A New Perspective in Arsenic Speciation: Simultaneous Separation of 17 Inorganic and Organic Arsenic Species in Marine Biota by Means of HPLC/ICPMS

Ute Kohlmeyer, Jürgen Kuballa, Eckard Jantzen

GALAB, Max-Planck-Str. 1, D-21502 Geesthacht, tel +49 (0)4152 889400, fax -889401, www.galab.de, info@galab.de

Introduction

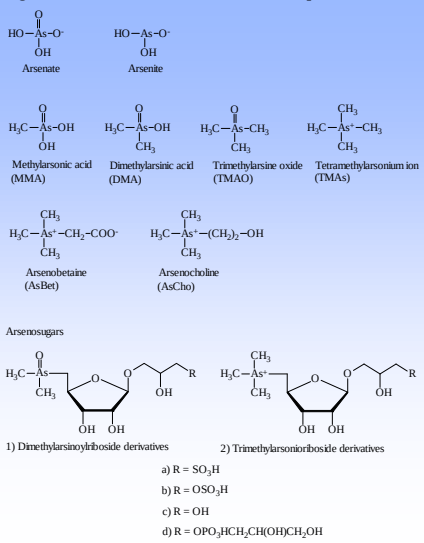
Marine organisms show a great variety of arsenic species, concentrations being much higher than those in seawater. Along with the inorganic forms of arsenic, namely arsenite and arsenate, a number of organic arsenic compounds like monomethylarsonic acid, dimethylarsinic acid, trimethylarsineoxide, the tetramethylarsonium ion, arsenobetaine, arsenocholine and several arsenosugars (derivatives of dimethylarsinoylribosides and trimethylarsonioribosides) are found (Figure 1).

Depending on the kind of organism, different patterns of arsenic species are observed. Arsenobetaine is the major compound in marine animals whereas arsenosugars and arsenate are dominant in marine algae.

Arsenic speciation is a means for metabolism studies as well as for the evaluation of the toxicity of seafood. In contrast to tin- and mercury species, inorganic arsenic is much more toxic than the organic forms of arsenic, which are considered to be only little- or even non-toxic.

The hyphenation of HPLC and ICPMS combines a powerful separation method with an element-specific detector at trace level. The challenge of arsenic speciation is based on the nature of arsenic forms: They differ in charge, molecule size and functional organic groups. Up to now, a complete characterization of a sample was only possible by running combinations of different separation mechanisms. With the method presented here, a separation of at least 17 species was achieved during a single chromatographic run on an anion-exchange column, using a nitric acid gradient and an ion-pairing reagent.

Figure 1 Chemical structures of arsenic compounds



Experimental

For each of the marine organisms – namely fish, bivalve and algae – one or two samples were analyzed as an example to represent the variety of arsenic species determinable with this method. The species were extracted from the biological matrix by means of a methanol/water mixture (3:1) and a dispersion unit within a few minutes. The extract was filtered and diluted 10 to 100-fold prior to injection into the chromatography system.

An ion-chromatograph, consisting of a gradient pump, a Rheodyne injection valve, capillary PEEK-tubing and a 200 µL injection loop, was connected to an anion-exchange column and coupled to an ICP-MS instrument, equipped with a Meinhard concentric nebulizer and a Scott-type glass spraychamber.

The separation was performed using a nitric acid gradient between pH 3,4 and pH 1,8. 0,05 mM benzene-1,2-disulfonic acid dipotassium salt was added as ion-pairing reagent.

At the outlet of the separation column, internal standard (10 µg/L rhodium in 0,01 M HNO_3) was added by means of a Y-connector in order to correct changes in instrument sensitivity over time.

Quantitation of the arsenic content of each species was based on an external three-point-calibration with the arsenic species available (evaluation by peak area). The concentration of the tetramethylarsonium-ion and the arsenosugars were evaluated applying the calibration equation of arsenocholine for TMAs and dimethylarsinic acid for the arsenosugars.

Spiking experiments were carried out for all sample extracts. The accuracy was tested with CRM DORM-2 (dogfish muscle), Table 1.

Results

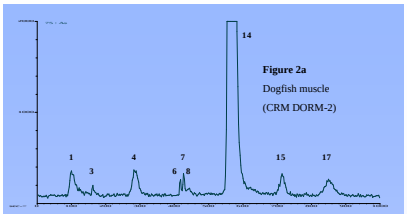


Figure 2a Dogfish muscle (CRM DORM-2)

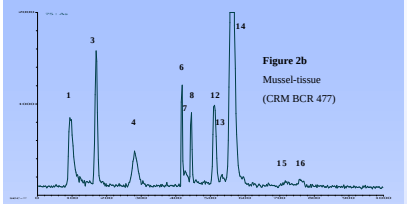


Figure 2b Mussel-tissue (CRM BCR 477)

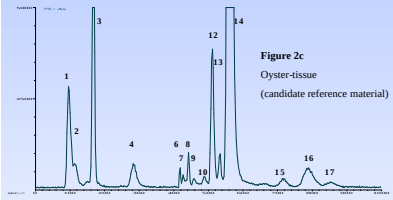


Figure 2c Oyster-tissue (candidate reference material)

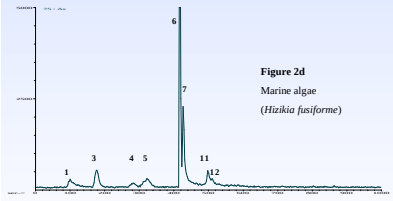


Figure 2d Marine algae (*Hizikia fusiforme*)

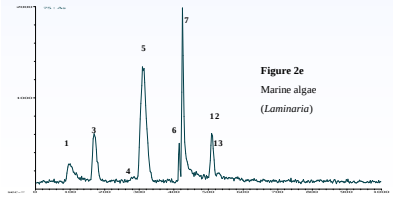


Figure 2e Marine algae (*Laminaria*)

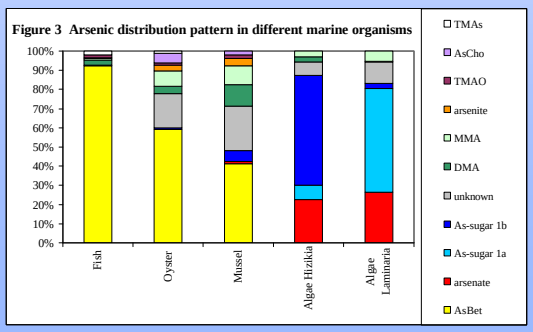
Table 1 Method characteristics

	Conc. range [µg.L^{-1}]	Slope (x10^6)	Intercept (x10^4)	R^2	Detection limit [µg.g^{-1}]	Results DORM-2 [µg.g^{-1}]	Certified value [µg.g^{-1}]
As(III)	0,02-0,6	10,1	-0,8	0,9998	0,008	0,05±0,01	
MMA	0,08-2,4	3,9	-1,7	0,9995	0,040	0,14±0,02	
DMA	0,08-2,4	4,6	-1,1	0,9999	0,035	0,49±0,03	
As(V)	0,08-2,4	2,4	-0,8	0,9997	0,080	0,05±0,02	
AsBet	1,0-30	5,1	25	0,9991	0,035	16,1±0,7	16,4±1,1
TMAO	0,08-2,4	5,0	-1,2	0,9991	0,035	0,30±0,03	
AsCho	0,08-2,4	5,0	0,2	0,9999	0,030	<0,03	
TMAs	0,08-2,4	5,0	0,2	0,9999	0,030	0,30±0,02	0,25±0,05

Table 2 Arsenic speciation – Results

Peak no.	t_R [min]	compound	**fish** µg.g^{-1} as As DORM-2	**mussel** µg.g^{-1} as As BCR477	**oyster** µg.g^{-1} as As candidate mat.	**algae** µg.g^{-1} as As *Hizikia fusif.*	**algae** µg.g^{-1} as As *Laminaria*
1	1,62	As(III)	0,05	0,35	0,71	-	-
2	1,89	*Unknown1*	-	-	0,13	-	-
3	2,82	MMA	0,14	0,84	2,10	1,75	2,74
4	4,71	DMA	0,49	0,94	0,97	1,32	0,27
5	5,21	As-sugar 1a	-	-	-	4,17	28,2
6	6,97	As-sugar 1b	-	0,49	0,17	31,4	1,37
7	7,12	As(V)	0,05	0,10	0,08	12,3	13,7
8	7,38	*Unknown2*	-	0,56	0,42	-	-
9	7,64	*Unknown3*	-	-	0,08	-	-
10	8,14	*Unknown4*	-	-	0,13	-	-
11	8,31	*Unknown5*	-	-	-	2,41	-
12	8,52	*Unknown6*	-	1,36	3,36	1,10	5,2
13	8,89	*Unknown7*	-	0,03	0,48	-	0,55
14	9,35	AsBet	16,1	3,49	15,1	-	-
15	12,1	TMAO	0,30	0,14	0,31	-	-
16	13,0	AsCho	-	0,17	1,24	-	-
17	14,2	TMAs	0,30	-	0,34	-	-
Sum of arsenic species			17,4	8,5	25,6	54,6	52,0
Total arsenic (digestion)			17,4	10,2	26,7	51,2	49,5
Extraction efficiency			100%	83%	96%	107%	105%

Figure 3 Arsenic distribution pattern in different marine organisms



Instrument settings

IC	Dionex 300, AGP Standard
Guard Column	IonPak AG7 (50 x 4 mm)
Analytical Column	IonPak AS7 (250 x 4 mm)
Flowrate	1 mL/min
Mobile phase A	0,5 mM nitric acid, 0,05 mM benzene-1,2-disulfonic acid dipotassium salt, 0,5%methanol
Mobile phase B	50 mM nitric acid, 0,05 mM benzene-1,2-disulfonic acid dipotassium salt, 0,5%methanol
Gradient programme	0-3 min 100% A (pH 3,4)
	3-4 min linear gradient to 50% A, 50% B
	4-12 min 50% A, 50% B (pH 1,8)
	12-13 min linear gradient to 100% A
	13-18 min 100 % A (pH 3,4)
ICP-MS	Agilent 7500s
Power	1210 W
Plasma gas flowrate	Ar, 15 l/min
Carrier gas flowrate	Ar, 1,0 L/min
Aux. Gas flowrate	Ar, 0,9 L/min
Peristaltic pump flowrate	50 rpm
Sampling Cone	Pt
Skimmer Cone	Pt
Monitored signals	^{75}As (500 ms), ^{103}Rh (500 ms), ^{35}Cl (10 ms)), ^{53}Cr (500 ms)

Conclusion

A reliable and stable HPLC/ICPMS method has been established: Preceded by a fast and efficient extraction, the method is capable of determining both inorganic and most of the organic arsenic species which have been discussed so far. It allows to get a clear overview of all major and minor arsenic compounds in different marine organisms in one chromatogram, including a clear separation of the toxic species from the less- and non-toxic compounds. Qualitative representation can be found in the retention time and quantitative representation in the peak area. Therefore, comparison of different samples for metabolism study and toxicity evaluation becomes much easier. In addition, low detection limits were achieved, especially for the toxic arsenite, the most critical compound in seafood.

Spiking experiments revealed, that the retention times of the compounds were constant in the different biological matrices, so that even unknown species could be directly compared in the marine organisms. The resolution of the separation proved to be very successful and, as a result, the risk of unrecognized co-elution was estimated to be negligible. A confirmation of the compound purity will be achieved by coupling of the chromatography to an ESI-MS/MS system in the near future. The coupling of this system to an ESI-MS/MS is also essential for characterization of the numerous arsenosugars, which could not be identified in this work because standard solutions are difficult to obtain.