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Short communication

Liquid chromatography/tandem mass spectrometry method for the quantification of deserpidine in human plasma: Application to a pharmacokinetic study

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ABSTRACT

A sensitive and rapid liquid chromatography/tandem mass spectrometric (LC/MS/MS) method was developed and validated for the determination of deserpidine in human plasma. The plasma samples were prepared using liquid–liquid extraction (LLE) with ethyl ether–dichloromethane (3:2, v/v). Chromatographic separation was accomplished on an Ultimate XB-C18 column. The mobile phase consisted of methanol–5 mM ammonium acetate–formic acid (72:28:0.036, v/v/v). Detection of deserpidine and the internal standard tropisetron was achieved by tandem mass spectrometry with an electrospray ionization interface in positive ion mode. The lower limit of quantification was 4.0 pg/ml. The linear range of the method was from 4.0 to 2000 pg/ml. The intra- and inter-day precisions were lower than 14.7% in terms of relative standard deviation (RSD), and the accuracy was within ±8.7% in terms of relative error (RE). This validated method was successfully applied for the evaluation of pharmacokinetics of deserpidine after a single oral administration dose of 0.25 mg deserpidine to 22 healthy volunteers.

1. Introduction

Hypertension is widespread and a major risk factor for stroke and heart disease. It may be possible for patients with hypertension or at high cardiovascular risk to be treated with blood pressure-lowering medication to reduce the likelihood of these adverse events and their complications [1,2]. The ganglionic blocking agents are frequently used to treat hypertension through depletion of tissue stores of catecholamines (epinephrine and norepinephrine) from peripheral sites [3]. Deserpidine (11-desmethoxyreserpine) is an effective ganglionic blocking agent, which was isolated from the root of *Rauwolfia canescens*. It is structurally similar to reserpine, whose chemical structures are illustrated in Fig. 1. Deserpidine has been used in the treatment of hypertension and psychosis [4–6]. The recommended dose in clinical settings is an oral administration of 0.25 mg once daily.

During recent years, mass spectrometry has repeatedly been proven to be a powerful technique for the rapid, quantitative determination of drugs and metabolites in physiologic fluids. Anderson et al. [7] developed a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method to quantify reserpine in equine plasma with the lower limit of quantification (LLOQ) of 10.0 pg/ml. Ke et al. [8] also reported an LC/MS/MS method to determine reserpine in mouse plasma. The method resulted in an LLOQ of 20.0 pg/ml.

So far, no method has been reported to determine deserpidine levels in biological samples. To characterize clinical pharmacokinetic profiles or evaluate formulation bioavailability and bioequivalence of deserpidine, it is important to develop a highly sensitive and simple method for quantification of deserpidine in human plasma. In this report, an LC/MS/MS method was described for this purpose. After validation, the method was successfully applied to a pharmacokinetic study.

2. Experimental

2.1. Chemicals and reagents

Deserpidine (99.8% purity) was kindly donated by Aodong Pharmaceutical Co. Ltd. (Jilin, China). Tropisetron hydrochloride (100% purity), used as the internal standard (IS), was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC-grade) was purchased from Sigma (St. Louis, MO, USA). Ammonium acetate and formic acid of HPLC grade were purchased from Tedia (Fairfeild, OH, USA). Other chemical reagents (analytical grade) were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China).

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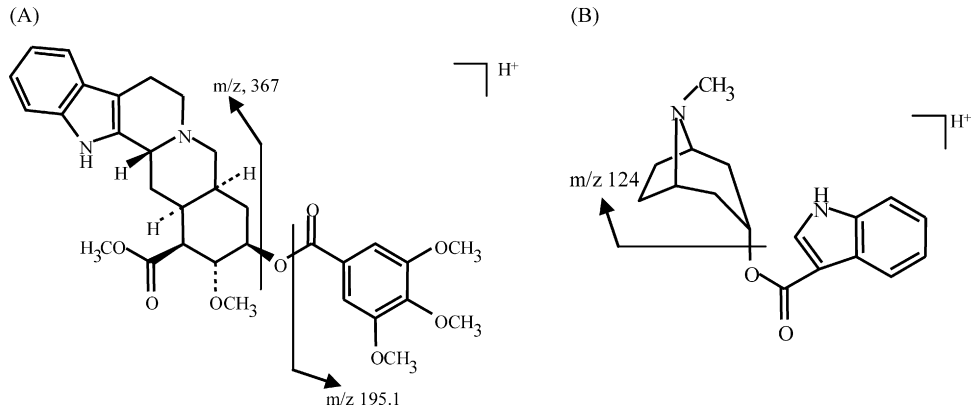

Fig. 1. Structures of (A) reserpine and (B) internal standard tropisetron.

Heparinized blank (drug free) human plasma was supplied by Shanghai Shuguang Hospital (Shanghai, China). Deionized water was obtained from a Millipore Milli-Q Gradient Water Purification System (Molsheim, France).

2.2. Instruments

An Agilent (Agilent, Waldbronn, Germany) 1100 liquid chromatographic system consisting of a G1311A quaternary pump, a G1379A degasser, a G1316A thermostatted column and a G1313A autosampler was used for solvent and sample delivery. An API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada), linked via an electrospray ionization (ESI) interface, was used for mass analysis and detection. Data acquisition was performed with Analyst 1.4.1 software.

2.3. LC/MS/MS conditions

Chromatographic separation was achieved on an Ultimate XB-C18 column (150 mm × 4.6 mm i.d., 5 μm; Welch Materials, Ellicott, MD, USA) with a SecurityGuard C18 guard column (4 mm × 3.0 mm i.d., 5 μm; Phenomenex, Torrance, CA, USA). The column temperature was maintained at 25 °C. A mixture of methanol–5 mM ammonium acetate–formic acid (72:28:0.036, v/v/v) was used as the mobile phase at a flow rate of 0.6 ml/min.

The mass spectrometer was operated in the positive ion mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 579.4 → (195.1 + 367.2) for deserpidine and m/z 285.3 → 124.2 for IS, respectively, with a scan time of 0.2 s per transition. The product ion spectra of deserpidine and tropisetron (IS) are shown in Fig. 2. The ionspray voltage was set at 4200 V. Source temperature was maintained at 550 °C. Nitrogen was used as the nebulizing gas (50 psi), auxiliary gas (60 psi) and curtain gas (10 psi). For collision activated dissociation (CAD), nitrogen was employed as the collision gas at a pressure of 4 psi. The optimized collision energies (CEs) at 50 and 37 eV were chosen for the fragment ions at m/z 195.1 and m/z 367.2 of deserpidine. The CE was set at 25 eV for IS.

2.4. Standard solutions and quality control (QC) samples

Two stock solutions of deserpidine were prepared separately by dissolving the accurately weighed reference compound in methanol to give a final concentration of 400 μg/ml. These two stock solutions were used for calibration standards and QC standards, respectively. The solutions were then serially diluted with methanol/water (50:50, v/v) to obtain the desired concentrations. A 400 μg/ml stock solution of internal standard tropisetron (calculated as the free base) was also prepared in methanol. This was diluted with methanol/water (50:50, v/v) to obtain a 50.0 ng/ml working solution. All of the solutions were stored at 4 °C and were brought to room temperature before use.

The analytical standard and QC samples were prepared by spiking blank heparinized human plasma (500 μl) with standard working solutions (100 μl) during validation and during each experiment for the pharmacokinetic study. Calibration samples were made at concentrations of 4.00, 10.0, 30.0, 100, 300, 800 and 2000 pg/ml. QC samples were prepared at the concentrations of 10.0, 100 and 1800 pg/ml. The analytical standards and QC samples were stored at −20 °C.

2.5. Sample preparation

To 500 μl of plasma sample, a 40 μl aliquot of the IS solution (50.0 ng/ml tropisetron), 100 μl of methanol/water (50:50, v/v) and 100 μl of 0.1 M NaOH were added. The mixture was extracted with 3 ml of ethyl ether–dichloromethane (3:2, v/v) by shaking for 10 min. After centrifugation at 2000 × g for 5 min, the upper organic layer was separated and evaporated to dryness at 40 °C under a stream of nitrogen in the TurboVap evaporator (Zymark, Hopkinton, MA, USA). The residue was reconstituted in 150 μl of the mobile phase, and then vortex-mixed. A 20 μl aliquot of the resulting solution was injected into the LC/MS/MS system for analysis.

2.6. Method validation

To ensure the accuracy, selectivity, reproducibility and specificity, the method was validated on the items described as follows [9]:

Selectivity was performed by analyzing the blank plasma from six different sources to test interference at the retention times of the analyte and internal standard.

The linearity of the assay method was determined by plotting the peak area ratios of deserpidine and IS against the concentrations of deserpidine in plasma in duplicate on three consecutive days.

Inter- and intra-day accuracy and precision for the assay were characterized by the performance of three levels of QCs run on three validation days, and on each day six replicates were analyzed together with an independently prepared calibration curve.

Recovery of deserpidine was evaluated by comparing the mean peak areas of the regularly prepared QC samples (n = 6) at 10.0, 100 and 1800 pg/ml with the mean peak areas of spiked-after-extraction samples, which represented the 100% recovery value.

The stabilities of deserpidine in human plasma were evaluated by analyzing replicates (n = 3) of plasma samples at the concentrations of 10.0 and 1800 pg/ml, which were exposed to different

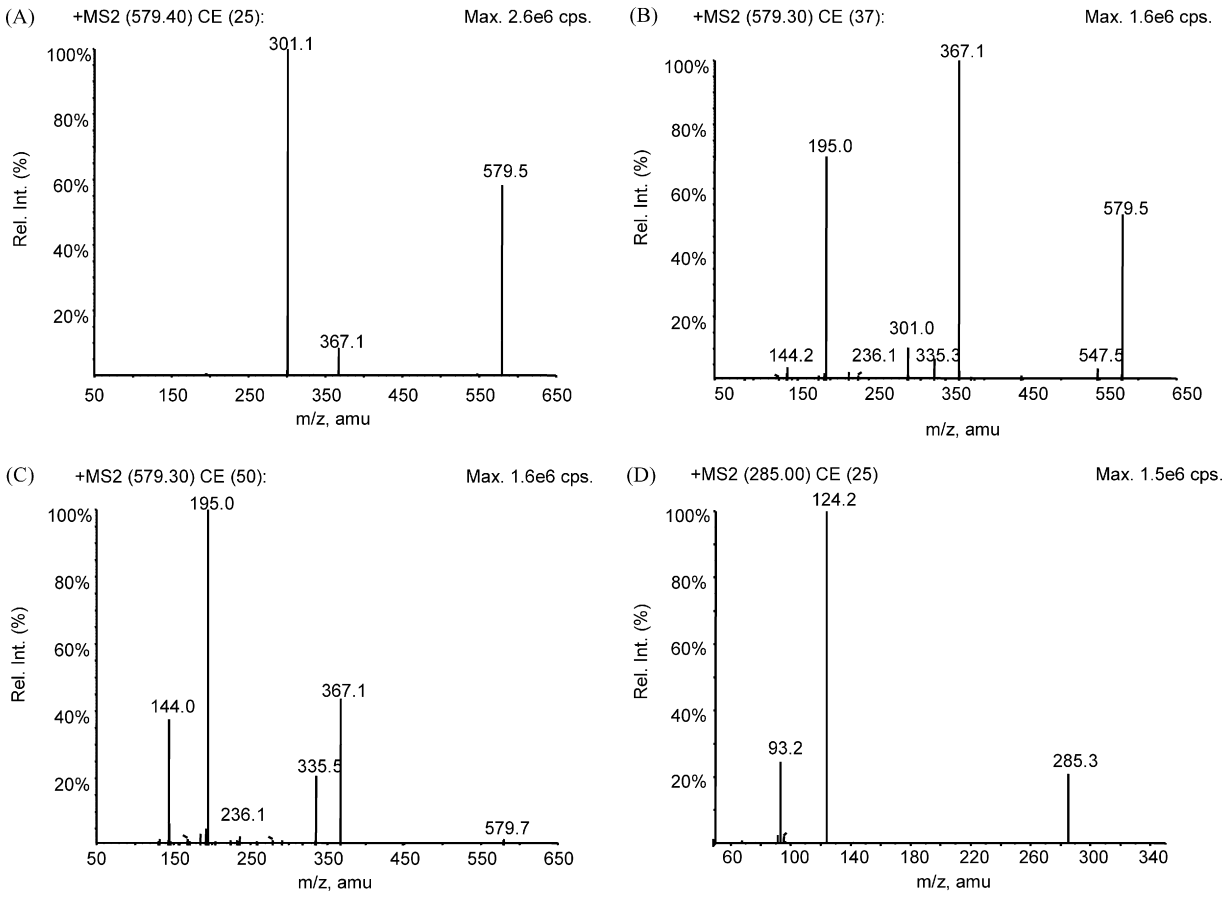

Fig. 2. Product ion mass spectra of $[M+H]^+$. (A) Deserpidine using a CE of 25 eV; (B) deserpidine using a CE of 37 eV; (C) deserpidine using a CE of 50 eV; (D) tropisetron using a CE of 25 eV.

conditions (time and temperature). The spiked plasma samples were analyzed after storage at ambient temperature for 2 h, in the autosampler for 24 h at room temperature after liquid–liquid extraction, at 20 °C for 25 days and after three freeze–thaw cycles from 20 °C to room temperature. The analytes were considered stable in plasma when 85–115% of the initial concentrations were found.

The matrix effect was evaluated at two concentrations (10.0 and 1800 pg/ml in plasma) as described in the literature [10].

2.7. Application of the assay

A pharmacokinetic study was performed in healthy Chinese volunteers approved by the Ethical Committee of the Second Affiliated Hospital of Liaoning University of Traditional Medicine. Single oral doses of 0.25 mg deserpidine were administered to 22 healthy volunteers. Blood samples (4 ml) were collected into sodium heparin containing tubes before, and 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 8.0, 12, 24, 48, 72, 96 and 120 h after dosing, and centrifuged at 2000 × *g* for 10 min to separate the plasma fractions. The plasma samples were stored at −20 °C until analysis.

3. Results and discussion

3.1. Optimization of the mass spectrometric condition

Because of a tertiary amino group in its structure, deserpidine produces a good mass spectrometric response in positive electrospray ionization (ESI) mode. In the Q1 full scan mode, the $[M+H]^+$ ion with a high MS response was generated and no other additive ions were observed. Fig. 2 shows the product ion spectrum of $[M+H]^+$, where three major product ions are observed at *m*/*z* 195, 301 and 367. The MS/MS parameters, including the declustering potential (DP), collision cell exit potential (CXP), gases (GS1, GS2) and collision-induced dissociation (CID) energies were optimized to identify the most stable and intense product ion for deserpidine. It was found that the product ion spectrum of the $[M+H]^+$ ion of deserpidine was dependent on the collision energy (CE). At low CEs (1028 eV), a major fragment ion at *m*/*z* 301 was formed. When a higher rate of collision energy (37 eV) was used, the parent ion was fragmented, forming a product ion of *m*/*z* 367. Increasing the CE value to 50 eV, the most abundant fragment ion at *m*/*z* 195 was generated. To improve the sensitivity, two major diagnostic fragment ions *m*/*z* 367 and *m*/*z* 195, were acquired in the multiple reaction monitoring (MRM) for deserpidine.

3.2. Optimization of the chromatographic condition

During method development, a number of reversed-phase C18 columns, such as Venusil XBP-C18, Venusil MP-C18, Capcell PAK-C18, Zorbax SB-C18 and Ultimate XB-C18 were tested to obtain an optimized response, suitable retention time and good peak shapes for deserpidine. The Ultimate XB-C18 column was selected, since it provided a symmetrical peak shape and high intensity for deserpidine. The composition of the mobile phase in particular, was optimized to achieve good sensitivity and a short run time. Methanol revealed a higher mass spectrometric response and lower background noise than acetonitrile and was chosen for the organic phase. The high organic solvent content shortened the chromatographic cycle time, and the acidic modifier

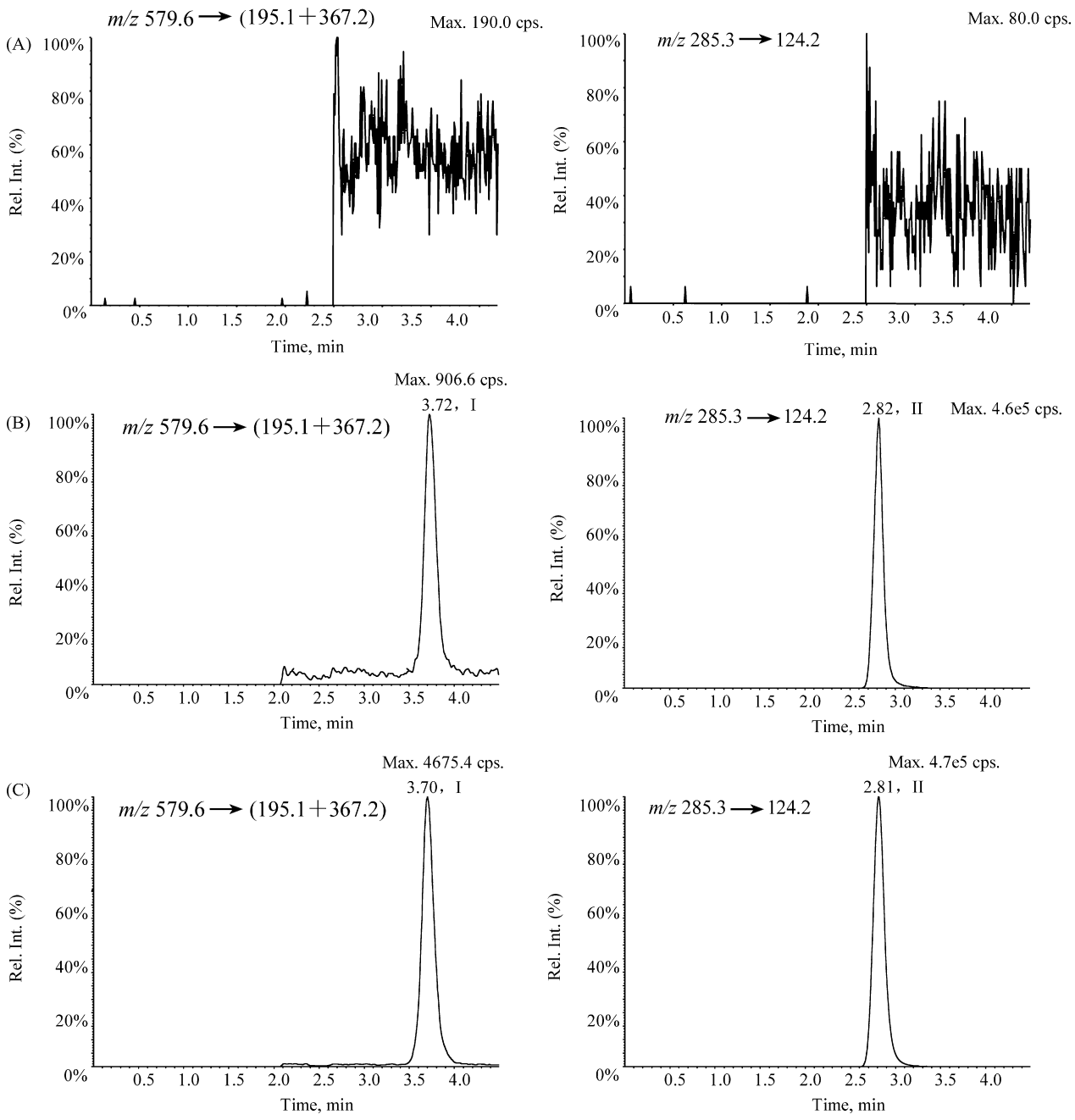

Fig. 3. MRM chromatograms of deserpidine and tropisetron (IS) in human plasma. (A) Blank plasma sample; (B) plasma sample spiked with deserpidine at 4.00 pg/ml and tropisetron (IS) at 4.00 ng/ml; (C) A plasma sample collected at 1.0 h after an oral dose of 0.25 mg deserpidine. Peaks I and II refer to deserpidine and tropisetron, respectively.

(formic acid) in the mobile phase improved sensitivity by promoting the ionization of the analytes. A mobile phase consisting of methanol–5 mM ammonium acetate–formic acid (72:28:0.036, v/v/v) was used in the experiment under the selected chromatographic condition; the retention times for both deserpidine and IS were <4.0 min.

3.3. Internal standard selection

In the study, reserpine, structurally similar to deserpidine, was firstly adopted as the internal standard, but a trace impurity of deserpidine in the reserpine reference standard affected the accuracy of the quantitation results, especially at LLOQ. To obtain satisfied accuracy and precision, tropisetron, a basic compound, was used as the internal standard.

3.4. Preparation of plasma samples

To separate the analyte from plasma, we attempted simple liquid–liquid extraction (LLE). In our experiment, different LLE conditions were evaluated including different organic extraction solvents and aqueous pH buffers to achieve the optimal extraction efficiency for both deserpidine and IS, along with minimal interference from endogenous substances. Three organic extraction solvents (ethyl acetate, ethyl ether and ethyl etherdichloromethane) were investigated. It was found that the supernatant was difficult to evaporate to dryness and it produced a high background signal when using ethyl acetate. Ethyl ether and ethyl ether–dichloromethane (3:2, v/v) yielded similar recoveries (about 50%) for deserpidine. Ether–dichloromethane was chosen for its stable analyte recovery property and its rapid evaporation

Table 1
Precision and accuracy of the LC/MS/MS method to determine deserpidine in human plasma ($n = 3$ days, six replicates per day).

Concentration (pg/ml)		RSD (%)		Relative error (%)
Added	Found	Intra-day	Inter-day	
4.00	4.38	5.2	1.5	8.7
10.0	10.5	3.0	9.4	4.5
100	100	9.5	11.2	−0.3
1800	1724	14.7	5.5	−4.2

characteristics. Buffers containing NaOH or phosphate buffers were tested during the extraction procedure. The use of 0.1 M NaOH could reduce unwanted interfering substances to a minimum. The recovery was increased from 50% to 68% when 0.1 M NaOH was used.

3.5. Method validation

3.5.1. Selectivity

The typical chromatograms of a blank, a spiked plasma sample with deserpidine at LLOQ and IS, and a plasma sample from a healthy volunteer 1 h after an oral administration are shown in Fig. 3. No interfering peaks from endogenous compounds were observed at the retention times of the analyte and IS. The retention times for deserpidine and IS were 3.72 and 2.82 min, respectively.

3.5.2. Linearity of calibration curves and lower limits of quantification

Calibration standards containing deserpidine over the concentration range of 4.0–2000 pg/ml were prepared from working solutions of deserpidine in duplicate. A typical regression equation was $y = 2.91 \times 10^{-4}x - 3.38 \times 10^{-4}$ with a correlation coefficient (r) of 0.9964, where y represents the peak area ratio of deserpidine to that of IS and x represents the plasma concentration of deserpidine.

The precision and accuracy data corresponding to the LLOQ are shown in Table 1. Under the achieved LLOQ of 4.0 pg/ml, the deserpidine can be determined in plasma samples until 120 h after a single oral dose of 0.25 mg deserpidine, which is sensitive enough to investigate the pharmacokinetic behaviors of deserpidine.

3.5.3. Precision and accuracy

Table 1 summarizes the inter- and intra-day precision and accuracy values for QCs. In this assay, the intra-day precision was 14.7% or less, and the inter-day precision was 11.2% or less for each QC level of deserpidine. The accuracy was within ±8.7%. These results above indicate that the present method has good accuracy, precision and reproducibility.

3.5.4. Recovery

The recoveries of deserpidine obtained from plasma were 68.4 ± 7.3%, 61.9 ± 6.9% and 64.8 ± 2.3% at concentrations of 10.0, 100 and 1800 pg/ml, respectively. Mean recovery for the IS was 74.0 ± 2.9%. The RSDs for all recoveries were <10.6% throughout the entire concentration ranges, demonstrating good consistency.

3.5.5. Stability

Deserpidine was stable in the reconstitution solvent when extracts were stored at room temperature for at least 24 h. Deserpidine in human plasma were stable for at least 2 h at room temperature, at −20 °C for 25 days and three freeze–thaw cycles.

3.5.6. Matrix effect

In our study, the estimation of the matrix effect was conducted following the procedures described above. The absolute matrix effects for deserpidine at concentrations of 10.0 and 1800 pg/ml were 101% and 102%, respectively. The relative matrix effects were 2.6% and 2.0%, respectively. The absolute and relative matrix effects for IS (4.0 ng/ml in plasma) were 98.0% and 1.9%, respectively. These results showed that ion suppression or enhancement from the plasma matrix was negligible under the current conditions.

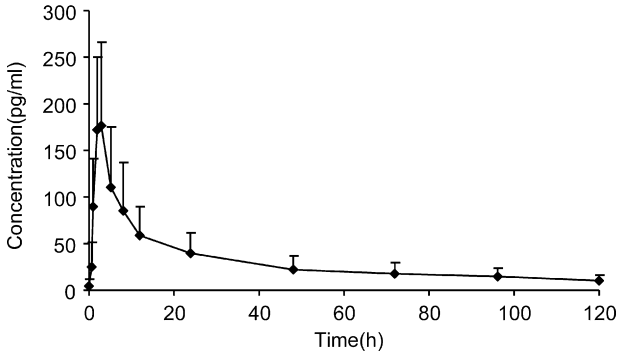

Fig. 4. Mean plasma concentration-time curve of deserpidine after an oral administration of 0.25 mg deserpidine to 22 volunteers ($n = 22$, mean ± SD).

3.6. Application of the method to pharmacokinetic study in healthy volunteers

To our knowledge, there was no publication on the pharmacokinetic study of deserpidine in humans. The validated method was applied to determine the plasma concentration of deserpidine after an oral administration of 0.25 mg deserpidine to 22 volunteers. Fig. 4 shows the mean plasma concentration–time curve of deserpidine. The mean peak concentration of deserpidine (172 pg/ml) occurred with 2 h after the oral dose. The elimination $t_{1/2}$ was estimated as 42.9 ± 17.8 h. The AUC_{0-t} and $AUC_{0-\infty}$ were found to be 3531 ± 1934 and 4193 ± 2350 pg h/ml, respectively.

4. Conclusion

The optimized method was validated to guarantee a reliable determination of deserpidine in human plasma. The method had an LLOQ of 4.0 pg/ml and proved to be highly sensitive and selective. It was then successfully applied to the evaluation of the pharmacokinetics of deserpidine in 22 healthy volunteers after an oral dose of 0.25 mg deserpidine.

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