Sensitive Determination of Microcystins from Environmental Waters Using On-Line SPE

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Overview

Purpose: Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are among the primary toxins produced by these species, which grow in lakes, ponds, and rivers used as drinking water sources. Microcystin contamination of drinking water at low nanomolar concentrations is considered a risk factor for cancer, and microcystin-LR has been associated with most of the incidents of toxicity involving microcystins. Therefore, the World Health Organization (WHO) has proposed a provisional guideline concentration of 1.0 µg/L for microcystin-LR in drinking water.[1]

Methods: Described here is a simple, fast, and effective target-cut on-line SPE method followed by HPLC with UV detection. This on-line SPE method differs from typical protocols in that the bound analyte on the SPE column is selectively eluted using a mobile phase gradient, just as the first dimension of a two-dimensional chromatography system is performed. This reduces the number of interferences for sample analysis. Here, the target-cut on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (-LR, -RR, and -YR) in drinking, tap, and lake water. The three target analytes were coeluted from the first column using chromatographic conditions that eliminated as many interferences as possible. The analytes were then sent to the analytical flow path and separated on the second column using the same type of stationary phase under different chromatographic conditions.

Results: This design takes advantage of the separation power of both columns and may eliminate interferences more efficiently than typical on- and off-line SPE methods. Sub-µg/L concentrations of microcystins-LR, -RR, and -YR spiked in water samples were determined, which exceeds the WHO requirement.

Introduction

The analytical approaches commonly used for microcystins include bioassay, chemical, and biochemical methods. Bioassays have been used in screening, but were found to be nonspecific and/or more time consuming. Biochemical methods, such as enzyme-linked immunosorbent assay (ELISA) and protein phosphatase inhibition assay (PPIA), are advantageous as screening methods due to their high sensitivity and ability to quickly treat a large number of samples, although they provide poor identification and have the potential for false positives. Reversed-phase high-performance liquid chromatography (HPLC) with UV detection, liquid chromatography tandem mass spectrometry (LC-MS), and capillary electrophoresis are chemical methods that have been used for the identification and quantification of microcystins.[2]

Methods

Liquid Chromatography

- Dionex UltiMate 3000 HPLC system including:
  - DGP-3600A Dual Gradient Analytical Pump
  - SRD 3600 Integrated Solvent and Degasser Rack
  - WPS-3000TSL Thermostatted Semipreparative Autosampler (with 2500 µL sample loop)
  - TCC-3200 Thermostatted Column Compartment equipped with two 2p–6p valves
  - VWD-3400RS Four Channel Variable Wavelength Detector (Without Flow Cell)
- Thermo Scientific Chromeleon Chromatography Data System software
- Thermo Scientific Orion 420A+ pH Meter

Standards

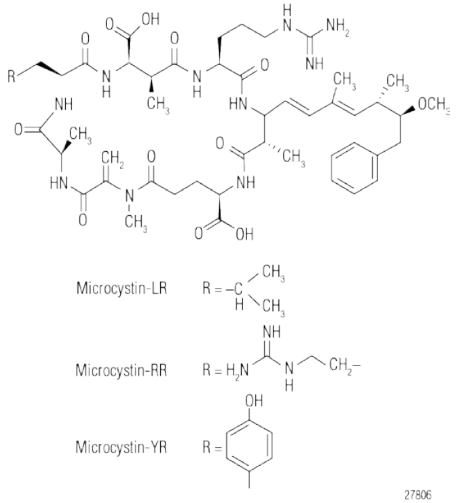
100 µg each of microcystins-LR (CAS 101043-37-2), -RR (CAS 111755-37-4), and -YR (CAS 101064-48-6), ≥ 95% (HPLC), Alexis Corporation.

Prepare stock standard solutions with 50 µg/mL concentrations by dissolving the standards with 2000 µL of methanol. Prepare the standard solutions used for the calibration curve by making appropriate dilutions of the stock standard solutions with water.

Samples

Tap water samples were collected at the Thermo Scientific Shanghai Applications Lab. The lake water sample was collected at Zhangjiang High Science and Technology Park located in the Pudong District of Shanghai. Bottled spring water samples were purchased from a supermarket in Shanghai. These samples were filtered through a 0.45 µm membrane (Millex-HN) prior to injection.

FIGURE 1. Structures of microcystins



Chromatographic Conditions
On-Line SPE

Column: Thermo Scientific Acclaim RSLC PA2, 3 µm Analytical (3.0 × 33 mm, P/N 066276)

Mobile Phase: A: 22.5 mM KH_2PO_4–2.5 mM K_2HPO_4 buffer (dissolve ~ 3.1 g of KH_2PO_4 and 0.44 g of K_2HPO_4 in 1 L of water)

B: CH_3CN in gradient[3]

Separation

Column: Acclaim™ PA2, 3 µm Analytical (3.0 × 150 mm, P/N 063705)

Mobile Phase: A: 0.05% (v/v) H_3PO_4 (dilute 0.6 mL of 85% H_3PO_4 to 1 L with water)

B: CH_3CN in gradient[3]

Valve Switching[3]

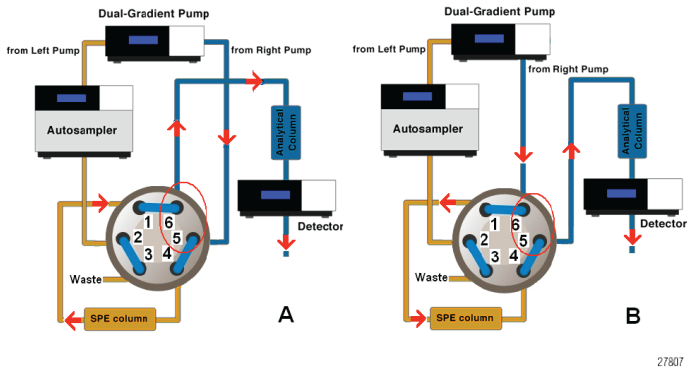
Flow Rate: 0.7 mL/min for both SPE and separation

Inj. Volume: 2500 µL on the SPE column

Temperature: 40 °C

UV Detection: Absorbance at 240 nm

FIGURE 2. Flow schematics for A) traditional and B) target-cut on-line SPE methods equipped with one 2p–6p valve for sample preparation and analysis



Comparison of Traditional and Target-Cut On-Line SPE Methods

The commonly used on-line SPE flow scheme (Figure 2A) couples the SPE column directly with the analytical HPLC column using one six-port (2p–6p) column valve. The filtered sample is injected directly onto the system and delivered to the SPE column. The SPE column is then switched into the analytical flow path to elute the bound analytes (6-1 position). The analytes are then separated on the analytical column and detected by the UV detector. For the target-cut on-line SPE method,[3] a small change in the flow scheme of the traditional on-line SPE mode reverses the flush direction on the SPE column (Figure 2B). The bound analyte on the SPE column is selectively eluted using a mobile phase gradient, Just before the front portion of the analyte peak elutes from the SPE column, the SPE column is switched into the analytical flow path.

Results

Microcystins Separation Using Target-Cut On-Line SPE

Figure 3 shows chromatograms of three types of water samples spiked with 1.0 µg/L each of microcystin-RR, -YR, and -LR standard using the traditional and target-cut on-line SPE methods, respectively.

FIGURE 3. Chromatograms of a) bottled spring water, b) tap water, and c) lake water, all spiked with 1 µg/L each of microcystin-RR, -YR, and -LR standard using A) traditional and B) target-cut on-line SPE methods

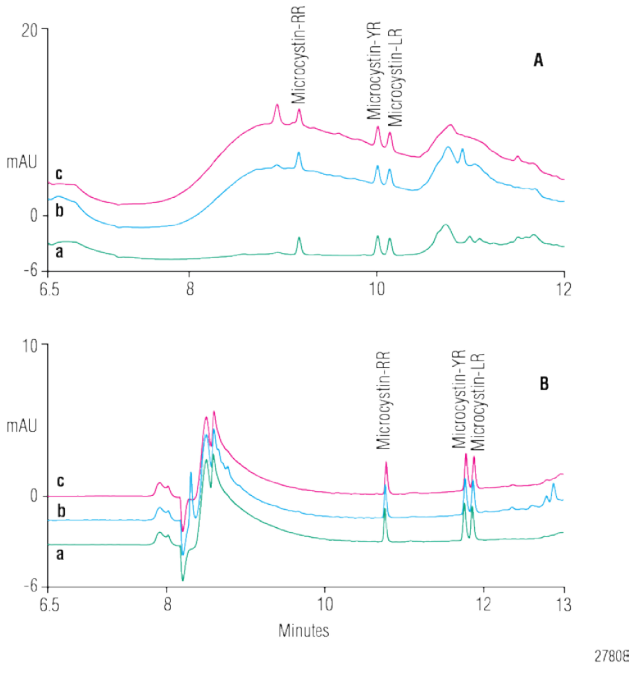
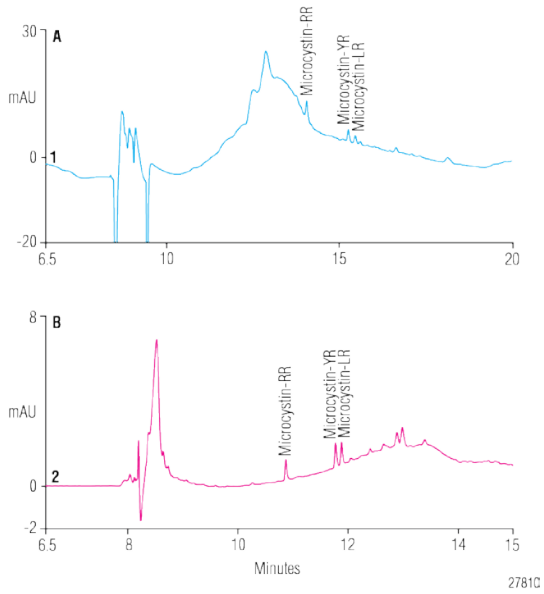


FIGURE 4. Chromatograms of a tap water sample spiked with 0.5 µg/L each of microcystin-RR, -YR, and -LR standard using different target-cut modes:

A) Valve-switching starts from microcystin-RR and ends at microcystin-LR, when they are eluted from the SPE column.

B) The three microcystins elute together from the SPE column.



As shown in Figure 4A, with the target-cut method, a large amount of interferences were still cut to the analytical flow path, which resulted in interference with the determination of microcystins at sub-µg/L concentrations. Figure 4B shows the target-cut method with a CH_3CN–phosphate buffer (pH 6.0) mobile phase to elute analytes in one peak from the SPE column, and the analytical column using CH_3CN–0.05% H_3PO_4 (v/v, pH 2.2) mobile phase.

Conclusion

This work describes a target-cut on-line SPE method that can fully recover low concentrations (< 1 µg/L) of three microcystins (-RR, -YR, and -LR) when added to three different water samples. These concentrations are less than the maximum concentrations recommended by WHO. This method is fully automated and easily configured on an UltiMate 3000 ×2 Dual HPLC system.

References

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2. Lee, H.S.; Jeong, C.K.; Lee, H.M.;Choi, S.J.; Do, K.S.; Kim, K.; Kim, Y.H. On-Line Trace Enrichment for the Simultaneous Determination of Microcystins in Aqueous Samples Using High-Performance Liquid Chromatography with Diode- Array Detection. *J. Chromatogr. A* **1999**, *848*, 179.
3. Dionex (now part of Thermo Scientific) Application Note 261: Sensitive Determination of Microcystins in Drinking and Environmental Waters, 2010 [Online] www.dionex.com/en-us/webdocs/88494-AN261-HPLC-Microcystins-Water-07Oct2010-LPN2607.pdf (accessed Apr. 19, 2012).

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