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Metabolomics and research

Exploring sex differences in zebrafish livers using a novel targeted discovery metabolomics approach

Michaela Schwaiger-Haber[1,2,3], Bashar Amer[4], Darshak Gadara[1,2,3], Cristina C. Jacob[4], Philip Remes[4], Susan S. Bird[4], Kevin Cho[1,2,3], Gary J. Patti[1,2,3]
[1]Departments of Chemistry and [2]Medicine, [3]Center for Mass Spectrometry and Metabolic Tracing, Washington University in St. Louis, St. Louis, MO, USA;
[4]Thermo Fisher Scientific, San Jose, CA, USA

Abstract

- New nominal mass instruments with high acquisition rates, sensitivity, and advanced features like MSn are emerging.
- Proposal of an alternative to untargeted analysis on high-resolution instruments.
- Spectra will contain all product ions.
- PRM Conductor helps with product ion selection for data analysis.
- Large sex differences between male and female zebrafish livers indicate the importance of separating them in statistical data analysis.

Introduction

- Targeted workflows using triple quadrupole (QQQ) instruments have recently gained more attention in the field of metabolomics [1].
- The targeted aspect, namely having to decide which metabolites to measure before data acquisition, seems to be a barrier for untargeted metabolomics
- New nominal mass instrument, Thermo Scientific™ Stellar™ Mass Spectrometer provides:
- Detection of all product ions, thus only precursors need to be known (can be identified or unknown compounds).
- High acquisition rates, and sensitivity.
- Features such as MSn, HCD, and CID, stepped collision energies.
- Software to help with method development.
- Zebrafish (*Danio rerio*) provides a premier model organism to study whole-body metabolism [2]. Using a targeted discovery metabolomics experiment, we measured liver extracts from adult male and female zebrafish.

Materials and methods

Sample preparation

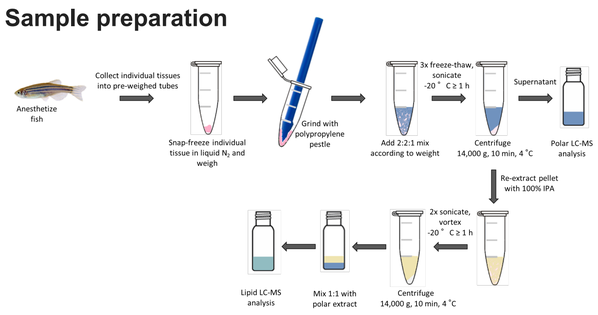

Figure 1. Sample preparation workflow for polar and lipid metabolite analysis from zebrafish.

Analytical method optimization

- Peak list obtained from high-resolution instrument in both positive and negative polarity using Thermo Scientific™ Compound Discoverer™ 3.4 software.
- Removal of in-source fragments, filtering to keep only one polarity.
- List can contain identified and unidentified compounds.
- Thermo Scientific™ AcquireX in combination with Compound Discoverer 3.4 software and Thermo Scientific™ LipidSearch™ software used for the identification.
- Additional MS3 data can be acquired for triglycerides (TGs).

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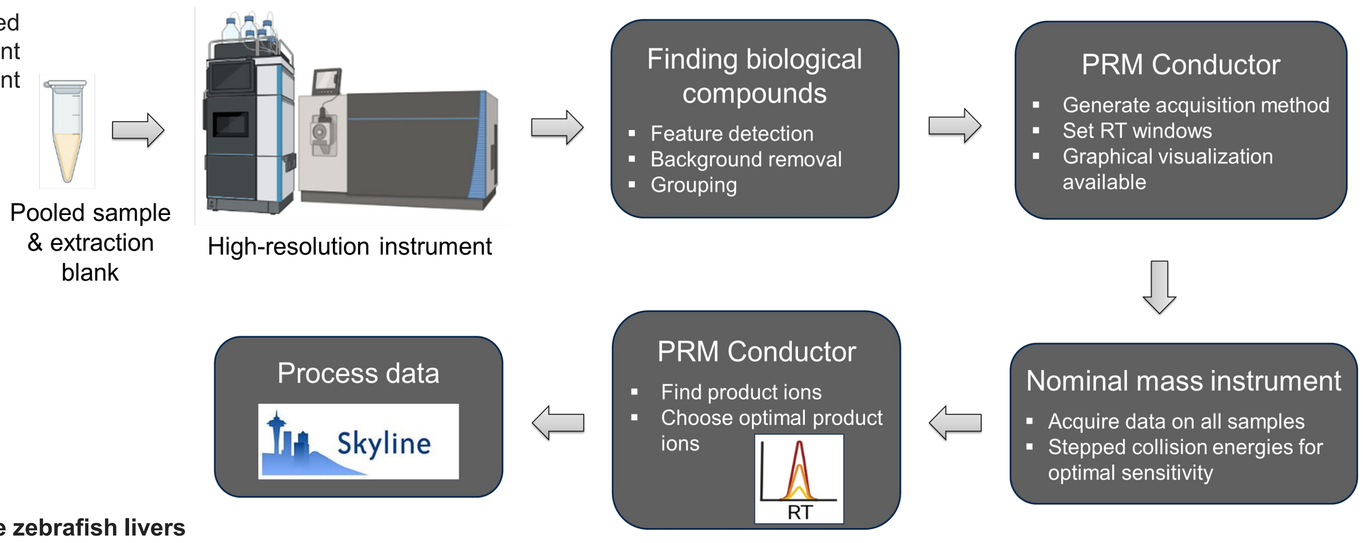
Results

Experimental workflow for Targeted Discovery Methodology

- Analysis of pooled sample and extraction blank on the high-resolution instrument
- Feature detection, grouping, etc. using Compound Discoverer 3.4 software
- PRM Conductor helps generate targeted acquisition methods with optimal RT windows
- Data acquisition for all samples on nominal mass instrument Stellar MS
- Stepped collision energies for optimal sensitivity, especially for unknowns
- Optimized collision energies can be used if available
- HCD and CID available (per precursor)
- PRM conductor helps to find optimal product ions and to generate a Skyline transition list
- Which product ions are the best (intensity and no interferences)

Figure 2. Workflow overview from a pooled sample run on a high-resolution instrument to a final nominal mass instrument acquisition method.



Sexual dimorphism in male and female zebrafish livers

- Major differences in lipid metabolite levels between male and female livers.
- The majority of compounds were higher in females, but eight lipids were lower in females, seven of which contained FA 18:2.
- TGs containing FA 18:2 follow the same pattern (lower in females).
- TGs containing FA 18:2 and FA 14:0 show biggest difference.

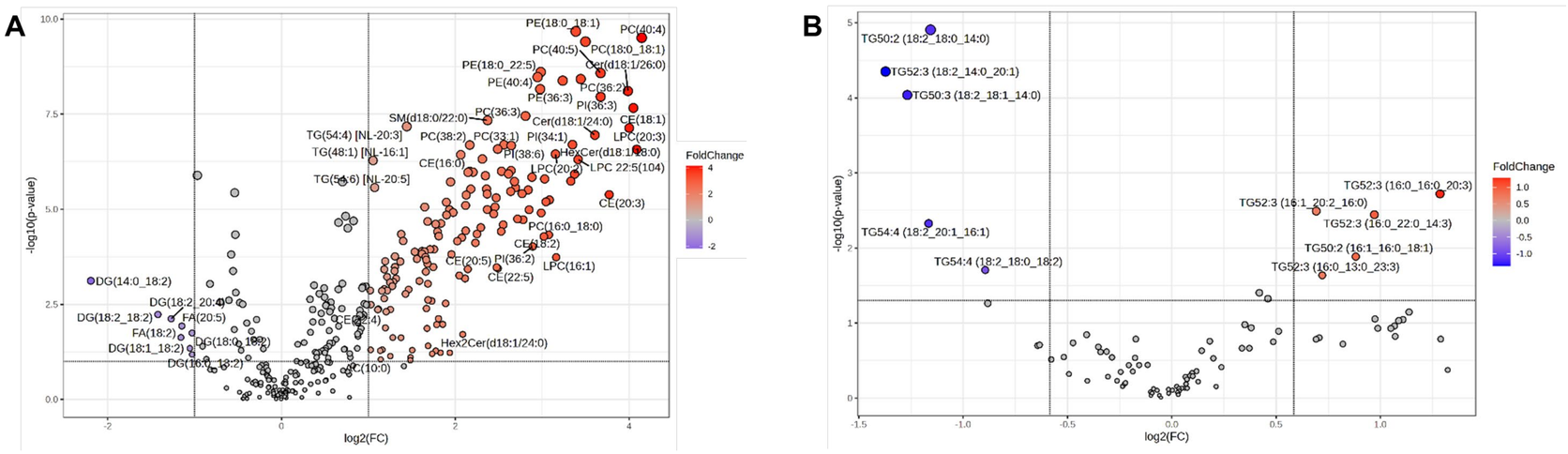
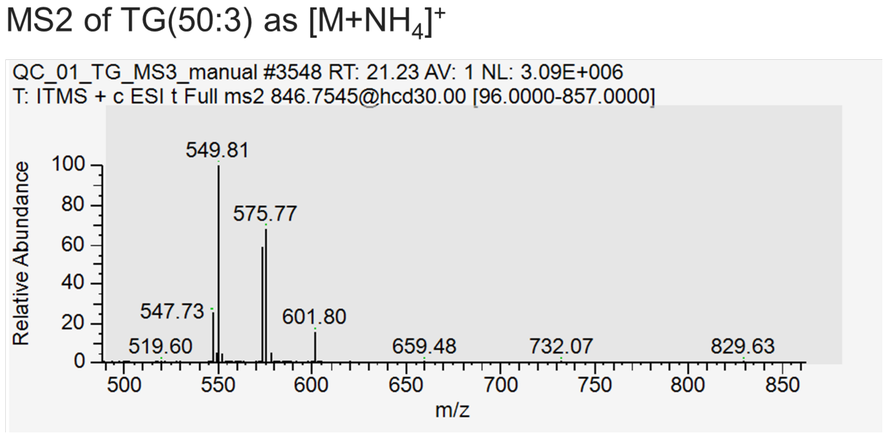


Figure 3. Volcano plot of lipids detected in female vs. male zebrafish livers. A) lipids from MS2 analysis, B) MS3 analysis of selected TGs. Generated using MetaboAnalyst 6.0.

TG characterization using MS3



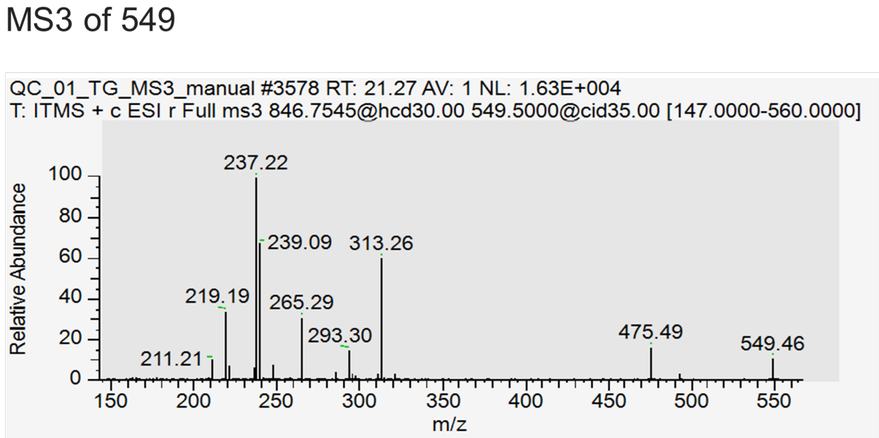


Figure 4. MS2 and MS3 spectra of TG(50:3) as an example for triglyceride structural characterization.

Conclusions

- Streamlined approach from untargeted metabolomics to a targeted discovery methodology
- All product ions are detected, which can help distinguish isomers
- Large biological sex differences indicate the importance of analyzing data from each gender separately

Acknowledgements & references

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Figure 2 was created using BioRender.com.

[1] Schwaiger-Haber et al., ACS Meas. Sci. 1, 1 (2021)
[2] Naser, Jackstadt et al., Cell Metab. 33, 7 (2021)

Conflict of interest

G.J.P. is a scientific advisory board member for Cambridge Isotope Laboratories and has a collaborative research agreement with Thermo Scientific. B.A., C.C.J., P.R., and S.S.B. work at Thermo Scientific. PO003608

All other authors do not have any conflicts of interest.

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