

Metabolome Variation between Strains of *Microcystis aeruginosa* by Untargeted Mass Spectrometry

Marianne Racine, Ammar Saleem and Frances R. Pick

Table S1. Possible MC variants for the three unknown metabolites from *M. aeruginosa* presented in Table 1. Corresponding molecular weights are expressed in Daltons (Da). List of MC variants from Spoof & Catherine (2017).

MC Variants	[M+H]	Molecular Formula
Unknown 1		
[Asp ³]MC-M(O ₂)R (<i>oxidation artefact</i>)	1031.4865	C ₄₇ H ₇₁ N ₁₀ O ₁₄ S
[d-Asp ³ , Dha ⁷]MC-HtyR		
[Asp ³ , DMAdda ⁵]MC-HtyR		
[Asp ³]MC-RY		
[Dha ⁷]MC-RY		
[Asp ³ , Dhb ⁷]MC-RY	1031.5197	C ₅₁ H ₇₁ N ₁₀ O ₁₃
[d-Asp ³]MC-YR		
[Dha ⁷]MC-YR		
[Asp ³ , (E)-Dhb ⁷]MC-YR		
[DMAdda ⁵]MC-YR		
Unknown 2		
[Met ¹]MC-LR	1055.5594	C ₅₁ H ₇₉ N ₁₀ O ₁₂ S
[Leu ¹ , NMeSer ⁷]MC-LR	1055.6136	C ₅₂ H ₈₃ N ₁₀ O ₁₃
Unknown 3		
[D-Asp ³ , ADMAdda ⁵ , Dha ⁷]MC-HilR		
[Gly ¹ , Asp ³ , ADMAdda ⁵ , Dhb ⁷]MC-LHAr		
[d-Asp ³ , ADMAdda ⁵]MC-LR	1009.5353	C ₄₉ H ₇₃ N ₁₀ O ₁₃
[ADMAdda ⁵ , Dha ⁷]MC-LR		
[Asp ³ , ADMAdda ⁵ , Dhb ⁷]MC-LR		
[MeAla ¹]MC-LR or [MeLeu ²]MC-LR		
MC-HilR		
MC-Lhar		
[d-Glu(OCH ₃) ₆]MC-LR	1009.5717	C ₅₀ H ₇₇ N ₁₀ O ₁₂
[Mdhb ⁷]MC-LR		
[Leu ¹ , Asp ³ , DMAdda ⁵]MC-LR		

Table S2. Major fragments of MC-LR detected in the standard solution at a RT of 2.84 min. *m/z* is presented as detected under positive ionization mode without mass correction.

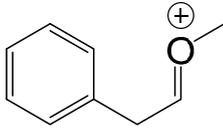
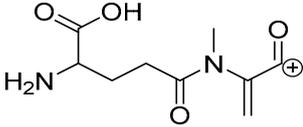
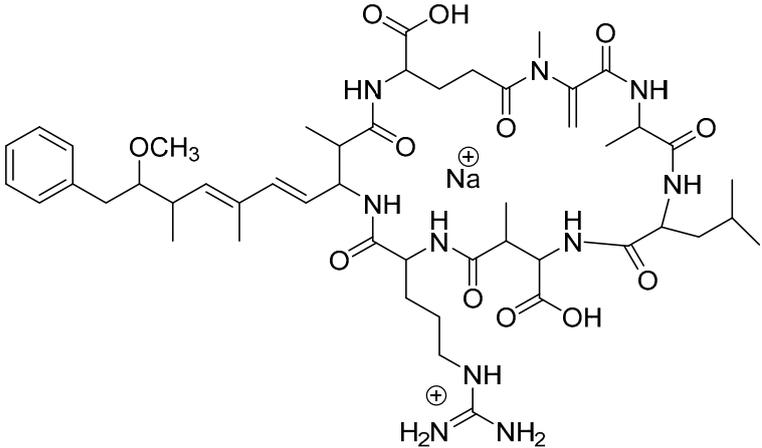
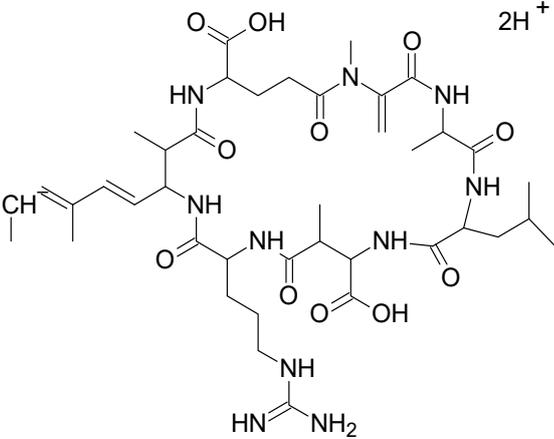
Fragment Structure	Molecular Formula	<i>m/z</i> (Da)
	C ₉ H ₁₁ O (ADDA moiety)	135.0808
	C ₉ H ₁₃ N ₂ O ₄	213.0799
	C ₄₉ H ₇₅ N ₁₀ NaO ₁₂	509.2528
	C ₄₀ H ₆₃ N ₁₀ O ₁₁ 2H ⁺	861.4587

Table 3. Major fragments of cyanopeptolin CPT911 detected in the samples at RT 2.13 min and 2.25 min. *m/z* is presented as detected under positive ionization mode without mass correction.

Fragment Structure	Molecular Formula	RT (min)	<i>m/z</i>
	C ₅ H ₁₂ N	2.25	86.0940
		2.13	157.0983
	C ₆ H ₁₃ N ₄ O	2.25	157.0983

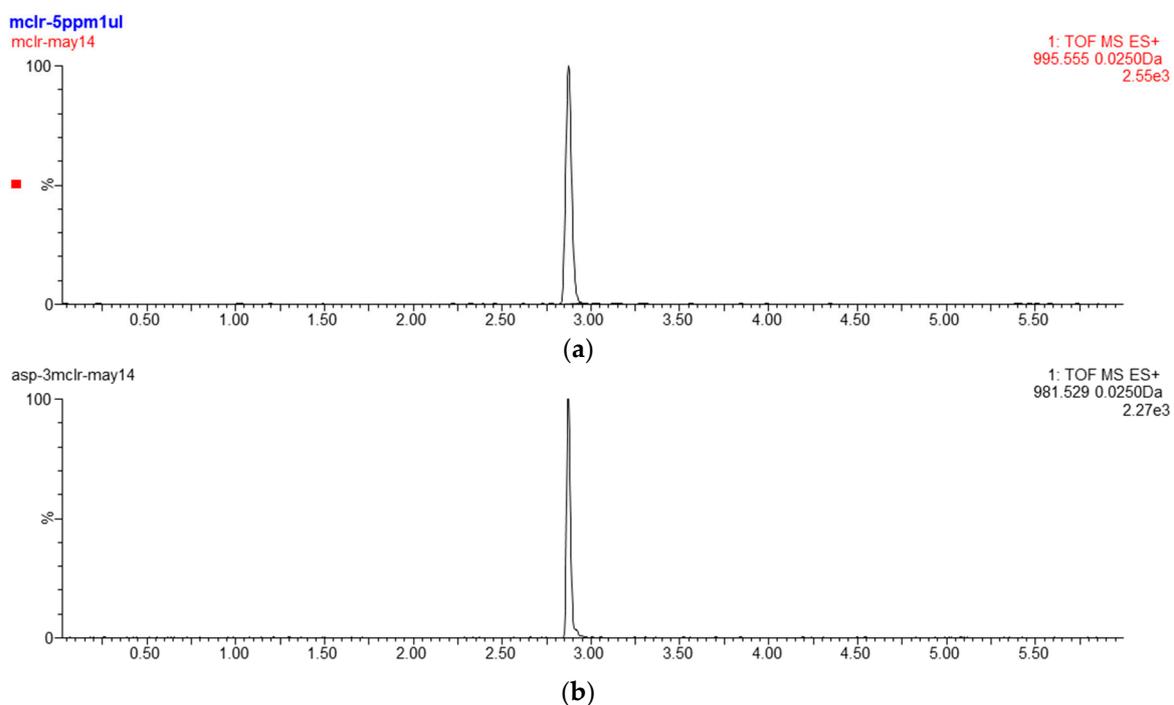


Figure S1. Separation of standards in a positive ionization mode (a) MC-LR (2.87 min); (b) [Asp³]-MC-LR (2.88 min). In this case, fragmentation is the only way to differentiate between the two metabolites.

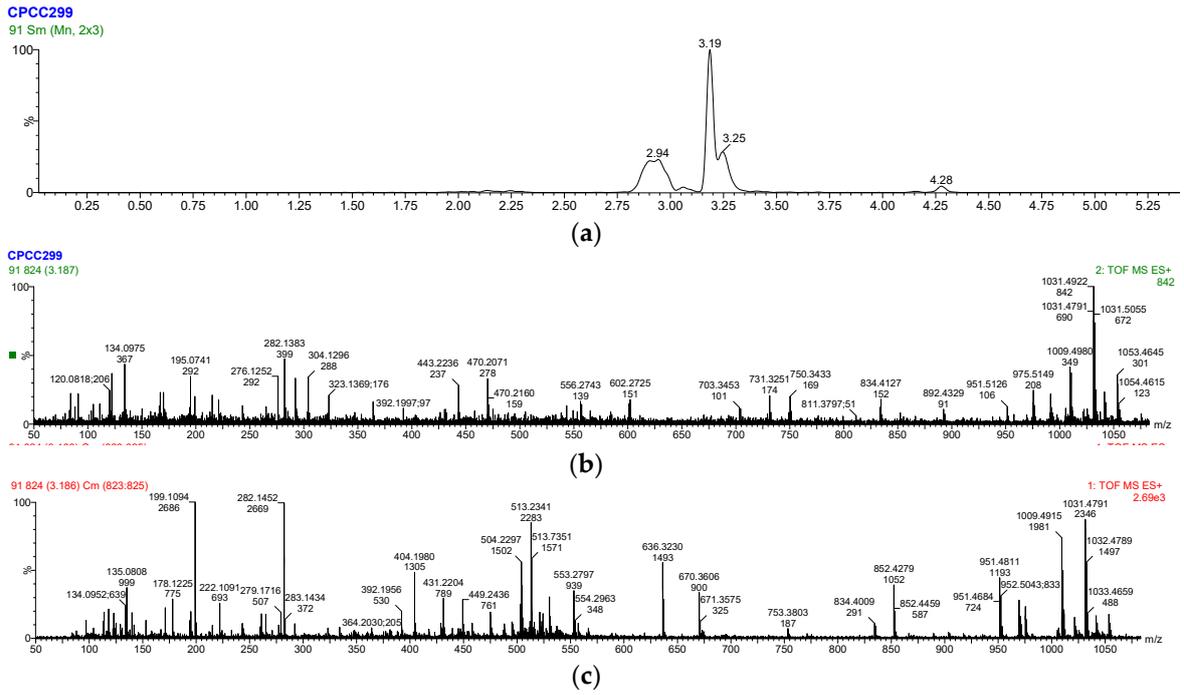


Figure S2. Unknown 1 separation chromatogram at RT 3.19 min (a), and spectra under high energy (b) and low energy (c) collision in positive ionization mode. The m/z of 134.0975 Da corresponds to the mass of the ADDA moiety present in all microcystins.

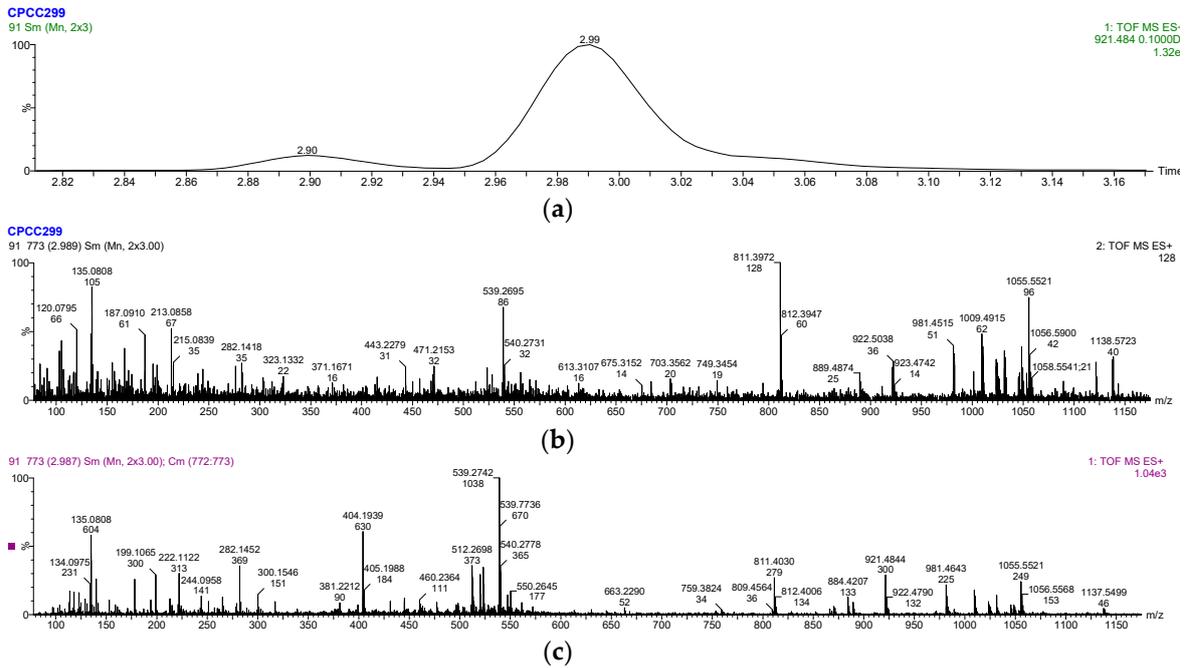


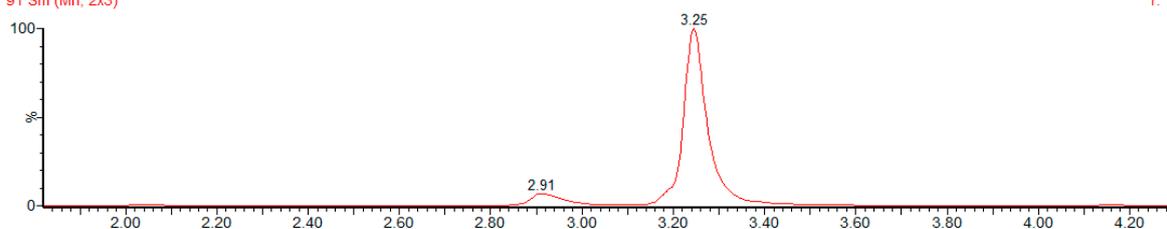
Figure S3. Unknown 2 separation chromatogram at RT 2.98 min (a), and spectra under high energy (b) and low energy (c) collision in positive ionization mode. The m/z of 135.0808 Da corresponds to the fragment mass of the ADDA moiety present in all microcystins.

CPCC299

21

91 Sm (Mn, 2x3)

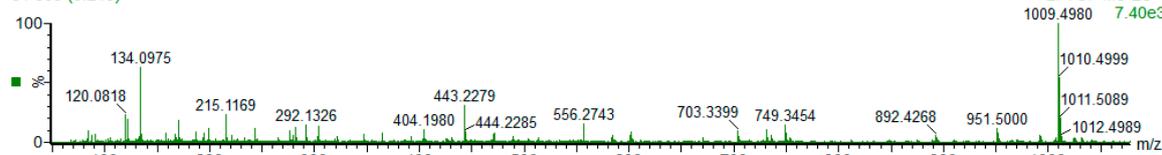
1: T



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12:13:58

91 839 (3.245)

2: TOF MS ES+
1009.4980 7.40e3

91 839 (3.243) Cm (836:843)

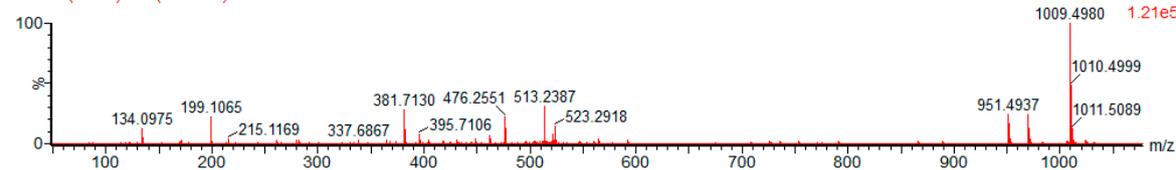
1: TOF MS ES+
1009.4980 1.21e5

Figure S4. Unknown 3 separation chromatogram at RT 3.34 min (a), and spectra under high energy (b) and low energy (c) collision in positive ionization mode. The m/z of 135.0975 Da corresponds to the fragment mass of the ADDA moiety present in all microcystins.

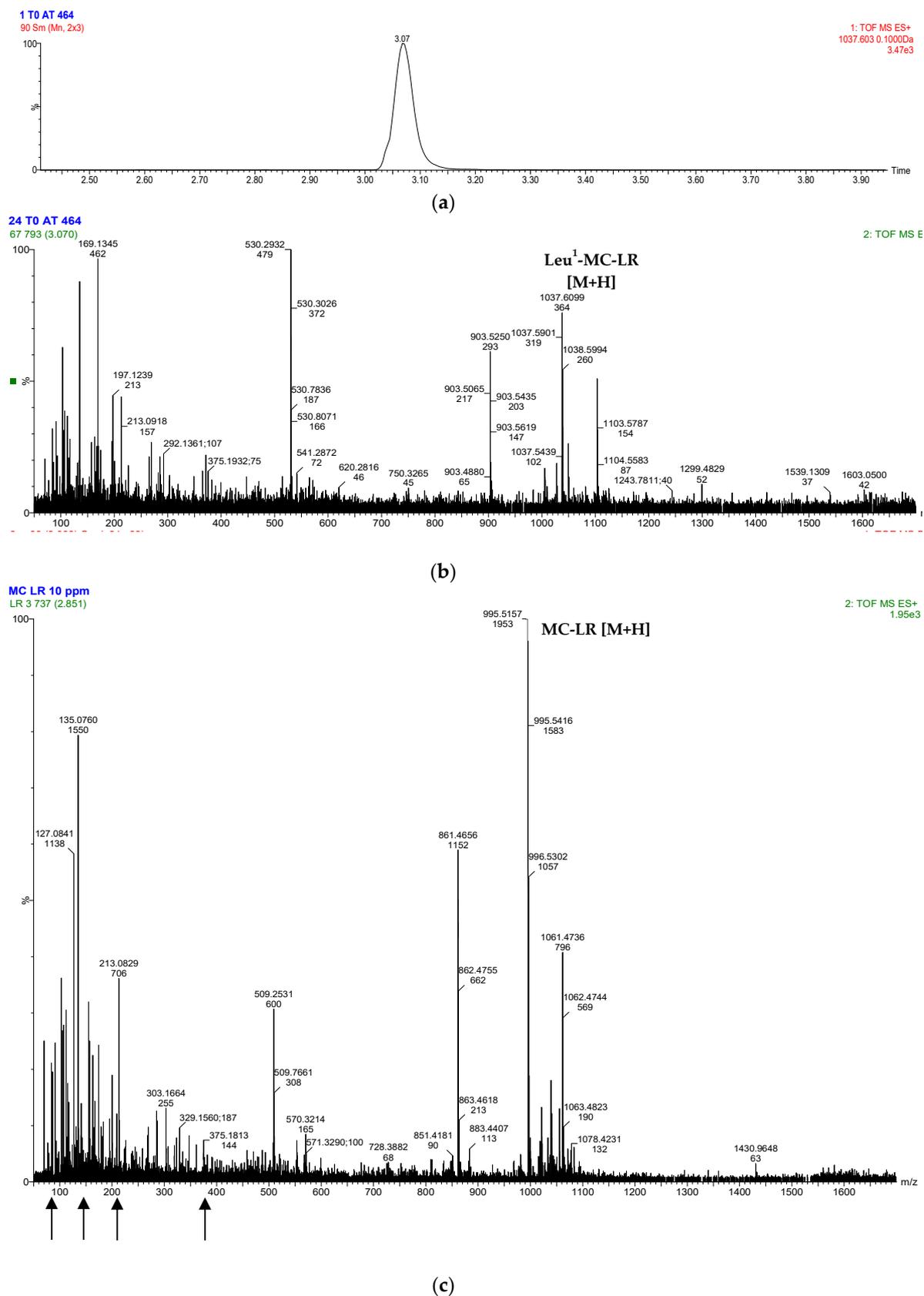


Figure S5. (a) Chromatography of [Leu¹]-MC-LR variant; (b) high energy spectrum of [Leu¹]-MC-LR; (c) high-energy spectrum of the standard solution of MC-LR. The arrows point to the common fragments between the two molecules.

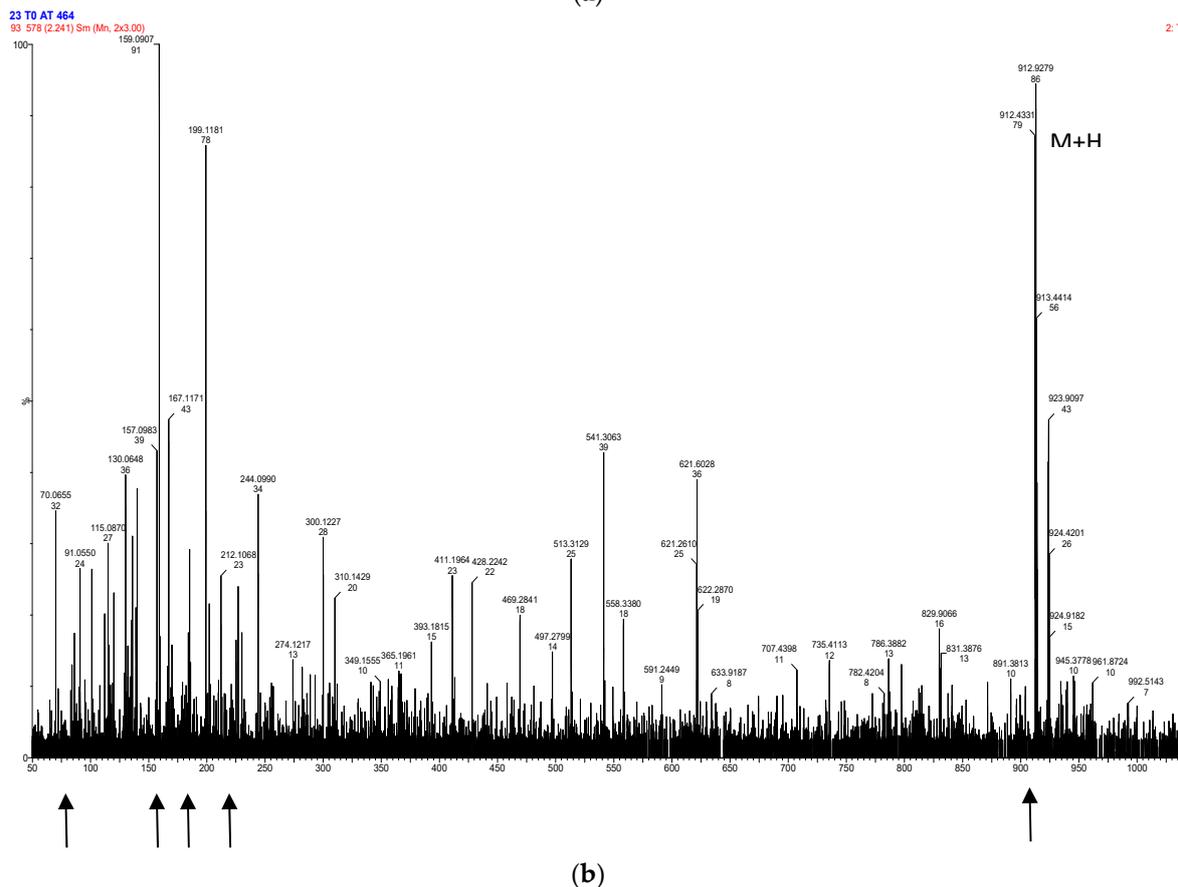
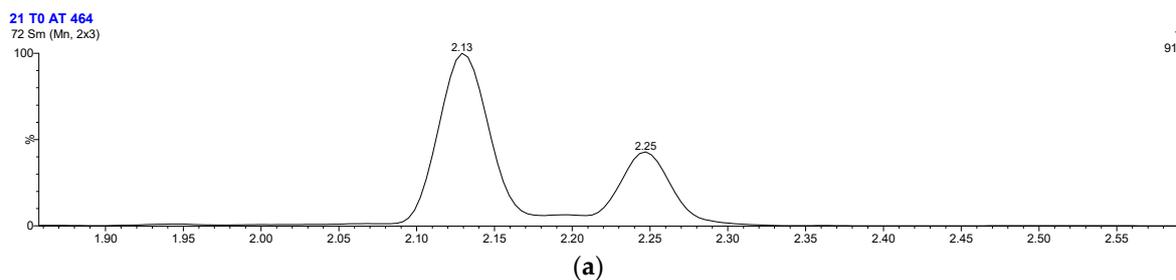


Figure S6. CPT911 detection in *Microcystis aeruginosa* strain CPCC464; (a) Separation of two isomers, CPT911A at RT 2.13 min and CPT911B at RT 2.25 min; (b) High energy spectra for CPT911B. Signals corresponding to identified fragments are indicated by an arrow. The pseudo molecule [M + H] represents the signal of the mass of the whole molecule under positive ionization mode.