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Magnetic solid phase extraction of mefenamic acid from biological samples based on the formation of mixed hemimicelle aggregates on Fe_3O_4 nanoparticles prior to its HPLC-UV detection

Asadollah Beiraghi [a], Kamyar Pourghazi [a,*], Mitra Amoli-Diva [a], Akbar Razmara [b]

[a] Faculty of Chemistry, Kharazmi (Tarbiat Moalem) University, Tehran, Iran
[b] Department of Chemistry, Faculty of Science, Payam Noor University, Marand, Iran

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ABSTRACT

A novel and sensitive solid phase extraction method based on the adsorption of cetyltrimethylammonium bromide on the surface of Fe_3O_4 nanoparticles was developed for extraction and preconcentration of ultra-trace amounts of mefenamic acid in biological fluids. The remarkable properties of Fe_3O_4 nanoparticles including high surface area and strong magnetization were utilized in this SPE procedure so that a high enrichment factor (98) and satisfactory extraction recoveries (92–99%) were obtained using only 50 mg of magnetic adsorbent. Furthermore, a fast separation time (about 15 min) was achieved for a large sample volume (200 mL) avoiding time-consuming column-passing process of conventional SPE. A comprehensive study on the parameters effecting the extraction recovery such of the amount of surfactant, pH value, the amount of Fe_3O_4 nanoparticles, sample volume, desorption conditions and ionic strength were also presented. Under the optimum conditions, the method was linear in the 0.2–200 ng mL^{-1} range and good linearity ($r^2 > 0.9991$) was obtained for all calibration curves. The limit of detection was 0.097 and 0.087 ng mL^{-1} in plasma and urine samples, respectively. The relative standard deviation (RSD %) for 10 and 50 ng mL^{-1} of the analyte ($n = 5$) were 1.6% and 2.1% in plasma and 1.2% and 1.9% in urine samples, respectively. Finally, the method was successfully applied to the extraction and preconcentration of mefenamic acid in human plasma and urine samples.

1. Introduction

Mefenamic acid (2-[2,3-dimethylphenyl)amino]benzoic acid) (Fig. 1) is one of the important non-steroidal anti-inflammatory drugs (NSAIDs) used to treat many diseases like rheumatoid arthritis, osteoarthritis, non-articular rheumatism and sport injuries [1,2]. On the other hand, overdoses of this drug can produce toxic metabolite accumulation causing acute hepatic necrosis, morbidity, and mortality [3]. So, drug analysis has an extensive impact on public health.

Although determination of NSAIDs can perform by various techniques and in spite of widespread application and use of mefenamic acid, small works have been reported for the quantitative determination of this drug in biological samples. Some reported methods are high performance liquid chromatography (HPLC) [4–6], spectrophotometry [7,8], capillary electrophoresis [9,10], and electrochemical detections [11,12].

In the past few years, a novel liquid–solid extraction technique termed mixed hemimicelles based on the formation of supramolecular self-assembly sorbents was developed [13]. In this method, sorbents produce by adsorption of ionic surfactants such as cetyltrimethylammonium bromide (CTAB) or sodium dodecyl sulfate (SDS) on the surface of mineral oxides sorbent such as alumina, silica, titanium oxide and ferric oxyhydroxides [14]. The formation of hemimicelles and/or admicelles aggregates can provide regions of different polarity allowing the adsolubilization of analytes with different nature. Adsolubilization is a phenomenon in which compounds can incorporate in the surfactant-adsorbed layer on adsorbents [15]. Hydrocarbon chains of surfactant provide hydrophobic or chain–chain interactions for hydrophobic analytes while, the polar groups absorb ionic analytes via electrostatic interaction or hydrogen bonding [16,17]. Therefore, using mixed hemimicelles in solid phase extraction (SPE) has many advantages such as high extraction yield, high breakthrough volume, and easy elution of analytes [17]. However, solid support also plays an important role in mixed hemimicelles solid phase extraction (MHSPE) and determines the loading capacity. Recently, some research groups have explored the possibility of applying nano-sized SPE adsorbents in MHSPE [18–20]. Magnetic nanoparticles

* Corresponding author. Tel.: +98 21 88848949; fax: +98 21 88820993.
E-mail addresses: kmrpourghazi@gmail.com, k.pourghazi@khu.ac.ir (K. Pourghazi).

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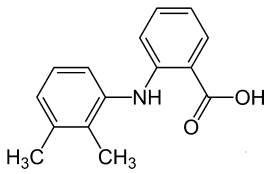

Fig. 1. Chemical structure of mefenamic acid.

(MNPs) such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) possess high surface area which can improve the adsorption capacity of analyte and superparamagnetism property allowing convenient separation of adsorbent from the solution by applying a strong magnetic field in a batch system. These properties make the magnetic nanoparticles an excellent candidate as solid phase extractor for extraction and separation of different analytes. However, few works were performed on the extraction and preconcentration of drugs using MHSPE [21–23] and to the best of our knowledge, neither magnetic separation nor MHSPE method have not yet been applied for determination of mefenamic acid in biological fluids.

In this work, a magnetic nanoparticles-based MHSPE procedure was developed which combines the advantages of both mixed hemimicelles extraction and magnetic nanoparticles adsorbent. The Fe_3O_4 nanoparticles were synthesized via a simple chemical co-precipitation method and then modified with a cationic surfactant to form a nano-sized SPE sorbent used for the extraction and preconcentration of mefenamic acid from biological samples. The analyte concentration in the eluent was determined by HPLC-UV detection. Factors affecting the formation of mixed hemimicelles and recovery of the analyte were investigated and optimized and the proposed method was successfully applied to the extraction and preconcentration of mefenamic acid in plasma and urine samples.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents were of analytical grade and used without further purifications. Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$), mefenamic acid, sodium chloride, glacial acetic acid, methanol, ammonia solution (25%, w/w), glycerol, chloroform, acetonitrile and nitric acid were purchased from Merck (Darmstadt, Germany).

A stock standard solution of the drug (1000 $\mu g\,mL^{-1}$) was prepared by dissolving appropriate amount of mefenamic acid in 5 mL of 0.1 M NaOH and diluting the solution to 100 mL with deionized water. This solution was stored in dark at 4 °C and the working standard solutions were prepared daily by appropriate diluting of stock solution with deionized water.

2.2. Instrumentation and chromatographic conditions

Mefenamic acid analysis was carried out using an Acme 9000 Young Lin HPLC system (Anyang, Korea) equipped with a Young Lin SP930D pump and a UV-Vis 730D detector. An L1-ODS-1 column (5 μm, 250 mm $\times$ 4.6 mm) was utilized and the injection volume was 20 μL. The mobile phase was methanol–water containing 0.1% glacial acetic acid (50:30, v/v) at a flow rate of 2 mL min^{-1} with isocratic elution and UV detection of mefenamic acid was performed at 280 nm. Phase characterization of the nanoparticles was performed by an Ital Structures APD 2000 X-ray diffractometer (XRD) (Riva Del Garda, Italy) using Cu Kα radiation source (λ = 1.54059 Å). The FT-IR spectrum of the prepared MNPs was obtained out by a Rayleigh WQF-510A FT-IR spectrometer (Beijing, China) with KBr method in the 400–4000 cm^{-1} range and a Metrohm 827 mV/pH meter (Herisau, Switzerland) with a combined glass electrode was used for adjusting and measuring pH.

2.3. Preparation of MNPs

Fe_3O_4 nanoparticles were prepared via a simple chemical co-precipitation method [24]. Briefly, $FeCl_3{\cdot}6H_2O$ (11.68 g) and $FeCl_2{\cdot}4H_2O$ (4.3 g) were dissolved in deionized water (200 mL) under nitrogen atmosphere with vigorous stirring at 85 °C. Then, ammonia solution (45 mL, 25%, w/w) was added to the mixture. Upon addition, color of the solution turned to black immediately and the solution was stirred at 3000 rpm for 15 min. The obtained Fe_3O_4 nanoparticles were separated from the reaction medium using an external magnet placing on the bottom of the beaker and the supernatant was decanted. Obtained participate was washed with deionized water (200 mL, five times) to remove excess amount of ammonia. Then, the suspension was completely decanted and washed sequentially with 0.02 M sodium chloride (100 mL, twice) and deionized water (200 mL, once). The prepared MNPs re-suspended in 200 mL deionized water with concentration of 20 mg mL^{-1}.

2.4. Recommended MHSPE procedure

A batch procedure was used for the extraction process. Magnetic nanoparticles (2.5 mL, 20 mg mL^{-1}) were added to CTAB solution (3 mL, 5 mg mL^{-1}) and the mixture was stirred for 3 min. Then 200 mL of sample solution containing 5 μg of the analyte was added to the suspension. The pH was adjusted to 10 and the mixture stirred for 5 min mechanically. The adsorbents were isolated from the solution by applying an external supermagnet. About 2 min takes the suspension became limpid and then the supernatant was decanted. The preconcentrated analyte was eluted with 2$\times$ 1 mL of methanol. The suspension was placed on a supermagnet to hold the adsorbent whist and the eluent was transferred to a test tube for subsequent HPLC-UV analysis. The blank solution was prepared under the same conditions as sample without adding the drug.

2.5. Real sample preparation

The developed MHSPE procedure was used for preconcentration and determination of mefenamic acid in human plasma and urine samples following oral administration of mefenamic acid capsules at single dose of 250 and 500 mg and multiple doses of 250 mg. The study was conducted on twelve male volunteers aged from 28 to 32 years who were judged to be in good health condition through medical history, physical examination and routing laboratory tests (blood biochemistry and urine analysis). Informed consent was obtained from all the subjects after explaining the aim and risks of the study. Volunteers were assigned to four groups (P-1V and P-2V for plasma and U-1V and U-2V for urine, three volunteers in each group) and received single dose of 250 and 500 mg of mefenamic acid under fasting conditions.

Blood samples (5 mL) were collected into centrifuge tubes about 30 min before administration and at the time of 1, 2, 4, 6 and 8 h after oral administration. The tubes were stored on ice for a maximum of 15 min before centrifugation. Blood samples were centrifuged at 10,000 rpm and plasma was withdrawn into another centrifuge tube and stored at −20 °C until analysis. Volunteers who had been assigned to the 250 mg group in the single-dose phase (P-1V) continued on the multiple-dose phase and received 250 mg mefenamic acid capsules three times daily for 3 days. This dose was chosen for the multiple-dose phase because it is similar to a commonly used starting dose in clinical practice. On day 4, the volunteers received a 250 mg mefenamic acid capsule one time and blood samples (P-3V) were drawn at 1, 2, 4, 6 and 8 h after administration. All other