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Development of a high-performance liquid chromatography method for the simultaneous quantification of four organoarsenic compounds in the feeds of swine and chicken

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ABSTRACT

A high-performance liquid chromatography (HPLC) method with UV detection was developed for the simultaneous determination of arsanilic acid, roxarsone, nitarsone, and carbarsone in the feeds of swine and chicken. Feed samples were extracted with methanol/1% acetic acid (90:10, v/v) in an ultrasonic bath and the protein was precipitated with 2% Cu_2SO_4. The samples were further purified by solid phase extraction (SPE) on SAX cartridges. Separation was performed on a Zorbax SB-Aq C18 HPLC column using an isocratic procedure with methanol and 1% acetic acid (3:97, v/v) at a flow-rate of 0.7 mL min^{-1}, and the UV detector was set at a wavelength of 260 nm. The recoveries of organoarsenic compounds spiked at levels of 2, 20 and 200 μg g^{-1} ranged from 81.2% to 91.3%; the inter-day relative standard deviation values were less than 7.0%. The limits of quantification for four organoarsenic compounds were 1.0–2.0 μg g^{-1}. This simple and fast method could be applied to the determination of multi-residues of organic arsenic compounds in animal feeds.

1. Introduction

Roxarsone, arsanilic acid, nitarsone, and carbarsone (Fig. 1) are the best known arsenic compounds. Arsanilic acid and roxarsone are used as animal feed additives for both pig and chicken, whereas nitarsone and carbarsone were used for controlling blackhead disease in turkeys. They are administered orally or mixed with animal feed. However, recent studies have disputed the beneficial effects of these compounds as growth promoters. In addition to these findings, a number of reports have pointed out that these additives can cause toxic effects when used at higher than recommended levels [1,2]. Roxarsone appears to ultimately decompose to water-soluble toxic arsenicals, primarily as inorganic arsenate [3,4]. To ensure confidence in the meat and egg industry and to avoid the misuse of this class of compounds, organoarsenic compounds in animal feeds must be monitored.

This work reported here was primarily concerned with the development of analytical methods capable of separating and determining organoarsenic compounds used in animal feeds. Methods such as HPLC with UV detection [5,6], gas chromatography–mass spectrometry (GC–MS) [7], capillary electrophoresis (CE) [8], liquid chromatography–mass spectrometry (LC–MS) [9,10], and inductively coupled plasma mass spectrometry (ICP-MS) [11–14] have been published describing the analysis of roxarsone or/and arsanilic acid. Only one method has described the simultaneous analysis of arsanilic acid, roxarsone, nitarsone and carbarsone using LC–MS [15]. However, in this work by Pergantis et al., organoarsenic compounds were only determined in standard solution, not in the complex matrices of animal feeds.

At present, no methods have been published for the simultaneous determination arsanilic acid, roxarsone, nitarsone and carbarsone by HPLC with UV detection and only one method for the determination of arsanilic acid and roxarsone in animal feeds using liquid chromatography–hydride generation coupled with atomic fluorescence spectrometry (LC–HG-AFS) [16].

The purpose of this study was to develop a rapid and sensitive HPLC method for simultaneous determination of four organoarsenic compounds in animal feeds. A simple sample preparation method including ultrasonic supported solvent extraction and SPE techniques have been established to decrease the total time of the analysis in this study.

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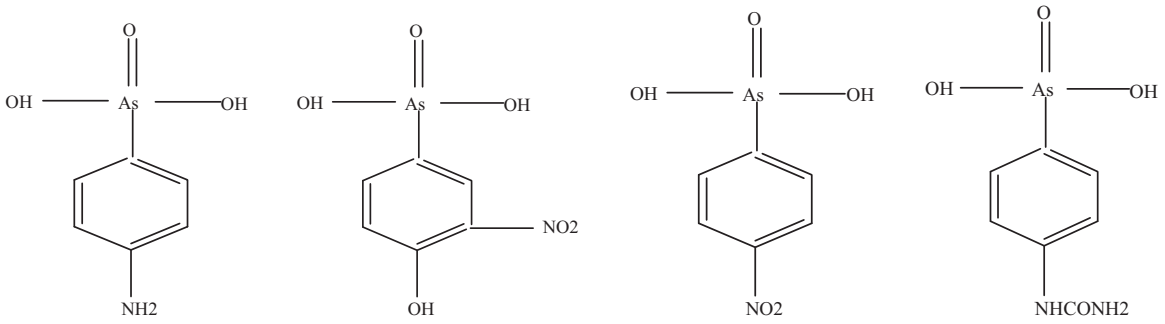

Fig. 1. Structures of organoarsenic compounds: arsanilic acid, roxarsone, nitarsone, and carbarsone.

2. Experimental

2.1. Chemicals and reagents

Analytical standards of arsanilic acid, roxarsone, nitarsone, and carbarson were purchased from Sigma–Aldrich (St. Louis, MO, USA). The four standards were of over 97% purity. Distilled water was further purified by passing it through a Milli-Q Plus apparatus (Millipore, Bedford, MA, USA). Methanol was HPLC-grade and obtained from Fisher (Bar-Bel, France). Other solvents of analytical reagent grade included trichloroacetic acid (TCA), acetic acid, formic acid, $PbAC_2$, and copper sulfate (Cu_2SO_4). The cartridge used for SPE was AccuBOND SAX (500 mg, 3 mL, Agilent, Milford, HA, USA). Other cartridges tested were Oasis HLB (60 mg, 3 mL, Waters Corp., Milford, MA, USA), and Oasis MAX (60 mg, 3 mL, Waters Corp.).

The porcine feed samples were supplied by Breeding Swine Testing Centre (Huazhong Agriculture University, Wuhan, China); the chicken feed samples were supplied by Feeds Inspection Point (Huazhong Agriculture University, Wuhan, China).

2.2. Standard solutions

Individual stock standard solutions were made by dissolving each pure standard in methanol to obtain 1000 $\mu g\,mL^{-1}$ concentration. Standard diluted solutions were mixed with methanol and 1% acetic acid (3:97, v/v). A 200 $\mu g\,mL^{-1}$ mixed standard fortification solution was prepared by combing 2.0 mL of each stock standard and dilute to 10 mL with methanol. Stock solution was prepared every 3 months and stored in amber vials at or below −20° C. Mixed fortification solution was prepared every 1-month and stored in amber vials at or below −20° C.

2.3. Sample preparation

A 5-g finely ground (1 mm) feed sample was transferred into a 50 mL centrifuge tube, spiked with the analytes and 15 mL methanol/1% acetic acid (90:10, v/v) were added to the sample. The mixture was shaken on a vortex system for 5 min, then sonicated in an ultrasonic bath for 10 min at room temperature, and the sample was then centrifuged at 8000 rpm for 10 min. The supernatant was transferred into another 50 mL centrifuge tube. The same extraction procedure was repeated again with 10 mL methanol/1% acetic acid (90:10, v/v). The two supernatant were combined. A 2 mL volume 2% Cu_2SO_4 was added to these extracts. After vortex-mixing for 2 min and centrifuging at 5000 rpm for 10 min, the supernatant was transferred and diluted to 30 mL with methanol. Three milliliters of the supernatant were collected and mixed with 6 mL water, which was ready for the clean-up procedure.

The SAX cartridge was pre-conditioned with 3 mL methanol and 3 mL water. All flow rates for conditioning and washing were set at 3 mL min^{-1}. The entire extracts were loaded onto the SPE column at flow rates of 1 mL min^{-1}. The column was washed with 3 mL water, 3 mL methanol, and 2 mL formic acid/acetonitrile (5:95, v/v), then dried by purging air at the rate of 10 mL min^{-1}. The analytes were eluted with 5 mL formic acid/methanol (5:95, v/v) at a flow rate of 1.0 mL min^{-1} into a 10 mL glass tube and evaporated to dryness under a stream of nitrogen at 40 °C. The dry residue was dissolved in 2 mL mobile phase. The flow diagram for sample preparation of organoarsenic compounds residues determination was seen in Fig. 2.

2.4. HPLC analysis

HPLC analysis was carried out on a Waters 2695 HPLC system coupled with UV detector. The chromatographic separation was accomplished on a Zorbax SB-Aq C18 column (250 × 4.6 mm, 5 μm) (Agilent Technology, USA) coupled with a 2 mm C18 guard-column at 40° C in a column oven. The mobile phase consisted of 1% acetic acid/methanol (97:3, v/v). A flow-rate and injection volume of 0.7 mL min^{-1} and 50 μL, respectively, were used. The UV detector was set at a wavelength of 260 nm for all the compounds.

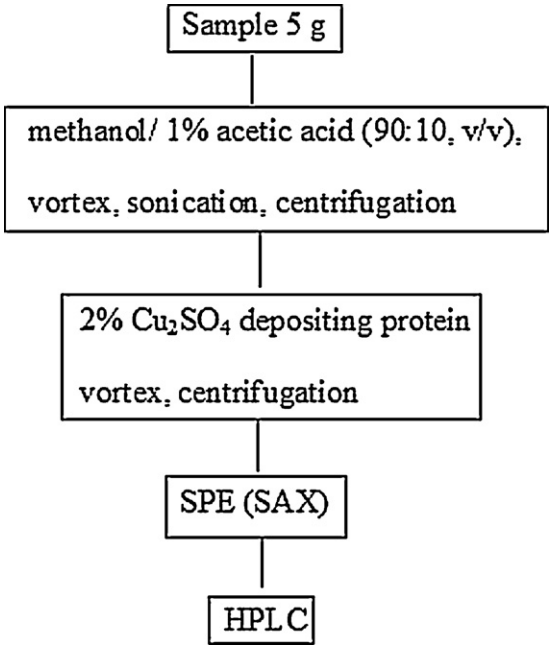

Fig. 2. Overview procedure for sample preparation of organoarsenic compounds residues determination.