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Carbon coated magnetic nanoparticles as a novel magnetic solid phase extraction adsorbent for simultaneous extraction of methamphetamine and ephedrine from urine samples

Arezou Taghvimi[a], Hamed Hamishehkar[b,*]

[a] Biotechnology Research Centre, Tabriz University of Medical Science, Tabriz, Iran
[b] Drug Applied Research Centre, Tabriz University of Medical Science, Tabriz, Iran

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ABSTRACT

This paper develops a highly selective, specific and efficient method for simultaneous determination of ephedrine and methamphetamine by a new carbon coated magnetic nanoparticles (C/MNPs) as a magnetic solid phase extraction (MSPE) adsorbent in biological urine medium. The characterization of synthesized magnetic nano adsorbent was completely carried out by various characterization techniques like Fourier transform infrared (FT-IR) spectroscopy, powder x-ray diffraction (XRD), scanning electron microscopy (SEM) and vibrating sample magnetometer (VSM). Nine important parameters influencing extraction efficiency including amount of adsorbent, amounts of sample volume, pH, type and amount of extraction organic solvent, time of extraction and desorption, agitation rate and ionic strength of extraction medium, were studied and optimized. Under optimized extraction conditions, a good linearity was observed in the concentration range of 100–2000 ng/mL for ephedrine and 100–2500 ng/mL for methamphetamine. Analysis of positive urine samples was carried out by proposed method with the recovery of 98.71 and 97.87% for ephedrine and methamphetamine, respectively. The results indicated that carbon coated magnetic nanoparticles could be applied in clinical and forensic laboratories for simultaneous determination of abused drugs in urine media.

1. Introduction

Ephedrine and methamphetamine are categorized as amphetamine type recreational drugs [1–5]. High doses of these alkaloids lead to addiction due to central nervous system stimulation potency of amphetamines. They might be used as doping agents in various sports by athletes so these drugs are classified in the list of forbidden drugs by the Medical Commission of the International Olympic Committee (MCIOC) [6–8]. Growing consumption trend of abused drugs and drug crimes especially among young people are a great concern [9,10], therefore, developing a novel and sensitive method for simultaneous detection of illicit drugs is indispensible [11]. Amphetamines could be quantified in various biological mediums such as plasma, hair, salvia and urine. Among different biological matrixes urine is more important due to its availability and useful role in medicinal and forensic diagnosis [12–14]. Direct analysis of urine samples is not feasible because of urine complex medium that causes low sensitivity and selectivity of the determination. On the other hand, presence of low doses of analytes in urine makes an extraction and pretreatment step important before determination. Liquid–Liquid extraction (LLE) [15] and solid phase extraction (SPE) are commonly used preconcentration methods in the extraction of different drugs from aqueous or biological samples [16]. Magnetic solid phase extraction (MSPE) is an interesting sample treatment method that takes the advantages of both nano adsorbent structure and magnetic property of solid phase adsorbent at the same time [17]. It is clear that nanoparticles have a high surface area to-volume-ratio that causes high extraction efficiency in compare with other adsorbent structures [18,19]. Moreover, magnetic property of nano adsorbent leads to comfort separation of adsorbent from sample media in the presence of external magnetic field without centrifuging or filtration step. These properties accelerate and simplify the extraction process. Magnetic Fe_3O_4 nanoparticle has been widely used in the drug extraction procedures through biological and environmental samples due to its unique magnetic property [20–22]. Carbon-based nanocomposites have attracted attention due to their biocompatibility and higher chemical and

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* Corresponding author.
E-mail address: Hamishehkarh@tbzmed.ac.ir (H. Hamishehkar).

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thermal stability. Furthermore, surface modification is an outstanding aspect of nano carbon materials which provides them extra capability as an adsorbent in aqueous or biological mediums [23].

In the present study, Fe_3O_4 is synthesized by chemical coprecipitation process then modified with carbon through a hydrothermal reaction. After fully characterization, magnetic nano material is utilized as a MSPE adsorbent for simultaneous extraction of ephedrine and methamphetamine from urine samples. Determination and quantification of the analytes were investigated by our newly developed high performance liquid chromatography equipped with an ultra violet detector (HPLC-UV).

2. Experimental

2.1. Reagents and materials

KH_2PO_4, $FeCl_2{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, KOH, NaOH, NH_3 (25%), and HCl were prepared from Merck Chemicals (Darmstadt, Germany). Methamphetamine hydrochloride stock solution 1000 μg/mL in methanol was obtained from Sigma-Aldrich (USA). Ephedrine hydrochloride stock solution 1000 μg/mL in methanol was kindly donated by the Zahravi Pharmaceutical Co. (Tabriz, Iran). D (+)-Glucose anhydrous was bought from AppliChem (USA). Acetonitrile, acetone and methanol (all in HPLC grade) were purchased from Duksan Co. Ltd. (Ansan, South Korea). Double distilled water was prepared from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran).

2.2. Characterization instruments of magnetic nano-adsorbent and analytical conditions

HPLC-UV analysis was performed on a Knauer (Germany) system equipped with a UV–vis detector (K-2600, Knauer, Germany) and pump (K-1001, Knauer, Germany) and a Knauer injector consisting of a 20 μL loop. Separation was conducted on an analytical C_{18} column (10 μm particle diameter, 4.6 mm i.d. × 25 cm) (Knauer, Germany) at room temperature. The mobile phase of the acetonitrile/phosphate buffer solution (10 mM) at a ratio of 15/85 (V/V) and final pH adjustment of 3.5 were used in the isocratic mode at a flow rate of 1.5 mL/min. A Fourier transform infrared (FT-IR) spectrometer (Tensor 27, Bruker, Germany) and powder X-ray diffraction (XRD) (D5000, Siemens, Germany) were applied to characterize the synthesized C/MNPs MNPs. Scanning electron microscopy (SEM) MIRA3 FEG–SEM (Tescan, The Czech Republic) was utilized for morphologic survey. Magnetization curves were recorded using a (VSM—4 inch, Daghigh Meghnatis Kashan Co., Iran) at room temperature.

2.3. Synthesis of magnetic nanoparticles and carbon coated magnetic nano adsorbent

Fe_3O_4 magnetic nanoparticles (MNPs) were synthesized by chemical coprecipitation method [24]. For this purpose, 10 mL of Fe^{+3} (100 mg) and Fe^{+2} (45 mg) solutions were purged under nitrogen protection for 30 min then, ammonia solution was added to the system gradually until the pH of the solution reached to 10–11. The temperature was then reached to 65 °C, and the solution was stirred for more two hours. Next, the solution was cooled down at room temperature, and the resultant black solids were separated by external magnet, washed repeatedly with double distilled water, and then dried at 75 °C for five hours in the oven (3493, Behdad, Iran). The synthesis of C/MNPs was carried out as [25] with some modification. Accordingly, 16 g of glucose powder was completely dissolved in 150 mL of 5 mg/mL Fe_3O_4 solution, and then the mixture was transferred into the 250 mL Teflon-sealed autoclave and heated for 5 h at 170 °C. After the autoclave was cooled downed, the mixture was separated with external magnetic field, washed with distilled water and ethanol for ten times. The resulted material was dried at 75 °C for five hours and applied as magnetic nano adsorbent for the MSPE procedure.

2.4. Urine samples preparation

A healthy volunteer took part in preparing drug free urine samples and the samples were kept in a polyethylene tube. Positive urine samples prepared from kindly cooperation of MAHAN therapy center (Tabriz, Iran). The pH of the urine samples were alkalized to 11 and centrifuged (universal 320, Pole Ideal Tajhiz Co., Iran) for 15 min to sediment white lipidic solid [13]. The supernatant was transferred into a clean tube and spiked with 0.1 μg/mL ephedrine and methamphetamine. All samples were stored at 4 °C and directly used for MSPE procedure.

2.5. MSPE procedure

The MSPE extraction process by magnetic nano adsorbent was conducted as follows: 40 mg of Fe_3O_4 carbon coated magnetic nanoparticles (C/MNPs) was dispersed in 5 mL of 0.1 μg/mL spiked ephedrine and methamphetamine urine sample and vortexed (LS-100, Labtron, Iran) for 2 min. The presence of hydrophilic groups of O—H and C=O on the surface of synthesized C/MNPs facilitates the dispersion of the adsorbent in the urine medium and increases the interaction of the analytes and the adsorbent [26]. The magnetic nano adsorbent was separated from urine medium by external magnetic field. The nano adsorbent is sonicated (30S, Farasout, Iran) in proper desorption solvent for 5 min to desorb extracted abused drugs from the adsorbent surface. The C/MNPs are separated by external magnet and 20 μL of sample was injected into the HPLC-UV system.

3. Results and discussion

3.1. Characterization of synthesized MNPs and C/MNPs

The SEM images show a complete equal size distribution of MNPs and C/MNPs, indicating uniform particle size synthesis of every nanoparticle separately. The estimated average particle size for the MNPs is about 27 nm. The average particle size of the little larger synthesized C/MNPs is about 47 nm, respectively (Fig. 1a). Fig. 1b shows the VSM characterization of MNPs and C/MNPs at room temperature, presence of S-like magnetization hysteresis loops offers superparamagnetization of Fe_3O_4 and nano composite. The M_s is 59.42 and 40.47 emug^{-1} for Fe_3O_4 and C/MNPs, respectively suggesting that carbon coating procedure did not greatly reduced the magnetic property of nanoparticles [27]. It proves the possibility of magnetic nano adsorbent dispersion in the urine medium and also the capability of rapid separation of magnetic nano adsorbent from the medium when an external magnetic field was applied. FT-IR spectrum is applied to investigate the surface chemistry of the synthesized MNPs and C/MNPs. Fig. 1c shows the FT-IR spectra of the synthesized MNPs before and after modification with carbon coated layer. It is clear that the presence of absorption peak at 571.26 cm^{-1} is corresponded to Fe—O band in Fe_3O_4 MNPs. In compare with bare Fe_3O_4 MNPs presence of carbon might prove by absorption peaks at 1641.19 and 1703.33 cm^{-1} related to the stretching frequencies of C=C and C=O of C/MNPs, respectively. Stretching and bending vibrating bands at 3439.08 and 1363.57 cm^{-1} are donated to O—H group [28]. Characterization of crystalline structures is commonly carried out by XRD pattern. Fig. 1d clears the XRD patterns of MNPs and C/MNPs, respectively. Diffraction lines at $2\theta = 30.30$, 36, 43.57, 53.50, 57.50 and 63 is attributed to (220), (311), (400), (422), (511), and (440) planes of