

Supplementary Materials: A Data-Independent Methodology for the Structural Characterization of Microcystins and Anabaenopeptins Leading to the Identification of Four New Congeners

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Microcystins							Diminutive	Name
AA1	X	AA3	Z	AA5	AA6	AA7		
Ala	Leu	MeAsp	Arg	Adda	Glu	Mdha	6(Z)-Adda	6(Z)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid
Leu	Arg	Asp	Glu(OMe)	ADMAdda	Glu(OMe)	Dha	(H4)Tyr	1,2,3,4-tetrahydroxytyrosine
Ser	Tyr		Ala	6(Z)-Adda	Glu(OC3H7)	Dhb	(Z)-Dhb	Dehydrobutyric acid
Gly	Hty		Tyr	DMAdda		L-Ser	Aba	Amino-isobutyric acid
MeAla	Glu(OMe)		Har			L-MeSer	AcSer	Acetylserine
MeLeu	Phe		Phe			(Z)-Dhb	Adda	3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (2S,3S,8S,9S)-
Met	Glu		Trp			L-MeLan	ADM Adda	3-amino-9-acetoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid
Met(O)	Hil		Leu			Mdhb	Aha	Amino heptaonic acid
Phe	Trp		Aba			Thr	Ala	Alanine
(H4)Tyr			Met				Apa	Aminopenicillanic acid
							Arg	Arginine
							Asp	Aspartic acid
							BhTrp	Bromo hydroxy tryptophan
							BrMeTrp	Bromo methyl tryptophan
							BrTrp	Bromo triptophan
							Bu	Butyric Acid
							Cl,MeTrp	Chloro methyl tryptophan
							Dha	Dehydroalanine
							Dhb	Dehydrobutyric acid
							DM Adda	Desmethyl-Adda
							EtPh	Ethyl homophenylalanine
							Glu	Glutamic acid
							Glu(OC3H7)	Glutamate ethyl ester
							Glu(OMe)	Glutamate methyl ester
							Gly	Glycine
							Har	Homoarginine
							Hil	Homoisoleucine
							Hph	Homophenylalanine
							hTrp	Hydroxy tryptophan
							Hty	Homotyrosine
							Hty(OMe)	Homotyrosine methyl ester
							Ile	Isoleucine
							Leu	Leucine
							L-MeLan	N -methyl-lanthionine
							Lys	Lysine
							M dha	N-methyl-dehydroalanine
							M dhb	Methyl-dehydrobutyric acid
							MeAphfa	N -methyl-2-amino-6-(4'-hydroxyphenyl)hexanoic acid
							MeAla	Methyl alanine
							MeAsn	Methyl asparagine
							MeAsp	Methyl aspartic acid
							MeCht	Chloro hydroxy methyltryptophan
							MeGly	Methyl glycine
							MeHph	Methyl homophenylalanine
							MehTrp	Hydroxy methyltryptophan
							MeHty	Methyl homotyrosine
							Melle	Methyl isoleucine
							MeLeu	Methyl leucine
							MeSer	Methyl serine
							Met	Methionine
							Met(O)	Methionine sulfoxide
							Met(O2)	Methionine sulfides
							Phe	Phenylalanine
							Ser	Serine
							Trp	Tryptophan
							Thr	Threonine
							Tyr	Tyrosine
							Tyr(OMe)	Methyl tyrosinate
							Val	Valine

Figure S1. Configuration of amino acids (AA) in MCs and APs.

Table S1. Details on samples with sampling date and region of sampling in Canada and Europe.

Sample No.	Sampling Date	Region of Sampling
1	07-2017	Durham, ON
2	08-2017	Laurentides, QC
3	08-2017	York, ON
4	08-2017	Simcoe County, ON
5	08-2017	Wentworth County, ON
6	08-2017	Chaudière-Appalaches, QC
7	08-2017	Chaudière-Appalaches, QC
8	08-2017	Montérégie, QC
9	08-2017	Digby County, NS
10	09-2017	Chaudière-Appalaches, QC
11	08-2017	United Kingdom
12	09-2017	France

Table S2. Confidence of identification by levels and number of features obtained at each step of identification using Compound Discoverer 3.0 software.

Sample No.	Features	Exact Mass (Level 5)		Isotopes, Adducts, RT ¹ , Molecular Formula, SD ² (Level 3)		Distinctive Fragments (Level 3)		Confirmed with Fragmentation (Level 2)	
		MCs	APs	MCs	APs	MCs	APs	MCs	APs
1	4,28 6	23	53	14	20	4	0	2	0
2	4682	107	83	47	41	12	1	2	0
3	5983	65	101	37	51	17	5	6	0
4	4212	45	78	21	28	3	6	2	0
5	4370	54	95	28	32	6	8	1	5
6	3173	47	60	3	24	3	3	0	2
7	3245	52	65	29	19	9	1	1	1
8	4027	116	92	34	26	7	2	2	0
9	4171	51	74	23	21	11	1	1	0
10	2960	38	49	18	21	10	2	1	1
11	1576	12	42	9	19	5	8	5	6
12	5909	102	104	18	38	10	7	7	3

¹ Retention time ² Standard deviation.

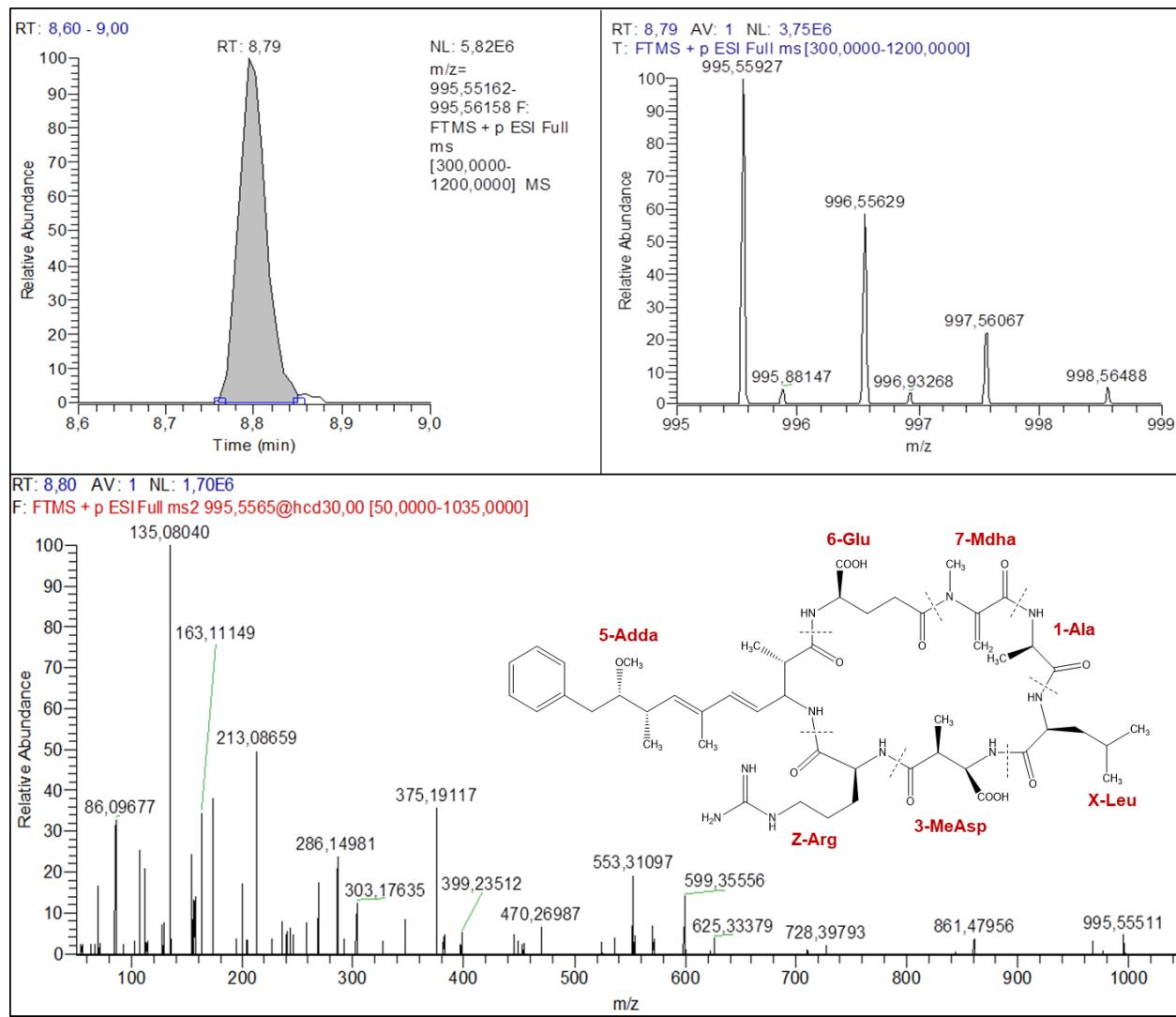


Figure S2. Chromatogram, isotopic pattern and fragmentation spectra of MC-LR with RT at 8.79 min.

Table S3. Number of MCs combinations using fragmentation spectra and identification of amino acids.

Identification ¹	[GluOMe ⁶]MC-LR		[M(O) ¹]MC-LR		[M(O) ¹ , GluOMe ⁶]MC-LR		[Asp ³]MC-RHar		[Leu ¹ , Ser ⁷]MC-HtyR	
	Comb. ²	Amino Acids ³	Comb.	Amino Acids	Comb.	Amino Acids	Comb.	Amino Acids	Comb.	Amino Acids
Exact mass	1316		856		823		934		482	
ADDAA	878	ADDAA (6Z)Adda	434	ADDAA (6Z)Adda	408	ADDAA (6Z)Adda	636	ADDAA (6Z)Adda	300	ADDAA (6Z)Adda
Amino acid 6	322	GluOMe	142	Glu	142	GluOMe	170	Glu	160	Glu
Z	82	Arg	42	Arg	42	Arg	28	Har	48	Arg
Amino acid 3	42	MeAsp	20	MeAsp	20	MeAsp	18	Asp	24	MeAsp
X	10	Leu	6	Leu	6	Leu	10	Arg	4	Hty
Amino acid 1	6	Ala	6	M(O)	6	M(O)	6	Ala	2	Leu
		Mdha		Mdha		Mdha		Mdha		
Amino acid 7	6	Dhb	6	Dhb	6	Dhb	6	Dhb	2	Ser
		(Z)Dhb		(Z)Dhb		(Z)Dhb		(Z)Dhb		

¹ Identification level: exact mass and different amino acids identified using the MS/MS spectra.² Number of possible MCs combinations after each level of identification. ³ Identified amino acids at each site of the MC structure.

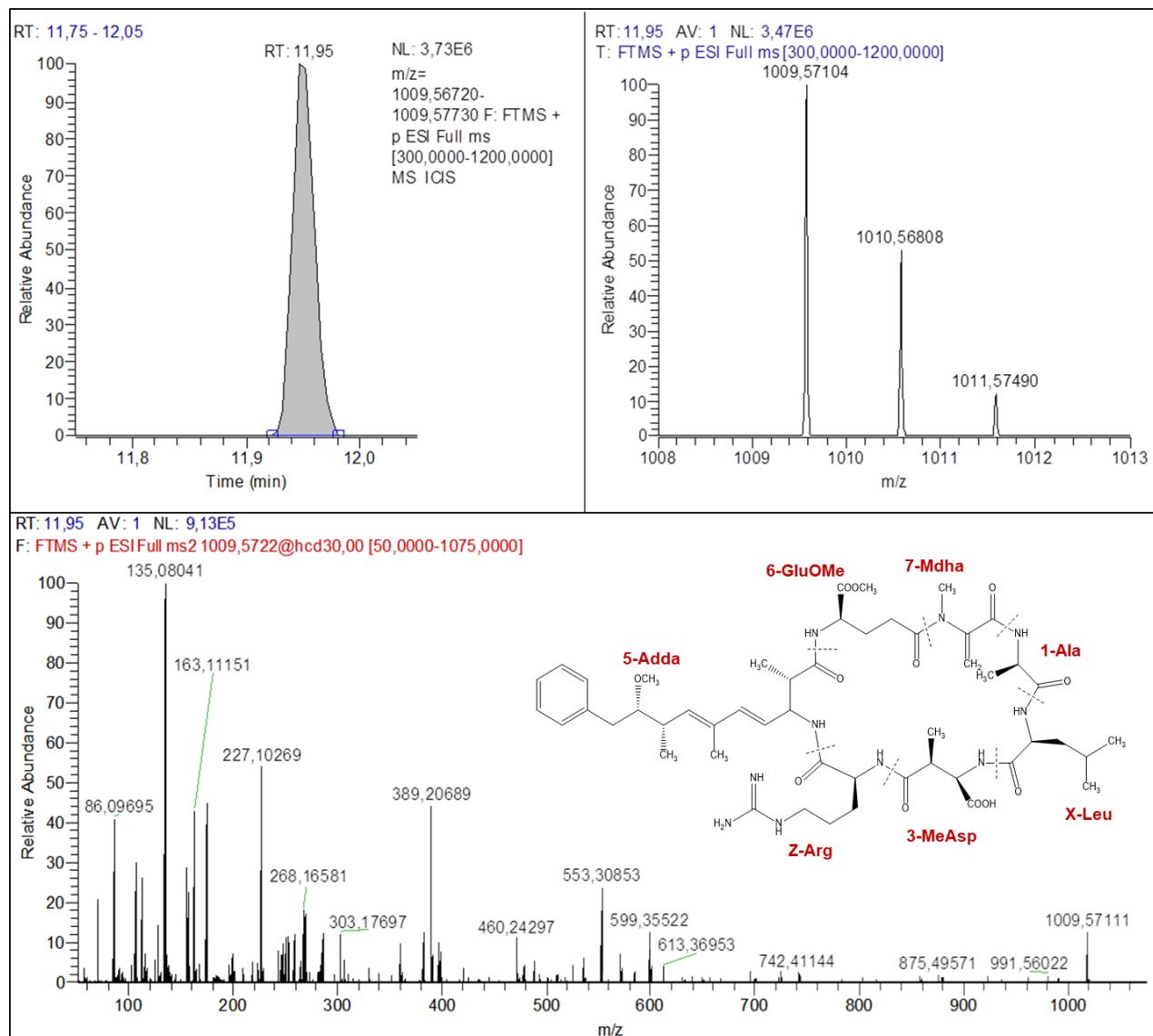


Figure S3. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 1009.57104 identified as [GluOMe⁶]MC-LR with RT at 11.95 min.

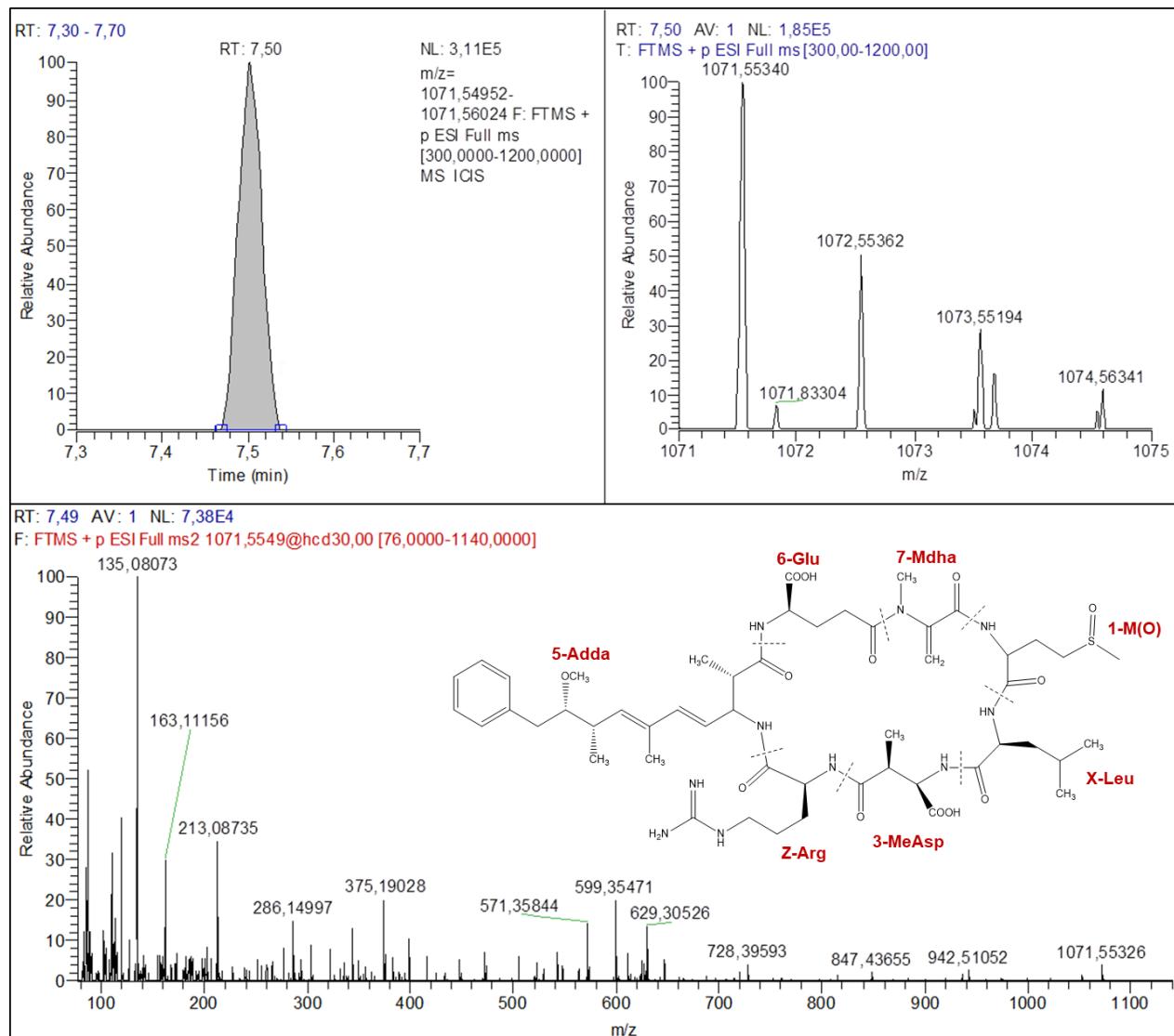


Figure S4. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 1071.55340 identified as $[M(O)^1]MC-LR$ with RT at 7.50 min.

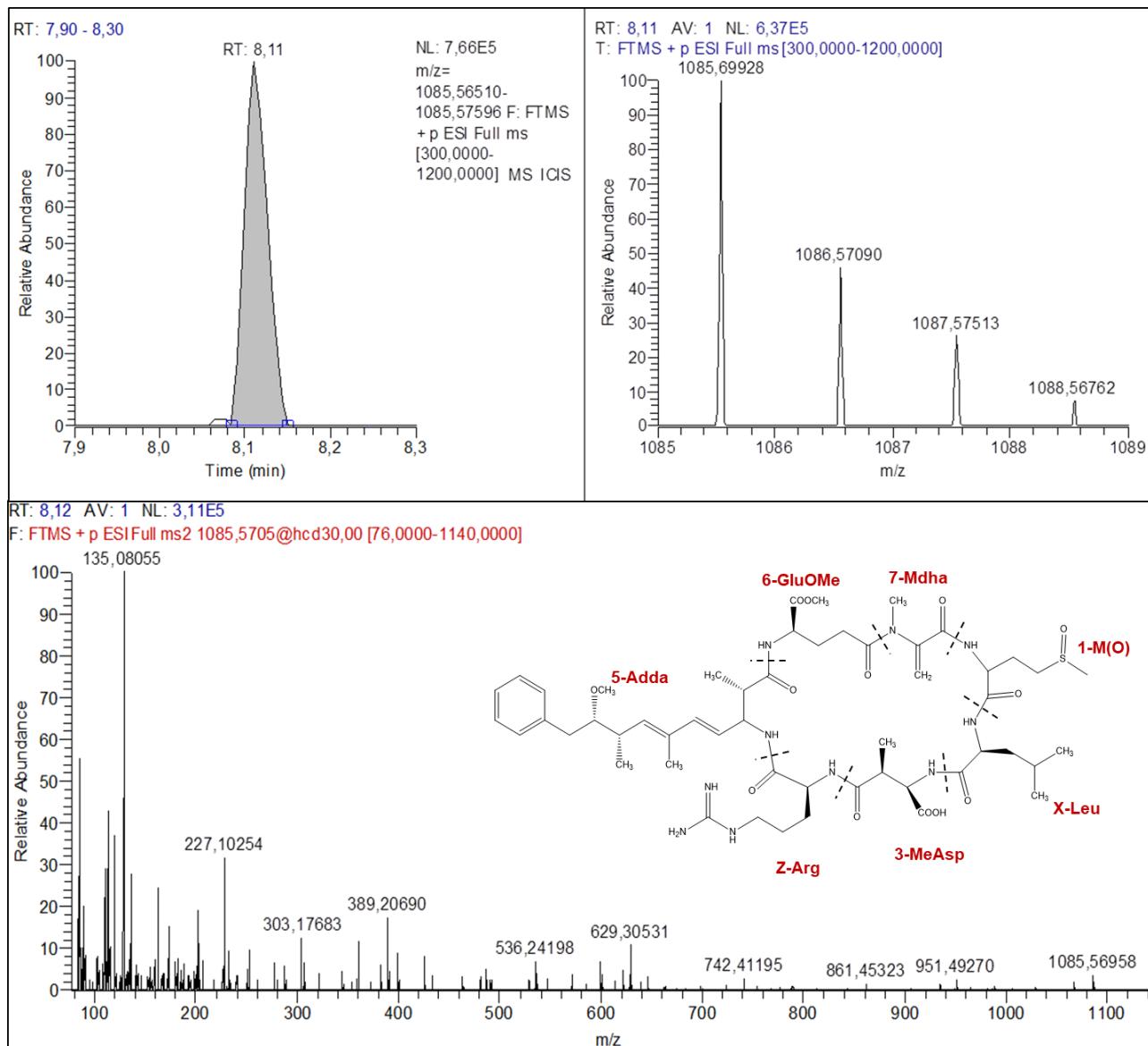


Figure S5. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 1085.56928 identified as $[M(O)^1, \text{GluOMe}^6]\text{MC-LR}$ with RT at 8.11 min.

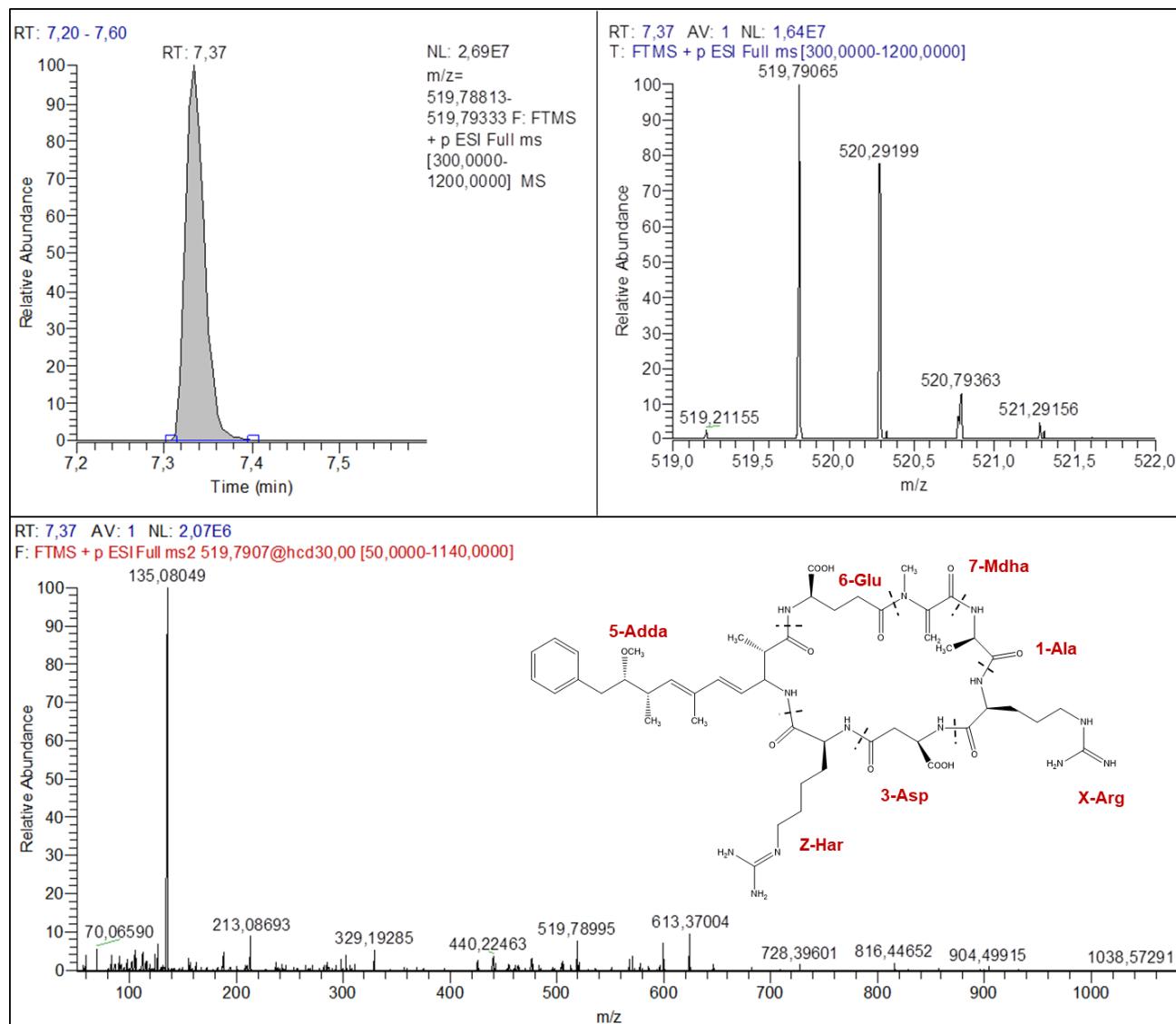


Figure S6. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 1038.57291 identified as $[\text{Asp}^3]\text{MC-RHar}$ with RT at 7.37 min.

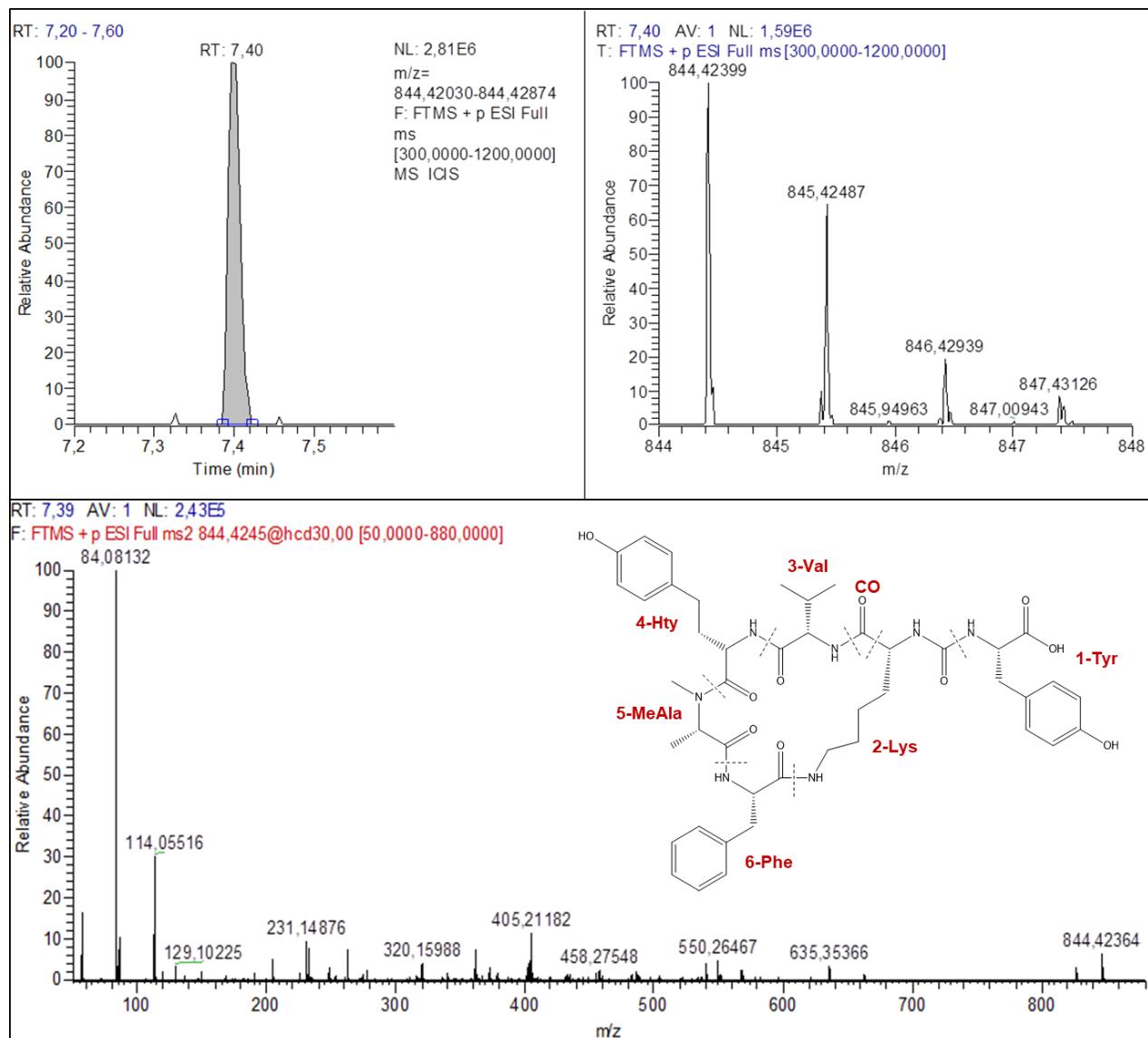


Figure S7. Chromatogram, isotopic pattern and fragmentation spectra of AP-A with RT at 7.40 min.

Table S4. Number of AP combinations at each level of identification using fragmentation spectra and identification of amino acids.

Identification	AP-C		AP-F		Ferintoic acid A		Oscillamide Y		AP731		AP803	
	Comb. ¹	Amin o Acids ³	Comb	Amin o Acids	Comb	Amin o Acids	Comb	Amin o Acids	Comb	Amin o Acids	Comb	Amin o Acids
	2	3
Exact mass	13		56		31		43		5		11	
Amino acid 1	13	Leu Ile	26	Arg	13	Trp	26	Tyr	1	Phe	8	Leu Ile
Amino acid 3	2	Val	2	Leu Ile	1	Val	2	Leu Ile	1	Val	2	Met

¹ Identification level: exact mass and different amino acids identified using the MS/MS spectra.² Number of possible APs combinations after each level of identification. ³ Identified amino acids at each site of the AP structure.

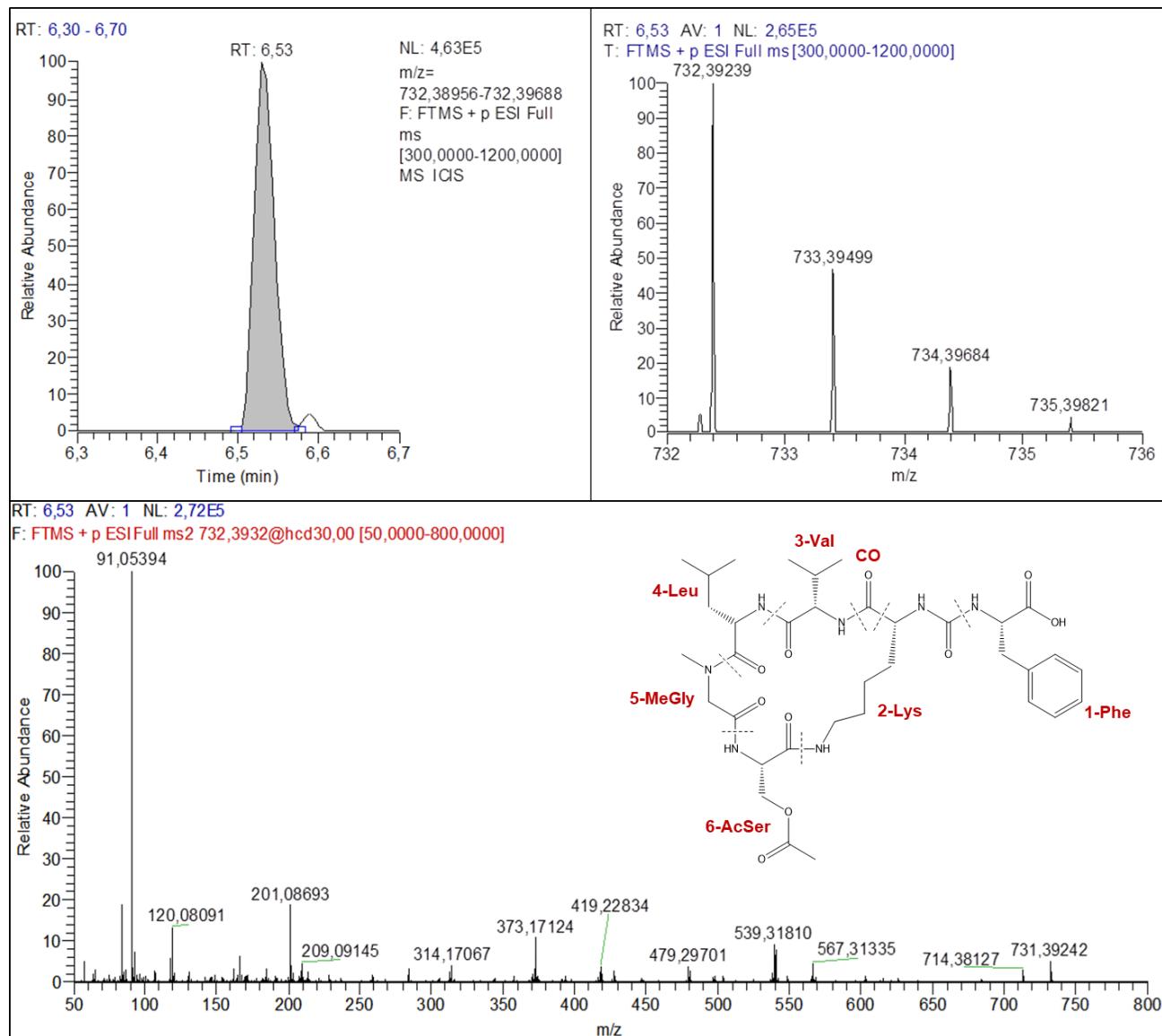


Figure S8. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 732.39224 identified as AP731 with RT at 6.53 min.

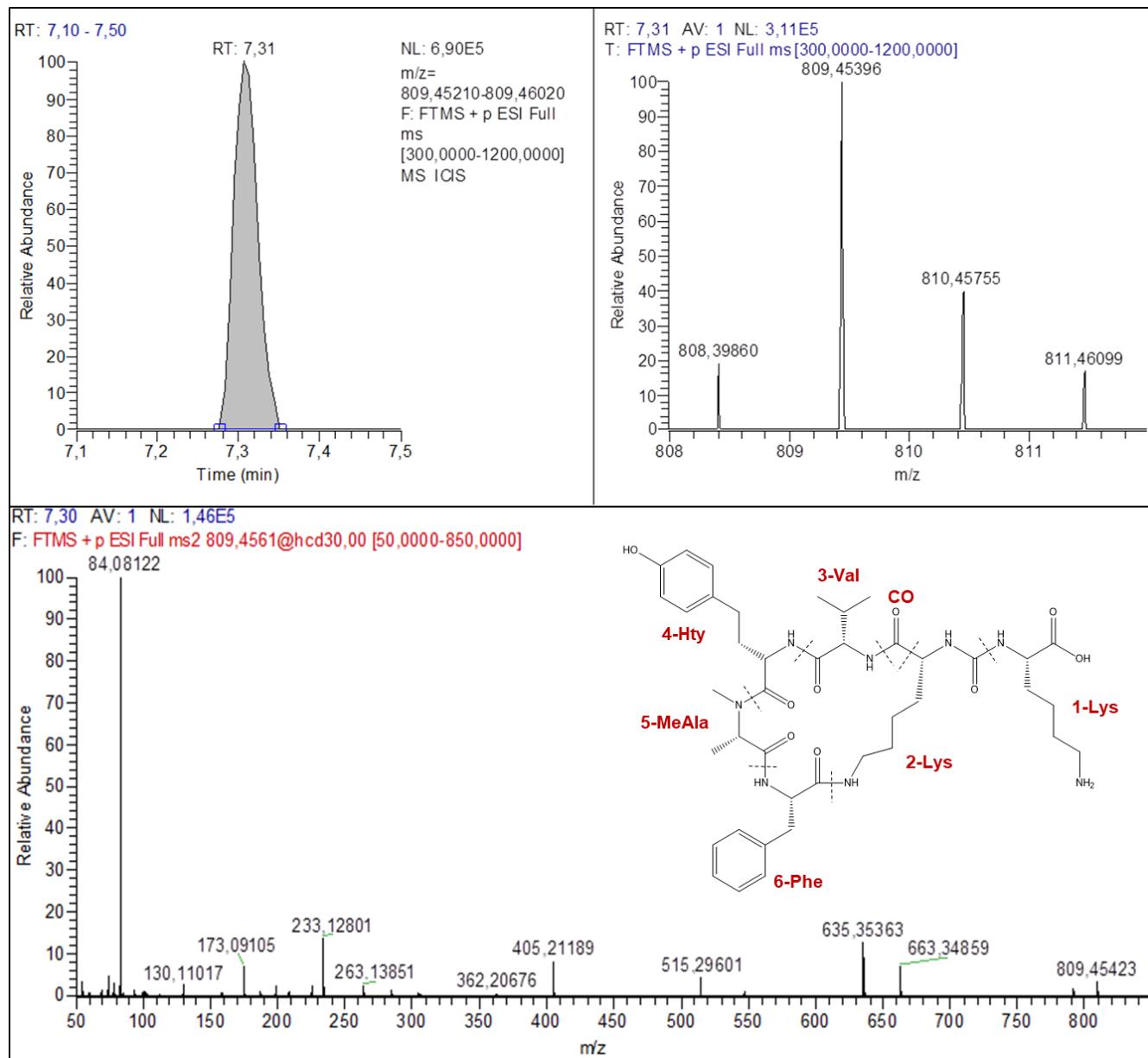


Figure S9. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 809.45396 identified as AP-C with RT at 7.31 min.

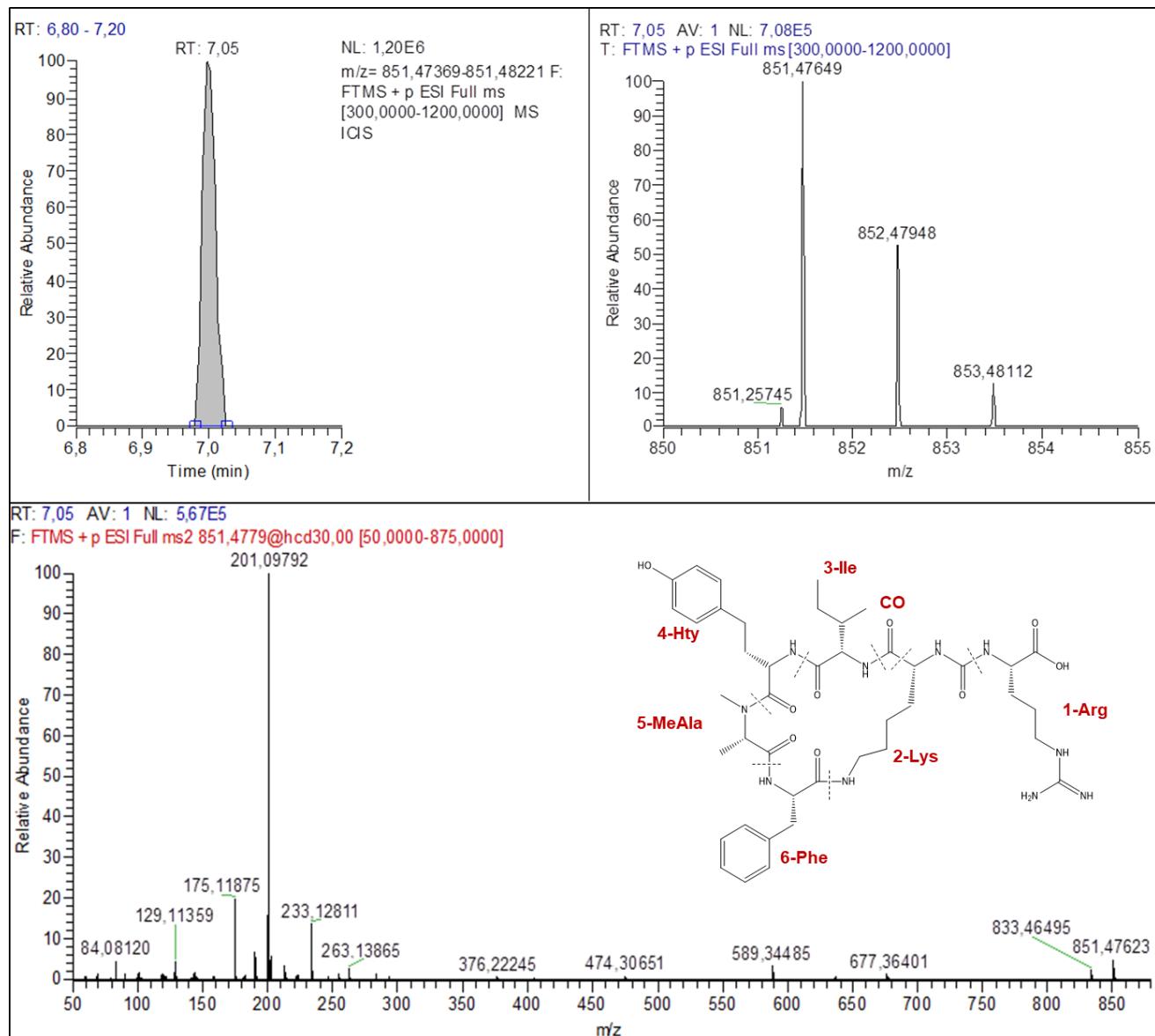


Figure S10. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 851.47649 identified as AP-F with RT at 7.05 min.

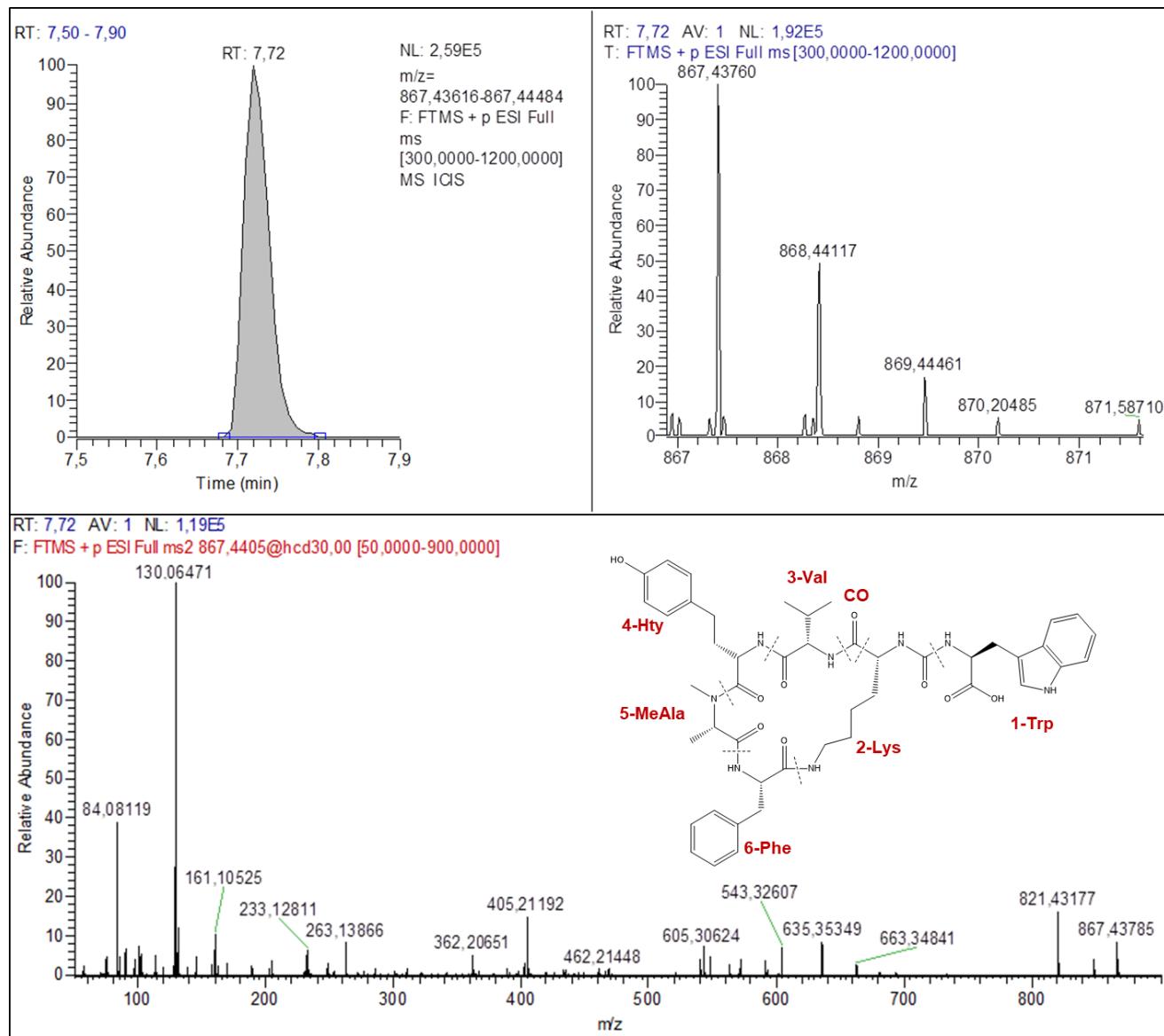


Figure S11. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 867.4376 identified as ferintoic acid A with RT at 7.72 min.

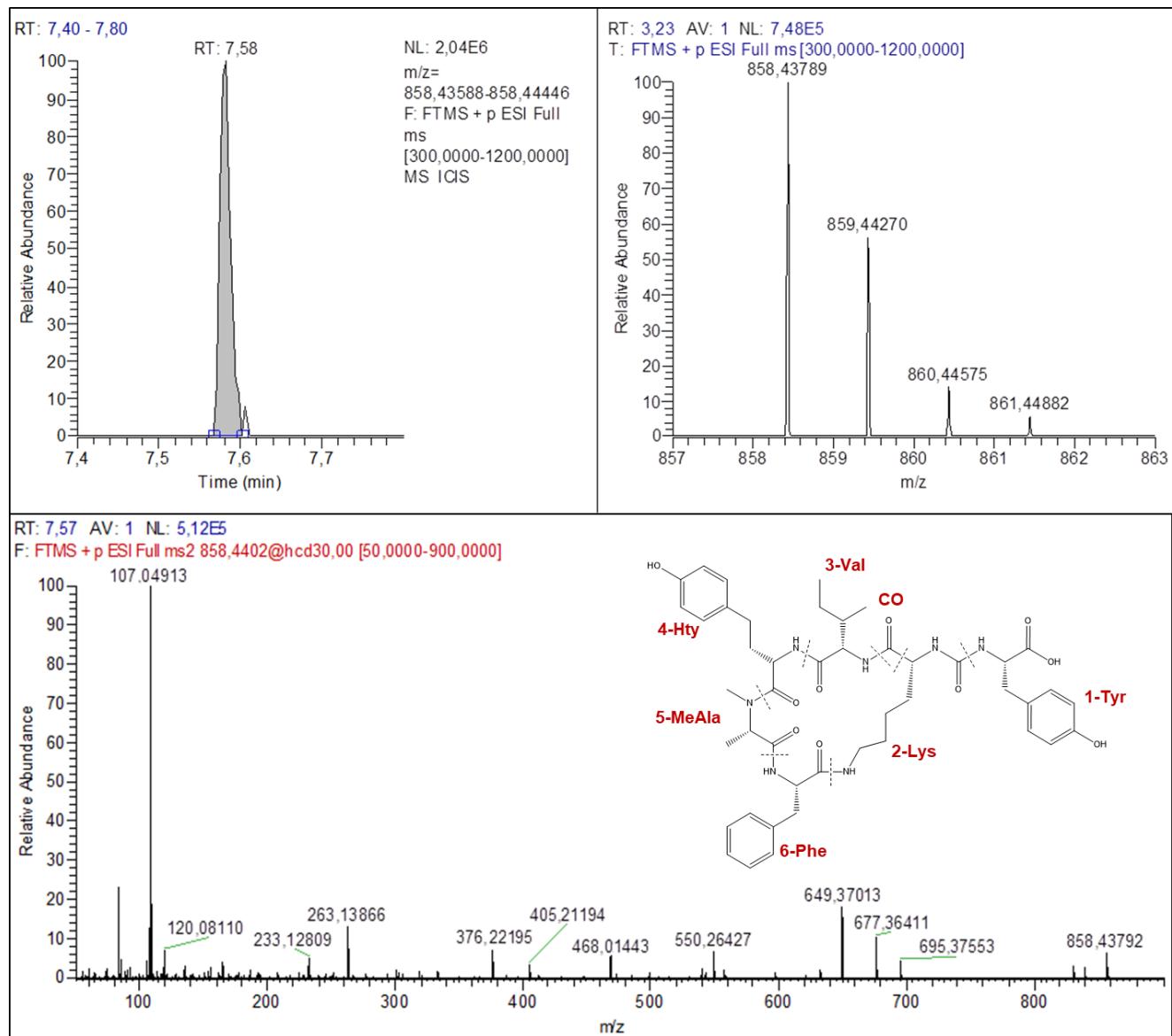


Figure S12. Chromatogram, isotopic pattern and fragmentation spectra of feature m/z 858.43789 identified as oscillamide Y with RT at 7.58 min.

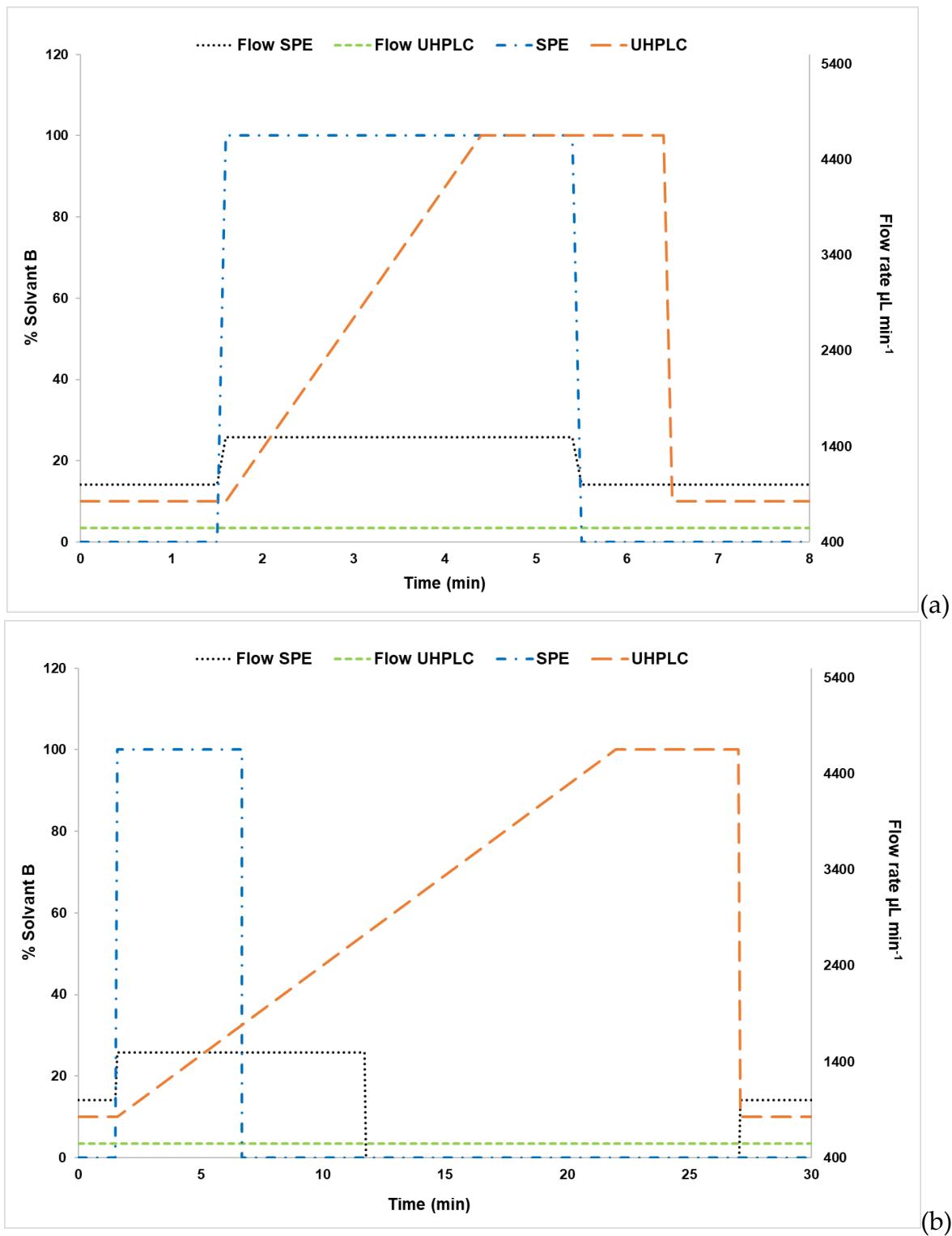


Figure S13. Details on the on-line SPE—UHPLC chromatographic gradient program for quantification (a) and suspect screening methods (b).

Table S5. Ionization and HRMS acquisition parameters.

UHPLC/Ionization.		Target Analysis (Quantification)			Suspect Screening (Identification and Semi-Quantification)		
Spray voltage	+3500 V	Full Scan	PRM	DIA (Full Scan)	DIA (MS/MS)	PRM	
Capillary temperature	350 °C	Scan range (<i>m/z</i>)	150–1200	Inclusion list	300–1400	300–1400 divided in 22 windows	Inclusion list
Vaporizer temperature	250 °C			CYN: 1.5–3.0			
Sheath gaz flow	60 arbitrary unit			ANA-a: 1.5–3.0			
Auxiliary gaz flow	15 arbitrary unit	Scan time (min)	3.0–7.0	HANA-a: 2.0–3.1 MC-LA: 3.7–4.5 MC-LY: 3.7–4.7	1–27	1–27	4–15 (depending of the selected feature)
		Resolving power (FWHM <i>m/z</i> 200)	70,000	17,500	35,000	17,500	17,500
		AGC target (ions)	1×10^6	1×10^5	1×10^6	1×10^5	1×10^5
		Max injection time (ms)	200	50	100	50	50