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Enantioselective extraction of (+)-(*S*)-citalopram and its main metabolites using a tailor-made stir bar chiral imprinted polymer for their LC-ESI-MS/MS quantitation in urine samples

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ABSTRACT

This paper reports the application of a chiral imprinted polymer (CIP)-coated stir bar for the selective extraction of (+)-(*S*)-citalopram (SCIT) and its main metabolites, (+)-(*S*)-desmethylcitalopram (SDCIT) and (+)-(*S*)-didesmethylcitalopram (SDDCIT), from urine samples. The developed device has been demonstrated to be capable of selectively extracting the three target analytes from urine samples without saturating the imprinted sites. A CIP-coated stir bar sorptive extraction procedure (CIP-SBSE) is proposed for the isolation of SCIT, SDCIT and SDDCIT followed by their subsequent analysis using liquid chromatography ion trap mass spectrometry (LC-ITMS). Deuterated SCIT-d6 was used as an internal standard. The method was validated using a standard procedure, which revealed that a quantification of 5 ng mL^{-1} was obtained in urine samples and that the accuracy and precision were within the established values while no matrix effect was observed.

1. Introduction

The antidepressant citalopram (CIT) is a potent and highly selective serotonin reuptake inhibitor (SSRI) that is primarily prescribed for the treatment of depression and other central nervous system diseases, such as anxiety disorder, panic disorder, obsessive–compulsive disorder, social phobia or post-traumatic stress disorder [1]. CIT was first introduced into therapy as a racemic drug because it has one chiral centre and therefore exists in the (−)-(*R*) and (+)-(*S*) forms. However, in vitro studies in rat brains have shown that the pharmacological effect of citalopram primarily lies on the (+)-(*S*)–CIT (SCIT) enantiomer, whereas the (−)-(*R*)–CIT (RCIT) enantiomer is considered to be pharmacologically inactive and could counteract the activity of the (*S*)-enantiomer [2]. Additionally, compared to the racemate, preclinical studies have postulated that the SCIT is more efficacious [3] and even 150-times more potent than the RCIT [4], which was initially assumed to be pharmacologically inactive but is currently known to counteract the action of the SCIT without causing pharmacokinetic interactions [5,6]. Based on these premises and on the growing trend to develop drugs that comprise a single enantiomer rather than a mixture of them [4], SCIT has begun to be marketed as a single-enantiomer drug.

SCIT is metabolised by partial N-demethylation to (+)-(*S*)-demethylcitalopram (SDCIT) and (+)-(*S*)-didemethylcitalopram (SDDCIT) by hepatic metabolism [7]. These compounds are primarily eliminated by the kidneys, and approximately 35% of the dose is excreted in urine [8]. From the literature available, it seems that mainly the *S*-enantiomer of DCIT is responsible for the clinically relevant 5-HT reuptake inhibiting properties of the drug [9]

In clinical practice, the determination of SCIT and its metabolites in urine samples could be useful for defining an efficient and safe dose and for detecting the adherence and compliance to the treatment.

To date, the separation of the *R* and *S* enantiomers of CIT and their metabolites has been based on chromatographic and electrophoretic methods [10]. With regard to chromatographic methods, the majority of the developed liquid chromatography methods are based on the use of cyclodextrin, macrocyclic glucopeptide or protein-based chiral stationary phases [11–19]. It is also possible to resolve both enantiomers by adding chiral selectors to the background electrolyte, such as carboxymethyl-gamma-cyclodextrin [20], beta-cyclodextrin [21,22] or macrocyclic antibiotics [23]. Another option for resolving the enantiomers has been to derivitise

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Table 1
Data acquisition parameters for the smart parameter setting (SPS) used in the LC/ITMS for detecting the analytes. Compound stability and trap drive level are fixed at 100% for all compounds, and the fragmentation width was 10.0 (*m/z*).

Compound name	Retention time (min)	Precursor ion $[M+H]^+$	MRM transition (*m/z*)	Fragmentation amplitude (V)	Cut-off (*m/z*)
SDDCIT	7.1	297.0	297.0→261.9	88	0.48
SDCIT	7.8	311.0	311.0→261.9	84	0.60
SCIT	8.6	325.0	325.0→261.9	80	0.51
SCIT-d6	8.6	331.0	331.0→261.9	89	0.50

the analytes with a chiral reagent to form diastereoisomeric derivatives followed by their chromatographic separation in an achiral column [24].

Within the field of methodologies for synthesising host-guest systems, there are numerous works that appear in the literature related to the generation of molecularly imprinted polymers (MIPs) for its application to stereoselective separation processes [25–27], which are primarily used as stationary phases in chromatography [28] and electrophoresis[29,30]. The interaction between the template molecule and the imprinted cavity plays a crucial role in chiral separation, and the MIPs synthesised using the non-covalent approach are suggested by most authors for chiral recognition [31,32].

Recently, imprinted polymer based stir bar coating with high affinity towards drugs of biological significance [33,34], triazine herbicides [35] or minor constituents in food samples [36] have been developed.

The purpose of this study was to evaluate the applicability of this CIP-coated stir bar for the enantiospecific biological sample preconcentration and clean-up before the subsequent analysis of the selected target compounds. The capability of the developed CIP coating for the simultaneous extraction of SCIT and its metabolites SDCIT and SDDCIT was also investigated, and the influence of their presence in the same matrix was evaluated. The proposed method could be beneficial in selective and simple urine sample treatment as a valuable tool from the analytical point of view.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents used in this work were analytical grade. Ammonium formate (99%) and HPLC-MS grade acetonitrile, which were used as the mobile phase, were supplied by Acros Organics (New Jersey, USA) and Scharlab (Barcelona, Spain), respectively. Dry toluene was purchased from Panreac (Barcelona, Spain), and HPLC grade methanol and dichloromethane were obtained from Scharlab (Barcelona, Spain). Sodium carbonate and sodium chloride were obtained from Merck (Darmstadt, Germany). All solutions were prepared using ultra-high purity water (UHP) prepared from tap water that was pre-treated using Elix reverse osmosis cartridges before filtration by a Milli-Q system from Millipore (Bedford, MA, USA).

(+)-(*S*)-citalopram oxalate was purchased from Trademax (Shangai, China). Optically pure (+)-(*S*)-enantiomers of DCIT and DDCIT were kindly donated by Lundbeck A/S (Copenhagen, Denmark). The deuterated internal standard *S*-citalopram-d6 oxalate (SCIT-d6) with a purity greater than 98% was obtained from LGC Standards (East Greenwich, Rhode Island, USA).

2.2. LC-ITMS analysis

Chromatographic separation was performed on an Agilent 1100-series binary pump system. A Zorbax Eclipse XDB-CN column ($150 \times 4.6\ mm^2$, 5 μm) from Agilent Technologies (Palo Alto, CA, USA) was used, and the mobile phase consisted of a mixture of ammonium formate (30 mM) and acetonitrile (30:70, v/v) with a flow rate of 0.6 mL min^{-1} at a constant temperature of 25 °C.

The ion trap mass spectrometer was a MSD Trap XCT Plus equipped with a G1948A electrospray ionisation source operating in the positive ion mode (ESI +). The operating conditions of the ESI interface were as follows: drying gas (N_2) temperature of 350 °C, drying gas (N_2) flow rate of 11.0 L min^{-1}, nebuliser gas (N_2) pressure of 60 psi and a capillary voltage of −3200 V. Full-scan MS and MS/MS spectra were obtained by scanning over mass ranges from 100 to 500. The multiple-reaction monitoring (MRM) transitions, using the protonated molecular ions $[M+H]^+$ as precursor ions, and the MRM parameters are also summarised in Table 1.

2.3. Urine samples

Urine samples were collected from a volunteer who was treated daily with Esertia® (10 mg escitalopram). The urine samples were collected in sterile containers (Deltalab Eurotubo, Barcelona, Spain) 0 h, 6 h and 12 h after administering the unique dose of the day and maintained at −42 °C until analysis. Control urine samples were collected before the volunteer donor was treated. Before extraction, the urine sample was centrifuged at 2500 rpm for 10 min at room temperature. The supernatant was diluted 1:10 in a 20 mM carbonate buffer solution (pH of 10.5) that contained 9% (w/v) sodium chloride.

2.4. Standards and quality control (QC) samples

Stock solutions that contained 1 mg mL^{-1} of the individual analytes and the deuterated internal standard were prepared in methanol. All solutions were maintained in the dark at −42 °C in a freezer. From the standard stock solution of each analyte, aqueous working standard solutions that contained each compound at a final concentration of 10 mg L^{-1} were prepared and stored in a refrigerator at 4 °C during use. The stock solution of the deuterated internal standard was also diluted in water to obtain a stock solution that contained 10 mg L^{-1} of the standard and was stored at 4 °C.

Calibration standards were freshly prepared in triplicate on the day of analysis by adding the appropriate volume of the working standard solutions to 10 mL of the blank urine samples to yield the following concentrations: 5, 20, 50, 200, 400, 1000, and 2000 ng mL^{-1}.

QC samples were prepared at the lower limit of quantification of 5 ng mL^{-1} (LLOQ), 15 ng mL^{-1} (low QC, three times the LLOQ), 150 ng mL^{-1} (middle QC) and 1600 ng mL^{-1} (high QC, 80% of the ULOQ) in blank urine.

2.5. CIP-SBSE procedure

CIP-coated stir bars were synthesised as previously reported [33]. Once the stir bar was prepared, the extraction of the analytes from the sample was performed by directly immersing a CIP-coated stir bar into 9 mL of a urine sample that was diluted in a 20 mM

carbonate buffer solution (pH of 10.5) that contained 9% NaCl (w/v) for 300 min while stirring at 300 rpm. The CIP-coated stir bars were subsequently cleaned with MilliQ water, dried under nitrogen and stirred in a dichloromethane:toluene (1:3, v/v) mixture for 15 min. Finally, the stir bars were dried and immersed in 3 mL of methanol at 55 °C under stirring at 300 rpm for 90 min to desorb the analytes. The methanol extract was evaporated to dryness under nitrogen, and the residue was reconstituted with 1 mL of the mobile phase. Subsequently, 10 μL of a stock solution that contained 10 mg L^{-1} of the deuterated analogue, SCIT-d6, was added.

3. Results and discussion

3.1. LC–MS/MS conditions

During method development, the first step was to optimise the chromatographic and detection conditions to enhance sensitivity with a good separation of the analytes. With this objective, different experiments were conducted using different mobile phase compositions on Zorbax Eclipse XDB-CN and Zorbax Eclipse XDB-C8 columns with dimensions of 150 × 4.6 mm^2 and a 5 μm particle size. The use of the Zorbax Eclipse XDB-CN column with a mobile phase that contained an aqueous phase of 30 mM ammonium formate (pH of 3) and acetonitrile as an organic modifier in isocratic elution (30:70, v/v) with a flow rate of 0.6 mL min^{-1} allowed a satisfactory separation of the compounds to be obtained in less than 10 min (Fig. 1). No additional equilibration time was required for the sample injection. To reduce the variability in the assay, the deuterated analogue of *S*-citalopram, SCIT-d6, was used. The retention times obtained under the described conditions are presented in Table 1.

The optimisation of the ion trap mass spectrometer parameters was conducted by infusing 20 μg mL^{-1} of each standard in a control urine sample that was previously extracted following the sample preparation procedure presented above. The precursor ions of the analytes resulted from the protonation of the molecular ion $[M+H]^+$ and were selected to generate the MS/MS spectra. The optimum fragmentation amplitude for each analyte was determined by increasing this parameter until the precursor ion intensity was reduced to 5–20% of its major product ion response. Improved sensitivity for these analytes was obtained using manual fragmentation cut-off values rather than the default value (27% from the *m/z* ratio of the precursor ions).

The multiple reaction monitoring mode (MRM) with an isolation width of 4 *m/z* for each analyte was used for data acquisition.

The mass transitions were selected according to their stabilities and intensities. The detection of the ions was performed by monitoring the transitions shown in Table 1. The LC–MS/MS ion trap chromatogram and the product ion spectra generated from the precursor ions at the expected retention time are shown in Fig. 1. As precursor ions, the protonated molecular ions $[M+H]^+$ were selected at 325.0 *m/z* for SCIT, at 311.0 *m/z* for SDCIT, at 297.0 *m/z* for SDDCIT and at 331.0 *m/z* for SCIT-d6. No ion-source fragmentation was observed. The most characteristic fragmentation route involved the loss of H_2O then yielding to an ion at 306.9 *m/z* for SCIT, 292.9 *m/z* for SDCIT and 279.9 *m/z* for SDDCIT, which was followed by loss of the end-of-chain amine towards the 261.9 *m/z* fragment. The formation of the 108.9 *m/z* ion observed for all the analytes could be due to the 1-fluoro-4-methylbenzene unit [37]. An alternative pathway yielded a 279.9 *m/z* ion, which was due to the loss of $HN(CH_3)_2$ from the precursor ion $[M+H]^+$ [38].

3.2. Sample preparation and CIP-SBSE procedure

The evaluation of the influence of the matrix on this method in comparison with standards prepared in water was conducted by

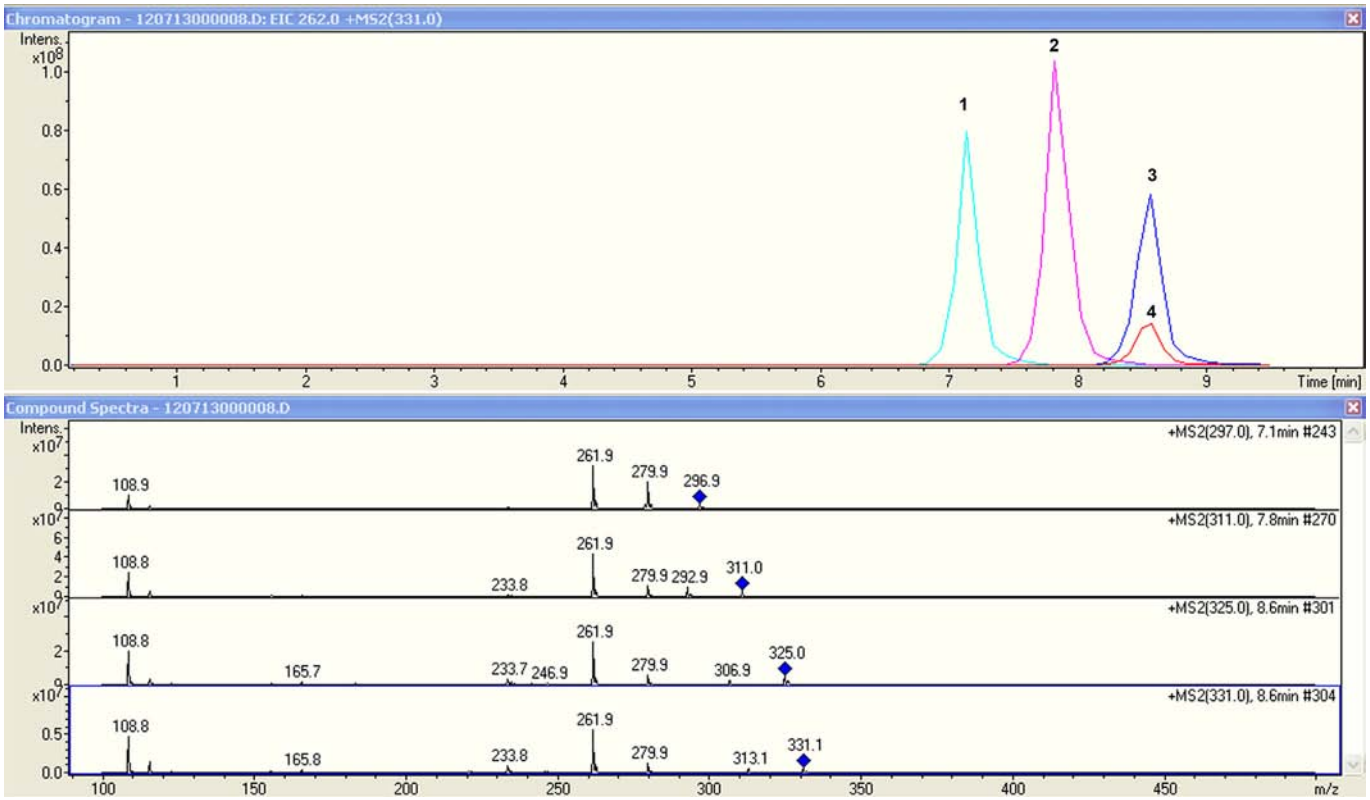

Fig. 1. LC–MS/MS ion trap segmented chromatogram (MRM) and MS/MS spectra of 500 ng mL^{-1} of each analyte and 100 ng/mL of the deuterated IS. 1: (+)-(*S*)–DDCIT, 2: (+)-(*S*)–DCIT, 3: (+)-(*S*)–CIT, and 4: (+)-(*S*)-CIT-d6.

doping the control urine samples with different concentrations of the analytes before applying the developed procedure. The urine samples were centrifuged at 2500 rpm for 10 min at room temperature to precipitate the urinary sediment. Initially, the supernatant was directly extracted using the developed CIP-SBSE procedure, but a considerable loss of sensitivity was observed due to an important matrix effect. Therefore, several trials were conducted using urine samples that were diluted in water in different proportions; the trials revealed that a 1:10 dilution allowed a satisfactory suppression of the matrix effect to be obtained. The 1:10 dilution of the samples should not be problematic for the quantification of SCIT, SDDCIT and SDDCIT in urine because their excreted levels are usually at the mg L^{-1} levels [39]. Note that the extraction and desorption processes were not influenced by passing from the aqueous solutions to urine samples and no interfering peak was observed in any chromatogram obtained after the extraction of the blank urine samples.

The CIP-SBSE procedure was described and justified by Gómez-Caballero et al. [33]. However, this procedure was only validated for the selective extraction of SCIT. Therefore, in the present work, the suitability of this procedure for a reproducible extraction of SDCIT and SDDCIT was also verified. Note that to improve the limit of quantification of the method, a final step was added that consisted of evaporating the methanol used in the desorption step. Then, the dry residue was reconstituted with 1 mL of the mobile phase.

3.3. Study of the selective recognition capability

The MIP-stir bar chiral recognition capability for different concentration levels of the metabolites SDCIT and SDDCIT was initially tested. This was performed comparing the signal of *S* enantiomers with the signal of the racemic mixtures at a concentration that ranged from 5 to 2000 ng mL^{-1}. A comparison study was carried out from the slope data of the linear regressions. In this sense, the slope of the calibration of each *S* metabolite was compared with the calibration slope of the racemic mixture. It was found that the residual standard deviations of each straight line do not differ significantly. This assumption was based on lower *F* experimental values than tabulated, i.e, experimental values of 1.94 for SDCIT and 2.80 for SDDCIT versus a tabulated *F* value of 7.39. Consequently it can be stated that data come from the same population[40].

In addition a two sided *t*-test was performed comparing the experimental *t* value with the tabulated one considering (n_1+n_2-4) degrees of freedom and a confidence level of 95% ($n_1=n_2=5$ calibration levels). The t experimental values were 1.36 for SDCIT and 0.94 for SDDCIT, lower than the tabulated *t* value 2.45. This results indicate that the MIP-stir bar presents enantioselective recognition capability for the metabolites SDCIT and SDDCIT.

Previous studies conducted by our research group have demonstrated that the CIP-coated stir bar was capable of recognising the target SCIT enantiomer with a high specificity [33]. In addition, in this work, this good selectivity was corroborated through by extending this study to the extraction of SCIT in the presence of SDCIT and SDDCIT. As shown in the chromatogram in Fig. 1, these metabolites are also extracted by the enantiospecific coating.

Moreover, the effect of the presence of SDCIT and SDDCIT on the recognition capability of this stir bar towards SCIT was also evaluated at different concentration ratios according to the relationship between the excreted urine levels of CIT, DCIT and DDCIT during the day in patients under a daily dose of CIT: between 0 and 6 h after the ingestion of the drug, CIT:DCIT:DDCIT relationship was 1:1:1; 6–12 h after the ingestion, the ratio was 2:1:1; and 12–24 h after, it was 1:2:2 [39]. Considering these ratios, individual stock solutions that contained 10 mg L^{-1} of SCIT, SDCIT or SDDCIT were prepared. In addition, three stock solutions were prepared as follows: solution A contained 10 mg L^{-1} of SCIT, SDCIT and SDDCIT; solution B contained 10 mg L^{-1} of SCIT, 5 mg L^{-1} of SDCIT and 5 mg L^{-1} of SDDCIT; and solution C contained 10 mg L^{-1} of SCIT, 20 mg L^{-1} of SDCIT and 20 mg L^{-1} of SDDCIT.

Different aliquots of the urine samples ($n=3$) were doped with the individual solution of 10 mg L^{-1} SCIT, SDCIT or SDDCIT to obtain a final concentration of 5, 20, 50, 200, 400, 1000 or 2000 μg L^{-1}. The procedure was repeated with solutions A, B and C in an attempt to obtain final SCIT concentrations of 5, 20, 50, 200, 400, 1000 or 2000 μg L^{-1}.

The CIP-coated stir bars were immersed in the doped urine control samples that were previously diluted 1:10 in a 20 mM carbonate buffer solution (pH 10.5) that contained 9% (w/v) NaCl, and the extraction procedure was conducted as described above. In this manner, the calibration curves were constructed applying a weighting factor of $1/x^2$ to the data. As shown in Table 2, the correlation coefficients (r^2) were greater than 0.987 in all the cases.

The agreement between the slopes of the calibrated straight lines was calculated according to the Student's *t*-test ($p<0.05$). This statistical test revealed no significant difference ($t_{calculated} < t_{tabulated}$) between the slopes of the calibration curve of SCIT in the absence of these metabolites and in the presence of different ratios of SDCIT and SDDCIT. Therefore, it was concluded that at these concentration levels, the presence of the metabolites did not alter the extraction capability of SCIT, and it was consequently assumed that the metabolites did not cause saturation of the imprinted sites with the subsequent significant decrease of the retention capacity. In the same manner, the extraction capability of SDCIT and SDDCIT was not altered in the presence of the remainder of the analytes because there was not a statistically significant difference between the slopes (Table 3).

As a conclusion, it can be stated that the CIP-coated stir bar is enantioselective towards SCIT, SDCIT and SDDCIT. Although these

Table 2
Summary of the linearity, detection limits and quantification limits of SCIT, SDCIT and SDDCIT in urine samples in the absence and presence of the remainder of the compounds.

Compound	Individual calibrations	SCIT:SDCIT:SDDCIT (1:1:1)	SCIT:SDCIT:SDDCIT (2:1:1)	SCIT:SDCIT:SDDCIT (1:2:2)	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
SCIT	$y=0.0020x+0.0587$ $r^2=0.9877$[a]	$y=0.0023x-0.0634$ $r^2=0.9959$	$y=0.0020x+0.114$ $r^2=0.9922$	$y=0.0022x+0.1290$ $r^2=0.9870$	2	5
SDCIT	$y=0.0054x+0.0080$ $r^2=0.9941$[b]	$y=0.0053x+0.0032$ $r^2=0.9911$	$y=0.0061x+0.0058$ $r^2=0.9993$	$y=0.0056x+0.0030$ $r^2=0.9958$	2	5
SDDCIT	$y=0.0055x+0.0105$ $r^2=0.9891$[c]	$y=0.0051-1.322$ $r^2=0.9969$	$y=0.0053+0.0133$ $r^2=0.9967$	$y=0.0051x+0.0086$ $r^2=0.9883$	2	5

[a] Calibration prepared from individual solution of SCIT.
[b] Calibration prepared from individual solution of SDCIT.
[c] Calibration prepared from individual solution of SDDCIT.

Table 3
Experimental Student's *t*-test values for comparing the slopes of the regression lines. The tabulated Student's *t*-value was 2.45 ($p=0.05$).

	SCIT	SDCIT	SDDCIT
SCIT:SDCIT:SDDCIT (1:1:1)	1.37	0.20	0.47
SCIT:SDCIT:SDDCIT (2:1:1)	0.22	1.44	0.32
SCIT:SDCIT:SDDCIT (1:2:2)	1.23	0.32	0.75

main metabolites are simultaneously extracted by the CIP-coated stir bar, their presence did not affect the extraction of the parent drug in the investigated concentration range. Therefore, the developed CIP-coated stir bar procedure is suitable for the enantiospecific sample pre-concentration of SCIT, SDCIT and SDDCIT in urine samples and their subsequent analysis by LC-ITMS.

3.4. Method validation

The developed method for the quantification of SCIT, SDCIT and SDDCIT in urine samples was validated using the standard procedure to ensure adequate selectivity, linearity, sensitivity, accuracy, precision, carry-over and matrix effects of the assay and the stability of the analytes [41].

The selectivity of the method was confirmed by the analysis of blank urine samples from ten different lots. No interfering signals were observed at the retention time and with the same m/z ratio of the compounds of interest.

As previously reported, the linearity of the method was validated for concentrations of SCIT, SDCIT and SDDCIT in urine between 5 and 2000 ng mL^{-1}. The LOD, which was 2 ng mL^{-1} for all the analytes, was defined as the lowest concentration that presented acceptable chromatography and with the presence of precursor and product ions with the ion ratios within $\pm$20% of standards. The LLOQ, which was defined as the lowest concentration used in the calibration with a trueness and precision of 100% $\pm$ 20%, was validated as 5 ng mL^{-1} in urine for all the analytes (Table 2).

The method accuracy was evaluated in terms of precision and trueness[42] by analysing ten replicates ($n=10$) of the QC samples at the four different spiking levels including at LLOQ, low QC, middle QC and high QC under within-laboratory reproducibility conditions. Five MIP-stir bars were used for each spiking level. Precision was calculated in terms of global inter-day precision and estimated as RSD (%) of 10 determinations. Trueness was calculated in terms of recovery. Measurement uncertainty was assessed at the four different spiking levels covering the whole dynamic range, with a confidence interval of 95%. For each level, the uncertainty (U) was evaluated using the within-laboratory reproducibility relative standard deviation (Table 4).

As shown in Table 4, the measured concentration of the analytes fell within the acceptable range of recovery of 89–107%. The precision determined at each concentration level did not exceed an RSD of 12% at the LLOQ level.

Sample carry-over was assessed by injection blank samples after calibration standard at the upper limit of quantification (2000 ng mL^{-1}). The carry-over signal in the blank sample was below the LOD ($n=10$) for all the analytes. In this way, it can be stated that the carry-over effect was not detected and at least 20 cycles of extraction could be performed without loss of effectiveness of stir bar.

The matrix effect was determined in the control urine samples from ten different lots of blank urine samples that were spiked with the analytes at concentrations of 15 and 500 ng mL^{-1}. The matrix effect was measured by comparing the responses from the post-extraction spiked samples with those from the standard solutions. Because the calculated ratios varied from 86 to 91% and 89 to 96% respectively, it could be concluded that there is no matrix effect. In addition, when the calibration curves based in the aqueous standards and the calibration curves of the diluted urine samples were compared, the slope of the equation did not significantly vary and the recoveries of the analytes were minimally affected by the matrix effect.

Finally, the stability of the analytes and of the deuterated internal standards was evaluated. The stability results revealed that the stock solutions were stable after 1 month at 4 °C (97–106%) and 3 months at −42 °C (96–105%). The stability of the analytes in the urine samples was verified by analysing the QC samples after storage for 6, 12 and 24 h at room temperature (103–108%), after long-term storage for 60 days at −42 °C (92–102%) and after going through freeze and thaw cycles (refrozen for 12 h when completely thawed) (87–99%).

Table 4
Summary of the trueness and precision results for the LC/MS/MS method in urine samples ($n=10$) for the analysed quality control samples.

Compound	Concentration level	Recovery (%)	Uncertainty (U) (%)	Global inter-day precision (RSD) (%)
(+)-(*S*)–CIT	LLOQ	104.4	4.2	8.9
	Low	100.0	4.2	9.2
	Middle	89.5	3.1	7.7
	High	102.9	4.0	8.5
(+)-(*S*)–DCIT	LLOQ	103.4	4.3	9.1
	Low	105.5	4.3	9.0
	Middle	100.7	4.6	10.0
	High	101.2	5.3	11.6
(+)-(*S*)–DDCIT	LLOQ	106.6	5.2	10.8
	Low	101.4	4.4	9.6
	Middle	97.3	5.0	11.3
	High	98.9	1.3	2.9

Table 5
Concentrations of the detected target analytical compounds in urine samples from depressed patients.

Sampling interval	(+)-(*S*)–CIT (ng mL^{-1})	(+)-(*S*)–DCIT (ng mL^{-1})	(+)-(*S*)–DCIT (ng mL^{-1})
0–6 h	1095 ± 44	907 ± 41	481 ± 22
6–12 h	476 ± 63	713 ± 91	468 ± 23
12–24 h	91 ± 13	116 ± 11	302 ± 7

*Mean ± SD.

3.5. Application to real samples

The effectiveness of the proposed method for the determination of SCIT, SDCIT and SDDCIT in real samples was investigated by performing analyses of urine samples from a patient diagnosed with depression. This patient was under daily treatment with Esertia® (10 mg escitalopram). Urine samples were collected 0 h, 6 h and 12 h after the administration of the first dose of the day and were monitored. With regard to the mean concentrations found in these urine samples (Table 5), it can be concluded that this method is suitable for a reliable quantification of analytes at clinical levels and therefore, it could be useful in the detection of non-adherence to treatment, which is commonly observed in long-term treatments and results in suboptimal medication and poor disease control.

4. Conclusions

This study demonstrates the suitability of a recently developed enantioselective molecularly imprinted polymer (CIP)-based stir-bar coating SBSE for the selective extraction of SCIT and its main active metabolites SDCIT and SDDCIT from urine samples. No saturation of the binding sites occurred when SCIT was simultaneously extracted with its main metabolites at concentrations close to the ones expected in real samples. Moreover, the absence of a matrix effect has also been demonstrated, which can be attributed to the highly specific CIP devices. The developed SBSE-CIP method meets the current requirements of bioanalytical method validation and has been successfully applied for the quantification of the analytes in real urine samples.

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