

PEPCY PRACTICAL GUIDELINES

TITLE: Analysis of anabaenopeptin-B by high performance liquid chromatography with photodiode array detection

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1. PURPOSE

To describe the conditions required to analyse (identify and quantify) anabaenopeptin-B (ABPN-B) by high performance liquid chromatography with photodiode array detection (HPLC-PDA) in the context of ABPN-B-containing crude, semi-pure and purified extracts of cyanobacteria from laboratory cultures and environmental samples.

2. INTRODUCTION

Anabaenopeptins (ABPNs) are a family of low molecular weight peptides produced by some cyanobacteria¹, of which ABPN-B is commonly encountered in the aquatic environment². Unlike some other cyanobacterial peptides (e.g. microcystins), the UV spectra of ABPNs are not specific to this family of compounds. HPLC has been used successfully to identify ABPN-B with PDA detection, however methods such as MALDI-TOF MS and LC-MS can also be used to analyse these compounds.

3. REQUIREMENTS

Materials

ABPN-B (stored at – 20°C until use)

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

HPLC grade: Acetonitrile, ACN (e.g. Rathburn)

Trifluoroacetic acid, TFA (e.g. Fisher)

Methanol (e.g. Rathburn)

C18 reversed phase HPLC column (e.g. Waters Symmetry 5µ 3.9 x 150 mm) with pre-column insert or guard column.

Equipment

HPLC (dual pump) with PDA detector and associated equipment.

Software for the analysis of HPLC chromatograms

Freezer (-20°C)

4. PROCEDURE

Solutions

50 % (v/v) aqueous methanol
MilliQ water + 0.1 % (v/v) TFA (HPLC solvent A)
ACN + 0.1 % (v/v) TFA (HPLC solvent B)

Sample preparation

A stock of ABPN-B should be prepared in 50 % methanol.
The stock should be diluted in 50 % methanol before analysis (e.g. 10 µg ml⁻¹).
Particles should be removed from samples before analysis (e.g. centrifuged).

Chromatography

The HPLC solvents should be degassed, the system primed, the column oven set to 40°C and correct column with pre-column fitted, all according to manufacturers instructions.

Set the detector to monitor chromatograms at 225nm.

Starting with 100 % HPLC solvent B gradually (over 30 to 45 min) increase the proportion of HPLC solvent A to reach starting conditions (below).

Inject 10 to 25 µl volumes of sample onto the column and elute according to the following gradient:

Time (min)	% HPLC solvent A	% HPLC solvent B
0 (starting conditions)	75	25
15	60	40
16	0	100
18	0	100
19	75	25
25	75	25

Note the retention time and PDA spectrum of the ABPN-B standard and compare to peaks in samples.

The concentration of peaks matching the retention time and spectra of the standard can be calculated as follows

$$\frac{\text{Peak area sample}}{\text{Peak area standard}} \times \text{concentration of standard} = \text{ABPN-B concentration}$$

5. REFERENCES

1. Harada, K. (2004) Production of secondary metabolites by freshwater cyanobacteria. *Chem. Pharm. Bull.* **52**, 889 - 899
2. Welker, M., Brunke, M, Preussel, K., Lippert, I. and von Döhren, H. (2004). Diversity and distribution of *Microcystis* (cyanobacteria) oligopeptide chemotypes from

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