Quantification of labile and stable non-polar arsenolipids in commercial fish meals and edible seaweed samples

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Electronic supplementary information

Total arsenic

ESI-Table 1 Sequential extraction method A performed on 27 herring and blue whiting samples, and capelin and dulse seaweed3 in mg kg^{-1}, total arsenic (n=3).

	n[A]	Water	Hexane	MeOH/DCM	Residue	Sum	Total	%
H1	15	2.6 ± 0.2	0.17 ± 0.06	0.75 ± 0.13	0.31 ± 0.04	3.8 ± 0.2	4.27 ± 0.03	89.0
H2	2	3.59 ± 0.08	0.18 ± 0.01	0.60 ± 0.02	0.23 ± 0.01	4.61 ± 0.06	5.29 ± 0.05	87.1
H3	2	1.63 ± 0.01	0.14 ± 0.02	0.51 0.01	0.18 ± 0.02	2.46 ± 0.03	3.13 ± 0.01	78.7
H4	2	4.05 ± 0.05	0.11 ± 0.01	0.60 ± 0.01	0.26 ± 0.01	5.01 ± 0.06	5.47 ± 0.14	91.6
H5	2	4.08 ± 0.01	0.12 ± 0.03	0.53 ± 0.02	0.27 ± 0.01	5.01 ± 0.05	6.07 ± 0.16	82.6
H6	2	2.91 ± 0.01	0.024 ± 0.001	0.72 ± 0.01	0.23 ± 0.01	3.88 ± 0.01	4.39 ± 0.23	88.4
H7	2	2.43 ± 0.14	0.11 ± 0.03	1.12 ± 0.05	0.42 ± 0.02	4.09 ± 0.15	4.39 ± 0.02	85.0
H8	2	1.99 ± 0.01	0.05 ± 0.01	0.67 ± 0.05	0.54 ± 0.07	3.25 ± 0.04	4.81 ± 0.08	81.7
H9	2	1.98 ± 0.13	0.026 ± 0.001	0.75 ± 0.01	0.48 ± 0.01	3.23 ± 0.13	3.98 ± 0.05	77.1
H10	2	3.51 ± 0.05	0.13 ± 0.01	0.56 ± 0.01	0.31 ± 0.01	4.51 ± 0.05	4.71 ± 0.06	95.8
H11	2	2.78 ± 0.03	0.25 ± 0.02	0.64 ± 0.08	0.39 ± 0.08	4.05 ± 0.11	4.46 ± 0.05	90.8
H12	2	2.52 ± 0.05	0.17 ± 0.09	0.64 ± 0.09	0.28 ± 0.01	3.61 ± 0.03	4.25 ± 0.04	84.8
H13	2	3.19 ± 0.08	0.13 ± 0.01	0.53 ± 0.09	0.25 ± 0.02	4.10 ± 0.02	4.23 ± 0.11	97.0
H14	2	1.68 ± 0.07	0.09 ± 0.01	0.69 ± 0.04	0.20 ± 0.01	2.65 ± 0.13	3.70 ± 0.92	71.7
BW1	2	17.59 ± 0.09	0.017 ± 0.005	1.24 ± 0.06	0.36 ± 0.01	19.2 ± 0.16	19.0 ± 0.4	100.9
BW2	2	17.48 ± 0.02	0.017 ± 0.001	0.86 ± 0.01	0.37 ± 0.03	18.71 ± 0.01	18.8 ± 0.7	99.5
BW3	2	18.6 ± 1.1	0.025 ± 0.001	1.04 ± 0.01	0.29 ± 0.02	19.9 ± 1.1	19.3 ± 0.2	103.3
BW4	2	16.75 ± 0.13	0.036 ± 0.004	0.90 ± 0.06	0.38 ± 0.01	18.1 ± 0.1	19.0 ± 0.1	94.9
BW5	2	13.36 ± 0.05	0.040 ± 0.001	0.77 ± 0.03	0.33 ± 0.05	14.5 ± 0.1	15.1 ± 0.1	96.3
BW6	2	15.45 ± 0.17	0.047 ± 0.001	0.81 ± 0.09	0.37 ± 0.03	16.7 ± 0.3	16.7 ± 0.1	99.7
BW7	2	16.33 ± 1.2	0.026 ± 0.001	0.83 ± 0.06	0.41 ± 0.04	17.6 ± 1.3	16.9 ± 0.1	104.1
BW8	2	16.48 ± 0.66	0.030 ± 0.001	0.77 ± 0.01	0.34 ± 0.01	17.6 ± 0.7	16.65 ± 0.2	105.9
BW9	2	15.16 ± 0.36	0.024 ± 0.002	0.80 ± 0.05	0.48 ± 0.01	16.5 ± 0.4	15.11 ± 0.4	109.0
BW10	2	18.85 ± 0.02	0.029 ± 0.002	0.93 ± 0.04	0.29 ± 0.25	20.1 ± 0.2	18.4 ± 1.5	109.1
BW11	2	16.60 ± 0.20	0.035 ± 0.011	0.81 ± 0.02	0.33 ± 0.03	17.8 ± 0.2	19.3 ± 0.4	92.2
BW12	2	19.24 ± 0.01	0.032 ± 0.013	1.10 ± 0.11	0.49 ± 0.02	20.9 ± 0.1	19.2 ± 0.5	108.5
BW13	2	21.16 ± 0.20	0.017 ± 0.001	1.18 ± 0.02	0.54 ± 0.04	22.9 ± 0.2	20.33 ± 0.9	112.6
SW1	3	7.7 ± 0.18	<LOQ	0.12 ± 0.02	-	7.8 ± 0.2	7.9 ± 0.5	99
SW2	6	-	-	-	-	-	8.4 ± 0.4	
SW3	3	-	-	-	-	-	7.6 ± 0.1	
Tort-2	3	16.26 ± 0.13	ND	0.48 ± 0.07	1.35 ± 0.07	18.08 ± 0.02	19.6 ± 0.3	92

[A]Method A was performed in triplicate for SW1, H1 and TORT-2. Subsequently was performed in duplicate for all fish meal samples, exception H1 which was monitored with each batch in addition to the triplicate already measured.

The sum of species tends to be higher for the extractions were water is the first extraction step. This is thought to be because of the method of analysis, where the water fraction was analysed for totAs by diluting 1 mL of extraction solution to 10 mL and measuring directly by the ICP-MS, whereas the MeOH/DCM fraction was evaporated to dryness and digested in a microwave in acid before analysis. The carbon enhancement would then be more prominent for the water fraction containing more matrix, including all the AB.

ESI-Table 2. Herring sample monitored with every batch as a QC (Herr1) conc. in mg kg^{-1}

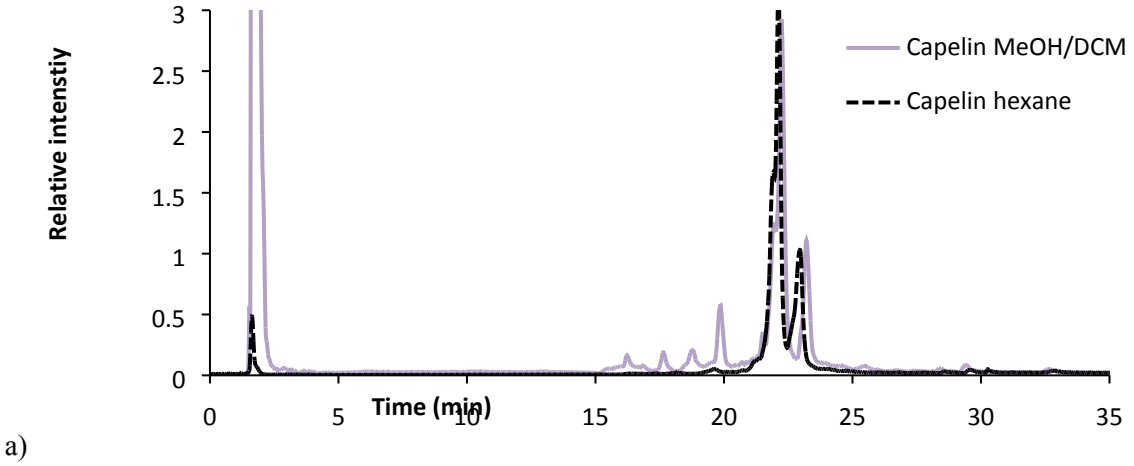
	Water	**Hexane**	**MeOH/DCM**	**Residue**	**Sum**
Method a)	2.07	0.24	0.63	0.23	3.17
	2.38	0.19	0.73	0.29	3.59
	2.19	0.19	0.82	0.32	3.52
	2.42	0.10	0.75	0.34	3.60
	2.63	0.11	0.65	0.32	3.71
	2.81	0.09	0.86	0.31	4.06
	2.76	0.15	0.79	0.29	3.99
	2.83	0.14	0.84	0.35	4.17
	2.72	0.12	0.58	0.29	3.72
	2.93	0.07	0.46	0.32	3.78
	2.90	0.24	0.86	-	3.99
	2.76	0.22	0.64	0.24	3.85
	2.49	0.25	0.86	0.32	3.93
	2.55	0.22	0.82	0.35	3.94
	2.51	0.15	0.94	0.34	3.94
Average	**2.6 ± 0.2**	**0.17 ± 0.06**	**0.75 ± 0.13**	**0.31 ± 0.04**	**3.8 ± 0.2**
Method b)	0.23	0.61	2.40	0.06	3.30
	0.24	0.62	2.13	0.03	3.03
	0.26	0.58	2.40	0.09	3.33
	0.28	0.58	2.34	0.26	3.46
	0.29	0.58	2.41	0.26	3.53
	0.27	0.60	2.27	0.25	3.40
	0.44	0.57	2.27	0.17	3.45
	0.39	0.59	2.41	0.27	3.65
	0.40	0.56	2.36	0.27	3.59
Average	**0.31 ± 0.07**	**0.59 ± 0.02**	**2.33 ± 0.09**	**0.18 ± 0.09**	**3.4 ± 0.2**

ESI-Table 3. Herr1 extracted with Method A (here "single" extraction) compared to where 3 extraction steps were quantified (n=3).

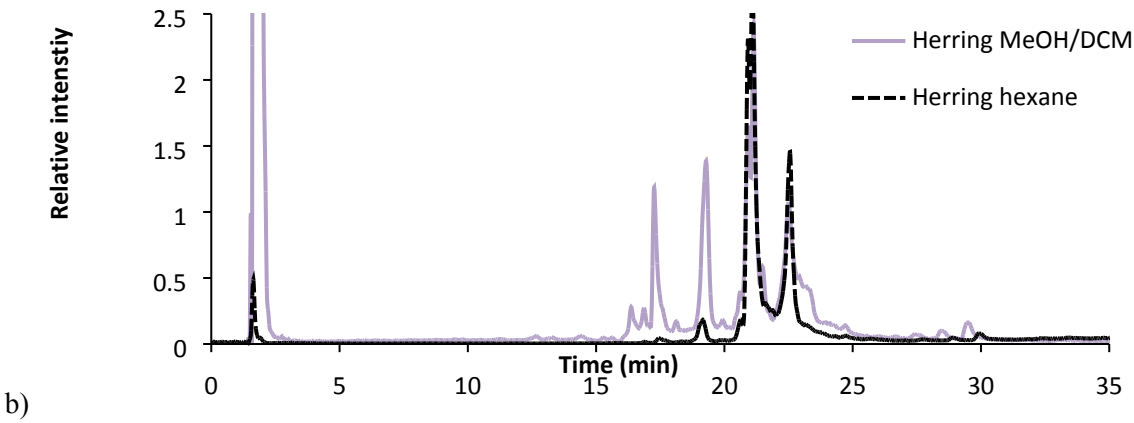
			Water phase	Hexane phase	MeOH/DCM phase	Residue	Tot As	Recovery
Single extraction	Extract	[As] ($\mu g.kg^{-1}$)	2.800	0.127	0.829	0.317	4.073	92.8 %
		SD	0.027	0.028	0.032	0.028	0.075	
Multiple extraction	1^{st} Extract	[As] ($\mu g.kg^{-1}$)	2.598	0.071	0.528	0.256	4.061	92.5%
		SD	0.185	0.016	0.096			
	2^{nd} Extract	[As] ($\mu g.kg^{-1}$)	0.539	0.005	0.048			
		SD	0.072	0.007	0.024			
	3^{rd} Extract	[As] ($\mu g.kg^{-1}$)	0.083	< 0.000	0.014			
		SD	0.034	-	0.005	0.037	0.597	

Two extractions were considered sufficient for the hexane and MeOH/DCM fractions, where little to no arsenic remains in the third fraction, ESI-Table 3. For the water phase, the first extract was considered to have fully extracted the arsenic. The remaining arsenic in 2^{nd} extract fits with what would be expected to remain since after centrifuging 15-25% of the liquid was still embedded in the residue, partly because of insufficient separation of solid and liquid sample. If the sample were evaporated directly these water-soluble arsenicals would still be left in the residue. For the samples this second extraction was performed as a washing step before further sequential extraction steps were performed. This step removed most of the remaining water-soluble arsenicals (very little found in 3^{rd} extraction, ESI-Table 3). Because the first extraction is considered quantitative (and its dilution factor fully known and noted) the second extraction was discarded.

Speciation – additional figures

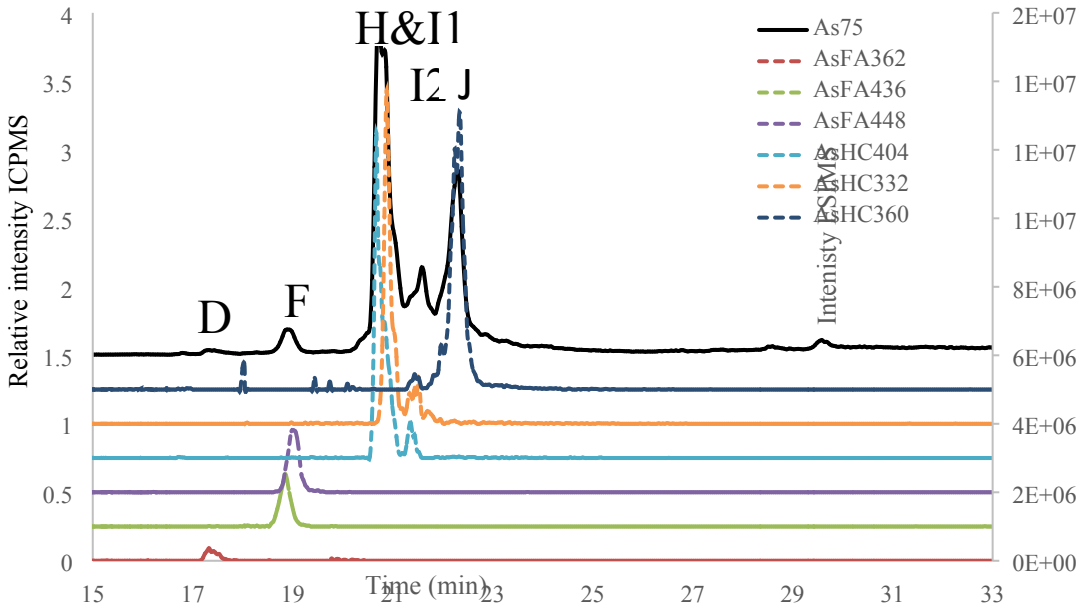


a)

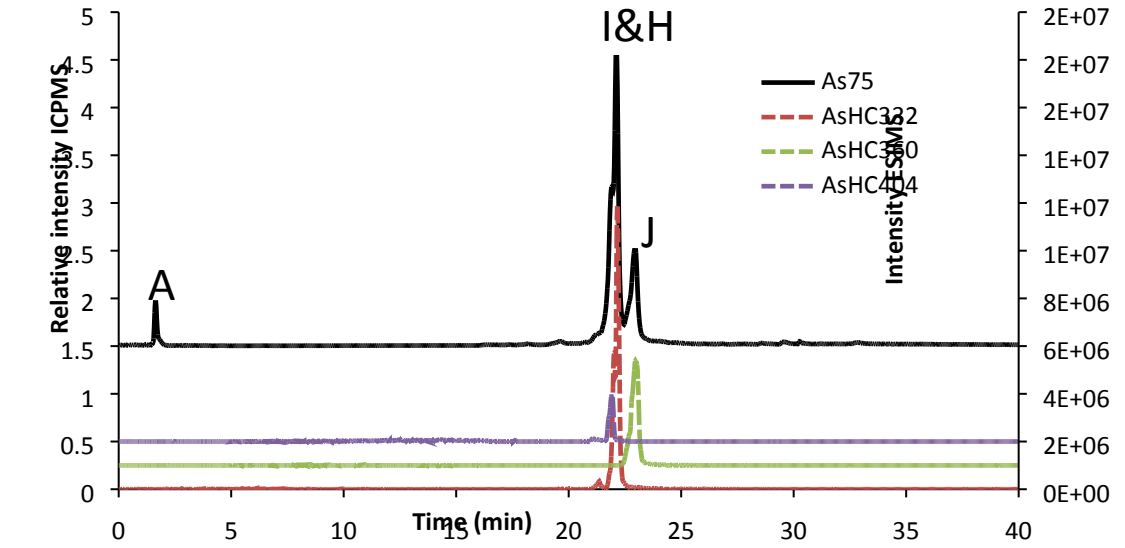


b)

ESI-Fig 1 *Hexane and MeOH/DCM fractions overlaid, ICPMS signal (m/z 75), a) capelin b) herring*

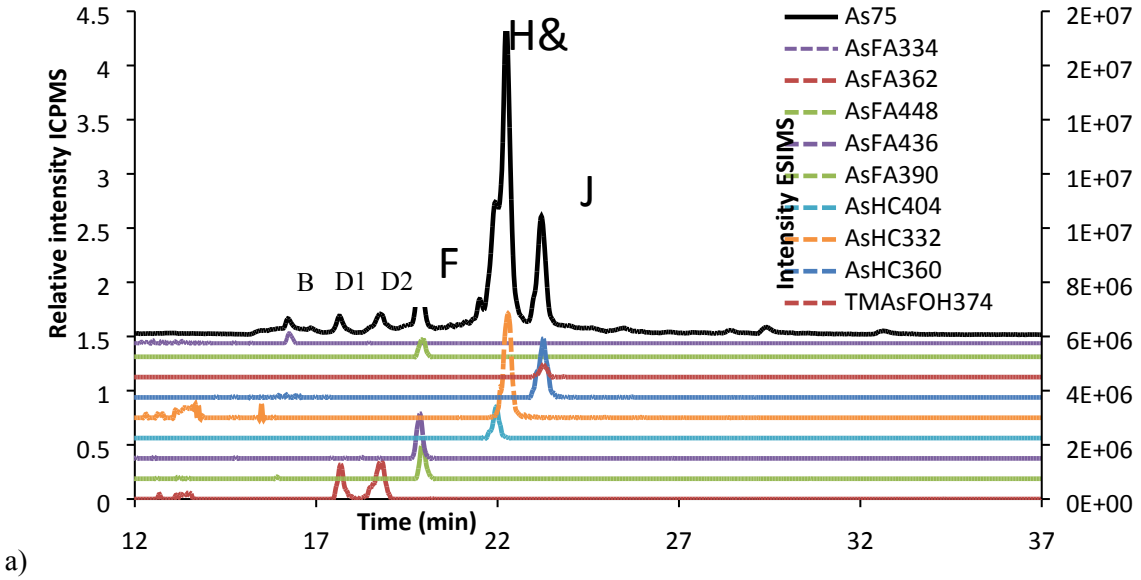


a)

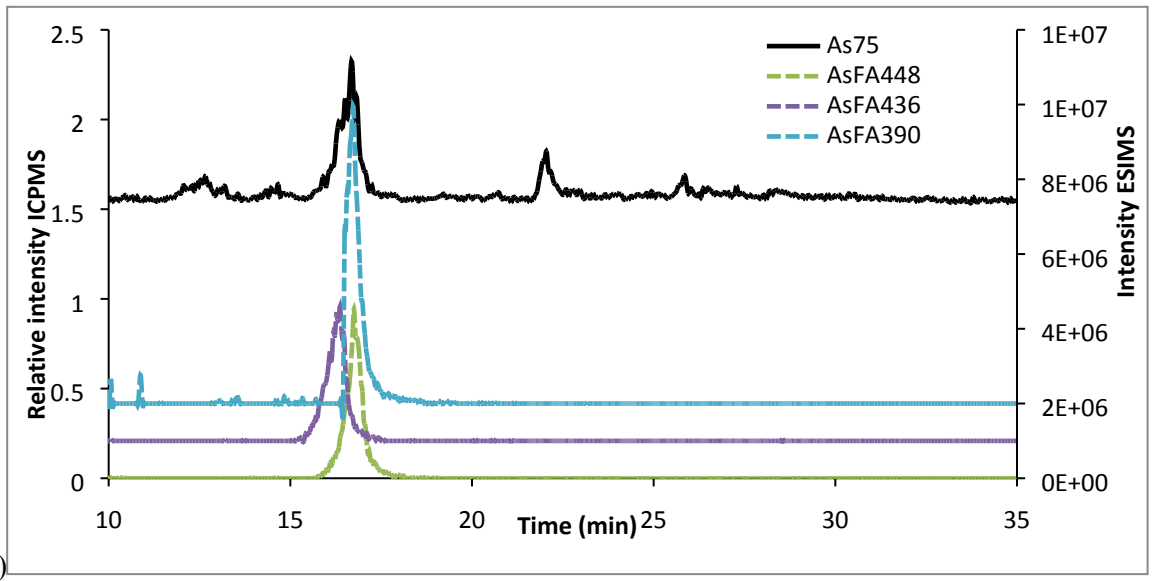


b)

ESI-Fig 2 *Hexane fraction, ICPMS (black) and ESIMS signal (colours) a) herring b) capelin. See Table 2 & 3 for peak letters.*



a)



b)

ESI-Fig 3. *MeOH/DCM fraction signals from ICPMS (black) and ESIMS signal (colours) a) capelin see Table 2 & 3 for peak letters, b) blue whiting*

In ESI-Fig a) the AsFA362 is a saturated AsFA, but shows for capelin a branched behaviour, indicating that different isomers exist (The bottom line (red)). Only a small portion of blue whiting total AsLp concentration was accounted for when analysed for speciation *ESI-Fig 2*b). This was not investigated further.

Speciation – quantification & identification

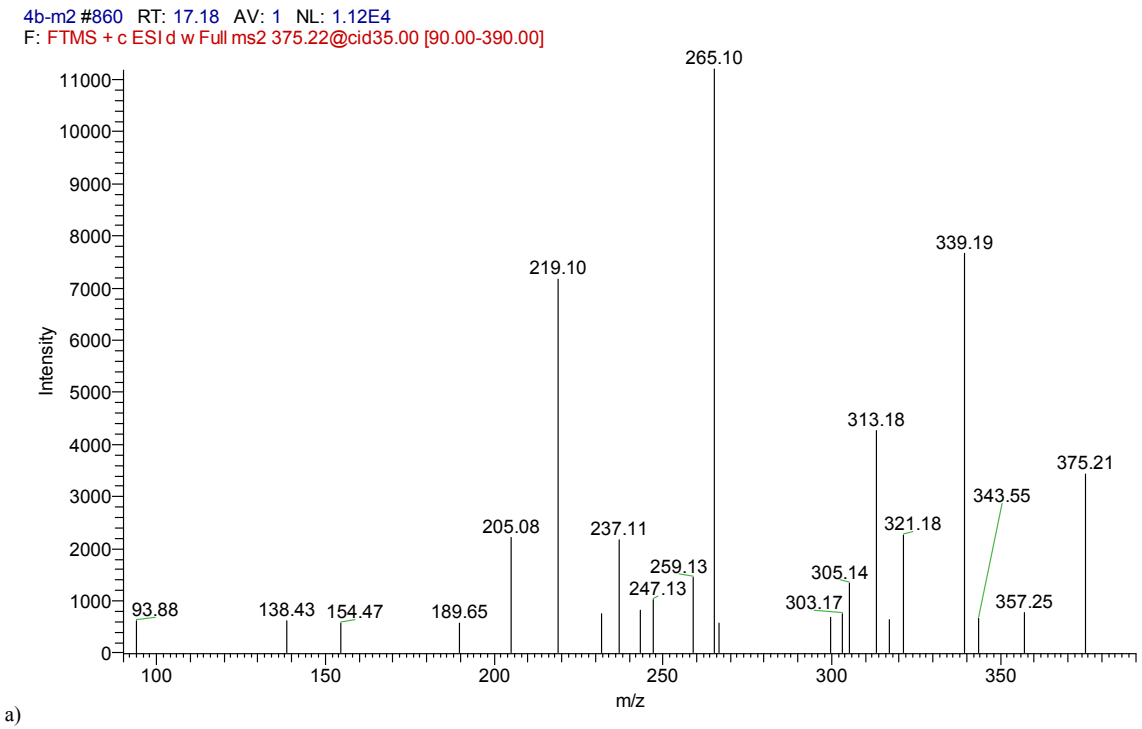
ESI-Table 4. Conc. of As peaks and identification of species in MeOH/DCM fraction of blue whiting, total As concentration of lipid fraction from Table 1

	Formula	Rt (min)	conc. (mg kg^{-1})	Mass (M+H)	Δm/z* (ppm)
U		12.7	0.014		
U		14.7	0.005		
AsFA448	C24 H38 O3 As			449.2025	-1.431
AsFA436	C23 H38 O3 As	16.7	0.059	437.2008	-5.336
AsFA390	C19 H40 O3 As			391.2183	-1.26
U		22.0	0.015		
U		25.8	0.006		
U		28.4	0.005		
	Sum:		0.104		

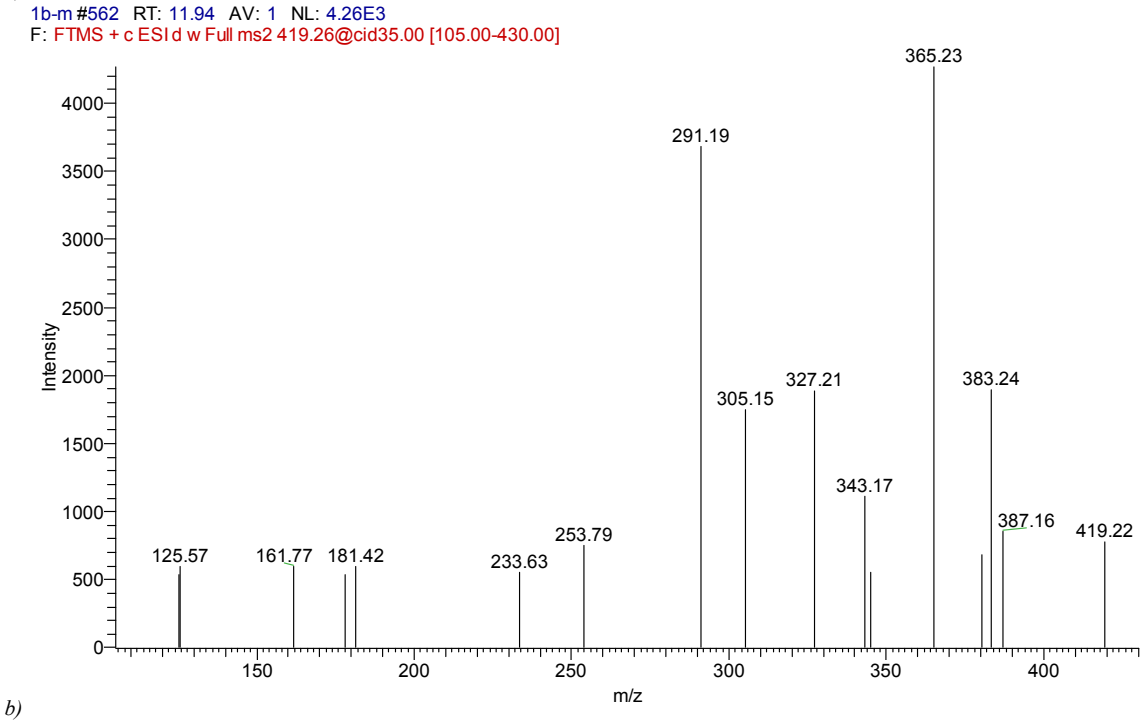
*$(m/z_{found} - m/z_{calc})*10^6/ m/z_{calc}$

ESI-Table 5. MS/MS data for Dulse, typical AsHC pattern and data for AsFA374

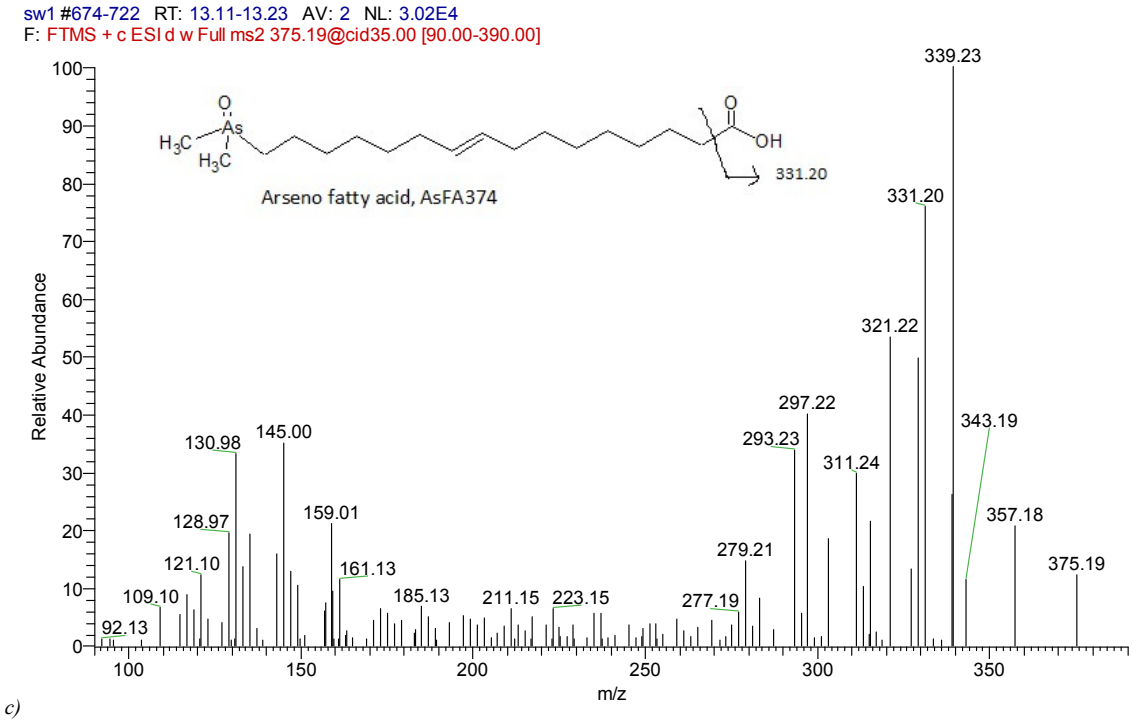
	Mass (M+H)	Formula (M+H)	Δm/z (ppm)
AsHC360	102.9522	C2 H4 As	-1.536
	104.9679	C2 H6 As	-0.935
	122.9784	C2 H8 O As	-1.162
AsHC358	122.9782	C2 H8 O As	-2.788
	104.9678	C2 H6 As	-1.793
	102.9524	C2 H4 As	0.018
AsFA374	375.1875	C18 H36 O3 As	0.046
	331.1976	C17 H36 O As	-0.102
	159.0146	C6 H12 As	-2.505
	144.999	C5 H10 As	-2.057
	130.9832	C4 H8 As	-3.269


4b-m2 #860 RT: 17.18 AV: 1 NL: 1.12E4
F: FTMS + c ESI d w Full ms2 375.22@cid35.00 [90.00-390.00]
265.10
219.10
339.19
313.18
375.21
343.55
205.08
237.11
321.18
259.13
305.14
247.13
303.17
357.25
93.88
138.43
154.47
189.65
Intensity
m/z

a)


1b-m #562 RT: 11.94 AV: 1 NL: 4.26E3
F: FTMS + c ESI d w Full ms2 419.26@cid35.00 [105.00-430.00]
365.23
291.19
305.15
327.21
383.24
343.17
387.16
419.22
253.79
125.57
161.77
181.42
233.63
Intensity
m/z

b)



c)

ESI-Fig 4. MSMS fragmentation from the ESIMS a) TMAsFOH374 in capelin, b) TMAsFOH419 in herring, c) AsFA374 for dulse sample (SW1).

Position of double bond is for AsFA375, ESI-Fig4, is here placed same as in palmitoleic acid (C16:1), but this is a guess.