

PEPCY PRACTICAL GUIDELINES

TITLE: Routine extraction of microcystin-LR, cyanopeptolin A, cyanopeptolin C and cyanopeptolin 970 from lyophilized cyanobacterial biomass.

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1. PURPOSE

To extract cyanopeptides including microcystin-LR, cyanopeptolin A, cyanopeptolin C and cyanopeptolin 970 and unknown peptides from lyophilized cyanobacterial cells.

2. INTRODUCTION

The cyanopeptolins are cyclic peptides containing the amino acid 3-amino-6-hydroxy-2-piperidone (Ahp). To investigate their production in culture experiments, relevance for risk assessment and ecological impact and as with other cyanopeptides (e.g.¹) they must be extracted from cyanobacterial biomass before quantification.

3. REQUIREMENTS

Materials

Lyophilised cyanobacterial cells e.g. *Microcystis* PCC 7806 (stored at – 20°C until use)

Purified water (e.g. 18MΩ Millipore MilliQ or equivalent)

HPLC grade: Methanol (e.g. Rathburn)

Ice

Equipment

Freezer (-20°C)

Extraction vessel with sealable lid/cap (e.g. 1.5 ml microcentrifuge tube)

Sample agitator (e.g. vortex mixer, ultrasonicator)

4. PROCEDURE

Solutions

50 % (v/v) aqueous methanol

Sample preparation

1. Weigh the quantity of cyanobacterial biomass to be extracted

2. Add 50 % methanol to give a concentration of about 10- 20 mg dry weight ml⁻¹
3. Mix sample vigorously with vortex mixer and either shake at 1400 rpm for 1 hour, ultrasonicate in a sonic bath for 15 min, or ultrasonicate with probe for 2 min (NB, sample must be kept on ice for ultrasonication treatments)
4. Centrifuge sample, decant supernatant and store separately
5. Re-extract the pellet as before (steps 2-4) a further 3-4 times
6. Pool all supernatants and dry under a stream of N₂ at 40 °C

5. REFERENCES

Fastner, J., Flieger, I. and Neumann, U. (1998) Optimized extraction of microcystins from field samples – a comparison of different solvents and procedures. *Water Research* 32:3177-3181