

Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed *Laminaria*

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Algae contain high amounts of arsenic in the form of arsenosugars. The metabolism and toxicology of these arsenic species are not yet fully understood. Three sets of experiments have been conducted in which the alga *Laminaria* was ingested by 2 to 5 healthy volunteers. Total arsenic concentrations in urine and in blood, packed blood cells and serum have been determined using ICP-MS and HGAFS, respectively. Neutron activation analysis was used for the determination of the total arsenic content in algae samples. Speciation analysis of urine and serum samples has been carried out using HPLC-ICP-MS. HPLC-ES-MS/MS has been used for structural confirmation. The stability of the arsenosugars in simulated gastric fluid was studied for both boiled and unboiled seaweed. A maximum level of arsenic in urine appears within 15 to 25 h after ingestion. Total arsenic and speciation analysis revealed no marked increase in arsenic blood, serum and packed cells levels up to 7 h after ingestion. Dimethylarsinic acid (DMA), methylarsonic acid (MA) and dimethylarsinoylethanol (DMAE) have been positively identified in urine sampled after algae intake. Another 5 species remain unknown. In simulated gastric fluid incubated with algae, the larger share of the arsenosugars degrade within a short time span into a compound with a mass of 254 Da.

Introduction

Arsenic levels in edible seaweeds amount to 200 mg kg⁻¹ (dry mass). Such concentrations are several orders of magnitude higher than the arsenic levels in seawater (1–2 µg L⁻¹).¹ Algae possess the ability to convert toxic inorganic arsenate from the surrounding seawater into arsenic-containing carbohydrate derivatives, denoted as arsenosugars. It is supposed that this synthesis of organoarsenic compounds by the algae is the result of a detoxification process. A pathway has been described by Francesconi and co-workers.^{2–5} This involves a twofold reduction and oxidative methylation of the inorganic arsenic species into dimethylarsinic acid (DMA), and binding to the ribose moiety of *S*-adenosylmethionine. Although about 16 arsenosugars have been identified so far, four predominate. For ages, algae have been a major part in the diet of Chinese, Japanese and Korean people. However, arsenosugars do not solely appear in algae, but also in crustaceans (lobster, crab...) and bivalves (mussels, oysters, clams...).⁶ As a consequence, arsenosugars do enter the diet of Western people as well. Along with their dietary intake, questions concerning possible toxic aspects of these algae may arise. It is well known that the toxicity of arsenic is highly dependent on the chemical form. Inorganic arsenic compounds (arsenite and arsenate) are highly toxic, whereas organic forms of arsenic, such as arsenobetaine (AB) and tetramethylarsonium ion (TETRA) are considered innocuous. DMA and methylarsonic acid (MA) are believed to have an intermediate toxicity.¹ Whereas the metabolism and excretion pattern of inorganic arsenic and several organic arsenicals such as arsenobetaine in humans are well known, not much information on the impact of arsenosugars on humans is available. It has been shown that DMA is the main metabolite after ingestion of arsenosugar-containing food. From a toxicological point of view, this may raise questions as the toxicity of DMA is higher than that of arsenosugars.

Moreover, speciation analysis in urine reveals several unknown metabolites.

It is the aim of this study to investigate urine, blood and seaweed samples in order to gain information about the identity of the urinary metabolites as well as about the metabolism of the arsenosugars in the stomach, gastrointestinal tract, blood and urine. During the past 2 years, several studies were conducted in which volunteers ingested a portion of boiled or non-boiled *Laminaria*. *Laminaria* was chosen because it contains three different arsenosugars and because it is, together with *Porphyra*, the predominant alga in the Chinese cuisine. We would like to report not only on some findings which confirm those of other researchers, but also on some new ones, which may give better insight in the metabolism of arsenosugars by man.

Experimental

Reagents, standards and samples

All reagents were of *pro analysis* grade unless otherwise mentioned. (NH₄)₂HPO₄ was from Shanghai Chemical Reagent (Shanghai, P.R. China). Pyridine was from Shanghai Chemical Reagent (Shanghai, P.R. China) and from Vel (Leuven, Belgium). Methanol (HPLC grade) was purchased from Tian Jin Si You Biochemical (Beijing, P.R. China) and from Panreac (Belgium). Ammonium acetate, acetonitrile, formic acid and diethyl ether were from Merck-Eurolab (Leuven, Belgium). Ammonium oxalate monohydrate (99.5%) and sodium borohydride (>97%) were purchased from Fluka (Steinheim, Germany). Sodium hydroxide was bought from Carlo Erba (Milano, Italy). Hydrochloric acid 37% (Panreac, Barcelona, Spain), nitric acid 65% and sulfuric acid >95% (both from Vel, Leuven, Belgium) were further purified by sub-boiling distillation. Perchloric acid 70% was purchased from Vel (Leuven,

Table 1 List of arsenic species

Compound	Chemical formula	Mol. mass/Da
1. Arsenic acid (As ^v)	H ₃ AsO ₄	142
2. Arsenous acid (As ⁱⁱⁱ)	H ₃ AsO ₃	126
3. Dimethylarsinic acid (DMA)	(CH ₃) ₂ As(O)OH	138
4. Methylarsonic acid (MA)	CH ₃ As(O)(OH) ₂	140
5. Dimethylarsinoylethanol (DMAE)	(CH ₃) ₂ As(O)CH ₂ CH ₂ OH	166
6. Arsenobetaine (AB)	(CH ₃) ₃ As ⁺ CH ₂ COO ⁻	178
7. Arsenocholine (AC)	(CH ₃) ₃ As ⁺ CH ₂ CH ₂ OHBr ⁻	165
8. Trimethylarsine oxide (TMAO)	(CH ₃) ₃ AsO	136
9. Tetramethylarsonium ion (TETRA)	(CH ₃) ₄ As ⁺ I ⁻	135

10. Arsenosugar OH (OH)	R = OH	328
11. Arsenosugar PO ₄ (PO ₄)	R = OP(O)(OH)OCH ₂ CH(OH)CH ₂ OH	482
12. Arsenosugar SO ₃ (SO ₃)	R = SO ₃ H	392
13. Arsenosugar SO ₄ (SO ₄)	R = OSO ₃ H	408

Belgium), creatinine was obtained from Acros (Geel, Belgium) and pepsin from Sigma (Bornem, Belgium). Deionised water was used throughout the whole experiment. 18 MΩ cm deionised water from a Milli-Q system (Millipore, Bedford, MA, USA) was used. Arsenic(v) stock solutions were prepared from Na₂HAsO₄·7H₂O (Sigma, St. Louis, MO, USA). Dimethylarsinic acid sodium salt trihydrate (DMA) 99% was bought from BDH Laboratory Supplies (Poole, UK). Arsenobetaine (AB) and arsenocholine (AC) were bought from Argus Chemicals (Italy). Methylarsonic acid disodium salt (MA) 99.0% was purchased from Chem Service (West Chester, USA). Trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TETRA) were provided by the Commission of the European Communities, Standard, Measurement and Testing Programme. The four arsenosugars 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropylene glycol (arsenosugar OH), 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl 2,3-hydroxypropylphosphate (arsenosugar PO₄), 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropanesulfonic acid (arsenosugar SO₃) and 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl hydrogensulfate (arsenosugar SO₄) and dimethylarsinoylethanol (DMAE) were donated from Prof. Dr K. A. Francesconi (Karl-Franzens University, Graz, Austria). The arsenic species are listed in Table 1. *Laminaria* was bought at a Chinese supermarket in Beijing and stored at room temperature until analysis.

Apparatus

The total arsenic content in urine was measured by means of ICP-MS on an Elan 6000 (PE Sciex, Toronto, Canada). The sample introduction system of this instrument consists of a

cross flow nebuliser and a Scott double pass Ryton spray chamber.

Urinary creatinine was determined by reversed phase HPLC with UV detection on a Waters 600 pump and a Waters 486 UV detector (Waters, Milford, USA).

The arsenic content in blood, serum and packed blood cells was determined by HGAFS using a Millenium Excalibur 10.055 (PSA, Orpington, UK) system.

The HPLC system used in combination with ICP-MS was equipped with a Model 625 metal-free pump (Alltech, Deerfield, IL, USA). Sample introduction was done using a 6-port valve (Valco) and a 50 μL injection loop (Rheodyne, Cotata, CA, USA). A PRP-X100 guard and analytical anion exchange column (Hamilton, Reno, NV, USA) and an Ionpac CS10 guard and analytical cation exchange column (Dionex, Sunnyvale, CA, USA) were used for all chromatographic experiments. The chromatographic conditions are listed in Table 2. The outlet of the HPLC column was connected to the nebuliser of the ICP-MS via a 1/16 in PEEK capillary tube (0.254 mm id). The ICP-MS instrument, which was used in combination with HPLC was either an Elan 5000 or an Elan 6000 (PE Sciex, Toronto, Canada).

A Quattro Micro triple quadrupole system (Micromass, Manchester, UK) in hyphenation with an Alliance 2690 HPLC system (Waters, Milford, USA) was used for HPLC-ES-MS/MS measurements. The outlet of the HPLC column was connected to a tee piece, which diverted 60% of the effluent to the waste.

Ingestion of seaweed and collection of blood and urine samples

Prior to ingestion, *Laminaria* was thoroughly cleaned with tap water and rinsed with deionised water. The volunteers ingested

Table 2 HPLC separation conditions

Technique	Anion exchange	Cation exchange
Column	Hamilton PRP-X100	Dionex Ionpac CS-10
Dimensions guard column	25 × 2.3 mm id, 12–20 μm	50 × 4 mm id, 10 μm
Dimensions analytical column	250 × 4.1 mm id, 10 μm	250 × 4 mm id, 10 μm
Flow rate/ml min ⁻¹	1	1
Buffer	Method 1: 20 mM (NH ₄) ₂ HPO ₄ (pH 7) in 3% MeOH Method 2: 2 mM NH ₄ Ox (pH 6) Method 3: 3 mM NH ₄ Ox (pH 6.5) in 3% MeOH Method 4: 30 mM NH ₄ Oac (pH 6) in 3% MeOH Method 5: 30 mM NH ₄ Oac (pH 6) in 20% MeOH	6:20 mM pyridine (pH 2.7)

^a Ox: oxalate; Oac: acetate.

a portion of 20–25 g (dry mass) of algae either dried or wetted within a time span of 30 min. The total arsenic content in the *Laminaria* is $43.2 \pm 0.4 \text{ mg kg}^{-1}$.⁷ The volunteers refrained from eating arsenic-rich food (fish, crustaceans, molluscs, mushrooms...) 3 days prior to and during the course of the experiment. Heparine vacuum tubes (Venosafe VF-1095 HL Lithium Heparin, Terumo Europe NV, Leuven, Belgium) were used to collect blood for the determination of arsenic in whole blood. Anti-coagulants free vacuum tubes (Venoject II autosep Gel + Clot. Act., Terumo Europe NV, Leuven, Belgium) were used to collect blood destined for the analysis of arsenic in serum (both determination of the total concentration and speciation analysis) and packed cells. Urine was collected in acid-cleaned 500 mL PE bottles. All samples were immediately stored at 4 °C. Prior to speciation analysis, all serum and urine samples were centrifuged and the supernatant thus obtained filtered through a 0.22 µm PVDF syringe filter.

In a first study, unboiled seaweed was ingested by 5 male volunteers: four ethnic Chinese, aged 21, 24, 28 and 45, respectively, and one ethnic European, aged 25. Urine was collected before and up to 120 h after uptake. The total amount of urinary arsenic *versus* time was determined using inductively coupled plasma mass spectrometry (ICP-MS). Creatinine was determined in order to normalise the results for the discrepancies in the urinary behaviour. HPLC-ICP-MS was used for speciation analysis of urine samples.

In a second experiment, unboiled *Laminaria* was eaten by two male ethnic European volunteers, aged 21 and 27. Blood was drawn before and up to 7 h after ingestion. Urine samples were collected up to 60 h after ingestion. Speciation analysis was carried out using both ICP-MS and electrospray mass spectrometry (ES-MS/MS).

In a third experiment, boiled *Laminaria* was ingested by two volunteers (one ethnic Chinese, aged 24, and one ethnic European, aged 27) and serum samples were analysed with hydride generation atomic fluorescence spectrometry (HGAFS).

Incubation of *Laminaria* in SGF and extraction of arsenicals from the seaweed

Simulated gastric fluid (SGF) was prepared according to US Pharmacopeia guidelines.⁸ To prepare SGF, 2.0 g NaCl and 3.2 g pepsin (from porcine stomach mucosa, with an activity of 800 to 2,500 units per mg of protein) were dissolved in 7 mL conc. HCl and water was added to make up to 1 L. This test solution has a pH of about 1.2. 500 mL solution was heated to 37 °C to incubate boiled as well as unboiled *Laminaria*. The mixture was mechanically shaken at this temperature. In the case of boiled *Laminaria* (boiling time 3 min), the algae was first chewed for about 1 min in order to mimic the eating process as well as possible. At different time intervals between 1 min and 4 h a portion of the incubated algae was taken. The *Laminaria* was cleaned, dried and freeze-dried. The dried products were cooled with liquid nitrogen for grinding in a dismembrator. The resulting powders were extracted according to a method similar to that of Shibata and Morita.⁹ A 0.1 to 0.2 g portion was weighed into 12 mL centrifuge tubes. 10 mL of a 1 + 1 H₂O/MeOH mixture was added. The suspension was vortex-mixed, ultrasonicated during 15 min and centrifuged at 5000 rpm during 15 min. The supernatant was transferred into a Petri dish. This procedure was repeated another two times. The collected supernatant fractions were left drying overnight in an oven at 40 °C. The residue was diluted with a minimal amount of deionised water, weighed and stored at 4 °C prior to analysis.

INAA for analysis of total arsenic in algae samples

The total arsenic content in *Laminaria* was measured with instrumental neutron activation analysis (INAA) by irradiation

in the Thetis reactor of Ghent University. Samples were irradiated at a thermal neutron flux of $1.2 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ during 7 h. Iron flux monitors were used to correct for the discrepancies in neutron flux between samples as a result of different positioning *versus* the reactor core. As₂O₃ was used as a standard. The 559 keV γ-rays emitted by the ⁷⁶As (*t*_{1/2} 26.3 h) isotope were measured with a Ge(Li) detector coupled to a multi channel analyser (Canberra, Meriden, CT, USA). The accuracy of the measurement was tested by the analysis of a certified reference material, NIST SRM 1571 orchard leaves (National Institute of Standards and Technology, Gaithersburg, MD, USA), which has a certified value of $10 \pm 2 \text{ mg kg}^{-1} \text{ As}$. The value obtained using our method was $12.4 \pm 0.4 \text{ mg kg}^{-1} \text{ As}$ (*n* = 4).

ICP-MS for analysis of total arsenic in urine samples

The arsenic content in urine was measured by means of ICP-MS. External calibration using an arsenic standard solution was applied. The ICP-MS measurement conditions were optimised on a daily basis. Average values for nebuliser gas flow (1 L min⁻¹), rf power (1300 Watt) and lens voltage (9 V) were obtained. The ion intensities at *m/z* 75 (As⁺) as well as 77 (Se⁺, ArCl⁺) and 82 (Se⁺) were monitored in order to gauge possible ³⁵Cl-based interferences. All standards and samples were diluted with 0.14 M HNO₃ to which 3% MeOH was added in order to improve the signal intensity. The urine samples were diluted 40-fold. Urinary creatinine was measured in order to normalise the different urine samples of each volunteer. The method is based on that of Archari *et al.*¹⁰ 50 µL of a 50-fold diluted urine was chromatographed with 50 mM sodium acetate (pH 6.5) in 98 + 2 water/acetonitrile on a Waters C18 column using a flow rate of 1 mL min⁻¹ and UV detection.

HGAFS for analysis of total arsenic in blood samples

The arsenic content in blood, serum and packed cells was analysed by HGAFS. All samples were digested in open vessels using a mixture of HNO₃, H₂SO₄ and HClO₄ (7:2:1) and a 3-step temperature program. First the sample was heated at 85 °C for 2 h, followed by a heating step at 130 °C (3 h) and 230 °C (3 h). The length of the digestion program was also dependent on the amount of sample added to the vessel. On average, 3 mL serum, 2 mL blood and 1 mL packed cells were digested with 10 mL of the acid mixture. The final solution was diluted to 25 mL with 3 M HCl to which 0.1 g L⁻¹ KI and 0.02 g L⁻¹ ascorbic acid was added in order to reduce all arsenic to its trivalent state. More details can be found in Zhang *et al.*¹¹ Hydride generation was realised by mixing a flow of the sample at a speed of 9 mL min⁻¹ with a stream of 7 g L⁻¹ NaBH₄ in 4 g L⁻¹ NaOH at 4.5 mL min⁻¹. The accuracy of the measurement was tested by the analysis of a certified reference material, freeze-dried human serum,¹² which has a certified value of $19.6 \pm 4 \text{ ng g}^{-1} \text{ As}$. The value obtained with the method we used was $24.0 \pm 1.8 \text{ ng g}^{-1} \text{ As}$ for 3 samples.

HPLC-ICP-MS for speciation analysis of arsenic in urine and serum

Speciation analysis of undiluted urine and serum was carried out using HPLC-ICP-MS. The ICP-MS measurement conditions were optimised on a daily basis. The ion intensities at *m/z* 75 and 51 (ClO⁺) were monitored. Average values for the important settings are listed above. Dwell time was 800 ms for As and 200 ms for the signal at *m/z* 51. The chromatographic conditions are listed in Table 2.

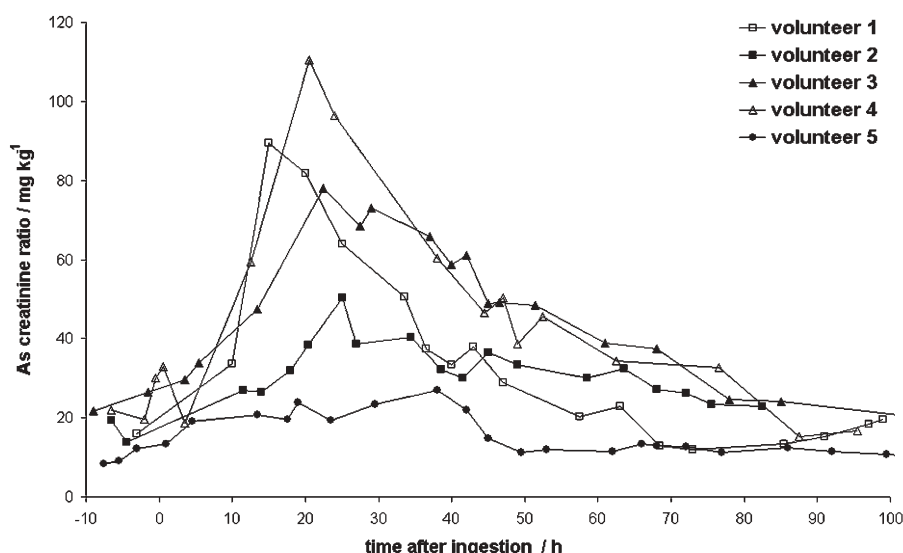


Fig. 1 Urinary creatinine-normalised arsenic concentrations of five volunteers before and after ingestion of *Laminaria*.

HPLC-ES-MS/MS for speciation analysis of arsenic in urine and *Laminaria* extracts

The same columns were used as with HPLC-ICP-MS. Depending on the arsenic concentration, 20–100 μL was injected on the column. The photo multiplier was set at 650 V. Desolvation temperature and desolvation gas flow were set at 350 $^{\circ}\text{C}$ and 650 L h^{-1} , respectively. An intermediate capillary voltage value of 3.0 kV was used. All samples were analysed using multiple reaction monitoring (MRM). In MRM, both quadrupoles are set at fixed masses of parent ion and fragment ion, respectively, and collision induced dissociation (CID) occurs. A typical parent–daughter transition is monitored. The method has been successfully applied for the analysis of arsenic in seaweed extracts.⁷

Results and discussion

A plot of total urinary arsenic/creatinine ratio *versus* time of ingestion for five individuals (experiment 1) is shown in Fig. 1. It can be seen that for volunteers 1 to 4 a maximum arsenic/creatinine ratio is obtained within 15 to 25 h after ingestion. Only low concentrations are reached for volunteer 5, a small

maximum can be seen at 38 h. Volunteers 1 to 4 are ethnic Chinese, whereas volunteer 5 is an ethnic European. This might explain the different background level of arsenic in urine. From the plot, it might be concluded that volunteer 5 behaves different in comparison to the other volunteers. However, in absolute amounts, the urinary arsenic levels of volunteer 5 are not significantly below the others. The highest value was found in the urine of volunteer 4 (228 ng mL^{-1}), followed by volunteer 2 (158 ng mL^{-1}), volunteer 3 (141 ng mL^{-1}), volunteer 1 (72 ng mL^{-1}) and volunteer 5 (70 ng mL^{-1}). On the other hand, the mean urinary creatinine value of volunteer 5 was higher than for volunteers 1, 2 and 3. Volunteer 4 showed the highest mean creatinine value. All concentration levels return to background levels after about 80 h. The results correlate well with those obtained by Le *et al.*,¹³ Ma and Le¹⁴ and Francesconi *et al.*¹⁵ Le *et al.* found that 9 volunteers who ingested 9.5 g of nori showed maximum concentrations 10–60 h after ingestion. Their values, however, were not normalised *versus* creatinine. Using non-normalised data we also obtained a broader time span. According to Ma and Le, the highest concentrations in the urine of the 4 volunteers who ingested 9.5 g of nori were found between 22 and 38 h after ingestion. In the study by Francesconi *et al.* one volunteer obtained the

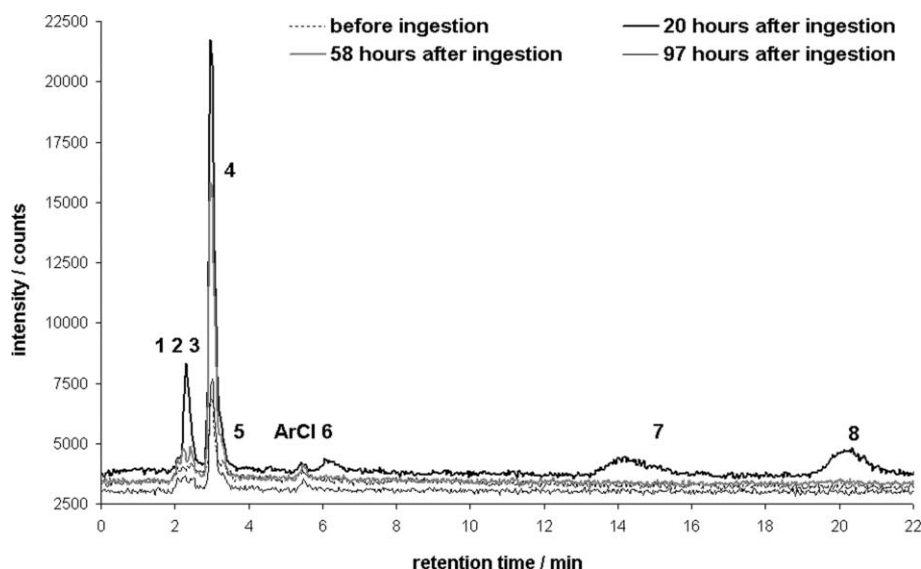


Fig. 2 Chromatogram of urine of 1 volunteer at different sampling times relative to ingestion of *Laminaria*. Detection with ICP-MS. For HPLC conditions: see Table 2 (Method 1).

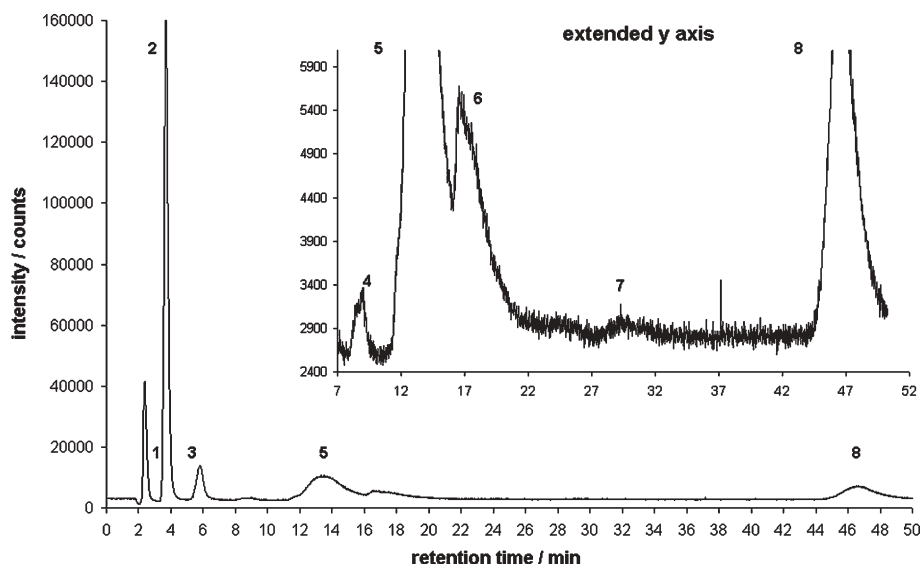


Fig. 3 Chromatogram of urine of 1 volunteer 17 h after ingestion of *Laminaria*. Detection with ICP-MS. For HPLC conditions: see Table 2 (Method 4).

maximum normalised arsenic value 27 h after ingestion of a pure arsenosugar.

Fig. 2 shows four anion exchange HPLC-ICP-MS chromatograms of urine from a Chinese volunteer obtained at different points of time relative to seaweed intake. A total of 8 arsenic containing peaks can be observed. Similar chromatograms were obtained for the other 3 Chinese volunteers. The urine of the European volunteer showed a slightly different pattern. The reason for this is not clear. None of the 5 volunteers had problems in digesting the seaweed or felt uncomfortable during the course of the experiment. Le *et al.* and Ma and Le noted that different individuals metabolise arsenosugars in different ways. In our study, however, the chromatograms of the 5

volunteers are quite similar. The chromatograms of the 4 Chinese people are identical, whereas a slight change can be seen in the chromatogram of volunteer 5. By spiking the urine samples with standards, it could be concluded that peak 4 is DMA, and that peak 5 (right shoulder of peak 4) is MA. By analysing the signal at m/z 51 (ClO^+), it could also be assumed that the peak at a retention time of 5.5 min is due to ArCl^+ interference. The results correlate well with those obtained by Francesconi *et al.* They noted 4 major and 4 minor peaks in the urine of a 47 year old male volunteer, 27 h after the ingestion of a pure arsenosugar. Differences in the mobile phase composition make a direct comparison difficult, but most probably the four major peaks found in their study are identical to peaks 2, 4, 7 and 8 in our chromatograms. None of the peaks in the anion exchange chromatogram, except for those in the void volume, match with the original arsenosugars in the seaweed extract. Because arsenosugar OH shows no retention in anion exchange mode, cation exchange chromatography was applied. No peak was found in the urine at the retention time of arsenosugar OH. From the first experiment it can thus be concluded that the arsenosugars are completely transformed into DMA, MA and unknown metabolites.

Fig. 3 shows the anion exchange HPLC-ICP-MS chromatogram of urine from volunteer 1 (experiment 2) collected 17 h after ingestion of *Laminaria*. The similarity with the urine samples of the five volunteers from the first experiment is good. The time needed to elute all arsenicals from the column is longer because the elution strength of 30 mM ammonium acetate at pH 6 is lower than 30 mM ammonium phosphate at pH 7. The urine samples were also subjected to HPLC-ES-MS/MS and further on, fractions obtained after HPLC separation (both anion and cation exchange) were analysed using direct infusion ES-MS. Fig. 4 shows a HPLC-ES-MS/MS chromatogram of the same urine sample separated with 30 mM ammonium acetate (pH 6) to which 20% MeOH was added. Two MRM transitions are recorded for both DMA (4a) and MA (4b). The detection of the molecular ion signal as well two daughter fragments unambiguously proves the presence of both compounds.

Cation exchange chromatography was also applied on selected urine samples. Using both ICP-MS (figure not shown) as well as ES-MS/MS (Fig. 5) the presence of dimethylarsinoylethanol (DMAE) could be confirmed. In Fig. 5, two MRM transitions of DMAE in a urine sample are shown. Moreover, spiking revealed an increase in intensity at the same retention time (not shown). Francesconi *et al.*¹⁵

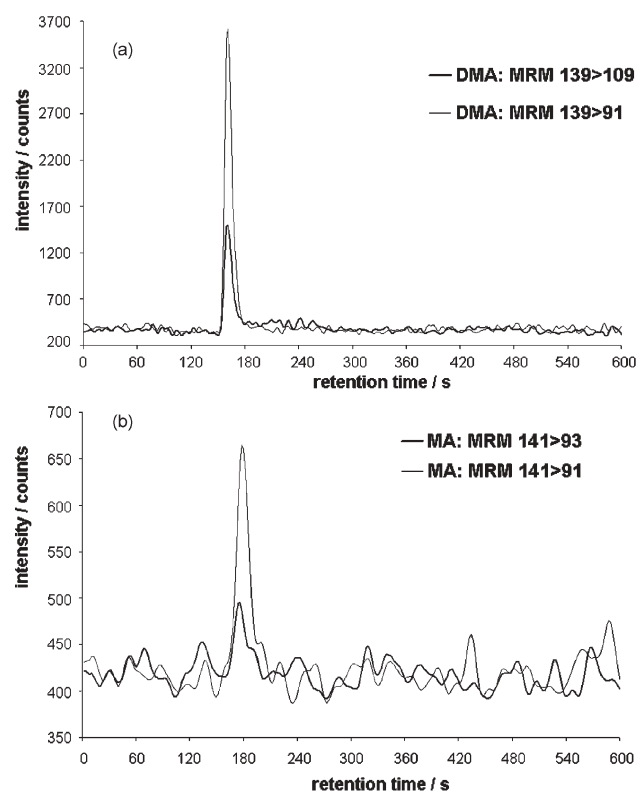


Fig. 4 Chromatogram of urine of 1 volunteer 17 h after ingestion of *Laminaria*. Detection of DMA (4a) and MA (4b) with ES-MS/MS. For HPLC conditions: see Table 2 (Method 5).

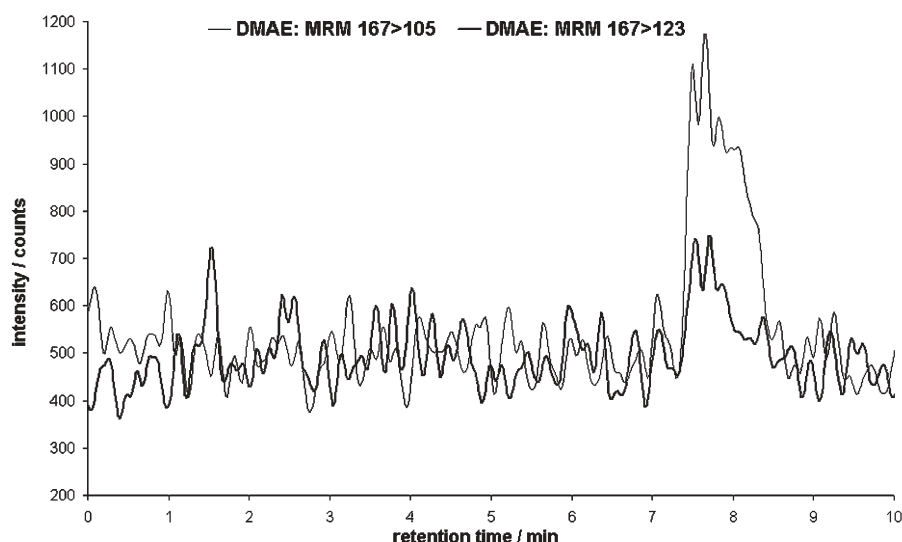


Fig. 5 Chromatogram of urine of 1 volunteer at 17 h after ingestion of *Laminaria*. Detection of DMAE with ES-MS/MS. For HPLC conditions: see Table 2 (Method 6).

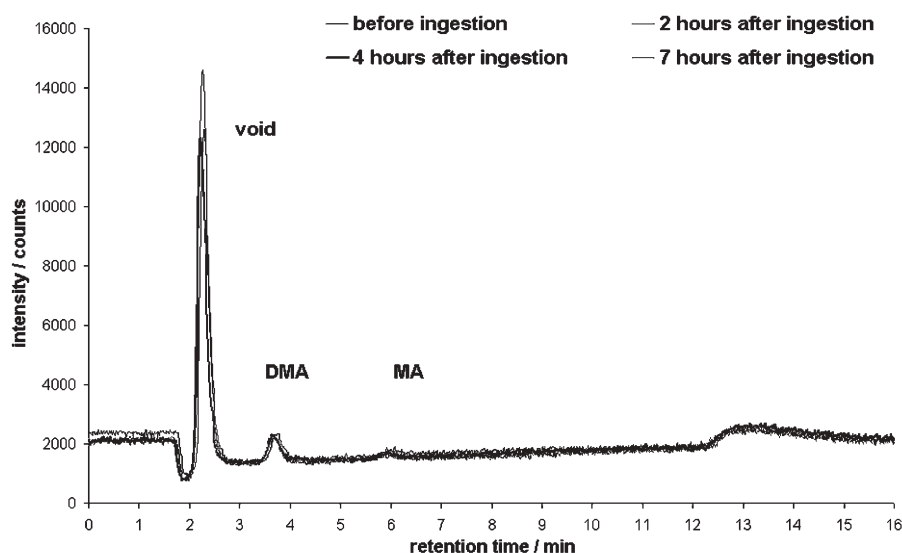


Fig. 6 Chromatogram of serum of 1 volunteer at different sampling times relative to ingestion of *Laminaria*. Detection with ICP-MS. For HPLC conditions: see Table 2 (Method 4).

were first to report the presence of DMAE in urine after ingestion of arsenosugars by means of HPLC-ICP-MS and HPLC-ES-MS on a single quadrupole instrument. In their paper, Francesconi *et al.* postulate that DMAE is likely to be originating from the degradation of the arsenosugars in the gut microflora, rather than from the addition of the $\text{CH}_2\text{CH}_2\text{OH}$ moiety to DMA. Their hypothesis is supported by experimental evidence.¹⁶

Serum samples of both volunteers were also subjected to speciation analysis. Fig. 6 shows a HPLC-ICP-MS chromatogram of 4 serum samples from volunteer 2. The samples were taken before and 2, 4 and 7 h after ingestion of the algae. Surprisingly no change in the arsenic profile could be seen. It was estimated that the residence time of food in the stomach and small intestines is shorter than 7 h. Moreover, uptake of arsenic species by the blood is believed to be fast and its elimination efficient in case of normal renal function. The peak in the void volume is not due to arsenite as spiking with this compound showed two closely eluted peaks. To find out whether or not arsenic was taken up by the packed cell fraction, whole blood, serum and packed cells were analysed for their

total arsenic content. Therefore, in a third experiment, blood samples were collected before and 4 and 7 h after ingestion of *Laminaria*. The results are shown in Table 3. In whole blood as well as in serum and packed cells, the concentration of arsenic does not significantly change. Only one sub-sample was available. It was measured in threefold.

The good agreement between our findings and those of Francesconi *et al.* possibly implies that the different arsenosugars

Table 3 Arsenic concentration in the different blood compartments of two volunteers

		Concentration $\pm$ sd/ng g ⁻¹		
		Before	After 4 h	After 7 h
Volunteer 1	Blood	4.4 $\pm$ 0.5	4.1 $\pm$ 0.5	5.7 $\pm$ 0.3
	Packed cells	8.5 $\pm$ 1.4	6.0 $\pm$ 0.7	9.9 $\pm$ 2.2
	Serum	2.2 $\pm$ 0.3	2.2 $\pm$ 0.3	2.4 $\pm$ 0.3
Volunteer 2	Blood	1.9 $\pm$ 0.1	1.8 $\pm$ 0.1	1.7 $\pm$ 0.2
	Packed cells	2.0 $\pm$ 0.2	2.5 $\pm$ 0.1	2.0 $\pm$ 0.1
	Serum	0.8 $\pm$ 0.5	1.1 $\pm$ 0.6	2.0 $\pm$ 1.0

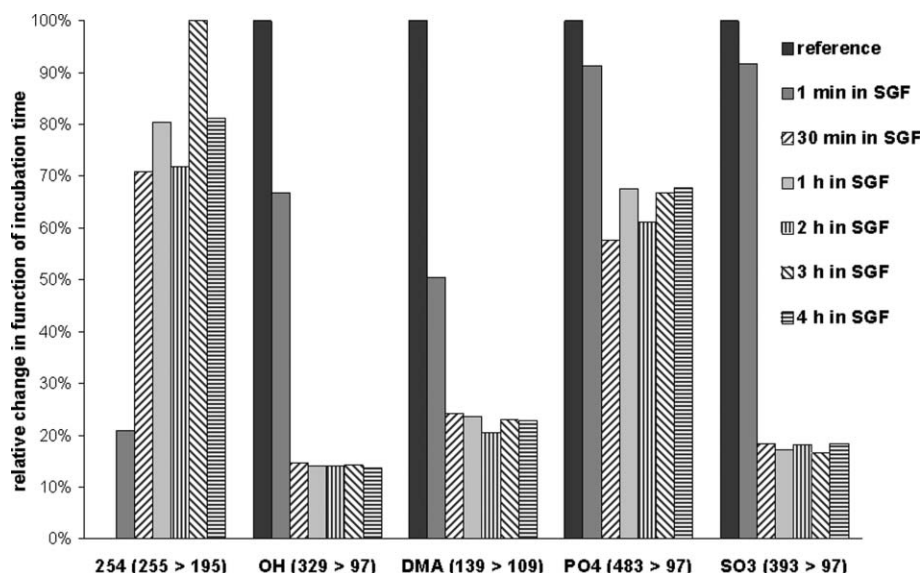


Fig. 7 Relative change of arsenic species in boiled *Laminaria* as a function of incubation time in simulated gastric fluid. Detection with ES-MS/MS and MRM transitions shown between brackets. For HPLC conditions: see Table 2 (Method 2).

give rise to the same metabolites. It might therefore be expected that the arsenosugars are first transformed into a compound, which is identical for all arsenosugars, and is then further metabolised in the body. Gamble *et al.*¹⁷ have published a paper on the stability of arsenosugars in an acid environment and in simulated gastric fluid (SGF). They reported that solutions of arsenosugars are slowly degraded (1.4% per h at 38 °C) into a compound with mass 254 Da. Their measurements were done using both HPLC-ICP-MS and HPLC-ES-MS/MS. The degradation involves an acid hydrolysis of the acetal function at C1 of the ribose and leads to a compound which is identical to all arsenosugars. Fig. 7 shows a plot of the relative change in concentration of the individual compounds present in *Laminaria*. Whereas INAA measurements show that the total arsenic content in each sample remains stable between 14.3 and 16.3 mg kg⁻¹ As, the amount of arsenosugars and DMA decreases and the compound with mass 254 Da ($[M + H]^+ m/z$ 255 in positive mode of ES-MS/MS) increases. As the ion intensity is different for these 5 compounds, the results are shown as relative changes. The MRM transition recorded were 255 > 195 for the compound with mass 254 Da, 329 > 97 (arsenosugar OH), 139 > 109 (DMA), 483 > 97 (arsenosugar PO₄) and 393 > 97 (arsenosugar SO₃). Compared to Gamble *et al.*, the breakdown of the arsenosugars seems to proceed more rapidly. Urine samples have been analysed for the presence of this 255 compound as well. However, it was not found. Therefore this compound is believed to be further metabolised to compounds which can be observed in the chromatograms in Figs. 2 and 3. The results for the incubation experiment of non-boiled *Laminaria* are similar. However, it could be seen that the breakdown of the arsenosugars was slower and that DMA remained stable.

Conclusions

DMA, MA and DMAE in urine are positively identified as metabolites of arsenosugars. The presence of the protonated mass $[M + H]^+$ and of two daughter ion fragments of each compound in the HPLC-ES-MS/MS chromatograms unambiguously confirms this. Possibly all arsenosugars are first transformed into a compound of mass 254 Da, before they are further metabolised. The arsenic levels in the different blood

compartments do not significantly change within 7 h after ingestion.

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