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Short communication

Rapid and sensitive liquid chromatography–tandem mass spectrometry method for the quantitation of levodropropizine in human plasma

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Abstract

A rapid and sensitive LC–MS–MS method for quantifying levodropropizine in human plasma after oral administration of a single-dose (60 mg/day) was developed and validated. The sample preparation used liquid–liquid extraction with a mixture of dichloromethane–diethyl ether (2:3, v/v) in a basic environment. The retention time of levodropropizne and zolmitriptan (used as internal standard) was 1.6 and 1.4 min, respectively. The assay was linear over the range 0.25–500 ng/mL with a LOQ of 0.25 ng/mL. The intra- and inter-day precision were <8.1% and <11.5%, respectively, and the accuracy was in the range 87.6–112%. The levodropropizine concentration profile in human plasma was determined.

Keywords: Levodropropizine; Zolmitriptan

1. Introduction

Levodropropizine (*S*(−)-3-(4-phenyl-1-piperazinyl)-1,2-propanediol), the (−)-enantiomer of dropropizine, was used as an antitussive drug in clinical on the central nervous system, which exhibits an antitussive activity comparable to dropropizine, with a reduced sedative effect and seems to be attributable to the (+)-enantiomer [1,2].

For pharmacokinetic study, various analysis methods including GC–MS [3] and HPLC [4] have been developed for the determination of levodropropizine in biosamples. However, these methods suffered from some disadvantages, such as low sensitivity, extensive sample preparation and derivatization before analysis, relatively long analytical time and high LOD value (1 ng/mL at a signal-to-noise ratio of 3).

Liquid chromatography with tandem mass spectrometry (LC–MS–MS) has been widely employed for the analysis of drug compounds in biological fluids because of its excellent specificity, speed, and sensitivity [5,6]. To our knowledge, LC–MS–MS method has not been applied for the determination of levodropropizine. In the present paper, we reported the development and validation of a rapid, sensitive and specific LC–MS–MS method for the determination of levodropropizine in plasma with a small volume sample (0.2 mL). The method had been successfully applied to a pharmacokinetic study in human given by oral administration of levodropropizine (60 mg/day).

2. Experimental

2.1. Reagents and chemicals

Levodropropizine (99.8%) and zolmitriptan (99.5%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol was HPLC grade, all other chemicals were analytical grade and used without further purification. The dis-

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tilled water, prepared from demineralized water, was used throughout the study.

2.2. Sample preparation

All the following preparations and determination were executed in light protective environment (Based on BP 2002).

Human plasma samples were harvested from blood (1 mL) by centrifugation at 2000 × *g* for 5 min, and stored at −20 °C prior to analysis. Liquid–liquid extraction was employed as follows: internal standard (100 μL of 10 ng/mL zolmitriptan in methanol and water (1:1, v/v)) was added to 200 μL plasma in a 10 mL capped test-tube. Extraction was performed with 100 μL of 1 mol/L NaOH solution and 2 mL of dichloromethane and diethyl ether (2:3, v/v), vortex-mixing the tube for 1 min, followed by centrifugation for 5 min at 2000 × *g*. The upper organic layer was decanted into another 10 mL test-tube and dried under nitrogen gas. The residue was reconstituted in 200 μL of the mobile phase and 20 μL was injected for analysis.

2.3. LC–MS–MS

The HPLC system (Agilent 1100 series) consisted of a binary pump, an autosampler, a column oven (set at 30 °C) and a Nucleosil C_{18} analytical HPLC column (4.6 mm × 100 mm, 5 μm; Dalian Johnsson Separation Science and Technology Corp, Dalian, China). The mobile phase was prepared by mixing 10 mM ammonium acetate (contained 1% formic acid, v/v) with methanol (55:45, v/v). Chromatography was performed at a flow-rate of 0.5 mL/min. Detection was performed on an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray ionization (ESI) for ion production. A valco valve was used to minimize introducing the pre-eluent into the ion source.

ESI was performed in positive-ion mode with nitrogen as nebulizing (gas 1), heater (gas 2) and curtain gas. High-flow gas flow parameters were optimized by making successive flow injections while introducing mobile phase into the ionization source at 0.5 mL/min. Optimum values for nebulizer, heater and curtain gas flow-rates were 40, 30 and 20 units, respectively. The TurboIonSpray temperature was set at 500 °C. The instrument response was optimized for levodropropizine and internal standard by syringe pump infusion of a constant flow (10 μL/min) of a solution of the two dissolved in mobile phase into the stream of mobile phase eluting from the column.

The LC–MS–MS detector was operated at unit resolution in the multiple-reaction-monitor (MRM) mode using the transitions of the protonated molecular ions of levodropropizine at *m*/*z* 237 → *m*/*z* 120 and zolmitriptan at *m*/*z* 288 → *m*/*z* 58. The values of declustering potential (DP) and collision induced dissociation (CID) energy of levodropropizine and internal standard were 75 V, 50 V and 38 eV, 44 eV, respectively.

2.4. Construction of standard curves

Standard stock solutions of levodropropizine were prepared in methanol at 1 mg/mL and were kept at −20 °C. The stock solution was made fresh by diluting with methanol and water (1:1, v/v) to obtain the concentrations required for preparation of standard working solutions of 0.25, 0.5, 2, 10, 40, 125 and 500 ng/mL. A working solution of internal standard was prepared at 10 ng/mL. For quality control (QC), solutions of levodropropizine at concentrations of 0.5, 40 and 400 ng/mL in methanol and water (1:1, v/v) were prepared. The calibrators were weighted according to $1/x$ (x = (concentration)^2) least-squared regression, and standard curves were constructed using linear regression of the peak area ratios of levodropropizine against internal standard obtained from LC–MS–MS analysis of standard solutions against actual standard concentrations.

2.5. Validation procedures

Quantification was based on the ratios of the peak areas of levodropropizine against those of the internal standard. The linearity, precision and accuracy were evaluated through determining replicate (n = 6) QC samples on three different days. Three different calibration curves using seven plasma concentrations (0.25–500 ng/mL) of levodropropizine were prepared to validate the linearity of the method.

Accuracy and precision of the method were determined through establishing intra- and inter-day accuracy and coefficient of variance (CV) on QC samples (0.5, 40 and 400 ng/mL). QC samples used were at the nominal concentrations of 0.5, 40 and 400 ng/mL. Inter- and intra-day variability was tested on three different days using calibration curves obtained daily.

The extraction recovery at three QC plasma samples concentrations of levodropropizine (0.5, 40 and 400 ng/mL) and internal standard (10 ng/mL), in quadruplicate, was determined by comparing the peak areas for levodropropizine or internal standard from plasma samples extracts to that of the analyte added to extracted blank plasma samples.

The stability of levodropropizine at three QC plasma sample concentrations (0.5, 40 and 400 ng/mL) and internal standard (10 ng/mL) after stored in 4 °C for 24 h, in quadruplicate, was determined by comparing the concentrations back-calculated by the standard curve and the nominal concentrations of 0.5, 40 and 400 ng/mL.

2.6. Application of method

Levodropropizine levels in human plasma of 22 healthy volunteers have been measured in a pharmacokinetic study to investigate the profile of levodropropiznine after a single administration. For each one, blood sampling (1 mL) was performed at 0, 0.17, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 12 and 24 h after oral administration of levodropropizine (60 mg/day).

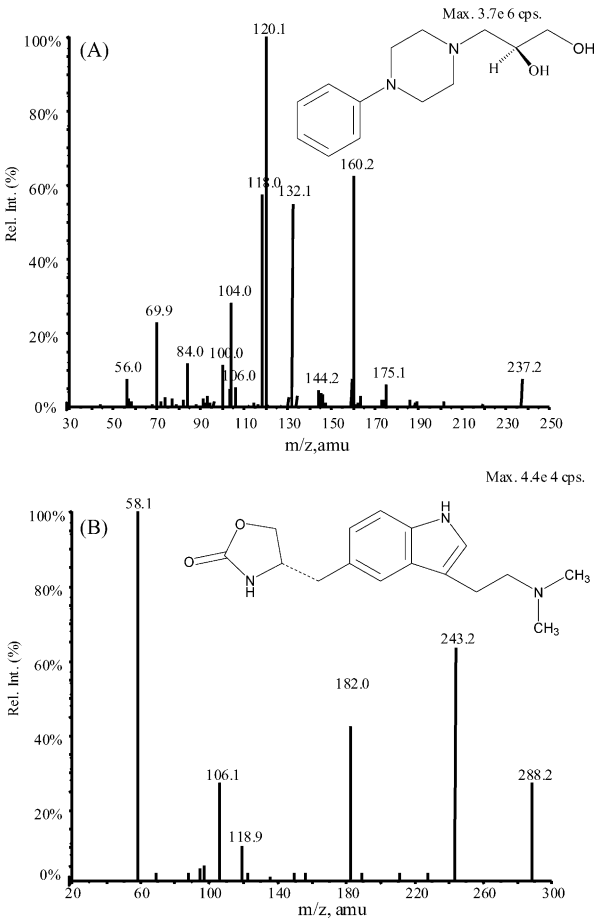

Fig. 1. Full-scan product ion spectra of $[M+H]^+$ and the structures for (A) levodropropizine and (B) zolmitriptan.

3. Results and discussion

3.1. LC–MS–MS

The chemical structures of levodropropizine and internal standard as well as the full-scan product ion spectra of $[M+H]^+$ are shown in Fig. 1. Strong signals were observed for the $[M+H]^+$ ion using ESI source. The MS parameters were optimized to maximize the response for levodropropizine in the positive ion mode.

Various combinations of acetonitrile, methanol, acetic acid and formic acid were investigated with a view to optimizing the mobile phase for sensitivity, speed and peak shape. Additions of formic acid and ammonium acetate reduced matrix effects without decreasing response and were included in the final mobile phase. A better response was achieved when 10 mM ammonium acetate was added into the mobile phase instead of pure water. A number of C_{18} columns (Nova-Pak, Hypersil and Zorbax) were evaluated. Compared to Nucleosil, they gave poor chromatography or matrix effects. Under the optimum assay conditions, analyte and internal standard were free of interference from endogenous substances and gave retention times of 1.6 and 1.4 min, respectively. This similarity of retention time reduced potential matrix effects and the run time for each sample (2.2 min), thus allowed a very high sample throughput (150–200 samples per day).

We found that zolmitriptan was a suitable internal standard because it is also a basic compound which forms a positive ion molecule easily through obtain of a proton by chemical ionization and has a good recovery under basic conditions during liquid–liquid extraction with dichloromethane and diethyl ether (2:3, v/v).

With these optimal conditions, no interference peak was identified for levodropropizine and the internal standard throughout the study. The matrix effects were evaluated by comparing the peak areas of levodropropizine (0.5, 40 and 400 ng/mL) and internal standard (10 ng/mL) in six QC samples (prepared by six different drug free plasmas) with those of the standard solutions, which were prepared in the same way as QC samples except water substituted for drug-free plasma. For levodropropizine, the relative error values calculated by mean peak area from the six samples were −8.9, −6.9 and −4.5% at concentrations of 0.5, 40 and 400 ng/mL, respectively; whereas the relative error values for zolmitriptan was −1.8%. Representative MRM chromatograms of blank plasma, plasma spiked with levodropropizne at 0.25 ng/mL and internal standard at 10 ng/mL, and a human plasma sample 24 h post-oral administration, were shown in Fig. 2. Low level of background and a stable baseline was maintained throughout.

3.2. Method validation

Quantification was conducted in the MRM mode at *m/z* 237 → *m/z* 120 for levodropropizine and *m/z* 288 → *m/z* 58 for internal standard. Good linearity was achieved over the range 0.25–500 ng/mL, with all coefficients of correlation greater than 0.99.

The resulting precision and accuracy data are presented in Table 1. The precision (CV) of the assay was less than 15% for each spiked calibrator concentration. The intra- and inter-day variability is shown in Table 1. For all the calibrators and QC samples, the precision CV of the assay was less than 11.5% and assay accuracy was in the range 87.6–112%. The lower limit of detection of the method was 0.05 ng/mL (S/N > 3). The LOQ was 0.25 ng/mL; at this concentration the precision was determined to be 11.5% and the accuracy was 112% (Table 1). The samples were stable when stored at 4 °C for 24 h

Table 1
Precision and accuracy for the determination of levodropropizine in human plasma (data are based on assay of six replicates on three different days)

Added concentration (ng/mL)	Found concentration (ng/mL)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Relative error (%)
0.500	0.499	8.04	11.4	−0.41
40.0	42.2	5.97	2.87	5.44
400	386	7.00	5.93	−3.60

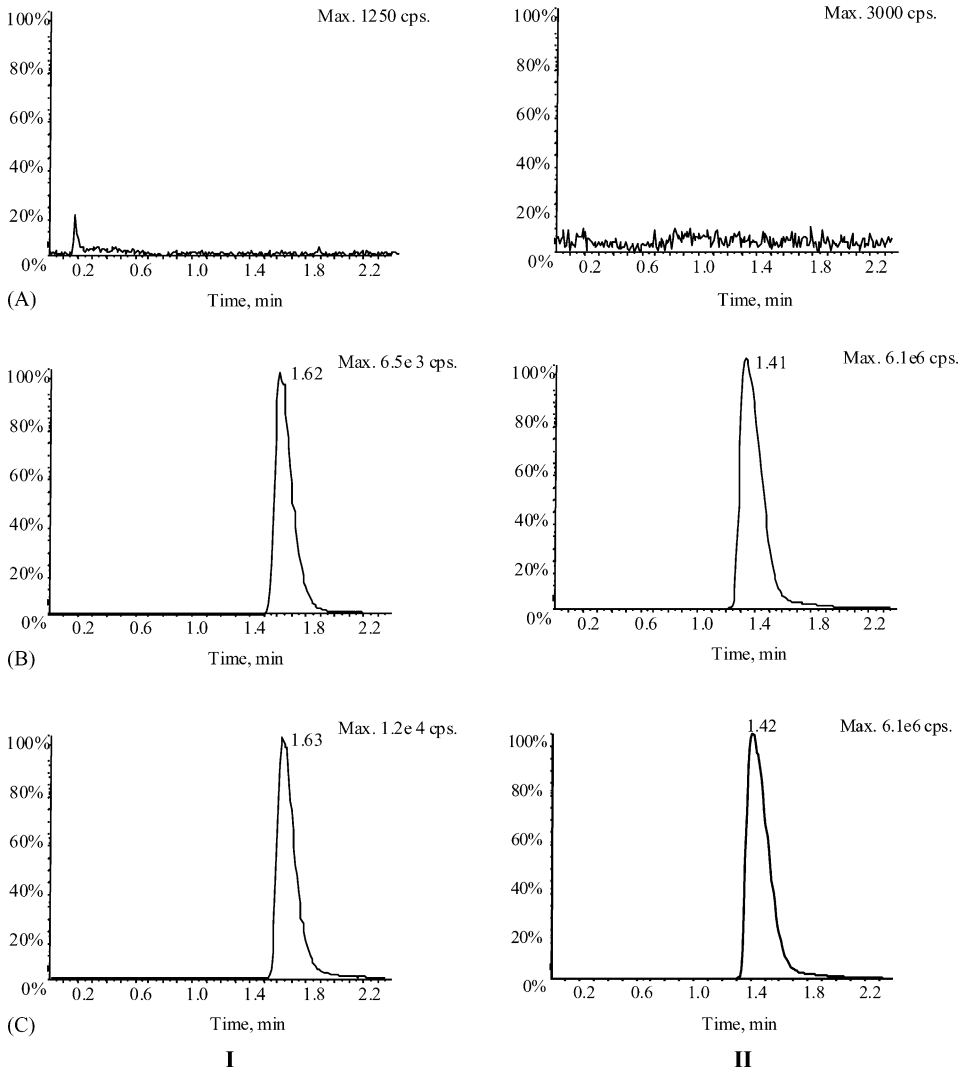

Fig. 2. Representative single reaction monitoring chromatograms of (A) blank plasma, (B) plasma spiked with levodropropizine and zolmitriptan at the limit of quantitation (0.25 ng/mL) and (C) a plasma sample 24 h after an oral administration (60 mg/day) of levodropropizine to healthy volunteers. Peak I, levodropropizine; Peak II, zolmitriptan.

Retention times and chromatographic profiles were reproducible in the method development. The stock solution of levodropropizine was stable for more than 1 month when stored at −20 °C and protected from light. The plasma samples were found to be stable for more than 1 month at the same condition. The QC samples after processed and stored at 4 °C for 24 h were also stable. The extraction recoveries of levodropropizine were 40.3 ± 4.0, 48.1 ± 2.8, and 44.3 ± 2.2% at concentrations of 0.5, 40 and 400 ng/mL, respectively. The extraction recovery of the internal standard at working concentration of 10 ng/mL was 62.8 ± 8.7%.

3.3. Method application

The applicability of the method was successfully demonstrated in human study. Fig. 3 shows the time course of levodropropizine plasma levels measured by this highly sensitive LC–ESI/MS method, administered levodropropizine orally at a dose of 60 mg/day. The mean C_{max} was 268.9 ± 109.6 ng/mL, the mean T_{max} was 0.76 ± 0.46 h and the mean $t_{1/2}$ was 2.66 ± 1.11 h.

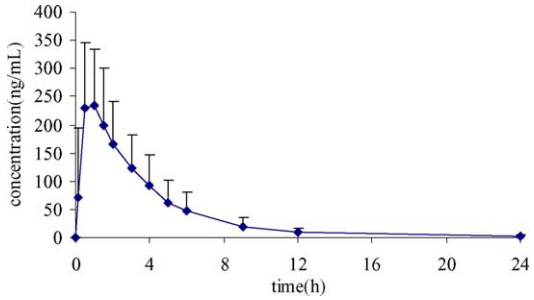

Fig. 3. Plasma concentration–time profile for levodropropizine after oral administration of levodropropizine. Data are mean ± S.D. for 22 healthy volunteers.

4. Conclusions

A highly sensitive, selective and rapid method for the determination of levodropropizine in human plasma was reported using high-performance liquid chromatography with tandem mass spectrometric detection. The sensitivity was sufficient to determine the drug in human plasma after oral administration making it suitable for use in pharmacokinetic studies. The method allows high sample throughput due to the short run time and relatively simple sample preparation procedure.

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