

Laboratory analysis of microcystins in samples from environmental waters

Prepared by: Marianne Reilly and Geoffrey A. Codd, University of Dundee.

Date: 30 April 2007

1 Introduction

This protocol provides instructions for the processing and storage of environmental samples for microcystin analysis by HPLC and ELISA.

Analysis will be performed by

(A) HPLC and ELISA, as appropriate, for total microcystin content (TM), soluble microcystin content (SM), and particulate microcystin content (PM),

(B) Further analysis for the microcystin content of single colonies and filaments will be performed by ELISA.

2 Experimental

Technical note: Samples for **(A)** and **(B)** should be processed immediately after collection or stored at around +4°C. They should **not** be frozen before processing.

2.1 Materials

- (a) 1L measuring cylinder
- (b) 1L glass bottles
- (c) Filter discs (e.g. Whatman GF/C, 70mm diameter)
- (d) "Buchner" vacuum funnel or similar
- (e) 1.5ml microcentrifuge tubes
- (f) 1.0ml pipette tips
- (g) Plastic Petri dishes
- (h) Glass Petri dish
- (i) Methanol HPLC grade, e.g. HPLC grade methanol, product number RH1019, from Rathburn (Walkerburn, Scotland, UK)
- (j) Water/de-ionized purified to 18.2 MOhm cm (e.g. Milli-Q Plus quality).
- (k) Isolute C₁₈ SPE cartridges. Size 1 gram sorbet in 3 ml reservoir

- (l) Parafilm

2.2 Special Equipment

- (a) -20°C freezer
- (b) Vacuum filtration apparatus
- (c) Oven set at 80°C
- (d) LVE (large volume extraction) kit for unattended loading of large sample volumes, made of PTFE tubing and adaptors for column connection, part number 121-2090, from Argonaut Technologies Peristaltic pump or Vacuum manifold.
- (e) Freeze-drying apparatus
- (f) Balance capable of measuring 0.1mg
- (g) Microcentrifuge

2.3 General Procedures

(A) Sample processing for HPLC and ELISA

- (a) In a glass Petri dish, carefully place some GF/C filters in the 80°C oven for a minimum of 24 hours.
- (b) Determine the dry weight of a GF/C filter disc to the nearest 0.1mg. **Record weight.**
- (c) Measure 1L of a mixed environmental water sample with the measuring cylinder.
- (d) Prepare the vacuum filtration apparatus and filter the 1L of mixed environmental water sample through the GF/C filter.
In the case of high biomass preventing the filtration of 1L, filter an appropriate reduced volume e.g. 250ml, 100ml.
Record volume filtered.
- (e) Retain the filtrate in a labelled 1L glass bottle for solid phase extraction (SPE) of microcystins. Store at +4°C, until SPE can be performed (see section I)

- (f) Place the GF/C filter disc in a labelled plastic Petri dish and store at -20°C over-night or until frozen.
- (g) Place Petri dish containing filter disk in freeze-drier. Freeze dry. This process usually takes around 24 hours, depending on efficiency of the apparatus.
- (h) Weigh freeze-dried GF/C filter disks. **Record weight.** Store at -20°C. These samples will be analysed at Dundee for PM microcystins by HPLC and ELISA.
- (i) Into 3 labelled 1.5ml microcentrifuge tubes, pipette 1ml of mixed environmental water sample. Store 2 tubes at -20°C. These samples will be analysed, at Dundee for TM by ELISA.
- (j) Take remaining 1ml microcentrifuge sample and centrifuge at 5000g for 5 min.
- (k) Remove supernatant and place into new labelled 1.5ml microcentrifuge tube, store sample at -20°C. This sample will be analysed at Dundee by ELISA for SM microcystins.
- (l) Take the 1L of filtered environmental water sample (from section e), allow to reach room temperature before SPE. This sample will be analysed at Dundee for SM microcystins by HPLC and ELISA.
- (m) Using vacuum manifold or peristaltic pump, condition a labeled SPE cartridge, Isolute C₁₈, 1g in a 3ml reservoir, with 10 ml HPLC grade methanol followed by 10 ml of purified water. **Do not let the cartridge dry during conditioning and sample application.**
- (n) Apply the sample (from section l) at a flow rate not exceeding 10 ml/min (visible drops).
- (o) Allow all sample to pass through Isolute C₁₈ cartridge, continue to pass air through cartridge for a further 2 min to dry cartridge.
- (n) Store at -20°C until cartridges can be freeze-dried.
- (o) Freeze-dry Isolute C₁₈ SPE cartridges. Then seal both ends with parafilm and store at -20°C, until dispatch to Dundee.

Filter-disks, samples in microcentrifuge tubes and SPE cartridges containing materials for analysis should be stored at -20°C before sending to Dundee in batches at intervals to be arranged.

B Samples for single colony and filament ELISA

Samples of environmental water, blooms, scums and laboratory isolates should not be frozen, but dispensed in approx 1ml volumes into sterile 1.5ml microcentrifuge tubes, stored at +4°C, and posted to Dundee as soon as possible.