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Supplementary Material

Isolation and purification of arsenolipids from natural marine sources for use in speciation and toxicological studies

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Isolation and purification of arsenolipids from natural marine sources for use in speciation and toxicologic studies

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Table S1. ES-MS/MS source parameters and method details.

Source parameters		**Aquisition method**							
Drying gas temperature [°C]	290	Mode	Compound group or m/z range	Precursor ion	Product ion	Dwell time [ms]	Fragmentor voltage [V]	Collision energy [V]	Cell Accelerator Voltage [V]
Drying gas (N_2) flow [L/min]	15	MRM	AsHCs	$[M+H]^+$	105	25	380	30	7
Nebulizer [psi]	45	MRM	AsFAs	$[M+H]^+$	105	25	380	25	7
Sheath gas temperature [°C]	380	MRM	Mono/di-acyl AsSugPLs	$[M+H]^+$	237	25	380	35	7
Sheath gas flow [L/min]	12								
Polarity	Positive	SCAN	50-1200			250-500	380	30	7
Capillary [V]	4500								
Nozzle voltage [V]	1500								
Ion funnel RF high [V]	150								
Ion funnel RF low [V]	60								

Table S2. HR-ESMS source parameters and method details.

Source parameters	**Acquisition methods**
• Polarity: positive • Drying gas (N_2) temperature: 450 °C • Capillary voltage: 3500 V • Capillary temperature: 300 °C	• Full MS: resolution of 70 000 full width at half-maximum (fwhm); scan range of *m/z* 120−1200; automatic gain control (AGC) target, 3 x 10^6, and a maximal injection time of 100 ms. • dd-MS^2: isolation window, *m/z* 4.0; resolution of 17 500 fwhm; AGC target, 1 × 10^5; maximal injection time, 100 ms; loop count, 5; intensity threshold, 8 × 10^4, and normalized collision energies of 15, 30 or 50 eV were used. • All ion fragmentation (AIF): resolution 35 000 at fwhm; scan range of m/z 80 – 1200; AGC target, 3 x 10^6; maximal injection time of 100 ms and normalized collision energy of 30 eV were used.

Table S3. Total arsenic contents of individual and bulk salmon oil samples.

	Total As [µg As/kg; n=3]				
Sample (Supplier)	**Lachs I** Scheidler	**Lachs II** Scheidler	**Lachs III** Scheidler	**Lachs IV** LIPROMAR	**Bulk** Mix
[%] of bulk	23	22	22	33	100
	665 ± 4	564 ± 5	553 ± 2	741 ± 8	643 ± 10

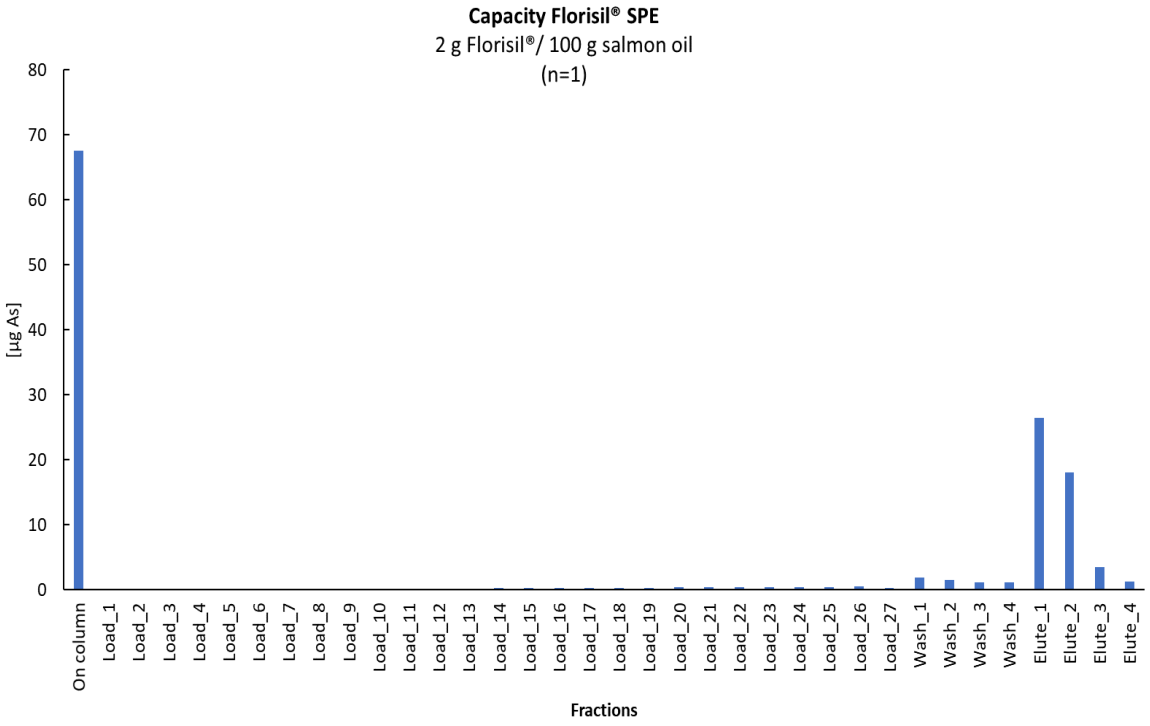

Figure S1. Capacity of the Florisil® SPE column. For the determination of the column capacity, a column (6 mL plastic syringe sealed with cotton wool) was filled with 2 g of dry Florisil® (60-100 mesh). Salmon oil (100 g, 68.0 µg As) dissolved in DCM/acetone 4:1, v/v containing 1% formic acid (v/v) to a volume of 300 mL was loaded on to the SPE column. Fractions (10 mL) of load (no. 1-27, 6.4 µg As); wash (no. 1-3, 4.5 µg As), DCM/acetone, 4:1 (v/v) – 1% (v/v) formic acid); wash (no. 4, 1.2 µg As), acetone and elute (no. 1-4, 49.3 µg As), 10% (v/v) ammonia/acetone were collected.

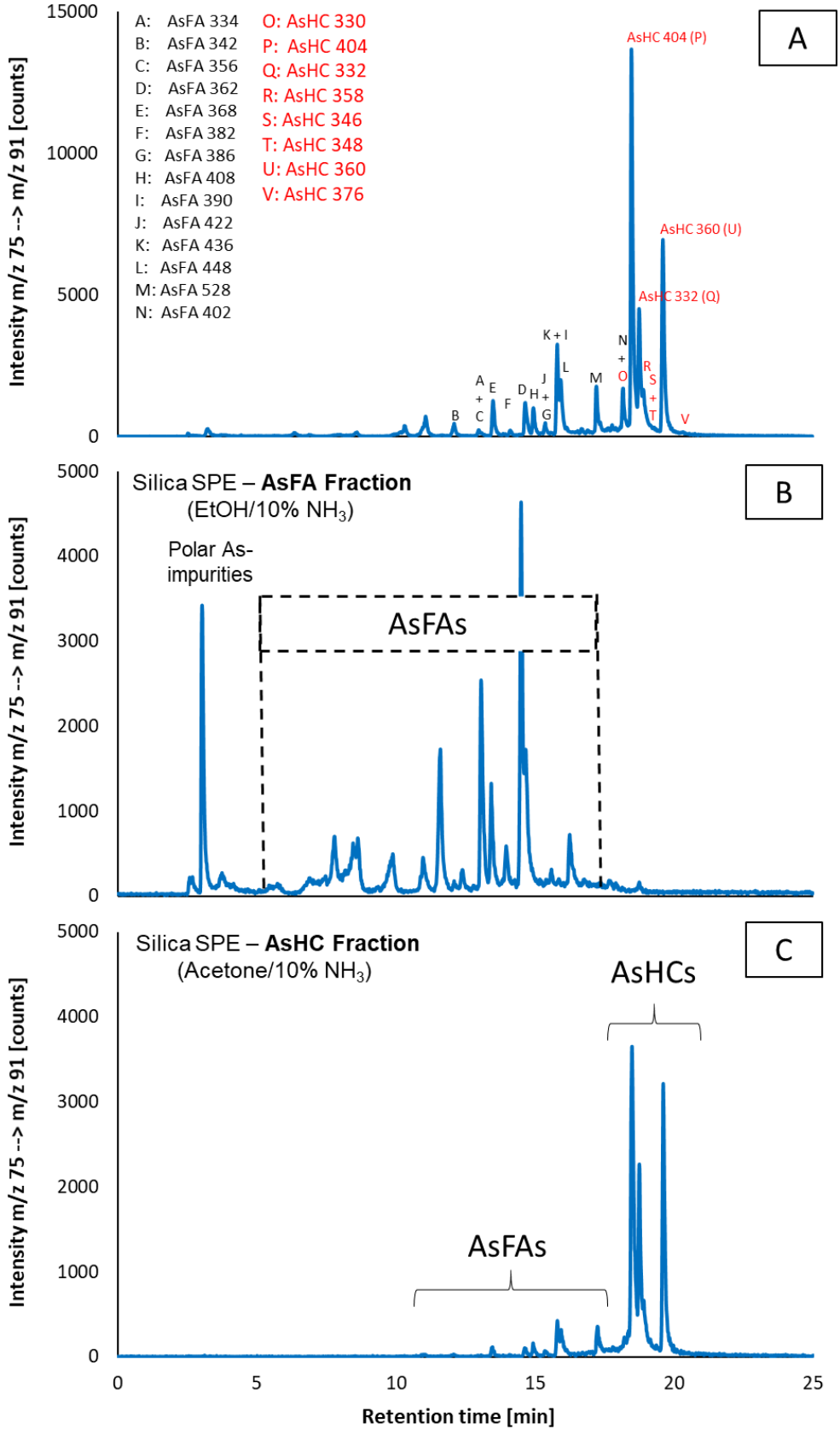

Figure S2. RP-HPLC/ICP-MS/MS chromatograms of (A) a hydrolyzed extract of the first acetone/ammonia elute fraction of Florisil® SPE of salmon oil, and fractions of AsFAs (B) and AsHCs (B) obtained after on-column separation using silica SPE. Chromatographic conditions: column, ACE C18; gradient elution with mobile phases A: water/25 mM formate at pH 9.2 and B: MeOH/25 mM formate at pH 9.2. Gradient: 0-1 min, 30% B; 1-15 min, 30% - 100% B, and 15-21min, 100% B. Runtime was 25 min; flow rate, 1 mL/min at 40°C and injection vol. was 10 µL.

Table S4. Mass balance silica SPE of Wakame lipid extracts (n=5).

Fraction	Conditions	Vol.	Wakame_1			Wakame_2			Wakame_3			Wakame_4			Wakame_5		
		[mL]	[µg As]	[%]	Mass [g]	[µg As]	[%]	Mass [g]	[µg As]	[%]	Mass [g]	[µg As]	[%]	Mass [g]	[µg As]	[%]	Mass [g]
On column			509	100.0	2.43	542	100.0	2.29	483	100.0	2.13	838	100.0	2.59	822	100.0	2.34
Load	Sample	1000	22	4.6	0.75	23	4.3	0.52	19	4.0	0.49	21	2.7	0.84	16	2.1	0.88
Wash_1	DCM/acetone/FA	1000	18	3.7	0.76	19	3.5	0.66	17	3.6	0.58	23	2.9	0.07	19	2.5	1.06
Wash_2	DCM/acetone/FA	1000	3	0.5	0.08	2	0.4	0.11	2	0.4	0.06	3	0.3	0.85	2	0.3	0.07
Wash_3	MeOH	1000	29	6.0	0.97	25	4.9	0.76	24	5.0	0.74	48	6.1	0.02	35	4.6	0.78
Wash_4	EtOH	1000	24	4.9	0.03	22	4.3	0.02	17	3.5	0.03	73	9.3	0.07	56	7.3	0.00
Elute_1	**Ethanol/ammonia**	**1000**	**287**	**59.3**	**0.07**	**306**	**58.3**	**0.08**	**275**	**57.7**	0.07	**450**	**57.0**	**0.11**	**469**	**61.0**	0.05
Elute_2	Ethanol/ammonia	1000	82	16.9	0.09	102	19.4	0.17	96	20.1	0.08	134	16.9	0.06	133	17.3	0.11
Elute_3	Ethanol/ammonia	1000	12	2.6	0.04	17	3.2	0.04	17	3.6	0.05	24	3.0	0.02	25	3.2	0.05
Elute_4	Ethanol/ammonia	1000	4	0.9	0.02	6	1.1	0.02	6	1.4	0.02	9	1.1	0.01	9	1.2	0.02
Elute_5	Ethanol/ammonia	1000	3	0.6	0.01	3	0.6	0.01	3	0.7	0.01	5	0.6	2.77	4	0.6	0.00
Off column			485	95.3	2.82	524	96.8	2.39	477	95.9	2.14	789	94.1		769	93.5	3.03
Recovery* (mean ± SD)			95 ± 2%														
Elute_1-5** (mean ± SD)			82 ± 2%														
Elute_1** (mean ± SD)			59 ± 2%														
Mass Elute_1 (mean ± SD)			0.08 ± 0.02 g														

*Calculation based on arsenic loaded on the column.

**Calculation based on arsenic eluted off the column.

Table S5. Pool of ethanol/ammonia silica SPE elute fractions of lipid extracts of Wakame samples 1-5.

Samples	Pool Fractions	Replicate No.	As [µg]	Mean As [µg]	Mass [mg]
Wakame 1 - 5	Elute_1	1	1614	1624	304
		2	1633		
	Elute_2	1	499	499	468
		2	500		
	Elute_3	1	85	86	219
		2	88		
	Elute_4	1	30	30	69
		2	30		
	Elute_5	1	17	16	31
		2	16		

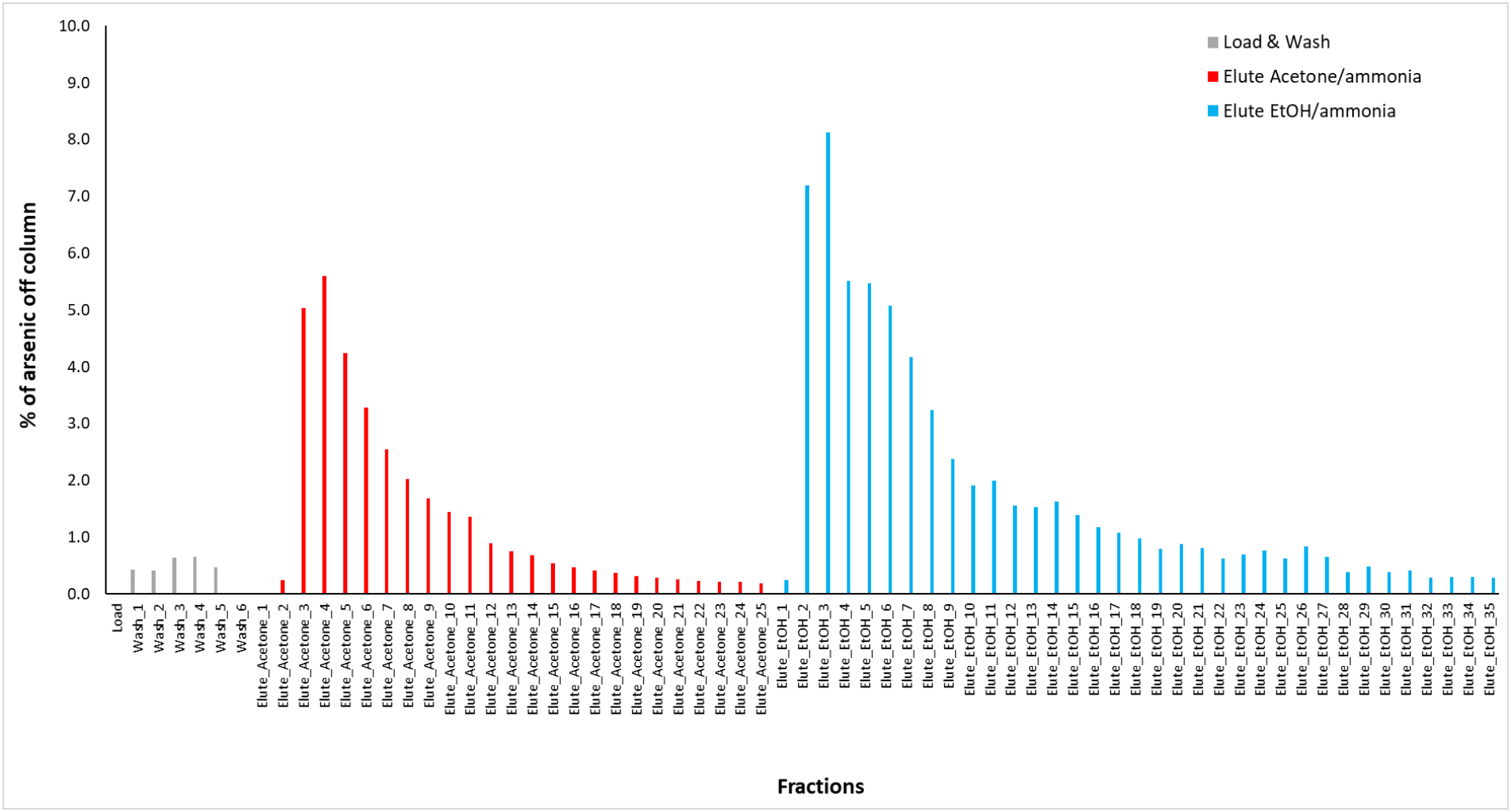

Figure S3. Distribution of arsenic in load (10 mL), wash (6 x 25 mL), and elute fractions of acetone/ammonia (25 x 10 mL) and ethanol/ammonia (35 x 10 mL) of Florisil® SPE of Wakame pool elute fraction 1. Arsenic concentration in fractions was determined by FIA-ICP-MS/MS. The ICP-MS/MS was operated with the setup used for arsenolipid analysis.

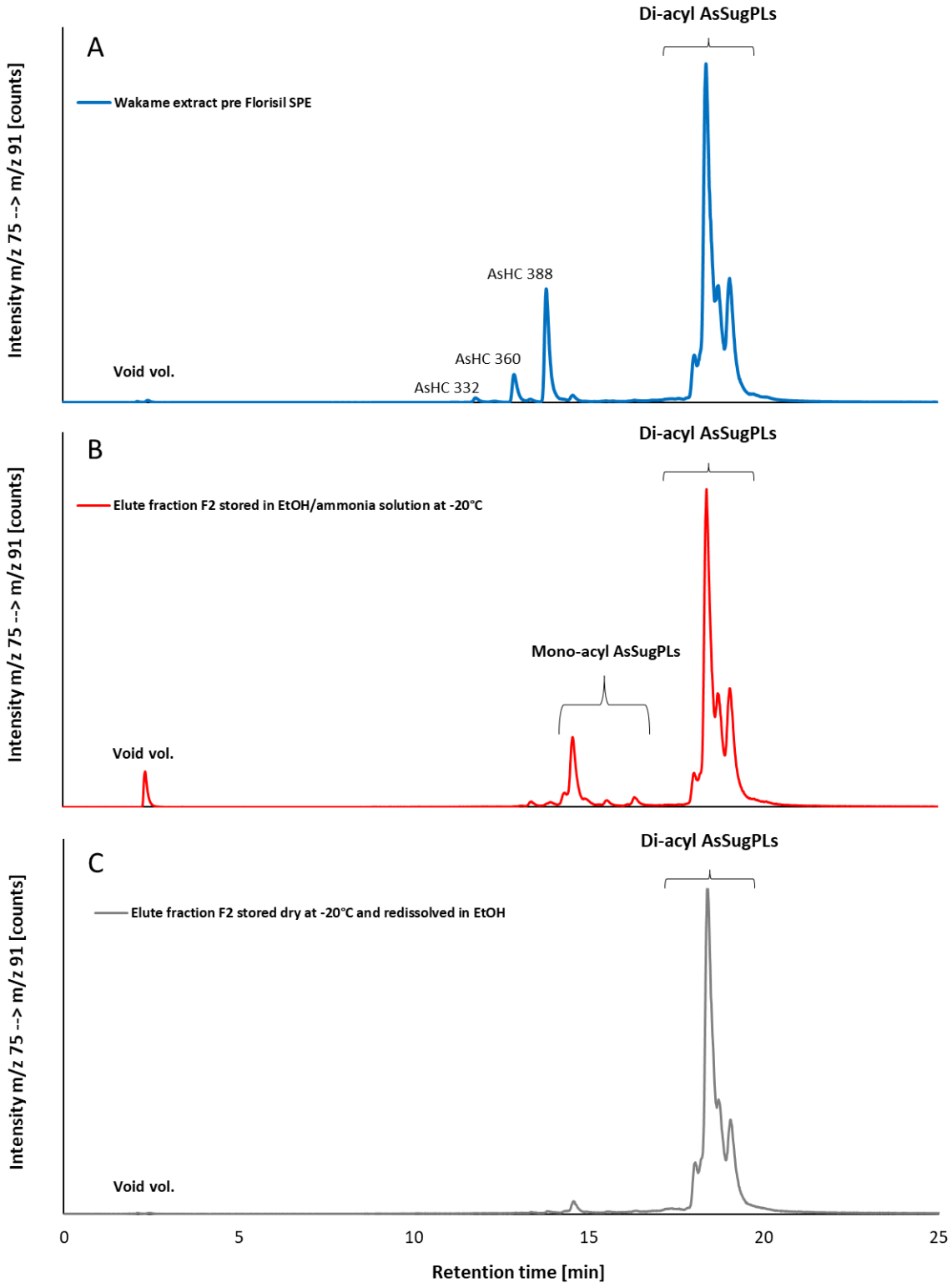

Figure S4. Transformation/degradation of di-acyl AsSugPLs under alkaline conditions. (A) HPLC/ICP-MS/MS chromatogram of the Wakame extract (DCM/acetone 1:1, 1% formic acid) applied on a Florisil® SPE column. (B) HPLC/ICP-MS/MS chromatogram of an ethanol/10% (v/v) ammonia elute fraction (F2) stored in solution at -20°C for 3 weeks after the Florisil® SPE; mono-acyl AsSugPLs have been found also 4 days after the Florisil SPE in the same fraction (data not shown). (C) HPLC/ICP-MS/MS chromatogram of ethanol/ammonia fraction F2, which has been immediately evaporated after the Florisil® SPE and stored dry at -20°C for 3 weeks and redissolved in ethanol for measurements. Chromatographic conditions: column, Shodex Asahipak C8P-50 4D; gradient elution, 0 –1 min: 30% MeOH, 1–5 min: 30–60% MeOH, 5–15 min: 60–100% MeOH, 15–28.5 min holding at 100% MeOH followed by equilibration for 6.5 min; flow rate, 0.8 mL/min at 50°C and injection vol. was 10 µL

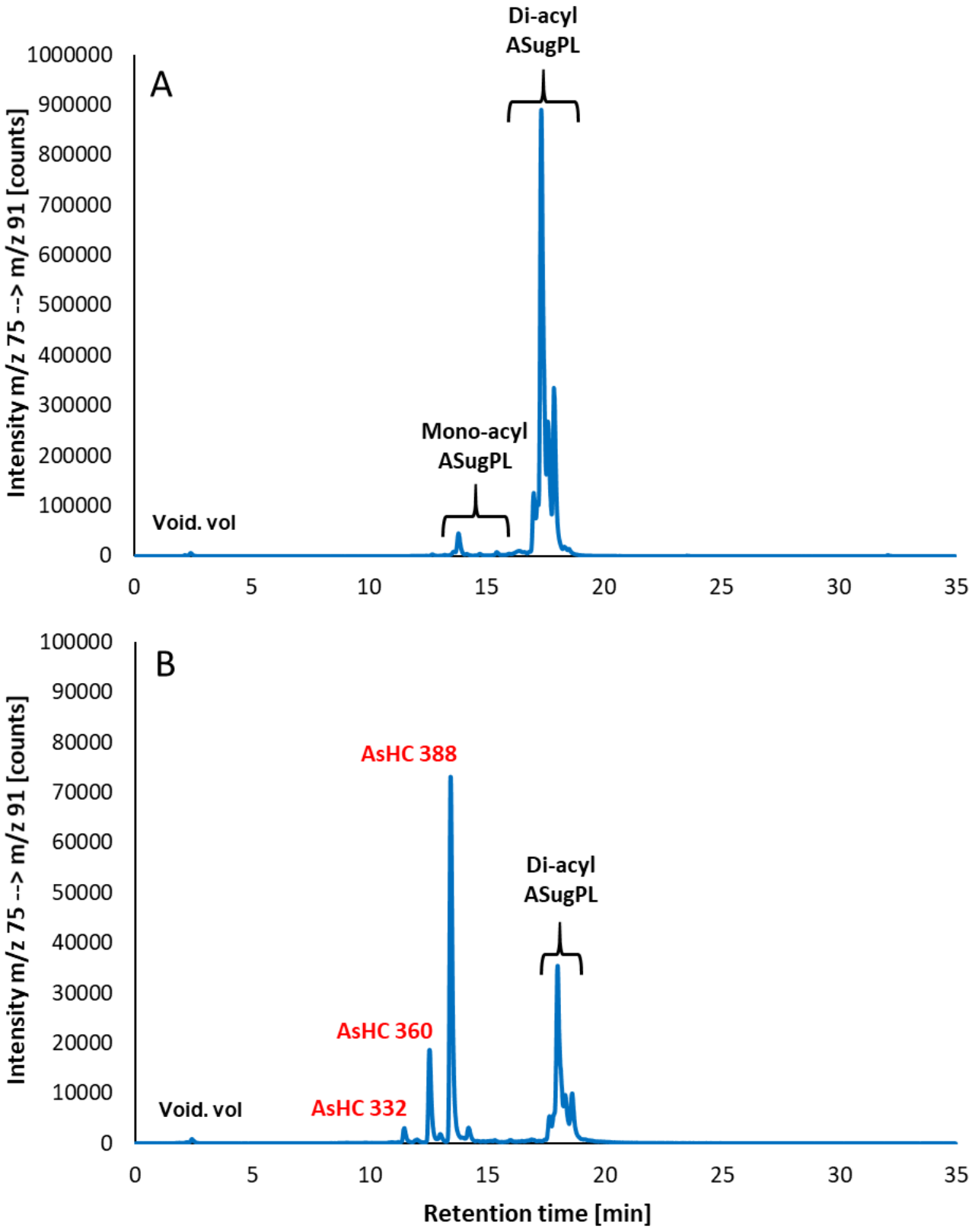

Figure S5. HPLC/ICP-MS/MS chromatograms of pooled ethanol/ammonia fractions 2-16 (A) and acetone/ammonia fractions 2-25 (B) of Florisil® SPE developed for the separation of AsHCs and di-acyl AsSugPLs in Wakame. Chromatographic conditions, see Figure S4.

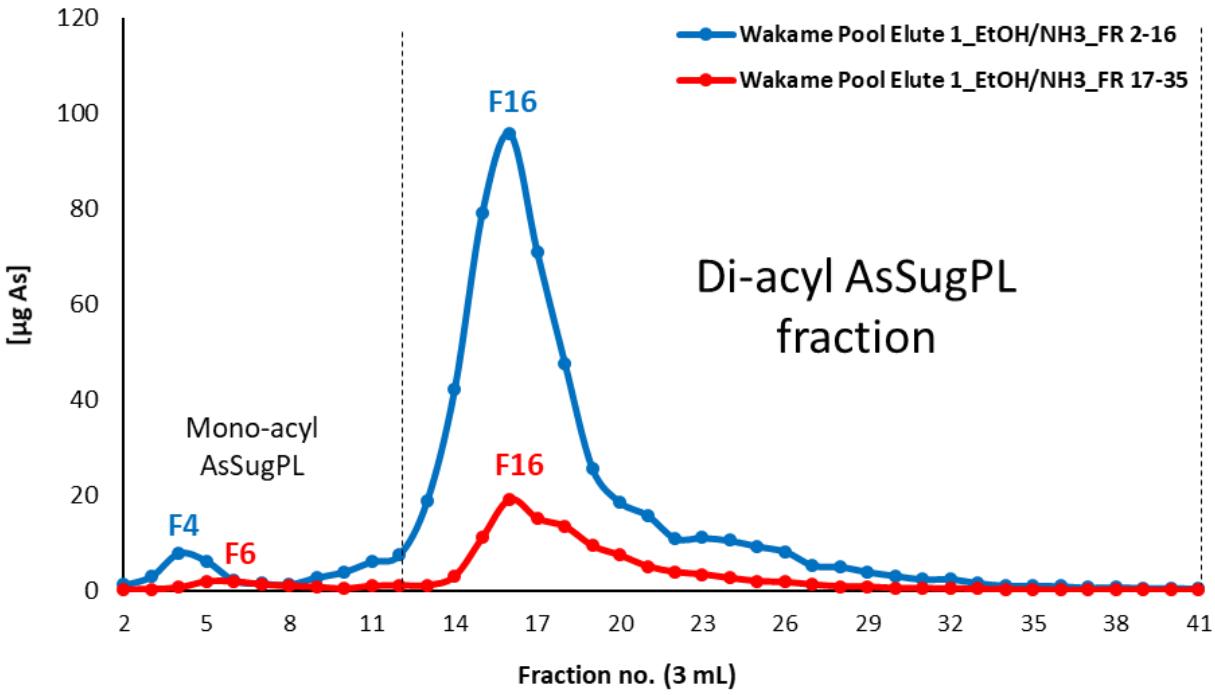

Figure S6. Distribution of arsenic in collected fractions obtained by preparative RP-liquid chromatography of puirifed Wakame extracts.

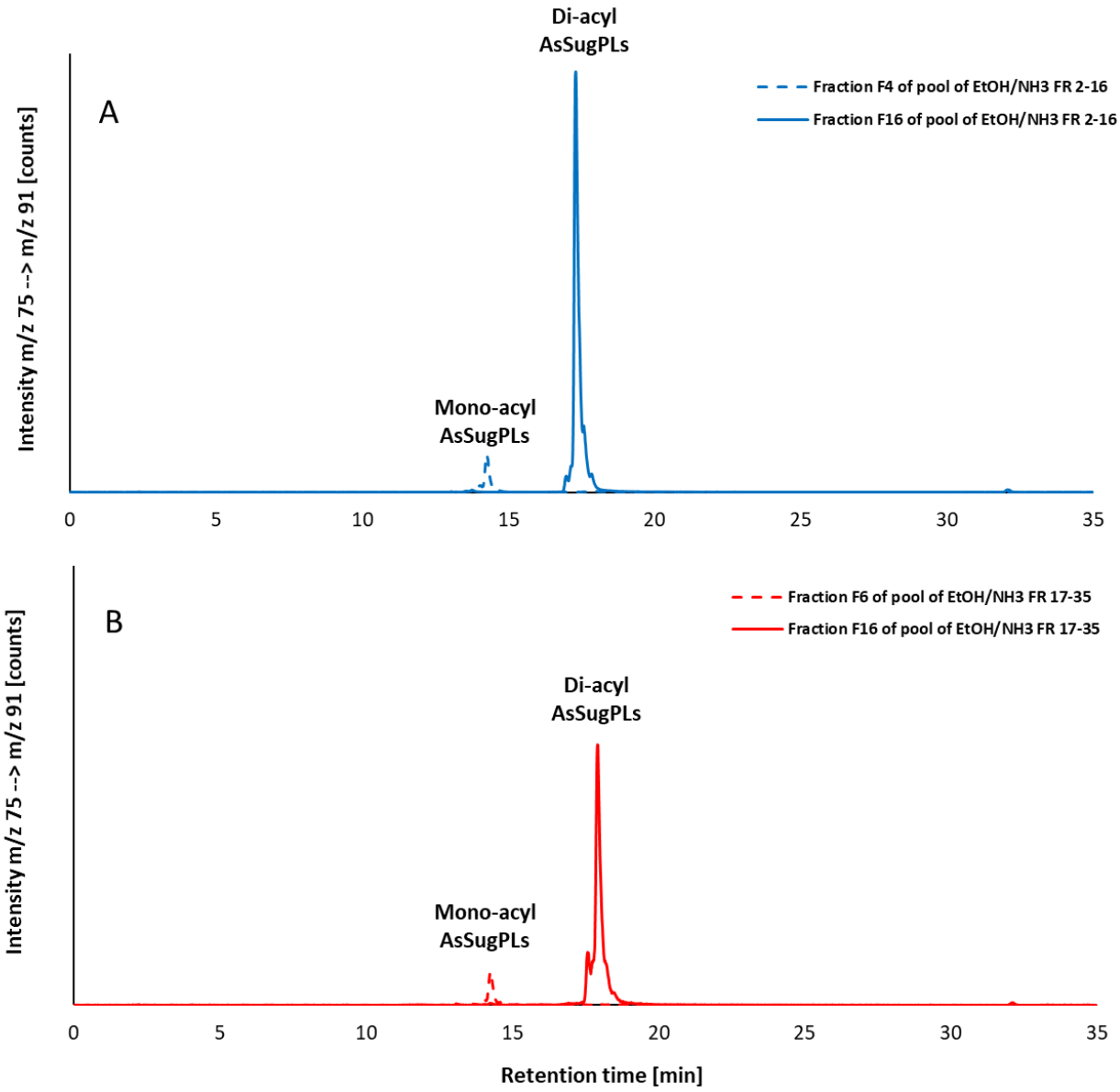

Figure S7. RP-HPLC/ICP-MS/MS chromatograms of selected fractions obtained by preparative RP liquid chromatography. Chromatographic conditions, see Figure S4.

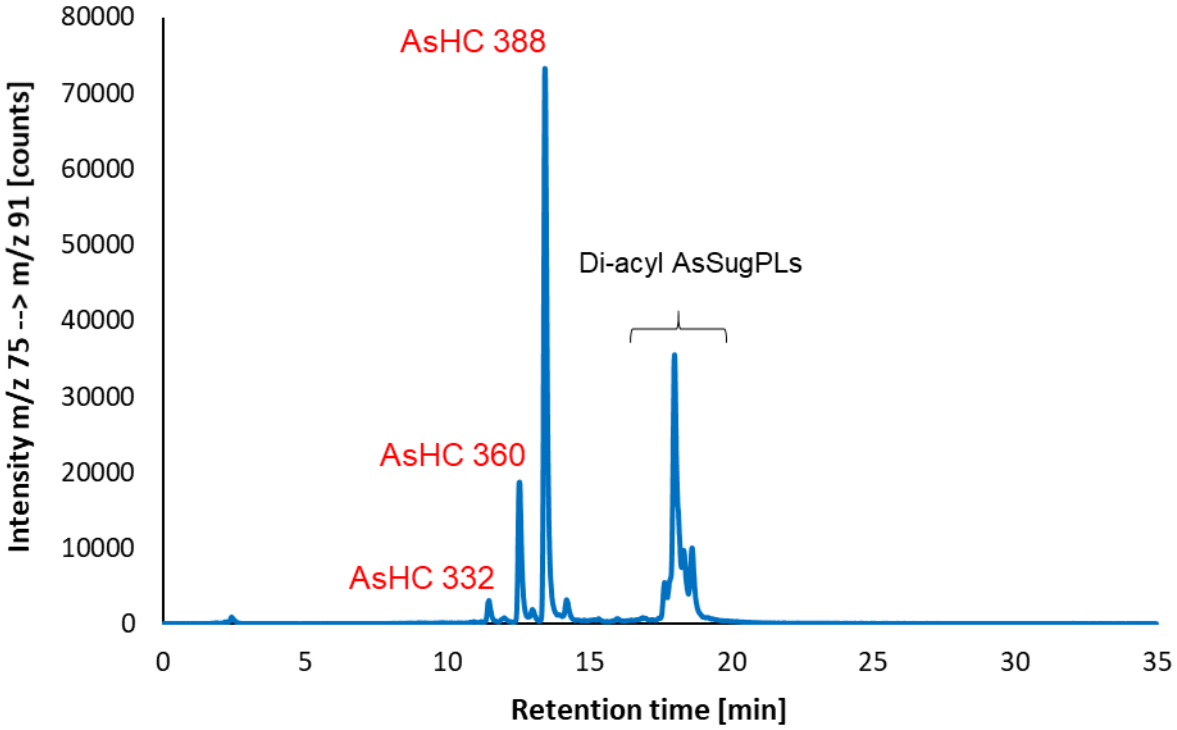

Figure S8. RP-HPLC/ICP-MS/MS chromatogram of pooled acetone/ammonia fractions of Wakame extracts subjected to SPE using Florisil®. Chromatographic conditions, see Figure S4.

Table S6. Characterization of the final purified extract of arsenic containing hydrocarbons obtained from salmon oil by HPLC/ICP-MS/MS/ES-MS/MS and HPLC/HR-ESMS. Total arsenic (mean, n=2, RSD <5%) was measured by ICP-MS/MS after microwave assisted acid digestion.

Arsenic hydrocarbons							
				Accurate molecular mass***			
Compound	Code	RT* [min]	Molecular formula	Found [u]	Calculated [u]	Δm/m [ppm]	Extract [%]
AsHC 330	1	17.4	$C_{17}H_{35}OAs$	331.1981	331.1977	1.4	1
AsHC 404	3	17.7	$C_{23}H_{37}OAs$	405.2137	405.2133	0.9	14
AsHC 406	-	-	$C_{23}H_{39}OAs$	407.2286	407.2290	-0.8	traces
AsHC 332	2	17.9	$C_{17}H_{37}OAs$	333.2136	333.2133	0.8	23
AsHC 358	4	18.0	$C_{19}H_{39}OAs$	359.2293	359.2290	0.8	8
AsHC 408	-	-	$C_{23}H_{41}OAs$	409.2448	409.2446	0.6	traces
AsHC 346	5	18.3	$C_{18}H_{39}OAs$	347.2292	347.2290	0.7	2
AsHC 372	-	-	$C_{20}H_{41}OAs$	373.2449	373.2446	0.8	traces
AsHC 360	6	18.8	$C_{19}H_{41}OAs$	361.2448	361.2446	0.5	47
AsHC 374	-	-	$C_{20}H_{43}OAs$	375.2606	375.2603	0.9	traces
AsHC 386	-	-	$C_{21}H_{43}OAs$	387.2602	387.2603	-0.3	traces
SUM**							95
Mass [mg]							28
Arsenic [µg]							2190

*Retention time (RT) obtained by HPLC/ICP-MS/MS (see Figure 2C)
**Sum arsenolipids identified by HPLC/HR-ESMS.
***Protonated accurate molecular mass.

Table S7. Characterization of the final purified extract of arsenic containing fatty acids obtained from salmon oil by HPLC/ICP-MS/MS/ES-MS/MS and HPLC/HR-ESMS. Total arsenic (mean, n=2, RSD <5%) was measured by ICP-MS/MS after microwave assisted acid digestion.

Arsenic fatty acids							
				Accurate molecular mass***			
Compound	Code	RT* [min]	Molecular formula	Found [u]	Calculated [u]	Δm/m [ppm]	Extract [%]
AsFA 306	7	6.7	$C_{13}H_{27}O_3As$	307.1257	307.1249	2.6	0
AsFA 328	8	7.6	$C_{15}H_{25}O_3As$	329.1101	329.1092	2.7	3
AsFA 334	9	10.0	$C_{15}H_{31}O_3As$	335.1567	335.1562	1.5	4
AsFA 356	11	10.0	$C_{17}H_{29}O_3As$	357.1414	357.1405	2.3	
AsFA 342	10	8.9	$C_{16}H_{27}O_3As$	343.1254	343.1249	1.5	3
AsFA 368	12	10.5	$C_{18}H_{29}O_3As$	369.1411	369.1405	1.5	8
AsFA 370	13	11.1	$C_{18}H_{31}O_3As$	371.1570	371.1562	2.3	1
AsFA 382	14	11.4	$C_{19}H_{31}O_3As$	383.1570	383.1562	2.1	2
AsFA 362	15	12.2	$C_{17}H_{35}O_3As$	363.1876	363.1875	0.3	13
AsFA 408	16	12.6	$C_{21}H_{33}O_3As$	409.1729	409.1718	2.6	5
AsFA 376	17	13.0	$C_{18}H_{37}O_3As$	377.2038	377.2031	1.7	1
AsFA 422	18	13.2	$C_{22}H_{35}O_3As$	423.1878	423.1875	0.8	3
AsFA 434	19	13.6	$C_{23}H_{35}O_3As$	435.1891	435.1875	3.7	1
AsFA 390	20	13.9	$C_{19}H_{39}O_3As$	391.2191	391.2188	0.8	19
AsFA 436	21	13.9	$C_{23}H_{37}O_3As$	437.2035	437.2031	0.8	
AsFA 448	22	14.1	$C_{24}H_{37}O_3As$	449.2034	449.2031	0.6	8
AsFA 450	23	14.3	$C_{24}H_{39}O_3As$	451.2203	451.2188	3.3	2
AsFA 404	24	14.4	$C_{20}H_{41}O_3As$	405.2350	405.2344	1.3	2
AsFA 418	25	15.2	$C_{21}H_{43}O_3As$	419.2506	419.2501	1.1	2
AsFA 430	26	15.2	$C_{22}H_{43}O_3As$	431.2512	431.2501	2.5	
AsFA 528	27	16.1	$C_{30}H_{45}O_3As$	529.2664	529.2657	1.3	3
AsFA 446	28	16.2	$C_{23}H_{47}O_3As$	447.2814	447.2814	-0.1	1
Unidentified							13
Void vol.							5
SUM**							82
Mass [mg]							85
Arsenic [µg]							520

*Retention time (RT) obtained HPLC/ICP-MS/MS (see Figure 2B).
**Sum arsenolipids identified by HPLC/HR-ESMS.
***Protonated accurate molecular mass.

Table S8. Characterization of the final purified extract of di-acyl arsenosugar phospholipids obtained from brown alga *Wakame* by HPLC/ICP-MS/MS/ES-MS/MS and HPLC/HR-ESMS. Total arsenic (mean, n=2, RSD <5%) was measured by ICP-MS/MS after microwave assisted acid digestion.

Di-acyl arsenosugar phospholipids							
				Accurate molecular mass***			
Compound	**Code**	**RT***	**Molecular formula**	**Found**	**Calculated**	**Δm/m**	**Extract**
		[min]		**[u]**	**[u]**	**[ppm]**	**[%]**
AsSugPL 930	E	20.0	$C_{43}H_{84}O_{14}AsP$	931.4904	931.4887	1.7	7
AsSugPL 956	F	20.3	$C_{45}H_{86}O_{14}AsP$	957.5069	957.5044	2.6	3
AsSugPL 982	G	20.7	$C_{47}H_{88}O_{14}AsP$	983.5222	983.5200	2.2	9
AsSugPL 944	H	20.7	$C_{44}H_{86}O_{14}AsP$	945.5066	945.5044	2.3	
AsSugPL 958	I	21.5	$C_{45}H_{88}O_{14}AsP$	959.5216	959.5200	1.6	39
AsSugPL 984	J	21.7	$C_{47}H_{90}O_{14}AsP$	985.5374	985.5370	1.7	9
AsSugPL 986	K	23.3	$C_{47}H_{92}O_{14}AsP$	987.5531	987.5513	1.8	9
AsSugPL 1014	L	25.7	$C_{49}H_{96}O_{14}AsP$	1015.5848	1015.5826	2.1	11
Unknown 1	UK 1	19.9					2
Unknown 2	UK 2	21.1					1
Unknown 3	UK 3	22.3					2
Unknown 4	UK 4	22.9					1
Unknown 5	UK 5	23.6					2
Unknown 6	UK 6	24.3					1
SUM**							96
Mass [mg]							20
Arsenic [µg]							345

*Retention time (RT) obtained by HPLC/ICP-MS/MS (see Figure 3C).
**Sum arsenolipids identified by HPLC/HR-ESMS.
***Protonated accurate molecular mass.

Table S9. Characterization of the final purified extract of arsenic containing hydrocarbons obtained from brown alga *Wakame* by HPLC/ICP-MS/MS/ES-MS/MS and HPLC/HR-ESMS. Total arsenic (mean, n=2, RSD <5%) was measured by ICP-MS/MS after microwave assisted acid digestion.

Arsenic hydrocarbons							
				Accurate molecular mass***			
Compound	**Code**	**RT***	**Molecular formula**	**Found**	**Calculated**	**Δm/m**	**Extract**
		[min]		**[u]**	**[u]**	**[ppm]**	**[%]**
AsHC 332	A	17.9	$C_{17}H_{37}OAs$	333.2136	333.2133	1.0	3
AsHC 346	-	18.1	$C_{18}H_{39}OAs$	347.2292	347.2290	0.7	1
AsHC 360	B	18.7	$C_{19}H_{41}OAs$	361.2447	361.2446	0.1	20
AsHC 386	-	19.0	$C_{21}H_{43}OAs$	387.2606	387.2603	0.8	2
AsHC 374	-	19.3	$C_{20}H_{43}OAs$	375.2607	375.2603	1.2	2
AsHC 388	C	19.8	$C_{21}H_{45}OAs$	389.2760	389.2759	0.2	69
SUM**							97
Mass [mg]							17
Arsenic [µg]							237

*Retention time (RT) obtained HPLC/ICP-MS/MS (see Figure 3B).
**Sum arsenolipids identified by HPLC/HR-ESMS.
***Protonated accurate molecular mass