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Simultaneous determination of citalopram, fluoxetine and their main metabolites in human urine samples by solid-phase microextraction coupled with high-performance liquid chromatography

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Abstract

A liquid chromatography method was developed for the determination of some frequently prescribed selective serotonin re-uptake inhibitors (SSRI) – citalopram and fluoxetine – and its main metabolites – demethylcitalopram, didemethylcitalopram and norfluoxetine – in human urine samples, using a previous stage of solid-phase microextraction. All the extraction parameters influencing adsorption (extraction time, temperature, pH, ion strength and organic modifier addition) and desorption (desorption time and desorption solvent mixture composition) of the analytes on the fiber have been studied. A satisfactory reproducibility for extraction from urine samples (R.S.D. < 10%) was obtained. The linearity for urine ranged from 0.05 to 2 mg l^{-1} with limits of detection close to 0.01 mg l^{-1}, which cover the typical urinary concentrations obtained for citalopram, fluoxetine and their metabolites.

Keywords: SPME–HPLC; Antidepressant; Fluoxetine; Citalopram; Metabolites; Urine

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are a class of drugs indicated for the treatment of depression and other psychiatric disorders such as anxiety, obsessive compulsive disorder, post-traumatic disorder and pre-menstrual dysphoric disorder as well as for cases in which selective inhibition of serotonin (5-HT) reuptake is desired [1,2]. A reduction of the brain levels of 5-HT was involved in the aetiology of these diseases. The antidepressant effect of SSRIs derives from the property to block the 5-HT transporter increasing 5-HT concentration in the presynaptic neurons of the central nervous system [3]. It has been reported that this generation of antidepressants is safer with regard to severe side effects such as cardiovascular [4] and anticholinergic adverse effects [5] and overdose [6] when compared with tricyclic antidepressants. Furthermore, all the above contribute to the safety of the drug with regard to drug–drug interactions with other substrates [7]. These advantages have led to its increasing use in the treatment of depression [8].

Fluoxetine (FLX) (*N*-Methyl-3-(*p*-trifluoromethylphenoxy)-3-phenylpropylamine), is a very effective SSRI in all of the indications shown above [1,9] since it also has high affinity for another receptor (5-HT_{2C}) implicated in antidepressant pharmacololgy. Fluoxetine is mainly demethylated to norfluoxetine (NFLX), which shares the property of blocking the serotonin (5-HT) transporter. Due to the activity of NFLX as SSRI, it contributes to the long duration of the action of FLX [10]. Both FLX and NFLX have long elimination half-lives ranging from 1 to 6 days and from 5 to 6 days, respectively, and about 11% of the dose is excreted as unchanged FLX and about 7% as NFLX [11]. Citalopram (CIT) (1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile), is the most selective SSRI drug for the inhibition of serotonin reuptake [9]. It is relatively free of cytochrome p450 interactions and has

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low potential for drug–drug interactions, which increases its usefulness in drug combination therapy. Citalopram is metabolized by partially *N*-demethylation to demethylcitalopram (DCIT) and didemethylcitalopram (DDCIT) [12]. The elimination half-life of CIT and DCIT was found to vary between 23 and 45 h [11] and 66 and 92 h, respectively, while the half-life of the DDCIT metabolite has not been reported [13]. The percentage of CIT excreted in urine as unchanged is about 12% [11]. In view of the above considerations, the development of a simple and reproducible method for the determination of FLX and CIT and their main metabolites NFLX, DCIT and DDCIT in urine could be very useful for toxicological and therapeutic purposes such as the monitorization of the treatment prescribed in the clinical practice.

Any chronic disease is a risk situation for non-adherence to treatment. This results in suboptimal medication and poor disease control. Adherence and compliance are directly related to therapeutic success, which is further complicated in mental disorders such depression. There is a wide spectrum of non-adherent behaviour, from outright refusal to take prescribed medication to occasional, inadvertent missing of doses. Specifically, it has been shown that multiple dosing and chronic diseases (requiring long-term treatment) are associated with higher rates of nonadherence [14]. Therefore, one of the main aims of this research is also to probe the suitability of the proposed method for a reliable quantification of analytes at clinical levels.

To date several analytical methods have been developed for the analysis of FLX, CIT and in some cases also their metabolites in biological fluids. Most of these methods are based on reversed phase high-performance liquid chromatography coupled to UV [7,15–20] and to a lesser extent on fluorescence detection [21,22] and they have been applied in the quantification of these compounds in plasma and serum samples. There are also other works related to the quantification of FLX and CIT with other antidepressant drugs in biological matrices such as plasma [23,24], serum [25,26], blood [27,28], urine [29–31] and hair [32,33] using liquid chromatography–tandem mass spectrometry (LC–MS).

Gas chromatography is also applied in SSRI quantification coupled to nitrogen–phosphorous [34,35], flame ionization detector [36] and mass spectrometry detection for plasma [37] and urine samples [38–40].

Plasma, serum and blood sample preparation is usually performed by liquid–liquid extraction [7,15,18,20,22,27] or solid-phase extraction [17,21,24]. For the analysis of urine samples liquid–liquid extraction [40] and SPE [38] are also used, but these procedures involve multiple steps and they are labour intensive and time consuming [41]. Solid-phase microextraction (SPME) is a solvent-less, simple and selective technique that allows the simultaneous extraction and preconcentration of analytes from a sample matrix. SPME has been successfully applied to determine a wide range of drugs in biomedical analysis [42,43]. It has been mainly applied in combination with GC; recently, SPME has been used to determine SSRIs in environmental water [44] and in urine coupled to gas chromatography mass spectrometry [39]. However, in the past few years a growing interest for SPME coupled to LC has been observed as demonstrated by a number of recently published papers [45–47].

In the present paper, a rapid and accurate method using solid-phase microextraction coupled to HPLC–DAD is proposed for the isolation, separation and quantification of FLX, CIT and their main metabolites NFLX, DCIT and DDCIT in human urine samples. The method proposed should be useful at clinical levels in studies examining fluoxetine and citalopram therapeutic failure, patient compliance, or screening in clinical urine samples. The optimization of the method is fully discussed and the validation parameters are presented.

2. Experimental

2.1. Reagents and stock solutions

All reagents were analytical grade of the highest purity available. Fluoxetine and norfluoxetine hydrochloride were purchased from Sigma (St. Louis, MO, USA) while citalopram, demethylcitalopram and didemethylcitalopram were kindly donated by Lundbeck A/S (Copenhagen, Denmark). Tetramethylamonium chloride (TMACl), used as ion-pair reagent in the mobile phase, was obtained from Merck (Darmstadt, Germany) and the acetonitrile was from Scharlab (Barcelona, Spain). LC-grade water was prepared by purifying demineralized water in a Milli-Q water filtration system (Millipore, Milford, MA, USA). For the optimization of SPME process sodium chloride, ammonium hidroxyde and ammonium chloride were from Merck (Darmstadt, Germany) and acetonitrile and methanol from Scharlab (Barcelona, Spain) were used.

Stock solutions were prepared in methanol from Scharlab (Barcelona, Spain) to contain 1 mg ml^{-1} of each compound and they were preserved at −42 °C in a freezer. An aqueous reference solution containing the mixture of all these compounds to a final concentration 10 mg l^{-1} was prepared from the standard stock solution of each one. Working standard solutions were prepared by diluting the appropriate volume of the 10 mg l^{-1} reference solution up to 10 ml with water.

2.2. Instrumentation

Chromatographic experiments were performed using a Hewlett Packard (Palo Alto, CA, USA) HPLC system model series 1050 pump with a DAD detector model 1040.

Commercially available polymeric coated fibers housed in the appropriated manual holder (Supelco, Bellefonte, PA, USA) were used for the development of SPME. 4 ml screw-cap vials supplied with a PTFE-lined septum (Kimble Glass, Vineland, NJ, USA), a 0.7 cm stir bar and a magnetic stirrer (IKA, Staufen, Germany) were used for magnetic stirring. The SPME interface (Supelco, Bellefonte, PA, USA) consisted of a standard six-port Rheodyne valve equipped with a fiber desorption chamber (total volume: 60 μl), installed in place of the sample loop. In order to select the most suitable fiber, three commercially available coatings, Polyacrylate 85 μm (PA), Polydimethylsiloxane/Divinylbenzene 60 μm (PDMS-

DVB) and Carbowax/Templated Resin 50 μm (CW/TPR), were purchased also from Supelco (Bellefonte, PA, USA).

2.3. Chromatographic and detection conditions

Chromatographic separation of the compounds was achieved using a Spherisorb ODS2 (15 cm × 0.4 cm, 5 μm) column protected with a ODS guard column cartridge (1 cm × 0.4 cm) both from Tracer (Barcelona, Spain). The mobile phase consisted on a mixture of TMACl (pH 4; 0.15%):Acetonitrile (50:50, v/v) at a flow rate of 1 ml min^{-1}. It was filtered through a 0.22 μm Millipore membrane filter type GVWP and degassed by a Selecta Ultrasound System (Selecta, Barcelona, Spain).

The analytes were simultaneously determined by DAD at 230 nm. The time of analysis was lower than 8 min with good resolution coefficients.

2.4. Sample collection and pre-treatment

Urine samples were collected in sterile containers (Deltalab Eurotubo, Barcelona, Spain) and stored at −42 °C until analysis. Samples were centrifugated at 2500 rpm for 10 min at room temperature and the supernatant was diluted with water in the proportion 1:5. 3 ml of the dilution were put into 4 ml screw-cap vials where the extraction was to be carried out.

2.5. Solid-phase microextraction procedure

The extraction was performed using a manual SPME fiber holder. Each day before sample analysis, in order to ensure good selectivity and sensitivity results, the fiber was conditioned in the interface (dynamic mode) with mobile phase for approximately 20 min. Then the fiber was immersed in water with magnetic stirring for 5 min and dried for 5 min. After conditioning, the fiber could be used for extraction.

An aliquot of 3 ml of standard solutions or diluted urine sample was put in a 4 ml screw-cap vial and 40 μl of NH_3/NH_4Cl buffer (pH 9.5, 2 M) and 150 μl of acetonitrile were added. The sample solution was stirred with stirring at a controlled speed before and during the extraction. The fiber was immersed in the sample for 15 min at room temperature (21 ± 1 °C).

After sample extraction, the SPME fiber was withdrawn into the needle, removed from the septum and introduced into the desorption chamber full of mobile phase. In order to desorb the analytes by static mode, the fiber was soaked in the chamber for 5 min. Then, the valve was changed to the inject position and the fiber was exposed to the mobile phase stream for 40 s.

Since the method required the introduction of the fiber in organic solvents and buffers, the fiber was cleaned with water for 5 min and dried for 5 min prior to starting the next extraction.

3. Results and discussion

3.1. Selection of the adequate fiber coating

Preliminary experiments were performed in order to determine which fiber coating displayed the strongest affinity for the antidepressants and their metabolites. Three fiber assemblies were examined: Polyacrylate 85 μm (PA), Polydimethylsiloxane/Divinylbencene 60 μm (PDMS/DVB) and Carbowax/Templated Resin 50 μm (CW/TPR) coated fibers. Stock solutions containing 1 mg l^{-1} of each analyte in water were used for this. The extraction was performed in 60 min and the desorption was carried out in dynamic mode for 5 min. As shown in Fig. 1, with the less polar PA fiber there was no signal for CIT, DCIT and DDCIT while PDMS/DVB fiber exhibited low extraction. The most polar CW/TPR fiber permitted the best extraction efficiency of FLX, CIT and their metabolites and so was selected for further experiments.

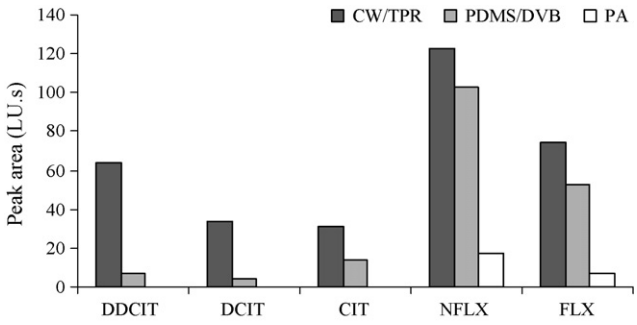

Fig. 1. Extraction efficiency of different SPME fibers: black square: Carbowax/Templated Resin (CW/TPR); grey square: Polydimethylsiloxane/Divinylbencene (PDMS/DVB); white square: polyacrylate (PA). SPME procedure: 3 ml of standard containing 1 mg ml^{-1} of each compound in water; extraction time, 60 min; extraction temperature, 21 ± 1 °C; desorption mode, dynamic during 5 min.

3.2. Optimization of the extraction process

3.2.1. Extraction time and temperature

The amount of analyte adsorbed by the fiber is a function of the distribution constant between the fiber and the solution, the thickness of the adsorbing phase and the diffusion coefficient of the analyte. SPME is based on the equilibrium of the analyte between the sample matrix and the coating of a fiber, that is why the extraction efficiency increases with the extraction time until the equilibrium. Stock solutions containing 1 mg l^{-1} of each analyte in water were extracted with CW/TPR fiber for periods of time ranging from 5 to 60 min. Fig. 2 shows the extraction time profiles, established by plotting the area versus the extraction time. As can be observed, all the compounds except NFLX reached the equilibrium in 15 min. Nevertheless, a satisfactory extraction was obtained for NFLX in 15 min. It is possible to obtain good extraction yields and reliable analysis also in non-equilibrium conditions as long as the exposure time of the fiber is kept exactly constant [28,29]. Therefore as a compromise between extraction efficiency and analysis time, an extraction time of 15 min was chosen for further experiments.

Since the temperature of extraction influences the mass transfer rates and the partition coefficients of the analytes, it plays an important role in their adsorption [48]. Therefore, extraction efficiency was studied using the CW/TPR fiber at room temperature, 40 and 70 °C. Extraction at temperatures higher than ambient produced a response decrease, probably due to the fact

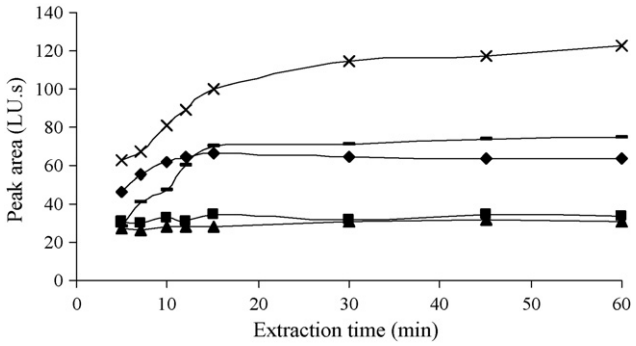

Fig. 2. The effect of equilibrium time on the chromatographic response of the analytes. SPME procedure: 3 ml of standard containing 1 mg ml^{-1} of each compound in water; fiber type, CW/TPR; extraction temperature, 21 ± 1 °C; desorption mode, dynamic during 5 min. Each point is the average of three data points: (♦) DDCIT, (■) DCIT, (▲) CIT, (X) NFLX, (−) FLX.

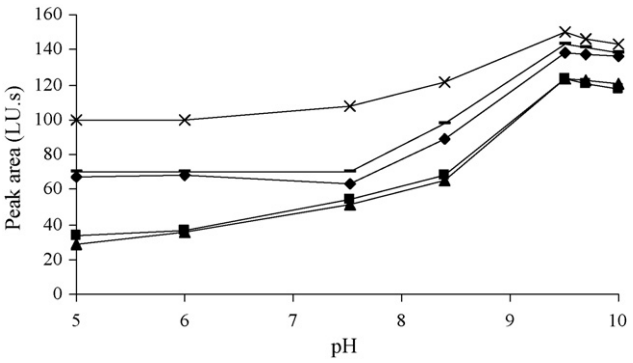

Fig. 3. The effect of pH on extraction efficiency. SPME procedure: 3 ml of standard containing 1 mg ml^{-1} of each compound in water; fiber type, CW/TPR; extraction time, 15 min; extraction temperature, 21 ± 1 °C; desorption mode, dynamic during 5 min. Each point is the average of three data points: (♦) DDCIT, (■) DCIT, (▲) CIT, (X) NFLX, (−) FLX.

that the adsorption is an exothermic process [45]. Thus all the following experiments were performed at room temperature.

3.2.2. Ion strength and pH

The distribution constant and therefore the extracted amount depend on the characteristics of the matrix such as ionic strength and pH. The experiments were carried out using NaCl as ionic salt, since its addition caused fewer problems during the fiber cleaning process [47]. The effect of ionic strength was studied by preparing standards with NaCl at concentrations between 0 and 300 g l^{-1}. However, a response decrease was observed with increasing NaCl from 0 to 50 g l^{-1}, while no significant change was observed with higher NaCl concentrations. This negative effect of salt addition on response has also been observed for the SSRI sertraline [28,31]. For this reason, the extraction process was carried out without ionic salt addition.

The extractability of any analyte into the SPME fiber also depends to a certain extent on the solution pH. If the solution pH is such that the analyte is present in a non-ionic form, it is preferably extracted by the polymeric coating of the fiber. pH ranged between 5 and 10 was studied in order to prevent the degradation of the CW/TPR fiber coating [48]. This was performed by adding 40 μl of NH_4OH/NH_4Cl solution (2 M) adjusted to yield the desired pH.

FLX, CIT and their metabolites are basic compounds and they are present in their undissociated form at basic pH. Fig. 3 clearly indicates that as expected, a response increase was observed by increasing the pH until 9.5 showing that the undissociated form of the analytes is preferentially extracted. Therefore this pH was selected for further studies.

3.2.3. Organic modifier

The effect of the organic modifier solvent content of the sample was evaluated by adding to the samples methanol or acetonitrile at percentages between 0 and 20%. The results obtained with methanol showed that a percentage higher than 7% involves a fall in the extractability. On the other hand, higher recoveries were observed in the presence of acetonitrile for FLX and NFLX while the signal of CIT, DCIT and DDCIT decreased at percentages above 5% (Fig. 4), so as a compromise an acetonitrile percentage (5%) was selected and 150 μl were added to the samples.

3.3. Optimization of the desorption process

The desorption modes include both static and dynamic mode. The choice of dynamic versus static mode depends on the desorption rate: for fast desorbing analytes dynamic mode should be used, while for slow desorbing analytes better peak shapes are obtained using static desorption [49].

Two modes of desorption, both with an extraction time of 15 min, were evaluated for CW/TPR fiber with samples containing 1 mg l^{-1} of each compound.

In dynamic mode, the fiber was placed into the desorption chamber. Then, the valve was switched from the load to the inject position and the mobile phase, at a flow of 1 ml min^{-1}, passed through the chamber for a time ranging from 2 to 7 min. Although the recoveries were satisfactory, very broad chromatographic peaks were obtained.

In static mode, several experiments were carried out with several solvents at different periods of time ranging from 1

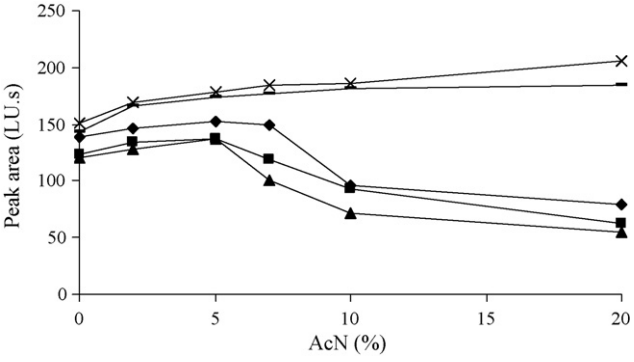

Fig. 4. Influence of the acetonitrile organic modifier on the extraction efficiency. SPME procedure: 3 ml of standard (pH 9.5) containing 1 mg ml^{-1} of each compound in water; fiber type, CW/TPR; extraction time, 15 min; extraction temperature, 21 ± 1 °C; desorption mode, dynamic during 5 min. Each point is the average of three data points: (♦) DDCIT, (■) DCIT, (▲) CIT, (X) NFLX, (−) FLX.

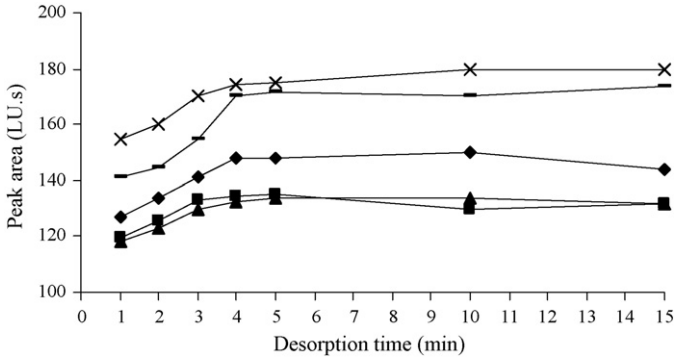

Fig. 5. Influence of desorption time on the extraction efficiency. SPME procedure: 3 ml of standard (pH 9.5) with 5% of acetonitrile containing 1 mg ml^{-1} of each compound in water; fiber type, CW/TPR; extraction time, 15 min; extraction temperature, 21 ± 1 °C; static desorption. Each point is the average of three data points: (♦) DDCIT, (■) DCIT, (▲) CIT, (X) NFLX, (−) FLX.

to 15 min. The desorption solvents evaluated were acetonitrile, methanol, water and mobile phase by injection of 0.5 ml of the solvent into the desorption chamber with a luer-tipped glass syringe. In order to evaluate the carry over, the fiber was left in the chamber after each experiment and a second chromatographic run was performed in dynamic mode leaving the interface valve in the inject position. The best results were reached in static desorption by soaking the fiber in mobile phase into the desorption chamber of the interface for 5 min (Fig. 5). Finally, the valve was changed to the inject position and the fiber was exposed for 40 s to the mobile phase stream. At this desorption and injection time there was no evidence of carry over and the preservation of chromatographic efficiency was ensured.

After desorption, as the fiber had been introduced in organic solvents and buffers during the extraction process, it was immersed in water with magnetic stirring for 5 min and dried for 5 min. In this way, the fiber was able to be used for later extractions.

3.4. Analytical assessment of the method in urine

Once the study on extraction and desorption conditions was completed, the method was applied to urine samples. In order to evaluate the matrix effect on this method, control urine samples were doped with different concentrations of the analytes and the developed SPME–HPLC–DAD procedure was applied. Urine samples were centrifugated at 2500 rpm for 10 min at room temperature in order to precipitate the urinary sediment. Initially, the supernatant was directly extracted by SPME but a considerable loss of sensitivity was observed due to an important matrix effect. The dilution of the urine could suppress the matrix effect and prevent the contamination of the SPME fibers [39]. Therefore, several trials were made with diluted urine in water in different proportions observing that a 1:5 dilution was enough to obtain a satisfactory suppression of the matrix effect. The 1:5 dilution of the samples should not be a problem for the quantification of FLX, CIT and their metabolites in urine samples because the prescription doses of these antidepressants are 20–60 mg/day so the urine levels excreted

Table 1
Summary of validation results: linearity, sensitivity, precision and accuracy of the proposed analytical method for three concentration levels

Compound	Slope (mAU s/mg l^{-1})	Intercept (mAU s)	Correlation coefficients	LOD (mg l^{-1})	Intraday						Interday					
					0.1 mg l^{-1}		0.5 mg l^{-1}		1.5 mg l^{-1}		0.1 mg l^{-1}		0.5 mg l^{-1}		1.5 mg l^{-1}	
					R.S.D.%	M.V.[a] (mg l^{-1})	R.S.D.%	M.V.[a] (mg l^{-1})	R.S.D.%	M.V.[a] (mg l^{-1})	R.S.D.%	M.V.[a] (mg l^{-1})	R.S.D.%	M.V.[a] (mg l^{-1})	R.S.D.%	M.V.[a] (mg l^{-1})
DDCIT	22.71 ± 0.62	2.56 ± 0.70	0.998	0.014	7.98	0.11	6.76	0.52	4.95	1.65	9.63	0.10	6.57	0.54	6.55	1.64
DCIT	21.55 ± 0.89	1.60 ± 1.00	0.997	0.014	7.39	0.10	5.67	0.51	5.72	1.50	9.98	0.09	6.78	0.53	7.68	1.59
CIT	23.66 ± 0.43	0.74 ± 0.48	0.999	0.013	9.66	0.11	5.23	0.53	4.64	1.47	9.36	0.10	8.76	0.54	8.26	1.42
NFLX	31.89 ± 1.17	2.27 ± 1.32	0.997	0.010	8.41	0.09	8.12	0.52	7.18	1.53	9.80	0.10	7.91	0.49	7.15	1.60
FLX	31.13 ± 0.71	1.41 ± 0.81	0.999	0.010	8.11	0.09	6.45	0.48	4.60	1.47	7.60	0.10	8.01	0.47	7.94	1.58

[a] M.V.: measured value.

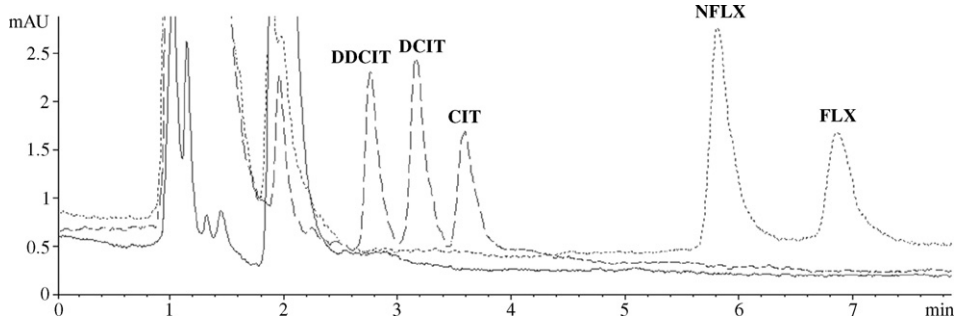

Fig. 6. SPME–HPLC–DAD chromatograms obtained from: a blank urine sample from a healthy volunteer (normal line); urine from volunteer 1 (DDCIT: 0.66 mg l^{-1}; DCIT 0.84 mg l^{-1}; CIT 0.48 mg l^{-1}) (dotted line); urine from volunteer 2 (NFLX 1.07 mg l^{-1}; FLX 0.50 mg l^{-1}) (. . . line).

are usually at mg l^{-1} levels [39]. It is worth noting that the extraction and desorption process were not influenced by passing from aqueous solutions to urine samples and no interfering peaks were found in chromatograms obtained after extraction of blank urine samples. Numerous tests carried out on blanks who were healthy non-medicated people and no interference was observed. The blank chromatogram featured in Fig. 6 corresponds to a healthy volunteer and shows the absence of interference from endogenous compounds. With regard to the presence of exogenous compounds possible interferences would be numerous, depending upon the type of pharmacological treatment. Nevertheless, the HPLC–DAD equipment software enables the peak purity to be estimated by means of various tests (ratigram generation, spectral similarity curves, threshold curves) so in the case of a particular interference taking place due to the co-elution of two substances, this could be easily detected.

The linearity of the response was examined by the analysis of seven doped urine samples after SPME extraction over the range 0.05–2 mg l^{-1} for each compound. As shown in Table 1, all the correlation coefficients were better than 0.997 confirming that the responses were linear in the concentration range studied. The estimated detection limits ranged 0.014–0.010 mg l^{-1}, below the usual urinary levels in patients under daily treatment [24].

Intra- and inter-day precision and accuracy are also reported in Table 1. For all analytes and for both concentration tested, the precision at 0.1 and 1.5 mg l^{-1} presented coefficients of variation below 10%. The accuracy of the assay based on the deviation of the mean measured value from the theoretical (doped) value ranged from 90 to 110%.

3.5. Urine sample analysis

The effectiveness of the proposed method for the determination of these antidepressants and their main metabolites in urine samples was tested by performing analyses of urine samples from two depressed patients. These patients had been diagnosed and had been undergoing treatment with therapeutic doses of FLX or CIT at the Hospital Santiago Apostol (Vitoria-Gasteiz) for at least 1 year. Based on the observation that about 90% of the steady-state concentration of a chronically administered drug is achieved after four half-life intervals [13], stable plasma fluoxetine and citalopram concentrations could be assured.

Volunteer 1 was under daily treatment with Seropram® (30 mg citalopram day^{-1}). Urine samples were collected between 0 and 6 h, 6 and 12 h and 12 and 24 h after the administration of the first dose of the day. Gross urine volumes from each 6 h period collection were recorded and urine collections were monitored. Volunteer 2 was being chronically treated with Prozac Weekly® (90 mg $week^{-1}$), an enteric-coated fluoxetine hydrochloride that delays release into the bloodstream. First morning urine samples were taken for the 7 days following the dose administration.

Table 2
Urine concentrations in two depressed patients under daily treatment

Volunteer	Sampling interval[a] (h)	CIT (mg l^{-1})	DCIT (mg l^{-1})	DDCIT (mg l^{-1})	FLX (mg l^{-1})	NFLX (mg l^{-1})
1	0–6	0.66 ± 0.01	0.58 ± 0.01	0.52 ± 0.02	–	–
	6–12	0.72 ± 0.01	0.48 ± 0.01	0.45 ± 0.02	–	–
	12–24	0.49 ± 0.01	0.83 ± 0.01	0.68 ± 0.02	–	–
2	0–24	–	–	–	0.69 ± 0.05	0.45 ± 0.02
	24–48	–	–	–	0.70 ± 0.01	0.60 ± 0.09
	48–72	–	–	–	1.42 ± 0.03	1.72 ± 0.01
	72–96	–	–	–	1.06 ± 0.07	1.51 ± 0.11
	96–120	–	–	–	1.20 ± 0.04	1.91 ± 0.21
	120–144	–	–	–	0.70 ± 0.05	1.21 ± 0.08
	144–168	–	–	–	0.52 ± 0.03	1.11 ± 0.05

Values are mean ± S.D. ($n = 3$).

[a] Time between ingestion of the drug by the patient and the collection of the urine.

The proposed method allowed the separation and quantification of CIT, DCIT and DDCIT in urine samples of volunteer 1 and FLX and NFLX in urine samples of volunteer 2. The mean concentrations found in these urine samples are shown in Table 2 and the chromatograms obtained for some of these samples can be seen in Fig. 6. Stable urine concentrations of the analytes could suggest that the steady-state concentration in plasma has been achieved. These results confirm that the proposed method is adequate to quantify CIT, NFLX and their main metabolites in urine samples of patients receiving therapeutic doses of citalopram or fluoxetine.

4. Conclusions

For the first time, an SPME–HPLC–DAD method for rapid quantification of FLX, CIT and their metabolites NFLX, DCIT and DDCIT in both water and human urine samples was developed. The chromatographic separation of the analytes was achieved in 8 min by means of a simple isocratic elution. The CW/TPR fiber was found to be the most suitable coating for the extraction of these compounds and several parameters influencing adsorption and desorption of the analytes on this fiber have been studied. Although a previous dilution of the urine sample was required in order to reduce the matrix effect, the detection limits obtained permitted the quantification of the analytes in human urine in an accurate and precise way. The application of the method to real samples proves its effectiveness in quantifying FLX, CIT, NFLX, DCIT and DDCIT in urine samples of patients treated with therapeutic doses of FLX or CIT.

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