Towards an automated untargeted method for microcystins analysis using two dimensional liquid chromatography and ion mobility/quadrupole time of flight mass spectrometry

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Outline

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- Introduction to microcystins
- Targeted analysis of microcystins
  - Advantages of 2DLC
  - MS, MS/MS, Target enhancement
- Untargeted analysis of microcystins
  - Waters Xevo G2XS - QToF (DDA)
  - Waters Vion IMS - QToF (HDMS^E)

Introduction

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- **Microcystins** are cyclic peptides produced by cyanobacteria
- They are **produced by overgrowth of algae**, especially at higher water temperatures – **Climate change, invasive species, global trade and agricultural practices can exacerbate** the problem
- Microcystins can be **toxic for plants and animals including humans**
- WHO action limit = **1000 ng/L (1ppb)** and methods are proposed including US EPA Methods 544 and 545 and European ISO 20179:2005(E) guideline
- Sensitive detection is needed

Microcystins

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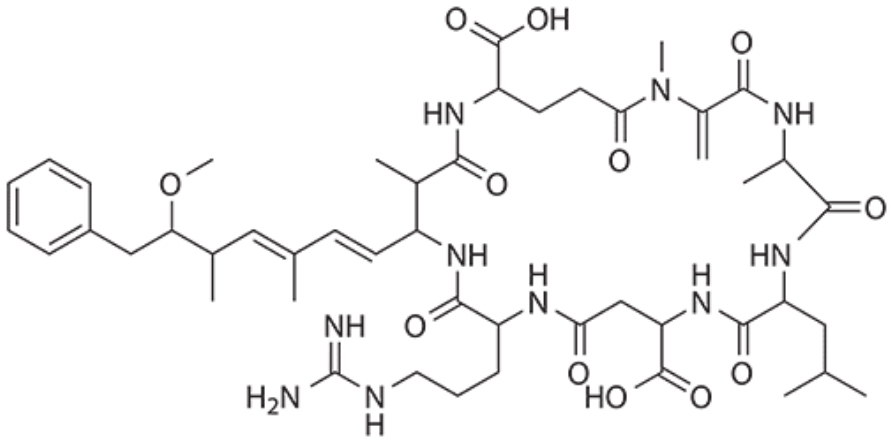
- Twelve different variants monitored at MOECC
- Several cases of human sickening/death (Toledo OH, 2014)

- Ontario Drinking Water Quality Standard set at 1.5 µg/L for Microcystin-LR
- From 2004-2015: > 2000 samples from drinking water monitoring program + > 2000 samples from algal blooms



Microcystin-LR

Microcystin variants

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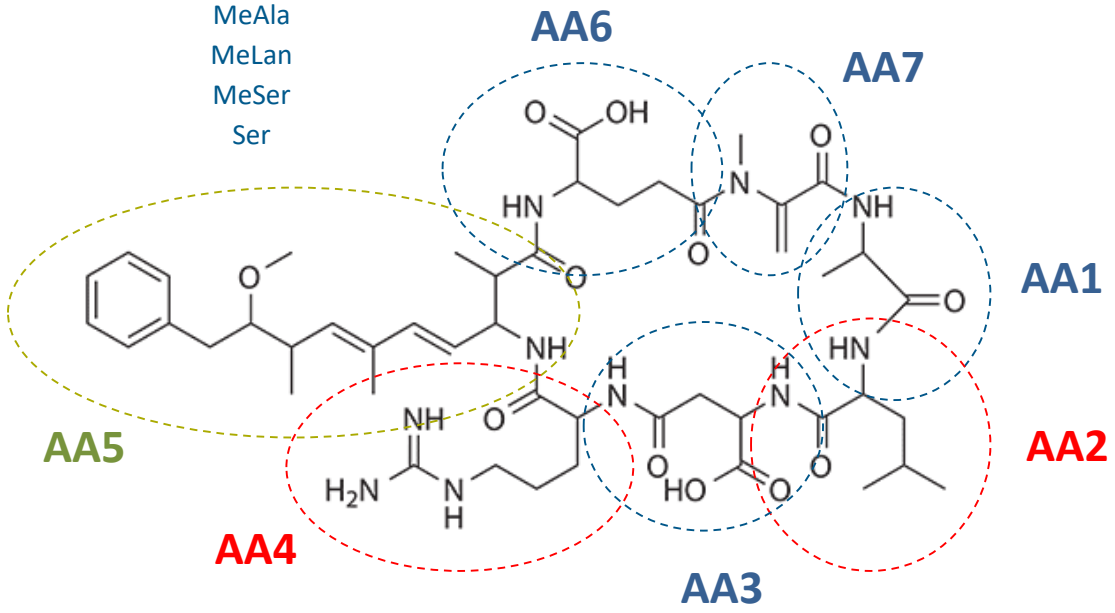
- Over 100 different variants reported in literature!

AA1	AA2	AA3	AA4	AA5	AA6	AA7
Ala	(H_4)Y	**MeAsp**	Aba	**Adda**	**Glu**	**Mdha**
Gly	Aha	Asp	Ala	(6Z)Adda	Glu(OMe)	Dha
Leu	Ala		Apa	ADMAdda	Glu(C_3H_7O)	Dhb
Ser	Arg		Arg	DMAdda		dihydro
	Glu		Butyrine	EtAdda		MeAla
	Glu(OMe)		Glu(OMe)			MeLan
	Hil		Har			MeSer
	Hph		His			Ser
	hR		hR			
	Hty		Hty			
	Leu		Leu			
	M(O)		M(O)			
	Phe		Met			
	Trp		Phe			
	Tyr		Trp			
	Val		Tyr			
			Tyr(OMe)			
			Val			



Microcystins common diagnostic fragments

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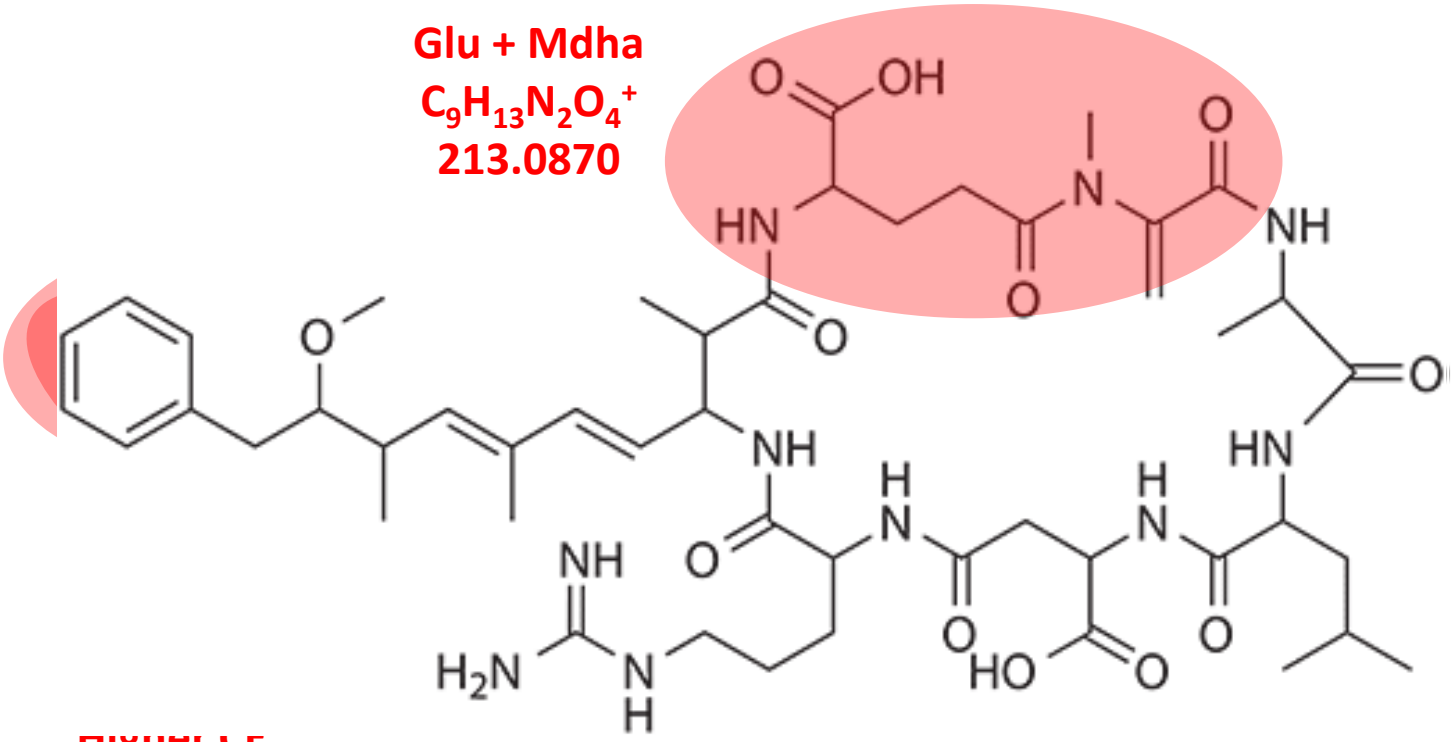
Glu + Mdha
$C_9H_{13}N_2O_4^+$
213.0870



Higher CE

Microcystins sample preparation

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Current MOECC method

Day 1: -Biomass filtration
-Cell lysis

Day 2: -Extraction
-Filtration
-Desorption
-Concentration

Day 3: -Preparation for LC-MS/MS analysis

Automated method

- 3 days
- 2 analysts
- 500 ml of sample

Day 1: -10 ml aliquot cell lysis (freeze/thaw)
-Preparation for online sample prep and instrumental
rnight)

Time and resources consuming method

Lab pr bled!

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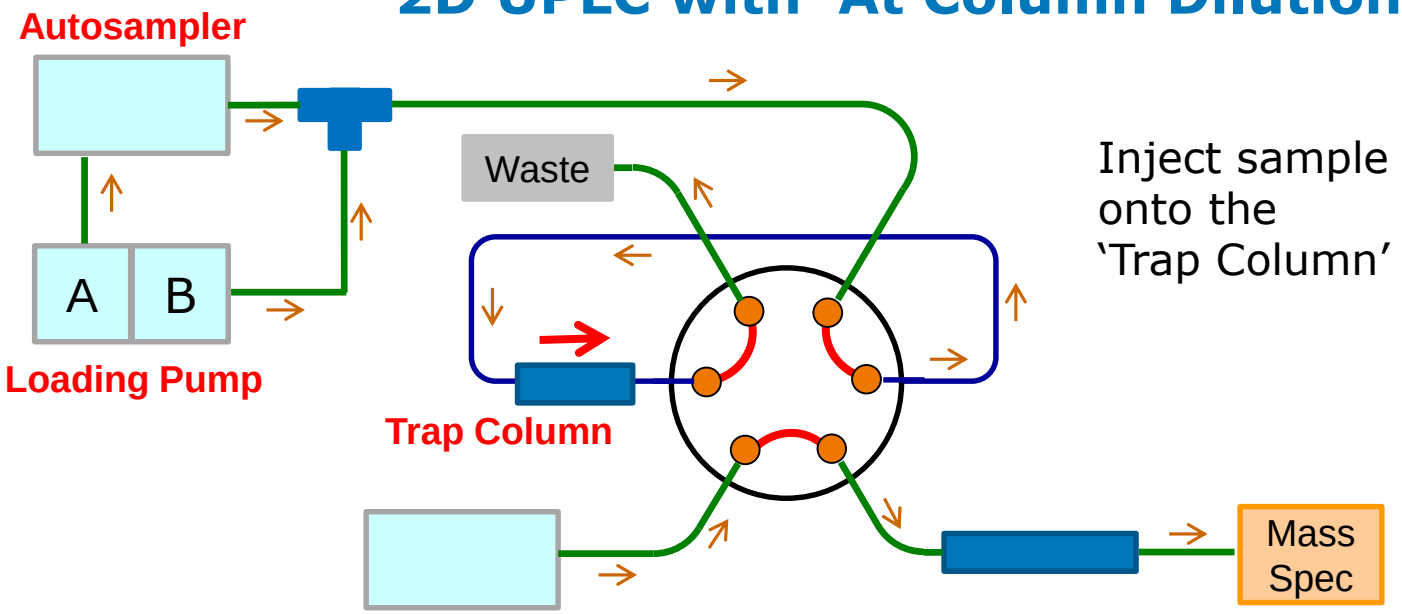
Targeted analysis of microcystins using 2D LC-QToF (Xevo G2-XS)

2D UPLC with 'At Column Dilution'

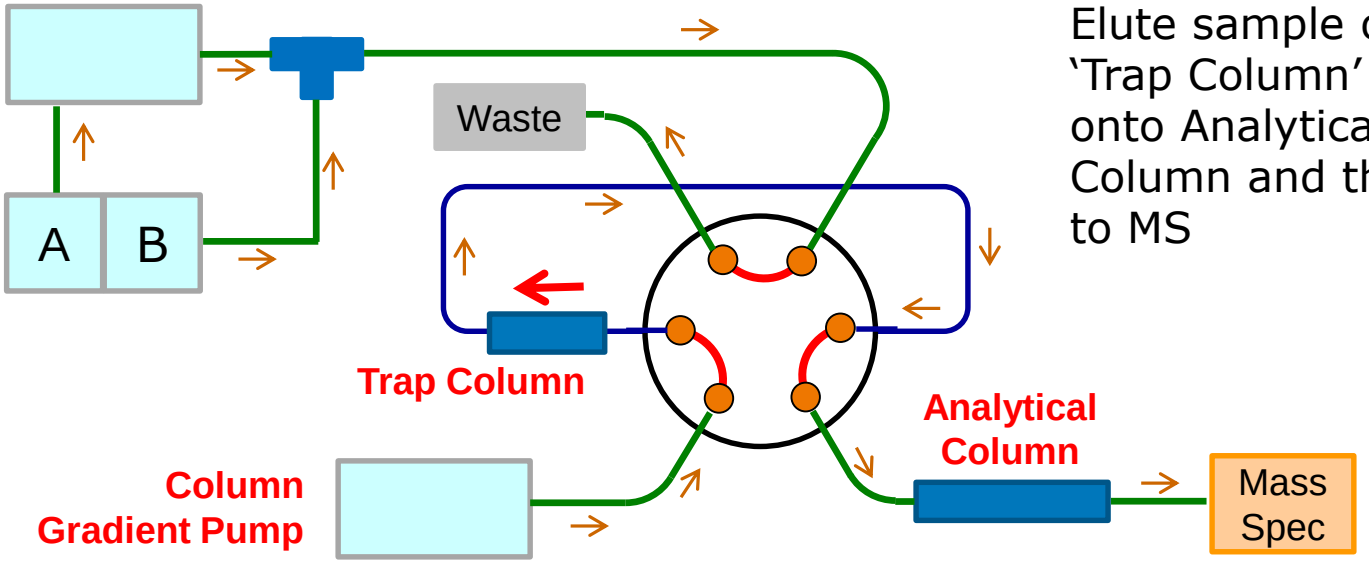
Loading Pump:
A pushes sample out of the autosampler

B dilutes the flow coming from the autosamper with aqueous to improve trapping



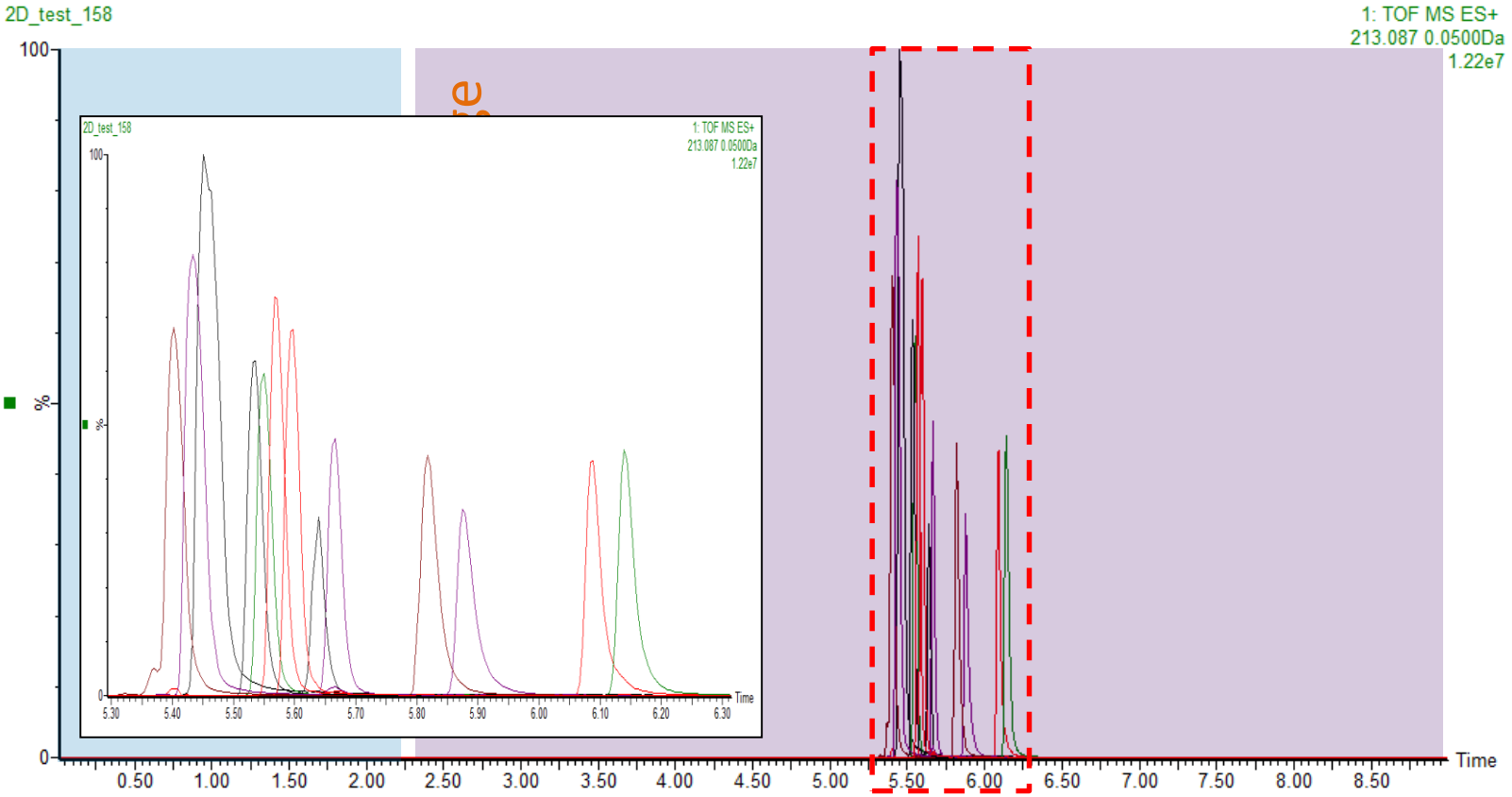
Inject sample onto the 'Trap Column'

After the valve switch, flow from the column pump goes through the trap and onto the analytical column connected to the mass spec



Elute sample off 'Trap Column' onto Analytical Column and then to MS

2D LC conditions



Advantages of 2DLC for microcystin analysis: Maintained chromatographic resolution

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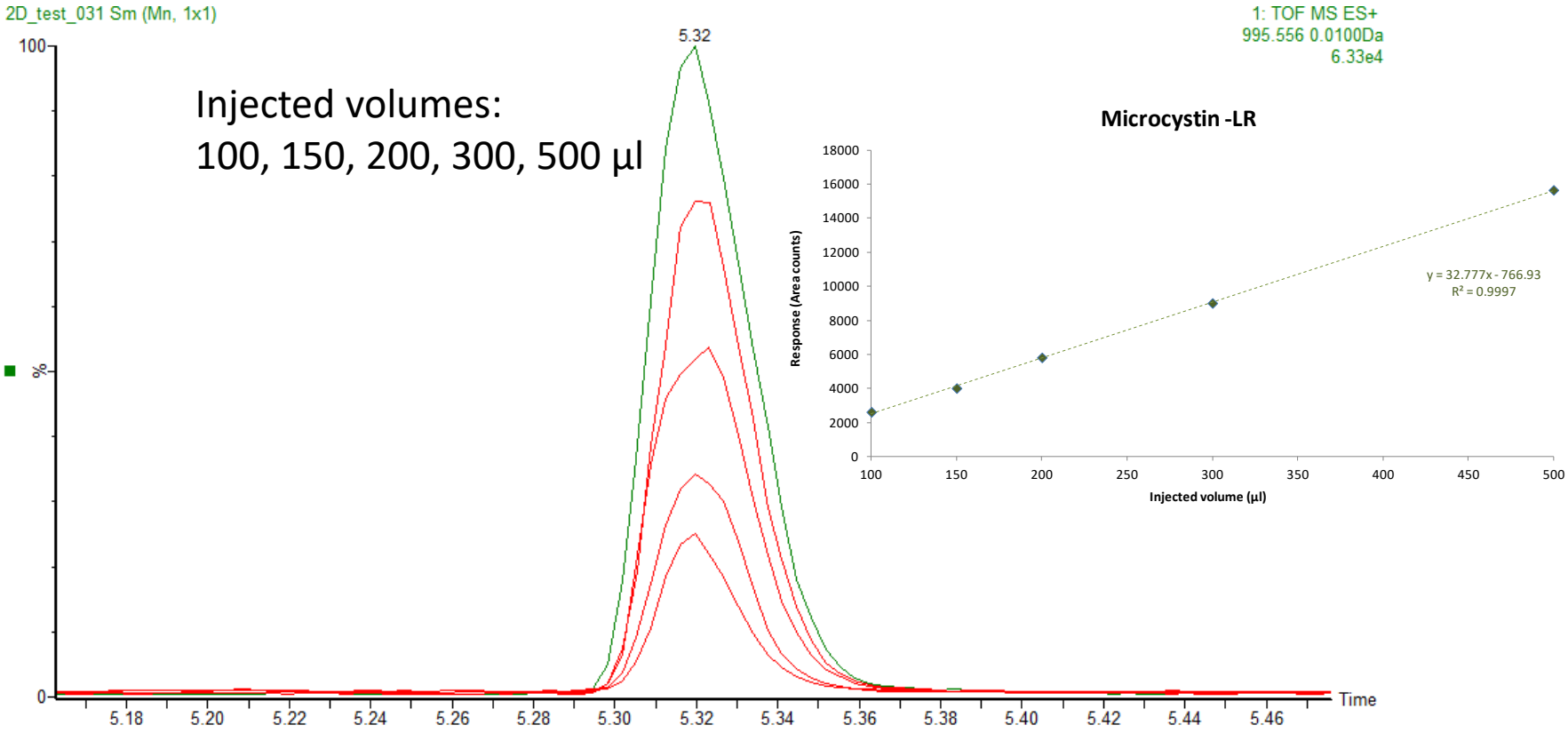

Microcystin sensitivity

- XIC from Tof Full Scan

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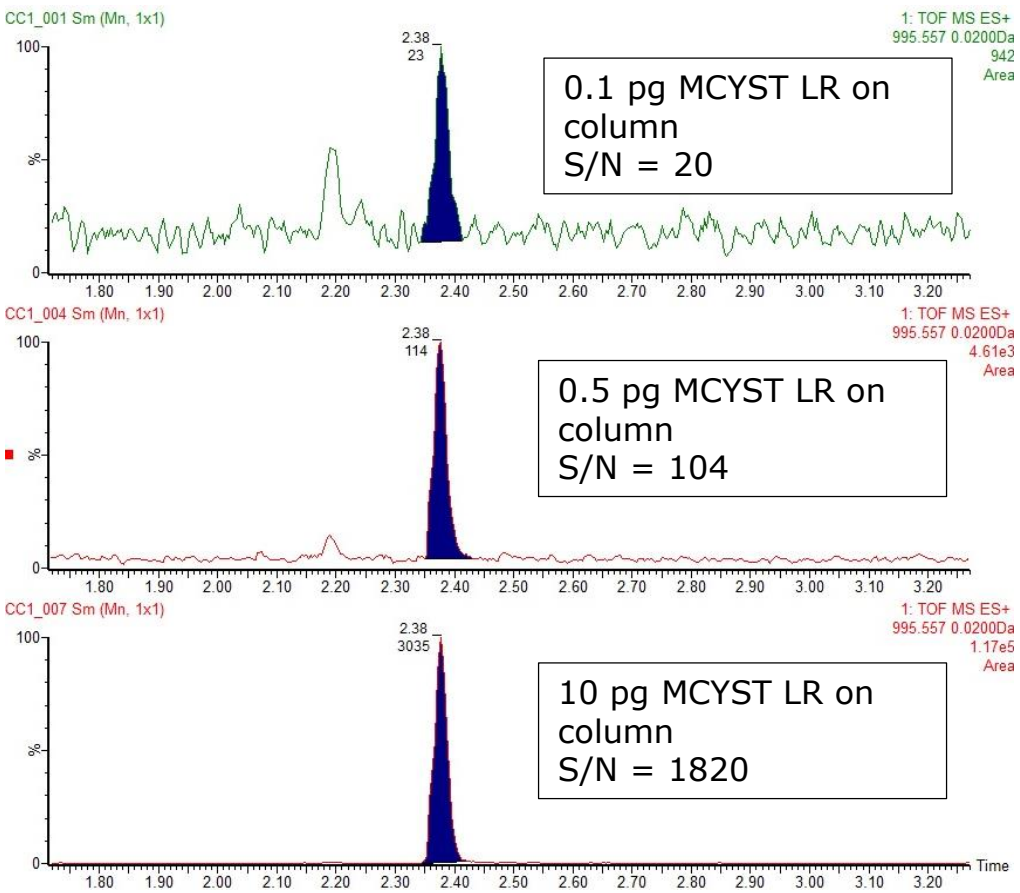
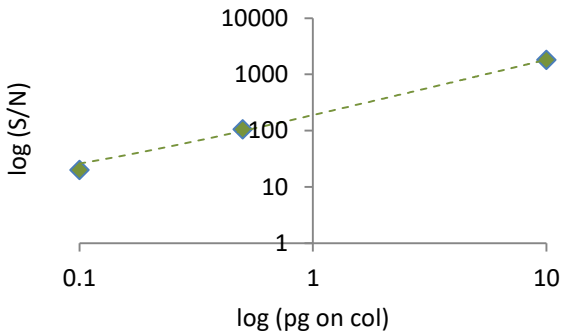


pg on col	S/N	Response Factor
0.1	20	200
0.5	104	210
10	1820	182



0.1 pg is already in the linear dynamic range!

Microcystin LR mass accuracy

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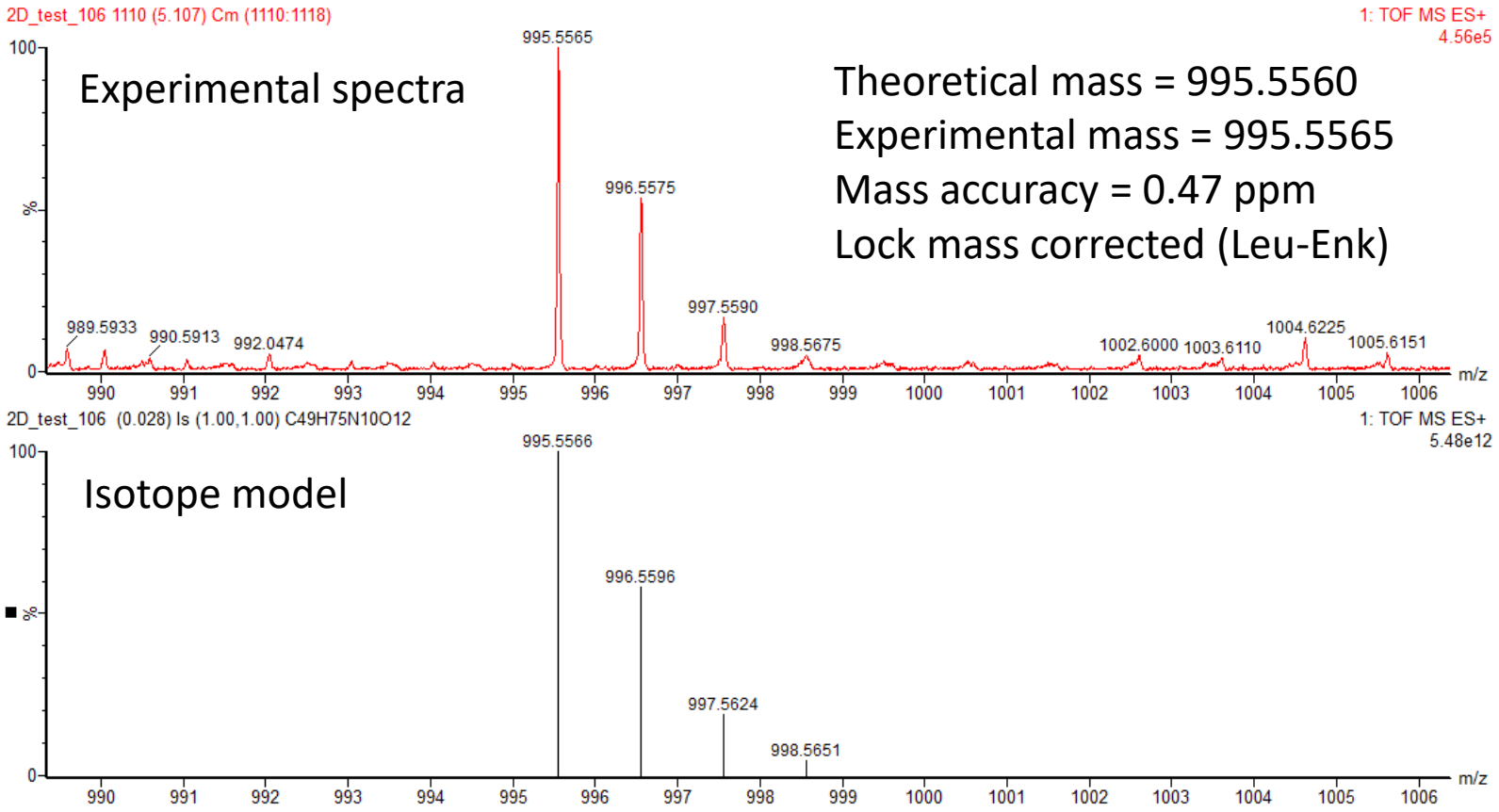
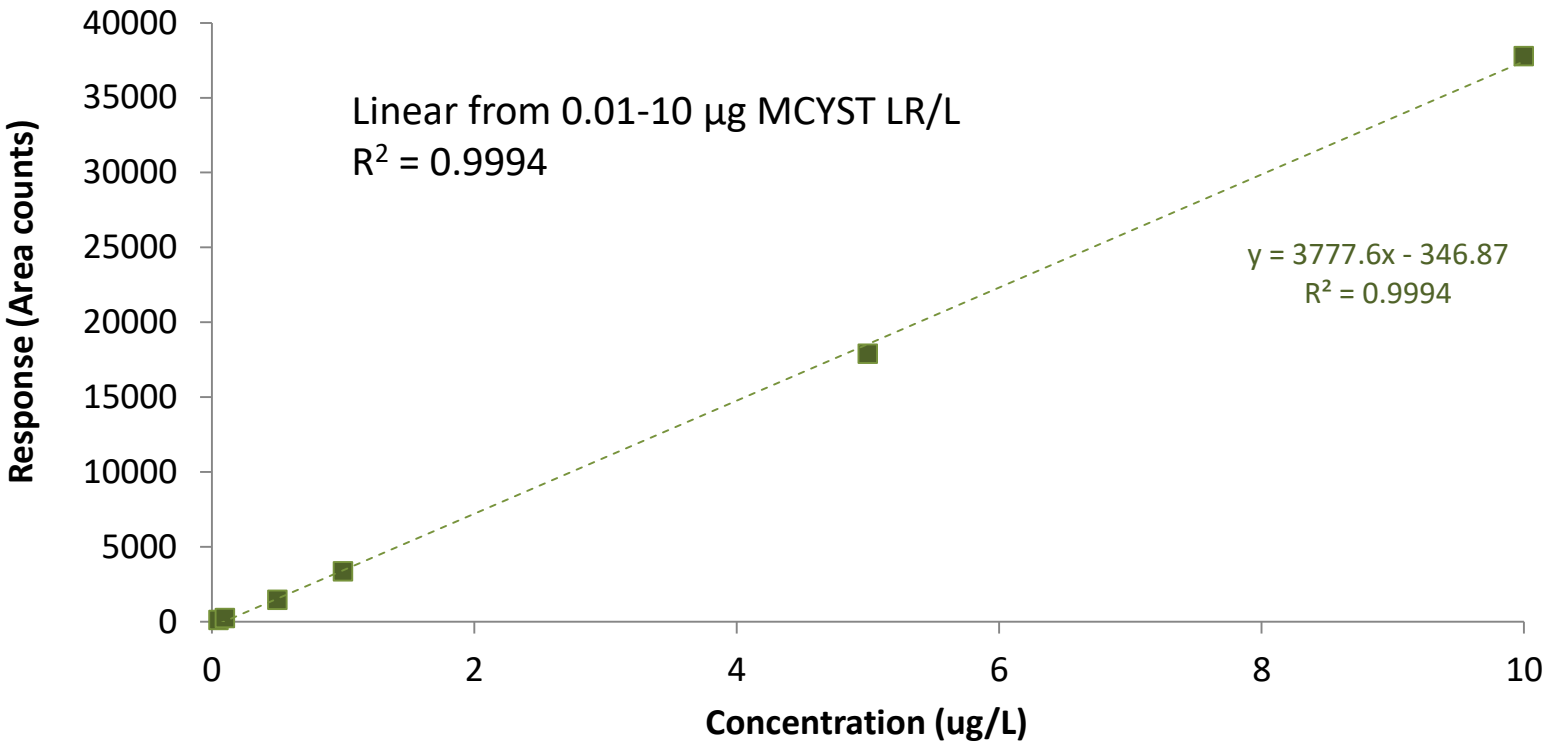


Theoretical mass = 995.5560
Experimental mass = 995.5565
Mass accuracy = 0.47 ppm
Lock mass corrected (Leu-Enk)

Microcystin LR linearity



Targeted analysis of microcystins in complex matrix @ 1ppt (500 µl inj.): MS vs MS/MS

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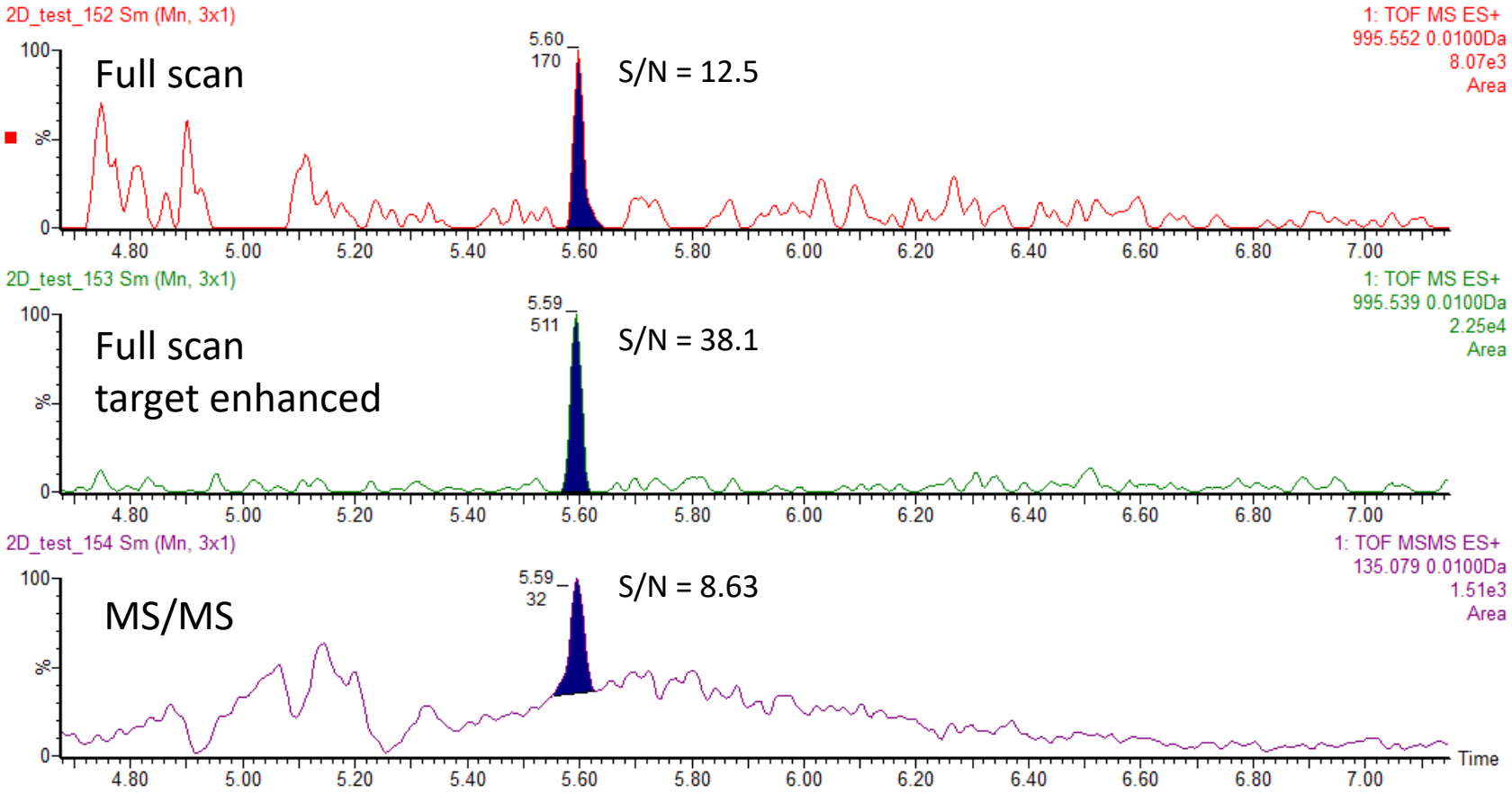

Targeted discovery of microcystins in real sample

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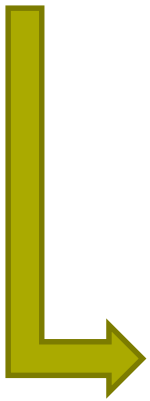
- Sample from algal bloom (2015 season) with high levels of microcystins determined with MOECC3450 method.
- All 12 monitored variants were detected by LC-MS/MS.
- Sample reanalized by 2DLC-QToF in full scan MS (no TE), XICs for the 100 variants described in literature[1].

[1]Timo Niedermeyer, Tubingen University
https://figshare.com/articles/_Microcystin_congeners_described_in_the_literature/880756



High resolution full scan data enables **retrospective analysis**: Inject once, reinterpret your data anytime!

Targeted analysis of microcystins in authentic sample

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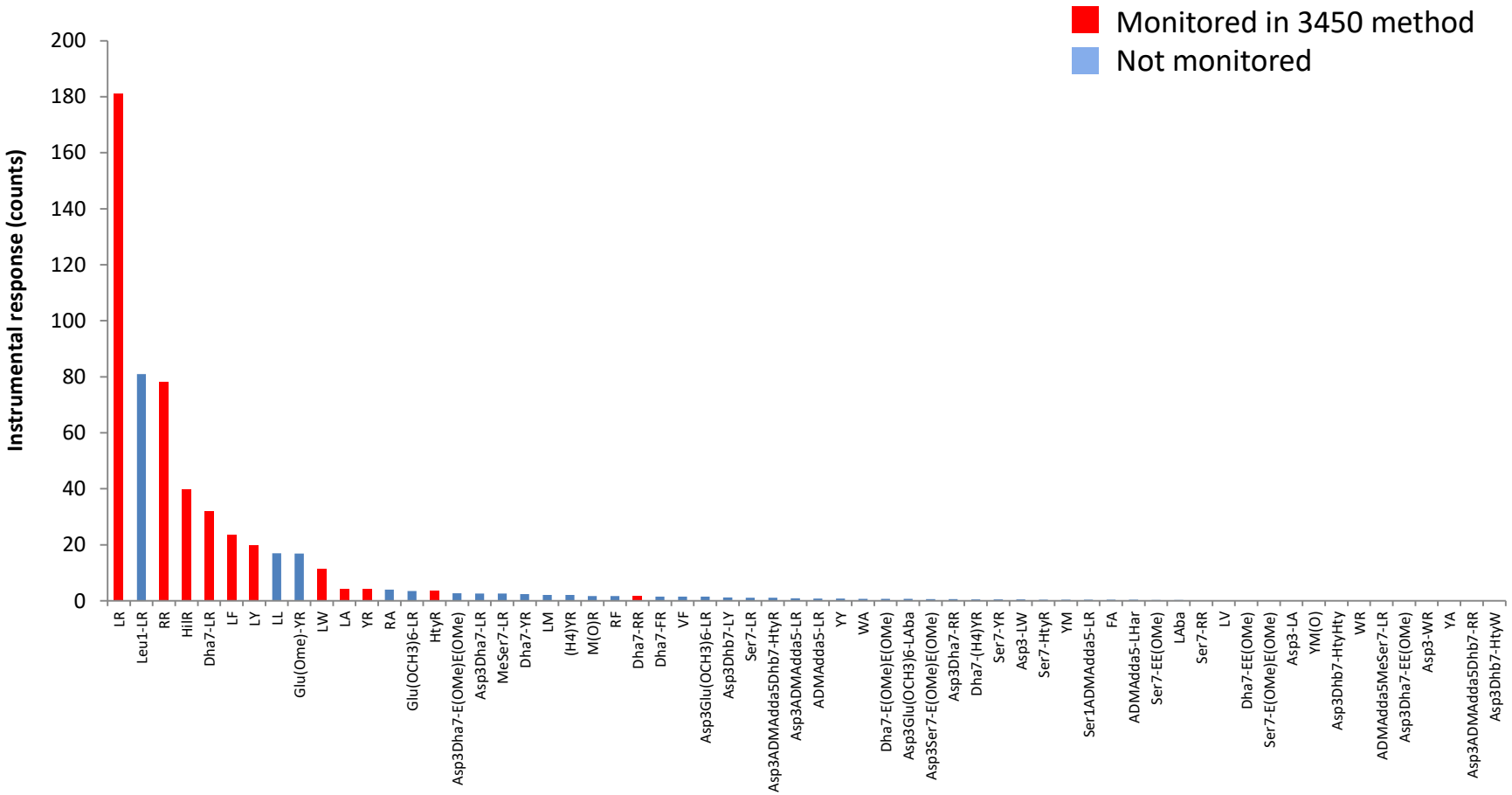

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Untargeted analysis of microcystins using a Xevo G2-XS (DDA acquisition)

Untargeted analysis of microcystins: DDA

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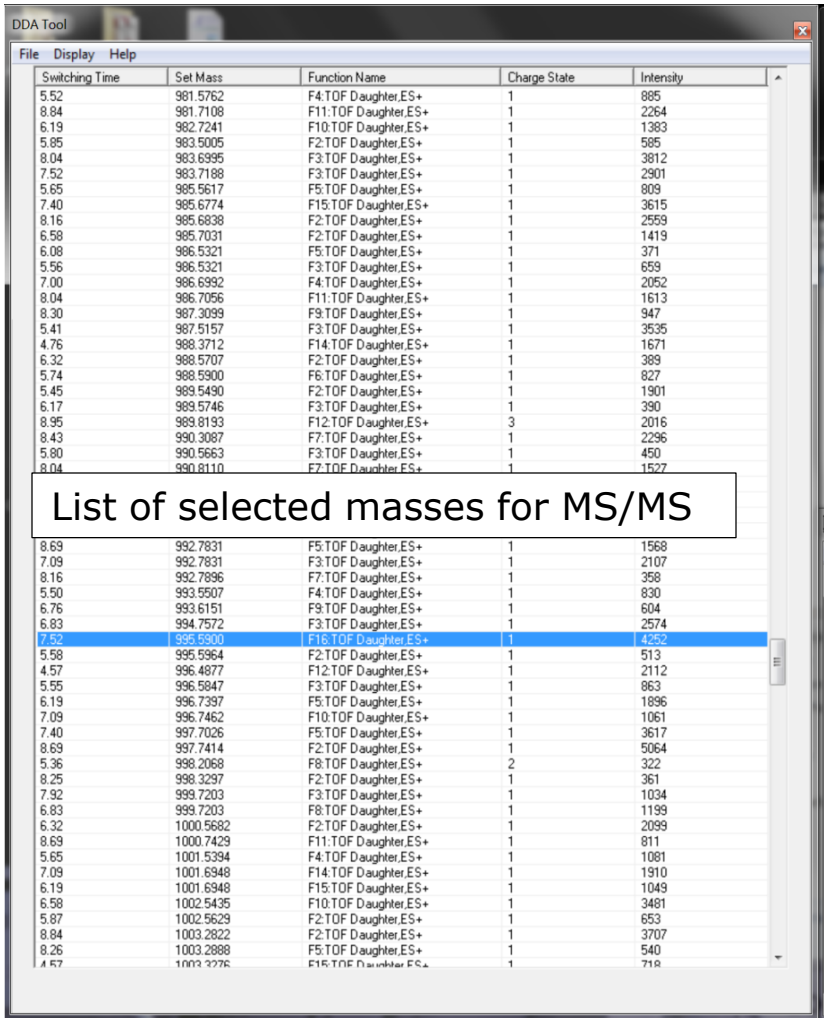

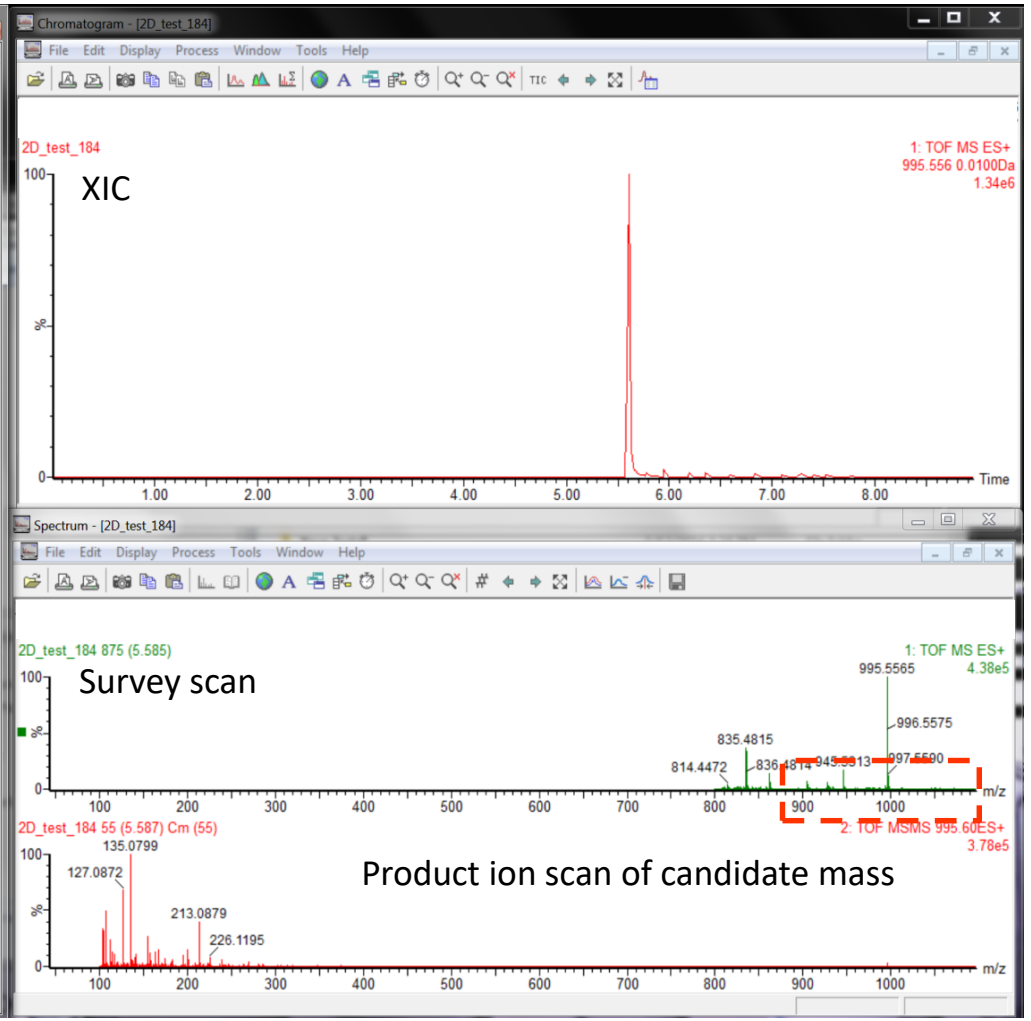

Untargeted analysis of microcystins: DDA

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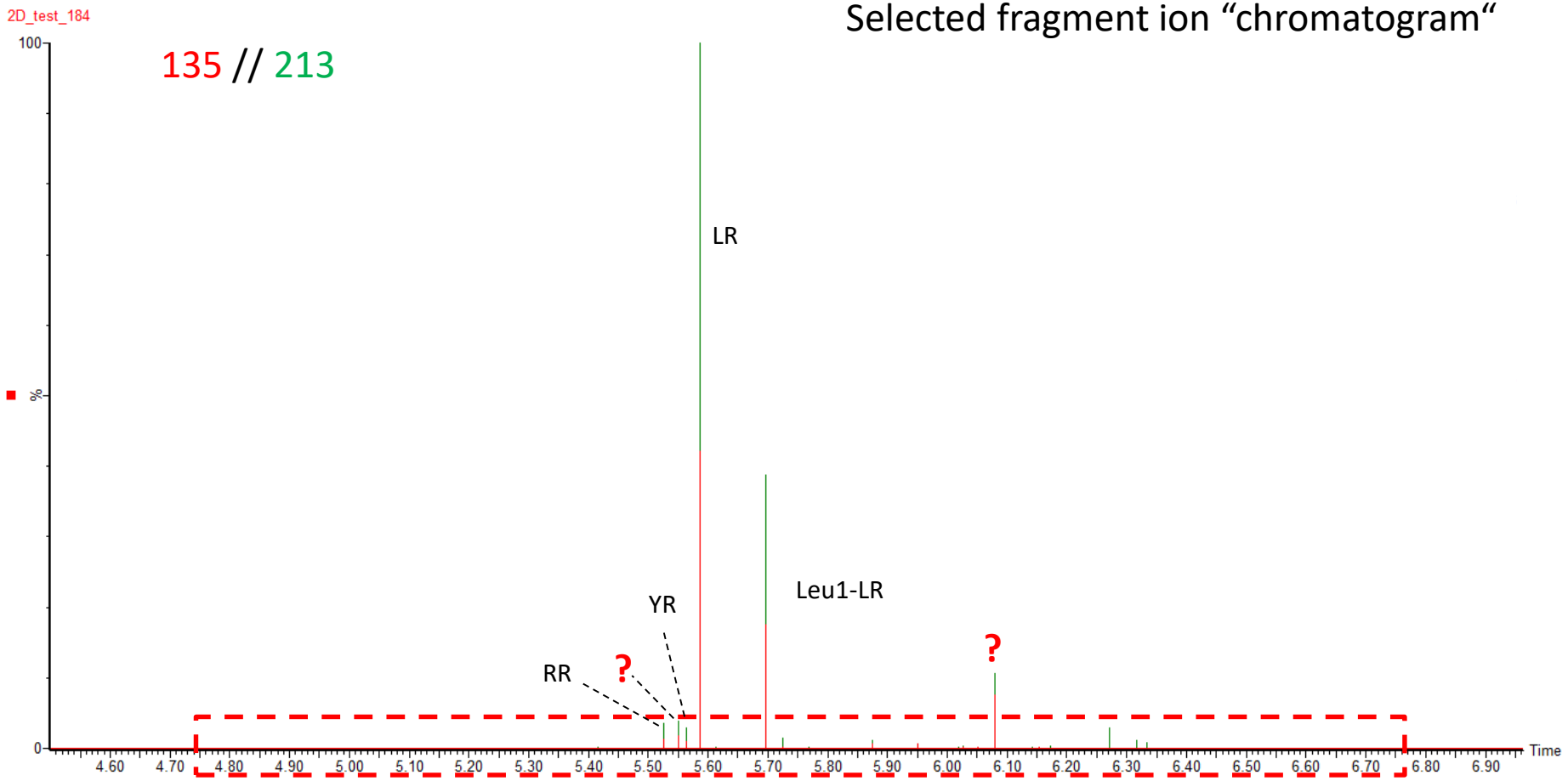
X7 diluted 1:10 DDA lock mass aplied 100 ul DDAtool

Selected fragment ion “chromatogram“



Untargeted analysis of microcystins: DDA

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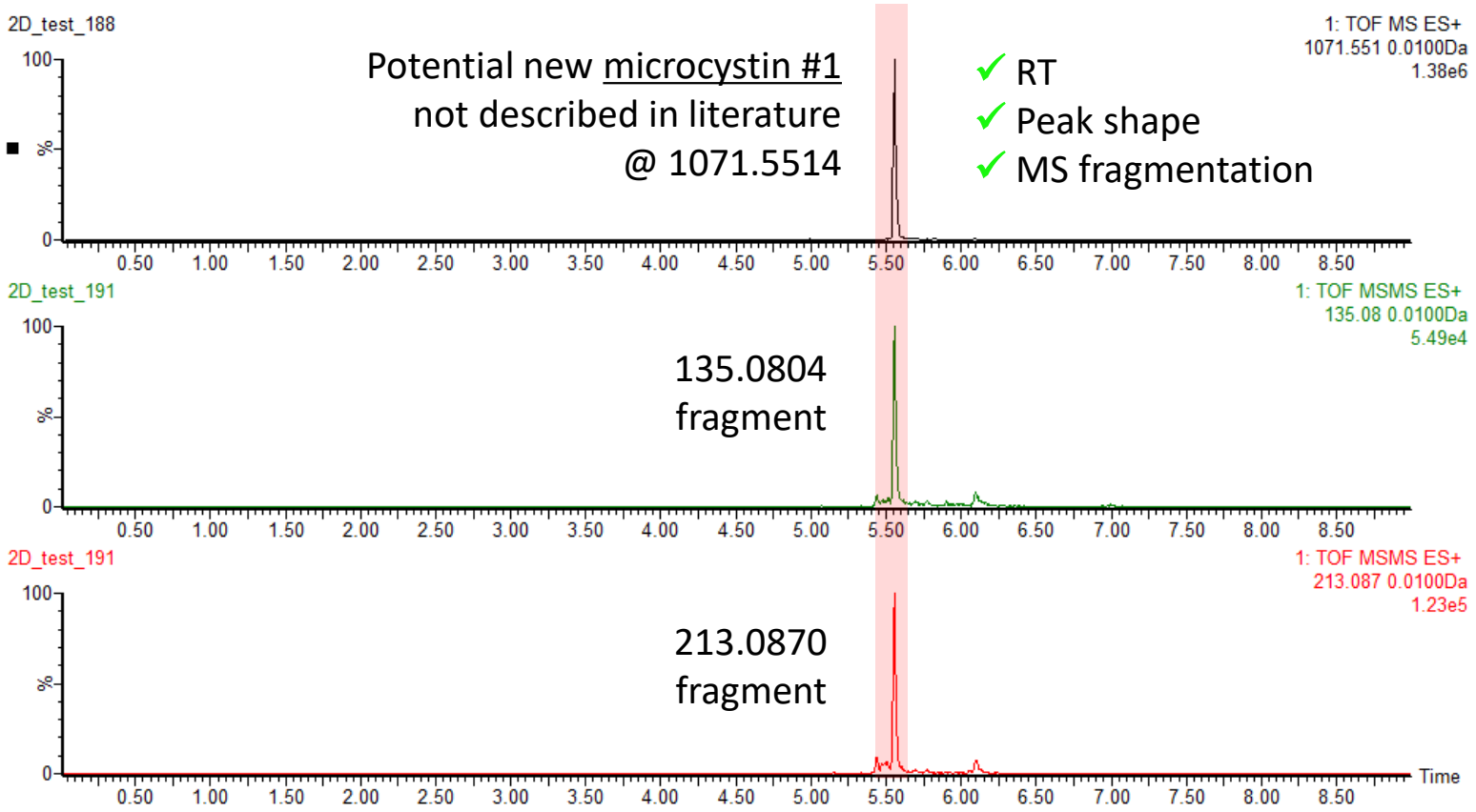

Untargeted analysis of microcystins: DDA

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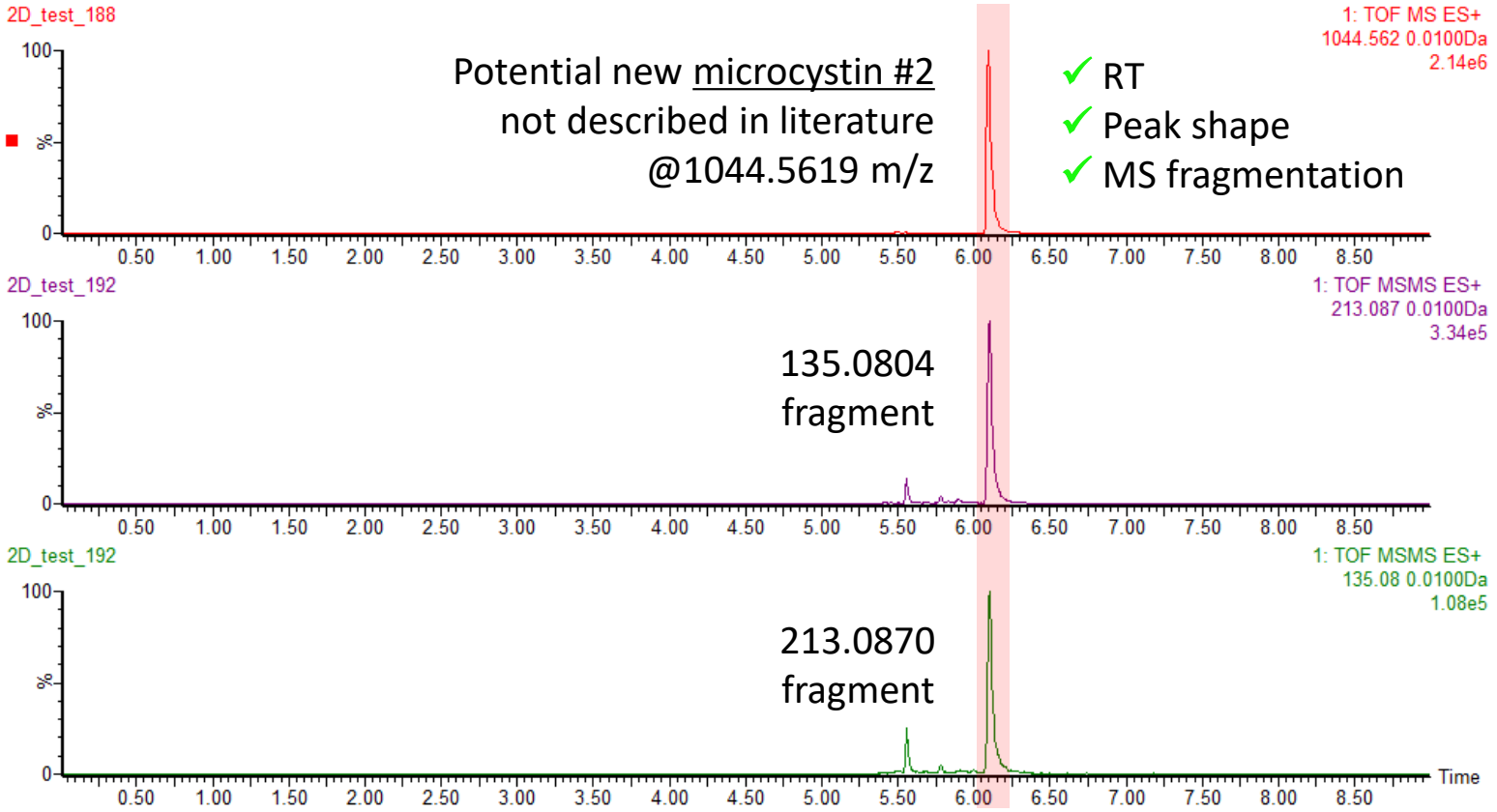

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Untargeted analysis of microcystins using a IMS QToF (HDMS^E acquisition)

Vion™ IMS QTof

Untargeted analysis of microcystins: Data independent acquisition (MS^E)

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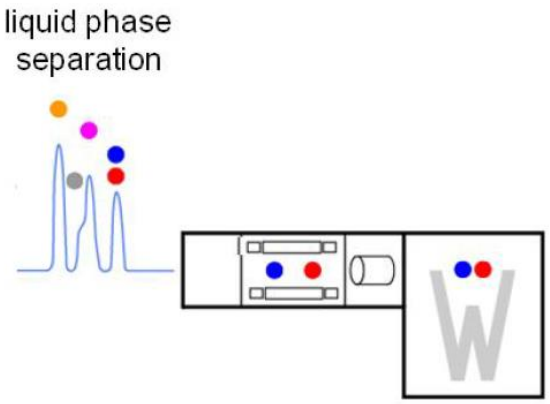

Ion Mobility Spectrometry

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- Separation of ionic species as they drift through a gas under the influence of an electric field
- Rate of drift is dependent on ion's mobility through the gas
- Mobility is dependent on factors such as:
    - Mass
    - Charge
    - Interaction Cross Section

What is CCS (Collisional Cross Section)?

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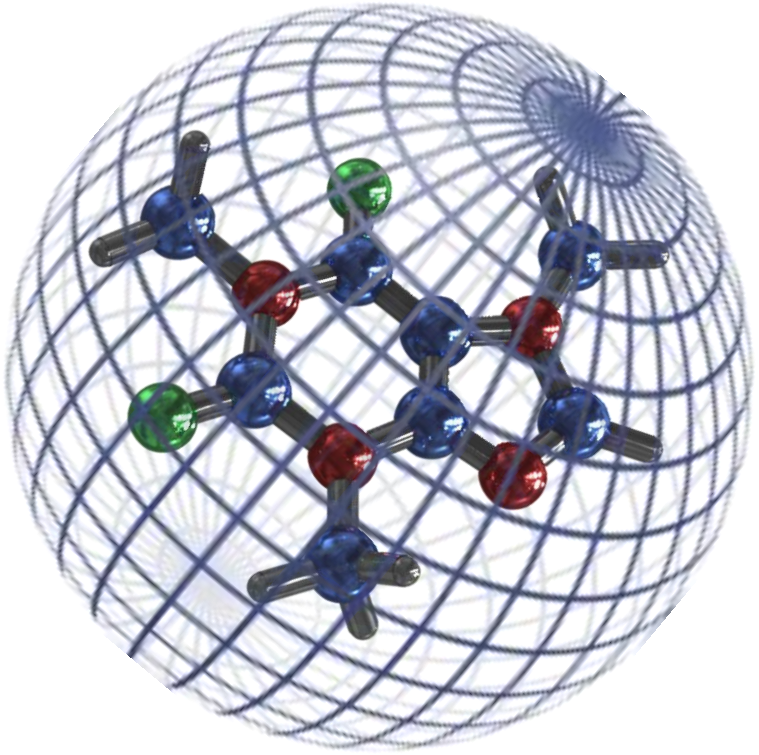
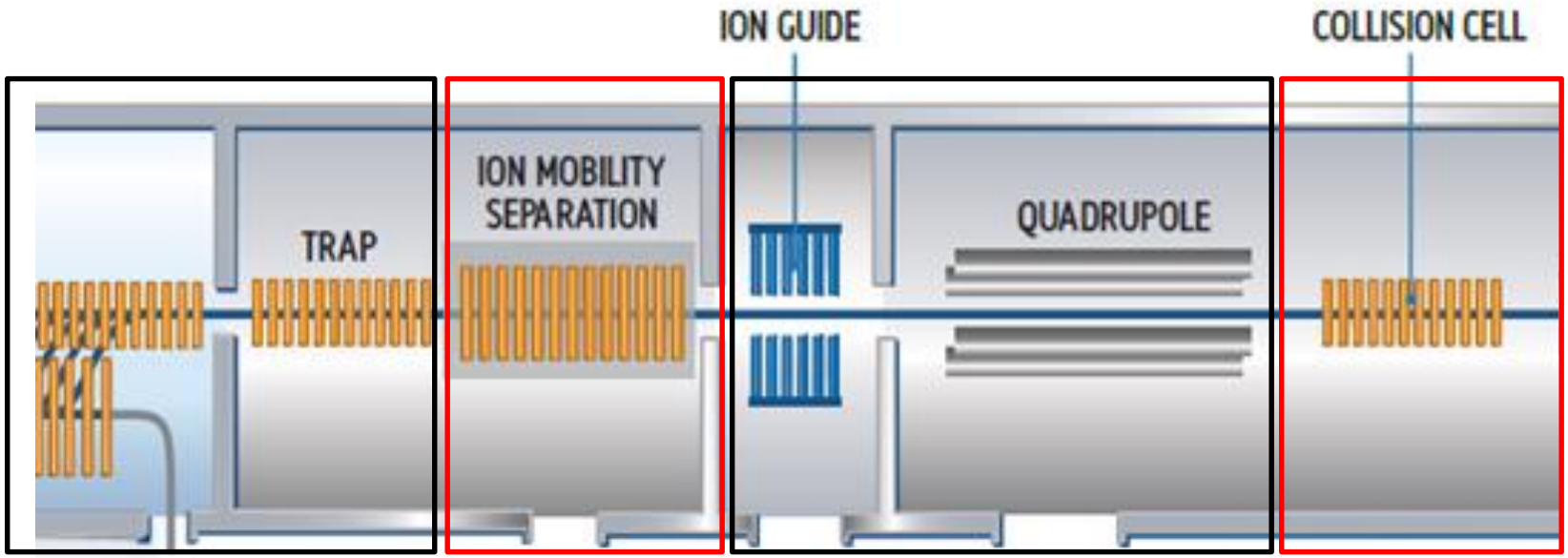
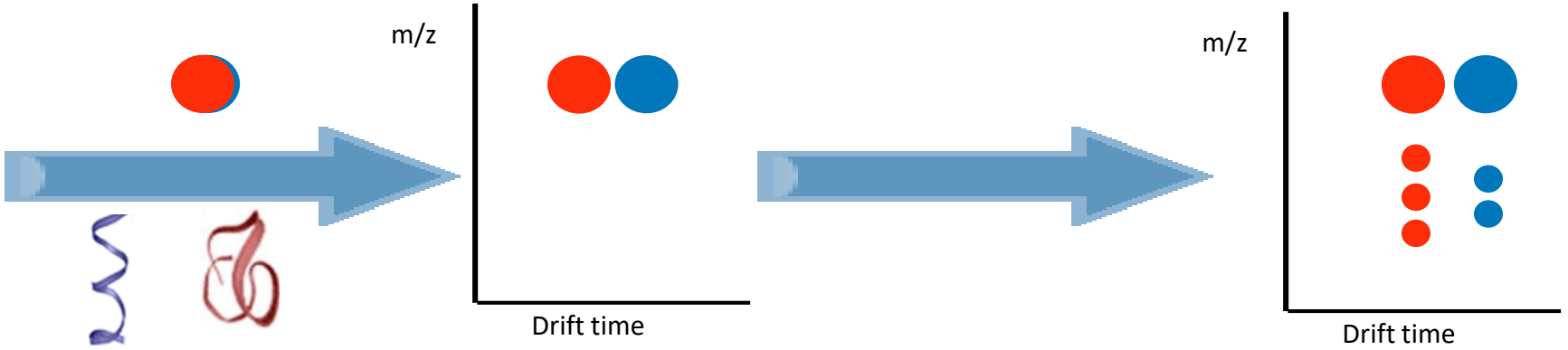
- CCS is related to:
  - chemical structure
  - 3D conformation
- CCS is a robust and precise physicochemical property of an ion



HDMS^E Product Ion Acquisition





Co-eluting precursor ions

Mobility separation

Drift time aligned precursors and products

IMS spectral clean up

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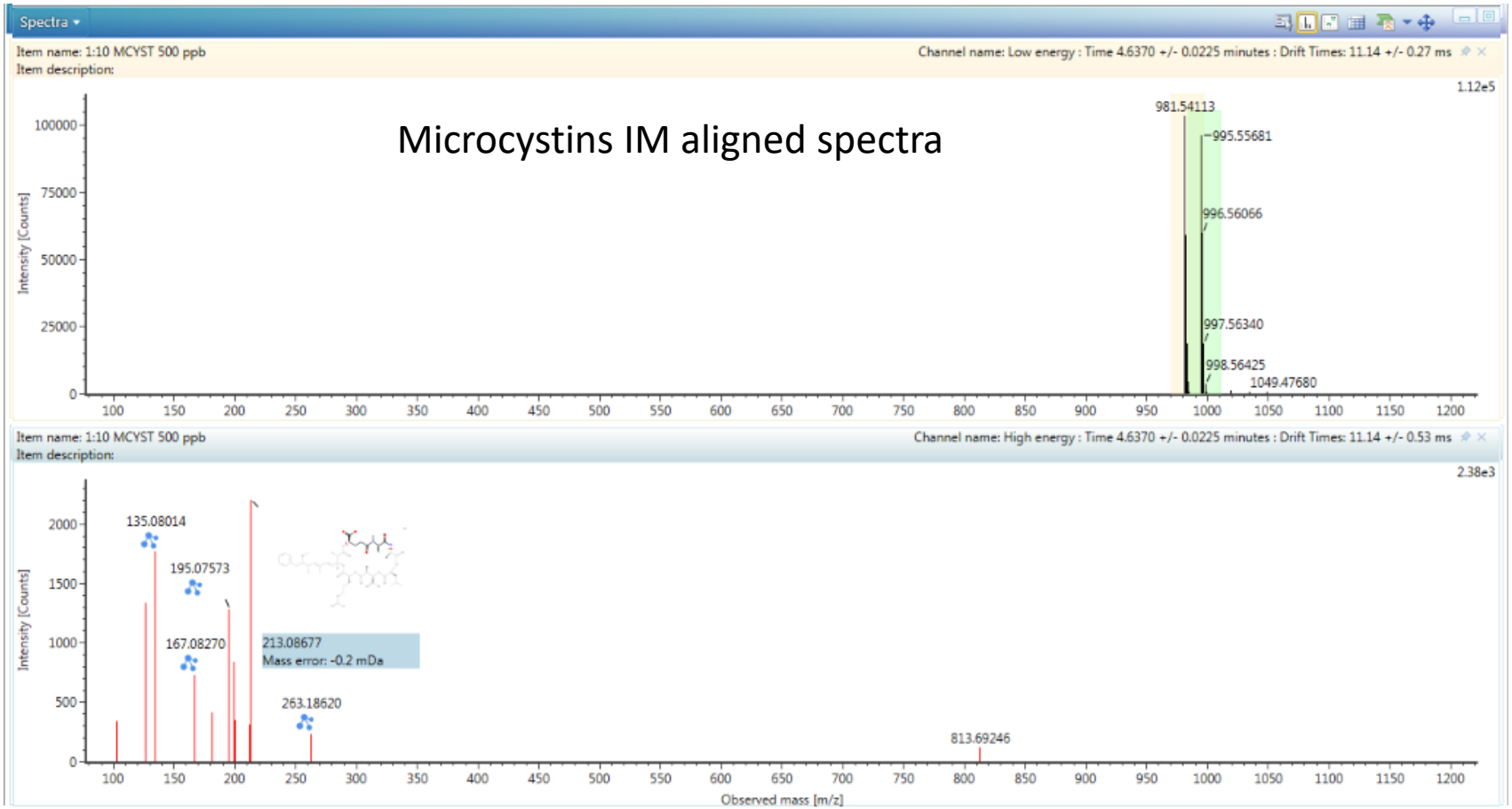

Microcystins IM aligned spectra

Scientific library set up

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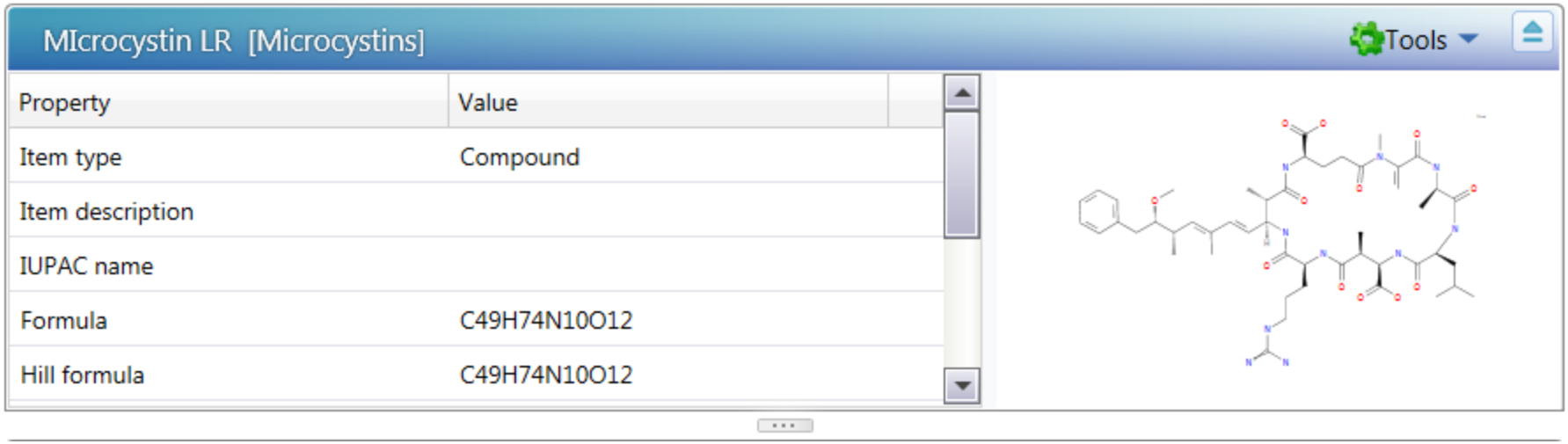
- A scientific library was generated containing 12 microcystins, anatoxin and nodularin including:
  - Formula
  - Common fragments (135.0802, 135.1168, 213.0870)
  - Structure (where available)



Scientific library set up

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- Microcystins CCS, m/z and RT ranges were determined

	Component name	Identification status	Observed m/z	Mass error (mDa)	Mass error (ppm)	Expected RT (min)	Observed RT...	Observed drift (ms)	Observed CCS (Å²)	Expected CCS (Å²)	CCS delta (%)
1	Anatoxin	Identified	166.1232	0.6	3.6	1.85	1.84	4.35	136.04	136.82	-0.57
2	Microcystin dmLR	Identified	981.5400	-0.4	-0.4	4.63	4.63	10.91	306.96	309.93	-0.96
3	Microcystin dmRR	Identified	512.7824	0.0	0.0	3.95	3.95	6.19	345.06		
4	Microcystin HilR	Identified	1009.5730	1.3	1.2	4.75	4.75	11.26	318.06	319.35	-0.41
5	Microcystin HtyR	Identified	1059.5502	-0.7	-0.7	4.56	4.56	11.30	319.46	321.00	-0.48
6	Microcystin LA	Identified	910.4935	1.5	1.6	5.85	5.85	10.63	298.06	300.31	-0.75
7	Microcystin LF	Identified	986.5246	1.3	1.3	6.60	6.60	11.09	312.51	314.39	-0.60
8	MIcrocystin LR	Identified	995.5559	-0.1	-0.1	4.64	4.64	11.08	312.25	314.90	-0.84
9	Microcystin LW	Identified	1025.5365	2.2	2.2	6.42	6.42	11.33	320.16	322.03	-0.58
10	Microcystin LY	Identified	1002.5200	1.7	1.7	5.91	5.91	11.24	317.16	318.59	-0.45
11	Microcystin RR	Identified	519.7902	0.1	0.1	4.03	4.04	6.26	348.69		
12	Microcystin WR	Identified	1068.5522	0.9	0.8	4.78	4.78	11.43	323.35	326.60	-1.00
13	Microcystin YR	Identified	1045.5345	-0.8	-0.8	4.53	4.54	11.32	319.94	324.16	-1.30
14	Nodularin	Identified	825.4502	-0.3	-0.4	4.62	4.39	10.60	297.24	300.06	-0.94

m/z (singly charged)
800 - 1200

RT
3.5-7 min

CCS
290-360 Å^2

Algal bloom sample HDMS^E analysis

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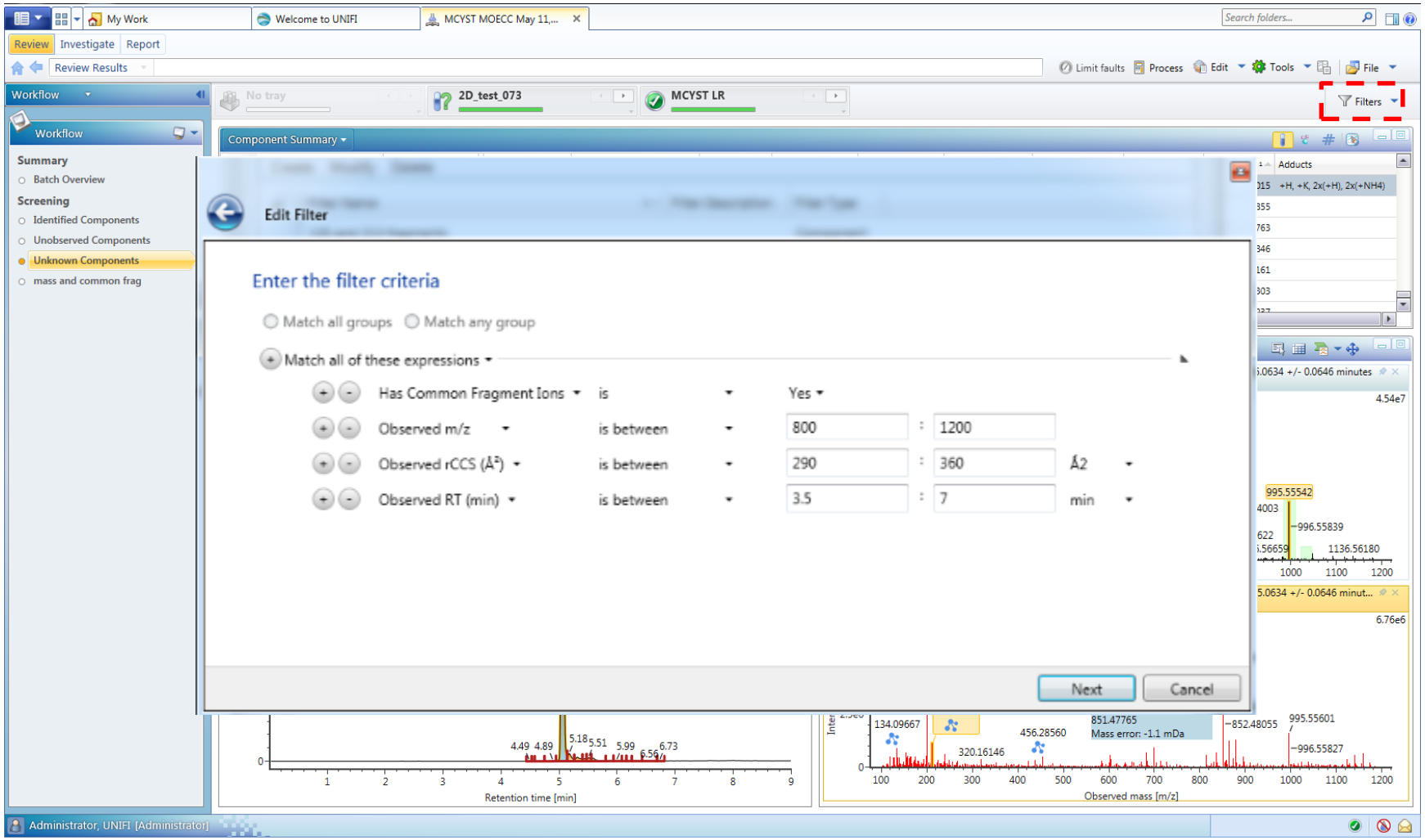
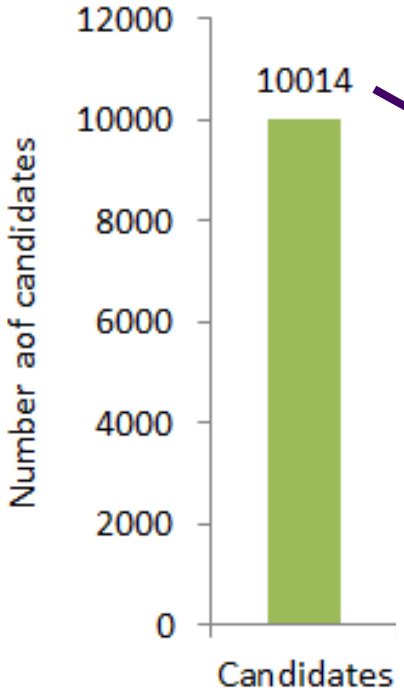


HDMS^E candidates filtering



- 98.5% of the initial candidates were discarded
- Filtered final candidates with cleaner spectra after IMS

Conclusions

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- 2DLC allows large volume injections, increasing sensitivity and maintaining chromatographic resolution.
- Xevo G2-XS QToF has shown excellent sensitivity (0.1 pg LR on column with S/N = 20), mass resolution (> 25,000), mass accuracy (< 2-3 ppm) and dynamic range (> 4 orders of magnitude) for microcystin analysis.
- Advanced acquisition methods such as DDA or MS^E can be useful tools for untargeted analysis of new microcystin variants not yet described in literature.
- Ion mobility offers a new dimension of separation, orthogonal to mass spectrometry and chromatography. This technique is very useful to screen unknown compounds.
- Mobility aligned mass spectra has higher selectivity than regular mass spectra, eliminating chemical interferences and facilitating MS identification and structure elucidation.

Acknowledgements

- De Watergroep, Belgium

- Ministry of Environment and Climate Change, Toronto

Thank You for Your Attention

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