

# Simultaneous Monitoring of Selective Serotonin Reuptake Inhibitors in Human Urine, Plasma and Oral Fluid by Reverse-Phase High Performance Liquid Chromatography

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**A simple and rapid reverse-phase high-performance liquid chromatography method is developed for the simultaneous determination of selective serotonin reuptake inhibitors (sertraline, citalopram, paroxetine and fluoxetine) in urine, plasma and oral fluid. Separation was performed on a Crestpack-18 column (4.6 × 250 mm × 5 μ) within 17.5 min. The mobile phase was composed of water (50 mL) [with glacial acetic acid (0.15 mL) + triethyl amine (0.30 mL)]–acetonitrile (40 mL)–methanol (10 mL), delivered isocratically (0.6 mL/min) at 270 nm. Liquid–liquid extraction was performed for isolation of analytes from biofluids. The developed methodology was validated in terms of sensitivity, linearity, accuracy, precision, stability and selectivity. The calibration curves were linear in the range of 5–1,000 ng/mL for all the compounds in three matrices, with coefficients of determination between 0.9991 to 0.9998. The average extraction recoveries for all the four analytes were above 90%. The limits of detection and limits of quantification were in the ranges of 0.02–1.20 and 0.12–2.51 ng/mL, respectively. The intra-day and inter-day variation coefficients were less than 8.0 and 11%, respectively. Moreover, the results were compared statistically for each analyte in three matrices and found to be equivalent, which signifies the absence of matrix effect. Thus, the method can be applied for the determination of selective serotonin reuptake inhibitors in urine, plasma and oral fluid for routine therapeutic and toxicological screening.**

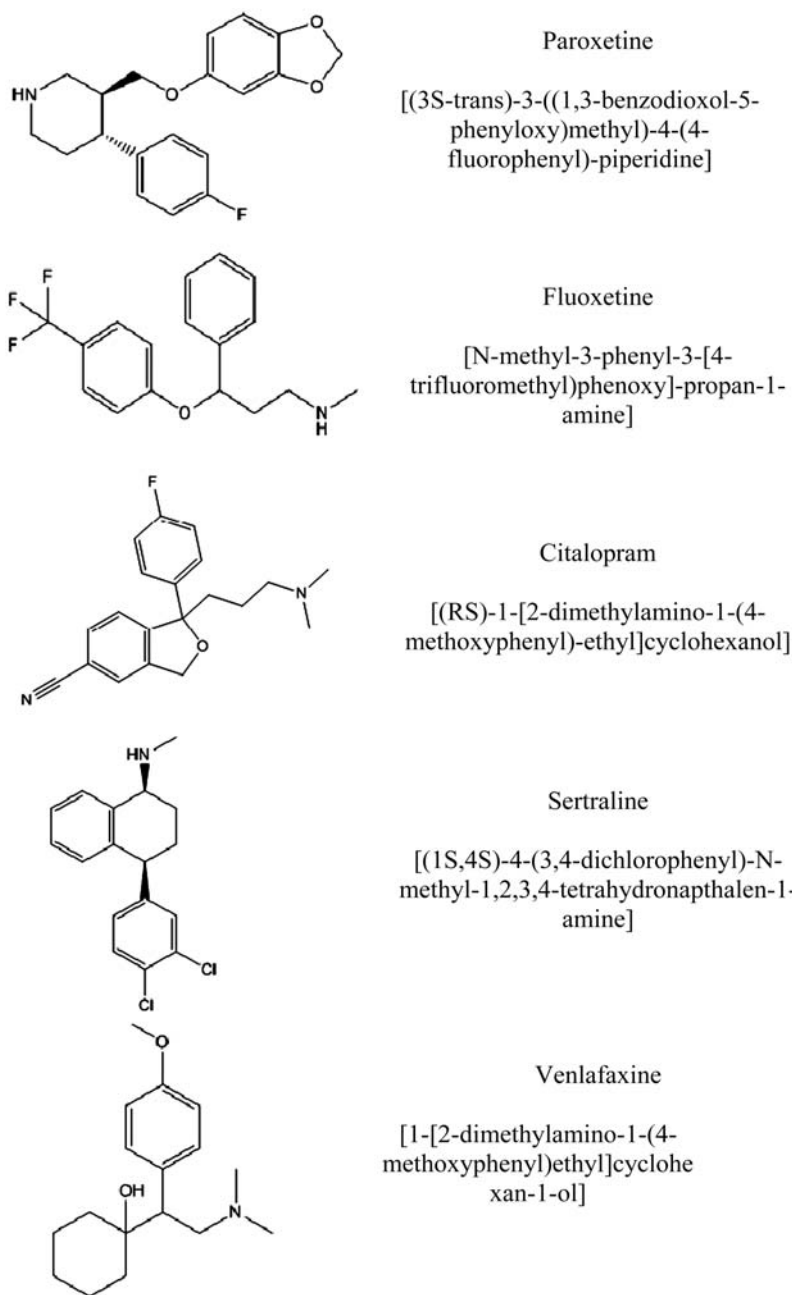
## Introduction

An antidepressant is a psychiatric medication used to alleviate mood disorders like depression and anxiety. These are chronic, recurring and potentially life-threatening illness with a broad range of symptoms and disorders. Nowadays, antidepressant drugs are the first choice to cure such disorders. This group covers drugs such as monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), tetracyclic antidepressants (TeCAs), selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) (1). SSRIs are the most often prescribed drugs, which are comparable to TCAs in their clinical efficacy, but because of their favourable pharmacological profile, they are considered safe, potent and well-tolerated (2, 3). The commonly administered drugs of this class are sertraline (SER), paroxetine (PAR), fluoxetine (FLU) and citalopram (CIT) (Figure 1). The primary risks associated with SSRIs are treatment-emergent suicidal

behaviour and withdrawal symptoms, especially in children and adolescents. Cases have also been observed in which suicidal and accidental deaths have occurred involving antidepressant drugs that are frequently abused (4, 5, 6). In addition, they are also considered to be date-rape drugs that have used for the purpose of drugging unsuspected victims for raping or robbing (7). Previous studies have indicated that these drugs may interact with transcription factors known as clock genes responsible for the addictive properties of drugs (8, 9). Thus, it is worthy to develop a sensitive, rapid and reliable method for their determination in different biological fluids for therapeutic and toxic drug monitoring.

Several methods have been published for the determination of one or more antidepressants in biological fluids for therapeutic monitoring or toxicological purposes. Liquid chromatography (LC) is considered to be the most demanding and sensitive technique for the separation and detection of SSRIs from various biological matrices. The use of tandem mass spectrometry (MS-MS) for LC detection has allowed limits of detection up to the nano level (10, 11). Gas chromatography has also been applied using different detectors, such as nitrogen-phosphorus (12), flame-ionization (13), or MS (14). Capillary electrophoresis was recently proposed for the analysis of FLU at clinically relevant levels in human urine (15, 16). Micellar electrokinetic capillary chromatography (MEKC) has been employed for the determination of antidepressant drugs (17, 18). Cyclic voltammetry techniques, such as cyclic, linear sweep, differential pulse and square wave voltammetry, have been preferred for the rapid detection of antidepressant drugs (19). High-performance liquid chromatography (HPLC) is used in clinical and toxicological laboratories. It offers analytical specificity superior to that of immunoassays and other chromatographic techniques. Moreover, it is a prevalent technique that provides a wide range of applicability in combination with many detectors for single to multi-component analysis (20–24). HPLC does not require pre-analytical treatments like derivatization of analytes before analysis and provides sensitivity similar to other sophisticated techniques. Its simplicity of analysis and cost-effectiveness makes this technique a primary choice.

The estimation of drugs in biofluids is the basis of this clinical study, and it is equally relevant for toxicological studies. Biological fluids such as urine, blood and oral fluid are very complex because these contain proteins, salts, acids, bases, and numerous organic compounds that can interfere with the



**Figure 1.** Chemical structures of selected SSRI drugs.

analytes of interest. Thus, various types of extraction procedures are required to eliminate such interferences, because a small sample may result in a highly comprehensive identification. Various techniques are used for the preparation of samples, such as solid-phase extraction (25, 26), solid-phase microextraction (27), liquid-phase microextraction (21) and stir bar sorptive extraction (28). However, liquid-liquid extraction (LLE) is most often used for the extraction of antidepressant drugs and does not require expensive instruments and techniques to process. In addition, the development of the LLE procedure is simple and provides similar sensitivity to other extraction techniques (12, 29, 30). Different extraction media

have been reported, such as like *n*-hexane (31), toluene (12), diethyl-ether (32) or a mixture of hexane with butanol (24), chloroform–2-isopropanol–*n*-heptane (29), ethyl-acetate (33) and iso-amyl alcohol (34) in different ratios. The use of a mixture of *n*-heptane with ethyl acetate has also been reported. Sometimes a back-extraction under acidic conditions (HCl) is applied, followed by a direct injection on the HPLC system (34, 35).

We designed a method for the simultaneous estimation of SER, CIT, PAR and FLU in biological fluids like urine, blood and oral fluid, using reversed-phase (RP)-HPLC. So far, no direct method has been reported for the simultaneous monitoring of

these analytes in three different matrices. The assay described here offers a short chromatographic run at a low flow rate in a small sample volume and is sensitive, specific, rapid and economical. Additionally, the emphasis was directed toward an approach with a single-step LLE procedure. The developed method may find wide applications in the quantitative evaluation of these analytes in said matrices, which are forensically and clinically significant.

## Experimental

### Standard and reagents

SER, PAR, FLU and venlafaxine (VLF) were provided by Zydus Cadila Pharmaceutical (Ahmedabad, Gujarat, India). CIT was provided by Torrent Pharmaceuticals (Ahmedabad, Gujarat, India). HPLC-grade methanol, acetonitrile and water were purchased from Abhishek Enterprises (Ahmedabad, Gujarat, India). All analytical grade solvents and reagents were purchased from Abhishek Enterprises (Ahmedabad, Gujarat, India).

### Biological samples

Urine and oral fluid were collected from healthy volunteers in normal conditions, and blood was provided from a local blood bank, from which plasma was separated for analysis after centrifugation. The samples were stored in appropriate polytetrafluoroethylene (PTFE) flasks at  $-20^{\circ}\text{C}$  until analysis.

### Chromatographic conditions

The RP-HPLC system was purchased from Jasco (Tokyo, Japan). It composed of a pump (PU-2080 Plus) and Crestpack-18 column ( $4.6 \times 250 \text{ mm} \times 5 \mu$ ) was used at ambient temperature. The mobile phase was composed of water (50 mL) [with glacial acetic acid (0.15 mL) + triethyl amine (0.30 mL)]–acetonitrile (40 mL)–methanol (10 mL) and was delivered isocratically at a flow rate of 0.6 mL/min. Sample injection volume was 20  $\mu\text{L}$ , injected via a Rheodyne injection valve (provided from Jasco). Detection was achieved at a wavelength of 270 nm by ultraviolet (UV) detector (UV-2075 Plus). Degassing of the solvents was achieved by ultrasonication before use. The software (ChromNAV) used for the data acquisition was supplied by the manufacturer only.

### Standard and sample preparation

Standard or stock solutions of all analytes were prepared by dissolving accurately weighed amounts (1 mg in 10 mL) of each reference compound in methanol to yield 100  $\mu\text{g}/\text{mL}$  of each. A 100  $\mu\text{g}/\text{mL}$  of stock solution of VLF [internal standard (IS)] in methanol was also prepared and further diluted to prepare the working solution. Working standards of the analytes were prepared in methanol within the range of 5–1,000 ng/mL. All stock solutions were stored at  $-20^{\circ}\text{C}$ , protected from light, and were found to be stable for three months.

Routine daily calibration curves were prepared by the addition of each standard series of analytes to blank urine, plasma and oral fluid. Aliquots of 0.05, 0.1, 0.5, 1, 2 and 4  $\mu\text{L}$  of each working solution and 1 mL of blank urine/plasma/oral fluid

were added to each test tube, which resulted in final drug concentrations of 5, 10, 50, 100, 200 and 400 ng/mL in each biological fluid. Quality control (QC) samples that were run in each assay were prepared in the same way, and final concentrations were between 10–400 ng/mL. Each spiked sample was processed as described previously.

### Sample preparation

#### Urine

The extraction consisted of the addition of 50  $\mu\text{L}$  of IS and 20 mg of tartaric acid to 1 mL of spiked urine to adjust the PH to 3. The solution was then extracted with two 3-mL portions of ether, after which the extracts were washed with 3 mL of water. The aqueous solution was retained for the presence of analytes of interest. To this aqueous solution was added a small amount of dilute ammonia solution to adjust the PH to 8, followed by the addition of 5 mL chloroform. After vigorous shaking and centrifugation, the organic layer was separated and evaporated to dryness under a constant air flow at room temperature. The residue was reconstituted with 100  $\mu\text{L}$  of methanol and chromatographed.

#### Plasma

For protein binding disruption, dilution of the 1-mL spiked plasma sample was used containing 50  $\mu\text{L}$  of IS with phosphate buffer of pH 2.5 (4 mL, 25 mM). This was followed by the centrifugation step (at 1,500 rpm) for 5 min. The supernatant was thereafter transferred for the extraction procedure. A similar extraction procedure was followed for the plasma sample as for the urine sample (except for the addition of tartaric acid).

#### Oral fluid

Denaturation of protein was conducted by the addition of 0.5 mL of trifluoroacetic acid to 1 mL of spiked oral fluid containing 50  $\mu\text{L}$  of IS. This was followed by centrifugation, and the supernatant was then administered for further extraction. Two hundred microliters of ammonium bicarbonate (0.2M, pH 9.3) and 1.25 mL ethyl acetate–heptane (4:1, v/v) were added to the supernatant. The mixture was shaken (15 min) and centrifuged (1,500 rpm) for 5 min. The organic phase was removed and evaporated at room temperature. The residues were re-suspended in 100  $\mu\text{L}$  of methanol and chromatographed.

### Method validation

The recovery of the drugs was determined at three different concentrations (QC samples) in blank urine, plasma and oral fluid samples. Biological samples with the drugs were extracted in triplicate according to the proposed procedures. The concentrations of these samples were calculated on the basis of calibration curves constructed from the reference standard solutions of the drugs (not submitted to the extraction procedures) prepared at the same concentrations.

To determine the intra-assay precision, aliquots ( $n = 6$ ) of blank urine, plasma and oral fluid containing the standard solution of the drugs at three concentrations (QC samples) were analyzed. To determine the inter-assay precision, blank urine,

plasma and oral fluid samples containing the standard solution at the same concentrations were analyzed on six consecutive days. Linearity was obtained by analyzing blank urine and plasma samples ( $n = 3$ ) containing standard solutions of drugs at QC levels. The standard curves were constructed by least-squares linear regression of the peak-area ratios of the drugs to the IS versus the respective standard concentration.

The limit of detection (LOD) is the minimal concentration from which the presence of the analyte can be deduced with reasonable statistical certainty. The limit of quantification (LOQ) is the minimal measured content of the identified analyte in a sample that can be quantified with a specified degree of accuracy and within-laboratory reproducibility. LOD was estimated by spiking urine with a decreasing concentration of analytes until the response of three times the background noise was obtained, and the LOQ was estimated by spiking urine with a decreasing concentration of analytes until the response of ten times the background noise was obtained.

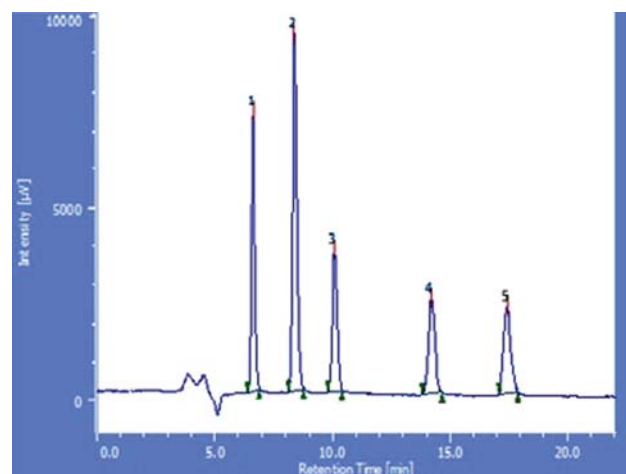
Selectivity was studied by accessing the absence of interference in the same chromatographic windows as the examined drugs in urine, plasma and oral fluid. Analysis of blank matrices was used to demonstrate the selectivity of the method. The accuracy (or percent relative error) was expressed as the absolute recovery and was measured by the comparison of peak area ratios of extracted samples of spiked biological matrices to standard solutions that were not extracted.

## Result and Discussion

The bioavailability of SSRIs after oral administration is approximately 80%. The peak plasma concentration occurs between 2 and 8 h after dosing. To some extent, they are also excreted in urine as a parent compound (36). The presence of SSRIs in oral fluid shows their long-term exposure, which has been successfully used in the bioanalytical studies as an alternative matrix to blood and urine (37, 38). Many methods have been provided for the determination of SER, FLU, CIT and PAR in biological fluids, obtained from patients after proper administration. It has been observed that the LOD values of the proposed method were in good agreement with those obtained from real biological samples for analytes of interest (39–42).

RP-HPLC separations were performed on stationary phase with modified silica. This silica is fairly reactive, which allows variations in the nature of the stationary and mobile phases to incorporate a wide range of selectivity and sensitivity. The suitable mobile phase was selected by a number of preliminary experiments that resulted in satisfactory separation with the selected analytical column (Crestpack-18).

Moreover, the mobile phase was delivered isocratically, which shows some advantages over gradient analysis, which requires long assay time with complex method development procedures (43). The aqueous component in the mobile phase showed an effect on the retention of analytes (ionizable), because the ionized form of the solute does not partition well into the hydrophobic stationary phase. Thus, the aqueous component of the mobile phase was kept high, which aided the proper separation of the analytes of interest (hydrophobic) during reverse-phase separation (because the stationary phase is also hydrophobic in nature) (44). Additionally, it was also



**Figure 2.** Typical RP-HPLC chromatogram of the examined SSRIs (10 ng/mL CIT, FLU, PAR and SER) in the presence of VLF (10 ng/mL) as the IS. RTs: 1, VLF, 6.6 min; 2, CIT, 8.3 min; 3, FLU, 10.0 min; 4, PAR, 14.1 min, 5, SER, 17.3 min.

observed after a series of experiments that the number and ratios of organic modifiers play a very important role in the resolution of the analytes. Thus, it was also determined that methanol should be used in the mobile phase, which often results in an improved peak shape as an organic modifier (45). Also, the antidepressants studied in this work are compounds with basic properties in the usual working pH range of a C-18 column (2.5–7.5). In this pH range, the drugs were in the cationic form, because of the protonation of the amine groups in the molecules. Thus, triethylamine (TEA) was added as a cationic additive, which gave rise to a positively charged stationary phase, repelling protonated basic drugs and suppressing the tailing of basic analytes (46). Glacial acetic acid was used to maintain the pH of the mobile phase (pH = 5.75) (47).

VLF was chosen as the IS because it shows similar chromatographic behaviour to the drugs of interest with no interference. This was completely resolved from the other four antidepressants. The separation of the four selected analytes and the IS was achieved in less than 17.5 min with the flow rate of 0.6 mL/min. Resolution factors ranged from 0.9 to 1.20, indicating an excellent separation. Under the described chromatographic conditions, the retention times (RT) for the examined compounds were (1) 6.6 min (VLF as IS), (2) 8.3 min (CIT), (3) 10.0 min (FLU), (4) 14.1 min (PAR) and (5) 17.3 min (SER). A typical chromatogram of a standard solution is shown in Figure 2.

## Validation

### Linearity and range

Linearity was determined from the results of replicate injections of six different concentrations ranging from 5–1000 ng/mL. Calibration curves were constructed through plotting the peak area ratios of the drugs to the IS versus the respective standard concentration. The correlation coefficients ranged from 0.9991 to 0.9998.

**Table I**  
Regression and Sensitivity Data for All Analytes in Standard Solutions, Urine, Plasma and Oral Fluids

Analytes	Parameters*	SER	PAR	FLU	CIT
Standard	Range (ng/mL)	5–1,000	10–1,000	10–1,000	5–1,000
	Correlation coefficient ( $r^2$ )	0.9980	0.9986	0.9998	0.9990
	LOD (ng/mL)	0.22	0.02	0.65	1.20
	LOQ (ng/mL)	0.67	0.12	1.98	2.51
Urine	Correlation coefficient ( $r^2$ )	0.9986	0.9984	0.9991	0.9985
	LOD (ng/mL)	0.30	0.70	0.89	0.51
	LOQ (ng/mL)	0.90	1.20	1.92	1.10
Plasma	Correlation coefficient ( $r^2$ )	0.9980	0.9984	0.9990	0.9986
	LOD (ng/mL)	0.52	0.61	0.81	0.51
	LOQ (ng/mL)	0.98	1.22	2.41	1.76
Oral fluid	Correlation coefficient ( $r^2$ )	0.9979	0.9983	0.9985	0.9982
	LOD (ng/mL)	0.70	0.80	0.52	0.60
	LOQ (ng/mL)	1.80	2.23	1.01	1.82

\*LOD =  $3.3 \times \text{SD}/\text{slope}$ ; LOQ =  $10 \times \text{SD}/\text{slope}$ .

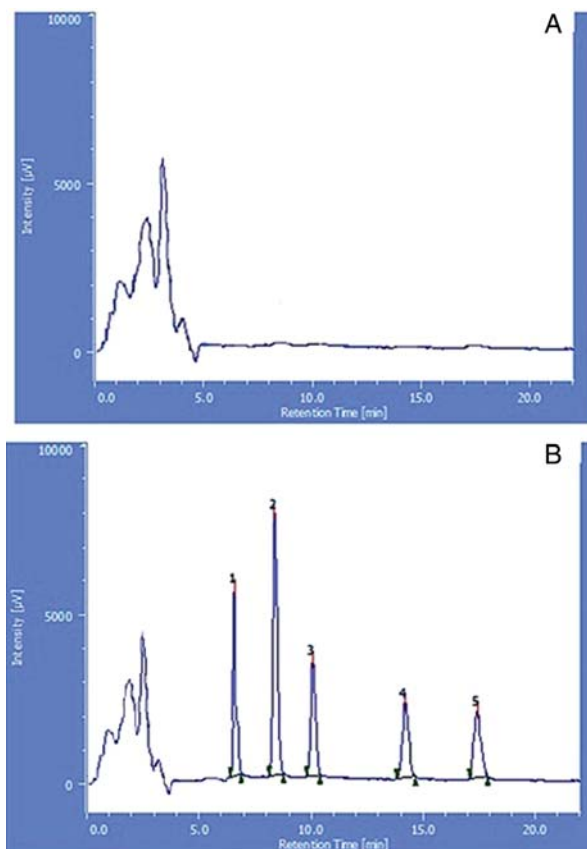
Biological assays were validated in the same manner, ranging from 5–1,000 ng/mL in urine, plasma and oral fluid. The calibration graphs were compared with those of calibration standards and few or slight significant differences were found between them, which suggests no or very few matrix interferences. In all three matrices, the correlation coefficients were greater than 0.9978, as shown in Table I. These results were in good agreement with the well-established method proposed for the therapeutic drug monitoring of similar analytes (29). The most recently published clinical studies showed that the optimal plasma concentrations mostly ranged between 50–400  $\mu\text{g}/\text{L}$  for therapeutic studies (48, 49). Thus, the range selected for the assays is suitable for therapeutic and toxicological monitoring of the analytes in biofluids.

#### Selectivity and sensitivity

To identify the selectivity of the method, representative chromatograms of mixed working solution, blank (uncontaminated) extracts and spiked blank sample extracts were compared (Figures 3, 4 and 5). All peaks evidencing RTs identical to their corresponding standards were evaluated using a UV detector (270 nm). The LODs and LOQs were in the range of 0.02–1.20 and 0.12–2.51 ng/mL, respectively. In case of biological assay, they were found to be 0.30–0.89 and 0.90–1.92 ng/mL in urine, 0.52–0.61 and 0.98–2.41 ng/mL in plasma, 0.52–0.80 and 1.01–2.23 ng/mL in oral fluid, respectively (Table I). Indeed, the LOD found for SER, CIT, PAR and FLU is suitable for clinical and toxicological drug monitoring.

#### Accuracy and precision

The accuracy for all three biological fluids was accessed by means of recovery percentage, performing six determinations of QC samples. Results were found between 76–104%. To determine the intra-day precision, aliquots ( $n=6$ ) of spiked urine, plasma and oral fluid were determined by QC samples at three different concentrations, as described previously. To determine the inter-day precision, aliquots ( $n=6$ ) of of spiked urine, plasma and oral fluid at the same concentrations were analyzed. In experiments performed to determine the

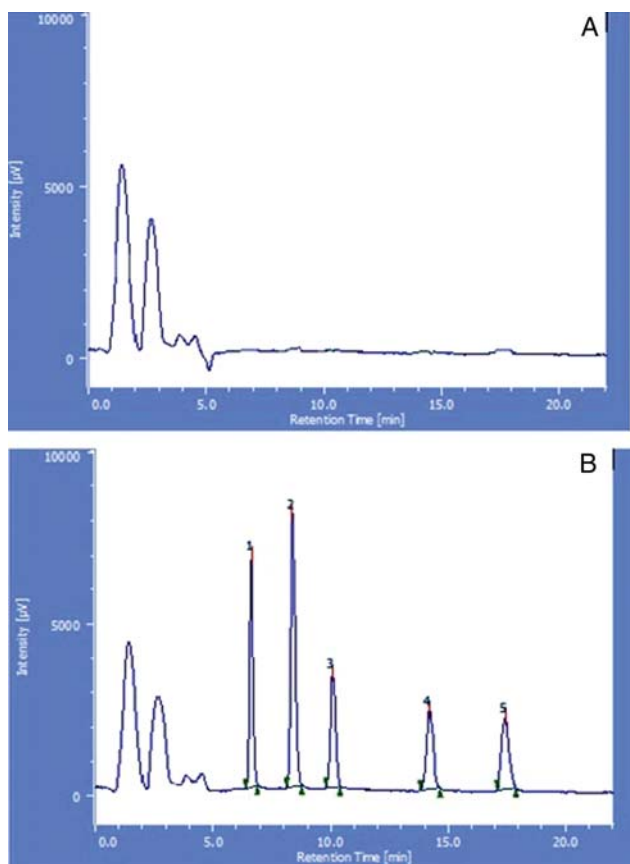


**Figure 3.** HPLC chromatogram of SSRIs in human plasma: blank (A); spiked with 10 ng/mL of CIT, FLU, PAR and SER in the presence of VLF (10 ng/mL) as the IS (B). RTs: 1, VLF, 6.6 min; 2, CIT, 8.3 min; 3, FLU, 10.0 min; 4, PAR, 14.1 min, 5, SER, 17.3 min.

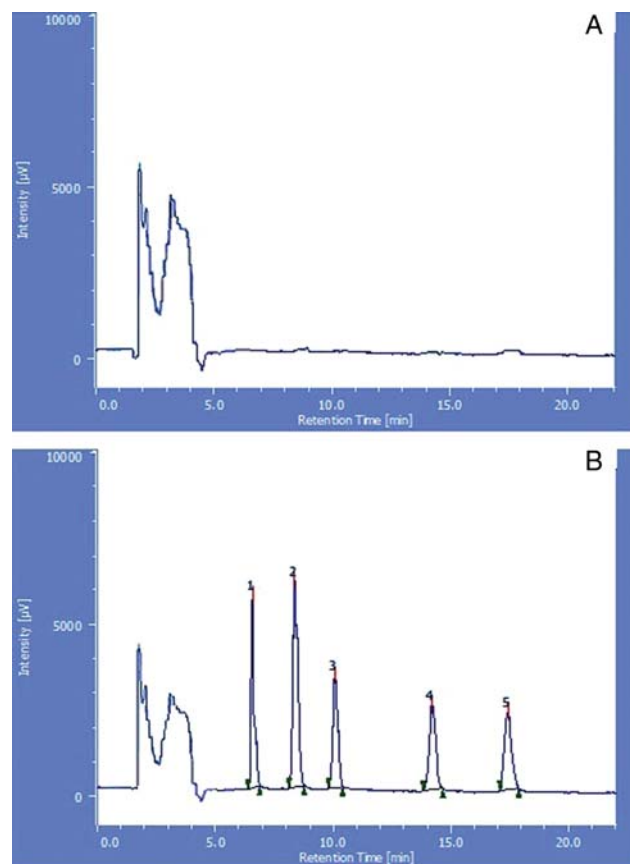
repeatability, it was remarkable that the relative standard deviation (RSD) was  $<8.0\%$ . The reproducibility was also satisfactory, with RSD  $<11.0\%$  for all analytes in three matrices. The results are summarized in Table II.

#### Recovery

Extraction recovery was determined by comparing the peak area ratios of extracted samples of spiked matrices against non-extracted standard solutions at QC levels. Despite high protein binding efficiency and differences in the chemical structures of the analytes, the extraction recoveries were found to be satisfactory. The mean recovery rates for SER were 103% in urine, 91.0% in plasma and 95% in oral fluid; recovery rates for PAR were 100.03% in urine, 102.2% in plasma and 92% in oral fluid; recovery rates for CIT were 96.2% in urine, 99.5% in plasma and 94% in oral fluid; recovery rates for FLU were 102.3% in urine, 100.8% in plasma and 96.4% in oral fluid. The protein binding disruption has been shown to be the most important and variable factor influencing extraction. Therefore, the use of tartaric acid (urine), phosphate buffer (plasma) and trifluoroacetic acid (oral fluid) for protein denaturation played an important step in obtaining efficient recovery. This is a simple and reproducible procedure for purification of biological samples that is easily applicable in routine laboratory



**Figure 4.** HPLC chromatogram of SSRIs in human urine: blank (A); spiked with 10 ng/mL of CIT, FLU, PAR and SER in the presence of VLF (10 ng/mL) as the IS (B). RTs: 1, VLF, 6.6 min; 2, CIT, 8.3 min; 3, FLU, 10.0 min; 4, PAR, 14.1 min, 5, SER, 17.3 min.



**Figure 5.** HPLC chromatogram of SSRIs in human oral fluid: blank (A); spiked with (10 ng/mL) of CIT, FLU, PAR and SER in the presence of VLF (10 ng/mL) as the IS (B). RTs: 1, VLF, 6.6 min; 2, CIT, 8.3 min; 3, FLU, 10.0 min; 4, PAR, 14.1 min, 5, SER, 17.3 min.

procedures. A similar extraction yield was obtained after LLE in the range of 83–96% was applied for the screening of similar analytes for toxicological and therapeutic investigative purposes (29).

#### Stability

Spiked samples (5 ng/mL) were stored in a freezer at  $-20^{\circ}\text{C}$  and analyzed at selected times by the proposed RP-HPLC method over a period of 90 days for urine and plasma, and 60 days for saliva. At  $-20^{\circ}\text{C}$ , at the end of the observation period, a decrease was measured at approximately 20% in urine and plasma, and at approximately 35% in oral fluid for all four analytes. Moreover, a stability study of all analytes over the same period revealed that the standard solution in methanol stored at  $4^{\circ}\text{C}$  can be used without any remarkable degradation. A significant decrease in concentration occurred at room temperature in both plasma and urine samples for all analytes during the 90-h intervals and in saliva at the end of the study period of 60-h intervals. The results of the stability study through freeze-thaw cycles over seven successive days for spiked samples stored at  $-20^{\circ}\text{C}$  showed that all compounds showed stability after five freeze-thaw cycles in urine and plasma and after three freeze-thaw cycles in oral fluid (Supplementary Figures 1, 2 and 3). The data suggest that quantitative results

concerning long-term stored samples should be interpreted with caution in forensic cases, especially when they are stored at room temperatures, because it was observed that the storage conditions were an important aspect in the recovery of drugs.

#### Applications

This method can be successfully applied to the analysis of real urine, plasma and oral fluid samples in clinical and forensic analysis of SSRIs. All three matrices were analyzed as described previously. The chromatograms show no interference from endogenous components of any of the matrices. These drugs are neither offered together in formulation nor administered together, but a multi-analyte method is a useful tool in bio-analysis and pharmaceutical analysis of these drugs. The key feature of this method is its applicability to the detection of new-generation antidepressant drugs in alternative biological matrices like oral fluid, which can also be an alternative to urine and blood for drugs-of-abuse testing. Future applications of the developed method could include the determination of alternative biological matrices like sweat and hair. To the best of our knowledge, this method is suitable for pharmacokinetic, clinical and toxicological studies of the four drugs.

**Table II**

Evaluation of Accuracy and Precision of SSRIs in Urine, Plasma and Oral Fluid by the Proposed Method

Analyte	Intra-day (n = 5)			Inter-day (n = 5)		
	Added (ng/mL)	Found (ng/mL) ± SD*	Recovery <sup>†</sup> (%) ± RSD	Added (ng/mL)	Found (ng/mL) ± SD	Recovery (%) ± RSD
Urine						
SER	10	8.5 ± 0.2	85.9 ± 2.9	10	8.0 ± 0.1	4 ± 3.8
	100	88.9 ± 0.7	88.9 ± 6.5	100	98.8 ± 0.7	98.8 ± 7.0
	400	398.4 ± 1.5	99.6 ± 4.2	400	397.8 ± 1.0	99.4 ± 4.0
PAR	10	8.2 ± 0.1	82.0 ± 7.9	10	8.0 ± 0.3	80.0 ± 7.8
	100	97.3 ± 0.2	97.3 ± 2.4	100	96.2 ± 0.4	96.2 ± 0.42
	400	403.8 ± 0.4	100.7 ± 7.4	400	401.1 ± 0.1	100.2 ± 7.3
CIT	10	10.4 ± 0.8	104.0 ± 6.3	10	10.2 ± 0.7	102.0 ± 4.2
	100	98.9 ± 1.8	98.8 ± 4.8	100	101.2 ± 0.5	101.2 ± 10.5
	400	402.3 ± 2.3	100.5 ± 6.6	400	397.9 ± 4.1	99.4 ± 7.8
FLU	10	9.70 ± 4.9	97.0 ± 7.2	10	8.6 ± 4.2	86.0 ± 8.0
	100	102.8 ± 2.8	102.8 ± 5.4	100	101.8 ± 5.0	101.8 ± 9.4
	400	403.2 ± 2.1	100.8 ± 5.3	400	400.9 ± 0.7	100.2 ± 5.8
Plasma						
SER	10	9.1 ± 0.2	91.0 ± 2.8	10	8.20 ± 0.1	82.0 ± 3.0
	100	100.8 ± 0.6	100.8 ± 7.6	100	98.5 ± 0.5	98.5 ± 6.8
	400	398.4 ± 1.8	99.6 ± 6.3	400	397.7 ± 0.7	99.4 ± 5.2
PAR	10	10.2 ± 3.3	102.0 ± 2.4	10	10.3 ± 1.7	103.0 ± 7.5
	100	97.2 ± 2.0	97.2 ± 3.8	100	96.3 ± 2.3	96.3 ± 6.3
	400	396.8 ± 0.4	99.2 ± 4.5	400	396.6 ± 0.8	99.1 ± 5.5
CIT	10	8.2 ± 1.7	82.0 ± 5.3	10	8.9 ± 2.6	89.0 ± 3.7
	100	96.8 ± 2.2	96.8 ± 6.2	100	96.1 ± 4.9	96.1 ± 6.8
	400	402.5 ± 5.2	100.6 ± 4.9	400	401.2 ± 0.8	100.3 ± 7.4
FLU	10	10.5 ± 4.9	103.0 ± 3.0	10	10.2 ± 5.4	102.0 ± 10.2
	100	98.0 ± 2.5	98.0 ± 7.8	100	96.5 ± 6.2	96.5 ± 7.6
	400	403.2 ± 4.2	100.8 ± 7.3	400	402.0 ± 1.8	100.5 ± 9.4
Oral Fluid						
SER	10	8.2 ± 0.1	82.0 ± 3.8	10	8.0 ± 0.7	80.0 ± 5.8
	100	96.4 ± 0.2	96.4 ± 6.9	100	95.0 ± 0.6	95.0 ± 6.3
	400	396.2 ± 1.6	99.05 ± 7.1	400	375.8 ± 2.4	93.9 ± 6.8
PAR	10	9.20 ± 0.4	92.0 ± 6.2	10	7.70 ± 3.1	77.0 ± 7.2
	100	101.2 ± 2.6	101.2 ± 7.4	100	101.1 ± 0.6	101.1 ± 8.0
	400	382.0 ± 4.3	95.5 ± 5.8	400	386.7 ± 2.3	96.6 ± 9.5
CIT	10	7.90 ± 0.8	79.0 ± 7.3	10	7.8 ± 5.6	78.0 ± 4.8
	100	98.3 ± 0.9	98.3 ± 3.8	100	99.6 ± 4.0	99.6 ± 8.2
	400	379.2 ± 4.2	94.7 ± 5.2	400	394.4 ± 0.8	99.1 ± 9.9
FLU	10	8.20 ± 3.9	82.0 ± 5.0	10	8.10 ± 2.3	81.0 ± 5.0
	100	95.8 ± 2.3	95.8 ± 4.4	100	94.2 ± 0.8	94.2 ± 4.4
	400	403.1 ± 5.4	100.7 ± 6.3	400	398.2 ± 0.4	99.5 ± 2.0

\*Average of six replicate determinations and their SD.

<sup>†</sup>Recovery: Amount Found/Amount Added × 100.

## Conclusion

In this paper, the performance of a simple, laboratory developed HPLC assay for monitoring of new-generation antidepressants in biological fluids is characterized. This is the first time when a paper described a method for simultaneous detection of SER, PAR, FLU and CIT in three bio-fluids. The assay is based upon a single-step LLE (i.e., without back-extraction or multiple steps that are employed in many other assays). Despite its simplicity, the proposed method is demonstrated to be suitable and sufficiently reliable for diverse clinical and toxicological investigations of compliance, drug overdoses, drug-induced psychoses and substance abuse. The stability studies showed that the analytes were stable during long-term periods for sample preparation and analysis. The method will be successfully applied for the simultaneous determination of selected classes of antidepressant drugs in biological samples at therapeutic and toxicological levels.

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