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Short communication

Quantification of prochlorperazine maleate in human plasma by liquid chromatography–mass spectrometry: Application to a bioequivalence study

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ABSTRACT

A sensitive and specific method using a one-step liquid–liquid extraction with dichloromethane followed by liquid chromatographic–electrospray ionization-mass spectrometric was developed and validated to determine prochlorperazine maleate in human plasma using amitriptyline hydrochloride as an internal standard. The samples were separated using a Thermo Hypersil-Hypurity C18 reversed-phase column (150 mm × 2.1 mm i.d., 5 μm). A mobile phase containing 10 mM ammonium acetate (pH 3.6)–methanol–acetonitrile (27:68:5, v/v/v) was used isocratically eluting at a flow rate of 0.22 ml/min. The average extraction recovery of prochlorperazine and internal standard were 81.8 ± 2.2% and 79.5 ± 3.7%, respectively. Prochlorperazine maleate and internal standard were measured by electrospray ion source in positive selective ion monitoring mode. The method demonstrated that good linearity ranged from 0.20 to 6.40 ng/ml with r^2 = 0.9989. The limit of quantification for prochlorperazine maleate in the plasma was 0.20 ng/ml. The established method has been successfully applied to a bioequivalence study of two prochlorperazine maleate formulations in 18 healthy male Chinese volunteers.

1. Introduction

Prochlorperazine maleate [2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-10H-phenothiazine(Z)-2-butenedioate (1:2)] is a phenothiazine derivative which is used as anti-psychotics. It is now seldom used for the treatment of psychosis and the manic phase of bipolar disorder. It has a prominent antiemetic activity and is most often used for the (short-time) treatment of nausea and vomiting and vertigo. A recent research about emergency department treatment of migraine headache indicated that prochlorperazine was statistically superior to octreotide in clinical success rate and pain relief in migraine patients [1].

Early publications have described methods of analyzing prochlorperazine in biological samples. The techniques adopted include GC [2], HPLC–ED/FD [3], HPLC–UV [4–7]. However, the reported methods are not sensitive and specific enough. They are time-consuming and require complex sample preparation [2,4,5] with long run times (>4 min) [2–7]. Highly sensitive and selective methods without time-consuming sample pretreatment are desired for bioavailability and bioequivalence studies. The latest assays using LC/MS/MS were reported [8–10]. However, due to the low sensitivity (LLOQ of 2.00 ng/ml) [8], long retention time (near 5 min) [9], lacking chromatography and validation details [10], or requirement of special testing equipment (tandem mass spectrometry), those methods were not suitable in most laboratories for studies involving low oral dose for current formulations and samples in high-throughput.

In this paper we described a simple and accurate LC–MS method to determine plasma prochlorperazine maleate concentrations using a one-step liquid–liquid extraction (LLE). The established method was applied to the bioequivalence study.

2. Experimental

2.1. Reagents and chemicals

Prochlorperazine maleate standard (purity >99.5%) was kindly supplied by Jean-Marie Pharmacal Co., Ltd., Hong Kong. Amitriptyline hydrochloride standard (purity >99.6%) was purchased from Great Southwest Pharmaceutical Co., Ltd., Chengdu, China; Ultrapure water prepared by a Millipore Milli-Q purification system (Millipore Corp. Bedford, MA, USA) was used to prepare the mobile phase. Acetonitrile and methanol were HPLC grade. Other chemicals and solvents were of analytical grade.

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2.2. Instrumentation

A Shimadzu LC–MS 2010 system (Shimadzu, Kyoto, Japan) was used, equipped with LC-10AD VP low pressure gradient pump, CTO-10A VP column temperature oven, SCL-10AD VP system controller, and LC–MS solution chemstation (version 2.04). Separation was achieved on a Thermo Hypersil-Hypurity C18 column (150 mm × 2.1 mm, i.d., 5 μm, USA) at 45 °C. Compounds were eluted up to a total retention time of 4.0 min using an isocratic mobile phase consisting of 10 mM ammonium acetate (pH 3.6)–methanol–acetonitrile (27:68:5, v/v/v) at a flow-rate of 0.22 ml/min, and the injection volume was 5 μl. The operating parameters of electrospray ionization-mass spectrometry (ESI–MS) were as follows: capillary voltage was 4.5 kV; Q-array was 25 V; nebulizer nitrogen gas flow-rate was 1.5 l/min; drying N_2 flow was 10 l/min; curved desolvation line (CDL) temperature was 250 °C; the gas used was of high purity, and system control and data evaluation were carried out using LC–MS solution chemstation (Japan); detector voltage was 1.7 kV. The mass selective detector (MSD) was operated in the positive ionization mode with selected-ion monitoring (SIM) at *m*/*z* 374 $[M+H]^+$ for prochlorperazine and *m*/*z* 278 $[M+H]^+$ for amitriptyline.

2.3. Preparation of stock solutions and calibration standards

Primary stock solutions of prochlorperazine maleate (105.1 μg/ml) and amitriptyline hydrochloride (I.S. 100.0 μg/ml) were prepared in methanol and all the stock solutions were stored at 4 °C without any appreciable degradation for 4 weeks. Working solutions were prepared from the stock solutions by serial dilution. Routine daily calibration curves and quality control samples were prepared in drug-free plasma. The calibration standards were made to obtain concentrations of 0.20, 0.40, 0.80, 1.60, 3.20, and 6.40 ng/ml. For the preparation of quality control samples, an independent stock solution was prepared and further diluted, to achieve concentrations of 0.40, 1.60 and 3.20 ng/ml, respectively.

2.4. Sample preparation and extraction procedures

Frozen human plasma samples were thawed at ambient temperature. A 50 μl aliquot of amitriptyline hydrochloride (I.S. 10 ng/ml) standard solution and 50 μl NaOH (1 M) were added to 500 μl of each plasma sample and then vortex-mixed. The mixture was extracted with 1.2 ml dichloromethane, vortex-mixed for 3 min, and centrifuged at 14,000 rpm for 5 min at room temperature. The organic layer was collected and evaporated to dryness under a gentle stream of nitrogen gas at 50 °C. The dried residue was dissolved with 50 μl mobile phase. After centrifugation, 5 μl of the clear supernatant was injected into the LC–MS system.

2.5. Assay validation

2.5.1. Assay specificity and matrix effect

Specificity was studied by comparing chromatograms of six different batches of blank plasma obtained from six subjects with those of corresponding standard plasma samples spiked with prochlorperazine maleate and I.S. and plasma sample after oral doses of prochlorperazine maleate tablets. To evaluate the matrix effect on the ionization of analyte, i.e., the potential ion suppression or enhancement, prochlorperazine at three concentration levels were added to the extract of 500 μl blank plasma, evaporated and reconstituted with 50 μl mobile phase. The corresponding peak areas (A) were compared with those of the prochlorperazine standard solutions evaporated directly and reconstituted with the same mobile phase (B). The ratio (A/B × 100)% was used to evaluate the matrix effect. The matrix effect of the I.S. was also evaluated with the same method.

2.5.2. Linearity

Plasma samples were quantified using the ratio of the peak areas of prochlorperazine to that of the I.S. as the assay response. To evaluate the linearity, plasma calibration curves were prepared and assayed in triplicate on 3 consecutive days over the range of 0.20–6.40 ng/ml, encompassing the therapeutic range of prochlorperazine maleate. Calibration curves were calculated using the peak area ratio vs. analyte concentration.

2.5.3. Precision, accuracy and extraction recovery

The precision and accuracy of the assay were determined from quality control (QC) samples. The intra-day precision was determined by repeating the analysis of the standard 5 times a day, and the inter-day CVs was determined by repeating the analysis on 3 consecutive days. Sample concentrations were determined using calibration standards prepared on the same day. The extraction recovery was determined by calculating the peak areas obtained from blank plasma samples spiked with analyte before the extraction with those from blank plasma samples, to which analyte was added after the extraction.

2.5.4. Stability

The stability experiments aimed at testing all possible conditions in which the samples might be exposed to during sample shipping and handling. The (1) short-term room temperature, (2) long-term storage, (3) stock solution, (4) post-preparative, and (5) freeze/thaw stabilities were tested. To test the stability of prochlorperazine in the plasma, QC samples were stored under different conditions. The freeze–thaw stability test was performed by freeze–thawing for 3 times. Freezing was performed at −20 °C for 24 h and thawed at room temperature. During each cycle, triplicate of 250 μl aliquots were processed, analyzed, and the results were averaged. Short-term stability was tested at room temperature for 24 h, and long-term stability was examined at −20 °C for 20 days. The results of the freeze–thaw, and short- and long-term stability tests were compared with those of the QC samples' averaged intra-day analysis (time zero). Post-preparative stability was tested by comparing after-day analysis with the first intra-day analysis.

2.6. Application to bioequivalence study

The bioequivalence study was approved by the Ethical Committee of Second Xiangya Hospital of Central South University, and all subjects signed informed consent before participating. The described method was used to analyze the plasma samples of 18 healthy male Chinese volunteers with an average age of 26.6 years, an average weight of 65.1 kg and an average height of 169.9 cm. Subjects were enrolled in this study after performing a medical history assessment, a physical examination, and standard laboratory test. Each volunteer was administered 15 mg of prochlorperazine maleate orally (JM-prochlorperazine maleate Tab®, 5 mg/tab [Jean-Marie Pharm. Co., Ltd., Hong Kong] and Stemetil Tab®, 5 mg/tab [Sanofi-Aventls, France]) using a standard 2 × 2 cross-over model in randomized order. A 10-day washout period was allowed between administrations. Approximately 4 ml blood samples were collected from each volunteer using a cannula inserted into the median cubital vein at the following time: predose and then at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after the drug administration. The blood samples were collected in separate vacutainers containing heparin as anticoagulant and centrifuged immediately. Then plasma samples were frozen at −20 °C until required for LC–MS analysis.

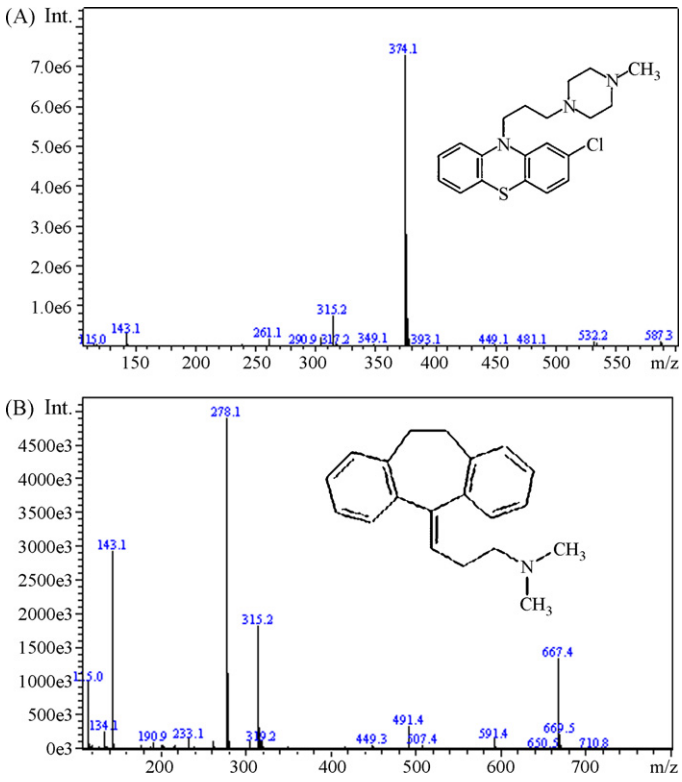

Fig. 1. ESI–MS positive ion scanning spectra and chemical structures of prochlorperazine (A) and amitriptyline (B).

Finally, C_{max} and T_{max} were determined using individual subject plasma concentration–time profiles. A non-compartmental model for extravascular input, provided by Drug and Statistics software (Version 2.0; Chinese), was used to calculate pharmacokinetic parameters, i.e., AUC_{0-24}, extrapolated $AUC_{0-\infty}$, and $t_{1/2}$. ANOVA was used to check the difference in the means of the pharmacokinetic parameters between the two preparations at a significant level of 0.05. Bioequivalence was determined by two one-sided *t*-tests [11].

3. Results and discussion

3.1. Chromatography and specificity

To develop this LC/MS-based method to quantify prochlorperazine maleate in human plasma, electrospray ionization (ESI) sources were evaluated in positive ion mode. ESI positive MS spectra for prochlorperazine and amitriptyline were dominated by the $[M+H]^+$ ions, i.e., *m*/*z* 374 for prochlorperazine and *m*/*z* 278 for amitriptyline. Typical positive ion scanning mass spectra of prochlorperazine and amitriptyline were shown in Fig. 1. The composition of the mobile phase was the critical factor to achieve good chromatographic peak shape and resolution. In the present study, 10 mM ammonium acetate (pH 3.6)–methanol–acetonitrile (27:68:5, v/v/v) was selected as an isocratic mobile phase. The retention time of prochlorperazine and amitriptyline was <4 min. The selection of amitriptyline hydrochloride as the I.S. was based on its chromatographic, extraction and MS behaviors. For example, the structure of amitriptyline is similar to prochlorperazine and they both contain nitrogen atoms in the molecules which are easily protonated during the ionization process to form a protonated molecular ion $[M+H]^+$.

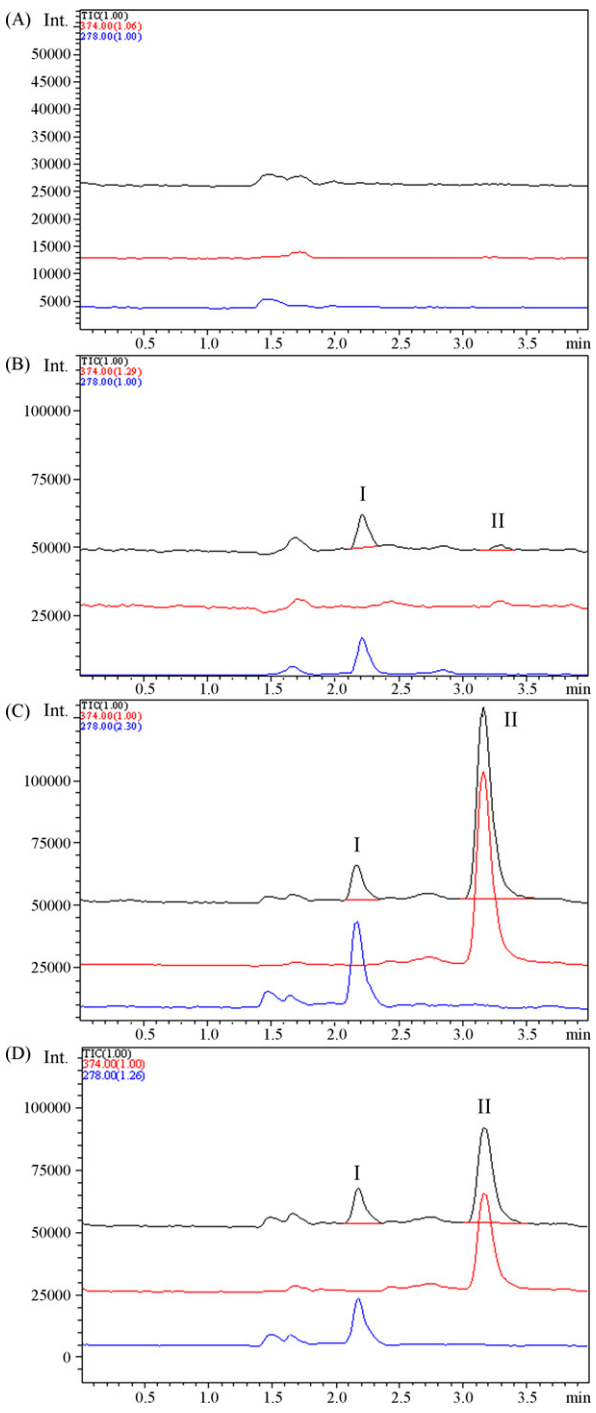

Fig. 2. Selective ion chromatograms of prochlorperazine and amitriptyline. (A) Blank plasma, (B) blank pasma spiked with 0.20 ng/ml (LLOQ) of prochlorperazine (II) and I.S. (I), (C) blank plasma spiked with prochlorperazine (II, 3.32 ng/ml) and I.S. (I, 10.00 ng/ml), (D) a plasma sample 6 h after a single dose of prochlorperazine maleate tablet (15 mg, 2.01 ng/ml) in healthy volunteers. The retention time of prochlorperazine and I.S. was 3.2 and 2.2 min, respectively.

Specificity was assessed by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. As shown in Fig. 2, no interference from the endogenous substance was observed at the retention time of prochlorperazine and I.S. In terms of matrix effect, all the ratios $(A/B \times 100)\%$ defined in Section 2 were between 85% and 115%, which means no matrix effect for prochlorperazine and I.S. in this method.

Table 1
Recovery, intra- and inter-day precision and accuracy of prochlorperazine maleate.

Concentration (ng/ml)	Recovery ($n=5$)		Intra-day ($n=5$)		Inter-day ($n=15$)	
	Mean (%)	CV%	Precision (R.S.D.%)	Accuracy (%)	Precision (R.S.D.%)	Accuracy (%)
0.40	79.8	7.6	7.9	101.5	11.8	104.8
1.60	81.4	8.1	9.2	96.9	9.9	96.9
3.20	84.2	7.6	8.2	100.5	10.9	99.0

Table 2
Stability data for prochlorperazine maleate.

Concentration (ng/ml)	24 h, room temperature ($n=5$)		−20 °C ($n=5$)		Freeze/thaw ($n=5$)	
	Mean	R.S.D. (%)	Mean	R.S.D. (%)	Mean	R.S.D. (%)
0.40	0.41	10.2	0.39	11.2	0.38	12.4
1.60	1.58	8.8	1.62	9.2	1.56	8.4
3.20	3.17	8.6	3.15	8.7	3.23	7.9

3.2. Linearity and lower limit of quantification

Calibration standards at 6 prochlorperazine maleate concentrations (ranging 0.20–6.40 ng/ml) were extracted and assayed. Least-squares linear regression was used to determine the plasma concentration from the peak area ratios (prochlorperazine vs. amitriptyline). The response was linear for prochlorperazine maleate throughout this concentration range and the correlation coefficients (r^2) were >0.99 for all standard curves using a $1/x^2$ weighted linear regression model. The typical equation was $y = 0.2530 \times C$ (ng/ml) $- 0.0381$ ($r^2 = 0.9989$). The lower limit of quantification (LLOQ) of prochlorperazine maleate, defined at S/N >10, was 0.20 ng/ml (Fig. 2). The precision of LLOQ were <9.1% and accuracy of LLOQ ranged 99.7–100.3%. During routine analysis each analytical run included a set of calibration samples, a set of QC samples in duplicate and the unknowns.

3.3. Precision, accuracy and extraction recovery

The extraction recovery, intra- and inter-day precision and accuracy values are shown in Table 1. The average extraction recovery of prochlorperazine and I.S. were 81.8 ± 2.2% and 79.5 ± 3.7%, respectively, and both the intra- and inter-day CVs were <12%.

3.4. Stability studies

The QC plasma samples were stable for 24 h at 4 °C and at room temperature (Table 2). In the long-term stability study, the plasma samples spiked with the QC plasma samples also showed no loss of the analytes when they were stored for 20 days at −20 °C (Table 2). The stock solutions were stable for at least 1 month. The difference between the time-0 QC plasma samples and the test solution in stock solution stability was less than 5% for both prochlorperazine maleate and amitriptyline hydrochloride. The post-preparative samples were stable at room temperature for at least 24 h including the residence time in the autosampler. The final stability test was demonstrated after 3 freeze–thaw cycles. No significant deterioration of the analytes was observed under any of these conditions (recovery ranged 95.0–102.5%, $n = 5$).

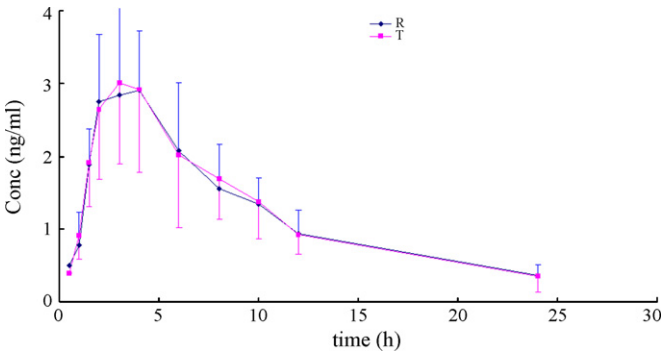

Fig. 3. Mean (±S.D.) plasma concentration–time profiles of prochlorperazine after the administration of 15 mg test and reference formulations to healthy Chinese male subjects.

Table 3
Pharmacokinetic parameters (mean ± S.D.) of prochlorperazine maleate, after the administration of an oral dose of 15 mg test or reference formulations to healthy Chinese male volunteers.

Parameters	Test formulation	Reference formulation
t_{max} (h)	3.2 ± 0.8	3.2 ± 0.9
C_{max} (ng/ml)	3.8 ± 1.0	3.6 ± 0.8
AUC_{0-24} (ng h/ml)	29.8 ± 4.6	29.5 ± 4.5
$AUC_{0-\infty}$ (ng h/ml)	34.5 ± 4.7	34.0 ± 5.8
$t_{1/2}$ (h)	8.4 ± 3.4	8.2 ± 2.2

3.5. Bioequivalence study

This method was successfully applied to the bioequivalence study of prochlorperazine maleate tablet in 18 healthy Chinese male volunteers. The mean plasma concentration–time profiles of prochlorperazine maleate after a single dose of 15 mg of either formulation were shown in Fig. 3. The pharmacokinetic parameters of the two prochlorperazine maleate formulations were shown in Table 3. The means and standard deviations of AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} for the test and reference formulations were similar, indicating that the pharmacokinetics of prochlorperazine maleate in the two formulations was similar. The 90.0% confidence intervals for the ratios of test drug to reference drug in terms of AUC_{0-t} and C_{max}, were within the range 80.0–125.0%, which is the range accepted by the State Food and Drug Administration [11].

4. Conclusion

A sensitive, rapid and specific LC–MS method using a simple liquid–liquid extraction procedure and isocratic elution has been described to determine prochlorperazine maleate in human plasma. The method has been successfully applied to a bioequivalence study by administering 15 mg of prochlorperazine maleate tablets to healthy Chinese male volunteers. The developed assay showed acceptable precision, accuracy, linearity, stability, and specificity. It can be easily repeated by most laboratories.

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