Multimodal urinary metabolomics comparing ion mobility mass spectrometry and NMR spectroscopy

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Introduction

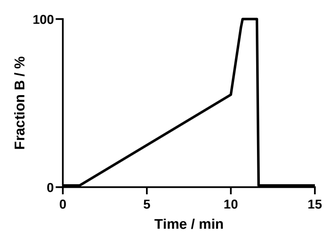
- Urine is a highly relevant sample matrix due to easy sample collection and possibility of temporary storage at -20°C or transport on ice
- Requires minimal sample preparation
- Endpoint of metabolism and not under homeostatic control
- Investigation of 30 urine samples of ataxia patients including controls
- Cohort previously measured using Bruker IVDr Methods on an Avance III 600MHz Spectrometer
- Additional measurements for 4D-Metabolomics on a Bruker timsTOF pro2
- Ion Mobility allows next generation level of annotation by Collisional Cross Section(CCS) and Parallel Accumulation Serial Fragmentation(PASEF)

Sample Preparation

- 30 biobanked samples of spontaneous urine from ataxia patients including controls
- Centrifuge sample 10 min at 5000 x g
- Aliquot 5µl of supernatant to pooled QC, 10µl to sample
- Divide pooled QC into 10µl QC samples
- Add 30µl LCMS-grade Water
- Centrifuge 10min at 5000 x g
- 30µl supernatant transferred into HPLC vials for RP+ and -

RPLC Method

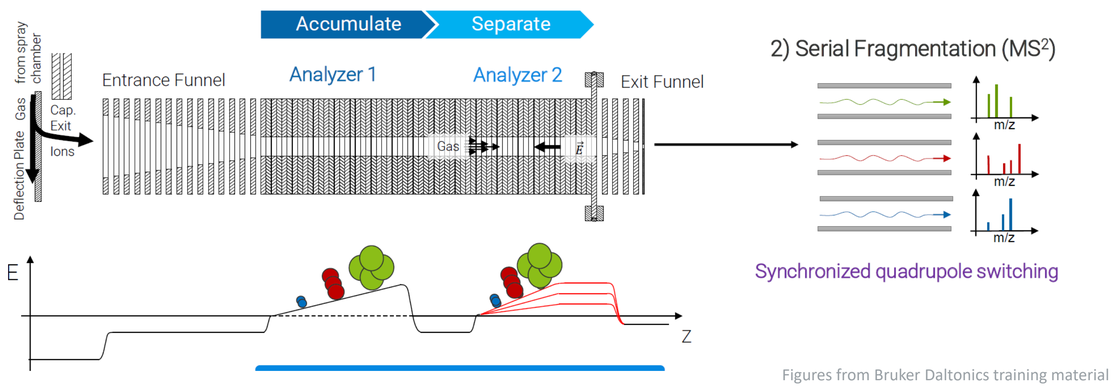
- Based on Open_LCMS Methods(1)
- Mobile Phase A:
  0.1% formic acid in water
- Mobile Phase B:
  0.1% formic acid in acetonitrile



Column	Waters ACQUITY UPLC HSS T3 2.1x 150mm, 1.8µm
Col. Comp.	40°C
Flow Rate	0.60 mL/min 1ml/min flushing

PASEF Stepping

- Parallel Accumulation Serial Fragmentation(PASEF) allows for high MS2 Coverage with full duty cycle
- Two TIMS ramps in series accumulate and separate ions respectively
- Stepping changes transfer parameters based on TIMS Ramp
- MS2 Stepping fragments ions at two levels of collision energy



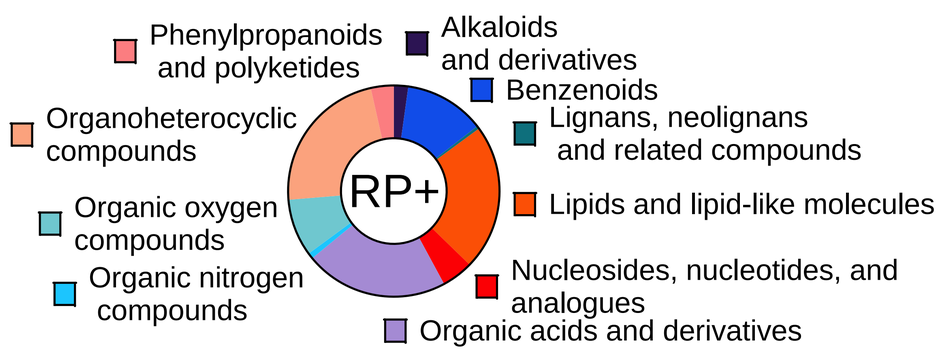
Figures from Bruker Daltonics training material

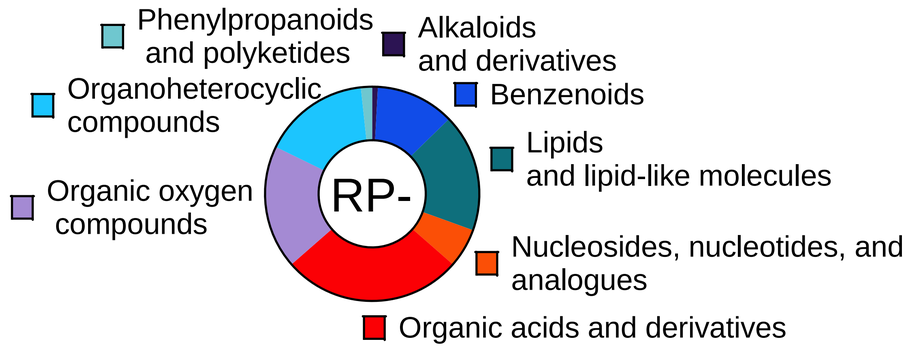
Annotation

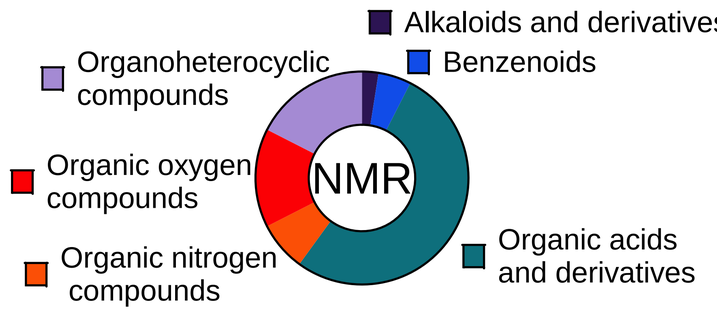
- Only consider features with < 40% QC Relative Standard Deviation(RSD)
- Annotation using MetaboScape Workstation 2024b
- filter for Annotation Quality > 4, minimum of two orthogonal measures
- Manual inspection of Extracted Ion Chromatograms and Mobilograms
- Filtering of duplicate annotations

Classification

- Classification of Annotations using ClassyFire Superclass based on structure(2)







Coverage

- 301 Annotations in RP+, 132 in RP-
- 40 quantified by ^{1}H-NMR through Bruker IVDr Methods
- Minimal overlap between modalities and measurement modes

Summary

- Out of 401 putatively identified metabolites using LCMS only 10 were also covered by NMR, 30 were exclusively identified by NMR
- NMR offers strong quantification and identification particularly of low molecular weight analytes but is held back by poor Limit of Detection(LOD)
- Highly diverse chemical composition requires multiple different analytical techniques to cover, selection of techniques necessary to meet each project's goals

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References:
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(**2**) *Djoumbou Feunang Y et al. Journal of Cheminformatics, 2016, 8:61.*
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