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Analytical Methods

Miniaturized graphene-based pipette tip extraction coupled with liquid chromatography for the determination of sulfonamide residues in bovine milk

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ABSTRACT

A miniaturized graphene-based pipette tip extraction (M-G-PTE) method coupled with liquid chromatography-ultraviolet detection was developed for rapid screening of sulfadimidine, sulfachloropyridazine, sulfamonomethoxine, and sulfachloropyrazine residues in bovine milk. Because of the large surface area and unique chemical structure of graphene, an M-G-PTE device packed with 3.0 mg graphene could handle 2.0 mL of milk samples at one time. This M-G-PTE device showed better adsorption performance for sulfonamides (SAs) than those packed with other adsorbents such as C_{18}, HLB, SCX, PCX, and multiwalled carbon nanotubes. Under the optimized conditions, good linearity was obtained in the range of 0.05–6.0 μg g^{-1}, with a correlation coefficient (r^2) of ⩾0.9991. The recoveries at three spiking levels ranged from 90.1% to 113.5% with relative standard deviations (RSDs) of ⩽3.9%. The proposed M-G-PTE method was simple, economical, sensitive, and produced less organic pollution. Thus, it could be applied to the rapid screening of SAs in complicated bovine milk samples.

1. Introduction

Sulfonamides (SAs) comprise a group of broad-spectrum antibiotics that are commonly used as veterinary drugs. The SAs residues in animal feed may cause hypersensitive allergic reactions and drug-resistance problems in human; some SAs are even potentially carcinogenic (Gentili, Perret, & Marchese, 2005; Koesukwiwat, Jayanta, & Leepipatpiboon, 2007a). To protect consumer health, the maximum residue limits (MRLs) for SAs in food have been established by the government agencies of many countries. The European Union Commission has laid down an MRL of 100 ng mL^{-1} for the total SAs in milk, and the Ministry of Agriculture of the People's Republic of China established an MRL of 100 ng g^{-1} (Gao, Luo, Ding, & Feng, 2010; Wang et al., 2012). Therefore, the development of rapid, simple, and inexpensive methods for the fast screening of SAs residues in milk is desired. Currently, several methods based on liquid chromatography (Huang, Qiu, & Yuan, 2009), gas chromatography (Reeves, 1999), and capillary electrophoresis (Santos, Lista, Simonet, Ríos, & Valcárcel, 2005) have been reported for the analysis of SAs, sample pretreatment is the key factor in guaranteeing the best analytical performance in these processes. In particular for the analysis of trace SAs in environmental and biological samples, sample-pretreatment procedures must be employed to isolate and preconcentrate the target analytes because of the complexity of sample matrices and the low levels of the target analytes.

To date, various sample-pretreatment techniques including solid-phase extraction (SPE) (Koesukwiwat, Jayanta, & Leepipatpiboon, 2007b), dispersive liquid–liquid micro-extraction (DLLME) (Wen, Li, Zhang, & Chen, 2011), solid-phase micro-extraction (SPME) (Wen, Zhang, Zhao, & Feng, 2005), and matrix solid-phase dispersion (MSPD) (Yan et al., 2012) have been proposed. Although each of these methods has its advantages, some drawbacks still exist in these procedures. The MSPD technique is a simple and efficient sample-pretreatment method, but time-consuming purification processes were usually required when pretreated high-fat samples. DLLME and SPME perform with high enrichment ability and low organic solvent consumption, but they suffer from low recovery and reproducibility. SPE is the most widely used sample-pretreatment technique; however, to satisfy the requirements of instrumental analysis and to handle suitable sample volumes, larger quantities of adsorbent and organic solvents were needed.

Recently, the miniaturization of these procedures and devices was proposed—for example, a miniaturized MSPD (Yan, Wang, Wang, & Yang, 2012) and micro-solid-phase extraction (Zhang,

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Low, & Lee, 2012)—to reduce the consumption of adsorbent, organic solvents, and time. Specifically, as a miniaturized form of SPE, the pipette tip solid-phase extraction (PT-SPE) technique has become an essential tool for the purification and concentration of proteins and peptides in the study of genomics, proteomics, and metabolomics (Tannu et al., 2004). Both the small amount of adsorbent in the pipette tip and the significant reduction in solvent consumption are favorable for reducing economic costs and environmental pollution. The PT-SPE method is specially designed for handling small volumes of biological samples; however, the current commercial (Kumazawa et al., 2007) and homemade (Alwael et al., 2011) adsorbents lack sufficient adsorption capacities to achieve adequate adsorption in one pass. Generally, the aspirating/dispensing cycle must be performed repeatedly to extract the analytes onto the adsorbent. Furthermore, the handling of large sample volumes seems impracticable for this method because of its low adsorption capacity and tedious repeat operation. The adsorbent is the core of SPE, and dominates the capacity and efficiency of extraction procedure. Therefore, it can be inferred that the problems above may be solved, if an appropriate adsorbent is developed.

Graphene, a two-dimensional structure consisting of a single layer of sp^2-networked carbon atoms, has attracted much interest research (Geim, 2009; Geim & Novoselov, 2007; Novoselov et al., 2004). The special structure of graphene can provide a large surface area (~2630 $m^2\ g^{-1}$) (Stoller, Park, Zhu, An, & Ruoff, 2008), which indicates a high adsorption capacity. Meanwhile, the extensive delocalized π-electron systems on the both sides of the planar sheets of graphene can form strong π-stacking interactions with aromatic rings (Allen, Tung, & Kaner, 2010; Dreyer, Park, Bielawski, & Ruoff, 2010), which makes graphene a good adsorbent for the extraction of benzenoid compounds. Furthermore, compared with carbon nanotubes, another type of carbonaceous material, the purity of graphene can be ensured by the quality of the raw graphite material (Ambrosi et al., 2012), because no metal catalysts are used in the synthesis of graphene. Therefore, graphene has great potential for use as a sorbent material in sample pretreatment. In recent years, graphene has been applied as the sorbent material in SPE (Huang, Jing, Wei, & Wu, 2011; Huang, Yu, Li, Wu, & Wei, 2012; Liu, Shi, Zeng, et al., 2011; Wang, Gao, Zang, Li, & Ma, 2012), SPME (Wu, Feng, Zhao, Wang, & Wang, 2012; Zou et al., 2011), and magnetic solid-phase extraction (Luo, Shi, Gao, & Feng, 2011; Wu, Zhao, Feng, Wang, & Wang, 2011). The ultrahigh specific surface area of graphene delivered excellent performance with the low consumption of adsorbent and organic solvent—favorable characteristics for miniaturization applications. Therefore, it's promising for graphene to be used as an adsorbent in PT-SPE.

The purpose of this work was to develop an M-G-PTE method using graphene as adsorbent for the determination of four SAs (sulfadimidine (SM_2), sulfachloropyridazine (SCP), sulfamonomethoxine (SMM), and sulfachloropyrazine (SPZ)) in bovine milk. An M-G-PTE device packed with 3.0 mg graphene has enough adsorption capacity to handle 2.0 mL sample volumes at one time with satisfactory results. The proposed method is simple, economical, sensitive, and produces less organic pollution. It can be applied to the rapid screening of SAs in complicated milk-product matrices.

2. Experimental

2.1. Chemicals and reagents

SM_2, SCP, SMM, and SPZ were purchased from Jingchun Reagent Co. (Shanghai, China). Graphite powder was purchased from Fuchen Chemical Reagent Co. Ltd. (Tianjin, China). Potassium permanganate ($KMnO_4$), hydrazine hydrate (80%), methanol, acetonitrile, acetone, dichloromethane, and ethyl acetate were purchased from Kermel Chemical Reagent Co. Ltd. (Tianjin, China). Sulfuric acid (H_2SO_4), lead acetate ($Pb(AC)_2$), and hydrogen peroxide (H_2O_2) were obtained from Huadong Chemical Reagent Co. Ltd. (Tianjin, China). Double-deionized water was filtered with 0.45 μm filter membrane before use.

2.2. Instrumentation and conditions

LC analysis was performed using a LC-20A system equipped with two LC-20AT Solvent Delivery Units, a SUS-20A gradient controller and a SPD-20A UV–Vis Detector (Shimadzu, Kyoto, Japan). A LC-solution workstation (Shimadzu, Kyoto, Japan) was utilized for data processing. The promosil C_{18} column (150 × 4.6 mm, 5 μm) was obtained from Bonna-Agela Technologies Co. (Tianjin, China). The mobile phase was water (containing 0.5‰ trifluoroacetic acid)–methanol (70:30, v/v) with a flow rate of 1.0 mL min^{-1}. The detection wavelength of the detector was set at 270 nm. An ultrasonic cleaner (KQ3200E, Kunshan Instrument Co., Jiangsu, China) was set at 40 kHz.

2.3. Synthesis of graphene

Graphite oxide (GO) was synthesized by a modified Hummers method (Hummer & Offeman, 1958). Graphite powder (1.0 g) was dispersed thoroughly in 95% H_2SO_4 (45 mL) under stirring in an ice bath, and $KMnO_4$ (3.0 g) was gradually added. The ice bath was removed and the mixture was stirred continuously at room temperature for 36 h. After this period, water (60 mL) was slowly added into the mixture, causing the color to turn from blackish green to brown. Note that the addition of water must be performed in an ice bath to prevent the temperature from exceeding 100 °C because the addition of water in sulfuric acid would release a large amount of heat. Then, the mixture was stirred for another 2 h, and 30% H_2O_2 (3 mL) was subsequently added. Upon H_2O_2 addition, the mixture immediately became golden yellow. Then, the mixture was washed with water to pH 7. Thus, GO was obtained as moist brown solid.

Graphene was prepared by the hydrazine reduction of GO. The obtained moist GO was dispersed in water (2 L) and ultrasonicated for 1 h. Then, hydrazine solution (16 mL, 80% in water) was added into the brown GO dispersion. After stirring for a few minutes, the dispersion was reduced by heating at 90 °C for 2 h. The product was filtered, washed successively with water and methanol, and finally, freeze-dried under vacuum.

2.4. Preparation of bovine milk sample

Bovine milk samples were purchased from local supermarkets in Baoding, China, and stored at 4 °C. The SAs standard solutions were directly spiked into the milk samples (50 g) over the range of 0.05–6.0 μg g^{-1} and stirred for 30 min to intermix completely. Then, 16% $Pb(Ac)_2$ solution (3.0 mL) was mixed with a plain or spiked sample (3.0 mL) and centrifuged at 4000 rpm for 15 min. The obtained supernatant was removed, mixed with 16% $Pb(Ac)_2$ solution (2.0 mL), and then centrifuged at 4000 rpm for 15 min; the supernatant was employed for further M-G-PTE processes.

2.5. Assembly of pipette tip cartridge and M-G-PTE procedure

As shown in Fig. 1, two dried pipette tips (100 μL and 1.0 mL) were employed for assembling the pipette tip cartridge. Graphene (3.0 mg) was packed in the smaller tip using degreased cotton at both ends to avoid adsorbent loss. After the tip of the larger pipette was cut, it was connected with the packed tip. Prior to extraction, the cartridge was pretreated successively with methanol (1.0 mL)