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Preconcentration and determination of chlordiazepoxide and diazepam drugs using dispersive nanomaterial-ultrasound assisted microextraction method followed by high performance liquid chromatography

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ABSTRACT

Benzodiazepines (BDs) are used widely in clinical practice, due to their multiple pharmacological functions. In this study a dispersive nanomaterial-ultrasound assisted- microextraction (DNUM) method followed by high performance liquid chromatography (HPLC) was used for the preconcentration and determination of chlordiazepoxide and diazepam drugs from urine and plasma samples. Various parameters such as amount of adsorbent (mg: ZnS-AC), pH and ionic strength of sample solution, vortex and ultrasonic time (min), and desorption volume (mL) were investigated by fractional factorial design (FFD) and central composite design (CCD). Regression models and desirability functions (DF) were applied to find the best experimental conditions for providing the maximum extraction recovery (ER). Under the optimal conditions a linear calibration curve were obtained in the range of 0.005–10 $\mu g\,mL^{-1}$ and 0.006–10 $\mu g\,mL^{-1}$ for chlordiazepoxide and diazepam, respectively. To demonstrate the analytical performance, figures of merits of the proposed method in urine and plasma spiked with chlordiazepoxide and diazepam were investigated. The limits of detection of chlordiazepoxide and diazepam in urine and plasma were ranged from 0.0012 to 0.0015 $\mu g\,mL^{-1}$, respectively.

1. Introduction

Benzodiazepines (BDs) are an important class of drugs with a broad range of therapeutic effects used as tranquillizers, muscle relaxant, anesthetics, hypnotics, and anticonvulsive. They are the most frequently approved drugs for the treatment of sleep disturbance, anxiety, and status epileptics [1]. Nowadays, there are over 50 derivatives of BDs available in the worldwide, with the vast majority being under the international control of the Convention on Psychotropic Substances. The major impact of BDs operates widely in the brain, affecting emotional reactions, memory, thinking, and coordination. In addition, to relieve tension in the preoperative period and to induce amnesia in surgical procedures, BDs are used in treatment of alcohol withdrawal [2]. Apart from their therapeutic applications, BDs are often harmed by drug addicts. Therefore, these compounds may be involved in cases of sudden death and be linked to different crimes [3]. Consequently, it is essential for laboratories to develop a rapid and sensitive analytical method for the analysis of these drugs in pharmaceutical preparations, clinical or criminal examinations and biological fluids. For these reasons, various analytical methods including spectrophotometric [4–6], spectrofluorimetric [7,8], chromatographic [9–13], capillary electrophoresis (CE) [14,15] and electrochemical methods [16–18] have been described in the literature for determination of BDs. Since chromatographic assays are widely utilized and inexpensive, it has been a good option for simultaneous separation and quantification in the analysis of BDs. Chromatographic techniques usually require complex isolation sample preparation to separate BDs from various complicated matrices. In recent years, sample preparation procedures such as liquid–liquid extraction (LLE) [19], solid phase extraction [20,21], and liquid phase microextraction [22] have been introduced for separation and preconcentration of drugs from different matrices. Despite of disadvantages including time consuming, high cost, however, the LLE and SPE methods often

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require large amounts of toxic organic solvents, some of which are hazard and contaminate the surroundings due to their high vapor pressure. For these reasons, in recent years the miniaturization and improvement of sample preparation methods of the analyte especially from complicated samples using alternatives is a challenge that has been discussed by several researchers [23,24]. In this regard, in 2006 the dispersive liquid–liquid microextraction (DLLME) with advantages including high preconcentration factor, rapidity, low cost, and simplicity was introduced as a novel sample preparation method for the extraction of organic compounds [25,26]. However, DLLME technique suffers from some drawback such as reduce partition coefficient of analytes into the extracting solvent with increase volumes (i.e., mL) of dispersion solvent and the consumption of the halogenated hydrocarbons (environmentally hazardous) as extraction solvents.

Combination of microextraction methods with ultrasound (US) radiation provides an efficient preconcentration technique including ultrasound-assisted emulsification–microextraction (USAEME) was used for extraction of various analytes at trace concentrations [27]. Sonication can be used to generate very fine emulsions from immiscible liquids, which produce the very large interfacial contact areas between the liquids and increase the mass transfer between two phases. This phenomenon leads to an upgrade in the extraction efficiency in a minimum time [28–31]. In these methods, the extractant is dispersed into the aqueous solution with the aid of ultrasound radiation.

On the other hand, due to unique properties of nanomaterial such as excellent mechanical, electrical, thermal, high adsorbent capacity and high surface area, recently the use of nanoparticles have been found in sample preparation methods. The applications of nanomaterials as adsorbent have several advantages in comparison with traditional adsorbents such as C_{18} and activated carbon (AC). In this work, ZnS nanoparticles loaded on activated carbon (AC) (ZnS-AC) as the new adsorbent for extraction of chlordiazepoxide and diazepam in urine and plasma samples was used. In this method, in order to accelerate the mass transfer of chlordiazepoxide and diazepam from aqueous media to solid phase, the ZnS-AC dispersed into aqueous media by vortex and ultrasonic device. Then the adsorbed analyte from the ZnS-AC eluted with desorption solvents and its concentration determined with HPLC-UV. There are several experimental factors affecting the dispersive nanomaterial-ultrasound assisted-microextraction (DNUM) procedure such as the amount of nanomaterial, vortex and ultrasound time, ultrasonic temperature, ionic strength, pH of aqueous sample and the kind and volume of the elution solvent were studied by experimental design, using a fractional factorial design (FFD) for screening and a central composite design (CCD) for determining the optimum values for the significant factors [32,33]. The best extraction conditions of DNUM and subsequent analysis of chlordiazepoxide and diazepam were validated in relation to the accuracy, precision, detection and quantification limits, and linearity. Finally, the proposed method was applied to the analyzing the chlordiazepoxide and diazepam in urine and plasma samples by HPLC-UV.

2. Experimental

2.1. Reagents and materials

Chlordiazepoxide and diazepam drugs standard were purchased from Sigma–Aldrich (Darmstadt, Germany). The stock solutions (200 mg L^{-1}) of chlordiazepoxide and diazepam were prepared by dissolving of them in methanol. The working solutions prepared by double distilled/deionized water (Milli-Q system, Millipore, MA, USA) of the stock solution. Methanol, ethanol, acetone, acetonitrile (HPLC-grade), $[Zn(CH_3COO)_2{\cdot}H_2O]$, $[CH_3CSNH_2]$, NaCl 99%, HCl 37%, and NaOH were purchased from Merck (Darmstadt, Germany). The standard solutions were kept at 4 °C and brought to ambient temperature just prior to analysis.

2.2. Preparation of zinc sulfide nanoparticle (ZnS-AC)

The ZnS nanoparticles were synthesized based on the reaction of the mixture of $[Zn(CH_3COO)_2{\cdot}H_2O]$ with $[CH_3CSNH_2]$ in aqueous solution. In the synthesis procedure, 10 mL of a 0.1 mol L^{-1} $[Zn(CH_3COO)_2{\cdot}H_2O]$ and 15 mL of a 0.2 M Na_2EDTA (as capping agent) were added into the solution (pH 6.0) under stirring. Then, gradually 4.0 mL (0.5 mol L^{-1}) of $[CH_3CSNH_2]$ as source for S^{2-} ions was added to it. Finally, double distilled water was added for dilution of the solution (100 mL) and the solution was stirred for 2 min for well mixing. The resulting mixture was kept at room temperature until the ZnS nanoparticles started to grows gradually. In the next step in order to deposition of the ZnS nanoparticles on the activated carbon (AC), 100 mL of the freshly prepared ZnS nanoparticles suspension was mixed with 30.0 g of the AC in a 250 mL Erlenmeyer flask under magnetic stirring for up to 1 h. The carbon-supported ZnS nanoparticles were filtered and washed with double distilled water and then dried at 120 °C in an oven for 10 h. A mortar was used to homogeneously grind carbon-supported ZnS nanoparticles powder.

2.3. Instrumentation and software

The KNAUER smartline HPLC system equipped with micro vacuum degasser, UV–vis Detector (2550: was set at 220 nm), Zorbax SB-C_{18} (150 mm × 4.6 mm, 5 μm) (Agilent) column, LPG system and EZCHROM software was used for chromatographic measurements. Determinations of chlordiazepoxide and diazepam drugs were performed at the optimum separation condition by HPLC with isocratic binary mobile phase consisting of acetonitrile: KH_2PO_4 buffer solution (5.0 mmol L^{-1}: pH 6.0) (70:30, v/v) with flow rate of 1.0 mL min^{-1}.

The pH measurements were performed by a digital InoLab pH meter (pH 730, Germany). Ultrasonic device (TECNO-GAZ, 60 Hz, 130 W, parma, Italy) is equipped with digital timer and temperature controller. The IKA multi-position magnetic stirrer (Staufen, Germany) was used for stirring of the solution. An automated Philips X'Pert X-ray diffractometer (Japan) with Co Kα radiation (40 kV and 30 mA) with 2θ values over 30–75° was used for recording the X-ray diffraction (XRD) pattern. Transmission Electron Microscopy (TEM) (JEM 100CX TEM, Tokyo, Japan) at an operating voltage of 80 kV was used to determine the morphology and dimensions of the adsorbent.

The STATISTICA statistical software (Stat Soft Inc., Tulsa, USA: version 7.0) was used for design and analysis of experimental runs. The coefficient of determination R^2 as the criteria for the quality of the polynomial model equation was determined by an *F*-test.

2.4. Sample collection and preparation

Blank urine and plasma samples (drug-free) was supplied by healthy volunteer in our lab, which not exposed to any drug for at least 4 months. Actual urine and plasma sample was collected from sick volunteers in Clinic of Parastar Hospital (Behbahan, Iran) and were kept frozen at −10 °C before analysis. The frozen urine and plasma samples were thawed at ambient temperature and centrifuged for 15 min at 4000 rpm in order to sediment the white lipid of these samples in the bottom of the conical test tube. Then, supernatants were decanted into clean glass tube and filtered through a 0.45 μm filter. The matrix effects were lowered by 2 time dilution of urine and 5 time dilution of plasma sample and subsequently the samples directly subjected to DNUM procedure. Then correctness