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Supplementary Material

New arsenic compound identified in rice grain: dimethylarsonyldimethylarsinic acid

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Electronic supplement for: **New arsenic compound identified in rice grain: dimethylarsonyldimethylarsinic acid**

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Tables S1 to S3

Figures S1 to S10

Table S1: *Instrumentation and main separation conditions used*

	US FDA	Graz	Graz
HPLC			
system	Agilent 1260	Agilent 1260	Dionex 3000
column	PRP X 100	Dionex AS14A	Dionex AS14A
eluent	10mM ammonium phosphate dibasic pH 8.25	30 mM Ammoniumcarbonate + 3 % (v/v) MeOH pH 10	30 mM Ammoniumcarbonate + 3 % (v/v) MeOH pH 10
flow rate (mL/min)	1	1	1
sample volume (µL)	100	50	20
ICPMS			
system	Agilent 7700 or 8800	Agilent 8800	
reaction gas(s)	He or O_2	O_2	
reaction gas(s) flow rate (mL/min)	0.3	0.3	
cones	nickel	nickel	
ESMS			
system		6440 TripleQuad	Exactive (Thermo)
polarity		positive	positive / negative
capillary voltage (V)			3500
mode		MRM	scan
MS/MS			automatic

Table S2: *other columns and separation conditions used during the experiments*

column	conditions
PRP-X100 4.1 x 250mm; 10µm	10mM ammonium phosphate dibasic at pH 8.25 (±0.05) (main paper see Figure 1)
	Mobile Phase A: 10mM Ammonium Nitrate pH=8.65 Mobile Phase B: 40mM Ammonium Nitrate pH=8.65 (0 min: 0% B, 5 min: 0% B, 6 min: 100% B, 11 min: 100% B, 12 min : 0% B) chromatogram see Figure S1
	Mobile Phase A: 2.5mM Ammonium Phosphate Dibasic pH=8.4 Mobile Phase B: 20mM Ammonium Phosphate Dibasic pH=8.4 (0 min: 0% B, 1 min: 0% B, 3 min: 100% B, 10 min: 100% B, 12 min: 0% B) chromatogram see Figure S2
	15mM AmFormate pH 4.6 chromatogram see Figure S3
	15mM AmFormate pH 5.5 chromatogram see Figure S3
	15mM AmFormate pH 9.3 chromatogram see Figure S3
PRP-X200 4.1 x 250mm; 10µm	5 mM pyridinium pH 2.6 chromatogram see Figure S4
DUAL ODS-CX10, 4.6 x 150mm; 5µm	50mM AmFormate pH 2.6 chromatogram see Figure S5
AS14A Dionex 4.6 x 250 mm	30 mM AmCarbonate + 3 % (v/v) MeOH pH 10.0 (main paper see Figure 3)

Table S3: *major HCD-fragments for Uk with elemental composition for $[M+H]^+$ 258.9285 ($C_4H_{13}As_2O_3$), $[M+H]^+$ 272.9448 ($C_5H_{15}As_2O_3$), $[M+H]^+$ 274.9056 ($C_4H_{13}As_2O_2S$), $[M+H]^+$ 290.8829 ($C_4H_{13}As_2OS_2$), $[M+H]^+$ 306.8598 ($C_4H_{13}As_2S_3$)*

$[M+H]^+$ 258.9285	$[M+H]^+$ 272.9448	$[M+H]^+$ 274.9054	$[M+H]^+$ 290.8830	$[M+H]^+$ 306.8600
$C_4H_{13}As_2O_3$	$C_5H_{15}As_2O_3$	$C_4H_{13}As_2O_2S$	$C_4H_{13}As_2OS_2$	$C_4H_{13}As_2S_3$
102.9527 (CH_4As^+) 0.4 ppm	102.9526 (CH_4As^+) 0.2 ppm	102.9527 (CH_4As^+) 0.4 ppm		
104.9683 ($C_2H_6As^+$) 3 ppm		104.9684 ($C_2H_6As^+$) 0.4 ppm	104.9685 ($C_2H_6As^+$) 0.4 ppm	104.9684 ($C_2H_6As^+$) 0.4 ppm
106.9473 (CH_4AsO^+) 0.7 ppm	106.9476 (CH_4AsO^+) 0.4 ppm	106.9477 (CH_4AsO) 1.9 ppm		
		108.9087 (H_2AsS^+) -0.5 ppm	108.9091 (H_2AsS^+) 2.8 ppm	108.9086 (H_2AsS^+) -1.4 ppm
116.9682 ($C_3H_6As^+$) 1.6 ppm	116.9682 ($C_3H_6As^+$) 1.1 ppm	116.9682 ($C_3H_6As^+$) 1.8 ppm		
118.9838 ($C_3H_8As^+$) 1.5 ppm	118.9838 ($C_3H_8As^+$) 1.6 ppm	118.9838 ($C_3H_8As^+$) 1.3 ppm	118.9838 ($C_3H_8As^+$) 0.2 ppm	118.9839 ($C_3H_8As^+$) 2.6 ppm
		122.9245 (CH_4AsS^+) 0.7 ppm		
		122.9784 ($C_2H_8AsO^+$) -1.4 ppm		
		136.9398 ($C_2H_6AsS^+$) -2 ppm	136.9400 ($C_2H_6AsS^+$) -0.8 ppm	136.9401 ($C_2H_6AsS^+$) 0.7 ppm
136.9944 ($C_3H_{10}AsO^+$) 1.2 ppm		136.9942 ($C_3H_{10}AsO^+$) 0.7 ppm		
138.9733 ($C_2H_8AsO_2^+$) -0.6 ppm		138.9733 ($C_2H_8AsO_2^+$) 1.2 ppm		
		150.9556 ($C_3H_8AsS^+$) -0.2 ppm	150.9556 ($C_3H_8AsS^+$) -0.2 ppm	150.9559 ($C_3H_8AsS^+$) 0.9 ppm
		152.9349 ($C_2H_6AsOS^+$) 0.3 ppm		
			154.9503 ($C_2H_8AsOS^+$)	

			-1.9 ppm	
164.8661 ($CH_3As_2^+$) 0.06 ppm	164.8662 ($CH_3As_2^+$) 0.6 ppm	164.8661 ($CH_3As_2^+$) -0.3 ppm		
				168.9127 ($C_2H_6AsS_2^+$) -3.9 ppm
				170.9278 ($C_2H_8AsS_2^+$) 0.4 ppm
			180.8962 ($C_2H_7As_2^+$) -1.1 ppm	
192.8972 ($C_3H_7As_2^+$) -0.9 ppm		192.8972 ($C_3H_7As_2^+$) -1.3 ppm		
			208.9288 ($C_4H_{11}As_2^+$) 0.2 ppm	
210.9079 ($C_3H_9As_2O^+$) 0.4 ppm	210.9082 ($C_3H_9As_2O^+$) 1.1 ppm	210.9079 ($C_3H_9As_2O^+$) 1.4 ppm		
			226.8852 ($C_3H_9As_2S^+$) 0.4 ppm	226.8852 ($C_3H_9As_2S$) 0.4 ppm
228.9186 ($C_3H_{11}As_2O_2^+$) 0.13 ppm	228.9187 ($C_3H_{11}As_2O_2^+$) 1.1 ppm	228.9186 ($C_3H_{11}As_2O_2^+$) -1 ppm		
	242.9338 ($C_4H_{13}As_2O_2^+$) -1.5 ppm	256.8958 ($C_4H_{11}As_2OS^+$) 0.2 ppm		
			272.8729 ($C_4H_{11}As_2S_2^+$) 0.2 ppm	272.8727 ($C_4H_{11}As_2S_2^+$) -0.1 ppm

Figures:

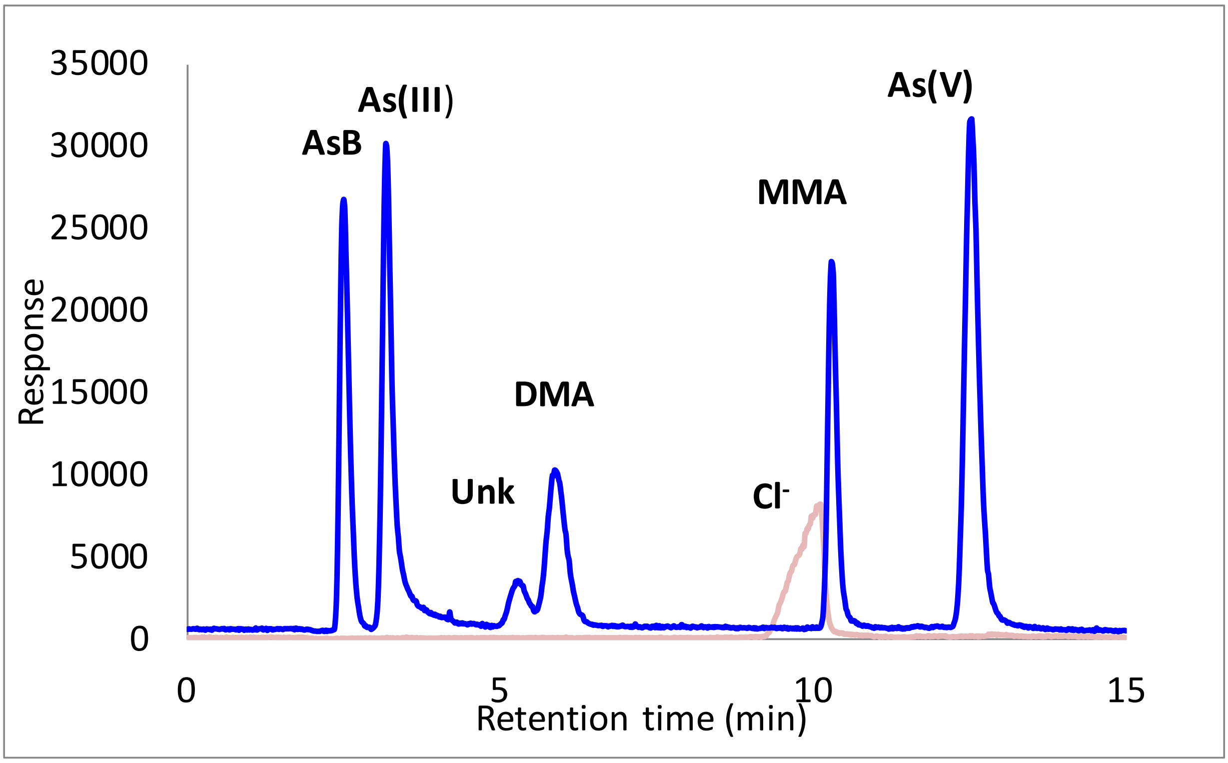

Figure S1*: separation of rice extract on PRP X 100 using a gradient of 10mM Ammonium Nitrate pH=8.65 and 40mM Ammonium Nitrate pH=8.65 (0 min: 0% B, 5 min: 0% B, 6 min: 100% B, 11 min: 100% B, 12 min: 0% B) ICPMS trace blue: ^{75}As, pink: ^{35}Cl*

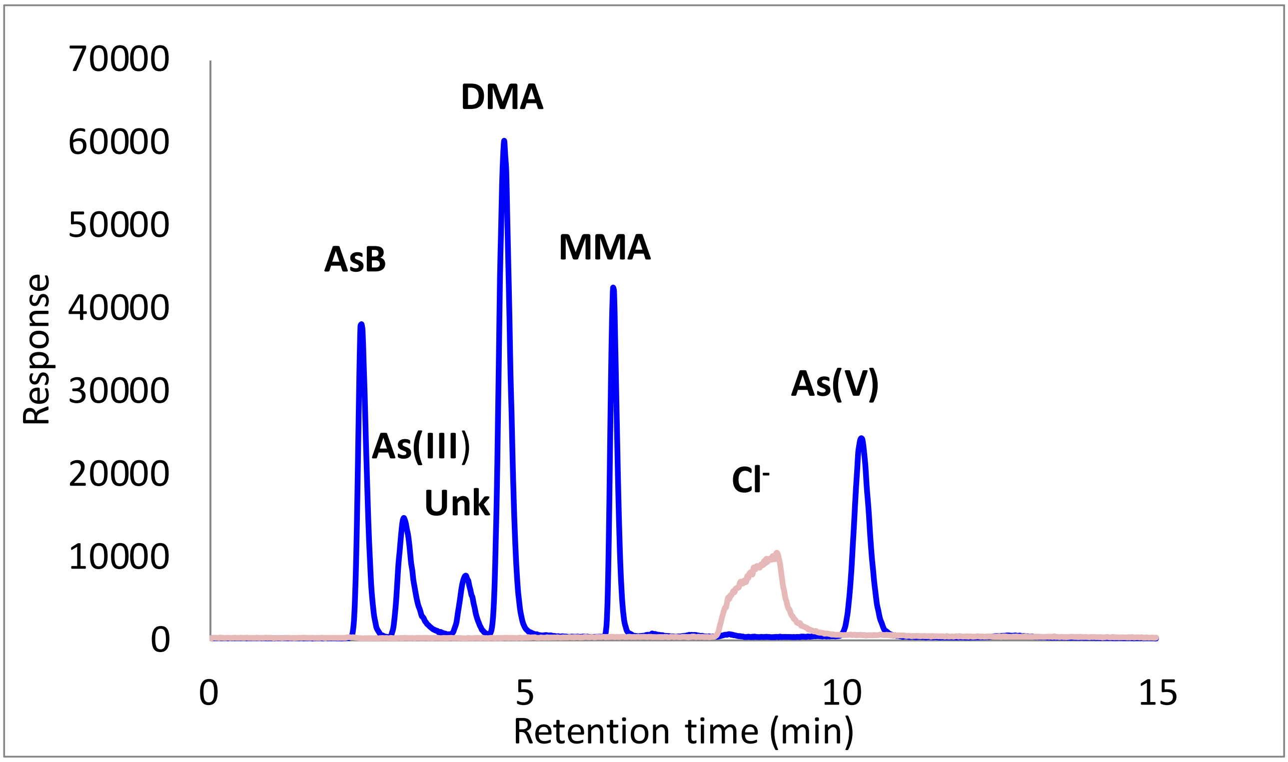

Figure S2: *separation of rice extract on PRP X 100 using a gradient of 2.5mM Ammonium Phosphate Dibasic pH=8.4 and 20mM Ammonium Phosphate Dibasic pH=8.4 (0 min: 0% B, 1 min: 0% B, 3 min: 100% B, 10 min: 100% B, 12 min: 0% B) ICPMS trace blue: ^{75}As, pink: ^{35}Cl*

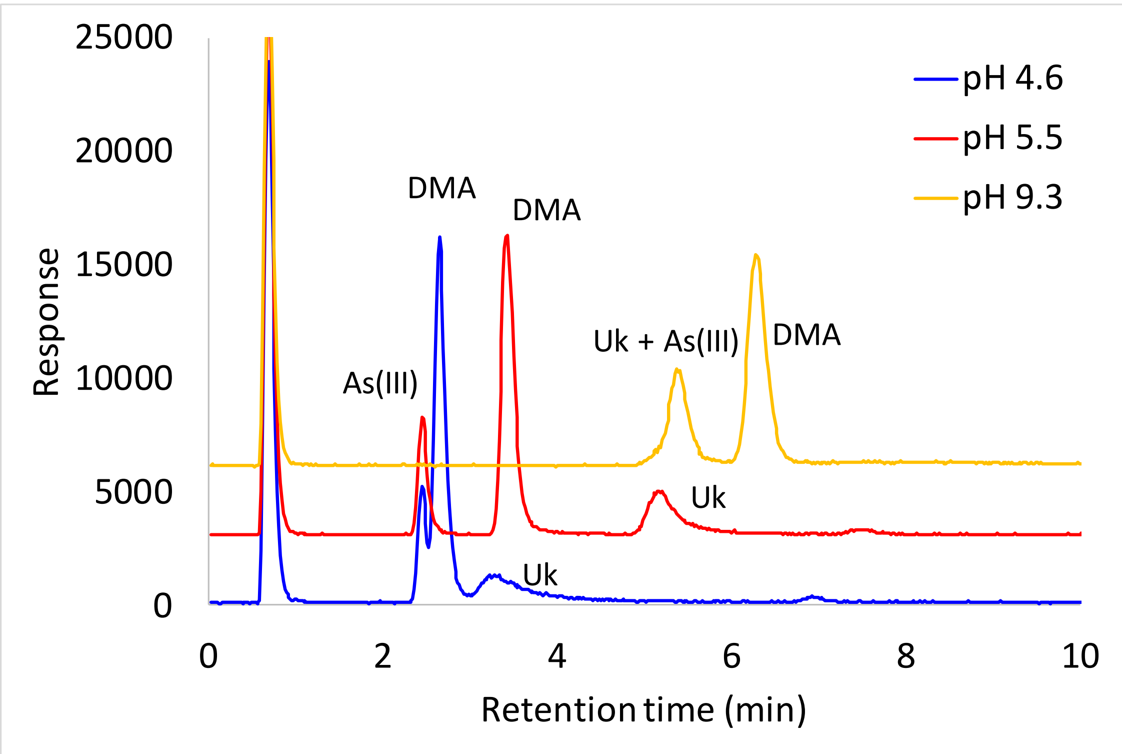

Figure S3: *extracted ion chromatograms of ^{75}As (ICPMS), separation of rice extract on PRP X 100 using a 15 mM ammonium formate at pH 4.6 (blue trace), 5.5 (red trace) and 9.3 (yellow trace)*

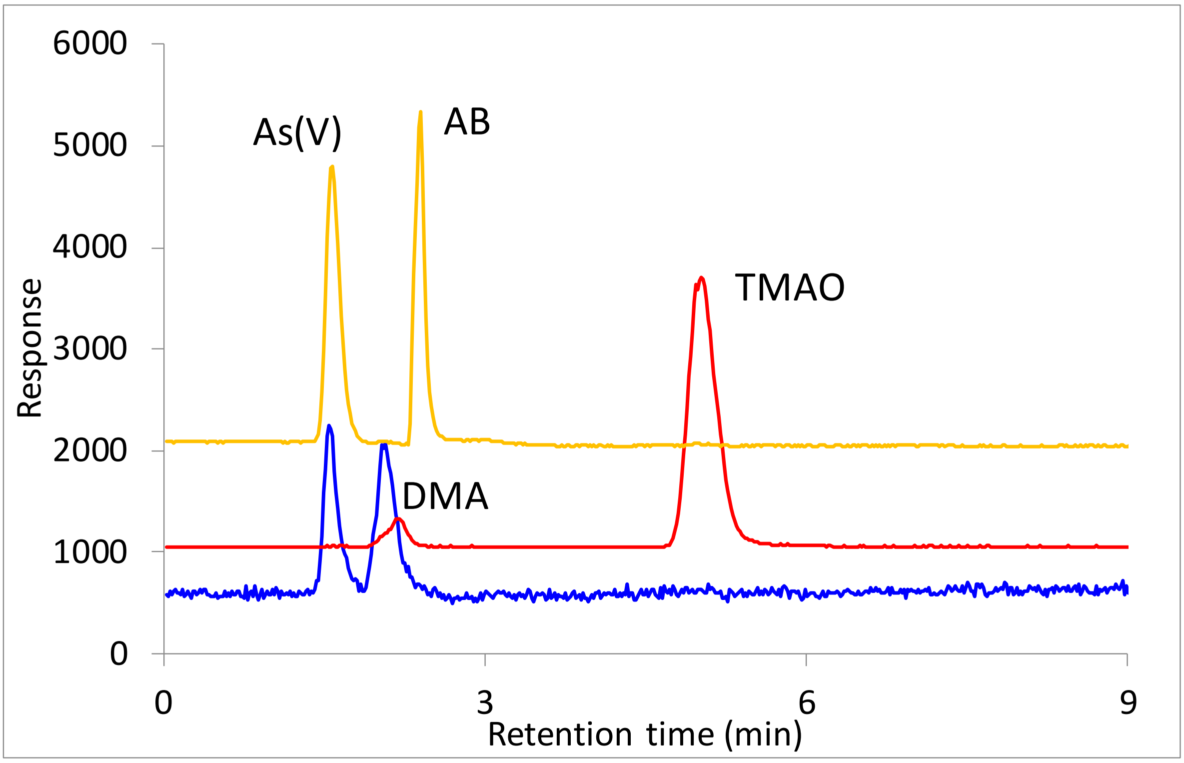

Figure S4: *separation of rice extract (enriched fraction of Uk) using PRP X 200 column, 5 mM pyridine pH 2.6, blue trace: fraction of Uk, red trace: TMAO standard containing traces of DMA, yellow trace: As(III) and AB standards. Uk seems to elute as double peak from a PRP X 200 column, potentially due to stability issues mentioned in paper*

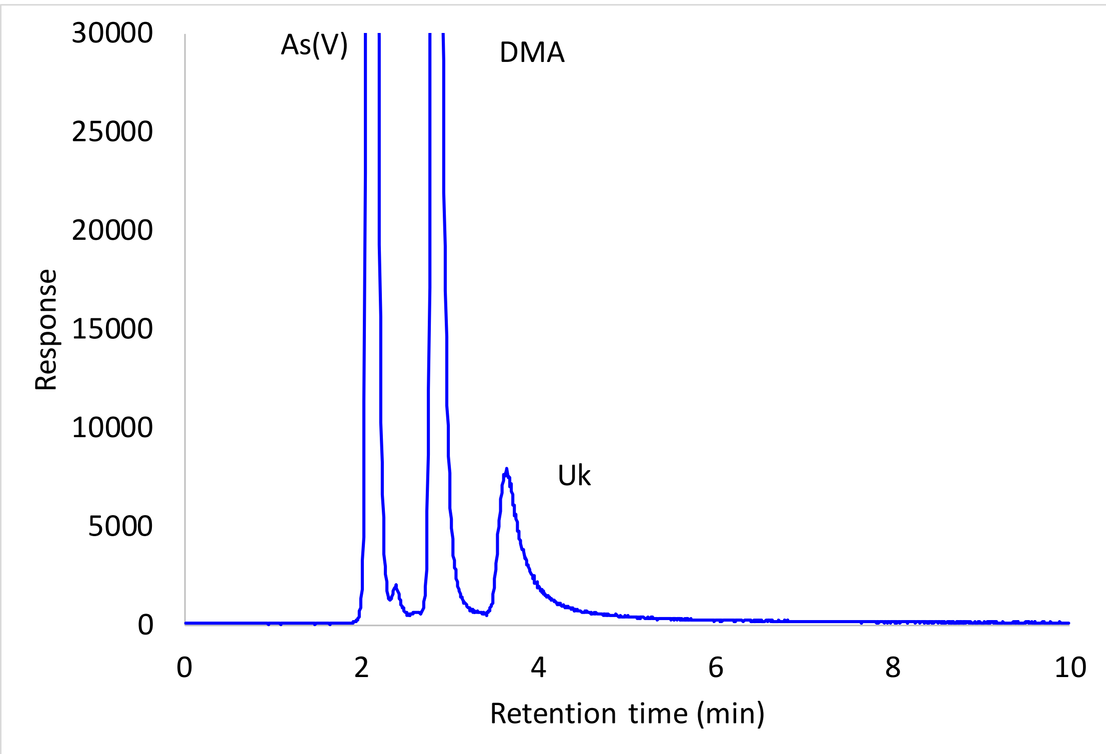

Figure S5*: separation of rice extract using Dual ODS-CX-10, 50 mM ammonium formate pH 2.6*

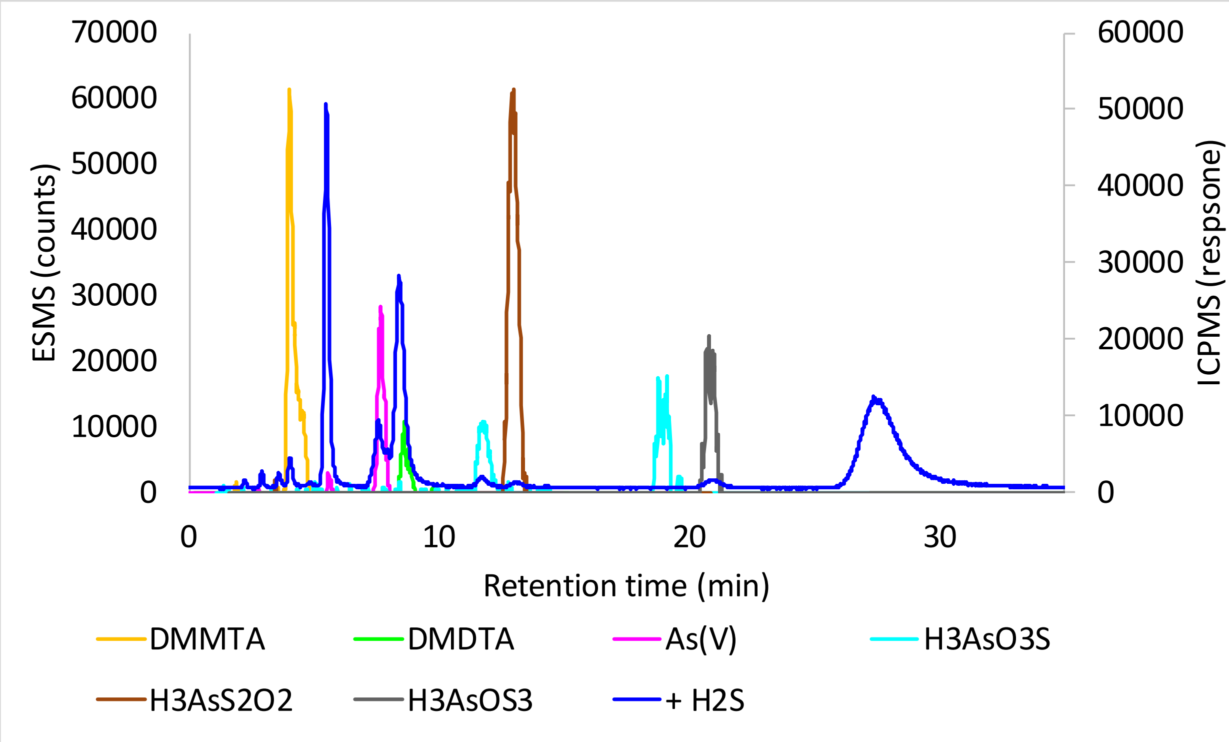

Figure S6: *ICPMS ^{75}As-trace (blue) overlayed with EIC (Exactive), rice extracted with NH_3 thiolated with H_2S, EICs in negative mode m/z 152.9361 (DMMTA, yellow), m/z 168.9132 (DMDTA, green), m/z 140.9174 (As(V), pink), m/z 156.8946 (H_3AsO_3S, turquoise), m/z 174.8863 ($H_3AsO_2S_2$, brown), m/z 190.8635 (H_3AsOS_3, grey)*

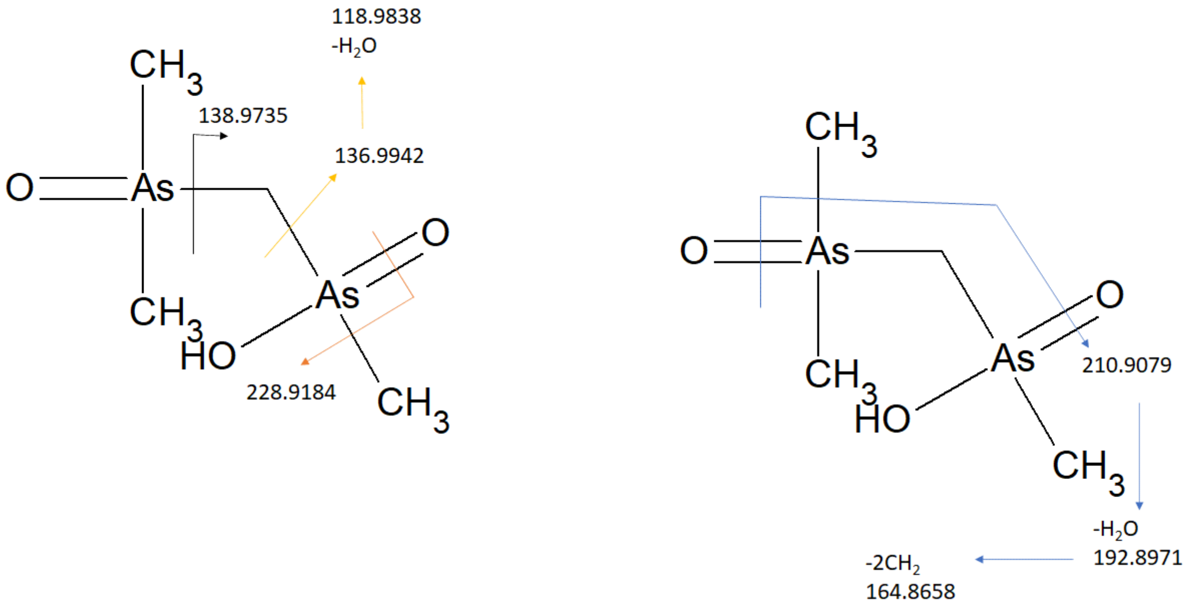

***Figure S7**: potential explanations for main fragments of Uk [M+H]+ 258.9285*

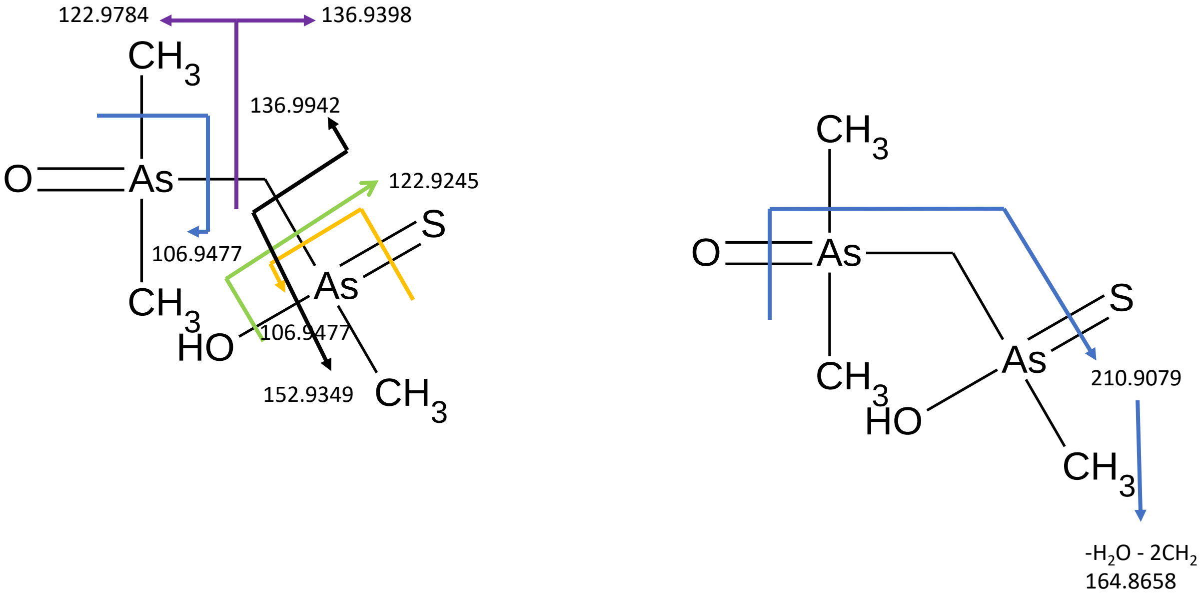

***Figure S8:** potential explanations for main fragments of Uk(S) [M+H]+ 274.9054*

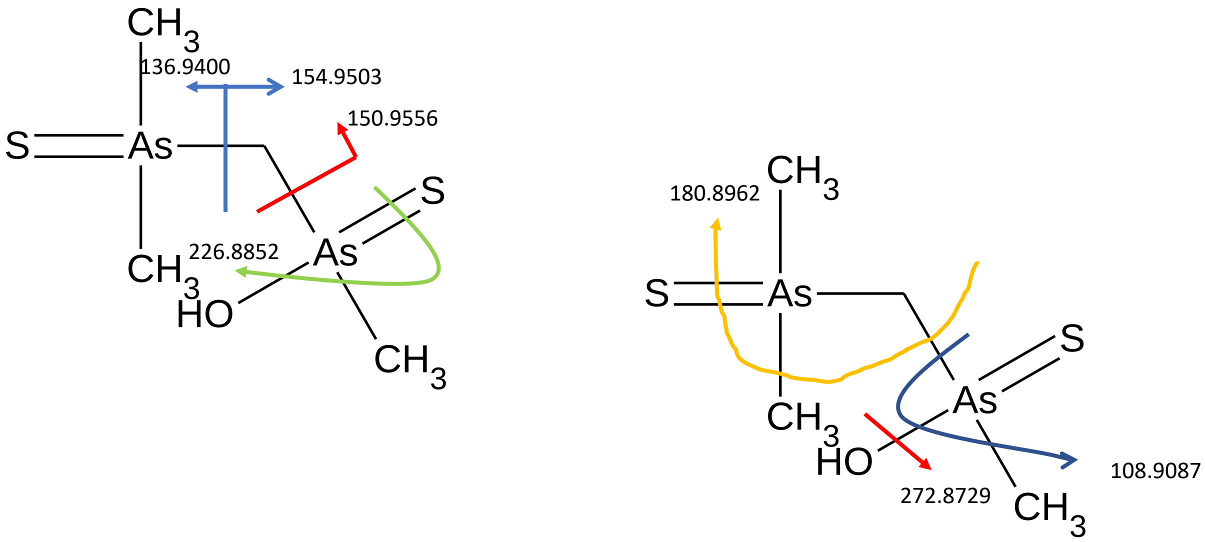

Figure S9: *potential explanations for main fragments of Uk(S2) [M+H]$^+$ 290. 8830*

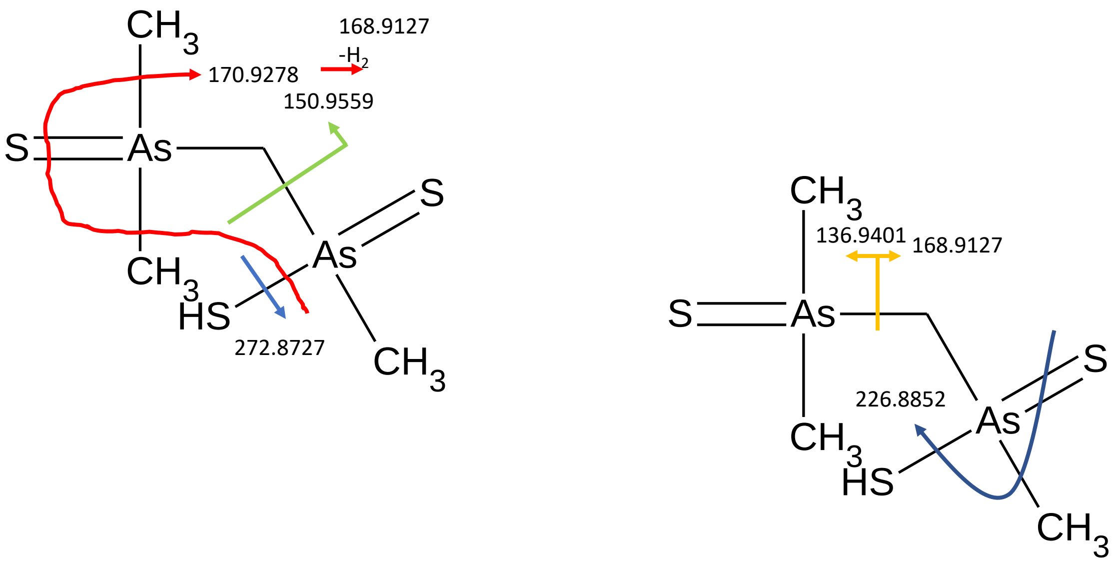

Figure S10: *potential explanations for main fragments of Uk(S3) [M+H]$^+$ 306.8600*