Analytica Chimica Acta 844 (2014) 80–89

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Dispersive micro-solid-phase extraction of benzodiazepines from biological fluids based on polyaniline/magnetic nanoparticles composite

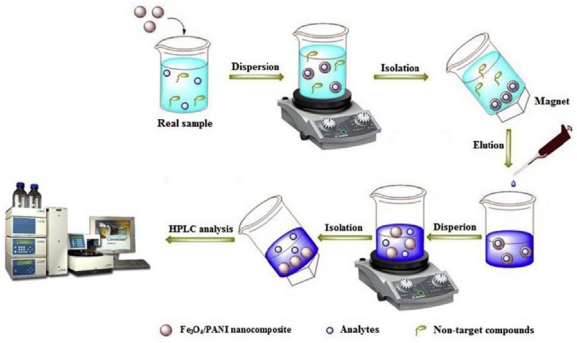
Ali Akbar Asgharinezhad, Homeira Ebrahimzadeh *, Fatemeh Mirbabaei, Narges Mollazadeh, Nafiseh Shekari

Faculty of Chemistry, Shahid Beheshti University, G.C., Evin, Tehran, Iran

HIGHLIGHTS

- Various conductive polymers were coated on the surface of Fe_3O_4 nanoparticles.
- These nanosorbents were used in dispersive μ-solid-phase extraction.
- Fe_3O_4/PANI nanocomposite depicted higher extraction efficiency than the others.
- A small amount of organic solvent and a very short extraction time were required.
- The results showed low LODs, good recoveries and wide linear dynamic ranges.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 April 2014
Received in revised form 2 June 2014
Accepted 6 June 2014
Available online 11 June 2014

Keywords:
Fe_3O_4-polyaniline nanocomposite
Dispersive-μ-solid phase extraction
Lorazepam
Nitrazepam
Preconcentration

ABSTRACT

In this study, diverse types of Fe_3O_4 nanocomposites modified by polyaniline, polypyrrole, and aniline–pyrrole copolymer were synthesized through chemical oxidative polymerization process for dispersive-μ-solid phase extraction (D-μ-SPE) in the presence of various dopants. The results showed that the nanocomposite modified by polyaniline with *p*-toluene sulfonic acid as a dopant demonstrated higher extraction efficiency for lorazepam (LRZ) and nitrazepam (NRZ). Also the synthesized magnetic sorbents were characterized. The nanocomposite sorbent in combination with high performance liquid chromatography–UV detection was applied for the extraction, preconcentration and determination of lorazepam and nitrazepam in urine and plasma samples. Different parameters influencing the extraction efficiency including: sample pH, amount of sorbent, sorption time, elution solvent and its volume, salt content, and elution time were optimized. The obtained optimal conditions were: sample pH, 6; amount of sorbent, 5 mg; sorption time, 5.0 min; elution solvent and its volume, 0.5 mM cethyltrimethyl ammonium bromide in acetonitrile, 150 μL; elution time, 2.0 min and without addition of NaCl. The calibration curves were linear in the concentration range of 1–2000 $\mu g\,L^{-1}$. The limits of detection (LODs) were achieved in the range of 0.5–1.8 $\mu g\,L^{-1}$ for NRZ and 0.2–2.0 $\mu g\,L^{-1}$ for LRZ, respectively. The percent of extraction recoveries and relative standard deviations ($n = 5$) were in the range of 84.0–99.0, 6.1–7.8 for NRZ and 90.0–99.0, 4.1–7.0 for LRZ, respectively. Ultimately, the applicability of the method was successfully confirmed by the extraction and determination of NRZ and LRZ in human urine and plasma samples.

* Corresponding author. Tel.: +98 21 29902891; fax: +98 21 22403041.
E-mail address: h-ebrahim@sbu.ac.ir (H. Ebrahimzadeh).

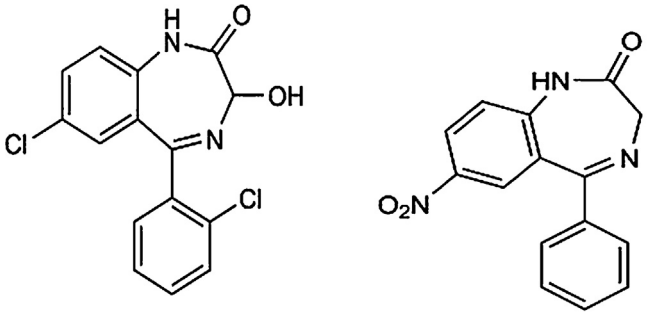
http://dx.doi.org/10.1016/j.aca.2014.06.007
0003-2670/

1. Introduction

Lorazepam (LRZ) and nitrazepam (NRZ) belong to a major class of pharmaceuticals named benzodiazepines (Fig. 1). Benzodiazepines are a class of the most frequently prescribed drugs for treating sleep disturbance and anxiety. They affect the central nervous system and exhibit hypnotic tranquilizing and anticonvulsant properties [1]. On the other hand, many unfavorable side effects including: drug dependency, impaired memory and concentration, depression, and loss of balance may occur. Overdoses of benzodiazepines can cause acute symptoms; therefore, it is desirable to monitor their concentrations in the human body [2].

Several analytical methods such as high-performance liquid chromatography (HPLC) [3,4], GC–MS [5], LC–MS [6], LC–tandem mass spectrometry [7,8], HPLC–MS [9] and voltammetry [10] have been applied for determining benzodiazepines and their metabolites in body fluids. Sample preparation prior to instrumental analysis is one of the most crucial steps in the overall analytical process. Generally, liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are considered as the most commonly used techniques for the preconcentration of compounds from various samples. Solid phase extraction has distinguished from many other extraction techniques due to the advantages such as lower cost, higher enrichment factor and less consumption of organic solvents [11]. Although SPE is being applied broadly, it suffers from some shortcomings such as solvent loss, large secondary wastes, a long procedure, and a need for complex equipment. Dispersive micro-solid phase extraction (D-μ-SPE) is categorized as a SPE technique. The D-μ-SPE exhibits some advantages over traditional SPE, such as convenience for efficiency of recovery; short time requirement and reduced solvent consumption [12,13]. Moreover, it is simple, economic and easy to perform [14,15]. Various sorbents can be employed with D-μ-SPE. Compared to traditional SPE sorbents, nanomaterials possess large surface area and short diffusion route, which may result in high extraction efficiency and rapid extraction dynamics. To counter the disadvantages of employing nanomaterials packed into a cartridge, such as high back pressure and long sample loading time; magnetic solid-phase extraction (MSPE), as a novel SPE method, has been introduced based on magnetic nanoparticles (MNPs) [16]. Magnetic nanoparticles are interesting and technologically substantial objects of physical and chemical researches with numerous promising applications [17,18]. They have large constant magnetic moments and can be easily collected by using an external magnetic field placed outside of the extraction container without additional centrifugation or filtration of the sample, which makes sampling and collection easier and faster [19]. MNPs such as Fe_3O_4 are good candidates for magnetic carrier technology by considering the main advantages: (1) MNPs can be produced in large quantity using a simple method; (2) it can be expected that their sorption capacity is high due to their large surface area; (3) they have strong magnetic properties and low toxicity [20,21]; and (4) these particles are super paramagnetic, that means metal-loaded sorbent can be easily separated from the treated water via an external magnetic field. However, the drawbacks of utilizing MNPs for sample preparation are their low selectivity toward target analytes, low stability in strong acidic aqueous media and low dispersibility in various sample matrices. Therefore, the modification of MNPs with a suitable coating has been proven to be one of the most efficient approaches. There has been an increasing interest in establishing new coating materials for MSPE [19,22]. Among different types of coating sorbents used for the extraction of organic analytes, conductive polymers have attracted a great deal of attention due to their multifunctional properties including hydrophobicity, acid–base character, π–π interaction, polar functional groups, ion exchange property, hydrogen bonding and electro-activity [23–25].

In a previous work, polypyrrole nanowires and MNPs were prepared by chemical oxidation and solvothermal methods, respectively, and then, the magnetic composite of them (mPPYs) was fabricated by a simple co-mixing method. The resulting interconnected network-like structures can offer better permeability to facilitate the mass transfer of analytes [26]. However, after the physical mixing some polypyrrole nanowires can be discarded due to the emersion of MNPs from network-like structure. In this paper, aniline and pyrrole conductive polymers and their copolymer were coated on the surface of Fe_3O_4 NPs. In the next step, the capability of these sorbents for the simultaneous preconcentration and determination of two widely used benzodiazepines as model compounds were examined with a new dispersive micro solid-phase extraction method. The applicability of the proposed method was investigated for the extraction and determination of LRZ and NRZ in plasma and urine samples.



Lorazepam
pKa = 1.3, 11.5 (20°)
Log k_{ow} (octanol/pH 7.4) = 2.4

Nitrazepam
pKa = 3.2, 10.8 (20°)
Log k_{ow} (octanol/pH 7.4) = 2.1

Fig. 1. Chemical structure and physiochemical properties of lorazepam and nitrazepam.

2. Experimental

2.1. Reagents and chemicals

Nitrazepam and lorazepam were kindly donated by Loghman Darou (Tehran, Iran) and used without further purification. Acetone, propanol, tetrahydrofurane (THF), aniline, pyrrole, *p*-toluene sulfonic acid (*p*-TSA), sodium chloride, sodium hydroxide, ferric chloride ($FeCl_3$) and ammonium ferrous sulphate ($(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$) were purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN) and methanol for HPLC were obtained from Caledon (Georgetown, Ont., Canada). Cethyltrimethyl ammonium bromide (CTAB) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water was prepared using a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. Instrumentation

Analyses of standard and test samples were performed by Wellchrom HPLC instrument from Knauer Company (Berlin, Germany). The HPLC instrument consisted of an online K-5020 degasser, a K-501 pump, a 6-port/3-channel injection valve equipped with a 20-μL loop, and a K-2501 UV detector. Eurochrom 2000 was the software used for data acquiring and processing. A capital HPLC column (Scotland, UK) ODS-H C18 (250 × 4.6 mm, i.d. 5 μm) was employed for all separations. The mobile phase was a mixture of potassium dihydrogen phosphate