Ontario



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

- 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



- Microcystins are cyclic peptides produced by <u>cyanobacteria</u>
- 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

- 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



Over 100 different variants reported in literature!





Microcystins common diagnostic fragments



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





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





2D LC conditions





Advantages of 2DLC for microcystin analysis: Maintained chromatographic resolution

2D test 031 Sm (Mn, 1x1) 1: TOF MS ES+ 5.32 995.556 0.0100Da 100-6.33e4 Injected volumes: **Microcystin -LR** 100, 150, 200, 300, 500 µl 18000 16000 14000 Response (Area counts) 12000 y = 32.777x - 766.93 10000 $R^2 = 0.9997$ 8000 6000 ** 4000 2000 0 100 150 200 250 350 400 450 300 500 Injected volume (µl) Time 5.20 5.22 5.24 5.26 5.28 5.30 5.32 5.34 5.40 5.42 5.18 5.36 5.38 5.44 5.46

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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



Microcystin LR mass accuracy

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Microcystin LR linearity





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



Targeted discovery of microcystins in real sample



- 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 literature¹.

¹Timo Niedermeyer, Tubingen University <u>https://figshare.com/articles/_Microcystin_congeners_described_in_the_literature/880756</u>

High resolution full scan data enables <u>retrospective analysis</u>: Inject once, reinterpret your data anytime!

Targeted analysis of microcystins in authentic Maters sample THE SCIENCE OF WHAT'S POSSIBLE. Monitored in 3450 method 200 Not monitored 180 160 Instrumental response (counts) 140 120 100 80 60 40 20 0 HtyR (H4)YR M(O)R Dha7-RR Dha7-FR Leu1-LR RR HilR RA Dha7-YR Ser 7-LR Ч Dha7-LR ц ≿ Ξ Glu(Ome)-YR ≥ P ΥR Glu(OCH3)6-LR Σ RF ¥ Ser7-YR Ser7-RR Asp3-LA Asp3-WR Asp3Dha7-E(OMe)E(OMe) Asp3Dha7-LR MeSer7-LR Asp3Glu(OCH3)6-LR Asp3Dhb7-LY Asp3ADMAdda5Dhb7-HtyR Asp3ADMAdda5-LR ADMAdda5-LR ≿ WA ž Ā LAba \geq YM(0) WR ¥ Dha7-E(OMe)E(OMe) Asp3Glu(OCH3)6-LAba Asp3Ser7-E(OMe)E(OMe) Asp3Dha7-RR Dha7-(H4)YR Asp3-LW Ser7-HtyR Ser1ADMAdda5-LR ADMAdda5-LHar Ser7-EE(OMe) Dha7-EE(OMe) Ser7-E(OMe)E(OMe) Asp3Dhb7-HtyHty ADMAdda5MeSer7-LR Asp3Dha7-EE(OMe) Asp3ADMAdda5Dhb7-RR Asp3Dhb7-HtyW



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

Untargeted analysis of microcystins: DDA

Waters

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Untargeted analysis of microcystins: DDA



Untargeted analysis of microcystins: DDA



ers

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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)





Ion Mobility Spectrometry



- 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)?



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



HDMS^E Product Ion Acquisition

Waters





IMS spectral clean up





Scientific library set up

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

MIcrocystin LR [Microcystins]		🧔 Tools 🔻 🧧
Property	Value	
Item type	Compound	
Item description		
IUPAC name		
Formula	C49H74N10O12	
Hill formula	C49H74N10O12	



Scientific library set up

• 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 (Å ²)	Expected CCS (Å ²)	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 Ų

Algal bloom sample HDMS^E analysis



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HDMS^E candidates filtering





Conclusions



- 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



