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LC-MS/MS Untargeted Metabolomics Data Acquisition

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Abstract

Scope:
To describe the procedure to acquire metabolomics data using our broad spectrum plateform.

Expected outcome/data:
Metabolite extracts are analyzed four times using HILIC and RPLC in positive and negative ionization modes.

Troubleshooting

1 Untargeted metabolomics was performed using a combination of HILIC- and RPLC-MS methods.

2 Metabolic extracts were analyzed four times using HILIC and RPLC separation in both positive and negative ionization modes.

3 Data were acquired on a Thermo Q Exactive HF mass spectrometer for HILIC (Thermo Fisher Scientific, Bremen, Germany) and a Thermo Q Exactive mass spectrometer for RPLC (Thermo Fisher Scientific, Bremen, Germany).

4 Both instruments were equipped with a HESI-II probe and operated in full MS scan mode.

5 MS/MS data were acquired on quality control samples (QC) consisting of an equimolar mixture of all samples in the study.

HILIC

6 HILIC experiments were performed using a ZIC-HILIC column 2.1 × 100 mm, 3.5 µm, 200Å (Merck Millipore, Darmstadt, Germany) and mobile phase solvents consisting of 10 mM ammonium acetate in 50/50 acetonitrile/water (A) and 10 mM ammonium acetate in 95/5 acetonitrile/water (B).

RPLC

7 RPLC experiments were performed using a Zorbax SBaq column 2.1 × 50 mm, 1.7 µm, 100Å (Agilent Technologies, Palo Alto, CA) and mobile phase solvents consisting of 0.06% acetic acid in water (A) and 0.06% acetic acid in methanol (B).

8 Data quality was ensured by (i) injecting 6 and 12 pool samples to equilibrate the LC-MS system prior to running the sequence for RPLC and HILIC, respectively, (ii) injecting a pool sample every 10 injections to control for signal deviation with time, and (iii) checking mass accuracy, retention time and peak shape of internal standards in each sample.