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SHORT COMMUNICATION

HPLC of Sertraline and Norsertraline in Plasma or Serum

Jignasha Patel, E. P. Spencer and R. J. Flanagan*

Poisons Unit, Guy's and St Thomas' Hospital Trust, Avonley Road, London SE14 5ER, UK.

A simple method for the measurement of sertraline and norsertraline in plasma or serum suitable for use in single-dose pharmacokinetic studies has been developed. Internal standard solution, aqueous fenethazine (10 mg/L) (20 μL), and Tris buffer (2 mol/L), pH 10.6) (100 μL) were added to plasma (200 μL). Sertraline, norsertraline and the internal standard were extracted into methyl *tert*-butyl ether (200 μL) by mixing (30 s) and centrifugation (11,000 r.p.m., 4 min). A portion (100 μL) of the extract was injected onto a Spherisorb S5SCX HPLC column (150 × 4.6 mm i.d.) which was eluted with methanol:water (19+1) containing ammonium perchlorate (40 mmol/L), final pH 7.0. Detection was by UV monitoring (215 nm). The concentration of each analyte in each sample was calculated from the calibration graph (peak-height ratio of analyte to that of the internal standard against analyte concentration) obtained after analysis of plasma samples containing known amounts of sertraline and norsertraline. The limit of accurate measurement of the assay was 10 μg/L) sertraline and 20 μg/L)norsertraline.

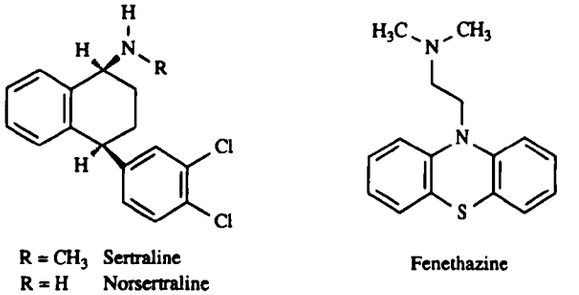
Sertraline [(1*S*,4*S*)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-naphthylamine, Fig. 1) is a 1-aminotetrahydronaphthalene derivative which selectively inhibits serotonin uptake into presynaptic nerve sites and exerts antidepressant effects in humans (Shelton, 1994). Sertraline is chemically unrelated to tricyclic, tetracyclic, or other marketed antidepressants. Sertraline undergoes extensive presystemic metabolism, norsertraline (*N*-desmethylsertraline, Fig. 1) being an important metabolite. However, norsertraline is about 10 times less potent than sertraline as an inhibitor of neuronal serotonin reuptake (Dollery, 1992). Sertraline is more than 98% bound to plasma protein and has a large volume of distribution (ca. 20 L/kg). Peak plasma concentrations are reached 6–8 h after an oral dose. The mean plasma elimination half-life in young adults is 26 h (22 h in males, 31 h in females). The half-life of norsertraline ranges from 62 to 104 h. Steady state plasma concentrations of sertraline range between 30 and 190 μg/L (Dollery, 1992). The ratio of the metabolite to the parent compound may be useful in assessing compliance and in the investigation of acute poisoning.

Methods for the measurement of plasma sertraline concentrations have included gas chromatography with mass spectrometric (Fouda *et al.*, 1987: Logan *et al.*, 1994; Rogowsky *et al.*, 1994), electron capture (Tremaine and Joerg, 1989), or nitrogen-selective detection (Levine *et al.*, 1994). High-performance liquid chromatography (HPLC), however, offers advantages of economy, simplified sample preparation and ease of measurement of norsertraline. Published HPLC methods for sertraline and norsertraline rely on the use of conventional alkyl-modified silica columns with aqueous acetronitrite and/or methanol eluents and UV detection (205–235 nm) (Gupta and Dziurdzy, 1994; Logan *et al.*, 1994; Rogowsky *et al.*, 1994; Wiener *et al.*, 1990; Wong *et al.*, 1993). Solid-phase sample preparation was used in the methods applied to human serum (Gupta and Dziurdzy, 1994; Rogowsky *et al.*, 1994).

Use of silica columns together with methanolic eluents containing an ionic modifier provides a simple HPLC system which has proved useful in the analysis of many basic drugs (Flanagan and Jane, 1985; Jane *et al.*, 1985). More recently, we have found that use of sulphopropyl (SCX)-modified packings with methanol or methanol:water eluents at an appropriate pH and ionic strength can in some cases provide enhanced stability and reproducibility as compared to unmodified silica (Croes *et al.*, 1995). The method described here uses a simple liquid–liquid extraction at an alkaline pH followed by analysis on an SCX column and is suitable for the measurement of the plasma sertraline and norsertraline concentrations attained after single oral dosage.

EXPERIMENTAL

Materials and reagents. Sertraline hydrochloride (CP-51,974-01) and norsertraline maleate (CP-62,508-11) were from Pfizer (Groton, CT, USA). The internal standard, fenethazine (10-(2-dimethylaminoethyl)phenothiazine, RP 3015: Fig. 1), was from Rhône-Poulenc (Paris, France). Fenethazine was used as a 10 mg/L (free base) solution in deionized water which was stored



R = CH_3 Sertraline
R = H Norsertraline

Fenethazine

Figure 1. Structural formulae of sertraline, norsertraline and fenethazine (internal standard).

* Author to whom correspondence should be addressed.

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at 4°C. Methanol and methyl *tert*-butyl ether (both HPLC grade) were from Rathburn (Walkerburn, UK). Tris(hydroxymethyl)methylamine (Tris), hydrochloric acid, perchloric acid (60% w/w) and sodium hydroxide were Analar grade. Tris solution (2 mol/L) was adjusted to pH 10.6 by dropwise addition of 1 mol/L hydrochloric acid or sodium hydroxide. Ammonium perchlorate was from Aldrich (Poole, UK). Non-sterile human serum was from the South Thames Blood Transfusion Service (Tooting, UK).

High-performance liquid chromatography. A Waters 510 reciprocating pump was used with a Spectra-physics SP8780XR autosampler (200 μL sample loop), a Waters 484 Tunable UV Absorbance detector set at 215 nm (time constant 2.0 s) and a Spectra-Physics ChromJet integrator linked via a LABNET system. The column was a stainless steel tube (150 × 4.6 mm i.d.) packed with Spherisorb S5 SCX (sulphopropyl-modified silica, 5 μm average particle size) (Phase Separations, Deeside, UK). The eluent flow-rate was 1.2 mL/min. The eluent was 40 mmol/L ammonium perchlorate in methanol:water (19+1) adjusted to apparent pH 7.0 with 1 mol/L methanolic sodium hydroxide or 1% (v/v) methanolic perchloric acid (Jenway model 3020 glass electrode calibrated against aqueous reference standards (pH 4, pH 7, Sigma)). Batches of eluent were filtered prior to use (Millipore 0.45 μm filter). Portions (10 μL) of a methanolic solution of sertraline (0.5 mg/L), norsertraline, and fenethazine (both 1 mg/L) were analyzed as necessary to monitor system performance.

Table 1 Interference study

Compound	Retention time relative to fenethazine
Tradozone	0.36
Lignocaine	0.45
Prazosin	0.49
Desethylamiodarone	0.50
Fluvoxamine	0.51
Mexiletine	0.51
Betaxolol	0.52
Labetalol	0.52
Norfluoxetine	0.52
Norsertraline	**0.54**
Norverapamil	0.57
Tocainide	0.57
Flecainide	0.58
Amiodarone	0.59
Diltiazem	0.60
Sotalol	0.61
Mianserin	0.62
Nadolol	0.64
Terazosin	0.64
Nortriptyline	0.66
Verapamil	0.67
Atenolol	0.70
Desipramine	0.70
Protriptyline	0.70
Norclomipramine	0.71
Fluoxetine	0.72
Flurazepam	0.72
Maprotiline	0.75
Sertraline	**0.78**
Trimipramine	0.81
Nordothiepin	0.82
Nordoxepin	0.85
Paroxetine	0.86
Amitriptyline	0.87
Viloxazine	0.87
Clomipramine	0.91
Imipramine	0.95
Chlorpromazine	0.97
Desacetyldiltiazem	0.98
Fenethazine	**100**
Dothiepin	1.08
Doxepin	1.12
Quinine/Quinidine	1.36
Codeine	2.03

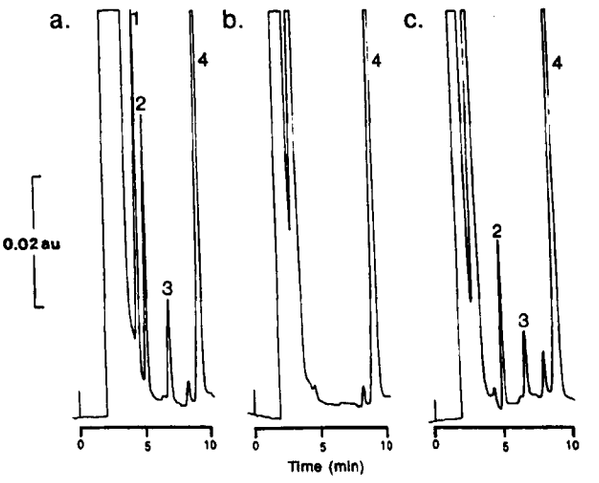

Figure 2. Chromatograms obtained on analysis of (a) the 50 μg/L sertraline 100 μg/L norsertraline calibration standard: (b) drug-free human plasma; (c) sample from a patient prescribed sertraline (200 mg/day) (sertraline and norsertraline concentrations 32 and 65 μg/L, respectively). Injection: 100 μL methyl *tert*-butyl ether extracts. See text for chromatographic conditions. Peaks: 1, lignocaine: 2, norsertraline: 3, sertraline: 4, fenethazine.

Sample preparation. Using respectively a Hamilton gas-tight glass syringe (1.0 mL) fitted with a Hamilton repeating mechanism and an Eppendorf Multipette fitted with a 5.0 mL syringe, 20 μL aqueous fenethazine solution (10 mg/L) and 100 μL Tris buffer (2 mol/L, pH 10.6) were added to a glass test tube (60 × 5 mm i.d.) (Dreyer tube). Using a semi-automatic pipette, 200 μL sample or standard were added and, after vortex mixing (5 s), 200 μL methyl *tert*-butyl ether were added using a 5.0 mL Hamilton gas-tight glass syringe fitted with a Hamilton repeating mechanism. After vortex mixing (30 s) and centrifugation (11,000 r.p.m., 4 min; Hettich EBA 12 bench centrifuge), the supernatant was transferred to a Chromacol glass autosampler vial (02-CVTG) using a disposable extended fine tip Pastette (Alpha Laboratories) and the vial was capped (Chromacol 8-ACT) and loaded onto the autosampler. The injection volume was 100 μL.

Calibration and quality assurance. A calibration stock solution containing sertraline and norsertraline (50 and 100 mg/L free base, respectively), was prepared in deionized water:methanol (3+1) and stored at 4°C until used. Using 1.0 mL and 2.5 mL Hamilton gas-tight syringes fitted with Hamilton repeating mechanisms, portions of this solution were dispensed into 100 mL volumetric flasks and diluted with analyte-free human serum to give calibration solutions containing 10, 20, 50, 100, 150, 200 and

Table 2 Intra-assay reproducibility ($n = 20$ in each case)

Analyte	Nominal (μg/L)	Mean (μg/L)	SD (μg/L)	RSD (%)
Sertraline	25	26.6	0.82	3.1
	125	135.1	4.55	3.4
	300	324.7	10.25	3.2
Norsertraline	50	49.4	1.21	2.4
	250	254.1	9.32	3.7
	600	617.6	20.56	3.3

Table 3 Inter-assay reproducibility ($n=10$ in each case)

Analyte	Nominal (μg/L)	Mean (μg/L)	SD (μg/L)	RSD (%)
Sertraline	25	25.2	1.55	6.2
	125	129.9	6.90	5.3
	300	305.7	13.40	4.4
Norsertraline	50	50.5	5.66	11.2
	250	246.9	12.06	4.9
	600	581.1	26.26	4.5

400 μg/L sertraline and 20, 40, 100, 200, 300, 400 and 800 μg/L norsertraline, respectively. The flasks were allowed to stand overnight (4°C) and the contents mixed thoroughly. Portions of the solutions were transferred to 3.0 mL hard plastic tubes, capped and stored at -20°C until required. Assay calibration was by analysis of these solutions and plotting the peak-height ratio of sertraline and norsertraline to the internal standard against sertraline and norsertraline concentration respectively.

Quality assurance specimens (25, 125 and 300 μg/L sertraline and 50, 250 and 600 μg/L norsertraline, respectively) were prepared similarly in analyte-free human serum by a second analyst using an independent stock solution and again stored in small portions at -20°C. These solutions were stable for at least 3 months at -20°C.

RESULTS AND DISCUSSION

An eluent pH of 7.0 was chosen in order to optimize resolution between the compounds of interest. Good peak shapes were obtained (Fig. 2). Potential interference from some drugs and drug metabolites was assessed by injection of 1 mg/L solutions of pure compounds in methanol (Table 1). The method should thus be used with caution if the presence of a drug or metabolite eluting near to either sertraline and norsertraline or the internal standard is a possibility. Some of these compounds (atenolol, for example) are poorly extracted under the conditions used and thus interference in sample extracts will be minimal. Lignocaine, a common constituent of blood-bank serum, was resolved from norsertraline (Fig. 2). Fenethazine is a convenient internal standard and is no longer prescribed. If unavailable, an alternative basic compound such as viloxazine (Table 1) should prove suitable.

Eluent (2 L batches) was re-used after brief sparging with helium to remove some methyl *tert*-butyl ether, and allowing to stand to re-equilibrate with atmospheric oxygen overnight. Water (5% v/v) was added to the eluent since this appeared to maximize column life if used in conjunction with a regimen whereby the column was inverted after every 80 sample injections or so and washed successively (5–10 column volumes) with (I) methyl *tert*-butyl ether, (ii) methanol, and (iii) methanol:water (1+1) containing

Table 4 Stability of sertraline and norsertraline after 3 freeze–thaw cycles ($n=6$ in each case)

Analyte	Nominal (μg/L)	Mean (μg/L)	SD (μg/L)	RSD (%)
Sertraline	25	25.8	1.3	5.2
	125	133.3	3.1	2.3
	300	319.3	5.6	1.8
Norsertraline	50	50.1	3.1	6.1
	250	254.5	5.3	2.1
	600	608.5	13.9	2.3

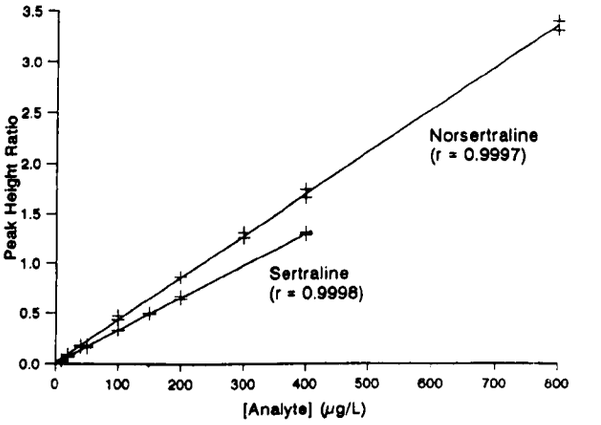

Figure 3. Typical calibration graph (peak-height ratio of analyte to internal standard vs. analyte concentration).

40 mmol/L ammonium perchlorate (pH 7.0).

The recovery of sertraline and norsertraline from human serum using the extraction procedure described was assessed by measurement of the peak-heights of sertraline and of norsertraline obtained on analysis of the QA solutions ($n=6$ at each concentration: 100 μL injections of methyl *tert*-butyl ether extracts). The results were compared to those obtained (peak-heights) on direct HPLC analysis of standard sertraline (25, 125 and 300 μg/L) and norsertraline (50, 250 and 600 μg/L) solutions prepared in HPLC eluent ($n+4$ at each concentration; 100 μL injections). Good calibration graphs were obtained ($r>0.999$ in both cases). The apparent recovery of sertraline in the QA solutions was 118, 114 and 119% and of norsertraline 112, 115 and 121%, respectively. This high recovery is probably due to concentration by evaporation of some of the extraction solvent prior to HPLC analysis.

The intra- and inter-assay reproducibility of the method was assessed by replicate analysis of the human serum QA specimens. The intra-assay relative standard deviations (RSDs) were less than 5% across the range 25–300 μg/L sertraline and 50–600 μg/L norsertraline (Table 2). The results showed an accuracy (calculated as % nominal) of between 106 and 108% (sertraline) and 99 and 103% (norsertraline) (Table 2). The inter-assay RSDs were 11.2% or less across these same ranges (Table 3). After three freeze–thaw cycles (-20°C to room temperature) the results given by the QA specimens (Table 4) were very similar to those obtained when the intra-assay RSDs were measured (Table 2).

Regression analysis of 8 sets of calibration standards analysed over 3 days was performed. Using the back calculated concentrations the mean and RSD for each standard were ascertained. The bias of the method (calculated as per cent nominal) was between 99.4 and 106% (sertraline) and 99.3 and 102% (norsertraline), with RSDs less than 8.5% for concentrations in the range 20 to 400 μg/L (sertraline) and less than 8% for concentrations in the range 40–800 μg/L (norsertraline). A typical calibration graph is shown in Fig. 3.

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