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Supporting Information

for the Paper

Mapping of arsenic species and identification of a novel arsenosugar in giant clams *Tridacna maxima* and *Tridacna derasa* using advanced mass spectrometric techniques

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Contents:

Standards of arsenic species:

Cacodylic acid (DMA) (puriss., >99%) and monosodium acid methane arsonate (MMA) (sesquihydrate) were obtained from Fluka, Buchs, Switzerland and Chem Service, West Chester, PA, respectively. Arsenobetaine (AsB) (BCR-626) was obtained from the Institute for Reference Materials and Measurements, Geel, Belgium.
Further arsenic species were derived from earlier studies at which they were either isolated from marine organisms (DMArsenoSugar-AminoSulfonate, -Glycol, -Phosphate, -Carboxyl, -Carbamate and –Methoxy) [ref. I, II] or synthesized (TMArsenoSugar-Glycol, -Phosphate, -Sulfonate, -Sulfate, -Methoxy [ref. III], DMAsSugar-Adenine [ref. IV], trimethylarsonio-propionate (AsB-2) and trimethylarsoniobutyrate (AsB-3) [ref. V], arsenocholine (AsC), tetramethylarsonium ion (TMA) and trimethylarsine oxide (TMAO) [ref. VI]).
DMAsSugarHydroxy and TMAsSugarHydroxy were prepared by acidic hydrolysis of DMAsSugarMethoxy and TMAsSugarPhosphate, respectively. [ref. VII]
An aqueous extract of kelp powder was used as source of DMAsSugarSulfonate, DMAsSugarSulfate, DMThioAsSugarGlycol, DMThioAsSugarPhosphate, DMThioAsSugar-Sulfonate and DMThioAsSugarSulfate. [ref. VIII]
DMAsSugarMannitol and dimethylarsinoylacetic acid have been identified in extracts of a granulated kelp sample. [ref. IX]

References:

I Edmonds, J.S., Francesconi, K.A., *J. Chem. Soc. Perkin Trans. I*, **1983**, 2375-2381.
II Francesconi, K.A., Edmonds, J.S., Stick, R.V., Skelton, B.W., White, A.H., *J. Chem. Soc. Perkin Trans. I*, **1991**, 2707-2716.
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IV Francesconi, K.A., Edmonds, J.S., Stick, R.V., *J. Chem. Soc. Perkin Trans. 1*, **1992**, 1349-1357
V Francesconi, K.A., Gailer, J., Edmonds, J.S., Goessler, W., Irgolic, K.J., *Comp. Biochem. Physiol. C*, **1999**, 122, 131-137.
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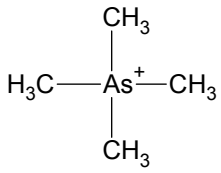
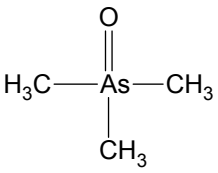
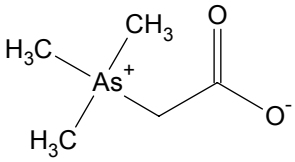
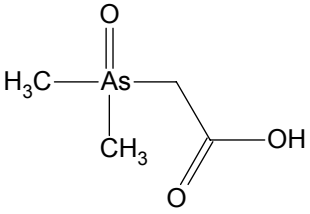
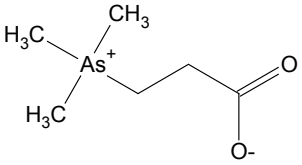
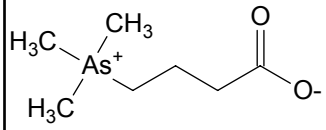
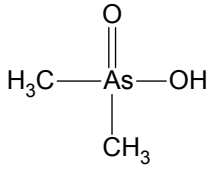
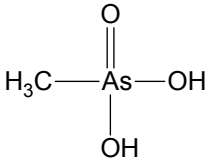
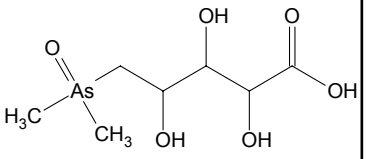
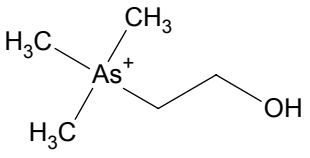
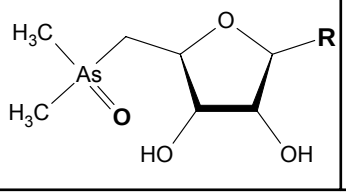
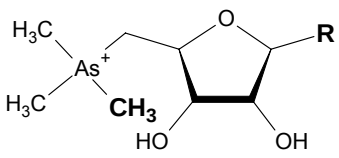
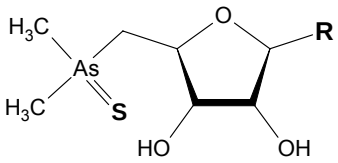
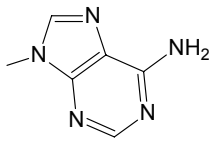
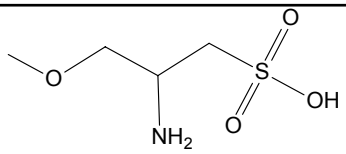
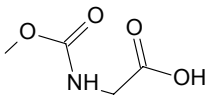
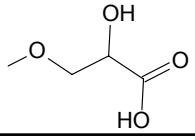
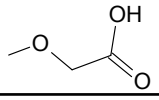
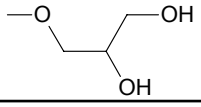
VII Nischwitz, V., Pergantis, S.A., *Anal. Chem.*, **2005**, 77, 5551-5563
VIII Nischwitz, V., Kanaki, K., Pergantis, S.A., *J. Anal. At. Spectrom.*, **2006**, 21, 33-40
IX Nischwitz, V., Pergantis, S.A., *J. Agric. Food Chem.*, **2006**, 54, 6507-6519

Tetramethyl-arsonium ion (TMA)		Trimethylarsine oxide (TMAO)	
Arsenobetaine (AsB)		Dimethylarsinoyl-acetic acid (DMAA)	
Trimethylarsonio-propionate (AsB-2)		Trimethylarsonio-butyrate (AsB-3)	
Dimethylarsinic acid (DMA)		Monomethyl-arsonic acid (MMA)	
5-Dimethyl-arsinoyl-2,3,4-trihydroxy-pentanoic acid (DMArsinoyl-Trihydroxypen-tanoic acid)		Arsenocholine (AsC)	

R	**DMAsSugar-** 	**TMAsSugar-** 	**DMThioAsSugar-** 
Adenine			
Amino-Sulfonate			
Carbamate			
Carboxyl			
Carboxyl-2			
Glycol			
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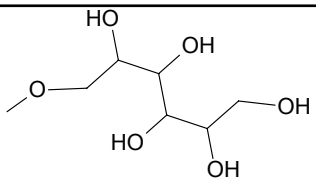
Mannitol			
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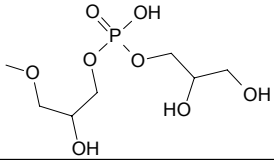
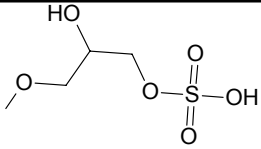
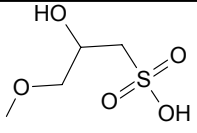
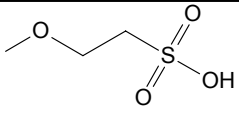
Phosphate			
Sulfate			
Sulfonate			
Sulfonate-2	(proposed structure in analogy to Carboxyl-2) 		

Table A Selected reaction monitoring (SRM) parameters (precursor ion → product ion (collision energy [eV]) for 32 organoarsenic species and glycine betaine.

Species	SRM 1	SRM 2
TMA	135 → 120 (22)	135 → 105 (22)
AsB	179 → 120 (21)	179 → 105 (30)
AsB-2	193 → 120 (28)	193 → 105 (28)
AsB-3	207 → 87 (20)	207 → 121 (20)
AsC	165 → 121 (23)	165 → 105 (23)
TMAO	137 → 107 (26)	137 → 122 (20)
TMAsSugarGlycol	327 → 193 (34)	327 → 120 (34)
TMAsSugarHydroxy	253 → 193 (25)	253 → 163 (25)
TMAsSugarMethoxy	267 → 75 (26)	267 → 120 (38)
TMAsSugarPhosphate	481 → 327 (30)	481 → 389 (30)
TMAsSugarSulfonate	391 → 235 (26)	391 → 293 (26)
TMAsSugarSulfate	407 → 327 (20)	407 → 193 (40)
DMA	139 → 91 (24)	139 → 109 (24)
DMAA	181 → 119 (15)	181 → 139 (15)
DMAsSugarAdenine	372 → 237 (20)	372 → 178 (28)
DMAsSugarAminoSulfonate	392 → 97 (30)	392 → 295 (25)
DMAsSugarCarbamate	356 → 237 (15)	356 → 97 (27)
DMAsSugarCarboxyl	343 → 97 (28)	343 → 237 (16)
DMAsSugarGlycol	329 → 97 (25)	329 → 237 (15)
DMAsSugarHydroxy	255 → 97 (24)	255 → 195 (24)
DMAsSugarMannitol	419 → 97 (38)	419 → 237 (20)
DMAsSugarMethoxy	269 → 97 (21)	269 → 105 (32)
DMAsSugarPhosphate	483 → 97 (40)	483 → 237 (25)
DMAsSugarSulfonate	393 → 97 (30)	393 → 237 (20)
DMAsSugarSulfate	409 → 329 (15)	409 → 97 (35)
DMThioAsSugarGlycol	345 → 97 (20)	345 → 253 (10)
DMThioAsSugarPhosphate	499 → 253 (15)	499 → 97 (27)
DMThioAsSugarSulfonate	409 → 97 (25)	409 → 253 (15)
DMThioAsSugarSulfate	425 → 97 (30)	425 → 253 (15)
MMA	141 → 91 (30)	-
DMAsSugarCarboxyl-2	313 → 97 (20)	313 → 237 (15)
DMArsinoylTrihydroxy-pentanoic acid	271 → 253 (18)	271 → 195 (25)
Glycine-betaine	118 → 58 (30)	118 → 59 (20)

Table B Quantification of arsenic species in extracts of 10 tissue fractions from a giant clam *Tridacna maxima* using anion exchange HPLC-ICPMS (method A). All contents are reported as μg As/g dry weight (n=1). Peak assignment is based on HPLC-ES-SRM data and matching retention times with calibration standards in HPLC-ICPMS.

RT [a]	Peaks	TM_1_1	TM_1_2	TM_1_3	TM_1_4	TM_1_5	TM_1_6	TM_1_7	TM_1_8	TM_1_9	TM_1_10
2.3	AsB	10.0 ± 0.2	18.4 ± 0.3	11.2 ± 0.2	26.6 ± 0.4	25.6 ± 0.4	24.4 ± 0.4	20.0 ± 0.3	19.8 ± 0.3	14.6 ± 0.2	7.6 ± 0.1
2.4	DMGlycol	14.5 ± 0.6	- [e]	9.5 ± 0.5	-	-	-	-	-	-	-
2.8	TMA	3.2 ± 0.2	0.1 ± 0.1	3.0 ± 0.1	0.15 ± 0.08	0.1 ± 0.1	0.9 ± 0.1	0.47 ± 0.08	0.1 ± 0.1	0.06 ± 0.05	0.06 ± 0.05
3.1	DMA	1.7 ± 0.2	-	1.6 ± 0.2	-	-	-	-	-	-	-
3.5	DMCarboxyl [b]	46.4 ± 0.8	0.6 ± 0.1	45.1 ± 0.7	0.24 ± 0.08	0.8 ± 0.1	10.4 ± 0.2	1.82 ± 0.08	2.8 ± 0.1	0.22 ± 0.05	0.50 ± 0.04
4.2	DMCarbamate	24.6 ± 0.4	0.3 ± 0.1	24.9 ± 0.4	0.11 ± 0.08	0.5 ± 0.1	5.6 ± 0.2	0.60 ± 0.08	1.42 ± 0.09	-	0.31 ± 0.04
5.2	DMSulfonate	9.3 ± 0.2	0.2 ± 0.1	8.2 ± 0.2	-	0.2 ± 0.1	2.0 ± 0.1	0.24 ± 0.09	0.6 ± 0.1	-	0.14 ± 0.05
5.9	Unknown1 [c]	5.9 ± 0.2	0.2 ± 0.1	4.8 ± 0.2	-	0.2 ± 0.1	1.1 ± 0.1	0.21 ± 0.09	0.4 ± 0.1	0.07 ± 0.05	0.11 ± 0.05
8.8	DMSulfate	84 ± 1	0.7 ± 0.1	82 ± 1	0.22 ± 0.08	1.6 ± 0.1	17.2 ± 0.3	1.69 ± 0.08	4.5 ± 0.1	0.10 ± 0.05	0.91 ± 0.04
10.6	Arsenate	8.9 ± 0.5	-	6.3 ± 0.4	-	0.2 ± 0.1	1.7 ± 0.1	0.35 ± 0.07	0.41 ± 0.08	-	0.10 ± 0.04
17.2	DMAdenine	3.6 ± 0.6	-	1.3 ± 0.5	-	0.5 ± 0.4	1.1 ± 0.4	0.4 ± 0.3	0.3 ± 0.3	-	0.2 ± 0.2
30.9	Unknown2	19.8 ± 0.3	-	17.9 ± 0.3	0.11 ± 0.08	0.3 ± 0.1	1.8 ± 0.1	0.39 ± 0.09	0.4 ± 0.1	-	-
Recovery [%] [d]		94 ± 2	92 ± 8	91 ± 1	90 ± 4	87 ± 5	89 ± 3	84 ± 4	91 ± 4	93 ± 4	87 ± 6

[a] Retention times [min]; additional peaks at 2.1 min for TM_1_1 (3.7 ± 0.2) and for TM_1_3 (3.3 ± 0.2) and at 12.0 min for TM_1_3 (0.7 ± 0.2)
[b] DMPhosphate, DMCarboxyl-2
[c] Identified as DMAsSugarSulfonate-2
[d] Sum of quantified species in % of total arsenic content in the extract
[e] not detected

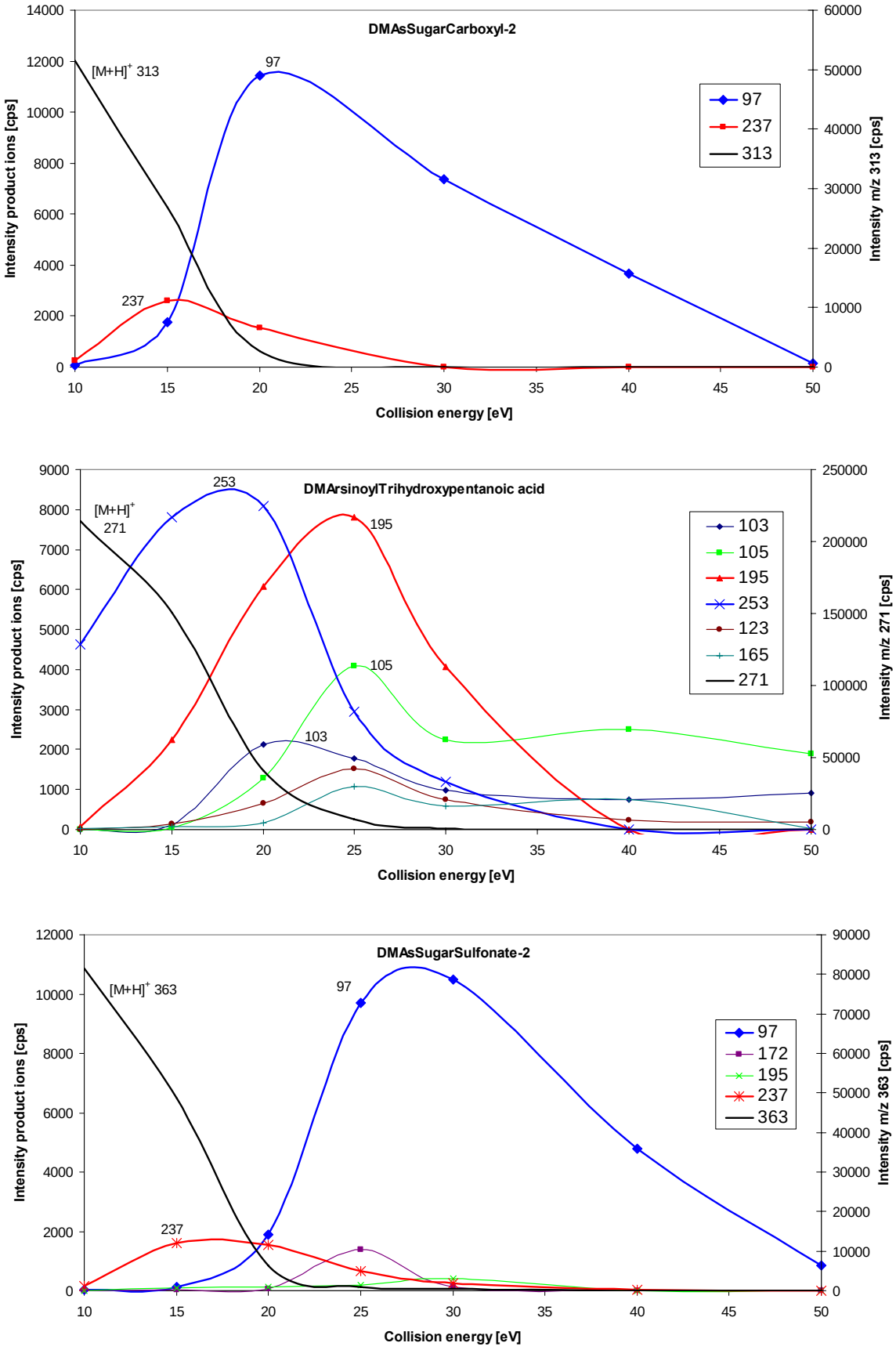


Figure A Collision induced dissociation (CID) breakdown curves for 3 arsenic species detected in *T. maxima* kidney extracts.

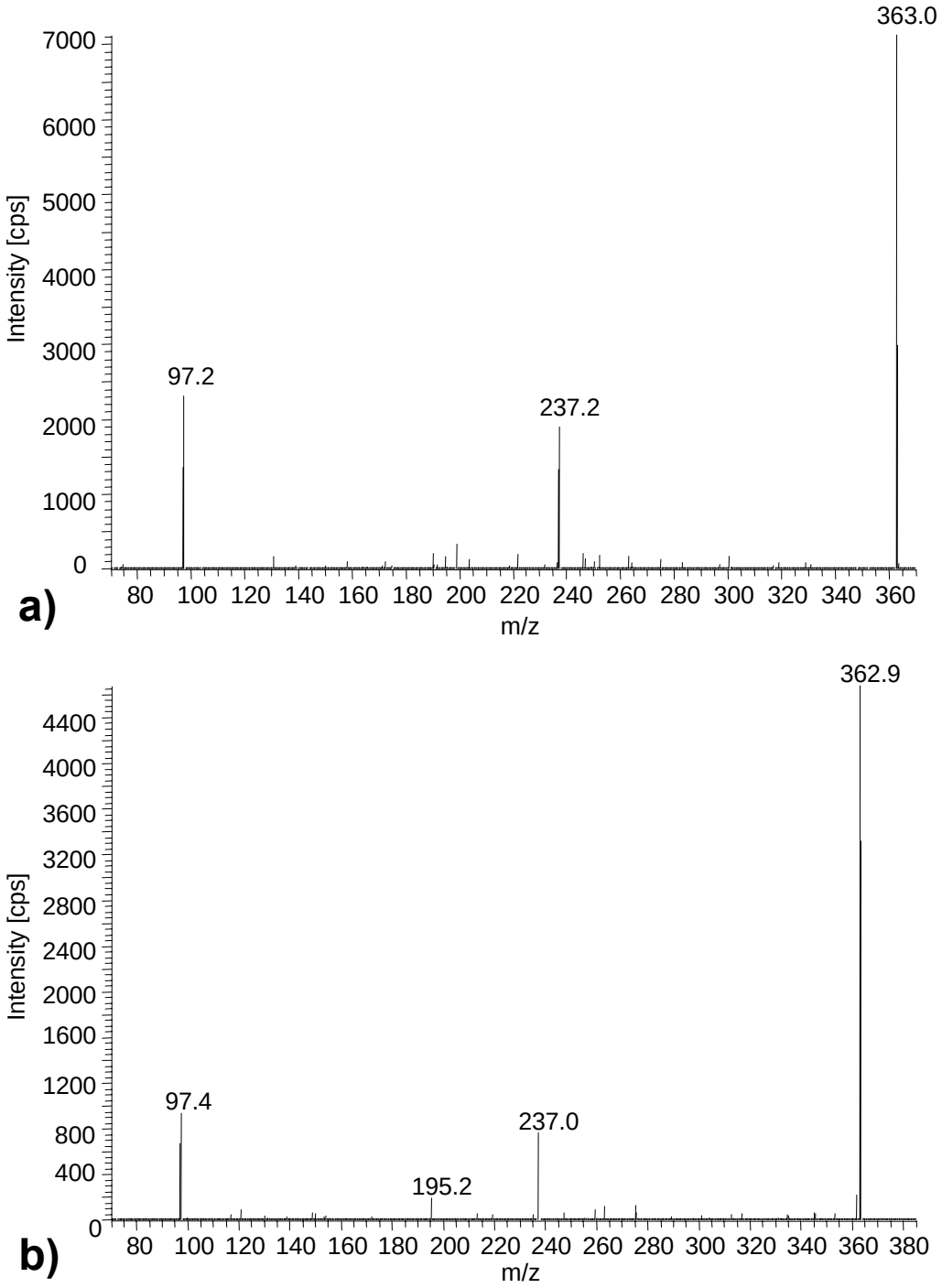


Figure B Product ion spectra of m/z 363 at 20 eV collision energy (*Tridacna* kidney extract) (a) and m/z 380 at 25 eV collision energy (same fraction of *Tridacna* kidney extract as in Fig. 4) (b) obtained from anion exchange HPLC-ES-MS/MS using 20 mM ammonium bicarbonate mobile phase at pH 10 with 10% methanol.

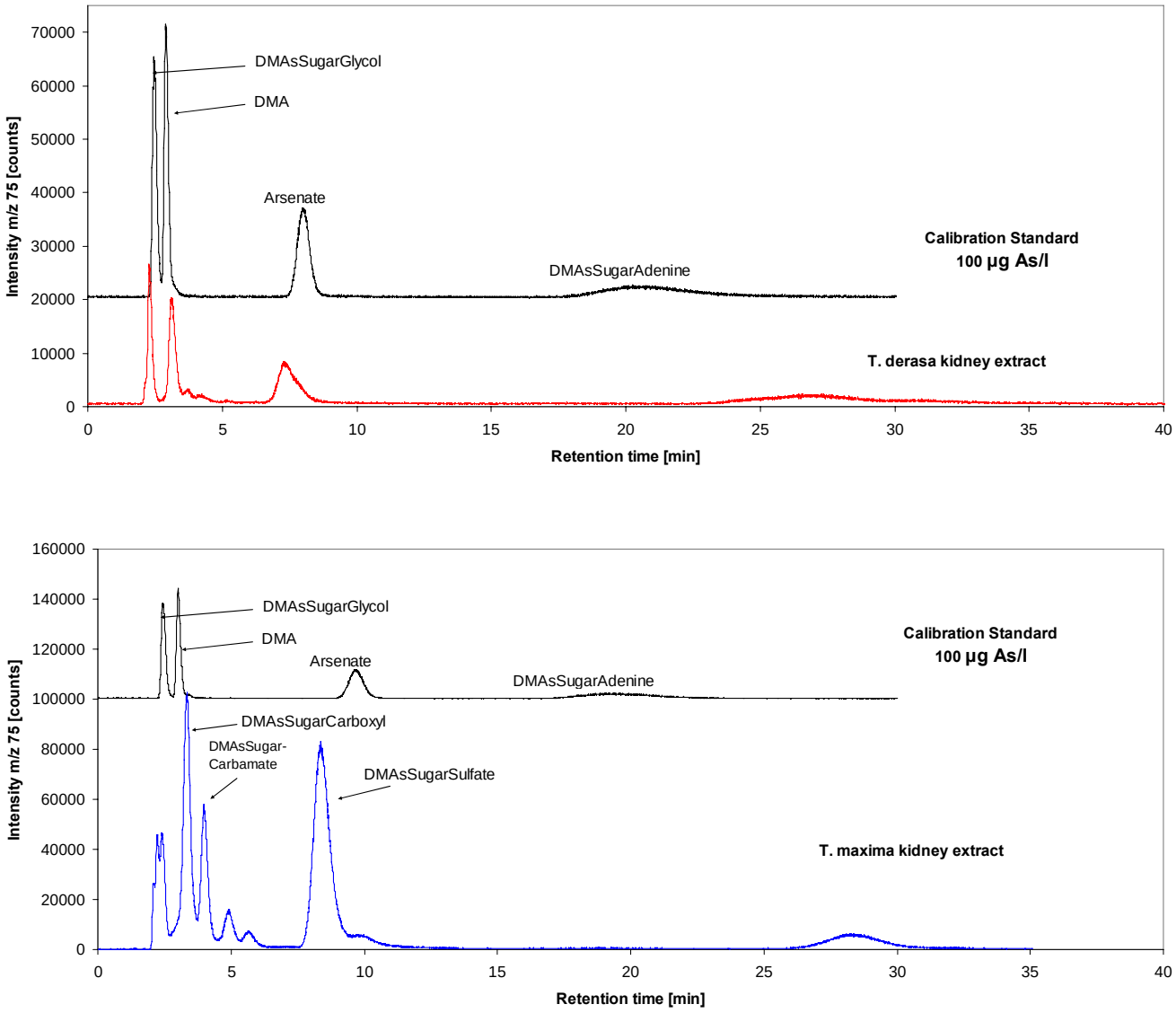


Figure C Anion exchange HPLC-ICPMS chromatograms (PRP-X100 column, 20 mM NH_4HCO_3, 3% methanol, pH 10; see experimental section for details) of an extract from *T. derasa* kidney (TD_1, upper graph) and an extract from *T. maxima* kidney (TM_1_1, lower graph). The samples were measured on different days, but the same calibration standard was analyzed on both days to indicate small changes in the retention behavior.