Development of a Simple and Rapid Sample Preparation Method for the Detection and Quantification of Algal Toxins in Surface Water.

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Introduction

Lakes of the Northern United States have recently seen a rise in occurrences of cyanobacteria blooms which can generate cyanotoxins in the presence of warming environmental conditions. With surface water a major source of drinking water, the presence of cyano bacterial blooms pose a potential threat to human health. Pilot studies have been implemented in order to develop suitable analytical methods for fast screening and quantification of individual toxic compounds.

Several non-spectroscopic methods are currently used for the detection of algal toxins, including microcystins (MCs) and anatoxins, which are responsible for health issues. These include the protein phosphatase inhibition assay and enzyme-linked immunosorbent assay (ELISA). More recently, high performance liquid chromatography (HPLC) tandem mass spectroscopy (MS/MS) and has become the method of choice for the analysis of specific MCs and other toxic metabolites. Ultra-high performance liquid chromatography (UPLC) has been found to further improve the overall analytical method with improved analyte separation, sensitivity and increase the speed of analysis.

The majority of published studies utilize solid-phase extraction (SPE) as a sample preparation technique, however, the process can be very time consuming. Direct solvent evaporation utilizing the GeneVac™ EZ-2 evaporator was therefore evaluated as a means to reduce lengthy sample preparation process and to improve efficiency.

A series of lake water samples were analyzed for the presence of four microcystins and anatoxin-a by UPLC-MS/MS. A novel direct evaporation method of sample preparation was evaluated and compared to SPE methods.

Method

Mass spectrometric conditions were optimised to maximize the sensitivity and selectivity of the targeted analytes.

Standards and Calibration Solutions

Stock solutions of Microcystins and anatoxin-a were prepared in methanol at a concentration of 1.0 µg/mL. Calibration standards of 100, 50, 25, 5, and 0.5 ng/mL were prepared from the original stock by serial dilution.

A surrogate compound (SUR), Leucine enkephalin acetate salt hydrate, was prepared in original stock and spiking solutions at a 0.9 mg/mL and 1.0 µg/mL, respectively.

Stock and spiking solutions of ketoprofen-d3 for use as internal standard (IS) were prepared at 1.0 mg/mL and 1.0 µg/mL, respectively.

Quality Control Samples

A set of quality control samples were prepared:

Laboratory control sample (LCS) – LC-MS grade water spiked with SUR and all analytes of interest at concentration of 1.25 ng/mL for all

Matrix blank (MBK) – LC-MS grade water spiked with SUR compound at 1.25 ng/ml

Calibration verification (CV) – analyte standard at 50 ng/mL

System blank (SBK) - LC-MS grade acetonitrile solvent

Sample Preparation

SPE method (non-acidic).

Unfiltered samples were subjected to an autoclave process and filtered to remove particulates.

A 200 mL aliquot of each water sample was extracted in triplicate using either Waters HLB (200 mg, 6cc) or a Biotage-Isolute C-18 (1 g, 6cc) SPE cartridges using identical methods [1]. All samples were spiked with SUR (1.25 ng/mL final concentration) and gently mixed by hand. The cartridges were pre-conditioned with methanol followed by UPLC grade water. Samples were loaded on the cartridges at a rate of ~ 5 mL/min and the analytes were eluted with 2.0 mL of methanol. The extracts were concentrated down to 1.0 mL under gentle nitrogen flow (~180 mL/min), spiked with IS and transferred into LC vials for subsequent analysis by UPLC-MS/MS.

Direct Evaporation

Unfiltered samples were subjected to an autoclave process and filtered to remove particulates.

A 20 mL aliquot of each water sample was pipetted into individual 50 mL glass tubes. All samples were spiked with SUR (1.25 ng/mL final concentration) and gently mixed by hand. This was followed by automatically evaporating water samples to dryness using the Genevac™ EZ-2 evaporator (Figure 1) using the aqueous setting. The residues were then reconstituted in 500 µL of methanol, spiked with the IS at a concentration of 50 ng/mL and sonicated for 5-10 seconds. Each sample was transferred into LC vials for subsequent analysis by UPLC-MS/MS.

Figure 1. Genevac EZ-2 Evaporator

UPLC and Mass Spectrometric Conditions

Quality control and water samples were analyzed using a Waters Acquity™ UPLC® coupled with an Acquity™ TQD™ tandem mass spectrometer.

SBK was periodically run between samples to monitor any analyte carryover.

Results

Utilizing non-acidic SPE methods unusually high recoveries for most microcystins were initially obtained (Table 1). It was observed that the introduction of water to the final solution contributed to this elevation. An attempt to improve analyte recoveries by varying the methanol/water ratio of the final solution was made. However, the targeted ratio was hard to maintain, mainly due to water being a sample matrix, and even the smallest deviation was found to influence analyte recoveries.

Table 1. SPE recovery results

	Anatoxin-a	MC-LA	MC-LR	MC-RR	MC-YR
% recovery, Waters HLB	77.9 +/- 14.7	46.5 +/- 8.9	192 +/- 7.0	266 +/- 2.6	172 +/- 7.9
% recovery, Biotage-Isolute C-18	123 +/- 7.1	18.9 +/- 26.7	74.8 +/- 28.1	119 +/- 25.5	77.9 +/- 44.1

In contrast the direct evaporation procedure, utilising the Genevac EZ-2, both simplified sample preparation and eliminated variability in percent water of the final solution. All samples being brought to complete dryness with methanol chosen as the final reconstitution solvent.

Method detection limit, precision and accuracy studies were conducted with results shown in Table 2. The limit of detection for all analytes was excellent and over a range of calibration standards (0.5-100 ng/mL) good correlations were obtained. Recoveries for SUR and QC samples were good, with correlation between analytical batches being within acceptable ranges. Results for QC samples can be seen in Table 3. For system and method blank samples results were below detection limits signifying that neither matrix contribution to signal enhancement nor analyte carryover were an issue.

Table 2. Direct evaporation: limits of detection, recovery, precision and accuracy study results.

	Anatoxin-a	MC-LA	MC-LR	MC-RR	MC-YR
Detection limit study (n=7)					
Concentration (ng/ml)	0.50	0.50	0.50	0.50	0.50
% Recovery	95.4 +/- 5.0	81.4 +/- 9.6	67.1 +/- 15.8	129 +/- 8.5	85.1 +/- 8.9
Method detection limit	0.0745	0.123	0.167	0.172	0.118
Precision and Accuracy study (n=4)					
Concentration (ng/ml)	1.25	1.25	1.25	1.25	1.25
% Recovery	111 +/- 3.5	75.2 +/- 3.2	103 +/- 1.8	113 +/- 2.2	80.0 +/- 7.5

Table 3. Direct evaporation QC sample results. Average recoveries with relative standard deviation. (ND = non detected)

	Anatoxin-a	MC-LA	MC-LR	MC-RR	MC-YR
Calibration Verification	122 +/- 3.5	126 +/- 11.8	88.5 +/- 11.7	99.7 +/- 1.8	83.5 +/- 6.6
Laboratory Control Sample	129 +/- 1.5	128 +/- 6.1	88.9 +/- 7.8	71.8 +/- 13.0	81.3 +/- 30.1
Matrix Spike Sample	63.4	75.0	62.0	71.8	67.6
System Blank	ND	ND	ND	ND	ND
Method Blank	ND	ND	ND	ND	ND

Elevated recoveries (> 100%) were observed in some LCS and CV standards subjected to direct evaporation. It was concluded that analyte degradation had occurred over the study period (three weeks), as has been previously described. We therefore advise that new calibration standards be prepared weekly from stock solution which had been stored at -20 °C. Excellent correlation was still obtained however, between the CV and LCS for target analytes, (with the exception of MC-RR), indicative of analyte stability during the evaporation step.

In the preliminary 14 water samples tested all MCs were below the level of concern (< 1.0 µg/mL) with only MC-RR being detected.

Conclusion

It has been shown that the direct evaporation sample preparation method has distinct advantages over solid phase extraction by eliminating the sample clean-up step, improving reproducibility, decreasing analysis time, minimizing waste generation and being more cost effective. In addition, minimal sample handling was required, reducing the risk of cross contamination and analyte loss. This method has been validated by testing a set of fresh water samples, detecting total microcystin-RR at trace level concentrations with all QCs results within expected criteria. Analyte sublimation or degradation was minimal for this method and as a result, recoveries for all analytes were excellent. Consequently, this methodology has been successfully utilized in routinely screening surface water samples with various MCs detected and reported in approximately 30% of the samples.

Note: The information presented in this paper does not constitute an endorsement of any instrument, consumable or manufacturer by the Authors, University, or the State of Connecticut.

References

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