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Interference by plastics additives in the HPLC determination of microcystin-LR and -YR

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Abstract

Plastics devices used for the field collection of water samples may contain plastics additives which will interfere with the HPLC determination of the cyanobacterial toxins microcystins. The presence of the additives resorcinol monobenzoate or 2,4-dihydroxybenzophenone can interfere with the determination of microcystin-LR. The presence of bisphenol A in plastics can interfere with the determination of microcystin-YR.

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1. Introduction

Reported incidences of toxin-producing freshwater cyanobacteria have increased with alarming frequency worldwide and a rapidly expanding literature has developed on this specific topic (Carmichael, 1992; Codd et al., 1994; Steffensen and Nicholson, 1994; Yoo et al., 1995; Watanabe et al., 1996). The most prevalent of the recognized cyanobacterial toxins are the microcystins, which are commonly found in Microcystis, but which have also been reported in other cyanobacteria, such as Anabaena, Oscillatoria and Nostoc (Rinehart et al., 1994). The microcystins produce liver damage in endothermic vertebrates (Foxall and Sasner, 1981; Runnegar and Falconer, 1981) and promote liver tumor growth (Fujiki et al., 1996).

The microcystins, first isolated and characterized in 1982 (Botes et al., 1982), consist of a class of closely related cyclic heptapeptides and HPLC is widely used
for the determination of individual microcystins of this hazardous group of compounds. With the increasing sensitivity of detection methods, the use of plastics for the collection and storage of water samples can cause interference problems in the HPLC determination of naturally occurring toxins. A problem became apparent when we were obtaining extraordinarily high values for microcystin-LR in lake water samples. This was traced to the gravity-type plastic coring device used in collecting the samples. A similar problem, but of lesser magnitude, was also encountered in the determination of microcystin-YR when a small diameter flexible tubing was used as an integrated water column sampler.

This paper identifies the plastics additives which were found to interfere with the HPLC determination of microcystin-LR and -YR.

2. Materials and methods

2.1. Field sampling and plastics extraction

The original objective of the study was to determine microcystin concentrations in lake sediments using a gravity type messenger-activated core sampler. Liner tubes (50 × 5 cm ID) made of clear cellulose acetate butyrate were placed inside a brass core tube. Upon retrieval, samples of water and sediment were removed from the liner within 0.5–1 h and placed in high density polyethylene (HDPE) sample containers and placed in a freezer (−30°C). Within 1–2 weeks samples were thawed and analyzed for microcystins using HPLC. Once contamination was suspected, all parts of the coring device, as well as other sampling devices and storage containers, were tested by placing them in distilled water at 20–22°C for 1 h. Extracts of the HDPE containers were made by leaving the water in the containers for several days. The extracts were concentrated on C18 cartridges and run on the HPLC as described below. The results of the extraction of various plastics components are shown in Table 1.

2.2. HPLC

The HPLC method described by Harada et al. (1988) was used. The system consisted of a Hewlett-Packard Series 1050 pump operated at 1 ml/min, a HP 1050 variable wavelength detector set at 238 nm, a HP 3396A integrator and a Nucleosil C18, 5 micron, 150 × 4.6 mm column. The mobile phase was methanol, 0.05 M pH 3 phosphate buffer (58:42), which gave elution times of the order of 6.5 min for microcystin-RR, 9 min for microcystin-YR and 11–12 min for microcystin-LR. For sample preparation, Waters Sep-Pak Plus C18 or Waters Sep-Pak Vac 3 cc (500 mg) C18 cartridges were used. Extract (100 ml) was acidified with 5 ml of glacial acetic acid and adsorbed onto the cartridge. The cartridge was washed with 20 ml of water followed by 20 ml of 10% methanol in water and the microcystins eluted with 20 ml 90% methanol:water (Tsuji et al., 1994). The eluate was taken to dryness in vacuo, the residue taken up in 1 ml of
methanol:water (60:40) and the solution syringe filtered through a 0.2 μm PTFE filter before HPLC. When 2 mg each of microcystin-RR, -YR and -LR were added to 100 ml of water and acidified with 5 ml of acetic acid, recoveries in the range of 80–100% were obtained using these cartridges. We found that some batches of C18 cartridges of some brands gave very poor recoveries (near 0%) of the microcystins and we strongly recommend testing each cartridge lot for recovery characteristics before use with unknown samples.

2.3. Chemicals

Bisphenol A, resorcinol monobenzoate and 2,4-dihydroxy-benzophenone were purchased from Aldrich Chemical Co., Milwaukee, WI and the microcystin-LR, -YR and -RR from Calbiochem-Novabiochem, La Jolla, CA.

2.4. Mass spectrometry

Mass spectra were obtained with a Hewlett-Packard Model 5988A quadrupole mass spectrometer.

3. Results and discussion

3.1. Interference of plastics and additives (Fig. 1) in the determination of microcystins

Table 1 shows the results from the extracts of some plastics in the determination of microcystins. Especially high values for microcystin-LR and -YR
were observed in some cases. The determination of microcystin-RR did not seem to be grossly affected here. In the case of both microcystin-YR and -RR, however, large peaks sometimes preceded the 'microcystin' peaks, but were separable. The HDPE containers used to store the water samples did not contribute significantly to the microcystin values even when the water was in contact with the container for several days.

3.2. ‘Microcystin-LR’

We consistently began to notice surprisingly high values for microcystin-LR (ca. 1000–2000 μg/l of lake water) whenever a particular rigid gravity type coring device was used for collecting samples of lake bottom sediment and benthic water samples. Intraperitoneal injection of 1 ml of lake water concentrate, estimated to contain 20 μg of ‘microcystin-LR’, into 20 g mice did not cause death or produce any hepatotoxic symptoms (LD₅₀ = 1 μg microcystin-LR/20 g mouse), indicating that the material was probably not microcystin-LR. Extraction of the various components of the coring device with distilled water for 1 h, revealed that the rigid clear plastic corer liner was responsible for the false reading of microcystin-LR. 100 ml of the corer liner extract was acidified with 5 ml of acetic acid and the material concentrated by passing it through a C18 cartridge as described above. Mass spectrometry of the extract indicated a compound with a molecular weight of 214 Da. A base peak at m/z 105 and a prominent peak at m/z 77 indicated the presence of an unsubstituted benzoyl and phenyl group. A molecular formula of C₁₃H₁₀O₃ corresponding to a monobenzoyl ester of a dihydroxybenzene or a dihydroxybenzophenone was indicated. Ash and Ash (1991) list resorcinol monobenzoate and 2,4-dihydroxybenzophenone as industrially used UV absorbing plastics additives. When these compounds were added to water at 2 μg/100 ml and the solutions acidified with acetic acid and concentrated through a C18 cartridge, they were recovered in +90% yield. Neither compound was separable from microcystin-LR on HPLC (Fig. 2A–E). However, Fig. 2(C) showed a slight
broadening on the trailing edge, indicating that resorcinol monobenzoate may slightly precede microcystin-LR. Resorcinol monobenzoate (1 μg) gave a HPLC response equivalent to 2 μg of microcystin-LR and 1 μg of 2,4-dihydroxybenzophenone gave a response equivalent to 0.85 μg of LR. The separation of resorcinol monobenzoate from 2,4-dihydroxybenzophenone was barely detectable, the resorcinol monobenzoate preceding the 2,4-dihydroxybenzophenone by about 12
s at a 12 min retention time. Mass spectra indicated that the plastics additive involved was resorcinol monobenzoate ($m/z = 214$ (M), 105 (base peak), 77) rather than 2,4-
dihydroxybenzophenone ($m/z = 214$ (M), 137 (base peak), 213 (intense), 105, 77).

3.3. ‘Microcystin-YR’

‘Microcystin-YR’ was associated with a small diameter flexible integrated water column sampler. A 1-h water extract of the device showed a microcystin-YR content corresponding to ca. 1000 µg/l. When the extract of the device was acidified and concentrated through a C18 cartridge, the concentrate gave a mass spectrum with a very strong peak at $m/z = 213$ and significant peaks at $m/z = 228$ and 119. The spectrum indicated bisphenol A, whose most significant peaks were $m/z = 228$ (M), 213 (base peak) and 119. Bisphenol A (2,2-bis(4-hydroxyphenyl)-propane) is used commercially to produce toughness and clarity in resins (Ash and Ash, 1991). Bisphenol A was recovered in quantitative yield when dissolved in water at 2 µg/100 ml, acidified and concentrated on a C18 cartridge. The substance did not separate from microcystin-YR on HPLC (Fig. 2F–H). 1 µg of bisphenol A gave a response equivalent to 0.6 µg of microcystin-YR.

The HPLC method of Harada et al. (1988), using pH 3 buffered aqueous methanol in an isocratic mode, has been a simple and useful assay system in our lake water studies for the commonly occurring more hydrophilic microcystins (-LR, -YR and -RR). The problem of interference in our determinations by additives in the plastics arose as a result of the use of plastic sampling devices. The use of HDPE storage containers appeared not to be the problem. The difficulty has been surmounted by testing various components and switching to those which did not contain the interfering additives. Considering the number and pervasiveness of plastics additives in use in the world today, any system containing suspect plastics would need to be tested for interfering substances under the prevailing HPLC conditions.

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