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Short communication

HPLC analysis of the second-generation antidepressant sertraline and its main metabolite *N*-desmethylsertraline in human plasma

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Abstract

A liquid chromatographic method with ultraviolet detection was developed for the analysis of the recent antidepressant sertraline and its main metabolite *N*-desmethylsertraline in human plasma. The analytes were separated on a C8 reversed phase column, using a mobile phase composed of acetonitrile and a 12.3 mM, pH 3.0 phosphate buffer containing 0.1% triethylamine (35:65, v/v). Clomipramine was used as the Internal Standard. Using a solid phase extraction procedure with C2 cartridges high extraction yields (>94%) and good purification from matrix interference were obtained. Good linearity was obtained in the 7.5–250.0 ng mL^{-1} range for sertraline and in the 10–500 ng mL^{-1} range for *N*-desmethylsertraline. The analytical method was validated in terms of precision, extraction yield and accuracy. These assays gave R.S.D.% values for precision always lower than 3.9% and mean accuracy higher than 90%. Thanks to its good selectivity, the method proved to be suitable for the analysis of plasma samples from patients treated with sertraline as either monotherapy or polypharmacy.

Keywords: Sertraline; *N*-desmethylsertraline; Human plasma; Liquid chromatography; Solid phase extraction

1. Introduction

Sertraline [(1*S*,4*S*)*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphtylamine, SRT, Fig. 1a] is an antidepressant drug belonging to the SSRI (selective serotonin reuptake inhibitor) class, which shows only minor inhibition of the norepinephrine and dopamine transporters [1]. It is particularly suitable for the treatment of major depression and obsessive-compulsive disorder in adult patients, however it is also used against panic fits with or without agoraphobia. The SRT daily dose usually ranges between 50 and 200 mg and the drug is slowly absorbed with an elimination half-life of 22–36 h; thus, once-daily administration is therapeutically effective [2]. Steady-state plasma levels range from 2.8 to 112 ng mL^{-1} with pronounced interindividual variability; peak plasma concentrations (C_{max}) of about 20 to 55 ng mL^{-1} occur approximately 4 to 8 h after administration of a single 100 mg dose [3], with food intake slightly increasing SRT bioavailability and C_{max} [4]. SRT undergoes extensive first-pass metabolism by the action of different cytochrome subtypes [5,6] to form *N*-desmethylsertraline (DSR, Fig. 1b), an active metabolite with longer half-life [7] which thus accumulates and reaches higher concentration in plasma than the parent drug at steady state [7]. Smokers tend to have lower levels of both SRT and DSR, while elderly people tend to have higher levels of both compounds [8]. For these reasons, the determination of plasma concentrations of the drug and its metabolite seems to be necessary in patients at risk, such as the elderly or poor metabolisers. Some papers can be found in the literature regarding the determination of SRT and DSR in human plasma, serum or brain (in postmortem samples) by gaschromatography–mass spectrometry (GC–MS) [9–11] or HPLC with UV detection [12,13]. Furthermore, SRT has been analysed as a single analyte by GC–MS [14] or together with other antidepressants using HPLC-DAD [15,16] or

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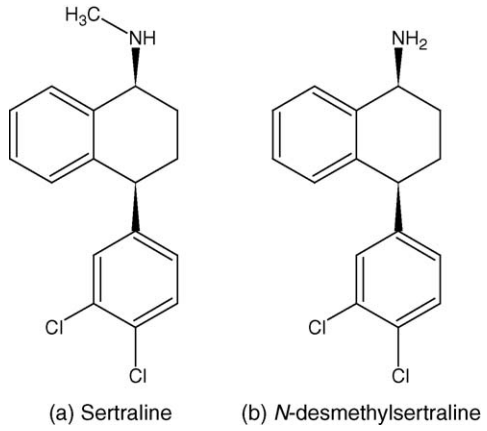

(a) Sertraline (b) *N*-desmethylsertraline

Fig. 1. Chemical structures of sertraline and desmethylsertraline.

HPLC coupled to spectrofluorimetric detection after derivatisation [17,18]. The pre-treatment of biological samples is usually carried out by means of liquid–liquid extraction (LLE) [13–17], which can be tedious and time-consuming (due to centrifugation and phase separation steps), as well as environmentally dangerous. However, in none of these contributions SRT was determined together with DSR in human plasma at usual therapeutic doses by HPLC-UV as described in the present work. It was the aim of this study to develop a rapid and reliable HPLC method with UV detection for the simultaneous analysis of SRT and DSR in human plasma using a solid-phase extraction (SPE) procedure for the sample pre-treatment.

2. Experimental

2.1. Chemicals

Sertraline [(1*S*,4*S*)*N*-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphtylamine] and *N*-desmethylsertraline [(1*S*,4*S*) -4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphtylamine] have been kindly donated by Pfizer Italia (Borgo S. Michele, Italy). Clomipramine hydrochloride, used as the Internal Standard (IS), has been kindly provided by Novartis Italia (Origgio, Italy). Methanol and acetonitrile were HPLC-grade; potassium dihydrogen phosphate, 85% (m/m) orthophosphoric acid, sodium carbonate and triethylamine were pure for analysis from Carlo Erba (Milan, Italy). Ultrapure water (18.2 MΩ cm) was produced by means of a Milli-Q apparatus from Millipore (Milford, MA, USA). Stock solutions (1 mg mL^{-1}) of SRT, DSR and the IS were prepared by weighing the appropriate amount of pure substance and dissolving it in methanol. The stock solutions were diluted daily with the mobile phase to obtain working standard solutions. All solutions were kept in the dark and all containers were low actinic or opaque.

2.2. Human plasma sampling

The blood samples were collected from healthy volunteers and from patients of the Borgo Panigale Mental Health Center (Bologna, Italy) who were subjected to therapy with SRT for at least 2 weeks, at daily doses between 50 and 200 mg. Blood samples were drawn 12 h after the last drug administration. Blood was stored in glass tubes containing EDTA, then centrifuged at 1400 × *g* for 15 min; the supernatant (plasma) was transferred to polypropylene tubes and stored frozen at −20 °C until analysis.

2.3. Chromatographic apparatus and conditions

Chromatographic analysis was carried out on a Jasco (Tokyo, Japan) PU-980 isocratic pump coupled to a Jasco UV-975 spectrophotometric detector. The separation was carried out on a Genesis C8 reversed phase column (150 × 4.6 mm I.D., 4 μm) from Jones Chromatography (Mid Glamorgan, UK). The mobile phase was a mixture of acetonitrile and a 12.3 mM, pH 3.0 phosphate buffer containing 0.1% triethylamine (35/65, v/v), flowing at 1.2 mL min^{-1}. Prior to use, the mobile phase was filtered and degassed; the injection loop volume was 20 μL and the UV detector was set at 220 nm.

2.4. Solid-phase extraction procedure

Solid-phase extraction (SPE) was carried out using Varian BondElut C2 cartridges (100 mg, 1 mL) on a Varian VacElut apparatus. Aliquots of 50 μL of IS standard solution (and analyte standard solution for blank plasma samples) were added to 250 μL of blank or patients' plasma. The resulting mixture was diluted with 500 μL of water and loaded onto a previously conditioned C2 cartridge. The cartrigdes were equilibrated with 1 mL of methanol 5 times and conditioned with 1 mL of water 5 times. After loading, the cartridges were washed with 1 mL of water 2 times, then with 1 mL of methanol/10 mM, pH 10.0 carbonate buffer (20/80, v/v) and finally with 50 μL of methanol. Elution was carried out with 1 mL of methanol. The eluate was brought to dryness (rotary evaporator), redissolved in 250 μL of mobile phase and injected into the HPLC system.

2.5. Method validation

2.5.1. Calibration curves, Limit of Quantitation, Limit of Detection

Analyte standard solutions (50 μL) at ten different concentrations, containing the IS at constant concentration were added to 250 μL of blank plasma. The resulting mixture was subjected to the sample pre-treatment procedure and injected into the HPLC. The procedure was carried out in triplicate for each concentration and the calibration curves were constructed by means of the least-square method. The Limit of Quantitation (LOQ) and the Limit of Detection (LOD) were calculated as the analyte concentrations which gave rise to chromatographic peaks whose height was equal to 10 or 3 times the baseline noise, respectively.

2.5.2. Extraction yield (absolute recovery)

The procedure was the same as that described under Section 2.5.1, except the points were at 3 different concentrations, corresponding to the lower limit, middle point and upper limit of each calibration curve (i.e. plasma concentrations of 7.5, 125.0 and 250.0 ng mL^{-1} for SRT; 10, 250 and 500 ng mL^{-1} for DSR). The analyte/IS peak area ratios thus obtained were compared to

the ratios obtained injecting standard solutions at the same theoretical concentrations, and the percentage recovery calculated.

2.5.3. Precision

The assays described under Section 2.5.2 were repeated six times within the same day to obtain repeatability (intraday precision) and six times over different days to obtain intermediate precision (interday precision) of the method, expressed as R.S.D.% values.

2.5.4. Accuracy

The assays described under Section 2.5.2 were carried out adding standard solutions of the analytes and the IS to human plasma samples taken from patients subjected to treatment with SRT. The percentage recovery of the spiked analytes was calculated.

3. Results and discussion

3.1. Chromatographic conditions

A mixture of a pH 3.0 phosphate buffer containing 0.1% trimethylamine and acetonitrile (65:35) at a flow rate of 1.2 mL min^{-1} proved to be suitable for the separation of SRT and DSR on a C8 reversed-phase column, within a run time of 14 min. A detection wavelength of 220 nm was chosen as a good compromise between the two relative absorbance maxima of the analytes at 208 and 275 nm. In fact, the maximum at 208 nm would confer higher sensitivity but selectivity would dramatically decrease; on the other hand, the maximum at 275 nm has a lower potential for interference but has a quite low absorbance value. Clomipramine was used as the internal standard (IS).

3.2. Solid-phase extraction procedure

Different kinds of sorbents were tested for the SPE procedure: Certify, diol, octyl, octadecyl, ethyl and hydrophilic-lipophilic balance (HLB). Certify and HLB cartridges did not allow for an appropriate purification of the biological samples; hydrophilic (diol) and lipophilic (octyl, octadecyl) cartridges, gave very low recoveries of the analytes. Weakly hydrophobic (ethyl) cartridges proved to be the most suitable. Washing was carried out with a mixture of basic buffer and methanol in order to keep the basic analytes in their non-protonated form. An aliquot of 50 μL of methanol was added as the final washing step since this volume of eluate did not contain any detectable amount of the analytes. The chromatogram of a blank plasma sample subjected to the SPE procedure is shown in Fig. 2a; the chromatogram of the same plasma sample spiked with 50 ng mL^{-1} of SRT, 100 ng mL^{-1} of DSR and 100 ng mL^{-1} of the IS is shown in Fig. 2b. As can be seen, peak shapes and resolution are good and sample clean-up is satisfactory.

3.3. Method validation

The method was validated in terms of linearity, extraction yield and precision. Good linearity ($r_c > 0.998$) was

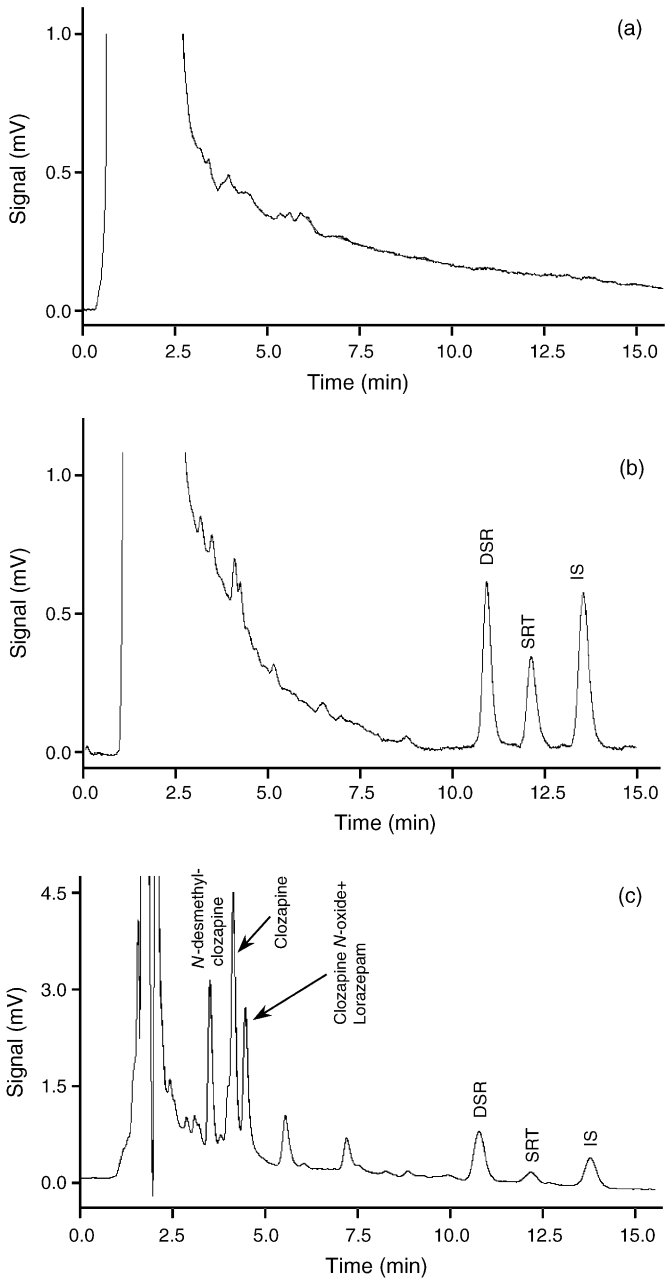

Fig. 2. Chromatograms of: (a) a blank plasma sample; (b) a blank plasma sample spiked with 50 ng mL^{-1} of SRT, 100 ng mL^{-1} of DSR and 100 ng mL^{-1} of the IS; and (c) a plasma sample from a patient subjected to therapy with 50 mg day^{-1} of SRT as well as clozapine, fluphenazine, brotizolam, lorazepam, flurazepam, acetylsalycilic acid, carvedilol and losartan.

obtained on spiked blank plasma samples in the following concentration ranges: 7.5–250.0 ng mL^{-1} for SRT; 10–500 ng mL^{-1} for DSR. Regression equations: $y = (-0.0085 \pm 0.0011) + (0.0124 \pm 0.0008)\ x$ for SRT; $y = (-0.0103 \pm 0.0010) + (0.0112 \pm 0.0009)\ x$ for DSR. The LOQ was 7.5 ng mL^{-1} for both analytes, while the LOD was 2.5 ng mL^{-1} for both analytes. Extraction yield assays also gave good results. In fact, mean absolute recovery was higher than 94% for both analytes. Precision values were very satisfactory: R.S.D.% values for repeatability (intraday precision) were lower than or equal to 3.5; R.S.D.% values for intermediate

Table 1
Validation parameters

Analyte	Concentration (ng mL^{-1})	Extraction yield (%)[a]	Repeatability (R.S.D.%)[a]	Intermediate precision (R.S.D.%)[a]
SRT	7.5	95	3.3	3.7
	125.0	95	2.4	2.9
	250.0	94	2.2	2.4
DSR	10	94	3.5	3.9
	250	95	2.4	2.7
	500	95	2.2	2.6
IS	100	92	2.4	2.5

[a] $n = 6$.

precision (interday precision) were lower than or equal to 3.9. Mean extraction yield for the IS was 92%, with an R.S.D.% lower than or equal to 2.5. The details of extraction yield and precision assays are reported in Table 1.

3.4. Selectivity

Assays were carried out to identify potentially interfering drugs. Standard solutions of compounds belonging to different therapeutic classes were injected: antidepressants (trazodone, fluvoxamine, paroxetine, fluoxetine, citalopram, imipramine, maprotiline, amitriptiline, reboxetine, venlafaxine), mood stabilisers (clonazepam, diazepam, flurazepam, lorazepam, brotizolam, carbamazepine), neuroleptics (clotiapine, haloperidol, chlorpromazine, zuclopenthixol, levomepromazine, fluphenazine) and atypical antipsychotics (risperidone, quetiapine, olanzapine, clozapine). None of these drugs had retention times similar to those of the analytes or the IS; thus, the method has proven to be quite selective. The anti-inflammatory drug indomethacin has also been tested, and it does not interfere in the determination; since its mean extraction yield after the SPE procedure is 94%, this compound can be used as an alternative IS in the uncommon event of polypharmacy with sertraline and clomipramine.

3.5. Application to patient plasma

The determination of SRT and DSR levels was carried out on the plasma of some patients undergoing monotherapy and polypharmacy obtaining good results; none of these samples posed any problem for the quantitation of the analytes, and no interference from the matrix was found in any of them. The chromatogram obtained injecting a plasma sample from a patient who was subjected to polypharmacy with nine drugs (clozapine, fluphenazine, brotizolam, lorazepam, flurazepam, acetylsalycilic acid, carvedilol and losartan as well as 50 mg day^{-1} of SRT) is shown in Fig. 2c. It can be seen that none of the other drugs interferes in the analysis even in this quite peculiar case, thus confirming the appropriate selectivity of the analytical procedure. SRT and DSR levels in this sample were found to be 31 ng mL^{-1} and 167 ng mL^{-1}, respectively; the peaks corresponding to clozapine, desmethylclozapine, clozapine *N*-oxide and lorazepam can also be seen in the chromatogram (clozapine *N*-oxide and lorazepam are co-eluting). Accuracy was assessed on patient plasma samples as detailed in Section 2. The results of these assays were satisfactory: mean accuracy resulted to be 92% for SRT and 90% for DSR.

4. Conclusion

The analytical method presented here is based on the use of a simple HPLC-UV apparatus and allows for the reliable determination of SRT and its main metabolite DSR in plasma of depressed patients undergoing therapy with SRT. The SPE procedure developed for this assay grants appropriate sample purification and gives good extraction yields (>94% for both analytes) with satisfactory precision (R.S.D. < 3.9%). Due to its good selectivity, the method can be used for patients subjected to polipharmacy. Compared to previously published pre-treatment procedures based on liquid–liquid extraction, the SPE procedure presented herein has demonstrated to be superior in terms of rapidity, feasibility, environmental compatibility, precision [15–17] and extraction yield [14]. The method seems thus to be suitable for the therapeutic drug monitoring (TDM) of SRT and DSR in plasma of depressed patients.

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References

[1] A.M. Comer, D.P. Figgitt, CNS Drugs 14 (2000) 391.
[2] D. Murdoch, D. McTavish, Drugs 44 (1992) 604.
[3] J. van Harten, Clin. Pharmacokinet. 24 (1993) 203.
[4] G. MacQueen, L. Born, M. Steiner, CNS Drug Rev. 7 (2001) 1.
[5] Z.-H. Xu, W. Wang, X.-J. Zhao, S.-L. Huang, B. Zhu, N. He, Y. Shu, Z.-Q. Liu, H.-H. Zhou, Brit. J. Clin. Pharmacol. 48 (1999) 416.
[6] R.S. Obach, L.M. Cox, L.M. Tremaine, Drug Metab. Dispos. 33 (2005) 262.
[7] M.V. Rudorfer, W.Z. Potter, CNS Drugs 7 (1997) 273.
[8] J. Lundmark, M. Reis, F. Bengtsson, Ther. Drug Monit. 22 (2000) 446.
[9] L.M. Tremaine, E.A. Joerg, J. Chromatogr. 496 (1989) 423.
[10] H.G. Fouda, R.A. Ronfeld, J. Chromatogr. 417 (1987) 197.
[11] D. Rogowsky, M. Marr, G. Long, C. Moore, J. Chromatogr. B 655 (1994) 138.
[12] H.L. Wiener, H.K. Kramer, M.E.A. Reith, J. Chromatogr. 527 (1990) 467.
[13] B.K. Logan, P.N. Friel, G.A. Case, J. Anal. Toxicol. 18 (1994) 139.
[14] M.K. Kyung, J.B. Hwa, C.M. Ho, W.J. Soo, P. Ki-Jung, C.B. Chul, J. Chromatogr. B 769 (2002) 333.
[15] K. Titier, N. Castaing, E. Scotto-Gomez, F. Pehourcq, N. Moore, M. Molimard, Ther. Drug Monit. 25 (2003) 581.
[16] C. Duverneuil, G.L. de la Grandmaison, P. de Mazancourt, J.C. Alvarez, Ther. Drug Monit. 25 (2003) 565.
[17] A. Lucca, G. Gentilini, S. Lopez-Silvia, A. Soldarini, Ther. Drug Monit. 22 (2000) 271.
[18] J. Patel, E.P. Spencer, R.J. Flanagan, Biomed. Chromatogr. 10 (1996) 351.