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LC-MS Applications in Metabonomics: from Toxicology to Disease Biomarkers

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Overview

Challenges in Metabonomics

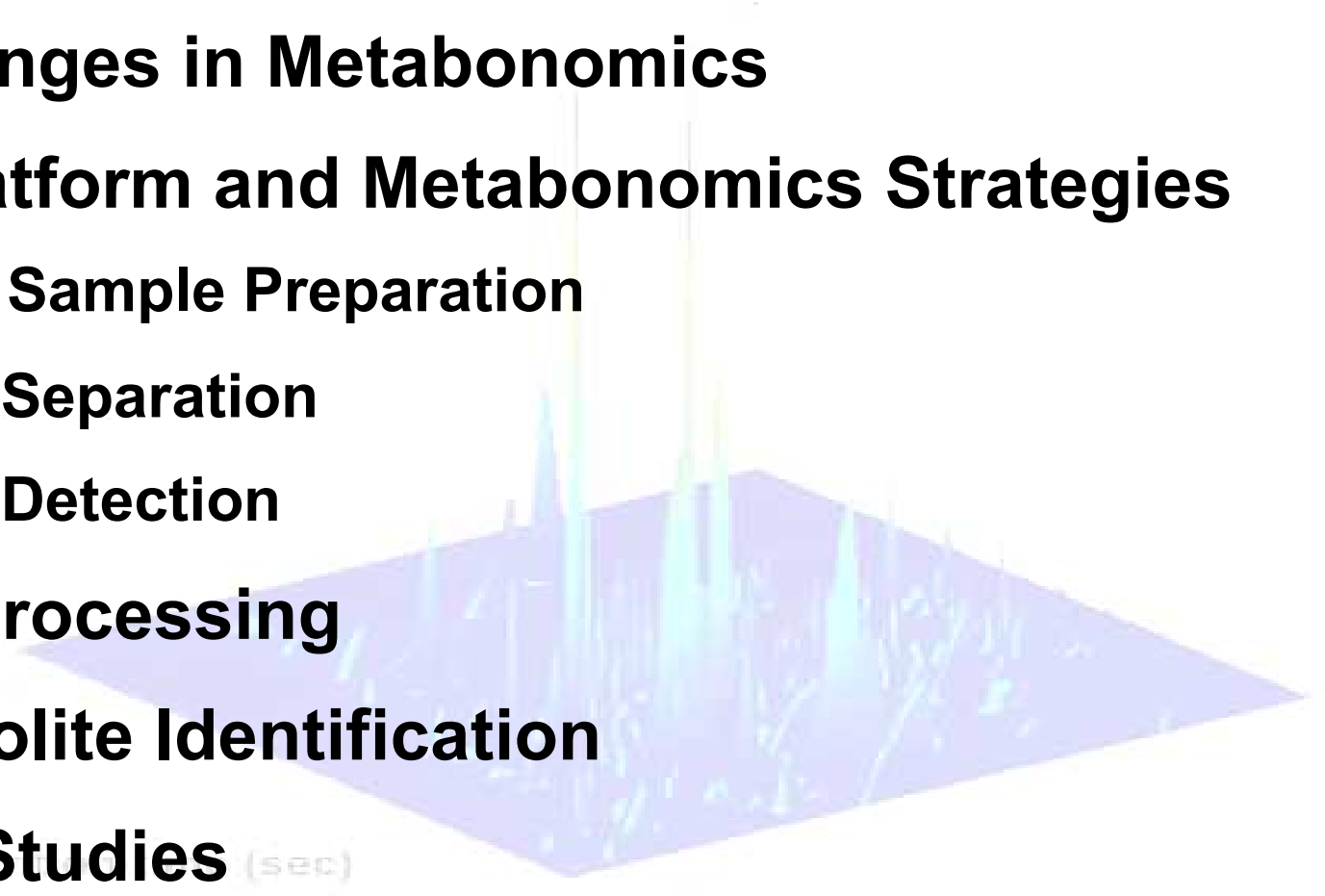
MS platform and Metabonomics Strategies

- **Sample Preparation**
- **Separation**
- **Detection**

Data Processing

Metabolite Identification

Case Studies



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Challenges in Metabonomics

Untargeted analysis

Hundreds of molecules in complex matrices

Wide concentration range of metabolites

Vast amount of data generated

Data analysis

Metabolite identification

Interpretation

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Why use MS?

- Complementary to NMR
- Reproducibility
- Sensitivity
- Dynamic range
- Sample throughput
- Structural information
- Quantitation
- Data analysis automation

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MS in Metabonomics

Cancer

- kidney & ovarian cancer, colorectal cancer, brain tumours

Toxicology

- COMET and COMET2

Disease biomarkers

- cardiovascular disease, diabetes

Nutrition

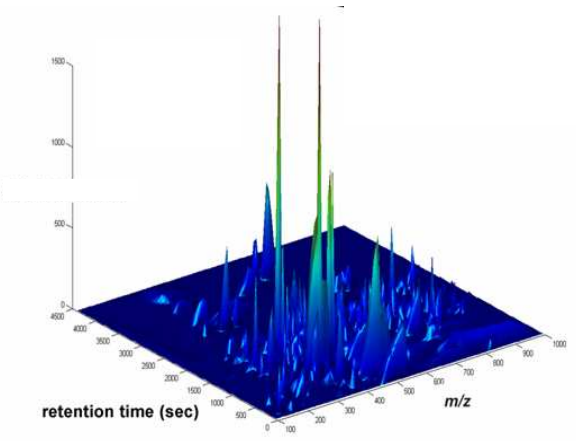
- Effects of green tea, flavonoids

Plants

- plant-host interactions, growth rate

Other organisms

- yeast, fungi



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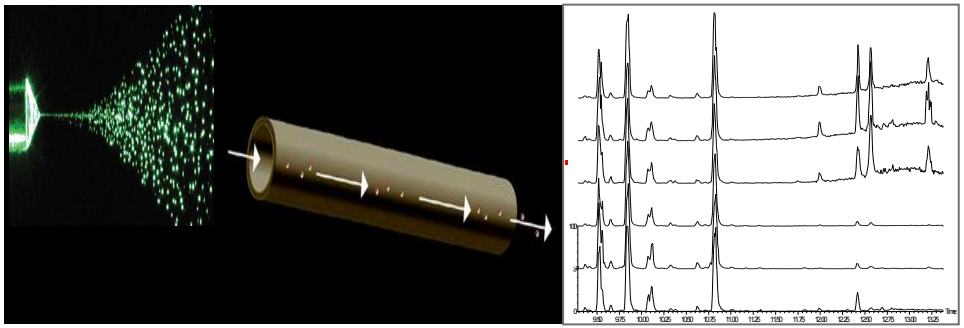
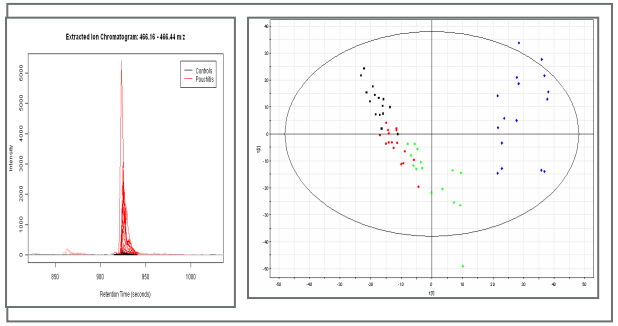
Metabonomics Platform

Sample
preparation

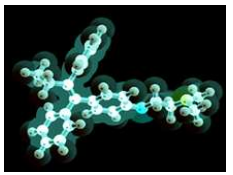


LC/GC-MS Analysis



Data Analysis

Metabolite
Identification



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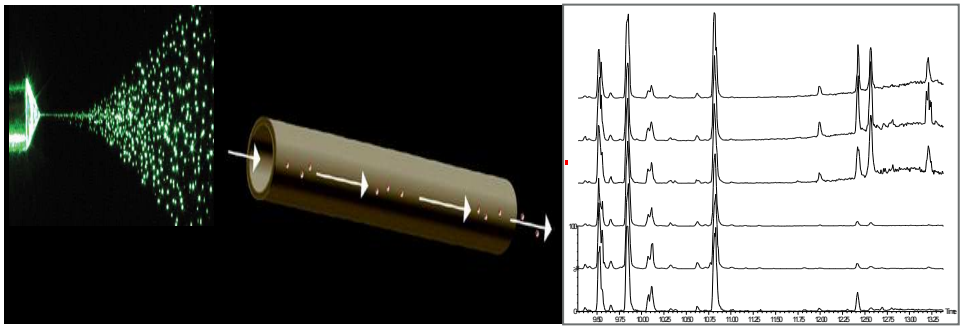
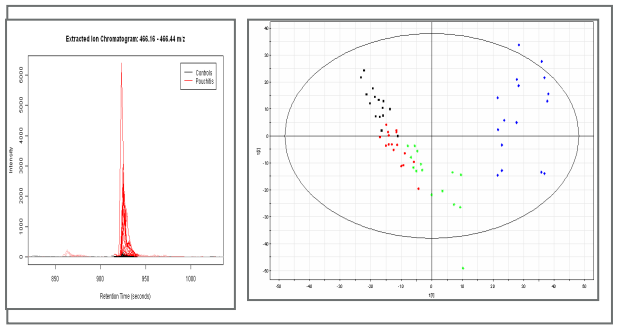
Metabonomics Platform

Sample preparation

Considerations

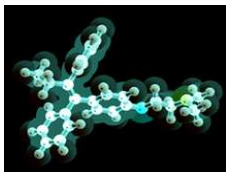


LC/GC-MS Analysis



Data Analysis

Metabolite Identification



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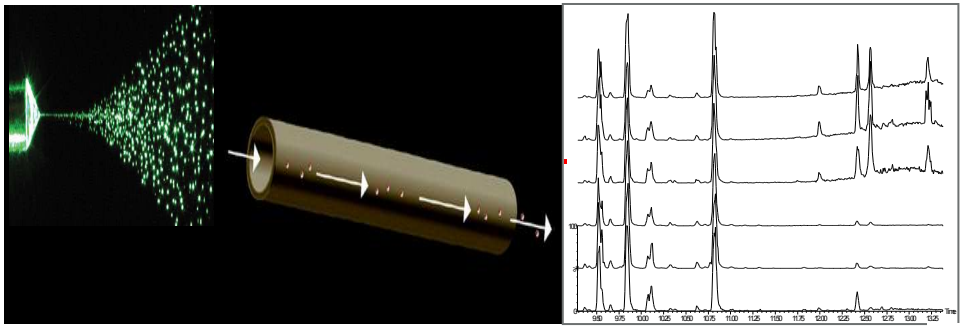
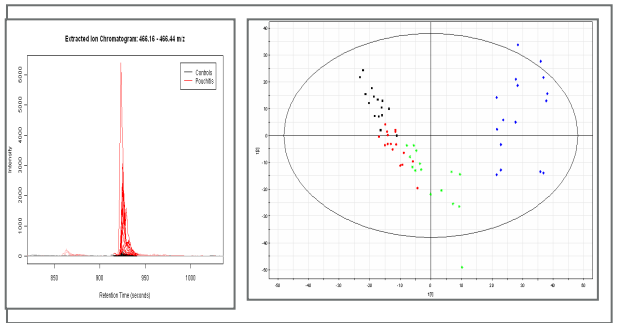
Metabonomics Platform

Sample type

Considerations



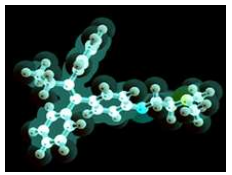


Chromatography Mass Spectrometer

Data Processing

Prior knowledge e.g. NMR data

Metabolite Identification



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Sample Preparation

Dependent on goal

• Untargeted analysis: minimal sample pre-treatment to prevent loss of metabolites

• Sample pre-concentration techniques i.e. SPE for low level metabolites

Serum/plasma

- **Methanol/acetonitrile protein precipitation**

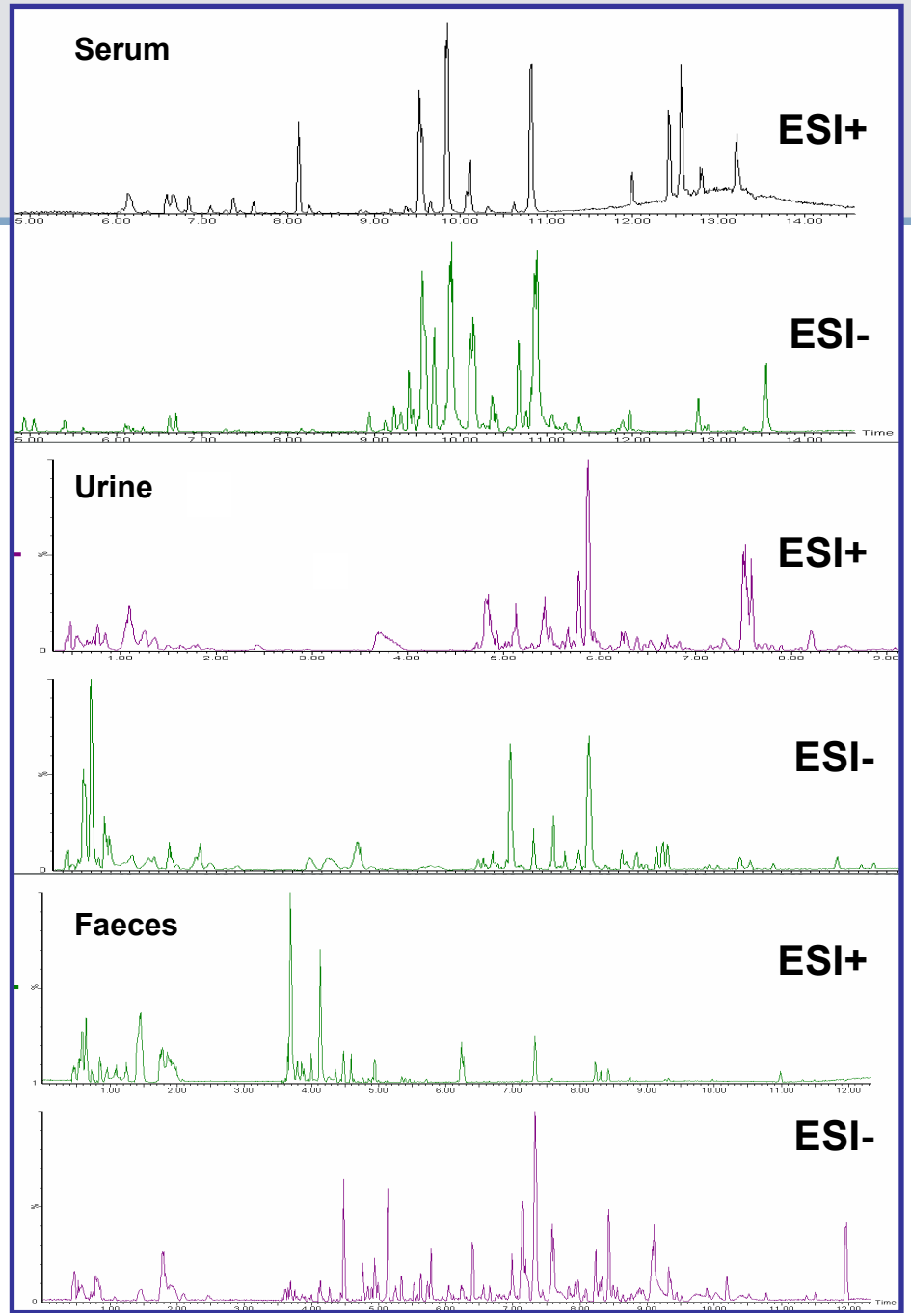
Urine

- **Centrifugation & dilution**

Faeces

- **Aqueous/organic extracts**

UPLC-MS Base Peak Intensity Chromatgrams



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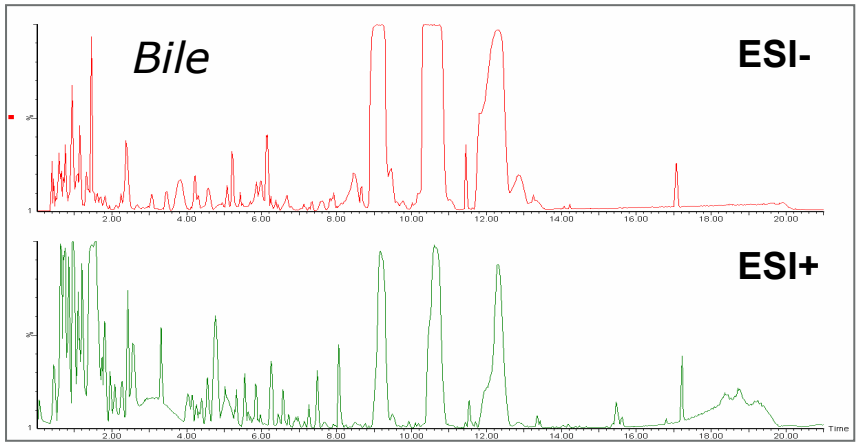
Sample Preparation

Bile

Dilution 1:4 with water

Centrifugation 13000rpm 10mins



Cerebrospinal fluid

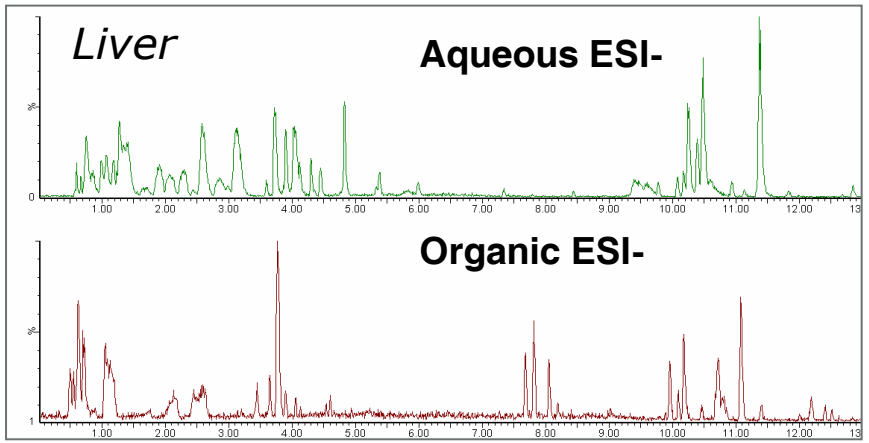
Methanol protein precipitation

(as serum/plasma)

Tissues e.g. liver

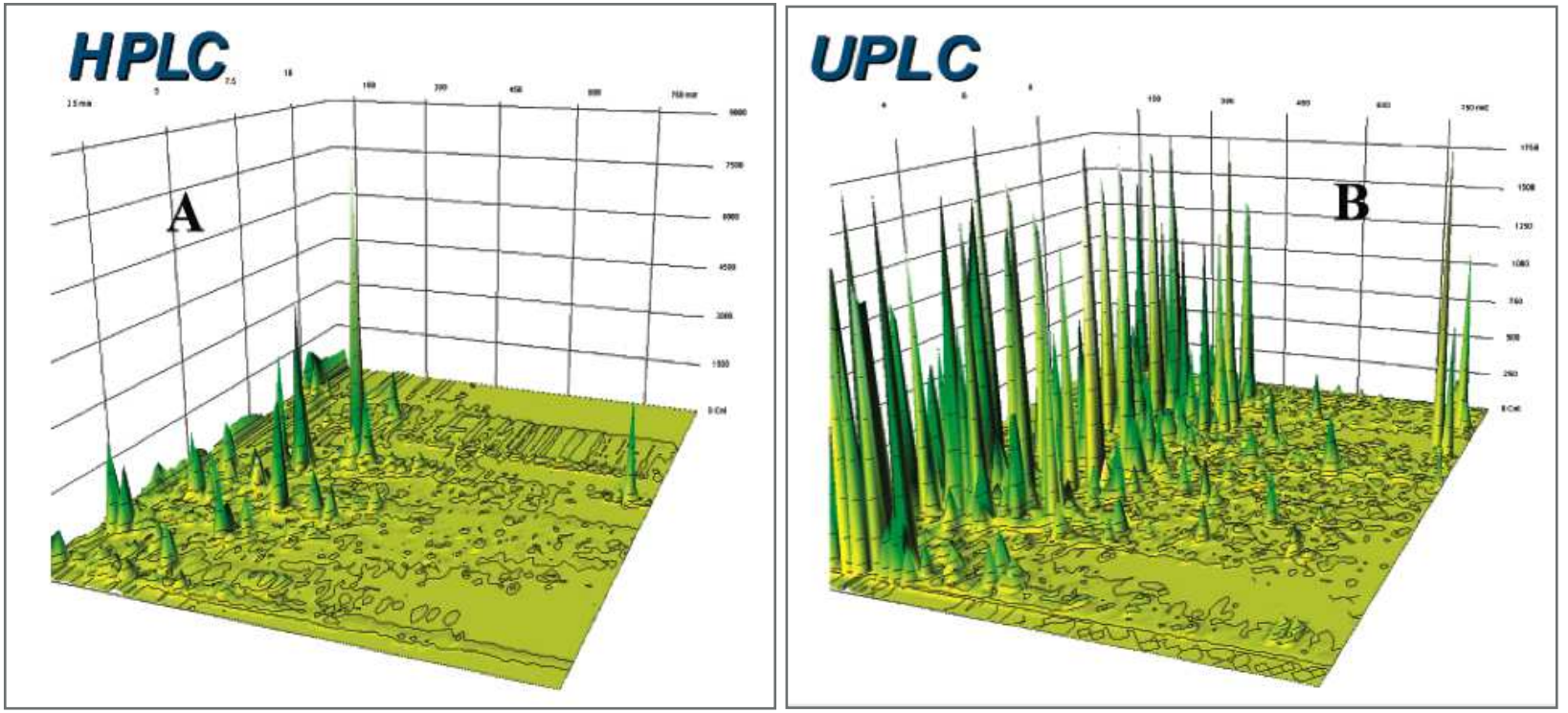
Chloroform: methanol extraction:

aqueous and organic extracts



Separation

Liquid chromatography



Wilson ID *et al.*,. J Proteome Res. 2005. 4(2):591-8.

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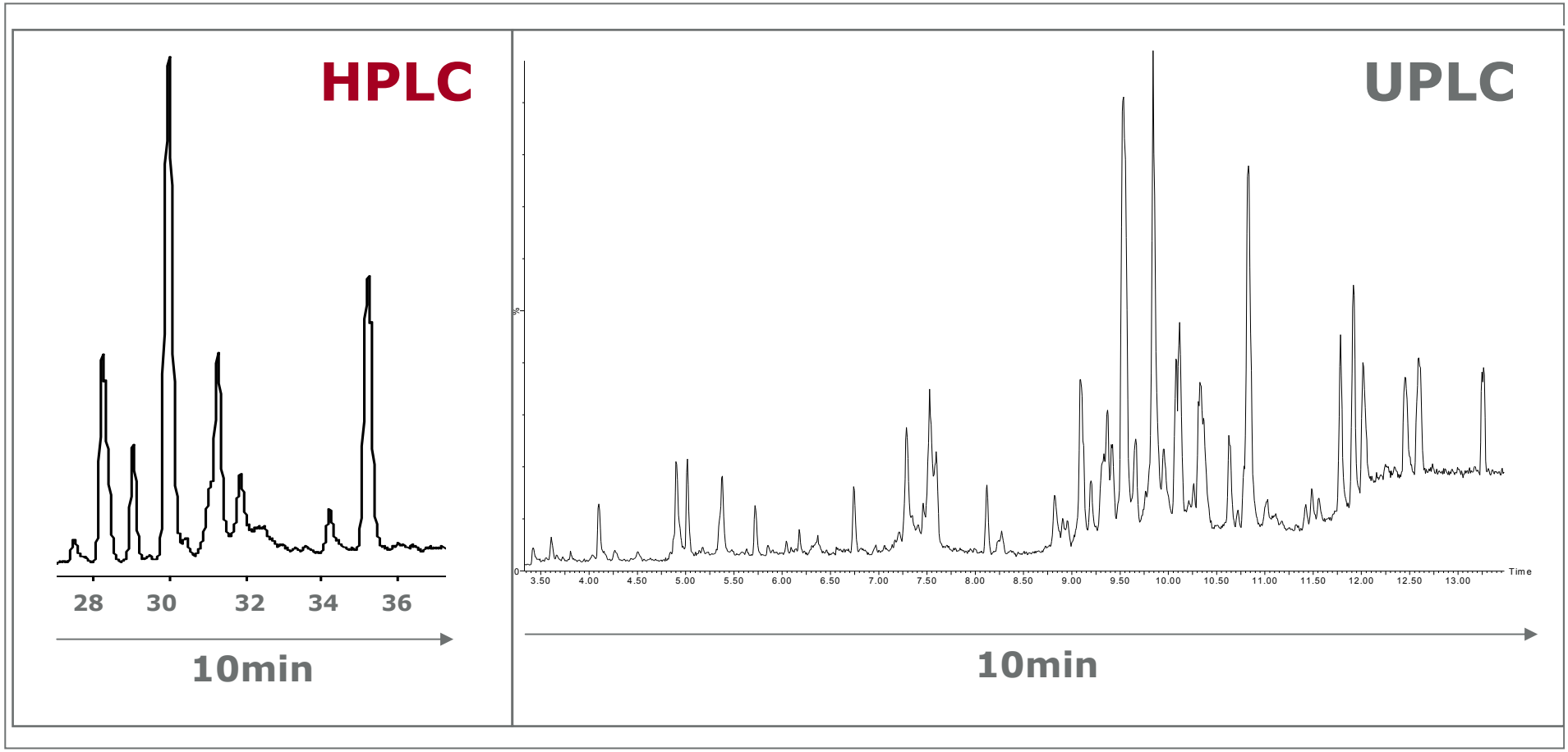
UPLC *vs* HPLC: Serum sample (methanol extract)

1.7um particle size columns can withstand higher backpressures and flow rates



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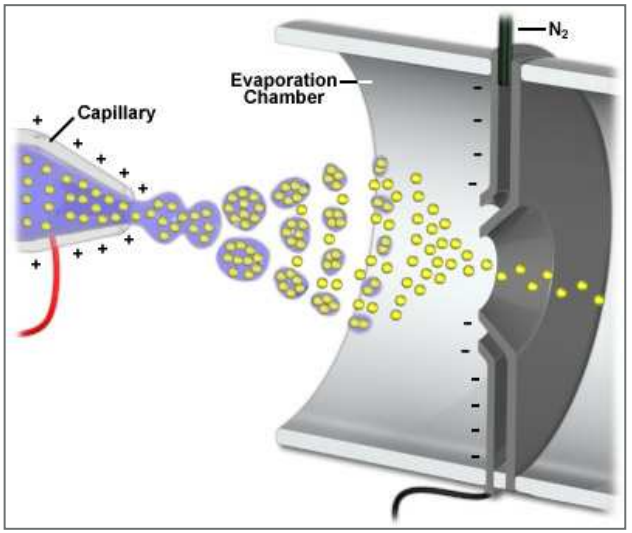
MS: Ionisation and Detection

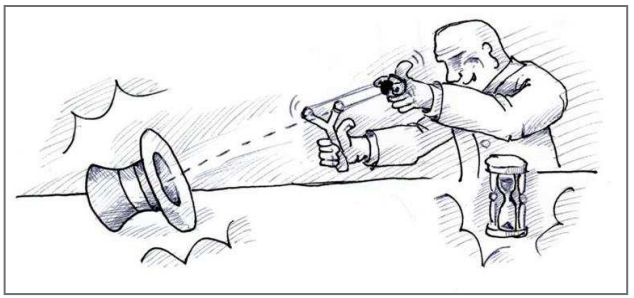
Electrospray ionisation (ESI)

- Can be interfaced to liquid chromatography
- Readily amenable to MS analysis
- No matrix necessary



Time of flight (ToF)

- good mass accuracy



Quadrupole time of flight (Q-ToF)

- good mass accuracy, MS/MS

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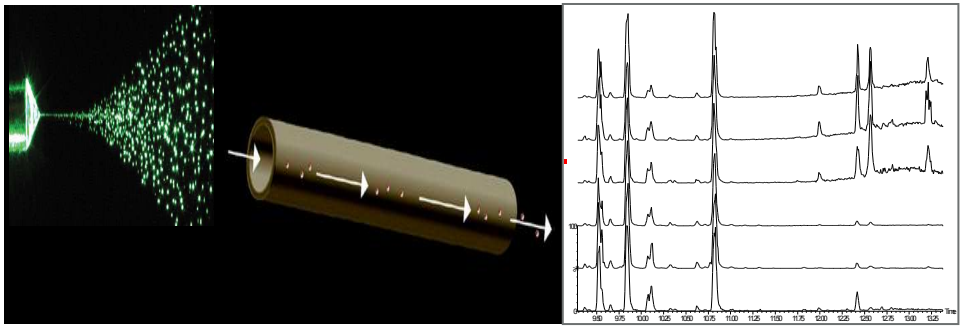
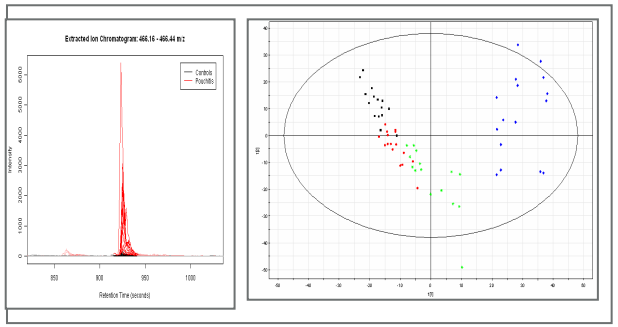
Metabonomics Platform

Sample type

Considerations



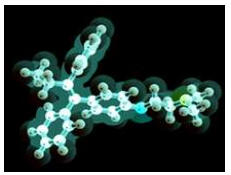


Chromatography Mass Spectrometer

Data Processing

Prior knowledge e.g. NMR data

Metabolite Identification



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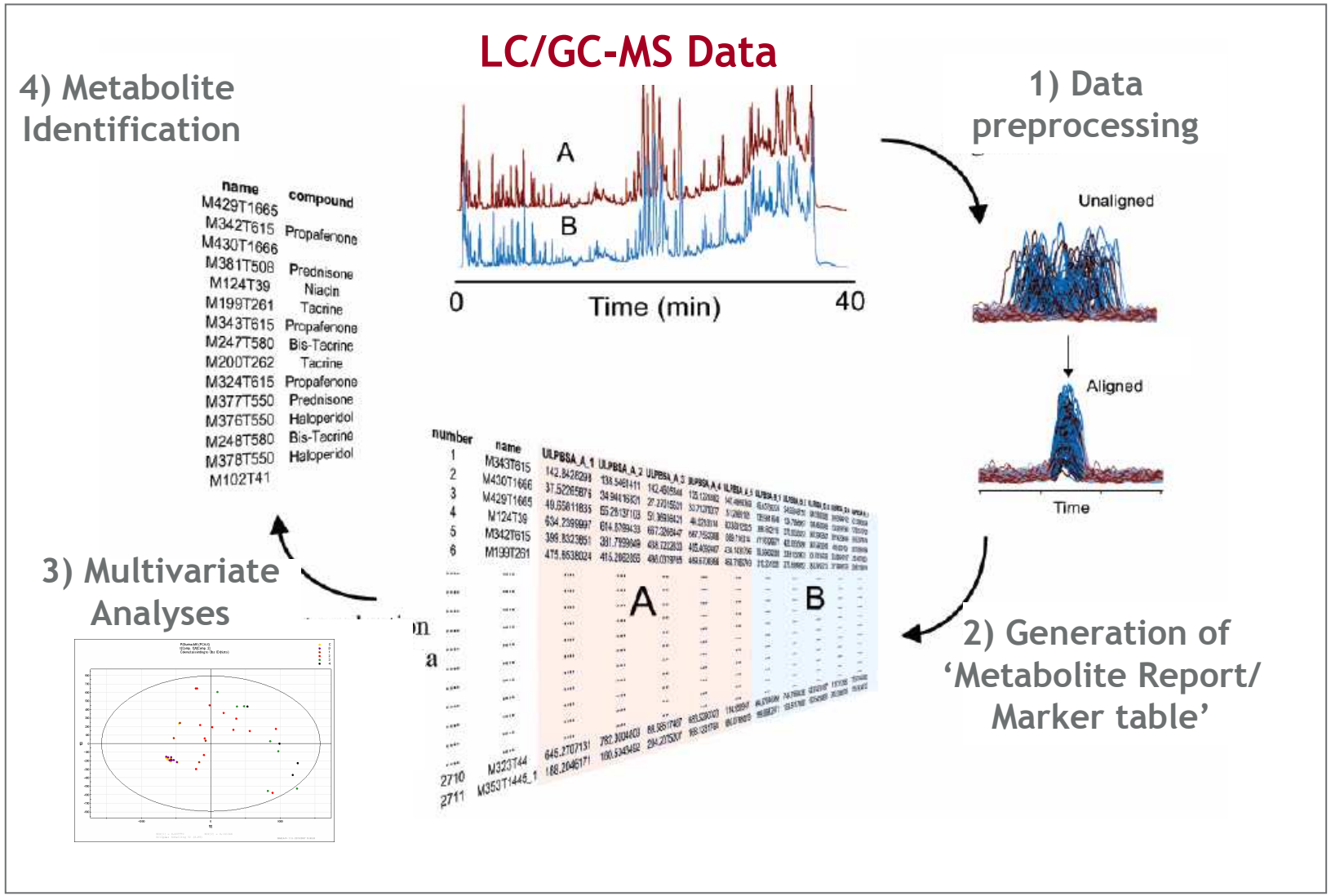
Data Processing Workflow



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Data Preprocessing

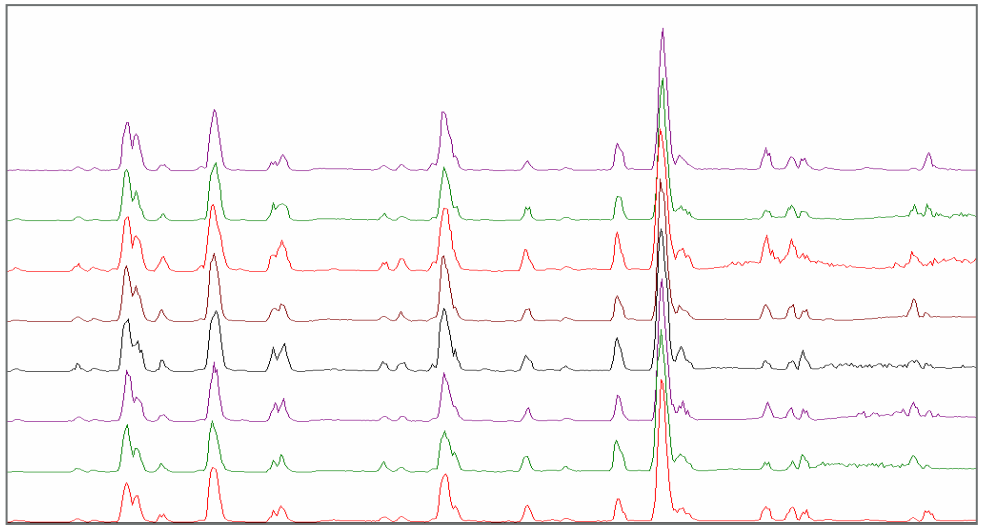
Objectives

Peak picking and alignment

'Matching' peaks across samples

Determination of differences between samples

Normalisation



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Data Preprocessing Challenges

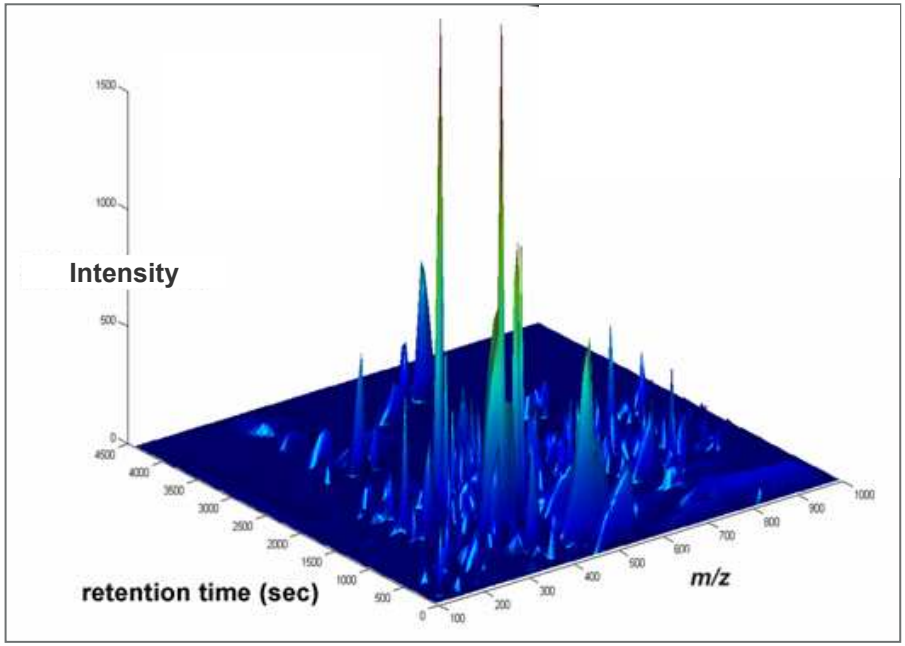
1) Dataset Complexity

Thousands of peaks

Isotopes, adducts, dimers, fragments

Noise

Positive & negative mode data



2) Peak Shifts

Temperature

Mobile phase changes

Stationary phase changes

Sample composition

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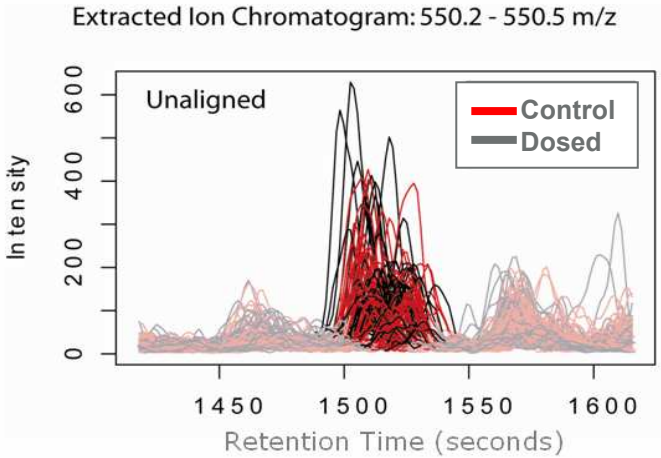
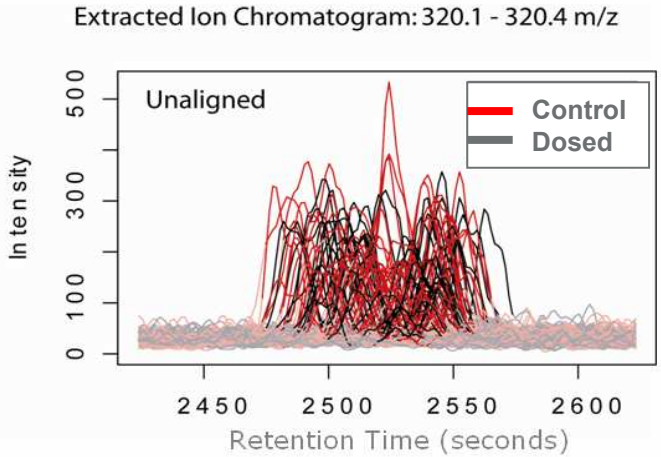
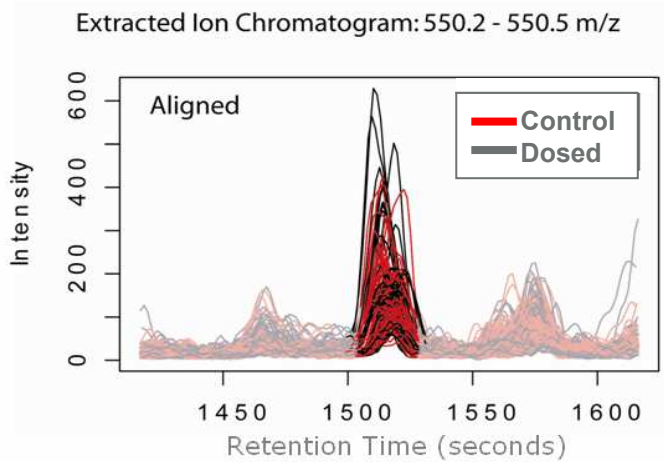
Importance of Alignment

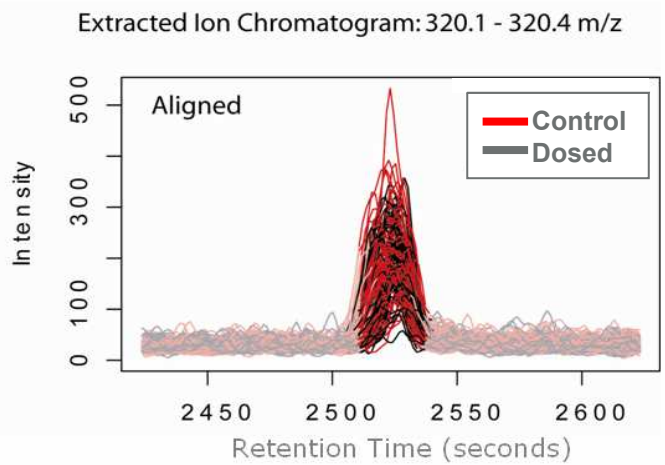
Unaligned





Aligned





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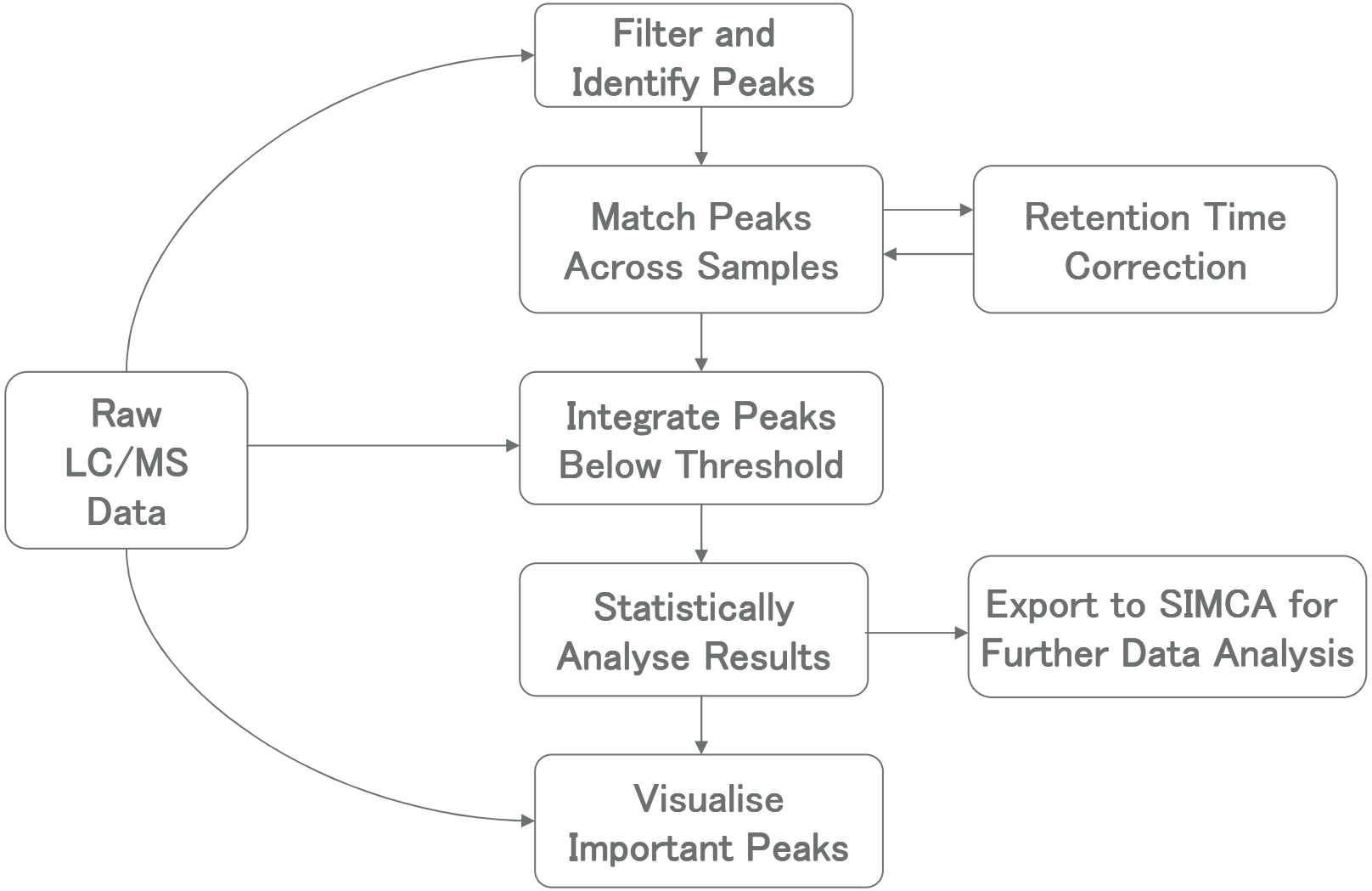
General Preprocessing Approach



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Software Options

Commercial Software

Markerlynx — Waters

MassHunter — Agilent Technologies

MarkerView — MDS SCIEX, Science advancing health

MassFrontier — Thermo SCIENTIFIC

Metabolic Profiler — BRUKER

Platform Independent Freeware

MZMINE

Katajamaa M, Oresic M. BMC Bioinformatics. 2005.18;6:179.
Katajamaa M, Miettinen J, Oresic M. Bioinformatics. 2006. 22(5):634-6.

MSFACTS

Duran AL, et al.,. Bioinformatics. 2003. 19(17):2283-93.

METALIGN

De Vos RC, et al.,. Nat Protoc. 2007;2(4):778-91.
Tikunov Y, et al.,. Plant Physiol. 2005 Nov;139(3):1125-37.

MET-IDEA

Broeckling CD, et al.,. Anal Chem. 2006 Jul 1;78(13):4334-41.

MATHDAMP

Baran R et al.,. BMC Bioinformatics. 2006. 7:530

XCMS

Smith CA, et al.,. Anal Chem. 2006. 78(3):779-87.

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Metabolite Identification

Query databases

Isolate metabolite of interest (prep LC)

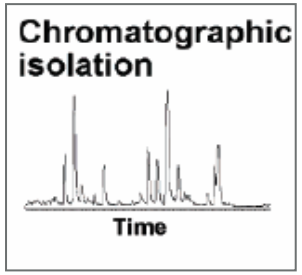
Obtain high accuracy mass data (FTMS)

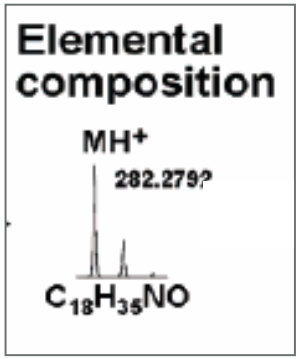
Fragmentation data (MS^E, MS/MS)

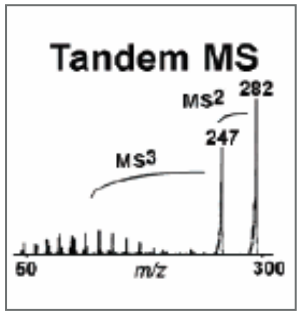
Other spectroscopic techniques for further characterisation (NMR)

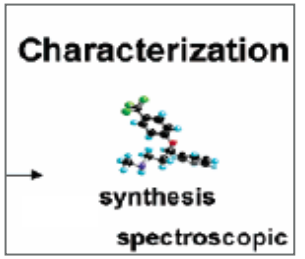
Purchase/synthesise standard

- **Compare retention time**
- **Accurate mass**
- **Fragmentation**









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Metabolite Identification

LipidBank

Suite of databases and associated software

ChemSpider™ Building a Structure Centric Community for Chemists

18.5 million unique chemical compounds

METLIN

> 23,000 metabolites

Must purchase a license

MDL® Metabolite Database

http://www.mdl.com//products/predictive/metabolite/

KEGG Kyoto Encyclopedia of Genes and Genomes

hmp human metabolome project

>6500 metabolites

Metabolome.jp

Plant metabolites and lipids

Tomato Metabolite Database

Tumor metabolome

Genome Proteome Metabolome®

Metabolic Database

GMD@CSB.DB
- The Golm Metabolome Database -
Hosted at Max Planck Institute of Molecular Plant Physiology

Custom MS libraries & metabolite profiling experiments

LIPID MAPS

BioCyc Database Collection

Includes MetaCyc

NIST National Institute of Standards and Technology

NIST: contains over 100,000 compounds (http://www.nist.gov/srd/nist1a.htm)

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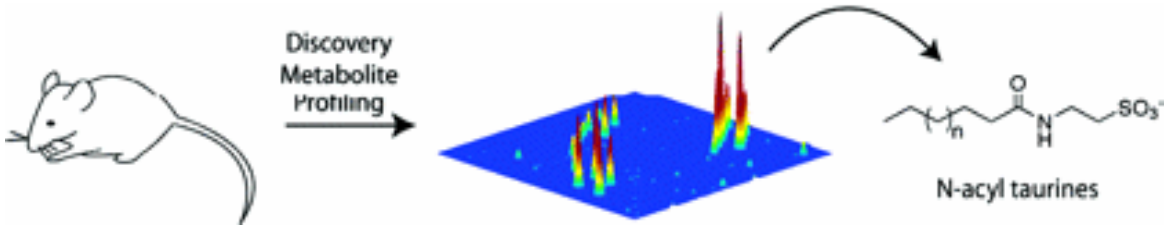
Identification of Novel Brain Lipids



Identification of enzyme substrates by untargeted LC-MS analysis of WT and KO mouse brain

Brain lipids regulated by FAAH *in vivo:*

- **known signalling molecules - anandamide**
- **novel family of taurine-conjugated fatty acids**

14332 *Biochemistry* **2004,** *43,* 14332−14339

Assignment of Endogenous Substrates to Enzymes by Global Metabolite Profiling†

Alan Saghatelian, Sunia A. Trauger, Elizabeth J. Want, Edward G. Hawkins, Gary Siuzdak, and Benjamin F. Cravatt*

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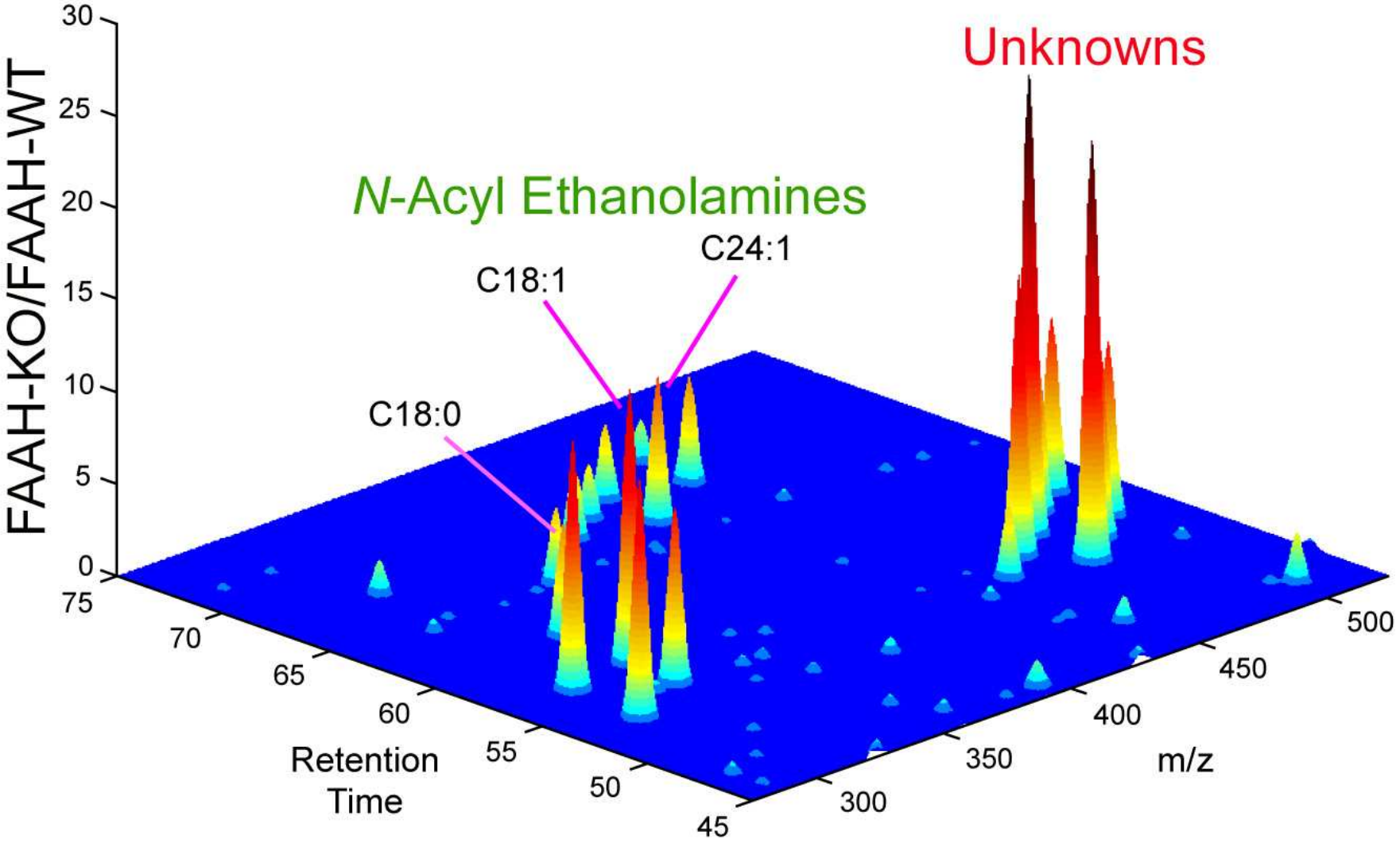
Unknown Brain Lipids

Regulated by Fatty Acid Amide Hydrolase (FAAH)



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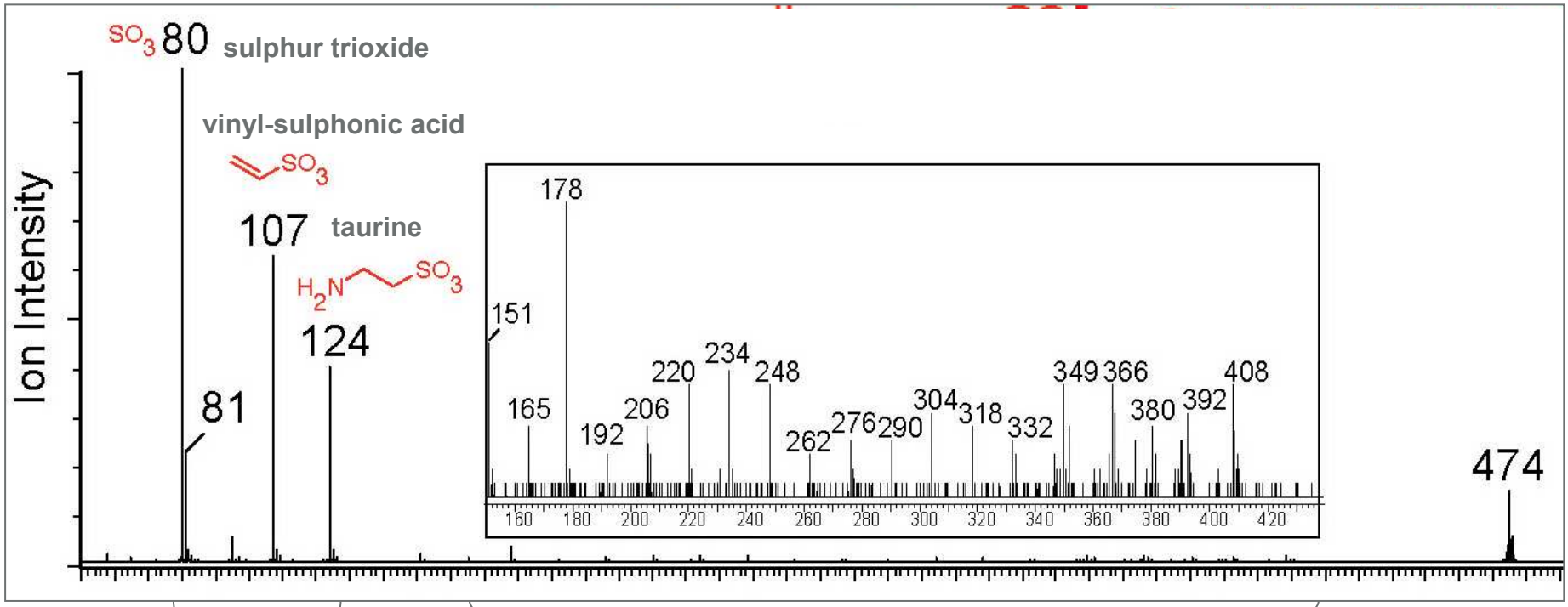
MS/MS: Structural Information

Highly related fragmentation patterns for the unknown metabolites



Indicative of taurine

serial loss of 14 mass units indicative of a lipid alkyl chain

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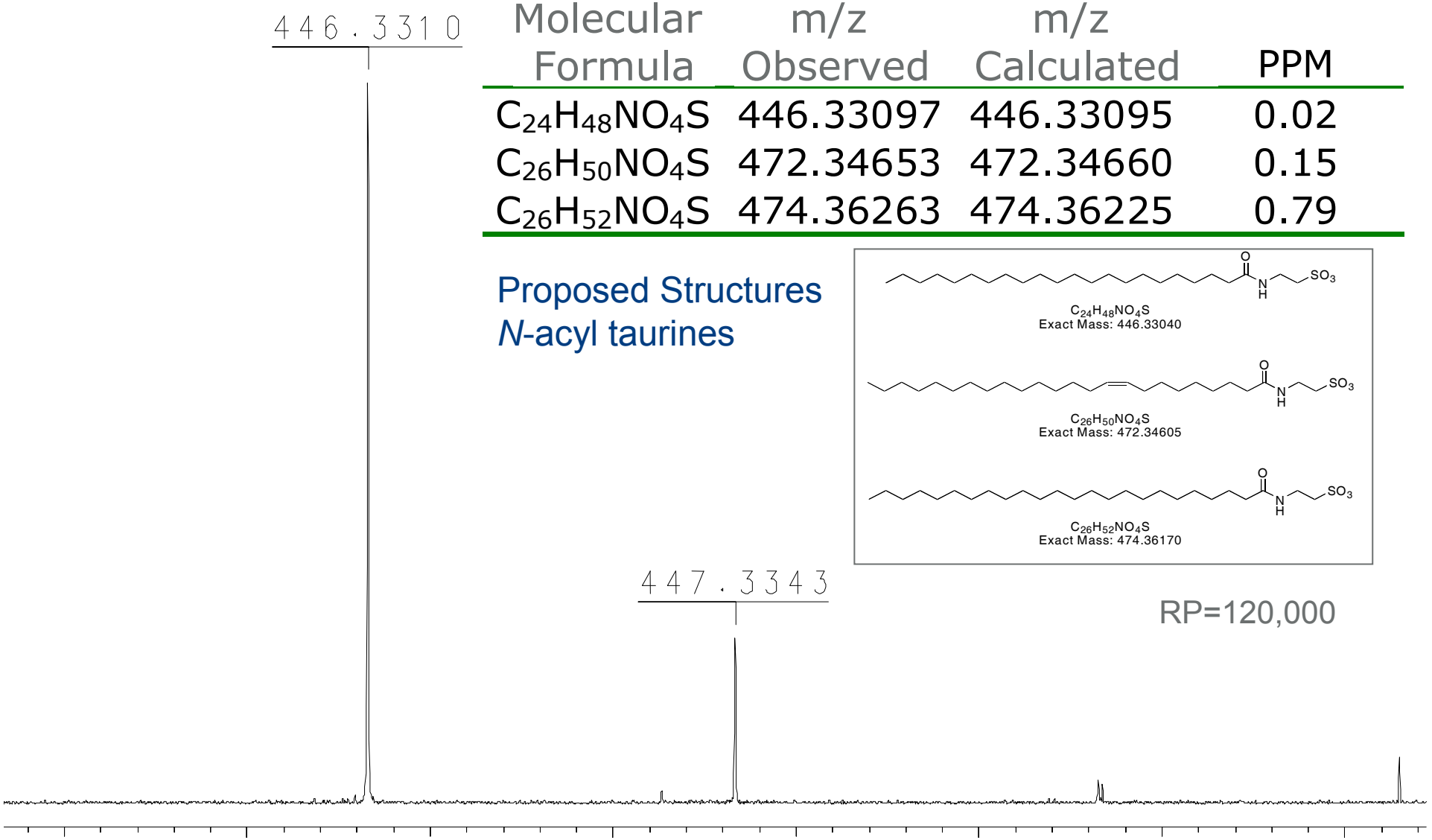
FTMS: Accurate Mass



Molecular Formula	m/z Observed	m/z Calculated	PPM
$C_{24}H_{48}NO_4S$	446.33097	446.33095	0.02
$C_{26}H_{50}NO_4S$	472.34653	472.34660	0.15
$C_{26}H_{52}NO_4S$	474.36263	474.36225	0.79

Proposed Structures
N-acyl taurines

RP=120,000

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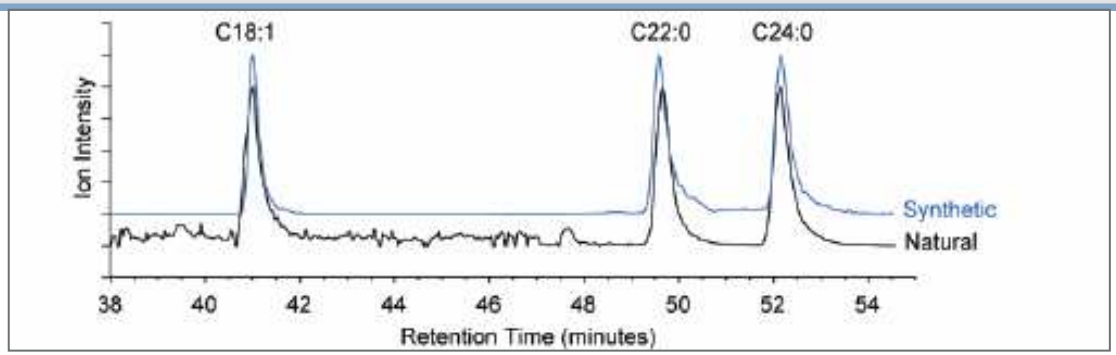
Confirmation of Identification

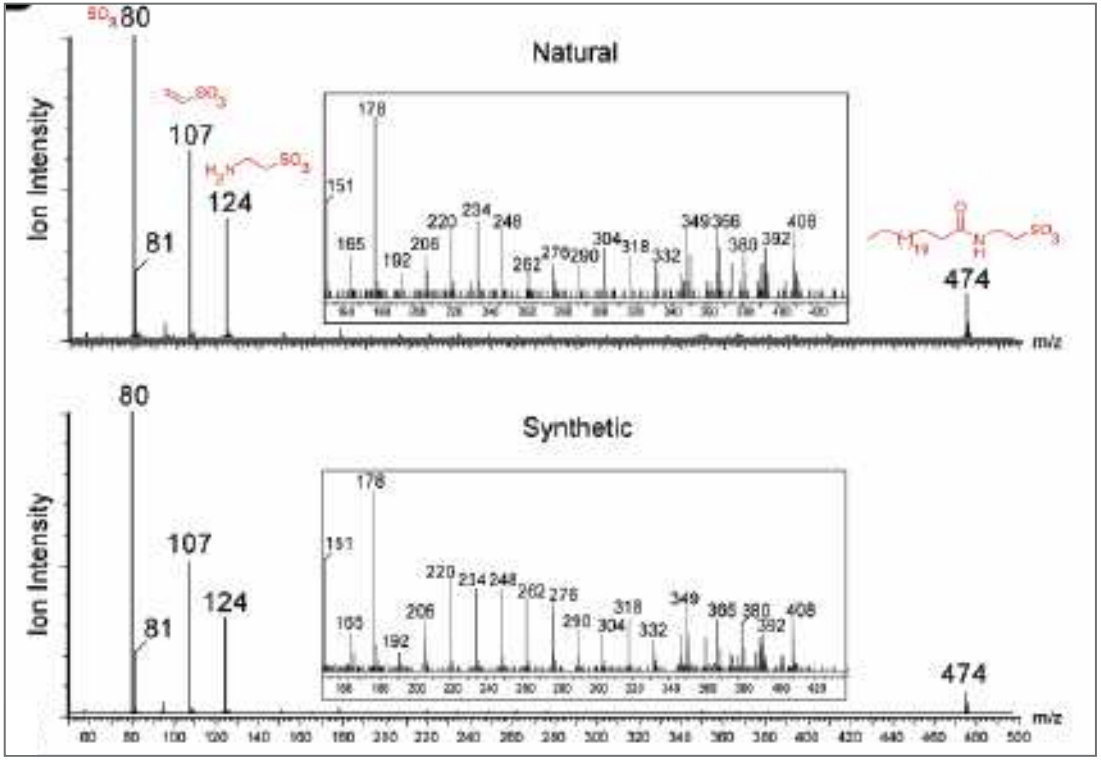
LC-MS

Co-migration of natural and synthetic NATs



MS/MS

Spectrum of endogenous metabolite matched the C24:0 NAT standard



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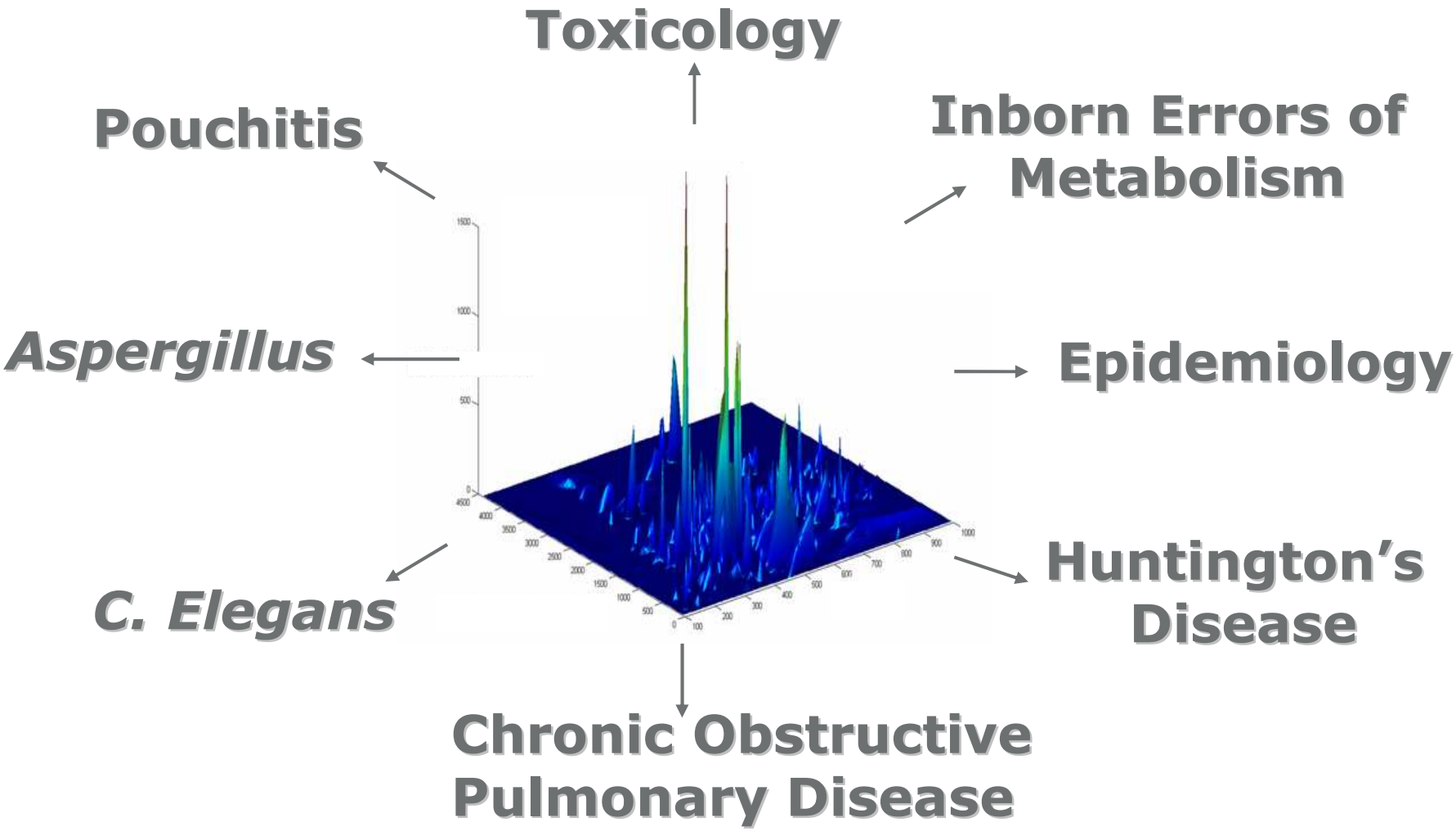
MS Studies in Biomolecular Medicine



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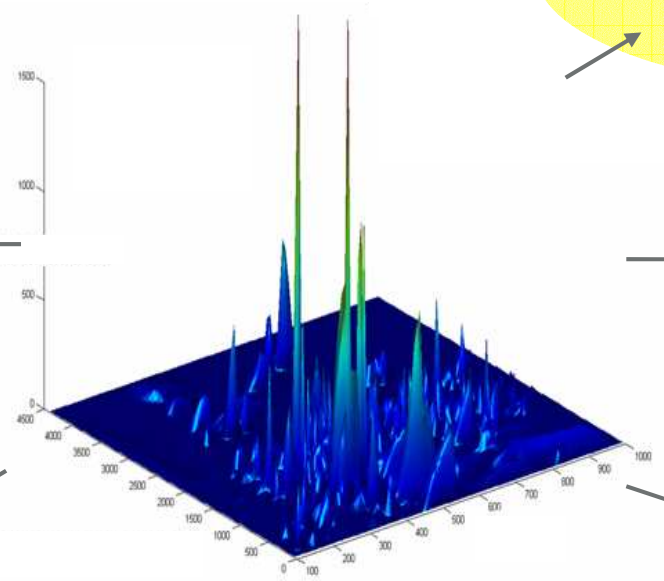
MS Studies in Biomolecular Medicine



Toxicology

Pouchitis

Inborn Errors of Metabolism

Aspergillus

Epidemiology

C. Elegans

Huntington's Disease

Chronic Obstructive Pulmonary Disease

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1) Toxicology: COMET 2 Project

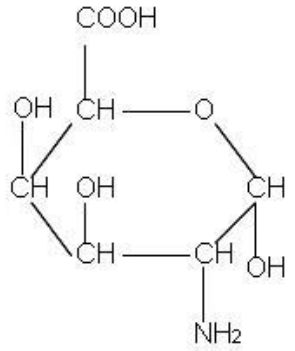
Pfizer Global R&D **Bristol-Myers Squibb**

Servier **Sanofi-Aventis** **Waters**

NMR and UPLC-MS Analyses

Galactosamine (galN)

Model hepatotoxin



Bromoethanamine (BEA)

Model nephrotoxin

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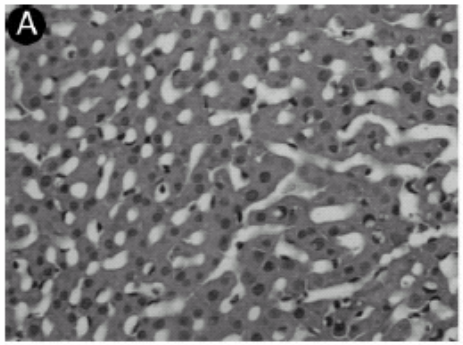
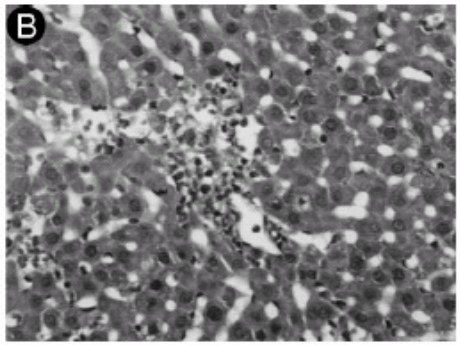
GalN-induced hepatotoxicity

- Selective hepatotoxin:produces dose-dependent, reversible liver damage
- Morphologically and biochemically similar to human hepatitis
- Severity of the response to galN is often quite variable
- Mechanism not yet fully resolved:
  - depletion of uridine nucleotide levels which inhibits RNA and protein synthesis (uridine or precursors protect)
  - alters gut permeability and increases bacterial translocation leading to endotoxemia (co-administration of LPS increases toxic response)
- Glycine protects against liver damage



Control



24 hr after GalN

R.F. Stachlewitz, et al. (1999) Hepatology 29:737

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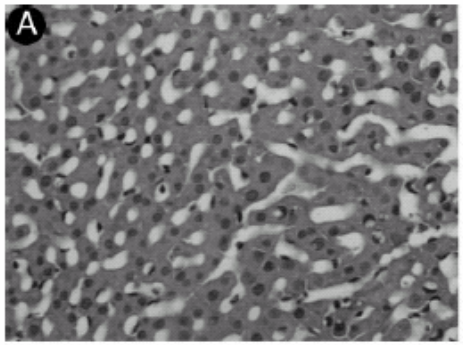
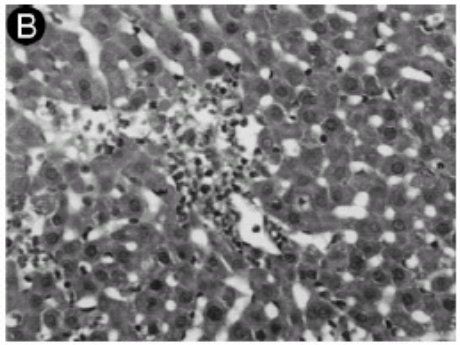
GalN-induced hepatotoxicity

- Selective hepatotoxin:produces dose-dependent, reversible liver damage
- Morphologically and biochemically similar to human hepatitis
- Severity of the response to galN is often quite variable
- Mechanism not yet fully resolved:
  - depletion of uridine nucleotide levels which inhibits RNA and protein synthesis (uridine or precursors protect)
  - alters gut permeability and increases bacterial translocation leading to endotoxemia (co-administration of LPS increases toxic response)
- Glycine protects against liver damage



Control



24 hr after GalN

R.F. Stachlewitz, et al. (1999) Hepatology 29:737

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UPLC-MS Strategy

Serum
methanol extraction

UPLC: HSS column (2.1 x 100mm, 1.7µm) gradient:
water + ACN 0.1% FA

MS: Q-ToF Premier, Negative mode
ESI
50-1000 *m/z*, MS(E)

<u>Data analysis</u>
Peak picking and alignment
generation of intensity plots
generation of marker table
SIMCA-P – PCA

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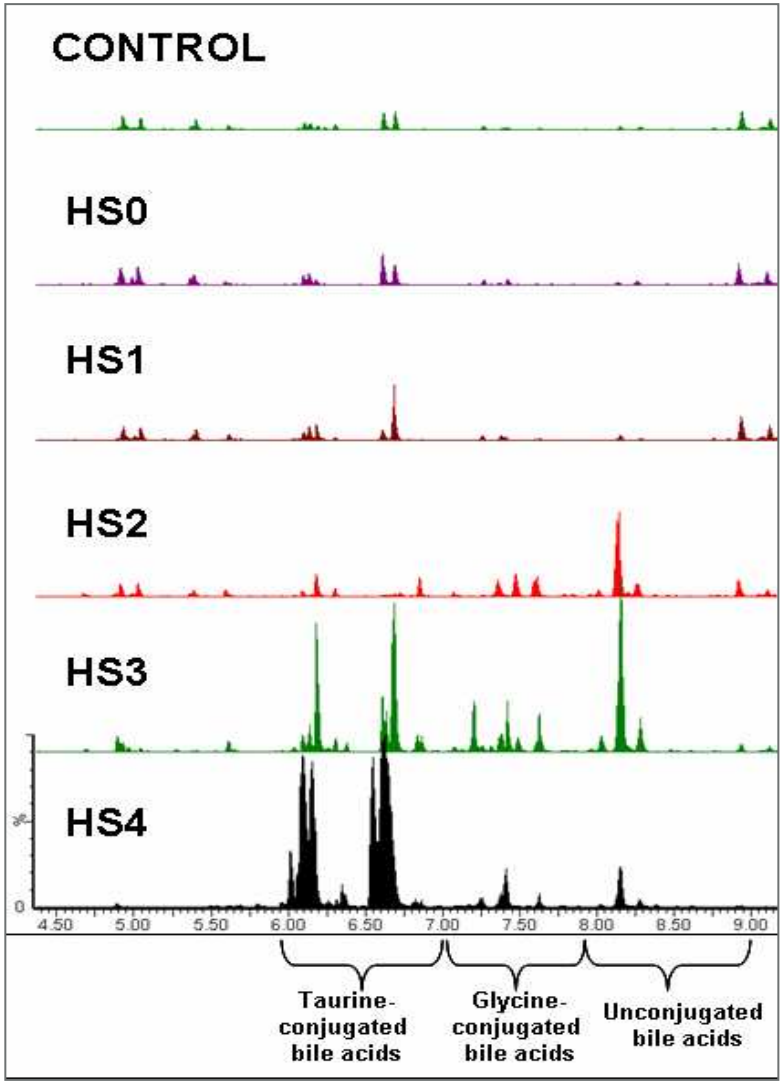
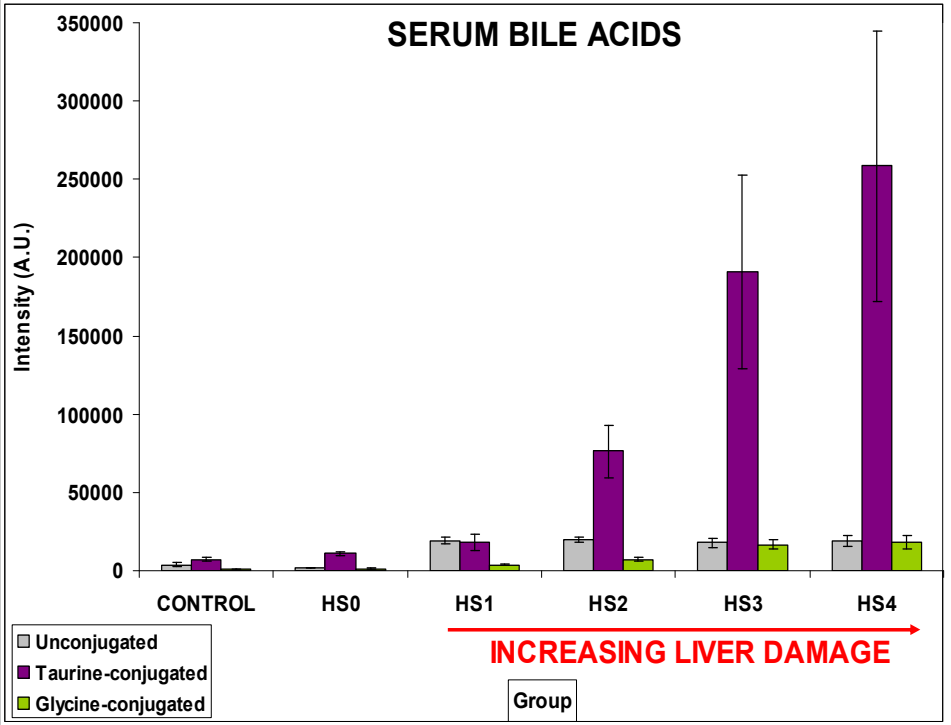
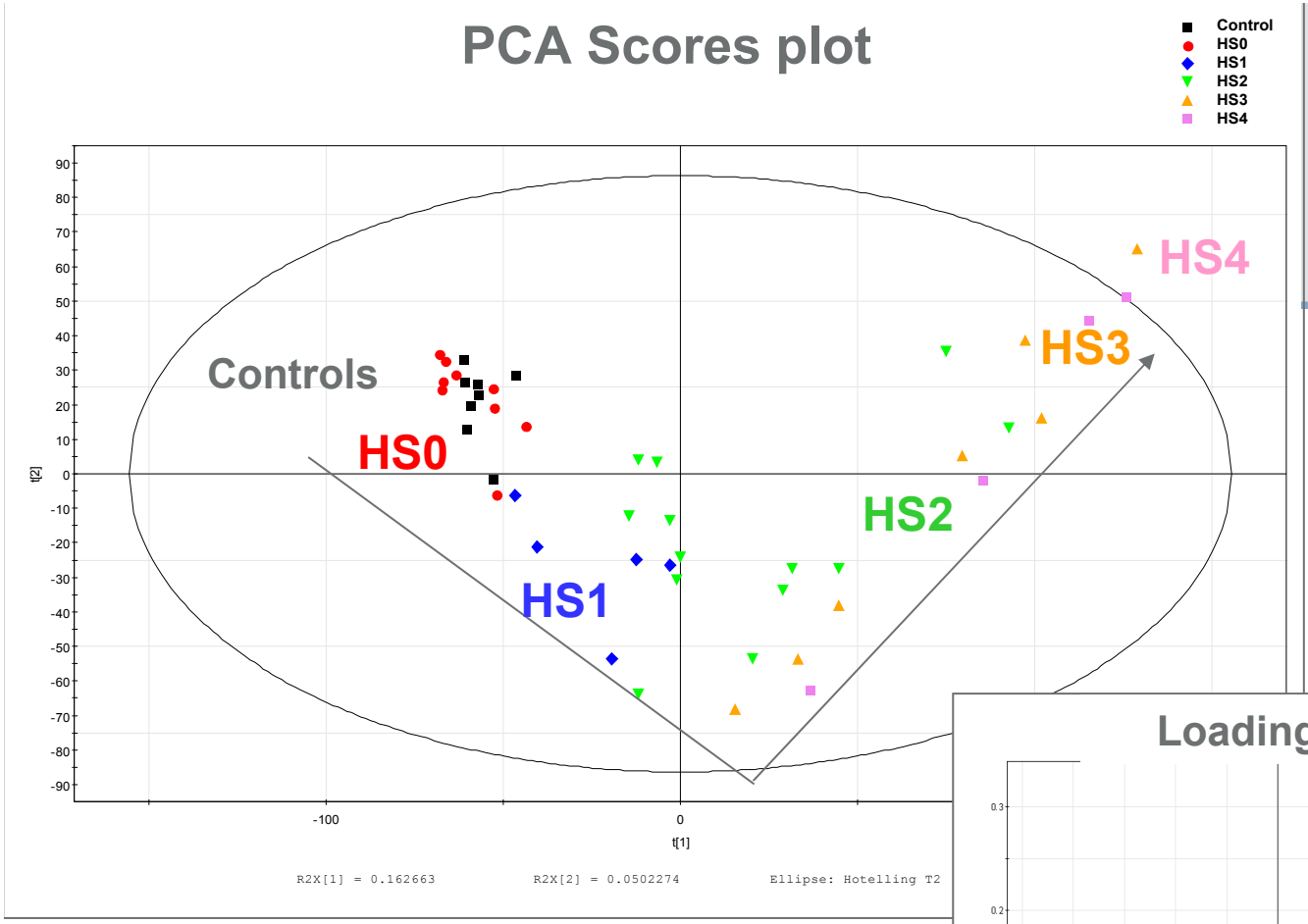
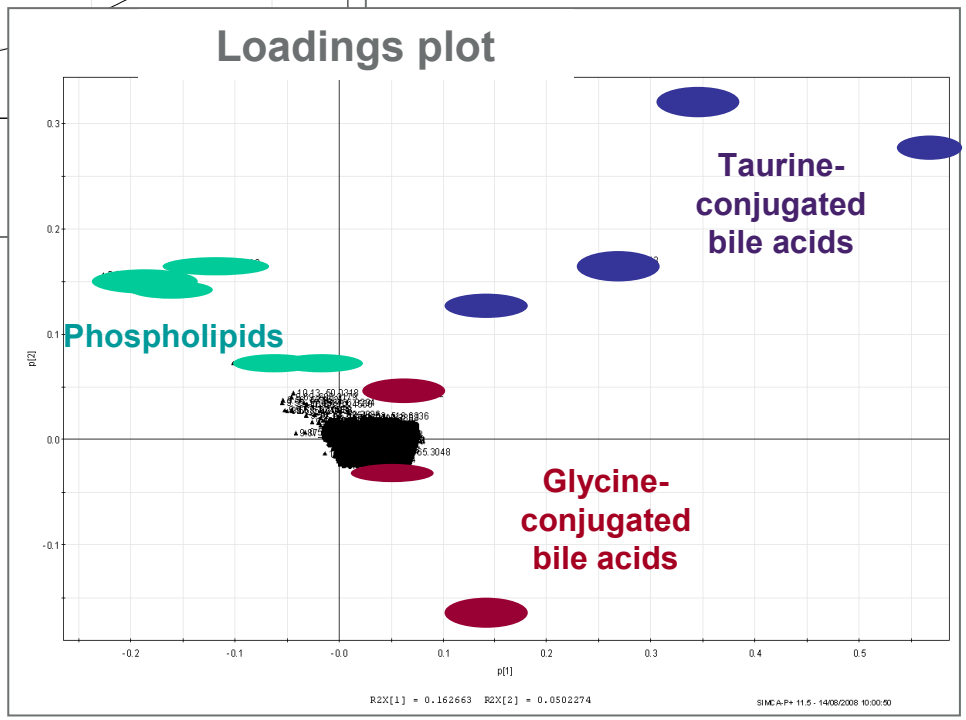
Intra-animal Variability






PCA Scores plot
Control
HS0
HS1
HS2
HS3
HS4
Controls
HS0
HS1
HS2
HS3
HS4
t[1]
t[2]
R2X[1] = 0.162663
R2X[2] = 0.0502274
Ellipse: Hotelling T2


Loadings plot
Taurine-conjugated bile acids
Phospholipids
Glycine-conjugated bile acids
p[1]
p[2]
R2X[1] = 0.162663
R2X[2] = 0.0502274

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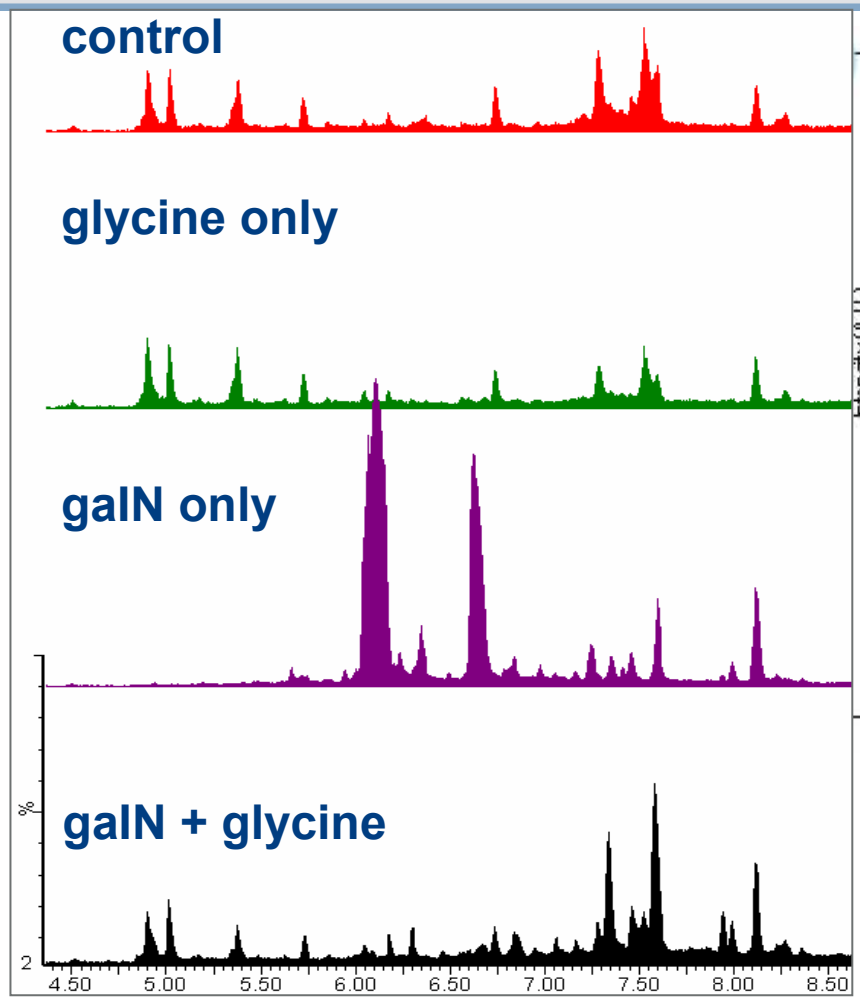
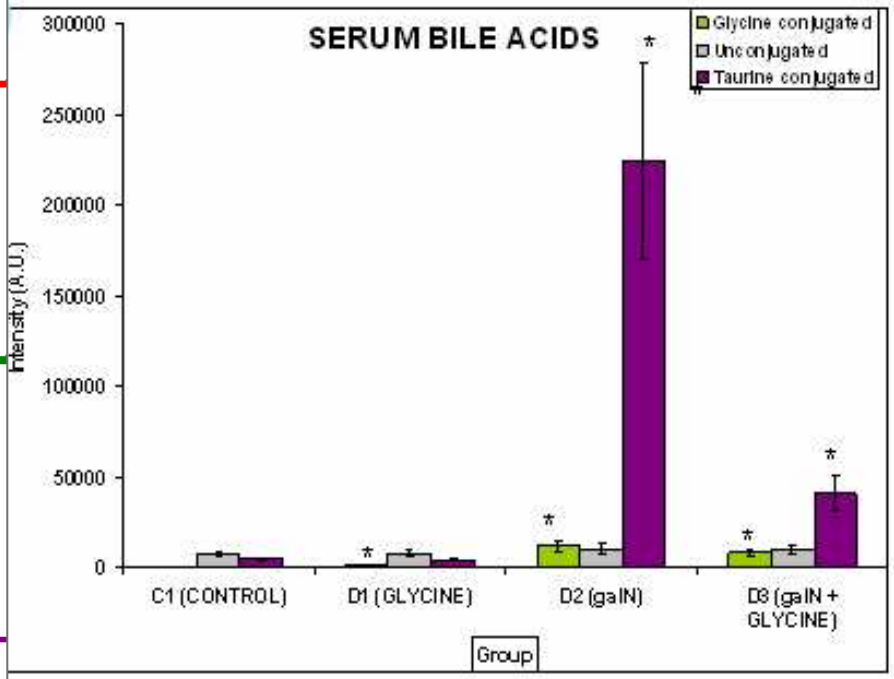
Protective Effect of Glycine





RT (mins)

Taurine-conjugated bile acids

Glycine-conjugated bile acids

Unconjugated bile acids

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2) Organic Acidurias

AIM: to evaluate the applicability of UPLC-MS for the identification of organic acidurias

Urine samples were screened from patients with five different organic acidurias

Urine
Diluted and Centrifuged

↓

UPLC: C18 column (2.1 x 100mm, 1.7µm)
gradient: water + ACN 0.1% FA

↓

MS: Q-ToF Premier, Positive and negative mode ESI
50-1000 *m/z*, MS(E)

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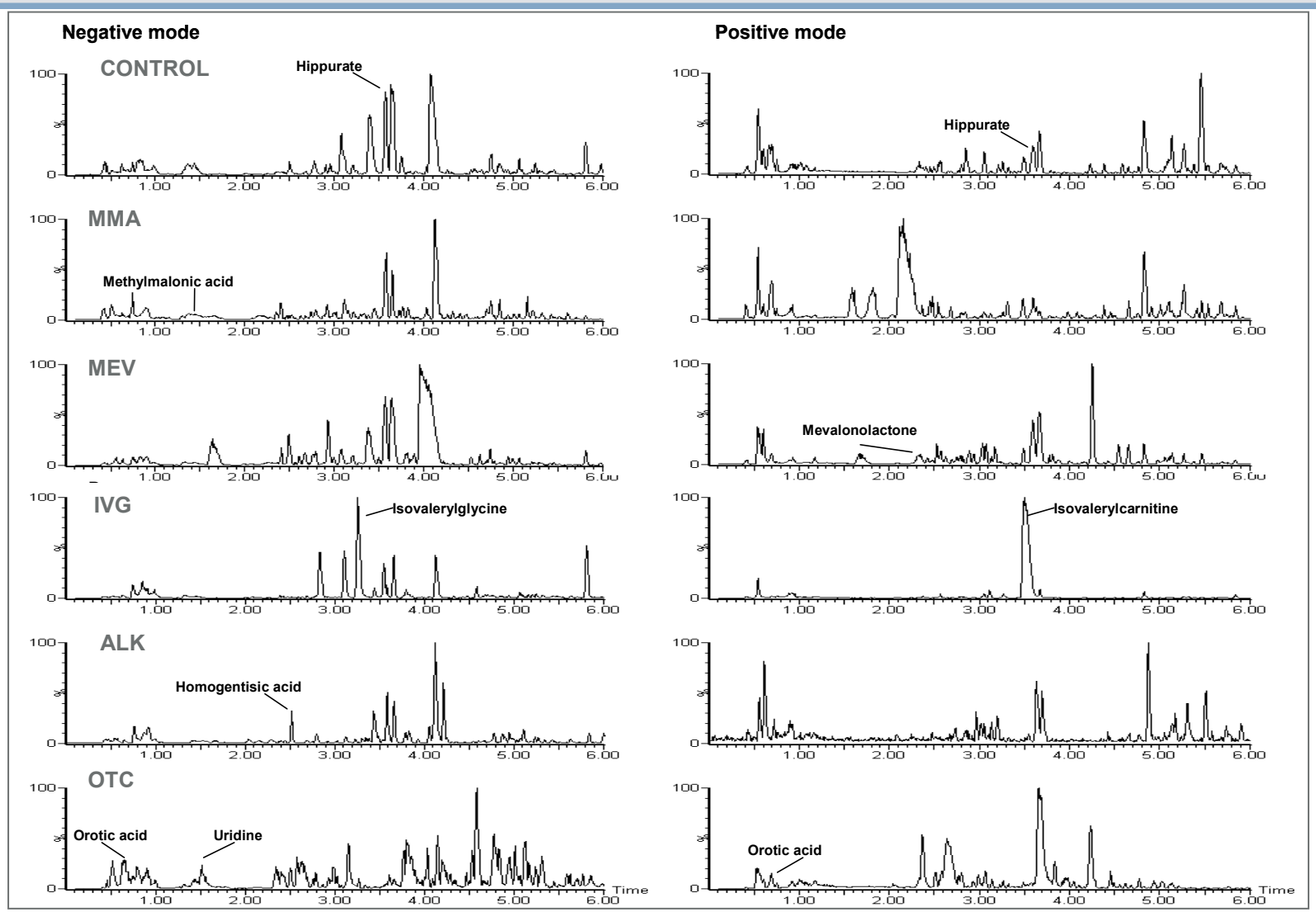
Urinary Metabolite Profiles



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3) Pouchitis

Ileo-anal pouches created in the management of some patients with ulcerative colitis

Proportion of these patients develop pouchitis

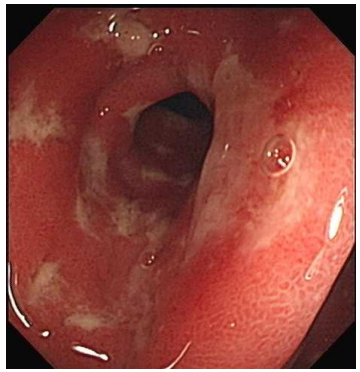
Inflammation of pouch lining

AIM: **to determine cause of pouchitis**

Metabonomic analysis of plasma, urine & faeces

NMR and UPLC-MS

UPLC-MS data processed using XCMS



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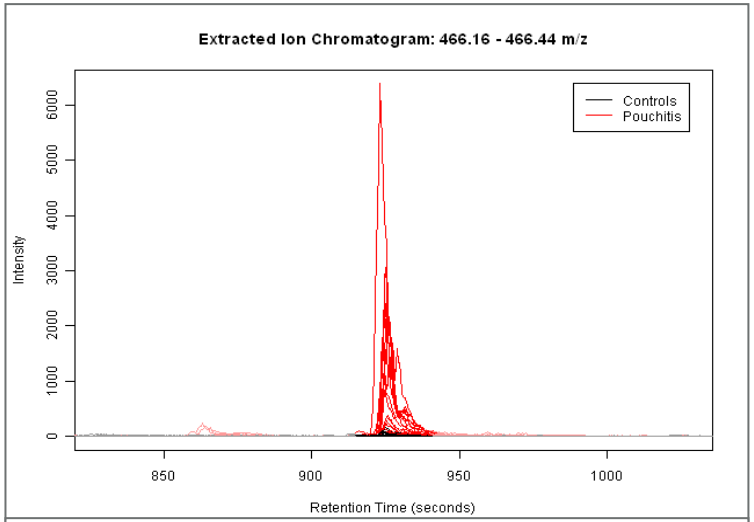
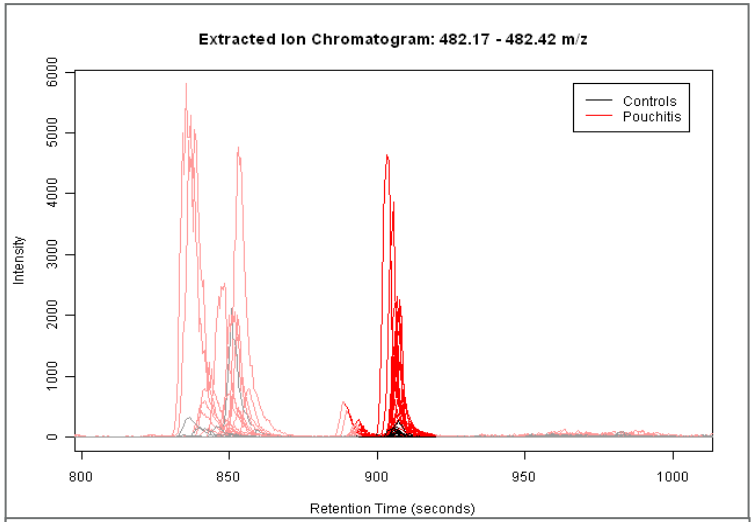
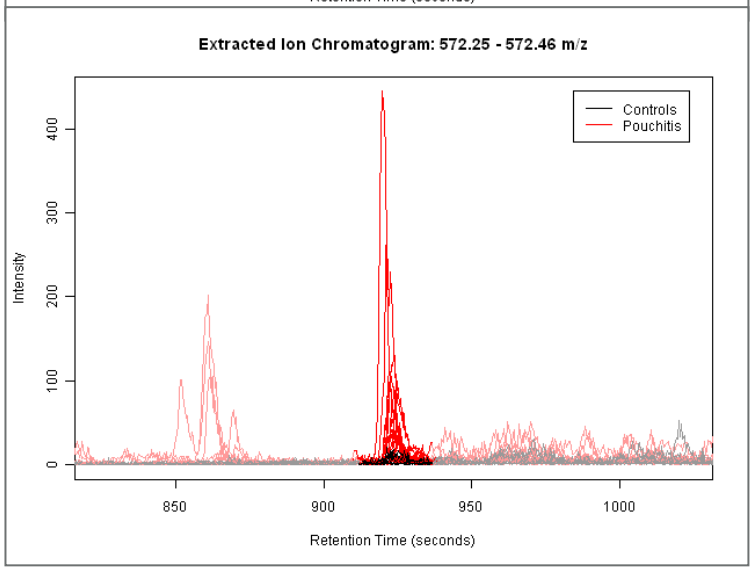
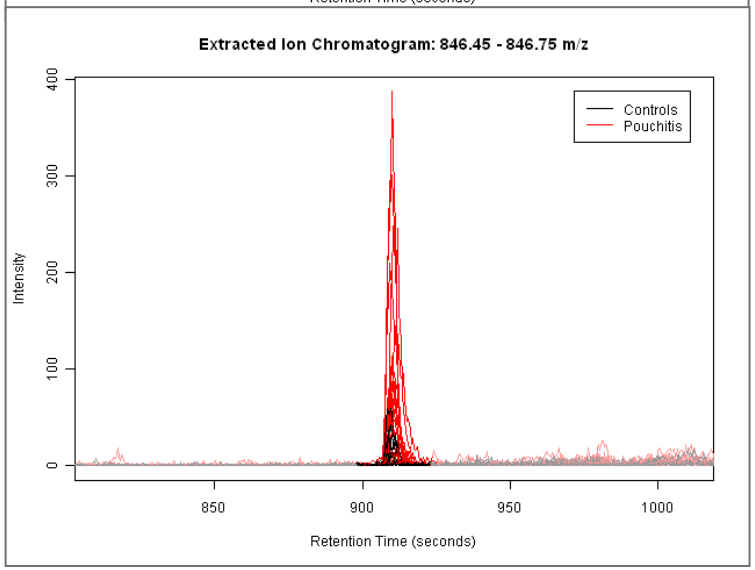
Elevated Faecal Lipids in Pouchitis









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Summary

Mass spectrometry-based metabonomics studies can offer

- **Sensitivity**
- **Reproducibility**
- **Sample throughput**
- **Complementary information to NMR**

Strategy varies depending on question being asked

Need to consider

- **Sample preparation**
- **Separation approaches**
- **Mass spectrometer**
- **Data analysis**

Challenges still remain in data analysis

- **Software & databases**

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