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Ultrasound-assisted combined with nano-sized molecularly imprinted polymer for selective extraction and pre-concentration of amitriptyline in human plasma with gas chromatography-flame detection

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ABSTRACT

A new process was developed for the selective extraction and pre-concentration of amitriptyline (AT) from human plasma using nano-sized molecularly imprinted polymer (MIP) with ultrasound-assisted extraction (UAE). The nano-sized AT imprinted polymer particles were synthesized using suspension polymerization in silicon oil and characterized by Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscope (SEM) methods. With the application of optimized values, linearity values in the ranges of 20–200 μg mL^{-1} and 35–200 μg mL^{-1} were obtained for AT with the correlation of determination values (r^2) 0.998 and 0.995 in water and plasma, respectively. The limits of detections (S/N = 3) for AT were found to be 0.7 and 1.2 μg mL^{-1} in water and plasma, respectively. The enrichment factors of AT in water and plasma were 52 and 40, respectively. The inter-day precisions (%) were in the range of 5.8–9.2%. Relative recovery rates ranged from 82.4% to 92.3%. The method was successfully applied to determine AT in the human plasma samples.

1. Introduction

Amitriptyline hydrochloride is a tricyclic antidepressant drug most commonly approved for the treatment of major depression [1]. This drug is chemically basic and in the form of hydrochloride salt (pK_a 9.4) in the market [2]. The function of these drugs is to block the reuptake of neurotransmitters, norepinephrine, and serotonin in the central nervous system [3]. The analytical methods described in the literature to analyze antidepressants in biological fluids usually use conventional sample pretreatment techniques that are laborious, time consuming, and require large amounts of organic solvents [4].

Currently, various methods are known for determining the serum levels of amitriptyline, in human plasma by high-performance liquid chromatography (HPLC) with ultraviolet (UV) and particle beam mass spectrometry (PBMS) [5] and HPLC-UV [6–10], by gas chromatography with mass spectrometry [11], in human urine by utilizing HPLC-UV [12], and in plasma by capillary zone electrophoresis (CZE) [13]. However, the sensitivity of these methods appears to be too low, leading to the need for a large sample volume. Kollroser and Schober [14] described a liquid chromatography with tandem mass spectrometry method that achieves better sensitivity. Proteins and other large biomolecules were removed during an on-line sample cleanup.

Molecularly imprinted polymers (MIPs) have attracted great interest in recent years [15]. During the preparation of MIPs, three-dimension structure cavities were generated after polymerization and template extraction [16,17]. As the cavities were complementary in size, shape, and chemical functionality to that of templates, MIPs possessed excellent recognition ability toward template molecules, and these specific binding affinities between the multifunctional MIPs and the target molecule have proven to be valuable for a variety of separation purposes in environmental remediation [18,19].

So far, synthesized MIPs have been widely used as selective sorbents in different methods such as solid phase extraction (SPE) [20,21], solid phase microextraction (SPME) [22,23], stir bar sorptive extraction (SBSE) [24,25], and dispersive liquid-liquid microextraction (DLLME) [26]. They are used as biosensors as well [27,28]. In these techniques, organic solvents are used to extract the analyte from the MIP, pre-concentrate, and analyze it.

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In this study, a new analytical method was proposed based on the selective extraction and pre-concentration of AT using nano-sized molecularly imprinted polymer (MIP) extraction from human plasma, and pre-concentration of AT with the lowest volume of organic solvent by ultrasonic-assisted extraction method followed by GC–FID determination. In previous studies, toxic organic solvents were used for the desorption of analytes from MIP; however, in the present study, no organic solvents were used, which is the novelty of this work. The amitriptyline (AT) in the sample blood plasma was absorbed by the synthesized nano-MIP. It was then eluted from the MIP by ultrasonic energy and N_2 gas and pre-concentrated (trapped) using just a few microliters of cold acetonitrile; it was then condensed and prepared for analysis by GC-FID.

2. Experimental

2.1. Reagents and solutions

The drugs used in this study (amitriptyline hydrochloride; 1-propanamine, 3-(10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene) N,N-methyl-hydrochloride) were obtained from Darouopakhsh Co. (Tehran, Iran), and methacrylic acid (MAA) as the functional monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linker, and 2,2-azobisisobutyronitrile (AIBN) as the initiator were purchased from Merck Chemical Company. Methanol, acetone, acetic acid, sodium hydroxide, and all the other chemicals used in this study were of analytical reagent grade and obtained from Merck (Germany). Double-distilled water was used throughout the experiments. Stock solution of target analytes, namely amitriptyline (AT), was prepared from methanol containing 1000 mg L^{-1} of the drug. The working solution (10 μg mL^{-1}) was prepared daily with the appropriate dilution of amitriptyline (AT) stock solution with double-distilled water. A drug-free human plasma sample was obtained from a healthy male volunteer.

2.2. Instrumentation

The analytes were separated and monitored by gas chromatography (Agilent 6890N, Agilent Technologies, CA, USA) equipped with a flame ionization detector (FID) and a hydrogen generator (CFH200, Peak Scientific). The injection port was kept at 300 °C and used in an uninterrupted mode and time (0.5 min). The FID temperature was maintained at 300 °C. Hydrogen was generated by a hydrogen generator for the FID at a flow-rate of 40 mL min^{-1}. Helium (99.999%, Gulf Cryo, United Arabic Emirates) was utilized as the carrier gas. The flow of zero air (99.999%, Air Products) for FID was 450 mL min^{-1}. The linear velocity of the carrier gas was 33 cm s^{-1}. The chromatographic separation was achieved on an HP-5 capillary column (30 m × 0.25 mm i.d., film thickness = 0.25 μm) (SGE Analytical Science, Forte, Australia). The oven temperature programming was as follows: 120 °C rising at 20 °C min^{-1} to 200 °C and rising at 10 °C min^{-1} to 280 °C (2 min).

The FT-IR spectra in KBr were recorded using a Spectrum RXI (PerkinElmer, USA), and a scanning electron microscopy (SEM, LEO 1430VP, UK) was used to characterize the nano-sized MIP.

2.3. Nano-sized MIP preparation

In order to prepare the MIP nano particles, ultrasound suspension polymerization in silicon oil was applied. Then, 0.08 mmol of AT, 4.6 mmol of MAA, 9 mmol of EGDMA, and 0.02 g of AIBN were dissolved in 5 mL of methanol. After that, 45 mL of silicon oil was deoxygenated by a nitrogen stream for 10 min. The pre-polymerization mixture was added to the silicon oil and then dispersed at 800 rpm for 15 min. The polymerized suspension mixture was mixed by an ultrasound mixer in order to break-up the suspension droplets into smaller droplets [29]. This process required approximately 15 min. Lastly, the obtained mixture was placed into a water bath, of which the temperature was fixed at 67 °C, for 12 h. The synthesized particles were filtered and washed with petroleum ether and toluene several times. To remove the remaining monomers from the polymer networks, the particles were washed with methanol. Then, to eliminate the AT from the polymer networks, the particles were washed with methanol/acetic acid (90:10, v/v) for 24 h. It was stirred for 24 h by a magnetic stirring device. The MIP was then dried at 40 °C for 1 h.

2.4. Nano sized-MIP SPE

To examine the absorption and desorption capacity of nano-molecular imprinted polymer, 200 mg portions of the nano-sized-MIPs were packed into an L-shaped glass tube and plugged with a small amount of glass wool at both ends. The nano-sized MIPs were pretreated with 2 mL of methanol and washed and then washed again with 10 mL deionized water prior to each SPE run. 10 mL of the working solution (10 μg mL^{-1} from AT in deionized water) with a pH of 7 was passed through the prepared packed nano-sized MIPs in the L-shaped glass tube. The packed nano-sized MIPs in the L-shaped glass tube were then washed with 3 mL of deionized water to remove the matrix interferences (Fig. 1). Next, in order to remove the remaining water from the MIP particles, one end of the L-shaped glass tube was attached to an N_2 gas stream for 15 min at a 0.7 mL min^{-1} rate.

2.5. Ultrasound-assisted procedure

As illustrated in Fig. 1, while the N_2 gas was passing through the L-shaped glass tube, it was put into an ultrasonic bath. Then the other end of the L-shaped glass tube was attached to a needle by a polyethylene tube, and the needle was placed into a conical bottom test tube containing 70 μL of acetonitrile. The conical bottom test tube was placed into an ice bath. The L-shaped tube was kept in 65 °C for 45 min as the N_2 gas was being passed through the tube in the ultrasonic bath. In the end, 1-μL of acetonitrile was taken using a 1-μL GC microsyringe (zero dead volume, PerkinElmer, USA) and injected into the GC system for analysis.

3. Results and discussion

3.1. Nano-sized MIP characterization

Fig. 2 shows the scanning electron microscopy (SEM) image of nano-sized MIP, prepared according to the methods and procedures discussed in Section 2.3. As illustrated in these images, it is possible to obtain real nano-sized MIP particles (around 80 nm) by means of the methods and techniques presented and discussed in this study.

Fig. 3(a)–(c) illustrates the FT-IR spectra of the unleached MIP, leached MIP, and non-imprinted polymer NIP respectively. The perceived strong stretching vibration band ~1733 cm^{-1} is due to the —C=O of carboxylic acid group of methacrylic acid, which is typically located at the polymeric particle surface. This band can be observed in all of the examined polymers. However, in the region of 1500–1700 cm^{-1} for NIP, there are no observed bands. For the unleached MIP at the previously discussed region of (~1620 cm^{-1}), a band is clearly noticed, which is considered to be the result of the —C=O, linked to AT, via coordination bonding. Since these kinds of —C=O groups are typically located in the interior parts of MIP particles, they are not as strong as the bands at ~1733 cm^{-1}. The observations show that MIP washing and AT removal result in a considerable reduction of the vibration band height of the