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Liquid chromatography–tandem mass spectrometry method for the quantification of deglymidodrine in human plasma

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Abstract

A simple, rapid and sensitive liquid chromatography–tandem mass spectrometry method was developed for quantification of deglymidodrine in human plasma. The plasma samples were pretreated by protein precipitation with trichloroacetate. The chromatographic separation was performed on reversed phase Aquasil C_{18} column, and the plasma extraction was eluted with a mobile phase solution consisting of acetonitrile (containing 0.02% formic acid) and water (containing 0.02% formic acid). The molecular ion of analyte was detected in positive ionization by multiple reaction monitoring. The mass transitions of m/z 198.4 → 148.1 and m/z 212.4 → 162.3 were used for detection of deglymidodrine and its internal standard, respectively. The assay exhibited linear ranges from 0.25 to 32 ng/ml for the analyte in human plasma. Acceptable precision and accuracy were obtained for concentrations of quality control (QC) samples. The proposed method has been successfully used to analyze human plasma samples for application in oral pharmacokinetic study.

Keywords: Deglymidodrine; Midodrine; LC–MS/MS; Pharmacokinetic; Plasma

1. Introduction

Midodrine, 2-amino-*N*-[2-(2,5-dimethoxy-phenyl)-2-hydroxyethyl] acetamide, is a peripheral a-receptor agonist capable of inducing venous and arterial vasoconstriction [1,2]. It has been approved in the United States for treatment of orthostatic hypotension, and several reports attest to its effectiveness in that setting [3,4]. Other studies suggest that midodrine may also be effective as a preventive measure in patients with vasovagal syncope [5,6]. Midodrine after oral or intravenous administration, undergoes an enzymatic hydrolysis in the systemic circulation, releasing its pharmacologically more active metabolite, deglymidodrine (Fig. 1), which loses the molecule of glycine present in midodrine [7]. Akimoto et al. studied the in vitro metabolism of deglymidodrine using human cytochrome P450 [8]. Oral midodrine may offer a useful treatment of hypotension associated with stunned myocardium [9].

Quaglia et al. have reported studies of deglymidodrine using high-performance liquid chromatography with LOQ 25 ng/ml [10]. A more sensitive determination method is needed for our oral pharmacokinetic study. In this paper we present a rapid and sensitive LC–MS/MS method for analysis of deglymidodrine in human plasma with simple extraction procedure, and for the oral pharmacokinetic study in human volunteers.

2. Experimental

2.1. Chemicals

Deglymidodrine (99.2% purity) was kindly supplied by Shanghai Institute of Materia Medica, Chinese Academy of Sciences, methoxamedrine (internal standard, IS) was purchased

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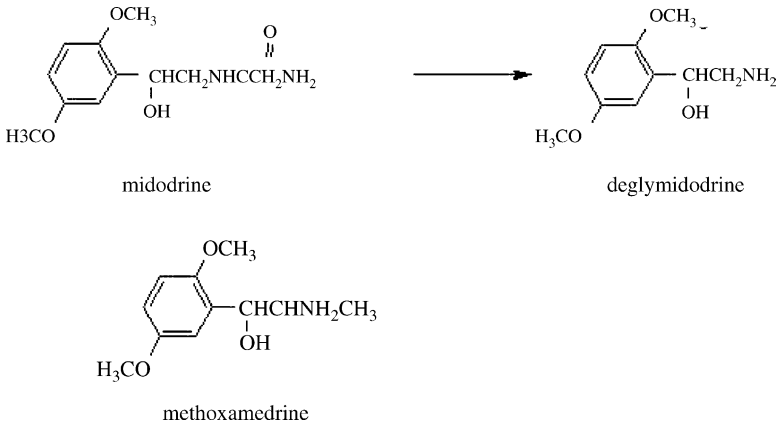

Fig. 1. Chemical structures of midodrine, deglymidodrine, and methoxamedrine (IS).

from Sigma (USA), midodrine tablet (2.5 mg, Lot 210631) was purchased from Nycomed Austria GmbH (Austria). Acetonitrile and methanol were of HPLC grade (Merck, Germany). Double-distilled water was used for the preparation of all solutions. All other reagents were of analytical grade.

2.2. Equipment and conditions

An Agilent (Agilent Technologies, USA) LC system was used consisting of an 1100 series pump and an autosampler. The chromatographic separation was carried out using an Aquasil C_{18} column (5 μm, 50 mm × 2 mm i.d., Thermo, USA) and a Symmetry C_{18} guard column at room temperature. The mobile phase consisted of 85% water (containing 0.02% formic acid) and 15% acetonitrile (containing 0.02% formic acid), which was pumped at a flow rate of 0.2 ml/min with isocratic elution. A 5 μl sample volume was injected to LC system.

Mass spectrometric detection was performed on an API 3000 triple quadrupole instrument (PE SCIEX, Toronto, Canada) using multiple reaction monitoring (MRM). A Turbo Ionspray interface in positive ionization mode was used. The precursor-to-product ion transitions m/z 198.4 → 148.1 for deglymidodrine and m/z 212.4 → 162.3 for internal standard were monitored. Data processing was performed with an Analyst 1.3.1 software package.

2.3. Sample preparation

To a 1.5 ml polypropylene test tube, 100 μl of plasma sample and 10 μl of IS (20 ng/ml) were added and vortex-mixed with 50 μl of 10% trichloroacetate for 1 min. After centrifuged at 10,000 rpm for 2 min, a volume of 5 μl of sample was injected into LC system.

2.4. Validation procedures

Standard stocks of deglymidodrine and methoxamedrine were prepared in methanol at a concentration of 0.1 mg/ml and stored at −4 °C. Calibration standards were prepared with blank plasma over the concentration of 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16, and 32 ng/ml for deglymidodrine. Quality control (QC) samples were prepared at nominal concentrations of 0.6, 15.0, and 25.6 ng/ml. Calibration and QC samples were treated at the same condition as the test samples.

Calibration curves were constructed by calculating peak area ratios (analyte/IS) as a function of plasma deglymidodrine concentration. These data were then fitted with a weighted ($1/x$) linear regression equation. The limit of detection (LOD) was defined as the lowest concentration with the ratio of signal-to-noise (S/N) ≥ 3.

The within-batch precision and accuracy was determined by analyzing six sets of quality control samples in a batch. The between-batch precision and accuracy was determined by analyzing six sets of quality control samples on three different batches. The quality control samples were randomized daily, processed and analyzed together with calibration samples. The precision of the method at each QC concentration was expressed as a coefficient of variation (CV) by calculating the standard deviation as the percentage of the mean calculated concentration, while the accuracy of the assay was determined as the ratio (percentage) of the mean with reference to the true value.

Recovery of analyte and internal standard were evaluated by comparing the mean peak areas of six processed samples at concentration of 0.5, 4.0, and 32 ng/ml to mean peak areas of six unprocessed of the corresponding concentration.

3. Results and discussion

3.1. Optimization of mass spectrometric and chromatographic conditions

In order to achieve the quantitative determination of deglymidodrine in plasma, the electrospray ionization interface parameters were optimized for maximum abundance of the molecular ions of the compounds. Acquisition parameters were determined by direct infusion into the mass spectrometer of 1 μg/ml solution of deglymidodrine and methoxamedrine (IS), at a flow rate of 10 μl/min. Variable mass spectrometric conditions (source temperature, ion spray voltage, collision energy, etc.) have been investigated. The positive ion electrospray mass

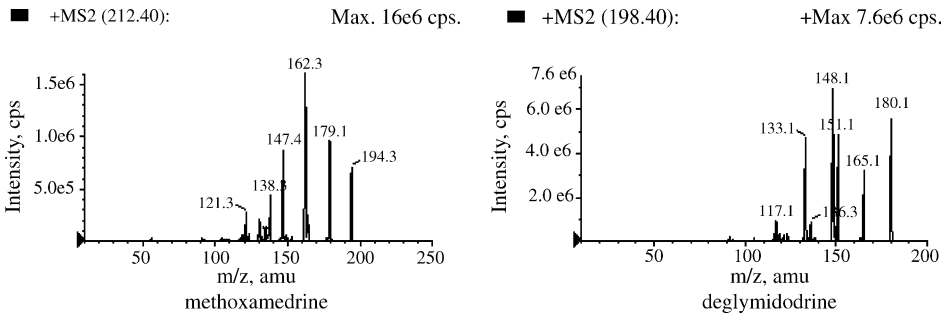

Fig. 2. Product ion mass spectra of $[M+H]^+$ of deglymidodrine and methoxamedrine.

spectrum (Fig. 2) of analyte and IS presented a molecular ions ($[M+H]^+$) at *m/z* 198.4, 212.4 and product ions at *m/z* 148.1, 162.3, respectively. It was found that the highest ion intensity for the molecular ion of analyte was achieved when the source temperature, ion spray voltage and collision energy were set at 400 °C, 2400 V, and 23 eV, respectively. An excellent specificity and sensitivity was attained under the present ion transition mass spectrometric conditions combined with liquid chromatographic separation. The limit of detection for deglymidodrine in human plasma was found to be 0.05 ng/ml. The percentage of acetonitrile in mobile phase was investigated. A low background interference and short retention time of analyte were observed when 15% acetonitrile to the mobile phase was set. The retention time was 2.36 min for analyte and 2.84 min for IS therefore a total run time of 3.5 min was achieved (Fig. 3).

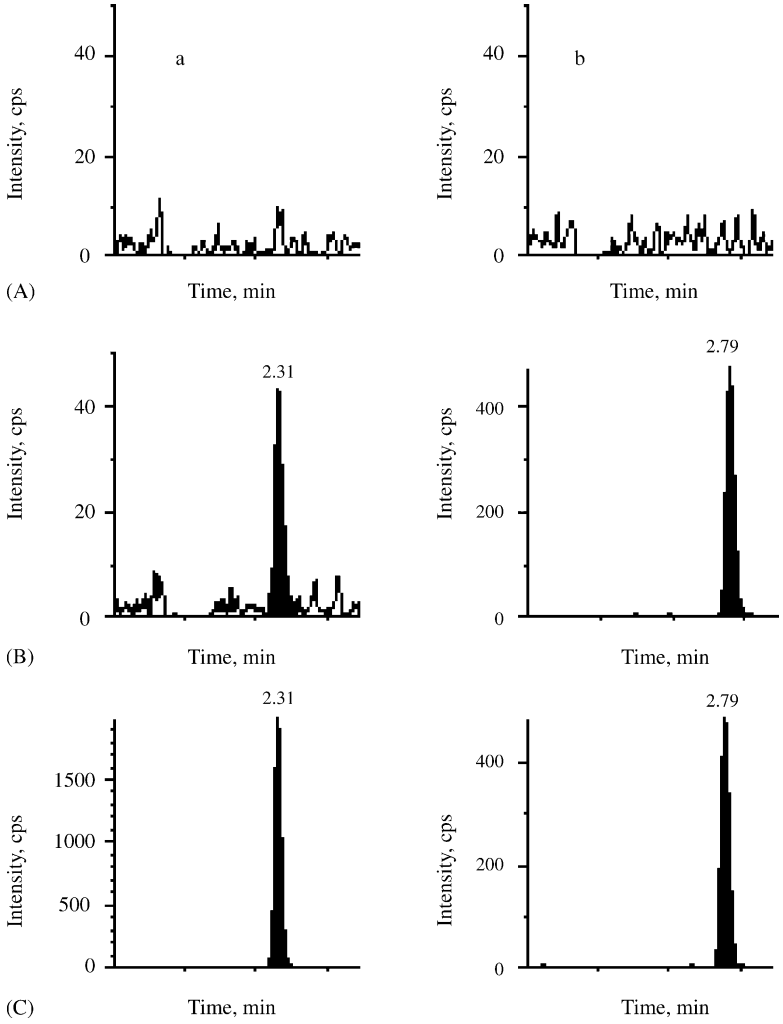

Fig. 3. Chromatograms of deglymidodrine and methoxamedrine (IS) in human plasma. (A) Blank plasma sample; (B) LOQ plasma sample with 0.25 ng/ml deglymidodrine; (C) plasma sample 0.75 h after oral administration of midodrine to human volunteer. (Peak a, b refer to deglymidodrine and IS, respectively.)

Table 1
Precision and accuracy of quality control samples for deglymidodrine in human plasma

Nominal concentration (ng/ml)	Within-batch ($n = 6$)		Between-batch ($n = 18$)	
	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)
0.6	3.51	91.5	4.68	100.4
15	3.17	93.7	3.42	98.3
25.6	2.84	90.7	4.59	98.0

Table 2
Stability of plasma samples ($n = 6$)

Nominal concentration (ng/ml)	Calculated concentration (ng/ml)								
	2 h	4 h	6 h	8 h	12 h	24 h	Average	S.D.	R.S.D. (%)
0.6	0.6	0.57	0.54	0.55	0.55	0.56	0.56	0.02	3.8
15	14.5	14.1	13.7	14.7	13.7	14.3	14.2	0.4	3
25.6	25.2	26.3	24.3	25.7	25.1	24.3	25.2	0.8	3.1

3.2. Validation procedures

The assay was linear over the validated concentration range from 0.25 to 32 ng/ml for deglymidodrine in human plasma (Fig. 4). The best fit for the calibration curve was obtained by using a weighting factor of $1/x$ for the analyte. The deviations from the nominal concentration were less than 8% at all concentrations. Correlation coefficient was at least 0.999.

The within- and between-batch assay data are presented in Table 1. Accuracies were 90.7–100.4% for all concentrations of quality control samples. Within-batch precision (expressed as coefficient of variation) was less than 3.51% and the mean between-batch precision was no more than 4.68%.

Recovery of analyte was investigated by adding three QC concentrations of deglymidodrine to blank human plasma and to equivalent volume of water then processed under same condition as test samples. The average recovery of analyte ranged from 94.6 to 106.4%, the recovery of internal standard was 97.7%.

Stability of deglymidodrine of processed samples was evaluated (Table 2). The room temperature storage at 2, 4, 6, 8, 12, and 24 h before analysis had little effect on the quantification. The matrix effect was examined and there was no signal suppression observed due to matrix effect during the ionization process by postcolumn infusion test.

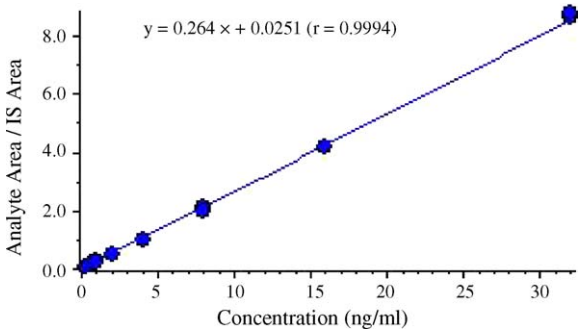

Fig. 4. Calibration curve for determination of deglymidodrine in human plasma.

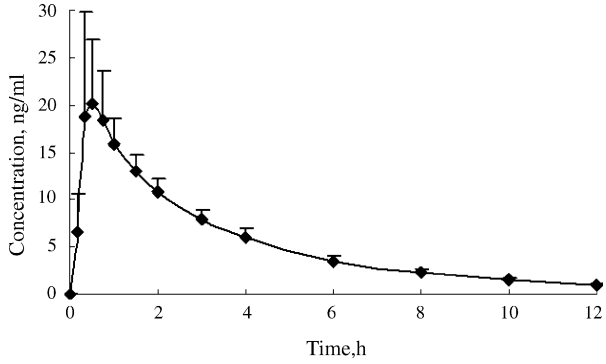

Fig. 5. Profile of mean plasma concentration of deglymidodrine vs. time after oral administration of midodrine to 20 healthy human volunteers.

3.3. Application

The described LC–MS/MS method was successfully applied to determine deglymidodrine concentration in human plasma samples from a bioequivalence study after a single oral dosing of midodrine capsule (10 mg) to 20 healthy male volunteers. Two milliliters of venous blood was draw and plasma was separated at the sampling times of 0 (predose) and 10, 20, 30, 45 min and 1.0, 1.5, 2, 3, 4, 6, 8, 10, and 12 h postdose. Fig. 5 shows plasma concentration profiles of deglymidodrine in the 20 subjects. C_{max} of the analyte was found to be 23.8 ± 9.4 ng/ml and T_{max} was reached at 20–45 min after oral administration.

4. Conclusions

The proposed liquid chromatographic–tandem mass spectrometric method enables a simple, rapid, and sensitive assay for the determination of deglymidodrine in human plasma with a run time less than 3.5 min. A simple sample pretreatment of protein precipitation was presented in the method. Mass spectrometric detection presents good sensitivity and selectivity of the proposed method. Recovery and precision studies successfully quantified deglymidodrine in human plasma samples. The

method had a limit of quantification of 0.25 ng/ml and was proved to be superior in sensitivity and speed of analysis with the analytical methods reported previously. Thus, the proposed method is suitable to support a wide range of pharmacokinetic study.

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