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Rapid and sensitive method for the determination of sertraline in human plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS)

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Abstract

A method for the determination of sertraline, a new antidepressant drug and a selective serotonin reuptake inhibitor (SSRI), in human plasma is described. Therapeutic drug monitoring (TDM) necessitates efficient, fast and reliable analytical methods validated by external quality control. We therefore devised a simple, rapid and sensitive isocratic reversed-phase liquid chromatographic-tandem mass spectrometric method equipped with Turbo Ion spray (TIS) source, operating in the positive ion and selective reaction monitoring (SRM) acquisition mode to quantify sertraline in human plasma. A new and superior procedure of solid-phase extraction (SPE) (compared to liquid–liquid extraction) was followed to extract sertraline and imipramine as internal standard (IS) from the human plasma. Sample preparation was performed using waters hydrophilic–lipophilic balance (HLB) cartridge and this method yielded extremely clean extracts with very good recovery, 81.47 and 85.79% for sertraline and IS, respectively. Both were analyzed by combined reverse phase liquid chromatography and tandem mass spectrometry (LC–MS/MS) with positive ion TIS ionization using SRM acquisition mode. The response of the LC–MS/MS method for sertraline was linear over the dynamic range of 0.5–60.0 ng/ml with correlation coefficient $r \geq 0.9996$. The coefficient of variance (%CV) was 8.53% at 0.5 ng/ml and the accuracy was well within the accepted limit of ±20% at lower limit of quantification (LLOQ) and ±15% at all the other concentrations in the linear range. This method was fully validated for the accuracy, precision and stability studies. The above findings indicate that the method is very much accurate and precise and can be successfully applied for bioequivalence studies in human subjects.
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1. Introduction

Depression is one of the most frequent of all major psychiatric illnesses. Major depressions are a common and disabling illness with a lifetime prevalence of 15–20% [1]. Antidepressant medication has been used to treat all forms of major depressive disorder. In spite of some contraindications, these agents are first line treatment when depressions are severe or when there are psychotic and melancholic symptoms [2]. Although clearly defined therapeutic ranges have not been established for majority of antidepressant drugs, therapeutic drug monitoring (TDM) can serve several purposes, including checking compliance and increasing safety and effectiveness of drugs by establishing well defined concentration–response relationships. Among all classes of antidepressants, the use of TDM has been well established only for tricyclic antidepressants (TCAs) because they exhibit a narrow therapeutic index and a plasma concentration–therapeutic response relationship. With the application of appropriate TDM, the antidepressant response to therapy with TCAs can improve two to threefold [3].

Selective serotonin reuptake inhibitors (SSRIs) are as effective as TCAs in the treatment of depression, but they are better tolerated. Over the past 15 years, particular attention has been focused on the ability of these medications to prevent new

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episodes in patients suffering from recurrent depression [4]. SSRIs have a wide therapeutic index and a flat dose-response curve [5], and therefore they do not fit the profiles of drugs that require TDM for safe and effective use. However, individual drugs of this class differ substantially in their pharmacokinetics and effects on cytochrome P450 (CYP) enzymes [6].

Many of these antidepressants are potent inhibitors of several different isozymes of the cytochrome P450 family of enzymes, so when these drugs are given simultaneously, they may mutually influence their disposition. In such cases, the risk of overdosing and adverse effects should be considered, and a laboratory measurement of plasma levels becomes mandatory. Understanding the similarities and differences in the pharmacology of SSRIs can guide the clinician to an optimal use of this important class of antidepressants.

Sertraline, a potent and latest generation antidepressant drug, selectively inhibits serotonin uptake into presynaptic nerve fibers. The analytical techniques employed in recent years for the estimation of sertraline in human plasma include HPLC, GC or in combination with MS i.e. liquid chromatography and tandem mass spectrometry (LC–MS/MS) and GC–MS/MS [7–18]. All these reported methods require either a lengthy extraction and/or derivatization procedure, yet the desired sensitivity is not achieved. Kim et al. [13] have reported a GC–MS/MS method with sensitivity of 0.2 ng/ml, but the extraction procedure involved deproteination, liquid–liquid extraction followed by derivatization. The upper limit of quantification (10.0 ng/ml) of their linearity range (0.2–10 ng/ml) for sertraline is inadequate to quantify the achieved C_{max} for 50 mg [7] as well as for 100 mg dose [8]. Also, these assay methods do not meet modern drug development needs, which require a rapid and accurate feedback of analytical information of pre-clinical and clinical pharmacokinetic studies. In our present method, using LC–MS/MS and solid-phase extraction (SPE) procedure, we have optimized and validated a linear dynamic range from 0.5 to 60 ng/ml for sertraline. The upper limit of quantification (60.0 ng/ml) is sufficient enough to quantify the expected C_{max} even for 100 mg dose. This SPE procedure with no drying and reconstitution step increases the sensitivity, specificity and throughput for the determination of sertraline [(1S,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-napthylamine] (Fig. 1) in human plasma.

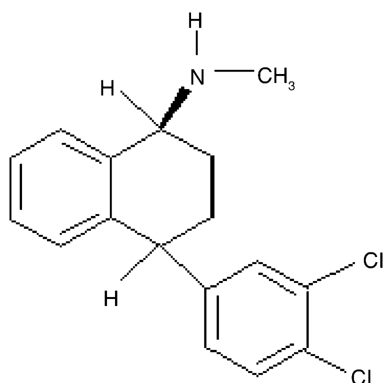

Fig. 1. Structure of sertraline.

2. Experimental

2.1. Materials and instrumental conditions

2.1.1. Reagents

Sertraline hydrochloride as well as imipramine hydrochloride (IS) was procured from Torrent Research Centre (Ahmedabad, India). Water used for the LC–MS/MS was prepared from Milli-Q water purification system (Bangalore, India). Acetonitrile, methanol of HPLC grade were purchased from JT Baker (Phillipsburg, USA). Ammonium acetate used for mobile phase buffer preparation was of molecular biology grade obtained from Sigma (Steinheim, Germany), while Suprapure formic acid used was procured from Merck (Darmstadt, Germany), Oasis HLB® SPE cartridge used for sample preparation was procured from Waters (Milford, Massachusetts).

Solutions of 5 mM ammonium acetate, pH 3.0 achieved with acetic acid was used as buffer in mobile phase; 0.1% formic acid in methanol was used for eluting sertraline and internal standard (IS) from the SPE cartridge.

Control human plasma was procured from Green cross blood bank (Ahmedabad, India) and was stored at −20 °C.

2.1.2. Instrumentation

Chromatographic instrument used was Perkin-Elmer PE 200 series pump, PE 200 series auto sampler and PE 200 series column oven, while triple quadrupole mass spectrometer used was API-4000, manufactured by MDS SCIEX, Canada. All the parameters of LC and MS were controlled by Analyst software version 1.4.

2.1.3. Liquid chromatographic conditions

Reversed-phase liquid chromatography (LC) was performed on a Perkin-Elmer PE 200 series a module incorporating a pump operating at 500 μl/min flow rate, auto sampler temperature was set at 5 °C. Chromatographic column used was from Thermo Electron Corporation Type Beta Basic C-8, 100 mm × 4.6 mm (Length x inner diameter), with 5 μm particle size and was maintained at 45 °C in column oven. The mobile phase consisted of buffer (5 mM ammonium acetate pH 3):acetonitrile in ratio of 20:80 (v/v).

2.1.4. Mass spectrometric conditions

The mass spectrometer equipped with TIS was run in positive mode and multiple reaction monitoring (MRM) mode to monitor the ions with *m/z* of 306.2 (parent ion) and 159.2 (product ion) for sertraline and 281.00 (parent ion) and 81.0 (product ion) for IS. For both sertraline and IS the parameters maintained were Gas 1 = GS1: 40.0 psig, Gas 2 = GS2: 50.0 psig, Ion spray voltage = IS: 5500.0 V, Turbo heater temperature = TEM: 450.0 °C, Interface heater = Ihe: ON, EP: 10.0 V, Collision activation dissociation = CAD: 5 psi, Curtain gas = CUR: 20 psi, while the Declustring potential DP, Collision energy CE and Cell exit potential CXP applied for sertraline was 48, 34 and 9 and 67, 27 and 14 for IS, respectively. The sertraline analysis data were acquired and quantified using Analyst software version 1.4.

2.1.5. Preparation of standard stocks and plasma samples (control and standards)

Main stock solution of 100 μg/ml for sertraline hydrochloride and imipramine hydrochloride (IS) were prepared by dissolving in methanol. These stock solutions were further diluted to get an intermediate concentration of 10 μg/ml for sertraline and 1 μg/ml for IS, respectively. Working solution of sertraline of different concentrations required for spiking plasma calibration and quality control samples were subsequently prepared using the main stock of 100 μg/ml and intermediate stock of 10 μg/ml. IS working solution of 40 ng/ml was prepared using the intermediate stock of 1 μg/ml and was used as internal standard in plasma samples preparation. All the main stock, intermediate stock and working stock solutions were prepared and stored at 2–8 °C until use. The control samples of blank plasma (free of sertraline) stored under refrigeration, were completely thawed before use. The spiking of these blank plasmas was carried out using the working solution of sertraline to obtain the desired concentration of sertraline in calibration and quality control samples. The spiked QC samples were stored at −70 °C for stability studies.

2.1.6. Sample extraction

Samples of blank human plasma were taken out from freezer maintained at −70 °C and kept at room temperature for 30–45 min for thawing. The samples were vortexed adequately using a vortex mixer before pipetting. Using a micropipette, 0.475 ml of plasma was transferred into 1.5 ml microtubes tube and to which 25 μl of the working stocks solutions of sertraline were spiked to prepare the required calibration standard and quality control samples. To these tubes, 50 μl of working solution of IS 40 ng/ml was added and vortexed to mix. To the same tube 500 μl of Milli-Q water was added and vortexed. The samples were loaded on HLB cartridge previously conditioned with 1 ml methanol and followed by 1 ml water, then the cartridge with sample was washed with 2 ml water followed by 1 ml of 5% methanol prepared in water and then again with 1 ml water by the application of vacuum. Sertraline and IS was eluted with 1.0 ml of 0.1% formic acid in methanol and 10 μl of eluate was directly injected in the LC–MS in partial loop mode.

3. Results

3.1. Specificity and sensitivity

The above rapid and specific method of extraction gave very good specificity and sensitivity for the analysis of sertraline and IS in the blank plasma. The chromatographic behaviour and peak shape was excellent for sertraline and IS. The retention time (RT) was short for both which makes it suitable for routine analysis. This SPE method gave clean chromatograms free of background interference. Test for specificity was carried out in different lots of blank plasma; four different lots of buffered blank plasma, four different lots of heparinised blank plasma, one lot of lipemic blank plasma and one lot of haemolysed blank plasma were studied. The mean area observed at the RT of sertraline in blank plasma was less than 20% of the LLOQ (0.5 ng/ml) standard area, where as the mean area observed at the RT of IS in blank plasma was less than 5% of IS area in sample preparation as shown in Table 1. The sensitivity results of intra- and inter-assay accuracy and precision at LLOQ level in Table 4 supports the specificity results. The aim of performing specificity check with these many different types of plasma samples is to ensure the quality of the results of study sample analysis. Fig. 2 represents the blank plasma specificity. The peak response of sertraline at LLOQ is shown in Fig. 3.

Table 1
Specificity in 10 different lots of plasma

Sample name	Sertraline		Imipramine (IS)	
	Area	Precentage area	Area	Precentage area
LLOQ-1	26123	NA	740531	NA
LLOQ-2	24747	NA	725495	NA
LLOQ-3	30706	NA	705539	NA
LLOQ-4	29113	NA	723061	NA
LLOQ-5	25422	NA	742797	NA
Mean	27222	NA	727485	NA
Blank-1[c]	3018	11.09	3101	0.43
Blank-2[c]	2883	10.59	4459	0.61
Blank-3[c]	4378	16.08	7061	0.97
Blank-4[c]	3838	14.10	4660	0.64
Blank-5[d]	2912	10.70	5945	0.82
Blank-6[d]	3864	14.19	6315	0.87
Blank-7[d]	5521	20.28	4734	0.65
Blank-8[d]	3403	12.50	6770	0.93
Blank-9[e]	5515	20.26	15788	2.17
Blank-10[f]	4101	15.07	5644	0.78
Mean[a]	3943	14.49	6447[b]	0.89

NA: not applicable.
[a] The mean area observed at the RT of sertraline are <20% of the mean area of LLOQ.
[b] The mean area observed at the RT of IS are <5% of the mean area of IS.
[c] Buffered blank plasma.
[d] Heparinised blank plasma.
[e] Lipemic blank plasma.
[f] Haemolysed blank plasma.

3.2. Linearity

The linearity of the method was determined by analysis of standard plots associated with a nine-point standard calibration curve. Best-fit calibration curves of peak area ratio versus concentration were drawn. The concentration of the sertraline was calculated from the simple linear equation using regression anal-

Table 2
Summary of five different linearity parameters for sertraline Linearity was plotted using weighting factor 1/concentration, i.e. $1/x$

Linearity	Intercept	Slope	Correlation coefficient (r)
1	0.0044	0.068	0.9996
2	−0.005	0.068	0.9997
3	0.0087	0.085	0.9998
4	0.009	0.109	0.9996
5	0.0079	0.087	1.0000

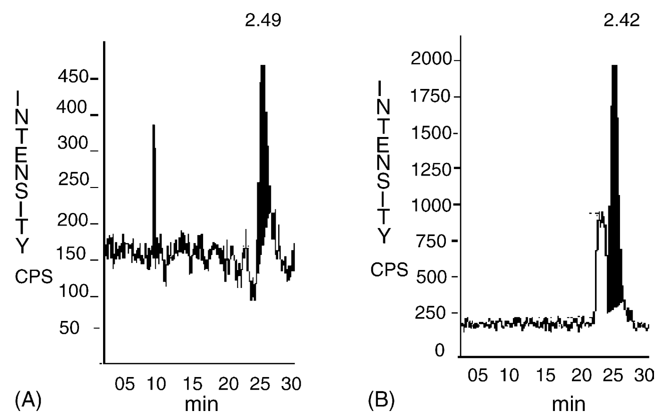

Fig. 2. Representative chromatogram of human blank plasma specificity for sertraline (A) and IS (B), CPS: counts per second.

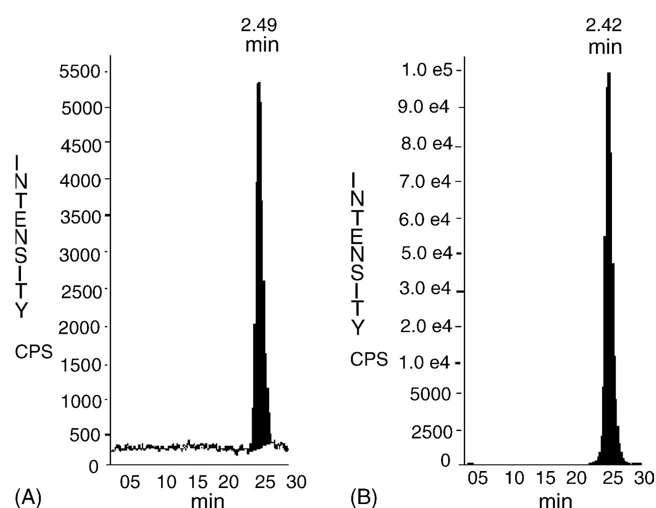

Fig. 3. Representative chromatogram of LLOQ (Q.5 ng/ml) for sertraline (A) and imipramine (B) in human plasma.

Table 5
Stability results of sertraline

Stability experiments	Storage condition	Mean % change
Stock solution	Room temperature (24 h)	−1.96
Stock solution	2–8 °C (21 days)	3.26
Bench top	Room temperature (6 h)	0.67
Process (extracted sample)	Autosampler (5 °C, for 40 h)	0.75
Freeze and thaw	After third cycle	4.33
Long-term	−70 °C for 71 days	−5.47

ysis of spiked plasma calibration standard with the reciprocal of the drug concentration as a weighting factor (1/concentration, i.e. $1/x$); $y = mx + c$, as indicated in Table 2.

The calibration curves were linear from 0.5 to 60.0 ng/ml with correlation coefficient of $r \geq 0.9996$. The observed mean back calculated concentration with accuracy (% nominal concentration) and precision (%CV) of five linearities are given in Table 3.

3.3. Recovery

The percentage recovery of sertraline was determined by comparing the mean area of five replicates of extracted quality control samples with mean area of freshly prepared unextracted quality control samples i.e. low quality control (LQC = 1.5 ng/ml), middle quality control (MQC = 15.0 ng/ml) and high quality control (HQC = 45.0 ng/ml) samples. The overall mean recovery calculated for sertraline and the IS was 81.47 and 85.79%, respectively.

3.4. Precision and accuracy

The intra-assay precision and accuracy was calculated for LLOQ (0.5 ng/ml), LQC, MQC and HQC levels after five repli-

Table 3
Back calculated concentration of calibration standards (CS) from respective calibration curves of sertraline

Linearity	Concentration (ng/ml)								
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7	CS-8	CS-9
Spiked concentration ng/ml	0.5	1	3	6	12	24	36	48	60
n	5	5	4	5	5	5	5	5	5
Mean (ng/ml)	0.49	1.04	2.91	5.85	12.15	24.2	35.86	48.48	59.49
Accuracy (%)	98.6	104.42	97.03	97.46	101.24	100.85	99.61	101.01	99.15
CV (%)	2.15	6.28	4.44	6.3	4.55	2.48	0.59	0.69	1.33

Table 4
Sertraline intra- and inter-assay precision and accuracy

Analyte	Precision (CV%)			Accuracy (%)	
	Concentration (ng/ml)	Intra-assay[a] ($n = 5$)	Inter-assay[b] ($n = 20$)	Intra-assay[a] ($n = 5$)	Inter-assay[b] ($n = 20$)
Sertraline	0.5	12.77	8.53	98.10	102.37
	1.5	4.17	6.34	102.60	101.17
	15	2.04	6.39	102.20	97.67
	45	0.83	1.98	99.90	99.38

n: total number of observations for each concentration.
[a] Average of five replicate observations at each concentration.
[b] Average of 20 observations recorded over four different analytical runs (five replicates/run).

Table 6
Comparison of analytical methods reported for sertraline in biological matrix for routine analysis

Sr. No.	Biological matrix and processing volume	Extraction procedure (steps)	Reconstitution volume	Injection volume (μl)	Analytical run time	Quantification limit	Detection technique	Reference
1	Human plasma (0.5 ml)	Liquid–liquid extraction	200 μl	50	6.0 min	0.5 ng/ml	LC–MS/MS	[7]
2	Human plasma and red blood cell (0.5 ml) each	Liquid–liquid extraction, followed by back extraction	200 μl	150	18–20 min	15 nmole, 221 nmole	HPLC, UV	[16]
3	Human plasma (1.0 ml)	Liquid–liquid extraction, drying, derivatization, drying	2.5 ml	150	25.4 min	10 ng/ml	HPLC, fluorescence	[15]
4	Serum (1.0 ml)	Solid-phase extraction	100 μl	50	11 min	10 ng/ml	HPLC, UV	[12]
5	Human plasma	Deproteination, liquid–liquid extraction, derivatization	–	–	–	0.2 ng/ml	GC–MS	[11]
6	Human plasma (0.5 ml)	Solid-phase extraction[a]	–	10	3.0 min	0.5 ng/ml	LC–MS/MS	Present method

[a] Present SPE extraction procedure has a final eluate volume of 1 ml with no drying and reconstitution step. 10 μl of the final eluate is directly injected into LC–MS/MS.

cates, each on the same analytical run and inter-assay precision and accuracy was calculated after repeated analysis in four different analytical batches. The results are given in Table 4.

3.5. Stability study

Stability experiments were performed exhaustively to evaluate the sertraline stability in stocks solutions and in plasma samples under different conditions, simulating the same conditions which occurred during study sample analysis: In plasma at room temperature stability, extracted sample stability (process stability), freeze thaw stability and long term stability. The results obtained were well within the acceptable limit. IS stock solution was also found to be stable.

Stock solution of sertraline and IS were stable at room temperature for 24 h and at 2–8 °C for 21 days. Sertraline in control human plasma at room temperature were stable at least for 6 h. Sertraline in the final solid phase extract was found to be stable in autosampler up to 40 h (process stability). Sertraline was found to be stable for at least three freeze and thaw cycles. The sertraline spiked plasma samples stored at −70 °C for long-term stability experiment were found stable for at least 71 days. The values for the percent change for the above stability experiment are compiled in Table 5.

4. Results and discussion

The method described is sensitive and highly reproducible for the analysis of sertraline in human plasma using LC–MS/MS coupled with TIS source. This is evident from the established high specificity, sensitivity and throughput. The basic underlying advantage of this optimized method is that, it utilizes only 0.5 ml of plasma and a very short SPE extraction procedure. The method involves minimum usage of organic solvent with no extra steps of drying, only 10 μl of the final eluate is directly injected into LC–MS, hence no extra labour of derivatization, as required in GC or GC–MS to increase the sensitivity. This method gives exceptionally good intra- and inter-assay precision and accuracy with quantitative recoveries. The retention time of both sertraline and IS is low enough 2.49 and 2.42 min making the run time of 3.0 min only. The method was more selective and specific because the data acquisition was performed with MRM mode and thus the need to separate sertraline and internal standard chromatographically is not mandatory. This short extraction procedure and analytical run time increases the throughput and validates the method for all the stability studies for sertraline and IS and renders it suitable for routine volunteers sample analysis. Table 6 summarizes the salient features of some methods reported in literature for the routine analysis of sertraline in human plasma, blood and serum.

5. Conclusion

After extensive research on the method development and studying the results of all the validation parameters, we conclude that the above validated method can be highly useful for the therapeutic drug monitoring both for analysis of routine samples of single dose pharmacokinetics and also for the clinical trial samples with precision, accuracy and high throughput. The LLOQ of 0.5 ng/ml achieved with the present method involves no drying or reconstitution step. A further advantage of this SPE method observed and proved that, the limit of quantification is low enough to monitor at least five half life of sertraline concentration with good inter-assay reproducibility (CV) for the quality control runs with patient samples.

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