Determination of Roxarsone, an Arsenic Animal-Feed Additive, and Its Transformation Products in Chicken Manure by CE-DIHEN-ICPMS and μHPLC-HEN-ICPMS

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1 Abstract

Separation of roxarsone and its transformation products in chicken manure is effected using capillary electrophoresis (CE) and microscale-high performance liquid chromatography (μHPLC). Both ultraviolet (UV) and inductively coupled plasma mass spectrometry (ICPMS) are used for detection. Although UV detection is not selective enough for environmental samples, it makes method optimization prior to coupling CE and μHPLC to ICPMS rugged and time-efficient. Direct injection high efficiency nebulizer (DIHEN) and high efficiency nebulizer (HEN) are explored as the sample introduction device for CE-ICPMS and μHPLC-ICPMS, respectively.

2 Introduction

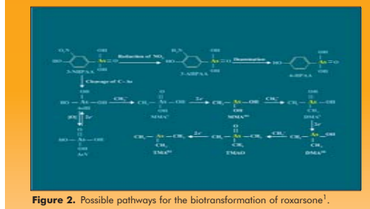


Figure 2. Possible pathways for the biotransformation of roxarsone[1].

Oxidative Aromatic ring fission (not shown): If roxarsone were to undergo such a reaction sequence, arsonoalkyl acids would be produced. The arsonoalkyl acids could then undergo conversion to arsine as demonstrated by Challenger and Higginbottom[12].

[1] Adapted from http://www.epa.gov/nerlesd1/chemistry/labmonitor/labresearch.htm

Arsenic animal-feed additives have been extensively used in the United States for their growth-promoting and disease-controlling properties. In particular, most broiler chickens are fed roxarsone (3-nitro-4-hydroxyphenylarsonic acid) to control coccidiosis. Disposal of the resulting arsenic-bearing wastes is currently unregulated, and they are frequently used to fertilize crop lands. Figure 1 depicts the potential pathways of arsenic exposure from agricultural use of roxarsone. Because of the high use of roxarsone in certain geographic regions, it is important that the environmental fate of this compound and its transformation products be studied in order to understand their possible impacts on human health and the environment. The U.S. Environmental Protection Agency's Office of Research and Development (ORD) has undertaken such a study in conjunction with the U.S. Geological Survey.

Because of the different toxicity levels associated with each arsenic species, it is of utmost importance to identify and measure the individual species existing in the environment. ORD's National Exposure Research Laboratory in Las Vegas is therefore developing analytical methods to speciate arsenic-containing compounds thought to be relevant to roxarsone transformation and fate. To develop a robust speciation method using a simplified sample preparation of chicken manure, CE and μHPLC coupled with on-line ICPMS are being investigated with different sample introduction devices (i.e., HEN and DIHEN). Several researchers have conducted speciation of arsenic compounds by CE-ICPMS[2-4] and HPLC-ICPMS[5-10], but to our knowledge there are few investigations conducted using chicken wastes[5,6]. Compounds investigated in the present study include roxarsone (3-NHPAA) and some likely transformation products, specifically arsenite (AsIII), arsenate (AsV), monomethylarsonate (MMA), dimethylarsinate (DMA), 3-amino-4-hydroxyphenylarsonic acid (3-AHPAA), and 4-hydroxyphenylarsonic acid (4-HPAA). Possible pathways for the biotransformation of roxarsone is shown in Figure 2[11].

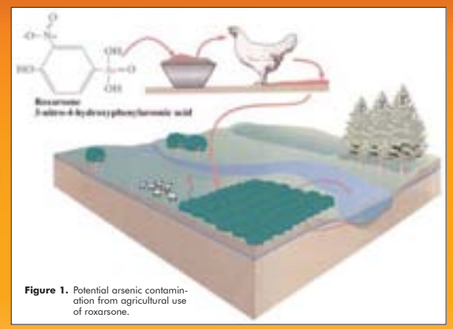
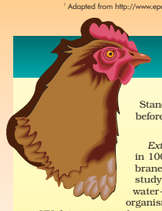


Figure 1. Potential arsenic contamination from agricultural use of roxarsone.

3 Experimental



Reagents and standards. All chemicals used were of analytical grade if not purified. Standard solutions were prepared by appropriate dilution with DI water and filtered (0.45 μm) before use.

Extraction of environmental samples. Samples of chicken manure (1 g in 10 mL DI and 1 g in 100 mL) were suspended in water, sonicated, and filtered through a 0.45-μm Teflon membrane prior to analyses by CE-ICPMS and μHPLC-ICPMS. Because the main focus of this study is to determine the potential exposure of humans and aquatic organisms to arsenic, the water-extractable portion of the manure samples, which is presumably most bioavailable to organisms, was analyzed. A cleanup step was added to the extraction procedure when employing UV determination because of the lack of selectivity of the detector. This cleanup step was necessary in order to remove proteins and other materials that interfered badly with the UV signals of the arsenic analytes and appreciably degraded the chromatograms and electropherograms.

CE, HPLC, and ICPMS Instrumentation. Electrophoretic separations were carried out using a P/ACE 5500 capillary electrophoresis from Beckman Instruments (Fullerton, CA) equipped with a UV/vis diode array detector and voltage supply of up to 30 kV (positive and negative). Applied voltage was set at 15 and 30 kV during UV and ICPMS detection, respectively. The capillary column used was fused silica (Beckman, Fullerton, CA, U.S.A.), 75-μm ID x 57 cm for CEUV and 75-μm ID x 140 cm for CE-DIHEN-ICPMS experiments. Capillary temperature was set at 22°C in all measurements. The reason for fixing the temperature was that the biggest part of the CE capillary was exposed at room temperature during the CE-DIHEN-ICPMS experiments.

An analytical microbore reversed phase HPLC 150 x 1.0 mm ID stainless steel column (Aqua, Phenomenex), packed with 3 μm C_{18} material, was used throughout this study. A 5-μL poly-ether-etherketone (PEEK) loop was used for HPLC sample loading. A syringe pump (Model 100 DM, Isco) was used to deliver pulse-free microflows of eluents to the chromatographic column at a flow rate of 40 μL/min. PEEK tubing was used to transfer column eluates to the high efficiency nebulizer.

The ICPMS system used was a VG PlasmaQuadII STE controlled by PlasmaLab software (Winsford, Cheshire, UK). HEN and DIHEN were purchased from Meinhard Associates, Inc. (Santa Rosa, CA).

CE-ICPMS interface. The interface was a modified design of Bendahl et al.[13] constructed with a PEEK tee. The fused silica capillary from the CE system was threaded through the colinear ends of the tee just outside the torch box and sealed in place with PEEK nuts and ferrules. The tip of the CE capillary was inserted into the sample capillary of the nebulizer about 2 mm behind the tapering such that make-up liquid flowed through unobstructed. Make-up liquid (1% HNO_3) was introduced by a syringe pump (Harvard Instruments) at 30 μL/min through the lower end of the tee where a platinum tube (Hamilton, Reno, NV) was anchored using Tefzel tubing sleeve and ferrule. A silver-coated copper wire was soldered on the platinum tube while the other end of the wire is connected to the back of the CE system for grounding. All connectors and fittings were purchased from Upchurch Scientific (Oak Harbor, WA).

4 Results and Discussion

CE technique. Several CE buffers were investigated, and 20 mM phosphate buffer at pH 5.6 produced successful separation of seven arsenic species in a single run. CEUV using a 6-sec injection (equals 29.5-nL volume) separated the compounds in about 13 min (Figure 3).

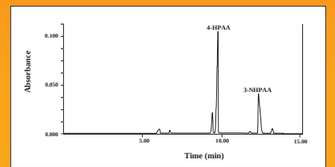


Figure 3. Separation of seven arsenic compound standards by CEUV at pH 5.6 in 10% 20 mM phosphate buffer, 192 nm, 22°C, 15 kV, 57-cm x 75-μm ID column. Volume injected = 29.5 nL.

Preliminary analysis of the same standard mixture conducted by CE-DIHEN-ICPMS using a 40-nL injection (20 seconds) produced the same order of elution as in the CEUV electropherogram except for the last species (AsV). Non-detection of As(V) may be due to coelution with roxarsone or possibly loss of sensitivity (too much peak broadening) because of column length (Figure 4). Obviously, separation time was increased because of the longer column used to accommodate interface design.

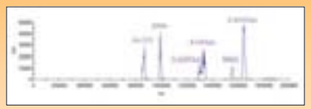


Figure 4. Preliminary separation of seven arsenic compound standards in 10% 20 mM phosphate buffer by CE-DIHEN-ICPMS at pH 5.6, 22°C, 30 kV, 140-cm x 75-μm ID column. Volume injected = 40 nL.

Using the same CE-DIHEN-ICPMS conditions as above, a 20-nL chicken manure extract in 10% 20 mM phosphate buffer was analyzed, and only roxarsone was detected in the sample extract (Figure 5). The anticipated possible transformation products were not detected, possibly due to the very low volume of sample injected. Unfortunately, instrument malfunction deterred us from optimizing the system's conditions to effect separations before this conference started.

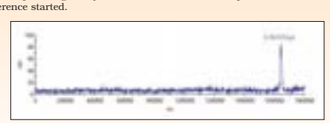


Figure 5. Preliminary analysis of fresh chicken-manure extract in 10% 20 mM phosphate buffer by CE-DIHEN-ICPMS at pH 5.6, 22°C, 30 kV, 140-cm x 75-μm ID column. Volume injected = 20 nL.

HPLC technique. Ion-pairing reversed-phased μHPLC was selected as the chromatographic method of choice to separate the arsenic analytes. Among several tetralkylarsonium ion-pairing salts tested, methyl-tributylammonium hydroxide offered the desired degree of selectivity to separate both inorganic and organic arsenic species of interest in the same chromatographic run. As shown in Figure 6, upon addition of a small amount of methanol in the mobile phase, it was possible to separate a mixture of 8 arsenic compounds in less than 40 minutes.

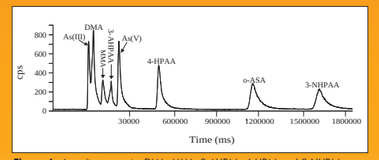


Figure 6. Arsenite, arsenate, DMA, MMA, 3-AHPAA, 4-HPAA and 3-NHPAA were separated on a C_{18} column (2.0 mm ID x 150 mm, Aqua, Phenomenex Inc.) with a mobile phase which contained methyl-tributylammonium hydroxide as ion-pairing agent and 4% v/v methanol. The concentration of the ion pairing agent was kept at 1 mM and the pH of the solution was adjusted to 4. o-Arsanilic acid served as an internal standard.

Preliminary analysis of chicken manure extracts (1 g of chicken manure in 100 mL deionized water) by μHPLC-HEN-ICPMS revealed the presence of roxarsone as the major arsenic compound (Figure 7). Small amounts of other arsenic containing species with retention times similar to DMA, As(III), 3-AHPAA, and 4-HPAA, and which coeluted with the standard compounds (Figure 8), were also present in the chromatogram. None of these compounds, including roxarsone, were present in control samples of chicken manure. Since the manure samples were collected shortly after excretion, it is likely that transformation of roxarsone into the identified arsenic compounds took place in vivo. In addition to the arsenic compounds identified in the extracts, there is a possibility that other arsenic compounds were also present, but they could not be identified because the extracts were highly diluted.

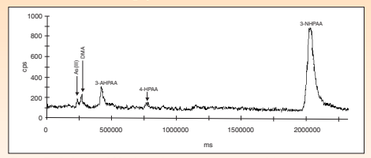


Figure 7. 5 μL of the 100-mL unspiked manure extract was injected on the Aqua C_{18} column and eluted with a methanol-rich mobile phase which contained 1 mM methyl-tributylammonium hydroxide. Roxarsone (3-NHPAA) was identified as the major arsenic compound followed by 3-AHPAA, 4-HPAA, DMA and As(III).

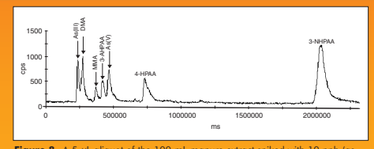


Figure 8. A 5-μL aliquot of the 100-mL manure extract spiked with 10 ppb (as As) standard mixture of As(III), DMA, MMA, 3-AHPAA, As(V), 4-HPAA and 3-NHPAA was injected on the C_{18} column and eluted 1 mM methyl-tributylammonium hydroxide which contained 5% methanol. Coelution of standard compounds and arsenic species extracted in the 1 g sample of chicken manure was observed.

For More Information

An extensive discussion of the environmental and analytical chemistry of arsenic can be found at http://www.epa.gov/nerlesd1/chemistry/labmonitor/labresearch.htm.

Acknowledgements

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