

PEPCY PRACTICAL GUIDELINES

TITLE: Routine small scale extraction of cyanostatins (microginins) from lyophilised cyanobacterial biomass

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1. PURPOSE

To extract cyanostatins from lyophilised cyanobacterial material for quantification.

2. INTRODUCTION

Cyanostatins and microginins are a family of linear peptide bioactive compounds produced by cyanobacteria mainly found in strains of *Microcystis* and in *Microcystis* containing bloom samples^{1,2}, but also *Planktothrix*³. 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. Sano, T., Takagi, H., Morrison, L. F., Metcalf, J. S., Codd, G. A. and Kaya, K. Leucine aminopeptidase M inhibitors, cyanostatin A and B, isolated from cyanobacterial water blooms in Scotland. *Phytochem.* 66, 543-548
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3. Sano, T. and Kaya, K. (1997) A 3-amino-10-chloro-2-hydroxydecanoic acid-containing tetrapeptide from *Oscillatoria agardhii*. *Phytochem.* 44, 1503-1505