Supporting Information for:

Comprehensive multi-technique approach reveals the high diversity of microcystins in field collections and an associated isolate of *Microcystis aeruginosa* from a Turkish lake

Toxicon

Mete Yilmaz[a], Amanda J. Foss[b], Christopher O. Miles[c], Mihriban Özen[a,d], Nilsun Demir[e], Muharrem Balcı[a,f], Daniel G. Beach[c]
[a]Bursa Technical University, Department of Bioengineering, 16310, Bursa, Turkey
[b]GreenWater Laboratories/CyanoLab, 205 Zeagler Drive, Palatka, FL, 32177, USA
[c]Measurement Science and Standards, National Research Council Canada, Halifax, NS B3H 3Z1, Canada
[d]Uludağ University, Department of Biology, 16059, Bursa, Turkey
[e]Ankara University, Department of Fisheries and Aquaculture Engineering, 06110, Ankara, Turkey
[f]Istanbul University, Department of Biology, 34134, İstanbul, Turkey
*Corresponding author: mete.yilmaz@btu.edu.tr, Tel: +90 224 300 3416

Tables

Figures

Table S1 Multiple reaction monitoring (MRM) transitions for targeted analysis of MCs (14 variants) and NOD-R used in LC-MS/MS (method A)

#	Analyte	Segment Time (min)	Transition (*m*/*z*)	MDL (μg g^{-1}) Stations	MDL (μg g^{-1}) Culture	CE%
7	[D-Asp^3]MC-RR	2.0-6.5	513.0→291.3, 426.3, 445.9, 498.9, 503.9	0.10	0.3	24
9	MC-RR	2.0-6.5	520.0→298.4, 440.4, 452.9, 455.4, 503.9, 511.0	0.05	0.1	20
	NOD-R	6.5-8.5	825.5→599.5, 674.5, 776.5, 781.5	0.05	0.1	28
22	MC-YR	8.5-15.5	1045.5→599.5, 710.4, 1027.6	0.05	0.1	20
23	MC-HtyR	8.5-15.5	1059.5→ 599.5, 584.5, 567.4, 484.4	0.05	0.1	16
26	MC-LR	8.5-15.5	995.5→553.4, 599.5, 866.6, 967.6, 977.6	0.05	0.1	18
27	[D-Asp^3]MC-LR	8.5-15.5	981.5→539.4, 599.5, 953.6, 963.6	0.04	0.1	14
28	[Dha^7]MC-LR	8.5-15.5	981.5→539.4, 599.5, 953.6, 963.6	0.04	0.1	14
29	MC-HilR	15.5-17.2	1009.5→484.4 , 567.4, 599.4	0.07	0.2	20
31	MC-WR	17.2-19.0	1068.6→599.5, 626.4, 939.6, 1040.6, 1050.6	0.10	0.3	21
	[D-Leu^1]MC-LR	17.2-19.0	1037.6→599.5, 1019.6, 612.5	0.05	0.1	25
33	MC-LA*	19.0-21.5	908.5→780.0, 797.0, 878.0, 891.0	0.07	0.2	20
34	MC-LY*	21.5-22.4	1000.5 →872.0, 889.0 ,969.7 ,982.7	0.10	0.3	20
35	MC-LW*	22.4-25.0	1023.5→1005.6	0.11	0.3	20
36	MC-LF*	22.4-25.0	984.5→966.6	0.05	0.1	20
	d_7-MC-LR	8.5-15.5	1002.5→599.5	NA	NA	35

*negative ionization used

MDL = Method Detection Limit

refers to compound numbering of MCs detected in Figure 6.

Table S2 Cyanobacterial species observed and their biovolumes at each sampling site in Lake Uluabat.

23 June 2015						
Cyanobacterial species	**Site 1 biovolume ($mm^3 L^{-1}$)**	**Site 2 biovolume ($mm^3 L^{-1}$)**	**Site 3 biovolume ($mm^3 L^{-1}$)**	**Site 4 biovolume ($mm^3 L^{-1}$)**	**Mean biovolume ($mm^3 L^{-1}$)**	**Mean bivolume contribution (%)**
Aphanocapsa delicatissima	0.179	0.721	1.54	1.76	1.05	41
Aphanocapsa holsatica	0.128	0.776	0.370	0.379	0.413	16
Cuspidothrix issatschenkoi	0.019	0.133	0.127	0.0520	0.0830	3
Dolichospermum planctonicum	0.00200	0.111	0.0530	0.216	0.0960	4
Dolichospermum spiroides	0.00900	0.0660	0.190	0.712	0.244	9
Limnococcus limneticus	0	0	0	0	0	0
Limnothrix redekei	0.00400	0.0130	0.00600	0.00900	0.00800	0
Merismopedia sp.	0.00100	0.0110	0.00600	0.00300	0.00500	0
Merismopedia tenuissima	0	0.00100	0	0	0	0
Microsystis aeruginosa	0.0180	0.554	0.264	0.0220	0.215	8
Microcystis wesenbergii	0	0	0	0	0	0
Oscillatoria tenuis	0	0	0	0	0	0
Planktolyngbya limnetica	0.00100	0.0290	0.00900	0.0170	0.0140	1
Planktothrix isothrix	0.0470	0.141	0.741	0.759	0.422	16
Pseudoanabaena catenata	0.00400	0.00600	0.0240	0.0910	0.0310	1
Total	0.412	2.56	3.33	4.02	2.58	100

10 July 2015						
Cyanobacterial species	**Site 1 biovolume ($mm^3 L^{-1}$)**	**Site 2 biovolume ($mm^3 L^{-1}$)**	**Site 3 biovolume ($mm^3 L^{-1}$)**	**Site 4 biovolume ($mm^3 L^{-1}$)**	**Mean biovolume ($mm^3 L^{-1}$)**	**Mean bivolume contribution (%)**
Aphanocapsa delicatissima	0.0710	0.546	0.286	0.240	0.286	4
Aphanocapsa holsatica	0	0	0	0	0	0
Cuspidothrix issatschenkoi	0.0160	0.0480	0.127	0.0800	0.0680	1
Dolichospermum planctonicum	0.0330	0.101	0.0530	0.0550	0.0610	1
Dolichospermum spiroides	9.09	4.77	6.32	6.50	6.67	92
Limnococcus limneticus	0	0	0	0	0	0
Limnothrix redekei	0	0	0	0	0	0
Merismopedia sp.	0.00700	0.0210	0.00600	0.00800	0.0110	0
Merismopedia tenuissima	0	0.00100	0	0	0	0
Microsystis aeruginosa	0	0	0	0	0	0
Microcystis wesenbergii	0	0	0	0	0	0
Oscillatoria tenuis	0	0	0	0	0	0
Planktolyngbya limnetica	0.00700	0.00300	0.00500	0.0150	0.00800	0
Planktothrix isothrix	0.0420	0.0640	0.0670	0.0710	0.0610	1
Pseudoanabaena catenata	0.0110	0.0960	0.0650	0.162	0.0840	1
Total	9.28	5.65	6.93	7.13	7.25	100

Table S2 continued.

22 July 2015						
Cyanobacterial species	**Site 1 biovolume ($mm^3 L^{-1}$)**	**Site 2 biovolume ($mm^3 L^{-1}$)**	**Site 3 biovolume ($mm^3 L^{-1}$)**	**Site 4 biovolume ($mm^3 L^{-1}$)**	**Mean biovolume ($mm^3 L^{-1}$)**	**Mean bivolume contribution (%)**
Aphanocapsa delicatissima	0.288	0.180	0	0	0.117	2
Aphanocapsa holsatica	0	0	0	0	0	0
Cuspidothrix issatschenkoi	0	0	0.0250	0	0.00600	0
Dolichospermum planctonicum	0	0.0550	0	0	0.0140	0
Dolichospermum spiroides	2.66	4.18	3.79	7.04	4.42	83
Limnococcus limneticus	0	0	0	0	0	0
Limnothrix redekei	0	0	0	0	0	0
Merismopedia sp.	0.0320	0.0240	0.0290	0.0500	0.0340	1
Merismopedia tenuissima	0.00100	0.00100	0.00400	0.00300	0.00200	0
Microsystis aeruginosa	0.888	0.554	0.264	0.555	0.565	11
Microcystis wesenbergii	0	0	0	0	0	0
Oscillatoria tenuis	0	0.243	0	0	0.0610	1
Planktolyngbya limnetica	0.00100	0.00200	0	0	0.00100	0
Planktothrix isothrix	0	0.0710	0.0670	0.141	0.0700	1
Pseudoanabaena catenata	0.0200	0.0310	0.0120	0	0.0160	0
Total	3.89	5.34	4.19	7.79	5.31	100

6 August 2015						
Cyanobacterial species	**Site 1 biovolume ($mm^3 L^{-1}$)**	**Site 2 biovolume ($mm^3 L^{-1}$)**	**Site 3 biovolume ($mm^3 L^{-1}$)**	**Site 4 biovolume ($mm^3 L^{-1}$)**	**Mean biovolume ($mm^3 L^{-1}$)**	**Mean bivolume contribution (%)**
Aphanocapsa delicatissima	0.420	1.08	0.240	0.821	0.640	11
Aphanocapsa holsatica	0.0390	0.0780	0.0780	0.0760	0.0680	1
Cuspidothrix issatschenkoi	0.0270	0.373	0.0530	0.00500	0.115	2
Dolichospermum planctonicum	0	0	0.111	0.325	0.109	2
Dolichospermum spiroides	0.0660	3.45	0.929	3.63	2.02	35
Limnococcus limneticus	0.0360	0.0720	0.0360	0.00400	0.0370	1
Limnothrix redekei	0.00700	0.0260	0	0.0390	0.0180	0
Merismopedia sp.	0.0210	0.0340	0.0230	0.0450	0.0310	1
Merismopedia tenuissima	0.00200	0.00300	0.00300	0.00100	0.00200	0
Microsystis aeruginosa	0.277	1.66	1.39	1.35	1.17	20
Microcystis wesenbergii	0	0.453	0.453	0.442	0.337	6
Oscillatoria tenuis	0	0	0.485	0.474	0.240	4
Planktolyngbya limnetica	0.00100	0.0320	0.0130	0.00900	0.0140	0
Planktothrix isothrix	0.0710	1.98	0.141	1.80	0.998	17
Pseudoanabaena catenata	0.0120	0	0.	0	0.00300	0
Total	0.979	9.24	3.96	9.02	5.80	100

Table S2 continued.

20 August 2015						
Cyanobacterial species	**Site 1 biovolume ($mm^3 L^{-1}$)**	**Site 2 biovolume ($mm^3 L^{-1}$)**	**Site 3 biovolume ($mm^3 L^{-1}$)**	**Site 4 biovolume ($mm^3 L^{-1}$)**	**Mean biovolume ($mm^3 L^{-1}$)**	**Mean bivolume contribution (%)**
Aphanocapsa delicatissima	1.56	2.52	0.661	2.16	1.73	58
Aphanocapsa holsatica	1.55	0	0.776	0.815	0.785	26
Cuspidothrix issatschenkoi	0	0	0	0.0530	0.0130	0
Dolichospermum planctonicum	0	0	0	0.0550	0.0140	0
Dolichospermum spiroides	0.133	0	0	0.199	0.0830	3
Limnococcus limneticus	0	0.0180	0	0.0140	0.00800	0
Limnothrix redekei	0	0	0	0.0130	0.00300	0
Merismopedia sp.	0.00100	0.00300	0.00200	0.00400	0.00300	0
Merismopedia tenuissima	0	0	0	0	0	0
Microsystis aeruginosa	0.0250	0.277	0.0280	0.554	0.221	7
Microcystis wesenbergii	0	0	0	0	0	0
Oscillatoria tenuis	0	0	0	0	0	0
Planktolyngbya limnetica	0.00100	0.0190	0.0110	0.0180	0.0120	0
Planktothrix isothrix	0.0710	0.0710	0.0710	0.141	0.0890	3
Pseudoanabaena catenata	0.0120	0.0250	0.0190	0.0690	0.0310	1
Total	3.35	2.93	1.57	4.10	2.99	100
3 September 2015						
Cyanobacterial species	**Site 1 biovolume ($mm^3 L^{-1}$)**	**Site 2 biovolume ($mm^3 L^{-1}$)**	**Site 3 biovolume ($mm^3 L^{-1}$)**	**Site 4 biovolume ($mm^3 L^{-1}$)**	**Mean biovolume ($mm^3 L^{-1}$)**	**Mean bivolume contribution (%)**
Aphanocapsa delicatissima	1.62	4.44	5.59	2.10	3.44	70
Aphanocapsa holsatica	0.0390	0.0780	0.116	0.0390	0.0680	1
Cuspidothrix issatschenkoi	0	0.0530	0	0.0270	0.0200	0
Dolichospermum planctonicum	0	0	0	0	0	0
Dolichospermum spiroides	0.0660	0.531	0	0.199	0.199	4
Limnococcus limneticus	0	0.0720	0	0.0360	0.0270	1
Limnothrix redekei	0.00700	0.0790	0.00700	0.0260	0.0300	1
Merismopedia sp.	0.00100	0.0110	0.00600	0.0120	0.00800	0
Merismopedia tenuissima	0	0	0.00100	0.00100	0.00100	0
Microsystis aeruginosa	0.554	1.66	0.554	0.139	0.727	15
Microcystis wesenbergii	0.226	1.13	0	0	0.339	7
Oscillatoria tenuis	0	0	0	0	0	0
Planktolyngbya limnetica	0	0.00800	0.0300	0.00300	0.0100	0
Planktothrix isothrix	0	0	0	0.0710	0.0180	0
Pseudoanabaena catenata	0.0120	0.0190	0.0500	0.0690	0.0380	1
Total	2.53	8.08	6.35	2.72	4.92	100

Table S3 Polymerase chain reaction (PCR) screening results for Lake Uluabat plankton tow samples. PCRs targeting the non-species-specific *mcyA* and *mcyE* regions of MC synthetase were performed with general PCR primers and shown under the *mcyA* and *mcyE* columns, respectively. PCRs targeting the *mcyE* region of *Microcystis*, *Anabaena*/*Dolichospermum* and *Planktothrix* are shown under the *mcyE-Mic*, *mcyE-Anab* and *mcyE-Plank* columns, respectively. + denotes presence and - denotes absence.

Sampling site	*mcyA*	*mcyE*	*mcyE-Mic*	*mcyE-Anab*	*mcyE-Plank*
23 June 2015					
Site 1	+	+	+	-	-
Site 2	+	+	+	-	-
Site 4	+	+	+	-	-
10 July 2015					
Site 3	-	-	-	-	-
22 July 2015					
Site 1	+	-	+	-	-
Site 2	+	+	+	-	-
Site 4	+	+	+	-	-
6 August 2015					
Site 1	+	+	+	-	-
Site 2	+	+	+	-	-
Site 3	+	+	+	-	-
Site 4	+	+	+	-	-
20 August 2015					
Site 2	+	-	+	-	-
3 September 2015					
Site 1	+	+	+	-	-
Site 2	+	+	+	-	-

Table S4 Results of the targeted MCs analysis (LC-MS/MS method A). Other variants were present, but not targeted in the original analysis due to a lack of available standards for calibration. NOD-R and [D-Leu^{1}]MC-LR were not detected in any samples. All results are in μg g^{-1} d.w.

Sampling dates (d/m/y)	Sites	[D-Asp^{3}] MC-RR	MC-RR	MC-YR	MC-HtyR	MC-LR	[D-Asp^{3}] MC-LR	[Dha^{7}] MC-LR-	MC-HilR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
23.06.15	St1	ND	1.9	0.73	0.07	2.0	0.08	0.07	0.07	ND	ND	ND	ND	0.09
23.06.15	St2	ND	5.7	2.4	0.14	11	0.20	0.31	0.19	0.17	ND	0.13	ND	0.11
23.06.15	St4	ND	0.19	0.06	ND	0.26	ND	ND	ND	ND	ND	ND	ND	0.10
10.07.15	St3	ND	0.07	ND	ND	0.09	ND	ND	ND	ND	ND	ND	ND	ND
10.07.15	St3-DUP	ND	0.11	0.07	ND	0.14	ND	ND	ND	ND	ND	ND	ND	ND
22.07.15	St1	0.34	25	15	1.0	29	0.81	0.96	0.69	1.6	ND	0.13	0.24	0.14
22.07.15	St2	0.46	31	25	0.81	45	1.0	1.5	1.2	2.3	ND	0.24	0.24	0.19
22.07.15	St4	0.46	27	22	0.76	34	1.1	1.2	1.0	2.5	ND	0.15	0.21	0.24
06.08.15	St1	0.36	24	15	0.58	35	0.74	1.1	1.3	1.4	ND	0.16	0.21	0.19
06.08.15	St2	1.86	90	58	3.2	150	3.1	4.3	7.0	9.0	0.08	0.57	0.24	0.45
06.08.15	St3	ND	7.0	5.8	0.18	8.9	0.25	0.31	0.27	0.12	ND	ND	ND	0.09
06.08.15	St4	0.76	40	32	0.97	48	1.1	1.5	2.7	3.3	ND	0.32	0.83	0.21
06.08.15	St4-DUP	0.65	36	30	0.91	45	1.0	1.3	2.6	3.2	ND	0.28	0.27	0.21
20.08.15	St2	0.69	45	28	1.5	63	1.2	1.6	3.1	2.8	ND	0.44	ND	0.22
03.09.15	St1	0.74	39	34	1.2	48	1.4	1.4	1.8	3.0	0.08	0.10	0.18	0.13
03.09.15	St2	0.34	26	22	0.62	38	0.70	0.86	2.6	1.4	ND	0.35	0.20	0.15
03.09.15	St2-DUP	0.68	46	32	0.49	57	1.0	1.2	2.8	2.4	ND	0.39	0.17	0.15
AQUAMEB-24		7.2	140	140	0.7	95	6.7	1.8	21	19	ND	ND	ND	ND

ND = not detected, below the method detection limit, DUP = Lab duplicate

Table S5 Pre-extraction lab fortified (0.10 μg g $^{-1}$) matrix spike returns for variants added to the Site 3 sample collected on 10 July 2015.

Variant	% Return
[D-Asp^3]MC-RR	86%
MC-RR	70%
NOD-R	120%
MC-YR	142%
MC-HtyR	114%
MC-LR	104%
[D-Asp^3]MC-LR	120%
[Dha^7]MC-LR	110%
MC-HilR	76%
MC-WR	104%
[D-Leu^1]LR	142%
MC-LA	150%
MC-LY	114%
MC-LW	84%
MC-LF	94%

Table S6 Microcystin variants detected in the *Microcystis* culture (AQUAMEB-24) and Lake Uluabat using Methods A and B. The variant ID corresponds to the list in Figure 6, with ions ($[M-H]^-$, $[M+H]^+$, $[M+2H]^{+2}$) extracted and interpreted. The retention times (RT; min) are reported for Method A (culture and site 2, 6 August 2015 extract) and Method B (culture). Concentrations were determined using Method A for variants with calibration standards available, but all other data was estimated from peak area of extracted ions and external curves.

Identity	ID	M-H	Method: Sample ID: M+H/M+2H	A Culture RT	A Station RT	B Culture RT	A Culture μg g^{-1}	A 6 Aug 15 (St2) μg g^{-1}
[DMAdda^5]MC-RR	**1**	1022.6	512.8	2.52	2.39	2.53	0.2	0.6
[DMAdda^5]MC-(H4)YR	**2**	1033.5	1035.5	3.20	3.10	5.36	0.4	0.1
[DMAdda^5]MC-YR	**3**	1029.5	1031.5	3.91	3.83	5.81	1.3	1.0
dmMC-RR	**4**	1022.6	512.8	4.00	3.87	4.09	0.3	0.2
[DMAdda^5]MC-LR	**5**	979.5	981.5	4.45	4.16	6.00	0.4	0.9
dmMC-RR	**6**	1022.6	512.8	4.50	4.34	4.33	0.3	0.4
[D-Asp^3]MC-RR	**7**	1022.6	512.8	4.76	4.70	4.36	7.2[a]	1.9[a]
[Mser^7]MC-RR	**8**	1054.6	528.8	4.84	4.80	4.51	1.7	0.9
MC-RR	**9**	1036.6	519.8	5.16	5.15	4.60	140[a]	90[a]
[Dha^7]MC-RR	**10**	1022.6	512.8	5.46	ND	4.64	3.3	1.8
MC-M(O)R	**11**	1027.5	1029.5	6.54	6.53	6.60	2.8	2.3
[D-Asp^3]MC-(H4)YR	**12**	1033.5	1035.5	7.46	ND	6.43	3.1	0.1
MC-(H4)YR	**13**	1047.5	1049.6	8.30	8.17	6.81	41	7.5
[Mser^7]MC-YR	**14**	1061.5	1063.5	8.73	8.85	6.96	1.3	0.3
dmMC-LR	**15**	979.5	981.5	8.76	9.03	6.85	0.30	0.2
[Dha^7]MC-(H4)YR	**16**	1033.5	1035.5	8.79	ND	6.76	1.2	ND
MC-(H2)YR	**17**	1045.5	1047.6	9.15	ND	7.02	0.1	ND
[Mser^7]MC-LR	**18**	1011.5	1013.6	9.50	9.03	6.99	1.6	1.1
[D-Asp^3]MC-YR	**19**	1029.5	1031.5	10.17	9.56	6.73	7.8	0.5
dmMC-LR	**20**	979.5	981.5	10.37	9.65	7.02	0.2	0.1
MC-MR	**21**	1011.5	1013.5	10.87	10.75	7.17	2.7	0.1
MC-YR	**22**	1043.5	1045.5	11.11	10.99	7.14	140[a]	58[a]
MC-HtyR	**23**	1057.5	1059.5	11.50	11.48	7.37	0.7[a]	3.2[a]
MC-Y(OMe)R	**24**	1073.5	1075.5	11.52	11.37	7.24	8.2	1.1
[Dha^7]MC-YR	**25**	1029.5	1031.5	11.70	11.26	7.12	2.9	0.6
MC-LR	**26**	993.5	995.6	12.78	11.95	7.30	95[a]	150[a]
[D-Asp^3]MC-LR	**27**	979.5	981.5	13.92	12.47	7.06	6.7[a]	3.1[a]
[Dha^7]MC-LR	**28**	979.5	981.5	14.28	13.50	7.29	1.8[a]	4.3[a]
MC-HilR	**29**	1007.5	1009.6	16.86	15.89	7.58	21[a]	7.0[a]
MC-FR	**30**	1027.5	1029.5	17.04	16.38	7.66	9.0	2.2
MC-WR	**31**	1066.5	1068.6	18.22	17.79	7.83	19[a]	9.0[a]
MC-HphR	**32**	1041.5	1043.5	ND	18.11	7.94[b]	ND	2.6
LA	**33**	908.5	910.5	ND	21.30	14.79[b]	ND[a]	0.08[a]
LY	**34**	1000.5	1002.5	ND	22.70	15.08[b]	ND[a]	0.57[a]
LW	**35**	1023.6	1025.5	ND	23.07	16.89[b]	ND[a]	0.24[a]
LF	**36**	984.6	986.5	ND	23.05	17.51[b]	ND[a]	0.45[a]

[a]Determined via LC-MS/MS Method A. [b]Not detected in culture, RT from Station 2 (6 Aug 2015)

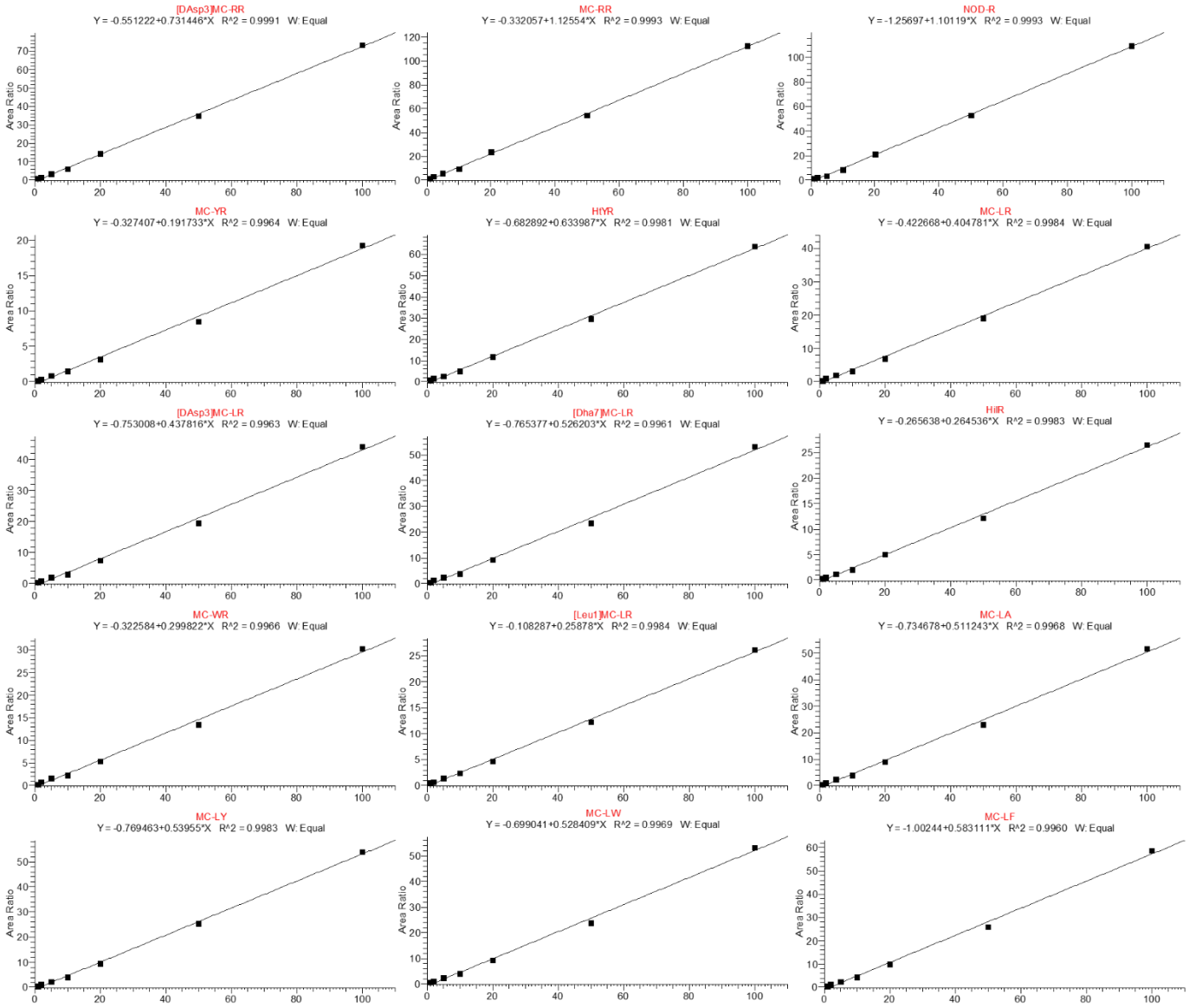

Figure S1 Calibration Curves utilized in the quantification of targeted microcystins (Method A). The internal standard (*d*$_7$-MC-LR) was utilized to develop the IS curves and in quantification.

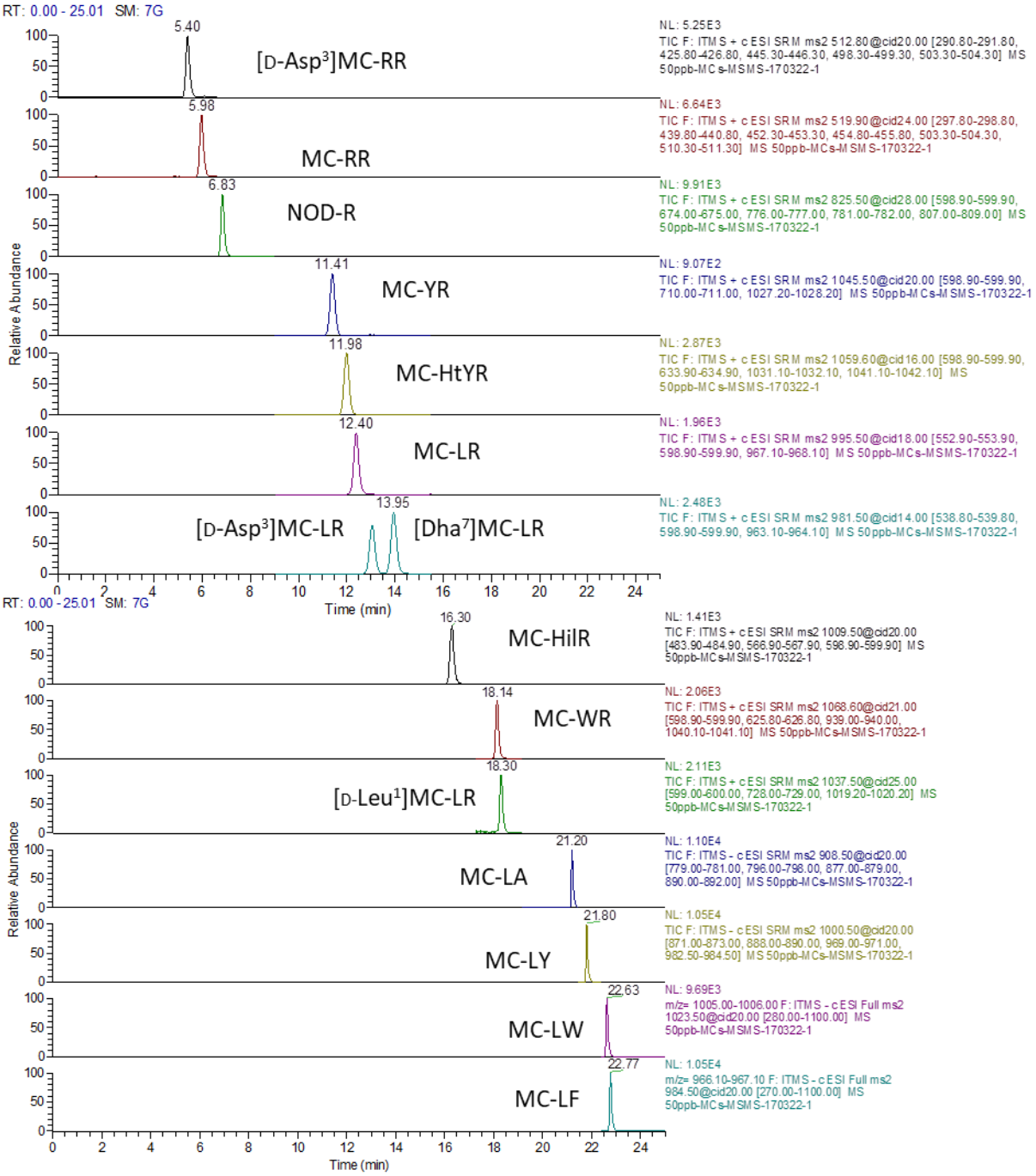

Figure S2 Chromatograms of the standards used to calibrate the targeted MS/MS analysis (Method A).

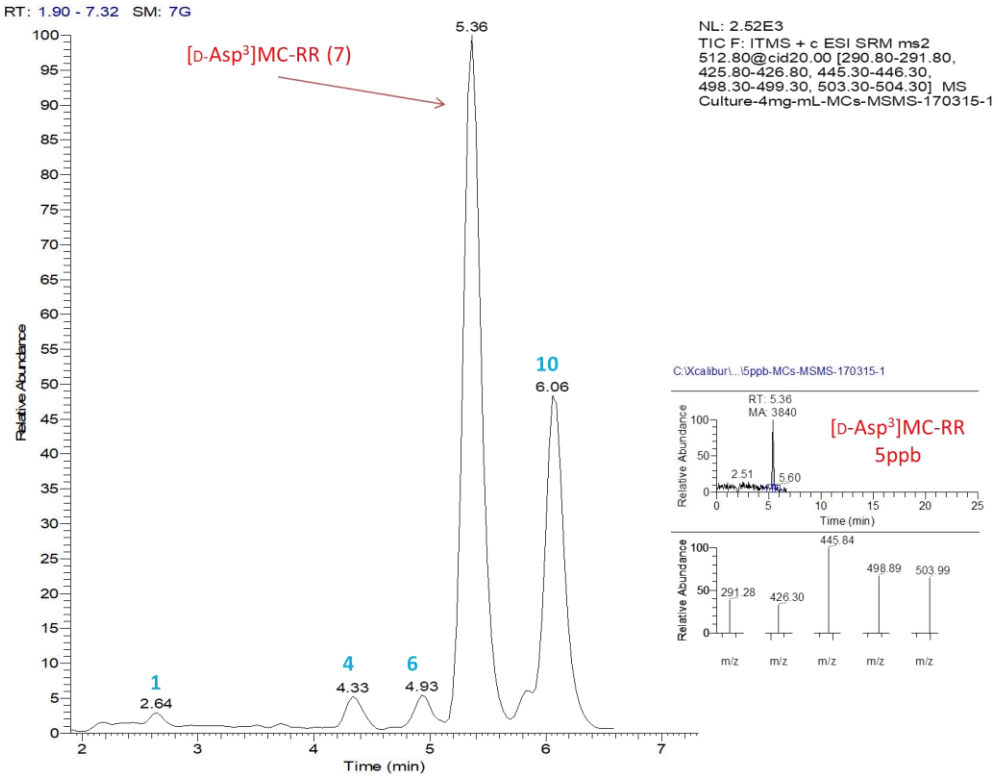

Figure S3 The Method A targeted analysis of dmMC-RR (including confirmed [D-Asp³]MC-RR) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL. In the chromatogram, peak numbers correspond to Figure 6.

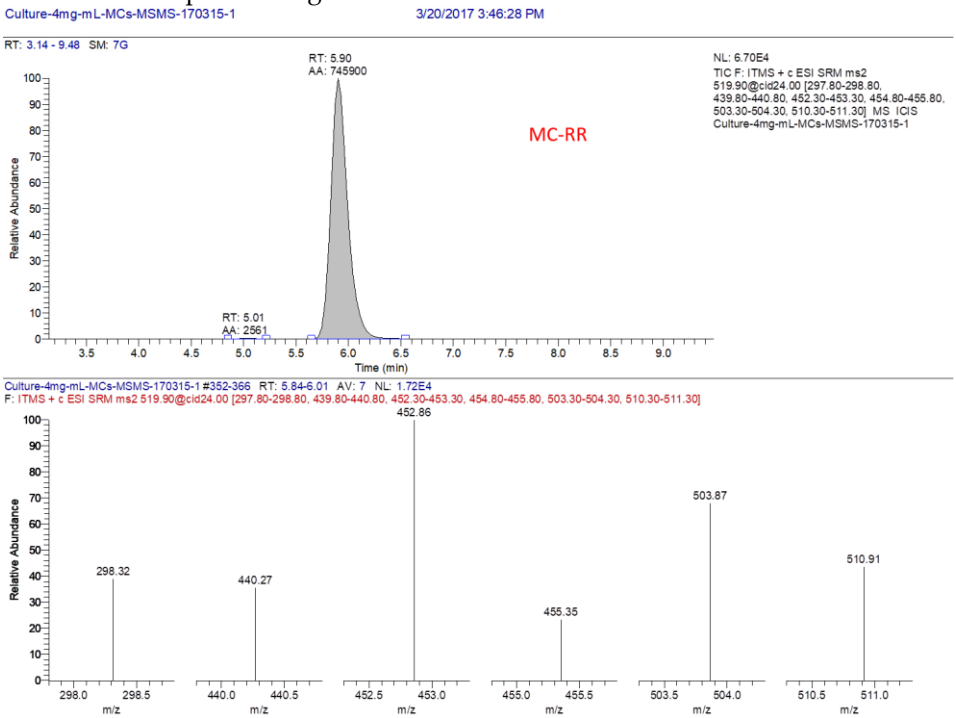

Figure S4 The Method A targeted analysis of MC-RR (**9**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL. Further dilution of the extract was required in order to quantitate.

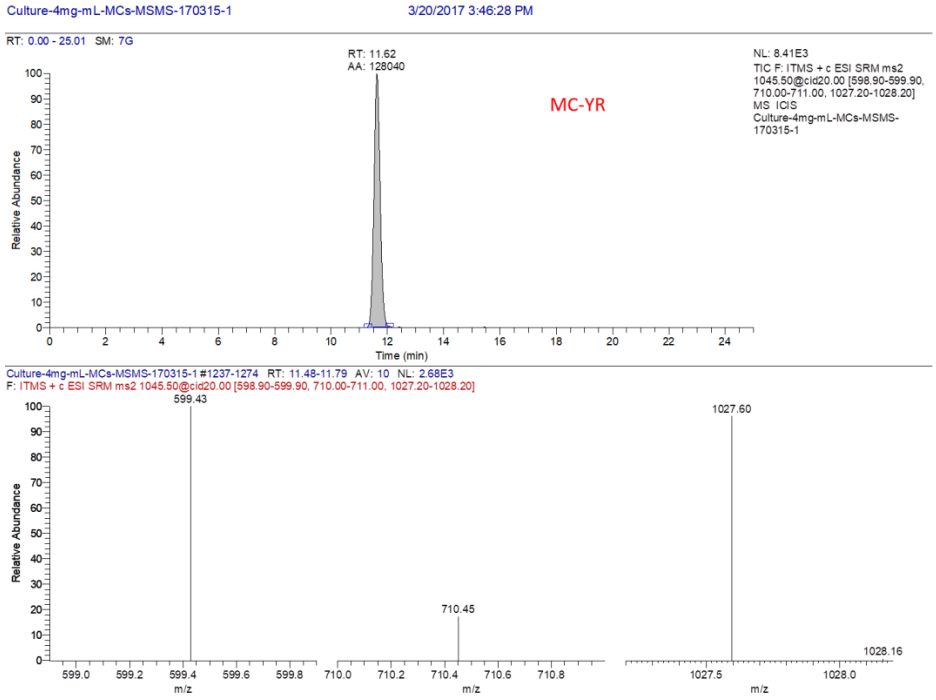

Figure S5 The Method A targeted analysis of MC-YR (**22**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL.

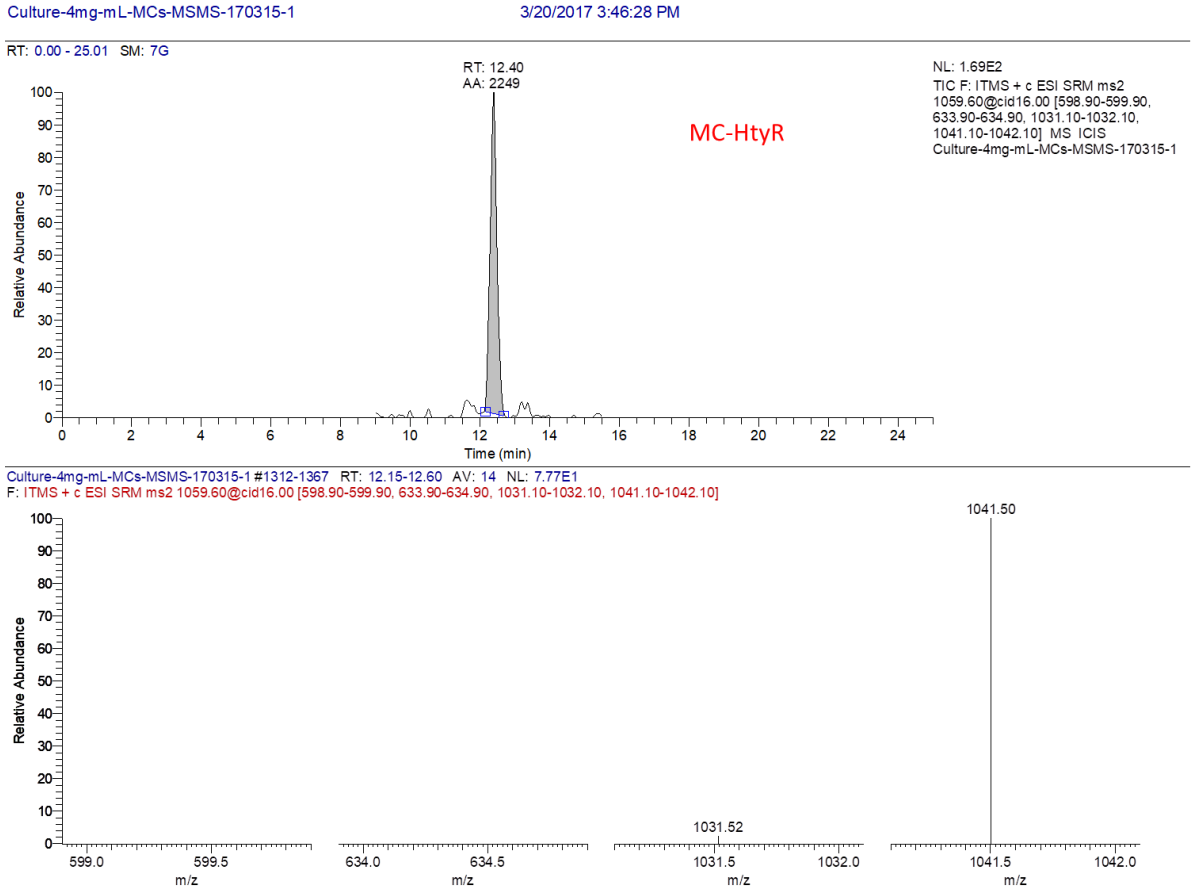

Figure S6 The Method A targeted analysis of MC-HtyR (**23**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL.

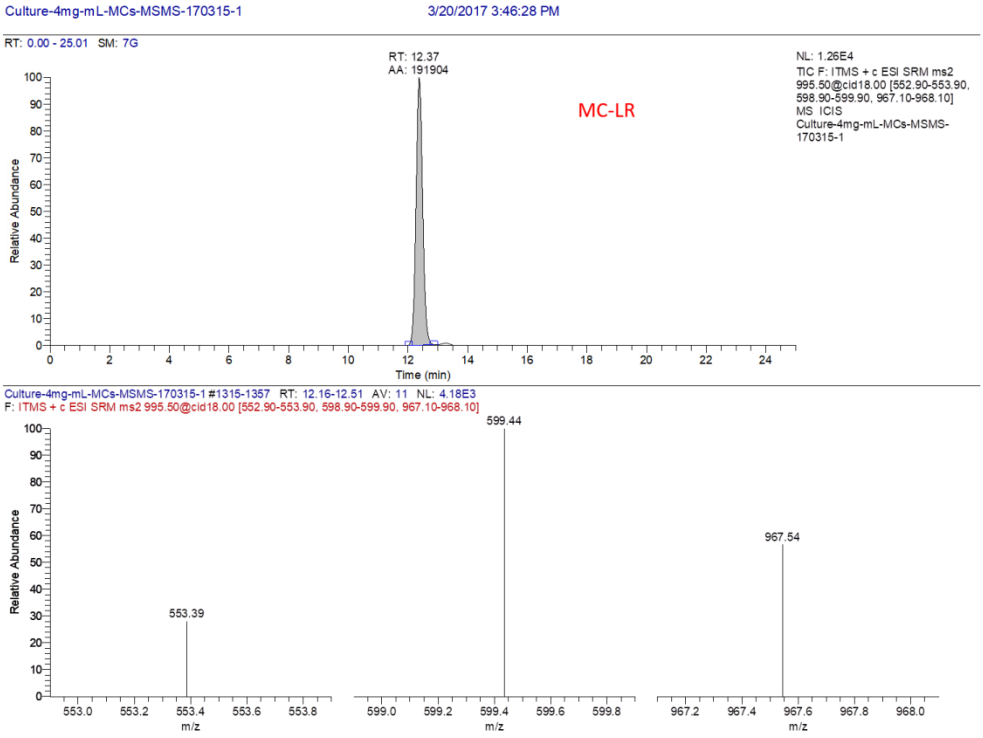

Figure S7 The Method A targeted analysis of MC-LR (**26**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL.

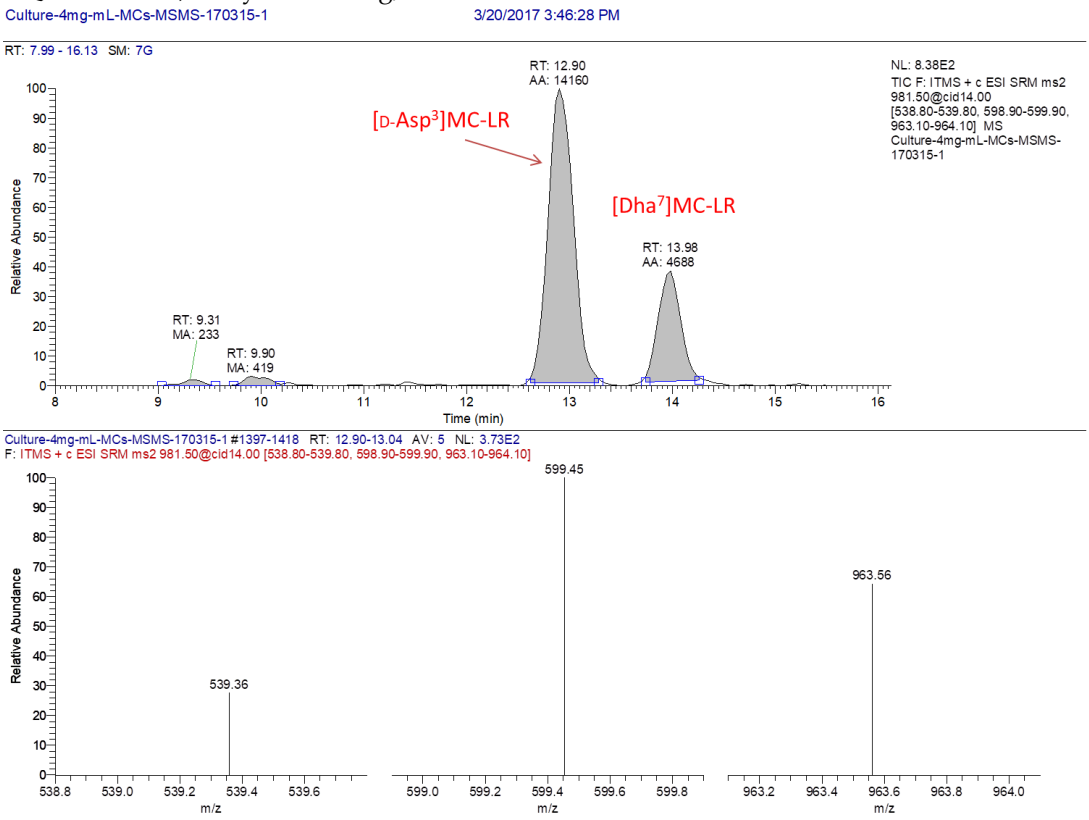

Figure S8 The Method A targeted analysis of dmMC-LR (including confirmed [D-Asp^3]MC-LR (**27**) & [Dha^7]MC-LR (**28**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL.

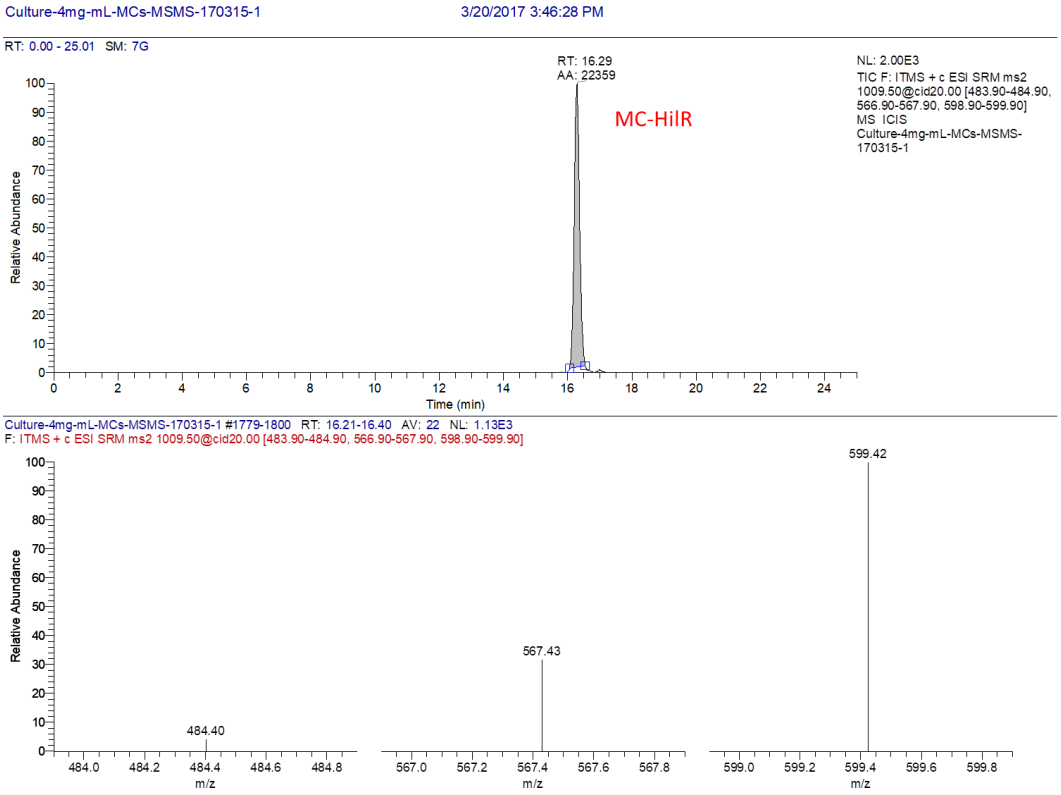

Figure S9 The Method A targeted analysis of MC-HilR (**29**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL.

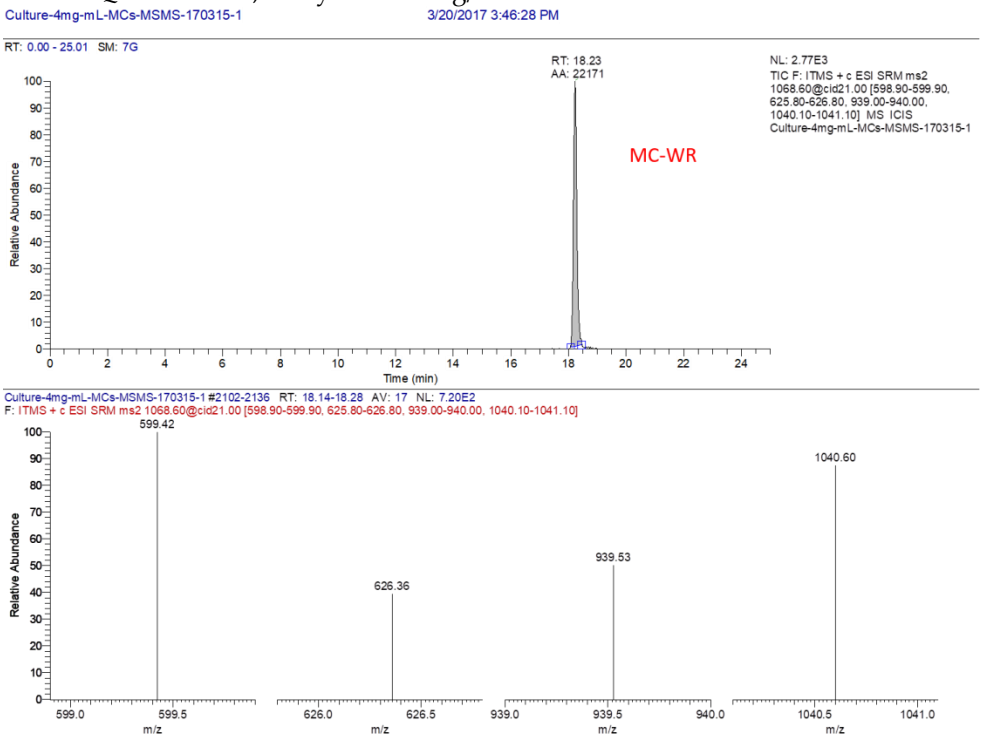

Figure S10 The Method A targeted analysis of MC-WR (**31**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL.

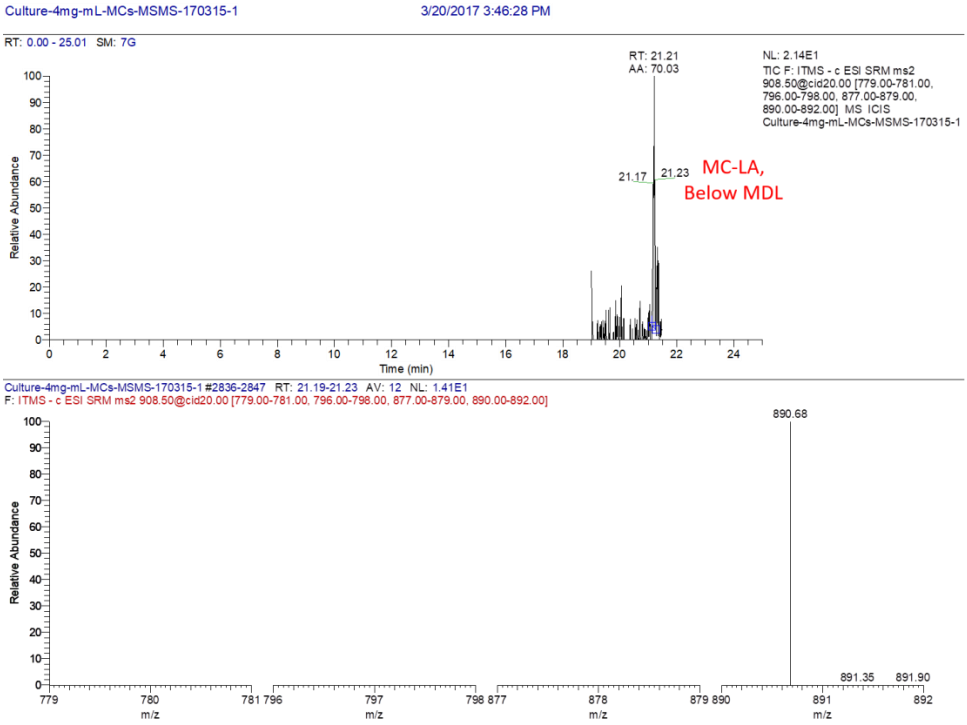

Figure S11 The Method A targeted analysis of MC-LA (**33**) LC-MS/MS chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 4 mg/mL. The observed peak at the RT of MC-LA was below the official Method Detection Limit and not reported.

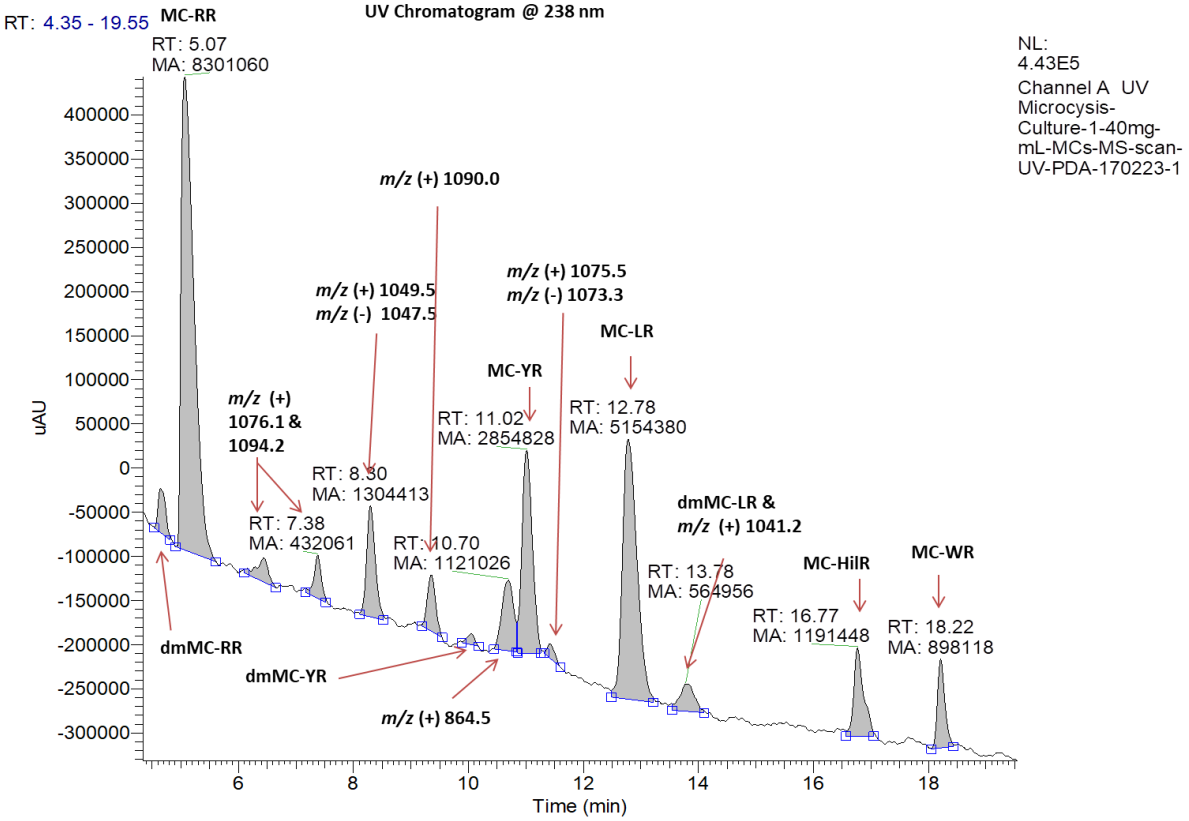

Figure S12 The Method A LC-UV chromatogram of the *Microcystis* strain AQUAMEB-24, analyzed at 40 mg/mL. Peaks of interest were further analyzed with LC-HRMS.

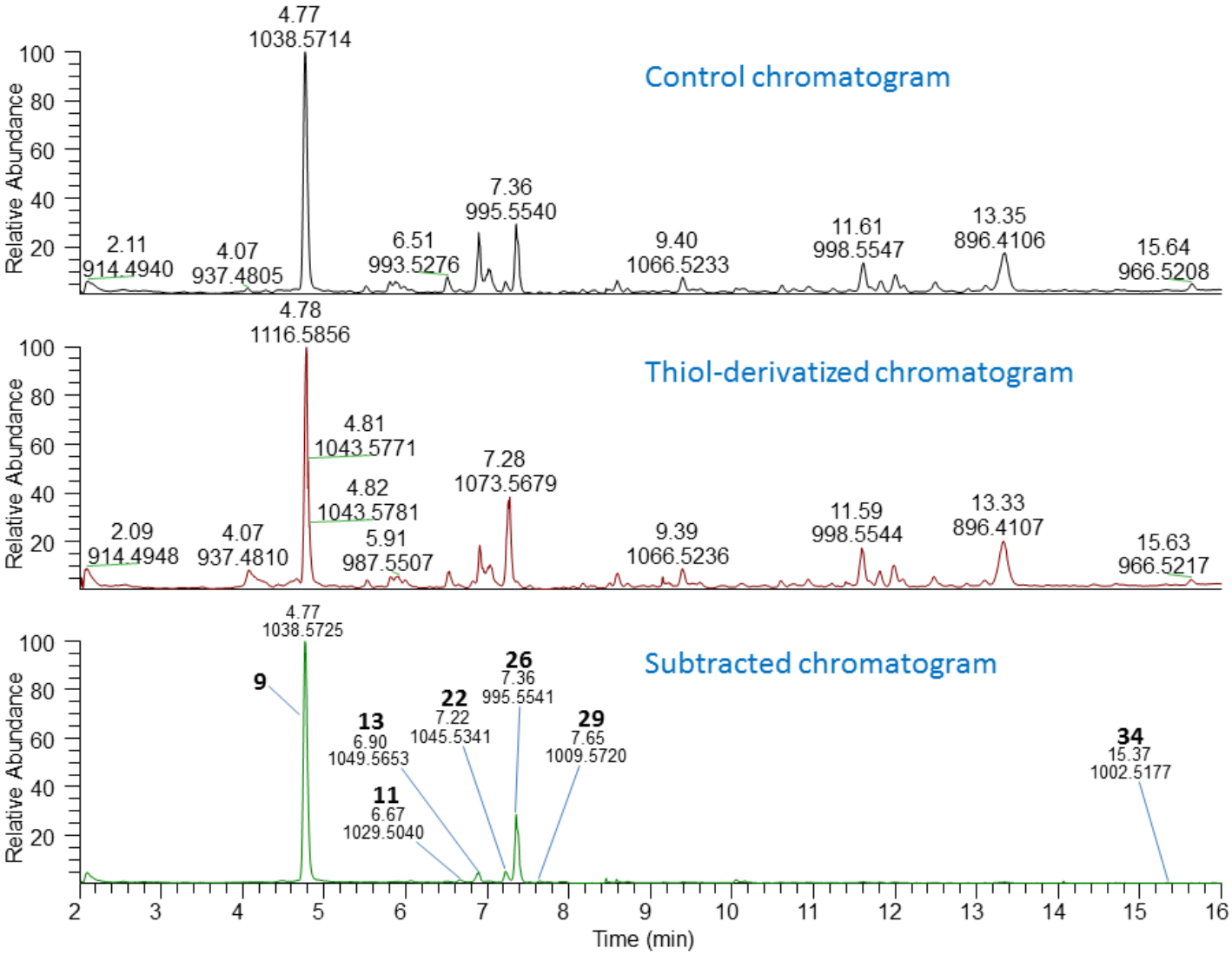

Figure S13 Thiol derivatization LC-HRMS (full scan, positive ionization) chromatograms of the HP-20 concentrate from Station 2 (6 August 2015): top, control extract, middle, extract derivatized with 1:1 d_0-/d_4-mercaptoethanol; bottom, difference chromatogram (top minus middle chromatograms). Numbers in bold are the compound numbers (Figure 6) while the retention times (min) and m/z (for $[M+H]^+$) values are also indicated

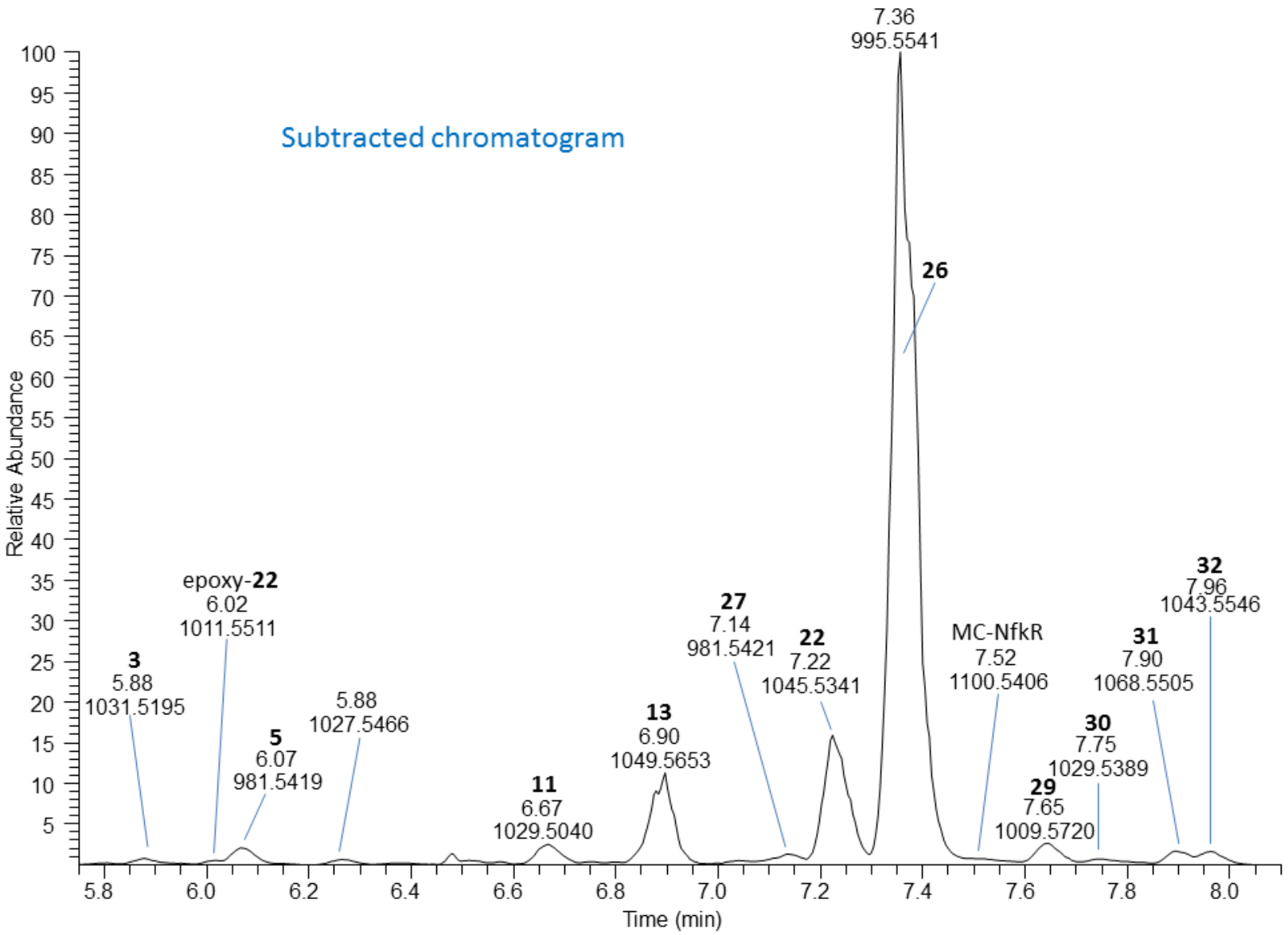

Figure S14 Expansion (5.75–8.10 min) of the thiol derivatization LC-HRMS (full scan) subtraction chromatogram from Figure S13.

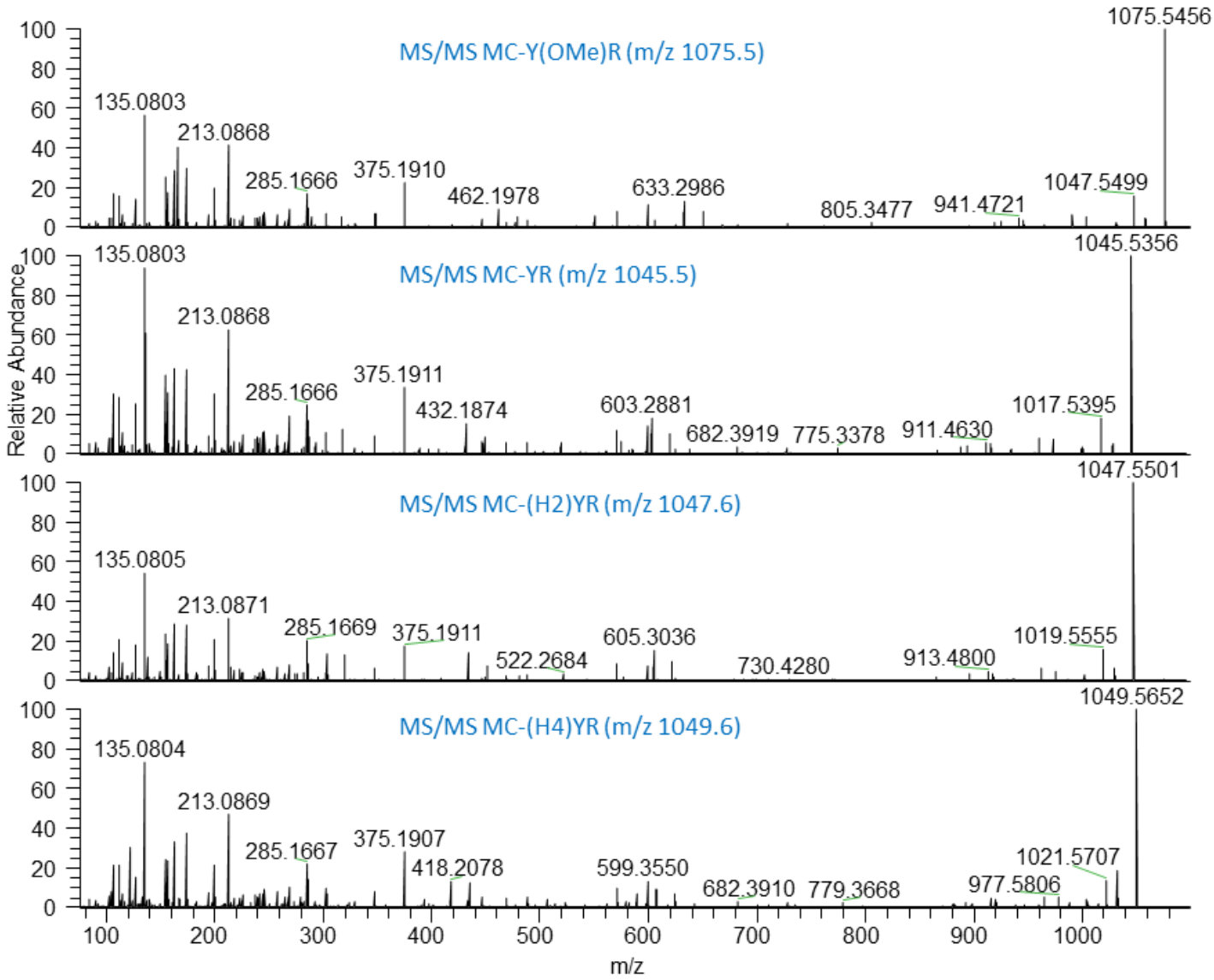

Figure S15 LC-HRMS/MS spectra (positive mode) of MC-Y(OMe)R (**24**), MC-YR (**22**), MC-(H2)YR (**17**) and MC-(H4)YR (**13**) from the HP-20 concentrate from Station 2 (6 August 2015).

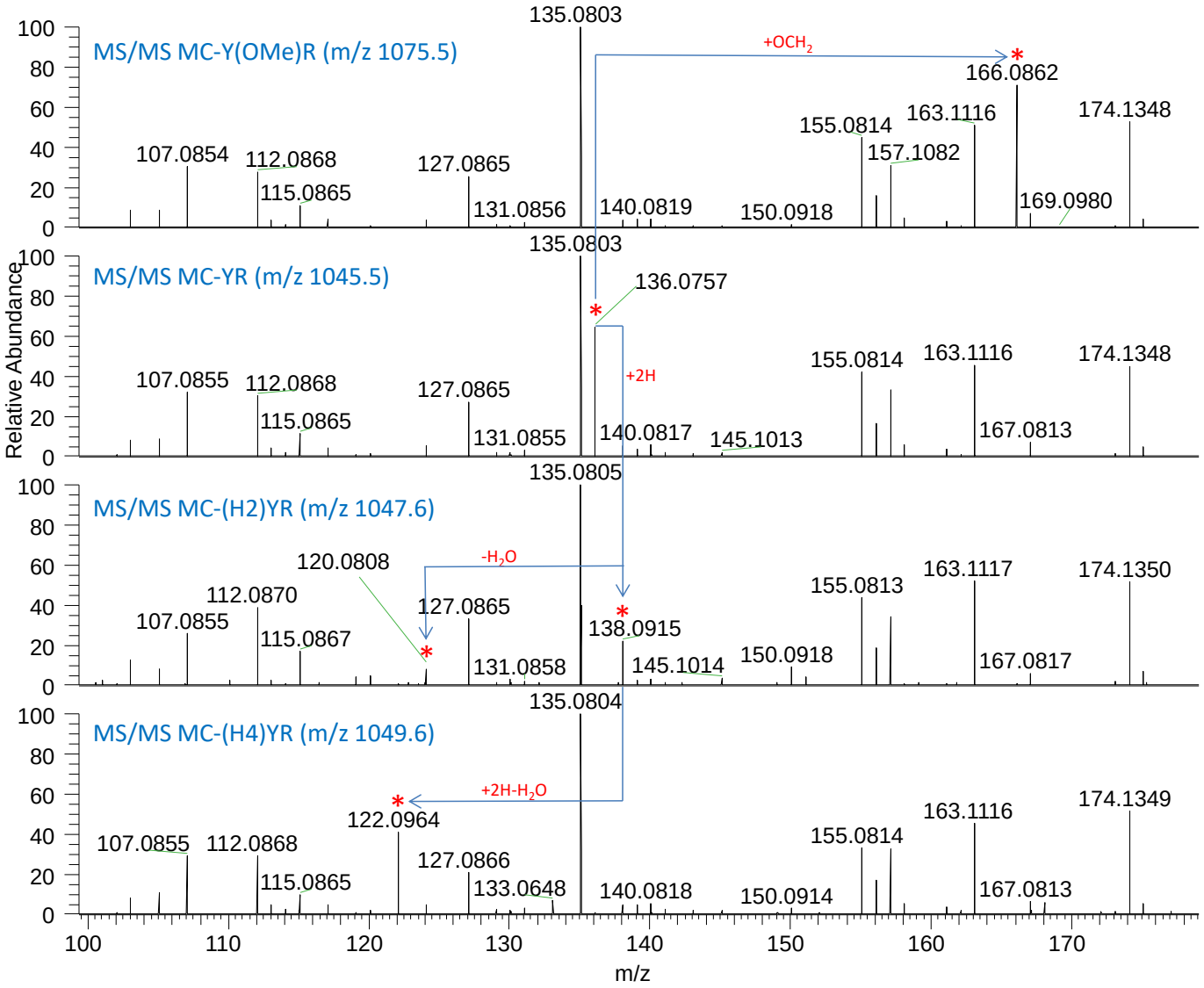

Figure S16 Expansion of LC-HRMS/MS spectra (positive mode) of MC-Y(OMe)R (**24**), MC-YR (**22**), MC-(H2)YR (**17**) and MC-(H4)YR (**13**) from Figure S15. Fragments marked with asterisks arise solely from amino acid 2.

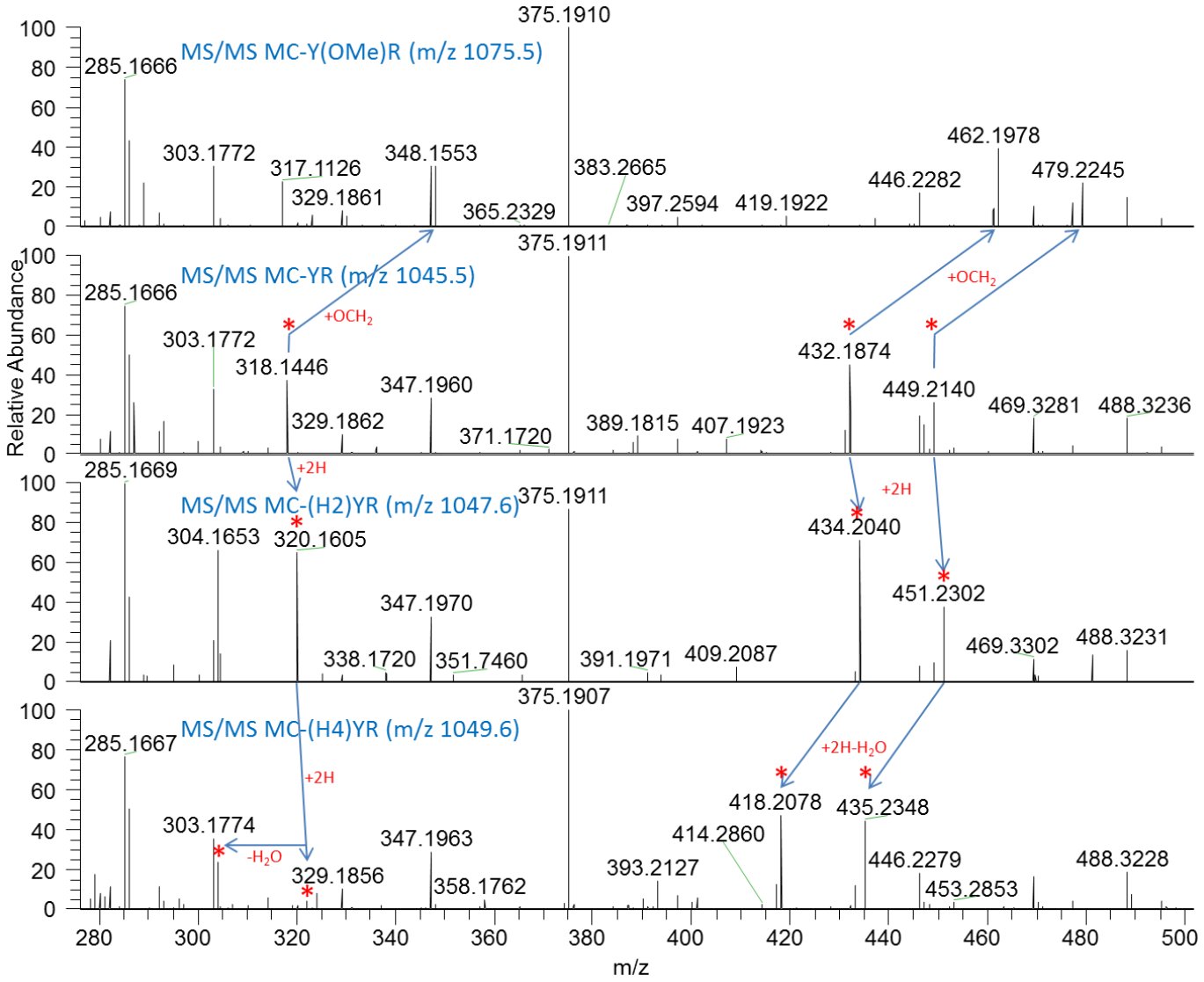

Figure S17 Expansion of LC-HRMS/MS spectra (positive mode) of MC-Y(OMe)R (**24**), MC-YR (**22**), MC-(H2)YR (**17**) and MC-(H4)YR (**13**) from Figure S15. Fragments marked with asterisks contain amino acid 2.

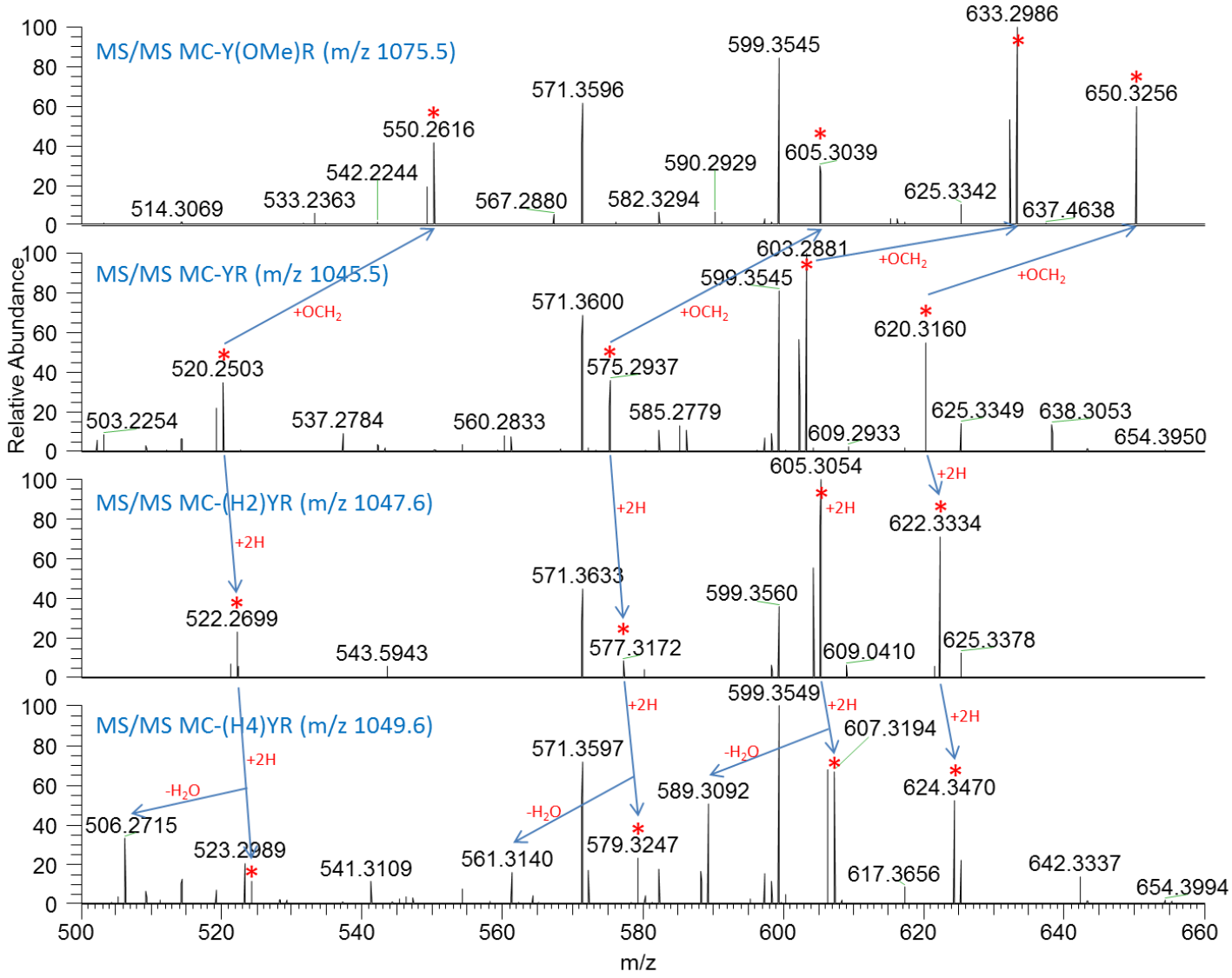

Figure S18 Expansion of LC-HRMS/MS spectra (positive mode) of MC-Y(OMe)R (**24**), MC-YR (**22**), MC-(H2)YR (**17**) and MC-(H4)YR (**13**) from Figure S15. Fragments marked with asterisks contain amino acid 2.

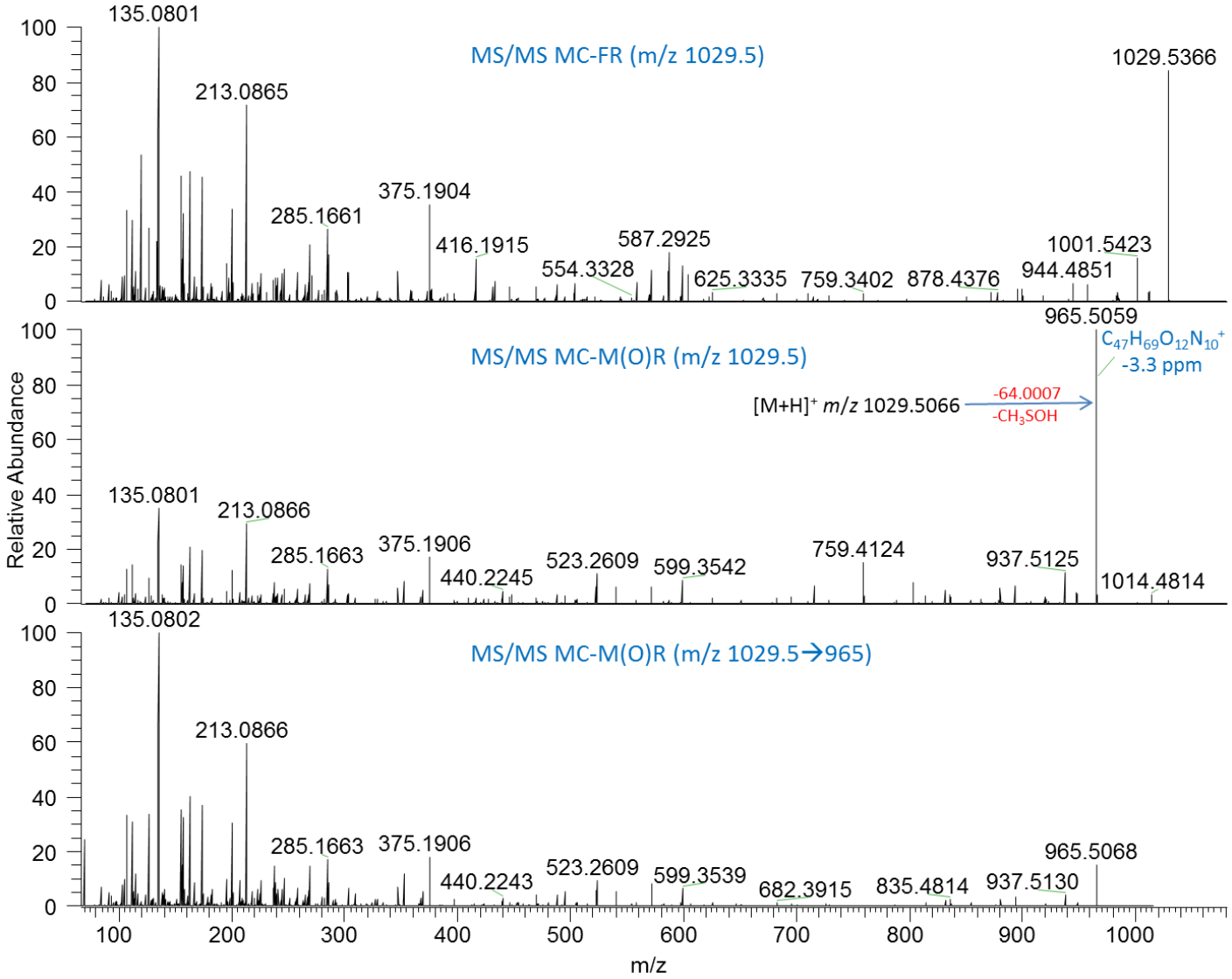

Figure S19 LC-HRMS/MS spectra (positive mode) of *m/z* 1029.5 (MC-FR (**30**) and MC-M(O)R (**11**)), and LC-HRMS/MS/MS spectrum of *m/z* 1029.5→965.5 (MC-M(O)R (**11**)), from the HP-20 concentrate from Station 2 (6 August 2015).

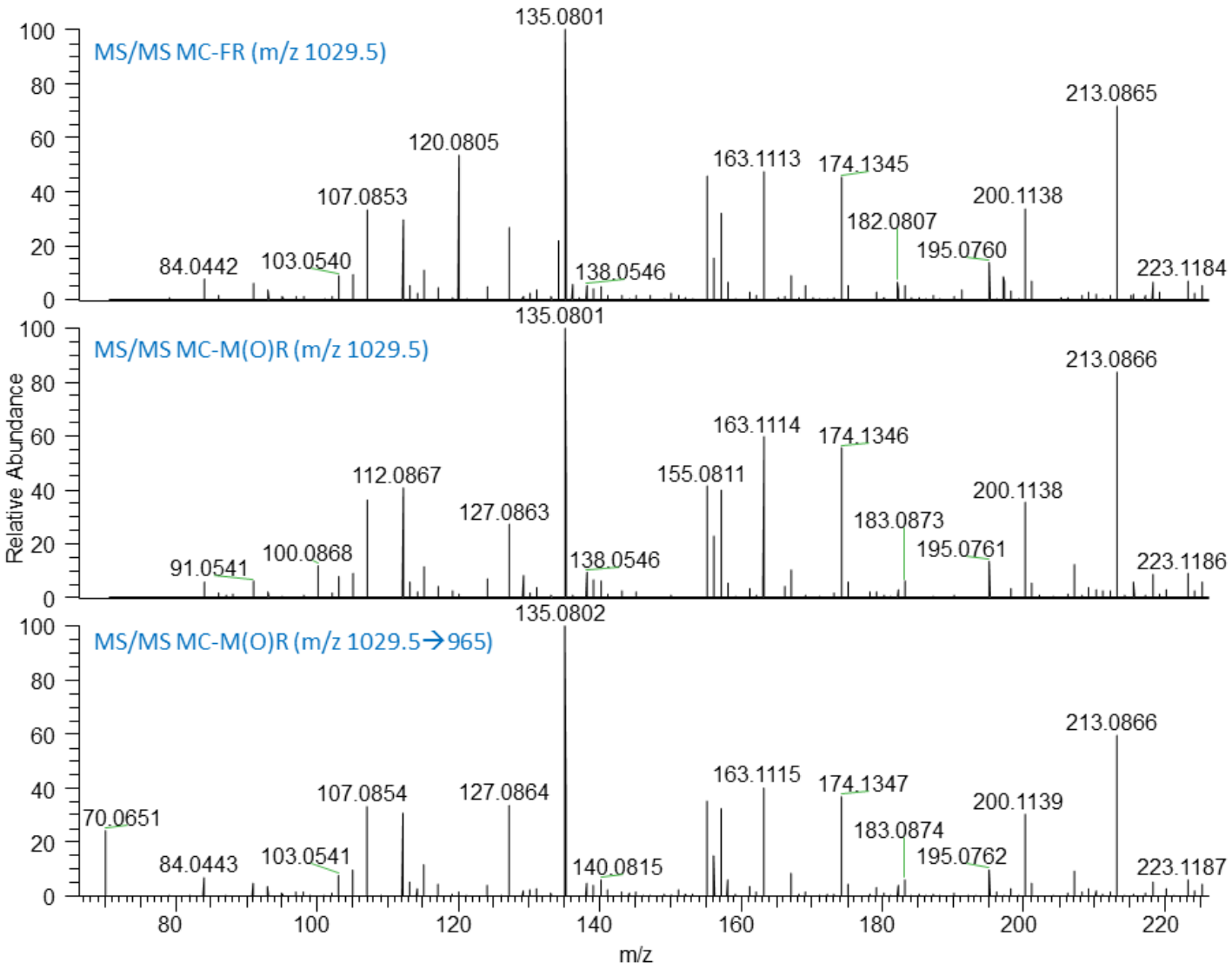

Figure S20 Expansion of LC-HRMS/MS spectra (positive mode) of *m*/*z* 1029.5 (MC-FR (**30**) and MC-M(O)R (**11**)), and LC-HRMS/MS/MS spectrum of *m*/*z* 1029.5→965.5 (MC-M(O)R (**11**)), from the HP-20 concentrate from Station 2 (6 August 2015) from Figure S19.

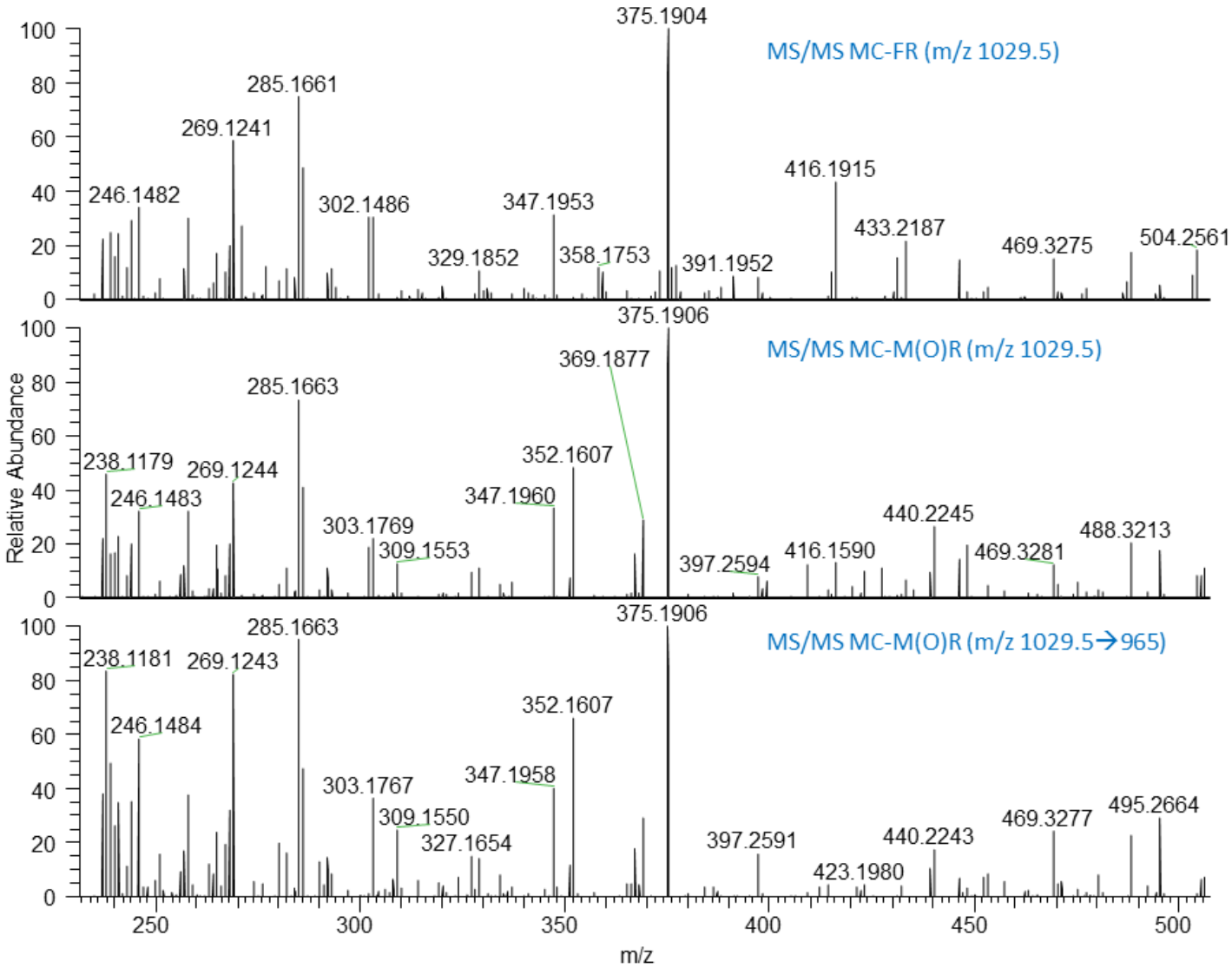

Figure S21 Expansion of LC-HRMS/MS spectra (positive mode) of *m/z* 1029.5 (MC-FR (**30**) and MC-M(O)R (**11**)), and LC-HRMS/MS/MS spectrum of *m/z* 1029.5→965.5 (MC-M(O)R (**11**)), from the HP-20 concentrate from Station 2 (6 August 2015) from Figure S19.

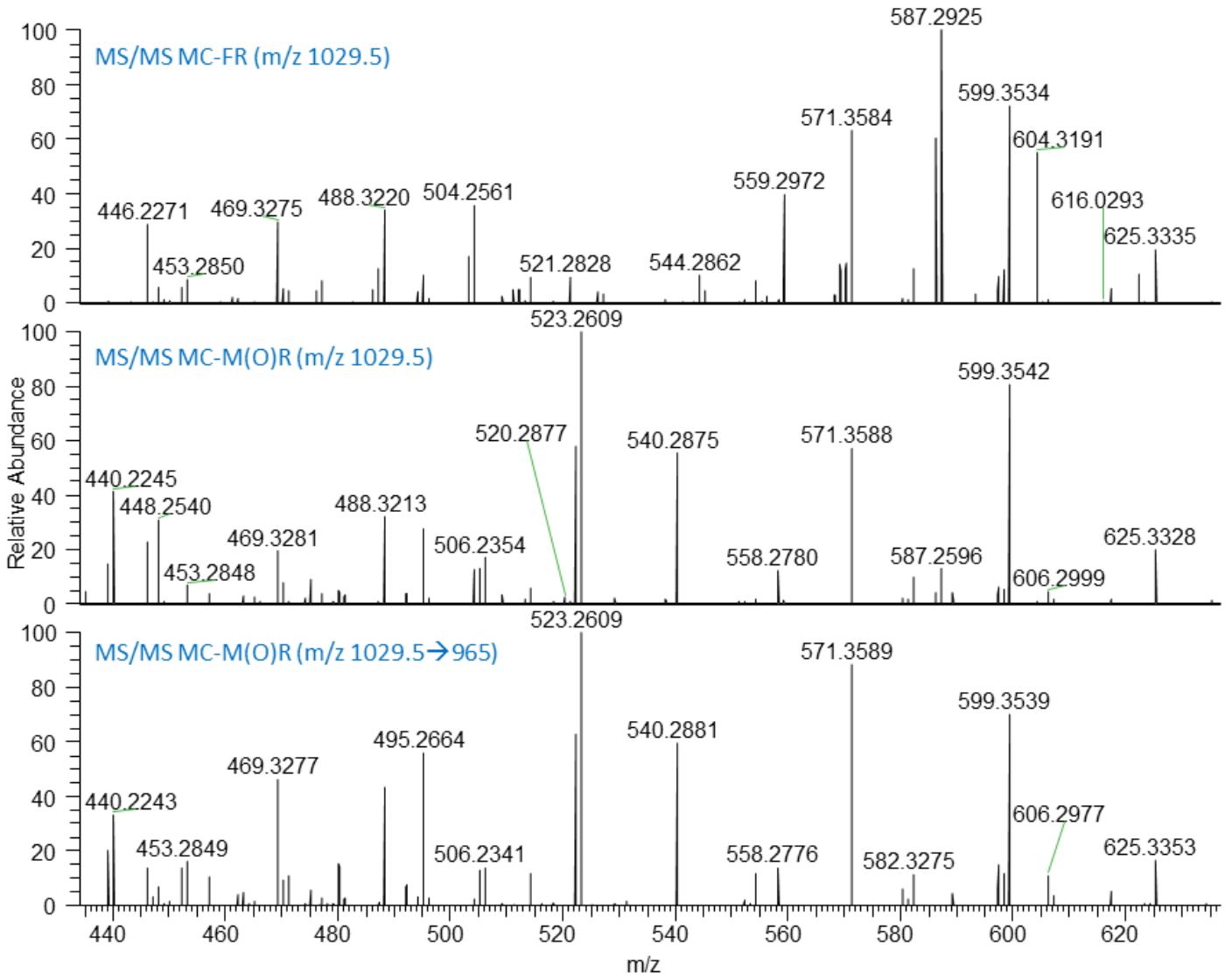

Figure S22 Expansion of LC-HRMS/MS spectra (positive mode) of *m/z* 1029.5 (MC-FR (**30**) and MC-M(O)R (**11**)), and LC-HRMS/MS/MS spectrum of *m/z* 1029.5→965.5 (MC-M(O)R (**11**)), from the HP-20 concentrate from Station 2 (6 August 2015) from Figure S19.

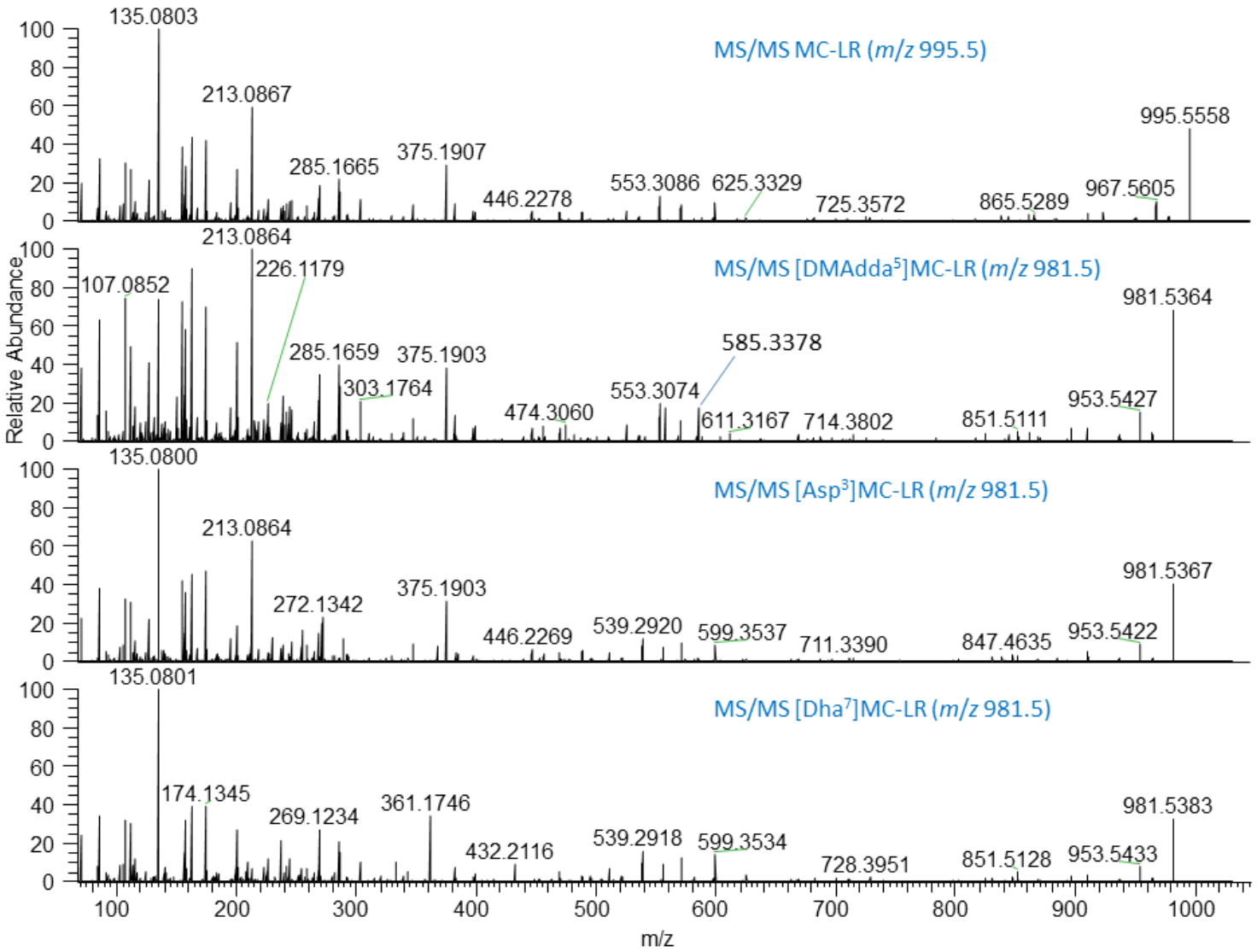

Figure S23 LC-HRMS/MS spectra (positive mode) of MC-LR (**26**) (*m/z* 995.5) and its three major dmMC-LR congeners (**5**, **27** and **28**) (*m/z* 981.5), from the HP-20 concentrate from Station 2 (6 August 2015).

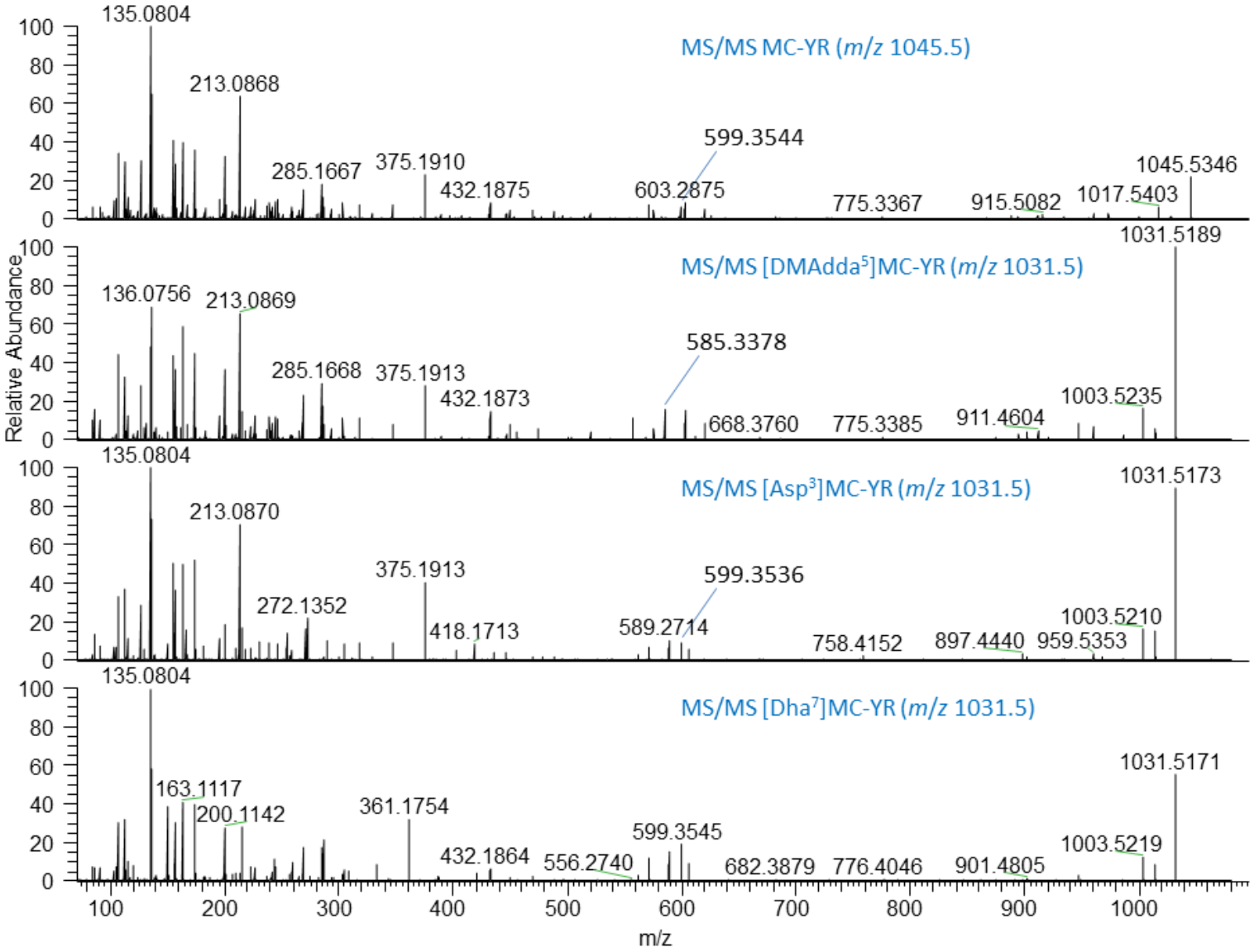

Figure S24 LC-HRMS/MS spectra (positive mode) of MC-YR (**22**) (*m*/*z* 1045.5) and its three major dmMC-YR congeners (**3**, **19** and **25**) (*m*/*z* 1031.5), from the HP-20 concentrate from Station 2 (6 August 2015).

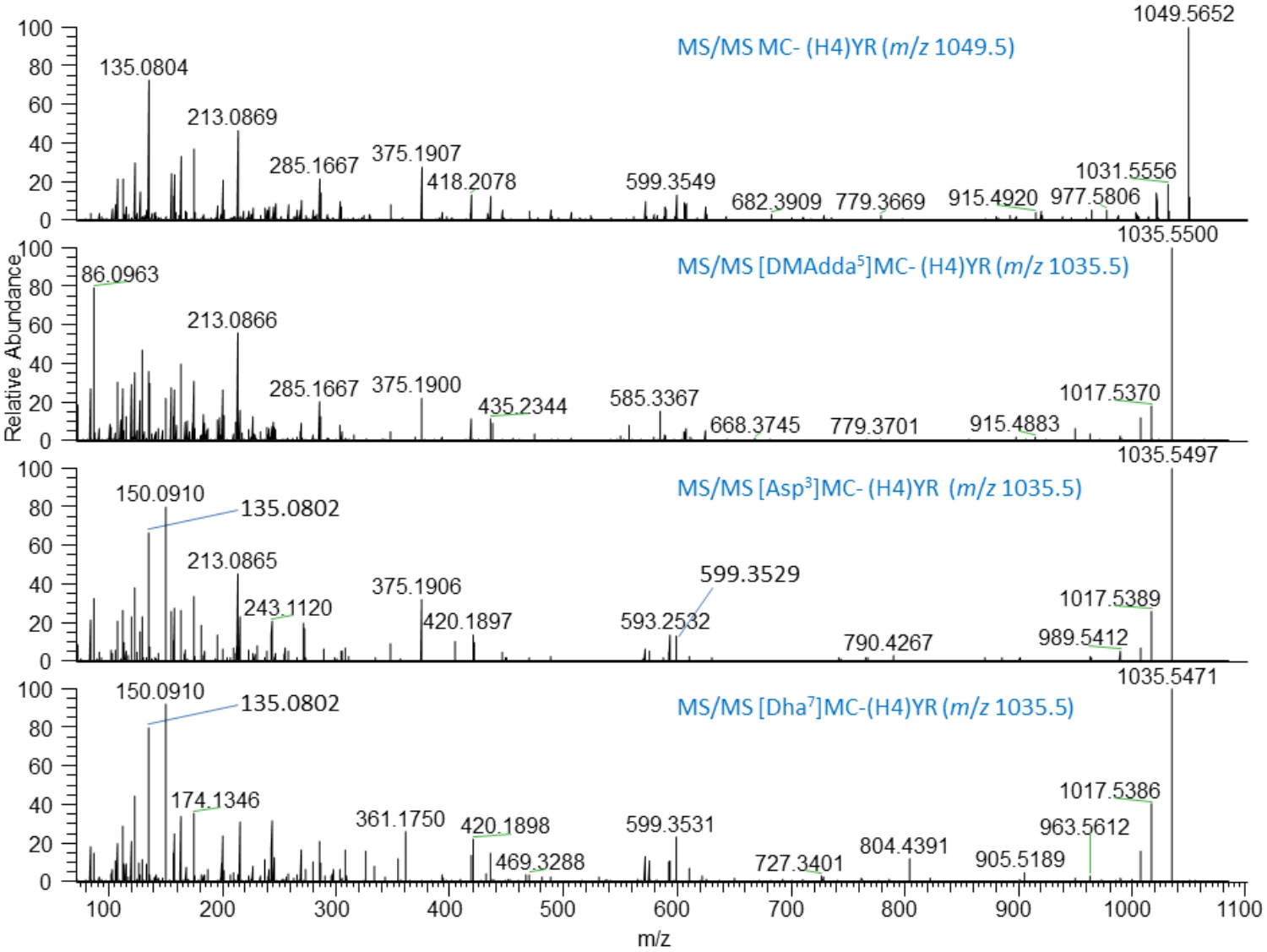

Figure S25 LC-HRMS/MS spectra (positive mode) of MC-(H4)YR (**13**) (*m/z* 1049.5) and the three major dmMC-(H4)YR congeners (**2**, **12** and **16**) (*m/z* 1035.5), from the HP-20 concentrate from Station 2 (6 August 2015).

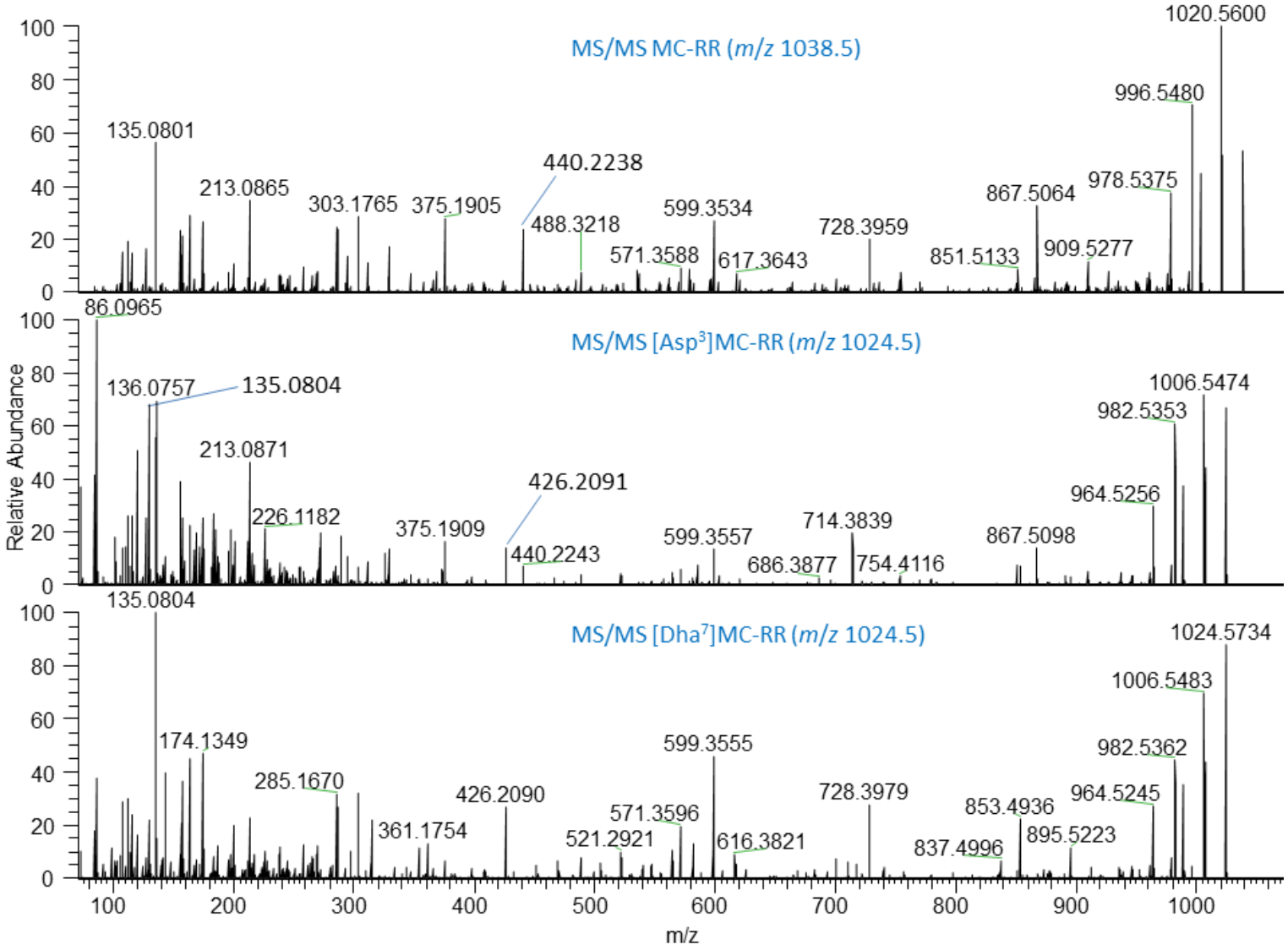

Figure S26 LC-HRMS/MS spectra (positive mode) of MC-RR (**9**) (*m*/*z* 1038.5) and the two major dmMC-RR congeners (**7** and **10**) (*m*/*z* 1024.5), from the HP-20 concentrate from Station 2 (6 August 2015).

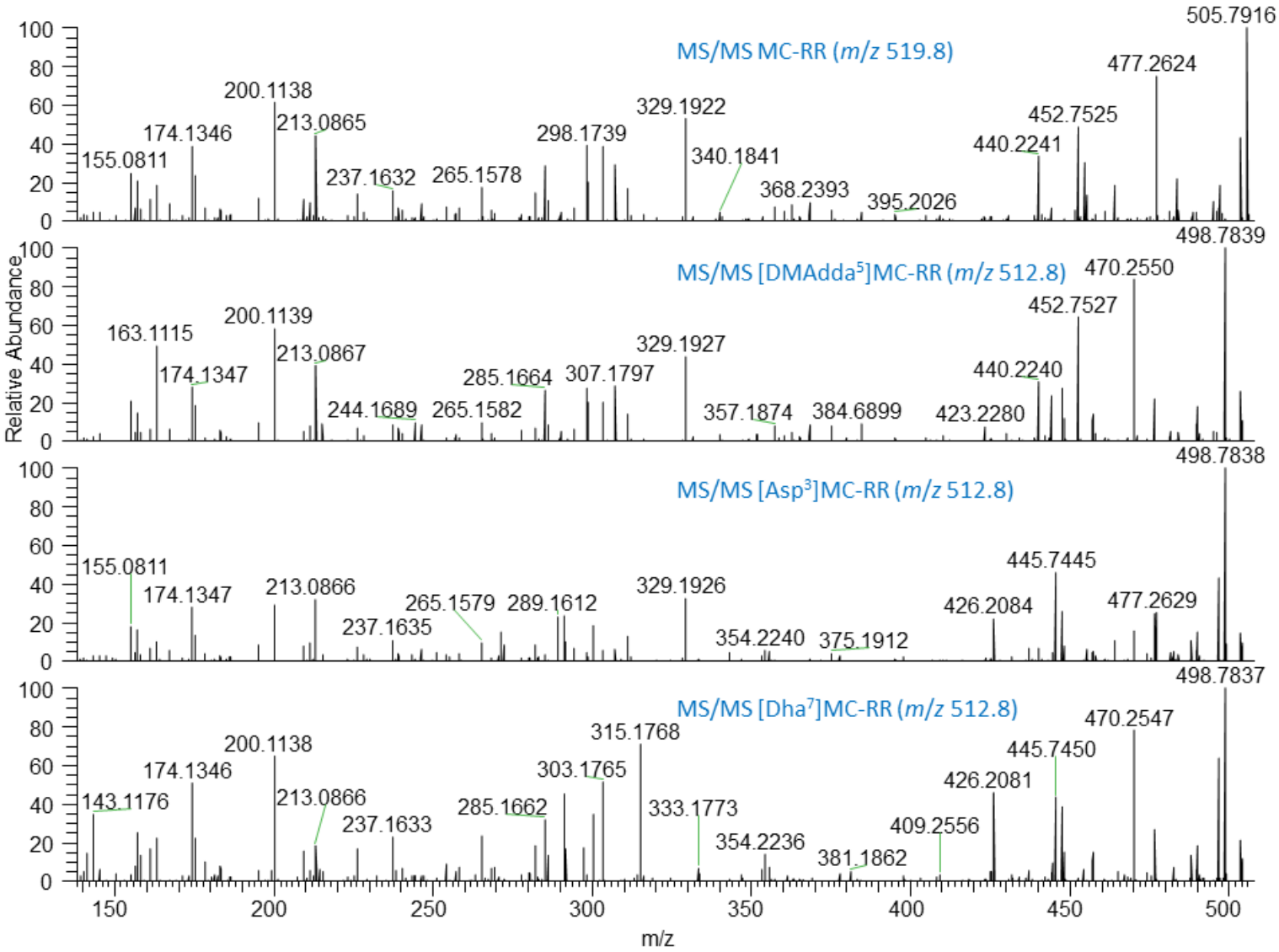

Figure S27 LC-HRMS/MS spectra (positive mode) of MC-RR (**9**) (*m/z* 519.8) and the three major dmMC-RR congeners (**1**, **7** and **10**) (*m/z* 512.8), from the HP-20 concentrate from Station 2 (6 August 2015).

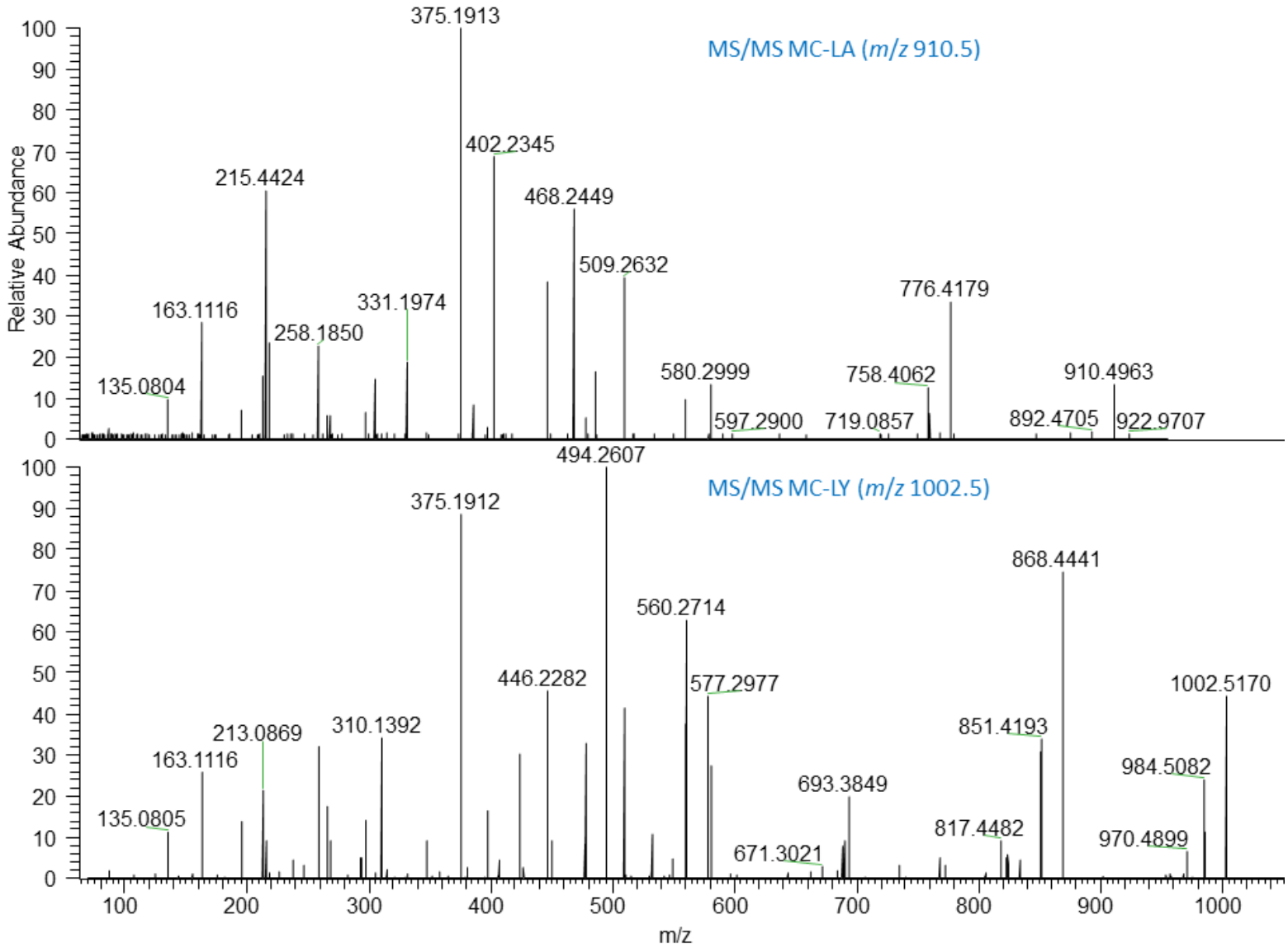

Figure S28 LC-HRMS/MS spectra (positive mode) of MC-LA (**33**) (*m/z* 910.5) and MC-LY (**34**) (*m/z* 1002.5), from the HP-20 concentrate from Station 2 (6 August 2015).

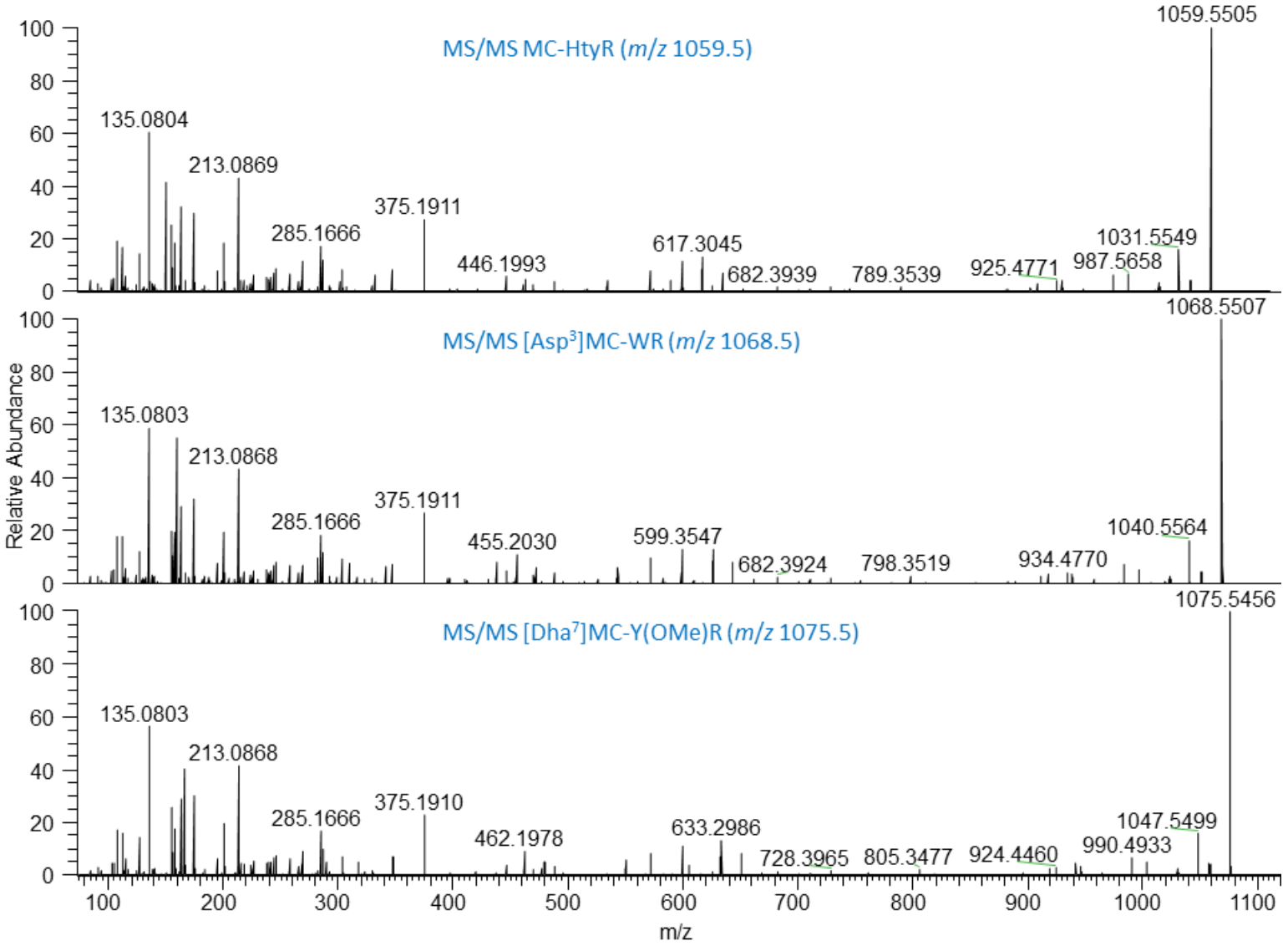

Figure S29 LC-HRMS/MS spectra (positive mode) of MC-HtyR (**23**) (*m/z* 1059.5), MC-WR (**31**) (*m/z* 1068.5) and MC-Y(OMe)R (**24**) (*m/z* 1075.5), from the HP-20 concentrate from Station 2 (6 August 2015).

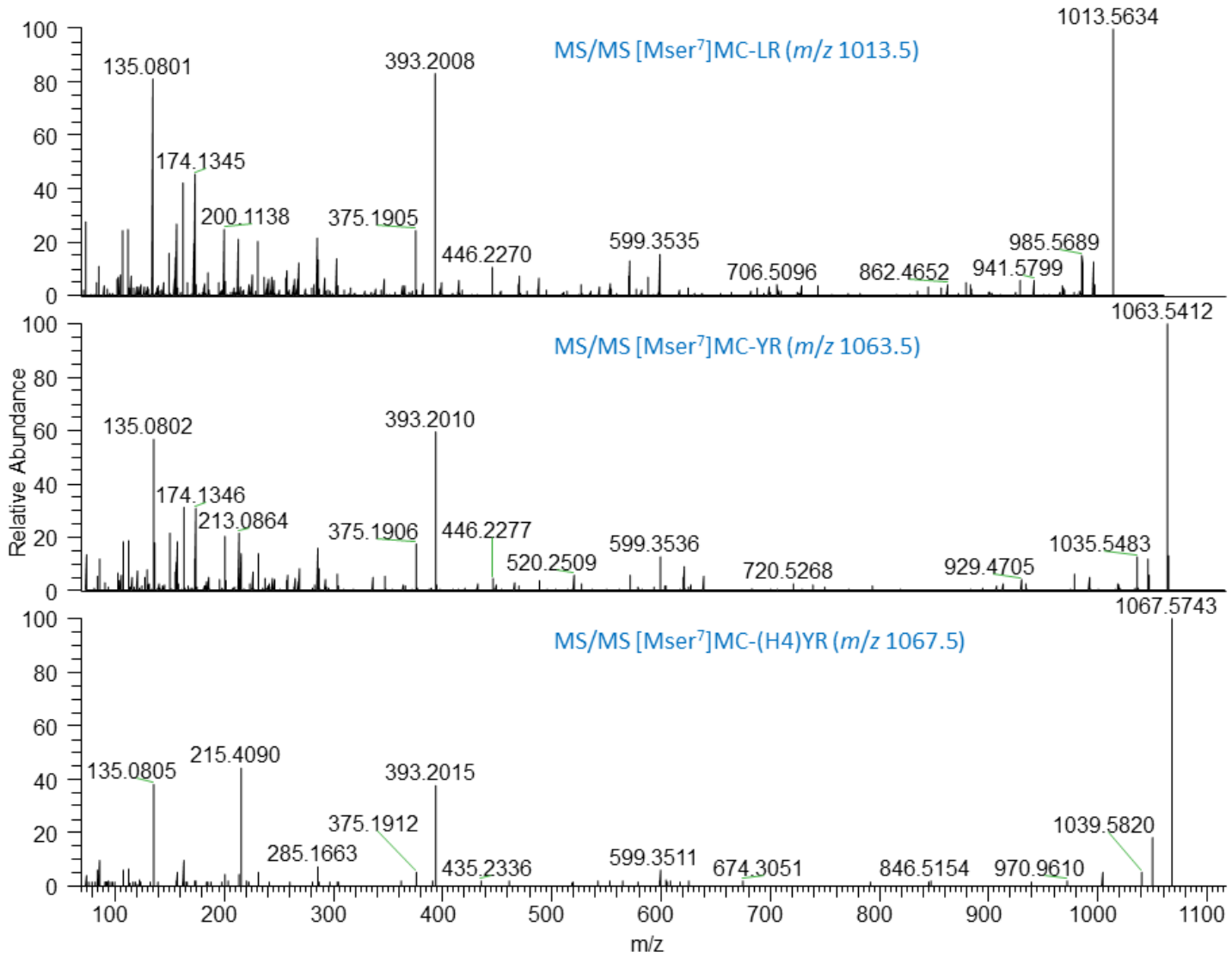

Figure S30 LC-HRMS/MS spectra (positive mode) of [Mser[7]]MC-LR (**18**) (*m/z* 1013.5), [Mser[7]]MC-YR (**14**) (*m/z* 1063.5) and putative [Mser[7]]MC-(H4)YR (*m/z* 1067.5), from the HP-20 concentrate from Station 2 (6 August 2015). Note the low S/N for the latter compound due to its low abundance.

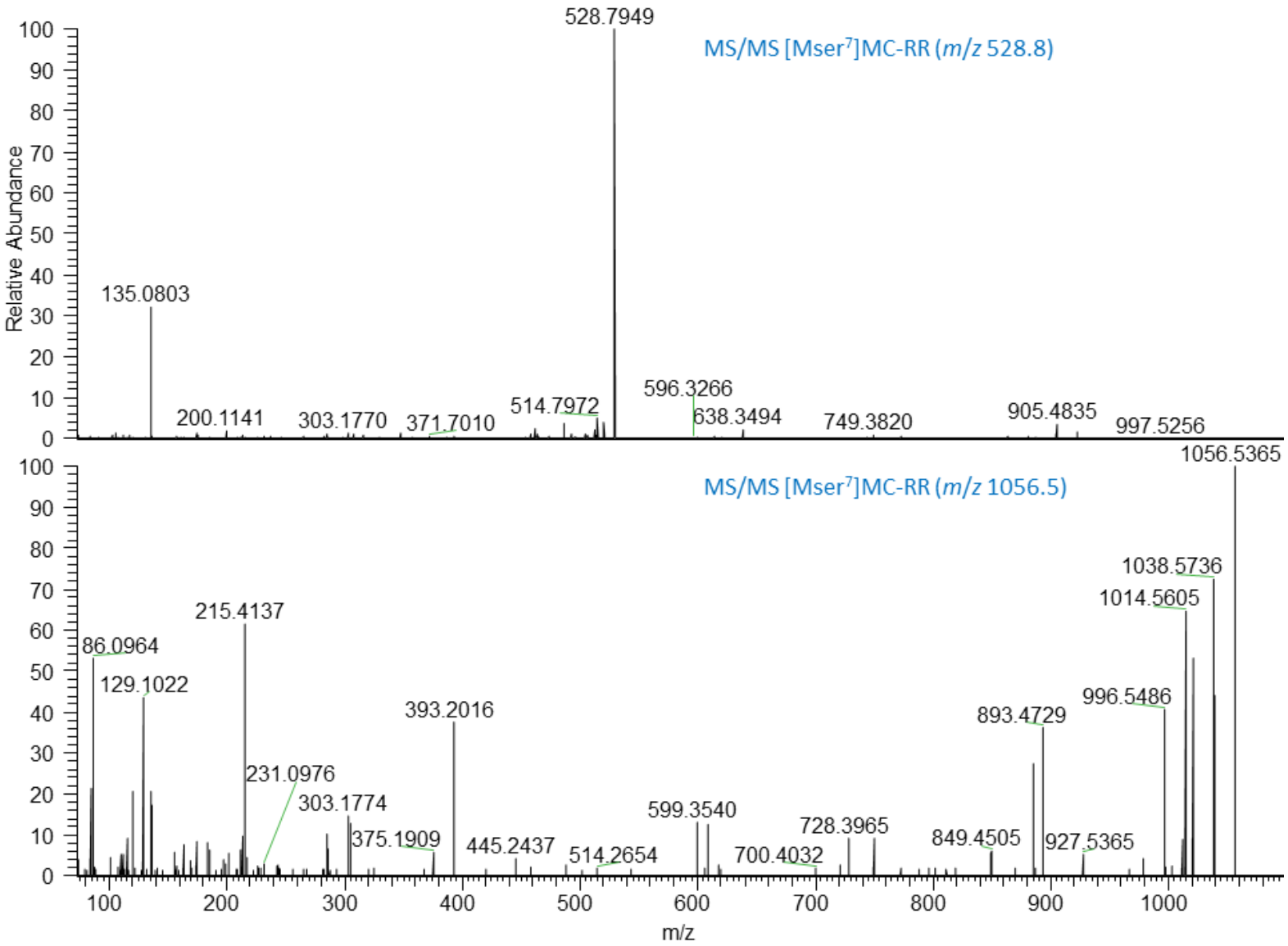

Figure S31 LC-HRMS/MS spectra (positive mode) of [Mser^{7}]MC-RR (**8**) (*m/z* 528.8 and 1056.5), from the HP-20 concentrate from Station 2 (6 August 2015).

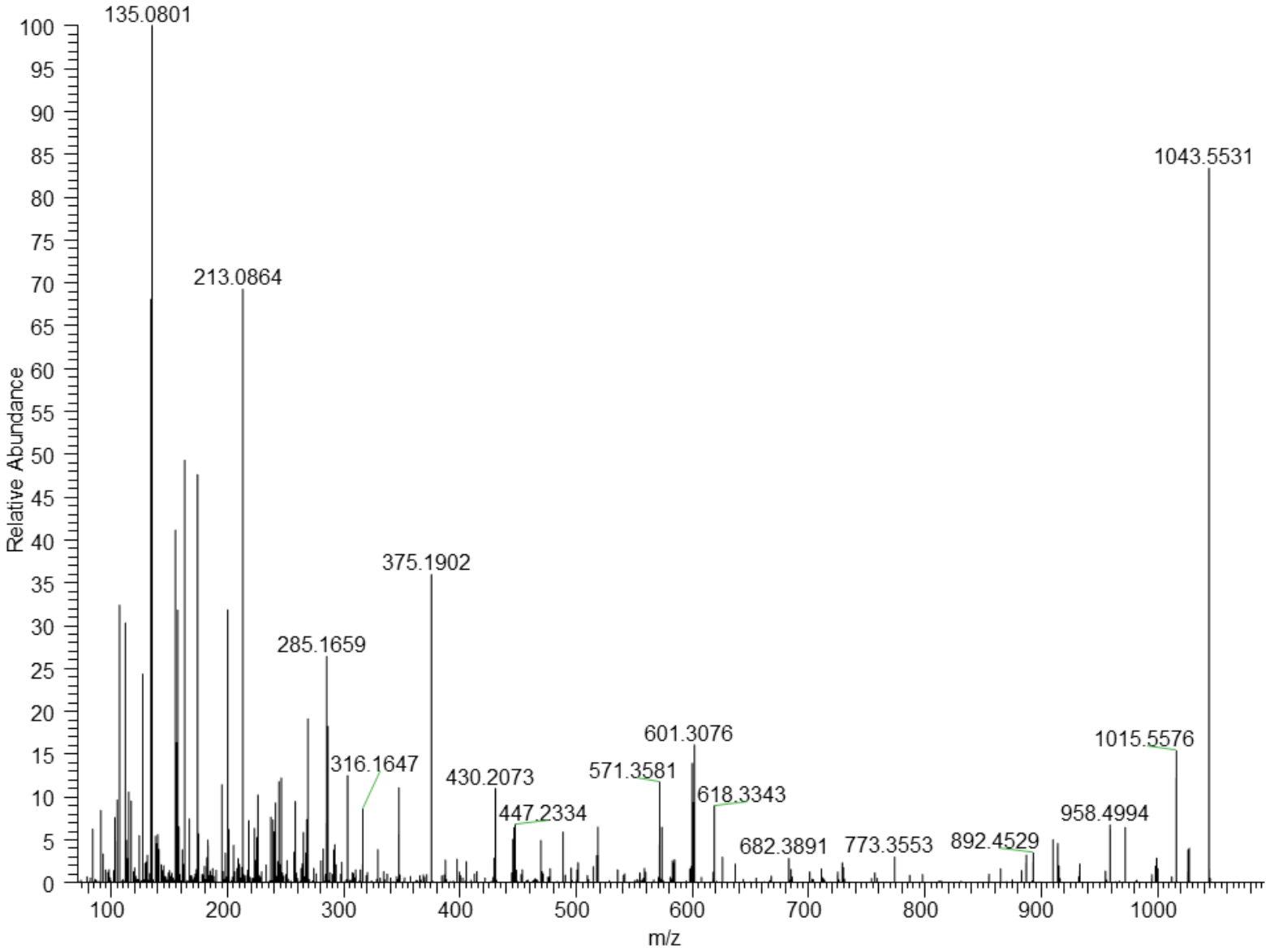

Figure S32 LC-HRMS/MS spectrum (positive mode) of MC-HphR (**32**) (*m/z* 1043.6), from the HP-20 concentrate from Station 2 (6 August 2015).

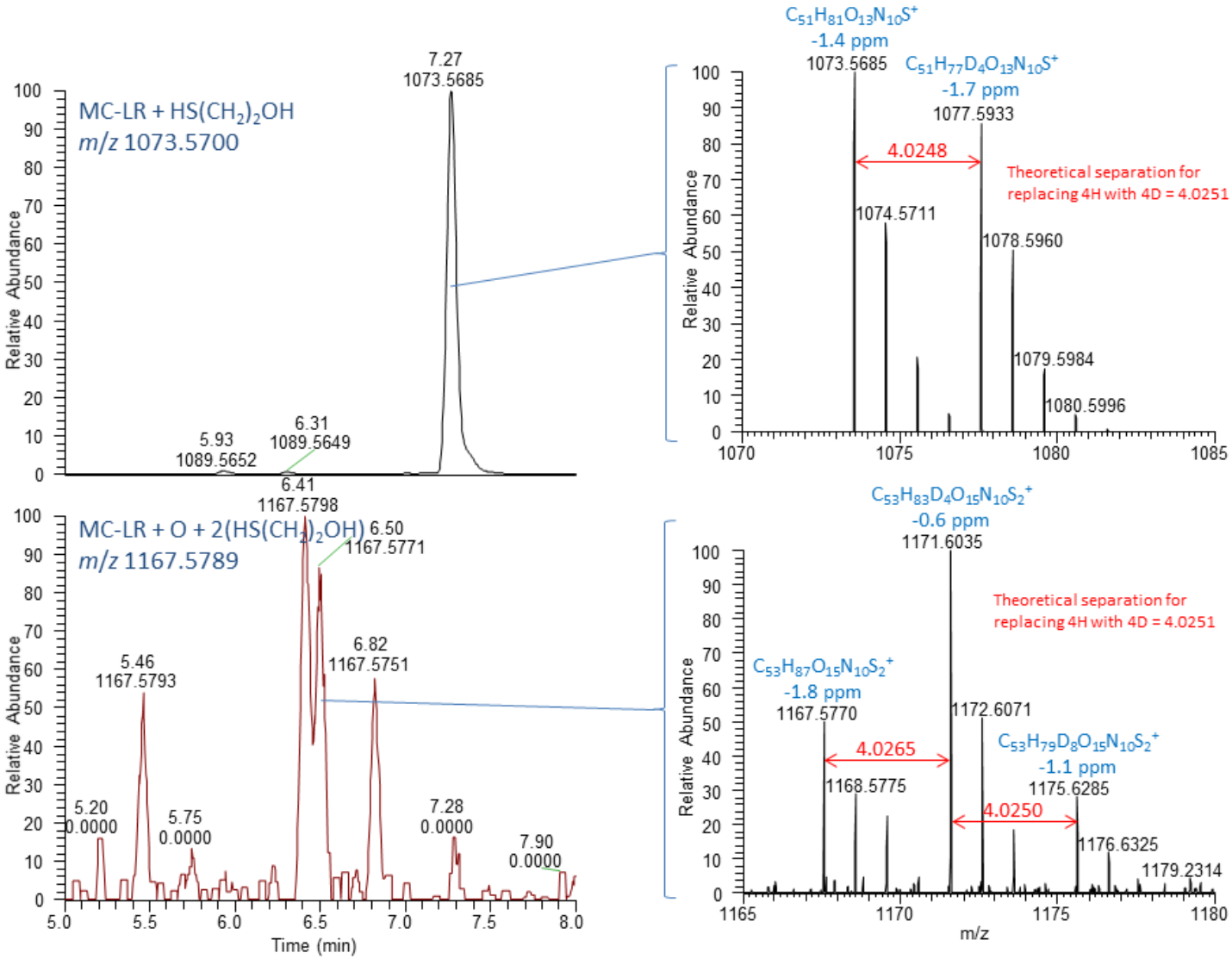

Figure S33 Left, extracted ion LC-HRMS chromatograms (positive mode) at *m/z* corresponding to the d_0-/d_4-mercaptoethanol adduct of MC-LR (top) and the corresponding double adduct of epoxyMC-LR (bottom), from the HP-20 concentrate from Station 2 (6 August 2015). Right, the corresponding mass spectra showing their isotope distributions for $[M+H]^+$ and the calculated atomic compositions.

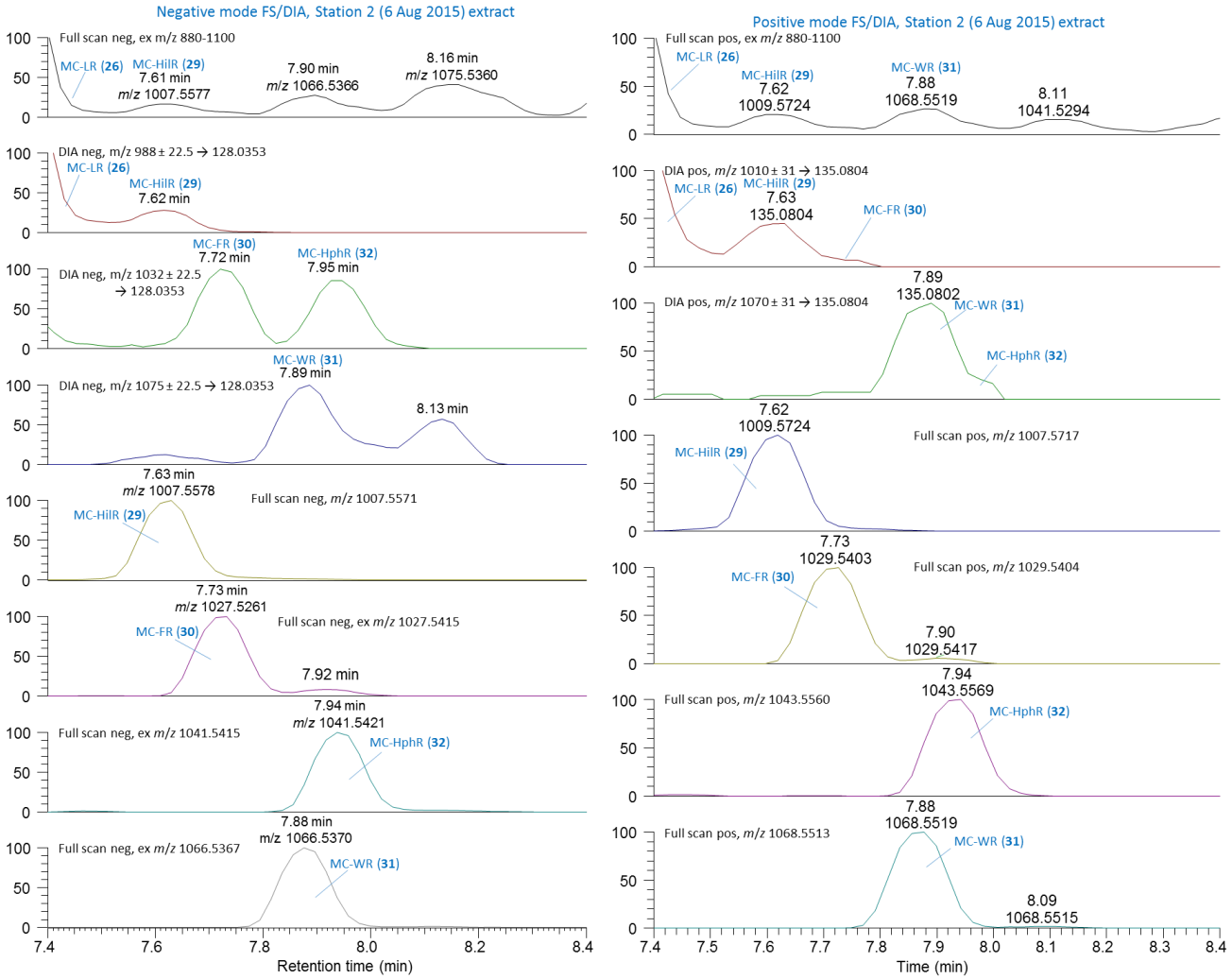

Figure S34 FS/DIA LC-HRMS/MS chromatograms (left, negative mode; right, positive mode) of the HP-20 extract from Station 2 on 6 August 2015. The top panel shows the full scan MS, and the bottom 4 panels show extracted ion chromatograms at *m/z* for **29–32** (± 5 ppm). The panels in between show the DIA chromatograms for mass ranges covering **29–31** extracted for product ions characteristic for microcystins (*m/z* 128.0353 and 135.0804 for positive and negative ionization modes, respectively).

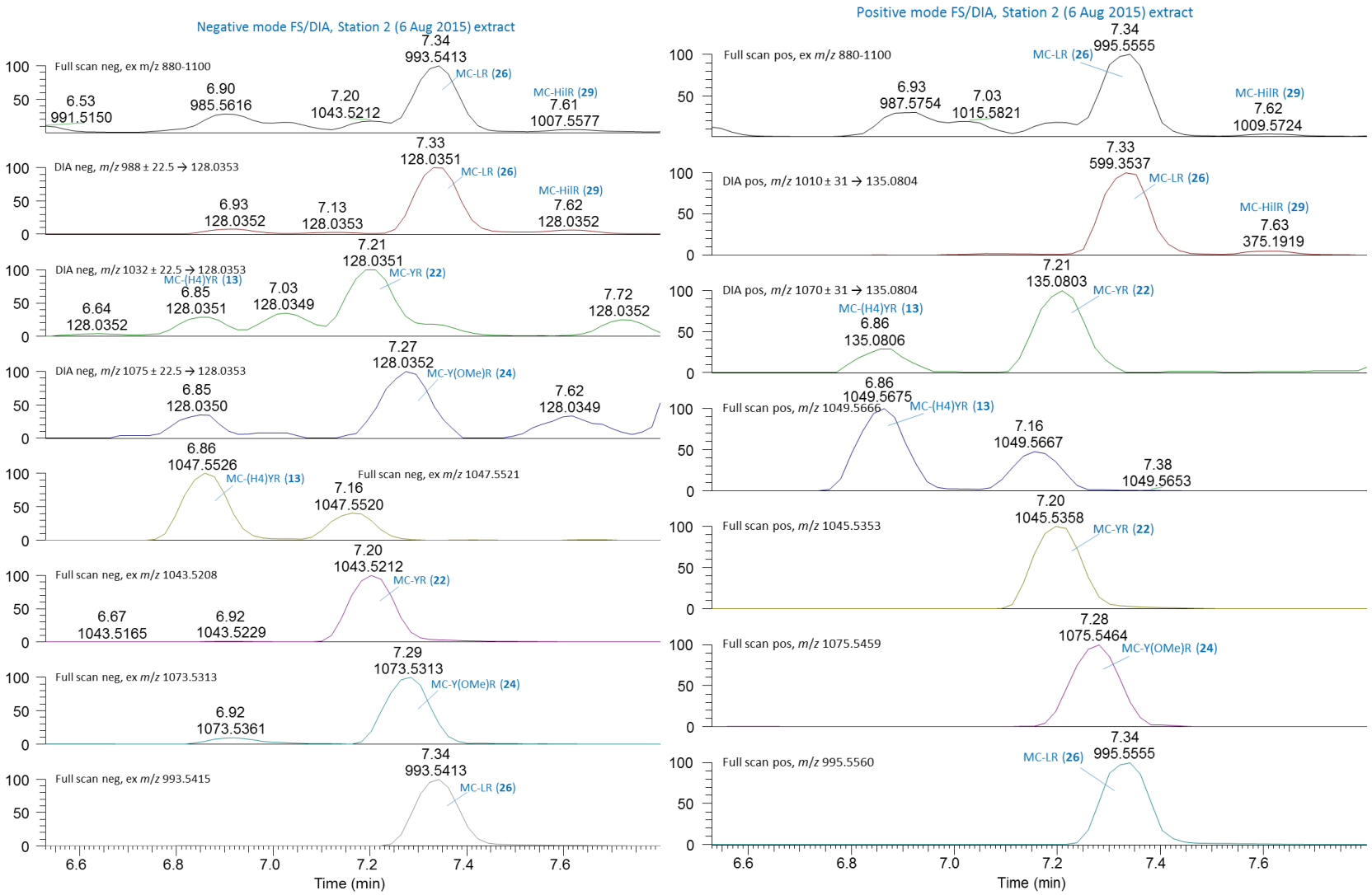

Figure S35 FS/DIA LC-HRMS/MS chromatograms (left, negative mode; right, positive mode) of the HP-20 extract from Station 2 on 6 August 2015. The top panel shows the full scan MS, and the bottom 4 panels show extracted ion chromatograms at *m/z* for **13**, **22**, **24** & **26** (± 5 ppm). The panels in between show the DIA chromatograms for mass ranges covering **13**, **22**, **24** & **26** extracted for product ions characteristic for microcystins (*m/z* 128.0353 and 135.0804 for positive and negative ionization modes, respectively).

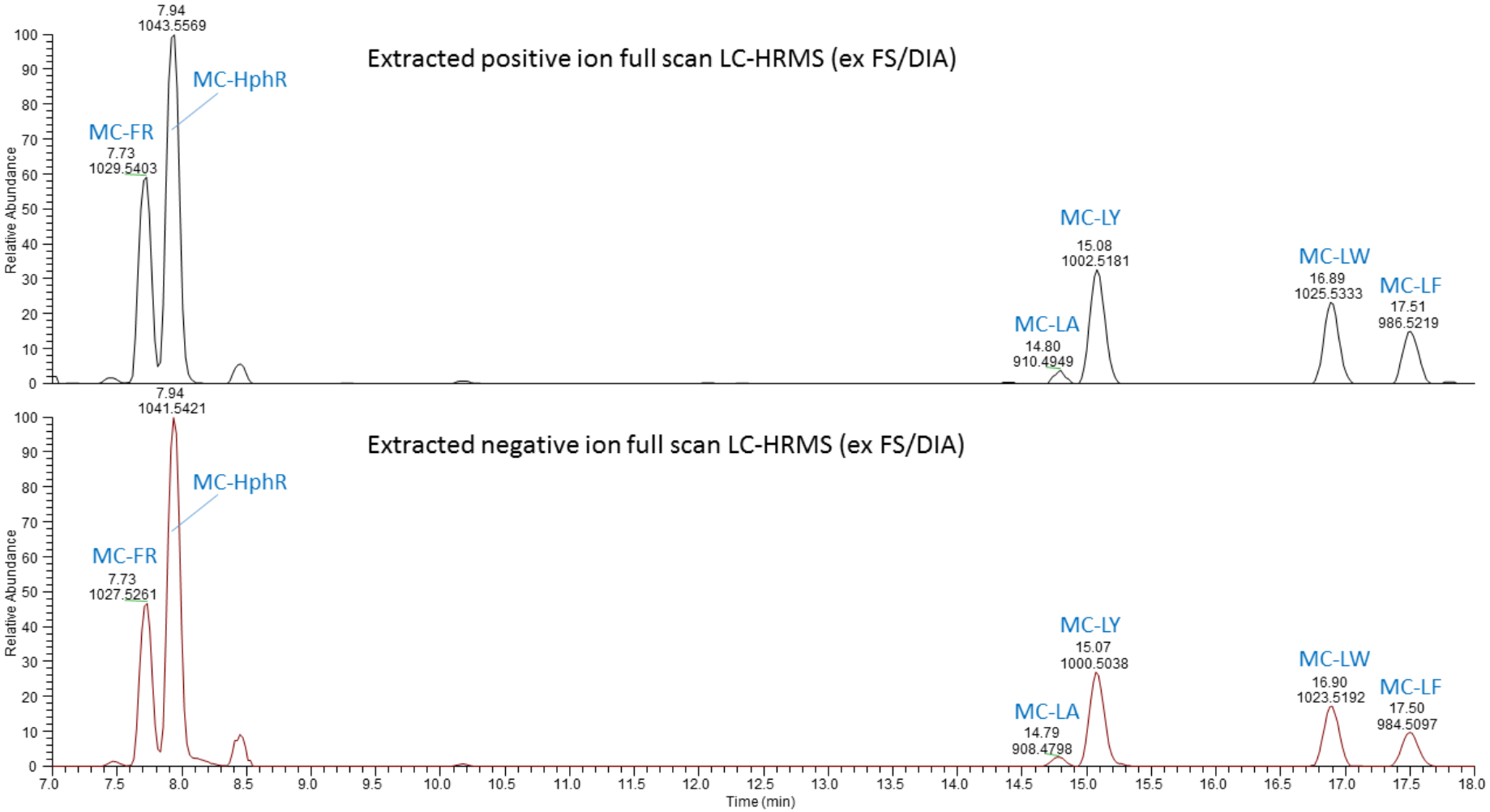

Figure S36 LC-HRMS (method B) full scan chromatograms from FS/DIA analysis of extract from Station 2 (6 August 2015), extracted for the exact *m*/*z* values (± 5 ppm) corresponding to MC-FR (**30**), MC-HphR (**32**), MC-LA (**33**), MC-LY (**34**), MC-LW (**35**) and MC-LF (**36**). The top panel shows the positive extracted ion chromatogram, while the lower panel shows the corresponding negative ion chromatogram. Peaks are labeled with the compound name, retention time (min), and the measured value of *m*/*z* (*z* = 1 in all cases).