Integration of metabolomic and lipidomic workflows for studying biological samples

Adriana Zardini Buzatto[1], Shuang Zhao[1], Ulrike Schweiger Hufnagel[2], Aiko Barsch[2] and Liang Li[1,*]

[1]Department of Chemistry, University of Alberta, Edmonton, AB, Canada; [2]Bruker Daltonik GmbH, Bremen, Germany *liang.li@ualberta.ca

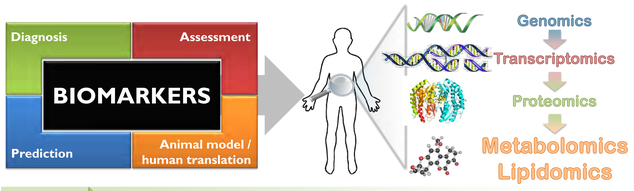
Overview

OBJECTIVES • Poof-of-concept study for the integration of previously developed untargeted metabolomic and lipidomic workflows, including sample preparation, LC-MS analysis, data processing and identification.

EXPERIMENTAL • Liquid-liquid extraction of serum and urine samples with dichloromethane and methanol; the aqueous layer was used for metabolomics and the organic layer, for lipidomics. Both layers were analyzed by LC-MS, followed by data alignment, filtering, identification, normalization and statistics.

RESULTS • The integrated workflow provided suitable results and reduced the sample volume requirement, as well as overall analysis time.

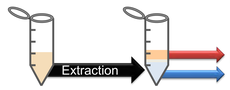
Introduction



Metabolites — Polar, hydrophilic small molecules (<1000 Da)
Functions: cell structure, signalling, defense, enzymatic co-factors, development, reproduction

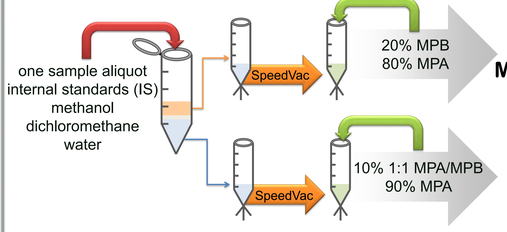
Lipids — Hydrophobic, low polarity small molecules (<1500 Da)
Functions: cell structure, signaling, defense, energy storage

MOTIVATION: **(1)** Small sample volumes require integration of preparation techniques • **(2)** Integrated sample preparation allows for high throughput • **(3)** Overlap between detection by different workflows is reduced.



Metabolomics LC-MS
Lipidomics LC-MS

Methods



RPLC-ESI-QqTOF (positive and negative ionization)

Bruker C18 T-Rex Elute M-column (100-mm X 2.1-mm)

Bruker Elute UHPLC with 5 µL injection

Bruker Impact II ESI-QqTOF-MS

Metabolomics[1]
- MPA: 0.1% formic acid in water
- MPB: 0.1% formic acid in acetonitrile
- 22 min gradient, 8 min equilibrium, 30° C

Lipidomics
- MPA: 10 mM NH4COOH in 50:40:10 methanol/acetonitrile/water
- MPB: 10 mM NH4COOH in 95:5 isopropanol/water
- 24 min gradient, 10 min equilibrium, 45° C

Experimental design

Dilution test
- Final volume fixed at 30 µL, sample volume varied
- Evaluated dilution factors: 1:10, 1:5, 1:2.5 and 1:1

Metabolomics
- 6 extraction replicates: aqueous fraction
- Positive and negative ionization

Lipidomics
- 6 extraction replicates: organic fraction
- Positive and negative ionization

Data processing

Alignment
- Bruker Metaboscape 4.0
- Mass re-calibration, peak picking, alignment, deisotoping
- Filtering (>80% of injections per group)

Normalization
- Lipidomics: internal standardization by 14 deuterated lipids (lipid class and retention time match), summed intensity ratio and auto-scaling
- Metabolomics: summed intensity and auto-scaling

Statistics
- MetaboAnalyst 4.0
- Volcano plots, dendogram, PCA

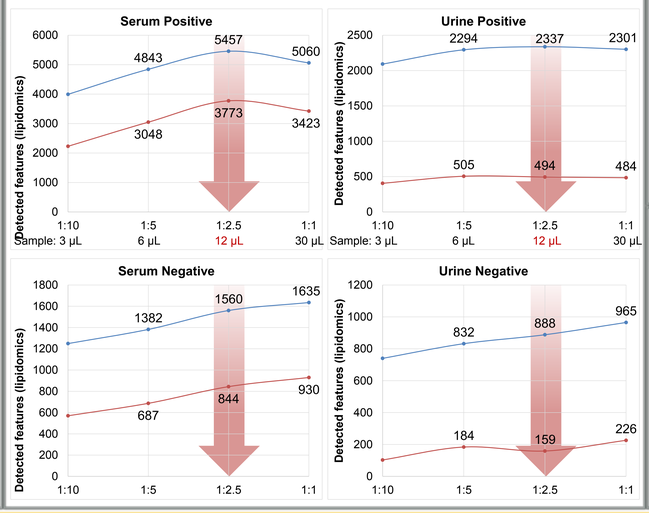
Identification

Metabolomics
- **Positive:** m/z error <5 mDa, mSigma <100, MS/MS score >200
  1. Bruker HMDB metabolite library (retention time and MS/MS)[1]
  2. MoNA[2] and MSDial LipidBlast[3] MS/MS libraries
- **Putative:** MyCompoundID (m/z tolerance of 5 mDa)[4]

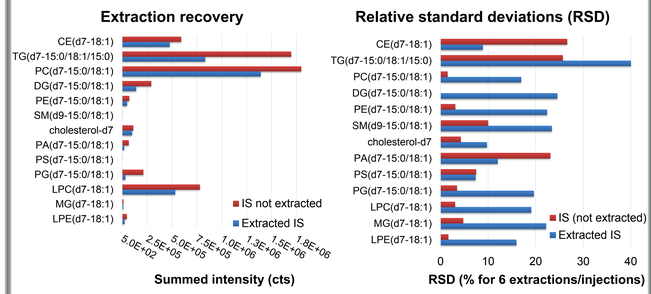
Lipidomics
- **Positive:** m/z error <5 mDa, mSigma <100, MS/MS score >100
  - MSDial LipidBlast, MoNA and HMDB MS/MS libraries[1,2,3]
- **Putative:** LipidMaps[5] (m/z match; tolerance of 5 mDa)

Dilution test: lipidomics

– Biological fluid + internal standard mix (IS, 14 deuterated lipids)
– Biological fluid after subtraction of features also detected for the extracted IS

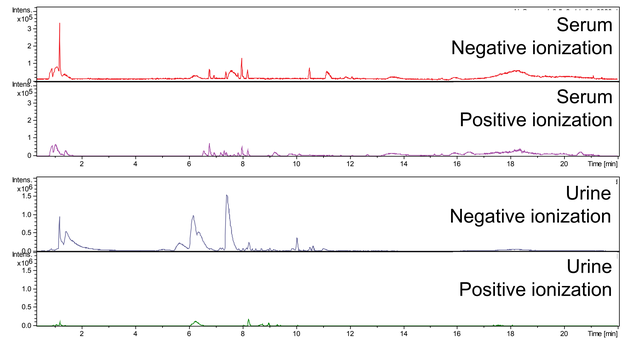


Extraction of lipid internal standards

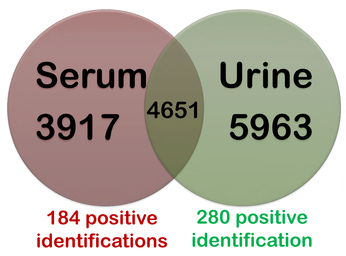


Results

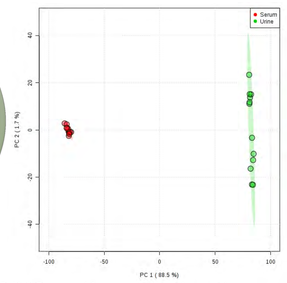
Metabolomics



Detected features



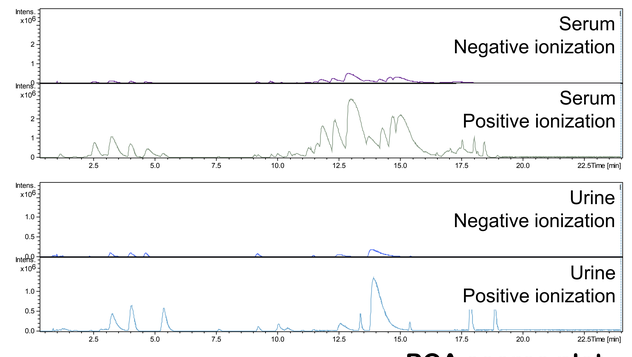
PCA scores plot



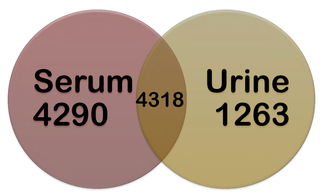
- **331 positive identifications by MS/MS and/or retention time**
- **2049 putative identifications (m/z tolerance of 5 mDa, no metabolic reactions)**

Results

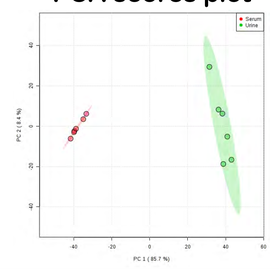
Lipidomics



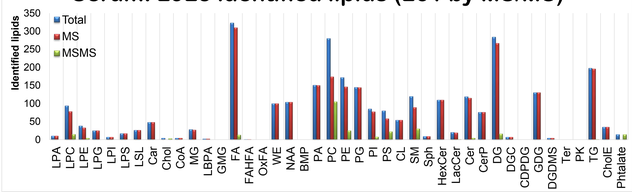
Detected features



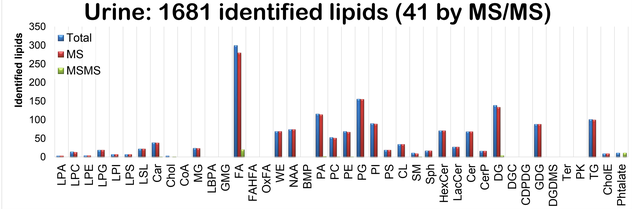
PCA scores plot



Serum: 2925 identified lipids (261 by MS/MS)



Urine: 1681 identified lipids (41 by MS/MS)



Conclusions

- The integrated workflow for lipidomics and metabolomics was suitable for the analysis of serum and urine samples.
- The metabolomics methodology allowed de identification of X metabolites.
- The combined sample preparation resulted in the detection of more than 17000 features for serum and 16000 for urine from 12.0 µL of sample.
- The lipidomics methodology allowed the identification of 2925 lipids for serum and 1681 for urine.

Acknowledgements

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References

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[3] Kind T, Liu KH, Lee DY, deFelice B, Meissen JK, Fiehn O, Nature Methods 2013, 10, 755-58.
[4] LipidMaps database, https://www.lipidmaps.org/
[5] MyCompoundID database, http://www.mycompoundid.org