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Pitfalls in microcystin extraction and recovery from human blood serum

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ABSTRACT

Background: Microcystins (MCs) contaminate water bodies due to cyanobacterial blooms all over the world, leading to frequent exposure of humans to MCs through consumption of meat, fish, seafood, blue-green algal products and water, accidental ingestion of contaminated water and scum during recreational activities and inhalation of cyanobacterial aerosols. For monitoring of human exposure, sensitive screening methods are needed. However, during the analytical process of various matrices, such as human serum, some problems appear to regularly occur during sample preparation and storage, leading to MC loss and thus to underestimation of the true MC concentration. The aim of the current study was therefore to assess the pitfalls of the MC-extraction method from human serum with more detail.
Methods: Six MC congeners (MC-LR, -YR, -RR, -LA, -LW, -LF) and defined equimolar MC mixtures thereof were spiked into human serum, and quantified using the commercially available Adda-ELISA subsequent to standard extraction (methanol extraction with subsequent SPE). To detect the potential influence of sample storage and preparation/storage materials, different types of material such as glass, standard polypropylene and surface-treated polypropylene were compared.
Results: Loss of MC during preparation and storage is largely dependent on (1) the handling of the stored material, (2) the 'surface' of the storage material and (3) the hydrophobicity of the MCs.
Conclusions: The pitfalls described for MC analysis with the ELISA are primarily associated with sample preparation and clean-up and thus also apply to other analytical techniques for MC detection beyond the ELISA used. It can be concluded that ELISA-based methods are suitable tools for the detection of MCs in human sera and other samples.

1. Background

Microcystins (MCs) are toxic cyclic peptides produced by certain cyanobacterial genera such as Anabaena, Microcystis, Nostoc and Planktothrix that contaminate water bodies due to cyanobacterial blooms all over the world [1]. This leads to the frequent exposure of humans to MCs through consumption of meat, fish, seafood, blue-green algal products and water, accidental ingestion of contaminated water and cyanobacterial scum during recreational activities and inhalation of cyanobacterial aerosols [2–5]. In order to monitor human exposure, sensitive screening methods are needed. In the past, ELISA has been demonstrated to be a robust routine tool for MC quantification in environmental samples and increasingly ELISAs are also used to detect MCs in tissue and blood samples from various species including humans [6–11]. However, some problems regularly occur during sample preparation, leading to MC loss and therefore to underestimation of the true MC concentration [2,11–16].

The aim of the current study was to assess the pitfalls of the MC-extraction from complex media with more detail. For this, MCs (MC-LR, -YR, -RR, -LA, -LW, -LF and defined equimolar MC mixtures thereof) were spiked into human blood serum as a surrogate for very complex sample media and quantified using the commercially available Adda-ELISA. To detect the potential influence of sample storage and preparation materials, the effect of different types of material (i.e. glass, standard polypropylene and surface-treated polypropylene) on MC recovery were compared.

Abbreviations: MeOH, methanol; SPE, solid phase extraction; MC, microcystin; LOD, limit of detection; RT, room temperature; HPLC, high performance liquid chromatography.

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2. Materials and methods

2.1. Materials

Unless otherwise stated, materials were purchased as follows: Abraxis, Warminster, PA, USA (ELISA kit), Waters GmbH, Eschborn, Germany (Oasis® HLB 6 cc (200 mg) extraction cartridges), Enzo Life Sciences GmbH, Lörrach, Germany (MC-LR, MC-YR, MC-RR, MC-LA, MC-LW and MC-LF), Carl Roth, Karlsruhe, Germany (Rotilabo®-screw neck ND8 vials (clear and brown glass 1.5 mL) with Butyl/PTFE seal in screw cap, cat# KE26.1, KE30.1, TY73.1; yellow pipette tips, cat# 2395.1; cristal pipette tips, cat# 2393.1), Eppendorf, Wesseling-Berzdorf, Germany (Protein LoBind tube 2.0 mL, cat# 0030 108.132; 300 µL pipette tips (for multichannel pipetting), cat# 022492047; blue pipette tips, cat# 0030000919), Sarstedt, Nümbrecht, Germany (micro tube 1.5 mL low binding, cat# 72.706.600; standard PP micro tube 1.5 mL, cat# 72.690.001; 5 mL pipette tips, cat# 70.1183.002), Biozym Scientific, Hessisch Oldendorf, Germany (micro tube 2.0 mL, clear, low binding, cat# 71028), Corning, Kaiserslautern, Germany (MAXYMum Recovery microtube 1.7 mL, cat# MCT-175-L-C), and Sigma–Aldrich GmbH, Seelze, Germany (all other chemicals and materials).

2.2. Preparation and storage of samples

MC-LR and MC-RR were dissolved in 100% MeOH in glass vials to a nominal concentration of 100 µM using vigorous mixing, shaking for 30 min at 4 °C and sonication (5 min). The real concentration was determined via detection of absorbance at $\lambda_{238\,nm}$ and using the molar absorption coefficient of 39,800 mol L^{-1} cm^{-1} [17] after calibration of the spectrophotometer with potassium dichromate in 1 mM perchloric acid [18]. Due to lack of validated coefficients for each of the MC congeners used in this study, all MC congeners were prepared as if they had the same absorption coefficient as MC-LR. Although some investigators used specific extinction coefficients (Wayne Carmichael, personal communication), using the same coefficient for the major MC congeners represents a widely accepted approach because the differences seem to be quite small (personal communication with Jussi Meriluoto and Linda Lawton) and thus will not impact the overall findings (for a more detailed discussion of this issue see [11,19]). The structural differences among the six MC congeners employed in this study are depicted in Fig. 1.

Dilution series of MCs were performed in glass vials. Human serum was spiked with the different MC congeners (1–100 µg/L) or a defined equimolar mixture of them (total of 1, 10 or 100 µg/L) in glass vials. Samples thus generated were then distributed to glass vials, standard polypropylene (PP) tubes or 'low-binding' PP tubes for the detection of handling and storage effects of the materials (Fig. 2). Samples were stored short-term at −20 °C until further use.

2.3. Extraction and purification of toxins from human serum

Spiked and control human serum samples were submitted to a methanol/hexane extraction as described by Hilborn and co-workers with some modifications [10], see Fig. 2. Subsequent to the organic solvent extraction and clean-up, solid phase extraction (SPE) was performed using Oasis® HLB 6 cc (200 mg) cartridges, according to the manufacturer's instructions (Waters GmbH,

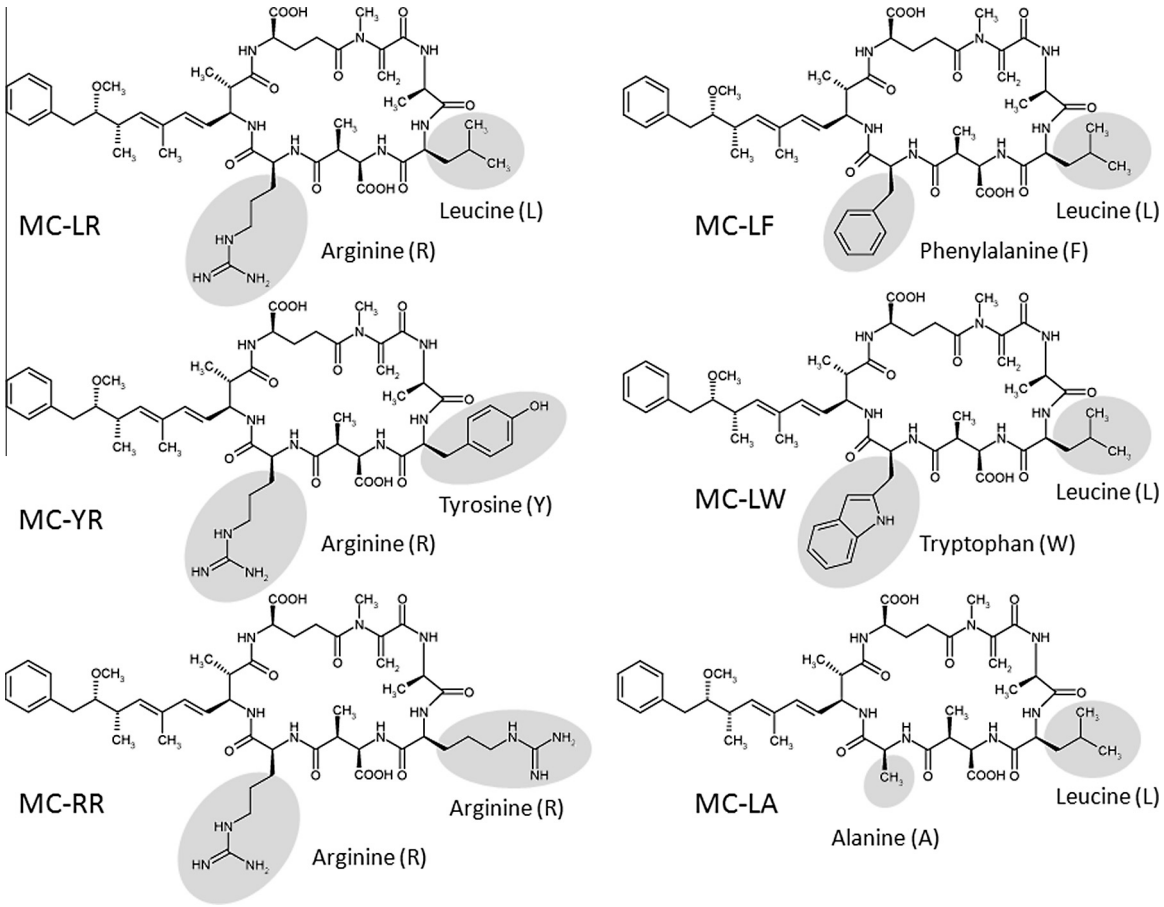

Fig. 1. Structures of MC congeners used in this study. The variable amino acids (labeled in gray), largely determining the relative hydrophobicity of the whole molecule, are also reflected in the name and abbreviation of the respective MC-congener.