Validation of targeted and non-targeted methods of analysis, Tartu, Estonia
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Advantages of Ultra Performance Liquid Chromatography–High Resolution Mass Spectrometer for the Analysis of Cyanotoxins in Water for Human Consumption

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INTRODUCTION

The presence of cyanobacteria in water intended for human consumption may represent a risk to human health due to the possible production of secondary metabolites, called cyanotoxins, toxic for animals and humans.
This work presents the comparison between two different mass spectrometric techniques (UPLC-HRMS/MS and LC-MS/MS) that allow the simultaneous detection of 21 cyanotoxins, of different classes (including 12 Microcystins, 5 Microginins, 2 Cyanopeptolins, and 2 Anabaenopeptins)

Figure 1. Bloom of cyanobacteria

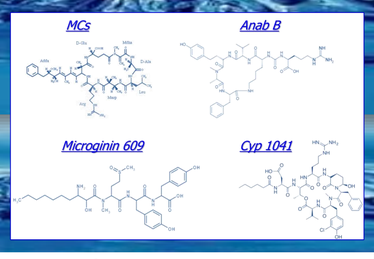

Figure 2. Structures of Microcystins, Anabaenopeptin B, Microginin 690, Cyanopeptolin 1041

EXPERIMENTAL METHOD

Drinking water samples were extracted and analyzed using a triple quadrupole and a High Resolution Q-TOF mass spectrometer and the results were compared.

250 ml of sample
+ 1 ng/mL
of Nodularin (I.S.)
filtered on Black
Ribbon filters.

EXTRACTION ON SPE CARBOGRAPH 4 CARTRIDGES:

Eluition: 1 mL CH_3OH + 6 mL CH_2Cl_2/CH_3OH (80:20 v/v) 10 mM TFA

Recovery: 1 mL H_2O/C_2H_3N 70:30 v/v

LC-MS/MS METHOD

- API 3000 (Atmospheric Pressure Ionization)
- Source TIS (Turbo Ion Spray)
- TIS 5500 V (positive ionization mode)
- Temperature: 450 °C
- Curtain gas flow: 10 u.a.
- Nebulizer gas flow: 12 u.a.
- Turbo gas flow: 7 u.a.
- Alltima C18 column
- 50 µl injected

	Retention [min]	Flow [µl/min]	%B	%C	Curve
1	0.000	200	15.0	0.0	
2	0.000	200	15.0	0.0	5
3	14.000	200	65.0	0.0	5
4	15.000	200	80.0	0.0	2
5	17.000	200	100.0	0.0	5
6	20.000	200	100.0	0.0	1
7	21.000	200	15.0	0.0	2
8	27.000	200	15.0	0.0	1

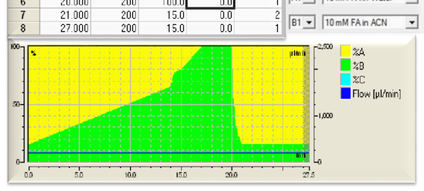

Figure 3. *LC-MS/MS* Method

UPLC-HRMS METHOD

- Xevo G2 (Qtof)
- Scan Mass: 50 to 1200 Da
- Polarity: ES+
- Analyser Mode: Sensitivity
- Source Temperature (°C): 130
- Desolvation Temperature (°C): 500
- Desolvation Gas Flow (L/Hr): 1000.0
- Column Temperature (°C): 40
- Column: BEH C18 1.7 µm
- 10 µl injected

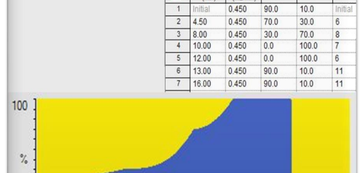

Figure 4. UPLC-HRMS Method

RESULTS AND DISCUSSION

- LC-MS/MS method : reproducibility better than 17% and LODs were in the range of 0.003– 0.032 µg/L for all the analytes; a good linearity was achieved, with correlation coefficients in the range 0.9925 ≤ R^2 ≤ 0.9998.
- UPLC-QTOF method : recovery percentages above 85%, with relative standard deviations ≤16% and LODs between 0.001 and 0.047 µg/l for the intended purposes at the concentrations of interest; a good linearity was achieved, with correlation coefficients in the range 0.9902 ≤ R^2 ≤ 0.9999

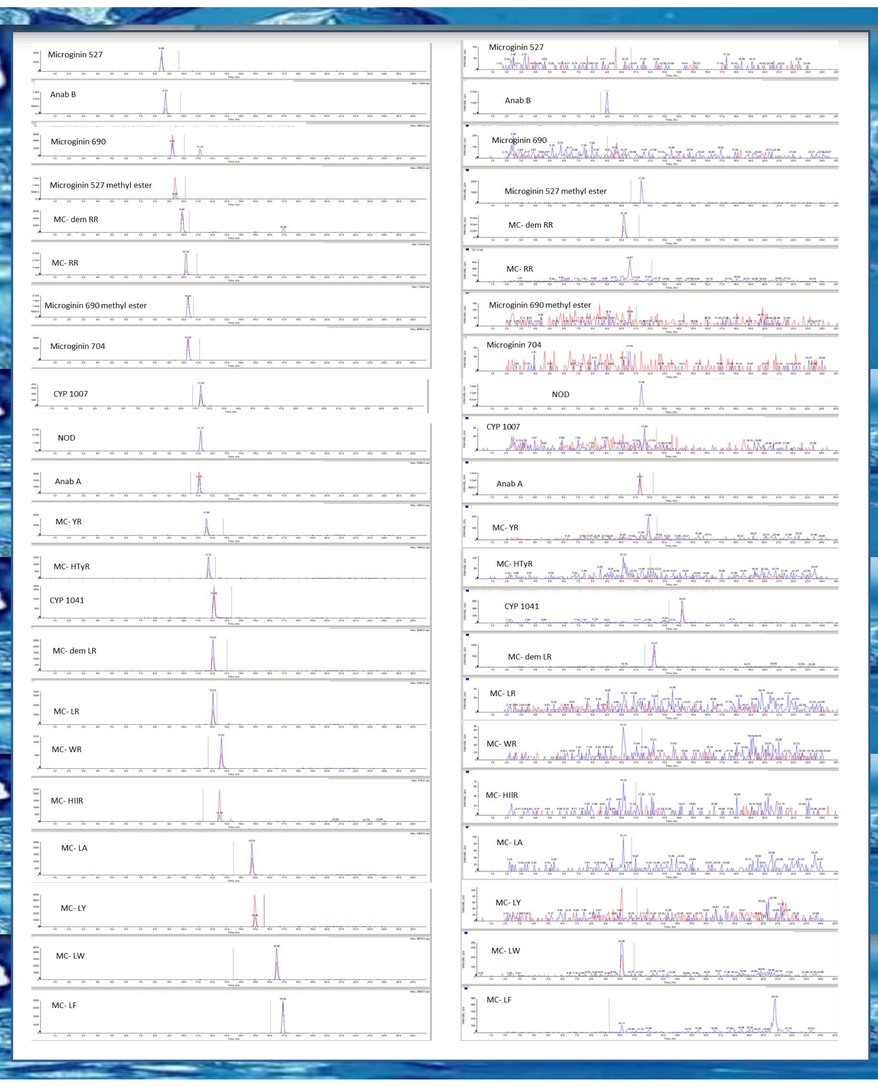

Figure 5. MRM LC/MS/MS chromatogram resulting from analysis of standard solution containing 0.1µg/L of all the analytes and 1 µg/L of Nodularin (chromatogram left) and raw water sample spiked with 1 µg/L of Nodularin. It is reported in Blue colour the quantitation transition and in red the qualification transition for each analytes (chromatogram right)

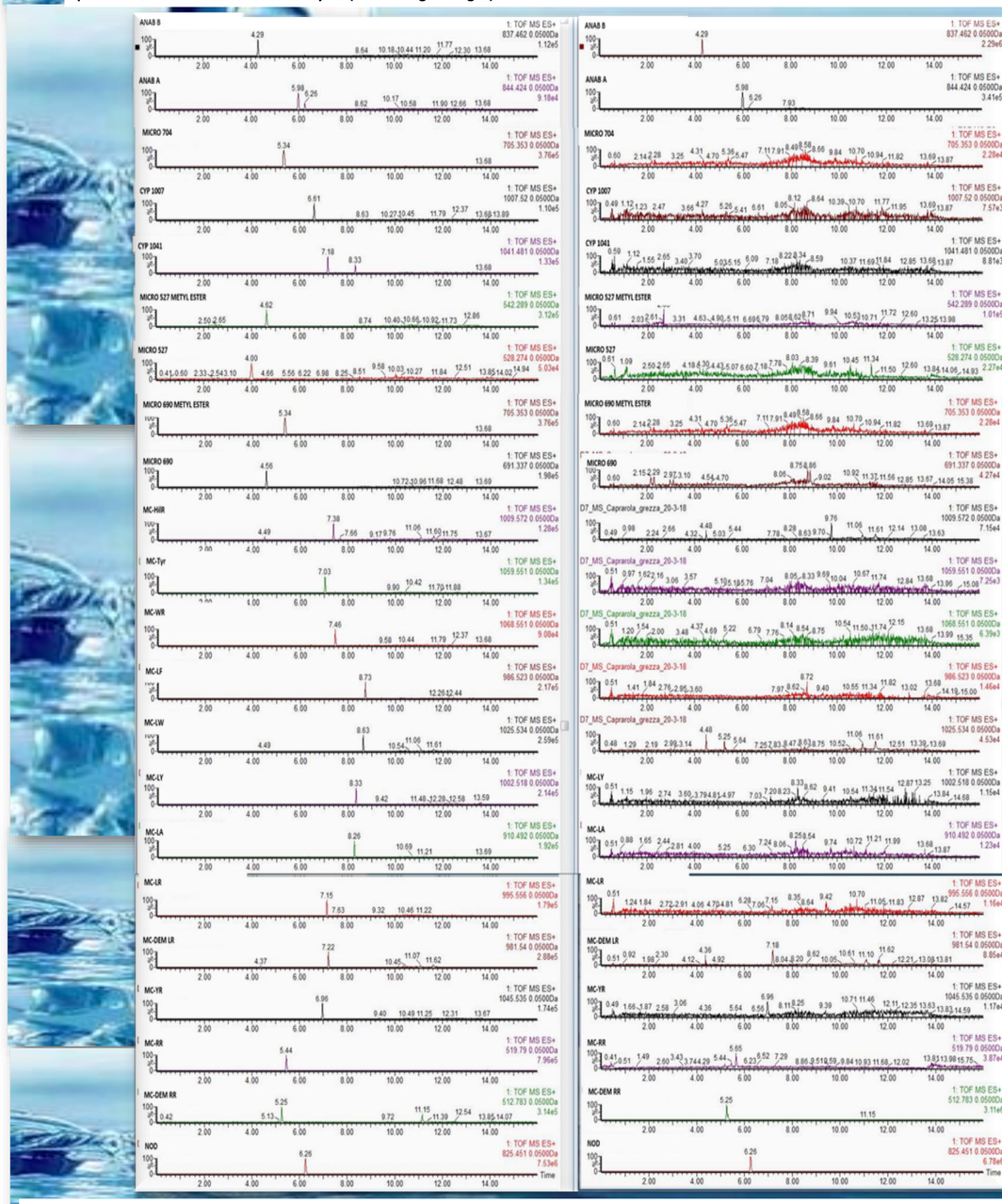

Figure 6. UPLC/HRMS chromatogram resulting from analysis in MS mode with a full scan 50-1200, of standard solution containing 0.1µg/L of all the analytes and 1 µg/L of Nodularin (chromatogram left) and raw water sample spiked with 1 µg/L of Nodularin (chromatogram right)

CONCLUSIONS

Both methods have been proven to be robust, precise and accurate with recovery percentages above 85% and with relative standard deviations ≤17%, fit for the intended purposes at the concentrations of interest; a good resolution has been obtained with both methods.
The performance and reliability of the method was proven to raw, treated and distributed water samples, with LODs 0.001 to 0.047 µg/L, at least 20-fold lower than the guideline value proposed by the WHO for drinking water (1.0 µg/L for microcystin-LR).
The advantages obtained by UPLC-HRMS/MS method are the shorter analysis times (16 minutes vs 27 minutes) and a lower injection volume (10 µl vs 50 µl).
Furthermore, this method allows the simultaneous identification of target and non-target compounds, allowing to detect the presence of other compounds potentially harmful to human health.