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Determination of four benzodiazepine residues in pork using multiwalled carbon nanotube solid-phase extraction and gas chromatography–mass spectrometry

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Abstract

A solid-phase extraction (SPE)/GC–MS method using multiwalled carbon nanotubes (MWCNTs) was developed for the determination of four benzodiazepine residues including diazepam, estazolam, alprazolam and triazolam in pork. The analytes were extracted by ultrasonic assistant extraction using acetonitrile, concentrated and purified by MWCNTs packed cartridge, and determined by GC–MS. Ultrasonic extraction conditions, which included temperature, time, power, and solvent volume, were optimized. Comparative studies showed that MWCNTs were superior to C18 for the adsorption of drugs. Parameters influencing SPE efficiency, such as sample amounts, variety and volume of the eluent, were also optimized. Electron ionization (EI) operating in time program-selected ion monitoring mode (SIM) was used for GC–MS identification. The analytes were quantified with external standard calibration curve method. Lower limit of detection and quantification was obtained by the new method. Linear calibration curves were obtained in the concentration range from 10 ng/mL to 500 ng/mL for diazepam and from 20 ng/mL to 1000 ng/mL for estazolam, alprazolam and triazolam with calibration coefficients of 0.99 and above. The average recoveries for the four benzodiazepines spiked in pork were 75% to 104% and their relative standard deviations were between 1.3% and 10%. The limits of detection were 2 μg/kg for diazepam and 5 μg/kg for estazolam, alprazolam and triazolam in pork, respectively.

Keywords: Multiwalled carbon nanotubes; Solid-phase extraction; GC–MS; Benzodiazepines; Pork

1. Introduction

Benzodiazepines are a large group of drugs that are used as muscle relaxants, anticonvulsants, sedative-hypnotics and anxiolytics. The basic chemical structure of benzodiazepines consists of a seven-membered ring fused to an aromatic ring, with four main substitution groups that can be modified without loss of activity. According to some investigation, these drugs have been applied in veterinary cases too. For example, diazepam has been used for tranquillising horses and relaxing their muscle [1]. Benzodiazepines have also been used as anaesthesia, ataractic in pig and dog, etc. [2–4]. In some countries, veterinary tranquillizers are developed for preventing mortality and avoiding weight loss during the period of transportation of the animals, especially swine [5,6]. Benzodiazepines are found probable cause of over dosage-dependence, excessive sedation and anesthesia, coma and death of some people, which have been frequently encountered both in clinical diagnosis and forensic toxicological cases. Moreover, the presence of benzodiazepines residues in edible tissues is raising a high risk to most of the consumers. Hence there is a requirement to establish relative laws and regulations to restrict the use of benzodiazepines in livestock. In fact, some countries have set up similar laws and regulations to prohibit the development of benzodiazepines to domestic animals [7]. Therefore, a novel method is required for the monitoring of benzodiazepines misuse.

However, there are few methods at present for the examination of benzodiazepine residual in animal origin foods. Dirk

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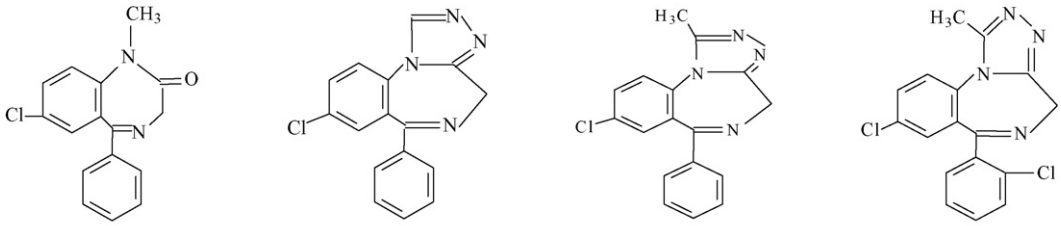

Fig. 1. Molecular structure of four benzodiazepines.

Höhne et al. has reported an analytical method, which identified and quantified the residues of 11 benzodiazepines and 12 metabolites in meat and other tissues, blood, serum, and urine. Benzodiazepines were analyzed by capillary gas–liquid chromatography using electron-capture detection after the Amberlite XAD-2 isolation procedure [8]. Current methods for the identification and determination of trace analytes in biological samples in clinical and forensic toxicology aspects were mainly concentrated on gas chromatography (GC) [9,10], gas chromatography/mass spectrometry (GC/MS) [11,12], high-performance liquid chromatography (HPLC) [13] and HPLC/MS [14]. Prior to the employment of these instrumental protocols, drugs were commonly extracted and cleaned-up by appropriate procedures to remove possible interfering contents of the matrices. Recently, solid-phase extraction (SPE) approach, in which adsorbents have been treated as media for the retaining of target compounds, followed by selective elution, has been broadly utilized due to the advantages of less organic solvents, rare emulsion, and minimize time, etc. [15]. Several reports on the solid-phase extraction of benzodiazepines in urine, serum and plasma [10,12,14] has become popular in many tasks. However, it could not be applied directly to animal tissue analysis or high-throughput detection of test samples because of the matrice difference. Our group has reported a procedure which uses shaking extract method for extraction, C18 SPE columns for the clean-up and GC/MS for the simultaneous determination of four benzodiazepines included diazepam, estazolam, alprazolam and triazolam in pork [16]. However, the method has few shortages. For example, the shaking extraction takes longer time and the results of C18 SPE for clean-up are dissatisfactory, especially for diazepam.

In order to improve these deficiencies, we have done further researches. According to the investigated references, we discovered that multiwalled carbon nanotubes (MWCNTs), a new carbon adsorption material, have obvious advantage as SPE adsorbent for the determination of other compounds. Therefore we have tried to use MWCNTs as a SPE adsorbent for the determination of benzodiazepines in pork samples.

Carbon nanotubes (CNTs) have attracted great attention because of their unique properties in structure, mechanics, electrics, and electromechanics [17–21] since the initial discovery by Iijima [22,23]. CNTs are considered as a sheet of graphite rolled into a tube. Depending on the carbon atom layers formed in the wall of nanotubes, these nanotubes are classified as singlewalled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes. On the basis of their peculiar electronic, metallic and structural characteristics, they have been exploited in analytical and other fields such as biosensor [24], modified electrode [25–27], field-effect transistors [28] and so on. Because CNTs have a large specific surface area, they should possess excellent adsorption ability in theory. The results of primary study demonstrated that CNTs had a unique feature with a notable enrichment efficacy as an absorbent to dioxins [29]. Cai et al. has applied MWCNTs for the pre-concentration of these trace compounds including phthalate esters, bisphenol A, 4-*n*-nonylphenol and 4-tert-octylphenol in environmental water specimens, and favorable results were obtained finally [30,31]. There are no reports for veterinary medicine application.

In this paper, we present a new and rapid procedure utilizing ultrasonic assistant extract method for the extraction, MWCNTs SPE columns for the clean-up and GC/MS for the simultaneous determination of four benzodiazepines included diazepam, estazolam, alprazolam to triazolam (structures showed in Fig. 1) in pork. In comparison with the former procedure, the new procedure using MWCNTs SPE columns has achieved better recovery for all the drugs, especially for diazepam. The application of ultrasonic assistant extraction technology greatly reduced pretreatment time. The absorption capability of MWCNTs was proven obviously higher than that of C18. Lower limit of detection and quantification was obtained by the new method.

2. Experimental

2.1. Reagents and solutions

Diazepam, estazolam, alprazolam and triazolam standards (purity >99.0%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Four stock standard solutions of 1.0 mg/ml were prepared in methanol. The working solutions were prepared by further diluting with methanol as required. Ethyl acetate and methanol were HPLC grade (Fluka, Buchs, Switzerland), and all other solvents were analytical grade from Beijing Chemical Reagents Co. (Beijing, China). Deionized water was purified through water purification system (Elix-3+Milli-QA, Millipore, France). 4.35 g K_2HPO_4 was added to water and the solution was made up to 500 ml with water to prepare the phosphate buffer (pH 7.0) for clean-up. The solution was adjusted to pH 7.0 with phosphate. MWCNTs were purchased from the Chemical Engineering Department of Tsinghua University (Beijing, China). The MWCNTs was already dried at 130 °C for 3 h. The