Untargeted metabolomics of cerebrospinal fluid with IM-HRMS and NMR – a comparison

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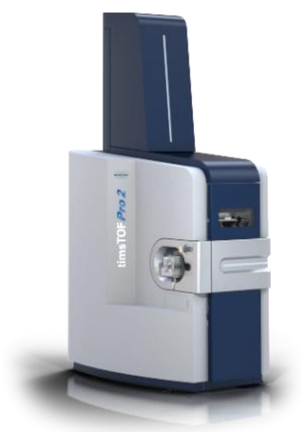
Introduction

- Cerebrospinal fluid (CSF) is an important biofluid for metabolomics of diseases of the central nervous system (CNS) [1]
- CSF is increasingly used to assess diseases like Parkinson's disease, multiple sclerosis or Alzheimer's disease [2]
- Application of 4D metabolomics workflows greatly increases the identification of additional metabolites, e.g. isomers and isobaric compounds, due to the addition of ion mobility giving specific collision cross section (CCS) values for the analytes [3]

Data Acquisition

- LC system: Elute plus
- Columns: Intensity Solo C18 100 mm * 2 mm * 2 µm (RP)
  BEH Amide 150 mm * 2.1 mm * 1.7 µm (HILIC)
- Source: VIP-HESI
- MS: timsTOF Pro 2 system
- MS mode: PASEF (data dependent MS/MS) + tims Stepping
  positive (+) and negative (-) modes
- Annotation: Metaboscape 2024b
  Target Lists + Spectral Libraries
  match for at least 2 of the following:
  m/z, mSigma, MS/MS spectra, CCS



Source: bruker.com/en/products-and-solutions/mass-spectrometry/timstof/timstof-pro-2.html (20.08.2024)

Sample preparation

- 100 µL CSF (3 fresh frozen samples)
- Add 300 µL ice-cold CAN,
- Vortex + centrifugation (15 min, 14,000 rpm)
- 50 µL supernatant for HILIC measurements
- 300 µL supernatant: evaporation to dryness
- Reconstitute in 60 µL MQ water : ACN (9:1)
- Vortex + centrifugation (15 min, 14,000 rpm)
- 50 µL supernatant for RP measurements

Results

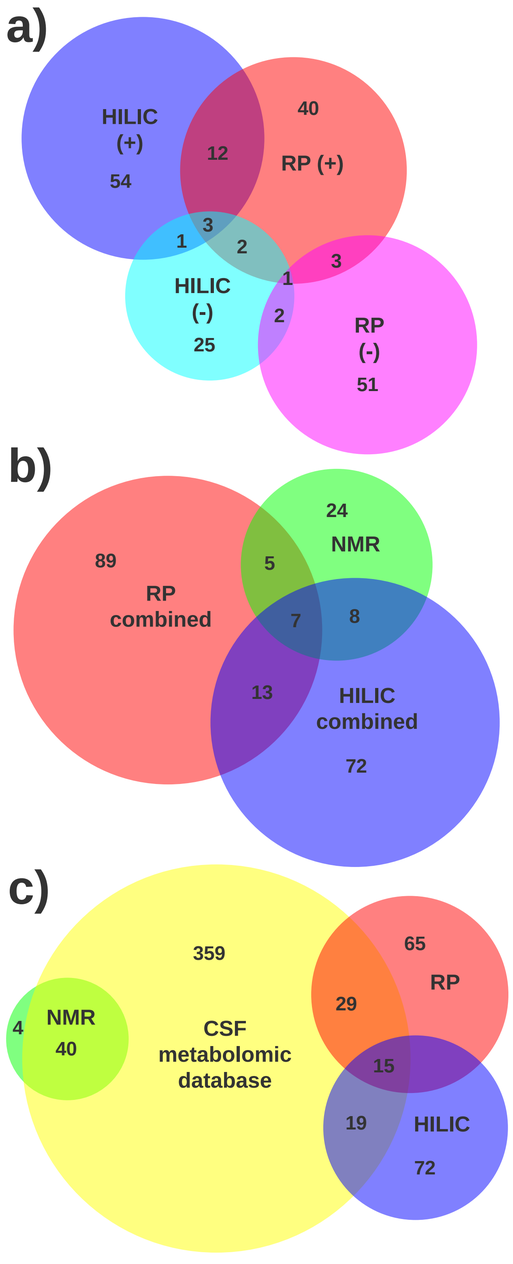


Figure 1: Venn diagrams (created with DeepVenn [4]) comparing the number of annotations between **a)** four different LC-IM-HRMS methods (3 freshly drawn CSF samples), **b)** LC-IM-HRMS data and proton NMR spectroscopy results from 71 biobanked CSF samples [5] and **c)** matching of LC-IM-HRMS/NMR annotations with the CSF metabolomic database (overlaps between LC-IM-HRMS/NMR not shown)

- In total, 186 analytes were identified across all four liquid chromatography coupled with ion mobility and high resolution mass spectrometry (LC-IM-HRMS) methods (Fig. 1)
- In 71 biobanked CSF samples, 45 analytes were identified by proton NMR spectroscopy [5]

➢ **LC-IM-HRMS** allows for identification of previously unknown substances

- **NMR** best covers highly abundant, polar, small molecules (*e.g.* formic acid, acetone, urea…) (Fig. 2)
- **LC-IM-HRMS** best covers lipid classes (*e.g.* phosphatidylcholines, sphingomyelins, fatty acids > C12…)

➢ Both technologies can cover different pathways (Fig. 3)

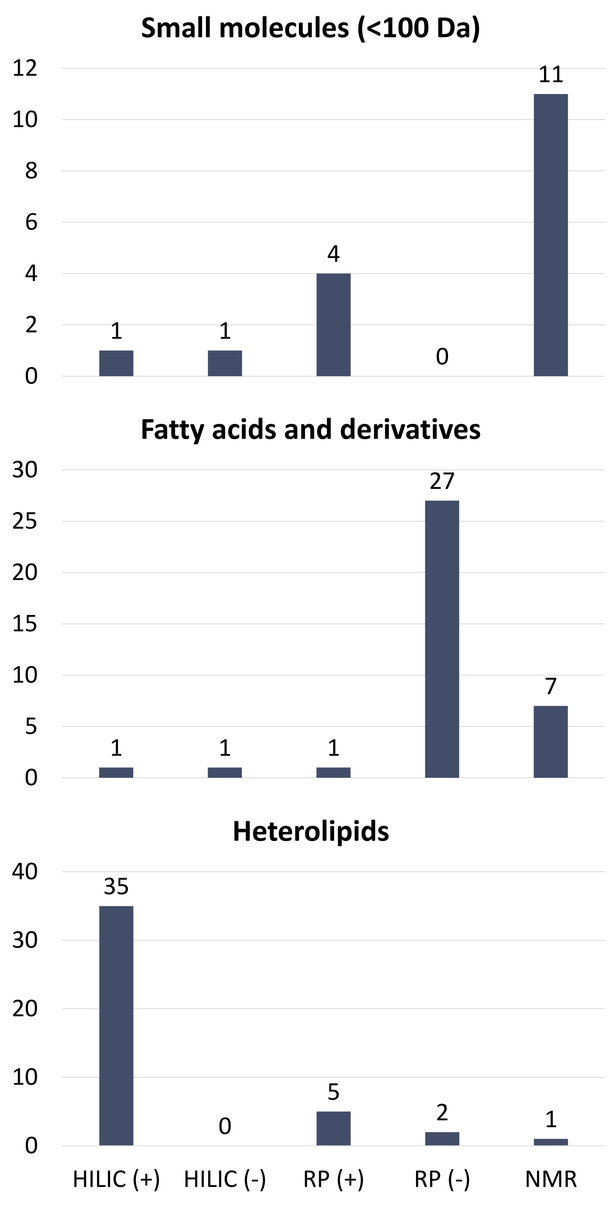


Figure 2: Number of metabolites of different classes (**upper**: small molecules with a molar mass <100 Da, **middle**: fatty acids and derivatives thereof, **lower**: heterolipids) as identified by different LC-IR-HRMS methods (3 fresh frozen CSF samples) and proton NMR spectroscopy (71 biobanked CSF samples) [5]

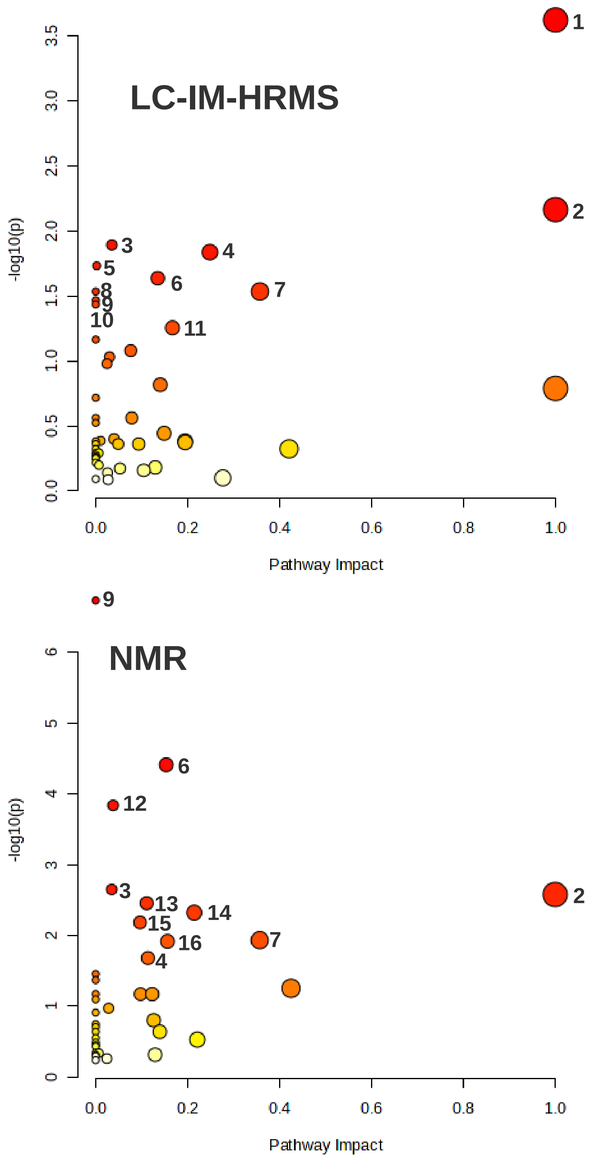


Figure 3: Pathway analysis of metabolites identified by four combined LC-IM-HRMS methods (**upper graph**) and proton NMR (**lower graph**) [5] **1**= Caffeine met., **2**= Phenylalanine, tyrosine and tryptophane synth., **3**= Galactose met., **4**= Alanine, aspartate and glutamate met., **5** = Lysine degradation, **6**= Glyoxylate and dicarboxylate met., **7**= Phenylalanine met., **8**= Valine, leucine and isoleucine synth., **9**= Biosynth. of unsaturated fatty acids, **10**= Ascorbate and aldarate met., **11**= Tyrosine met., **12**= Valine, leucine and isoleucine degradation, **13**= Butanoate met., **14**= Glycine, serine and threonine met., **15**= Glycerophospholipid met., **16**= Pyruvate met.

Summary

- **Combination** of LC-IM-HRMS methods and NMR gives a **comprehensive picture of the metabolome** of a sample
- Select methods have individual strengths (and weaknesses) -> **tailoring of analysis package** to scientific problem possible to increase efficiency
- Outlook: Implementation of **Lipidomics protocols** in our Core Facility to increase lipid phenotyping (*e.g.* di- and triacylglycerides, steroids)

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