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Measurement of Molecular Species of Arsenic and Tin Using Elemental and Molecular Dual Mode Analysis by Ionspray Mass Spectrometry

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Ionspray mass spectrometry has been employed for dual mode, molecular and elemental, detection and quantification of tributyltin and arsenobetaine. A selectable degree of ion–solvent declustering and molecular fragmentation in the ion source–mass spectrometer interface region permitted detection of fully intact molecular species, or fragments of the molceular species to any selected degree. Complete dissociation of the molecular species in the interface region enabled detection of the underlying element of interest. Tandem mass spectrometry (MS–MS) was used in the molecular detection mode to increase selectivity of the analysis. Instrumental parameter adjustment permitted dual mode detection for the same sample on subsequent injections. Dual mode detection using FI was performed to determine the tributyltin concentration in the National Research Council of Canada (NRCC) harbour sediment reference material PACS-1. Measured tributyltin concentrations were 1.24 ± 0.04 μg g^{-1} of Sn in the sediment (MS–MS mode) and 1.29 ± 0.04 μg g^{-1} of Sn in sediment (elemental mode), compared with the certified value of 1.27 ± 0.22 μg g^{-1} of Sn in sediment. The dual mode detection system was coupled to two forms of liquid chromatography, cation exchange and ion-pairing, to determine arsenobetaine concentration in the NRCC dogfish muscle reference material DORM-2, as part of the NRCC certification process. Results of the two detection modes with both chromatographic methods were consistent, and yielded a mean arsenobetaine concentration of 16.6 ± 0.6 μg g^{-1} of As in the material. Although the certification process is not complete, this result was consistent with an expected arsenobetaine level slightly higher than previously measured values of 15.7 ± 0.4 μg g^{-1} of As in for DORM-1, the NRCC predecessor reference material to DORM-2. Additionally, an intermediate mode of interface fragmentation coupled with MS–MS enabled the detailed mapping of possible collisionally induced dissociation channels for arsenobetaine.

Keywords: *Organotin; organoarsenic; tributyltin; arsenobetaine; speciation; ionspray mass spectrometry*

Recently in the literature there has been an increasing number of reports on the use of two atmospheric pressure ionization (API) techniques, electrospray (ES) and ionspray (IS), as ion sources for mass spectrometric analyses of monatomic and molecular elemental species.[1–16] Both ES and IS, the pneumatically assisted version of ES, are 'soft' ionization techniques which produce gas-phase analyte ions directly from pre-existing ions in solution. They have been employed extensively, often coupled with LC, for analyses of large organic moleclues such as proteins, peptides and other biomolecules by MS and tandem mass spectrometry (MS–MS). MS-MS, a molecular detection technique, facilitates elucidation of molecular structure through characteristic fragmentation patterns of a precursor molecule as a result of collision induced dissociation (CID). The ES and IS sources for mass spectrometric detection offer unique opportunities for analysis of molecular forms of elemental species that standard inorganic detection techniques do not provide. ES-MS(–MS) and IS-MS(–MS) are capable of performing as dual mode detectors: as elemental analyzers, as well as in their more typically utilized molecular modes. Such techniques find natural application in elemental speciation analysis. Since the toxicity, bioavailability and transport of various elements are dependent on the exact molecular chemical form of the element present in a sample or system, it is essential to determine the particular individual species present in the sample or system selectively.

This requirement of individual species determination has resulted in the coupling of element specific detectors with a variety of separation techniques, of which HPLC has been the most frequently employed. Separation techniques such as HPLC serve the purposes of separating the analytes of interest from the sample matrix, as well as separating the individual elemental species to be detected or measured. The most frequently utilized element specific detectors for HPLC separations have been AAS, ICP, AES and ICP-MS.[17] Although techniques such as HPLC–ICP-MS offer the advantages of high selectivity and sensitivity as well as low detection limits, species identification is based on chromatographic retention times compared with those of available standards. Since no molecular information is available from a standard elemental detector, identification of different species of the same element depends entirely on the separation technique. Species which co-elute require modification of the separation conditions and, again, comparison with separations of standards, often a tedious or impossible proposition for certain species. For real samples and the presence of complicated sample matrices, retention times of chromatographic peaks may shift compared with those of a mixture of standard substances, requiring determination of the proper chromatography and retention time matching for the particular matrix. Chromatographic peaks which are not identified by retention time matching against standard substances resist identification, and will continue to do so until the appropriate standard substances become available. For these reasons, and particularly since speciation analysis is becoming increasingly involved with larger relative molecular mass species of the elements of interest, a technique that combines molecular as well as elemental detection capabilities is required.

Triorganotins are utilized as fungicides, herbicides and insecticides owing to their toxicity to a variety of living organisms. The trialkyltin compounds are extremely toxic to a number of aquatic organisms and have been found to accumulate in sediments. Tributyltin {TBT, $[CH_3(CH_2)_3]_3Sn$} or Bu_3Sn leaches into the marine environment mainly through its effective use in antifoulant paints for ocean-going vessels. The risks associated with TBT have been formally recognized, but despite its ban or restriction as a component of antifoulants in a number of countries, TBT continues to enter the marine

environment in large amounts. Gas chromatography has been the most frequently used separation technique for speciation of organotin compounds, and is often coupled with one of the element specific detection methods discussed above, though these are not the most common GC detectors for these analyses. Derivatization procedures are used in conjuction with GC, but the derivatization process and resulting clean-up procedures involved are problematic and result in long analysis times.[18,19] HPLC has been coupled with AAS,[20] ICP-AES[21] and ICP-MS[22] for organotin speciation. However, these techniques involving HPLC depend entirely on the chromatographic separation and retention time matching against standards for species identification and offer no molecular or structural information for confirmation of detection of tin species. Hence, it would be advantageous to develop new speciation techniques for determination of organotin compounds. Organotin standard substances have been studied by IS-MS(–MS) and ES-MS[4,7,8,10,15] and quantification of TBT in the National Research Council of Canada (NRCC) sediment reference material PACS-1 was accomplished by IS-MS(–MS).[10] The analytical power of dual mode detection by IS-MS(–MS) is demonstrated in the present study by performance of quantification of TBT in this same reference material in both molecular and elemental modes of detection. This includes presentation of the first quantification of a tin species by elemental mode IS-MS, or ES-MS.

Arsenic occurs in the environment and in biological systems in a number of different organic and inorganic molecular forms or species. The quaternary arsonium compounds, arsenobetaine (AsB), arsenocholine (AsC) and the tetramethylarsonium ion $(CH_3)_4AS^+$ are essentially non-toxic. The formulae of the arsenic species of interest to this study are shown in Table 1. AsB has been the most extensively studied organoarsenic species owing to the high levels of this compound found in many edible fish, shellfish and seafood products. For arsenic speciation studies a variety of hyphenated analytical techniques, involving the coupling of separation techniques with element selective detectors, have been reported in the literature.[23,24] Soft ionization techniques, such as field desorption,[25–27] thermospray[28] and electron impact,[29] have been used for introduction of organoarsenicals for MS detection. Fast atom bombardment[27,30,31] and desorption chemical ionization[32] MS permitted probing of organoarsenic molecular structure by characteristic fragmentation induced in the ion source. Atmospheric pressure chemical ionization and ES-MS(–MS) have facilitated deeper elucidation of CID characteristic fragmentation patterns.[9] Dual mode, elemental and molecular, HPLC–IS-MS(–MS) has been used to study a number of organoarsenic species, including dimethylarsinyl-riboside derivatives (arsenosugars), as standards and in real samples.[14] That study presented the first coupling of HPLC with elemental mode IS-MS, or ES-MS, for analysis of standards and real samples. Quantification of AsB in the dogfish muscle reference material DORM-1 has been performed by HPLC–ES-MS(–MS).[11] The present study details the power of dual mode detection by demonstration of the coupling of IS-MS(–MS) to cation exchange and ion-pairing chromatography for molecular mode and elemental mode quantification of AsB in the NRCC reference material DORM-2, the successor to DORM-1. Presented are the first elemental mode IS-MS, or ES-MS, quantification results involving coupling with HPLC.

Table 1 Formulae of organoarsenic species

Compound	Formula
Dimethylarsinic acid (DMA)	$(CH_3)_2AsO(OH)$
Trimethylarsine oxide (TMAO)	$(CH_3)_3AsO$
Tetramethylarsonium ion (TMAs)	$(CH_3)_4As^+$
Arsenobetaine (AsB)	$(CH_3)_3As^+CH_2COO^-$
Arsenocholine (AsC)	$(CH_3)_3As^+CH_2CH_2OH$

EXPERIMENTAL

Chromatographic Systems

For arsenic speciation two chromatographic methods, ion-pairing and cation exchange, were employed. Ion-pairing chromatography was performed using a diisobutyl n-octadecylsilane based Zorbax SB-C_{18} analytical column (150×2.1 mm, 5 μm particle size). For arsenic standard substances the mobile phase consisted of 10 mmol l^{-1} octanesulfonate in a water–methanol (60+40) mixture adjusted at pH 2.7 with acetic acid, delivered isocratically at 200 μl min^{-1}. For analysis of DORM-2 dogfish muscle reference material the mobile phase was adjusted to a water–methanol (80+20) mixture and the flow rate was increased to 250 μl min^{-1}. For DORM-2 analyses an Applied Biosystems Aquapore C_{18} guard column (30×2.1 mm, 7 μm particle size) was also installed. Cation exchange chromatography was performed using a silica based Chrompack Ionospher C analytical column (100×3 mm, 5 μm particle size) with sulfonic acid functional groups. The mobile phase consisted of 15 mmol l^{-1} pyridinium formate in a water–methanol (80+20) mixture adjusted at pH 2.7 with formic acid,[14,33] delivered isocratically at 1 ml min^{-1}. For arsenic speciation studies the chromatographic mobile phases were delivered by a Perkin-Elmer Series 200 LC pump, while for tributyltin FI quantification experiments a Shimadzu LC-10AD HPLC pump was employed. The post-column effluent was split such that only 20 μl min^{-1} were pumped through the ionspray capillary. For arsenic speciation studies a Rheodyne 8125 low dead volume injector with a 5 μl (ion-pairing chromatography) or 20 μl (cation exchange chromatography) stainless steel sample loop was used. For tributyltin quantification a Rheodyne 7520 fixed loop injector with a 0.5 μl sample loop was employed with a carrier solution flow rate of 20 μl min^{-1}. For infusion experiments analyte solution was delivered by a Harvard Apparatus Model 22 syringe pump at 5 μl min^{-1}.

Ionspray Source

Previous in-house investigations of elemental cations and anions, as well as oxo-anions, indicated the possibility of memory effects in the ionspray source. Hence, for the present study and other concurrent elemental analysis studies, the ionspray source was modified to reduce possible memory effects owing to adsorption of analytes on the fused silica capillaries normally used to carry solution through the ion spray device. The use of Teflon and stainless steel solution transfer tubing in the ionspray source offerred no indication of adsorption of the analytes of interest in this study. A 0.1 cm i.d. stainless steel capillary carried solution from the splitter T and served as the needle probe. Coaxial to the solution carrying capillary was a 0.4 cm i.d. stainless steel tube that delivered the nebulizing gas at 0.95 l min^{-1} for molecular mode operation, and at 1.44 l min^{-1} for elemental mode analyses. The solution carrying capillary protruded approximately 2 mm from the nebulizer tube. The ionspray probe was oriented at a severely oblique angle to the curtain plate (or sampling plate) of the mass spectrometer enabling sampling of only the edge of the ionspray aerosol cone into the mass spectrometer, resulting in enhanced ionspray signal stability and sampling of less highly clustered analyte molecules. The two modes of analysis required different ionspray needle probe potentials: approximately 4.5 kV for molecular mode, and an increase of approximately 0.5 kV for elemental mode.

Mass Spectrometer

A triple quadrupole PE-SCIEX API 300 mass spectrometer (Fig. 1) was modified to permit elemental analysis as well as the standard molecular analysis performed with such an instrument. This system utilized a differentially pumped atmosphere-to-vacuum interface. Ions from the ionspray source were sampled into the mass spectrometer system through a dry nitrogen curtain gas between the curtain plate (or sampling plate) and the orifice. The pressure due to curtain gas flow in this region was minimally higher than atmospheric pressure. The curtain gas assisted in the declustering of ion–solvent clusters and the desolvation of ionspray droplets, as well as in preventing contaminants from entering the mass spectrometer. Ions underwent a free jet expansion through the orifice into a differentially pumped region maintained at a pressure of approximately 1 Torr (1 Torr = 133.3 Pa). The potential difference between the orifice and the electrically grounded skimmer is referred to as the declustering potential. Although a number of source and interface parameters were adjusted to select a particular mode of operation, the curtain gas flow and the declustering potential were the most critical. Upon passing through the skimmer, ions entered an rf-only quadrupole (Q_0) maintained at ≈ 5 mT. In the Q_0 region, collisional focusing caused energetic cooling of the ions and forced them onto the axis of the mass spectrometer system,[34] resulting in increased sensitivity.

There were two forms of molecular mode operation: detection of fully intact molecular species and detection of collisionally induced fragments *via* MS–MS. In fully intact molecular mode, source and interface parameters were adjusted such that solvent clusters were stripped from the molecules of interest, yet the molecules were not fragmented. In this mode, the instrument was operated as a single quadrupole device with only the first quadrupole (Q_1) performing mass analysis for the purpose of monitoring particular ion masses as a function of time [single ion monitoring (SIM)], or to scan the full mass range. The second and third quadrupoles (Q_2 and Q_3, respectively) were operated in rf-only mode, resulting in transmission, but not mass analysis, of ions. For MS–MS analyses the same source and interface parameters were employed as for fully intact molecular mode. In this mode, mass analysis was performed in both Q_1 and Q_3, while the enclosed rf-only Q_2 quadrupole was filled with nitrogen collision gas to create a high pressure collision cell.[35] Q_1 was operated to allow transmission of only masses corresponding to particular molecules of interest, the precursor ions. These precursor ions impinged upon the collision gas in Q_2 with a collision energy given by the dc offset potential difference between Q_1 and Q_2, multiplied by the charge of the precursor ion exiting Q_1. CID of the precusor ion occurred, resulting in characteristic fragment or product ions for that specific collision energy. The third quadrupole (Q_3) was operated as a mass analyzer for the purpose of monitoring specific fragment ions as a function of time [single or multiple reaction monitoring (SRM or MRM, respectively)], or to scan the full mass range. In elemental mode, source and interface parameters were adjusted such that not only were solvent clusters stripped from the molecules of interest, but the molecules were fragmented or dissociated to their underlying elements of interest. As in fully intact molecular mode, the instrument was operated as a single quadrupole device with only the first quadrupole (Q_1) performing mass analysis for the purpose of monitoring particular ion masses as a function of time, or to scan the full mass range.

The mass spectrometer was usually operated such that in full scan mode the valleys between peaks differing by 1 Da were less than 10% of the more intense peak. Full widths at half height were 0.6 Da. Full scan spectra were obtained using 0.1 Da steps with 5 ms dwell times. SIM and MRM chromatograms were acquired with 250 ms dwell times. For TBT determination, SIM and MRM experiments involved setting Q_1 for selective transmission of m/z 120 (elemental mode) or m/z 291 (molecular modes), corresponding to the most abundant isotope of tin.

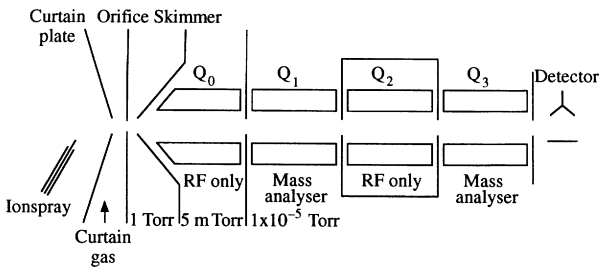

Fig. 1 Schematic diagram of the ionspray tandem mass spectrometer system.

Chemicals

Tributyltin chloride was obtained from Aldrich Chemicals (96% purity, Milwaukee, WI, USA). This substance was dissolved in distilled, de-ionized water to form a 10^{-2} mol l^{-1} stock solution of TBT (as the molecule). Distilled, de-ionized water was purified in-house with a Waters Milli-Q purification system (Millipore, Bedford, MD, USA). The aqueous standard substances used to prepare arsenic standard solutions have been described previously.[14,33] The species represented were As^V, MMA (monomethylarsonic acid), DMA, TMAs, AsB and AsC. Aqueous mixtures of these standards were prepared at levels of 1 μg ml^{-1} or 200 ng ml^{-1} each (as species concentration) for HPLC–IS-MS(–MS) experiments. For infusion experiments the individual arsenic and tin standard substances were prepared at the 1 μg ml^{-1} level in water–methanol (50 + 50) with 20 μl of 0.1 mol l^{-1} HCl added for each 5 ml of solution (to aid signal stability). HPLC grade chemicals were purchased from Aldrich or Fisher Scientific (Nepean, Ontario, Canada). The harbour sediment reference material, PACS-1, and the dogfish muscle reference material, DORM-2, were obtained from the NRCC (Ottawa, Ontario, Canada).

Sample Preparation

Extraction of TBT from the sediment material followed the butan-1-ol method given by Siu *et al.*[10] Briefly, 4 g of PACS-1 were placed in each of three 50 ml glass centrifuge tubes. Employing the method of standard additions, appropriate spikes were added to each tube as well as 8 ml of butan-1-ol. The mixtures were placed in an ultrasonic bath for 1 h, and then centrifuged at 2000 rev min^{-1} for 10 min. The butan-1-ol phase was removed from each centrifuge tube and diluted to 25 ml with methanol containing 1 mmol l^{-1} ammonium acetate, the FI carrier solution.

Extraction of arsenic species from the DORM-2 reference material proceeded *via* the method employed by Beauchemin *et al.*[29] for DORM-1. Briefly, 2 g of DORM-2 were placed in each of three 50 ml glass centrifuge tubes. Employing the method of standard additions, appropriate spikes were added to each tube as well as methanol (20 ml) and chloroform (10 ml) . The mixtures were placed in an ultrasonic bath for 30 min, and then centrifuged at 2000 rev min^{-1} for 10 min. The orange methanol–chloroform liquid containing the extract was removed from each tube and placed in 125 ml separatory funnels. The entire process was repeated, and the two extracts for each spike level combined in separatory funnels. Water (20 ml) and chloroform (20 ml) were added to the separatory funnels, which were then shaken vigorously and allowed to

stand while phase separation occurred. The lower chloroform phases contained little arsenic and hence were drained and set aside. The upper water–methanol phases were drained and allowed to evaporate overnight with the assistance of a dry nitrogen gas flow. The yellow–brown residues of extraction were dissolved in 1 ml of water and sonicated for 10 min. Sample clean-up was performed to reduce the level of hydrophobic biogenic substances in the samples and therefore reduce matrix effects for ionspray sample introduction. This was accomplished through use of a Varian Mega Bond Elut C_{18} solid phase extraction (SPE) cartridge. Through this process the redissolved 1 ml of extract was diluted to 10 ml in water.

RESULTS AND DISCUSSION

Dual Mode Detection of TBT

The ionspray or electrospray mass spectra of inorganic and low relative molecular mass organometallic cations generally contain high degrees of ion–solvent clustering which may dominate the spectra. These clusters and their relationship to the ionic species in solution have been studied in detail by a number of groups.[36–41] Results have been presented which detail source–interface fragmentation of inorganic cations[1–7,12,13,16] or low relative molecular mass organometallic cations[4,7,8,10,14–16] to reveal the underlying element of interest in a bare or slightly clustered elemental form. A number of these studies presented relatively clean full scan spectra which were dominated by singly charged, completely declustered elemental ions, regardless of the charge of the analyte in solution.[1,3,7,12–16] One of the most important factors in obtaining such spectra was the use of an elevated declustering potential, which produced more energetic collisions between ion–adduct molecules and the curtain gas in the atmosphere-to-vacuum interface of the mass spectrometer system. Kebarle and co-workers[36–39] have explained the observation of singly charged metals as being due to gas phase charge reduction. Another factor critical to obtaining complete dissociation to the underlying element of interest is an elevated curtain gas flow. Such a curtain gas flow assists in the declustering of ion–adduct molecules and the desolvation of ionspray droplets, permitting optimized utilization of the declustering potential for fragmentation or dissociation of molecules. This mode of ionization was applied for elemental mode analysis in the present study.

Three modes of detection (fully intact molecular, MS–MS and elemental) are demonstrated in Fig. 2 for infusion of a 1 μg ml^{-1} (as Sn) standard solution of tributyltin (TBT). The full scan, fully intact molecular mode detection of the TBT^+ ion, $[CH_3(CH_2)_3]_3Sn^+$ or Bu_3Sn^+, at *m/z* 291 is shown in Fig. 2(*a*). A declustering potential of 25 V provided sufficiently energetic collisions in the differentially pumped region to eliminate TBT–adduct clusters, yet resulted in no fragmentation of the TBT molecular ion. For most organometallic species, observation of the uncomplexed, yet unfragmented, molecule occurs only for a very narrow range of declustering potentials and interface parameters. Attaining detection in this manner is desirable for analytical purposes since the TBT signal is not diluted over a range of clusters or fragments. Optimization of the fully intact molecular signal included adjustment of the declustering potential, the curtain gas flow, the ionspray potential and nebulizer gas flow, and the ion optics up to Q_1. The background signal intensity across the full scan mass range is significant owing to the soft mode of detection used. While it is possible to optimize the system for maximum TBT^+ signal, it is not possible to obtain selective fragmentation of other molecules simultaneously, which therefore contribute to the background mass spectrum.

The MS–MS mode of detection and the availability of

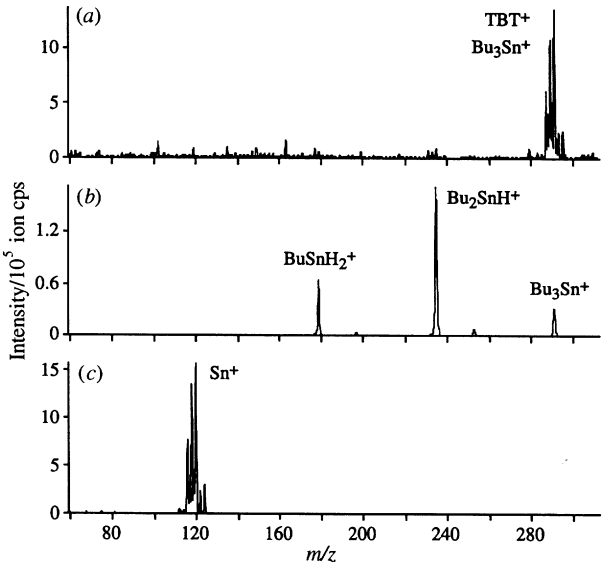

Fig. 2 Detection of TBT in three modes: (*a*) molecular, with 25 V declustering potential; (*b*) MS–MS, product ions of *m/z* 291 with 10 eV collision energy; and (*c*) elemental, with 250 V declustering potential. TBT concentration was 1 μg ml^{-1} (as Sn).

molecular structure information in this mode are demonstrated in Fig. 2(*b*). All instrumental parameters up to and including Q_1 were identical with those used in Fig. 2(*a*), including the declustering potential and the curtain gas flow. In this mode Q_1 was not scanned, but was operated to allow selective transmission of only the TBT^+ molecule, while Q_3, the second mass analyzing quadrupole, was scanned across the mass range. This resulted in a product ion spectrum for TBT^+. The dc quadrupole offset potential difference between Q_1 and Q_2 was 10 V, providing a collision energy of 10 eV for the singly charged TBT^+ molecule with the nitrogen collision gas. This collision energy was chosen since it resulted in the highest intensity for any fragment or product ion (*m/z* 235), an important consideration for analytical purposes, particularly for quantification at low levels. The product ion at *m/z* 235 is $[CH_3(CH_2)_3]_2SnH^+$ (or Bu_2SnH^+), corresponding to loss of a butene group, $CH_2CHCH_2CH_3$, as suggested by Siu *et al.*[8,10] Ion path parameters downstream of the collision cell were optimized for maximum sensitivity for this product ion, while retaining mass resolution in Q_3. Maximum sensitivity for the product ion at *m/z* 235 was achieved at a collision energy for which complete dissociation of the precusor ion, TBT^+, did not occur. This is a common phenomenon in MS–MS. Small increments in collision energy permit access to further CID channels, distributing the total ion signal over an extended range of dissociation products. The other major product ion in the MS–MS spectrum is $[CH_3(CH_2)_3]SnH_2^+$ (or $BuSnH_2^+$), at *m/z* 179, corresponding to further loss of a butene group. Origin of the minor peaks at *m/z* 197 and *m/z* 253 is uncertain, but may be due to water adducts of the product ions as a result of water impurity in the N_2 collision gas. Increasing the collision energy by 10 eV resulted in complete dissociation of TBT^+ and the appearance of a product ion at *m/z* 123 (SnH_3^+), corresponding to subsequent loss of another butene group. Under conditions corresponding to the fully intact molecular mode, increasing the declustering potential to ≈100 V resulted in detection of this same set of fragment ions under single-MS conditions. The background level in the MS–MS mode is extemely low, particularly compared with the fully intact molecular mode [Table 2 lists signal-to-background (S/B) ratios for the three modes of detection]. This is due to the high selectivity of the MS–MS process, and

Table 2 S/B and DL for TBT and AsB for three modes of detection. S/B values are for infusion of standards. DLs are for FI of PACS-1 (TBT) and cation exchange chromatographic analysis of DORM-2 (AsB)

	TBT		AsB	
Mode	S/B	DL/fg	S/B	DL/fg
Molecular	350	—	200	—
MS–MS	90550	64	92650	415
Elemental	13550	64	7550	415

offers decreased detection limits (DL), particularly for MRM experiments in which precusor and product ion masses are specifically selected. Hence the MS–MS mode and MRM experiments are the methods used most often for organic analyses.

Elemental mode detection is demonstrated in Fig. 2(*c*), a full scan single-MS spectrum. The only feature in the spectrum is the tin isotopic distribution pattern near *m/z* 120, resulting in a spectrum similar to that obtained by ICP-MS. A declustering potential of 250 V was used and the curtain gas flow was increased 52% to 1.44 l min^{-1}. The increased curtain gas flow assisted in solvent cluster removal and desolvation of ionspray droplets, leaving more of the collision energy in the orifice–skimmer differentially pumped region available for molecular fragmentation. It also shortened the mean free path of the molecules and their fragments in the interface region, resulting in enhanced dissociation. The degree of fragmentation attainable is equally a function of the number of collisions the molecules experience and the energies of the collisions. In general, elemental fragments or significantly dissociated molecules passing through the skimmer and into Q_1 have experienced a substantial electric field between the orifice and the skimmer. This results in fragment kinetic energies too large for efficient quadrupole mass analysis with good mass resolution. In the literature, there are several examples of mass spectral resolution being sacrificed in favour of a higher degree of fragmentation and increased sensitivity. Collisional focusing[34] in Q_0 of this mass spectrometer energetically cooled the fragment or elemental ions such that they trickled out of Q_0 and into the mass analyzing quadrupole, Q_1, with approximately 1 eV of kinetic energy, enabling improved mass resolution. This same collisional focusing forced the elemental or fragment ions onto the axis of the mass spectrometer as they were energetically cooled, permitting increased transmission and hence higher sensitivities than in other studies. Expansion of the tin isotope distribution pattern in Fig. 2(*c*) reveals a small peak at *m/z* 121, where no tin isotope exists. This is due to the fact that TBT^+ readily dissociates to SnH_3^+, but in this configuration the hydrogen is bound substantially tighter to the tin atom. Under the fragmentation conditions employed for this spectrum there is a low level of SnH^+ detected since the declustering potential is sufficient to dissociate two of these hydrogens, but not sufficient to fragment the SnH^+ molecule completely. Operation at a higher declustering potential ($\approx$350 V) permitted elimination of the final hydrogen, but resulted in a sensitivity compromise of approximately 25%. This is the first report of the use of IS-MS (or ES-MS) for predominant detection of Sn^+ at *m/z* 120 compared with detection of SnH_3^+ at *m/z* 123.

Dual Mode Quantification of TBT

To investigate the dual mode quantification possibilities of IS-MS(–MS), TBT concentrations were determined in the NRCC harbour sediment reference material PACS-1, using both MS–MS and elemental modes of detection for FI. Siu *et al.*[10] have previously used IS-MS(–MS) to measure TBT concentrations in this same reference material, but their determinations were limited to the MS–MS mode only.

Siu *et al.*[10] indicated that dilution of the PACS-1 extract into a polar methanol–acetonitrile FI carrier solution has two major advantages. Firstly, TBT ionization was promoted since TBT compounds, such as acetates, are polar and are expected to dissociate readily, by heterolytic cleavage of the TBT–acetate bond, into TBT^+ and a counter ion. This is critical, since for a particular compound to be observed from an electrospray or ionspray source, the compound must be ionized in the solvent being sprayed. Secondly, this carrier solution is very favourable to the ionspray process. The efficiency of transfer of a particular ion from the liquid to the gas phase depends, among other factors, upon ion evaporation at atmospheric pressure. The lower surface tension of methanol-containing droplets facilitates disruption of the bulk liquid surface of a droplet, allowing for more rapid evaporation of solvent. Hence the sprayed droplets more readily achieve the critical electric field on their surfaces necessary for ion transfer to the gas phase. The result is improved stability and sensitivity of signal.

The triplicate injection, dual mode quantification of TBT in PACS-1 sediment reference material is shown in Fig. 3. In each of the figures, the first three peaks correspond to triplicate injections of unspiked extracts, while the second and third sets of triplicate injections correspond to extracts including spikes of 1.48 and 2.96 μg g^{-1} of Sn in sediment, respectively. Elemental mode detection is shown in Fig. 3(*a*), and MS–MS mode detection is displayed in Fig. 3(*b*). In the elemental mode, all operating conditions were the same as for Fig. 2(*c*), except that the declustering potential was increased to 350 V to prevent detection of the tin hydride. In this mode of operation the declustering and fragmentation conditions were sufficient to dissociate most compounds present in the PACS-1 extract. This mode of analysis is analogous to ICP-MS detection in that the harsh ionization conditions are expected to dissociate any concomitant compounds which may be present at the detected mass of interest. As in ICP-MS analyses, sample

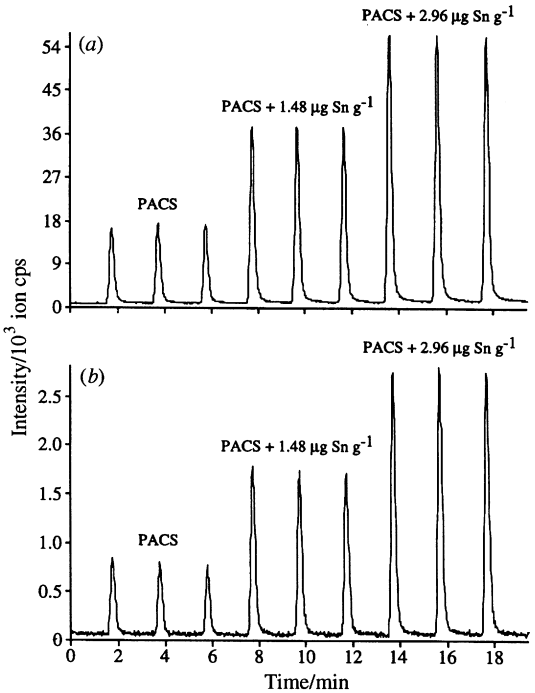

Fig. 3 Dual mode FI quantification of TBT in sediment reference material PACS-1: (*a*) elemental, single ion monitoring of *m/z* 120; and (*b*) MS–MS, selected reaction monitoring of Q_1 at *m/z* 291, Q_3 at *m/z* 235 with 10 eV collision energy.

preparation is critical for elemental mode IS-MS since any other tin species extracted from the sediment, and which the interface region was capable of dissociating to elemental constitients, would be detected in the elemental mode. Fortunately, the extraction method utilized was exclusive for TBT and there were no other tin species present in the extract. Otherwise, using FI with the elemental mode would not be sufficient for unambiguous determination and a separation technique would become neecessary. For five replicate analyses the TBT concentration in PACS-1 was determined to be 1.29 ± 0.04 μg g^{-1} of Sn in sediment by elemental mode IS-MS, compared with the certified value of 1.27 ± 0.22 μg g^{-1}. Background subtracted FI peak areas were calculated to determine the measured TBT concentrations.

In the MS–MS mode detection displayed in Fig. 3(*b*), single reaction monitoring (SRM) was utilized to monitor a specific precursor ion–product ion transition as a function of time. Q_1 was operated to transmit selectively only ions of *m/z* 291, corresponding to TBT^+, while Q_3 analyzed only product ions of *m/z* 235, corresponding to CID loss of a butene group. A Q_2 collision energy of 10 eV was selected for optimum product ion production at *m/z* 235. Scans were performed for many other tin species, but none was detected. The high specificity of this mode of detection allows for a less ambiguous analysis, which is often necessary for complex samples. Although the TBT extraction used for this analysis was specific for TBT, such extractions are not available or practical for all analytes of interest. For five replicate analyses the TBT concentration in PACS-1 was determined to be 1.24 ± 0.04 μg g^{-1} of Sn in sediment by MS–MS mode ionspray mass spectrometry, compared with the certified value of 1.27 ± 0.22 μg g^{-1}.

Experiments in which the spike was added after the extraction yielded identical TBT concentrations. The above experiments corresponded to the actual injection of 12.7 pg of TBT (as Sn) and detected concentrations of 25.4 ng ml^{-1}. Further experiments were conducted to determine the levels of detection possible, for this sample, using both modes of detection. For both elemental mode and MS–MS mode it was possible to dilute the PACS-1 extract by a factor of 200 and obtain an S/B of 2:1, corresponding to injection of 63.5 fg of TBT (as Sn) and a detected concentration of 127 pg ml^{-1} (DLs are listed in Table 2). The agreement between the two modes of detection for quantification of TBT in PACS-1, their agreement with the certified value and these levels of detection indicate that dual mode ionspray mass spectrometry is a viable technique for elemental speciation studies.

Dual Mode Detection of Arsenobetaine

Three modes of detection (fully intact molecular, MS–MS and elemental) are demonstrated in Fig. 4 for infusion of a 1 μg ml^{-1} (as the molecule) standard of arsenobetaine (AsB). The corresponding arsenic concentration was 421 μg ml^{-1}. The previously described acid addition to the sample matrix assisted in protonation of the AsB molecule. Although AsB is a zwitterion it exists as a cation in acidic solutions. The full scan spectrum of Fig. 4(*a*) shows fully intact molecular mode detection of the protonated AsB molecule at *m/z* 179. A declustering potential of 30 V was employed and Q_1 single-MS scanning parameters were the same as for Fig. 2(*a*). As in Fig. 2(*a*), the declustering and interface parameters were adjusted such that the solvent adducts were stripped from the fully intact molecule, yet little molecular fragmentation occurred. The only other significant feature in this spectrum is the protonated methanol dimer at *m/z* 65, originating from the sample matrix. Use of a different solvent in the standard solution generally yields analogous detection of a corresponding solvent molecule.

In Fig. 4(*b*) the MS–MS mode of detection is displayed for

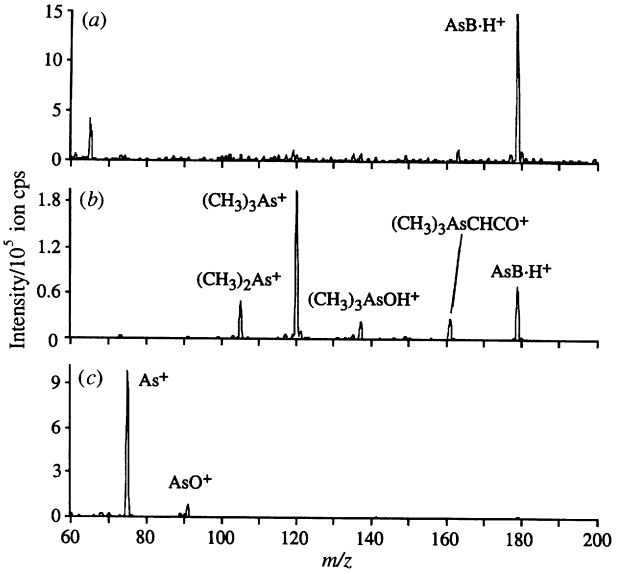

Fig. 4 Detection of AsB in three modes: (*a*) molecular, with 30 V declustering potential; (*b*) MS–MS, product ions of *m/z* 179 with 27 eV collision energy; and (*c*) elemental, with 300 V declustering potential. AsB concentration was 1 μg ml^{-1} (as the molecule).

a collision energy of 27 eV. This collision energy was chosen since it yielded the highest sensitivity for any product ion, the fragment at *m/z* 120. All instrumental parameters up to and including Q_1 were identical with those used in Fig. 4(*a*). Q_1 was operated to allow selective transmission of only the $AsB \cdot H^+$ precursor molecule, and Q_3 was set to perform full mass scans. The resultant product ion spectrum for $AsB \cdot H^+$ is significantly more complicated than that offerred by TBT in Fig. 2(*b*). For TBT, the CID characteristic fragmentation followed one route only, loss of butene groups. For AsB a more diverse set of CID channels is apparent. The most intense product ion is the trimethylarsine ion, $(CH_3)_3As$, at *m/z* 120, representing loss of CH_3CO_2 or loss of CH_2CO followed by loss of OH. The product ion at *m/z* 105, $(CH_3)_2As$, corresponds to the further loss of a methyl group and appeared only as a result of dissociation of the trimethylarsine ion. Another major, though less intense, dissociation channel was loss of water to yield the product ion $(CH_3)_3AsCHCO$ at *m/z* 161. The other major CID characteristic fragment ion, at a collision energy of 27 eV, was $(CH_3)_3AsOH$ at *m/z* 137, representing loss of CH_2CO. Further dissociation of these product ions and the study of minor, less intense, product ions will be presented in the next section.

Elemental mode detection of AsB is demonstrated in Fig. 4(*c*), which is a Q_1 single-MS, full scan spectrum. The spectrum was acquired with a declustering potential of 300 V and the curtain gas flow was increased in the same manner as the TBT case. The dominant features in the spectrum are a peak for As^+ at *m/z* 75, and a peak for AsO^+ at *m/z* 91. Otherwise the spectrum is extremely clean, though at lower masses the background is increased owing to fragmentation of other substances in the solution. In ICP-MS analyses, detection of the bare arsenic ion presents a problem owing to the $ArCl^+$ interference at *m/z* 75. No such interference exists for IS-MS detection in the elemental mode, suggesting this technique as complementary to ICP-MS, specifically for *m/z* values at which ICP-MS experiences interferences. The oxide at *m/z* 91 is difficult to eliminate, and is extremely dependent on the position of the ionspray probe, as well as on the interface and ionspray parameters. Small changes in ionspray probe position, nebulizer gas flow, ionspray potential, curtain gas flow rate and interface potentials may have profound effects on oxide

formation and detection. In general the same situation exists for any element which has a high affinity for oxide formation. This is in contrast to the situation for TBT, where detection of the bare metal ion, Sn^+, was accomplished with little effort. Positioning the ionspray needle probe at very oblique angles to the curtain plate, and spraying past the aperture in the curtain plate, such that only the edge of the aerosol cone was sampled into the mass spectrometer, was empirically found to facilitate reduction of oxides. The S/B values for the three modes of detection are listed in Table 2.

Moderate Dissociation of Arsenobetaine in the Interface

The fully intact molecular and elemental modes shown in Fig. 4 demonstrate dissociation and fragmentation in the source–interface region at two extremes. Moderate declustering potentials may be selected which produce substantial, but not total, dissociation of the molecular species. Operation in such a mode provides another level of information on molecular structure by, in effect, offerring another quasi-degree of MS. A Q_1 single-MS full scan spectrum acquired for a 1 μg ml^{-1} (as the molecule) standard of AsB with a declustering potential of 150 V, is shown in Fig. 5. Mass spectral resolution was compromised somewhat to enhance sensitivity owing to the fact that the total ion signal was spread over a number of fragment ion masses under these dissociation conditions. The *m/z* values detected range from the fully intact protonated molecule at *m/z* 179 to the bare elemental arsenic ion at *m/z* 75. The MS–MS product ions of Fig. 4(*b*) reside in the moderate declustering potential spectrum of Fig. 5.

To probe more extensively into AsB molecular structure and dissociation, source and interface conditions were adjusted to optimize production of a particular fragment, upon which MS–MS was then performed at a number of different collision energies. Every fragment produced in the source–interface region and then studied by MS–MS was subjected to extreme MS–MS conditions to verify that it contained arsenic. Under such extreme CID conditions bare As^+ was observed at *m/z* 75, but the conditions necessary to achieve such extensive fragmentation were detrimental to signal intensity and resolution, rendering the CID spectra of little use other than to verify the presence of As^+. For example, source and interface conditions were adjusted to provide optimum sensitivity for the previously discussed major fragment at *m/z* 161. MS–MS was then performed with Q_1 operating such as to permit only transmission of ions of *m/z* 161. With Q_3 operating in full scan mode, product ion spectra for *m/z* 161 were produced. It was found that *m/z* 161 dissociated preferentially, and exclusively, by loss of a methyl group to yield $(CH_3)_2AsCHCO$ at *m/z* 146. This CID product ion, in turn, lost another methyl group to yield $CH_3AsCHCO$ at *m/z* 131. The CID reaction then proceeded to yield a fragment at *m/z* 103, which could correspond to CH_3AsCH (loss of CO) or AsCO (loss of CH_3CH). Observation of the next fragment at *m/z* 91 could represent CH_3AsH or AsO, but the technique is not capable of offerring further elucidation of the dissociation pathway. As the collision energy was increased from 0 to 10 eV the first product of $AsB \cdot H^+$ to appear was $(CH_3)_3As$ at *m/z* 120, indicating that loss of CH_3CO_2 was the preferred mechanism. At 15 eV collision energy, the previously discussed product ion at *m/z* 137 appeared, which could lose OH to yield this same fragment at *m/z* 120. Another observed path of dissociation for *m/z* 137 was sequential loss of methyl groups to yield $(CH_3)_2AsOH$ at *m/z* 122 and CH_3AsOH at *m/z* 107. All dissociation pathways were verified by precursor ion scans. In such a scan, Q_3 is operated to permit only transmission of product ions of a particular *m/z* and Q_1 is scanned over the selected full *m/z* range to indicate which precursor ions resulted in the selected fragment. The dissociation mechanisms discussed above are only tentatively suggested and have only involved fragment ions previously proposed by other groups.[9,26,27,30–32] Such operation of the IS-MS(–MS) system clearly permitted more extensive probing into the structure and dissociation of the AsB molecule than previous studies were able to achieve. Similar dissociation pathways have been mapped out for the less complicated TMAs, DMA and AsC molecules, but are not included in this paper.

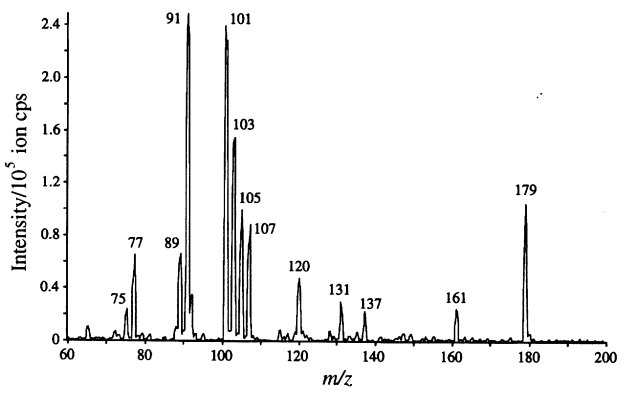

Fig. 5. Moderate fragmentation of AsB in the interface region, with 150 V declustering potential.

LC–IS-MS(–MS) of Arsenic Standard Substances

The cation exchange chromatography performed in this work has been described in detail elsewhere,[14] hence only the ion-pairing chromatography will be described in this section. Three modes of detection (fully intact molecular, MS–MS and elemental) for ion-pairing separation of five co-injected arsenic species are demonstrated in Fig. 6. The arsenic species were at concentrations of 200 ng ml^{-1} each (as the molecule), corresponding to injections of 4 ng of each molecule, or approximately 2 ng of As for each species. Effluent splitting prior to the ionspray probe resulted in approximately 0.4 ng of each

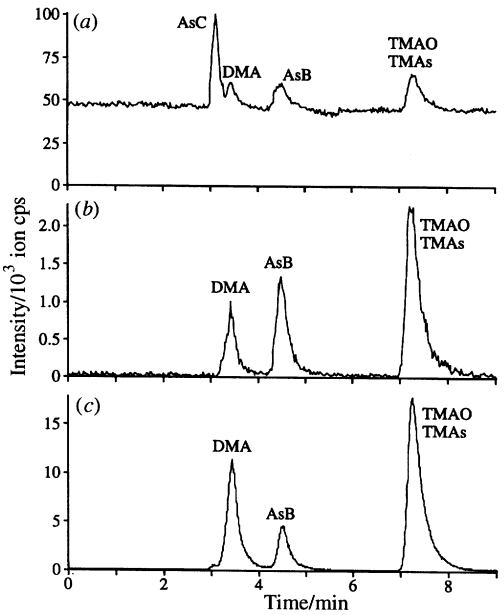

Fig. 6 Ion-pairing LC–IS–MS(–MS) detection of five co-injected arsenic species in three modes: (*a*) molecular, with 35 V declustering potential; (*b*) MS–MS, with 25 eV collision energy; and (*c*) elemental, single ion monitoring of *m/z* 75 with 350 V declustering potential. Figs. (*a*) and (*b*) are the summed, or TIC chromatograms, for the separate chromatograms corresponding to the arsenic species.

molecule, or ≈0.2 ng As for each species, reaching the detector. Seven arsenic species were included in the samples injected, but As^V and MMA were not detectable in the positive ion mode under the experimental conditions used. Using continuous infusion of AsB in the mobile phase, the IS-MS(–MS) system was optimized for detection of AsB in each of the three modes of detection. Sprayer position was optimized for AsB detection in the elemental mode, since of the five species detected AsB was the most difficult to dissociate to bare elemental As^+. The sprayer position was not changed when switching between detection modes. In each of the three modes, the 10 mmole l^{-1} octansulfonate mobile phase was responsible for suppression of the sensitivity, owing to matrix effects, by approximately a factor of five as compared with infusion of AsB in the 50% methanol solution previously described. However, the low pH of the mobile phase promoted cation formation by protonation of the DMA, TMAO and AsB molecules which were detected as protonated molecules in the molecular modes. Protonation was not necessary for the permanent cations TMAs and AsC.

The total ion current (TIC) chromatogram for fully intact molecular detection is displayed in Fig. 6(*a*). The TIC chromatogram is the sum of the individual chromatograms corresponding to the single ion monitoring of TMAs (*m/z* 135), $TMAO \cdot H^+$ (*m/z* 137), $DMA \cdot H^+$ (*m/z* 139), AsC^+ (*m/z* 165), and $AsB \cdot H^+$ (*m/z* 179). Only Q_1 was utilized as a mass analyzer, and the declustering potential was 35 V for optimized AsB sensitivity. The TIC chromatogram for MS–MS detection of five individual CID transitions, with a collision energy of 25 eV, is shown in Fig. 6(*b*). For DMA, TMAO and AsB detection the precusor ion was the protonated molecule. For the individual SRM chromatograms the fragment with the highest sensitivity under these CID conditions was monitored as a function of time. All instrumental parameters up to and including Q_1 were identical with those employed in Fig. 6(*a*). The chromatogram for single-MS elemental mode detection of As^+ at *m/z* 75, using a declustering potential of 350 V is given in Fig. 6(*c*). The chromatograms in Fig. 6 each display less than five chromatographic peaks since TMAs and TMAO co-eluted at a retention time of 7.2 min. Additionally, column problems resulted in inconsistent retention of AsC. AsC eluted in the void volume or at retention times greater than 20 min. The reason for this behaviour is unknown, though the column expired shortly thereafter (these chromatograms were obtained after the AsB quantification work). Hence, AsC was detected in fully intact molecular mode and as a shoulder on the leading edge of the peak for DMA in the elemental mode data, while in the MS–MS mode AsC was not detected in the 9 min data acquisition. The elemental mode chromatogram in Fig. 6(*c*) is analogous to LC detection by ICP-MS in that no further selectivity is available. In order to detect TMAs and TMAO unambiguously the chromatography would require modification. However, molecular detection modes provide the specificity necessary for unambiguous detection of the various species.

Although the gross features of the three chromatograms presented in Fig. 6 are similar, relative intensities for chromatographic peaks differ depending on the detection mode. These differences in sensitivities for the various species indicate factors which must be considered when employing molecular ionization techniques for analysis of more than one species in a single sample. Several of these factors have been presented in a recent publication,[14] and will not be repeated in the present discussion. It is sufficient to summarize that for any given solution or mobile phase, the ionspray (or electrospray) and mass spectrometer interface parameters selected will promote more intense detection of some species than others. Since many compounds react differently to the ionspray process, analyses which target a single particular species often significantly improve detection for that particular analyte.

The fully intact molecular mode single ion chromatograms for the five arsenic species are shown in Fig. 7. The TIC chromatogram presented in Fig. 7(*a*) is the same as the chromatogram in Fig. 6(*a*). In fully intact molecular mode the five single ion chromatograms provide selective detection of the five arsenic species, and in particular, the ability to distinguish chromatographic peaks corresponding to TMAs and TMAO. The single ion chromatograms for *m/z* 135 (TMAs) and *m/z* 137 (TMAO) indicate that the chromatographic peaks corresponding to these species contribute peak heights of 12 000 and 16 000 cps (counts s^{-1}), respectively, towards the total peak height of 48 000 cps in Fig. 6(*a*). However, fully intact molecular mode detection offers the highest DLs of the three detection modes owing to elevated background levels, and hence is very limited for analytical and quantification purposes. In elemental mode, Fig. 6(*c*), the elevated declustering potential resulted in a minimization of the background level, therefore increasing S/B values. The high selectivity of MS–MS also results in high S/B values.

The extracted ion chromatograms for the individual MS–MS transitions are shown in Fig. 8. The TIC chromatogram presented in Fig. 8(*a*) is the same as in Fig. 6(*b*). Each of the SRM chromatograms is the result of the monitoring of the most intense CID fragment of a particular precursor molecule, after only that particular precursor molecule was transmitted through the first analyzing quadrupole, Q_1. For example, the chromatogram in Fig. 8(*c*) corresponds to Q_1 operating such as to permit only transmission of ions of *m/z* 179 ($AsB \cdot H^+$), and Q_3 detecting only the *m/z* 120 fragment ions. For DMA, TMAO and AsB detection the precursor ions were the protonated molecules. Owing to the co-elution of TMAs and TMAO it is not possible to claim that these species do not interfere with one another in some manner, but it may be stated that there is no interference between DMA and AsB and either of the other two species. These individual chromatograms represent extremely selective detection of specific arsenic species, a fact that presents several advantages for chromatographic detection. The specificity offerred by IS-MS(–MS) detection for chromatographic separations may be used to reduce the necessity of complete chromatographic resolution of all species. This results in shorter analysis times and less

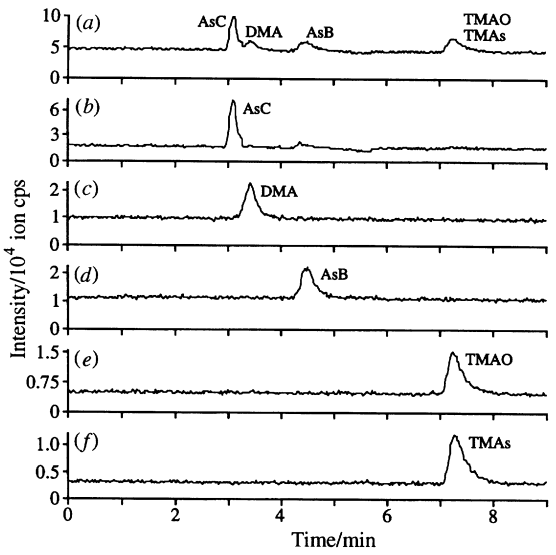

Fig. 7 Fully intact molecular mode ion-pairing LC–IS–MS detection of five co-injected arsenic species: (*a*) TIC chromatogram; (*b*) AsC, *m/z* 165; (*c*) DMA, *m/z* 139; (*d*) AsB, *m/z* 179; (*e*) TMAO, *m/z* 137; and (*f*) TMAs, *m/z* 135. The declustering potential was 35 V.

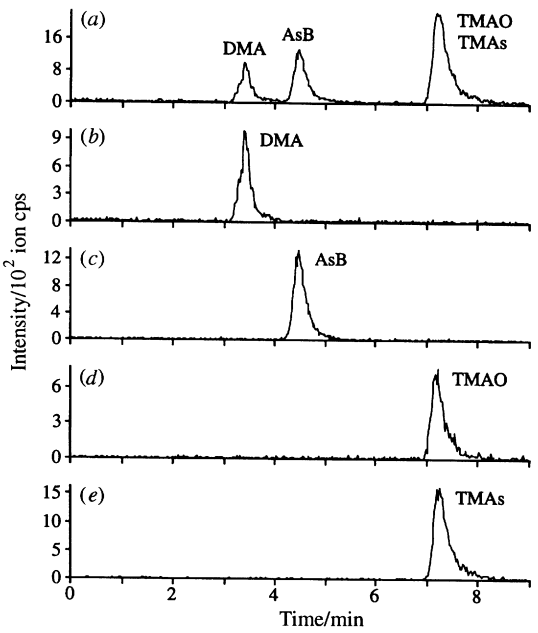

Fig. 8 Molecular mode ion-pairing LC–IS–MS(–MS) detection of four co-injected arsenic species: (*a*) TIC chromatogram; (*b*) DMA, Q_1 at *m/z* 139, Q_3 at *m/z* 121; (*c*) AsB, Q_1 at *m/z* 179, Q_3 at *m/z* 120; (*d*) TMAO, Q_1 at *m/z* 137, Q_3 at *m/z* 122; and (*e*) TMAs, Q_1 at *m/z* 135, Q_3 at *m/z* 120. The collision energy was 25 eV.

complex chromatography. For ionspray or electrospray, further advantages may be realized with the possibility of reducing the ionic strengths of the mobile phases, thus reducing matrix effects, and therfore promoting increased sensitivity and reduced detection limits.

Dual Mode Quantification of Arsenobetaine

To test the dual mode quantification capabilities of IS-MS(–MS) further, AsB concentration was determined in the NRCC dogfish muscle reference material DORM-2, using both MS–MS and elemental modes of detection coupled with LC. Again, Siu *et al.*[11] have previously used IS-MS(–MS) to measure the AsB concentration in DORM-1, the predecessor to DORM-2, but their determinations were limited to the MS–MS mode only.

Quantification of AsB in DORM-2 was performed using both cation exchange and ion-pairing chromatography. MS–MS and elemental modes of detection were employed for each chromatography. Infusion of AsB in the appropriate mobile phase was used to optimize the IS-MS(–MS) system for AsB detection for each method. Owing to the expectation that AsB would be present at significant levels in the DORM-2 reference material, sample dilution prior to injection was considered. In fact, sample dilution was necessary owing to severe column overload in the void volume, particularly since AsB eluted close to the void volume. This was especially true for ion-pairing chromatography, in which the myriad other substances in the sample caused the AsB retention time to be reduced (compared with the retention time of the standard substance) to the point where AsB detection was interfered with by matrix effects from the void volume. As a result, a mobile phase with increased aqueous content, compared with the mobile phase used for standards separations, was employed for ion-pairing chromatography. For cation exchange chromatography the sample was diluted a further factor of 1+49 in water while for ion-pairing chromatography the dilution factor was 1+4.

For each mode of detection, and for each chromatographic method, five replicate measurements were made. In all cases, the standard deviation of the variance of the background subtracted peak areas (for the same spike level) was less than 5%, and in two cases was under 3%. Results of dual mode determination of AsB in DORM-2 are shown in Table 3. Ion-pairing results were identical for MS–MS and elemental modes of detection. For cation exchange chromatography the dual mode results were within statistical error of each other. The results of the two chromatographic methods, in either detection mode, were also consistent. These results were included in the NRCC certification procedure for AsB in DORM-2. Since the process is not yet complete, a final certified value for the AsB concentration in DORM-2 is not yet available. However, it is expected that the AsB concentration will be slightly higher than previously measured value[11,29] of 15.7 ± 0.4 μg g^{-1} of As in material for DORM-1, the NRCC predecessor reference material to DORM-2. The results presented above are consistent with this expectation.

For cation exchange chromatography the above quantification measurements represented detection of AsB at a concentration of 133 ng ml^{-1} (as As), corresponding to an injected amount of 2.7 ng, and 66 pg of As reaching the detector. For ion-pairing chromatography all levels were a factor of ten higher. Further experiments were conducted to determine the levels of detection attainable employing these chromatographic methods and ionspray mass spectrometry detection. For both MS–MS and elemental modes of detection for cation exchange chromatography it was possible to detect, at a 2:1 S/B, concentrations of 830 pg ml^{-1} (as As), which corresponded to 16.6 pg of AsB (as As) injected, and 415 fg reaching the detector (detection limits are listed in Table 2). These levels of detection were a significant improvement over the values presented by Corr and Larsen[14] using the same cation exchange chromatography. In that work crude extracts were injected with no prior sample clean-up procedure occurring. This indicates the significance of presenting samples in a matrix as amenable as possible to the ionspray process.

CONCLUSIONS

The agreement between the two modes of detection (elemental and MS–MS) for quantification of TBT and AsB in certified reference materials, and the detection levels attainable for these samples, indicates that ionspray mass spectrometry is a viable technique for elemental speciation studies. The molecular structure and dissociation information provided by the molecular modes of this technique, as illustrated by the AsB example, indicate that ionspray mass spectrometry has significant potential for the development of future speciation methodologies. The dual mode capability of the technique offers possibilities that other methods of detection do not provide and hence this technique may prove important in furthering the state-of-the-art in speciation analysis.

The author thanks Michael Siu of NRCC for many helpful discussions and for the DORM-2 reference material.

Table 3 Quantification results for determination of AsB in DORM-2. Concentration units μg g^{-1} of As in the material; $n=5$ replicates for each detection mode for each chromatographic method

Mode	Cation exchange	Ion-pairing	Both chromatographic methods
Elemental	16.6 ± 0.5	16.4 ± 0.7	16.5 ± 0.6
MS–MS	17.1 ± 0.5	16.4 ± 0.6	16.7 ± 0.6
Overall	16.8 ± 0.6	16.4 ± 0.6	16.6 ± 0.6

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