

PEPCY PRACTICAL GUIDELINES

TITLE: Preparation of gravimetric cyanopeptide standards

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1. PURPOSE

To describe the procedures required to prepare, weigh and aliquot cyanopeptides for use as analytical standards.

2. INTRODUCTION

To determine the concentration of a cyanobacterial peptide, reference can be made to a quantitative standard of the pure material. Where these are not available, standards can be made from pure peptide by obtaining an accurate weight for the dry substance or by using the absorption coefficient, if available.

3. REQUIREMENTS

Materials

- Pure peptide, > 0.5 mg (stored at – 20°C until use)
- Purified water (e.g. 18M Ω Millipore MilliQ or equivalent)
- HPLC grade methanol (e.g. Rathburn)
- Tin capsules (e.g. Elemental Microanalysis 5 x 3.5 mm)
- Silica gel with moisture indicator (e.g. Merck brown 1 to 4mm)
- Ice

Equipment

- Freezer (-20°C)
- Freeze-drier unit (e.g. Edwards Super Modulyo)
- Desiccator attached to vacuum line
- Microbalance to 0.0001 mg (e.g. Sartorius)
- Microbalance implements, forceps, spatula etc (e.g. Carlo Erba Strumentazione).
- Glass tile
- 96-well plate and lid
- Glass vials and lids
- Calibrated pipettes

Note:The balance should be positioned on a weighing bench, in an environment free from large vibrations and draughts.

The desiccator must be positioned close to the balance.

4. PROCEDURE

Solutions

50 % (v/v) aqueous methanol

Sample preparation

If not already dry in powder form, the purified peptide should be reconstituted in MilliQ water and lyophilised.

The dried peptide powder should then be stored in the desiccator containing dry silica gel for at least 24 hours before being weighed.

Weigh

- a. Turn on balance and calibrate according to manufacturer's instructions.
- b. Ensure microbalance implements and glass tile are clean and dry.
- c. Keep all implements on glass tile when not in use.
- d. Remove 3 tin capsules from container using forceps and place on glass tile.
- e. Zero balance, place an empty tin capsule onto the balance, record weight, remove from balance and place into a well of the 96-well plate (this is the control capsule).
- f. Ensure balance settles to zero. Place another tin capsule on the balance, record weight and remove from the balance.
- g. Remove the peptide powder from desiccator and fill the weighed tin capsule $\frac{3}{4}$ full with sample to be weighed: the powder.
- h. Holding the tin capsule with the forceps tap the forceps gently with the spatula to settle and compact the powder.
- i. Ensure the balance has settled to zero, reweigh the filled capsule, record weight, remove from balance and place into a well of the 96-well plate.
- j. Repeat steps f. to i. with the remaining tin capsule.
- k. Cover and store 96-well plate in desiccator for 24 hours.
- l. Repeat steps a. to c. and reweigh the tin capsules (step f.) until a constant weigh has been achieved on 3 consecutive occasions over 24 to 72 hours.

Aliquot

When an accurate weight has been obtained the tin capsule should be dropped into a glass vial containing a known volume e.g. 1 ml of 50 % (v/v) aqueous methanol or other suitable solvent.

Holding the vial over ice to minimise evaporation (but not in the ice), the samples should be dispensed into desired quantities (e.g. 20 replicates containing 10 μg each).

Aliquots (e.g. 4 replicates) can be dried and reconstituted, or diluted before quantification (e.g. HPLC-PDA) and statistical analysis (e.g. ANOVA).