Application of a screening method for cyanobacterial toxins in natural samples

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Introduction

Cyanobacterial toxins as microcystins (MCs), nodularins (NODs), Paralytic Shellfish Poisoning (PSP) toxins, anatoxins (ANAs), and cylindrospermopsins (CYNs) with over 100 known varieties, occur worldwide associated with human and animal lethal poisoning.

In contrast to all analytical methods for toxin determination are based on LC/MS-MS measurements with Multiple Reaction Monitoring (MRM) the application of Precursor Ion mode allows the coverage of all these structural variants. Although in MRM mode enables a higher sensitivity, a lot of information regarding structural changes is missed. The new generation of Q-TRAPs combines the advantages of quadrupoles having a high selectivity with the high sensitivity of ion-trap systems.

Here we published results, showing the suitability of Precursor Ion mode for detection of cyanobacterial toxins extracted from phytoplankton.

Table 1: Retention times, MRMs, precursor ions and LOD of cyanobacterial toxins

	period 1				period 2				period 3			
Toxin	t_R (min)	mass transition (*m/z*)	LOD (pg)	Toxin	t_R (min)	Precursor ion (*m/z*)	LOD (pg)	Toxin	t_R (min)	Precursor ion (*m/z*)	LOD (pg)
NEO	1.4	316 > 298	1.0	**ANA**	5.2	91.0	700	**MC-RR**	12.3	135.0	20
dcSTX	1.4	257 > 239	9.0	**CYN**	5.4	194.0	9	**MC-YR**	14.9	135.0	15
STX	1.4	300 > 282	10.0	**doCYN**	5.5	194.0	no standard	**MC-LR**	15.5	135.0	15
B 1	1.5	380 > 300	3.0					**MC-LA**	19.7	135.0	18
dcGTX 2/3	1.6	353 > 273	25.0					**MC-LW**	21.0	135.0	100
GTX 2/3	1.6	396 > 316	12.0					**MC-LF**	22.0	135.0	100
GTX 1/4	1.7	412 > 332	6.0					**NOD**	14.6	135.0	6

t_R Retention time; LOD: S/N = 3

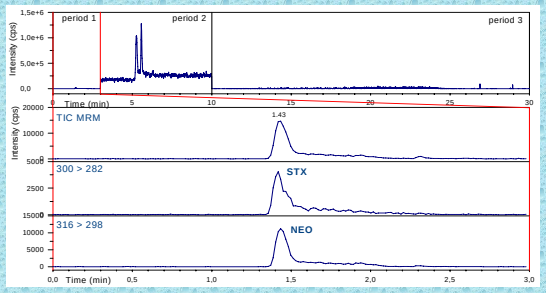

Table 2: MS parameter for detection of PSP toxins

MS parameters	
MRM mode	
transition:	$[M+H]^+$ / $[M+H - H_2O]^+$
300 / 282	STX
316 / 298	NEO
IS:	5000 V
CAD:	high level
Temp.:	550 °C
Gas 1:	50 L h^{-1}
Gas 2:	70 L h^{-1}
CUR:	25 L h^{-1}
CE:	30 eV
DP:	40 eV

Fig 1: MRM chromatogram of STX and NEO at t_R 1.43 min from *Aphanizomenon flos-aquae* extract

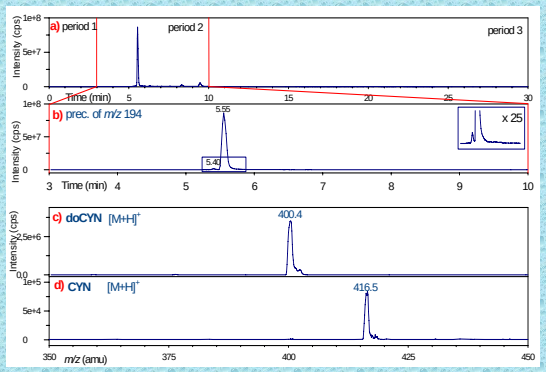

Table 3: MS parameter for detection of CYNs

MS parameters	
Precursor Ion mode	
protonated ion:	$[M+H]^+$
precursor ion (*m/z*):	194.0
scan range (*m/z*):	350-450 amu
IS:	5200 V
CAD:	high level
Temp.:	550 °C
Gas 1:	50 L h^{-1}
Gas 2:	70 L h^{-1}
CUR:	25 L h^{-1}
CE:	50 eV
DP:	80 eV

Fig 2: Precursor Ion spectrum of total run (a); of time period 2 (b) containing CYN at t_R 5.40 min and doCYN at t_R 5.55 min; Precursor Ion chromatogram of doCYN (c) and CYN (d) from *Lyngbya wollei* extract

Material and Methods

Lyophilised phytoplankton samples of *Aphanizomenon flos-aquae* [Fig 1], *Lyngbya wollei* [Fig 2], *Nodularia spumigena* [Fig 3] and *Microcystis aeruginosa* [Fig 4] were extracted with a methanol - 0.1 M acetic acid (1:1) solution using ultrasonic bath and ultrasonic stick. An HPLC system equipped with Agilent 1100 series components was applied for the separation. LC/MS-MS experiments were performed using a 4000 Q Trap (ABI Sciex, Darmstadt, Germany) equipped with a turbo ion-spray source. The chromatographic separation was carried out with a Luna column (3 µm, 150 mm x 3.0 mm; Phenomenex, USA) using two eluents containing 50 mM formic acid and 2 mM ammonia formate in water (eluent A) or methanol / water (95/5, eluent B) and gradient elution.

Based on the retention times [Table 1], three time periods with different detection modes and parameters [Table 2-5] were generated for the different toxin classes. Only PSP toxins were detected in MRM mode, due to no known daughter ion usable in Precursor Ion mode. For ANAs, CYNs, MCs and NODs a fragment characteristic for each toxin group was utilized to analyse the compounds in Precursor Ion mode [Table 1].

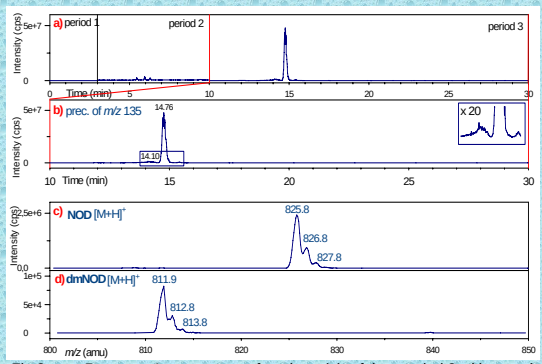

Table 4: MS parameter for detection of NODs

MS parameters	
Precursor Ion mode	
protonated ion:	$[M+H]^+$
precursor ion (*m/z*):	135.0
scan range (*m/z*):	800-850 amu
IS:	5500 V
CAD:	high level
Temp.:	550 °C
Gas 1:	50 L h^{-1}
Gas 2:	70 L h^{-1}
CUR:	15 L h^{-1}
CE:	90 eV
DP:	175 eV

Fig 3: Precursor Ion spectrum of total run (a), of time period 3 with experiment for NODs containing dmNOD at t_R 14.10 min and NOD at t_R 14.76 min (b), Precursor Ion chromatogram of NOD (c) and dmNOD (d) from *Nodularia spumigena* extract

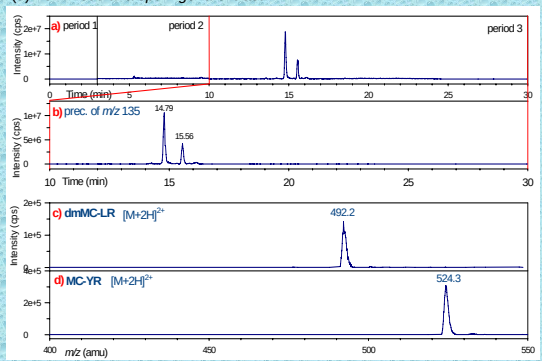

Table 5: MS parameter for detection of MCs

MS parameters	
Precursor Ion mode	
protonated ion:	$[M+2H]^{2+}$
precursor ion (*m/z*):	135.0
scan range (*m/z*):	400-550 amu
IS:	5500 V
CAD:	high level
Temp.:	550 °C
Gas 1:	50 L h^{-1}
Gas 2:	70 L h^{-1}
CUR:	15 L h^{-1}
CE:	35 eV
DP:	40 eV

Fig 4: Precursor Ion spectrum of total run (a); of time period 3 with experiment for MCs containing dmMC-LR at t_R 14.79 min and MC-YR at t_R 15.56 min (b); Precursor Ion chromatogram of dmMC-LR (c) and MC-YR (d) from *Microcystis aeruginosa* extract

Conclusions

It could be shown, that a qualitative screening method using Precursor Ion mode is well suited for detection cyanobacterial toxin variants, which allows a very rapid screening of putatively toxic cyanobacterial samples of uncertain taxonomic composition and unknown toxin profile.