Oxidative cascade reactions forming polyhydroxy theaflavins and theacitrins in the formation of black tea thearubigins: Evidence by tandem LC-MS

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Supplementary information

Table S1: Assignment of sharp well-resolved peaks in LC-MS chromatograms of thearubigins

Compound	Name	Experimental mass	Retention time	MS^2
56	Bis flavanol gallaate	761.3	11.8	591.2, 609.2
7, 33	Theacitrin gallate	759.3	32.9	607.3, 741.2
	Ellagoyl-galloyl glucose	633.2	14.7	301.1
7, 33	Theacitrin gallate	759.3	33.4	607.3, 741.2
1	EGCG	457.2	20.6	305.1, 331.1
34	Theacitrin di-gallate	911.2	22.7	893.2, 759.2
	EC-ECG	729.3	24.5	407.2, 441.2 ECG derivative
	Quercitin-glycoside derivative	593.2	26.2	413.2, 293.1
2	Epicatechin gallate	441.1	29.7	289.1
22	Flavonol diglycoside	609.3	30.3	301.1
	Unassigned	775.2	31.6	739.4, 543.3
55	Kaempferol rutinoside	593.2	35.0	285.1
	Unassigned	753.2	36.2	601.3, 409.2 some gallate
8	Theaflavin	563.2	46.1	462.9 and many more
	Unassigned	901.3	46.4, 49.2	775.3 some deoxy hexose derivative
9, 10	Theaflavin gallate	715.2	49.1, 50.6	563.2, 407.2
11	Theaflavin 3,3´ digallate	867.2	51.2	715.3, 527.2, 563.2

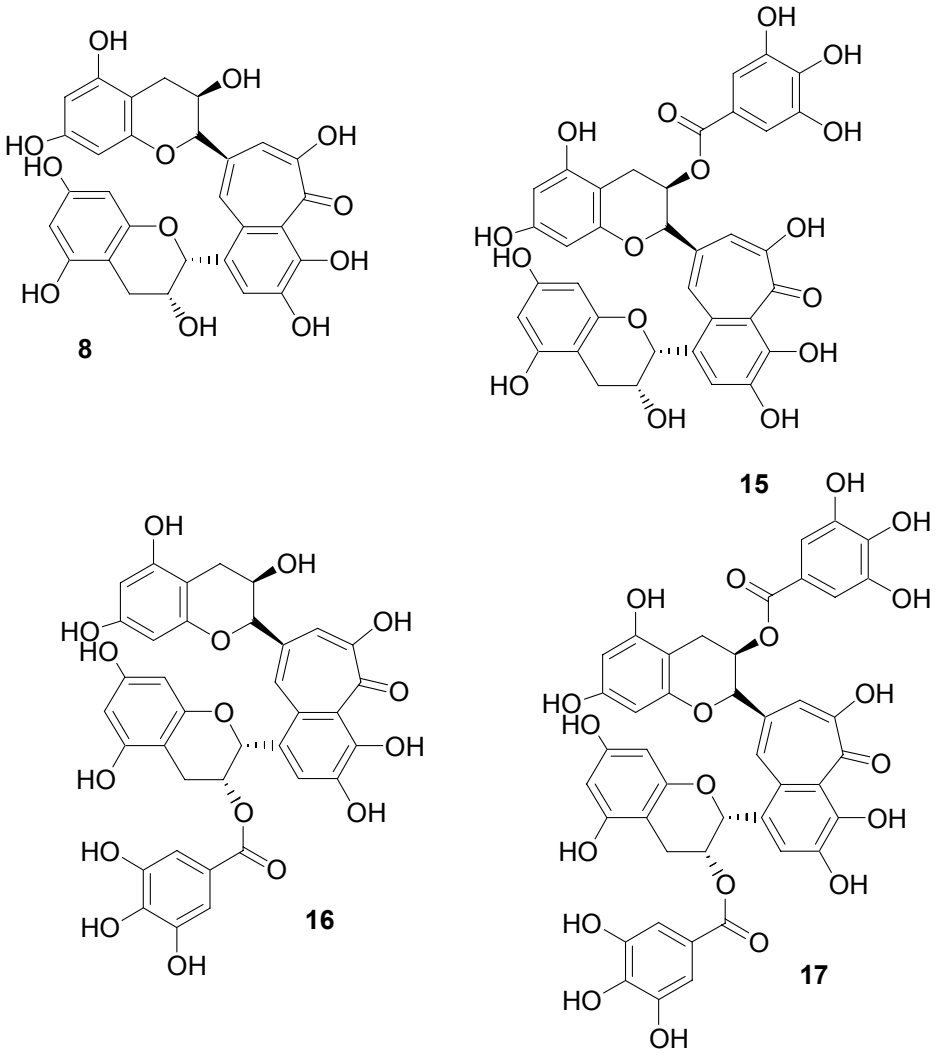
Table S2: Assignment of found masses to tea components described in the literature (see Ref. 15) by searching LC-MS data

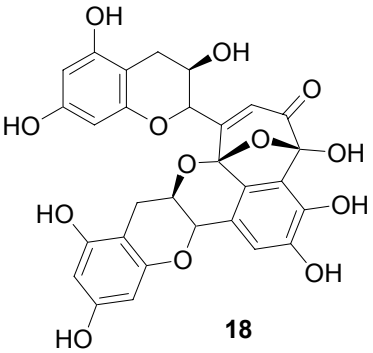
Compound	Trivial name	Molecular formula	Theoretical mass [a] [M–H]	Retention time [min] [b]	MS^2 first peak refers to base peak
1	(-)-Epigallocatechin-gallate	$C_{22}H_{18}O_{11}$	457.0764	20.6	305.1, 331.1
2	(-)-Epicatechin-gallate	$C_{22}H_{18}O_{10}$	441.0816	29.7	289.1
3	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	305.0656	Not found	
4	(-)-Epicatechin	$C_{15}H_{14}O_6$	289.0707	Not found	
5	(+)-Gallocatechin	$C_{15}H_{15}O_7$	305.0656	Not found	
6	(+)-Catechin	$C_{22}H_{18}O_6$	289.0707	Not found	
7	Theacitrin-3-gallate	$C_{37}H_{28}O_{18}$	759.1192	33.4	427.2, 520.8
8	Theaflavin	$C_{29}H_{24}O_{12}$	563.1184	46.1	462.9
12	Theasinensin	$C_{30}H_{26}O_{14}$	609.1239	31.5	439.2, 540.9, 287.1, 331.1
9	Theaflavin 3-gallate	$C_{36}H_{28}O_{16}$	715.1294	50.6	563.2, 407.2
10	Theaflavin-3`-gallate	$C_{36}H_{28}O_{16}$	715.1294	49.2	563.2
11	Theaflavin 3, 3`-digallate	$C_{43}H_{32}O_{20}$	867.1403	51.2	715.3, 563.3, 527.2
18	Dehydrotheaflavin	$C_{29}H_{22}O_{13}$	577.0977	20.4	Poor quality MS^2
19	Dehydrotheaflavin	$C_{29}H_{22}O_{13}$	577.0977		
20	Theanaphthoquinone	$C_{29}H_{24}O_{12}$	563.1184		See theaflavin (isomer of)
21	Dibenztropolone	$C_{50}H_{38}O_{21}$	973.1822	19.3	765.3, 935.2
22	Quercetin diglycoside	$C_{27}H_{30}O_{16}$	609.1450		Isomer of theasinensin **9**
23	Malvin [a]	$C_{29}H_{35}O_{17}$	655.1869	14.6	323.0
24	Quercetin	$C_{15}H_{10}O_7$	301.0343	28.1	284.1, 232.8, 185.0
25	Myricetin glycoside	$C_{21}H_{20}O_{13}$	479.0820	25.2, 26.1	316.1 two isomers found (in some teas 4)
26	Quinic acid ester	$C_{36}H_{32}O_{21}$	799.1352	Not found	
27	Chalcan–flavan dimer-gallate	$C_{43}H_{38}O_{24}$	937.1667	11.5	Poor quality MS^2
28	Chalcan–flavan dimer	$C_{36}H_{32}O_{20}$	783.1403	Not found	
29	Oolongtheanin gallate	$C_{36}H_{28}O_{17}$	731.1243	30.2	Poor quality MS^2
30	Oolongtheanin	$C_{29}H_{24}O_{13}$	579.1133	Not found	

31	Stereoisomer of **30**	$C_{29}H_{24}O_{13}$	579.1133	Not found	
32	Theacitrin	$C_{30}H_{24}O_{14}$	607.1082	Not found	
33	Theacitrin-gallate[a)]	$C_{37}H_{28}O_{18}$	759.1192	32.8	427.2, 520.8
34	Theacitrin-digallate	$C_{44}H_{32}O_{22}$	911.1302	21.8	591.3, 743.2, 759.2, 775.2
36	Theasinensin-digallate	$C_{44}H_{34}O_{22}$	913.1458	21.8	761.2, 743.2, 591.3
37	Theasinensin-digallate	$C_{44}H_{34}O_{22}$	913.1458	Not found	
38	Theasinensin-trigallate	$C_{66}H_{50}O_{33}$	1369.211	Not found	
39	Theaflavate	$C_{36}H_{28}O_{15}$	699.1345	Not found	
40	Theaflavate	$C_{36}H_{28}O_{15}$	699.1345	Not found	
41	Theaflavin-gallate	$C_{27}H_{20}O_{12}$	535.0871	Not found	
42	Theaflavin	$C_{29}H_{24}O_{12}$	563.1184 (see **12**)	Not found	
43	Theaflavic acid	$C_{21}H_{16}O_{10}$	427.0660	11.9	409.1, 360.9, 301.1, 383.1, 261.0, 193.0
44	Theaflavic acid	$C_{21}H_{16}O_{10}$	427.0660	Not found	
45	Theaflavin	$C_{29}H_{24}O_{12}$	563.1184 (see **12**)	Not found	
46	Theanaphthoquinone	$C_{28}H_{22}O_{11}$	533.1078	Not found	
47	Diastereomer of **46**	$C_{28}H_{22}O_{11}$	533.1078	Not found	
48	No trivial name	$C_{45}H_{34}O_{21}$	909.1509	22.6, 29.5, 11.5, 12.7	Poor quality MS^2
49	No trivial name	$C_{44}H_{34}O_{23}$	929.1407	30.2, 32.2	Poor quality MS^2
50	No trivial name	$C_{45}H_{34}O_{22}$	925.1458	51.2	Poor quality MS^2
51	No trivial name	$C_{30}H_{24}O_{14}$	607.1082	Not found	
52	No trivial name	$C_{58}H_{48}O_{25}$	1143.24.01	Not found	
53	No trivial name	$C_{57}H_{46}O_{23}$	1097.2346	Not found	
54	Quercetin glycoside derivative	$C_{27}H_{30}O_{15}$	593.110	38.6	524.9, 442.2 (possibly a gallate)
55	Ellagoyl-galloyl-glucose	$C_{27}H_{22}O_{18}$	633.0731	14.7	301.0, 271.1, 331.1

a) Masses quoted here are two four digital figures since they originate from TOF data (compare to five figures for ESI-FT ICR data in previous apper); b)The description "not found" relates to MS peaks with an absolute intensity below 1000 counts in EICs created for this particular mass.

Figure S1: Structures of previously reported polyphenols from black tea in tables 1 and 2 (see reference 12)





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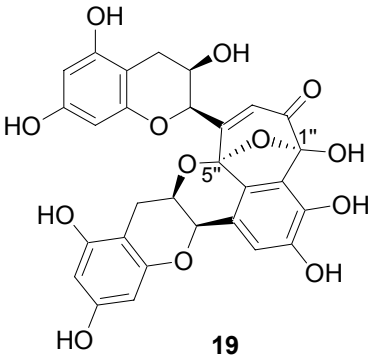

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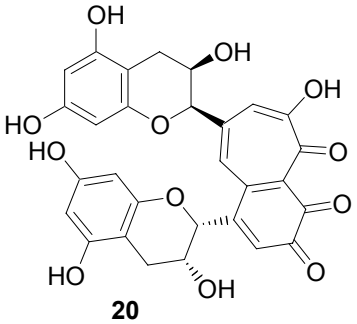

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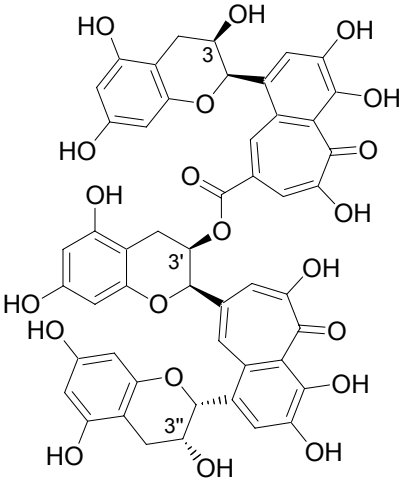

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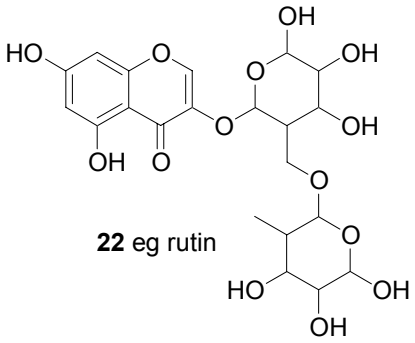
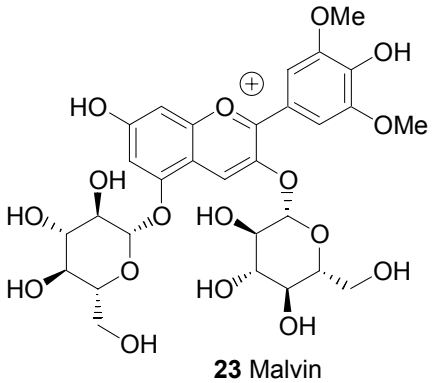


22 eg rutin



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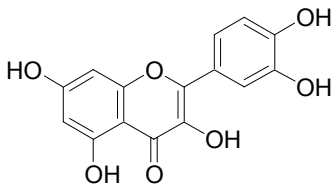

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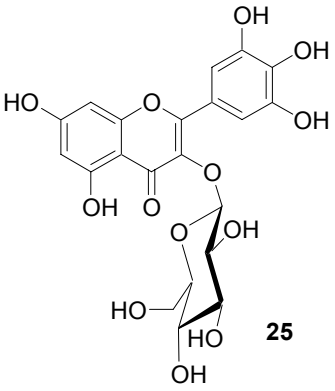

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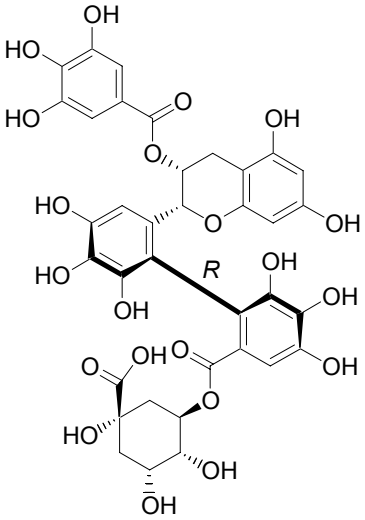

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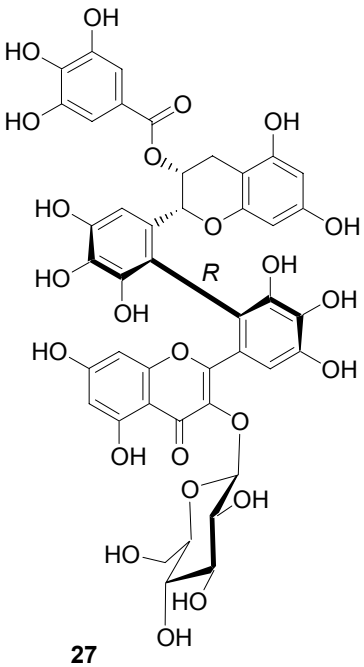

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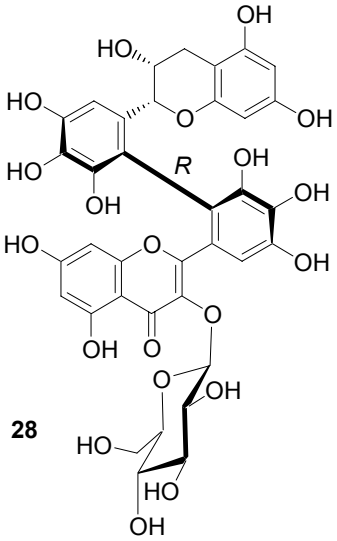

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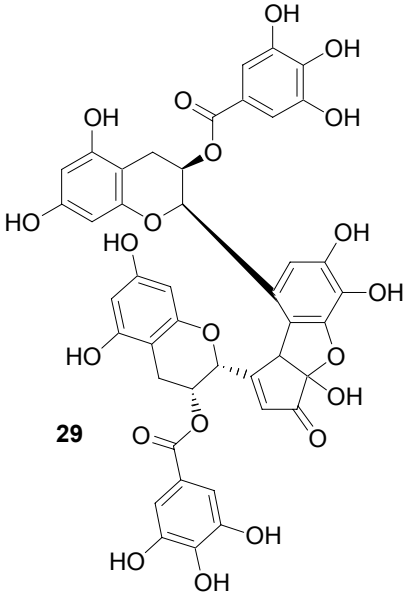

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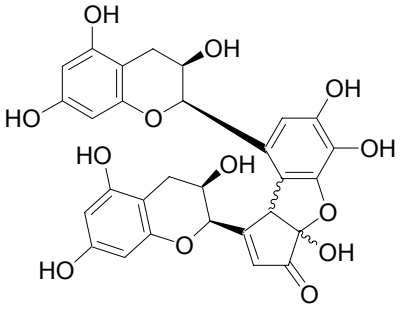

30 and **31**

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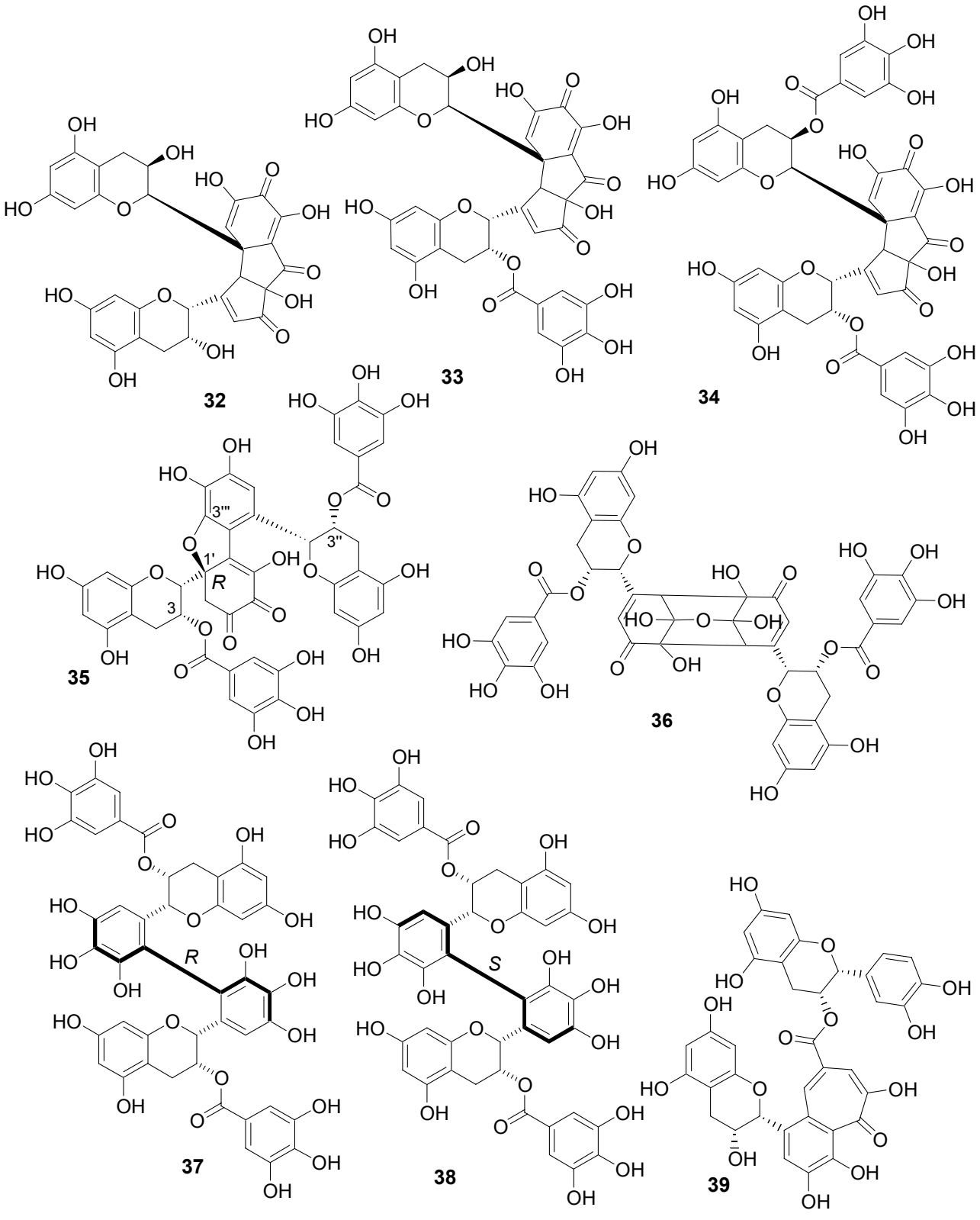
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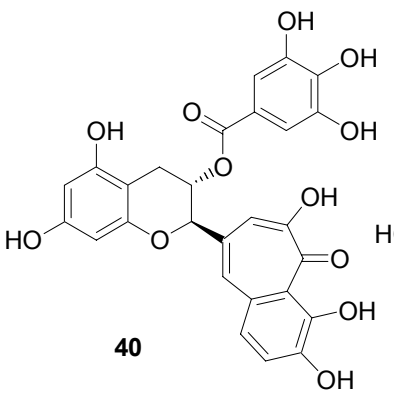

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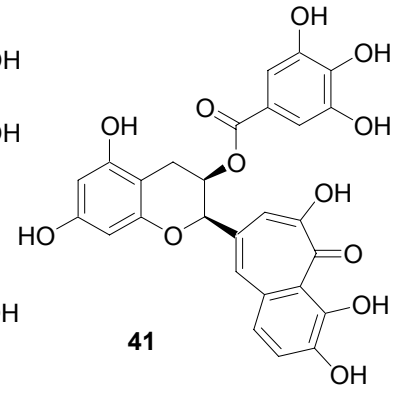

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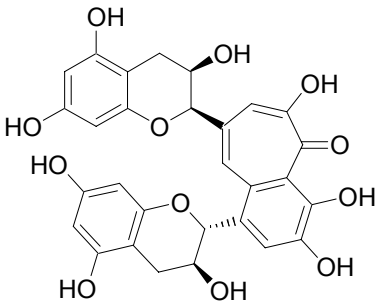

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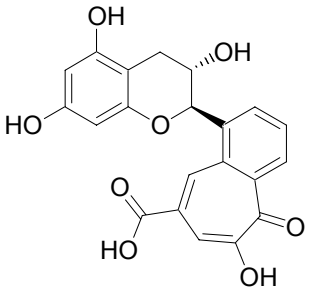

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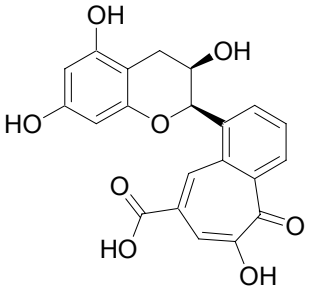

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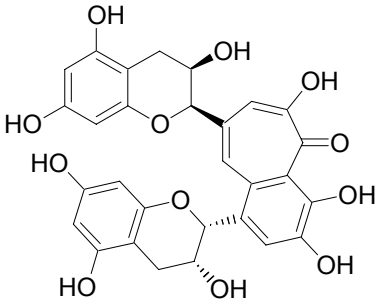

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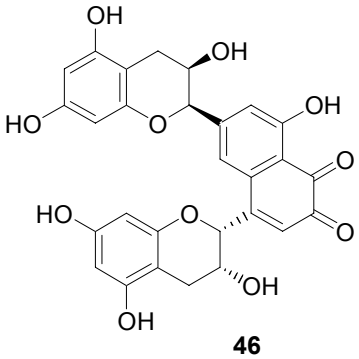

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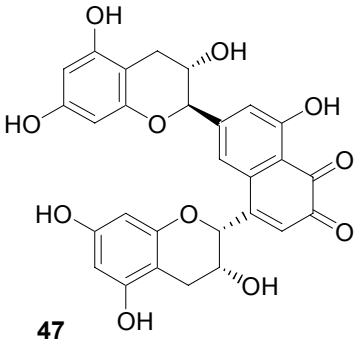

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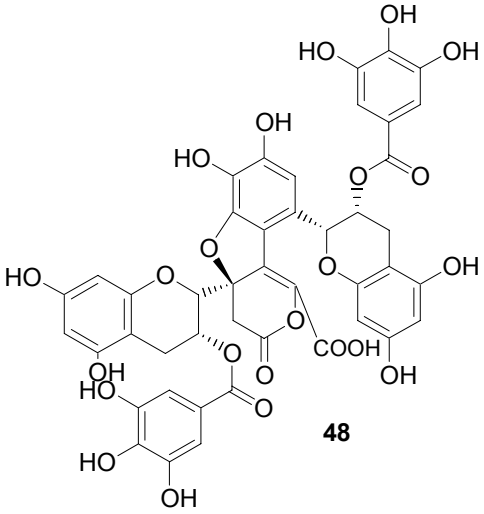

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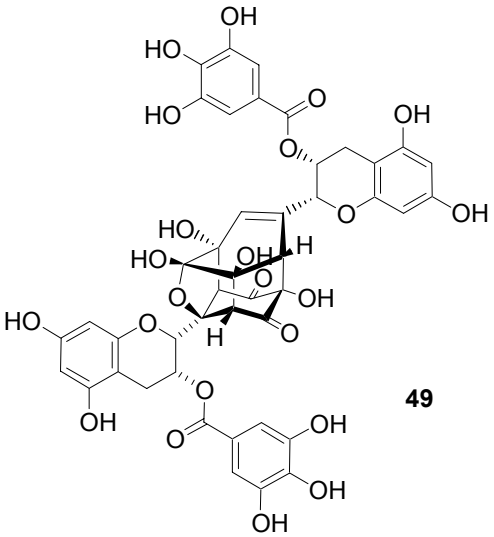

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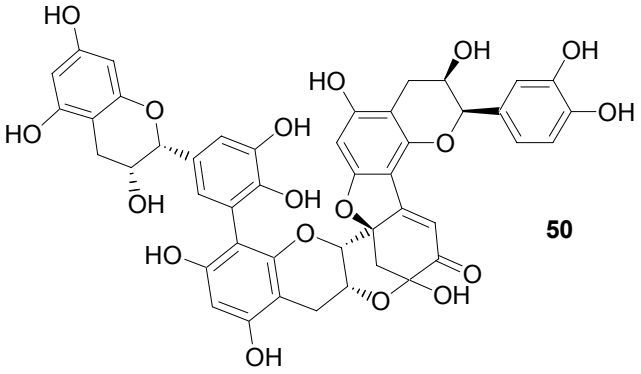

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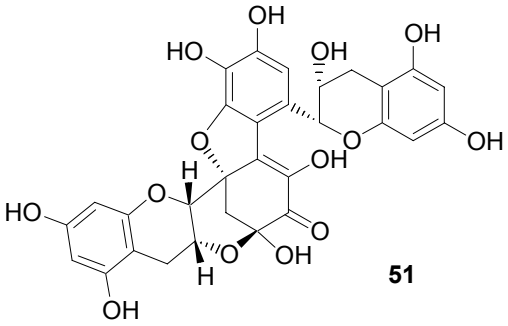

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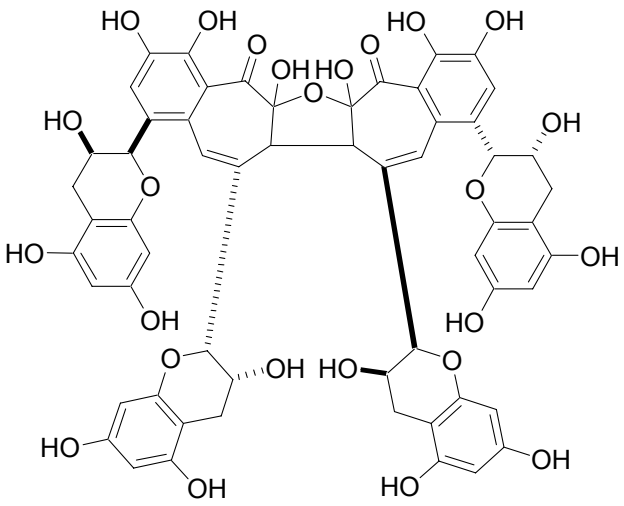

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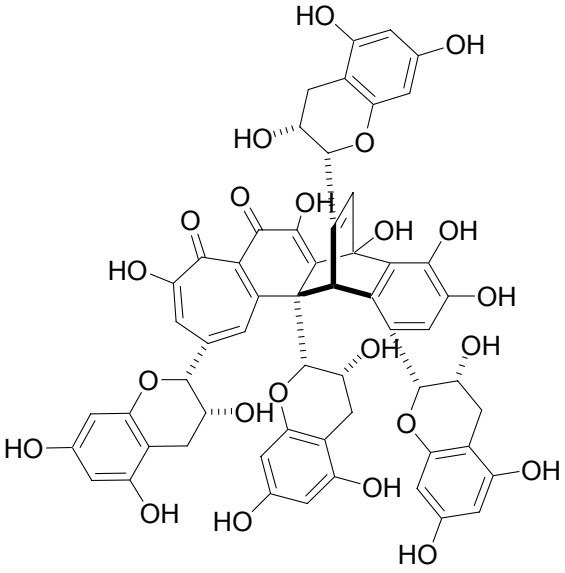

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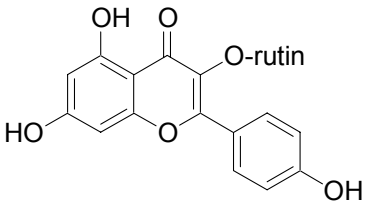
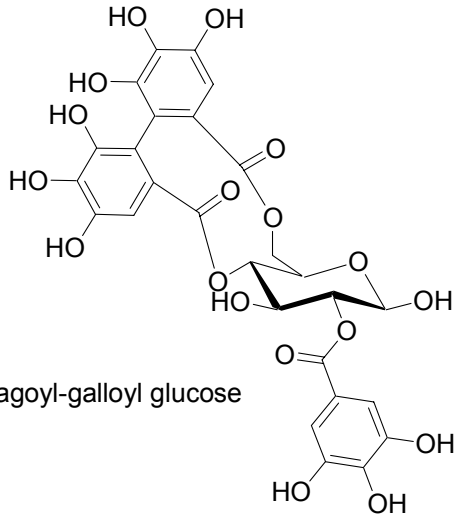


54
Kaempferol rutinoside



55
ellagoyl-galloyl glucose

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Figure S2: a) Total ion chromatogram in negative ion mode of sample TR XII, b) EIC of ion at *m/z* 609.2, c) EIC of ion at *m/z* 563.2, d) EIC of ion at *m/z* 441.1, e) EIC of ion at *m/z* 973.2.

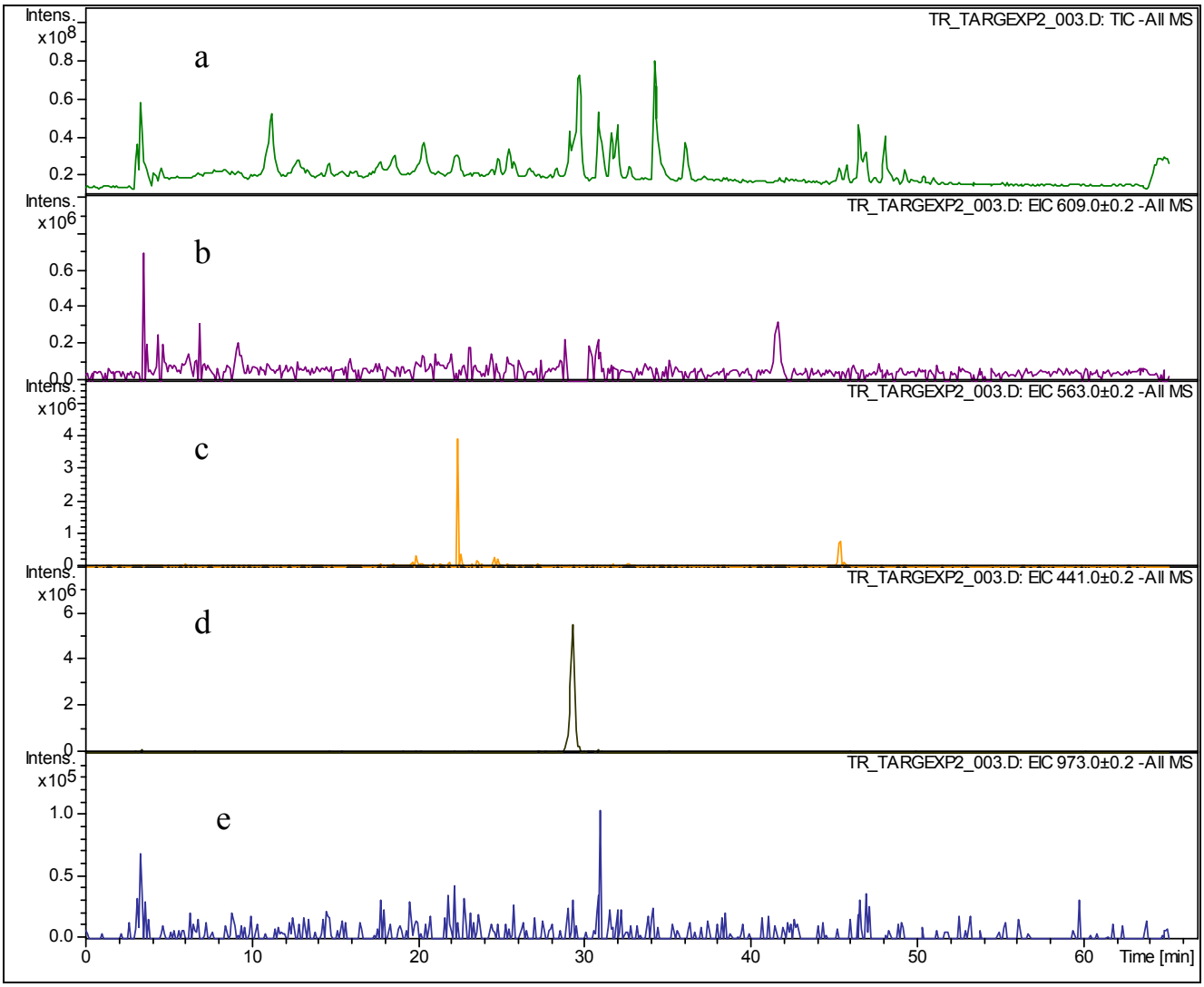

Figure S3: a) TIC of all MS^n in negative ion mode of sample TR VI, b) constant neutral loss chromatogram of neutral loss at *m/z* 18 (water), c) constant neutral loss chromatogram of neutral loss at *m/z* 15 (methyl), d) constant neutral loss chromatogram of neutral loss at *m/z* 44 (CO_2).

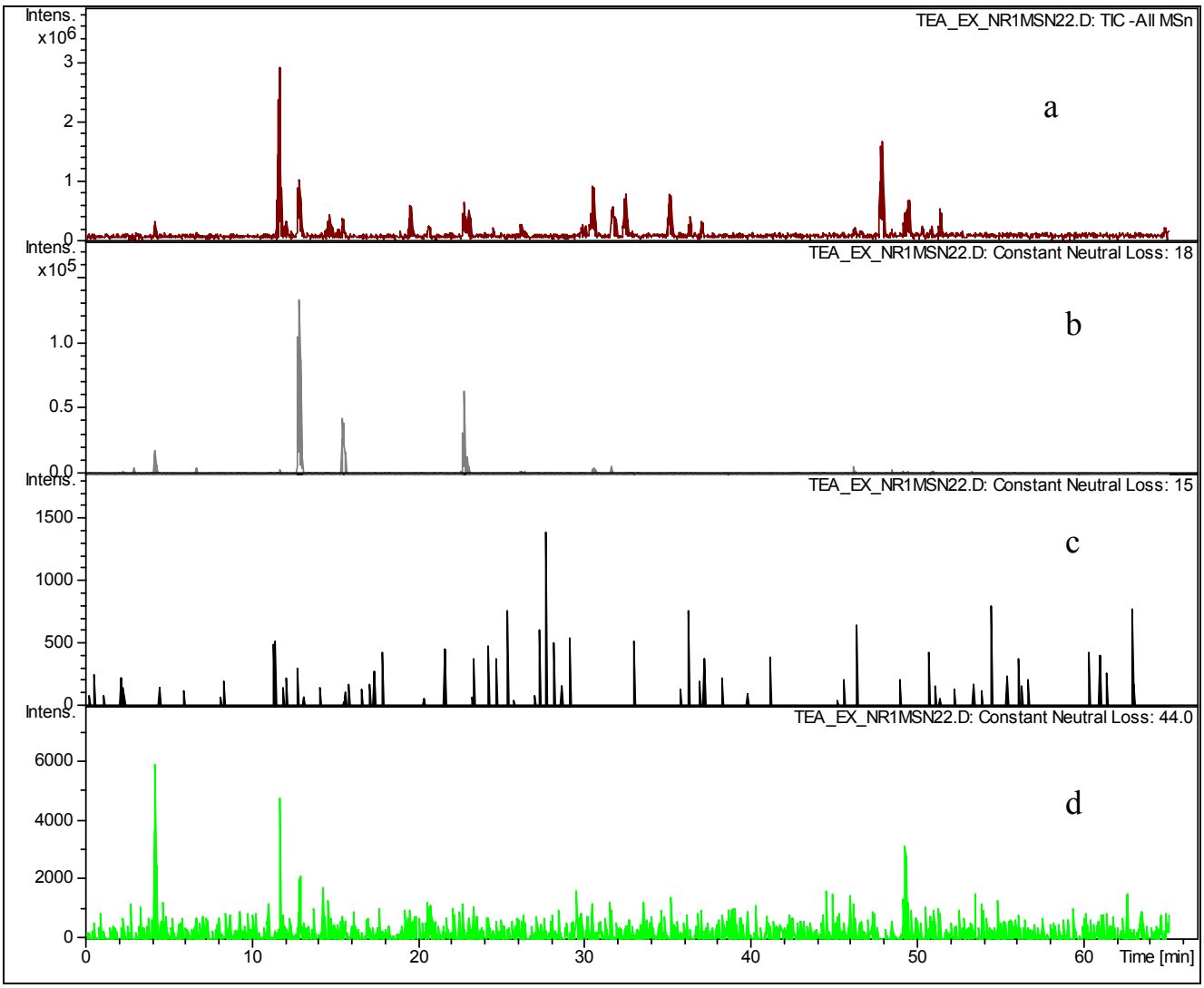

Figure S4: EIC chromatograms extracted from all MS^n data for RDA fragment ions in homologous series **A**: a) EIC of fragment ion at *m/z* 425.1, b) EIC of fragment ion at *m/z* 441.1, c) EIC of fragment ion at *m/z* 457.1, d) EIC of fragment ion at *m/z* 473.1, e) EIC of fragment ion at *m/z* 489.1.

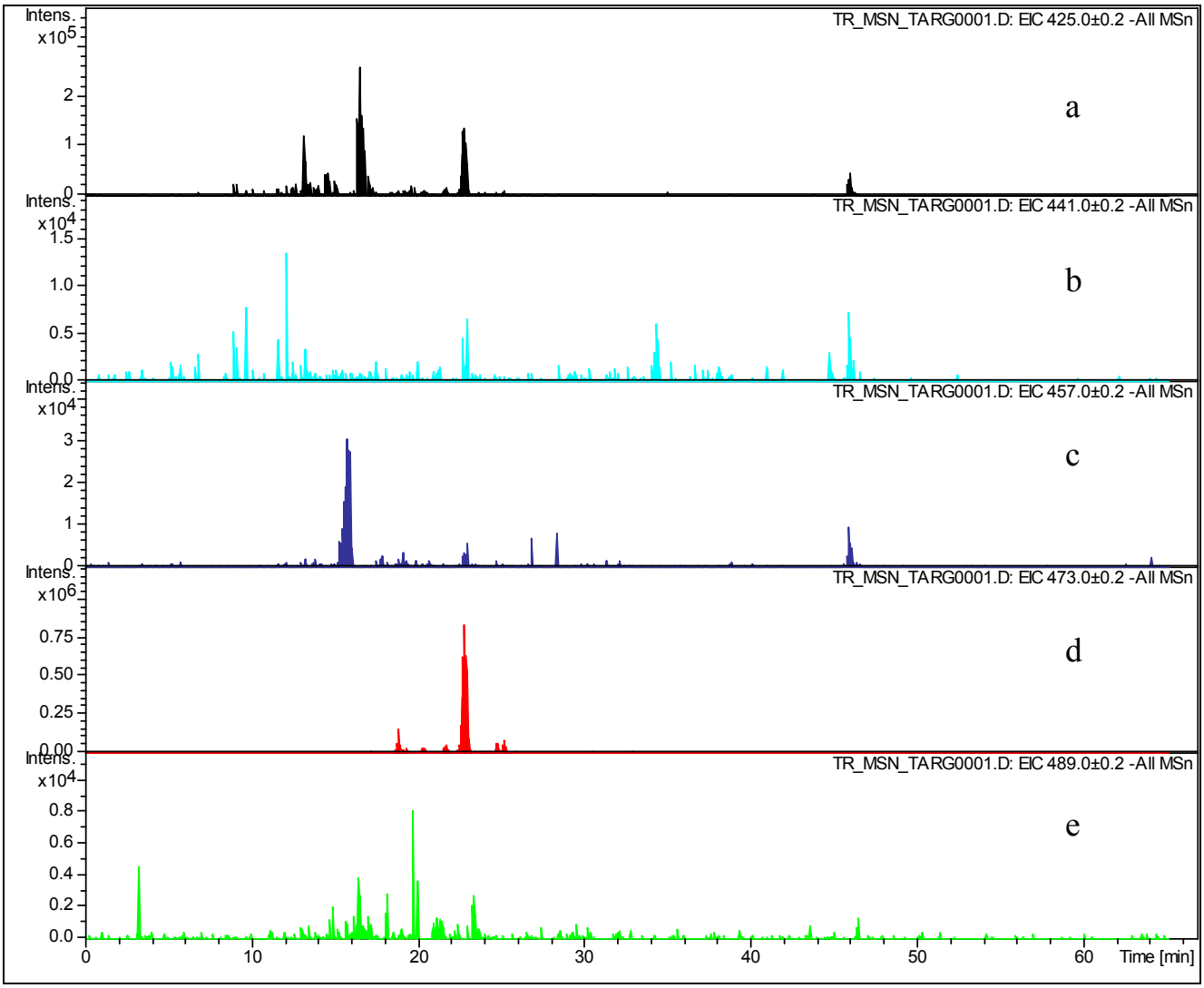

Figure S5: EIC chromatograms extracted from all MS^n data for degallated fragment ions in homologous series **C**: a) EIC of fragment ion at *m/z* 715.2, b) EIC of fragment ion at *m/z* 731.2, c) EIC of fragment ion at *m/z* 747.2, d) EIC of fragment ion at *m/z* 763.2, e) EIC of fragment ion at *m/z* 779.2.

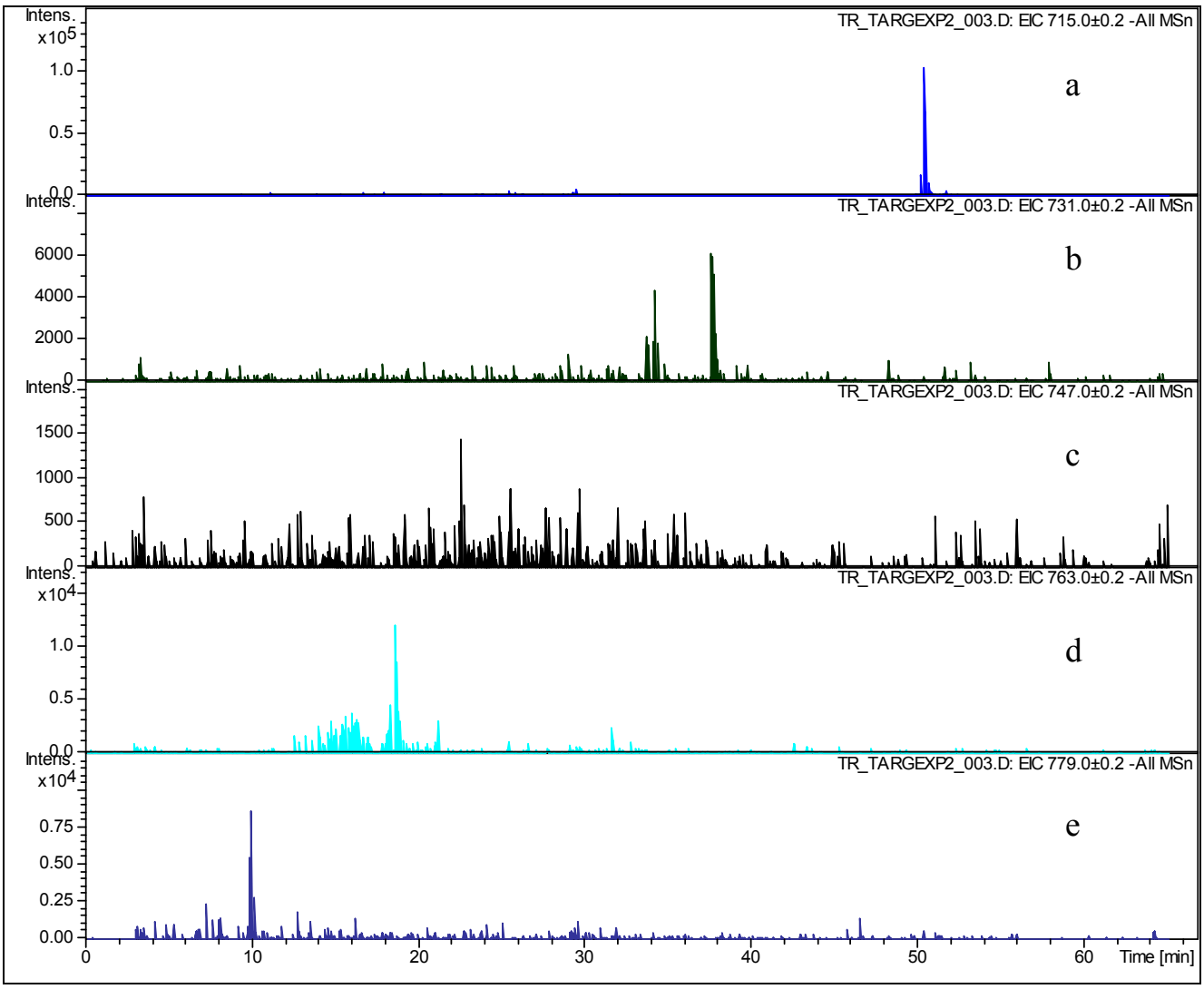

Figure S6: Selected tandem MS^2 spectra from LC–MS runs in negative ion mode for three regioisomers of **11+O1** in series **C** showing fragment ion at *m/z* 731.2 originating from parent ion **11+O1** at *m/z* 883.2.

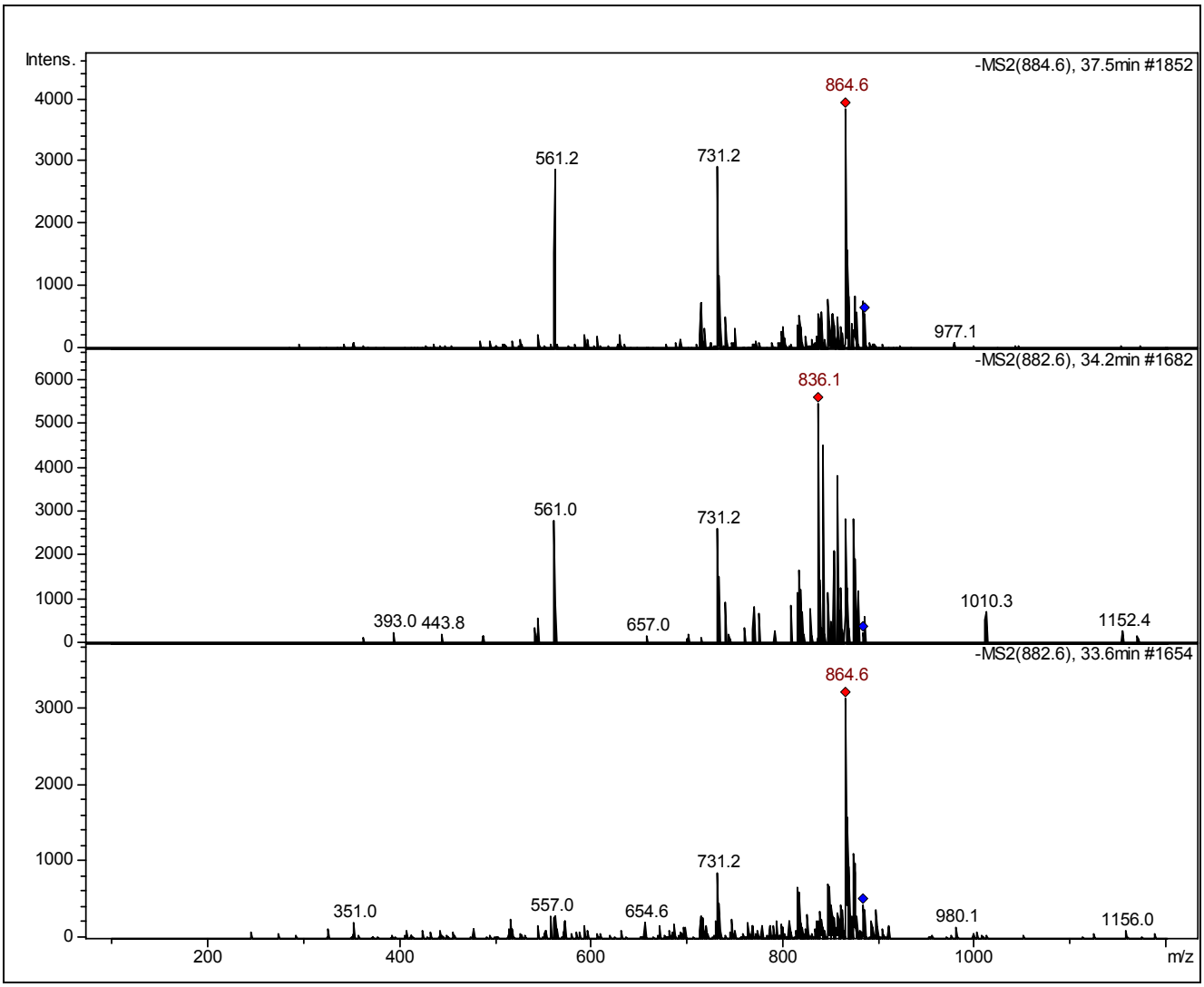

Figure S6 continued: Selected tandem MS spectra from LC–MS runs in negative ion mode for three isomers in series **C** showing fragment ion at *m/z* 763.2. originating from parent ions **11+O3** at *m/z* 915.2.

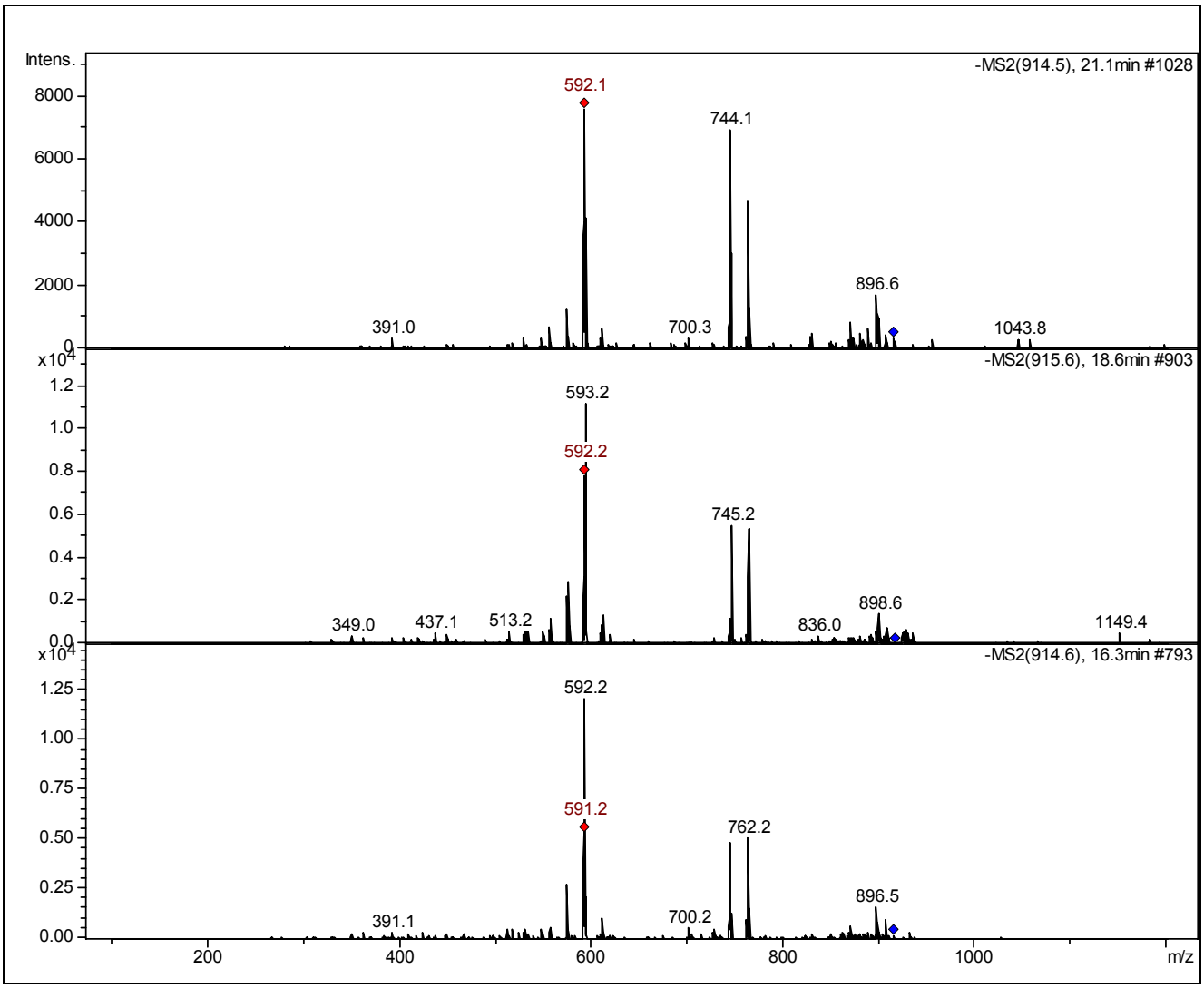

Figure S7: EIC chromatograms for parent ions in homologous series **D**: a) EIC of ion **7** at *m/z* 759.2, EIC of ions **7+O1** at *m/z* 775.2, b) EIC of ions **7+O2** at *m/z* 791.2, c) EIC of ions **7+O3** at *m/z* 807.2, d) EIC of ions **7+O4** at *m/z* 823.2.

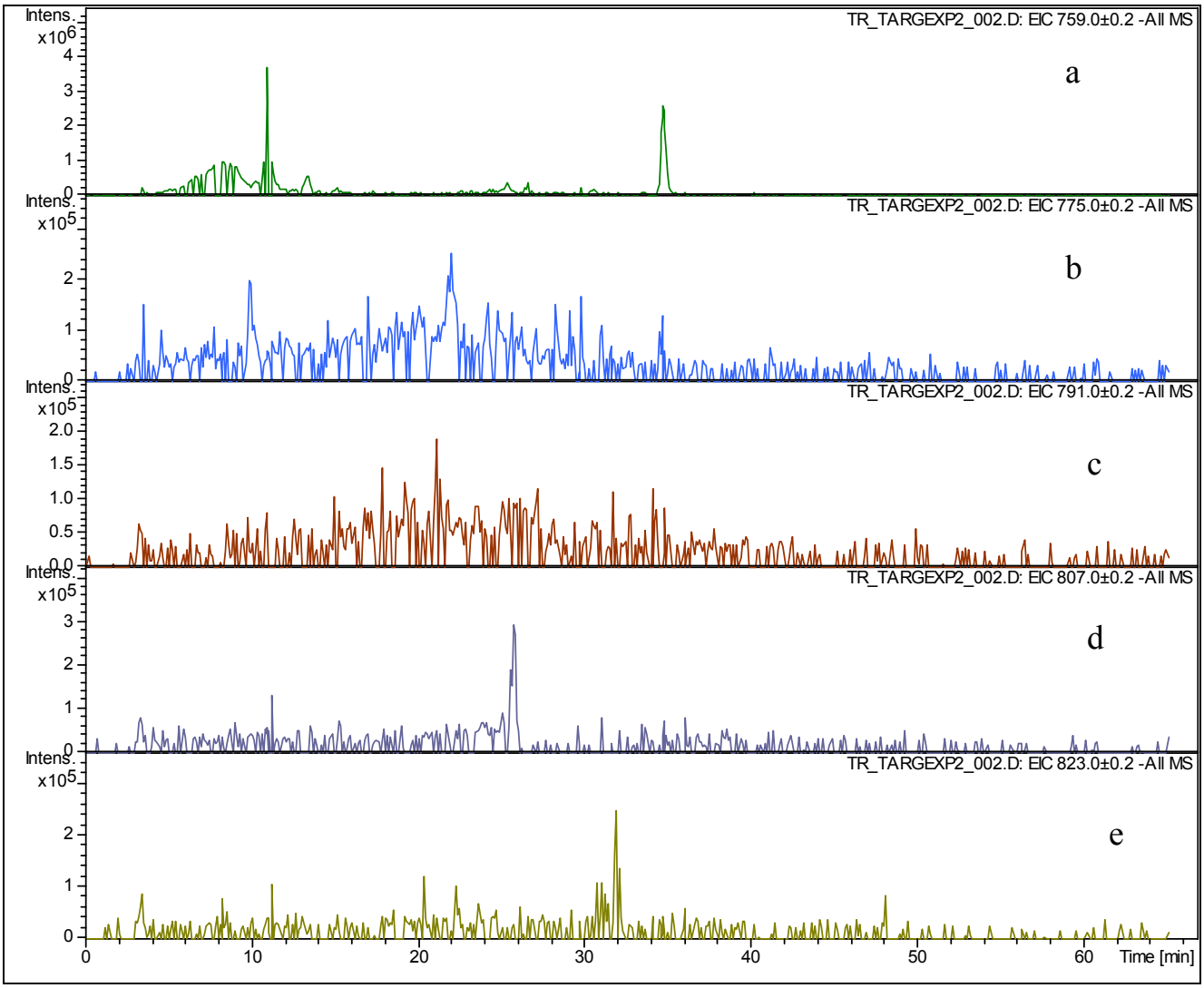

Figure S8: EIC chromatograms extracted from all MS^n data for degallated fragment ions in homologous series **D**: a) EIC of fragment ion at *m/z* 607.2, b) EIC of fragment ion at *m/z* 623.2, c) EIC of fragment ion at *m/z* 639.2, d)EIC of fragment ion at *m/z* 655.2, e)EIC of fragment ion at *m/z* 671.2.

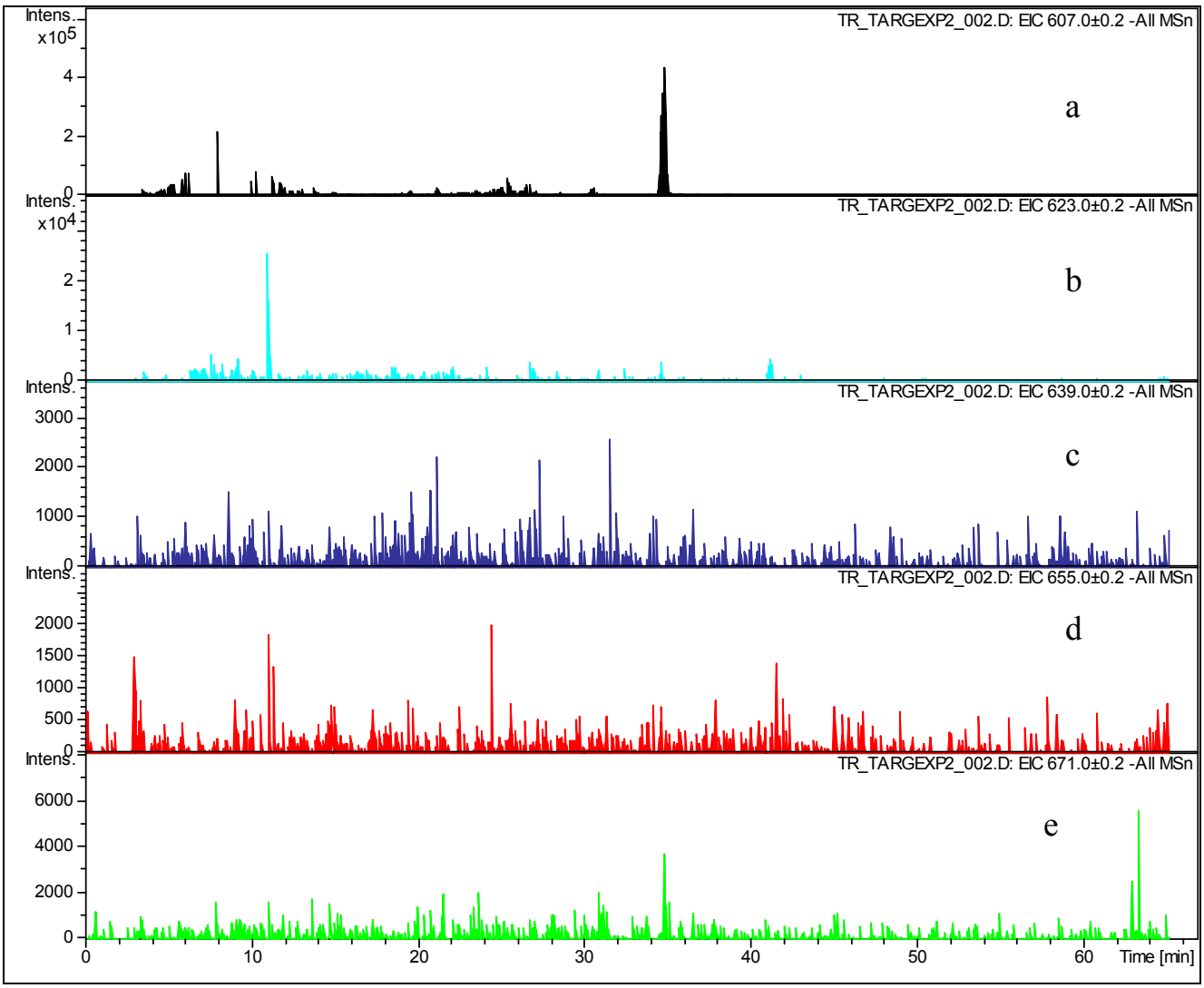

Figure S9: Selected MS^n spectra for compounds from series **D**: a) MS^2 spectrum of **7**, b) MS^3 spectrum of **7**, c) MS^2 spectrum of **7**+**O1**.

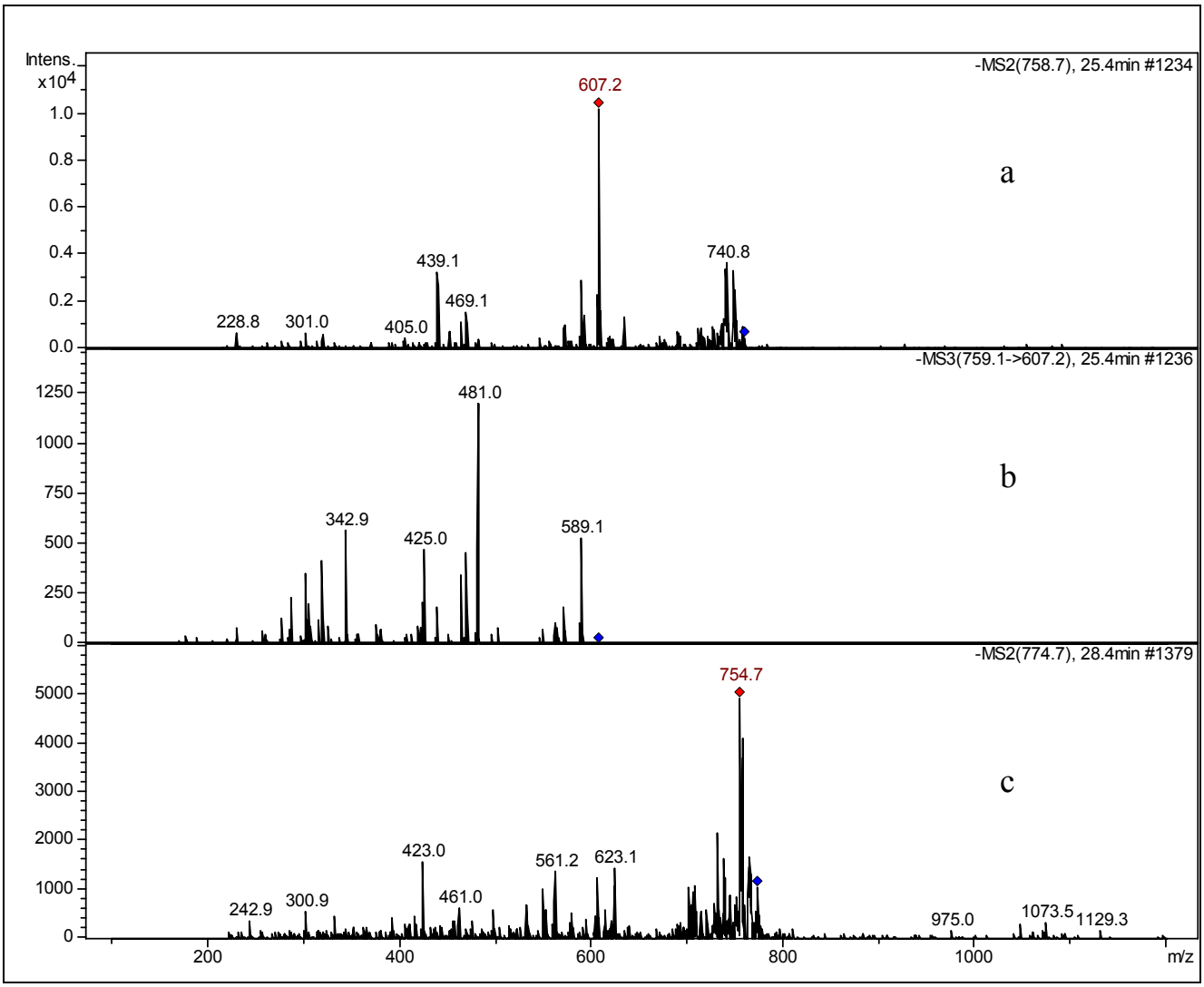

Figure S10: a) TIC of all MS^n in negative ion mode of sample TR VI treated with $KMnO_4$, b) constant neutral loss chromatogram of neutral loss at *m/z* 15 (methyl), c) constant neutral loss of 18 water, d) constant neutral loss chromatogram of neutral loss at *m/z* 44 (CO_2), d) constant neutral loss of 28 (CO).