

PEPCY PRACTICAL GUIDELINES

TITLE: Routine, small scale extraction of anabaenopeptins (ABPNs) from lyophilised cyanobacterial biomass

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1. PURPOSE

To extract ABPNs from lyophilised cyanobacterial laboratory culture material or lyophilised cyanobacterial bloom samples for quantification.

2. INTRODUCTION

ABPNs are a commonly occurring family of cyclic bioactive compounds produced by several cyanobacterial genera^{1,2}. To investigate their production, relevance for risk assessment and ecological impact they must be extracted from cyanobacterial biomass before quantification.

3. REQUIREMENTS

Materials

Lyophilised cyanobacterial cells (stored at – 20°C until use)
Purified water (e.g. 18M Ω 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

70 % (v/v) aqueous methanol

Sample preparation

1. Weigh the quantity of cyanobacterial biomass to be extracted

2. Add 70 % methanol to give a concentration of no more than 50 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

1. Fastner, J., Erhard, M. and von Dohren, H. (2001) Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *Appl. Environ. Microbiol.* 67, 5069-5076
2. Welker, M., Christiansen, G. and von Dohren, H. (2004) Diversity of coexisting *Planktothrix* (Cyanobacteria) chemotypes deduced by mass spectral analysis of microcystins and other oligopeptides. *Arch. Microbiol.* 182, 288-298