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ScienceDirect
Analytica Chimica Acta 586 (2007) 399–406
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Multiwalled carbon nanotubes as a solid-phase extraction adsorbent for the determination of three barbiturates in pork by ion trap gas chromatography–tandem mass spectrometry (GC/MS/MS) following microwave assisted derivatization

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Received 10 July 2006; received in revised form 20 October 2006; accepted 4 December 2006
Available online 9 December 2006

Abstract

A new method was developed for the rapid screening and confirmation analysis of barbital, amobarbital and phenobarbital residues in pork by gas chromatography–tandem mass spectrometry (GC/MS/MS) with ion trap MSD. The residual barbiturates in pork were extracted by ultrasonic extraction, cleaned up on a multiwalled carbon nanotubes (MWCNTs) packed solid phase extraction (SPE) cartridge and applied acetone–ethyl acetate (3:7, v/v) mixture as eluting solvent and derivatized with CH_3I under microwave irradiation. The methylated barbiturates were separated on a TR-5MS capillary column and detected with an ion trap mass detector. Electron impact ion source (EI) operating MS/MS mode was adopted for identification and external standard method was employed for quantification. One precursor ion *m/z* 169 was selected for analysis of barbital and amobarbital and *m/z* 232 was selected for phenobarbital. The product ions were obtained under 1.0 V excitation voltage. Good linearities (linear coefficient $R > 0.99$) were obtained at the range of 0.5–50 $\mu g\,kg^{-1}$. Limit of detection (LOD) of barbital was 0.2 $\mu g\,kg^{-1}$ and that of amobarbital and phenobarbital were both 0.1 $\mu g\,kg^{-1}$ (S/N $\geq$ 3). Limit of quatification (LOQ) was 0.5 $\mu g\,kg^{-1}$ for three barbiturates (S/N $\geq$ 10). Satisfying recoveries ranging from 75% to 96% of the three barbiturates spiked in pork were obtained, with relative standard deviations (R.S.D.) in the range of 2.1–7.8%.

Keywords: Barbiturates; Ultrasonic extraction; Multiwalled carbon nanotubes; Microwave assisted derivatization; Gas chromatography–tandem mass spectrometry; Pork

1. Introduction

Barbital, amobarbital and phenobarbital, the three derivatives of barbituric acid (2,4,6-trihydroxypyrimidine), are effective sedative hypnotics for clinical diagnosis [1]. Their broad applications are still popular now due to the effect of appeasing anxiety, reducing blood pressure, decelerating heart rate, and so on, whether in inhalation anaesthetic and ataractic in the veterinary cases or in the aspects of chemical protecting drugs among the field of animal butchery or horse-racing. However, the three barbiturates are frequently abused as the animal feed additives in that they can make the livestock (e.g. swine) more sleepy and moveless, as a result decrease the raising-cost and accelerate the growth rate. Related laws and regulations have been established to prohibit the world-wide use of the three chemicals in the area of the animal feed additives and the animal drinking water [2].

Traditional methods for the analysis of barbiturates are generally conducted by capillary gas chromatography–mass spectrometry (GC–MS) [3–7]. The GC–MS is sensitive for quantitative analysis of the three barbiturates with a limit of quantification (LOQ) 1 $\mu g\,kg^{-1}$ according to our previous study [7]. Generally, a methyl derivatives procedure was needed before

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0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.12.003

the GC/MS analysis [3–7], and direct heating incubation derivatization method [6,7] or method of extracting methylation from the hydrochloric acid solution [3–5] was commonly adopted. However, these methods become less competitive whenever a rapid response time is requested. So that the well-known microwave assisted derivatization come to be a nice substitution for their rapidity [8,9]. Admittedly, microwave, an excellent energy suppler, more distinct than the conventional methods due to the fact of its notably time-saving to reach the perfect effects of derivatization, enabled the whole derivatized reaction duration shortening to only a few minutes. Previous research [3–7] showed that in the determination of the barbiturates residues by quadrupole GC–MS operated in the selected ion monitoring mode (SIM), ions at a low mass region such as *m/z* 169 and 232 were commonly applied as the representative ones due to its extensive ability of fragmentation [3–7]. At such a low mass region, interference from samples and high background noise may become problematic to GC/MS/SIM. To be mentioned, MS/MS or tandem mass spectrometry methods are characterized by high specificity and sensitivity due to their efficiency in excluding interfering matrices. The technique of tandem-in-time MS, as a bench-top ion trap, was also widely applied in many occasions of residual determination [10–12].

In recent years, carbon nanotubes (CNTs), a kind of adsorbent with amazing effects and low cost, has attracted more and more attention in the enrichment of pollutants from environmental samples, such as bisphenol A, 4-*n*-nonylphenol, and 4-*tert*-octylphenol [13]; phthalate esters [14]; dioxin [15]; and heavy metal ions of Cd, Mn and Ni [16]. The previous researches indicated that CNTs may have great analytical potential as an effective solid-phase extraction adsorbent towards a wide range of chemical compounds. Consequently, the feasibility on the clean-up of three barbiturates from the complex matrix of pork utilizing the multiwalled carbon nanotubes (MWCNTs)-packed SPE cartridge was presented in this paper. The analytical performance of the MWCNTs SPE was evaluated by comparison with C18 SPE that reported in literatures [6,7] and has been applied for the analysis of pork samples.

2. Experiments

2.1. Apparatus, chemicals, and pork sample

Analysis was performed on a Trace GC Ultra gas chromatograph coupled with an ion trap Polaris Q MSD, and equipped with a split/splitless injector, an autosampler AS 3000, the Xcalibur Data System and the NIST05 libraries (Thermo Electron Corporation, USA). A TR-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used (Thermo Electron Corporation, USA). Helium was used as carrier gas (purity > 99.995%). Digital ultrasonic cleaner (KQ-250DE, Kunshan, Jiangsu, China), nitrogen evaporator (CNM MST-1, China) and a household microwave oven (Haier MM-2270MG, Qingdao, China) were also used in this study.

Barbital, amobarbital and phenobarbital standards (purity > 99.0%) were provided by the courtesy of the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, hexane, ethyl acetate and acetone were HPLC grade (Fluka Co. Ltd., Switzerland). Other reagents were analytical grade (Beijing Chemical Reagents Company, Beijing, China). Standard stock solutions of three barbiturates (1000 mg mL^{-1}) were prepared in methanol separately and stored at 4 °C. Series of working standards were obtained by appropriate dilution of standard stock solutions with methanol. 0.1 mol L^{-1} K_2HPO_4 buffer (pH 7.4, 7.2 and 7.0) and 0.1 mol L^{-1} NaAc buffer (pH 7.0 and 6.7) were used. Anhydrous sodium sulfate and potassium carbonate (K_2CO_3) were baked for 6 h at 450 °C and kept in desiccator.

MWCNTs were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) and were dried for 3 h at 130 °C to removing the adsorbed water beforehand and kept in desiccator. New solid-phase extraction (SPE) cartridge tube (empty, 6 mL, polypropylene, with 20 μm polypropylene upper and lower frits) was purchased from Agela Technologies Co. Ltd. (Shanghai, China).

Blank pork samples were prepared based on the Ref. [7] description and proved to be free of the three barbiturates by the GC/MS method [6].

2.2. Ultrasonic extraction

An aliquot (2 g) of spiked ground pork sample or blank sample was put into a 50 mL plastic centrifuge tube, followed by adding anhydrous sodium sulfate 2 g and 25 mL acetonitrile, blended completely. The centrifuge tube was ultrasonic extracted for 30 min at 30 °C under the condition of 90% ultrasonic power, then centrifuged at 4000 rpm for 5 min. The extractive solution was decanted into a 100 mL separatory funnel. The above ultrasonic extraction operation was repeated once. The extractive solution was combined, mixed thoroughly and defatted with 20 mL hexane, the remaining extractive solution was evaporated to near dryness and re-dissolved in 7.5 mL buffer solution composed of 5 mL 0.1 mol L^{-1} K_2HPO_4 (pH 7.4) and 2.5 mL 0.1 mol L^{-1} NaAc (pH 7.0).

2.3. MWCNTs SPE clean-up

2.3.1. Preparation of MWCNTs SPE cartridge

The MWCNTs-packed cartridge was prepared in the following step: 20 μm polypropylene lower frits was placed at the bottom of a new bare tube (6 mL, polypropylene), 0.250 g MWCNTs was packed, then 20 μm polypropylene upper frits was placed on the top. The MWCNTs packed height was controlled about 1 cm. The flow rate of the solutions was maintained 0.5–1 mL min^{-1} under vacuum conditions. The MWCNTs-packed cartridge was washed with 10 mL ethyl acetate, acetone and methanol step by step.

2.3.2. Determination of breakthrough volumes for MWCNTs SPE cartridge

The breakthrough volume of the SPE cartridge was measured by continuously monitoring the concentration of three barbiturates in eluate at the outlet of the cartridge. MWCNTs with different average external diameter (20–40, 40–60 and