Development and Validation of an Improved Method for the Quantitation of Sertraline in Human Plasma using LC–MS–MS and Its Application to Bioequivalence Studies

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Abstract

A rapid and sensitive LC–MS–MS method for the quantitation of sertraline in human plasma was developed and validated. Sertraline and the internal standard, telmisartan, were cleaned up by protein precipitation from 100 µL of plasma sample, and analyzed on a TC–C18 column (5 µm, 150 × 4.6 mm i.d.) using 70% acetonitrile and 30% 10 mM ammonium acetate (0.1% formic acid) as mobile phase. The method was demonstrated to be linear from 0.1 ng/mL to 50 ng/mL with the lower limit of quantitation of 0.1 ng/mL. Intra- and inter-day precision were below 4.40% and 3.55%. Recoveries of sertraline at low, medium, and high levels were 88.0 ± 2.3%, 88.2 ± 1.9%, and 90.0 ± 2.0%, respectively. The method was successfully applied to a bioequivalence study of sertraline after a single oral administration of 50 mg sertraline hydrochloride tablets.

Introduction

Depression is a common psychiatric illness, and many people suffering from identifiable depression need psychiatric treatment or psychosocial intervention every year (1). Although the mechanism of antidepressants is not very clear, many antidepressants have been studied in clinical trials, including tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, and serotonin-norepinephrine reuptake inhibitors (2). Sertraline [(1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthyl(methyl)- amine], is a potent inhibitor of serotonin reuptake into presynaptic nerve fibers in the central nervous system (3) and has been clinically used for the treatment of depression and obsessive-compulsive behaviors (4). Due to the poor compliance, considerable genetic variability in metabolism, and the clinical heterogeneity of depression (3), the development for a robust therapeutic drug monitoring (TDM) method is quite challenging.

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Various analytical methods have been reported for the analysis of sertraline in biological samples such as capillary electrophoresis (5), gas chromatography-mass spectrometry (GC–MS) (1,6–11), high-performance liquid chromatography (HPLC) with fluorescence (12–13), diode array and UV detection (14–18), and LC–MS–MS (4,19–24). However, these methods have experienced some shortcomings that limited their applications to high sample throughput or pharmacokinetic studies in human. For example, several methods based on HPLC with UV or diode array detection (14–18) have long analytical run time more than 10 min. The sensitivity is relatively low (≥ 5 ng/mL) and even large amount of plasma (≥ 0.5 mL) have been used. Although GC–MS or LC–MS–MS methods have been developed for the determination of sertraline, they posses complex extraction with indispensable procedure of drying or derivatization (1,6–11,19,21), time-consuming chromatographic separation (4,19–21,23–24), and low sensitivity (1,4,6–7,9–11,19–24) due to their simultaneous determination of the metabolites of setraline and other drugs.

In this paper, a LC–MS–MS method was developed that provides a sensitivity limit of 0.1 ng/mL using 100 µL of plasma. The method exhibited excellent performance in terms of recovery and efficiency with a short run time of 2.8 min per sample, and simple sample preparation by protein precipitation. The method has been successfully applied to a bioequivalence study in 18 healthy volunteers treated with oral administration of 50 mg sertraline hydrochloride tablets.

Experimental

Materials and reagents

Sertraline hydrochloride (purity > 99.5%) and telmisartan (purity > 99.0%), the internal standard, were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol were HPLC-

grade, and all other chemicals were analytical grade and used without further purification. Distilled water, prepared from demineralized water, was used throughout the study. Blank human plasma from six different drug-free volunteers was obtained from Changchun Blood Donor Service (Changchun, China).

Calibration standards and quality controls

Stock solutions of sertraline and telmisartan (1.0 mg/mL, respectively) were prepared in methanol–water (50:50, v/v). Sertraline standard solutions with concentrations of 0.1, 0.3, 1, 5, 10, 20, and 50 ng/mL were prepared by dilutions of aliquots of the stock solution with methanol–water (50:50, v/v). Low, medium, and high concentration quality control (QC) solutions (0.3, 5, and 20 ng/mL) were prepared in a similar way. A working IS solution (telmisartan, 100 ng/mL) was also prepared in methanol–water (50:50, v/v). All the solutions were stored at 4°C.

LC–MS–MS conditions

The LC–MS system consisted of an Agilent 1100 series (Agilent Technologies, Palo Alto, CA) binary pump, an autosampler connected to a TC–C18 column (5 µm, 150 × 4.6 mm i.d., from Agilent Technologies) and an Applied Biosystems Sciex API-4000 Mass Spectrometer (Applied Biosystems Sciex, Ontario, Canada) with an ESI source. Oven temperature was maintained at 30°C. The mobile phase was formic acid–10mM ammonium acetate–acetonitrile (1:299:700, v/v/v) delivered at 1.0 mL/min. An approximately 1:1 split of the column eluent was included so that 0.50 mL/min entered the mass spectrometer.

The detector was operated at unit resolution in the multiple-reaction monitoring (MRM) mode using the transitions of the protonated molecular ions of sertraline at *m/z* 306.3 → 159.1 and telmisartan at *m/z* 515.2 → 276.1. MS parameters were optimized by syringe pump infusing of a solution containing analyte and IS in mobile phase. The determined parameters were as follows: curtain gas, gas 1, and gas 2 (nitrogen) had 15, 45, and 50 units, respectively; dwell time 200 ms; source temperature 450°C; ion spray voltage 1500 V. Declustering potential and collision energy were 38 V and 36 eV for sertraline and 96 V and 60 eV for telmisartan. The instrument was interfaced to a computer running Applied Biosystems Analyst version 1.3.2 software (Carlsbad, CA).

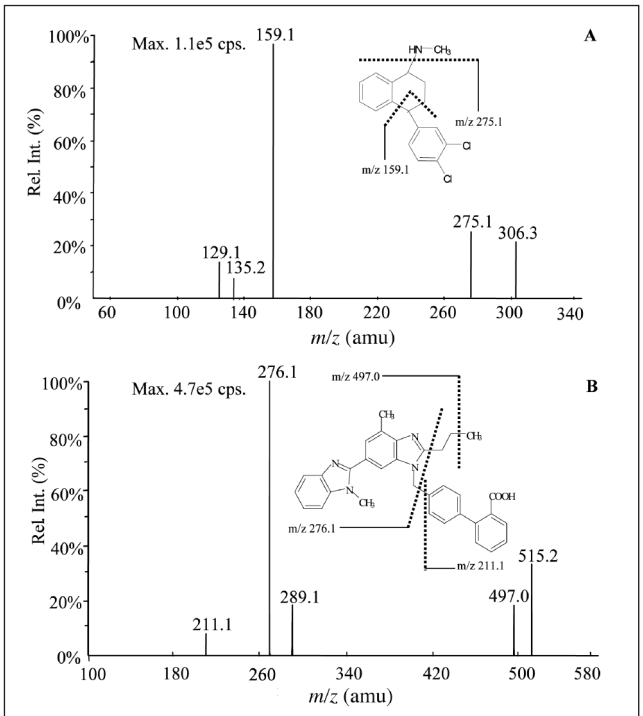

Figure 1. Full-scan product mass spectra and fragmentation pathways of sertraline (A) and telmisartan (B).

Sample preparation

Thawed samples were vortexed thoroughly at room temperature and were employed as follows: 100 µL IS solution, 100 µL methanol–water (50:50, v/v) or a standard or QC solution of sertraline and 1000 µL acetonitrile were added into 100 µL human plasma in a 1.5 mL Eppendorf tube. The mixture was vortex-mixed for 30 s and centrifuged at 12,000 × *g* for 10 min, the supernatant was transferred to an autosampler vial and 10 µL was injected into the LC–MS–MS system for analysis.

Validation

The method validation assays were performed according to US Food and Drug Administration (FDA) bioanalytical method validation guide (25). Three independent calibration curves based on seven spiked plasma samples (from 0.1 to 50 ng/mL) and six replicates at each concentration of 0.3, 5, and 20 ng/mL of sertraline were analyzed on three different days to validate the linearity of the method. Linearity was analyzed by weighted linear regression ($1/x^2$) of analyte-internal standard peak area ratios. Accuracy (as relative error) and precision (as RSD) were based on assay of six replicates of QC samples analyzed on three separate days. The lower limit of quantitation (LLOQ) was the concentration below which the inter-day RSD exceeded 20%. Recoveries of sertraline were determined by comparing peak areas of extracted QC samples with those of corresponding concentration QC solutions dissolved in the supernatant of the processed blank plasma. Stability tests of sertraline and IS including three freeze-thaw cycles, storage for 3 month at –20°C and at room temperature for 12 h were assessed by QC samples.

The matrix effects were evaluated by comparing the peak areas of sertraline in QC samples (0.3, 5, and 20 ng/mL) with those of the standard solutions prepared in the same way as QC samples except aqueous blanks substituted for drug free plasma.

Application of method

The pharmacokinetic study protocol in human is approved by the Ethic Committee of 302 Military Hospital of China. The method was applied to the evaluation of the bioequivalence of two tablet formulations of sertraline in 18 healthy adult male volunteers who received a single dose (50 mg sertraline hydrochloride tablets) in a two-period randomized crossover design with a 2-week washout period between doses.

Venous blood samples were collected before administration 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, and 120 h after dosing. Plasma samples were obtained by centrifugation of the whole blood at 3000 × *g* for 10 min and kept frozen at –20°C until analysis. Bioequivalence of the two formulations was assessed according to US-FDA methodology (26).

Results and Discussion

Mass spectrometry

Both analyte and IS responded best to the protonated molecular ions $(M+H)^+$. The full-scan product ion spectra and fragmentation pathways of sertraline and telmisartan are shown in Figure 1. Because the fragment ion at *m/z* 159.1 for sertraline and *m/z* 276.1 for telmisartan were the most abundant ion respectively, the precursor/product ion pairs at *m/z* 306.3 → 159.1 and 515.2 → 276.1 were selected for quantitation.

Table I. Accuracy and Precision for the Determination of Sertraline in Human Plasma*

Added Conc. (ng/mL)	Found Conc. (ng/mL)	Intra-day RSD(%)	Inter-day RSD(%)	Relative error (%)
0.10	0.099	4.38	3.21	–0.78
5.00	5.09	2.91	3.55	1.83
20.00	20.22	4.40	2.61	1.11

* 6 replicates per day on 3 different days.

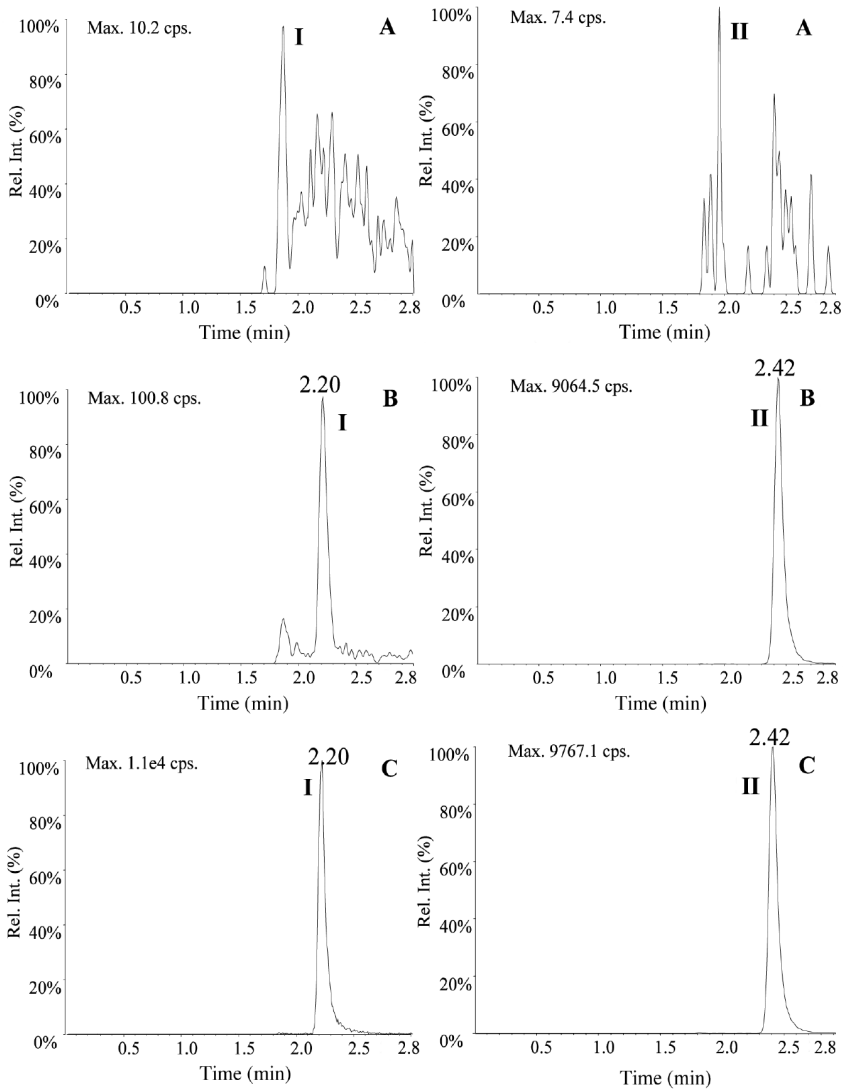

Figure 2. Representative MRM chromatograms ofblank plasma (A), the standard sample at LLOQ (0.1 ng/mL) (B), anda plasma sample at 4 h after an oral administration of 50 mg sertraline (C). Peak I, sertraline; peak II, telmisartan.

Chromatography

Sample pretreatment was performed by protein precipitation which was economical and convenient, with no extra steps of drying or derivatization. In the study, initially, both acetonitrile and methanol were used to precipitate protein, but acetonitrile showed more satisfactory efficiency in precipitating and extraction than methanol and had better response. Based on the strong enough response of sertraline, a comparatively large volume of acetonitrile (1000 µL) was used to provide an efficient protein precipitation and a clean chromatogram. At the same time, an insignificant matrix effect can be achieved. Moreover, the retention time of sertraline and IS are 2.20 and 2.42 min ensuring a run time of 2.8 min, which is shorter than most other methods previously mentioned (4–21,23–24).

Assay validation

The calibration curves were linear in the concentration range 0.1–50 ng/mL (r^2 > 0.9954). The present method offered an LLOQ of 0.1 ng/mL for sertraline using only 100 µL plasma, which was more sensitive than the reported methods (5–6,8–20, 22–24) and can improve the compliance of volunteers in the process of blood collection. Representative chromatograms of blank plasma, the standard sample at LLOQ (0.1 ng/mL) and a study sample containing a low concentration of sertraline are shown in Figure 2.

Intra- and inter-day precisions ranged from 2.91% to 4.40% and from 2.61% to 3.55%, respectively, and the accuracy was in the range of 90.6–112% (Table I).

Recoveries of sertraline at concentrations of 0.3, 5, and 20 ng/mL were 88.0 ± 2.3%, 88.2 ± 1.9%, and 90.0 ± 2.0%, respectively. The recovery of the IS was 86.4 ± 2.6% at a concentration of 100 ng/mL. No instability of analytes was observed under all the storage conditions evaluated with the nominal concentrations. Matrix effects were in the range of 102.0–108.4%, which showed no obvious ion suppression or enhancement from plasma matrix. Besides, both sertraline and IS gave a satisfactory recovery (> 88%) on the solvent extraction.

Pharmacokinetic study

The validated method was applied to the bioequivalence evaluation from 18 healthy volunteers treated with an oral dose of 50 mg sertraline hydrochloride tablets. The

mean plasma concentration versus time curve is shown in Figure 3. The main pharmacokinetic parameters of sertraline, similar to those reported in the literature (8,21–22), are shown in Table II. The elimination half-time ($T_{1/2}$) was estimated as 29.04 h and 29.92 h for the reference tablet and test tablet, respectively. And 3 subjects of the ratio of $AUC_{0-96\,h}$ to $AUC_{0-\infty}$ in this assay were less than 80%, which indicated that a 120 h (~ 4 half life) sample collection was meaningful. Approximately half of the samples (16 in 36) concentrations at 120 h were below 0.5 ng/mL, which indicated that the LLOQ of 0.1 ng/mL is necessary. The LLOQ of 0.1 ng/mL in this study was low enough to monitor the sertraline concentrations of samples collected within four half life time. In addition, the two tablets equivalent was found by calculating 90% confidence intervals of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ (after log-transformed) within the 80–125% required by the US-FDA (26).

Conclusions

A selective, sensitive and rapid method is developed for the determination of sertraline in human plasma using HPLC with detection by MS–MS. The LLOQ of 0.1 ng/mL is sufficient to determine the drug in human plasma after oral administration of 50 mg sertraline hydrochloride tablets with no drying or other extra steps in sample preparation process. The method allows high sample throughput (more than 200 samples per day) owing to the short run time and relatively simple sample preparation procedure. Finally, the method was successfully applied to the assay of human plasma samples from 18 volunteers taking a single dose.

Table II. Pharmacokinetic Parameters of Sertraline in Test and Reference formulations*

Parameter	Test formulation	Reference formulation
T_{max} (h)	5.22 ± 1.17	4.72 ± 1.18
C_{max} (ng/mL)	14.10 ± 6.49	14.51 ± 6.27
$T_{1/2}$ (h)	29.92 ± 7.43	29.04 ± 7.52
AUC_{0-t} (ng × h/mL)	457.86 ± 236.28	452.49 ± 275.10
$AUC_{0-\infty}$ (ng × h/mL)	502.95 ± 276.04	502.59 ± 324.23

*Data are mean ± SD, n = 18.

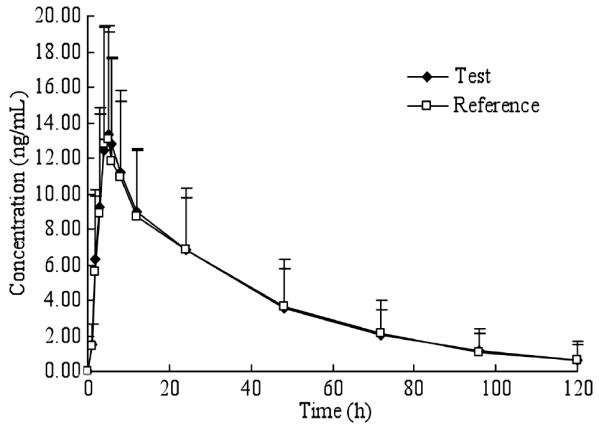

Figure 3. Mean plasma concentration-time curves for two sertraline tablet formulations in healthy volunteers (*n* = 18). Data are mean ± S.D.

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