Mass spectrometry imaging of small xenobiotics on *Danio rerio* : influence of molecular profiles modification as potential localization asset

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Overview

Objectives

- Develop a methodology to indirectly locate small xenobiotics hindered by ion suppression effect.
- Obtain information on the ions affected by the contamination.

Methods

- Biological model: *Danio rerio WTAB*
- Xenobiotic: Diazinon
- Contamination: 24 hours at 5µM.
- Sectioning: cryosectioning after gelatin embedding
- Mass spectrometry: MALDI-FT-ICR
- Data analysis: Receiver Operating Characteristics

Results

- Brain and liver were highlighted as potential localization of diazinon which correlates with literature.
- Some affected ions were identified.

Introduction

Biolocalization is an information of choice when studies want to better understand a xenobiotic's effect on an organism. When dealing with lipophilic xenobiotics present in small quantities, this information is often hindered, especially if ion suppression effect is taken into account. However, even a small quantity of a contaminant is enough to have a significant effect on the molecular profiles of different tissues.

In this study we show that a statistical analysis with Receiver Operating Characteristic (ROC) can compare data sets of contaminated and healthy tissues from Danio rerio in order to highlight discriminant signals. Regions of interest where the contaminant could be found are thus identified.

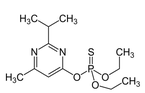
Methods

Samples

- Model: *Danio rerio*
- Type: wild type AB
- Control sample: 3
- Cont. sample: 3

Contamination

- Contaminant: diazinon
- Concentration: 5µM
- Time: 24 hours



Sectioning

- Instrument: Cryostar NX70 (Thermo)
- Embedding: Gelatin
- Thickness: 12µm

Matrix deposition

- Instrument: SunCollect II (SunChrom)
- Matrix: HCCA
- Flux: 5 ; 5 ; 10 ; 20µL/min
- Quantity: ~50 nmoles/mm²

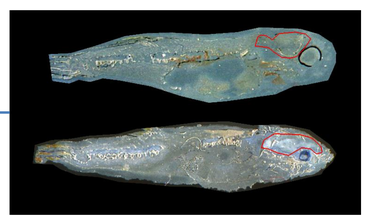
Mass spectrometry imaging

- Instrument: Solarix 9,4T (Bruker)
- Source: MALDI
- Analyzer: FT-ICR
- Mass range: 100 to 1500 Da
- Raster width: 40µm
- Resolution: 100,000 at 400*m/z*

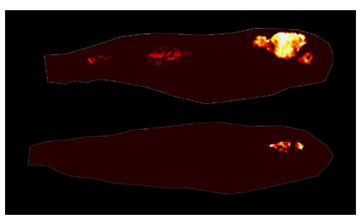
Data analysis

- Software: SCiLS Lab 2016b (SCiLS)
- Denoising: weak
- Normalization: Root Mean Square
- Statistical tools: ROC & Co-lolocalization

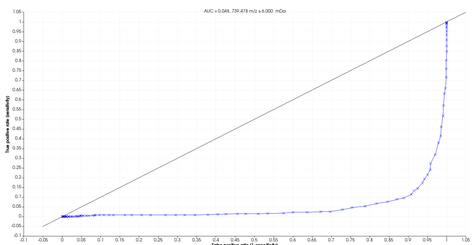
Results



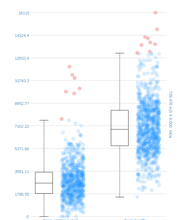
Optical image of a control fish (top) and a contaminated fish (bottom).



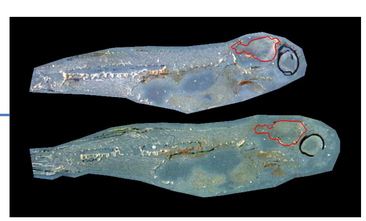
Distribution of 739.479 ± 6mDa *m/z*



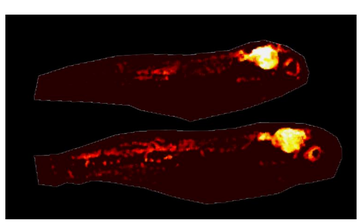
ROC curve ctrl vs cont. for ion 739.479



Intensity box plot



Optical image of two control fish.



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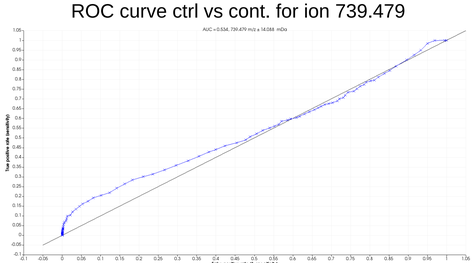
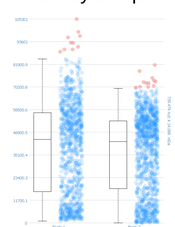


ROC curve ctrl vs ctrl for ion 739.479



Intensity box plot

Tissue	# of discriminant ions
Brain	**31**
Heart	1
Intestine	2
Kidney	0
Liver	**12**

- ROI of the analyzed tissue is drawn on the optical image
- ROC are generated for all set of tissues
- An ion considered discriminant if only the ROC between ctrl. and cont. is significant.
- Table show that the brain and the liver are affected by the presence of the contaminant

Name	Exact *m/z*	Formula	Ion
PE-Cer(d14:2(4E,6E)/22:1(13Z)(2OH))	739.4787	$C_{38}H_{73}N_2O_7PK$	$[M+K]^+$
PE-Cer(d16:2(4E,6E)/20:1(11Z)(2OH))	739.4787	$C_{38}H_{73}N_2O_7PK$	$[M+K]^+$

Result of Lipidmaps.org database identification

Conclusions

- This method highlighted the brain and the liver as potential localization for the diazinon, which is in correlation to the literature.
- Some ions marked as discriminant were also be identified, which could be interesting for the understanding of the action mechanism of the xenobiotic.

Perspectives

- Confirm the presence of diazinon in the determined regions by laser microdissection coupled with LC or GC-MS(/MS).
- Coupling of mass spectrometry imaging with ion mobility in order to distinguish the isobaric ions.