Electronic Supplementary Material (ESI) for Journal of Analytical Atomic Spectrometry.

Investigation of the Oxidative Transformation of Roxarsone by Electrochemistry Coupled to Hydrophilic Interaction Liquid Chromatography/Mass Spectrometry

Lisa Maria Frensemeier, Lars Büter, Martin Vogel and Uwe Karst*

University of Münster, Institute of Inorganic and Analytical Chemistry,

Corrensstraße 30, 48149 Münster, Germany

Supporting Information

Table S-1: Mass spectrometric parameters of the Exactive™ mass spectrometer for the generation of a mass voltammogram, the HILIC separation of the TPs and adduct formation with GSH illustrated in Figure 1 and 3 as well as Figure S-7.

Parameters	**Mass voltammogram**	**HILIC separation**	**GSH adduct formation**
mode	ESI(-)	ESI(-)	ESI(-)
scan range [*m*/*z*]	100-600	100-600	100-600
resolution	high	high	high
	(50,000 @ 2Hz)	(50,000 @ 2Hz)	(50,000 @ 2Hz)
polarity	negativ	negativ	negativ
microscans	1	1	1
lock masses	off	off	off
AGC* target	balanced	balanced	balanced
maximum injection time [ms]	100	250	100
sheath-gas flow rate	10.00	60.0	10.0
aux-gas flow rate	0.00	10.0	0.00
sweep-gas flow rate	0.00	0.00	0.00
spray voltage [kV]	4.00	4.00	3.50
capillary temperature [°C]	275	285	275
capillary voltage [V]	-32.5	-25.0	-57.5
tube-lens voltage [V]	-80.0	-80.0	-110
skimmer voltage [V]	-22.0	-24.0	-22.0

* automatic gain control

Table S-2: Mass spectrometric parameters of the iCAP Qc ICP-MS for the HILIC separation of the TPs in Figure 4.

Parameter	**Value**
spray chamber geometry	cyclonic (quartz)
spray chamber temperature [°C]	-3
nebulizer type	PFA MicroFlow ST
injector	1 mm
sampler material	Pt
skimmer material	Pt
power [W]	1550
cool gas flow rate [mL/min]	14.0
auxiliary gas flow rate [mL/min]	0.8
nebulizer gas flow rate [mL/min]	0.5
additional gas	O_2 (5% (v/v))
extraction lens voltage [V]	-157.4
detector voltage [V]	1787
dwell time [s]	0.1 (^{75}As)
collision gas	He (4.2 mL/min)

Table S-3: LC gradient profile for the separation of a) roxarsone and its TPs and b) the protein adduct formation of oxidized roxarsone with β-lactoglobulin A and human serum albumin. The mobile phases consisted of a) NH_4FA (solvent A, 50 mM, pH 3.0) and AcN (solvent B) as well as b) 0.1% formic acid in water (solvent A) and AcN (solvent B).

a)

t [min]	5	20	30	35	36	55
%B	85	75	5	5	85	85

b)

t [min]	2	6	9	12	15
%B	25	60	60	25	25

Table S-4: Mass spectrometric parameters of the micrOToF mass spectrometer for the adduct formation with proteins β-LGA and HSA displayed in Figures 6 and 7.

Parameter	**Value**
nebulizer pressure [bar]	1.8
dry gas flow rate [L/min]	8.0
dry temperature [°C]	180
capillary voltage [V]	4500.0
endplate offset [V]	-350.0
capillary exit voltage [V]	180.0
skimmer 1 voltage [V]	60.0
skimmer 2 voltage [V]	22.0
hexapole 1 voltage [V]	23.0
hexapole RF voltage [Vpp]	700.0
transfer time [µs]	50.0
pre-pulse storage time [µs]	30.0
lens 1 storage voltage [V]	40.0
lens 1 extraction voltage [V]	21.7
lens 2 voltage [V]	9.0
lens 3 voltage [V]	22.0
lens 4 voltage [V]	1.0
lens 5 voltage [V]	-35.5
m/z range	50-2500

Table S-5: Sum formulae, calculated as well as detected m/z, and relative mass deviations [ppm] of roxarsone and its TPs.

		Modification	Sum formula	Theoretical m/z	Detected m/z	Relative deviation [ppm]
		$[M-H]^-$	$C_6H_5O_6NAs$	261.9338	261.9334	1.5
		$[M-H_2O-H]^-$	$C_6H_3O_5NAs$	243.9233	243.9223	4.1
		$[M+Cl]^-$	$C_6H_6O_6NAsCl$	297.9105	297.9105	0.0
	ROX	$[2M-H]^-$	$C_{12}H_{11}O_{12}N_2As_2$	524.8750	524.8746	0.8
		$[2M-H_2O-H]^-$	$C_{12}H_9O_{11}N_2As_2$	506.8644	506.8639	1.0
		$[2M+Na-2H]^-$	$C_{12}H_{10}O_{12}N_2As_2Na$	546.8569	546.8567	0.4
		$[2M+K-2H]^-$	$C_{12}H_{10}O_{12}N_2As_2K$	562.8308	562.8302	1.1
	M1.1	$[M+O-H]^-$	$C_6H_5O_7NAs$	277.9287	277.9286	0.4
	M1.2	$[M+2O-H]^-$	$C_6H_5O_8NAs$	293.9237	293.9238	0.3
		$[M+2O-H_2O-H]^-$	$C_6H_3O_7NAs$	275.9131	275.9130	0.4
	M1.3	$[M+2O-2H-H]^-$	$C_6H_3O_8NAs$	291.9080	291.9082	0.4
M1		$[M+2O-2H-H_2O-H]^-$	C_6HO_7NAs	273.8974	273.8973	0.4
	M1.4	$[M+3O-H]^-$	$C_6H_5O_9NAs$	309.9186	309.9187	0.3
		$[M+3O-H_2O-H]^-$	$C_6H_3O_8NAs$	291.9080	291.9082	0.4
	M1.5	$[M+O+NH-H]^-$	$C_6H_6O_7N_2As$	292.9396	292.9398	0.7
	M1.6	$[M+O+NH-2H-H]^-$	$C_6H_4O_7N_2As$	290.9240	290.9242	0.7
	M1.7	$[M+2O+NH-2H-H]^-$	$C_6H_4O_8N_2As$	306.9189	306.9190	0.3
		As(V)	H_2AsO_4	140.9162	140.9170	5.7
^{i}As		As(V)-H_2O	AsO_3	122.9069	122.9063	4.9
		As(V)+FA	CH_4O_6As	186.9229	186.9228	0.5
	M2.1	$[M+O-HAsO_3-H]^-$	$C_6H_4O_4N$	154.1046	154.1043	1.9
	M2.2	$[M+2O–HAsO_3-H]^-$	$C_6H_4O_5N$	170.0095	170.0094	0.6
	M2.3	$[M+2O-2H-HAsO_3-H]^-$	$C_6H_2O_5N$	167.9938	167.9937	0.6
M2	M2.4	$[M+3O–HAsO_3-H]^-$	$C_6H_4O_6N$	186.0044	186.0042	1.1
	M2.5	$[M+2O+NH-2H–HAsO_3-H]^-$	$C_6H_3O_5N_2$	183.0047	183.0046	0.6
	M2.6	$[M+3O+NH-HAsO_3-H]^-$	$C_6H_5O_6N_2$	201.0153	201.0151	1.0
	M2.7	$[M+3O-2H+NH-HAsO_3-H]^-$	$C_6H_3O_6N_2$	198.9997	198.9994	1.5
	$M_{red}1$	$[M-2O+2H-H]^-$	$C_6H_7O_4NAs$	231.9597	231.9588	3.9
M_{red}		$[M-2O+2H-H_2O-H]^-$	$C_6H_5O_3NAs$	213.9487	213.9487	0.0
	$M_{red}2$	[M+NH-2O+2H-H]-	$C_6H_8O_4N_2As$	246.9706	246.9694	4.9
	$M_{red}3$	$[M+O-2H-2O+2H-H]^-$	$C_6H_5O_5NAs$	245.9389	245.9378	4.5
Dimer	dimer 1	$[2M-2H-H]^-$	$C_{12}H_9O_{12}N_2As_2$	522.8593	522.8591	0.4
	dimer 2	$[2M+2O+NH-4H-HAsO_3-H]^-$	$C_{12}H_7O_{11}N_3As$	443.9302	443.9301	0.2
	U1	unknown	n. d.	n. d.	157.9729	-
	U2	unknown	n. d.	n. d.	208.9044	-
U	U3	unknown	n. d.	n. d.	224.8782	-
	U4	unknown	$C_6H_3O_7N_3$	227.9887	227.9893	2.6
	U5	unknown	$C_8H_2O_5N_2As$	279.9096	279.9080	5.7

n. d. = not determined

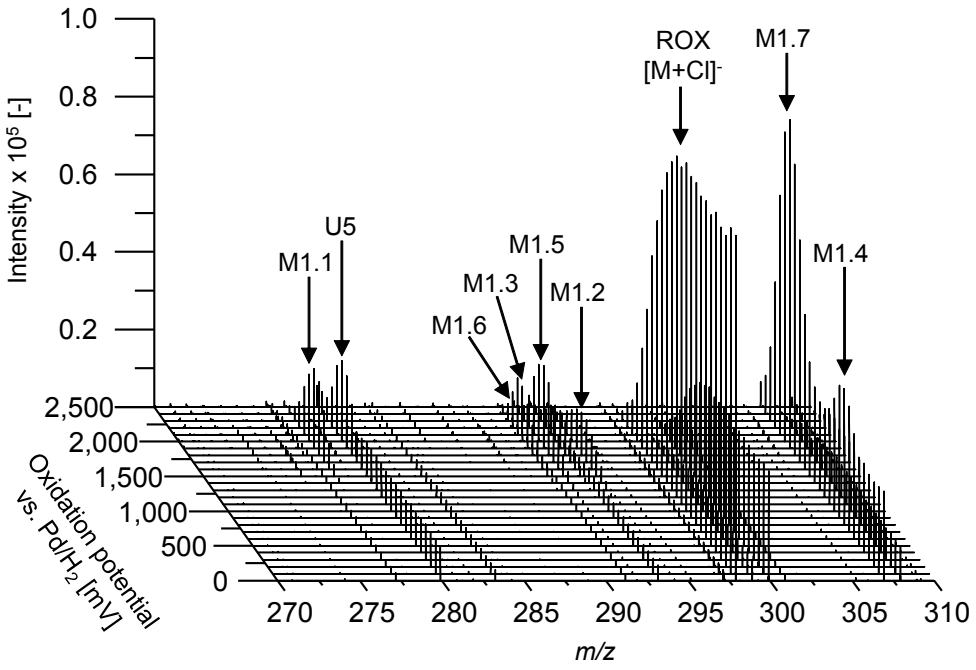

Figure S-1: Mass voltammogram (m/z 270-310) of roxarsone obtained with ESI(-)-MS in the potential range 0-2,500 mV vs. Pd/H_2. The section shows formation of M1 products and U5.

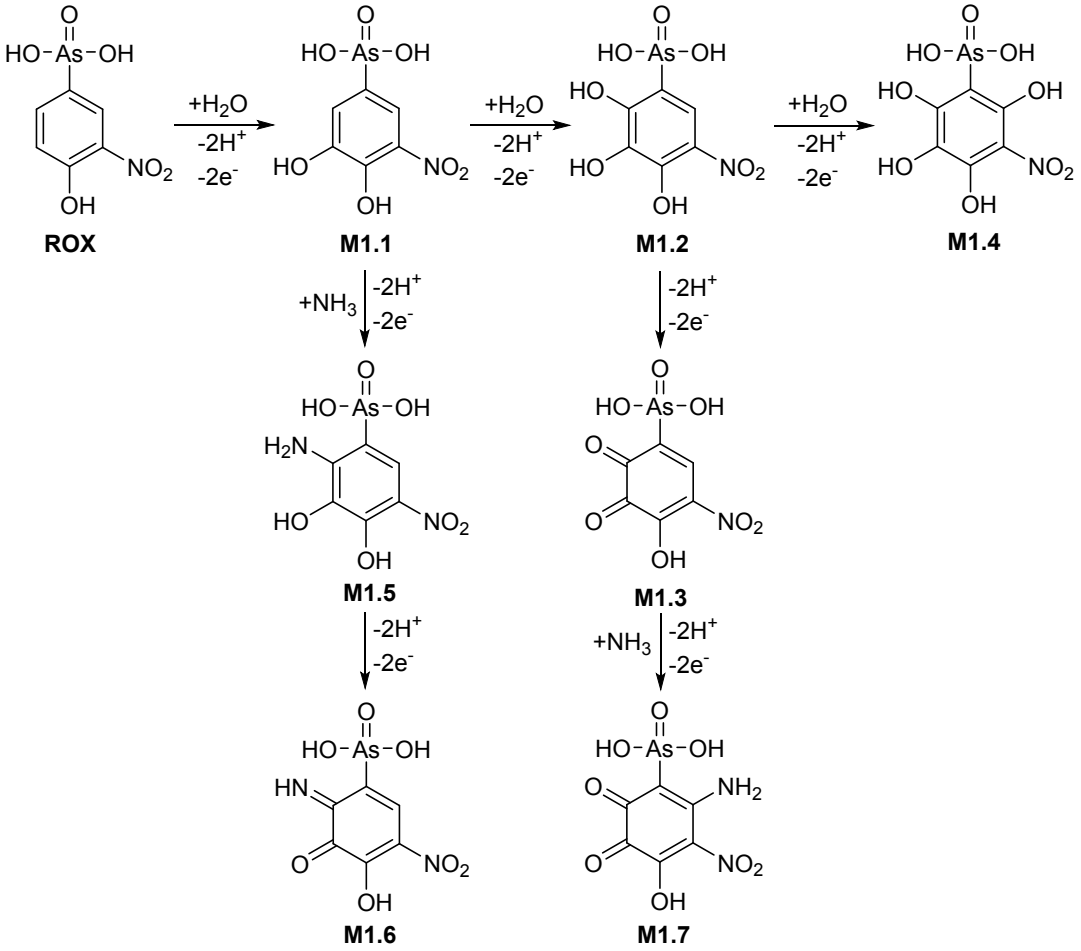

Figure S-2: Structure proposals and reaction pathway of type M1 oxidation products of roxarsone.

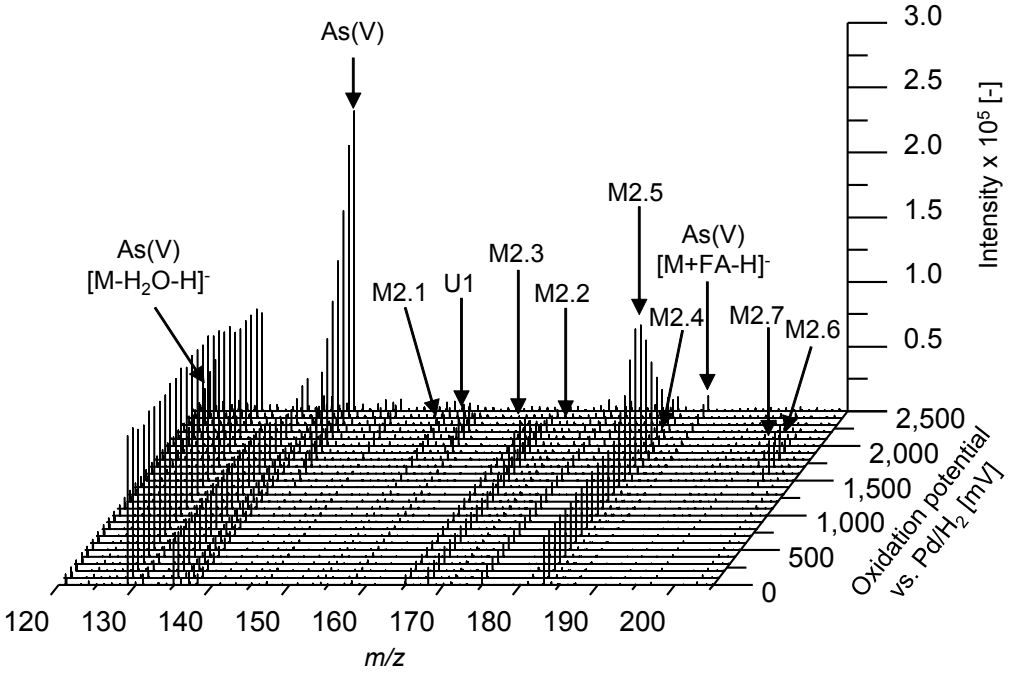

Figure S-3: Mass voltammogram (m/z 120-205) of roxarsone obtained by ESI(-)-MS in the potential range 0-2,500 mV vs. Pd/H_2. The section shows formation of M2 products, As(V) and U1.

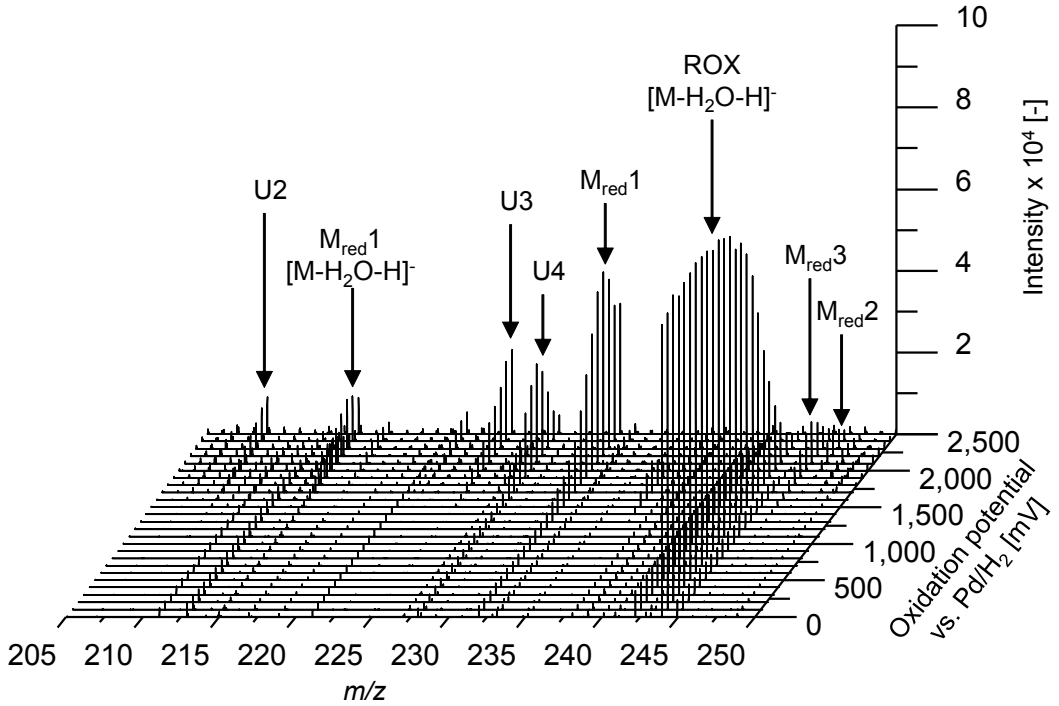

Figure S-4: Mass voltammogram (m/z 205-250) of roxarsone obtained by ESI(-)-MS in the potential range 0-2,500 mV vs. Pd/H_2. The section shows formation of M_{red} products, U2, U3 and U4.

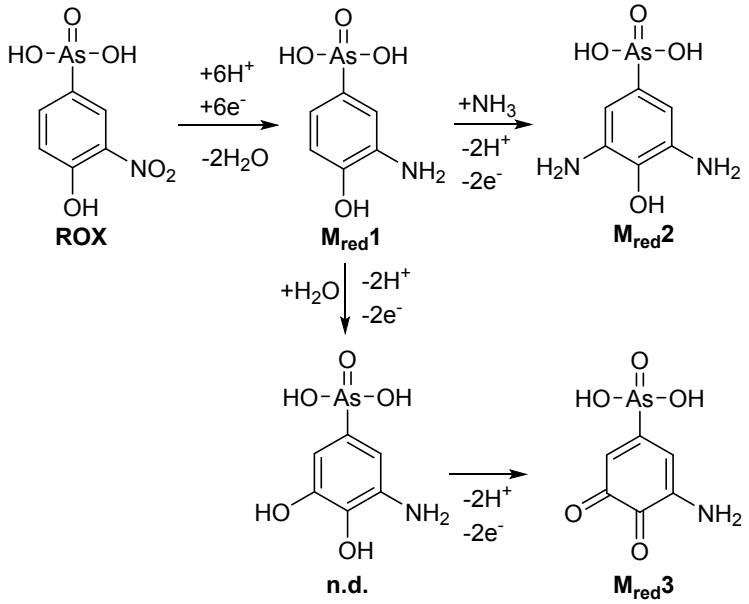

Figure S-5: Structure proposals and reaction pathway M_{red} formation.

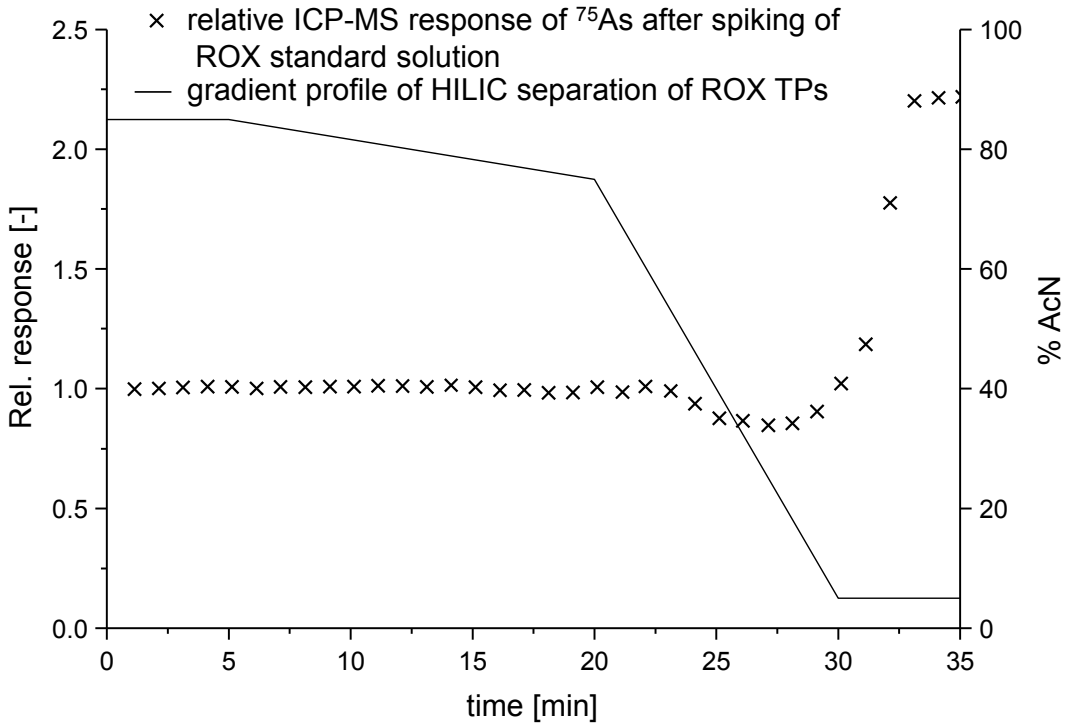

Figure S-6: Response function of the ICP-MS (iCap Qc) for the used gradient during HILIC separation of roxarsone and its TPs.

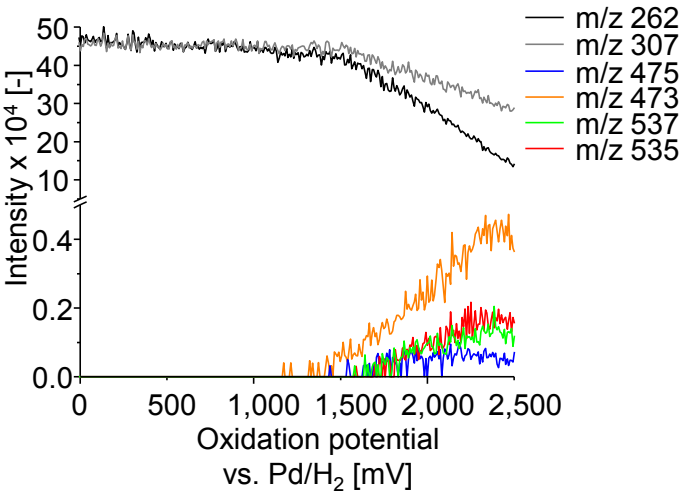

Figure S-7: Adduct formation of ROX with GSH. Depicted are the extracted ion chromatograms of ROX (m/z 262), GSH (m/z 306) and adducts GSH+168 (m/z 475) and GSH+230 (m/z 537) as well as their dehydrogenation products GSH+166 (m/z 473) and GSH+228 (m/z 535).

Table S-6: Sum formulae and calculated as well as detected m/z of roxarsone, GSH and detected adducts presented in Figure 8.

Modification	**Sum formula**	**Calculated m/z**	**Detected m/z**	**Relative deviation [ppm]**
ROX	$C_6H_5O_6NAs$	261.9338	261.9333	1.9
GSH	$C_{10}H_{16}O_6N_3S$	306.0754	306.0765	3.6
GSH+168	$C_{16}H_{19}O_{11}N_4S$	475.0766	475.0778	2.5
GSH+166	$C_{16}H_{17}O_{11}N_4S$	473.0609	473.0620	2.3
GSH+230	$C_{16}H_{22}O_{10}N_4AsS$	537.0267	537.0282	2.8
GSH+228	$C_{16}H_{20}O_{10}N_4AsS$	535.0111	535.0124	2.4