Determination of Sertraline in Human Plasma by High-Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometry and Method Validation

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Abstract

A sensitive, simple, and specific liquid chromatographic method coupled with electrospray ionization–mass spectrometry (MS) is presented for the determination of sertraline in plasma. With zaleplon as the internal standard, sertraline is extracted from the alkalized plasma with cyclohexane. The organic layer is evaporated and the residue is redissolved in the mobile phase of methanol–10 mmol/L ammonium acetate solution–acetonitrile (62:28:10, v/v/v). An aliquot of 20 μL is chromatographically analyzed on a Shimadzu ODS C18 column (5 μm, 150- × 4.6-mm i.d.) by means of selected-ion monitoring mode of MS. The calibration curve of sertraline in plasma exhibits a linear range from 0.5 to 25.0 ng/mL with a correlation coefficient of 0.9998. The limit of quantitation is 0.5 ng/mL. The intra- and interday variations (relative standard deviation) are less than 7.8% and 9.5% (_n_ = 5), respectively. The application of this method is demonstrated for the analysis of sertraline plasma samples in a human pharmacokinetic study.

Introduction

Sertraline, [(1-S, _cis_)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-_N_-methyl-1-naphthalene amine] (Figure 1A), is a new antidepressant of the selective serotonin reuptake inhibitor (1). It is a naphthalenamine derivative that differs structurally from classic tricyclic antidepressants (TCA) (1). It is known that sertraline is as effective as TCA and has minimal side effects, such as insomnia, nervousness, nausea, diarrhea, dry mouth, and dyspepsia. Because of its minimal side effects, sertraline has become one of the most widely used medications for the treatment of depression as an alternative to TCA. Several methods for sertraline determination, including high-performance liquid chromatography (HPLC)–UV (2,3) and gas chromatography (GC)–MS (4,5), have been reported. However, for HPLC–UV methods, a relatively complex sample preparation was needed, either solid-phase extraction (2) or re-extraction (3), to remove the interference in biological fluid and separate sertraline.

Moreover, the sensitivity is rather low; thus, this method cannot meet the need of the pharmacokinetics and bioavailability studies of sertraline. As for GC–MS (4,5), a sophisticated derivatization techniques is required for the selective and sensitive determination of sertraline, and this is not suitable for routine analysis in clinical studies. This paper describes a simple, rapid, sensitive, and specific LC–electrospray ionization (ESI)–MS method for the determination of sertraline in the concentration range 0.5–25 ng/mL in human plasma.

Experimental

Chemicals

Sertraline and zaleplon [internal standard, (IS)] were supplied by the Jiangsu Institute for Drug Control (Nanjing, China). The reference tablets were supplied by Pfizer Pharmaceuticals Ltd. (Dalian, China) and the test tablets were supplied by the Department of Pharmaceutical Development (Lian Huan, Pharmaceutical, Yangzhou, China). Methanol and acetonitrile were HPLC/spetro grade and purchased from Merck (Darmstadt, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

Instrumentation and chromatographic condition

The LC system was a 2690 HPLC (Waters, Milford, MA), consisting of a quaternary pump, mobile phase vacuum degassing unit, autosampler, temperature-controlled column

Figure 1. Structure of sertraline (A) and zaleplon (B).
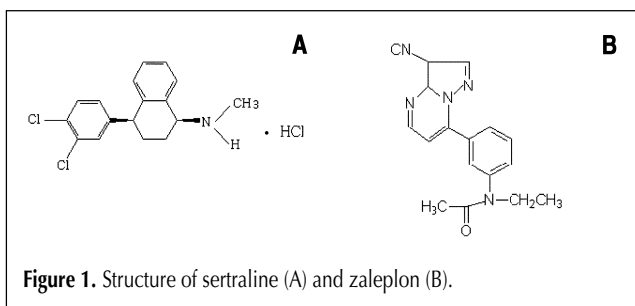
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compartment, and UV diode-array detector (DAD). A Shimadzu ODS C18 column (150- × 4.6-mm i.d., 5 µm) was used for all of the chromatographic separations. The mobile phase was methanol–10 mmol/L ammonium acetate solution–acetonitrile (62:28:10, v/v/v), run at a flow rate of 1 mL/min and split at the ratio of 1:1 before entering the ESI interface.

The MS analyses were performed with a Micromass three-quadruples MS equipped with an ESI source (Micromass, Manchester, U.K.). The responses of sertraline and zaleplon were measured in the positive ion mode with a capillary voltage of 3.65 kV, cone voltage of 15 V, extractor voltage of 1 V, source temperature of 100°C, desolvation temperature of 300°C, and desolvation flow of 400 L/h. The system was operated in the full scanning and then selected-ion monitoring modes, in which the target ions were $[(M+H)^+, m/z\ 306.2]$ for sertraline and $[(M+H)^+, m/z\ 306.1]$ for zaleplon.

Sample preparation

Twenty healthy, young, male volunteers were given a single oral dose of 50 mg sertraline in a random two-way cross-over design. Blood samples were collected at the time of 0, 1.5, 3, 4, 5, 6, 7, 8, 9, 10, 12, 24, 48, 72, and 96 h after oral administration of the tablets. The blood samples were immediately centrifuged at 3000 g for 10 min. The plasma was removed and stored at –20°C until the analysis was conducted.

Twenty microliters of the internal standard solution (1.0 µg/mL) was added to 0.5 mL of plasma, then vortex mixed for 30 s. Afterwards, 150 µL of 1.0 mol/L NaOH and 4 mL of cyclohexane were added and then vortex mixed for 3 min. Following centrifugation at 4000 rpm for 10 min, 3 mL of the organic layer was transferred to a 10-mL tube and evaporated to dryness in a water bath at 50°C under a nitrogen stream. The residue was redissolved in 100 µL of mobile phase. An aliquot of 20 µL was injected into the LC–MS system.

Calibration and quality control samples

Stock solutions of sertraline and zaleplon, with a concentration of 1 mg/mL, were prepared separately by dissolving 10 mg of each compound in methanol. Quality control samples were prepared by spiking blank human plasma of 0.5 mL with sertraline at three concentration levels (0.5, 4.0, and 15.0 ng/mL) on the day of blood sampling. The samples were stored at –20°C together with the unknown samples and were analyzed at each concentration level in each sample sequence.

Calibration samples were prepared by spiking 0.5 mL of blank human plasma with sertraline at 0.5, 1.0, 2.0, 4.0, 8.0, 15.0 and 25.0 ng/mL and 20 ng zaleplon [internal standard (IS)]. These calibration standards were then treated and analyzed in the same way as mentioned in the Sample preparation section. The ratio (Ai/As) of the peak areas of the analyte (Ai) and IS (As) in each fraction was calculated. The calibration curves were constructed by plotting the peak-area ratio versus concentration of sertraline, and the plot was then subjected to linear regression analysis.

Validation

The method was validated by analysis of human plasma quality control samples on 6 days. On the first day, 15 quality control samples (0.5, 4.0, and 15.0 ng/mL × 5) were analyzed. Over the following 5 days, the samples (0.5, 4.0, and 15.0 ng/mL × 1) were analyzed per day. The precision of the assay was studied by calculating the relative standard deviation (RSD) of replicate measurements at each concentration level of sertraline. Accuracy of the method was also investigated by calculating the difference from the theoretical value. To be acceptable, the calculated value of precision or accuracy should be less than 15% at all concentration levels.

The absolute recovery of extraction of sertraline was determined by comparing the peak-area ratio resulting from the sample after extraction with that obtained from the sample that contained the same amount of sertraline in the extracted plasma but that was not extracted again after the addition of the drug.

The limit of detection (LOD) was defined as the sample concentration resulting in a peak area of three times the noise level. The limit of quantitation (LOQ) was defined as the lowest drug concentration that can be determined with acceptable accuracy and precision. Over one month, the stability of sertraline was studied in both frozen plasma (–20°C) and processed samples left at room temperature (20°C ± 3°C) over 24 h.

Results and Discussion

At the start of this investigation, the authors tried to use the published method (3) to analyze sertraline in blood by HPLC coupled with UV detection. However, some endogenous interference gave a very similar response and could not be resolved perfectly from the analyte. More critically, the LOD reported in the reference was 7.5 ng/mL of sertraline, which was not sensitive enough to meet the requirement of detection in this investigation. As for GC–MS, it is reported that 0.1 ng/mL of sertraine in the human plasma can be detected (4). However, derivatization techniques are complex, time-consuming, and need a greater amount of serum—at least 1 mL (4,5). Speed and simplicity are important factors when considering the analysis of 600–700 samples from clinical trials; therefore, GC–MS is not suitable. In this paper, the HPLC–ESI-MS technology described provides a simple, rapid, selective, and highly sensitive alternate for the analysis of sertraline contained in biological fluids, especially for a large number of samples at low concentrations resulting from animal or human (or both) pharmacokinetic study.

Sample preparation

Liquid–liquid extraction (LLE) was necessary and important because this technique can not only purify but also concentrate the sample, although the usage of an MS detector can satisfy the demand of sensitivity to certain extent. Ethyl acetate, cyclohexane, and ether were all tested for the extraction. Cyclohexane was finally adopted because of no interfering peaks at the retention times of sertraline and IS, although its extraction efficiency is a little lower than ethyl acetate and ether. An amount of 150 µL NaOH (1.0 mol/L) was added to the mixture of plasma and cyclohexane in order to increase extrac-

tion efficiency because sertraline itself is an alkalic drug. The results of the absolute recovery of extraction test of sertraline are shown in Table I.

Selection of IS

It is necessary to use an IS to get high accuracy when MS is used for detection. Zaleplon, diazepam, and mirtazapine were investigated to find out which was the more suitable one. Zaleplon was adopted in the end because of its similarity of retention action, ionization, and extraction efficiency, as well as less endogenous interference at m/z 306.1 (structure shown in Figure 1B).

Analytical conditions and optimization

The sensitivity and selectivity in the HPLC–MS mainly depended on the analyte properties (i.e., how easily it is ionized) and the compositions of the mobile phase used. Increasing the percentage of organic solvent in mobile phase will strengthen the MS signal, and methanol generally gives a stronger signal than acetonitrile. However, acetonitrile can produce a more effective separation than methanol. Therefore, a mobile phase of methanol–10 mmol/L ammonium acetate solution–acetonitrile (62:28:10, v/v/v) was adopted. Under these conditions, the peak shape of sertraline and IS was good, and no significant endogenous interference appeared near the retention in the chromatogram; the retention time of sertraline and IS were 5.7 and 2.5 min, respectively. Such short retention times were also very important to ensure the analytical speed of a large number of samples from clinical trials.

Because sertraline is a weak base, positive ESI is the best option for the molecule to obtain a high sensitivity. Different cone voltage values from 10 to 40 V were tested in order to increase the sensitivity of detection, and the strongest response was obtained at 15 V. Other MS parameters (desolvation temperature, flow, and extractor voltage) were adjusted to get a maximum signal for the sertaline hydrogen adduct $[M+H]^+$. The ion at m/z 306.2 for sertraline was not detected in the mass spectrum of zaleplon (m/z 306.1). Therefore, the ions at m/z 306.2 and 306.1 were monitored by single ion monitoring as the target ions.

Specificity

Comparing the HPLC–MS chromatograms of blank and validation samples obtained from validation experiments with those of control human plasma from individual volunteers showed that the procedure has a very good specificity for sertraline and IS. The typical chromatograms of blank plasma,

blank plasma spiked with sertraline and IS, as well as the volunteer's plasma, are shown in Figure 2.

Assay performance

Assay performance of the present method was assessed by the following criteria: linearity, accuracy, precision, stability, recovery, LOD, and LOQ. Results for the calibration curve showed good linearity (r = 0.9998) over the concentration range 0.5–25 ng/mL with an equation of:

$$f = 0.1967c - 0.00101 \qquad \text{Eq. 1}$$

where c is the sertraline concentration in ng/mL and f is the sertraline area/zaleplon area. Calibration curve intercepts were not significantly different from zero. Accuracy values were within acceptable limits (Table II). The results of intra- and interday precision for control samples ranged from 1.5% to 9.5%, respectively (Table III). Sertraline is stable in plasma samples stored at –20°C for at least 1 month. The stability of sertraline in processed samples left at room temperature(20°C ± 3°C) over 24 h was also studied from our laboratory. The IS were also stable under these conditions. The LOD was 0.2 ng/mL and the LOQ was 0.5 ng/mL (signal to noise ratio = 3). All these results indicate that the method was reliable within the analytical ranges, and the use of the internal was very effective for reproducibility by LC–MS.
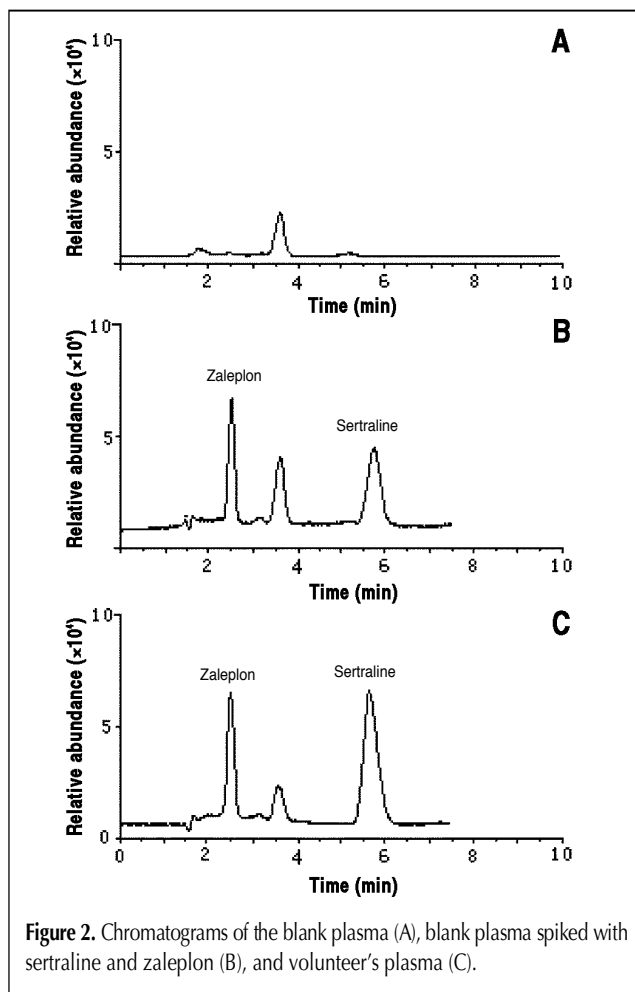

Figure 2. Chromatograms of the blank plasma (A), blank plasma spiked with sertraline and zaleplon (B), and volunteer's plasma (C).

Table I. Recovery of Sertraline from Plasma (n = 5)

Added (ng/mL)	Recovery (%)	%RSD
0.5	84.5 ± 9.6	11.4
4.0	94.3 ± 4.7	4.9
15.0	106.1 ± 2.2	2.0

Results of pharmacokinetic study

The applicability of this method has been demonstrated by the determination of sertraline in plasma from 20 subjects that each received an oral dose of 50 mg sertraline in a bioequivalent study. The developed procedure was sensitive enough to assure the quantitative analysis of sertraline in plasma, with acceptable accuracy and precision over a period of 96 h after a single oral administration. The pharmacokinetic parameters of sertraline were calculated using 3P97 program (the Chinese Society of Mathematical Pharmacology, Beijing, China), and a two-compartment open linear pharmacokinetic model was fitted to the plasma concentration data. Values for area under the curve (AUC) and the plasma elimination half-life of the β-phase ($T_{1/2}$) were calculated by the following equations:

$$AUC_{0 \to 96} = \sum (C_i + C_{i-1}) \times (t_i - t_{i-1})/2 \qquad \text{Eq. 2}$$

$$AUC_{0 \to \infty} = \sum (C_i + C_{i-1}) \times (t_i - t_{i-1})/2 + C_n/\lambda_n \qquad \text{Eq. 3}$$

$$T_{1/2} = 0.693/\lambda_n \qquad \text{Eq. 4}$$

where λ_n is the elimination rate constant of terminal phase. Data were expressed as mean ± standard deviation of sample. The mean plasma concentration-time curve of sertraline of the 20 volunteers is shown in Figure 3. The pharmacokinetic parameters of the test and reference tablets are listed in Table IV. The test tablets were found to be bioequivalent to the reference.

Table II. Accuracy for the Method for Determining Sertraline in Human Plasma (*n* = 5)*

Conc. added (ng/mL)	Conc. found (*x* ± s, ng/mL)	Accuracy (%)
0.5	0.54 ± 0.05	8.60
4.0	4.02 ± 0.21	3.77
15.0	14.40 ± 0.20	1.38

* Expressed as relative error (%).

Table III. Precision of the Method for Determination of Sertraline (*n* = 5)

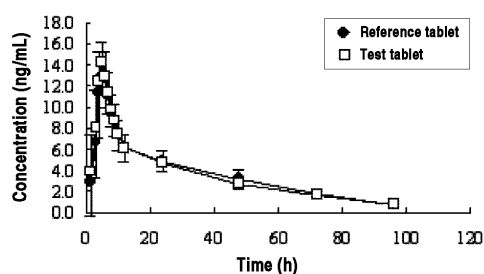| Conc. added (ng/mL)	Within-day		Between-day	
	x ± s	%RSD	*x* ± s	%RSD
0.5	0.55 ± 0.04	7.8	0.54 ± 0.05	9.5
4.0	3.84 ± 0.15	3.9	4.02 ± 0.21	5.2
15.0	14.28 ± 0.21	1.5	14.39 ± 0.25	1.7

Figure 3. Mean drug plasma concentration-time curve of sertraline in 20 volunteers after oral administration.

Table IV. Pharmacokinetic Parameters of 20 Healthy Male Volunteers after Oral Administration of 50 mg Sertraline

Parameters	Test	Reference
$T_{1/2}$/h	29.53 ± 5.40	29.32 ± 5.02
C_{max}/ng/L	15.86 ± 2.78	15.24 ± 2.52
T_{max}/h	4.8 ± 0.8	5.1 ± 1.3
$AUC_{0 \to 96}$/ng × h × mL^{-1}	338.0 ± 29.34	347.96 ± 41.0
$AUC_{0 \to \infty}$/ng × h × mL^{-1}	370.95 ± 31.68	381.78 ± 41.68

Conclusion

A simple, rapid, specific, and highly sensitive method for the determination of sertraline in plasma by HPLC–ESI-MS has been developed and validated for use for the analysis of sertraline in plasma samples resulting from a human pharmacokinetic study. It serves as a suitable alternative to other published methods for large numbers of biological samples because of its simple LLE processing and short analytical time.

References

1. G.K. McEvory, K. Litvak, and O.H. Welsh. "Sertraline". In *Drug Information*. American Hospital Formulary Service, Bethesda, MD, 1995, p. 1497.
2. D. Rogowsky, M. Marr, and G. Long. Determination of sertraline and desmethylsertraline in human serum using copolymeric bonded-phase extraction, liquid chromatography and gas chromatography-mass spectrometry. *J. Chromatogr B* **655(1):** 138–41(1994).
3. Y. Chen, H. Xiao, and R.J. Wu. Simultaneous determination of paroxetine and sertraline in human plasma by RP-HPLC [J]. *J. China Pharm. Univ.* **4(2):** 141–43 (2003).
4. K.M. Kim, B.H. Jung, M.H. Choi, J.S. Woo, K.J. Paeng, and B.C. Chung. Rapid and sensitive determination of sertraline in human plasma using gas chromatography-mass spectrometry. *J. Chromatogr. B* **769:** 333–39 (2002).
5. C.J. Zhu, J.F. Wu, and Z.W. Au. Bioequivalence evaluation of two sertraline tablet formulation in healthy male volunteer after a single dose administration. *Int. J. Clin. Pharmacol. Ther.* **37(3):** 120–24 (1999).