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Analytical challenges in Zebrafish metabolomics

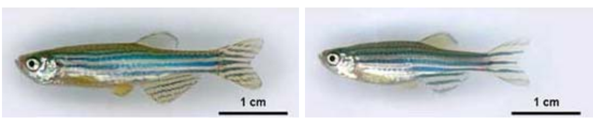
The zebrafish model organism is increasingly used for assessing drug and chemical toxicity and safety. Numerous studies confirm that mammalian and zebrafish toxicity profiles are strikingly similar. Zebrafish can be used to eliminate potentially unsafe compounds rapidly in the early stages of drug development and to prioritize compounds for further preclinical and clinical studies. In addition to toxicity testing, zebrafish models are used to study human diseases like cancer and cardiovascular -, muscular -, neurological- and metabolic diseases.

INTRODUCTION

Needless to say, the use of zebrafish as a model in different studies is a new analytical challenge. Very low amounts of sample are available (different tissues) and extremely low amounts of plasma. In these low sample amounts, determination of dosed chemicals or drugs needs to be performed as well as concentrations of endogenous metabolites, if mechanistic insight into the studies processes is required. For this reason, adaptation of conventional methods combined with new technology developments are required in order to expand use of zebrafish for chemical and drug screening or as a model for studying different disease states.

Zebrafish

Male Female



GOAL:

As a preparation step for further studies, assess the suitability of available metabolomics platforms for zebrafish applications:

1. GC-MS method
2. Lipids and free fatty acids LC-MS
3. Amino acid AccQ-Tag LC-MS

EXPERIMENTAL:

Zebrafish (Danio rerio, AB strain) samples:
The following organ tissues were collected from two adult fish which received no treatment. The tissues were selected based on relevance for future toxicity or disease model studies.

Liver (Hepatotoxicity)	Kidney (Nephrotoxicity)
Spleen (Immunotoxicity)	Brain (Neurotoxicity)
Heart (Cardiotoxicity)	Ovary (Reprotoxicity)

The tissue sample size varied from 2 to 100 mg depending on the organ. Organs from one fish were used for GC-MS and AccQ-Tag and organs from the second fish were used for the lipid analysis.

GC-MS: The available method was downsized for lower volumes of plasma or tissue extracts. 3-15 mg of tissue was extracted with methanol and, an aliquot of the extract was further derivatised and analysed.
Lipid and free fatty acids LC-MS: wet tissues were accurately weighed and extracted using Folch extraction, to obtain 1 mg/ml extract of tissue. 10 µL of the extract was derivatised and analysed.
Amino acids AccQ-Tag LC-MS: an aliquot of the same extract as was used for GC-MS was derivatised and analysed.
For both LC-MS methods, the Accela-LTQ-Orbitrap from ThermoScientific was used and the GC-MS data was acquired using the GC-MSD system from Agilent.

RESULTS

Several adaptations of our existing methods were needed in order to be able to handle the very low amounts of sample. The extractions are performed on wet tissue rather than lyophilized tissue. For soft tissues such as brain, ovary, liver etc this does not pose a problem and many metabolites can be extracted.
The tissue extracts were analysed with several methods and examples of the results are shown in Figures 1-3.

Ovaries do not contain many abundant lipids, but the extracts from other tissues contain many fatty acids and lipids. Lipid profiles comparable to those obtained from similar rodent tissues were acquired.

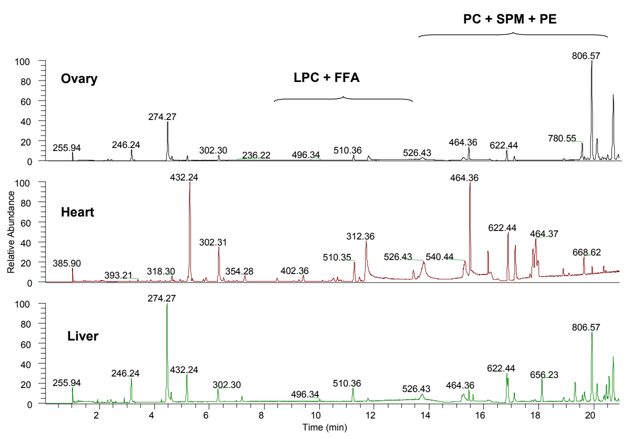


Figure 1. Base Peak chromatograms obtained with the polar lipid method for ovary, heart and liver. The retention time areas of elution of the different lipid classes are shown. Annotation above the peak is the m/z of the Base Peak. Examples of detected compounds are: lysophosphatidyl cholines, phosphatidyl cholines, saturated fatty acids, polyunsaturated fatty acids, phosphatidyl ethanolamines, sphingomyelins, unconjugated bile acids

Also here, the ovaries are very challenging because of the very low sample amount. Even after downsizing the GC-MS method, the ovary extract shows the least number of metabolites present in concentrations that we can detect. Also with this platform, the heart and liver extract chromatograms contain many metabolites. The identities were checked only briefly, but there is a large overlap with metabolites detected previously in similar tissues from rodents.

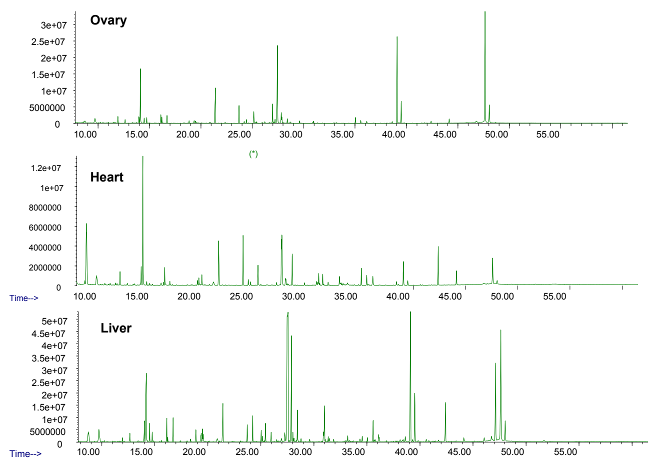


Figure 2. Total ion chromatograms acquired with the downsized GC-MS method for ovary, heart and liver extracts. Examples of detected metabolites are free fatty acids, sugars (inositol, glucose), small organic acids (lactate), cholesterol, cholic acid

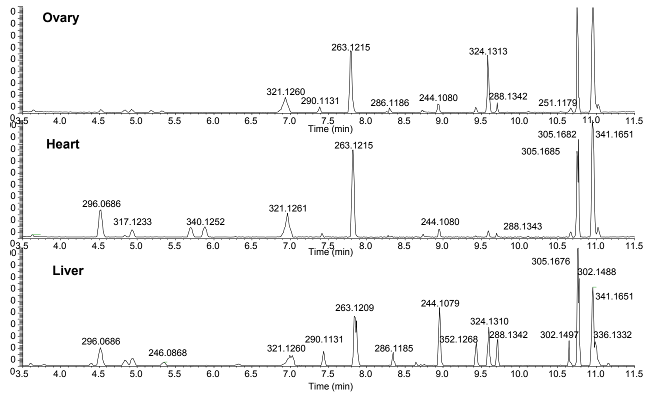


Figure 3. Base peak chromatogram from the AccQ-Tag method obtained from ovary, heart and liver extracts. Annotation above the peak is the m/z of the base peak. Examples of amino acids detected are Glycine, Leucine, Lysine, Proline, Serine, Threonine, Alanine, Glutamic acid, Arginine, Valine, Histidine

CONCLUSIONS AND FUTURE PLANS:

- Our lipid and amino acid platforms showed to be sufficiently sensitive to observe profiles comparable to those obtained with larger sample quantities, such as obtained in rodent studies
- After downsizing the GC-MS platform, we were able to obtain a lot of metabolite information from the low amount of tissue available.
- For GC-MS and the AccQ-TAG LC-MS a single extract can be split into two aliquots for analysis.
- Our methods will be used for zebrafish applications in toxicology, pharmacology and disease pathogenesis research and they will be optimized for embryonic and larval zebrafish applications.