LC-SWATH/MS Metabolomics Platform with Hyphenation of Extraction for the Analysis of Polar and Non-polar Metabolites in Biological Samples

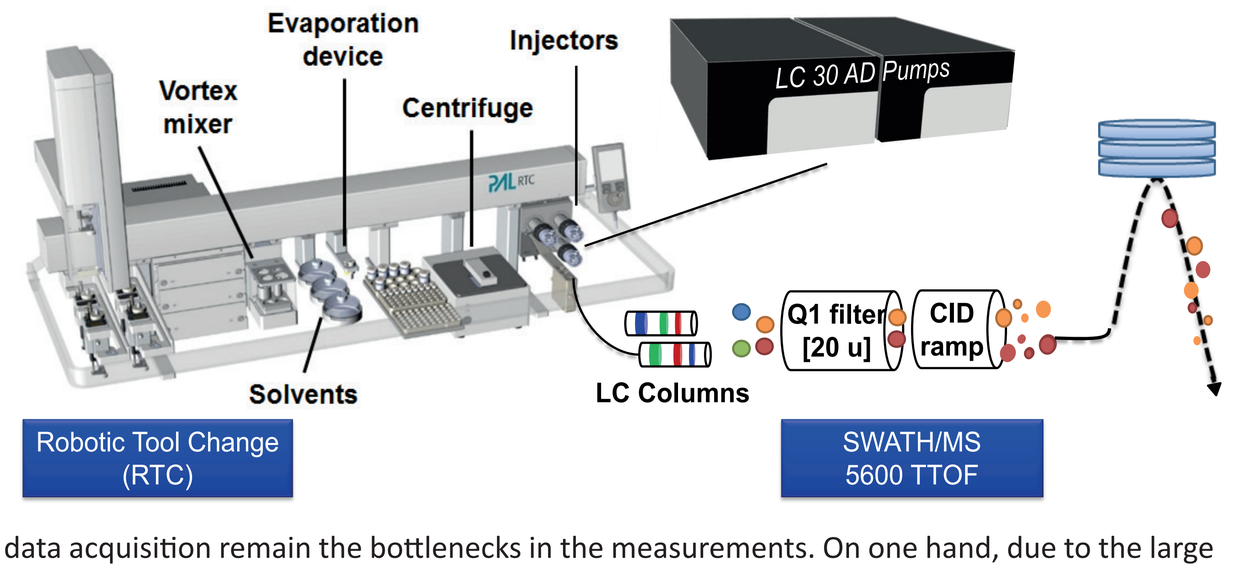
Michel Raetz[1], Renzo Picenoni[2], Guenter Boehm[2], Gérard Hopfgartner[1]

[1]Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva, Switzerland
[2]CTC Analytics, Zwingen, Switzerland

YOU 4, Short Oral 3

Overview

- Fully automated robotic sample preparation with hyphenated dual LC-SWATH/MS analysis for lipids and polar metabolites
- Automated SPE based lipid fractionation to equalize lipid abundances and reduce of runtimes
- SWATH/MS acquisition to record all the product ions originating from specific precursor ion windows in order to measrue lipids and polar metabolites
- Automated library search for polar metabolites using an in-house accurate mass MS/MS library



Introduction

Metabolomics studies are still challenging and technically demanding. Both sample preparation and data acquisition remain the bottlenecks in the measurements. On one hand, due to the large chemical space of the analytes and their broad dynamic range that need to be dealt with. On the other hand, metabolomics still suffer from limited selectivity due to the structural similarities within the low molecular weight compounds.
SWATH/MS opened new possibilities within Data Independent Aquisition (DIA) to gain selectivity for -omics studies as all fragments form all precursors in different isolation windows are recorded. The hyphenated setup allows full automation from sample preparation over chromatography to MS data acquisition and feature annotation. The integrated dual LC configuration with three different mobile phases enables a broad coverage of the metabolome. Additionally, the online SPE based lipid fractionation allows to equalize the dynamic range of the abundance of lipid classes.

Methods

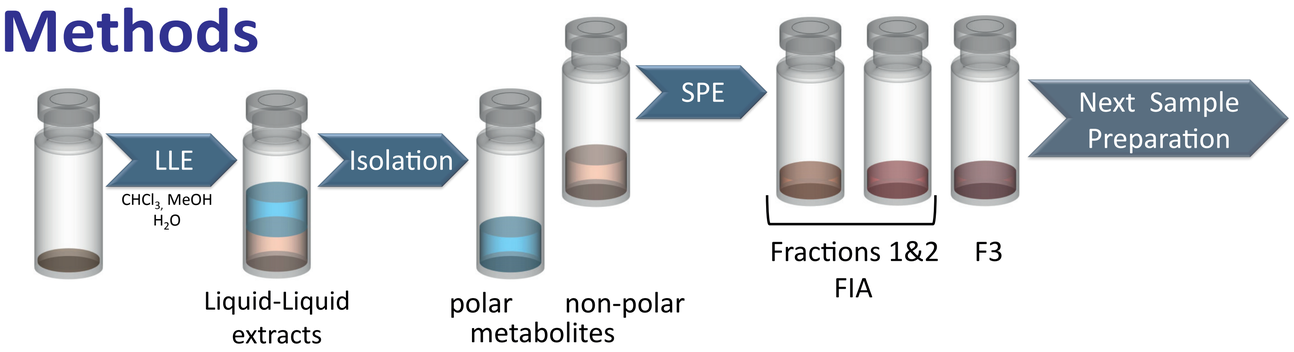
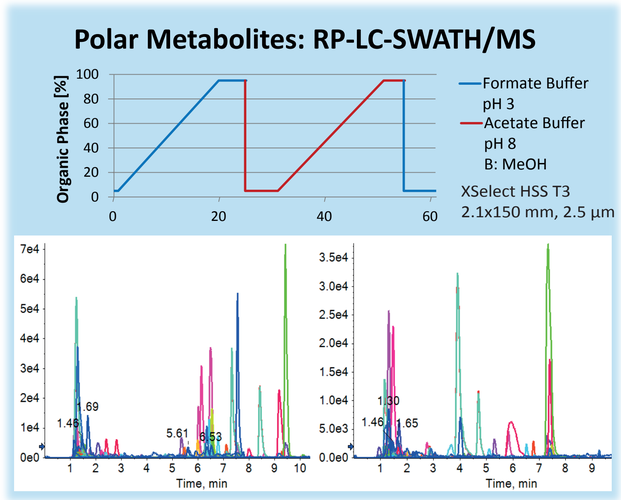


Figure 1: Workflow of the robotic sample preparation consisting of a Bligh and Dyer extraction, phase separation, and SPE using the RTC with hyphenation to a dual LC-SWATH/MS system.



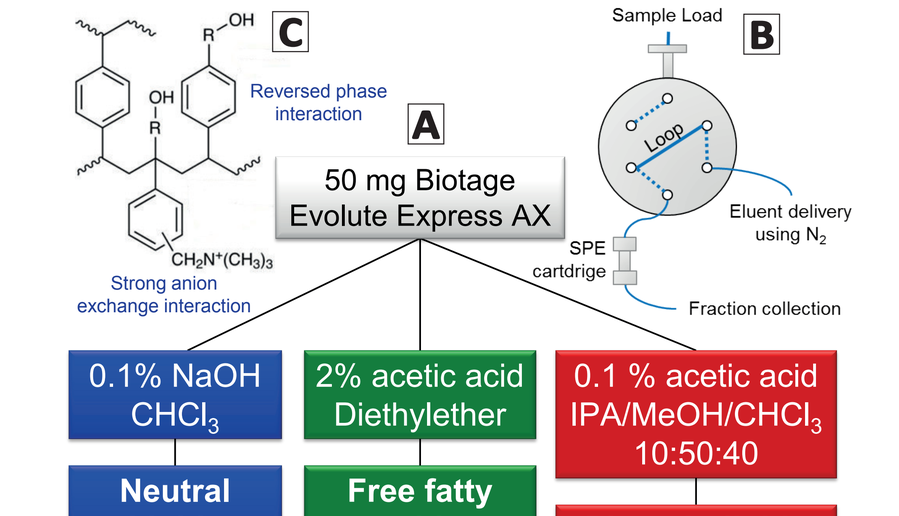


Figure 2: A – Experimental setup of the automated SPE fractionation of the lipid classes. **B –** Injection valve system used for the elution of the fractions from the self-packed cartridge by a stream of nitrogen as carrier. **C –** Mixed mode sorbent used to pack the cartridge.

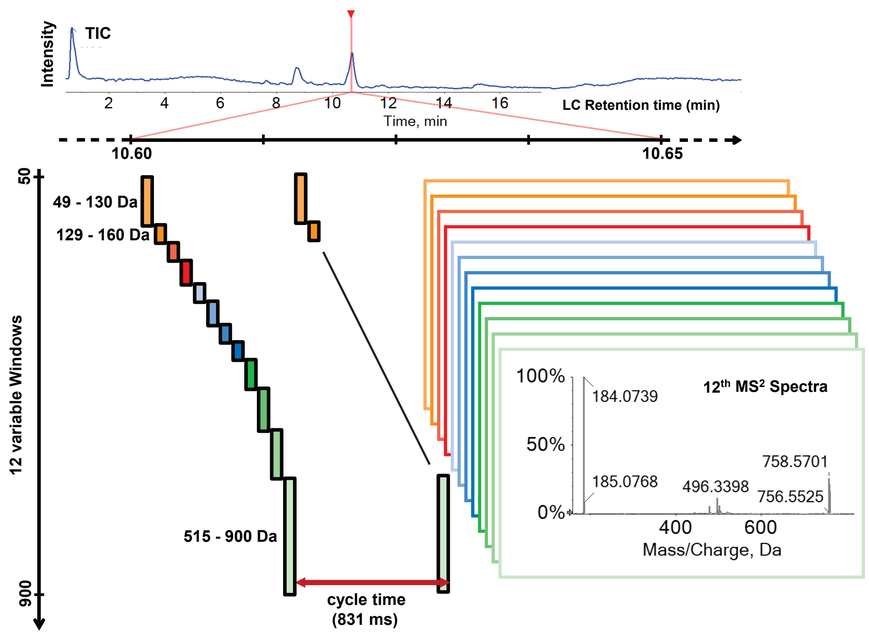
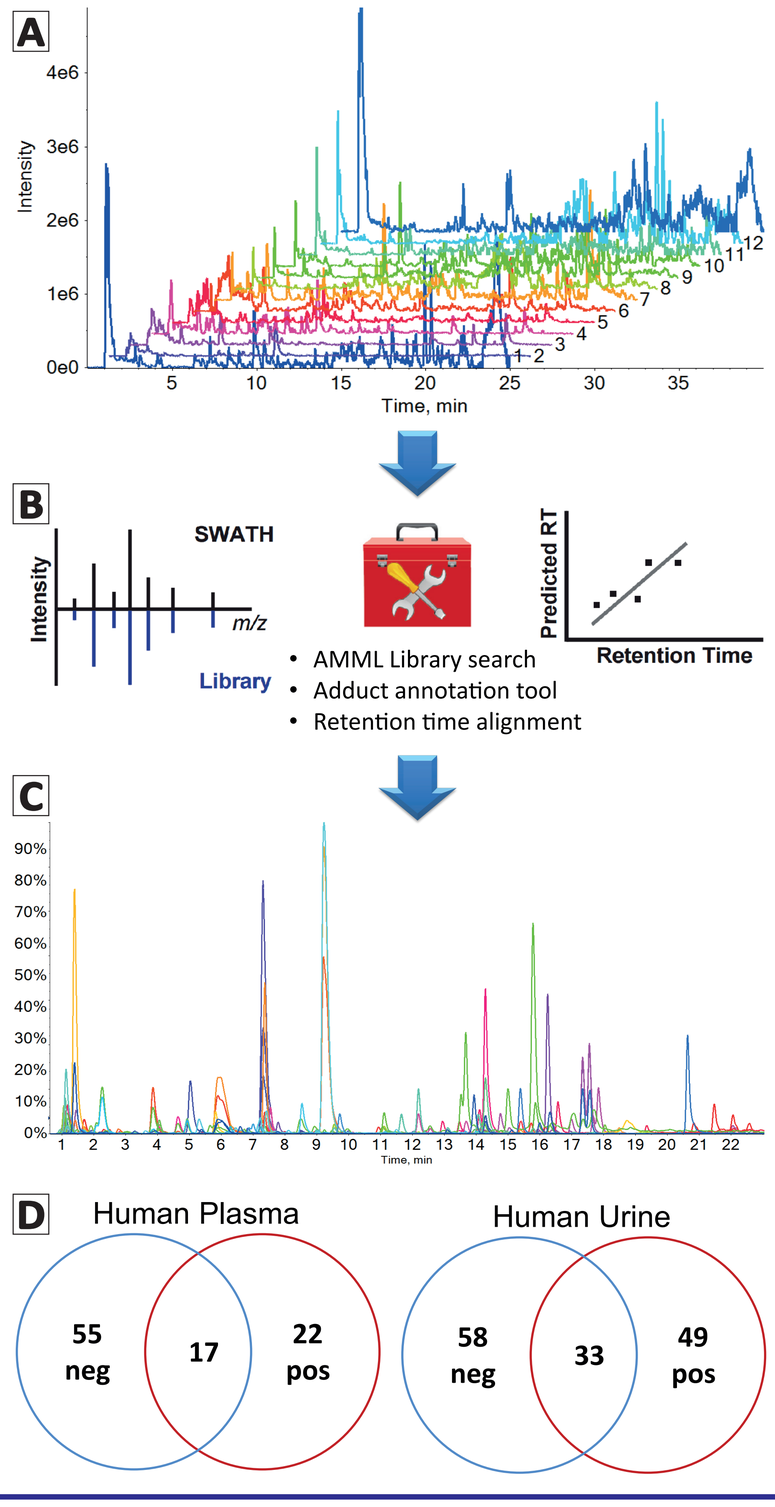


Figure 3: SWATH/MS setup applied for the analysis of polar metabolites in negative ion mode. Data were acquired on a Sciex 5600 TripleTOF.
Parameters: 500 °C, 5.2/-4.5 kV, DP 80, 12 SWATH windows with variable unit sizes created using swathTUNER, 831 ms cycle time, 50 ms accumulation time.

Results



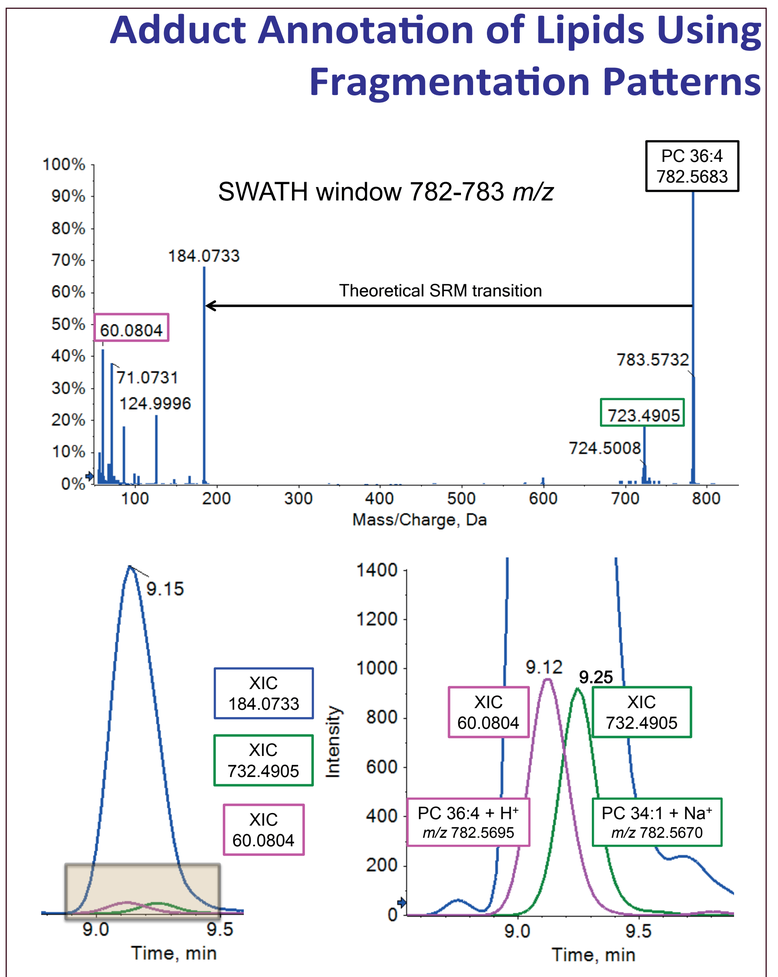


Figure 5: Top - MS2 spectrum from the SWATH Q1 window from 782-783 *m/z*. The traditionally used SRM transition from precursor to headgroup fragment ion is indicated. **Bottom –** XIC traces of selected fragment ions originating from co-eluting isobaric precursor ions (Δm 25 mmu).

Figure 4: A – Offset chromatogram of the 12 SWATH experiments for an undiluted human urine sample (5 µL injection volume). **B –** In-house tools for analyte indentification. **C –** Chromatogram of the identified analytes fulfilling the following criteria: Library score > 0.75, RT shift < 1%, difference in isotope intensity < 10%, mass accuracy <5 ppm. **D –** Venn diagrams for the number of annotations in human urine and plasma in positive and negative ion mode (pH 3 and 8).

Conclusion

- In-house processing tools allow fast data evaluation and increased confidence of identifications
- Full workflow automation enables single sample approach and possibility of adaption during data acquisition processes
- SWATH/MS acquisition enables quantification using any fragments on product ion level and increases selectivity when overlapping precursor ions are analyzed due to differences in fragmentation pathway of co-eluting precursors
- SWATH/MS allows reprocessing of all the data post-acquisition and reduces the rate of false analyte identification compared to SRM

Y. Zhang et al. The Use of Variable Q1 Isolation Windows Improves Selectivity in LC–SWATH–MS Acquisition. J. Proteome Res., 2015.
T. Bruderer et al. In silico prediction for the investigation of comedication interferences in quantitative LC–MS detection in the SRM mode. Bioanalysis. 2012.
R. Bonner et al. SWATH acquisition mode for drug metabolism and metabolomics investigations. Bioanalysis, 2016.

Acknowledgements

Sciex and Shimadzu for their support and valuable contribution.

MalarX
Systems Medicine of Malaria

SystemsX.ch
The Swiss Initiative in Systems Biology