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Liquid chromatography and mass spectrometry for the speciation of arsenic animal feed additives

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Abstract

A number of analytical techniques have been investigated for the determination of arsenic animal feed additive compounds. More specifically, liquid chromatography methods have been developed for the separation of these arsenicals. Micro liquid chromatography, which is a relatively new technique, shows a number of advantages for these analyses, especially when interfaced to mass spectrometers. Improved limits of detection, low solvent consumption, reduced amounts of stationary phase, as well as flow-rates easily accommodated by continuous-flow liquid secondary ion mass spectrometry and direct liquid introduction mass spectrometry, are some of the advantages we have observed when fabricating and using these columns. The off-line combination of micro liquid chromatography and electrothermal atomic absorption spectrometry improves the limit of detection for arsenic and shows potential for on-line coupling. Further use of the methods developed here may allow for a more detailed understanding of the fate and interactions of arylarsenicals in biological and environmental systems.

Keywords: Detection, LC; Arsenic compounds

1. Introduction

The use of arsenic in modern human medicine started in 1907 with the discovery of Salvarsan by Erlich [1]. While this arsenic compound was used as an anti-syphilis drug, other structurally related arsenicals were developed and used for a variety of medicinal applications. In 1946 it was shown that 3-nitro-4-hydroxyphenylarsonic acid (3-NHPAA) was capable of controlling cecal coccidiosis in poultry [2]. While investigating the therapeutic performance of this compound it was also discovered that it acted as a growth promoter, improved feed conversion, provided better feathering, increased egg production and pigmentation. In the years following, a number of other arsenic compounds were shown to have similar properties, these are *p*-arsanilic acid (*p*-ASA), 4-nitrophenylarsonic acid (4-NPAA), *p*-ureidophenylarsonic acid (*p*-UPAA) and benzenearsonic acid (Fig. 1). With the exception of the latter, these compounds are still in use today in Canada and the USA. Variation in the substituents on the aromatic ring results in differences in the growth-promoting and disease-controlling effects of the compounds. Thus, 3-NHPAA and *p*-ASA are approved as animal feed additives for both poultry and swine, whereas 4-NPAA and *p*-UPAA are approved only for controlling blackhead disease in turkey [3–5].

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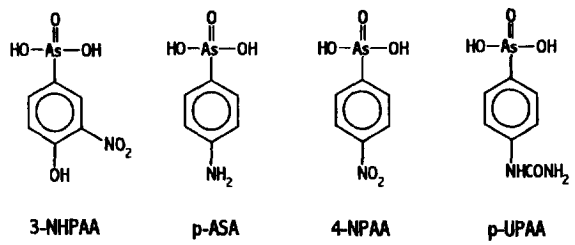

Fig. 1. Structures of arsenic compounds used as animal feed additives: 3-nitro-4-hydroxyphenylarsonic acid (3-NHPAA), *p*-arsanilic acid (*p*-ASA), 4-nitrophenylarsonic acid (4-NPAA), *p*-ureidophenylarsonic acid (*p*-UPAA).

However, recent studies have disputed the beneficial effects of these compounds as growth promoters [6,7]. 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 [8,9]. Nevertheless, the absence of microscopic changes in tissues from animals whose diets had been supplemented with these compounds within the allowable levels has been demonstrated [6,7].

The work reported here is primarily concerned with the development of analytical methods capable of separating and determining organoarsenicals used in animal feeds, as well as identifying their potential metabolites. New analytical methods are needed to investigate the interactions and fate of these compounds in the environment and food chain, particularly in view of their current usage. Presently there is very little information available on this subject.

So far only a number of methods have been developed for the determination of this class of compounds, most of these, however, are for target analysis of a few specific compounds and are not suitable for the detection of possible metabolites. For example, gas chromatography–flame ionization detection (GC–FID), which has been used for the determination of *p*-UPAA and *p*-ASA, is not suitable for the determination of 3-NHPAA, and is also interference prone [10]. A spectrophotometric method for the determination of *p*-UPAA in Carbasone has also been reported [11]. Thin-layer chromatography (TLC) has been used for the separation and identification of 3-NHPAA, *p*-ASA, 4-NPAA and *p*-UPAA; colouring reagents were used for visualization [12]. High-performance liquid chromatography (HPLC) has also been used to separate some arylarsenicals, but not specifically the ones used as animal feed additives [13–15]. Maruo et al. [13] used a 0.5 mm I.D. column packed with an anion exchange stationary phase to separate three arsenicals, *p*-ASA and two other compounds that are structurally similar to the arsenic animal feed additives: *o*-arsanilic acid and *o*-nitrophenylarsonic acid. The resulting ultraviolet (UV) chromatogram showed a separation that required over 40 min and resulted in broad peaks with baseline widths between 5 and 10 min. Others have attempted to separate the same compounds on columns similar to those used by Maruo et al. They employed non-suppressed ion chromatography; this resulted in co-elution of *o*-arsanilic acid and *o*-nitrophenylarsonic acid [14]. Recently a liquid chromatographic method was presented for the detection and quantitation of Roxarsone (3-NHPAA) in poultry feed. The drug is extracted with a phosphate buffer and is determined using solid-phase extraction in combination with reversed-phase liquid chromatography and UV detection [16]. HPLC coupled on-line to inductively coupled plasma mass spectrometry (ICP-MS) has been used recently for the determination of Roxarsone [17,18] as well as other arsenic animal feed additive compounds [17].

The four compounds used commercially as feed additives are non-volatile. Thus, in this work HPLC was selected as probably being the most efficient method for achieving their separation. Micro-HPLC separation was also evaluated and compared to results obtained on conventional and microbore-HPLC systems. The micro-LC columns used in this work were constructed and packed in our laboratory as part of continuing studies on the application of this technique, which appears to have many advantages [19]. UV, electrothermal atomic absorbtion spectrometry (ET-AAS), thermospray mass spectrometry (TSP-MS), direct liquid introduction mass spectrometry (DLI-MS), liquid secondary ion mass spectrometry (LSI-MS) and dynamic or continuous flow LSI-MS (CF-LSI-MS) were used as detectors

for the liquid chromatography separations, both in on-line and off-line modes.

2. Experimental

2.1. Instrumentation

Two different types of LC pump were used in these experiments. A Waters 510 reciprocating pump was employed for conventional HPLC columns. A dual syringe pump (Applied Biosystems, Model 140B) was used to provide the appropriate mobile phase gradient and flow-rate for the microbore and micro columns. This system consists of two independently driven syringe pumps with a volume of 10 ml each. A 200 μl mixer, used to form gradients, was part of the system. This pump is capable of delivering pulse-free solvent flows at rates as low as 1 μl/min. Pulse-free mobile phase flows are extremely advantageous for obtaining stable ion beams for the CF-LSI-MS detector.

Samples were introduced into the conventional column via a 20 μl stainless steel loop injector (Rheodyne 7125). This dual-mode injector was operated in the complete-filling mode, in order to improve accuracy and precision of the sample volume loaded. A 0.5 μl internal-chamber micro injector (Rheodyne 7520) was used for loading samples onto microbore and micro columns.

The conventional HPLC column used was a C_{18} Spheri-5 RP-18 (Brownlee Labs.) of 100 mm×4.6 mm I.D., containing 5 μm diameter packing material.

Microbore separations were achieved on a C_{18} Spheri-5 RP-18 (BrownLee Labs.), 250 mm×1 mm I.D., containing 5 μm diameter packing material.

Micro columns in a variety of lengths (80, 145, 200 mm) and 0.32 mm I.D. were fabricated in-house and packed with C_{18} packing material (Spherisorb ODS2, 3 and 5 μm diameter). For initial evaluation of the LC columns and mobile phases a UV detector (Waters, Lambda-Max Model 481) equipped with a 1 μl flow cell was used to monitor the separations, and allow for optimization of experimental conditions.

A Kratos Concept II H mass spectrometer was used for the LSI-MS and CF-LSI-MS studies. The DLI-MS experiments were carried out on a Nermag R10-10C quadrupole mass analyzer equipped with a desolvation chamber and a cryopump mounted on top of the source housing, in addition to the standard oil diffusion pumps. A Kratos MS 80 mass spectrometer was used for the TSP-MS analyses.

An Atomic Absorption Spectrometer (Varian Techtron Model AA 1275) equipped with an arsenic hollow cathode lamp (Spectra AA) operating at 8 mA, and a deuterium background corrector were used for the detection of arsenic in the LC fractions. The 193.7 nm arsenic resonance line was selected and used with a 1 nm bandwidth. The spectrometer was equipped with a GTA-95 (Varian) graphite furnace; pyrolytically coated graphite partioned tubes and argon purge gas were also employed.

In order to perform on-line micro-LC–MS experiments three interfaces were evaluated, a CF-LSI-MS probe and two different DLI couplings.

The CF-LSI-MS probe located inside the high-vacuum ion source chamber delivers the mobile phase via a quartz capillary tube to a mesh screen at the tip of the probe. The quartz capillary is in firm contact with the mesh, thus allowing the liquid to spread over the mesh by vacuum and capillary actions. A porous steel body in contact with the mesh acts as an absorbent for the excess solvent, and, thus, prevents potential peak broadening caused by the probe tip. The latter is in contact with the heated ion source block allowing for efficient heat transfer to the mobile phase, thus leading to its stable rate of evaporation. This feature is critical for maintaining a stable ion source pressure and ion currents.

Two DLI interfaces were used in these experiments. One was equipped with a nickel diaphragm having a 2–5 μm orifice. A liquid jet was formed by forcing the mobile phase to pass through this orifice. The probe was inserted into a heated desolvation chamber attached to the ion source. This allowed for the subsequent desolvation of the liquid jet droplets. The other DLI interface was constructed in-house. This interface is similar to a thermospray nebulizer, the main difference being its smaller dimensions. A quartz capillary (10 μm I.D.) is heated, thus converting the liquid mobile phase into a vapour jet, which

is introduced into the mass spectrometer ion source. For both DLI interfaces an electron beam was used to generate ions for subsequent mass analysis.

2.2. Chemicals

Stock solutions of 1000 mg/kg (As) of each of the following organoarsenicals were prepared in deionized water: 3-nitro-4-hydroxyphenylarsonic acid (ICN Biochemicals, Cleveland, OH, USA), *p*-arsanilic acid (Eastman Organic Chemicals, Rochester, NY, USA), 4-nitrophenylarsonic acid (Aldrich, Milwaukee, WI, USA) and 4-hydroxyphenylarsonic acid (Eastman Kodak, Rochester, NY, USA).

All solvents (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA) were filtered and degassed by using a 35 mm all-glass filter holder fitted with an HA 0.45 μm filter (Millipore, Bedford, MA, USA) for the aqueous solvents and a FH 0.5 μm filter (Millipore) for the organic solvents.

2.3. Preparation of micro-HPLC columns

A detailed description of column fabrication and packing is given in [20]. The following materials were used: 0.32 mm I.D. fused-silica (HP-ULTRA 2) for the column body and 0.050 mm I.D. fused-silica (Polymicro Technologies, Phoenix, AZ, USA) for coupling the micro-HPLC column to the detector. Columns were packed with silica-based C_{18} packing material (Spherisorb 3 or 5 μm ODS2). No noticeable difference in the chromatographic resolution achieved was observed for these two different particle sizes. The slurry packing procedure was evaluated in both the upward and downward directions, both methods produced columns with similar performance characteristics.

3. Results and discussion

Several combinations of HPLC techniques and detectors were evaluated for the determination of arsenic animal feed additives. Combinations of separation and detection techniques were selected in such a way that void volume would be minimized, for instance microscale LC coupled with small volume detectors.

3.1. Separation and determination of arylarsenicals by using HPLC–thermospray MS

Separation of a mixture of 3-NHPAA, *p*-ASA and 4-NPAA on a conventional C_{18} column (100 mm× 4.6 mm I.D., 5 μm particle size) was monitored by using UV detection. Optimization of this chromatographic separation was carried out by varying the methanol content of the mobile phase and its flow-rate. The resolution, R_s, obtained from each experiment for 3-NHPAA and 4-NPAA, along with two representative chromatograms, are presented in Fig. 2.

Thermospray MS was also used for the detection

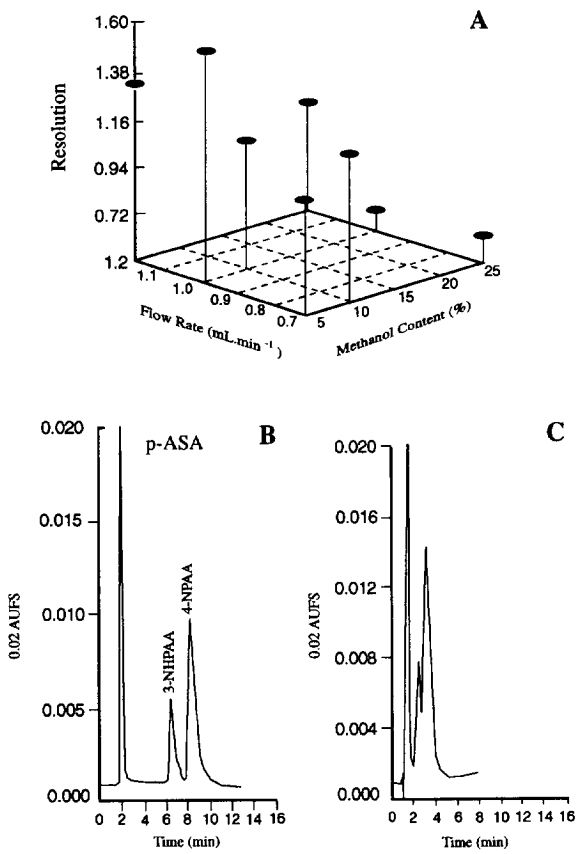

Fig. 2. (A) Three-dimensional graph showing the variation of resolution between 3-NHPAA and 4-NPAA when the methanol content of the mobile phase and the flow-rate are varied. (B) UV (λ=254 nm) chromatogram of arylarsenicals (22.5 ng As of each compound), 1 ml/min flow-rate, water (0.1% TFA)–methanol (95:5). (C) UV chromatogram (λ=254 nm) of arylarsenicals, 1 ml/min flow-rate, water (0.1% TFA)–methanol (75:25).

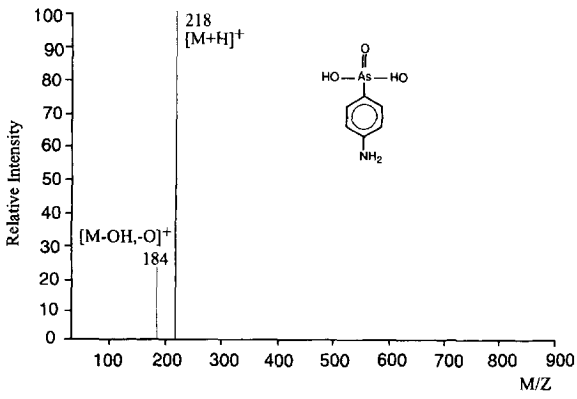

Fig. 3. Thermospray mass spectrum of *p*-arsanilic acid. Temperature settings: probe 129°C, vaporizer 185°C, source block 223°C and jet 120°C. Mobile phase as in Fig. 2B.

of these compounds. The protonated molecule was the base peak in the mass spectra of all samples analyzed. Fig. 3 shows the TSP mass spectrum of *p*-ASA, which is typical for all the arylarsenicals analyzed. TSP-MS provided little information concerning the analyte structure, but the molecular mass of the analyte could be determined. In addition, a discharge can be generated in the source of some TSP-MS systems, leading to some fragmentation. Collisional-induced dissociation (CID) can also be used to generate characteristic fragment ions if a MS–MS series is available. For our study only the TSP-MS was used.

All of the arsenicals studied here, the animal feed additives and a possible metabolite (4-hydroxyphenylarsonic acid) of 3-NHPAA, gave similar responses in terms of total ion current signal for the same amount of compound, when using the same set of TSP temperatures. Calibration curves for *p*-ASA and 4-NPAA, for instance, exhibit slightly different slopes, indicating that the sensitivities for the two compounds vary slightly. This variation, however, is not great enough to prevent us from believing that structurally related arylarsenicals will in fact be detected under similar TSP-MS conditions as used here. The linearity of the calibration curves supports the possibility of quantitation within a limited dynamic range. Clearly, the use of a suitable internal standard would greatly improve the precision of the method. The limit of detection (LOD) for these compounds in TSP-MS was calculated to be between 30 and 33 ng (1.5–1.6 ppm) of arsenic, depending on the compound analyzed. The calculated LOD is based on the signal being three times the intensity of baseline noise.

The results obtained here indicate that TSP-MS is a useful technique for the determination of arsenical animal feed additives. The non-volatile nature of the compounds has made their detection quite difficult when using other conventional MS techniques, such as desorption chemical ionization (DCI) [21]. This is due to the fact that the compounds pyrolyze in the ion source prior to their desorption, affording mass spectra that lack molecular mass information.

TSP-MS operation generally requires solvent flow-rates of 1–1.5 ml/min, clearly compatible with conventional analytical HPLC columns (3–5 mm I.D.). The successful coupling of microbore and micro-HPLC with TSP-MS is only possible if a make-up solvent is added post-column to generate a total liquid flow of 1–1.5 ml/min. However, this would worsen the LOD obtained when using microscale HPLC, possibly approaching values indicated above for the conventional column-TSP coupling.

3.2. Separation of arylarsenicals on a microbore C_{18} HPLC column

The first step towards the miniaturization of HPLC systems was the development of microbore LC columns [22,23]. We found that the arylarsenicals can be separated by using a microbore column (Spheri-5 C_{18}, 250 mm×1 mm I.D.) at a flow-rate of 80 μl/min (Fig. 4). The methanol content of the mobile phase has a pronounced effect on the resolution, as noted previously (Fig. 2A). Higher methanol percentage decreased the retention time of *p*-ASA and 4-NPAA as well as the overall resolution. Good resolution was achieved by using 10–15% methanol. The separation efficiency of this column is quite similar to that obtained on the conventional HPLC column (Fig. 2B). However, because of the reduced sample dilution on the microbore column, the LOD for *p*-ASA is 0.22 ng of arsenic, compared to 1.9 ng of arsenic on the conventional column using the same UV detector (λ=254 nm). Another advantage we observed is the considerable reduction

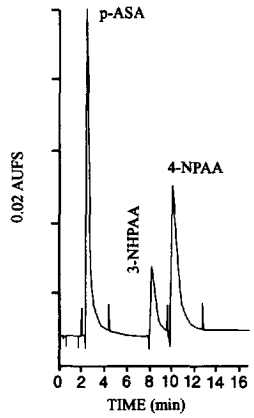

Fig. 4. UV (λ=254 nm) chromatogram of arylarsenicals (2.5 ng As of each compound); microbore C_{18} column, 80 μl/min flow-rate, water (0.1% TFA)–methanol (85:15).

in solvent consumption, leading to economic and environmental benefits.

3.3. Separation of arylarsenicals on micro C_{18} HPLC columns

The elution behaviour of the arylarsenicals on the micro-LC columns (Fig. 5) are similar to those observed on the conventional (Fig. 2) and microbore (Fig. 4) columns, both in terms of the overall time required for the separation and the efficiency of separation. Column length had a profound effect on the separation efficiency of the micro-LC column. As expected, the longest column (200 mm) provided the best resolution; however, the 145 mm long column also exhibited good resolution.

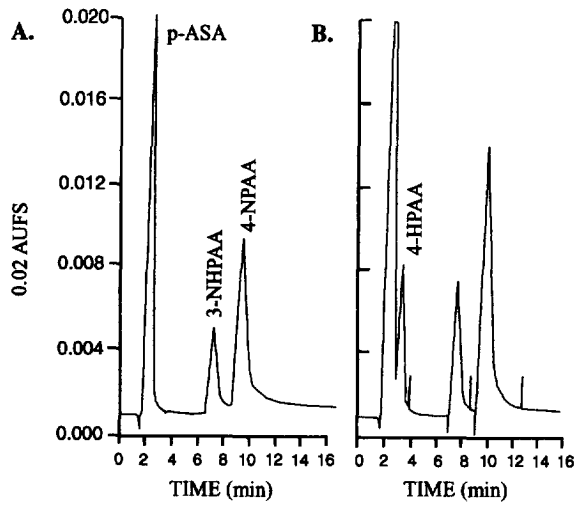

Fig. 5. UV (λ=254 nm) chromatogram of arylarsenicals (0.8 ng As of each compound); micro-HPLC column, 10 μl/min flow-rate, water (0.1% TFA)–methanol (85:15). (A) Compounds separated: *p*-ASA, 3-NHPAA and 4-NPAA. (B) 4-hydroxyphenylarsonic acid added to the analyte mixture of (A).

The LODs with UV detection improved by one and two orders of magnitude, respectively, over those obtained by using microbore and conventional HPLC columns. The LOD for *p*-ASA was calculated to be 0.02 ng of arsenic.

In order to evaluate the reproducibility of the retention times, seven repetitive separations were carried out using the micro-LC columns. The relative standard deviation of the retention times was found to be 0.9% for *p*-ASA, 1.2% for 4-HPAA, 1.6% for 3-NHPAA and 1.4% for 4-NPAA. These results demonstrate that the micro-HPLC columns, fabricated and packed in this study, can be used for the identification of arsenicals based on their retention times.

4-HPAA could be regarded as a possible metabolite of 3-NHPAA. Fig. 5B shows that it is quite well resolved from its potential precursor. 4-HPAA could also serve as an internal standard for quantitation of the other arsonic acids.

In terms of solvent consumption, a typical arsenical separation requires 0.14 ml, 1.12 ml and 14 ml of solvent on micro, microbore and conventional LC columns, respectively. This means that a 99% reduction in the volume of solvent is achieved by switching from conventional to micro-LC, and a 87.5% reduction when switching from microbore to micro-LC. These percentages can translate into long-term savings, both in terms of solvent consumption and solvent waste disposal.

3.4. Effect of temperature on the elution characteristics of the arylarsenicals

It has been shown that elevated LC column temperatures can improve separation of certain compound classes. Hansen et al. [24] have demonstrated enhanced separation efficiencies at 50°C for various inorganic and methylated arsenicals.

The temperature of the micro columns can be easily varied by immersing the column in a water

bath. Separations were carried out over a range of temperatures from 18°C to 57°C; the effect of temperature change is quite pronounced. At elevated column temperatures retention times and resolution are dramatically reduced (Fig. 6). This can be explained by the fact that temperature increases reduce mobile phase viscosity and increase diffusion rates, thereby changing mass transfer between mobile and stationary phases.

3.4.1. Detectors used for microscale HPLC

Because UV detectors are not very selective and only provide limited structural information about compounds eluting off HPLC columns, it is necessary to consider other more specific types of detectors for arylarsenicals. In this work we have evaluated a number of detectors based on their ability to provide structural and/or elemental information for the arsenicals used as animal feed additives. For example, MS techniques such as CF-LSI-MS and DLI can provide structural information, while element-specific detectors such as ET-AAS can be very useful for selective detection of arsenic. All of these detectors have the potential to be used either off-line or on-line.

3.5. ET-AAS as a detector for microscale-HPLC

ET-AAS was used in the off-line mode for the detection of arsenic compounds (*p*-ASA, 3-NHPAA, 4-NPAA) eluting off LC columns. To date there has not been a satisfactory LC interface for ET-AAS, which has received general acceptance [25]. Thus, in most applications, fractions of the LC eluent are collected and subsequently analyzed by ET-AAS. This method has been used extensively in conjunction with conventional HPLC columns [26], the main disadvantage being the low sensitivity of the method due to dilution in the LC column volume. In addition, the resolution achieved on the column is degraded. Both of these features make it very difficult to detect minor arsenic components in environmental samples. In this investigation we have compared the chromatograms obtained by using conventional, microbore and micro-HPLC coupled off-line to an ET-AAS detector.

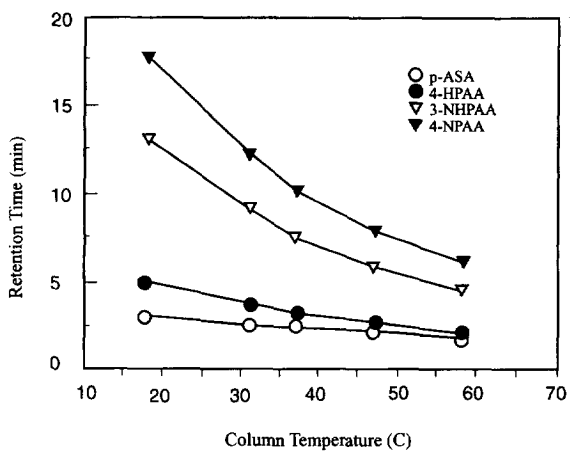

Fig. 6. Effect of temperature on the arylarsenical retention times.

Fractions were collected every 30 s from all three different column types. This procedure resulted in fraction volumes of 500 μl for conventional columns, 40 μl for microbore and 5 μl for micro columns. The resulting ET-AAS chromatograms are shown in Fig. 7.

The LOD for *p*-ASA on the three chromatographic systems was calculated to be 250 ng of arsenic for the conventional column, 2.5 ng of arsenic for the microbore column and 0.3 ng of arsenic for the micro column. Because the ET-AAS detector is mass dependent, rather than concentration dependent, an improvement in absolute LOD for arsenic on the micro column was observed. This is expected because all of the micro-LC eluent is injected into the graphite furnace, while only 2% and 37.5% of the eluent from the conventional and the microbore column are injected, respectively.

The micro column has the disadvantage that the eluent volume of each fraction is only sufficient for a single analysis, instead of two or three as is the case with the other two LC systems. This translates into potential problems in the accuracy and precision of the analysis. Of all three LC systems used, the microbore column seems to be the most suited for this application because it has a good LOD, and also allows for replicate determinations of each fraction collected.

3.6. Liquid secondary ion mass spectrometry

Fast atom bombardment (FAB-MS) has been recognized as a powerful tool in the mass spectral

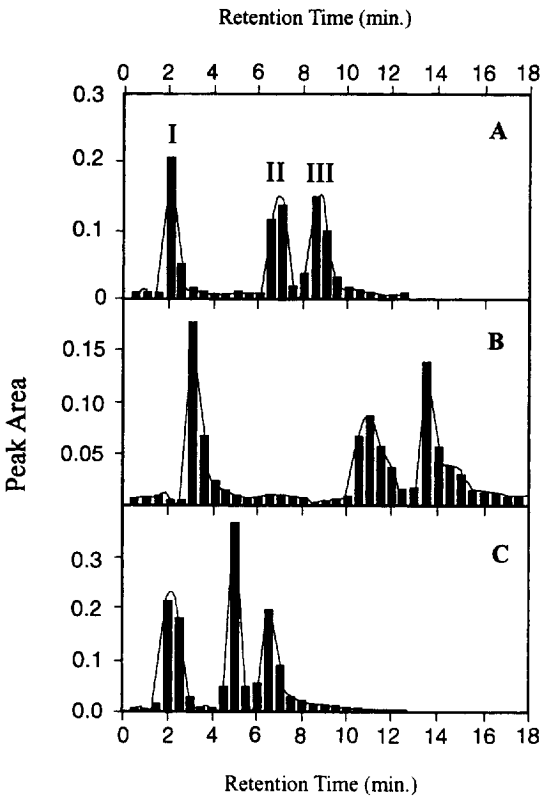

Fig. 7. ET-AAS chromatograms of arsenicals, separated on: (A) conventional-LC column, 500 μl fractions collected; (B) microbore-LC column, 40 μl fractions collected; (C) micro-LC column, 5 μl fractions collected. I=*p*-ASA, II=3-NHPAA, III=4-NPAA.

analysis of polar, thermally labile and/or involatile compounds. The operating principle of the method has been described in detail by Barber et al. [27]. In brief, the analyte is dissolved in a viscous matrix of low volatility such as glycerol, and is subsequently introduced into the ion source of the mass spectrometer, where it is bombarded with a beam of high energy (6–8 keV) neutral (>80%) species (Ar, Xe). A similar technique, liquid secondary ion mass spectrometry (LSI-MS) uses high energy Cs^+ ions (≥8 keV), instead of neutrals. Both methods yield similar results, although some differences can result, since the ion beam is generally more focused as compared to the neutral beam, is of higher energy and usually has a higher density of particles. Thus, in general, LSI-MS tends to be the more sensitive method. Spectra obtained in FAB and LSI-MS are affected by the nature of the matrix [28] and in some cases strong matrix–analyte interactions exist in the solution [29].

In this work we have utilized both LSI-MS and CF-LSI-MS to analyze the animal feed arsenicals. LSI-MS has the potential to be used as an off-line LC detector, while CF-LSI-MS can be used on-line.

In LSI-MS a variety of different matrices, 3-nitrobenzylalcohol (NBA), glycerol and thioglycerol, were investigated for their ability to promote ionization of the arsenicals. The mass spectra of 3-NHPAA, in both negative and positive ion detection mode are presented in Fig. 8; the matrix was NBA. The main feature of the positive ion spectrum is the formation of protonated molecules and adduct ions of the matrix and the analyte.

In negative ion mode, a triple pattern (de-protonated molecules and two de-protonated adducts of matrix and analyte) observed in the spectrum provides molecular mass information concerning the analyte. The general features of the mass spectra of all the arylarsenicals are similar to those observed for 3-NHPAA.

The use of CF-LSI-MS to detect the arsenicals is illustrated in the positive ion mass spectrum obtained for *p*-ASA (Fig. 9). In addition to the protonated molecules and matrix adducts, two characteristic fragment ions are also observed (*m/z* 201, 184). The mobile phase, used to deliver the analyte at a flow-rate of 5 μl/min to the ion source, consisted of 89% H_2O (0.1% TFA), 10% CH_3OH, and 1% glycerol. These conditions were selected because they satisfied both the requirements for the micro-LC column separation of the arsenicals as well as for the LSI-MS. The latter requires the presence of a matrix-substance, such as glycerol. Micro-HPLC chromatography of the arsenicals under these mobile phase conditions produced UV chromatograms which showed few effects associated with the presence of 1% glycerol. However, possible problems could be avoided by introducing the matrix solution after the LC separation has taken place (post-column mode of introduction).

The concentration of glycerol matrix is much lower than in the conventional standard probe LSI-MS (static) conditions, thus considerably reducing the signal from matrix ions (chemical noise). Another

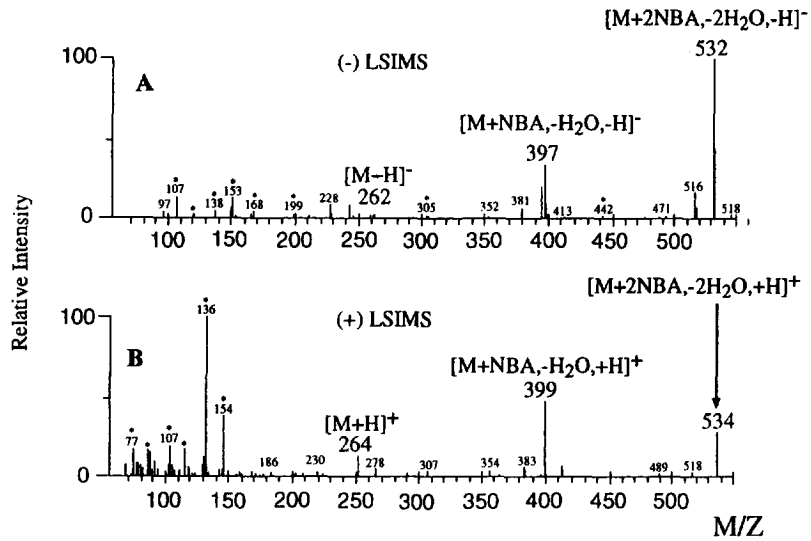

Fig. 8. LSI-MS mass spectra of 3-NHPAA. (A) Negative ion detection. (B) Positive ion detection. *=NBA matrix ions.

advantage of the CF-LSI-MS method is that with time the spectrum extracted from the total ion chromatogram changes through the cycle: pure matrix to sample plus matrix to pure matrix. This permits background subtraction, providing much cleaner mass spectra. The *p*-ASA spectrum shown in Fig. 9 has been cleaned up in this way. The LOD of *p*-ASA was determined to be approximately 10 ng of arsenic under the experimental conditions used in this study.

3.7. Direct liquid introduction-mass spectrometry

DLI-MS is another MS method with potential for coupling on-line with micro-LC. Two different nebulizers were used in this study. A heated capillary

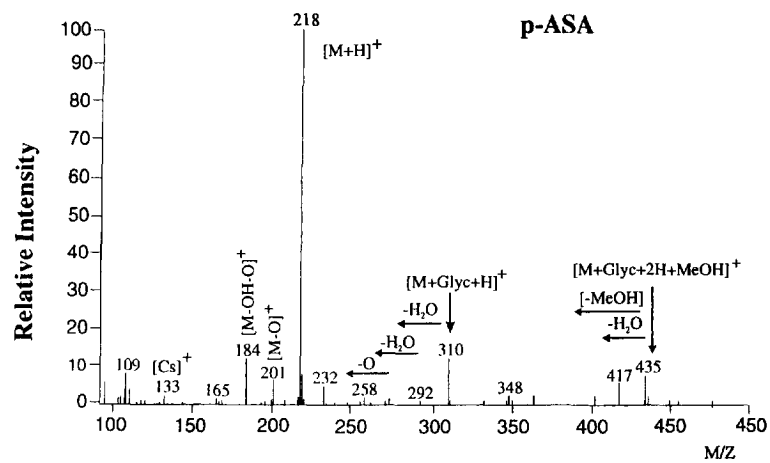

Fig. 9. Background subtracted CF-LSI-MS mass spectrum of *p*-ASA; mobile phase: water (0.1% TFA)–methanol–glycerol (Glyc) (89:10:1); flow-rate: 5 μl/min.

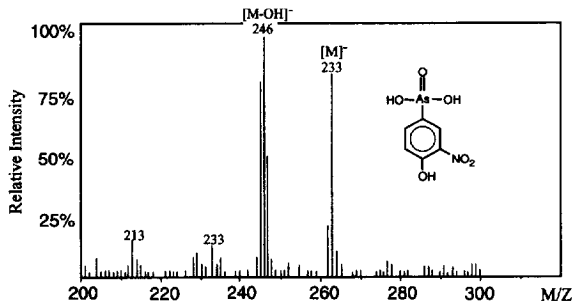

Fig. 10. DLI-MS of 3-NHPAA; mobile phase consisted of water (0.1% TFA)–methanol (90:10), 20 μl/min flow-rate, negative ion detection.

nebulizer was built in-house, while a diaphragm nebulizer with an associated desolvation chamber was obtained commercially (Delsi-Nermag).

When using the diaphragm nebulizer for the introduction of arsenicals, useful mass spectra could only be obtained in the negative ion mode. The main disadvantage of the method was the requirement for relatively large amounts of analyte, compared to the other methods discussed earlier. Spectra were obtained with approximately 1000 ng of arsenic, in the negative ion detection mode. As can be seen from the mass spectrum of 3-NHPAA (Fig. 10), extensive fragmentation has occurred and the molecular ion is not the base peak as was the case with most of the other MS methods discussed before. This is in accordance with our previous observations that these arylarsenicals are thermally labile and decompose/pyrolize upon extensive heating, in this case initiated by elevated temperatures of the desolvation chamber. Further attempts to optimize the desolvation chamber temperature did not reduce the observed decomposition.

The heated capillary interface was also tested. No meaningful spectra of the arsenicals were produced when using this nebulizer. Again, the absence of molecular mass information can be explained in terms of analyte decomposition, caused by extensive heating which is required in order to convert the CF-liquid into vapour. Organic test compounds, used for the initial set-up of this system, were analyzed successfully. These included caffeine, naphthalene and 2,6-dimethylphenol; spectra of these compounds consisted mainly of protonated molecules.

3.8. Comparison of methods used for arsenical detection

The performance characteristics of the various analytical methods developed in this study are compared. Of primary importance are their LODs, both in terms of concentration and absolute amounts of arsenic. Table 1 contains a summary of the LODs obtained for arsenic by using the various methods developed in this study. Even though micro-LC with UV detection provides the lowest LOD its lack of selectivity and information content limits its use for the analysis of 'real' samples. Only upon extensive

Table 1
Limits of detection (as arsenic) for arylarsenicals using different types of LC columns and detectors

Detection method	Column type	Flow (μl/min)	Concentration LOD (ng/μl)	Absolute LOD (ng)
Thermospray MS	Conventional	1000	1.5	30
UV	Conventional	1000	0.095	1.9
UV	Microbore	80	0.44	0.2
UV	Micro	10	0.04	0.02
ET-AAS (off-line)	Conventional	500/10[a]	12.5	250
ET-AAS (off-line)	Microbore	40/15[a]	5	2.5
ET-AAS (off-line)	Micro	5/5[a]	0.6	0.3
CF-LSI-MS	Micro	5	20	10
DLI-MS	Micro	20	1000	500

[a]Volume (μl) of fraction collected/Volume (μl) of fraction injected onto ET-AAS.

sample pre-treatment and availability of suitable standards can UV detection be useful. ET-AAS together with micro-LC is another very sensitive method; it is element selective and, on this basis, can be used for 'real' sample analyses. However, at present, only off-line coupling capabilities are available for ET-AAS. TSP-MS, even though it is not as sensitive, is quite useful because it provides molecular mass information regarding the arsenicals. CF-LSI-MS provides low absolute LODs as well as structural information on this class of compounds.

4. Conclusion

This work describes the development of analytical techniques which can be used for the determination of arsenic animal feed additives. More specifically, chromatographic methods have been developed for the separation of these arsenicals. In particular, micro-LC, which is a relatively new technique, shows a number of advantages for these analytes, especially when interfaced to MS. Improved limits of detection, low solvent consumption, lower use of stationary phase, as well as flow-rates easily accommodated by CF-LSI-MS and DLI-MS, are some of the advantages we have observed when fabricating and using these columns. Various mass spectrometric techniques were evaluated for the structural information they provided. Off-line coupling of micro-LC to ET-AAS not only improves the LOD for arsenic, compared to conventional column ET-AAS, but also offers improved selectivity over UV detection. These results open the possibility of on-line coupling, an area which requires extensive research before it becomes a reality.

Additional advancements can also be made in this area by coupling micro columns to MS–MS systems or alternative detectors such as ICP-MS. These aspects of micro-LC remain to be evaluated for their effectiveness in analysing not only arylarsenicals but also arsenic compounds encountered in the marine and terrestrial environment.

Further use of the methods developed here will allow for a more detailed understanding of the fate and interactions of arylarsenicals in biological and environmental systems.

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