HPLC-MS

This protocol is for the quantification of tryptophan, phenylalanine and tyrosine on HPLC-MS. Only done together with Anja or Rasmus.

Preparation

1. Liquid *E. coli* grown, and samples removed as needed for the experiment
2. Remove cells from media to measure concentration in media

Cell lysation

- Cells are lysed to determine the intracellular amino acid content.

HPLC preparations

- Prepare all samples by mixing
    - 5 µl sample
    - 33,5 µl borate buffer
    - 1,5 µl internal standard (derivatised amino acids)
- Add 10 µl Accq-taq. Wait 1 minute
- Heat 10 minutes at 55 °C
- Spin 5 minutes at 10,000 rpm to remove condensed solution from the lid.
- Transfer the 50 µl sample to HPLC glass vials, making sure to avoid air bubbles in the bottom.

HPLC-MS detection

Mass spectrometry gives a better detection, as it enables us to distinguish compounds based on m/z, not only retention time in the column. Thus we are able to distinguish between closely eluted amino acids, such as tyrosine and tryptophan.

Run with both internal and external standards to ensure correct quantification.

HPLC-MS set up and validated amino acid detection protocol provided by associate professor Henning Jørgensen.