Evaluation and optimization of rapid DDA and DIA screening methods for yeast sub-metabolome analysis on a high-resolution IM-Q-TOF mass spectrometer

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Overview

- We are aiming at the significant enhancement of analytical selectivity in non-targeted analysis by combining dansylation of metabolites and rapid UHPLC with prototype quadrupole broad band isolation directed by ion mobility drift separation.
- The optimized conditions were applied to extracts of Pichia Pastoris and the method was validated via standard addition of a multi-precursor/mixture.
- Figures of merit regarding linearity and precision of mass, precursor and fragment drift time as well as precursor/fragment ratios were determined for selected compounds.

Methods

Derivatization

- BBI_11_1µM_e_in_Pp
- BBI_10_1µM_d_in_Pp

Sample preparation

- Boiling EtOH extraction of metabolites

UHPLC

- Column: Acquity UPLC BSH CH1, 1.7 µm, 1.5 x 50 mm
- Flow: 1 mL/min
- Injection Vol: 5 µL

Derivation via Dansylation

- m/z and drift time vs. m/z trend line for dansylated compounds

IMS Settings

- Total drift time: 60 ms
- TQ mode drift time: 10 ms
- Drift Tube Entrance: 10 V
- Drift Tube Exit: 217.5 V
- IM Transient per frame: 16
- Cycle time: 0.96 s

DIA OTOF

Auto MS/MS mode was employed for generation of fragment spectra of dansylated compounds

DIA IM-All ions

- 0 and ramped CE was applied in alternating ion mobility frames

DIA IM-Q-broad band isolation

- Quadrupole broad band isolation was applied with 0 and ramped CE in alternating ion mobility frames

Results

- Ratio... 0.02 13 12 0.96 6.2 0.00 8.0 11
- Histidine fragment ion
- Histidine precursor ion
- Glycine fragment ion
- Glycine precursor ion

Conclusions

- Drift time directed broad band isolation allows the determination of small metabolites over several orders of magnitude. In comparison to all ions mode we observed less artefacts, higher linearity and a larger working range.
- Fragment/precursor ratios obtained via alternating frames with quadrupole and collision energy ramping are stable over the working range. Evaluation of fragment intensities even extends the working range and is promising in the context of fold change analysis in non-targeted approaches.
- The combination of retention time, accurate mass of precursor and fragment ions and their respective CCS will lead to highest confidence in identity confirmation of unknown compounds.

Acknowledgements: We thank H. Loffler and Dr. T. Wimmer for helpful discussions and for providing mass spectra. We further acknowledge financial support from the Austrian Science Fund (FWF) under grant P25225-N22 and the European Commission (EU) and acknowledge financial support from the European Community (FP7) under grant 245101.