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Rapid and sensitive determination of sertraline in human plasma using gas chromatography–mass spectrometry

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Abstract

A method for the determination of sertraline in human plasma using gas chromatography–mass spectrometry (GC–MS), with the selected ion-monitoring (SIM) mode, was described. The following was used in this study: (1) single liquid–liquid extraction at alkaline pH after deproteinization of plasma protein and (2) perfluoroacylation with HFBA, which has higher sensitivity (about 10-fold) compared with previous reported derivatization. The detection limit for the SIM of sertraline as an N-HFB derivative was 0.1 ng/ml, and its recovery was 80–85%. The linear response was obtained in the range of 0.2–10.0 ng/ml with a correlation coefficient of 0.999. The coefficient of variation (C.V.%) was less than 12.1% in the 1–30 ng/ml, and less than 18.2% at 0.2 ng/ml, and the accuracy was less than 10% at all of the concentration range. These findings indicate that this assay method has adequate precision and accuracy to determine the amount of sertraline in human plasma. After pharmacokinetics was performed with this assay method following oral administration of sertraline hydrochloride in man, moment analysis revealed that pharmacokinetic parameters for sertraline (C_{max}, 10.3 ng/ml; T_{max}, 8.0 h; $T_{1/2}$, 28.6 h) were similar to previously reported results. These results indicate that this simple and sensitive assay method is readily applicable to the pharmacokinetic studies of sertraline.

Keywords: Perfluoroacylation; Sertraline

1. Introduction

Sertraline;(1-*S*,*cis*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthalene amine (Fig. 1), 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 [2] and has minimal side effects, such as insomnia, nervousness, nausea, diarrhea, dry mouth and dyspepsia. In contrast, TCA commonly causes a number of troublesome side effects, including sedation, hypotension, cardiotoxicity, and urinary retention [3]. Moreover, risk of adverse effects in TCA increases sharply with increasing plasma levels [4], and they are especially

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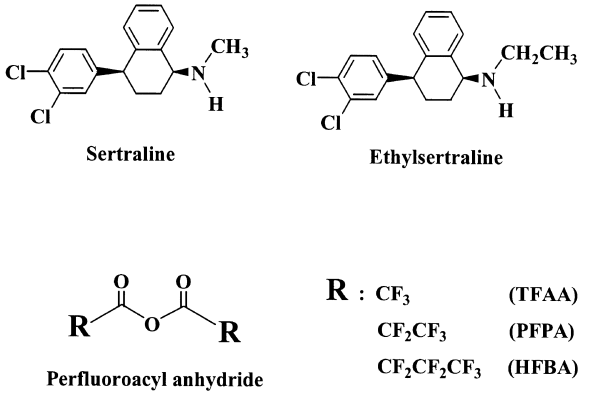

Fig. 1. The structures of sertraline, ethylsertraline, and three types of derivative reagents as the perfluoroacyl anhydride.

lethal in overdose [3].

Due to its minimal side effects, sertraline has become one of the most widely used medications for the treatment of depression as an alternative to TCA.

Recently, many methods have been developed for the determination of sertraline in biologic specimens. Almost all assays are based on the separation by gas chromatography [5–8] and high-performance liquid chromatography [9,10].

In order to remove the interference in biological fluid and separate sertraline, re-extraction at a different pH using another organic solvent [5,6,8] or solid-phase extraction [7] were proposed in previous studies. In addition, derivatization techniques like perfluoroacylation with TFAA; trifluoroacetic anhydride [6] or MBTFA; *N*-methyl-bis(trifluoroacetamide) [8] was used for the selective and sensitive determination of sertraline in the analysis of GC–MS.

However, it is not desirable to accept complex extraction methods, such as those in the re-extraction in the clinical studies which have large sample numbers. In the derivatization, TFAA-derivatization was influenced by the interference of the blank plasma, and it was not possible to achieve an acceptable detection limit for the clinical study in MBTFA-derivatization in our preliminary study.

Therefore, we developed and validated a rapid and sensitive determination method for sertraline in human plasma with a pg/ml level of detection limit (0.1 ng/ml). This method contains simple liquid–liquid extraction and highly sensitive HFBA-derivatization. We also compared sensitivity and efficacy in two other types of perfluoroacylation reagents, pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA) with TFAA.

The plasma concentration after oral administration of sertraline was also performed by this assay method for the pharmacokinetics of sertraline.

2. Experimental

2.1. Materials

Sertraline and ethylsertraline (internal standard, I.S.) were obtained from Hanmi Pharmaceutical (SungNam, South Korea). Perfluoroacylating reagents (TFAA, PFPA, and HFBA) were purchased from Sigma (St. Louis, MO, USA). Fig. 1 shows the structure of sertraline, ethylsertraline, and perfluoroacylating reagents. Na_2HPO_4, NaOH and Na_2SO_4 were purchased from Junsei Chemical (Tokyo, Japan). All solvents were HPLC grade and purchase from J.T. Baker (Phillipsburg, USA). The solvents were used without further purification. Standard solutions of sertraline and ethylsertraline at the concentrations of 1.0 mg/ml were prepared in methanol, and they were kept below 4 °C until they were used. Diluted standard solutions were prepared by serial dilution of the standard solutions (1.0 mg/ml) in methanol at the appropriate concentration.

2.2. Sample treatment

To plasma samples (1 ml), 2 ml of acetone and 40 ng of ethylsertraline (I.S.) were added. Then, the mixture was vortexed and centrifuged at 14 000 rpm for 10 min. After the removal of plasma protein, 3 ml of supernatant was separated, and 1 ml of Na_2HPO_4(0.05 *M*)–NaOH (0.1 *M*) buffer was added to adjust the pH 12.0. The extraction was performed by shaking the supernatant with 3 ml of diethylether. After it was shaken for 10 min with subsequent centrifugation (5 min at 2500 rpm), placing it in a dry ice–acetone bath separated the ether layer. The ether layer was evaporated under a stream of N_2 gas to dryness. Then, derivatizing reagent (20 μl), acetone (100 μl), and anhydrous Na_2SO_4 (about 20 mg) were added to the dried residues. The mixture

was placed in a heating block (50 °C) for 30 min and then taken to dryness under a gentle stream of N_2 gas. The residue was reconstituted with 40 μl of ethyl acetate, and 2 μl of aliquots were injected into the GC using an auto-sampler.

In order to select the most effective and sensitive derivatizing reagent, a residue of spiked plasma samples, which contained 1 ng/ml of sertraline and 40 ng/ml of ethylsertraline, was derivatized with TFAA, PFPA and HFBA, respectively, and injected into GC–MS.

2.3. Gas chromatography–mass spectrometry

A Hewlett-Packard GC–MS system consisting of a Model 5890 SERIES II gas chromatograph and a Model 5989B mass spectrometer was used. Samples were injected into a fused-silica capillary column coated with cross-linked methyl silicone (Ultra-1, 17 m×0.2 mm I.D., 0.11 μm film thickness) in the split-injection mode (1:5).

The oven temperatures were as follows: the initial temperature was 160 °C. It was increased to 220 °C at a rate of 10 °C/min and held there for 10 min. It was finally increased to 320 °C at a rate of 50 °C/min and held there for 2 min. The electron energy was 70 eV, the ion source temperature was 200 °C and the injector temperature was 280 °C. Helium, as a carrier gas, was set to a column head pressure of 25.5 kPa (column flow: 1 ml/min at 160 °C). The selected ion-monitoring (SIM) mode was used. The quantitation ions for sertraline and ethylsertraline were *m/z* 501 and *m/z* 515, respectively (Table 1). The dwell time for each ion was set at 100 ms.

2.4. Pharmacokinetics following oral administration of sertraline in man

Five healthy volunteers (male, mean age, 26.1±4.2 years; mean weight, 68.4±5.5 kg; mean height, 173.4±6.6 cm) participated in this study. The subjects were fasted overnight, and then they received at 07:00 h a single 50-mg dose of sertraline tablets (Zoloft from Pfizer, South Korea). Approximately 10 ml of blood samples were taken from a cubital vein via a butterfly cannula before oral administration of the sertraline tablet and at 0.5, 1, 2, 4, 6, 8, 10, 12, 25, 48, and 72 h after dosing. The plasma samples were separated immediately after centrifugation at 2500 rpm for 15 min and store at −20 °C until they were analyzed.

Table 1
Comparison of GC–MS data on SIM-mode for sertraline as their three types of derivatives

Derivatives	MW	RT (min)	Characteristic ions (*m/z*)	LOD[a] (ng/ml)
TFAA	402	9.53	*401*, 274	1.0
PFPA	452	9.36	*451*, 274	1.0
HFBA	502	9.58	*501*, 274	0.1

[a] Limit of detection.

Pharmacokinetic parameters include the following: the biological half life ($T_{1/2}$), the area under the plasma concentration–time curve from time zero to infinity (AUC), and the mean residence time (MRT). These parameters were calculated according to the standard method of moment analysis [11].

3. Results and discussion

3.1. Liquid–liquid extraction for sertraline in human plasma

To remove interference in the plasma, re-extraction in a different pH level was used in most of the GC–MS analysis [5,6,8]. Sometimes solid-phase extraction [7] or single liquid–liquid extraction at pH 7.6 with ethyl acetate [12] was used in the HPLC analysis; however, it could not detect the pg/ml or several ng/ml level of sertraline in our preliminary study. Therefore, single liquid–liquid extraction at pH 12.0 with diethylether was tested.

A certain extent of recoveries (71% at 1.0 ng/ml) for the extraction of sertraline from plasma was obtained by the single liquid–liquid, but the organic layer was not separated due to the plasma proteins. Thus plasma protein was removed with acetone and then liquid–liquid extraction was performed. Deproteinization with acetone before extraction achieved clear separation between ether and buffer, while the mean recovery increased up to 84.5%. Recovery test was also performed at 0.1 ng/ml, and the mean recovery was 80.7%. The test showed that the recovery was independent upon analyte concentration.

3.2. Selection of a suitable derivative for sertraline in GC–MS detection

We also evaluated the derivatization efficiencies of three derivatizing reagents (TFAA, PFPA and HFBA) for sertraline and ethylsertraline. Upon the perfluoroacylation, the active hydrogen atoms of sertraline and ethylsertraline were readily converted to their corresponding perfluoroacyl-derivatives. The total ion chromatograms in the SCAN mode did not show any peaks corresponding to partially derivatized sertraline or ethylsertraline of unexpected features (data not shown). This indicated that the derivatization was complete.

Table 1 shows the comparison of perfluoroacylation with TFAA, PFPA, and HFBA for sertraline and ethylsertraline. They have similar retention times and fragment patterns, but have different characteristic ions and LOD (limit of detection) value. However, when sertraline was derivatized with HFBA, it was separated completely with the peaks of blank plasma and has least interferences (Fig. 2). Moreover, peak area of derivatized sertraline was the largest in HFBA derivatization among three derivatization. Therefore, HFB derivatization was accepted in this work.

3.3. GC–MS properties of sertraline and ethylsertraline as their HFB-derivatives

The formation of molecular peaks of HFB-derivatized sertraline and ethylsertraline at m/z 501 and 515, respectively, is well-demonstrated in the full-scan spectra (Fig. 3). Minor peaks at $[M-169]^+$ and $[M-197]^+$ detected in the mass spectra of sertraline and ethylsertraline were derived from the losses of $CF_2CF_2CF_3$ and $COCF_2CF_2CF_3$ from molecular peaks, respectively. The peaks at m/z 486 and 274 were generated by the losses of CH_3 or C_2H_5 from molecular peaks and the fragment peaks at exocyclic C–N bonds, respectively. Peaks at m/z 238 and 203, which were derived from the losses of Cl and Cl_2 with a proton transfer from m/z 274, were prominent in sertraline and in ethylsertraline. Peak at m/z 159 was the fragmentation from $C_6H_3C1_2–CH_2$ by the proton transfer.

A molecular ion peak of HFBA-derivative (m/z 501) of sertraline and ethylsertraline (I.S., m/z 515) permitted enough sensitivity for the detection of sertraline in the plasma samples. Therefore, they were used as characteristic ions for the quantification. The detection limit for sertraline was 0.1 ng/ml and GC–SIM-MS response ratio (sertraline response/I.S. response) was linear with a correlation coefficient of 0.999 in the concentration range of 0.2–10.0 ng/ml.

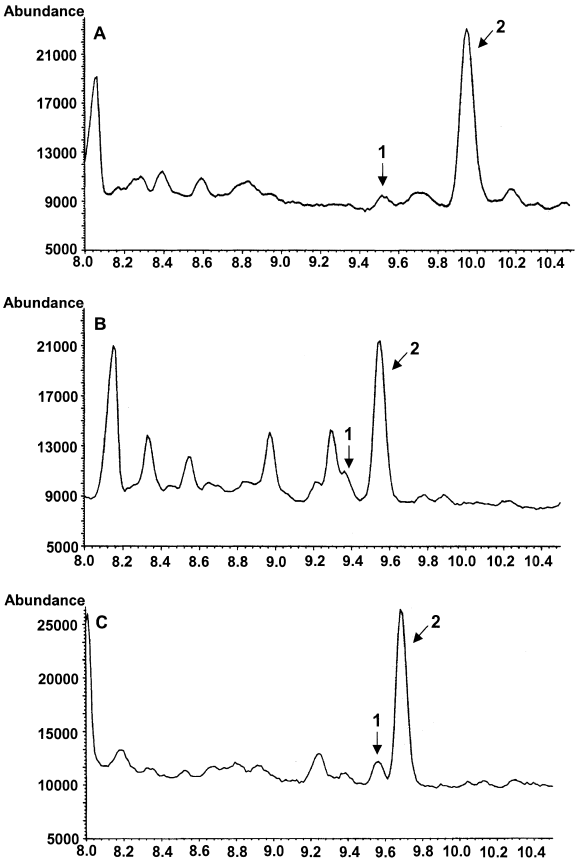

Fig. 2. Total ion current chromatograms of sertraline 1 ng/ml (1) and ethylsertraline 40 ng/ml (2) in plasma at selected ion-monitoring (SIM) mode as their TFA- (A), PFP- (B), and HFB-derivatives (C).

3.4. Validation of analytical procedure

The precision of the present method was assessed by analyses of triplicate aliquots of plasma fortified

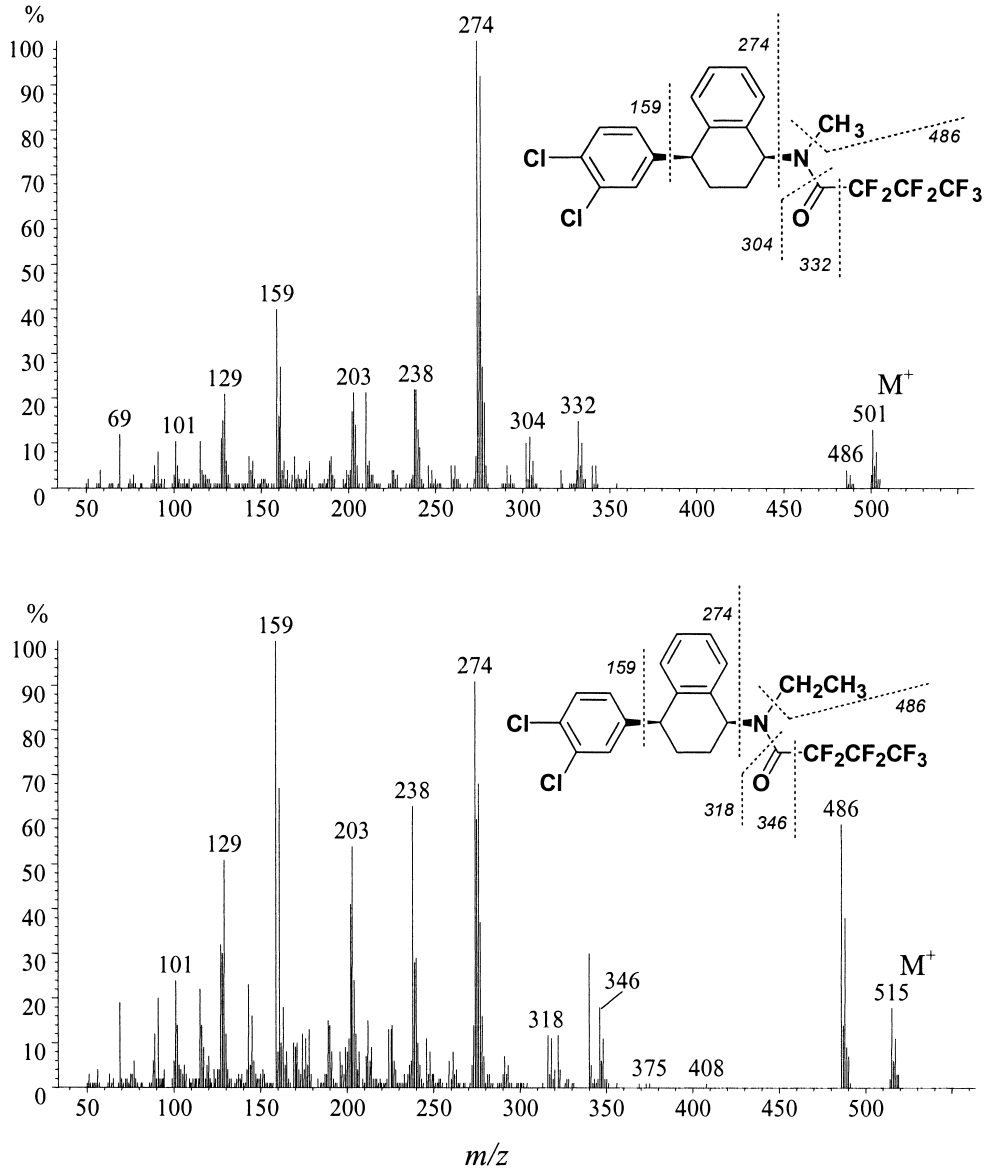

Fig. 3. Electron-impact mass spectra of sertraline (upper) and ethylsertraline (lower) as their HFB-derivatives obtained in the scanning mode at a rate of 0.48 scans/s with a mass range of m/z 50–550.

with three different concentrations. The intra- and inter-day assay variances obtained by GC–MS analyses with SIM are given in Table 2. C.V.% was less than 12.1% at 1–30 ng/ml and 18.2% at 0.2 ng/ml. The accuracy was less than 10% in the all-concentration range. The C.V.% at 0.1 ng/ml, the limit of detection (LOD), was 35%; therefore, the limit of quantification (LOQ) was determined to be 0.2 ng/ml. In keeping with the validation parameters, it is approved that the precision and accuracy of this method were adequate for the determination of sertraline in human plasma.

Table 2
The intra- and inter-day variations for sertraline

Concentrations added (ng/ml)	Intra-day variation ($n=4$)			Inter-day variation ($n=4$)		
	Concentrations found (mean±SD, ng/ml)	C.V. (%)	Accuracy (%)	Concentrations found (mean±SD, ng/ml)	C.V. (%)	Accuracy (%)
0.2	0.20±0.03	16.39	1.42	0.19±0.04	18.17	2.52
1.0	1.01±0.12	12.11	0.56	1.02±0.09	9.24	2.47
5.0	4.95±0.13	2.71	1.04	5.06±0.03	0.56	1.25
10.0	10.07±0.07	0.72	0.70	10.00±0.24	2.44	0.02
30.0	29.94±0.01	0.04	0.21	30.01±0.09	0.29	0.02

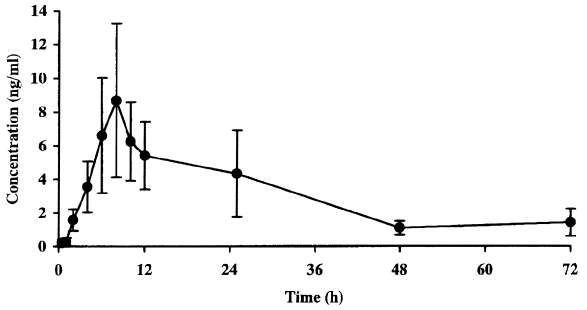

Fig. 4. Temporal plasma profile of sertraline (mean±SD $n=5$) after oral administration of sertraline hydrochloride in humans at a dose of 50 mg sertraline.

3.5. Pharmacokinetic study for sertraline after oral administration

Temporal plasma profile and pharmacokinetic parameters are shown in Fig. 4 and Table 3, respectively. The mean T_{max} was 8.0 h and the mean $T_{1/2}$ was 28.6 h. Those results were consistent with the previous reports [3,13]. It indicates that this simple and selective method for the determination of sertraline in human plasma was readily applicable to the clinical study for sertraline.

Table 3
Pharmacokinetic parameters following oral administration of sertraline hydrochloride at a dose of 50 mg sertraline/kg in man ($n=5$)

Parameters	Mean±SD
AUC (ng·h/ml)	267±121
MRT (h)	40.0±4.91
C_{max} (ng/ml)	10.3±2.47
T_{max} (h)	8.00±1.63
$T_{1/2}$ (h)	28.6±3.82

4. Conclusion

A major advantage of the present method is the selective and rapid recovery of sertraline from the plasma by deproteinization and by a liquid–liquid extraction. Moreover, the subsequent HFB-derivatization of the active hydrogen atom in the amino group enhanced the GC–SIM-MS properties of sertraline. It made the detection limit go down to 0.1 ng/ml and allowed the determination of sertraline with adequate precision and accuracy. In the pharmacokinetic analysis of sertraline in human plasma, T_{max} and $T_{1/2}$ from the moment analysis were similar to the previous reports. Therefore, it appears that the developed determination method for sertraline in human plasma is readily applicable to the clinical studies.

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