ARTICLE

JAAS
www.rsc.org/jaas

Arsenic speciation in marine certified reference materials Part 2. The quantification of water-soluble arsenic species by high-performance liquid chromatography-inductively coupled plasma mass spectrometry†

Raimund Wahlen,[a,b] Shona McSheehy,*[a] Christine Scriver[a] and Zoltán Mester[a]

[a]*Institute for National Measurement Standards, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R9*
[b]*LGC Ltd., Queens Road, Teddington, Middlesex, United Kingdom TW11 0LY*

Received 18th February 2004, Accepted 6th April 2004
First published as an Advance Article on the web 17th June 2004

This study describes the analysis of arsenicals in three marine based certified reference materials (CRMs), DORM-2, DOLT-2 and TORT-2, all produced by the National Research Council of Canada. Extraction protocols involving accelerated solvent extraction (ASE) and sonication were investigated and arsenicals were determined on-line by anion-exchange and cation-exchange high-performance liquid chromatography (HPLC). The major species in all three tissues was found to be arsenobetaine (AsB), corresponding to 90%, 67% and 73% of the water-soluble arsenic in DORM-2, DOLT-2 and TORT-2, respectively. Other species quantified were arsenocholine (AsC^+), tetramethylarsonium ion ($TMAs^+$), trimethylarsoniopropionate (TMAP), dimethylarsenic acid (DMA), monomethylarsenic acid (MMA), arsenosugar D (As-sug D), arsenate (As^V) and arsenosugar A (As-sug A). The major species detected in all three materials were AsB and DMA, which together accounted for 97.4% (DORM-2), 95.4% (DOLT-2) and 91.7% (TORT-2) of the sum of the water soluble As species. The agreement for the determination of AsB by the different methods was good for all three materials, whereas some discrepancy was evident in the results for DMA. When an ASE approach with 50% acetic acid in methanol was used for the extraction, between 22% (DOLT-2) and 58% (TORT-2) more DMA was determined compared with extraction by sonication with water. Some discrepancy was evident between the sum of the species extracted and both the total As determined in the extracts and the certified As content based on complete digestion of the materials. The sum of the species recovered by sonication corresponded to 84% (DOLT-2) to 94% (DORM-2) of the total As determined in the aqueous extract. In comparison with the certified As content of the three materials, the sums of the extracted species range from 46% (DOLT-2) to 76% (TORT-2) and 102% (DORM-2).

Introduction

The efficiency of extraction of a particular analytical method for organometallic speciation can be assessed in a number of different ways. The most commonly used methods are spike recovery experiments, analysis of a certified reference material and the establishment of mass-balance budgets. The use of certified matrix matched reference materials also serves as a means of estimating the extraction efficiency of an analyte from a matrix. However, the choice of CRMs for speciation analysis is currently still fairly limited and therefore a good match with the required analyte–matrix combination at the appropriate level may not be available. In addition, the use of CRMs may still give a false estimate of the extraction efficiency compared with real-life samples because most CRMs have been processed to a great degree (*e.g.*, fat removal, grinding, sieving, drying) to make them homogeneous prior to the certification campaign. They can also be very different from a sample of biological tissue taken from the environment in terms of particle size distribution, organic carbon content and lipid content. In such cases, the use of mass-balance budgets between the sum of the species and the total inorganic fraction of the elemental metal (either after complete digestion or in the extracts used for speciation) can be more appropriate. In order to apply this approach, it is mandatory that all of the species present can be extracted, separated and accurately quantified and that a reliable method for the complete digestion and accurate determination of the total metal content in this matrix is available for comparative purposes.

The performance of different digestion methods and analytical techniques used for the determination of total As are variable,[1] which may, in part, be due to effects such as the incomplete mineralisation of arsenobetaine (AsB) during digestion,[2] thereby significantly influencing the analytical result. The incomplete mineralisation of different arsenicals could cause a discrepancy if the total As is to be determined by a technique wherein different species generate different response factors. Differences in signal response can be overcome for the quantification of individual species by using standard addition calibration. Spike recovery experiments are usually only used when standard addition is not possible. This occurs mainly because the recovery of a superficial spike is, in most cases, a gross simplification of the interaction between analyte and matrix. Unfortunately, isotope dilution, a more powerful method of quantification, is not possible due to the mono-isotpic nature of arsenic.

An extraction procedure that is capable of quantitatively extracting arsenic from every tissue does not currently exist, and it is apparent from similar studies that it is the matrix composition that is the determining factor.[3,4] For white fish tissue, quantitative extraction seems to be relatively successful using a variety of extraction procedures.[3–7] For molluscs or particular organs of an organism, however, the extraction is rarely, if ever, quantitative,[3,4,8] even with more modern, aggressive extraction techniques.

† For Part 1 see ref. 12.

DOI: 10.1039/b402482f

 This journal is © The Royal Society of Chemistry 2004

Table 1 Total and speciated certified values (in μg g^{-1} As) for the CRM tissues investigated in this study

Tissue	Total As	AsB	TMAs+
DORM-2	18.0 ± 1.1	16.4 ± 1.1	0.248 ± 0.054
DOLT-2	16.6 ± 1.1		
TORT-2	21.6 ± 1.8		

Compared with other extraction techniques for As-speciation, accelerated solvent extraction (ASE) has only been used for speciation studies in the recent past. One of the first publications using this technique for As speciation (McKiernan *et al.*, 1999)[7] showed a comparison of the extraction of arsenicals from fish samples using ASE and a sonication method based on that proposed by Alberti and co-workers.[9] The comparison showed that both sonication and ASE are useful methods for the extraction of arsenicals from fish tissues. The authors concluded that the elevated temperatures and pressures used during ASE may lead to more efficient and more reproducible extraction compared with sonication techniques. Gallagher *et al.* (1999)[10] used ASE for the extraction of arsenosugars A, B and D from ribbon kelp with a 30 : 70 MeOH : H_2O mixture. One of the most recent publications for arsenicals is a study by Gallagher *et al.* (2002)[6] in which an ASE method using different MeOH : water mixtures was assessed using a CRM (DORM-2) and laboratory fortified blanks (LFB). Using the CRM, the retention of different arsenicals was verified on different dispersion media. Wahlen and Catterick (2004)[11] also used ASE for the extraction of arsenicals, amongst other organometallic species, from an oyster tissue CRM (BCR 710).

In Part I of this study[12] the aqueous soluble arsenic species were identified by molecular mass spectrometry. The purpose of the current study is to quantify the arsenic containing species found in three marine-tissue CRMs so as to provide a more comprehensive set of speciation values for further characterization. The CRMs investigated were a dogfish muscle tissue (DORM-2), a dogfish liver tissue (DOLT-2) and a lobster hepatopancreas (TORT-2), all produced by the National Research Council of Canada. These CRMs have been certified for total arsenic concentrations and DORM-2 has certified values for arsenobetaine and tetramethylarsonium ion (Table 1). In order to obtain a comprehensive set of quantitative data for the evaluation of these materials, at least three independent analytical methods were used for the extraction and separation of the different species.

Experimental

Apparatus

Ultrasonic extraction was performed using a Branson 2200 ultrasonic cleaner (Danbury, CT, USA). The extractant was separated from residue using an IEC HN-SII centrifuge (IEC Labsystems, Needham Hts, MA, USA). Accelerated solvent extraction was performed with a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA). A rotary evaporator set up with a Büchi 461 water bath, a Büchi RE 111 rotor and a Büchi condenser (Büchi, Switzerland) was used to eliminate methanol in ASE extracts.

The different liquid chromatographic approaches are summarised in Table 2. Anion-exchange HPLC separations were carried out using a Hamilton PRPx-100 (250 × 4.6 mm × 5 μm) column (Hamilton) with a PRPx-100 guard column (Hamilton) and a Supelcosil SAX (250 × 4.6 mm × 5 μm) column (Supelco) with a Supelguard SAX guard column (Supelco). Cation-exchange separations were carried out using a Supelcosil SCX (250 × 4.6 mm × 5 μm) column (Supelco) with a Supelguard SCX guard column (Supelco). A Dionex BioLC, Model LCM (Dionex Corp., Sunnyvale, CA, USA) fitted with a 100 μL injection loop was employed for the HPLC separations.

The ICP-MS instrument used in this work was the ELAN 6000 (PE-SCIEX, Thornhill, ON, Canada) equipped with a Ryton spray chamber and either a cross-flow or Meinhard nebulizer. ICP-MS measurement conditions (nebulizer gas flow, rf power and lens voltage) were optimized daily using a standard built-in software procedure. Typical examples are a nebulizer gas flow of 0.75 L min^{-1}, ICP rf power of 1100 W and a lens voltage of 8 V.

Standards and reagents

Standard stock solutions at a concentration of 1 mg mL^{-1} were prepared by dissolving the respective compound in water. Arsenic(III) and arsenic(V) standards were prepared from As_2O_3 and As_2O_5, respectively (Aldrich) in 0.2 M HCl. Dimethylarsinic acid standard solution was prepared from cacodylic acid, sodium salt (Sigma) and arsenobetaine was obtained from Fluka. Monomethylarsonic acid, arsenocholine and tetramethylarsonium ion were obtained from Tri Chemical Laboratory Inc. The chromatographic purity of the standards was assessed by HPLC-ICP-MS and for quantification purposes the standard compound was accepted as a pure substance. Arsenosugars A and D were isolated from macroalgae purchased in a Chinese food market, following the same multidimensional LC protocol outlined elsewhere.[12] The purified arsenosugars were analysed by anion-exchange and cation-exchange HPLC to verify chromatographic purity, identified by ES-MS and quantified by ICP-MS for use as standards. Working solutions were prepared on the day of analysis by the appropriate dilution of the stock solutions with water. The stock solutions were kept at 4 °C in the dark.

Water was purified to 18.2 MΩ cm resistivity using a NANOpure water purification system (Barnstead/Thermolyne, Dubuque, Iowa, USA). Analytical reagent grade chemicals (pyridine, methanol, acetic acid and formic acid) from Fisher (New Jersey, USA) were used throughout. Ammonium acetate and mono- and diammonium phosphates were from Anachemia (Montreal, Canada). Tartaric acid was obtained from Fisher (Certified ACS grade, New Jersey, USA) and ammonium

Table 2 HPLC protocols used in conjunction with ICP-MS detection

Protocol	Separation	Column	Dimensions	Guard column	Mobile phase
1	Anion exchange	Supelcosil SAX	250 mm × 4.6 mm, 5 μm	Supelguard SAX	0–5 min: 2.5 mM phosphate buffer 5–20 min: 2.5 mM–25 mM phosphate buffer 20–30 min 25 mM phosphate buffer
2	Anion exchange	Hamilton PRP X-100	250 mm × 4.6 mm, 5 μm	PRP X-100	2.2 mM ammonium carbonate–2.5 mM tartaric acid, 1% MeOH, pH 8.4
3	Anion exchange	Hamilton PRP X-100	250 mm × 4.6 mm, 5 μm	PRP X-100	0–5 min: 5 mM acetic acid–5 mM ammonium acetate 5–30 min: 50 mM acetic acid–50 mM ammonium acetate
4	Cation exchange	Supelcosil SCX	250 mm × 4.6 mm, 5 μm	Supelguard SCX	20 mM pyridine buffer, pH 3 with formic acid

carbonate from Baker and Adamson (NY, USA). The 25 mM phosphate buffer (pH 6.0) for HPLC protocol 1 was prepared by mixing 57.5 mL of a 0.2 M $NH_4H_2PO_4$ with 5.0 mL of a 0.2 M $(NH_4)_2HPO_4$ solution and making up with water to 0.5 L. More dilute solutions were prepared by dilution with water as appropriate. For HPLC protocol 2 the eluent was prepared by weighing the appropriate amounts of ammonium carbonate and tartaric acid and dissolving the salts in an appropriate volume of de-ionised water. Methanol was then added at 1% (v/v) and the pH adjusted using ammonia solution. For HPLC protocol 3, a 50 mM eluent was prepared by diluting 50 mL of both a 1 M acetic acid and a 1 M ammonium acetate stock solution to 1 L and diluting the 50 mM solution a further 10 times to obtain the 5 mM solution. For cation exchange chromatography (HPLC protocol 4) a 20 mM pyridine buffer was prepared and adjusted to pH 3 using formic acid. All eluents were de-gassed by sonication for 20 min prior to use.

Procedures

Extraction

For each extraction procedure performed, bottles of the respective CRMs were shaken for 10 min and left to settle for 5 min prior to sub-sampling.

Extraction 1: Sonication. A sub-sample of CRM (250–270 mg) was weighed into an extraction/centrifugation tube and suspended in 10 ml of water. The tubes were sonicated for 20 min at room temperature (24–26 °C) and centrifuged for 20 min at 2500 rpm. The supernatant was decanted into a clean dry vial and the residue re-extracted 3 more times with water (5 mL). The extracts were combined and filtered (0.45 μm) prior to analysis by HPLC-ICP-MS. Eight replicate extractions for each CRM tissue were carried out on at least 3 different days.

Extraction 2: Accelerated solvent extraction. A sub-sample of the CRM (250–270 mg) was weighed into an ASE extraction cell (11 mL). Due to the small sample sizes used in this study no dispersing agent was necessary for the extractions. Two different extraction approaches using 50 : 50 methanol–acetic acid were performed. A sequential extraction of the same sample was used to study the extraction profile of different As-species with time. Here, the extracts for consecutive extraction steps were collected in different vials. Secondly, a quantitative extraction method was used where all of the extract was collected in the same vial. The extraction conditions used are shown in Table 3. After the extraction, a known volume of the extract was rotary evaporated to remove the MeOH fraction and made up to volume with water. Seven replicate extractions for each CRM tissue were carried out on at least 3 different days.

Table 3 Instrumental parameters used for accelerated solvent extraction

	Quantitation extraction	Sequential extraction
Cell volume	11 mL	11 mL
Pre-heat	2 min	0 min
Heat	5 min	5 min
Static	3 min	0 min
Cycles	5	1
Flush % volume	40	0
Purge	100 s	100 s
Pressure	1500 psi	1500 psi
Temperature	100 °C	100 °C
Solvent	50% CH_3COOH in MeOH	50% CH_3COOH in MeOH

Extraction 3: Formic acid with sonication. A sub-sample of CRM (250–270 mg) was weighed into an extraction tube and suspended in 10 ml of formic acid (23 M). The tubes were sonicated for 2 h at 50 °C. The digest was then transferred to a volumetric flask and made up to 50 mL in volume with water. The extracts were filtered (0.45 μm) prior to analysis by HPLC-ICP-MS. Five replicate extractions for each CRM tissue were carried out on at least 3 different days.

Quantification by ICP-MS

For LC determinations, a total of 8 extracts of each CRM tissue were analysed for total As and speciated As on at least 3 separate days. The reason for the determinations on 3 separate days was to minimise any systematic errors, which may influence the results if all analyses were carried out on the same day. Random errors are reflected by an elevated coefficient of variation, giving the analyst a chance to correct sources of error or to eliminate the result as an outlier. For each chromatographic set-up used, the arsenic species were quantified by standard addition on two levels. The arsenosugars were also quantified by standard addition using purified species, isolated from an algal source. Chromatographic signals were processed using in-house software and standard additions were plotted in Excel.

The total arsenic in the species stock solutions, purified algal extracts and CRM extracts were quantified by standard addition on 3.

In all determinations, only best-fit lines where $R = 0.99$ or better were used for generating quantitative results.

Moisture determination

A 1 g portion of each tissue was weighed into a clean, dry, flat vessel and dried in an oven at 105 °C for 24 h. For each hour after this time the vessel was removed and weighed until no significant difference (*i.e.*, greater than 0.001 g) was found between two consecutive weighings.

HPLC recovery and effect of eluent

All solutions used to determine recovery and effect of eluent were collected from identical injections *via* the HPLC pump in 10 mL volumetric flasks. The CRM extracts (extraction 1), extracts plus standard additions of AsB and AsB (concentrations equivalent to standard additions in extracts), were collected from the anion exchange column employed in HPLC protocol 3. The concentration of ammonium acetate–acetic acid was increased from 50 to 200 mM to ensure the elution of all the arsenic into the volumetric flask. The extracts were also collected without an analytical column in place, employing water as eluent and then with the same gradient of ammonium acetate–acetic acid as that used in the HPLC collection. Blank injections were also collected in each case. The arsenic signal generated by ICP-MS was generated in triplicate for each solution.

Results and discussion

Determination of arsenic species in the CRMs

The data for the arsenic species detected in the aqueous extracts of the three CRMs are shown in Table 4. For the complete characterisation of arsenic species in the tissues, 2 anion exchange columns and 1 cation exchange column were used to ensure the chromatographic purity of the species being quantified. Fig. 1 illustrates the separation of each tissue with the 3 LC columns used in the analytical protocol. It can be observed that on one anion exchange column, arsenobetaine elutes shortly after the void volume (where AsC^+ and $TMAs^+$ elute) and TMAP elutes shortly after AsB (Fig. 1, column 2),

Table 4 Total arsenic and arsenic-containing species (μg g^{-1} As) in the CRM tissues extracted by sonication with water

CRM	Certified [As]	[As][a]	AsB (1)	DMA (1)	MMA (3)	Sug D (3)	Sug A (3)	As^V (3)	TMAP (4)	AsC^+ (4)	$TMAs^+$ (4)	Sum of species
DORM-2	18.0 ± 1.1	19.63 ± 0.69	17.64 ± 0.37	0.23 ± 0.03	0.015 ± 0.004	0.014 ± 0.005	nd	0.0063 ± 0.0015	0.154 ± 0.059	0.024 ± 0.010	0.266 ± 0.033	18.35 ± 0.37
DOLT-2	16.6 ± 1.1	9.15 ± 0.6	6.09 ± 0.32	1.24 ± 0.26	0.042 ± 0.007	0.142 ± 0.044	nd	0.0071 ± 0.0015	0.125 ± 0.012	0.030 ± 0.007	nd	7.68 ± 0.42
TORT-2	21.6 ± 1.8	19.65 ± 0.54	14.25 ± 1.08	0.84 ± 0.10	0.093 ± 0.069	0.231 ± 0.072	0.0225 ± 0.0148	0.0928 ± 0.0371	0.836 ± 0.072	0.043 ± 0.010	0.044 ± 0.009	16.45 ± 1.09

[a] Total As determined by ICP-MS with standard addition calibration in aqueous extracts (ultrasonication); all values reported as mean and one standard deviation. [b] nd: Species not found. [c] The HPLC protocol employed is noted for each species in brackets.

whereas on the second anion exchange column all these species elute together in the void volume. On the other hand, the second anion exchange column is more suitable for the separation of arsenosugars and arsenate found in the aqueous extracts. This effect of different stationary phases in columns of the same mode of separation has previously been reported.[13]

Up to 9 species could be separated and detected in the different materials and the contributions of the individual species to the sum of the species detected in an aqueous extract are shown in Fig. 2. Whilst AsB and DMA constitute the major species in all three tissues, the varying number and proportions of arsenic species is of interest. In different tissues of the same organism, such as the liver and muscle tissues from dogfish, there is a distinct difference in the proportions of different arsenicals. The dogfish liver contains Sugar D at a higher proportion than in the muscle tissue and the proportions of the major species AsB and DMA of 66.6% and 13.6%, respectively, in the dogfish liver are significantly different from the muscle tissue (AsB: 89.9%, DMA: 1.1%). On the other hand, $TMAs^+$ is detected in the muscle tissue but not the liver. Dogfish feed on crustaceans such as crabs and shrimps, and molluscs such as whelk, mussels and oysters, as well as small fish and squid. Certain components of such food are broken down in the liver. Because the diet of dogfish is fairly diverse and rich in arsenicals it is therefore not surprising that the liver contains a higher concentration of DMA, as a degradation product of AsB[14] or possibly as an accumulation from sea-water.

Sequential extraction

In order to determine the extraction profile of the main species in the different materials, a sequential extraction method was set up using accelerated solvent extraction (Table 3). The examples of results for DORM-2 and DOLT-2 are summarised in Fig. 3 below. Within 2 consecutive extraction steps of 5 min, 98.5–100% of the extractable AsB and DMA were recovered from both tissues. This data emphasises the efficient removal of these species from the tissues by the method described. The patterns shown in Fig. 1 and 2 indicate that a higher proportion of AsB and DMA (94.5% and 95.7%, respectively) is removed from the dogfish liver material in the first extraction step compared with 76% and 78.5%, respectively, from the dogfish muscle CRM. For both materials, there are only minor fractions (<1.5%) of AsB detectable after the second 5-min cycle. In order to assure the complete removal of both species, the extraction method used for quantitative purposes was set up to contain 5 consecutive 3 min cycles, as shown in Table 3.

Comparison between ASE and sonication

The data in Table 5 show good agreement between the three methods for AsB, regardless of the matrix material analysed. The AsB data for DORM-2 also agree well with the certified value and data reported by other authors.[4,5,14] However, significant differences in the data for DMA are observed for all of the CRMs analysed. Generally, the ASE approach recovers from 22% (DOLT-2), to 53% (DORM-2) and 58% (TORT-2) more DMA than ultrasonication with water. A comparison of the total As content in extracts of fish tissues by ASE and sonication has already been reported by McKiernan and co-workers.[7] The speciation results were also given, but expressed as relative percent area rather than quantitative data that could be used for comparative purposes here. Their data showed that sonication and ASE gave comparable results for total As as well as the relative proportions of AsB/AsC and DMA in DORM-2 and the other fish tissues examined (salmon, shark, tuna and whitefish). The only significant difference observable is in the extraction of DMA from tuna, where ASE yields a higher relative percent area, albeit with a considerable coefficient of variation. The difference between the DMA recoveries

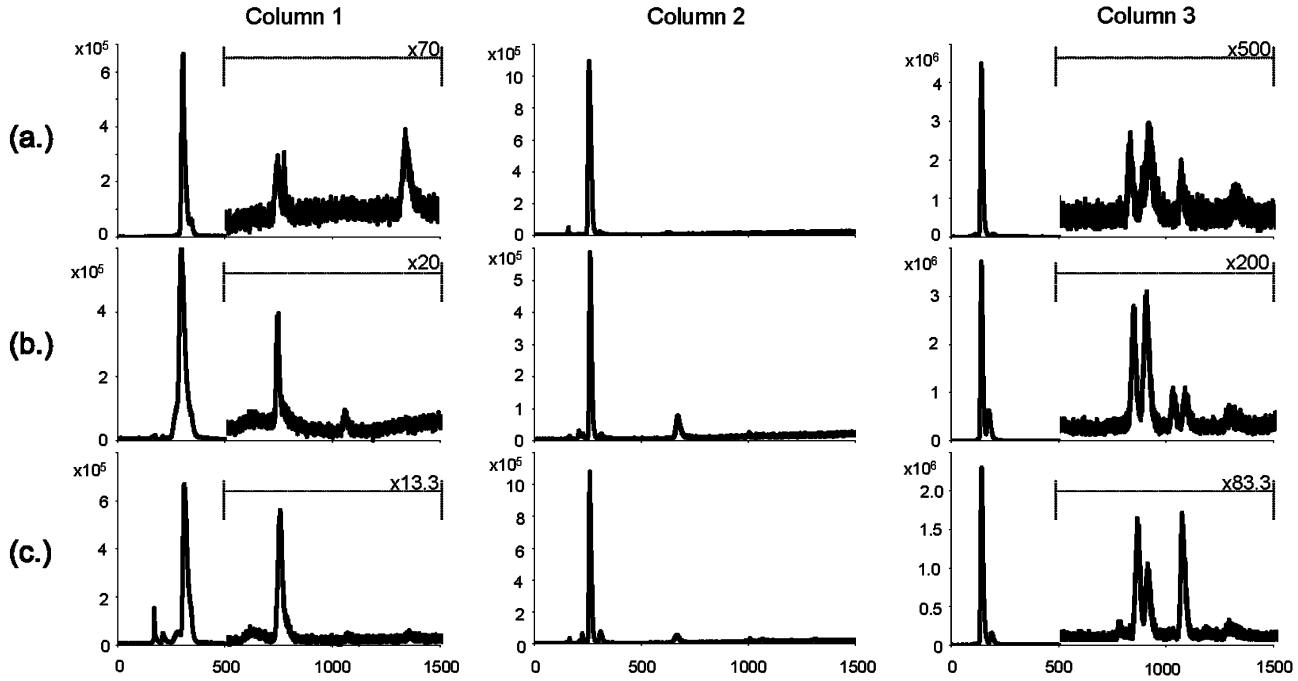

Fig. 1 The analytical separation: column 1—HPLC protocol 4, column 2—HPLC protocol 1, column 3—HPLC protocol 3 of the aqueous CRM extracts. (a) DORM-2, (b) DOLT-2, (c) TORT-2.

presented here and the data shown by McKiernan *et al.* could be due to the fact that the ASE procedure used for their work was carried out at ambient temperature rather than at 100 °C. The DMA data obtained for DORM-2 by ASE (0.48 ± 0.07 μg g^{-1} As) agree very well with data reported by Kohlmeyer *et al.*,[15] who determined 0.49 ± 0.03 μg g^{-1} As as DMA in this material. The fact that the recovery of DMA by ASE is higher in all of the materials at varying concentrations with respect to other species indicates that this is probably an extraction related effect rather than the result of incorrect quantitation due to co-elution with other species. Sonication with formic acid also yielded a higher extraction of DMA compared with sonication with water, indicating DMA specific extraction could be increased due to the acidic extraction medium rather than the ASE protocol. Kirby[4] used a microwave extraction approach to obtain DMA data for DORM-2 and TORT-2 (0.280 ± 0.004 μg g^{-1} As and 1.03 ± 0.10 μg/g As respectively) that fell in-between the sonication and ASE data presented here. The other As-species in the different materials were not determined by the ASE-LC-ICP-MS

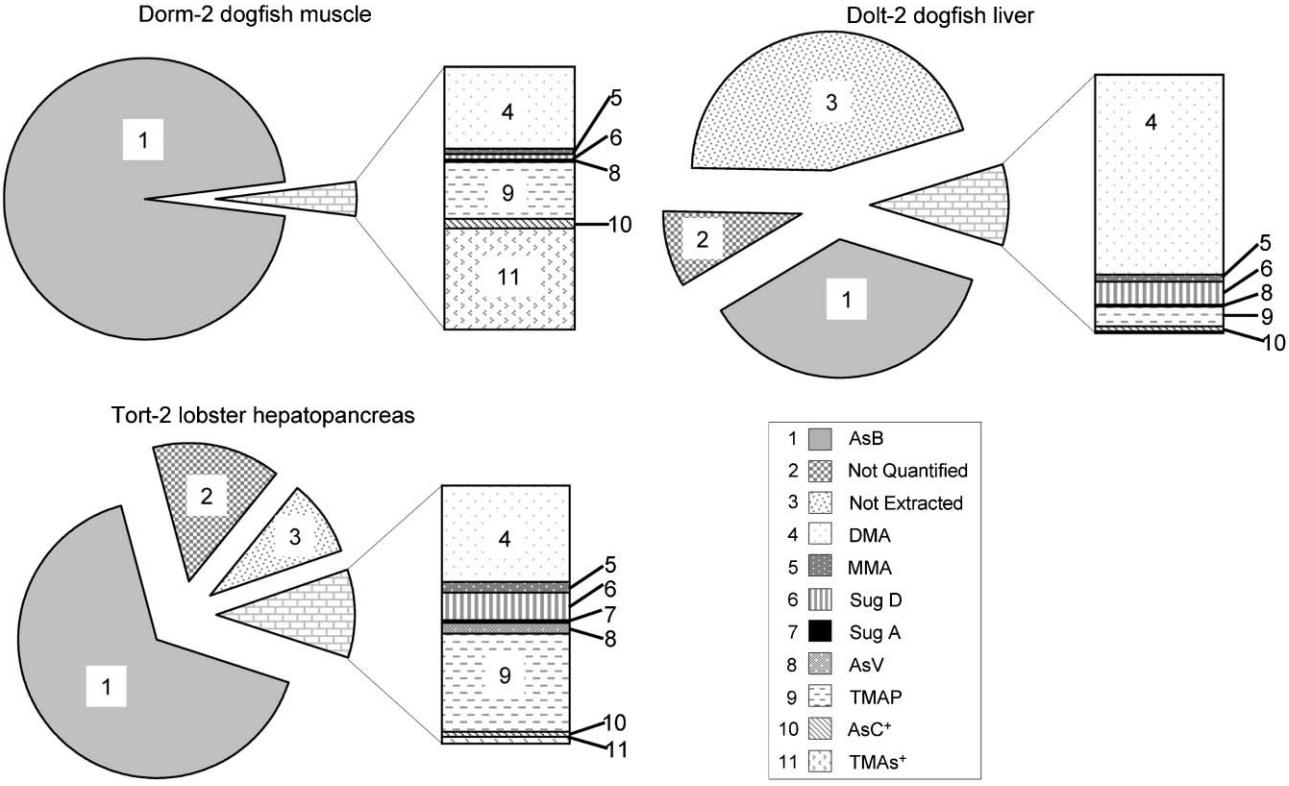

Fig. 2 Distribution of species in the three CRM tissues.

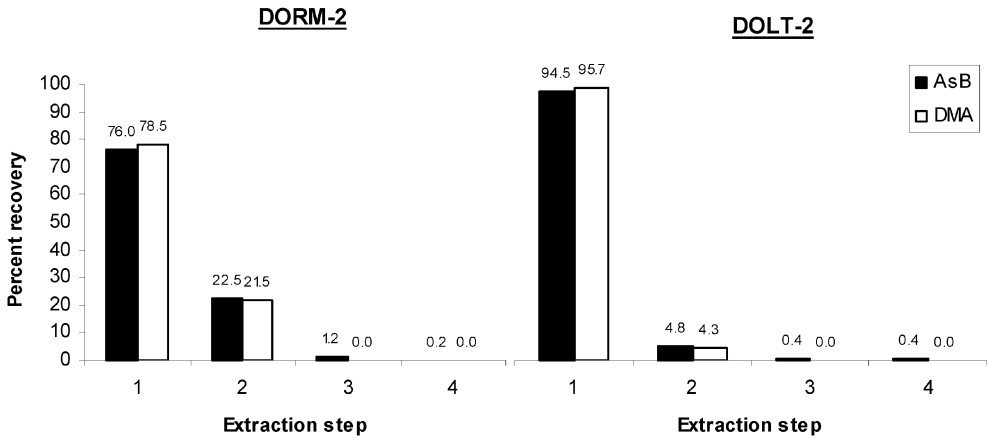

Fig. 3 Sequential extraction of DORM-2 and DOLT-2 by ASE (for conditions see Table 3).

combination, but the data in Table 4 indicate that AsB and DMA together make up from 91.7% (TORT-2) to 97.4% (DORM-2) of the sum of all the species detected.

Extraction efficiencies based on analysis of CRMs and mass-balance budgets

Using microwave-assisted extraction (MAE), Kirby reported extraction efficiencies of 103% for DORM-2 (dogfish muscle) tissue, 92% for TORT-2 (hepatopancreas) tissue and 75% for DOLT-1 (liver) tissue[4] based on the comparison of As determined in the methanol–water extracts with the certified total As, which are to date the greatest recoveries achieved for the latter two tissues. When this is converted to a mass-budget balance of the sum of the species recovered and compared to the total certified As content of these materials, the extraction efficiency for DORM-2 and TORT-2 decrease to 97% and 75%, respectively. A similar trend was observed in this study as the extraction of TORT-2 with water in an ultrasonic bath led to an extraction efficiency of 91%, whereas only 76% of the certified total value was accounted for by the sum of species. The sums of the species extracted by the aqueous sonication approach and quantified by LC-ICP-MS method 1 for DORM-2 and TORT-2 (18.4 μg g^{-1} As and 16.5 μg g^{-1} As, respectively) are in good agreement with the sums of As-species species recovered with the MAE approach used by Kirby (17.5 μg g^{-1} As and 16.1 μg g^{-1} As, respectively).

For DOLT-2, an extraction efficiency of only 55% was achieved with the water/sonication extraction protocol. Another extraction of DOLT-1, applying sonication to aid extraction, resulted in an extraction efficiency of 62%,[3] based on comparison of the 'water-soluble' As in the extract with the total As certified. The low extraction efficiency and relatively high CV (which reflects poor homogeneity) for the DOLT-2 tissue could partly be accounted for by the nature of the tissue, which is fibrous with a range of particle sizes (when compared with DORM-2 and TORT-2 which are both fine powders). Another factor, which is likely to cause differences in extraction efficiencies, is the cellular composition of each of the tissues. DOLT-2 (liver) and TORT-2 (hepatopancreas) tissues are likely to have cells that are quite similar as, during embryonic growth, the liver and pancreatic cells grow from similar, adjacent tissues[16] and this could possibly account for the low extraction efficiencies of these two CRMs.

When comparing the sum of the major species extracted by the ASE approach (AsB + DMA) with the total certified As content in the different materials, it is apparent that only the DORM-2 material yields quantitative recovery (96%). The recoveries based on the same principle for DOLT-2 and TORT-2 range from 41% to 75%, respectively. The sum of AsB and DMA recovered from DORM-2 by the MAE approach of Kirby and the sonication method here account for 95% and 94%, respectively. These data are encouraging for the ASE approach considering that only two of the 8–10 species quantified by the MAE and sonication approaches were used for the calculation of these recoveries. However, quantitation of the remaining species in ASE extracts of these samples is the subject of further ongoing investigations. Based on the indication that the ASE approach may recover a greater proportion of the DMA present in these tissues, it remains to be seen whether the extraction of the other As-containing species can also be enhanced.

Investigation of discrepancies in quantification

The major discrepancy noted in the mass balance comparisons is that the sums of the quantified species are consistently lower (84% (DOLT-2 and TORT-2) to 94% (DORM-2)) than the total As determined in the aqueous sonicated extracts. The fact that they are not lower by the same amount implies that it may be a tissue dependent effect. It is hypothesized that the systematic discrepancy could be due to one or a number of causes, such as irreproducible recovery from the analytical column, differences in the effect that the mobile phase can produce in the ICP-MS system, erroneously quantified standards or unidentified minor species.

The retention of species on the analytical column should not, in theory, cause errors in quantification unless there is a significant difference between the recovery of the sample alone and the recovery of the sample using standard addition. This was investigated by the collection of LC eluent (HPLC protocol 3) after an injection of each of the CRM extracts (water/sonication), with and without the chromatographic column and a subsequent comparison of the total As (by signal

Table 5 Comparison of AsB and DMA data in the tissues DORM-2, DOLT-2, and TORT-2 by different analytical protocols (all μg g^{-1} As)

Extraction	HPLC protocol	DORM-2		DOLT-2		TORT-2	
		AsB	DMA	AsB	DMA	AsB	DMA
Sonication with water	1	17.64 ± 0.37	0.23 ± 0.03	6.09 ± 0.32	1.24 ± 0.26	14.25 ± 1.08	0.84 ± 0.10
ASE with methanol–acetic acid	2	16.81 ± 0.89	0.48 ± 0.07	5.22 ± 0.45	1.60 ± 0.14	14.14 ± 0.62	1.98 ± 0.10
Sonication with formic acid	3	16.16 ± 0.53	0.33 ± 0.05	6.00 ± 0.25	1.66 ± 0.23	14.34 ± 0.60	1.84 ± 0.16

intensity) in each collection. Quantitative recovery was found for DORM-2, while recoveries of 70% and 79% were found for DOLT-2 and TORT-2, respectively, which supports the theory that matrix effects in some way contribute to incomplete recovery. The recovery of the analyte based on standard additions was also investigated. The AsB species used in the standard addition was recovered quantitatively from all three tissues. Therefore, for DOLT-2 and TORT-2 there is a difference between the recovery of the sample alone and the recovery of the sample with standard addition. It follows then, that for DOLT-2 and TORT-2, where recoveries of endogenous arsenic were low, the quantified result will be lower than the correct concentration in the extract. Even though the sum of species in DORM-2 was also less than the total arsenic quantified in the arsenic extracts, it does agree well with the certified value of arsenic. Quantitative recovery of arsenic in the DORM-2 extracts from the HPLC column suggest the discrepancy in this case is more likely caused by quantitation errors.

In a similar experiment, the effect of HPLC eluent on the arsenic signal intensity was also investigated. The total arsenic in the sample collected without the column in place was compared with the collection of the sample with only water as eluent in the LC pump. This was done with the aqueous/sonication extract of each tissue for HPLC protocol 3 employed in the study. For each tissue the arsenic signal generated in H_2O was only 60% of the signal found when the CRM was collected in the HPLC eluent. However, theoretically, this will only cause errors in quantification if the retention time of the species significantly changes during the set of standard addition injections, in such a way that the species elutes with a different concentration of HPLC eluent (due to the gradient elution). This should also only affect HPLC protocols 1 and 3 as the other two employ an isocratic elution. However, the retention times are repeatable for both of the HPLC protocols employing gradients. From Table 5 it is clear that there is no appreciable difference between results generated from HPLC protocols 1, 2 and 3 and so differences in the As signal generated from differences in HPLC eluent seem to contribute to a very minor error, if any, in the quantified results.

The possibility that erroneously quantified species could also cause discrepancies in the mass balance budget was also considered. The arsenic species stock solutions were checked with the arsenic standard used to quantify the total arsenic in the aqueous extracts and all were found to have concentrations that agreed with the appropriate dilutions. However, there could be trace impurities in the standards that could contribute to errors in species quantification. Another possibility for variations in the data reported may be due to different signal responses[17] of the individual species in the extracts.

Conclusions

The data generated show that a certification of the AsB content of the two CRMs which are not yet certified for this compound is feasible because there is very good agreement between three completely independent methodologies used for the analysis. The certification of DMA and other species in the three materials is currently not possible due to the discrepancy between the data from the various methods. An evaluation of DMA results reported in the literature also indicates that the quantitation of DMA in the materials is method-dependent and that a more conclusive intercomparison, preferably involving a greater range of different extraction, separation and detection methodologies, is required for a meaningful certification value. In addition to the variation in the DMA data highlighted here, a discrepancy between the sum of all detected As-containing species and the total As measured in the extracts was also confirmed during this study. Further work is also needed to elucidate the processes that affect the quantitation of total arsenic in marine tissues by ICP-MS.

It is likely that the discrepancy between the sum of species quantified and the total arsenic found in the extracts is due to matrix effects causing retention of arsenic in the extracts on the HPLC column. The recovery of arsenic in DOLT-2 and TORT-2 tissue is less than the recovery of a spike of arsenic in the tissue and this leads to a result lower than the accurate concentration in the extract. A more detailed set of experiments, performed with large number of replicates at various concentration levels, may shed more light on this problem, which is likely to be different for each tissue and HPLC protocol used. One solution may be extraction procedures that remove the compound of interest completely from the matrix (such as SPME). HPLC recovery and different signals generated in the ICP-MS due to different eluent concentrations induce method errors that are difficult to control. The production and augmentation of certified reference materials will provide the necessary diversity in matrices needed to support the analyst in quality control and the identification of sources of errors in newly developed analytical protocols.

Acknowledgements

R. W. acknowledges funding under a Glazebrook Fellowship Award for a research secondment to the National Research Council, Canada, sponsored by the Department for Trade and Industry (DTI) UK.

S. M. would like to thank NSERC for a Postdoctoral fellowship and the NRC for partial financial support.

References

1 P. Fecher and G. Ruhnke, *At. Spectrosc.*, 1998, **19**, 204.
2 Z. Slejkovec, J. T. van Elteren and U. D. Woroniecka, *Anal. Chim. Acta*, 2001, **443**, 277.
3 Z. Slejkovec, J. T. van Elteren and A. R. Byrne, *Talanta*, 1999, **49**, 619.
4 J. Kirby and W. Maher, *J Anal. At. Spectrom.*, 2002, **17**, 838.
5 W. Goessler, D. Kuehnelt, C. Schlagenhaufen, Z. Slejkovec and K. J. Irgolic, *J. Anal. At. Spectrom.*, 1998, **13**, 183.
6 P. A. Gallagher, S. Murray, X. Wei, C. A. Schwegel and J. T. Creed, *J. Anal. At. Spectrom.*, 2002, **17**, 581.
7 J. W. McKiernan, J. T. Creed, C. A. Brockhoff, J. A. Caruso and R. M. Lorenzana, *J. Anal. At. Spectrom.*, 1999, **14**, 607.
8 T. Dagnac, A. Padro, R. Rubio and G. Rauret, *Anal. Chim. Acta*, 1998, **364**, 19.
9 J. Alberti, R. Rubio and G. Rauret, *Fresenius' J. Anal. Chem.*, 1995, **351**, 420.
10 P. A. Gallagher, X. Wei, J. A. Shoemaker, C. A. Brockhoff and J. T. Creed, *J. Anal. At. Spectrom.*, 1999, **14**, 1829.
11 T. Catterick and R. Wahlen, *Rapid Commun. Mass. Spectrom.*, 2004, **18**, 211.
12 S. McSheehy and Z. Mester, *J. Anal. At. Spectrom.*, 2004, **3**, 373.
13 S. McSheehy, J. Szpunar, R. Morabito and P. Quevauviller, *Trends Anal. Chem.*, 2003, **22**, 191.
14 R. O. Jenkins, A. W. Ritchie, J. S. Edmonds, W. Goessler, N. Molenat, D. Kuehnelt, C. F. Harrington and P. G. Sutton, *Arch. Microbiol.*, 2003, **180**, 142.
15 U. Kohlmeyer, J. Kuballa and E. Jantzen, *Rapid Commun. Mass Spectrom.*, 2002, **16**, 965.
16 M. E. Horb, C.-N. Shen, D. Tosh and J. M. W. Slack, *Curr. Biol.*, 2003, **13**, 105.
17 E. H. Larsen and S. Sturup, *J. Anal. At. Spectrom.*, 1994, **9**, 1099.