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Solid-phase extraction Citalopram by C18 modified carbon nanotubes in water and Human Plasma samples and determination of using HPLC

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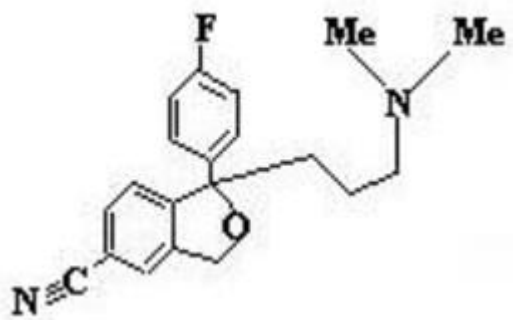
ABSTRACT

A simple method to pre-concentrated Citalopram on C18 modified carbon nanotubes in column has been applied as stationary phase which is used to measure the concentration of Citalopram in water samples by means of solid-phase extraction. To measure 250 mL water samples and 250mg C18 modified carbon nanotubes could be applied. Next step is to measure the Citalopram by injecting them to the gas chromatography with flame ionization. The advantages of applying HPLC with SPE in presence C18 modified carbon nanotubes are high sensitivity, High speed transformation of Citalopram and improving ration standard for river waters with Citalopram in the range of ppb or those with less than 10% of LOD. The quantity of extraction could be affected by sample's pH, amount of solvent, washing liquid type, solvent and flow rates of the sample solutions. The percentage recovery by SPE was 98.00 and the validation parameters proved the precision of the method and its applicability for the determination of citalopram in human plasma.

Introduction

Citalopram are involved in many in processes and frequently are released into the environment through industrial discharges. The neural 5- hydroxytryptamine (5-HT) [serotonin] secreted from the presynaptic cleft of brain binds on the serotonin transporter (SERT), thus resulting into depression. Basically, SERT has a molecular structure of 12 trans-membrane α-helices (TMHs) and intracellular amino- and carboxy-terminals belonging to a family of sodium/chloride-dependent transporters, which is the major pharmacological target in thetreatment of several clinical disorders, including depression. Interaction with a low-affinity allosteric site on SERT modulates various drugs (fluoxetine, imipramine, citalopram, venlafaxine, and duloxetine) affinity at the binding site. Among these drugs, citalopram is the best medication for depression due to its strong binding with 5-HT on SERT; by an allosteric mechanism [2]; resulting into inhibition of presynaptic reuptake of 5-HT and thereby its concentration level in the synaptic cleft is elevated. But it is a chiral molecule (Figure 1) with two enantiomers. S-(+)-Citalopram is twice therapeutically active than its racemic mixture. A search of literature indicates that enantiomers may differ in their pharmacological actions [3-5]. Therefore, the determination of enantiomeric purity is of great importance in pharmaceutical and pharmacological activities. The US Food and Drug Administration (FDA) have issued guidelines to pharmaceutical and agrochemical industries to specify the enantiomeric purity of the optically active compounds prior to their marketing [6] and hence, the last decade has seen a rise of modern electrochemical techniques [7] for such purposes. A thorough search of literature was carried out and only few papers are available on enantiomeric resolution of citalopram by HPLC on Chirobiotic V [8], acetylated 3-cyclobond [9], AGP [10], and Chiracel OD columns [11]. Our experience and literature dictate that amylose based chiral columns are very effective in enantiomeric separation of about 80 percent racemates [12]. In view of this, attempts have been made to resolve the enantiomers of citalopram on amylose chiral column under normal phase mode. The pharmacokinetic and pharmaco-dynamic studies involving plasma profile of citalopram; with respect to time; require chiral ratio of citalopram in human plasma. For chiral analysis of citalopram in human plasma, sample preparation is an integral part and about 80 percent chromatographers are using Solid Phase Extraction (SPE) as the sample preparation method in various biological samples [13]. Literature indicates few papers on solid phase extraction of citalopram in human plasma [15-17]. These methods have certain limitations such as time consuming, poor selective and less efficient. In view of all these facts, attempts have been made to develop fast, inexpensive, selective and reproducible SPE-HPLC methods for resolution of citalopram (Schematic 1) in human plasma. The results of these findings are discussed herein.



Schematic 1. The chemical structure of citalopram

Various types of solid-phase sorbents have been used, including C_{18} [21,22], polystyrene–divinylbenzene-based polymers [22,23], and various forms of carbon [18,20]. A

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number of these sorbents show relatively low recovery for some phenolic compounds [13,14,7,24,25].In this study, a C18 modified carbon nanotubes column has been applied as stationary phase which is used to measure the concentration of Citalopram in water samples by means of solid-phase extraction.

Experimental

Reagents

The racemic and optically active samples of citalopram were obtained from Sigma Chemical Co., USA. The solutions of these samples (1.0 µg/mL) were prepared in LiChrosolve methanol. Methanol, n-hexane, 2-propanol, diethyl amine (DEA) and acetic acid of HPLC grade were purchased from Fisher Scientific (Fairlawn, New Jersey, USA).

Phosphoric acid and sodium phosphate (Na_2HPO_4) of A.R. grade were obtained from Merck India. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., U.S.A.) water purification system. C18 Sep-Pak Classic (short body) cartridge was obtained from Waters, USA. pH was recorded with a pH meter (model 611, Orion Research Inc., USA). All HPLC experiments were carried out on an HPLC system of ECOM (Czech Republic) consisting of solvent delivery pump (model Alpha 10), manual injector, absorbance detector (Sapphire 600 UV-Vis.), Chromatography I/F module data integrator (Indtech. Instrument, India) and Winchrome software. Chiral column used was AmyCoat (150 x 46 mm, 3 µm silica particle size); a gift from Kromasil (Eka Chemicals Separation Products, Bohus, Sweden).

Apparatus

The stock solutions (1.0 µg/mL) of the racemic, S-(+) and R-(-) citalopram used in this study were prepared in LiChrosolve methanol. An aliquot of 5.0 µL of each solution was injected on to a HPLC system described above.

The mobile phases used in this study was n-hexane-2-propanol-DEA (95:05:0.2, v/v/v) at 0.5 mL/min flow rate. The mobile phase was filtered and degassed before use daily. The separations were carried out at room temperature with detection at 240 nm.

The order of elution of the enantiomers was confirmed. The chromatographic parameters such as capacity (k), separation (ά) and resolution (Rs) factors were calculated.

Solid-phase extraction equipment

A standard column 20mm glass vacuum filtration apparatus was utilized after being rebuilt according to The normal sintered piece of glass, acting as support for the glass fibre filters and SPE on C18 modified carbon nanotubes, This construction facilitated and reduced the time for cleaning of the extraction equipment. The vacuum source used was a MZ 2C vacuum pump (Germany).

Sample preparation and derivatization

Prior to the preconcentration step, the pH of sample was adjusted to 1.5 with sulfuric acid. A known volume of distilled or river water was spiked with Citalopram standards and was subsequently passed through a preconditioned SPE column at a flow-rate of 2–6 ml min^{-1}. When the sample had passed through, the cartridge was eluted with 2ml of methanol at the flow-rate of 0.2 ml min^{-1}. The cartridge was preconditioned by washing with 5ml of methanol and activated with 5ml of distilled water at pH 9. For those experiments where the pH effects were studied, citrate buffer (pH 2–10) was used for the adjustment. The derivatization procedure used was based on previous report by Rodr´ıguez et al. [31]. A volume of 2ml of a methanol solution Citalopram was mixed with 1ml of 5%K_2CO_3 and 2ml of *n*-hexane containing 200 µl of acetic anhydride and internal standard. The mixture was shaken for 1 min and the organic phase was allowed to be separate. The aqueous phase was then extracted with a further 1ml of *n*-hexane containing only internal standard. The two *n*-hexane portions were collected, mixed and dried over anhydrous sodium sulfate and injected into the HPLC system. To access lower detection limits in the sample solution at sub-ppb concentrations, the final extract was concentrated to 0.5 ml under a gentle stream of nitrogen.

Results and discussion

The CDS possesses a porous structure should significantly increase the available surface area of it, and therefore, increase the extraction capacity.

Evaluation of sorbent

To evaluate the ability of the CDS for the extraction of Citalopram from water sample.In general, Citalopram are amenable to HPLC without derivatization [17,7–9]. But at lower concentration, peak tailing and discrimination in the injector of capillary column might occur [9,10], especially when environmental samples are analyzed. To overcome these problems, Citalopram could be derivatized with a suitable derivatizing reagent [11–14]. Among the wide variety of derivatizing reagents used for this purpose, acetylating agents have been employed to the greatest extent [15–17].

Effects of different parameters such as the sample pH, the sample volume, flow rate of sample (Table 1) , the volume of eluting solvent, the capacity of sorbent and the linearity of recovery were evaluated using this sorbent. The sample pH is an important factor, which may affect on the recovery of Citalopram from water. To increase the extraction recovery of Citalopram by sorbents, it is necessary to acidify the sample [7]. At low pH, the acid–base equilibrium for the Citalopram compounds shifts significantly toward the neutral forms, which have greater affinities toward the sorbent, and the extraction efficiencies are, therefore, increased. To study the effect of sample pH on the recovery of Citalopram from water samples, 120 ml samples with same concentration in the 200–300µg l^{-1} levels at different pH values (1.5, 3, and 5) were preconcentrated using CDS as a sorbent. Fig1 shows the recovery obtained at each pH and clearly, the maximum recovery is obtained at pH 1.5. Higher recovery results at low pH could indicate that the ion exchange interactions have little contribution in retaining mechanisms. The pronounced recovery decrease for Citalopram in comparison with Citalopram at higher pH, justifies the non-ionexchange interactions.

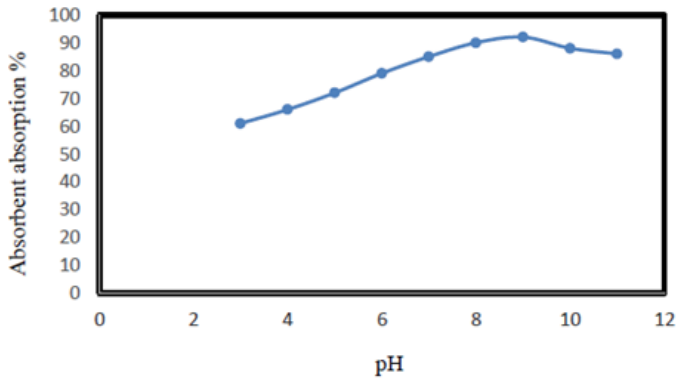

Fig 1. The extraction recoveries obtained for the studied Citalopramat different sample pH

In order to determine the volume of the sample that can be concentrated with acceptable recoveries for all the compounds studied, it was necessary to obtain the breakthrough volumes. Different volumes (100, 300, 500, and 1000 ml) of distilled water, at pH 9, were spiked with a solution containing Citalopram at the 200–500µg l^{-1} levels.

Table 1. Effect of flow rates of the sample solutions on the recovery percentage of Citalopram

Flow rate ml/min	Extraction% Citalopram
0.5	80.97
1	75.12
1.5	57.74
2	8.23
3	8.0
4	2.2

Table 2. The extraction recoveries obtained for the studied Citalopram at different volume of sample solution (*n* =4)

							Volume (ml)	Compound
	1000		500		300		100	
RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	
5.9	22.3	6.2	97.4	5.5	80.5	5.0	85.2	Citalopram

Table 3. The extraction recoveries obtained for the studied Citalopram at 200 ml volume of waste water sample solution Alum Pars manufacture spiked in the range between 2 and 3 µg1^{-1} using C18 modified carbon nanotubes [a]

	C18 modified carbon nanotubes		
LOD (%)	Recovery (%)	LOD (%)	RSD(%)
144	10.4	51	3.5
158	20.2	85	5.8
128	38.3	74	4.9
153	30.7	69	8.6
191	35.9	131	9.4

[a] The relative standard deviations (RSD) between 3.5–9.4% (*n* = 4).

Following the preconcentration step, the trapped analytes on the percolumn were eluted with 2ml of methanol. After derivatization and extraction with a total of 3ml of *n*-hexane, an aliquot of 2 µl was injected into the HPLC system. The recovery of Citalopram compounds and the repeatability for the different volumes are given in Table 2. Good recoveries were obtained for all compounds studied using 250 ml sample volumes. Of course, when samples of 500 ml were preconcentrated, the recoveries were, still, acceptable, except for Citalopram. Further experiments revealed that, for less polar compounds, i.e. 3-CP breakthrough volumes higher than 600 ml was obtainable. It was also found that flow rates up to 7ml min^{-1} for water samples loading on the percolumn had no effect on the recovery percentage.

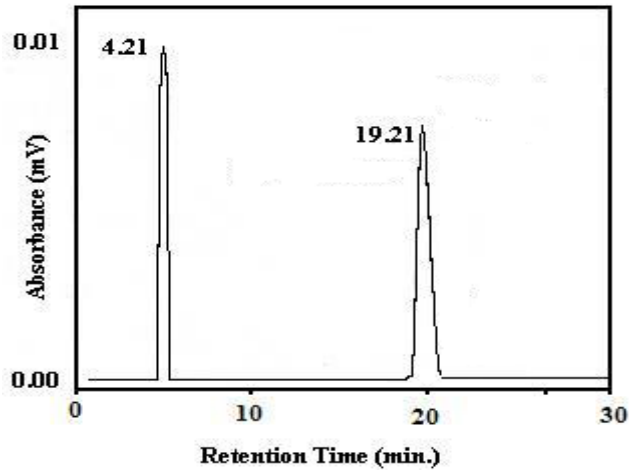

Figure 2. Chromatogram showing the resolution of citalopram (standard solution) Experimental conditions as given in text

To find the required volume of methanol to elute all Citalopram from the cartridge, elution volumes up to 4ml were examined. It was found that a volume of 1ml was sufficient to desorb the trapped pollutants from the SPE per column; of course includes the volume of solvent to saturate the packed cartridge.

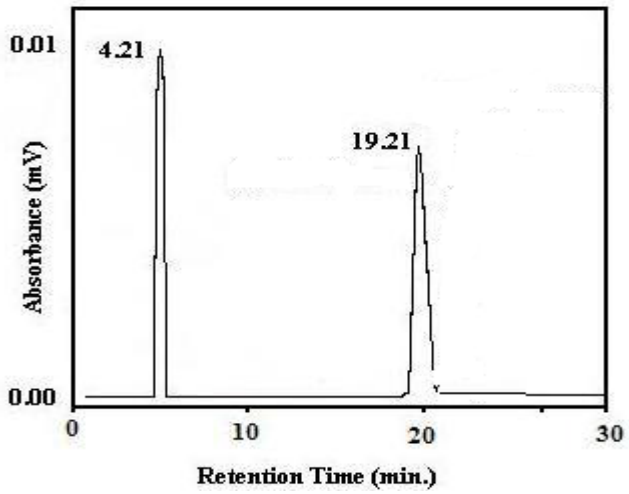

Figure 3. Chromatogram showing the resolution of citalopram (in human plasma) Experimental conditions as given in text

This relatively low volume of methanol eluted all compounds from the cartridge easily and other solvents were, therefore, excluded from any further examination. The low consumption of desorbing solvent is a clear advantage of this sorbent, which would be far more useful in on-line applications. In order to study the capacity of the sorbent and the linearity of recovery, each compound was determined using a river water sample spiked at much higher levels, i.e. 2–3 µg l^{-1}, by GC–MS. No significant differences were obtained, indicating that its capacity is sufficiently high. It also demonstrates that even the preconcentration of water samples spiked with such levels of concentrations has no negative influence on the recovery results.

Real sample

In order to study the effects of sample matrix on the performance of the sorbent, the recovery results were examined using real-life sample spiked with the Citalopram compounds at two different concentration levels. A wastewater sample from Alum Pars manufacture (Saveh, Iran) was spiked with the

selected Citalopram at 2–3 μg l^{-1} levels. After the SPE and derivatization step, an aliquot of final extraction was injected into the GC–MS system. The TIC traces obtained from SPE of 200 ml of river water spiked with a standard solution of Citalopram when CDS was used revealed that, in this case, the clean up process was more efficient. The capacity of CDS for retaining Citalopram were 50–125 mg g^{-1}, while for Citalopram was 30mg g^{-1}. Fig. 2 shows the gas chromatograms of the Alum Pars manufacture (Saveh, Iran) wastewater sample and the same sample spiked with a standard solution of Citalopram compounds. The limits of detection using 200 ml of water were calculated based on a signal-to-noise ratio of 3 and were in the range of 15–120 ng l^{-1}, using TIC mode (Table 3).

The base line and successful resolution of citalopram on C18 modified carbon nanotubes column (150 x 46 mm) column of Kromasil, Sweden. The typical chromatograms of the resolved citalopram enantiomers are shown in Figure 2 and 3 for standard solution and extracted from human plasma, respectively. The values of k α and Rs were 3.56, 4.00, 1.12 and 1.22, respectively. Linearity was in the concentration range of 100-500 μg/L with 10 ng as the limit of detection. A comparison of Figure 2 and 3 indicates that the peaks area of Figure 3 is less than Figure 2. As a result of extensive experiments the optimized chromatographic conditions were developed and reported herein.

Conclusions

The developed Using C18 modified carbon nanotubes as a SPE sorbent method was capable of handling various water samples with a reduced sample preparation time and solvent consumption compared to classical LLE The capability of this sorbent to extract Citalopram has been compared with the results obtained for commercial sorbents and this laboratory-made with a relatively small specific surface area, showed comparable breakthrough volumes for the studied compounds. A CDS sorbent was prepared and investigated with five Citalopram. It could be used more than 150 times. It exhibited fast equilibrium in the extraction for the porous structure of silica particles.

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