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An insight into the determination of trace levels of benzodiazepines in biometric systems: Use of crab shell powder as an environmentally friendly biosorbent

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ABSTRACT

A vortex assisted dispersive solid phase extraction approach (VADSPE) based on crab shell powder as biodegradable and biocompatible μ-sorbent was developed for simultaneous analysis of three benzodiazepines (BZPs): Oxazepam, Flurazepamand Diazepam, in biological matrixes included blood, nail, hair and urine samples. The effective parameters in VADSPE process, including the volume of uptake solvent, the dosage of sorbent, extraction time and back extraction time, were optimized using response surface methodology(RSM) based on central composite design(CCD). The suggested technique allows successful trapping of BZPs in a single-step extraction. Under the optimized extraction conditions, the proposed approach was exhibited low limits of detection (0.003–1.2 μg·mL^{-1}), an acceptable linearity (0.04–20 μg·mL^{-1}). Method performance was assessed by recovery experiments at spiking levels of 10 μg·mL^{-1}(n = 5) for BZPs in blood, nail, hair and urine samples. Relative recoveries were determined by HPLC, which were between 36%and 95.6%.

1. Introduction

Benzodiazepines are a large class of drugs with a wide spectrum of therapeutic result, involving soothing-hypnotics, anticonvulsants, muscle-relaxants and anxiolytics [1]. Benzodiazepines are now in the thick of the greatest customary prescribed drugs, which sharply intensify their possibility for the sexual attack, suicide, abuse in connection with crime and addiction driving under the impact of the considered drugs [2]. Hence, on the authority of the forensic and clinical toxicology significance of BZPs, predictable, rapid and sensitive analytical techniques are necessitated for the accurate parallel determination of BZPs in complicated matrices. Fig. S1 was supplied with Electronic Supplementary Material (ESM), which shows the chemical structure of target analytes. From the physicochemical perspective, BZDs have somewhat high octanol-water partition coefficient (e. g. for diazepam log$P_{o/w}$ = 2.8) which is related to the lipophilic composition [3].

Quite a few quantitative approaches have been well-expressed in the literature for the parallel determination of BZPs as well as: capillary electrophoresis (CE), liquid chromatography mass spectrometry, or tandem mass spectrometry, gas chromatography–mass spectrometry (GC–MS) and electrochemical approaches [4]. Generally, chromatographic methods, required isolation procedure of BZPs from complicated biological matrices. For this purpose, solid phase extraction, Liquid–liquid extraction (LLE), liquid phase microextraction (LPME) and solid-phase microextraction (SPME), have been progressed. SPME has benefited from the more advantages such as excellent enrichment factor, cost saving and less exhaustion of organic solvents [5,6].

Despite of the SPE is definitely the most widely employed technique for clean-up, it suffers from some drawbacks such as subordinate wastes, a time-consuming process, solvent loss and a requisite for the complicated apparatus. Dispersive micro- solid phase extraction (D-μ-SPE) classified as an SPE method. The D-μ-SPE indicates some benefits of conventional SPE, such as moderated solvent exhaustion, an excellent amenity for the efficiency of recovery, straightforward, cost-effective and easy to use. Various sorbents can be exerted with D-μ-SPE. Equate to long-established SPE sorbents, nanomaterials acquire short diffusion path and considerable surface area, which may result in great extraction efficiency and fast extraction dynamics. To overcome the serious drawbacks of utilizing nano-materials packed into a cartridge, such as lengthy sample loading time and higher back pressure; vortex assisted dispersive solid-phase extraction (VADSPE), as an efficient SPE mode, has been introduced based on a wide range of adsorbents

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including crab shell powder [7].

Crab shells powder contained chitin, calcium carbonate and proteins. Chitin, poly-β-(1, 4)-*N*-acetyl-D- glucosamine, is one of the structural ingredients in the external body skeleton of crabs. It is cellulose-like biopolymer, with a number of different functional groups, such as hydroxyls and amines, which increase the uptake efficacy of many drugs and chemicals and maximizes chemical loading. Chitosan, is the product of Chitin's de-acetylation and is the most important derivative of it. The mechanism of chitosan formation from chitin exhibited graphically in Fig. S2 [8]. It has captivated special attentiveness on account of its unique aspects, including adsorption capacity, large surface area, biodegradability, good biocompatibility, renewability non-toxic, film forming ability and hydrophobicity [9].

Due to the presence of a multitude of free hydroxyl and amino groups in its molecular structure and because of protonation of the–NH2 functional groups introduce into the C-2 position of the D-glucosamine reiterating division, it is capable of forming hydrogen bonding interactions, ion-exchange and electrostatic attraction. Further, in the acidic aqueous medium chitosan back bone suffers solubilization. This generates chitosan well afford as a unique pseudo-natural polymer which can be implemented as film, fibers and the hydrogel form [10]. In addition, the chitosan is an environmentally friendly, readily available, effective, low-cost material. Additionally it has capacity for the coupling easily, modification and has an excellent adsorption ability for the oil, grease, metals and other matters [11]. Accordingly chitosan has been extensively applied for miscellaneous fields including drug delivery, food sector, agriculture, separation, pharmaceutical, sewage treatment and medicine [12–14]. For these reason, this bio-available sorbent has been used.

In this research, a response surface methodology approach was applied to identify the optimum conditions for analysis during method development. The traditional method of optimization is the one-factor-at-a-time approach, which has been extensively employed via the past investigations. Even if, it is well approved that this technique persistently was unsuccessful to predict optimal separation conditions and demand a proportionate massive number of trials. It is predominately long-lasting, labor exhaustive, and can tend towards misconstruction of the results due to an inadequacy to contemplate feasible interactions between factors. Quite the opposite, multivariate optimization based on the statistical experimental model attitude affords superiorities including enhanced statistical construction of the results, cutback on the number of trials significantly and that refrain from ambiguous outcomes. Additionally, the impression of a particular criterion can be determined at various stages of the further characteristics, so the outcomes are logical over a wide series of experimental conditions [15].

The RSM is a capable model for optimizing an assortment of proceedings where several variables and interactions influence in the expected response. It can use the quantitative figures of the well-chosen experimental design to estimate manifold variables along with their interactions by set up a short-lived and less arduous mathematical style [16].

In some previous works [11,12], the considerable enhancement of analyte extraction which was accomplished in SPE by supplying an integrated method of vortex assisted solid phase extraction had been projected. Following to those works, we have tried to progress this method in order to develop method precision with lower detection limits. In addition, central composite design was applied to optimize the extraction parameters.

So, in the present work, crab shell has drawn a lot of our attention for pre-concentration and determination of three BZPs; oxazepam, flurazepma and diazepam in various biological matrix as a result of its unique aspect such as: green, low cost and biodegradability.

Indeed, our intent was to examine an eco-friendly adsorbent to isolate the target drugs from bio-matrixes. Cellulose-like backbone of the crab shells also played an important role in the benzodiazepine isolation. The results show that crab shells, a marine waste, could be used as a promising adsorbent in the bio-matrix treatment process. Therefore, a novel process of benzodiazepines isolation, which is environmentally friendly, practical and economical for actual sewage and bio-matrix treatment and easy to operate, should be developed.

Further, in this research for the first time, crab shell as an environmental-friendly adsorbent has been provided for the extraction of benzodiazepines from complicated matrix, due to its adhesives aspect for isolation of biomolecules.

Though in similar researches, the chitosan as an adsorbent mainly in simple environments such as aqueous solutions for extraction or removal of species had been reported. It is noteworthy that the porosity of crab shell is much more than the chitosan. So, in addition to chemical absorption, physical adsorption is involved in the extraction of target analytes because of its entrapment and adsorption properties. Furthermore, crab shell consisting of great reactive groups for chemical activation and cross-linking. Another highlighted feature of the considered biopolymer is high surface charge density, which may play the important role in the extraction procedure due to the electrostatic interactions. However, it suffers some drawbacks including the narrow pH stability range of crab shell back bone and poly-dispersion of the crab shell micro particles [17].

2. Experimental

2.1. Instrumentation

The HPLC system is composed of a Waters 600 E (Millipore, Milford, MA, USA), LC-600pump, C1Cheminert injector valve equipped with a 20 μL sample loop (Switzerland), a Waters 486 tunable UV–Vis detector and a Waters 746 integrator. A C18 column (125 mm length, 4.0 mm diameter, and 5 μm particle size) was applied for separation.

This column was packed in our laboratory with a Knauer packing system involving a Knauer pneumatic HPLC pump (Berlin, Germany), utilizing packing material (Eurospher 100, C18). The degassed mobile phase was a mixture of acetonitrile- methanol–pure water optimized on (80:10:10, v/v/v). The UV detection wavelength was set at 238 nm and the mobile phase flow-rate was 1 mL/min. The column was utilized at room temperature (22 ± 0.5 °C) [18].

2.2. Reagents and solutions

The HPLC-grade solvents including: methanol, pure water, acetonitrile and other analytical grade were purchased from Merck (Darmstadt, Germany). Crab shells were obtained from the supermarket, which was powdered by the ball mill (planetary mill, Iran) with the resolution scale of 2 μm.

Subsequently, the powdered shells soaked in an acidic solution (5% HCl) to remove calcium salt and other minerals for one hour at room temperature. After a washing step with deionized water to remove residual HCl, the powder was soaked in a hot alkaline solution (50% NaOH, 80 °C) for at least an hour, until it has been deacetylated. At this stage, the protein is also removed. After that, the powder was rinsed in deionized water, the pH was adjusted to neutral by HCl treatment and then the bio-sorbent was air-dried. The sorbent was stored in a suitable container until use [19].

Purified free BZPs (Fig. S1) with a purity of > 99% were purchased from Sigma-Aldrich (St. Louis, USA). Sodium hydroxide was purchased from Farabi Co. (Tehran, Iran). Stock solutions of discussed analytes (0.1 mg/mL) were prepared in methanol and kept at 4 °C. Standard sample solutions were supplied diurnal at various concentrations by diluting the stock standard solution with distilled water, which was purified on a Milli-Q ultra-pure water-purification system (Millipore, Bedford, MA, USA).