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In-tube solid-phase microextraction based on hybrid silica monolith coupled to liquid chromatography–mass spectrometry for automated analysis of ten antidepressants in human urine and plasma

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ABSTRACT

A rapid, sensitive and automated in-tube solid-phase microextraction-liquid chromatography–mass spectrometry (in-tube SPME/LC–MS) method was developed for the analysis of ten antidepressants in urine and plasma. A hybrid organic–inorganic silica monolith with cyanoethyl functional groups was prepared and used as a sorbent for in-tube SPME. Integration of the sample extraction, LC separation and MS detection into a single system permitted direct injection of the diluted urine or plasma after filtration. Under the optimized conditions, good extraction efficiencies for the targets were obtained with no matrix interference in the subsequent LC–MS. Automation of the sampling, extraction and separation procedures was realized under the control of a program in this study. The total process time was 30 min and only 30 μL of urine or plasma was required in one analysis cycle. Good linearities were obtained for ten antidepressants with the correlation coefficients (R) above 0.9933. The limits of detection ($S/N = 3$) for ten antidepressants were found to be 0.06–2.84 ng/mL in urine and 0.07–2.95 ng/mL in plasma. The recoveries of antidepressants spiked in urine and plasma were from 75.2% to 113.0%, with relative standard deviations less than 16.5%. The developed method was successfully used to analyze urine sample from ageing patients undergoing therapy with antidepressants.

1. Introduction

The antidepressants are a group of important drugs that are used for the treatment of psychiatric patients suffering from clinical depression. Therapeutic drug measurement for antidepressant agents in biofluids is important for quality assurance in preparations and for obtaining optimum therapeutic concentrations, while minimizing the risk of overdose and adverse effects [1,2]. For example, the therapeutic concentration range for most tricyclic antidepressants is approximately 100–300 μg/L, while toxic effects can occur when plasma concentrations exceed 500 μg/L [3].

In order to determinate the amount of antidepressants in different biological matrices such as plasma or urine for monitoring or toxicological purposes, HPLC conjunction with UV [4–6] or fluorescence (FL) detection [7,8] has been frequently used. In recent years, HPLC with mass spectrometry (MS) detection [9–11] has been favored by many analysts due to their higher sensitivity and ability to provide compound confirmation. Due to the complexity of biological samples, the sample pre-treatment process has become the bottleneck in method development and sample analysis in many cases [12].

In-tube solid phase microextraction (in-tube SPME) coupled to HPLC is the on-line mode of SPME coupling to liquid chromatography, which was put forward by Eisert and Pawliszyn in 1997 [13] and received wide acceptance since then [14]. By integrates sample extraction, concentration, and injection into one step, in-tube SPME provide an suitable sample preparation technique prior to HPLC and HPLC–MS [15]. Since it is fast to operate, easy to automate, solvent-free and requires small volume of the samples, in-tube SPME is especially suitable for biological sample analysis [16–18], and has also been used for antidepressants analysis in plasma and urine samples [19,20]. Automation analysis is becoming increasingly important in all areas of science. Due to the automation, in-tube SPME not only overcomes the problems of those traditional offline techniques but also provides better accuracy, precision and sensitivity. By simplifying and minimizing sample preparation, degradation of analytes was reduced and percentage of recovery increased. Furthermore, on-line automated method minimizes laborious repetitive work and eliminates the analyst's exposition to hazard and toxic solvents when robotic offline extraction tools are not available [21].

Up till now, organic polymer [22–24] and silica-based [25–27] monoliths have been introduced as extraction media for in-tube

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SPME. Monolithic materials show several attractive advantages including frit-free construction, easy preparation with good control of porosity, diverse surface chemistry [28–30] and satisfactory loading capacity superior to that of open tubular columns. Moreover, the monolithic porous structure offers convective mass transfer procedure [23], which is preferable in extraction process. Though organic polymer monoliths have excellent pH stability and good biocompatibility, the swelling in organic solvents might lead to the decrease of mechanical stability [31]. In comparison, silica-based monoliths offer high permeability, high mechanical strength, and good organic solvent tolerance. However, the preparation of conventional silica-based monoliths is time-consuming, and difficult to control, leading to poor reproducibility. As an attractive alternative, the organic–inorganic hybrid silica monoliths, which combine the advantages of silica with organic polymer monoliths, have been recently employed as extraction sorbent for in-tube SPME in dealing with water, milk and urine samples [30,32–33]. Cyanoethylsiloxanes exhibit both polar and polarizable characteristics. The cyano group is dipolar and strongly electron attracting, hence displaying dipole–dipole, dipole–induced dipole, and charge–transfer interactions. Furthermore, the unshared electron pair in the nitrile nitrogen may form intermolecular hydrogen-bonds with suitable hydrogendonor molecules. Malik et al. have proved that the cyanopropyl moiety in CN-PDMS coatings provided effective extraction of highly and medium polar analytes from aqueous media without requiring derivatization, pH adjustment or salting out procedures [34]. Therefore, a hybrid organic–inorganic silica monolith with cyanoethyl functional groups could be employed as a sorbent for extraction of antidepressants from urine and plasma samples appropriately.

Several reports have been published to develop on-line extraction techniques, allowing automation of samples [12,35,36]. These methods have been applied for the detection of only a few antidepressants [35] or the device was too complex [12]. The aim of this study was to develop a rapid, sensitive and automated in-tube solid-phase microextraction-liquid chromatography–mass spectrometry (in-tube SPME/LC–MS) method for the analysis of ten antidepressants in urine and plasma. A hybrid organic–inorganic silica monolith with cyanoethyl functional groups was prepared and used as a sorbent for in-tube SPME. Using this hybrid monolith, targets were selectively isolated from biological samples and the impurities were eliminated simultaneously with no matrix interference in the subsequent LC–MS. The present method showed high selectivity and sufficient accuracy to be used on antidepressants analysis in human urine and plasma.

2. Experimental

2.1. Chemicals and materials

Tetraethoxysilane (98%, TEOS) was purchased from the Chemical Plant of Wuhan University (Wuhan, China), 2-cyanoethyltriethoxysilane (CN-TEOS) was purchased from TCI development Co. (Shanghai, China), which were used directly without further purification. *N*-dodecylamine (98%) and anhydrous ethanol were purchased from Sinopharm Chemical Reagent (Shanghai, China). Acetonitrile (HPLC grade) was obtained from Tedia (Ohio, USA). Purified water was obtained with an Aike water purification equipment (Chengdu, China).

A set of eleven antidepressants (trazodone, clozapine, citalopram hydrobromide, doxepin, paroxetine, fluvoxamine, imipramine, amitriptyline, fluoxetine, sertraline, clomipramine) were obtained from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). The molecular structures of selected antidepressants are shown in Fig. 1. Other chemicals used in the experiment were of analytical grade, and were purchased from Shanghai Chemical (Shanghai, China). A mixed stock solution of all standards was prepared in acetonitrile at a concentration of 10 μg/mL and stored at 4 °C in the dark. The standard solution was diluted to the desired concentration for experiments.

2.2. Preparation of hybrid silica monolith

Fused-silica capillaries (O.D. 375 μm and I.D. 250 μm), purchased from Sino. Sumtech (Hebei, China), were activated with 1 M NaOH and then 1 M HCl. After rinsing with double distilled water, they were dried at 160 °C under N_2 flow for 5 h. The hybrid monolith was synthesized by hydrolysis and polycondensation of precursors via a two-step catalytic sol–gel process as described by Yan et al. [37]. The optimal preparation conditions were as follows: 180 μL of methanol, 25 μL of 2 M acetic acid, 110 μL of CN-TEOS and 110 μL of TEOS were mixed together in a 1.5 mL Eppendorf vial. After thorough vortexing, the mixture was left for hydrolysis at 60 °C for 5 h. After cooling to room temperature, 10 mg of *N*-dodecylamine was added to the solution. Then the pretreated capillary was filled to a certain length with the sol by a syringe. The capillary were sealed at the both ends with silicon rubber, and then was allowed to further react at 40 °C for 15 h. Subsequently, the capillary was rinsed with ethanol to remove the *N*-dodecylamine and soluble hydrolysis products, and then dried at 60 °C for 48 h. The total and effective lengths of the hybrid silica monolith were 20 and 15 cm, respectively.

2.3. Instrument and analytical conditions

Diffused IR spectra were determined using a Thermo Nicolet 670 FT-IR (Boston, USA). Elemental analysis was performed by an Elementar VarioEL elemental analyzer (Hanau, Germany). The microscopic morphology of the monolith was examined by a Quanta 200 scanning electron microscopy (SEM) (FEI, Holand). Nitrogen sorption experiments were carried out at 77 K using JW-BK surface area and pore size analyzer (JWGB Sci. & Tech., Beijing, China). The pore size distribution was measured by an Autopore IV 9500 mercury porosimeter (Micromeritics, Norcross, USA).

All the in-tube SPME/HPLC–MS experiments were carried out on a LCMS-2010EV HPLC-ESI/MS system (Shimadzu, Tokyo, Japan) that consisted of two LC-20AD pumps, a SIL-20A autosampler, a TCO-20A thermostated column compartment, a DGU-20A vacuum degasser, a FCV-12AH high-pressure flow channel selection valve units (valve B shown in Fig. 2), a SPD-20A UV–vis detector and a SHIMADZU LCMS-2010A single quadrupole mass spectrometer. Data acquisition and processing were performed with the LC solution Ver 3.0 Workstation.

2.3.1. LC–MS conditions

The column was Shim-pack VP-ODS (Shimadzu, 150 × 2.0 mm i.d., 5 μm) fitted with a C_{18} guard column (Shimadzu). The optimized mobile phase consisted of 0.2% formic acid solution/acetonitrile (70:30, v/v). The column oven temperature was maintained at 30 °C and the flow rate was 0.2 mL/min. Positive ion electrospray ionization (ESI) was employed for MS. Selected ion monitoring (SIM) was conducted to simultaneously monitor ions at *m/z* 372, 325, 278, 280, 330, 315, 281, 306, 319, 327 and 310 which corresponded to the protonated molecular ions of trazodone, citalopram, amitriptyline, doxepin, paroxetine, clomipramine, imipramine (IS), fluvoxamine, sertraline, clozapine and fluoxetine. Capillary voltage was 4.5 kV. Curved desolvation line (CDL) and heat block temperatures for the analysis were set at 250 and 200 °C, respectively. Nitrogen was set at 0.02 MPa and

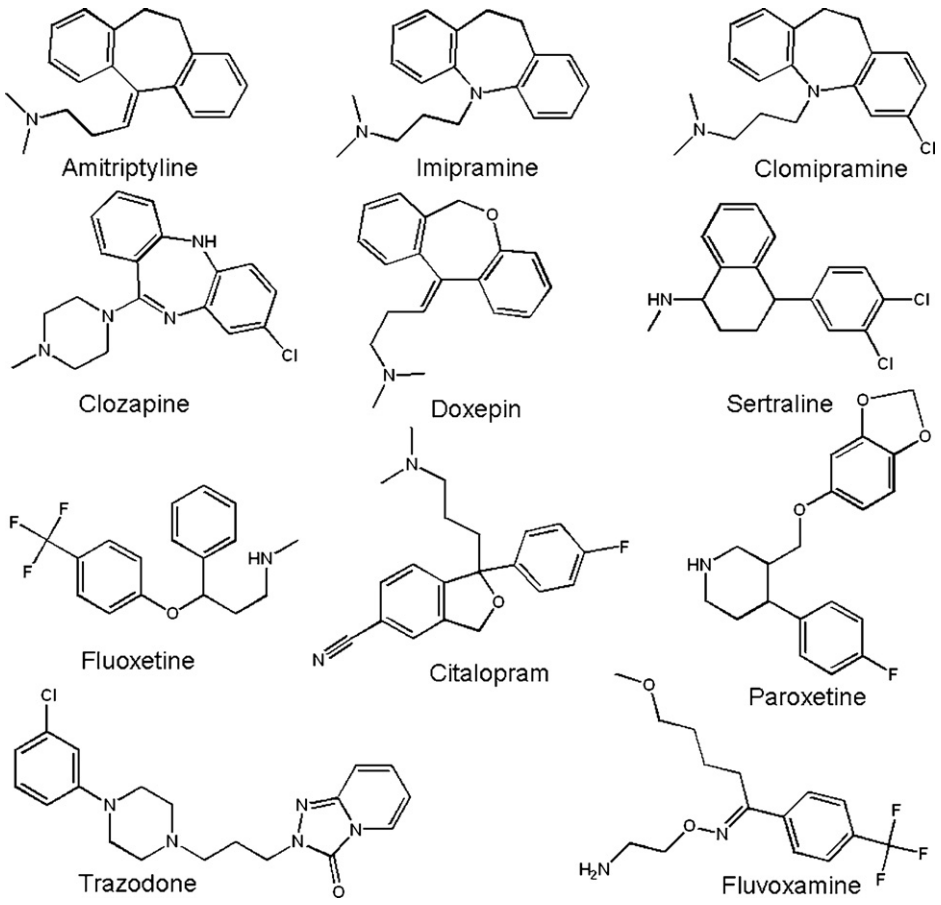

Fig. 1. Molecular structures of selected antidepressants.

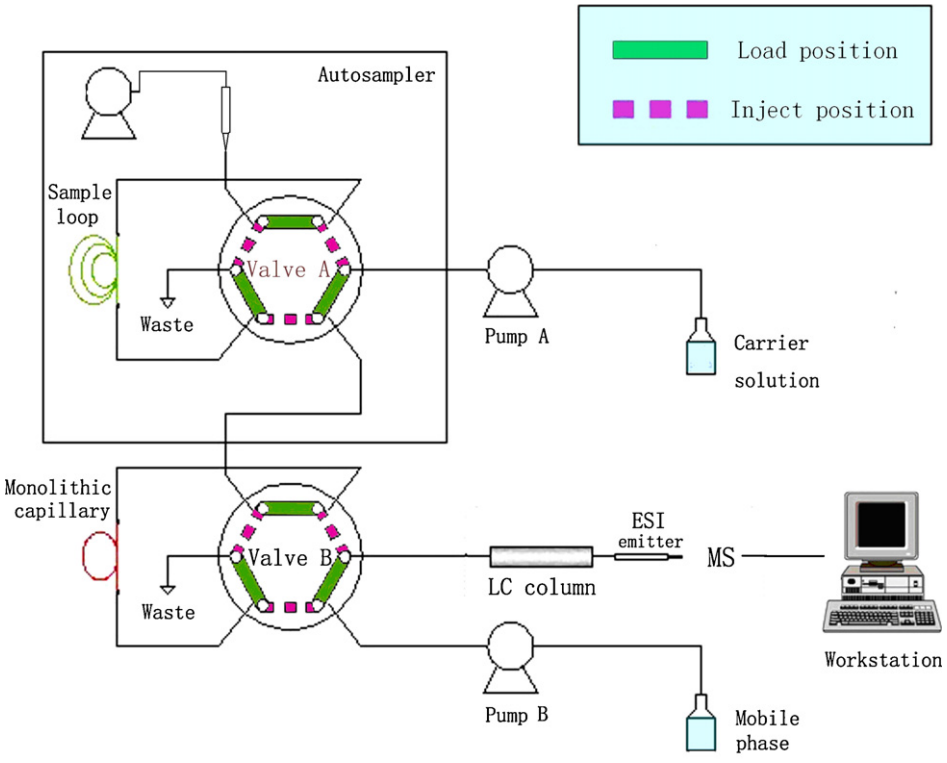

Fig. 2. Construction of automated in-tube SPME–HPLC/MS system.

Table 1
Program for automatic in-tube SPME/HPLC–MS procedures[a].

NO.	Time (min)	Action	Position	Event
1	0.00	Valve A	INJECT	Starting extraction, after sample injection
	0.00	Valve B	LOAD	
2	10.00	Valve A	LOAD	Washing the monolith with carrier solution for 2.0 min after 10.0 min of extraction
3	12.00	Valve B	INJECT	Desorption 5.0 min with mobile phase
4	17.00	Valve B	LOAD	After desorption, the monolith was conditioned by carrier solution until next extraction

[a]Carrier solution: 0.2% formic acid solution/acetonitrile (9:1, v/v); mobile phase: 0.2% formic acid solution/acetonitrile (7:3, v/v).

1.5 L/min for drying and nebulizer gases, respectively. The detector voltage was set at 1.6 eV.

2.3.2. LC–UV conditions

The investigation of extraction conditions were carried out using a SPD-20A UV–vis detector. The analytical column was a Kromasil ODS column (150 mm × 4.6 mm i.d., 5 μm, Sweden). The mobile phase was ACN-50 mM NaAc buffer solution (40:60, v/v; pH 4.5) with a flow rate of 1 mL/min. UV detection was set at 254 nm.

2.4. Urine and plasma sample preparation

Blank human urine or plasma samples were collected from healthy volunteers and stored at −20 °C before use. IS solution (50 μL of 50 ng/mL imipramine in H_2O) was added to 0.5 mL of urine or plasma samples which were spiked with known variable amounts of antipressants. 10 min was allowed for equilibration at room temperature, after being mixed with a vortex mixer. These samples were diluted with 0.5 mL ACN. After being mixed with a vortex mixer again, the samples were centrifuged at 0–4 °C for 5.0 min at 10,000 rpm. The supernatant was diluted with 5 mM phosphate solution (disodium hydrogenphosphate solution pH 7.0) to 5 mL, and then filtered through a 0.45 μm pore filter prior to on-line in-tube SPME/HPLC–MS analysis. Due to the high concentration of antidepressants in patient's urine, the urine of patient was diluted with 5 mM phosphate solution (pH 7.0) for 100 fold before sample preparation as described above. Blank samples were prepared in the same way as above but without the compound-spiking step.

2.5. Automatic procedures of in-tube SPME/HPLC–MS

The handling of in-tube SPME/HPLC–MS was pretty much the same as that described in our previous reports [22] except the whole extraction and separation procedures which were automated (Fig. 2). The hybrid silica monolith was connected at 1 and 4 position of valve B. Before the extraction, the carrier solution, 0.2% formic acid solution-ACN (9:1, v/v), was driven by a LC-10ADVP pump (Shimadzu, Tokyo, Japan) (pump A) to flow through the monolith and the flow rate was kept at 0.04 mL/min. At the same time, the sample loop was filled with a certain volume of sample solution by the autosampler accurately. Valve A was switched from the LOAD to INJECT position for a given time interval in the extraction step and then returned to the LOAD position immediately. The carrier solution then kept flowing through the monolith for 2.0 min to eliminate the residual sample solution and remove unretained matrix to waste. The analytical mobile phase then desorbed the extracted analytes from the monolith to the analytical column at a flow rate of 0.03 mL/min for 5.0 min by switching valve B to INJECT position. After switching valve B back to the LOAD position, the flow of the mobile phase was increased to 0.20 mL/min to initiate chromatographic separation. At the same time the monolith was conditioned by carrier solution until next extraction. The above-mentioned procedures can be programmed by LC–MS solution Version 3.0 Workstation (Table 1).

3. Result and discussion

3.1. Characterization of the cyanoethyl-functionalized hybrid silica monolith

The cyano-functionalized hybrid silica monolith was taken for Fourier-transform IR characterization. The absorption spectrum of the hybrid silica monolith displays readily identifiable peaks at 2938 cm^{-1}, which are characteristic of C–H stretching vibrations. The absorption peak observed at 2261 cm^{-1} is caused by –CN stretching vibration. The C–H bending band is at 1430 cm^{-1}. The stretching band at 805 cm^{-1} indicates the presence of Si–C bonds in the prepared hybrid silica monolith.

Fig. 3 displays the microscopic morphology of the hybrid silica monolith. It could be seen that the hybrid monolith pos-

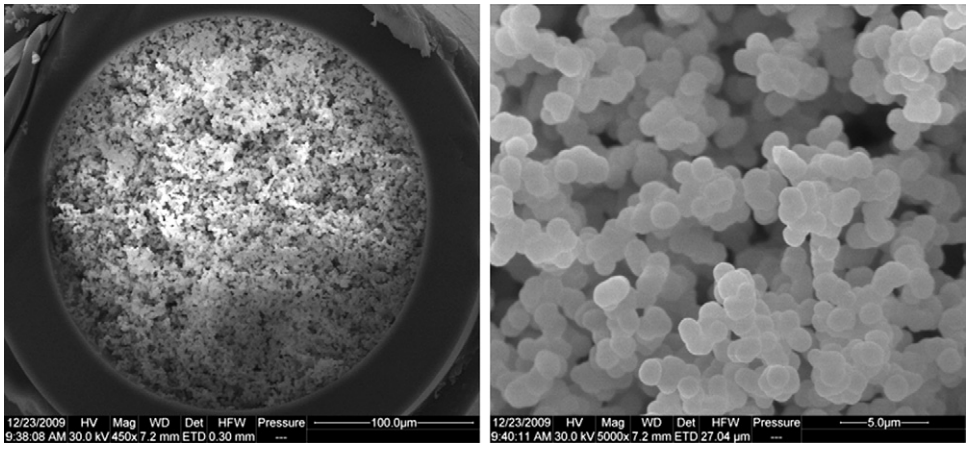

Fig. 3. Scanning electron microscope images of the cross section of the hybrid silica monolith: wide view (left) and close-up view (right).

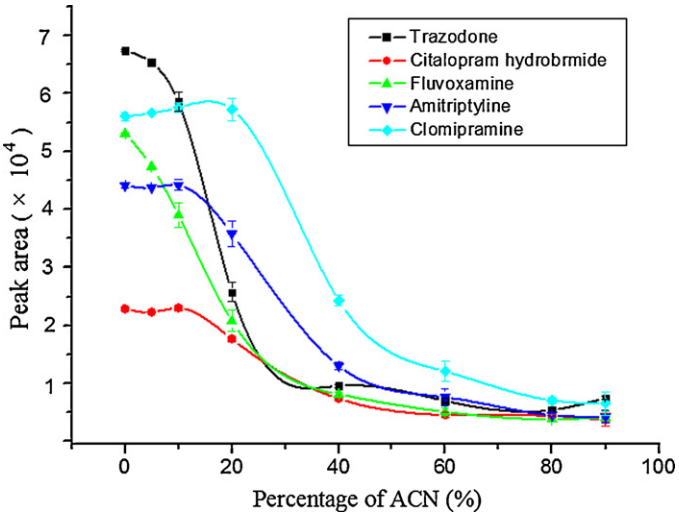

Fig. 4. The effect of ACN addition on extraction efficiency. Sample solutions of five antidepressants spiked at 0.2 μg/mL in 5 mM phosphate solution at pH 7 which contained different percentages (v/v) of ACN addition.

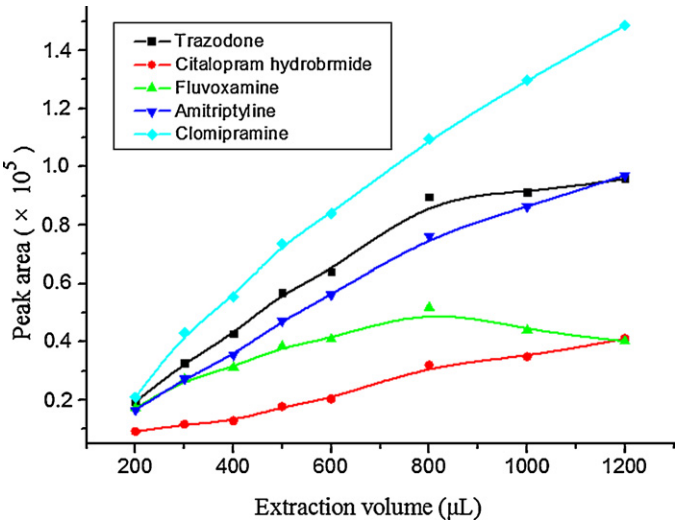

Fig. 5. Extracted sample equilibrium profile of antidepressants for in-tube SPME/HPLC-UV. The sample solutions were spiked at 0.1 μg/mL for five antidepressants.

sesses particle agglomeration with interconnecting macropores. The microglobules are interconnected to form larger clusters that yield continuous skeleton. It can also be observed that the monolith is attached tightly to the inner-wall of the capillary. The flow-through pores size distribution determined by mercury porosimeter was around 4 μm with a narrow size distribution, which results in high permeability and favorable mass transfer in extraction applications.

The C%, N% and H% (w/w) of the hybrid monolith were determined by elemental analysis to be 21.0%, 7.8% and 2.4% respectively, indicating that cyanoethyl groups have been successfully incorporated into the monolith during the sol–gel process. The calculated C, N and H contents were 20.9%, 8.1% and 2.3% based on the empirical formula $(SiO_2)_x(SiC_3H_4NO_{1.5})_y$ with complete hydrolysis and condensation, where x and y were the moles of TEOS and CN-TEOS, respectively. The observed C and N contents were in agreement with the stoichiometric C and N contents and much greater than that of traditionally bonded silica materials [38]. However, the amount of the cyano groups that are accessible for extraction is not known for the hybrid monolith because some cynao groups may be embedded in the bulk of the silica matrix. The specific surface areas and pore volumes from nitrogen adsorption–desorption experiments were 26 m^2/g and 0.05 cm^3/g, respectively.

3.2. Optimization of in-tube SPME conditions

The effect of sample solution pH on the extraction efficiency was experimented in the range from 3 to 9. No obvious change in extraction efficiency was found. The salt concentration (addition of NaCl from 0 to 160 mM) of the sample solution also showed no significant influence on the extraction efficiency. These results may be explained that the hydrophobic interaction and dipole–dipole interactions play a dominant role to the retention between the analytes and extraction phase. Therefore, the sample solution was adjusted to pH 7.0 without adding NaCl.

Standard sample solutions containing different levels of acetonitrile were extracted in the same conditions to compare extraction efficiency. As shown in Fig. 4, extraction efficiencies do not obviously decrease when the sample solution contains less than 10% (v/v) of acetonitrile. Therefore, 10% (v/v) of acetonitrile was spiked to sample solutions, in order to reduce the interference from urine or plasma samples to some extent.

Fig. 5 shows the equilibrium extraction volume profiles, which were obtained by increasing the extracting volume at constant extraction flow rate (0.04 mL/min) of sample solution. The extracted amount of these compounds increased rapidly with increasing extraction volume, indicating the remarkable enrichment capacity of the hybrid silica monolith towards these antidepressants. The extraction equilibriums of trazodone and fluvoxamine were achieved at 800 μL; while for other antidepressants the equilibrium was achieved over 1200 μL. To achieve sufficient sensitivity within a short time, extraction volume of 300 μL was selected for the subsequent analysis.

The chromatograms of eleven antidepressants (including IS) obtained by in-tube SPME/HPLC–MS and direct HPLC–MS analysis under optimal experimental conditions are shown in Fig. 6. In comparison with the chromatogram of direct injection, an obvious enhancement of the peak height was observed after extraction, indicating the remarkable preconcentration ability of the hybrid monolith. The enrichment factors were calculated by comparing the peak area obtained with in-tube SPME and without preconcentration. The extraction yields were based on the percentage of extracted amounts of antidepressants over the total amounts loaded. The enrichment factors and extraction yields were found to be 3.8–7.5 and 25.3–50.0% for eleven antidepressants, respectively (Table 2).

3.3. Reproducibility and stability of hybrid monolith

The column-to-column reproducibility was assessed by calculating the relative standard deviation (RSD) for five antidepressants during extraction. The intra-batch and inter-batch RSDs were in the range of 1.2–5.9% and 3.1–6.1%, respectively (Table 3). Moreover, the monolith showed high stability and could be used for extraction more than 100 times with no significant changes in column backpressure and extraction efficiency.

3.4. Analysis antidepressants in human urine and plasma

Under the optimized conditions, the proposed in-tube SPME/HPLC–MS method was applied for determination of ten antidepressants in human urine and plasma samples. Figs. 7 and 8

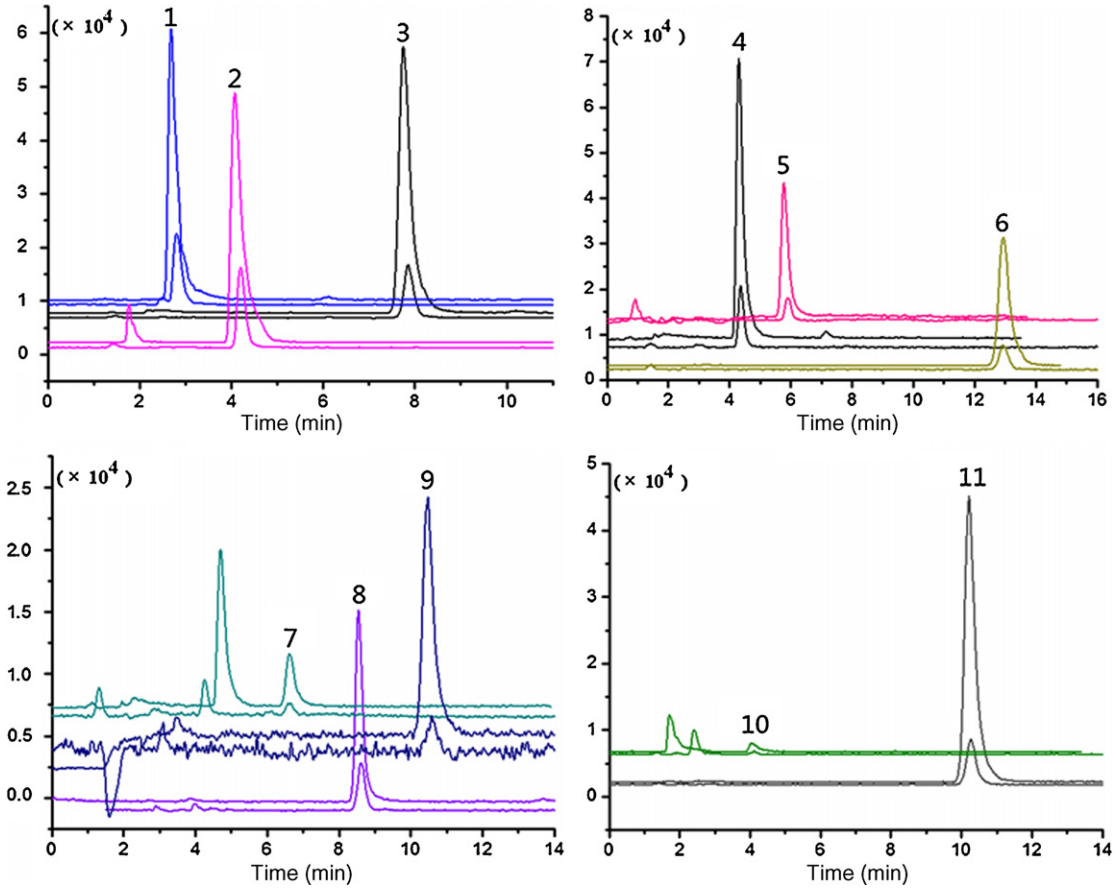

Fig. 6. Chromatograms of antidepressants obtained by direct injection and in-tube SPME/HPLC–MS using hybrid silica monolith. The direct injection volume was 20 μL. The extraction volume for in-tube SPME was 300 μL. The sample solution was spiked at 0.5 ng/mL for eleven antidepressants. The in-tube SPME conditions were the same as that described in Table 1. Peaks: 1, trazodone; 2, citalopram; 3, amitriptyline; 4, doxepin; 5, paroxetine; 6, clomipramine; 7, imipramine (is); 8, fluvoxamine; 9, sertraline; 10, clozapine; 11, fluoxetine.

show the chromatograms obtained from urine and plasma spiked at different concentrations of ten antidepressants, respectively. No interferences from the matrix were observed in the quantification of the analytes.

The relative recoveries, which may present the matrix effect, were calculated by comparing the peak area ratios of antidepressants from the spiked urine and plasma sample to those obtained from the working standard solutions (phosphate solution) at the same concentration. As shown in Table 4, the relative recoveries of ten antidepressants range from 40.5% to 125.3%. Relative recoveries deviating from 100% might be resulted from (1) the residue interferences in sample solution that could affect the ionization efficiency of analytes in ESI; (2) the urine and plasma matrices which would reduce the extraction efficiency of in-tube SPME. Therefore, to provide reliable results, matrix-matched calibration curves were chosen as reference curves.

The application of the in-tube SPME/HPLC–MS method for the determination of ten antidepressants was verified using an internal standard for quantification. The internal calibration in urine and plasma samples was performed by plotting peak area ratios (analytes/I.S.) versus the respective analytes concentration. Matrix-matched calibration curves were established with R above 0.9933. Detection limits (LODs) and quantification limits (LOQs) were calculated as the concentration corresponding to a signal 3 and 10 times the standard deviation of the baseline noise, respectively. As listed in Table 5, the LODs for ten antidepressants were found to be 0.06–2.84 ng/mL in urine and 0.07–2.95 ng/mL in plasma. The LOQs were found to be 0.19–9.45 ng/mL in urine and 0.23–9.83 ng/mL in plasma.

Table 2
Enrichment factors and extraction yields of antidepressants on the hybrid silica monolithic column.

Compounds	Enrichment factors	Extraction yields (%)
Trazodone	5.0	33.0
Clozapine	3.8	25.3
Citalopram	4.6	30.7
Doxepin	5.6	37.3
Paroxetine	7.0	46.7
Fluvoxamine	3.9	26.0
Amitriptyline	6.4	42.7
Fluoxetine	6.7	44.7
Sertraline	7.5	50.0
Imipramine	5.0	33.3
Clomipramine	6.7	44.7

Table 3
Column-to-column reproducibility of hybrid silica monolithic columns.

Precision (RSD, %)	Trazodone	Citalopram	Fluvoxamine	Amitriptyline	Clomipramine
Intra-batch (n = 5)	1.6	5.9	1.2	2.1	4.2
Batch-to-batch (n = 4)	4.8	5.6	6.1	3.1	3.7

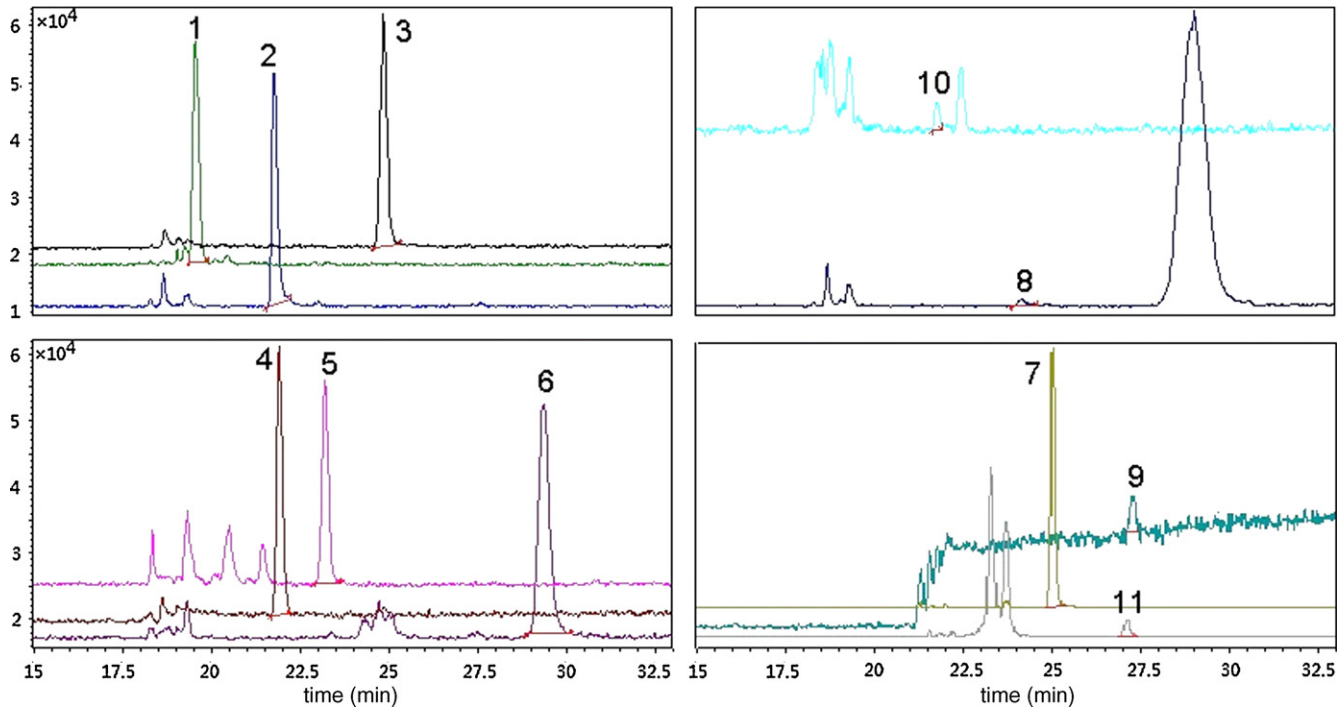

Fig. 7. Typical in-tube SPME/HPLC–MS chromatograms for urine sample spiked with 5 ng/mL ten antidepressants and 5 ng/mL IS (imipramine). The in-tube SPME conditions were the same as that described in Table 1. Peaks: 1, trazodone; 2, citalopram; 3, amitriptyline; 4, doxepin; 5, paroxetine; 6, clomipramine; 7, imipramine (is); 8, fluvoxamine; 9, sertraline; 10, clozapine; 11, fluoxetine. The exhibit time is the sum of extraction time (17 min) and retention time.

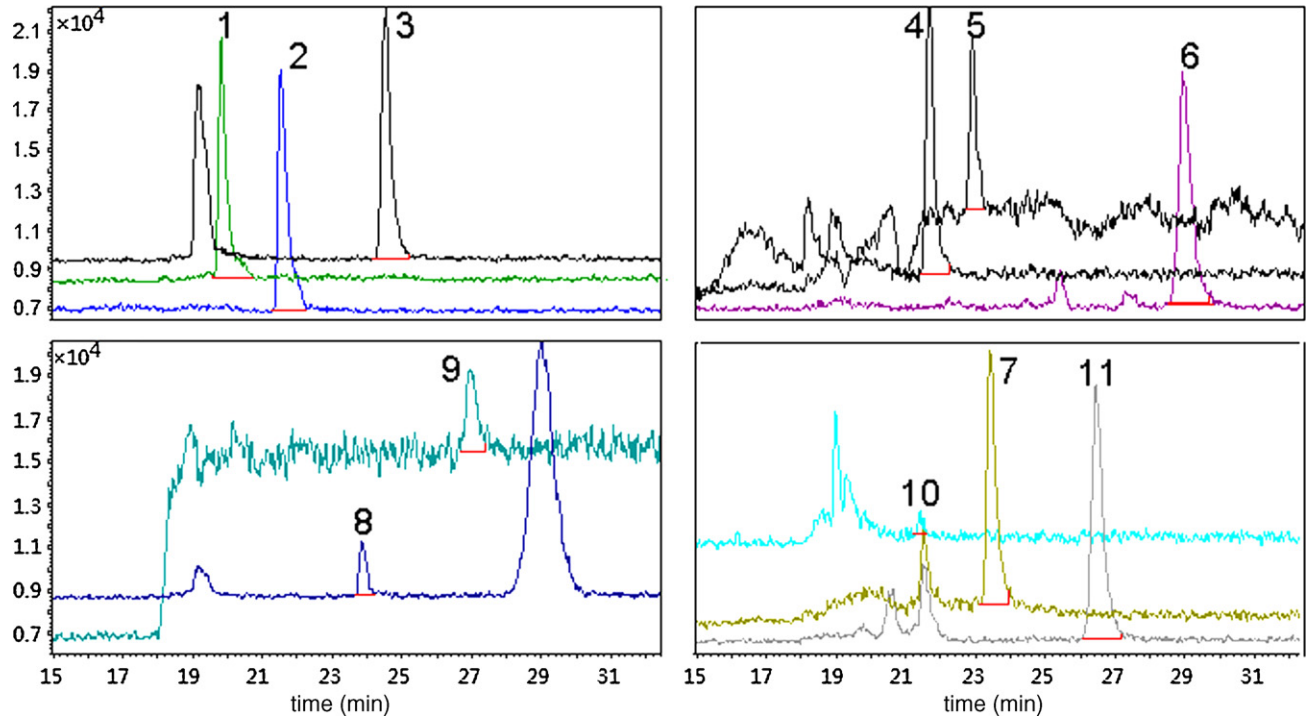

Fig. 8. Typical in-tube SPME/HPLC–MS chromatograms for plasma sample spiked with 5 ng/mL ten antidepressants and 5 ng/mL IS (imipramine). The in-tube SPME conditions were the same as that described in Table 1. Peaks: 1, trazodone; 2, citalopram; 3, amitriptyline; 4, doxepin; 5, paroxetine; 6, clomipramine; 7, imipramine (is); 8, fluvoxamine; 9, sertraline; 10, clozapine; 11, fluoxetine. The exhibit time is the sum of extraction time (17 min) and retention time.

The recoveries and intra- and inter-day RSDs of the proposed method were measured with ten antidepressants spiked at three different concentrations in urine and plasma samples. The recoveries were determined by comparing the calculated amounts of antidepressants in the samples (using matrix-matched calibration curves) with the total spiking amounts. The recoveries and RSDs data for antidepressants spiked in urine and plasma samples are summarized in Table 6. The intra- and inter-day recoveries were

Table 4
Relative recoveries ($n = 3$) of ten antidepressants spiked in urine and plasma samples.

Compounds	Relative recoveries (%)	
	Plasma	Urine
Imipramine	93.5	78.7
Amitriptyline	89.9	95.7
Trazodone	108.8	58.1
Citalopram	112.5	40.5
Doxepin	109.1	61.1
Paroxetine	97.0	67.6
Fluvoxamine	125.3	111.2
Fluoxetine	80.8	75.9
Sertraline	71.0	94.7
Clomipramine	75.8	97.7
Clozapine	111.5	94.4

between 83.0% and 113.0% for urine and 75.2% and 110.9% for plasma. The intra- and inter-day precision for recoveries of ten antidepressants were evaluated with the resulting RSDs less than 16.5%. In addition, the reproducibility of retention time under same analytical conditions was in the range of 0.3–0.8% ($n = 7$).

The in-tube SPME/HPLC–MS was used to analyze urine samples from an elderly patient undergoing therapy with fluoxetine (Prozac®, 20 mg/day). In comparison with the chromatogram of diluted blank urine without in-tube SPME, an obvious enhancement of the peak height was observed after in-tube SPME. Meanwhile, the impurity peaks (2–5 min) were weakened obviously, indicating the remarkable preconcentration and purification ability of the hybrid monolith (Fig. 9). Fluoxetine concentration found in the urine sample was 7.8 μg/mL. The RSDs ($n = 3$) for the determinations was 2.5%.

Table 5
Linear regression and LOD, LOQ data for SPME/HPLC–MS of the ten antidepressants from urine and plasma samples.

Sample	Compounds	Linear range (ng/mL)	Calibration curves			LOD (ng/mL)	LOQ (ng/mL)
			Slope	Intercept	R		
Urine	Trazodone	1–50	0.16	−0.015	0.9951	0.06	0.19
	Clozapine	10–200	0.0026	0.0022	0.9963	2.60	8.66
	Citalopram	1–50	0.17	0.0056	0.9998	0.06	0.19
	Doxepin	1–50	0.21	−0.0027	0.9994	0.16	0.53
	Paroxetine	1–500	0.074	0.042	0.9977	0.08	0.26
	Fluvoxamine	10–500	0.0045	0.0018	0.9992	2.84	9.45
	Amitriptyline	1–500	0.21	0.092	0.9986	0.08	0.28
	Fluoxetine	1–500	0.14	−0.032	0.9988	0.10	0.33
	Sertraline	10–200	0.054	0.016	0.9947	2.64	8.79
	Clomipramine	1–500	0.19	−0.022	0.9978	0.17	0.56
Plasma	Trazodone	1–200	1.6	0.45	0.9957	0.10	0.34
	Clozapine	20–500	0.047	0.093	0.9933	2.95	9.83
	Citalopram	1–200	2.1	0.18	0.9982	0.15	0.49
	Doxepin	1–500	2.1	1.5	0.9933	0.23	0.77
	Paroxetine	5–500	1.3	0.39	0.9980	1.50	5.00
	Fluvoxamine	5–500	0.34	0.20	0.9963	0.66	2.19
	Amitriptyline	1–500	2.6	0.67	0.9982	0.07	0.23
	Fluoxetine	1–500	2.2	0.94	0.9973	0.09	0.30
	Sertraline	10–500	1.1	0.59	0.9961	2.87	9.56
	Clomipramine	1–500	2.7	−0.91	0.9995	0.10	0.35

Table 6
The method accuracies (expressed as recoveries) and precisions at three different concentrations for on-line in-tube SPME/HPLC–MS of the ten antipressants in urine and plasma samples.

Compounds	Intraday recoveries (% RSD%, $n = 4$)			Interday recoveries (% RSD%, $n = 3$)		
	1 ng/mL	5 ng/mL	20 ng/mL	1 ng/mL	5 ng/mL	20 ng/mL
Urine						
Trazodone	83.7 (4.2)	98.1 (3.7)	90.9 (5.9)	97.1 (12.6)	97.5 (2.8)	101.0 (10.3)
Amitriptyline	97.4 (2.0)	85.7 (0.9)	83.0 (3.6)	101.7 (5.2)	88.5 (11.7)	89.0 (10.7)
Citalopram	84.8 (1.7)	101.3 (5.3)	103.3 (4.0)	101.1 (16.7)	99.5 (2.1)	107.3 (9.2)
Doxepin	87.8 (3.6)	92.7 (1.5)	93.9 (0.5)	99.1 (11.0)	94.3 (5.4)	98.2 (3.8)
Paroxetine	98.9 (3.8)	96.5 (1.3)	93.3 (3.8)	107.5 (13.0)	94.9 (6.3)	97.0 (3.5)
Fluvoxamine	109.0 (9.3)	90.3 (12.2)	89.9 (1.2)	113.0 (13.7)	90.1 (9.7)	87.2 (16.5)
Clozapine	97.3 (3.2)	88.1 (0.5)	86.9 (1.2)	100.1 (2.9)	92.0 (7.6)	94.5 (7.2)
Fluoxetine	85.4 (9.7)	89.2 (2.5)	93.9 (5.5)	98.4 (12.6)	91.9 (7.7)	95.4 (4.3)
Sertraline	98.3 (13.0)	85.8 (3.2)	90.9 (6.4)	102.0 (5.0)	92.1 (7.8)	93.8 (5.7)
Clomipramine	91.7 (8.4)	86.7 (4.1)	87.1 (3.2)	95.2 (4.5)	91.5 (8.0)	96.2 (8.3)
Trazodone	97.1 (2.7)	99.6 (4.0)	91.2 (6.8)	101.6 (4.4)	96.3 (14.2)	101.4 (15.3)
Plasma						
Amitriptyline	106.4 (3.9)	86.7 (3.7)	101.0 (3.6)	101.4 (5.5)	85.6 (4.3)	103.8 (9.4)
Citalopram	95.4 (12.3)	95.2 (2.2)	100.4 (4.0)	101.0 (6.1)	93.2 (6.9)	102.8 (12.2)
Doxepin	100.9 (5.5)	92.5 (2.2)	100.5 (4.3)	102.8 (4.1)	87.1 (5.7)	108.7 (9.7)
Paroxetine	84.5 (4.5)	89.1 (0.9)	85.8 (5.2)	94.3 (9.0)	84.7 (5.2)	86.8 (11.8)
Fluvoxamine	82.8 (5.4)	92.5 (3.7)	85.7 (4.8)	90.5 (9.7)	79.6 (9.7)	94.9 (2.8)
Fluoxetine	93.0 (7.5)	92.1 (3.4)	86.0 (3.6)	97.4 (3.9)	81.4 (8.0)	94.6 (2.1)
Sertraline	82.5 (10.0)	88.2 (10.1)	81.2 (5.4)	94.8 (11.3)	81.6 (6.8)	87.7 (8.0)
Clomipramine	102.5 (2.7)	89.0 (5.7)	91.2 (3.3)	104.1 (5.0)	83.9 (3.5)	93.9 (6.8)
Clozapine	–	89.9 (2.8)	106.0 (4.9)	–	75.2 (4.2)	110.9 (11.4)

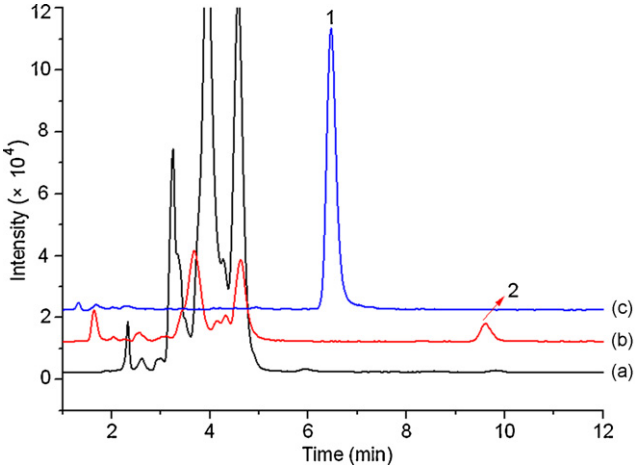

Fig. 9. Chromatograms of patient's urine collected 12 h after taking fluoxetine drugs obtained by direct injection (a) and in-tube SPME/HPLC–MS (b) using hybrid silica monolith. The direct injection volume was 20 μL. The extraction volume for in-tube SPME was 300 μL. The in-tube SPME conditions were the same as that described in Table 1. Peaks: 1. imipramine (IS); 2. fluoxetine.

4. Conclusion

A automated in-tube SPME/HPLC–MS method for analysis of ten antidepressants in human and plasma was developed. The combination of in-tube SPME with LC–MS allowed the development of a fast, sensitive and high-throughput method with a 30 min total analysis time. The proposed in-tube SPME based on cyanoethyl-functionalized hybrid silica monolith advocated an environmentally friendly, inexpensive, and rapid sample pretreatment technique. This automated method offers advantages over those previously methods minimizing laborious repetitive work, improving precision of the method and eliminating the analyst's exposition to toxic solvents.

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