Electronic supplement for

Sulfur fertilization influences the sulphur species composition in *Allium sativum:* sulfomics using HPLC-ICP-MS/MS-ESI-MS/MS

Andrea Raab, Marilena Ronzan, Joerg Feldmann
TESLA (Trace Element Speciation Laboratory), University of Aberdeen, Chemistry, Meston Walk, Aberdeen, AB243UE, Scotland, UK

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1. Composition of Hoagland solution

   Sulphur level 0.5 mM: 1.25 mM KNO_3, 1.50 mM $Ca(NO_3)_2$, 0.75 mM $MgSO_4$, 0.50 mM KH_2PO_4, 50 µM KCl, 50 µM H_3BO_3, 10 µM $MnSO_4$, 2.0 µM $ZnSO_4$, 1.5 µM $CuSO_4$, 0.5 µM $Na_2MoO_4.2H_20$, 0.1 mM Na_2O_3Si, and 72 µM Fe-EDTA pH 6.0

   Sulphur level 0.1 mM: Plant group exposed to 0.1 M sulphur: $MgSO_4$ was replaced by 0.75 mM $MgCl_2$

   Sulphur level 2 mM: Plant group exposed to 2 mM sulphur: $MgSO_4$ 2 mM $MgSO_4$ was used instead of 0.75 mM

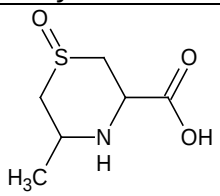
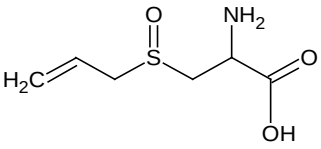
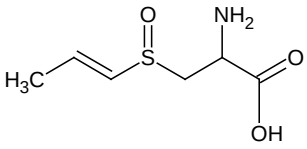
2. Details of compounds (ESI-MS spectra, EIC+ICP-MS/MS trace, fragmentation by ESI-MS/MS) Figures S1-S26

The following tables summarize the molecular structure, molecular weight, theoretical and measured $[M+H]^+$ and retention time of the compounds and contain the average compound or sulphur concentrations.

2.1. Cycloalliin, aliin & isoalliin

The identities of cyclo-alliin, alliin and isoalliin were deduced by comparison of their q-TOF-ESI-MS/MS spectra. ES-MS/MS spectra of the q-TOF instrument for alliin contained predominantly signals at *m/z* 137.0134 ($C_3H_7NO_3S$) and *m/z* 120.0108 ($C_3H_6NO_2S$) (Fig. S2). Isoalliin (Fig. S3) showed additionally a fragment at *m/z* 114.0547 identical to one of the major fragments of cyclo-alliin (C_5H_8NS). The different position of the double bond compared to alliin allows stabilisation of isoalliin-fragments by cyclisation. The other major fragments of cyclo-alliin were *m/z* 160.0456 (loss of water) and *m/z* 131.0128 ($C_5H_7O_2S$) (Fig. S1).

Table S1 Alliin and isoalliin content in root and bulb in mmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), cylcoalliin; in brackets compound average in % of total sulphur, database ID: ChemSpider.

	Cycloalliin*	Alliin	Isoalliin
			
Molecular formula	$C_6H_{11}NO_3S$	$C_6H_{11}NO_3S$	$C_6H_{11}NO_3S$
MW (g mol^{-1})	177.2214	177.2214	177.2214
$[M+H]^+$ (theoretical)	178.0532	178.0532	178.0532
$[M + H]^+$ (measured)	178.0538	178.0531	178.0534
RT (min)	2.2	2.5	3.2
Database ID	167735	78760	3677225
Root (0.1 mM S)		14 ± 15[a] (7.1 %)	4.1 ± 3.4[a] (2.0 %)
Root (0.5 mM S)		19 ± 16[b] (6.7%)	4.3 ± 4.3[b] (1.5 %)
Root (2 mM S)		79 ± 41[a,b] (17%)	30 ± 27[a,b] (6.4%)
Bulb (0.1 – 2 mM S)		21 ± 8.1 (16 %)	3.7 ± 2.1 (2.8 %)

*cycloalliin + methiin + methylcysteine, amount of associated sulphur see table S13
[a,b]: statistically significant difference between groups by One Way ANOVA $p < 0.01$

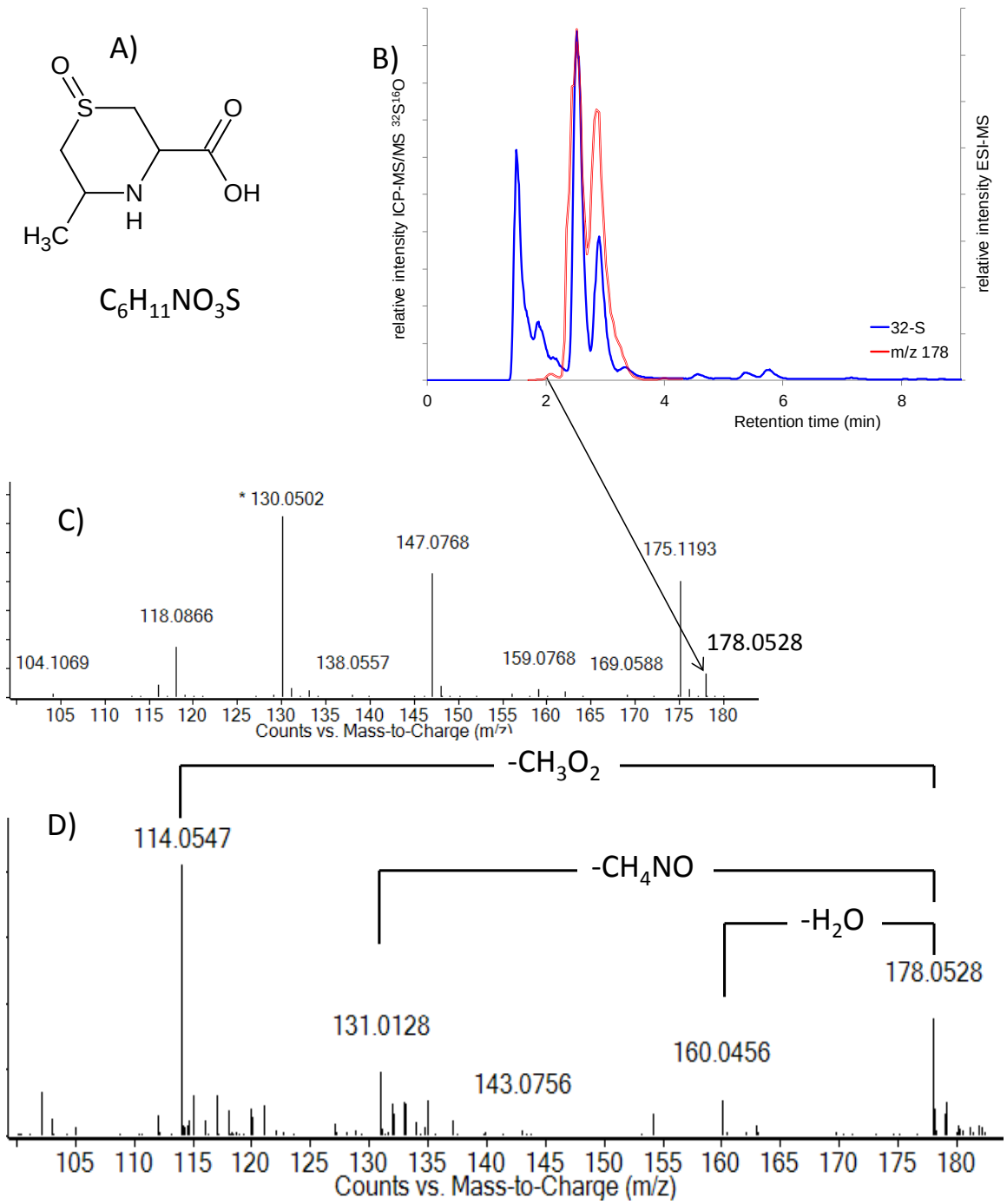


Fig. S1 **Cycloalliin;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

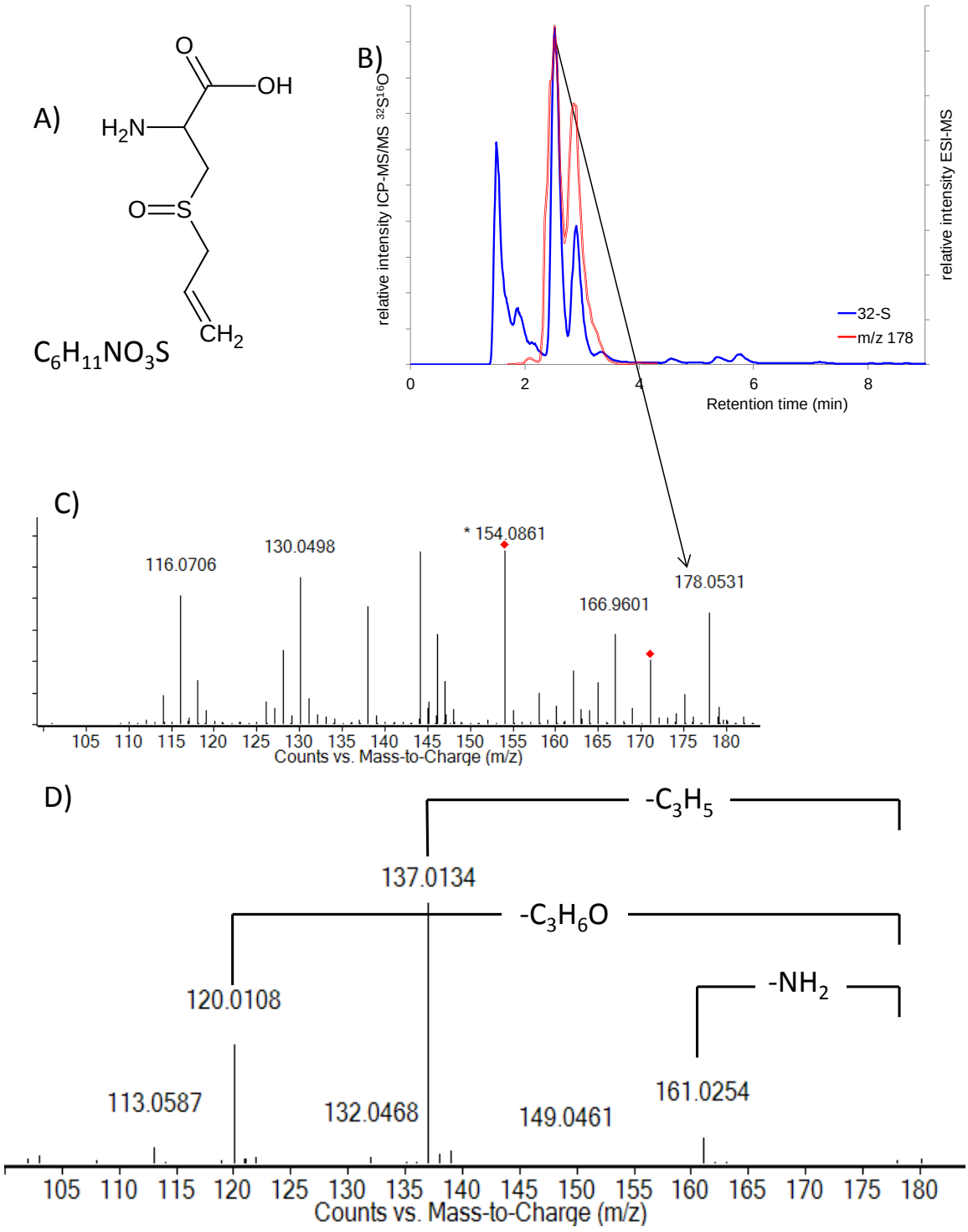


Fig. S2 **Alliin;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

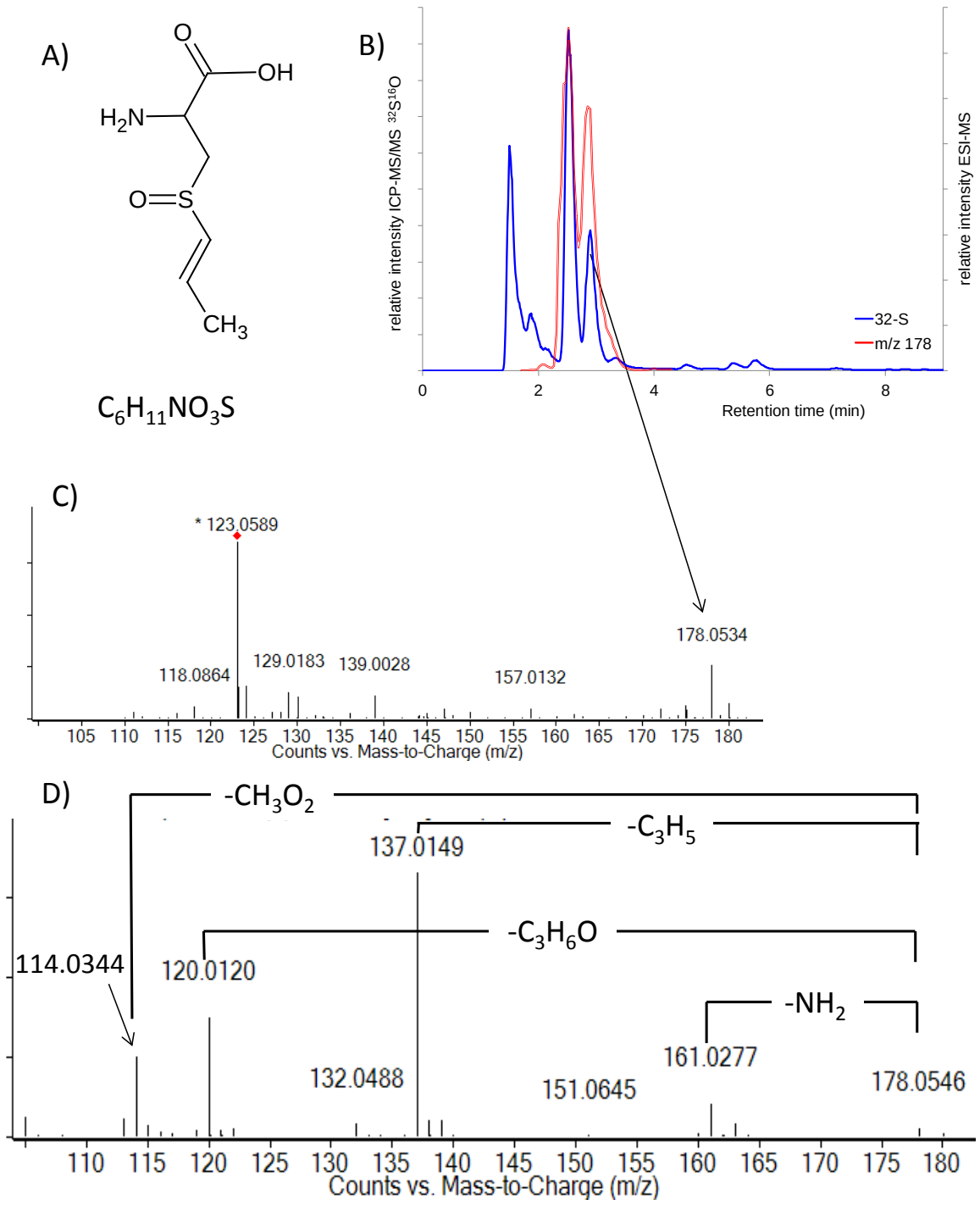
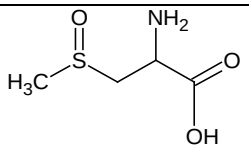
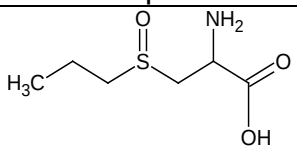
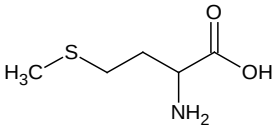


Fig. S3 **Isoalliin;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.2. Methiin, propiin and methionine

It was not possible to confirm the identity of methiin other than from the accurate *m/z* ratio (Δppm = 0.54). The signal was not intense enough in any of the samples for fragmentation by either Orbitrap ESI-MS or q-TOF-ESI-MS (Fig. S4). The retention time is similar to those published by others. Cycloalliin (Table S1) and methylcysteine co-eluted with methiin. Propiin (Fig. S5) eluted together with glutathione (Fig. S17) and methionine (Fig. S6). In addition to the accurate $[M+H]^+$, propiin and methionine showed the expected fragmentation patterns, with propiin fragmenting to *m/z* 134.0634 (loss of CH_2O_2), *m/z* 116.0530 (loss of CH_3O_3) and *m/z* 120.996 (loss of NH_2 and C_3H_7) (Fig. S5). Methionine fragments of *m/z* 104.0531 (loss of CO_2) *m/z* 133.0322 (loss of NH_2) (Fig. S6) confirmed its presence together with its accurate mass.

Table S2 methiin propiin and methionine, database ID: ChemSpider.

	Methiin*	Propiin$^+$	Methionine$^+$
			
Molecular formula	$C_4H_9NO_3S$	$C_6H_{13}NO_3S$	$C_5H_{11}NO_2S$
MW (g mol^{-1})	151.1842	179.2373	149.2113
$[M+H]^+$ (theoretical)	152.0376	180.0689	150.0583
$[M+H]^+$ (measured)	152.0375	180.0685	150.0583
RT (min)	2.2	3.7	3.7
Database ID	74136	78759	5907
Root (0.1 mM S)			
Root (0.5 mM S)			
Root (2 mM S)			
Bulb (0.1 – 2 mM S)			

*cycloalliin + methiin + methylcysteine, amount of associated sulphur see table S13
$^+$ propiin + glutathione + methionine, amount of associated sulphur see table S13

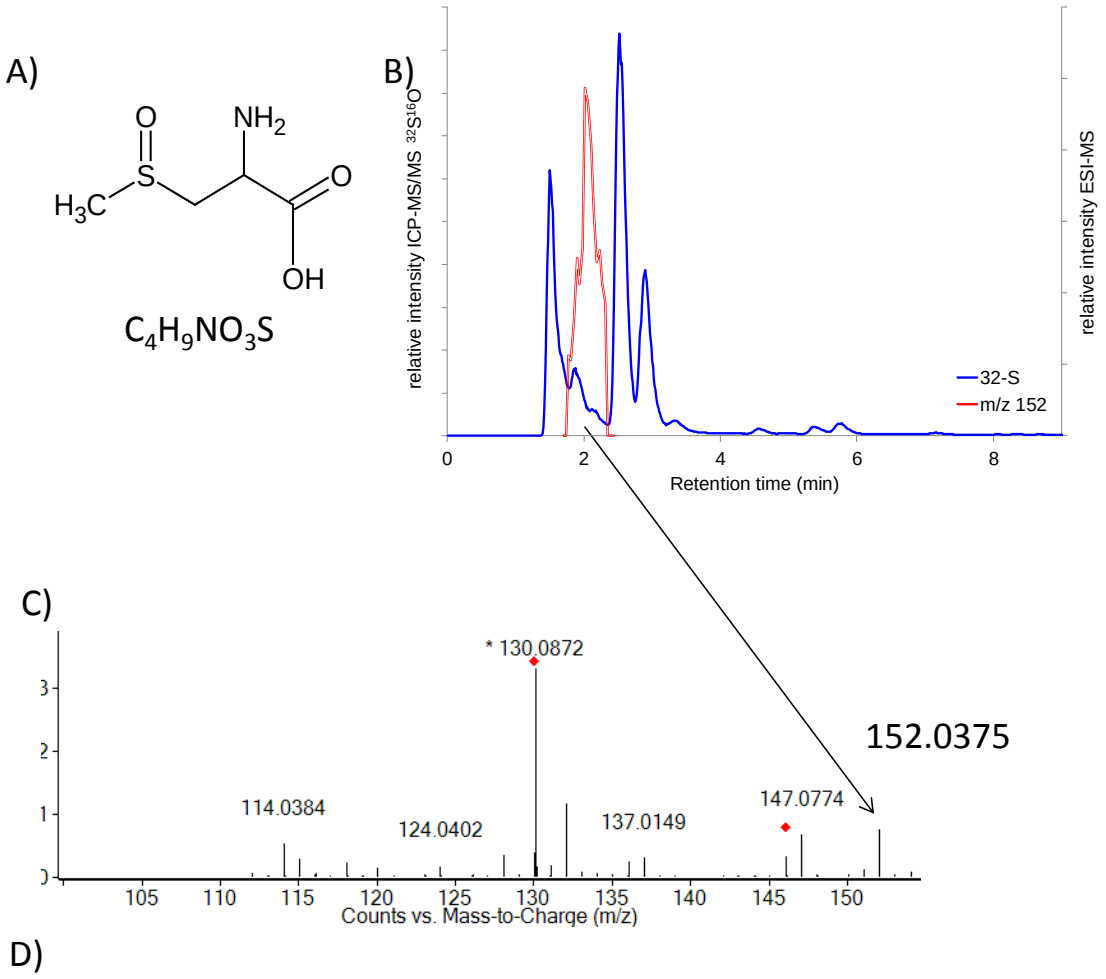


Fig. S4 **Methiin;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) no fragmentation; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

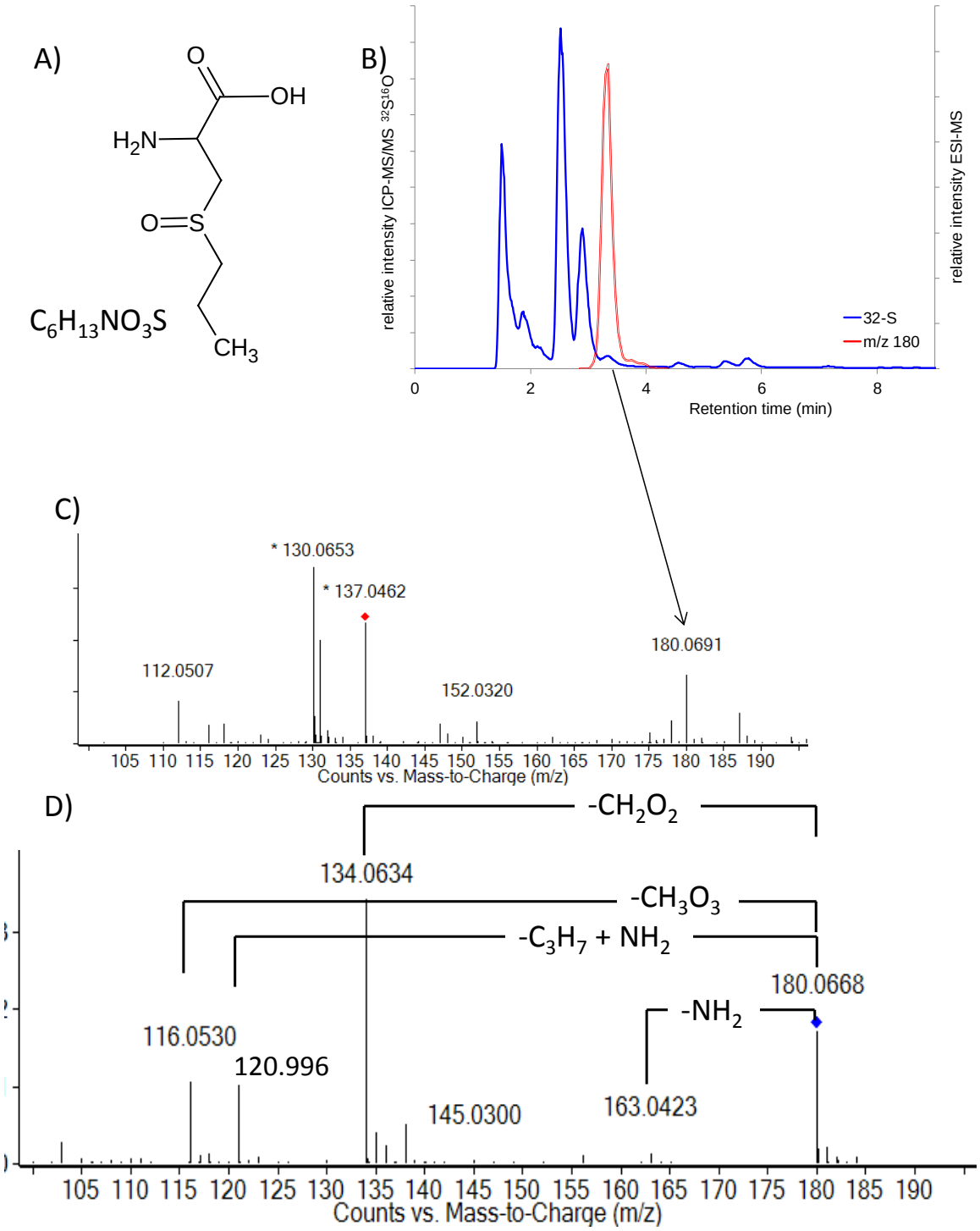


Fig. S5 **Propiin;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

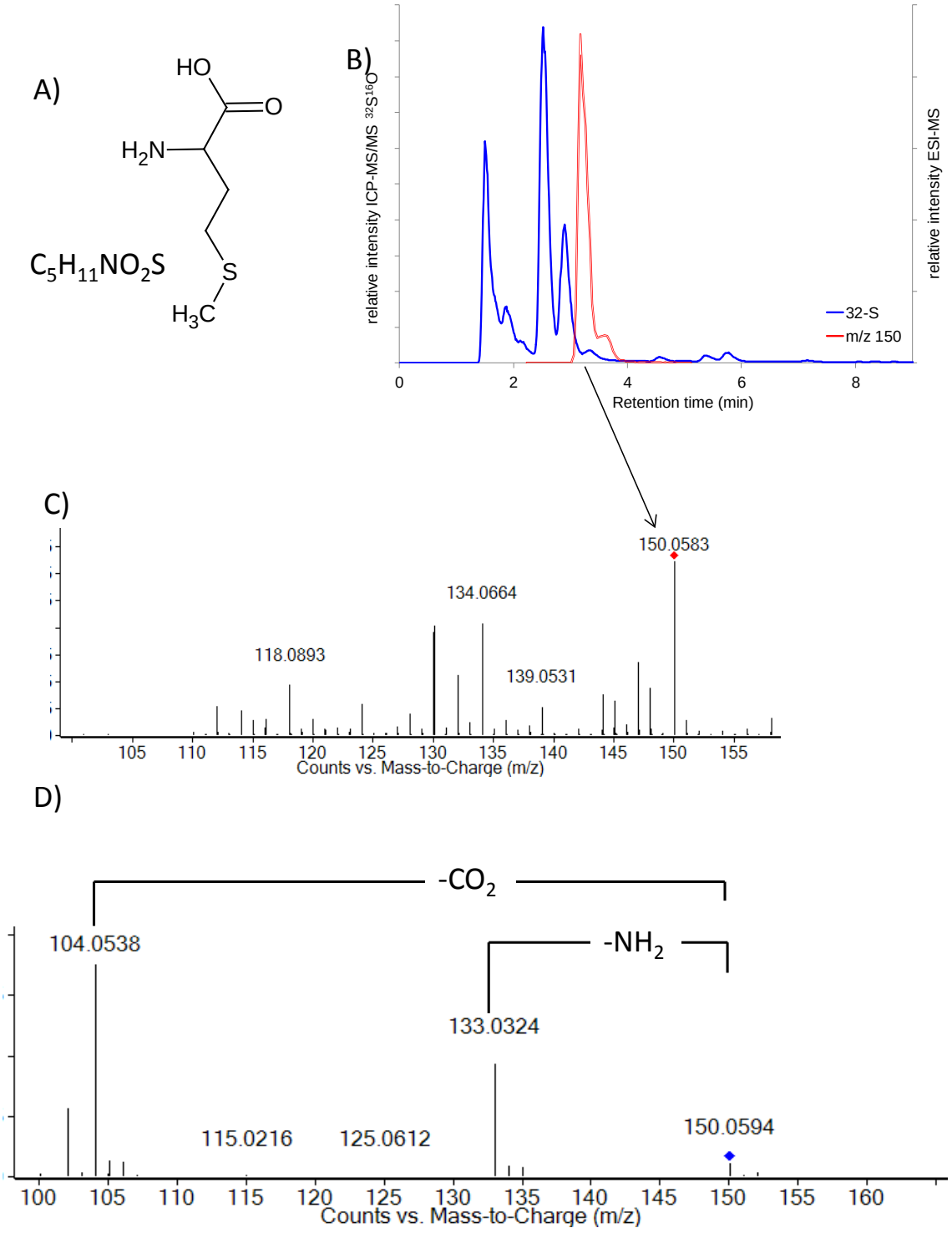
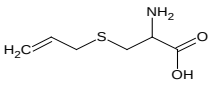
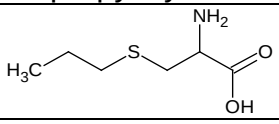
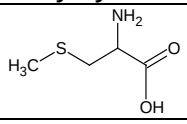


Fig. S6 **Methionine;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.3. S-allyl-cysteine (SAC, deoxyalliin) and derivatives

Methyl-cysteine showed a fragment at *m/z* 119.0344 (loss of NH_2) (Fig. S7) and no other significant fragments due to settings of the mass range for both Orbitrap ESI-MS and q-TOF-ESI-MS. S-allyl-cysteine showed fragments at *m/z* 145.0306 (amine loss), *m/z* 116.0525 (loss of CO_2) and *m/z* 105.0001 (loss of NH_2 and CO_2) (Fig. S8). SAC was also identified in garlic by Yamazaki *et al.*[1] eluting after GSMC identical to the retention behaviour under the conditions used here. S-propyl-cysteine did not occur in either bulbs or roots.

Table S3 S-allyl-cysteine in bulb and root in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), methyl cysteine, database ID: ChemSpider.

	S-allyl-cysteine	S-propyl-cysteine	Methylcysteine*
			
Molecular formula	$C_6H_{12}NO_2S$	$C_6H_{14}NO_2S$	$C_4H_9NO_2S$
MW (g mol^{-1})	161.2220	163.2379	135.1848
$[M + H]^+$ (theoretical)	162.0583	164.0739	136.0427
$[M+H]^+$ (measured)	162.0586		136.0423
RT (min)	7.4		2.2
Database ID	8488717	111437	28885
Root (0.1 mM S)	325 ± 195	-	
Root (0.5 mM S)	256 ± 100	-	
Root (2 mM S)	414 ± 149	-	
Bulb (0.1 – 2 mM S)	143 ± 231	-	

*cycloalliin + methiin + methylcysteine, amount of associated sulphur see table S13

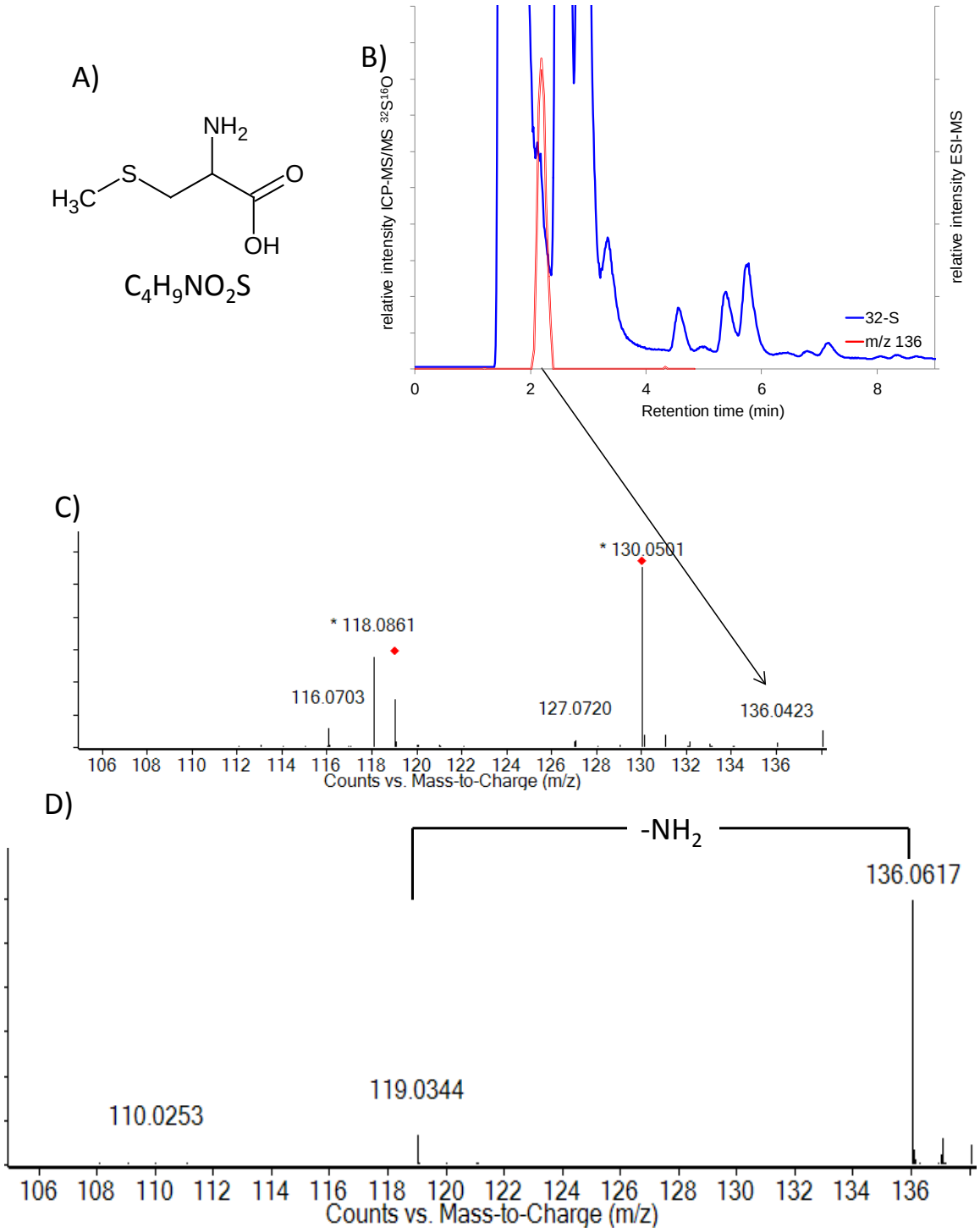


Fig. S7 **Methyl-cysteine;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

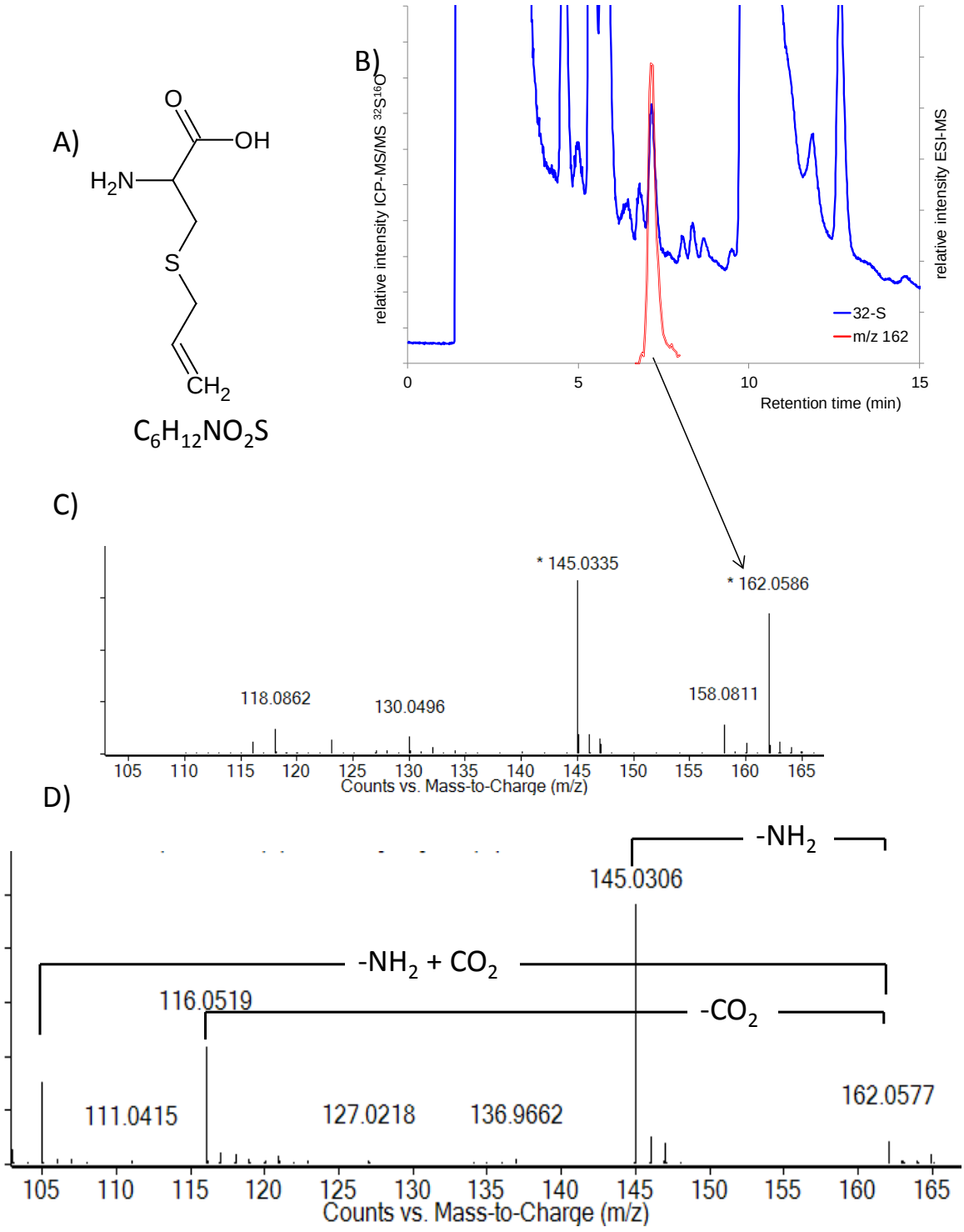
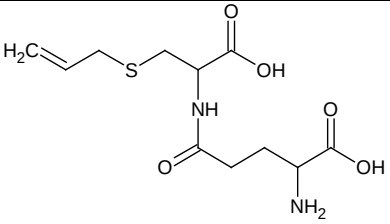
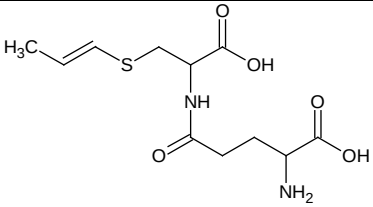


Fig. S8 **S-allyl-cysteine;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.4. γ-glutamyl-S-allyl-cysteine (GSAC) and γ-glutamyl-S-1-propenyl-cysteine (GSPC)

GSAC and GSPC were identified by their accurate *m/z* ratio and their fragmentation pattern. The assignment of the allyl respectively the propenyl-group to either compound was done based on comparison with published retention times, which all showed that compounds containing the allyl-group elute before the related compound containing a propenyl-group. The main fragment for both compounds was *m/z* 145.30313 ($C_6H_9O_2S$) followed by *m/z* 162.0573 ($C_6H_{12}NO_2S$, loss of γ-Glu) and *m/z* 130.0497 ($C_5H_8NO_3$, loss of propenyl/allyl-cysteine) (Fig. S9 and S10). Identification and quantification of both compounds in bulbs was uncomplicated. Both compounds showed clear signals in the ICP-MS/MS trace and the ESI-MS extracted ion chromatogram (Fig. S11b). GSAC in root was similarly clear to identify. GSPC identification/quantification was more complicated, since the extracted ion chromatogram from roots showed two clearly separated signals at m/z 291.1009 (with the same fragmentation pattern) at RT 17.0 and 17.6 min overlapping one broad sulphur signal from the ICP-MS/MS (Fig. S10 and S11a). The extracted ion chromatogram of bulbs showed one dominant signal at 17.6 min associated with one sulphur signal from the ICP-MS/MS. It was not possible to identify any other co-eluting sulphur compounds in root extracts and the existence of this double peak in the EIC remained unexplained (Fig. S11 comparison of bulb and root).

Table S4 GSAC and GSPC in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), database ID: ChemSpider.

	GSAC	GSPC
		
Molecular formula	$C_{11}H_{19}N_2O_5S$	$C_{11}H_{19}N_2O_5S$
MW (g mol^{-1})	290.3360	290.3360
$[M+H]^+$ (theoretical)	291.1009	291.1009
$[M+H]^+$ (measured)	291.1010	291.1009
RT (min)	14.7	17.7
Database ID	9368976	23254569
Root (0.1 mM S)	70 ± 41	164 ± 50[a]
Root (0.5 mM S)	61 ± 18	118 ± 59[b]
Root (2 mM S)	78 ± 16	263 ± 97[a,b]
Bulb (0.1 -2 mM S)	40 ± 16	650 ± 660

[a,b]: statistically significant difference between groups by One Way ANOVA $p < 0.05$

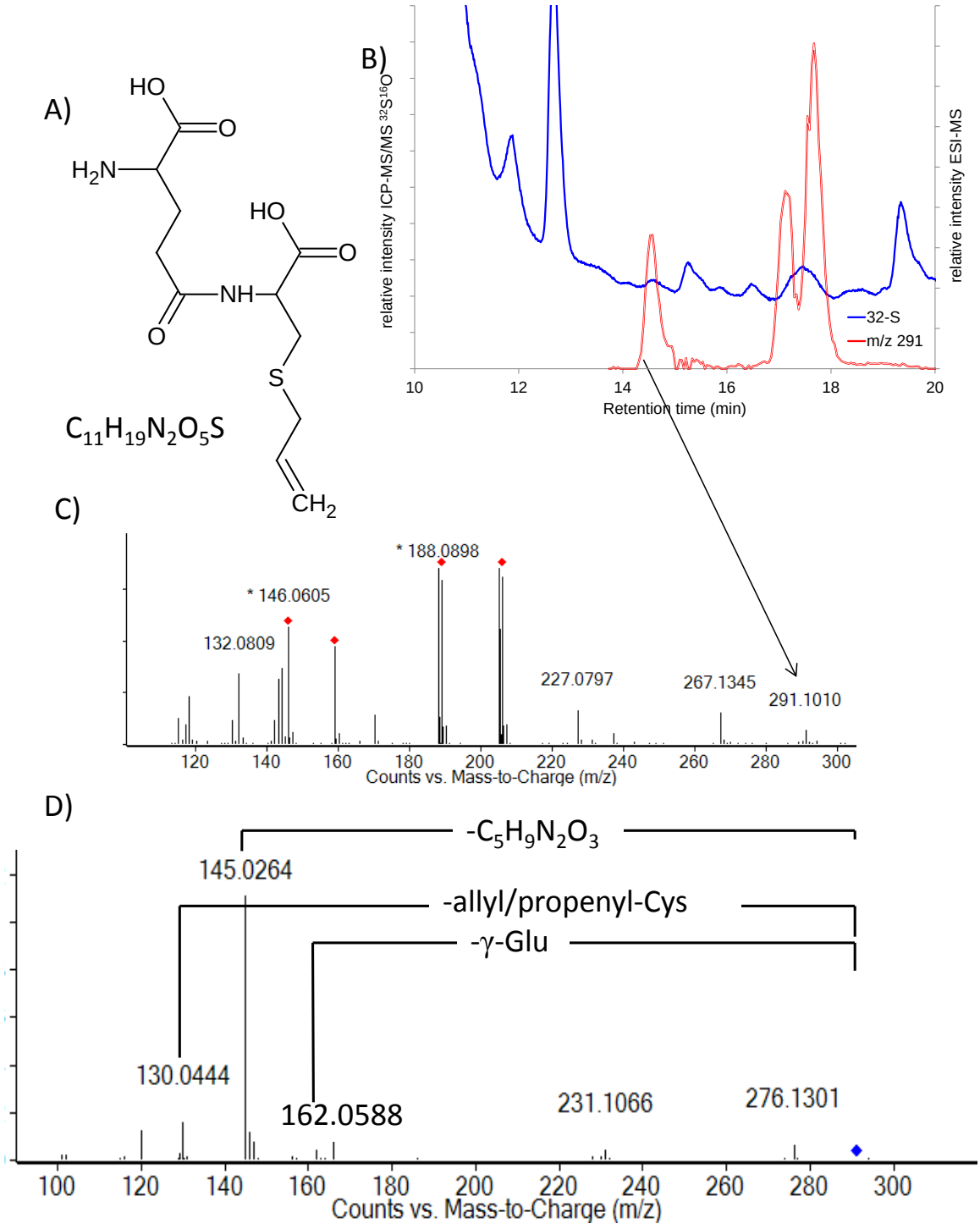


Fig. S9 **GSAC;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

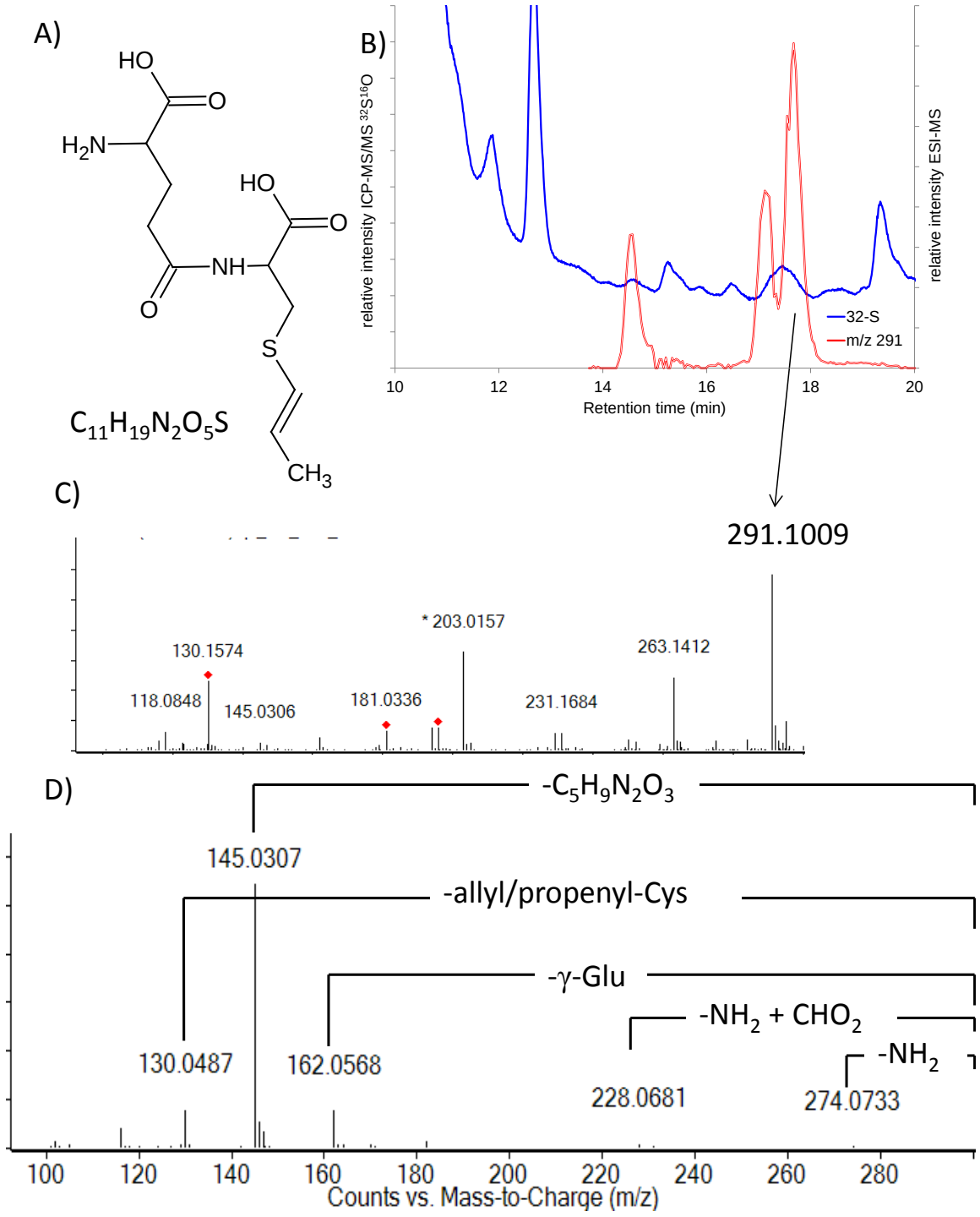


Fig. S10 **GSPC;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

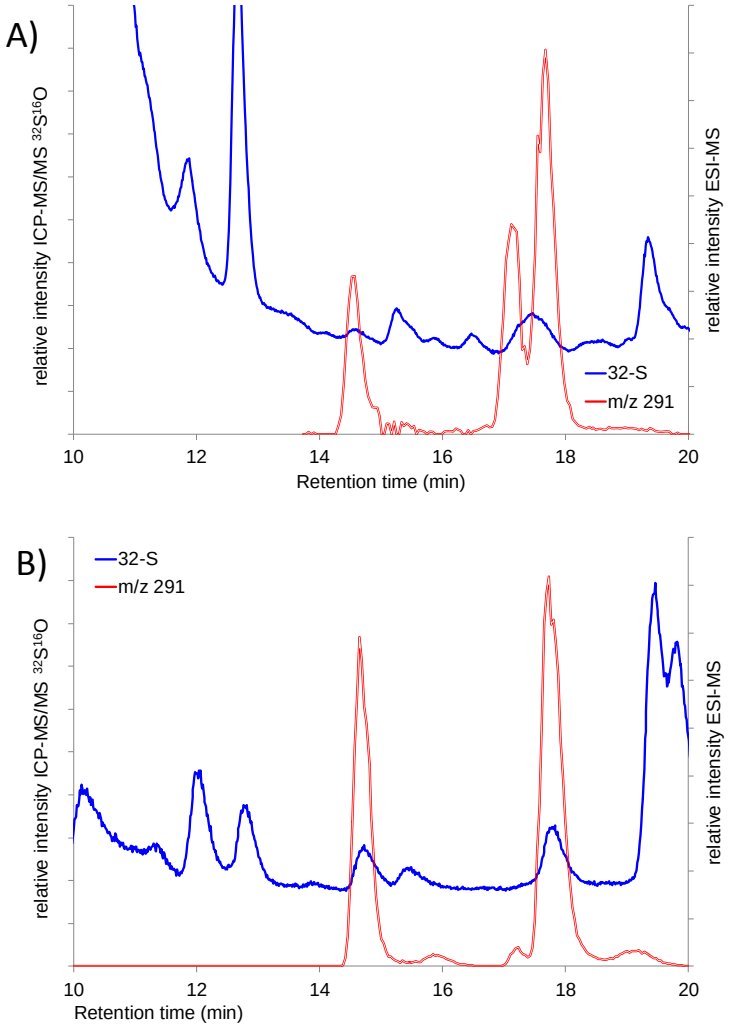
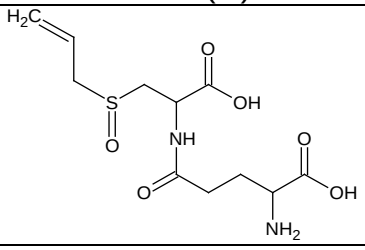
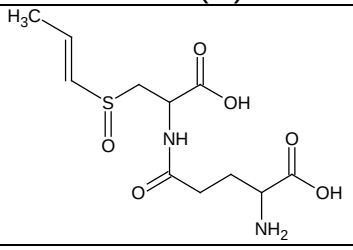


Fig. S11 Comparison of EIC 291.1009 (GSAC and GSPC) and ICP-MS/MS trace for root (panel A) and bulb (panel b).

2.5. γ-glutamyl-S-2-propenylcysteine sulfoxide GSAC(O) and γ-glutamyl-S-1-propenylcysteine sulfoxide GSPC(O)

The main fragments of oxidised GSAC and GSPC were *m/z* 130.0505 (γ-Glu) and *m/z* 217.081 (Fig. S12 and S13). Also the loss of γ-Glu at *m/z* 177.032 was present in both MS spectra, careful comparison of the fragment spectra showed that *m/z* 137.0147 (loss of $C_8H_{12}NO_3$), *m/z* 120.0120 ($C_3H_6NO_2S$) and *m/z* 204.0746 (loss of C_4H_7OS) were more intense in GSAC(O) than GSPC(O) (Fig. S14).

Table S5 GSAC(O) and GSPC(O) in root and bulb in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), database ID: ChemSpider.

	GSAC(O)	GSPC(O)
		
Molecular formula	$C_{11}H_{19}N_2O_6S$	$C_{11}H_{19}N_2O_6S$
MW (g mol^{-1})	306.3354	306.3354
$[M+H]^+$ (theoretical)	307.0958	307.0958
$[M+H]^+$ (measured)	307.0959	307.0955
RT (min)	4.3	4.9
Database ID	-	4476495
Root (0.1 mM S)	703 ± 398[a]	836 ± 635[b]
Root (0.5 mM S)	1236 ± 528	1627 ± 1317
Root (2 mM S)	3065 ± 2471[a]	1900 ± 856[b]
Bulb (0.1 – 2 mM S)	400 ± 792	160 ± 241

[a]: statistically significant difference between groups by One Way ANOVA $p < 0.001$
[b]: statistically significant difference between groups by One Way ANOVA $p < 0.05$

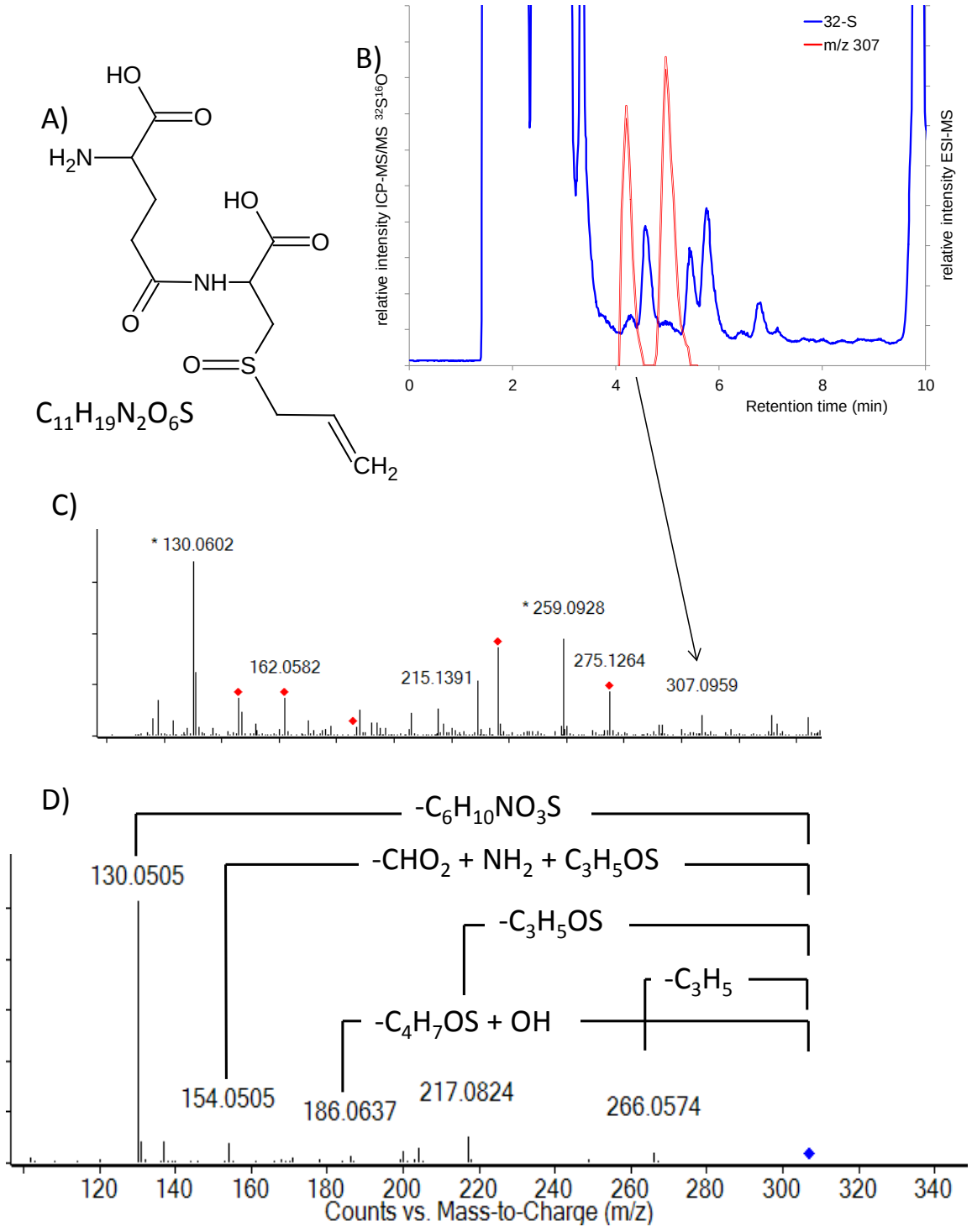


Fig. S12 **GSAC(O);** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

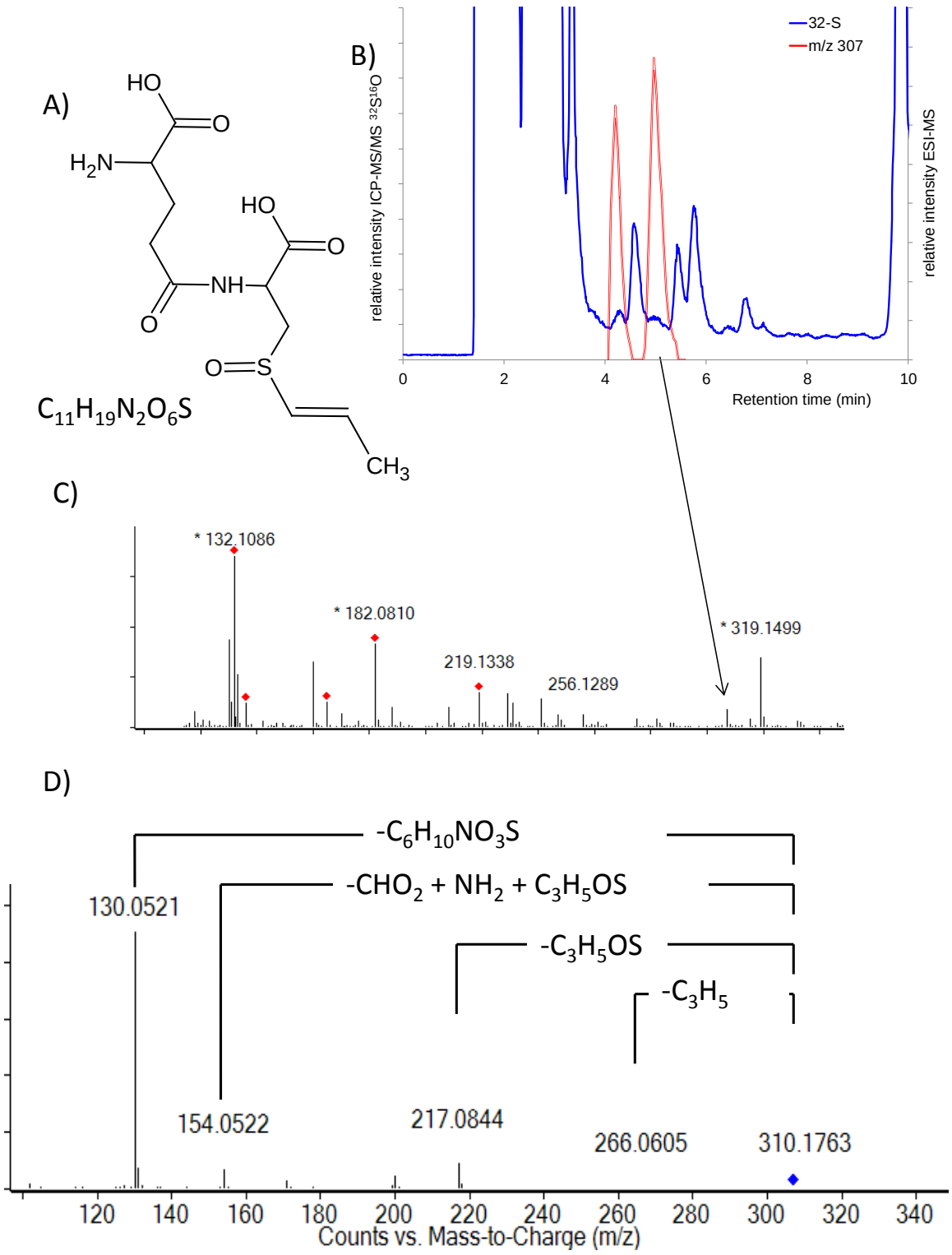


Fig. S13 **GSPC(O);** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

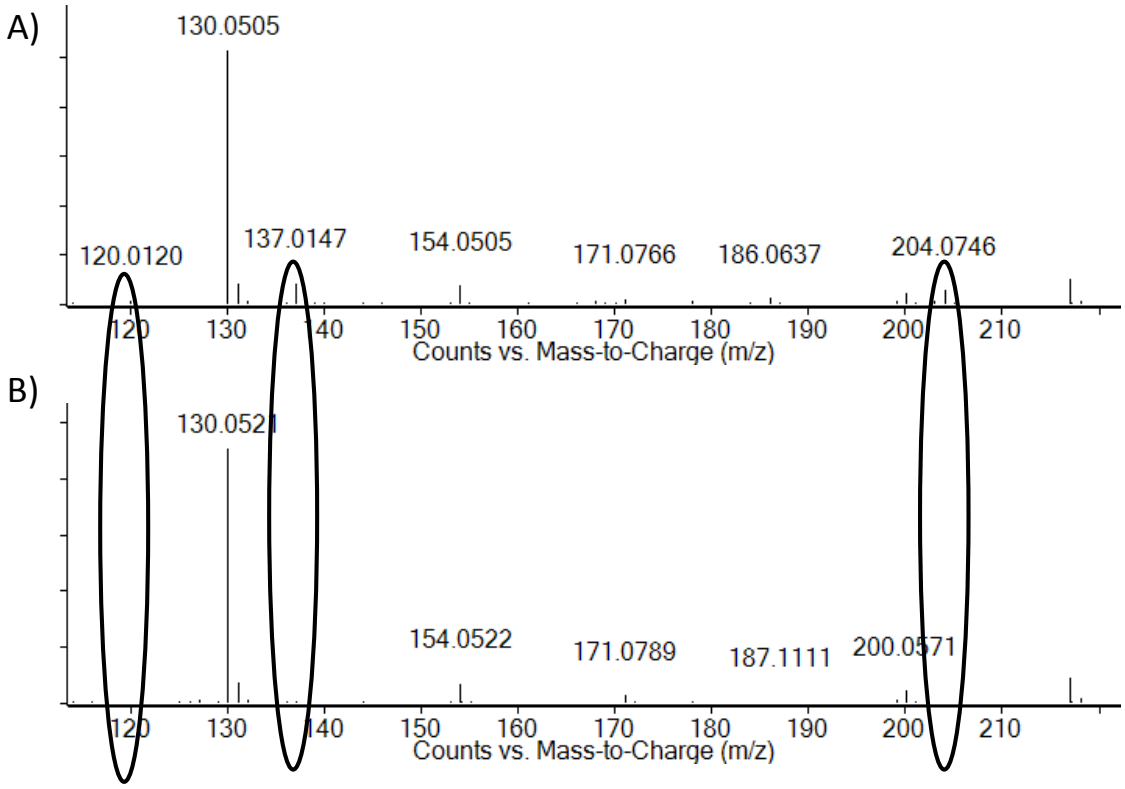
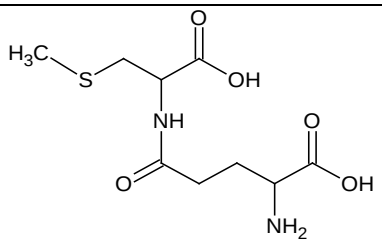
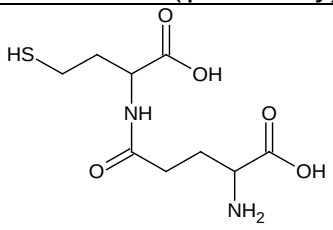


Fig. S14 Details of q-TOF ESI-MS/MS spectra of GSAC(O) and GSPC(O), main differences in occurring fragments marked by cycles.

2.6. γ-glutamyl-S-methyl-cysteine (GSMC) and γ-glutamyl-homocysteine (C264)

Three chromatographic peaks with the same molecular composition at *m/z* 265.0853 (GSMC) were clearly detectable in the extracted ion chromatogram. The detailed study of the ESI-MS/MS fragmentation patterns showed that one of the minor signals at 12.5 min was an in-source fragment of C393, whereas the signal at ca. 8.4 min was a genuine compound with the same *m/z* but a different fragmentation pattern not yet described in the literature (C264). GSMC showed a main fragment at *m/z* 119.0162 (loss of $C_5H_9N_2O_3$) (Fig. S15) and C264's main fragment was *m/z* 136.0468 (loss $C_5H_8NO_3$) (Fig. S16). To fragment this way C264 is likely to be γ-Glu-HCy (2-amino-5-[(1-carboxy-3-sulfanylpropyl)amino]-5-oxopentanoic acid).

Table S6 GSMC and γ-Glu-HCy in root and bulb in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), database ID: ChemSpider.

	GSMC	C264 (γ-Glu-HCy)
		
Molecular formula	$C_9H_{17}N_2O_5S$	$C_9H_{17}N_2O_5S$
MW (g mol^{-1})	264.2987	264.2987
$[M+H]^+$ (theoretical)	265.0853	265.0853
$[M+H]^+$ (measured)	265.0852	265.0851
RT (min)	6.0	8.6
Database ID	9218811	24751867
Root (0.1 mM S)	699 ± 750[a]	288 ± 207
Root (0.5 mM S)	1863 ± 1758[a]	278 ± 222
Root (2 mM S)	1695 ± 1528	237 ± 69
Bulb (0.1 – 2 mM S)	79 ± 48	69 ± 117

[a]: statistically significant difference between groups by One Way ANOVA $p < 0.05$

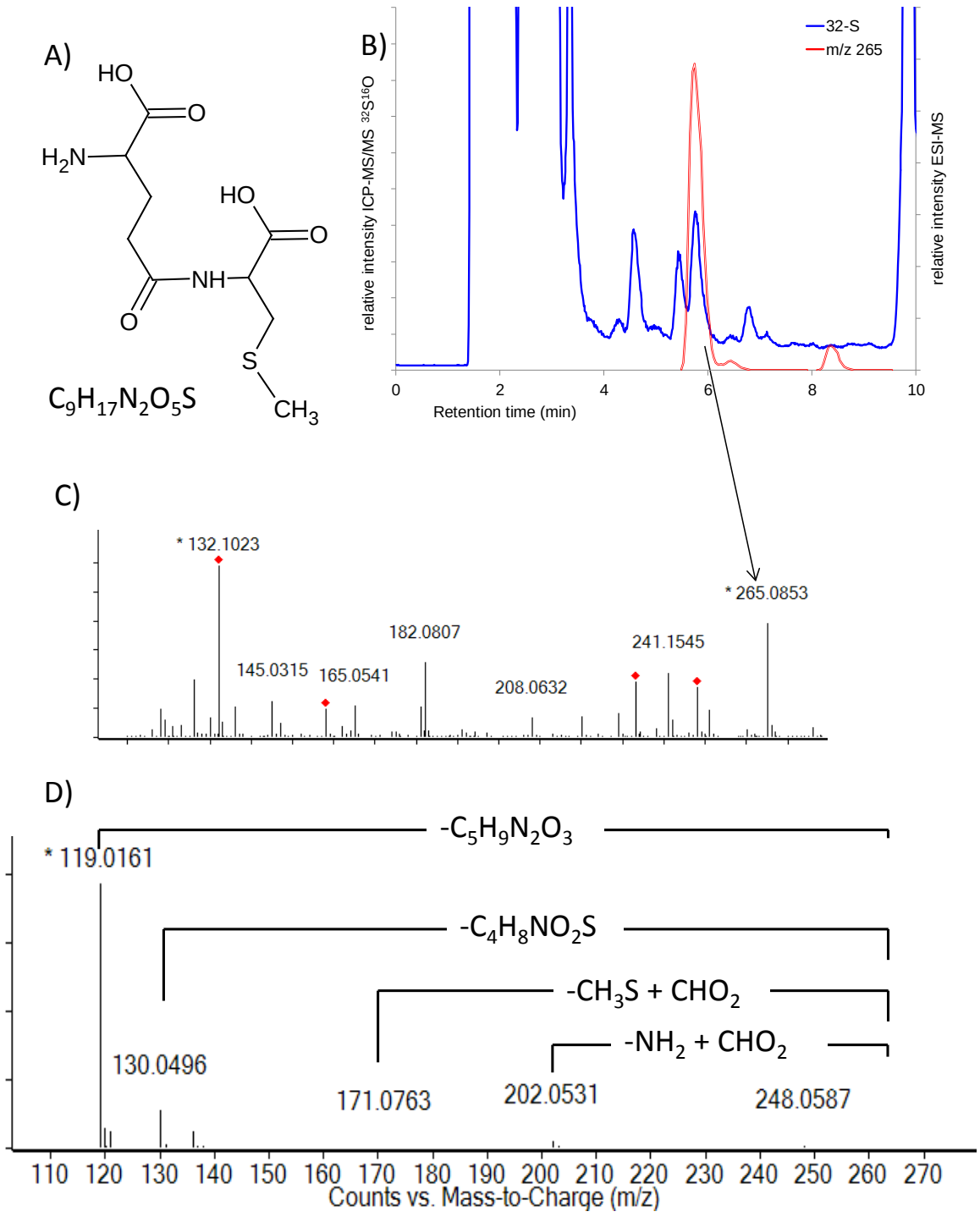


Fig. S15 **GSMC;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

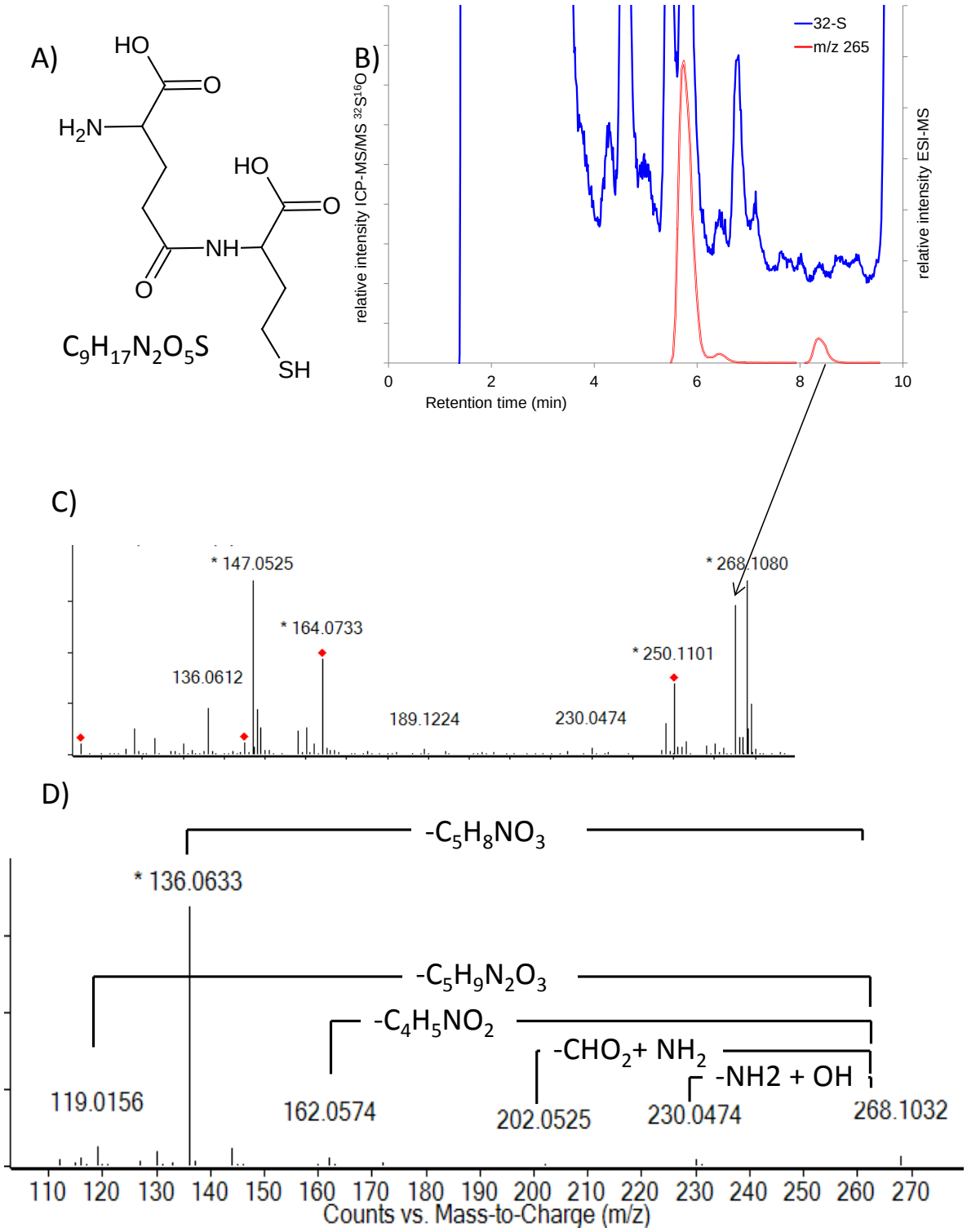
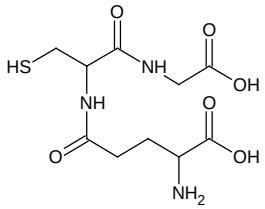
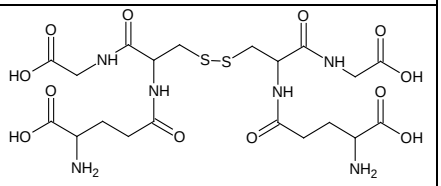


Fig. S16 **C264 (γ-Glu-HCy);** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.7. Glutathione (GSH and GSSG)

The molecular masses of reduced and oxidised glutathione were detectable by both ESI-MS instruments, but ESI-MS/MS fragments of the single charge molecular ions were only found in the data of the Orbitrap (shown in Fig. S17 and S18). The main fragments for GSH were *m/z* 179.0479 (loss of γ-Glu), *m/z* 162.0217 ($C_5H_8NO_3S$) and *m/z* 233.0580 (loss of Gly) (Fig. S17), whereas GSSG showed main fragments at *m/z* 484.1153 (loss of γ-Glu) and *m/z* 355.0731 (loss of 2 γ-Glu) (Fig. S18).

Table S7 GSSG in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), GSH, database ID: ChemSpider.

	GSH^+	GSSG
		
Molecular formula	$C_{10}H_{18}N_3O_6S$	$C_{20}H_{33}N_6O_{12}S_2$
MW (g mol^{-1})	307.3234	612.6311
$[M+H]^+$ (theoretical)	308.0911	613.1592
$[M+H]^+$ (measured)	308.0911	613.1594
RT (min)	3.7	6.4
Database ID	111188	950
Root (0.1 mM S)		174 ± 178
Root (0.5 mM S)		253 ± 137
Root (2 mM S)		275 ± 104
Bulb (0.1 – 2 mM S)		93 ± 88

$^+$ propiin + glutathione + methionine, amount of associated sulphur see table S13

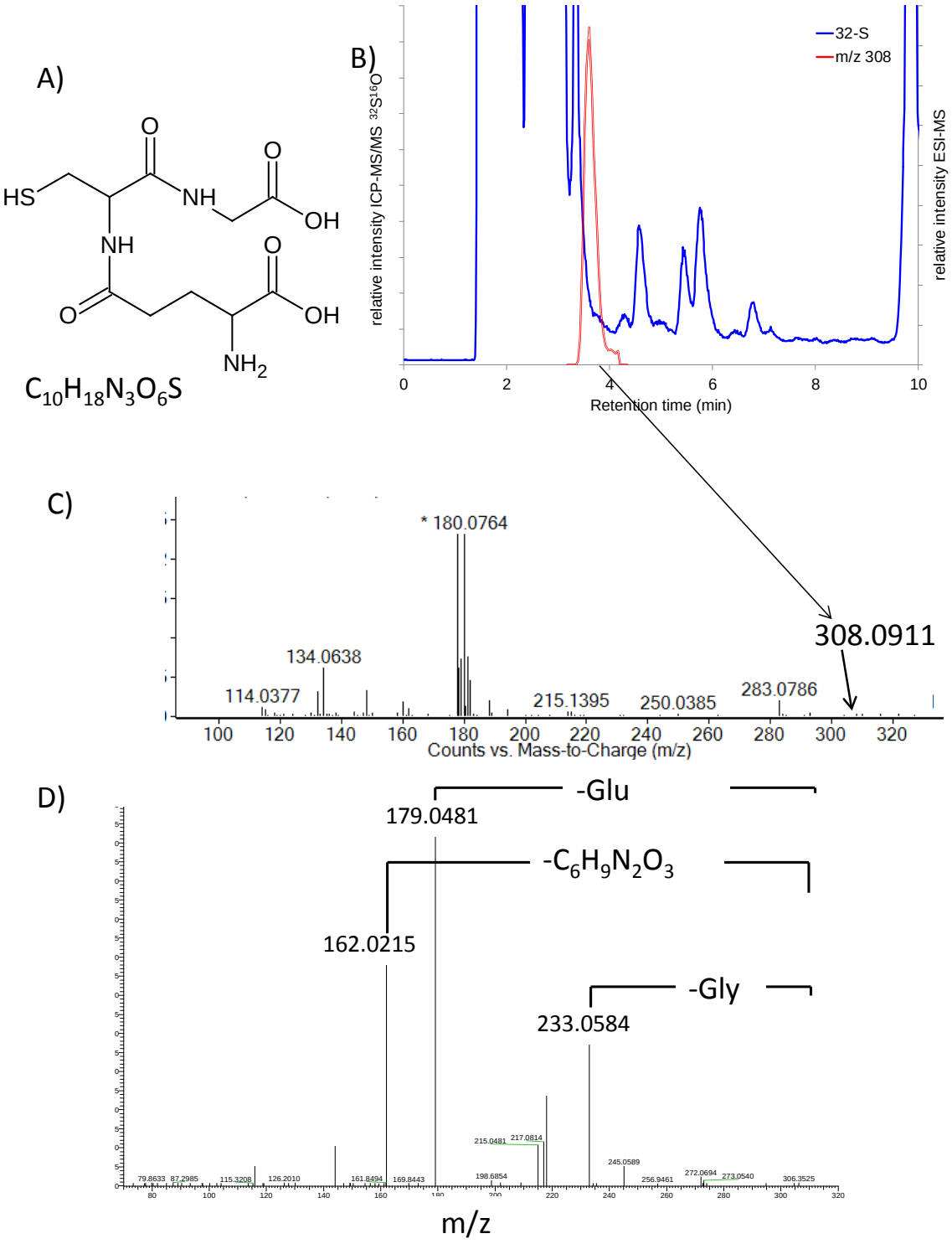


Fig. S17 **GSH;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) ESI-MS-spectra, panel D)) Orbitrap-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

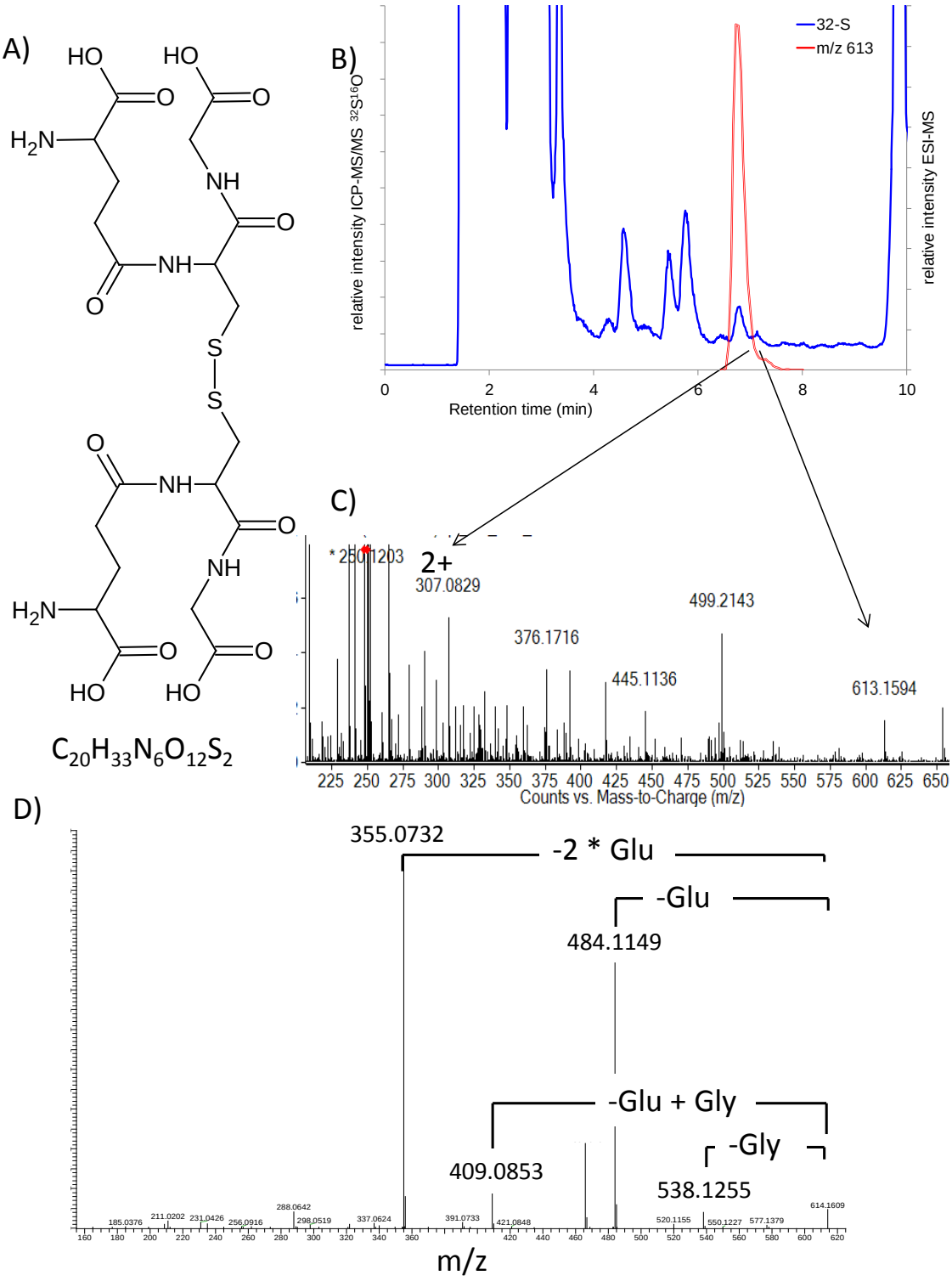
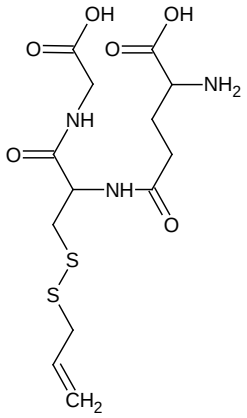
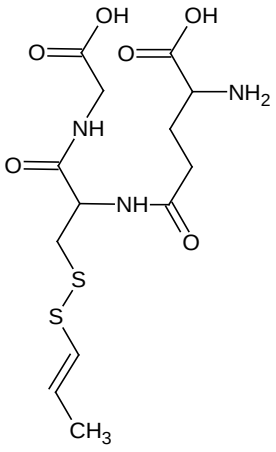
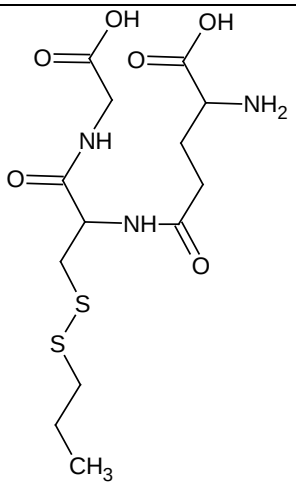


Fig. S18 **GSSG;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) MS-spectra, panel D) Orbitrap-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.8. S-1-propenylmercaptoglutathione, S-2-propenylmercaptoglutathione and S-propylmercaptoglutathione

Both compounds were described before by Nakabayashi *et al.*[2] Their main ESI-MS/MS fragments were *m/z* 251.0480 (loss of $C_5H_7NO_3$), *m/z* 177.0296 (loss of $C_8H_{13}NO_3S$), *m/z* 148.0218 ($C_5H_{10}NS_2$), *m/z* 130.0468 ($C_5H_8NO_3$) and *m/z* 104.9801 ($C_3H_5S_2$). We identified the same MS fragments using q-TOF-ESI-MS and Orbitrap ESI-MS as identified by Nakabayashi and co-workers using an FT-ICR-MS. The fragmentation spectra for both compounds also showed the by Nakabayashi et al identified characteristic ions for the allyl respectively propenyl compound (*m/z* 209.004 ($C_5H_9N_2O_3S_2$), Fig. S19, respectively *m/z* 217.0617 ($C_8H_{13}N_2O_3S$, Fig. S20). S-propylmercaptoglutathione ($C_{13}H_{23}N_3O_6S_2$), $[M+H]^+$ *m/z* 382.1101, measured *m/z* 382.1102) not yet mentioned in the literature was identified by ESI-MS, eluting shortly after S-2-propenylmercaptoglutathione. Its main ESI-MS/MS fragments were *m/z* 253.0673 (loss of $C_5H_7NO_3$), *m/z* 177.0296 (loss of $C_8H_{13}NO_3S$), *m/z* 307.0778 (loss of Gly) and *m/z* 130.0499 ($C_5H_8NO_3$) (Fig. S21).

Table S8 S-1-propenylmercaptglutathione and S-2-propenylmercaptoglutathione in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), S-propylmercaptoglutathione, database ID: ChemSpider.

	S-1-propenylmercapto-glutathione	S-2-propenylmercapto-glutathione	S-propylmercapto-glutathione*
			
Molecular formula	$C_{13}H_{21}N_3O_6S_2$	$C_{13}H_{21}N_3O_6S_2$	$C_{13}H_{23}N_3O_6S_2$
MW (g mol^{-1})	379.4523	379.4523	381.1028
$[M+H]^+$ (theoretical)	380.0944	380.0944	382.1101
$[M+H]^+$ (measured)	380.0948	380.0948	382.1105
RT (min)	23.8	26.5	27.3
Database ID	8083486	-	-
Root (0.1 mM S)	245 ± 92	1054 ± 720	
Root (0.5	236 ± 138	788 ± 724	

mM S)			
Root (2 mM S)	344 ± 91	1270 ± 321	
Bulb (0.1 -2 mM S)	282 ± 165	163 ± 93	

*co-eluting with one isomer of S-allyl/propenyl-PC-2, amount of associated sulphur see table S13

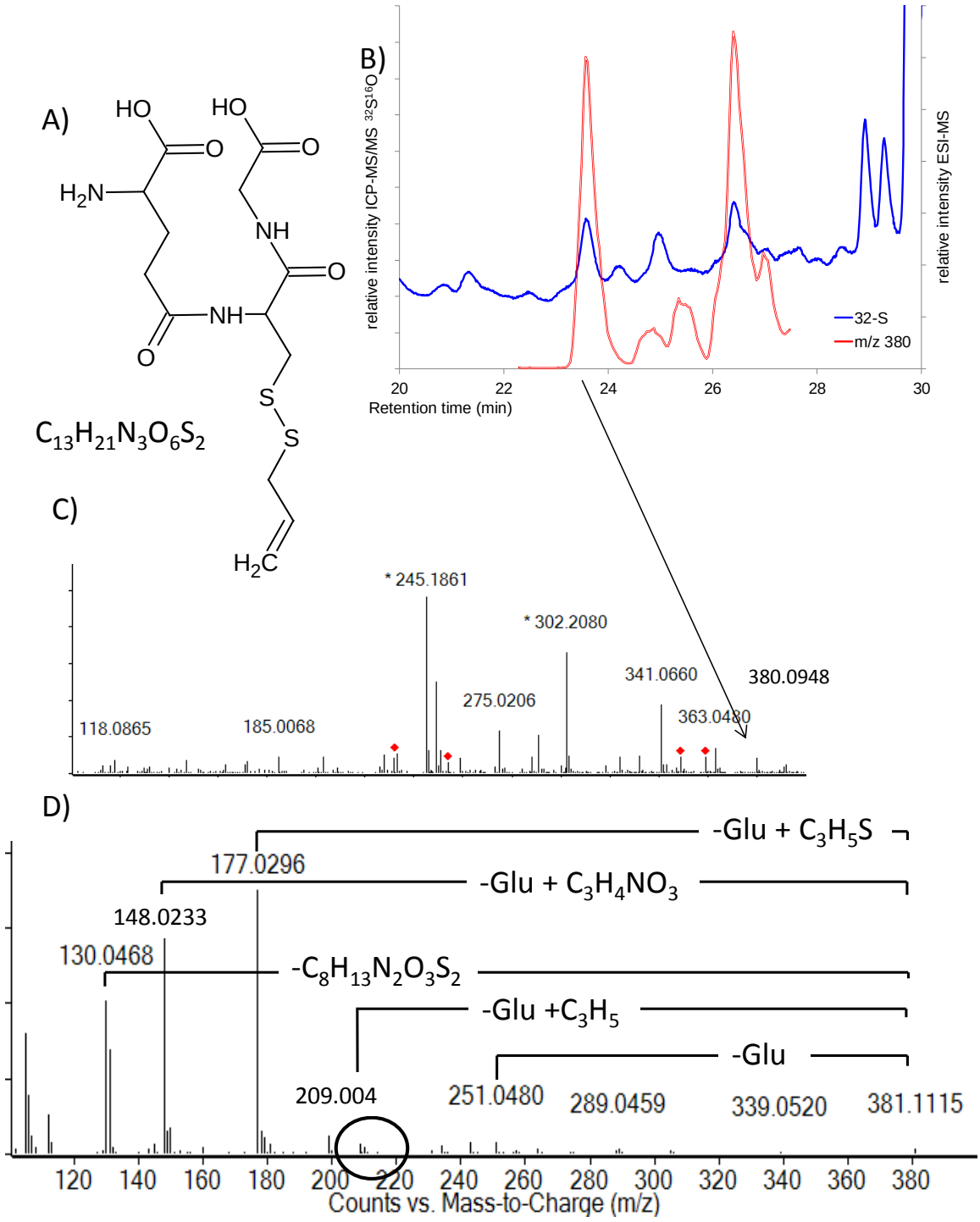


Fig. S19 S-1-propenylmercaptoglutathione; panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation; cycle marks specific fragment for this isomer.

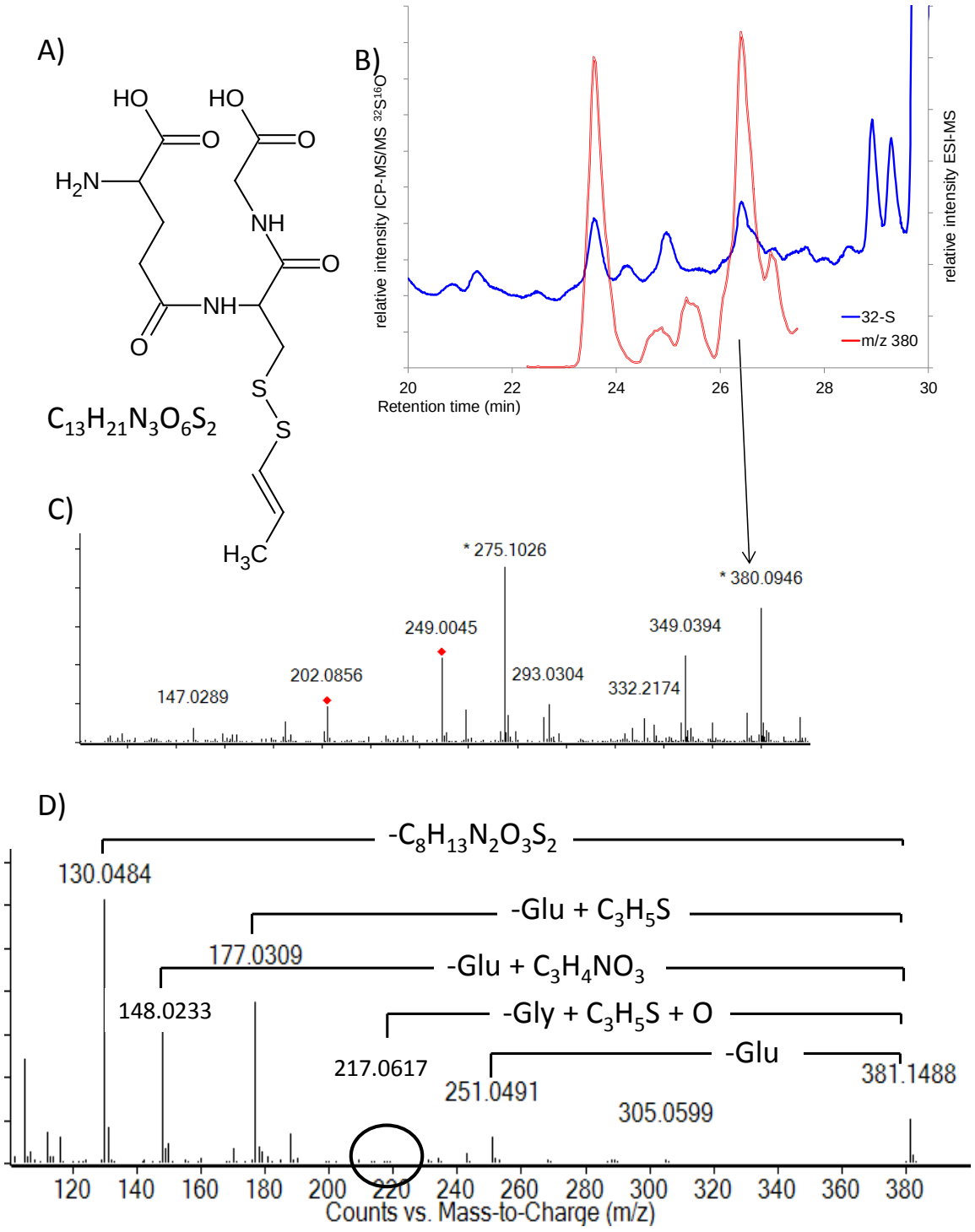


Fig. S20 S-2-propenylmercaptoglutathione; panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation; cycle marks specific fragment for this isomer.

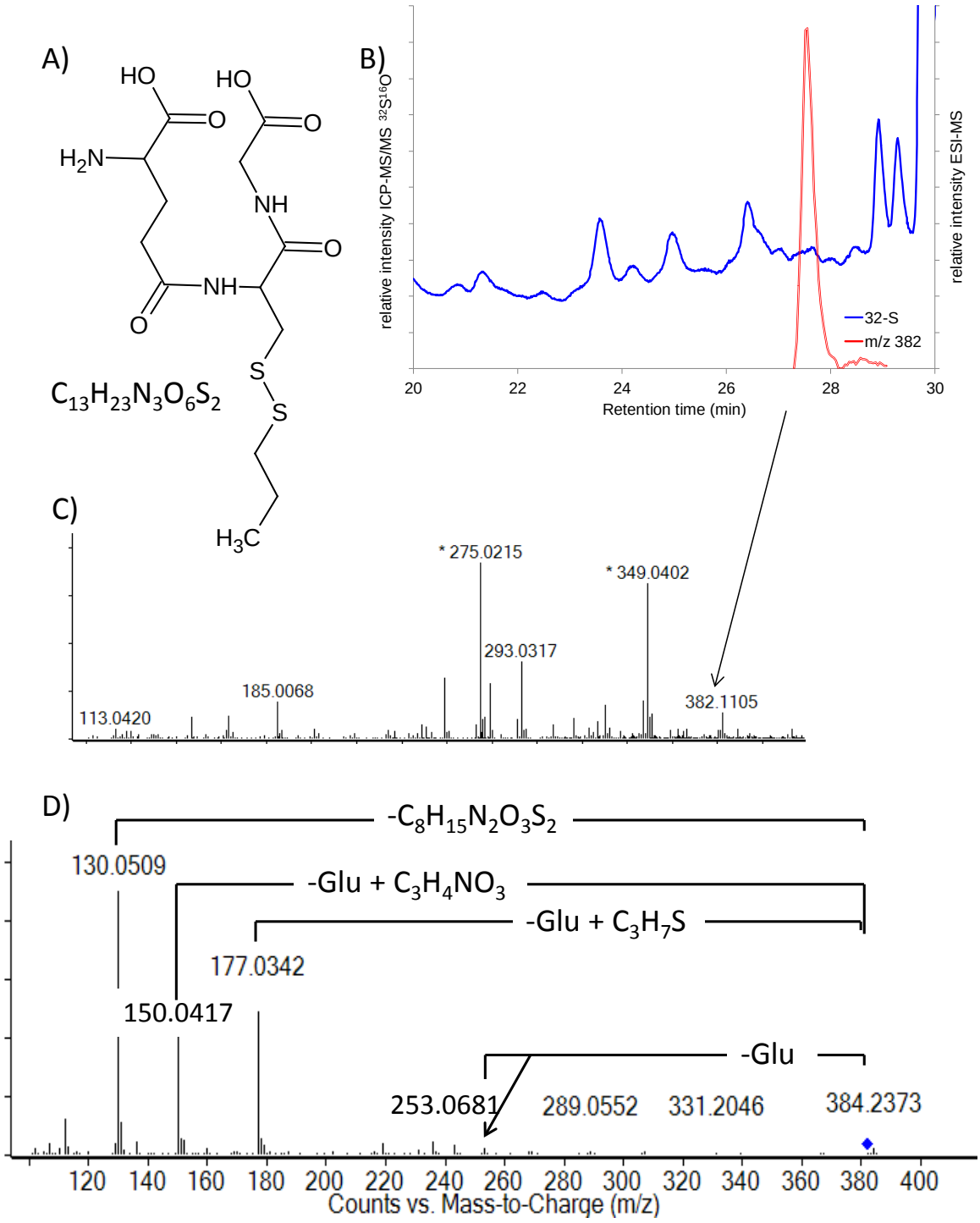
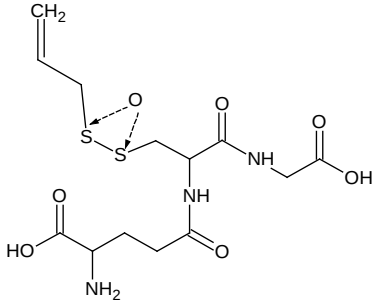
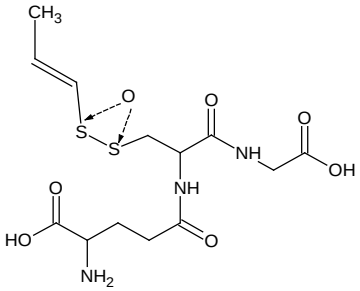


Fig. S21 **S-propylmercaptoglutathione;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.9. Oxidised forms of S-1-propenylmercaptoglutathione and S-2-propenylmercaptoglutathione

Just as the oxidised relatives of GSAC and GSPC are present oxidised forms of S-1 and S-2-propenylmercaptoglutathion were present in the extracts of root and bulb. The fragmentation patterns for 3 of the compounds by qTOF-ESI-MS were very similar. Compounds 1a and b showed more pronounced fragments at *m/z* 131.0459 ($C_4H_7N_2O_3$) and *m/z* 263.01 ($C_8H_{11}N_2O_4S_2$) (Fig. S22 and S23). Compound 2a showed a more pronounced fragment at *m/z* 120.0126 ($C_3H_6NO_2S$) (Fig. S23). The last of the mentioned fragments may indicate that for this compound the HS-group of GSH was oxidised. No further identification with regard to the influence of double bond position and oxygen position on retention time and therefore compound characterisation was possible.

Table S9 Oxidised forms of S-1-propenylmercaptglutathione and S-2-propenylmercaptoglutathione; 4 isomers (forms 1a, 1b, 2a and 2b) were found, double bond and position of SO-group can vary, compound 1a co-eluted with PC 2 oxidised. Sum of compounds (1b, 2a and 2b) in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group); quantification of individual compounds in Table S10, database ID: ChemSpider.

	S-1-propenylmercaptoglutathione oxidised	S-2-propenylmercaptoglutathione oxidised
		
Molecular formula	$C_{13}H_{21}N_3O_7S_2$	$C_{13}H_{21}N_3O_7S_2$
MW (g mol^{-1})	395.4517	395.4517
$[M+H]^+$ (theoretical)	396.0894	396.0894
$[M+H]^+$ (measured)	396.0894	396.0892
RT (min)	11.1 , 11.7, 13.6 , 14.3	
Database ID	-	-
Root (0.1 mM S)	265 ± 125*	
Root (0.5 mM S)	187 ± 96*	
Root (2 mM S)	395 ± 219*	
Bulb (0.1 – 2 mM S)	85 34*	

* S-1-propenylmercaptoglutathione and S-2-propenylmercaptoglutathione compounds 1b + 2a + 2b

Table S10 Oxidised forms of S-1-propenylmercaptoglutathione and S-2-propenylmercaptoglutathione compounds 1b, 2a and 2b in µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group) (mean ± standard deviation, n = 9 per group).

	compound 1 a (11.3 min)*	compound 1b (11.7 min)	compound 2a (13.6 min)	compound 2b (14.3 min)
Root (0.1 mM S)		104 ± 79	27 ± 3	24 ± 2
Root (0.5 mM S)		63 ± 28	26 ± 5	26 ± 10
Root (2 mM S)		153 ± 135	36 ± 6	43 ± 12
Bulb (0.1 – 2 mM S)		54 ± 30	24 ± 8	16 ± 5

*PC-2 + oxidised form of S-1-propenylmercaptoglutathione or S-2-propenylmercaptoglutathione compound 1a, amount of associated sulphur see table S13

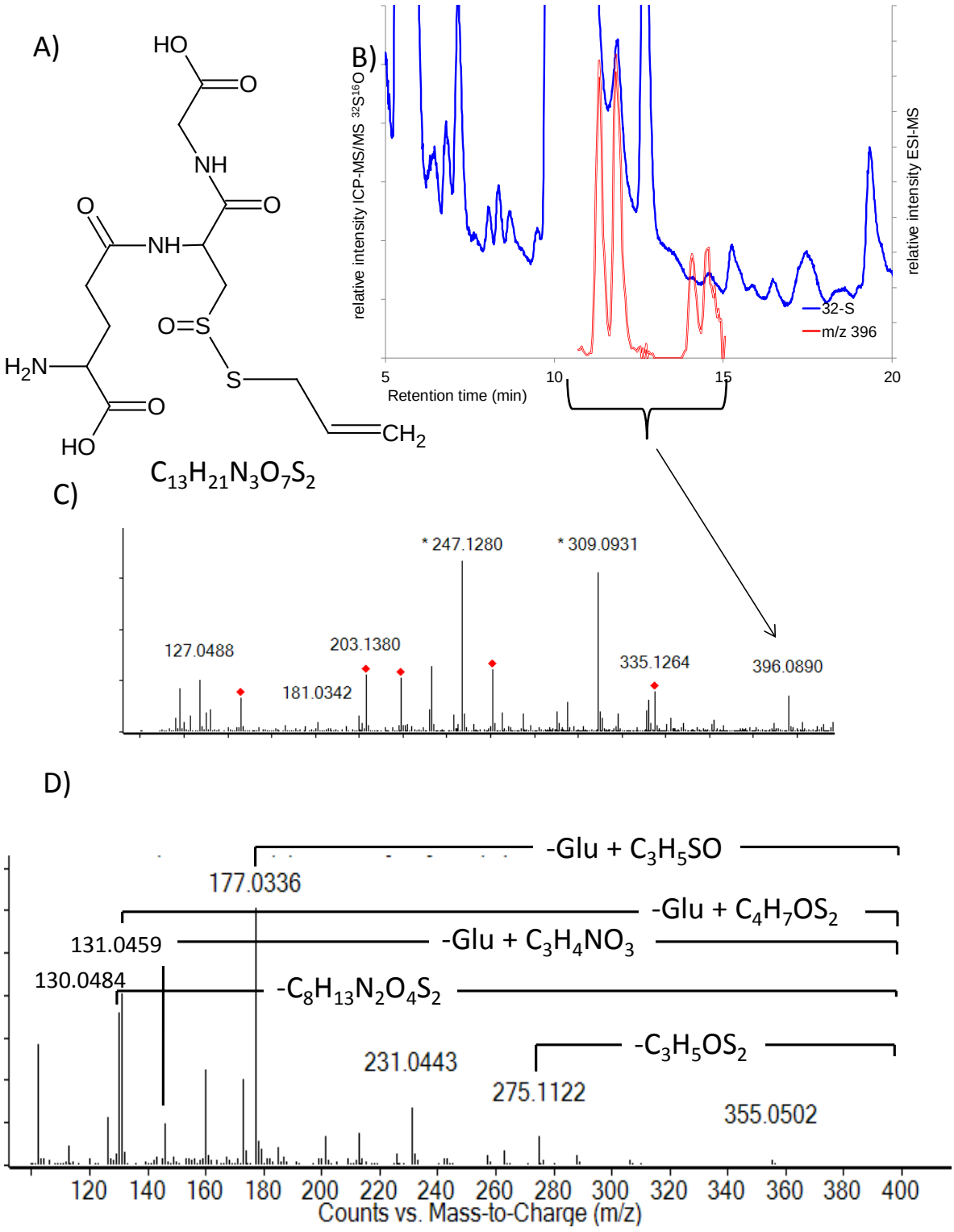


Fig. S22 oxidised forms of S-1-propenylmercaptoglutathione and S-2-propenylmercaptoglutathione; panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

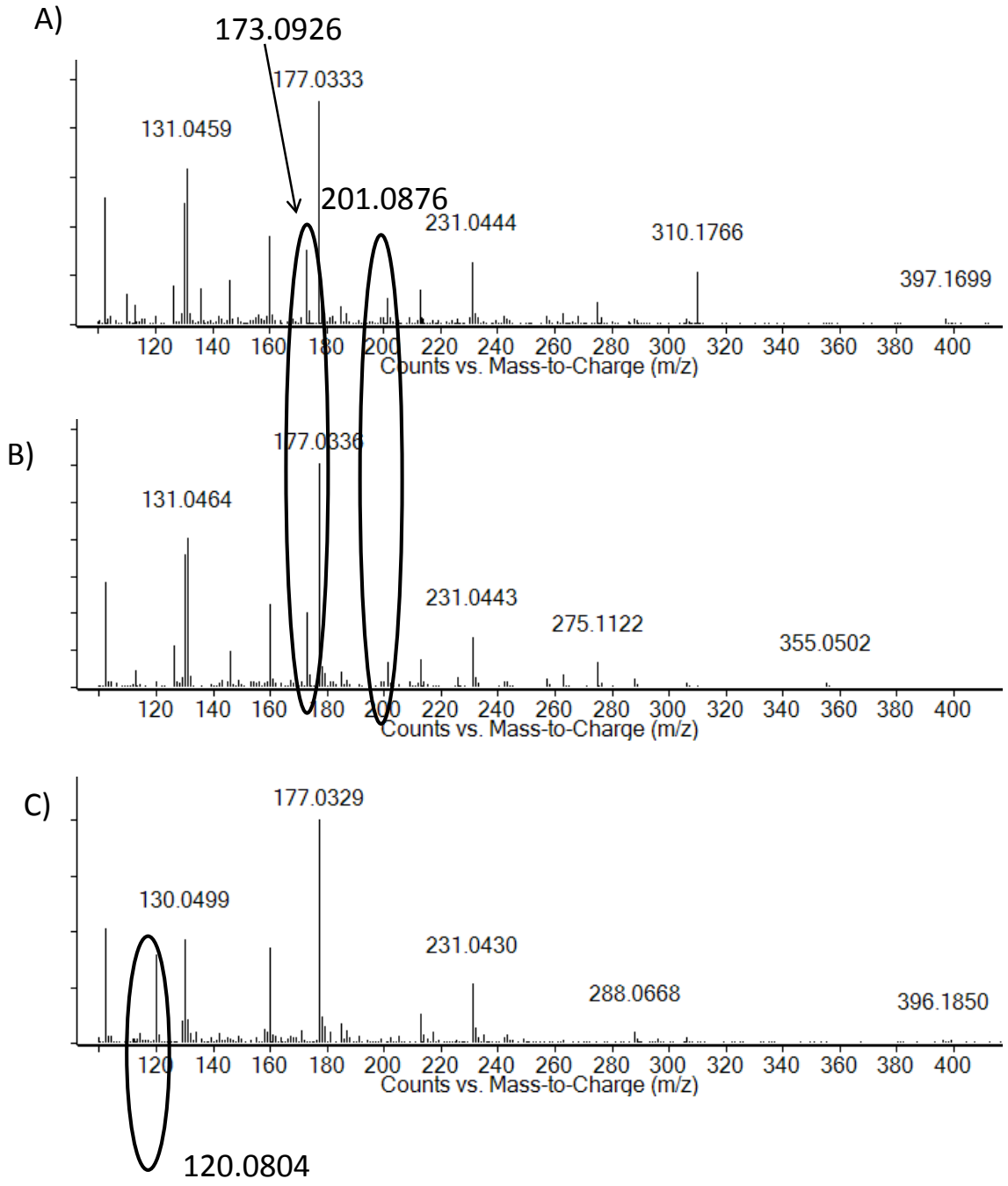
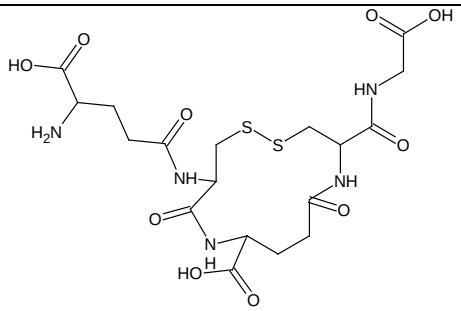
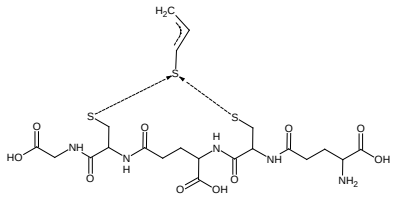


Fig. S23 Details of q-TOF ESI-MS/MS spectra of compounds 1a (panel A), 1b (panel B) and 2a (panel C) of oxidised forms of S-1-propenylmercaptoglutathione and S-2-propenylmercaptoglutathione; main differences in occurring fragments marked by cycles.

2.10. Phytochelatin 2 (PC-2) and derivatives

The main ESI-MS/MS fragments of oxidised PC-2 were *m/z* 231.0434 ($C_8H_{11}N_2O_4S$), *m/z* 306.0570 ($C_{10}H_{15}N_3O_6S$), *m/z* 409.0835 (loss of γ-Glu) and *m/z* 334.0522 (loss of γ-Glu + Gly) (Fig. S24). The yet undescribed, S-allyl/propenyl-containing PC-2 (C611) was present in both root and bulb in at least four different isomeric forms (Fig. S24), indicating that the S-allyl/propenyl-group can be bound to either SH-group of PC2. The main ESI-MS/MS fragments were *m/z* 408.0708 (loss of γ-Glu + Gly), *m/z* 483.0984 (loss of γ-Glu), *m/z* 380.0721 (loss of γ-Glu-Cys) and *m/z* 306.0571 ($C_{10}H_{15}N_3O_6S$) (Fig. S25).

Table S11 PC-2 oxidised and S-allyl/propenyl-PC-2 (mean ± standard deviation, n = 9 per group), database ID: ChemSpider.

	PC-2 oxidised*	S-allyl/propenyl-containing PC-2$^+$
		
Molecular formula	$C_{18}H_{27}N_5O_{10}S_2$	$C_{21}H_{33}N_5O_{10}S_3$
MW (g mol^{-1})	537.5645	611.7092
$[M+H]^+$ (theoretical)	538.1272	612.1462
$[M+H]^+$ (measured)	538.1270	612.1463
RT (min)	11.2	27.3-28.2
Database ID	-	-
Root (0.1 mM S)		
Root (0.5 mM S)		
Root (2 mM S)		
Bulb (0.1 -2 mM S)		

* PC-2 + S-1-propenylmercaptoglutathione compound1a, amount of associated sulphur see table S13

\+ S-allyl/propenyl-containing PC-2 + S-propymercaptoglutathione, amount of associated sulphur see table S13

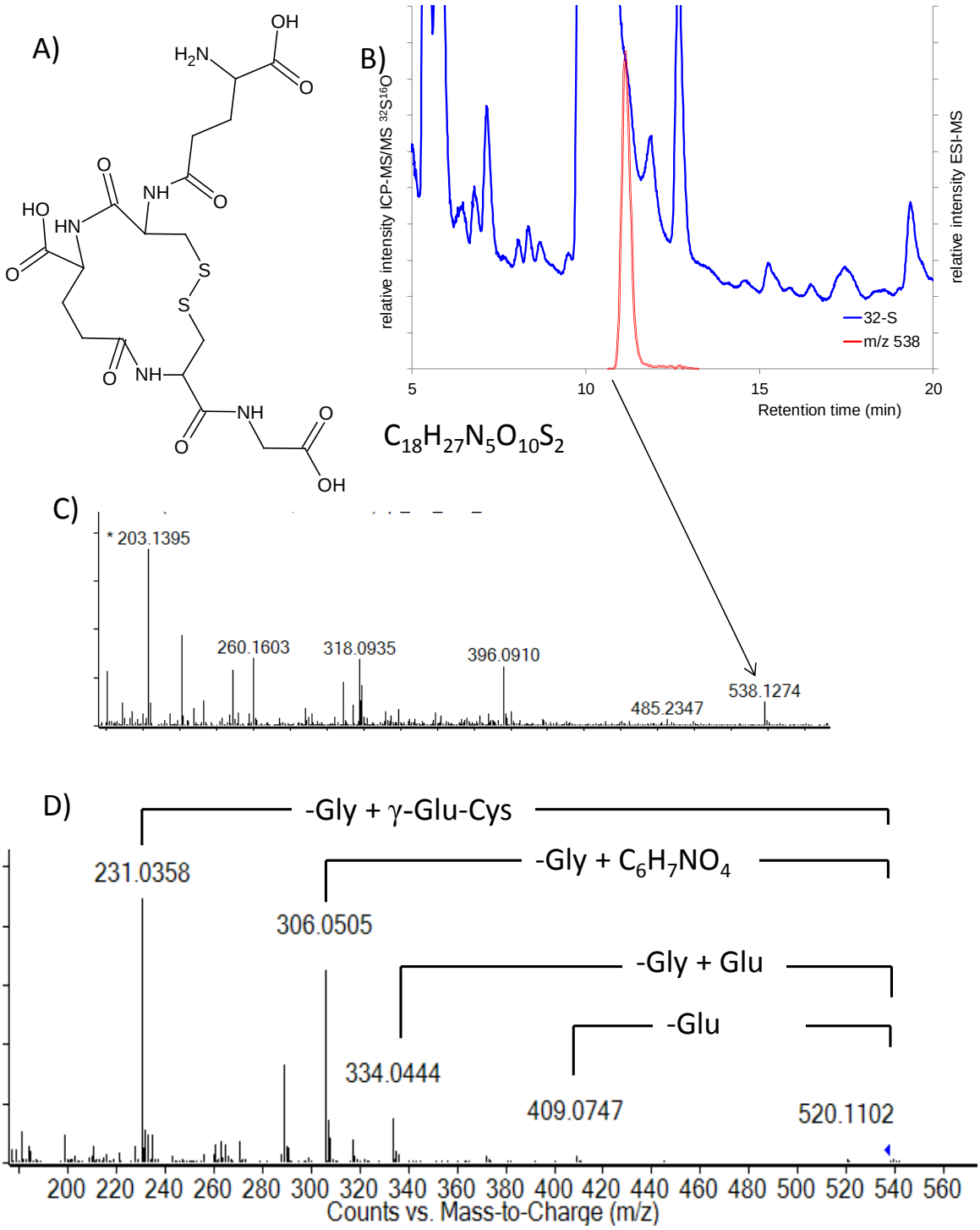


Fig. S24 **oxidised PC-2;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

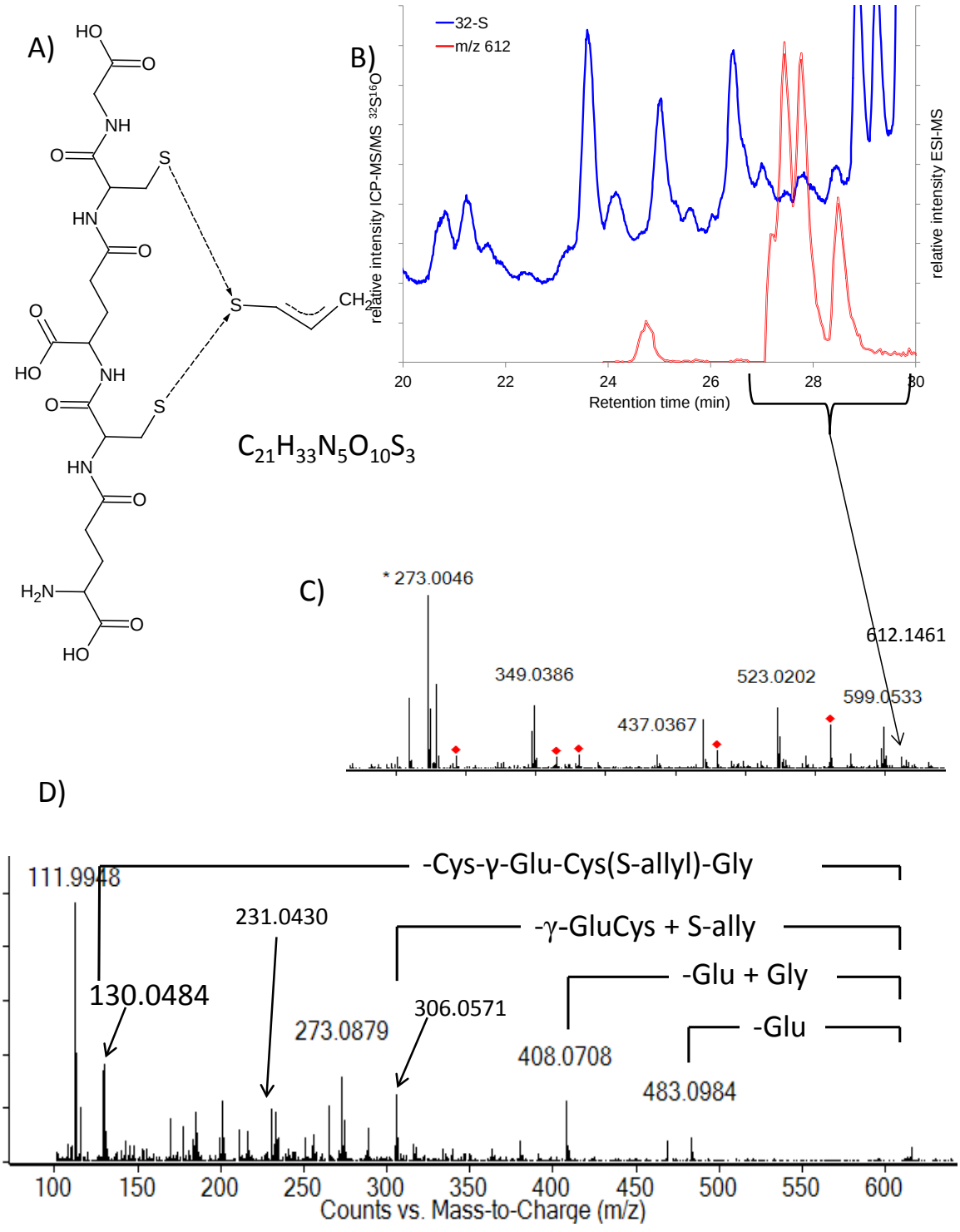
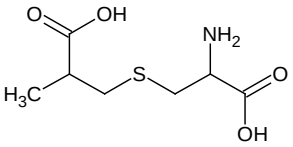
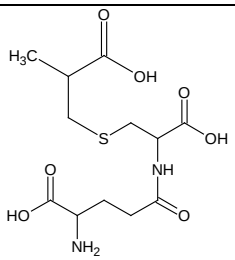
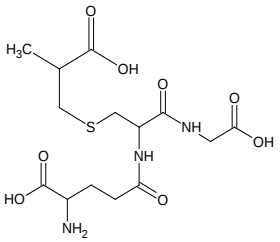


Fig. S25 **S-allyl/propenyl-PC-2;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

2.11. Precursor molecules for S-alk(en)ylcysteine sulfoxides

The main fragments of C207 were *m/z* 119.0161 ($C_4H_7O_2S$) and *m/z* 191.0372 (loss of NH_3) (Fig. S26). For C393 the main fragments were *m/z* 119.0161 ($C_4H_7O_2S$), *m/z* 130.0498 (γ-Glu), *m/z* 162.0582 ($C_6H_{12}NO_2S$), *m/z* 230.0484 ($C_9H_{12}NO_4S$) and *m/z* 319.0958 (loss of Gly) (Fig. S27).

Table S12 C207 and C393 µmol **compound** kg^{-1} d.w. (mean ± standard deviation, n = 9 per group), database ID: ChemSpider.

	C207 (2-amino-3-[(2-carboxypropyl)sulfanyl]-propanoic acid)	C336 (2-amino-5-({1-carboxy-2-[(2-carboxypropyl)sulfanyl]ethyl}amino)-5-oxopentanoic acid)	C393 (2-amino-5-({1-[(carboxy-methyl)amino]-3-[(2-carboxypropyl)sulfanyl]-1-oxopropan-2-yl}amino)-5-oxopentanoic acid)
			
Molecular formula	$C_7H_{13}NO_4S$	$C_{12}H_{20}N_2O_7S$	$C_{14}H_{23}N_3O_8S$
MW ($g\ mol^{-1}$)	207.2774	336.3614	393.4127
$[M+H]^+$ (theoretical)	208.0638	337.1064	394.1279
$[M+H]^+$ (measured)	208.0640		394.1280
RT (min)	5.7		12.7
Database ID	133465	-	18579450
Root (0.1 mM S)	669 ± 305[a]	-	83 ± 36[c]
Root (0.5 mM S)	1272 ± 859[b]	-	125 ± 104[d]
Root (2 mM S)	3214 ± 1827[a,b]	-	599 ± 375[c,d]
Bulb (0.1 – 2 mM S)	158 ± 74	-	201 ± 216

[a,b]: statistically significant difference between groups by One-way ANOVA $p < 0.05$
[c,d]: statistically significant difference between groups by One-way ANOVA $p < 0.01$

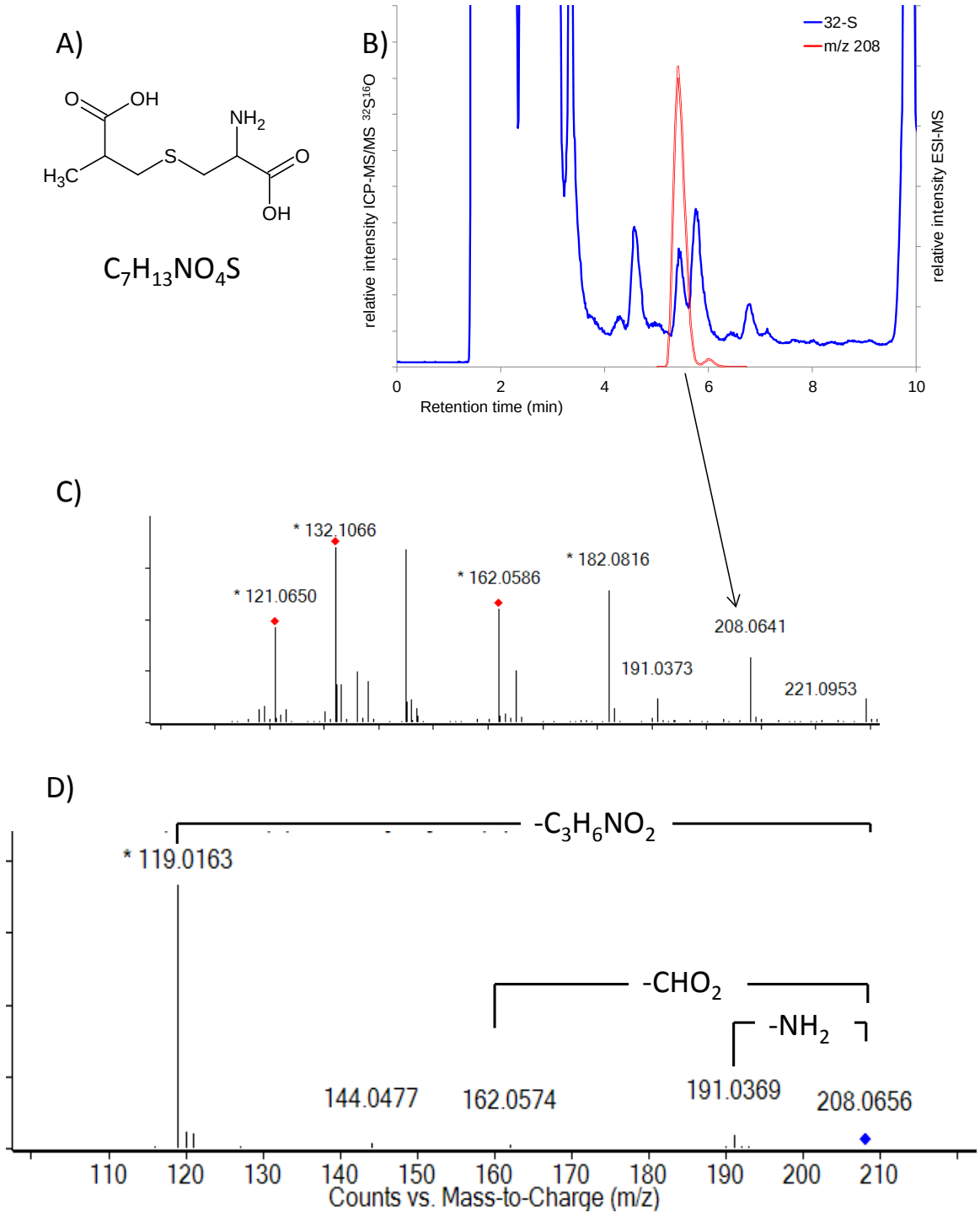


Fig. S26 **C207;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

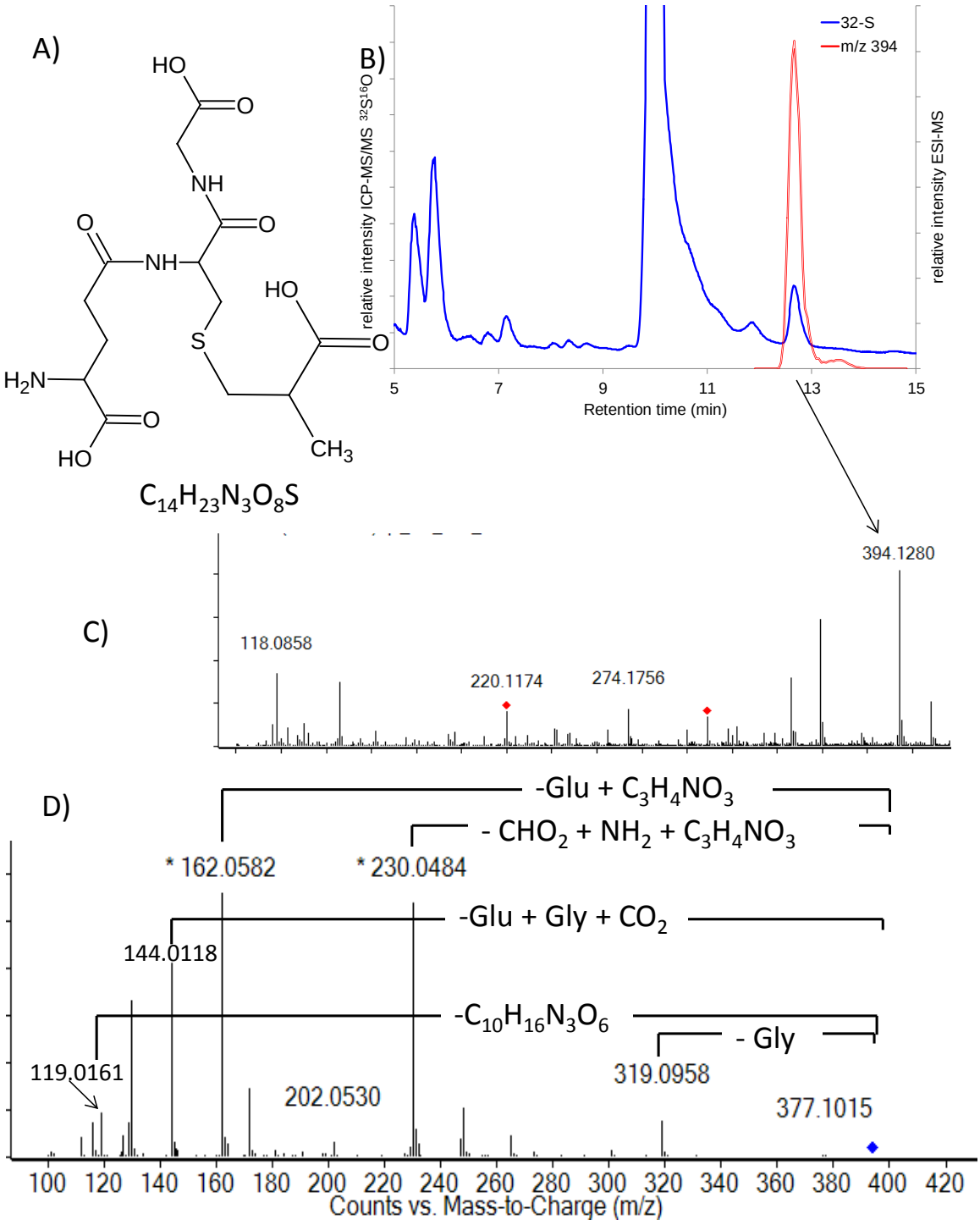


Fig. S27 **C393;** panel A) structure; panel B: EIC of m/z + ICP-MS/MS trace of root extract exposed to 2 mM S, panel C) q-TOF ESI-MS-spectra, panel D) ESI-MS/MS spectra with some annotated fragments, shown is the composition of the loss for the individual fragment calculated from the parent mass; for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

Table S13 Amount of sulphur associated with co-eluting compounds in mmol S kg^{-1} d.w. at the given retention times

	RT 2.2 min	RT 3.7 min	RT 11.1 min	RT 27.3 min
	cycloalliin methiin & methylcysteine	propiin, methinone & GSH	PC2 ox. & γ-glutamyl-S-2-propenylcysteine sulfoxide 1a	propyl-mercaptoglutathione & S-allyl/propenyl-containing PC-2
Root (0.1 mM S)	3.5 ± 3.6 (1.7 %)	1.2 ± 0.53[a]	0.19 ± 0.16	1.7 ± 1.1
Root (0.5 mM S)	6.0 ± 4.5 (2.2 %)	1.5 ± 0.64	0.09 ± 0.06	1.2 ± 1.1
Root (2 mM S)	10 ± 1.5 (2.2%)	3.2 ± 2.2[a]	0.32 ± 0.31	1.6 ± 0.8
Bulb (0.1 – 2 mM S)	0.88 ± 0.66 (0.67 %)	0.95 ± 0.43	0.03 ± 0.02	1.1 ± 0.6

3. Results from PCA and cluster analysis

3.1. Loading plot

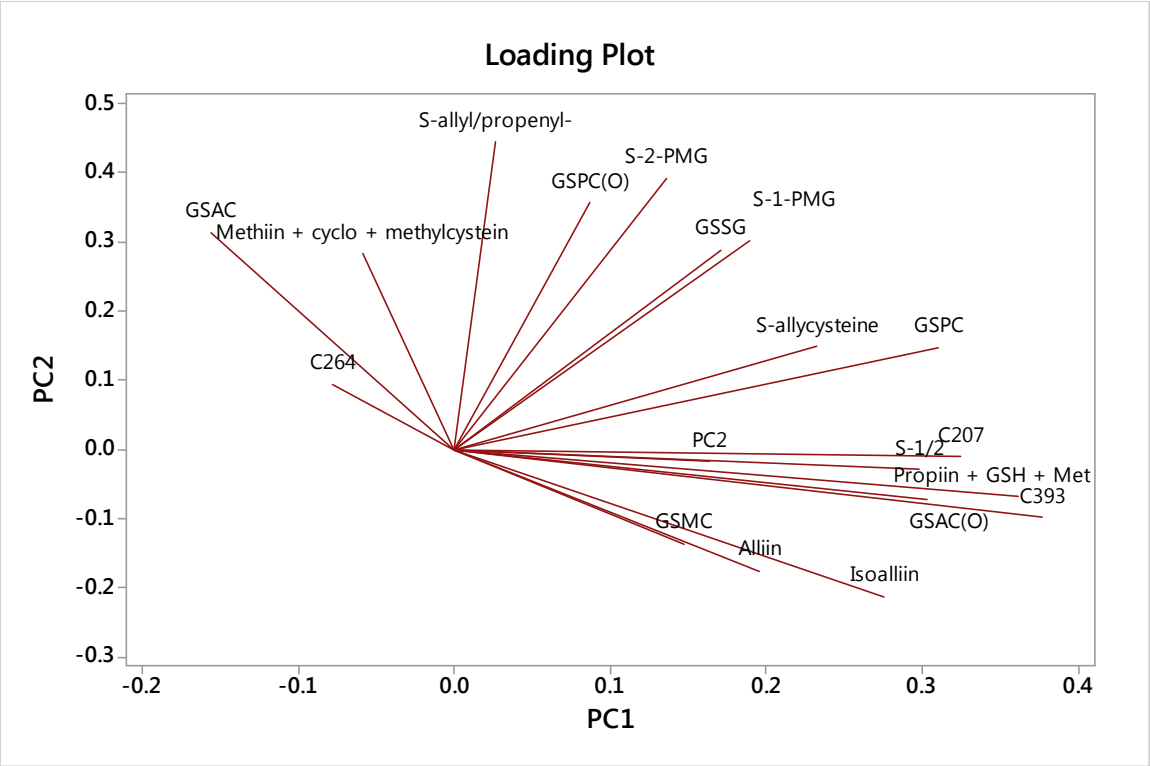


Fig. S28 Loading plot for the PCA in which all plants from the three different S-fertilization stage (0.1, 0.5 and 2 mM S) by utilisation of all identified low molecular weight S-containing metabolites.

3.2. Dendrogram

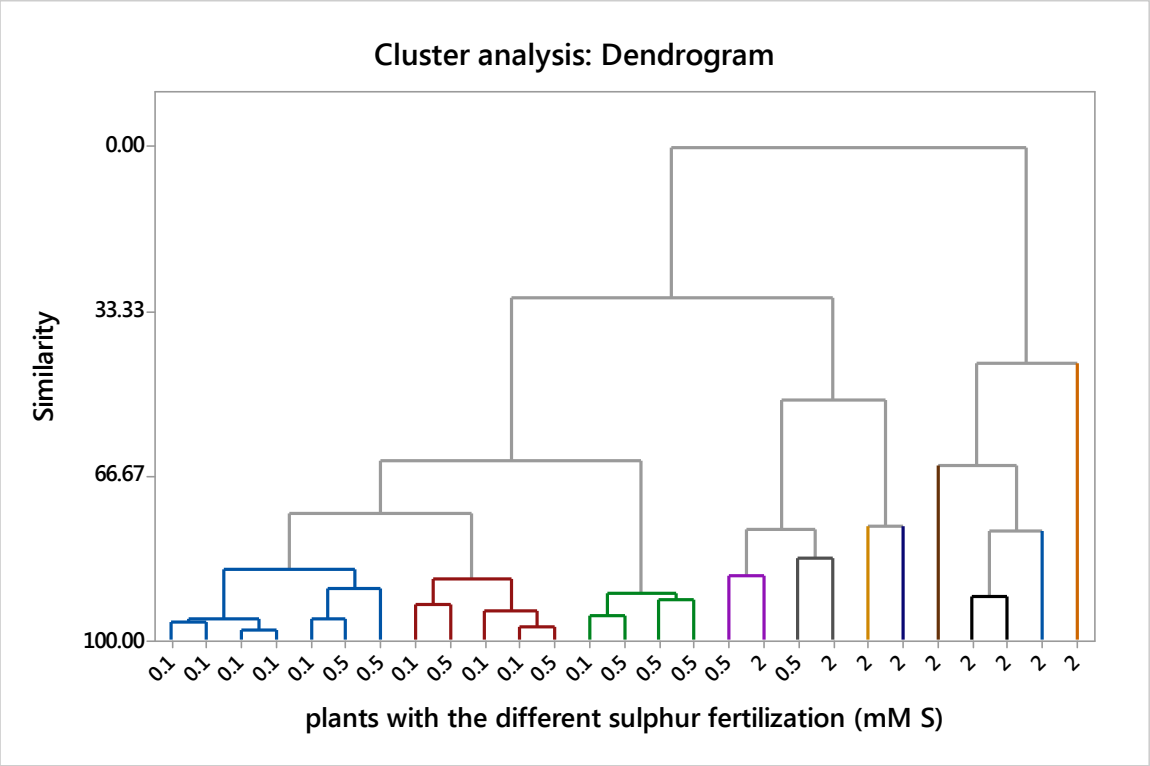


Fig. S29 Cluster analysis in which all plants from the three different S-fertilization stage (0.1, 0.5 and 2 mM S) by utilisation of all identified low molecular weight S-containing metabolites.

4. Example of HPLC-ICP-MS/MS traces for all plant parts fertilized with different amount of sulphur

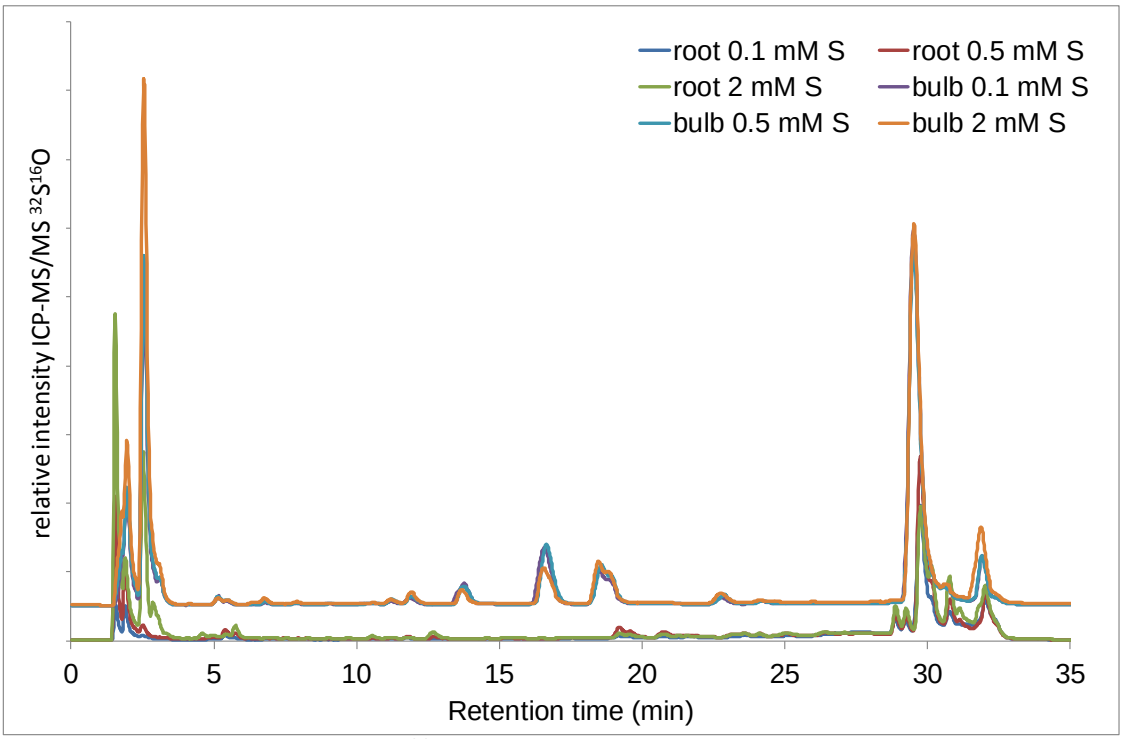


Fig. S30 comparison of ^{32}S-trace of root and bulb extracts (one each at different fertilizer concentrations); for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

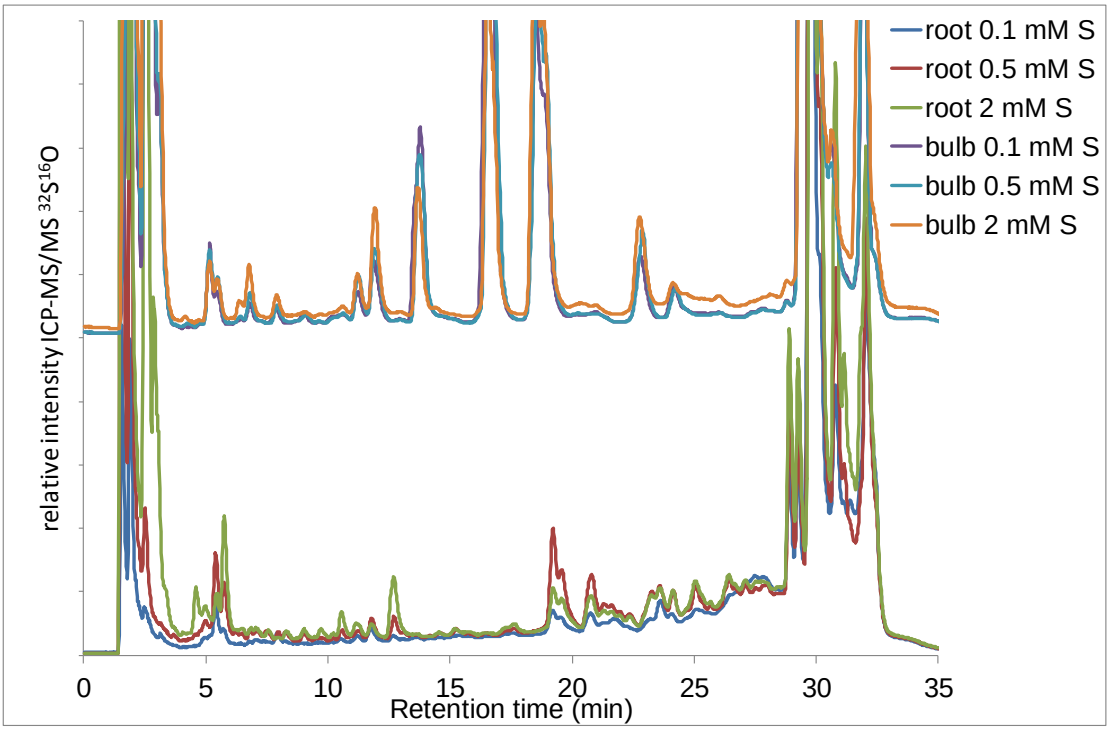


Fig. S31 comparison of ^{32}S-trace of root and bulb extracts (one each at different fertilizer concentrations); for HPLC + ICP-MS/MS and ESI-MS conditions see main text under instrumentation.

5. Example of peak area integration

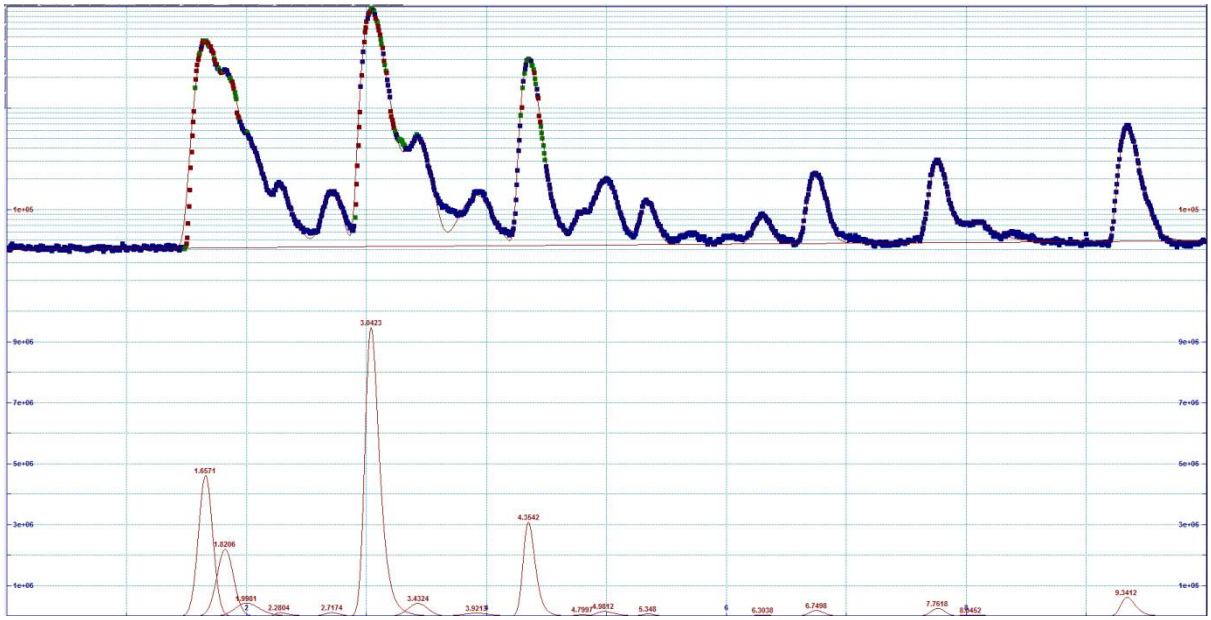


Fig. S28 Example of peak area integration using PeakFit. Top panel: measured signal (line) and calculated line (dots) using the described model for calculation in logarithmic y-scale, bottom panel: showing individual peaks (linear y-scale); R^2= 0.998 for peak area covered

1 Y. Yamazaki, T. Tokunaga, T. Okuno, Quantitative determination of eleven flavor precursors (S-alk(en)yl cysteine derivatives) in garlic with HPLC method, *Nippon Shokuhin Kagaku Kogaku Kaishi*, 2005, **52**, 160-166; in E. Block, Garlic and other Alliums – The Lore and the Science, Royal Society of Chemistry: Cambridge, U.K., 2010.

2 R. Nakabayashi, Y. Sawada, M. Aoyagi, Y. Yamada, M. Y. Hirai, T. Sakurai, T. Kamoi, D. D. Rowan, K. Saito, Chemical Assignment of Structural Isomers of Sulfur-Containing Metabolites in Garlic by Liquid Chromatography-Fourier Transform Ion Cyclotron Resonance-Mass Spectrometry, *J. Nutr*., 2016, **146**, 397S-402S.