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Application of High Performance Microfluidic Electrokinetic Liquid Chromatography-Mass Spectrometry (eHPLC-MS) in Separation and Analysis of Isomers of Microcystins

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INTRODUCTION

High performance microfluidic electrokinetic liquid chromatography (eHPLC) is an emerging electro-liquid phase micro-separation technology with dual separation mechanisms of electroosmotic flow and pressure flow. This boasts the performance characteristics of high column efficiency, high selectivity, high resolution and rapid separation. This is unique in complex, trace sample analysis [1]. Mass spectrometry (MS) detection is a universal detector widely used for material structure analysis and identification, with high sensitivity and high accuracy [2]. The Microsaic 4500 MiD®, a single quadrupole mass spectrometer, is used in this case due to its unrivalled ease of deployment and suitability for use with eHPLC applications (figure 1). Therefore, the separation and analysis technology based on eHPLC-MS not only has the high efficiency, speed and trace characteristics of electrokinetic chromatography, but also has the advantages of high selectivity and high sensitivity of the 4500 MiD®. Thus, providing more information-rich data about the tested substance [3].

Microcystins (MC), as a kind of biologically active cyclic heptapeptide compound, is a cell endotoxin produced by cyanobacteria. MC are synthesized in cells and are released into water after rupture. This is known to have a strong cancer-promoting effect. It is also the most widely polluted algal toxin. MC has a variety of isomers, among which the most common and most abundant in nature are MC-LR, and MC-RR, L/R refer to leucine and arginine, respectively). See figure 2 for the molecular structure. In 2004, the WHO recommended in the Guidelines for Drinking Water Hygiene that the concentration of MC-LR in drinking water should not exceed 1.0 μg/L [4].

At present, the separation and detection methods of microcystins mainly include high performance liquid chromatography, liquid chromatography-tandem mass spectrometry and enzyme-linked immunoassay. There are few reports on separation and analytical methods in electrochromatography. In particular, the combination of eHPLC-MS for the separation and analysis of microcystins isomers has not been reported.

EXPERIEMNTAL

A method for isolating and identifying two MC isomers (MC-LR, MC-RR) in microcystins based on eHPLC-MS is presented in this paper. For the experiments, a capillary column (total length 45 cm, effective length 20 cm) filled with 3.0 μm C18 packing was used. The mobile phase was acetonitrile-water (70: 30, v/v) + formic acid (0.1%, v/v). The experimental apparatus are shown in figure 1.

FIGURE 1. *The experimental apparatus used in this work*

RESULTS AND DISCUSSIONS

The chromatography from the eHPLC shows good separation between MC-RR and MC-LR. The resolution in positive separation mode is 1.54 and in the negative mode, the resolution is 12.2 (see Figure 3).

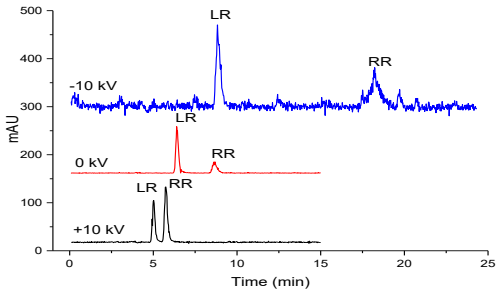

FIGURE 2. *Effect of applied voltage for separation of MC-LR and MC-RR by eHPLC-MS*

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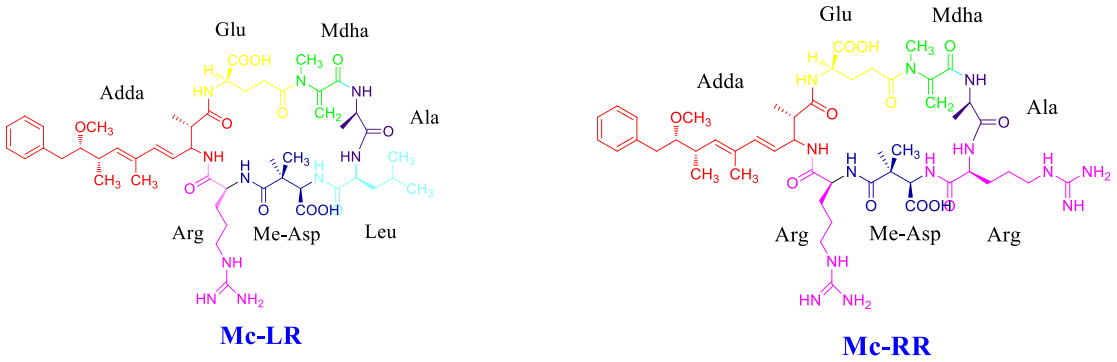

FIGURE 3. *The molecular structure of MC-LR and MC-RR*

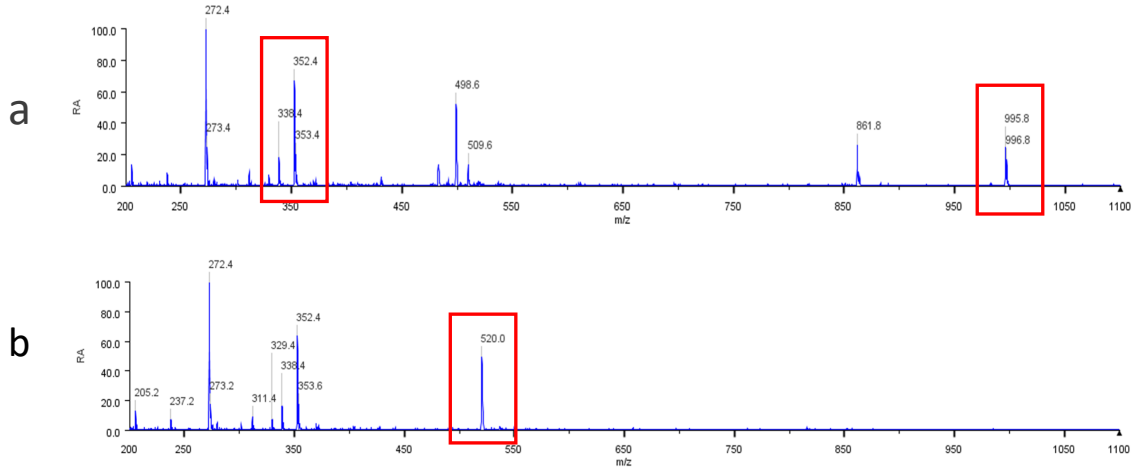

FIGURE 4. *Analysis of MC-LR (a) and MC-RR (b) by eHPLC-MS*

Mass spectrometry data showed that the parent ion of MC-LR $[M+H]^+$ (m/z 995.8), parent ion MC-LR $[M+2H]^{2+}$ (m/z 498.6), and the parent ion of MC-RR were $[M+2H]^{2+}$ (m/z 520.0) (see Figure 3). The method is accurate and reliable. The RSD of the retention time is 0.29%. The peak area RSD is 1.8%, which is significantly better than other detection methods.

Therefore the application of the 4500 MiD® as a detector for eHPLC in the analysis of microystins is suitable. The quality of drinking water from environmental pollution can therefore be characterised in this way.

CONCLUSIONS

The separation and analysis of microcystins by eHPLC-MS has been demonstrated in this application note. The capability of optimising separation with an applied voltage can be used to resolve the co-eluting compounds. Mass detection adds the capability to characterise molecules eluting from the eHPLC with little or no increase in footprint.

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