

Electronic Supplementary Information

Lipid-soluble arsenic species identified in the brain of the marine fish skipjack tuna (*Katsuwonus pelamis*) using a sequential extraction and HPLC/mass spectrometry

Michael Stiboller^a, Fabiana P. Freitas^b, Kevin A. Francesconi^a, Tanja Schwerdtle^c, António J. A. Nogueira^b and Georg Raber^{a#}

^aUniversity of Graz, Institute of Chemistry, Analytical Chemistry NAWI Graz, Universitätsplatz 1/I, 8010 Graz.

^bUniversity of Aveiro, Department of Biology & CESAM, 3810 Aveiro, Portugal

^cUniversity of Potsdam, Institute of Nutritional Sciences, Food Chemistry, Arthur-Scheunert-Alle 114-116, 14558 Nuthetal, Germany

#Corresponding author: georg.raber@uni-graz.at

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Table S1. Identification (HPLC/HR ESMS) and quantification (HPLC/ICPMS) of arsenolipids in BCR-627 tuna fish tissue.

Table S2. Arsenic species in brain and muscle tissues of skipjack tuna fish identified by HPLC/HR ESMS.

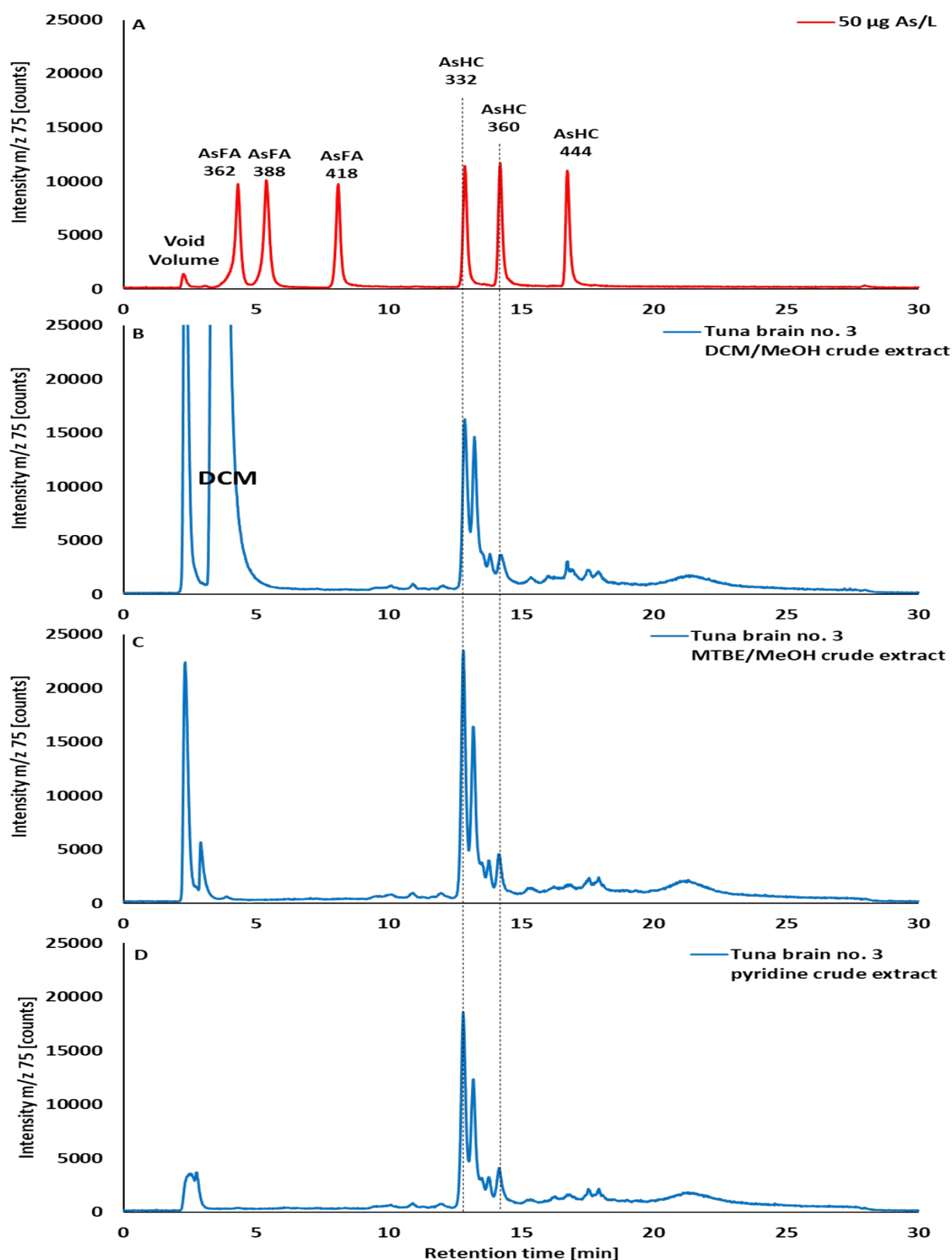


Figure S1. HPLC/ICPMS chromatograms of (A) a 50 μg As/L arsenolipid mixed standard solution and tuna brain no. 3 organic crude extracts of (B) DCM/MeOH, (C) MTBE/MeOH and (D) pyridine extractions. Column, ACE Ultra Core 5 SuperPhenylHexyl (250 x 4.6 mm; 5 μm); mobile phases, 25 mM $\text{NH}_4(\text{CH}_3\text{COO})/\text{water}$ pH 9.2 (pH adjusted with aqueous ammonia) and 25 mM $\text{NH}_4(\text{CH}_3\text{COO})/\text{MeOH}$ pH 9.2; gradient elution: 0-15 min from 60-100%, 15-25 min holding at 100%, and returning at 25.1min to 60% 25 mM $\text{NH}_4(\text{CH}_3\text{COO})/\text{MeOH}$ pH 9.2; flow rate, 1 mL/min; T, 40°C and injection volume 20 μL .

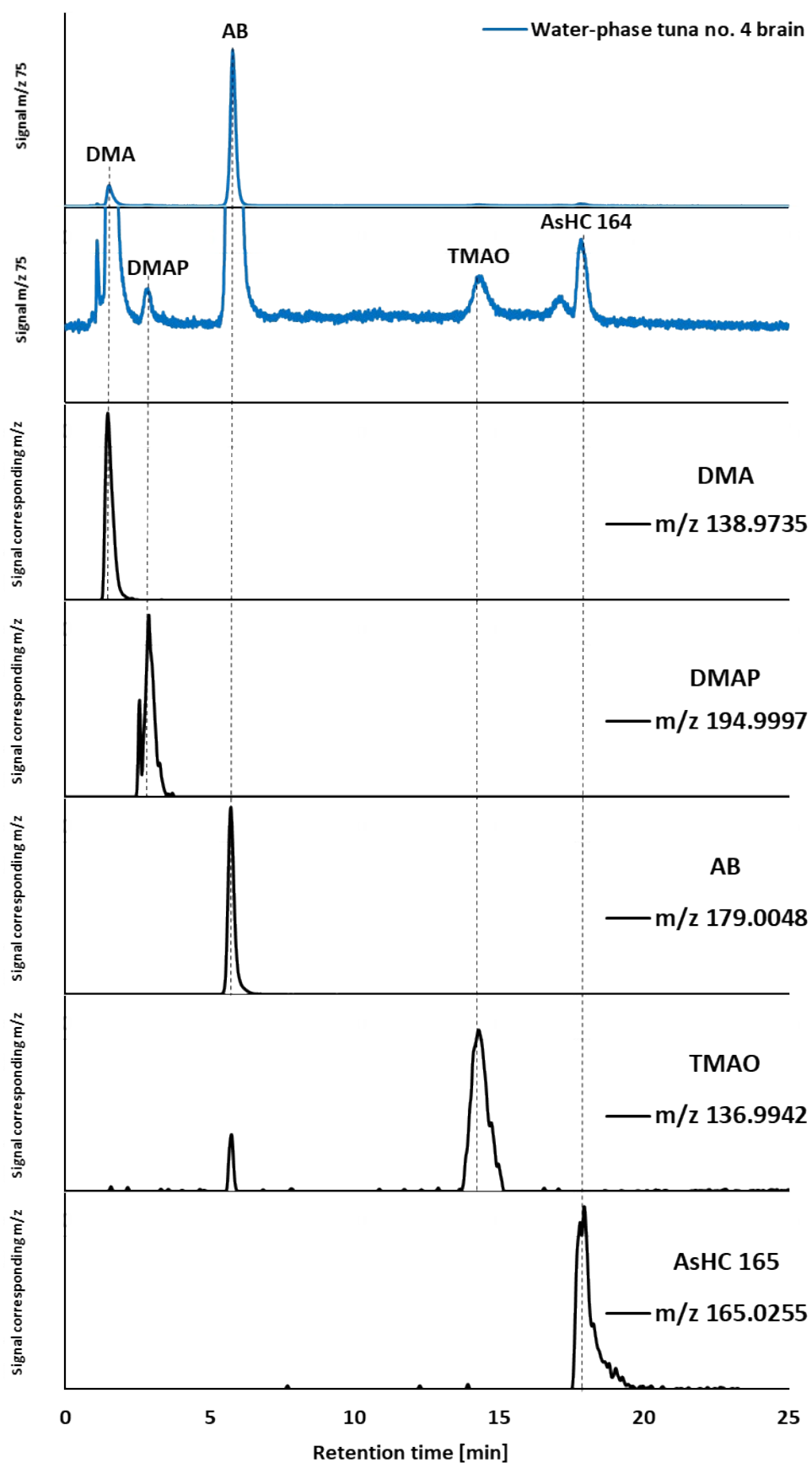


Figure S2. Overlay of cation-exchange HPLC/ICPMS chromatograms (blue) and cation-exchange HPLC/HR ESMS extracted ion chromatograms (black) of specific protonated m/z of DMA, AB, TMAO and AsHC 164 (extraction window ± 5 ppm) of brain water crude extract of skipjack tuna no. 4.

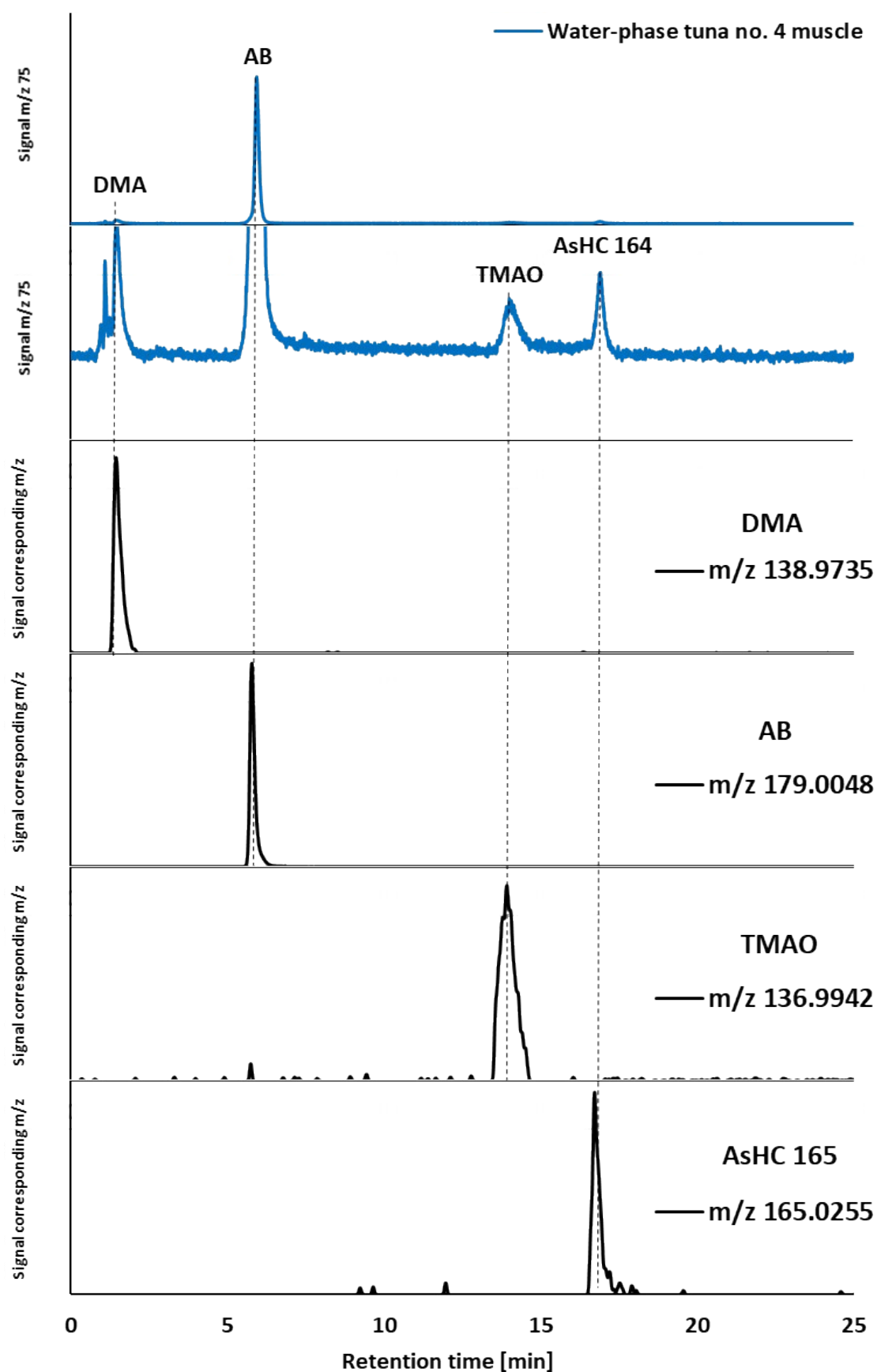


Figure S3. Overlay of cation-exchange HPLC/ICPMS chromatograms (blue) and cation-exchange HPLC/HR ESMS extracted ion chromatograms (black) of specific protonated m/z of DMA, AB, TMAO and AsHC 164 (extraction window ± 5 ppm) of muscle crude water extract of skipjack tuna no. 4.

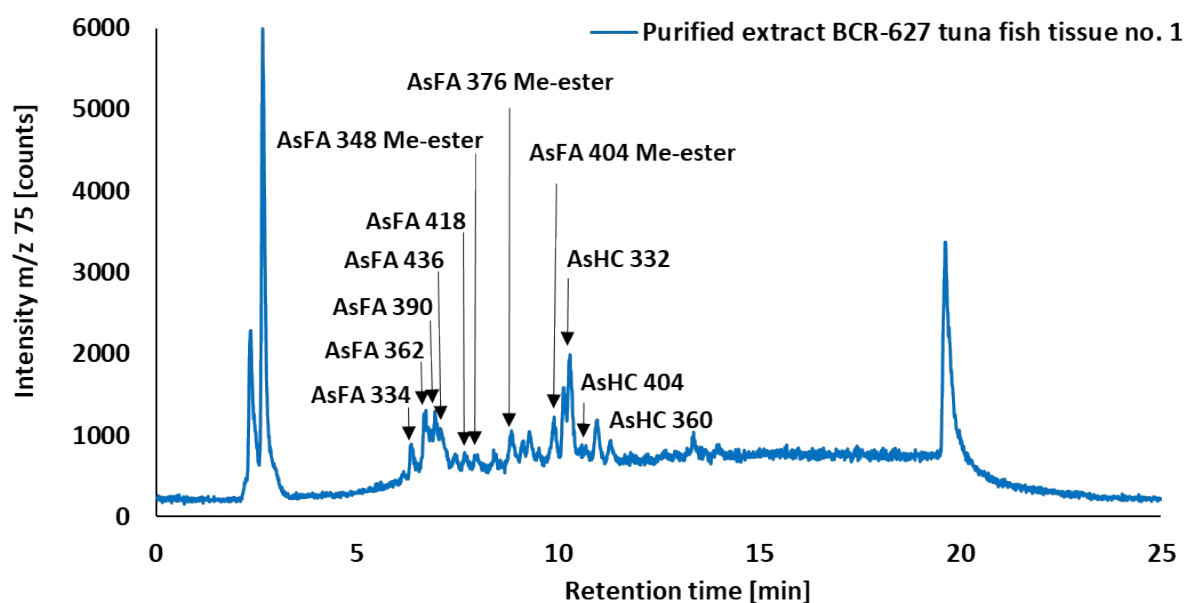


Figure S4. HPLC/ICPMS chromatogram of silica-purified pyridine extract of BCR-627 tuna fish tissue no. 1. Column, ACE Ultra Core 5 SuperPhenylHexyl (250 x 4.6 mm; 5 μ m); mobile phases, 25 mM $\text{NH}_4(\text{CH}_3\text{COO})/\text{water}$ pH 9.2 (pH adjusted with aqueous ammonia) and 25 mM $\text{NH}_4(\text{CH}_3\text{COO})/\text{MeOH}$ pH 9.2; gradient elution: 0-2 min from 0-20%, 2-4 min from 20-80%, 4-12 min from 80-100%, 12-17 min holding at 100% and returning at 17.1min to 25 min to 0% 25 mM $\text{NH}_4(\text{CH}_3\text{COO})/\text{MeOH}$ pH 9.2; flow rate, 1 mL/min; T, 40°C and injection volume 20 μ L.

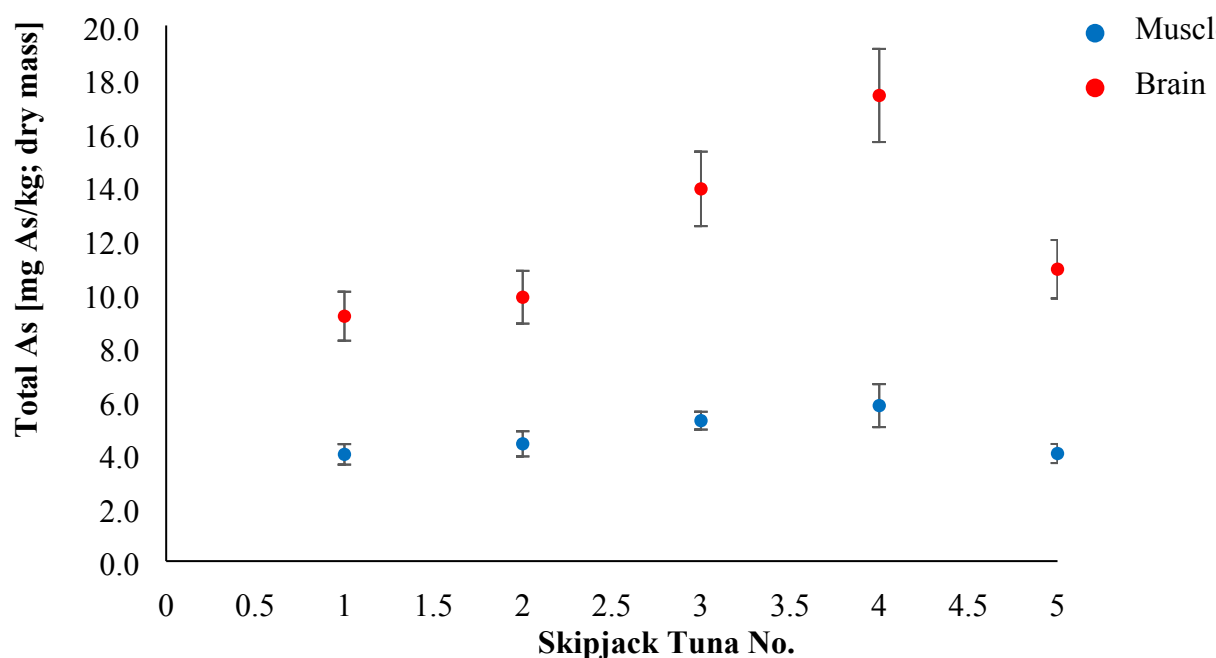


Figure S5. Mean total As concentrations of freeze-dried muscle (n=4) and brain tissues (n=2) of the natural marine fish Skipjack tuna (*Katsuwonus pelamis*). Error bars represent standard deviations of four and two replicates of muscle and brain tissues respectively. Error bars of brain tissues are calculated as $\pm 10\%$ (estimated precision of total As determination by ICPMS. The relative deviation of the total As content of the two brain replicates was $\leq 5\%$).

Table S1. Arsenolipids identified in purified extracts by HPLC/HR ESMS in the certified reference material BCR-627 tuna fish tissue (not certified for arsenolipids) after pyridine extraction and SPE-silica purification. Quantification of respective arsenolipids was performed by HPLC/ICPMS (mean \pm SD; n=3; dry mass).

Compound code	Formula [M+H] ⁺	Mass Theor.	Mass Exp.	Δm [ppm]	Concentration [μg As/kg]
AsFA 334	C ₁₅ H ₃₂ AsO ₃	335.1562	335.1565	-0.9	9 \pm 1
AsFA 362	C ₁₇ H ₃₆ AsO ₃	363.1875	363.1867	-2.2	20 \pm 2
AsFA 390	C ₁₉ H ₄₀ AsO ₃	391.2188	391.2190	0.5	15 \pm 2
AsFA 436	C ₂₃ H ₃₈ AsO ₃	437.2031	437.2039	1.8	16 \pm 1
AsFA 418	C ₂₁ H ₄₄ AsO ₃	419.2501	419.2506	1.2	10 \pm 1
AsHC 332	C ₁₇ H ₃₈ AsO	333.2133	333.2130	-0.9	28 \pm 3
AsHC 360	C ₁₉ H ₄₂ AsO	361.2446	361.2447	0.3	9 \pm 1
AsHC 404	C ₂₃ H ₃₈ AsO	405.2133	405.2133	0.1	6 \pm 2
AsFA 348 Me-ester	C ₁₆ H ₃₄ AsO ₃	349.1718	349.1718	0.1	10 \pm 1
AsFA 376 Me-ester	C ₁₈ H ₃₈ AsO ₃	377.2031	377.2023	-2.2	16 \pm 1
AsFA 404 Me-ester	C ₂₀ H ₄₂ AsO ₃	405.2344	405.2343	-0.3	6 \pm 2
Arsenolipid content					300 \pm 15
Total As (n=6)					4700 \pm 300

Table S2. Arsenic species identified by HPLC/HR ESMS in crude water extracts of brain and muscle tissues of skipjack tuna.

Abbreviation Arsenic Compound	Molecular Formula [M]	Theor. Molecular mass [M+H] ⁺	Muscle	Brain
			Exp. Molecular Mass [M+H] ⁺	Exp. Molecular Mass [M+H] ⁺
DMA	C ₂ H ₇ AsO ₂	138.9735	138.9732 (Δm = -1.9)	138.9733 (Δm = -1.5)
DMAp	C ₅ H ₁₁ AsO ₃	194.9997	Not detected	194.9995 (Δm = -0.8)
AB	C ₅ H ₁₂ AsO ₂	179.0048	179.0046 (Δm = -1.0)	179.0046 (Δm = -1.1)
TMAO	C ₃ H ₉ AsO	136.9942	136.9939 (Δm = - 2.7)	136.9940 (Δm = - 1.3)
AsHC 164	C ₅ H ₁₃ AsO	165.0255	165.0252 (Δm = -2.2)	165.0257 (Δm = 0.3)

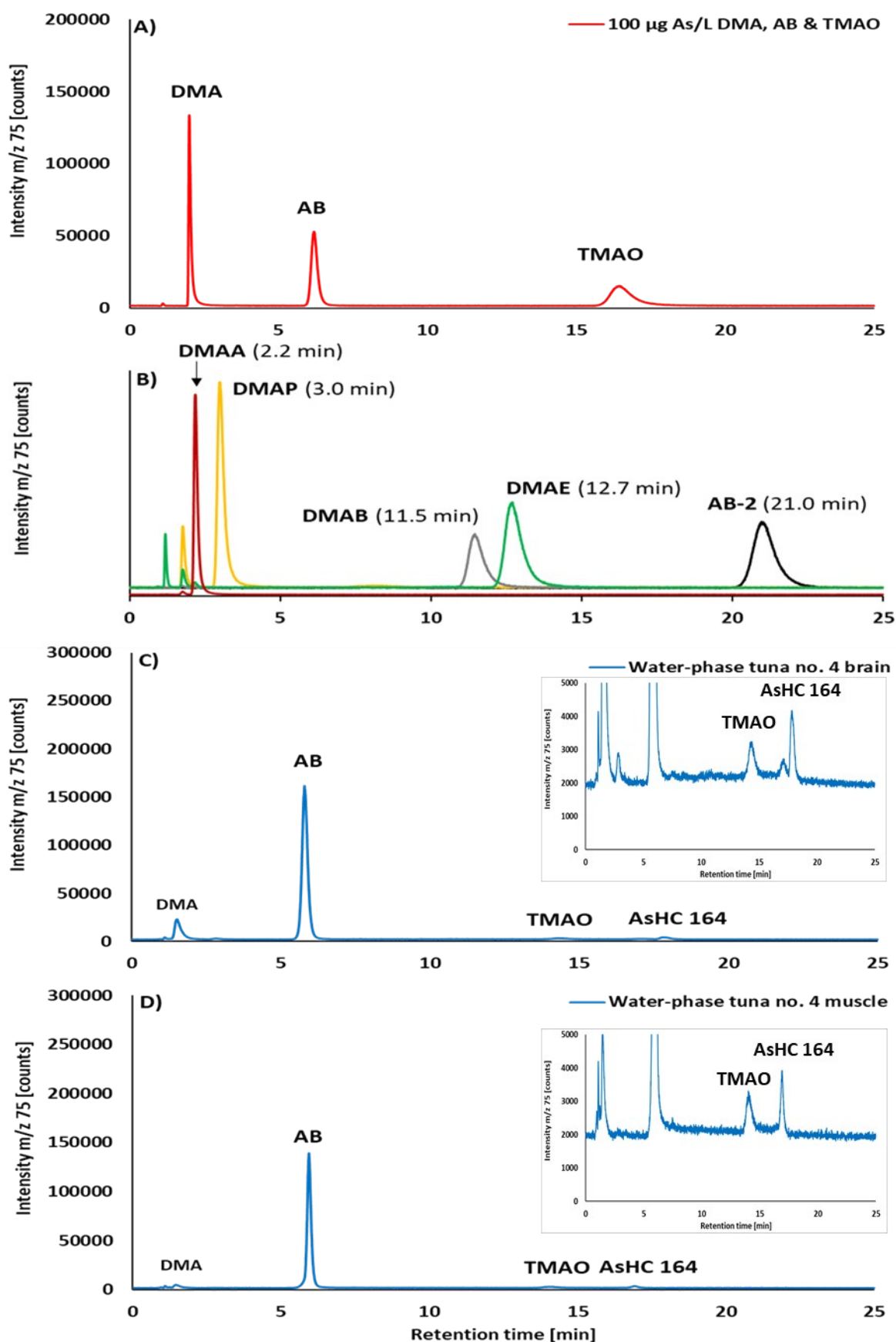


Figure S6. Cation-exchange HPLC/ICPMS chromatograms of A) DMA, AB and TMAO (each 100 $\mu\text{g As/L}$) calibration mixed standard solution, B) single standards of DMAA, DMAP, DMAB, DMAE and AB-2 (each 100 $\mu\text{g As/L}$), and water crude extracts of C) brain and D) muscle of skipjack tuna no. 4.

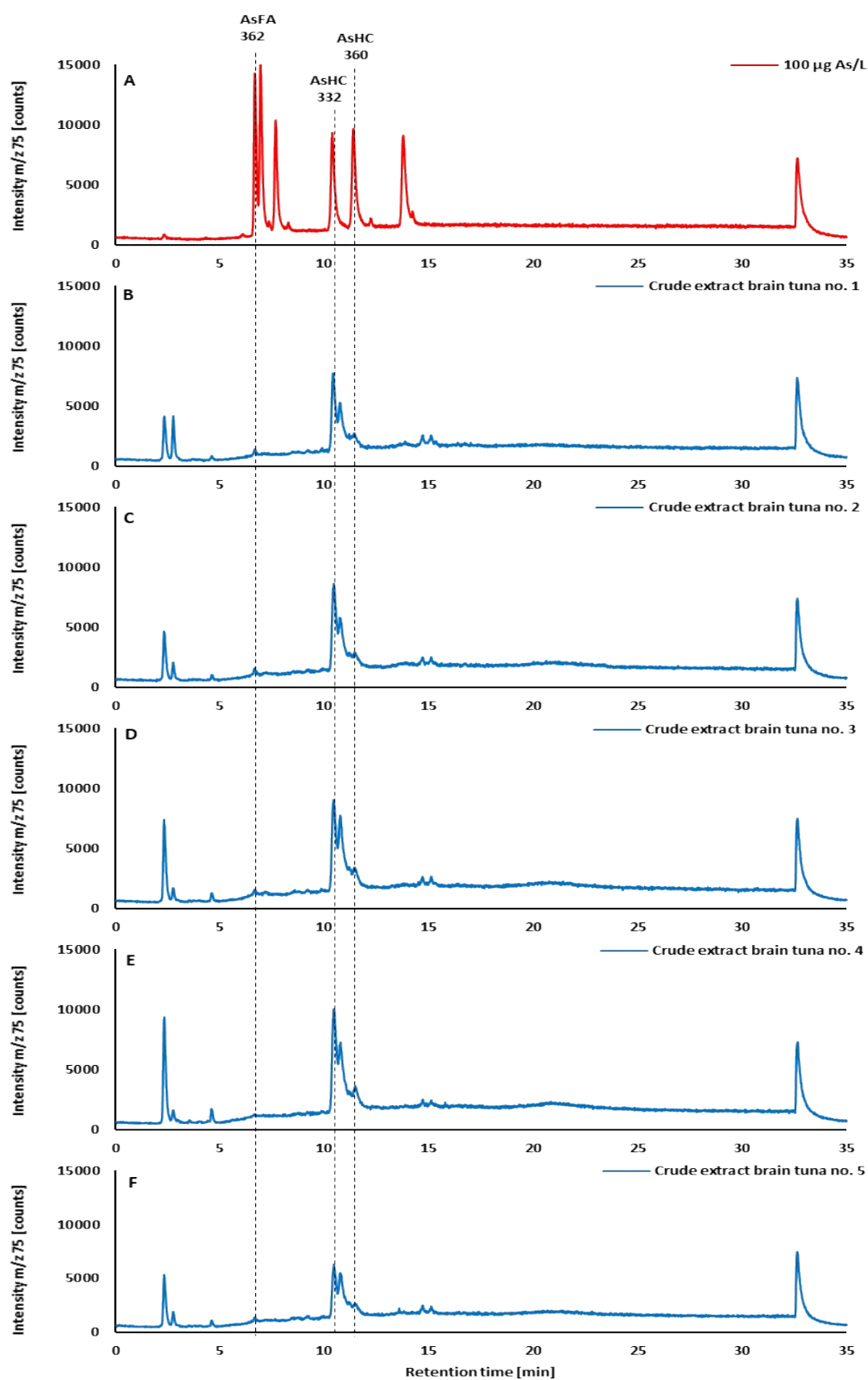


Figure S7. HPLC/ICPMS chromatograms of A) arsenolipid standard mixture and of B-G) pyridine crude extracts of brain tissues of skipjack tuna no. 1 -5.

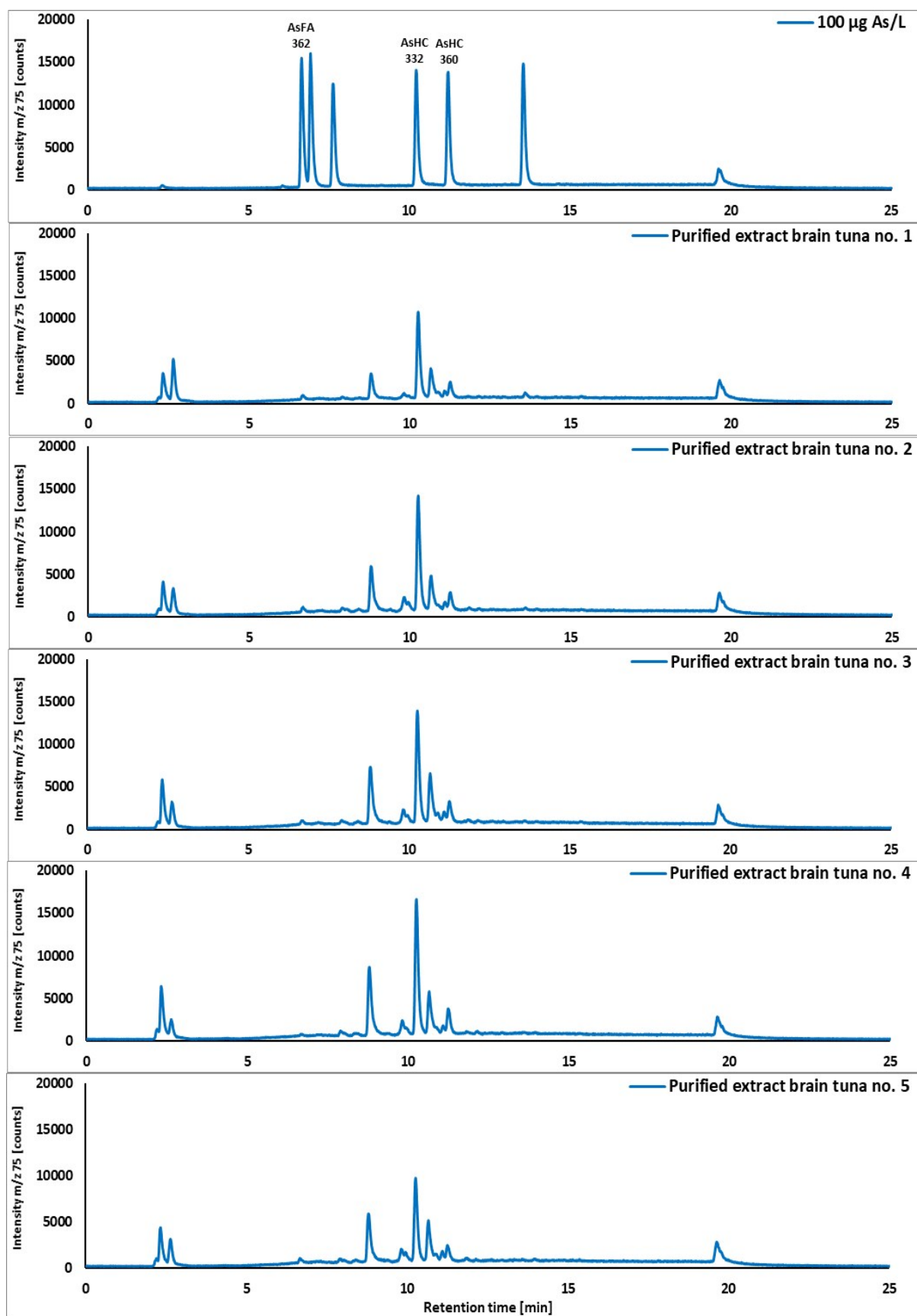


Figure S8. HPLC/ICPMS chromatograms of arsenolipid standard mixture and of silica-purified pyridine extracts of brain tissues of skipjack tuna no. 1 -5.

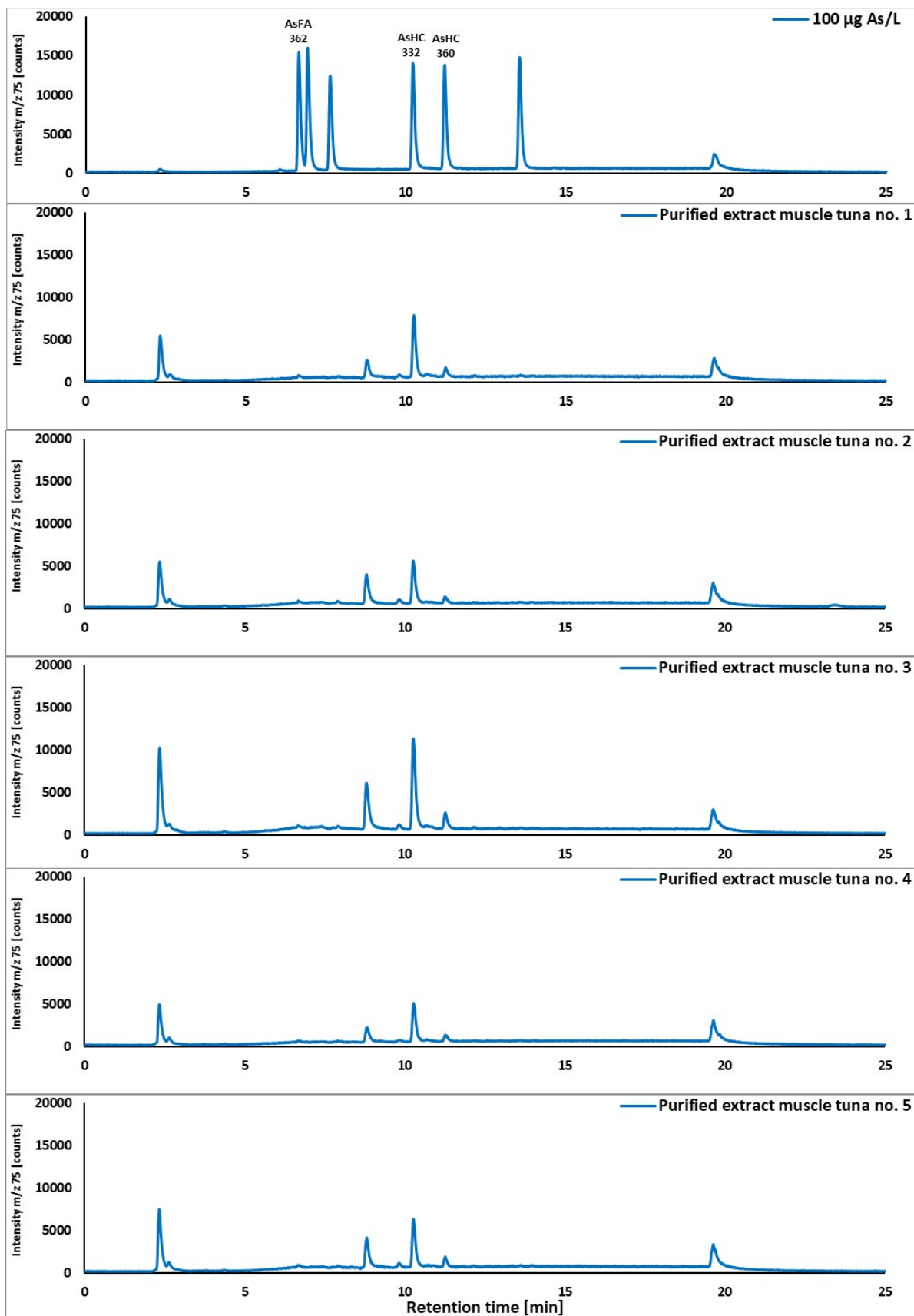


Figure S9. HPLC/ICPMS chromatograms of arsenolipid standard mixture and of silica-purified pyridine extracts of muscle tissues of skipjack tuna no. 1 -5.

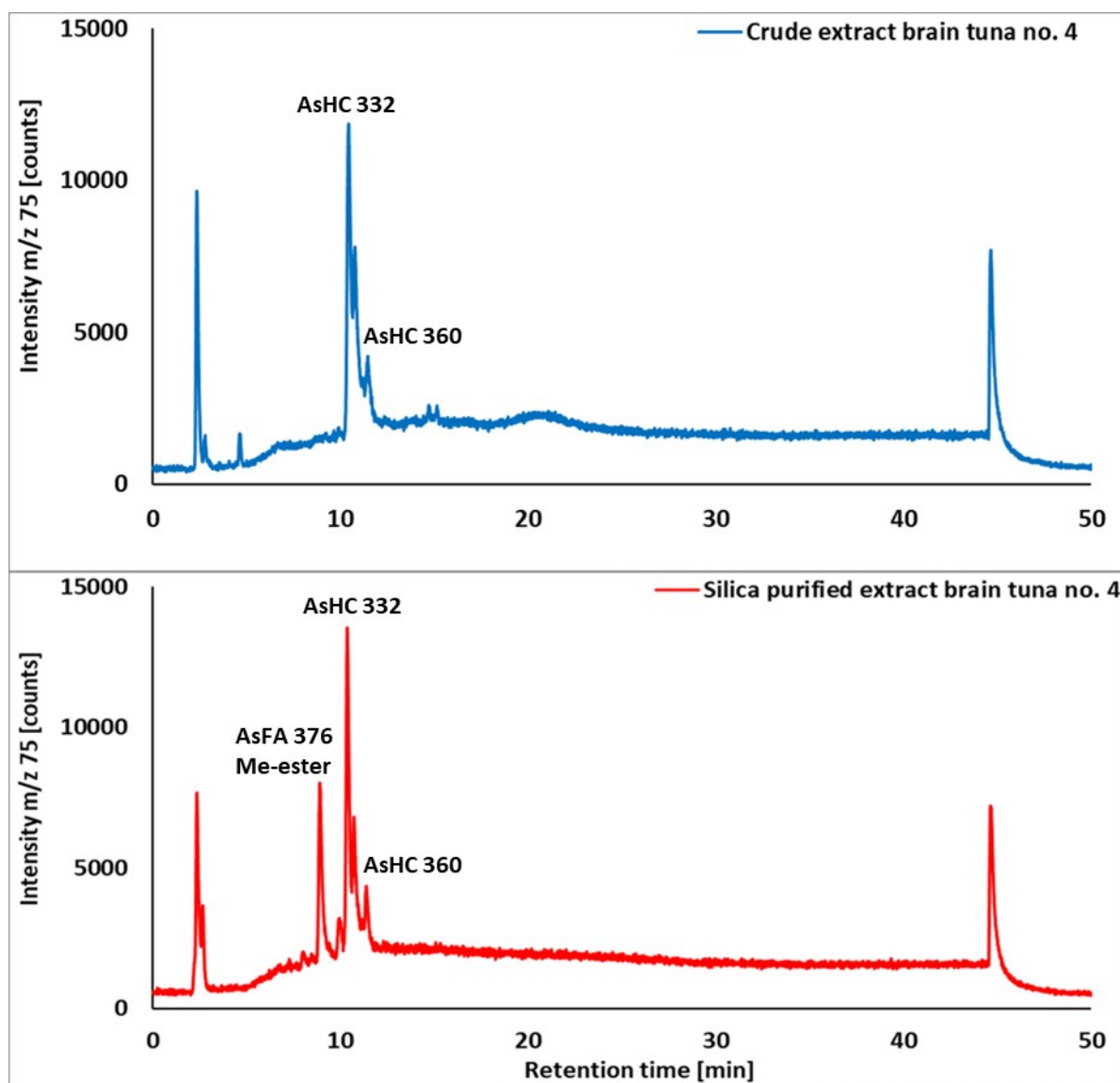


Figure S10. HPLC/ICPMS chromatograms of crude (blue) and silica-purified pyridine extracts (red) of brain tissue of skipjack tuna no. 4. Runtime set 50 min to exclude arsenolipids eluting after 25 min in crude and purified brain extracts.

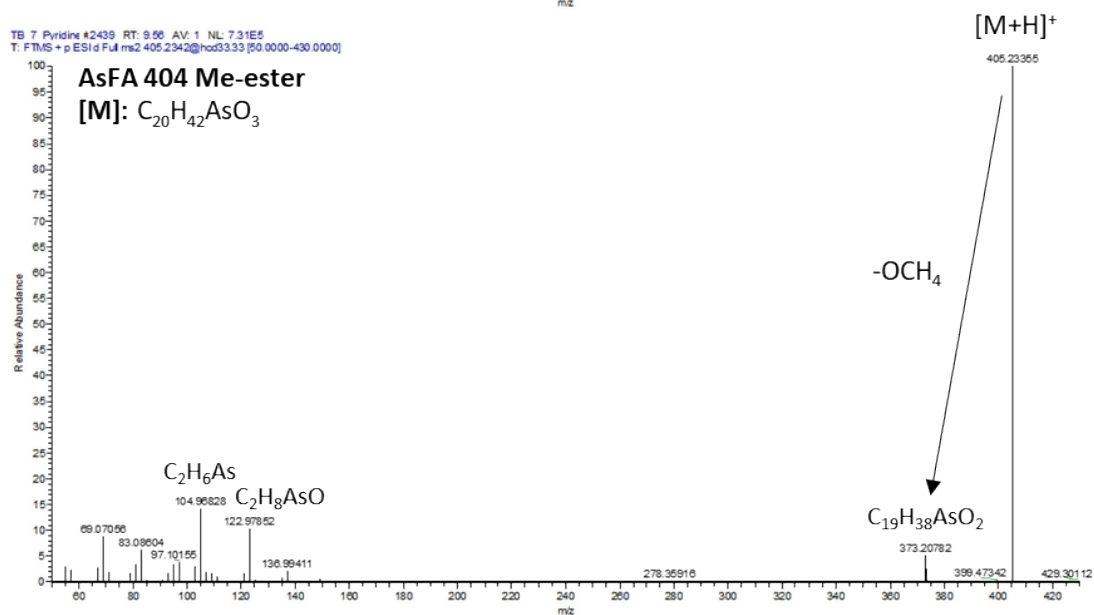
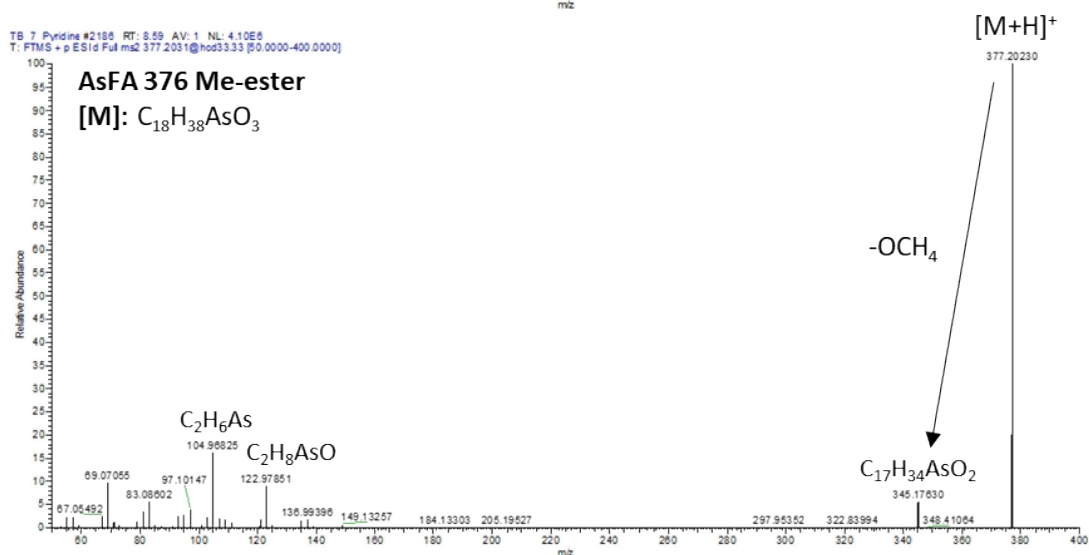
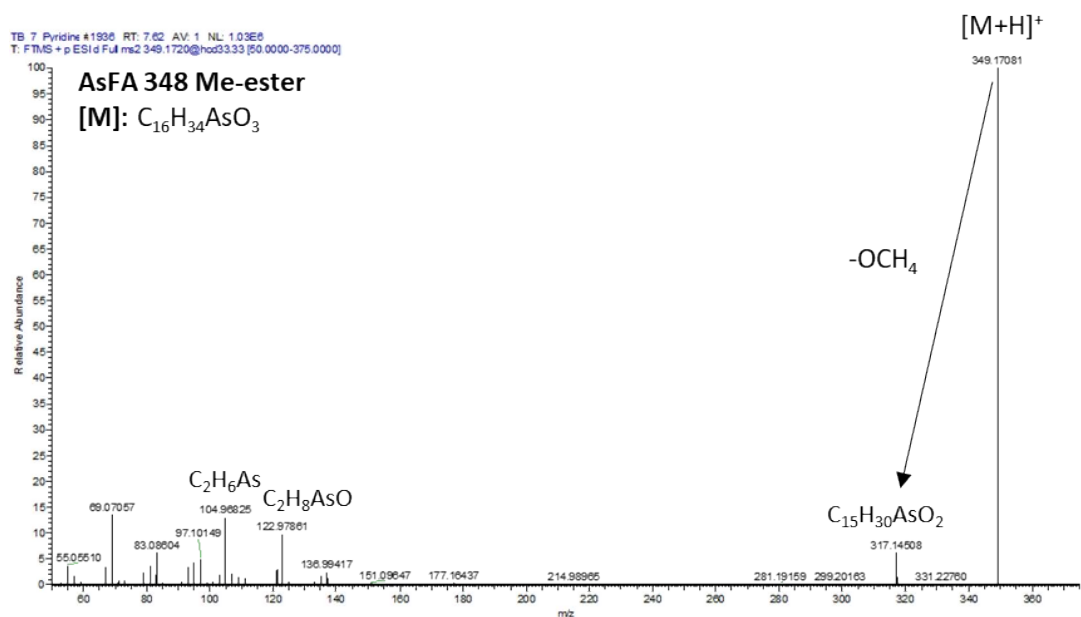


Figure S11. MS² spectra of AsFA Me-esters 348, 376 and 404 in silica-purified brain tissues of skipjack tuna obtained by HPLC/HR ESMS.