Quantification was performed using selected reaction monitoring (SRM) of the transitions of *m/z* 180 → *m/z* 162 for methylephedrine, *m/z* 414 → *m/z* 220 for noscapine and *m/z* 256 → *m/z* 167 for diphenhydramine (I.S.), respectively, with a scan time of 0.3 s per transition. The optimized collision energies chosen for methylephedrine, noscapine and I.S. were 20, 25 and 25 eV, respectively.

2.5. Preparation of standard and quality control samples

Standard stock solutions of methylephedrine and noscapine were prepared in methanol to give a final concentration of 400 μg/ml for each analyte. The solutions were then successively diluted with methanol–water (50:50, v/v) to prepare working solutions in the concentration range of 1.0–1000 ng/ml for each analyte. The I.S. working solution (0.50 μg/ml) was prepared by diluting the 400 μg/ml stock solution of diphenhydramine with water. All the solutions were stored at 4 °C and were brought to room temperature before use. All concentrations were calculated based on the free base form.

Calibration curves were prepared by spiking 50 μl of the appropriate standard solution to 0.5 ml of blank human plasma. Plasma concentrations were 0.10, 0.25, 0.5, 1.25, 3.75, 10, 25, 50 and 100 ng/ml for both analytes. All the quality control samples (QCs) used in the validation and during the pharmacokinetic study were prepared in the same way as the calibration standards before analysis. Plasma concentrations of QCs were 0.25, 3.75 and 75 ng/ml for both analytes. The spiked plasma samples (standards and QCs) were extracted on each analytical batch along with the unknown samples.

2.6. Sample preparation

To a 0.5-ml aliquot of plasma samples, 50 μl of methanol–water (50:50, v/v), 50 μl of the I.S. solution and 50 μl of 1 M sodium hydroxide solution were added. The mixed samples were then extracted with 3 ml of *n*-hexane–dichloromethane–isopropanol (2:1:0.1, v/v/v). The mixture was vortex-mixed for approximate 1 min, then shaken on a mechanical shaker for 15 min. After centrifugation at 3500 × *g* for 5 min, the upper organic layer was removed and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μl of the mobile phase, then vortex-mixed. A 20-μl aliquot of the resulting solution was injected onto the LC/MS/MS system for analysis.

2.7. Method validation

Plasma samples were quantified using the ratios of the peak area of each analyte to that of I.S. as the assay parameter. Peak area ratios were plotted against methylephedrine and noscapine concentrations and standard curves in the form of $y = A + Bx$ were calculated using weighted ($1/x^2$) least squares linear regression.

During prestudy validation, the calibration curves were defined in three separate days based on duplicate assays of the spiked plasma samples, and QCs using six replicate preparation of plasma samples at three concentration levels were

Table 1
Accuracy and precision for the analysis of methylephedrine and noscapine in human plasma

Drug	Concentration ($\mu g\,L^{-1}$)		R.S.D. (%)		Relative error (%)
	Added	Found	Intra-day	Inter-day	
Methylephedrine	0.250	0.249	3.9	5.1	0.0
	3.75	3.65	5.2	3.8	−0.2
	75.0	73.5	4.8	3.3	−2.5
Noscapine	0.250	0.247	4.6	6.3	0.0
	3.75	3.68	3.9	5.2	−0.2
	75.0	73.1	3.6	6.7	−3.0

measured on the same day (see Table 1). The accuracy and precision were calculated using one-way ANOVA [11]. The accuracy was expressed by relative error (RE). The precision was expressed by relative standard deviation (R.S.D.). The within-run and between-run mean square of variance was used to calculate the R.S.D. as follows:

$$\text{R.S.D. (\%)} = \frac{\sqrt{\text{mean square variance}}}{\text{mean}} \times 100$$

The lower limit of quantification, 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 6 replicates as follows: spiking 50 μl of the standard solution (1.0 ng/ml for both analytes) to 0.5 ml of blank human plasma.

The extraction recoveries of methylephedrine and noscapine at three QC levels were determined by comparing the peak area ratios of each analyte to I.S. in plasma samples that had been spiked with each analyte prior to extraction with samples, to which each analyte had been added post-extraction. The extraction recovery of the I.S. was determined in a similar way using medium QC as a reference.

Stability of processing (3 freeze–thaw cycles, benchtop for 2 h), chromatography (re-injection) and sample storage (−20 °C for 30 days) was assessed by analyzing replicates ($n = 3$) of QC samples (at the concentrations of 0.25 and 75.0 ng/ml for both analytes). The results were compared with those QC samples freshly prepared, and the percentage concentration deviation was calculated.

2.8. Pharmacokinetic study

The method was applied to determine plasma concentrations of methylephedrine and noscapine from a clinical trial in which 14 healthy male volunteers received a multicomponent formulation containing 20 mg DL-methylephedrine hydrochloride, 16 mg noscapine, 300 mg paracetamol and 1 mg of chlorpheniramine maleate. Blood samples were collected in heparinized 10-ml tubes before and 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 h post-dosing and centrifuged to separate the plasma fractions. The obtained plasma samples were stored at −20 °C until analysis.

3. Results and discussion

3.1. Mass spectrometry

To determine the methylephedrine and noscapine in human plasma using SRM mode, full scan and product ion spectra of each analyte and I.S. were investigated under the present HPLC conditions. The analytes and I.S. are moderate polar compounds, each containing an alkylamine group in their structures, therefore, they could be ionized under positive APCI or electrospray ionization (ESI) conditions. It was found that ESI could offer higher sensitivity for the analytes than APCI, especially for methylephedrine. But the precision (R.S.D.) of the peak areas for the standards of each analyte analyzed by LC–ESI/MS–MS was higher (>6%) than that by LC–APCI/MS–MS (<4%). Therefore, APCI was chosen as the ionization source in the experiment to improve the reproducibility of the assay.

By APCI, the methylephedrine and I.S. formed predominantly protonated molecules $[M + H]^+$ in full scan spectra. No sodium or other solvent adducts or dimmers were observed. But it was found that when methanol was employed as the organic additive in the mobile phase, the most abundant peak in the full scan Q1 mass spectrum of noscapine was the solvent adduct ion $[M + H + CH_3OH]^+$ (m/z 436). The relative abundance of $[M + H]^+$ was less than 10% and the other clustered ions, such as $[M + H + K]^+$ (m/z 453), $[M + H + Na]^+$ (m/z 437) were also observed. When the source CID was utilized, the intensity of clustered ions significantly decreased, whereas that of the $[M + H]^+$ increased. When 10 V source CID was applied, the intensity of $[M + H]^+$ increased 10 times compared with the condition that no source CID was used, while the solvent adduct ion showed only a weak signal. The most abundant peak in the full scan Q1 mass spectrum of noscapine was the $[M + H]^+$ ion in this condition, which improved the sensitivity of noscapine.

Fig. 1 displays the product ion spectra of $[M + H]^+$ ions from three compounds. Methylephedrine gave an intense product ion at m/z 162, formed by losing water. Noscapine showed an intense ion at m/z 220 corresponding to the loss of isobenzofuranone group from $[M + H]^+$ ion. Diphenhydramine showed a major fragment ion at m/z 167 corresponding to a neutral loss of $[HOCH_2CH_2N(CH_3)_2]$. These major fragment ions at m/z 162, m/z 220 and m/z 167 were

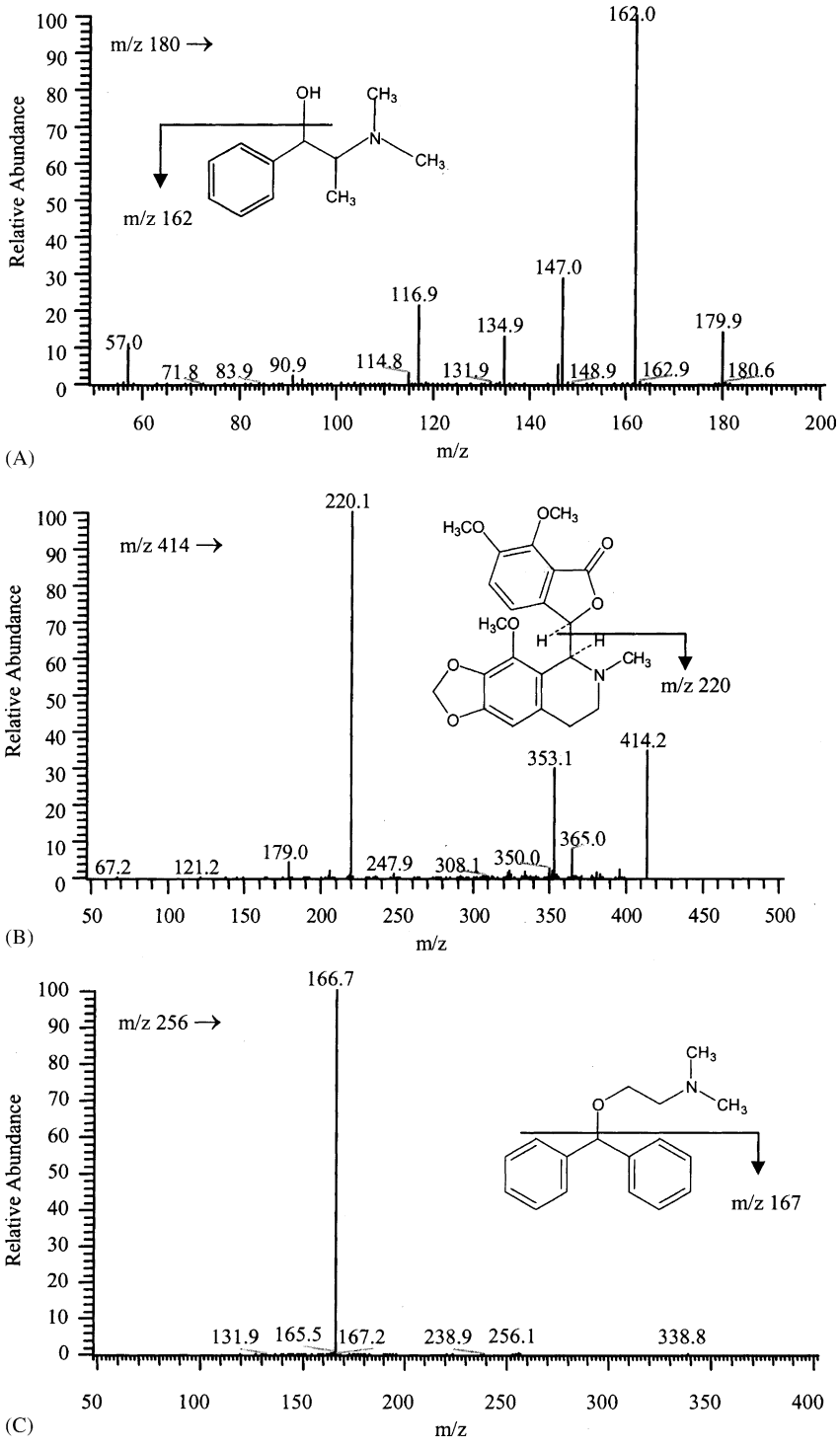

Fig. 1. Full scan product ion spectra of $[M + H]^+$ of methylephedrine (A), noscapine (B) and diphenhydramine (C).

chosen in the SRM acquisition for methylephedrine, noscapine and diphenhydramine, respectively. Compared with SRM Chromatograms of noscapine and IS, methylephedrine gave a relatively high chemical noise by using SRM transition of *m/z* 180 → *m/z* 162. During the initial development step of the LC/MS/MS method, the low-abundance fragment ions at *m/z* 147 or *m/z* 117 were also chosen as product ions for monitoring methylephedrine. Although background noise decreased, the sensitivity of the analyte was three to four times lower than that of using *m/z* 162 in SRM acquisition. As a result, the signal-to-noise of methylephedrine was not improved. When higher collision energy (35 eV) was used, $[M + H]^+$ ion was further fragmented and the obtained base ion (*m/z* 115) was also weak and unsuitable for quantitative measure-

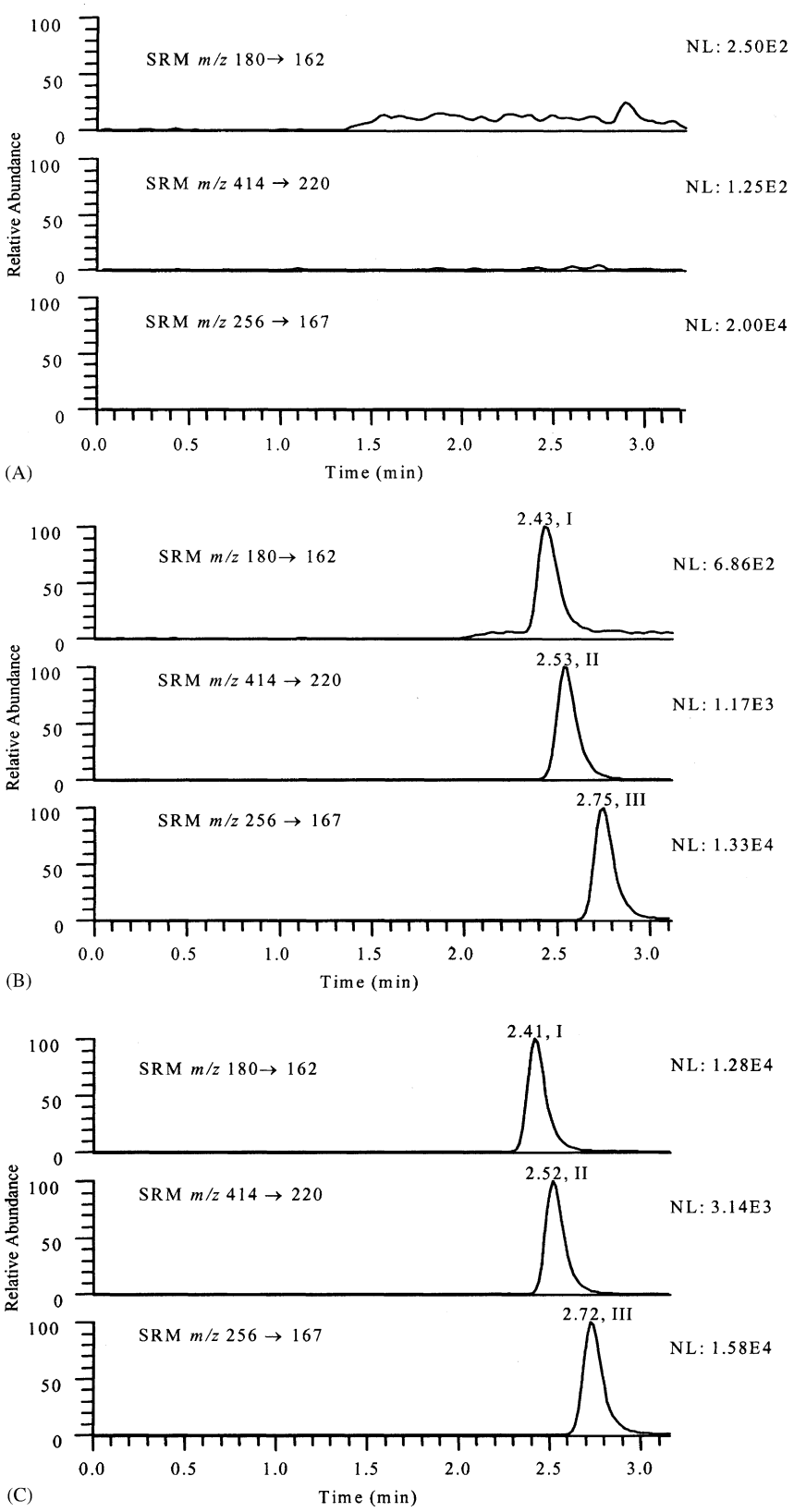

Fig. 2. SRM Chromatograms of methylephedrine (I), noscapine (II) and I.S. (III) in human plasma sample, (A) a blank plasma sample, (B) a plasma sample spiked with methylephedrine (0.1 ng/ml), noscapine (0.1 ng/ml) and I.S. (10 ng/ml), (C) a volunteer plasma sample 2 h after an oral dose of 20 mg DL-methylephedrine hydrochloride, 16 mg noscapine, 300 mg paracetamol and 1 mg of chlorpheniramine maleate.

Table 2
Accuracy and precision at the concentrations of LLOQ for methylephedrine and noscapine

	Concentration (ng/ml)		S.D.	R.S.D. (%)	Relative error (%)
	Added	Found (mean)			
Methylephedrine	0.100	0.100	0.004	3.9	0.9
Noscapine	0.100	0.0943	0.01	6.7	−6.4

ments. Furthermore, because of the relatively high concentration of methylephedrine in plasma, the selectivity of the present method was proved sufficient for methylephedrine. There are a number of reports in literature that quantification of similar drugs by LC/MS/MS using SRM transition with the neutral loss of water [5,12,13].

3.2. Chromatography

The analytes and I.S. are all basic compounds, therefore, a low amount of formic acid in the mobile phase could shorten the chromatographic cycle time and improve the peak shape, while it had no marked effect on the sensitivity of the analytes and I.S. Under the present chromatographic conditions, the retention times of all analytes were within 3.2 min. During sample preparation, it was necessary to dissolve the residues with the mobile phase to avoid chromatographic peak distortion.

The extraction method was slightly modified from the method for determination of chlorpheniramine and pseudophedrine [13]. It was found that the extraction recovery was mainly influenced by the pH of plasma sample, hence alkalic modifiers was used to adjust the pH of plasma samples. When plasma sample was adjusted to pH 11 with 1 M phosphate buffer, a high extraction recovery (about 85%) was achieved for methylephedrine, while that of noscapine was only about 60%. The extraction recovery of methylephedrine was slightly lower (83%), but that of noscapine was much improved (80%) when plasma sample was adjusted to pH 10 with 1 M sodium hydroxide solution. Therefore, sodium hydroxide solution (1 mol/l) was used as the alkaline modifier, and the *n*-hexane–dichlormethane–isopropanol (2:1:0.1, v/v/v) as the extraction reagent to get a good cleanup and adequate recovery values for the plasma samples. It was also found that the reproducibility of the extraction recovery was influenced by the shaking time. When it was set at 10 min, the R.S.D. of the extraction recovery for both drugs were >12%. The R.S.D. of the extraction recovery was less than 4% for both analytes when the shaking time was increased to 15 min. Therefore, the shaking time was set at 15 min to get a good consistency.

3.3. Method validation

3.3.1. Selectivity

Potential interference from endogenous compounds was investigated by analyzing human plasma of six different sources. Typical SRM chromatograms of a blank plasma sample, a blank plasma sample spiked with methylephedrine, noscapine at the LLOQ and I.S., and a plasma sample from a

Table 3
Stability of methylephedrine and noscapine plasma samples ($n = 3$)

Condition	Concentration (ng/ml)				Relative error (%)
	Added	Found (mean)	S.D.	R.S.D. (%)	
Three freeze–thaw cycles					
Methylephedrine	0.250	0.261	0.01	3.5	6.0
	75.0	74.0	2.0	2.7	−1.1
Noscapine	0.250	0.243	0.01	4.7	−0.9
	75.0	76.0	3.3	4.3	1.1
Post-freezing (−20 °C) for 30 days					
Methylephedrine	0.250	0.252	0.03	10.0	0.1
	75.0	73.4	7.3	10.0	−1.0
Noscapine	0.250	0.233	0.02	9.6	−7.2
	75.0	79.6	4.2	5.3	6.1
Post-treatment for 24 h (room temperature)					
Methylephedrine	0.250	0.262	0.02	8.2	2.8
	75.0	75.2	1.8	2.3	0.3
Noscapine	0.250	0.271	0.01	3.4	7.6
	75.0	77.2	2.9	3.8	2.9
Benchtop for 2 h (room temperature)					
Methylephedrine	0.250	0.242	0.01	3.0	−2.7
	75.0	77.2	2.9	3.8	2.9
Noscapine	0.250	0.241	0.01	4.4	−4.9
	75.0	80.3	2.8	3.4	7.0

healthy volunteer 2 h after an oral administration are shown in Fig. 2. No significant interference or ion suppression from endogenous substances was observed at the retention time of each analyte and I.S. The LC/MS/MS method has high selectivity because only the precursor and product ions derived from the analytes of interest are monitored. With the method, simultaneous multi-compound quantification is possible in a short chromatographic run time.

3.3.2. Linearity of calibration curves and lower limits of quantification

Excellent linearity was obtained over the concentration range of 0.1–100 ng/ml for both analytes in human plasma. The correlation coefficients for the calibration regression curves were 0.99 or greater. The typical equations of the calibration curves were as follows: $y = 4.192 \times 10^{-3} + 7.639 \times 10^{-3}x$ for methylephedrine and $y = 3.555 \times 10^{-3} + 1.382 \times 10^{-2}x$ for noscapine.

The current method had an LLOQ of 0.1 ng/ml for both analytes, which is sufficient for clinical pharmacokinetic studies following oral administration of therapeutic doses. The accuracy and precision at the concentrations of LLOQ for methylephedrine and noscapine was shown in Table 2.

3.3.3. Precision and accuracy

The method showed good precision and accuracy. Table 1 summarizes the intra- and inter-day precision and accuracy for methylephedrine and noscapine from the QC samples. In

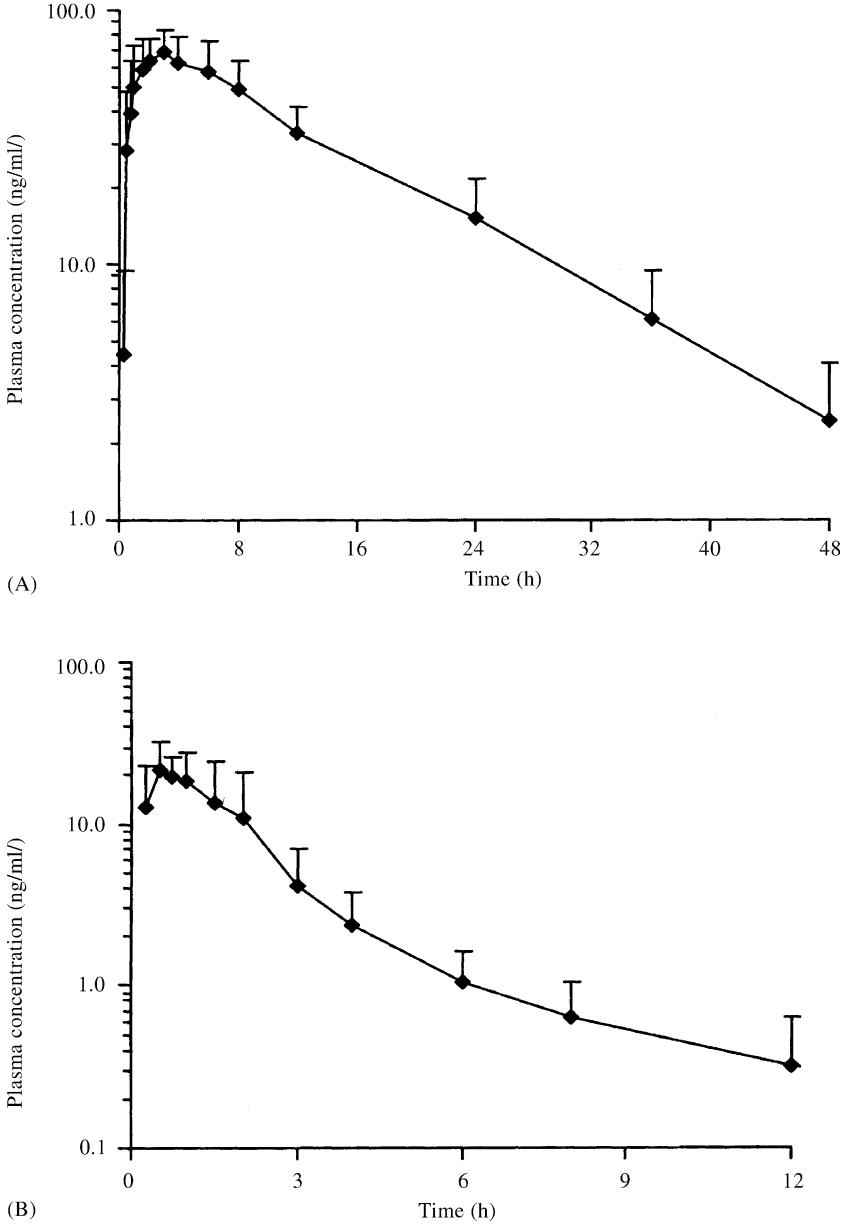

Fig. 3. Mean plasma concentration–time curves of methylephedrine (A) and noscapine (B) after an oral administration of a multi component formulation containing 20 mg DL-methyl ephedrine hydrochloride, 16 mg noscapine, 300 mg paracetamol and 1 mg of chlorpheniramine maleate to 14 healthy volunteers (each point represent the mean and S.D.).

this assay, the intra-run precision was less than 5.2% for each QC level of methylephedrine and less than 4.6% for each QC level of noscapine. The inter-run precision was less than 5.1% for methylephedrine and less than 6.7% for noscapine. The accuracy was within ±2.5% for methylephedrine and within ±3.0% for noscapine.

3.3.4. Extraction recovery and stability

Mean extraction recoveries for methylephedrine at 0.25, 3.75 and 75 ng/ml were 86.0, 82.4 and 79.2%, respectively. For noscapine, the recovery values at 0.25, 3.75 and 75 ng/ml were 84.5, 80.6 and 81.5%, respectively. Mean recovery for the internal standard (100 ng/ml) was 81.3%. All recoveries had R.S.D. less than 4% throughout the entire concentration ranges, showing good consistency.

Stability data are shown in Table 3. The results of stability experiments showed that no significant degradation occurred during chromatography, extraction and sample storage processes for methylephedrine and noscapine plasma samples.

3.4. Application of the method to a pharmacokinetic study in healthy volunteers

This validated analytical method was used to study pharmacokinetic profiles of methylephedrine and noscapine in human plasma after an oral administration of a multicomponent formulation containing 20 mg DL-methylephedrine hydrochloride, 16 mg noscapine, 300 mg paracetamol and 1 mg of chlorpheniramine maleate to 14 healthy volunteers. Profiles of the mean plasma concentration of methylephedrine and noscapine versus time are shown in Fig. 3.

4. Conclusions

An LC/MS/MS method with APCI interface was developed and validated for the simultaneous determination of methylephedrine and noscapine in human plasma. The method is very sensitive with an LLOQ of 0.1 ng/ml for both analytes. The method has proven to be fast and rugged, with each sample requiring less than 3.2 min of chromatographic run time. This method was suitable for pharmacokinetic studies of compound formulations containing DL-methylephedrine hydrochloride, noscapine, paracetamol and chlorpheniramine maleate.

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