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Identification and Quantification of Microcystins with Highly Sensitive Targeted MS/MS Approach with Velos Pro Mass Spectrometer

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Outline

- Introduction
- Peptides Quantification with Mass Spectrometer
- Peptides Separation with UHPLC
- Microcystins Identification /Quantification with Linear Ion Trap Mass Spectrometer
- Conclusion

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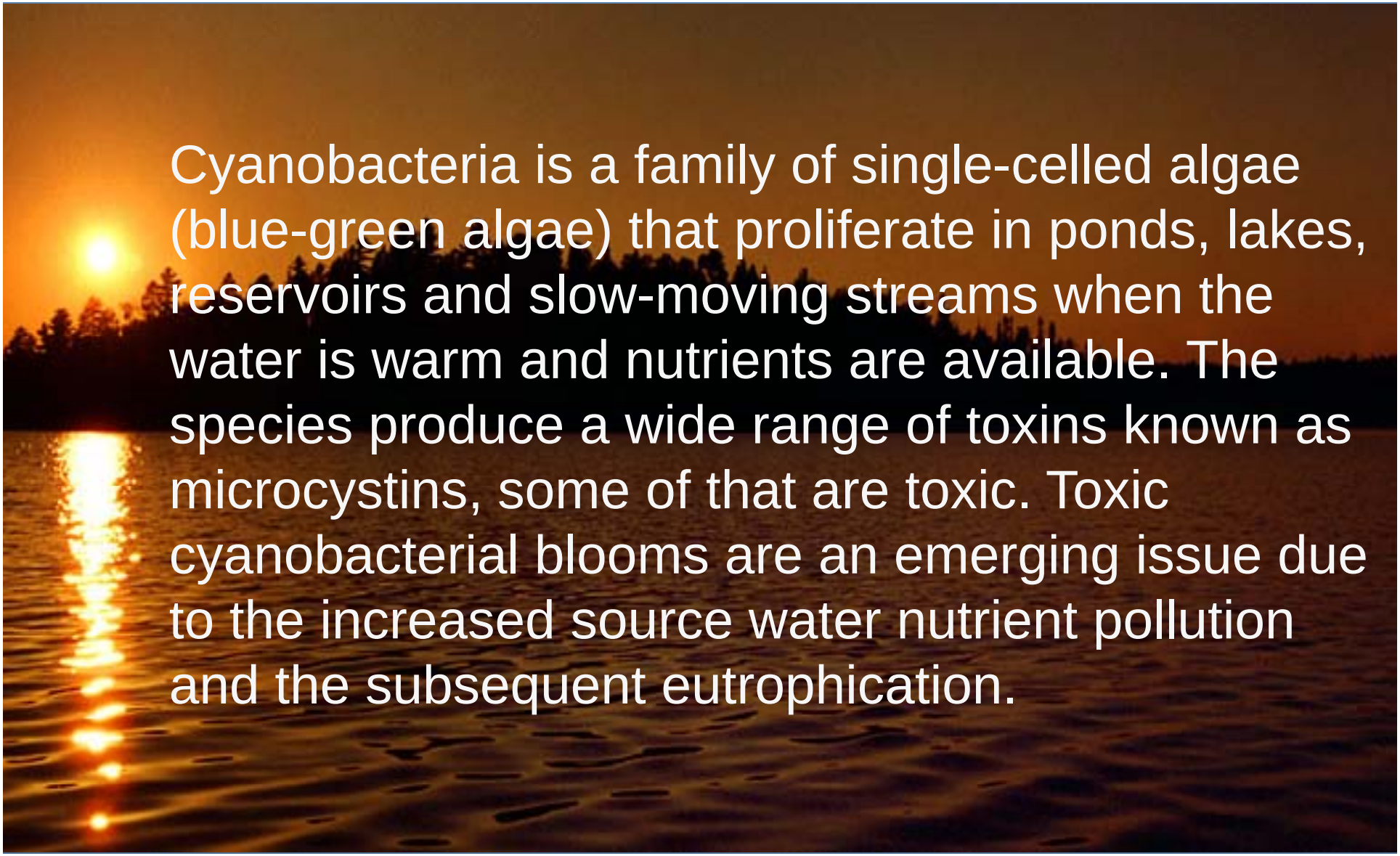
Sources of Microcystins



Cyanobacteria is a family of single-celled algae (blue-green algae) that proliferate in ponds, lakes, reservoirs and slow-moving streams when the water is warm and nutrients are available. The species produce a wide range of toxins known as microcystins, some of that are toxic. Toxic cyanobacterial blooms are an emerging issue due to the increased source water nutrient pollution and the subsequent eutrophication.

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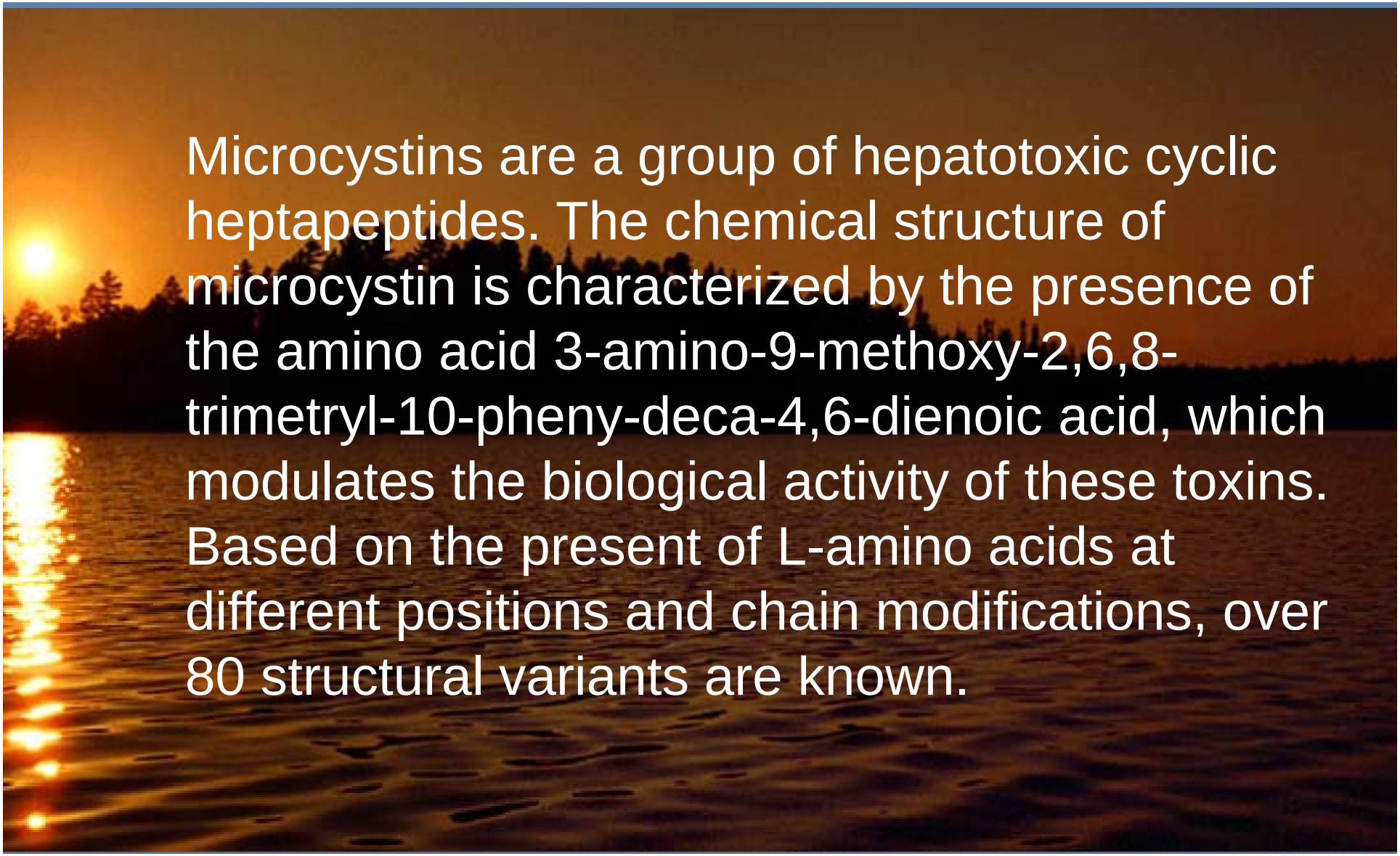
Properties of Microcystins



Microcystins are a group of hepatotoxic cyclic heptapeptides. The chemical structure of microcystin is characterized by the presence of the amino acid 3-amino-9-methoxy-2,6,8-trimetryl-10-pheny-deca-4,6-dienoic acid, which modulates the biological activity of these toxins. Based on the present of L-amino acids at different positions and chain modifications, over 80 structural variants are known.

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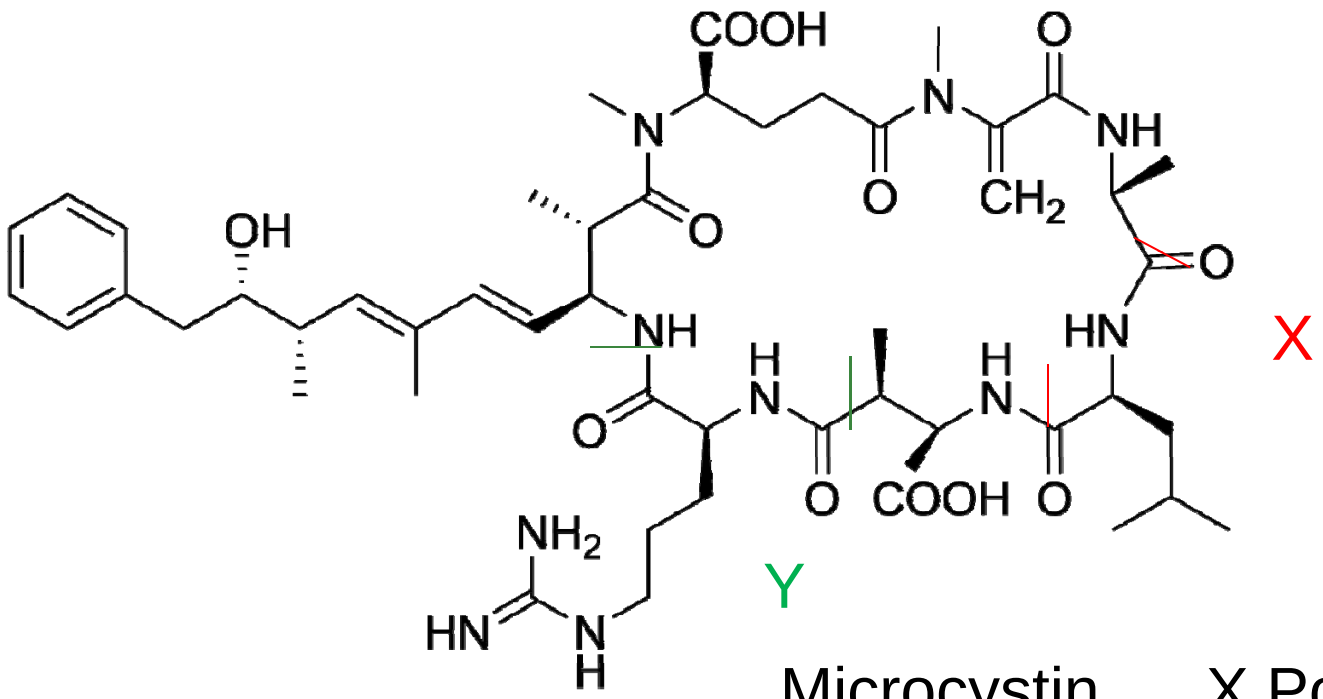
Compositions and Structures

MCs are cyclic peptides containing seven amino acids
Only two of seven are *variable*



Microcystin	X Position	Z Position
MC-LR	Leu	Arg
MC-RR	Arg	Arg
MC-YR	Tyr	Arg

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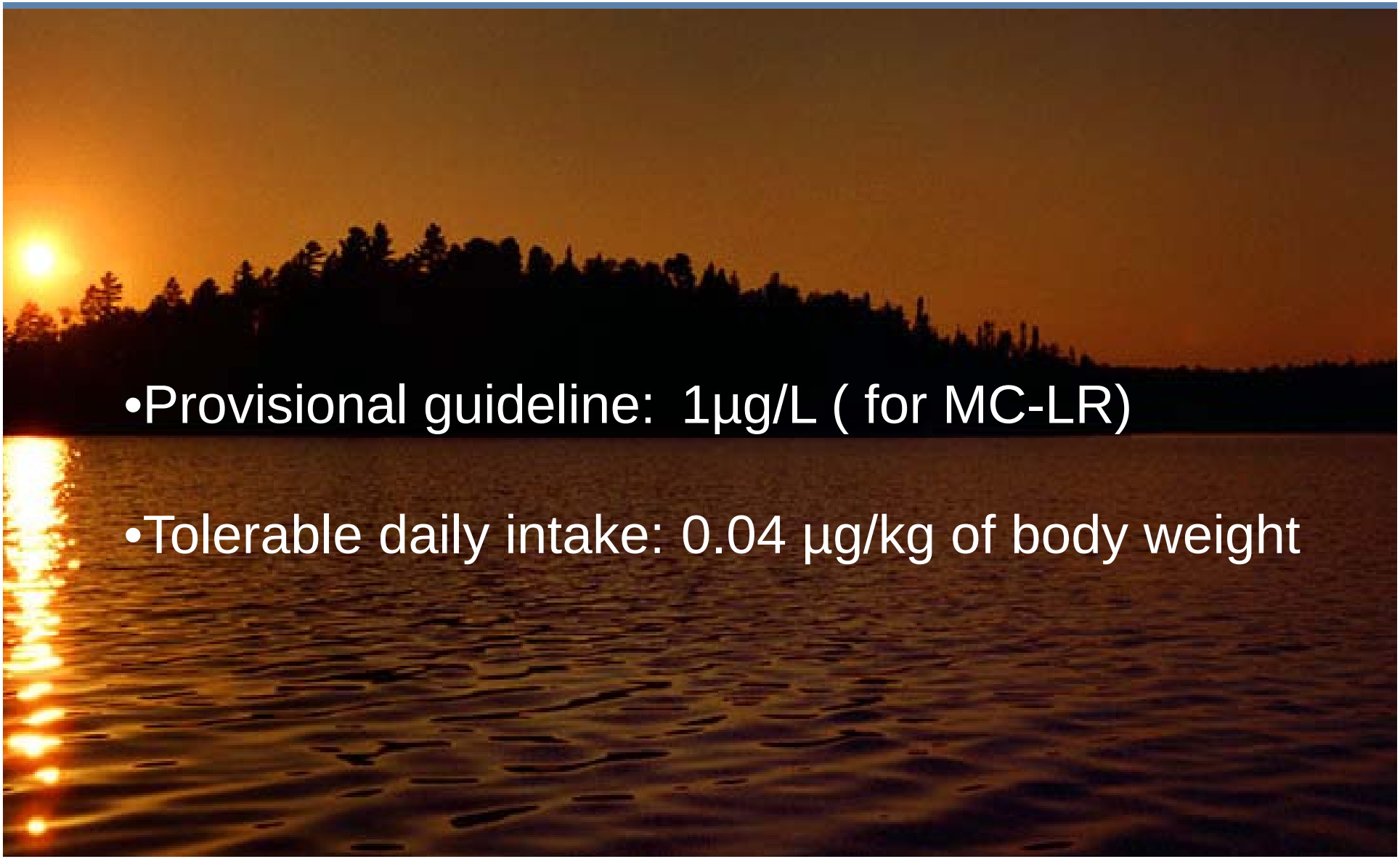
Guidance



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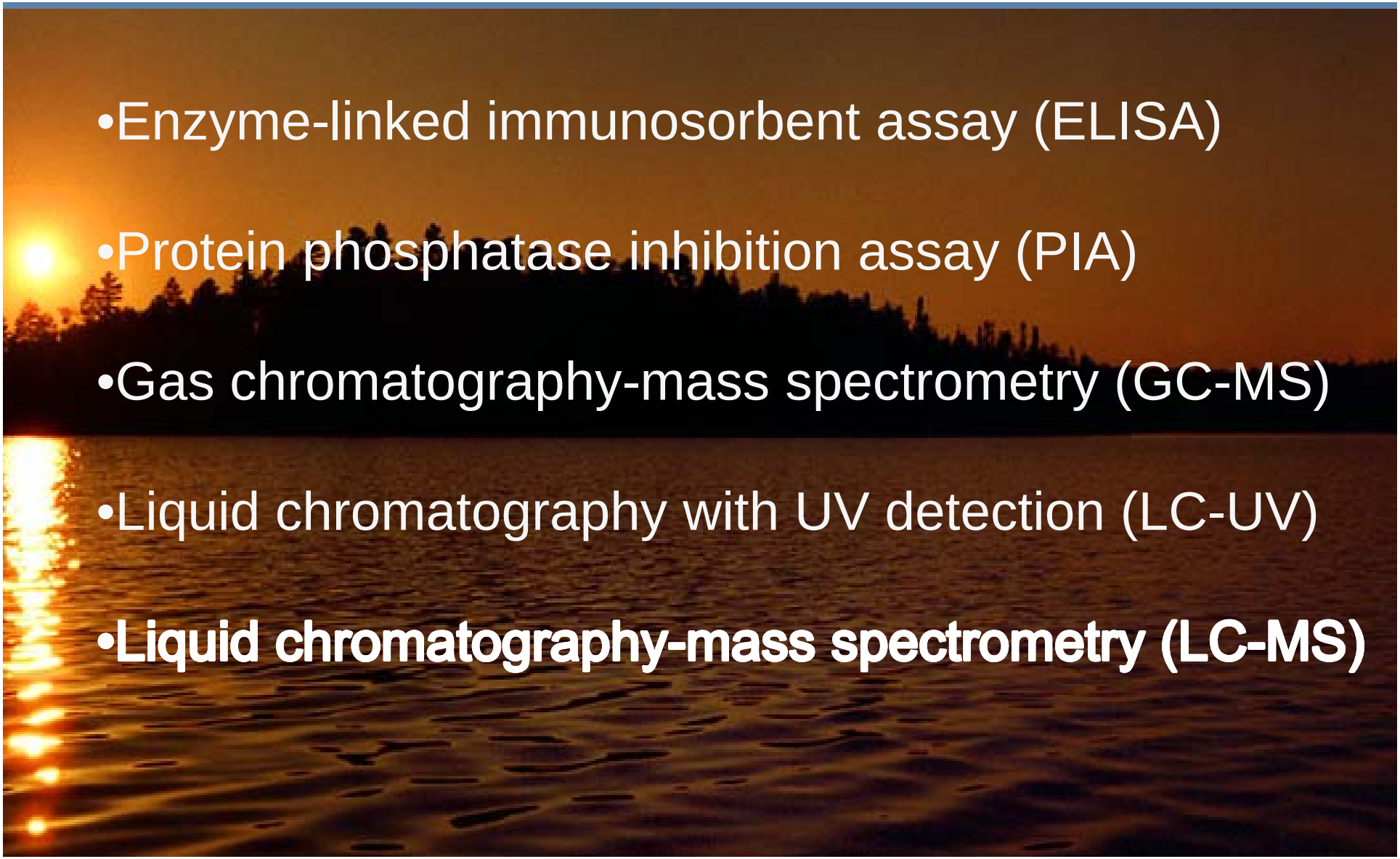
Microcystins Analysis Methodologies



- Enzyme-linked immunosorbent assay (ELISA)
- Protein phosphatase inhibition assay (PIA)
- Gas chromatography-mass spectrometry (GC-MS)
- Liquid chromatography with UV detection (LC-UV)
- **Liquid chromatography-mass spectrometry (LC-MS)**

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Advantages of LC/MS Method

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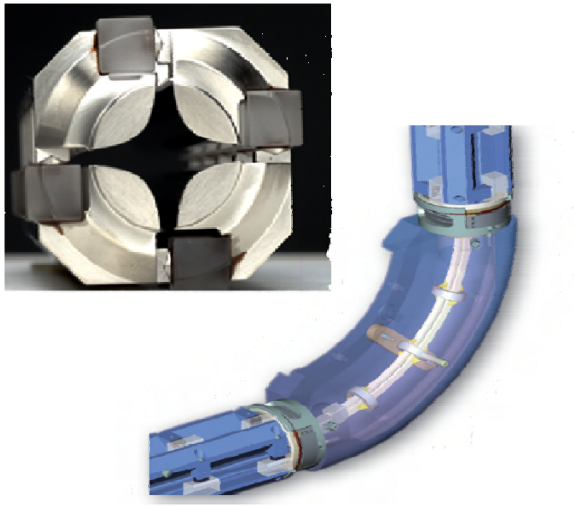
Microcystins Quantitation – Mass Spectrometry Approaches

1. Triple Quadrupole based methods

- *SRM*



2. Ion Trap based methods

- *Full Scan CID MS2*

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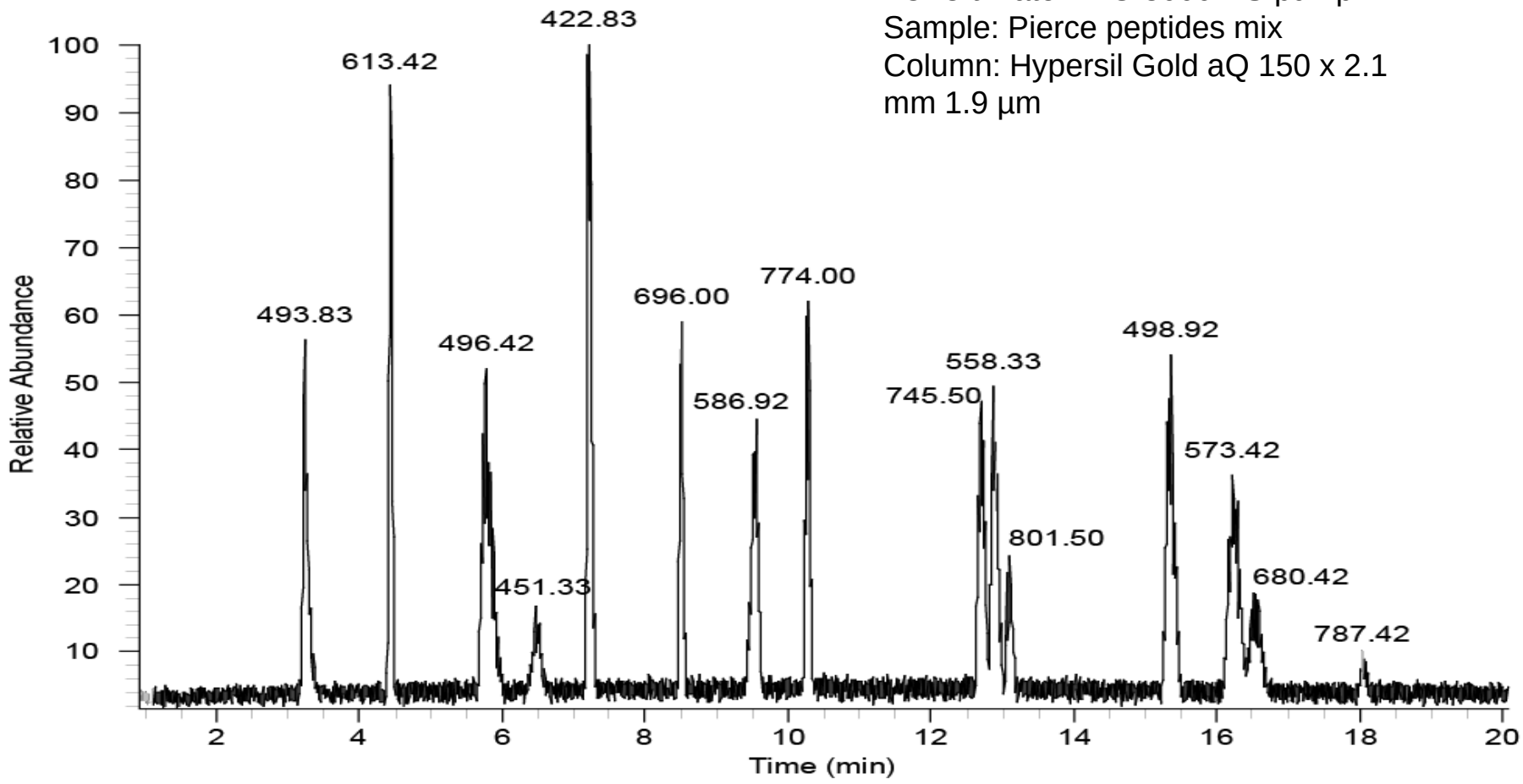
Peptides Analysis with UHPLC

Mass Spec: LTQ
LC: Ultimate HPG 3000 RS pump
Sample: Pierce peptides mix
Column: Hypersil Gold aQ 150 x 2.1 mm 1.9 µm



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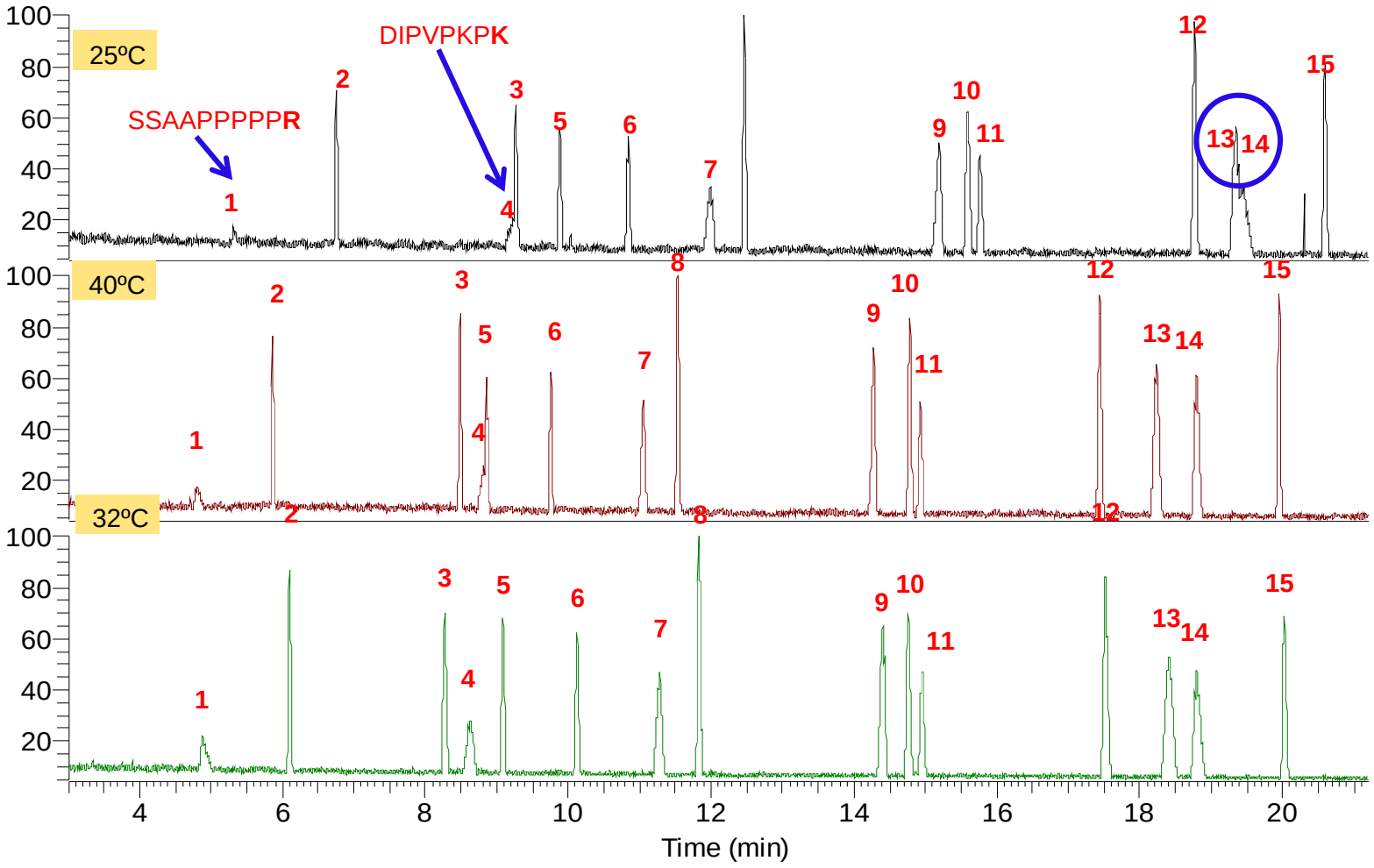
Temperature Effect on Peptides Separation



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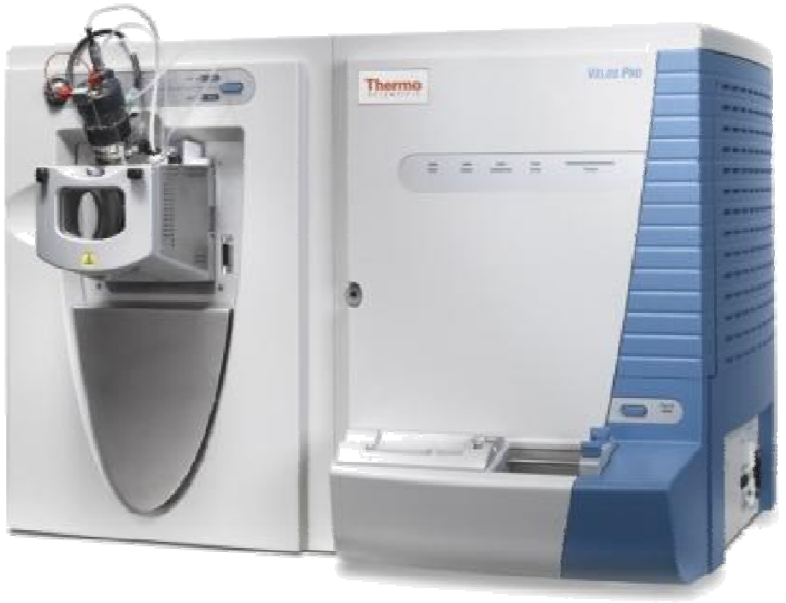
The Velos Pro Linear Ion Trap

Meet both Qualitative and Quantitative applications demands

1.The worlds fastest most sensitive ion trap for the most demanding applications

2.Wide dynamic range, great accuracy.

3.CID, PQD, ETD, and now Trap-HCD fragmentation increases flexibility for structural elucidation



Thermo Scientific Velos Pro Dual-Pressure Linear Ion Trap

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Microcystines - Experiment Conditions

- **Detector:** The Velos Pro
- **LC:** Ultimate HPG 3000RS
- **Sample:** MC-RR, MC-LR, MC-YR mix
- **Column:** Analytical column: Acclaim PepMap 100 C18, 150x1.0 mm, 3µm, 100 Å
  Guard cartridge: C18, 10x3.0 mm, 5µm, 120 Å
- **Column:** 40ºC
- **Mobile Phase:** A: 0.1% FA in water
  B: 0.1% FA in ACN
- **Targeted MS/MS :** 520 (150-1100)
  1045, 995 (285-1100)
- **Collision Energy:** 35%
- **Isolation window:** 2
- **Flow Rate:** 150 µL/min

Gradient

Time	% A	% B
0.1	98	2
1.5	98	2
2.0	80	20
3.0	60	40
7.4	40	60
7.5	2	98
7.9	2	98

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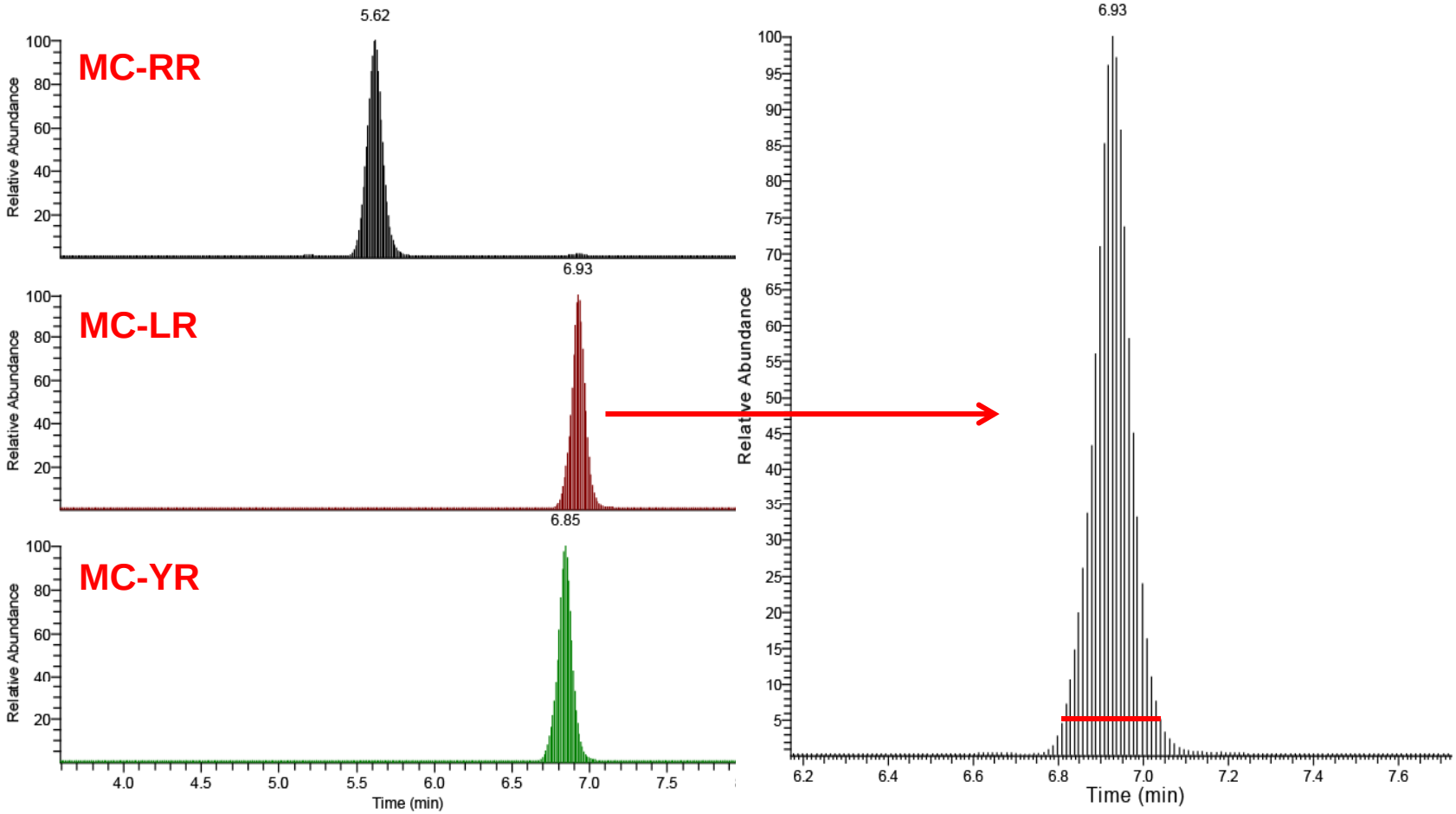
The Velos Pro - Fast Scan Speed for Quantiation



More than 20 data points for quantitation

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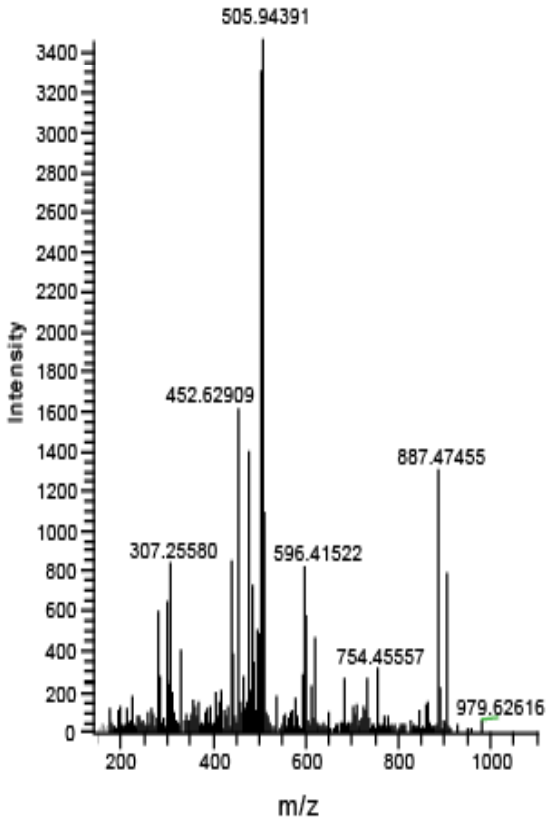
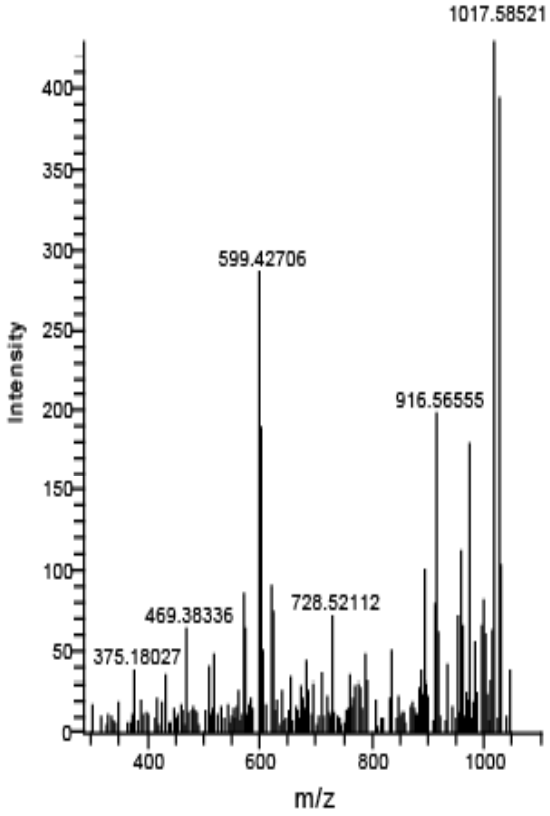
The Velos Pro - MSn Capability for Identification

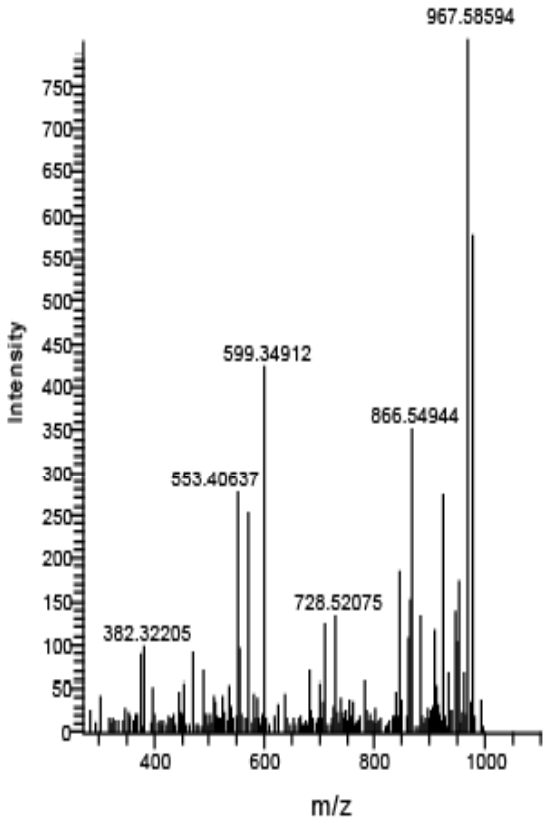
MC-RR



MC-YR



MC-LR



Extracted MS/MS Chromatogram & MS/MS Spectra

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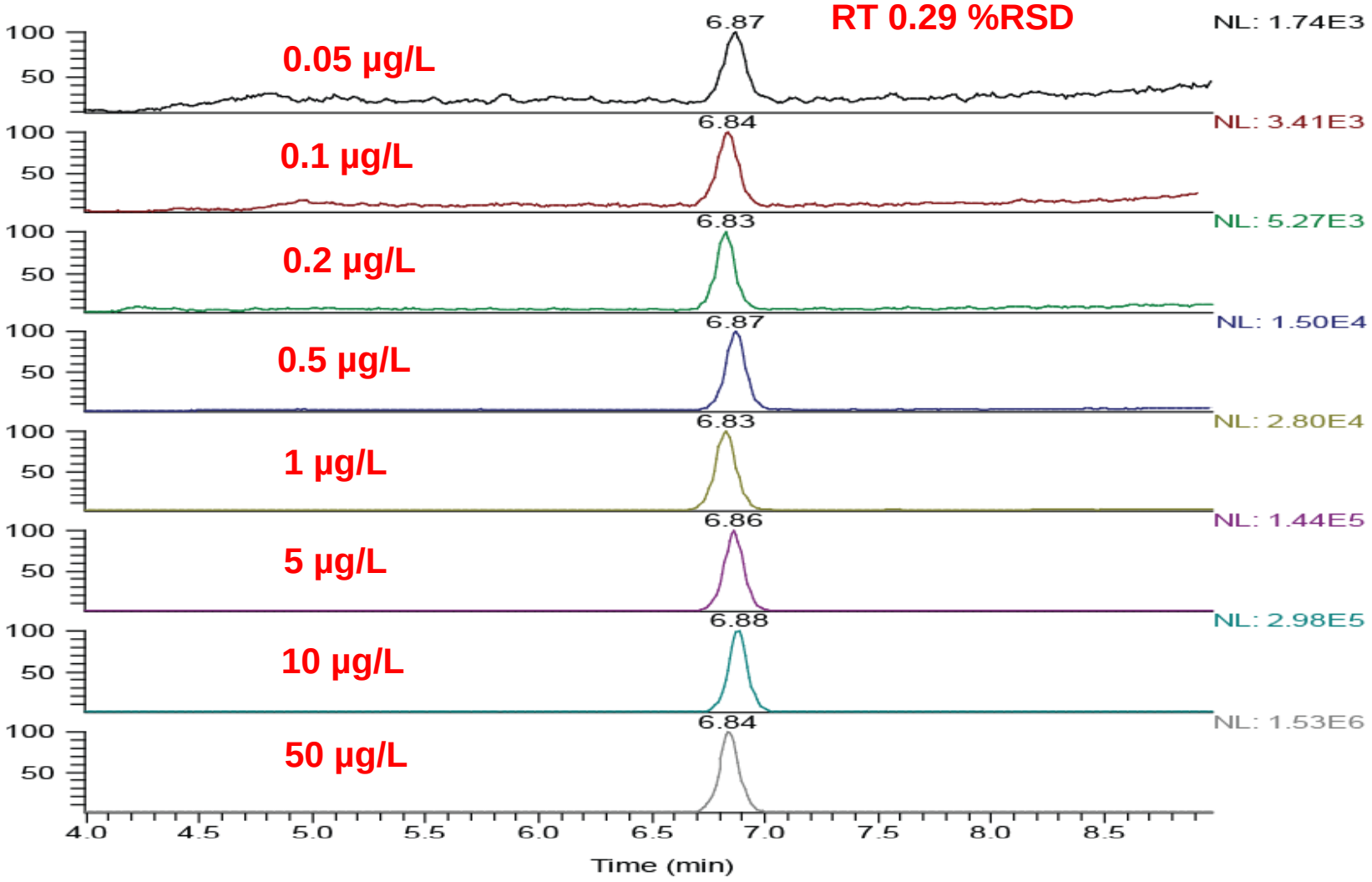
Wide Dynamic Range with High RT Precision



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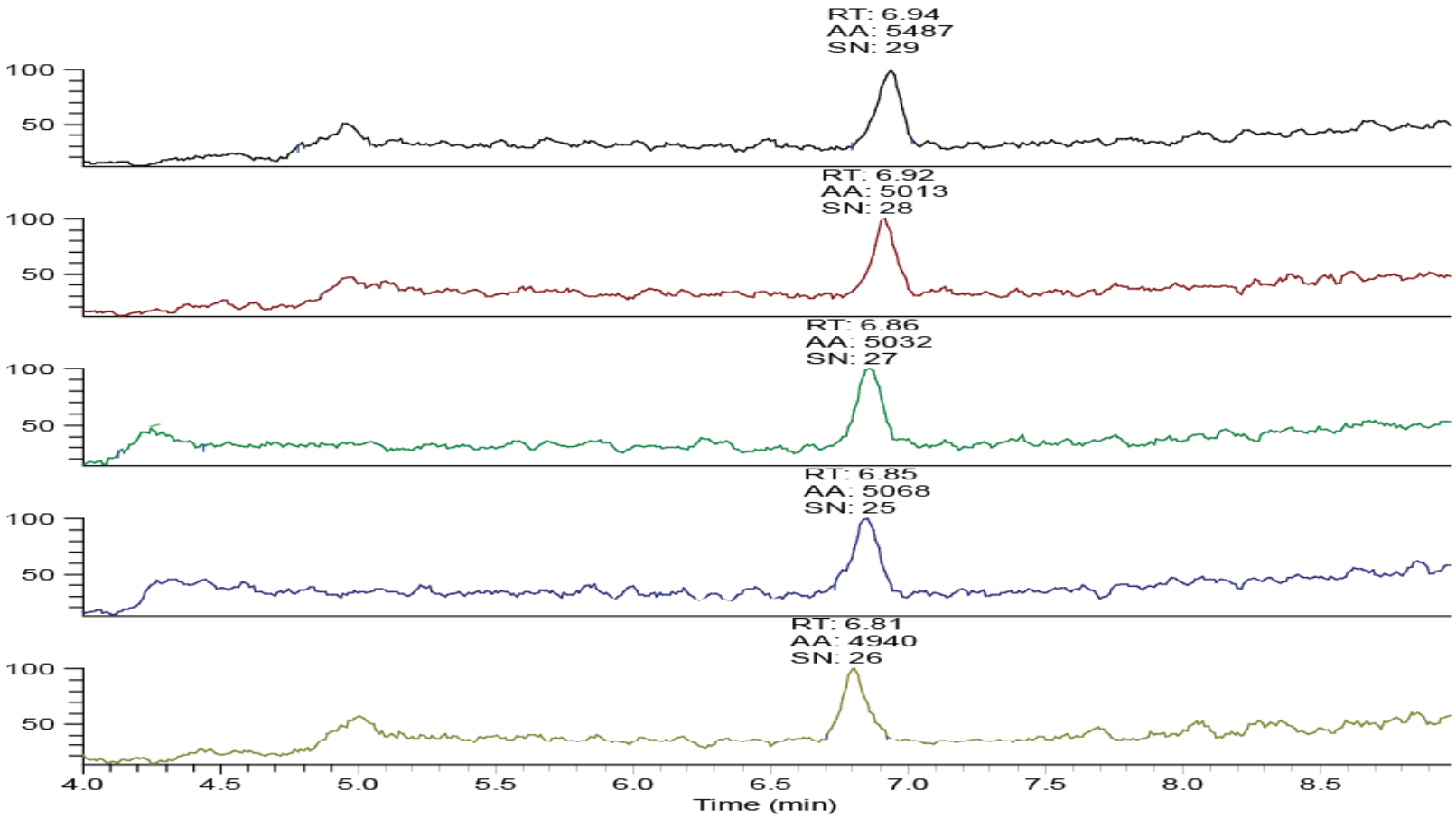
High Repeatability & Sensitivity



0.05 µg/L MC-LR (LOQ) with S/N bigger than 25

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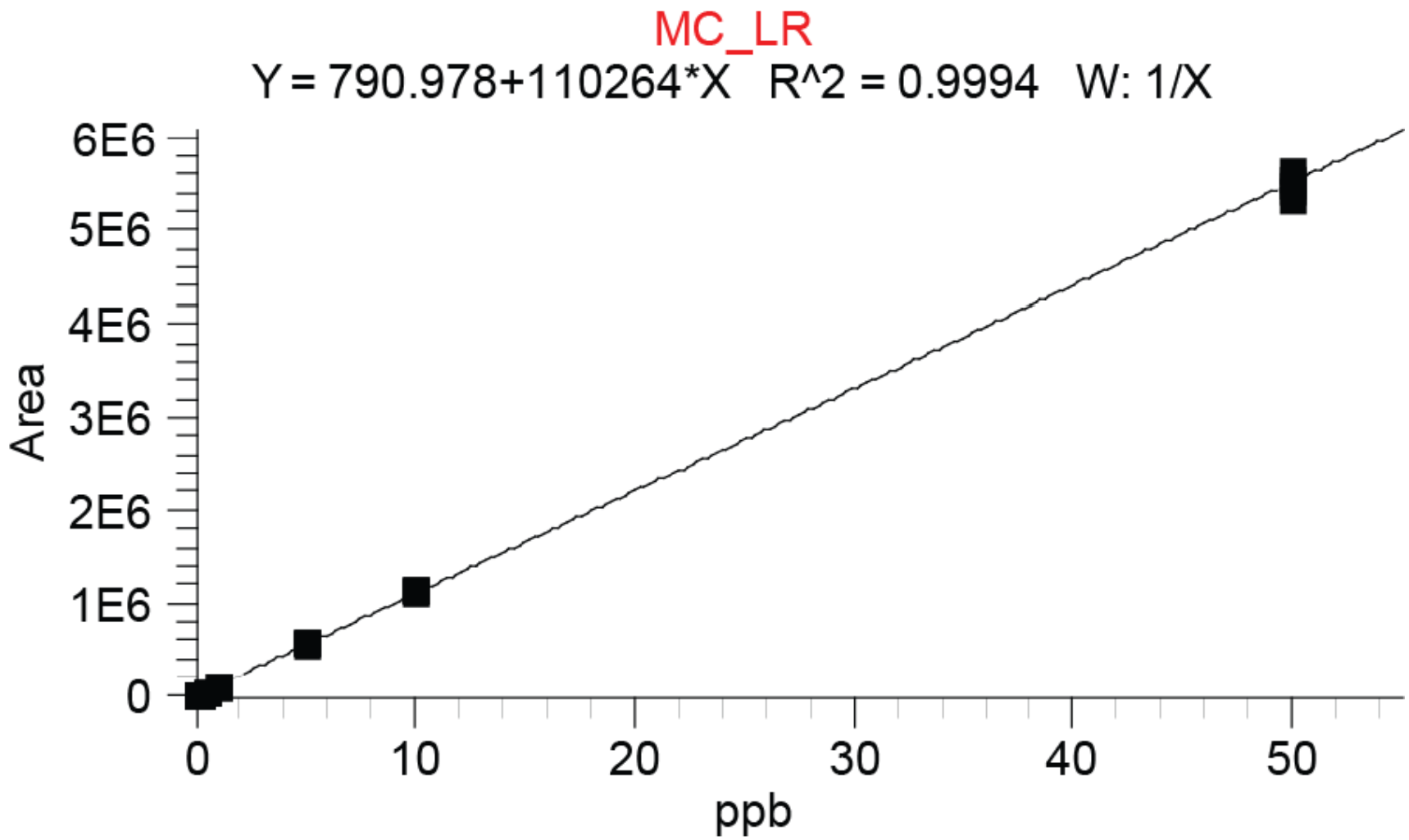
Example Calibration Curve for MC-LR



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Analysis Summary

Compounds	Linear range µg/L	R^2	Accuracy %	
			QC1 (0.5 µg/L)	QC2 (5 µg/L)
MC-RR	0.05-50	0.9986	95.0	99.0
MC-YR	0.05-50	0.9994	94.5	97.5
MC-LR	0.05-50	0.9994	98.8	99.0

Area Precision, %RSD n=5

Levels µg/L	MC-RR	MC-YR	MC-LR
0.05	16.01	10.5	6.91
0.10	2.82	5.88	3.97
0.20	3.54	5.25	4.89
0.50	4.86	8.54	3.03
1.00	5.84	1.76	4.25
5.00	2.28	2.13	2.47
10.00	4.54	1.30	1.31
50.00	2.40	1.76	2.66

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Conclusions

- **A simple, sensitive, LC-MS method for identification and quantitation of MCs was developed using a Ultimate 3000RS coupled with The Velos Pro mass spectrometer platform.**
- **With the fast scan speed and the MSn capability of The Velos Pro. MCs could be quantified and identified within one LC-MS analysis.**
- **The targeted MS/MS method using The Velos Pro is a highly selective and accurate quantiation approach.**
- **The method could detect MC concentration as low as 0.025µg/L with S/N bigger than ten.**
- **The limit of quantitation (LOQ) of 0.05µg/L was achieved for all three analyzed MCs.**