

# The Third Omic: metabonomics by MS and NMR

**Ian Wilson**

# Acknowledgements

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- **AstraZeneca: Rebecca Williams, Eva Lenz, Jennifer Granger, Tim Sangster, Lindsay Lai.**
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# Outline

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- **Introduction to Metabonomics**
- **Analytical Methods**
- **Method Development**
- **Applications in Animal Models**
- **Conclusions**

# What is metabonomics?

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- “the quantitative measurement of the the dynamic multiparametric response of living systems to pathophysiological stimuli or genetic modification”
- Its about seeing what *changes* in intermediary metabolites in response to something or other!

## And is it the same as metabolomics?

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- **Not really – but the terms are often used interchangeably, especially by newcomers.**
- **Metabolomics was originally defined as being about measuring all the metabolites in a cell sample.**
- **Use whichever term you like, but search on both!**

# Metabonomics/Metabolomics

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- **Whichever term you end up using, the aim is to produce “Global Metabolite Profiles”.**
- **I.e you do not pre-select the analytes**
- **If you are only analysing for one class of compounds (e.g amino acids) its not metabonomics!**

# What might metabonomics deliver?

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- **Markers of disease**
- **Markers of genetic difference**
- **Markers of pharmacology**
- **Markers of efficacy**
- **Markers of toxicity**
- **Markers of physiology**

# Where Does Metabonomics Fit?

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- **Genomics: transcriptomics –what should happen.**
- **Proteomics: the result of the transcription of RNA what really should happen**
- **Metabolites: what did happen!**
- **To understand a biological system you may need all 3 omics ie., SYSTEMS BIOLOGY**



# How is it done? Technology platforms

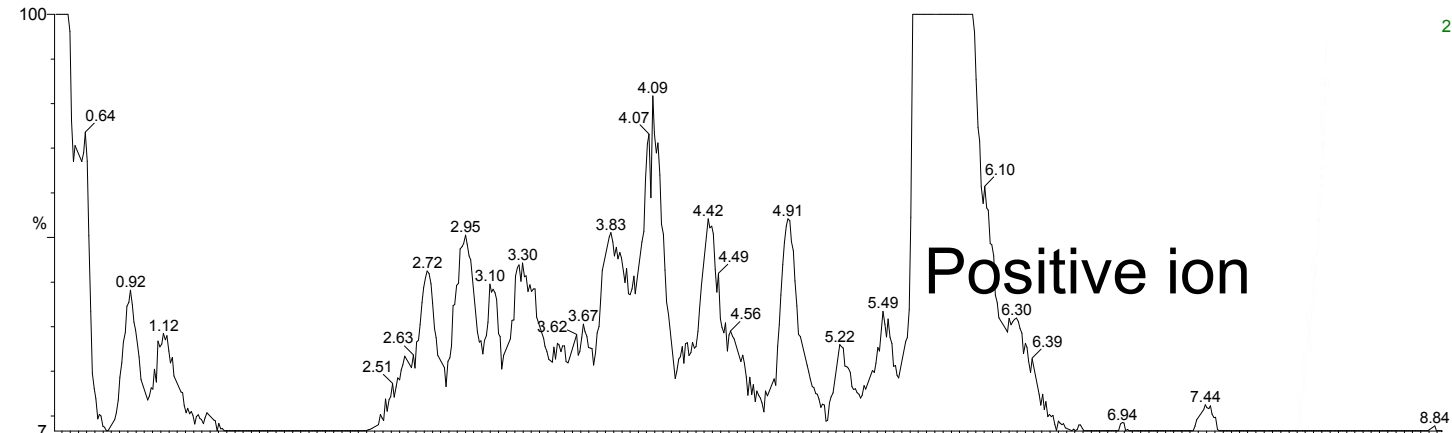
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- **NMR – both liquids and solids**
- **MS – direct infusion**
- **HPLC-MS**
- **GC-MS**
- **CE-MS**
- **Multivariate statistics/chemometrics for pattern recognition**

# Male: Black Mouse Urine LC-MS

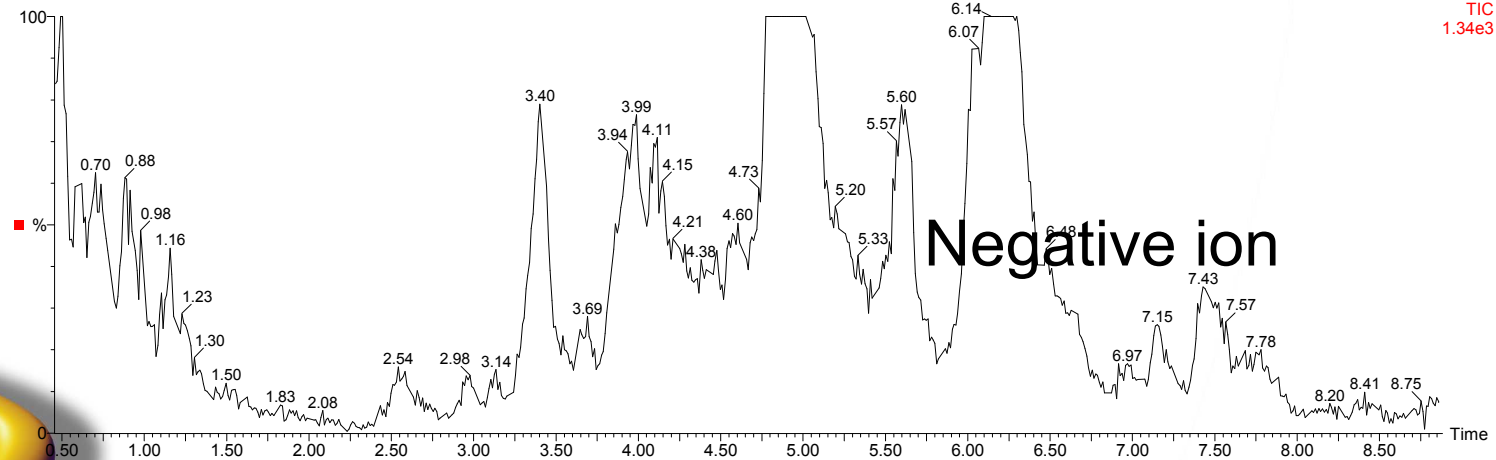
AM\_Male\_Black1  
H030305\_CS\_ES+\_021

1: TOF MS ES+  
TIC  
2.55e3

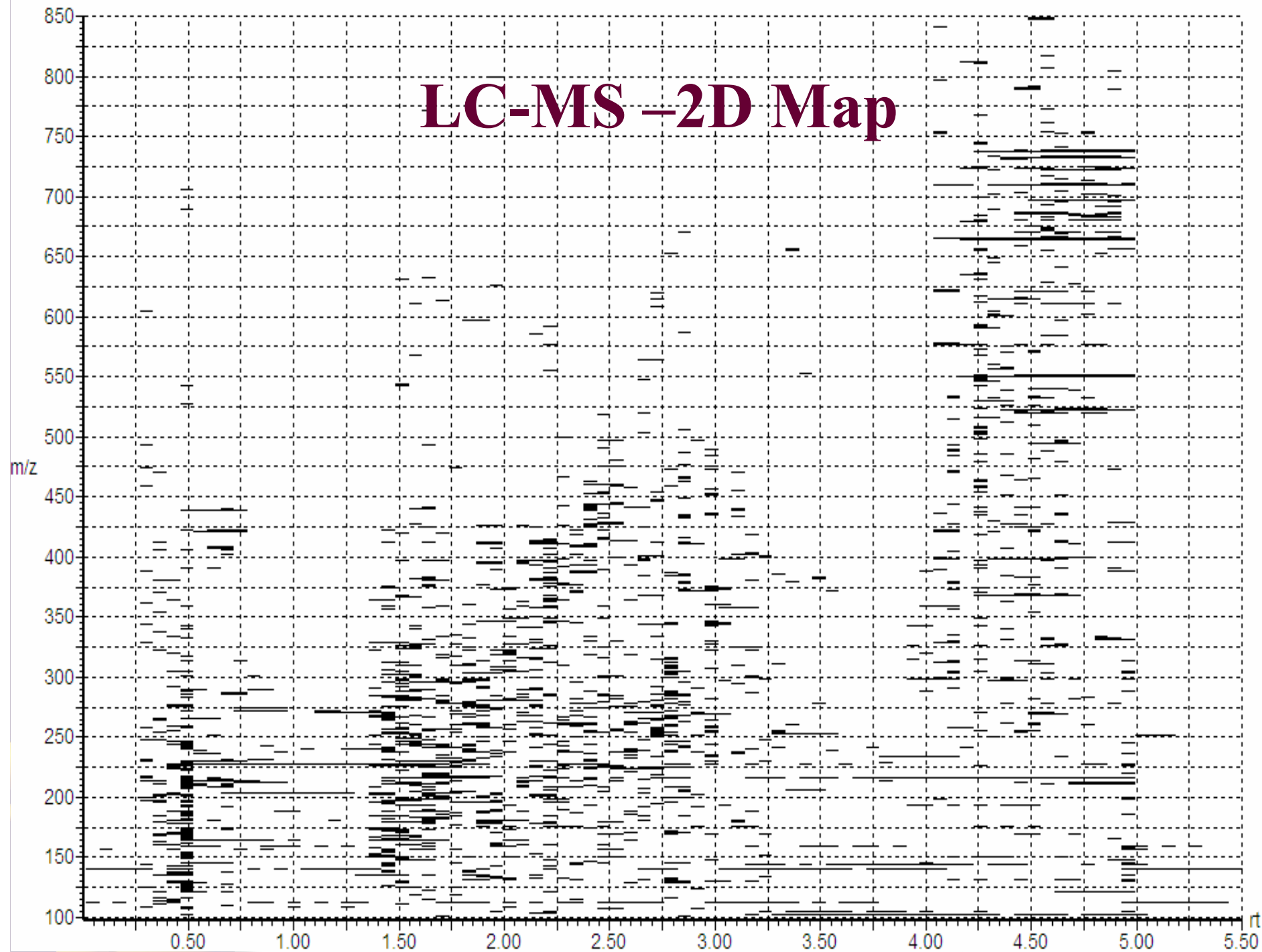


H030306\_CS\_ES-\_021

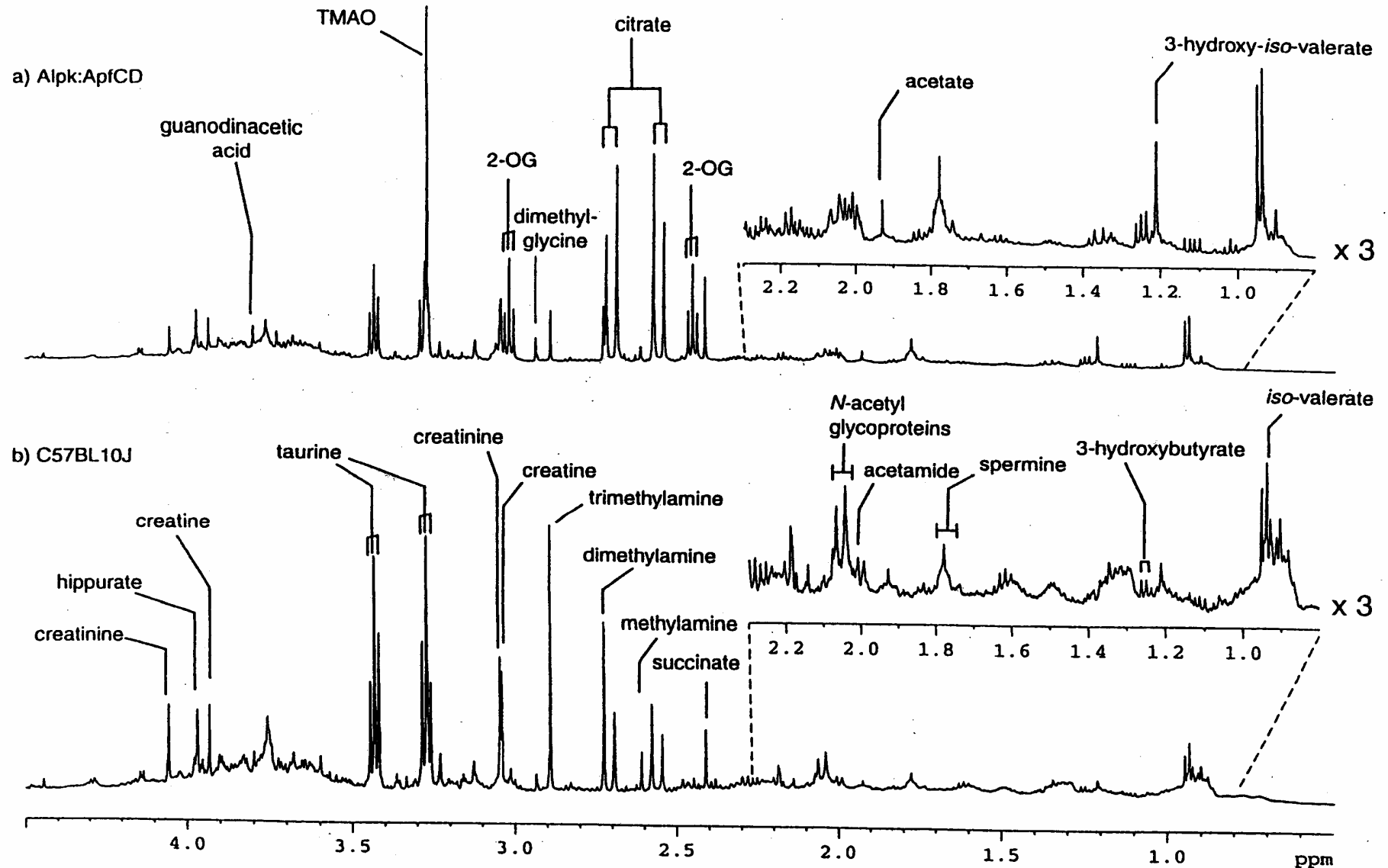
1: TOF MS ES-  
TIC  
1.34e3



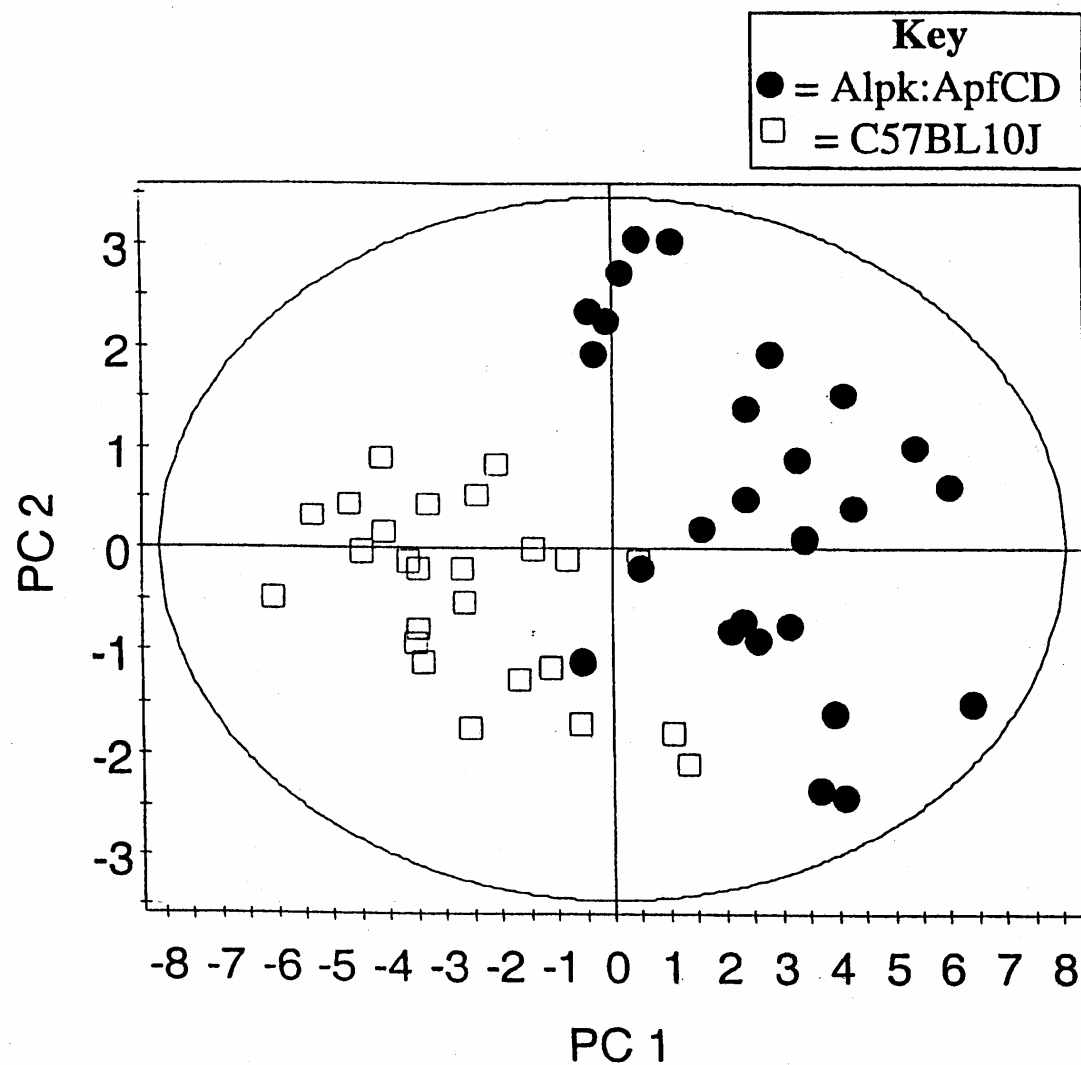
# LC-MS -2D Map



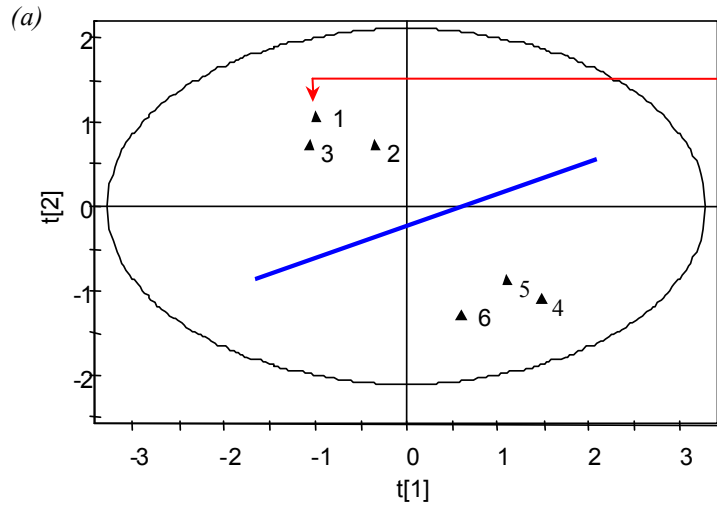
# NMR Spectra of Mouse Urines



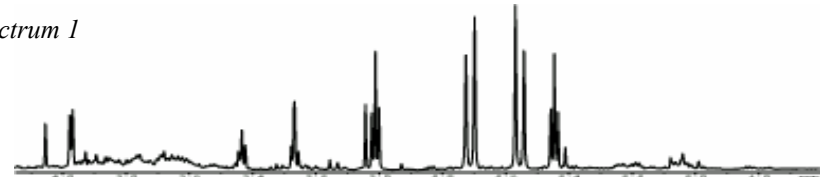
# PCA of Mouse Urines



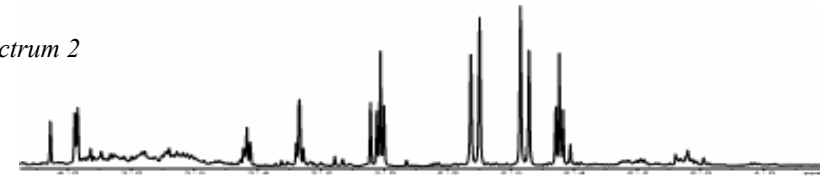
# How the PCA Works



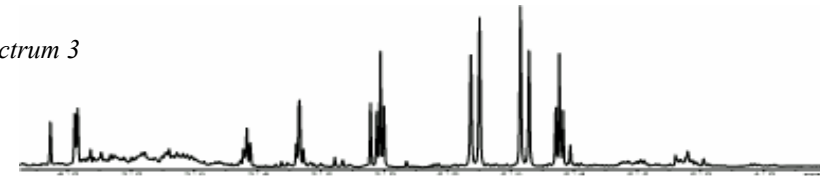
Spectrum 1



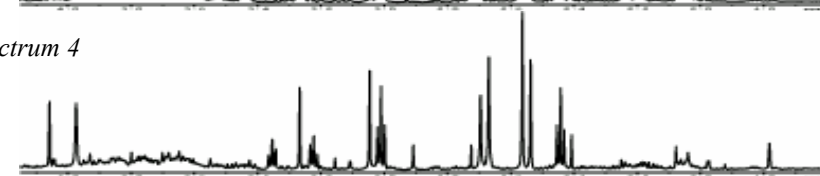
Spectrum 2



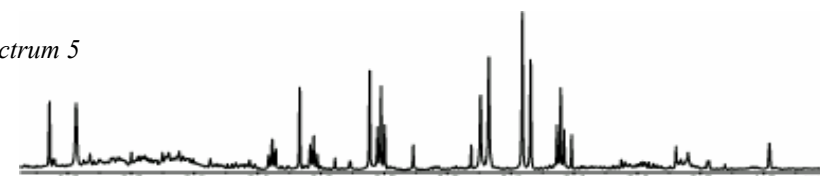
Spectrum 3



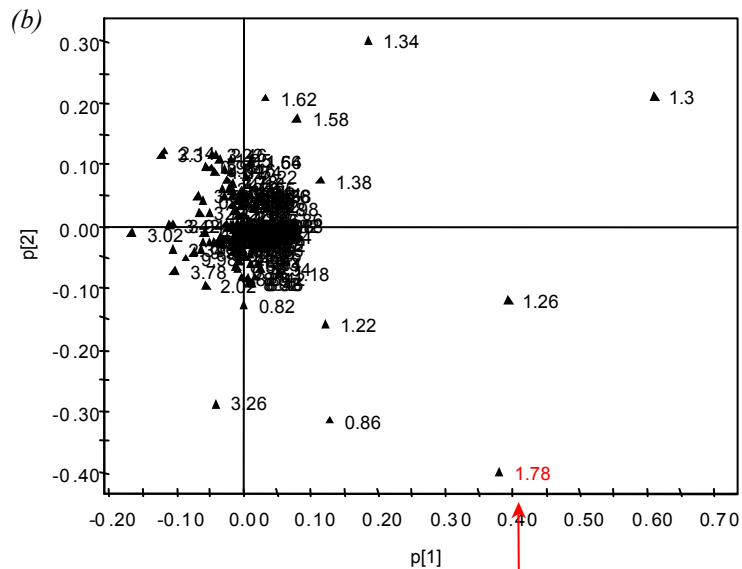
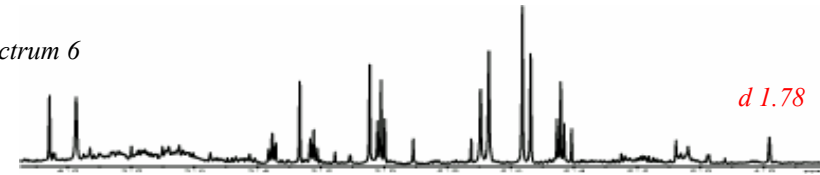
Spectrum 4



Spectrum 5



Spectrum 6



# Sample Types

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- **Plasma**
- **Urine**
- **Bile**
- **Saliva**
- **Milk**
- **Tissues: Tissue extracts**
- **Cell culture (media, cells, cell extracts)**
- **If you can get it, we can profile it!**

# Confounding Factors in Metabonomics

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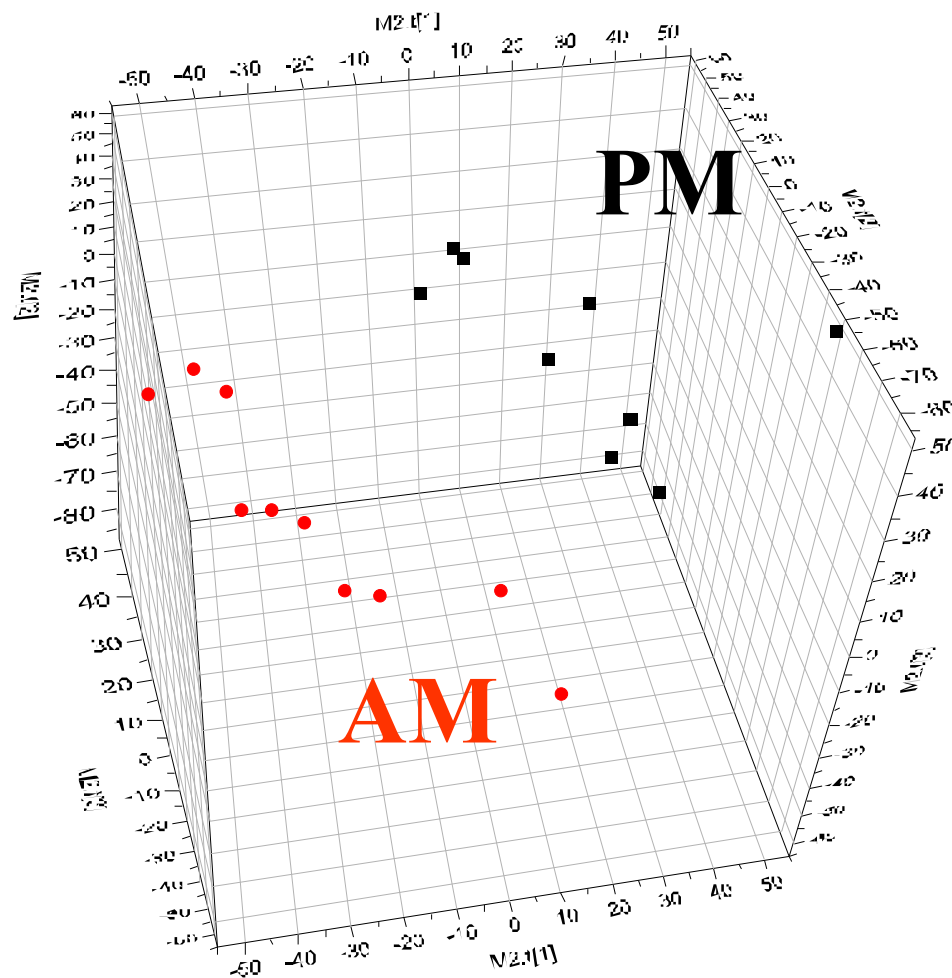
- **Strain, gender, diurnal variation, stress, diet, feeding, age, disease, pharmacology, microflora.**
- **The methodology can be very sensitive, you need to take care if you want to see the effects of toxicity/disease and not just normal variation.**
- **Analytical variability can add to the problem**



# am versus pm in the rat

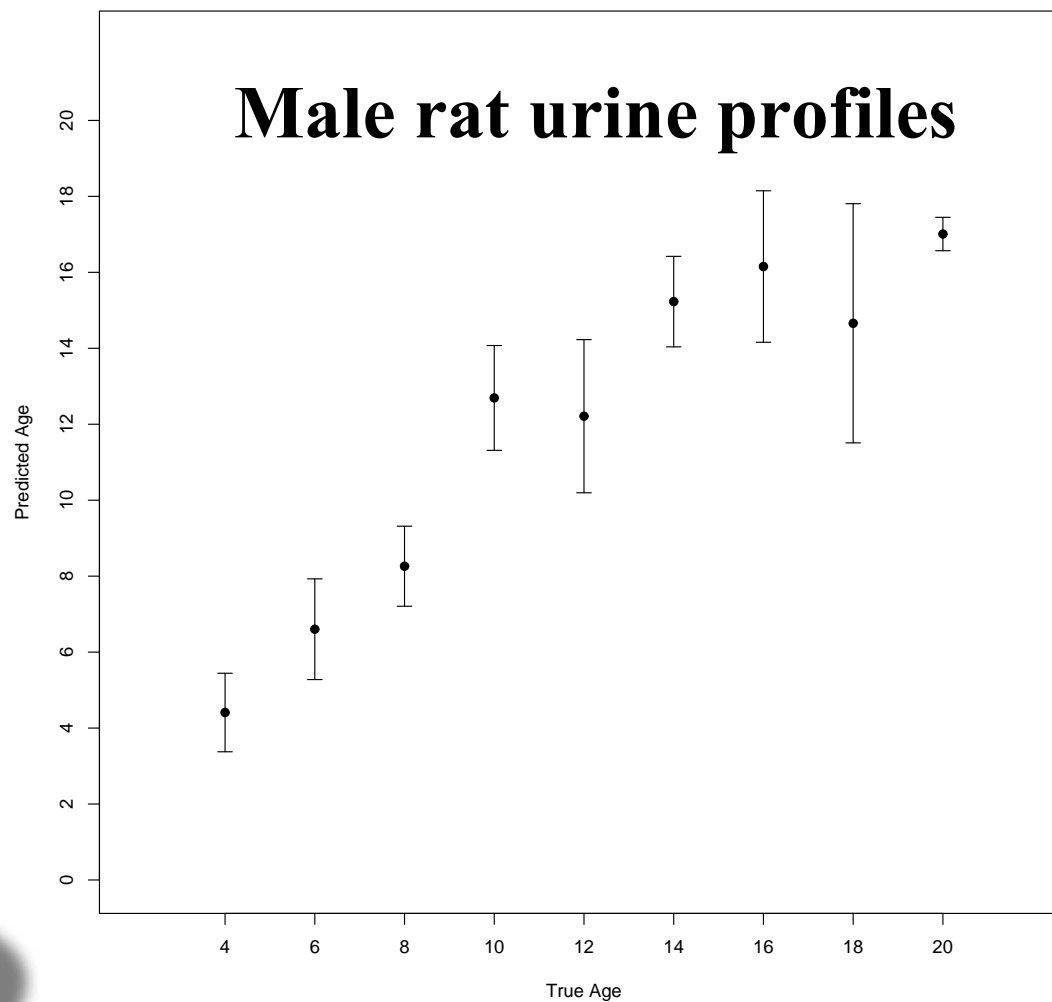
## Urine

## LC-MS



**Take care when you sample!**

# Changes in metabolites with age

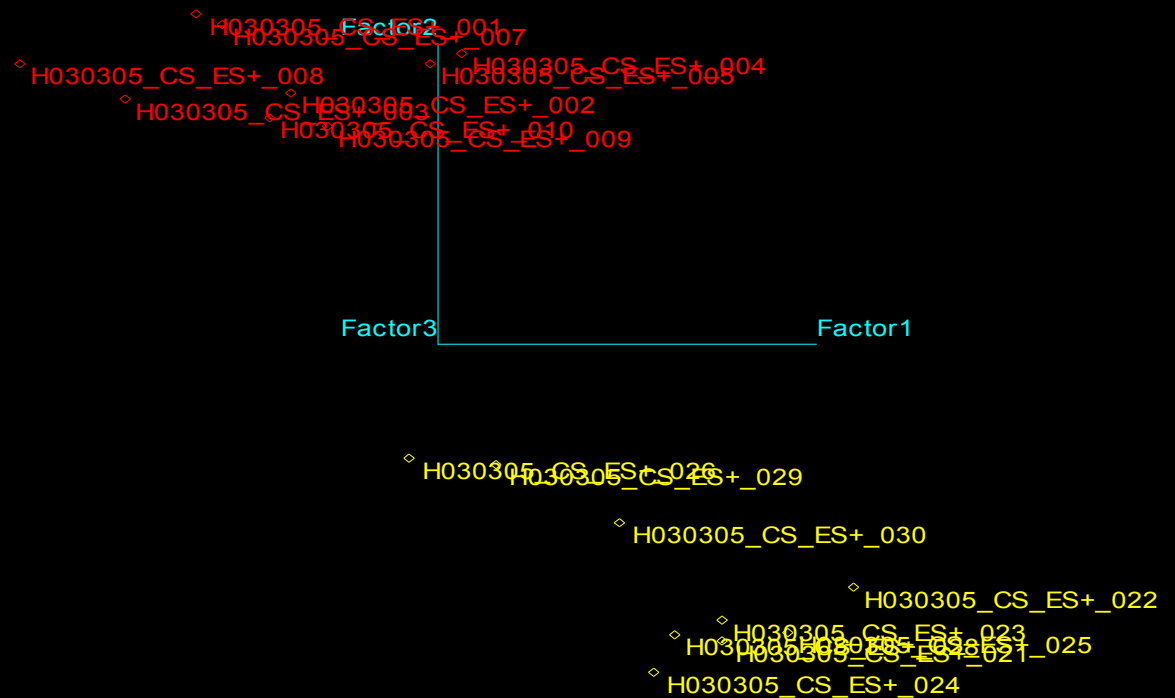


**$^1\text{H}$  NMR**

# Gender: Male vs female in black mice

**Yellow:**  
Male

**Red:**  
Female



# Strain in mice

**Yellow:**

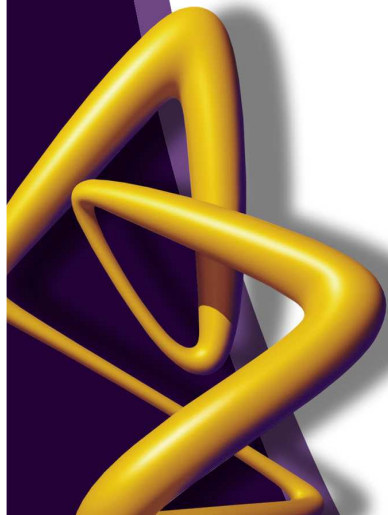
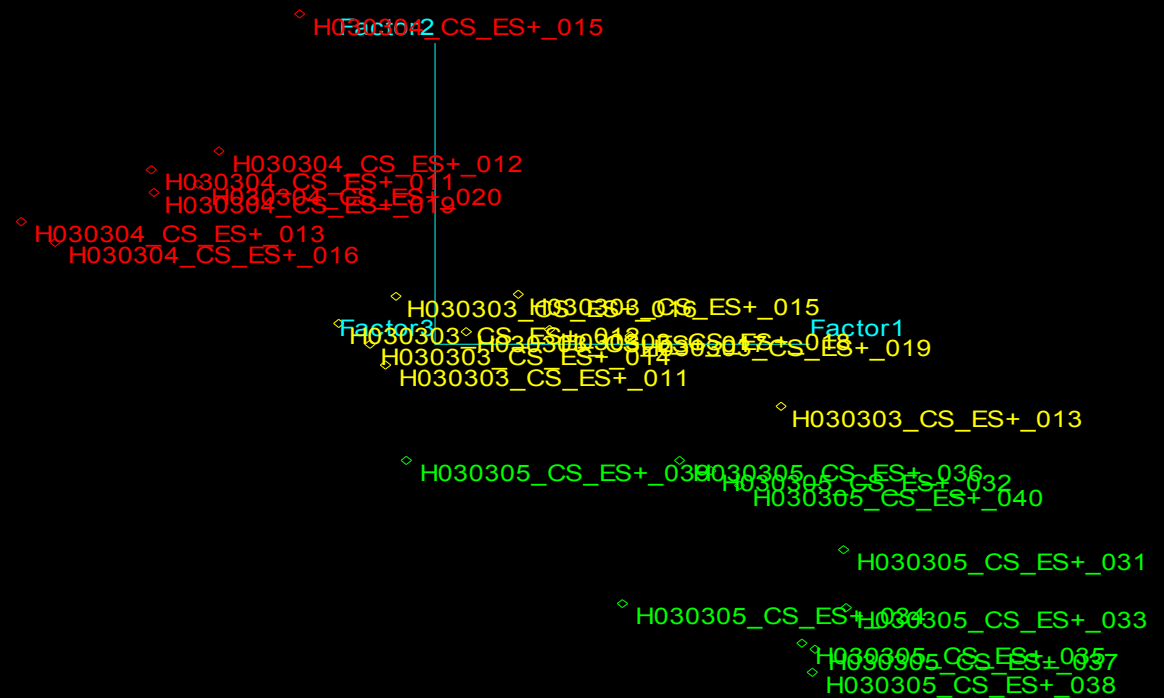
White

**Red:**

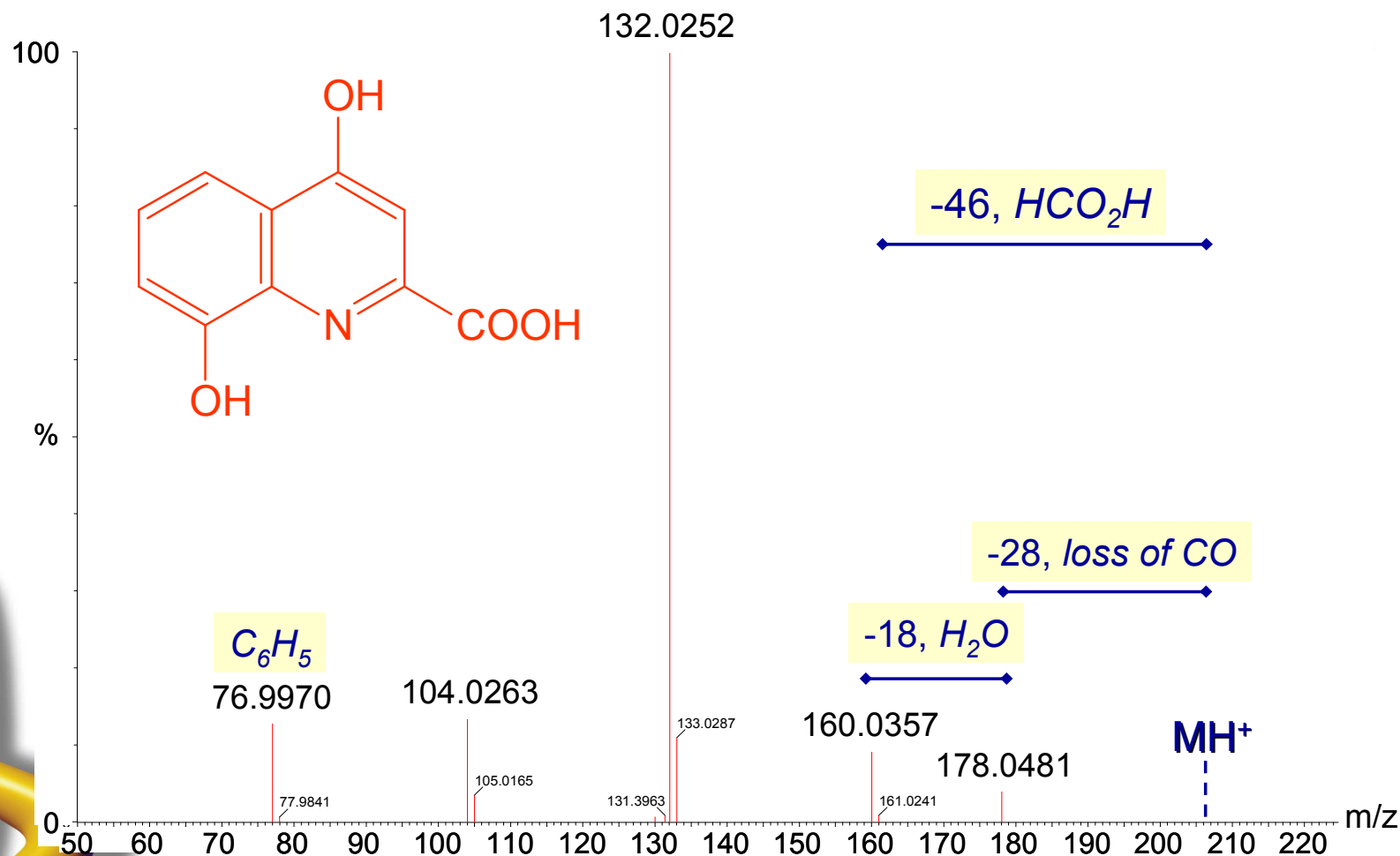
Nude

**Green:**

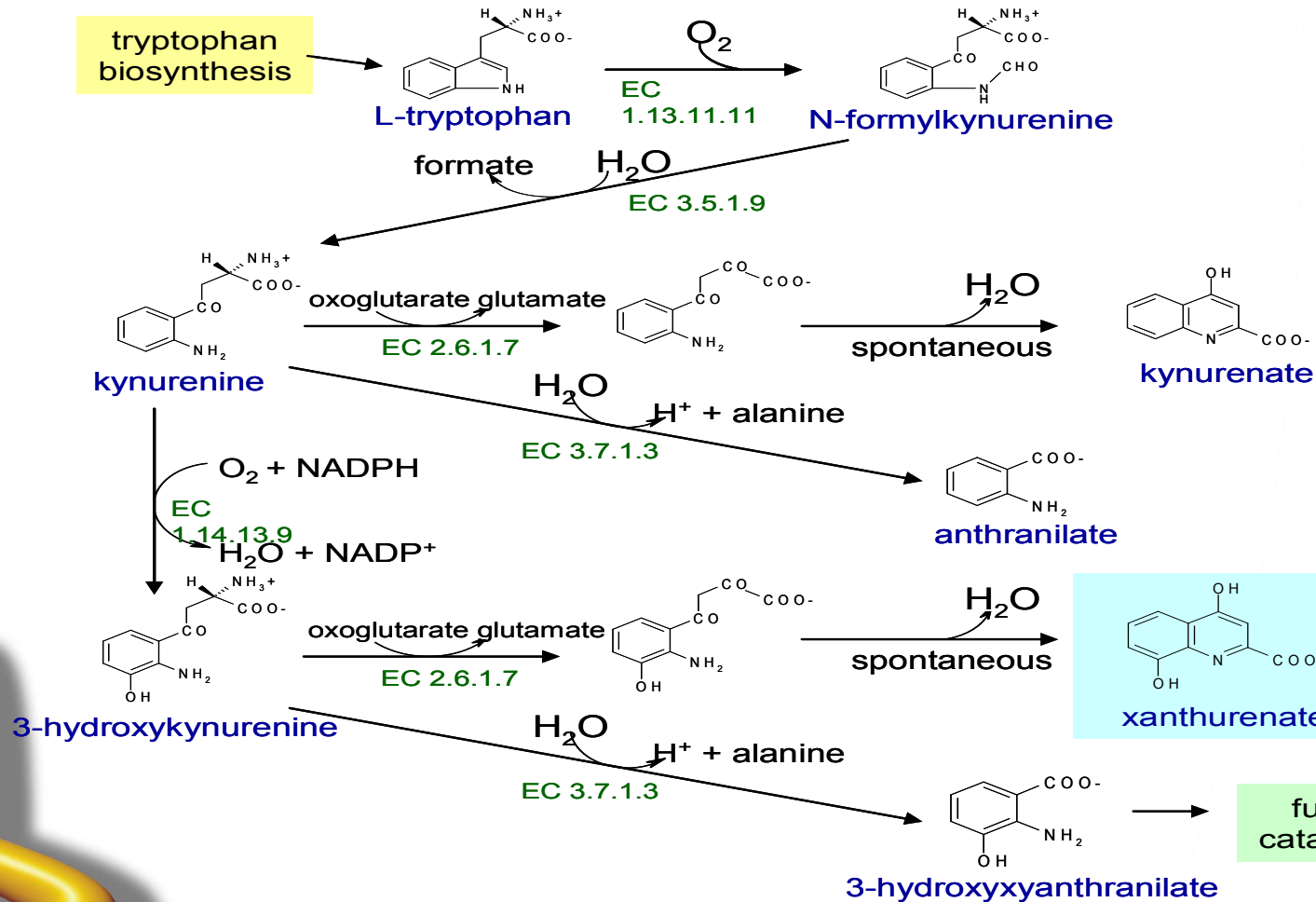
Black



# A marker of strain by MS/MS



# Metabolic Pathways for Markers



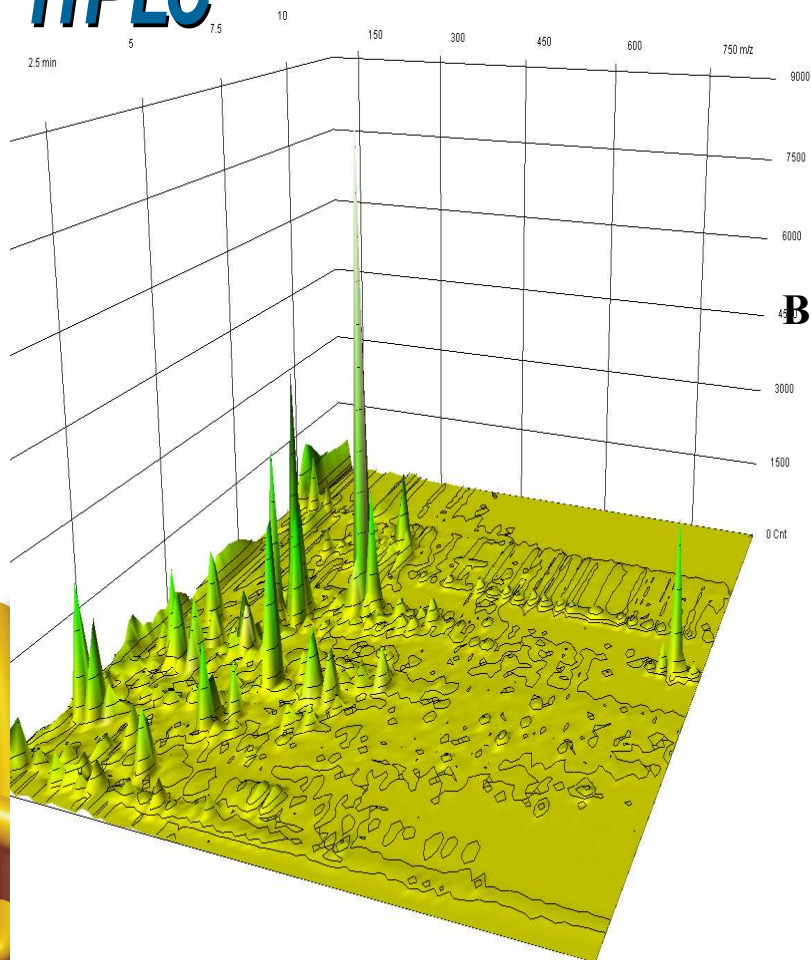
# UPLC-MS: The new HPLC

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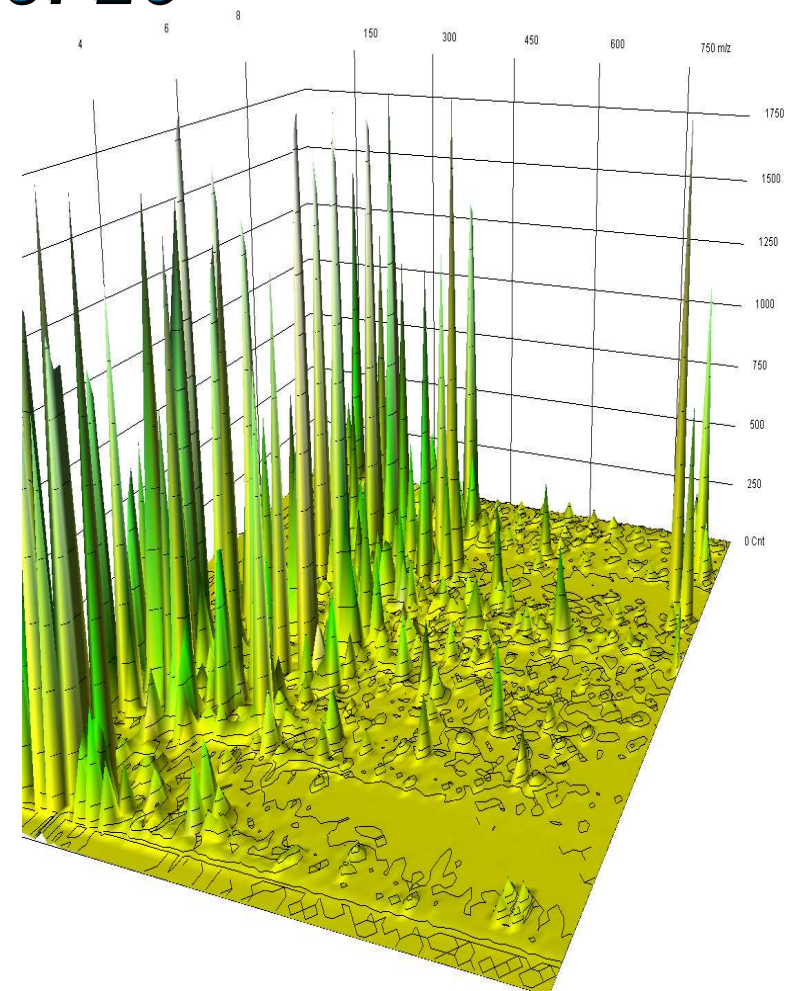
- “Ultra Performance” LC
- Small particles (1.7  $\mu\text{m}$ )
- High pressures (up to 15,000 psi)
- 2.1 mm diam. 10 cm columns
- “Normal” LC flow rates of  $\approx 0.5$  mL/min

# UPLC vs HPLC

## HPLC



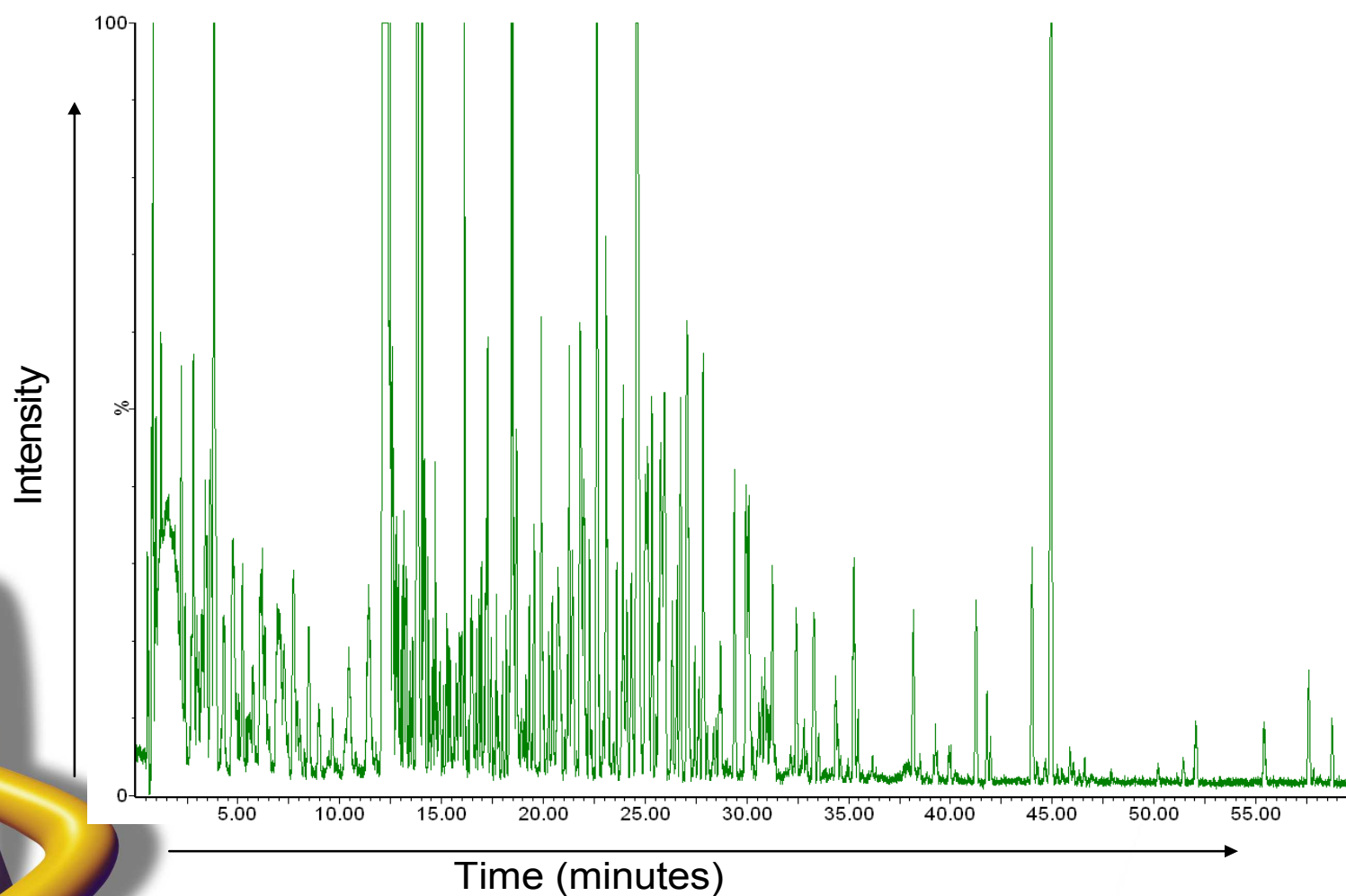
## UPLC



**Same Sample, Same Mass Spec, Same Day**



# Rat Urine UPLC at 90°C – 1Hr



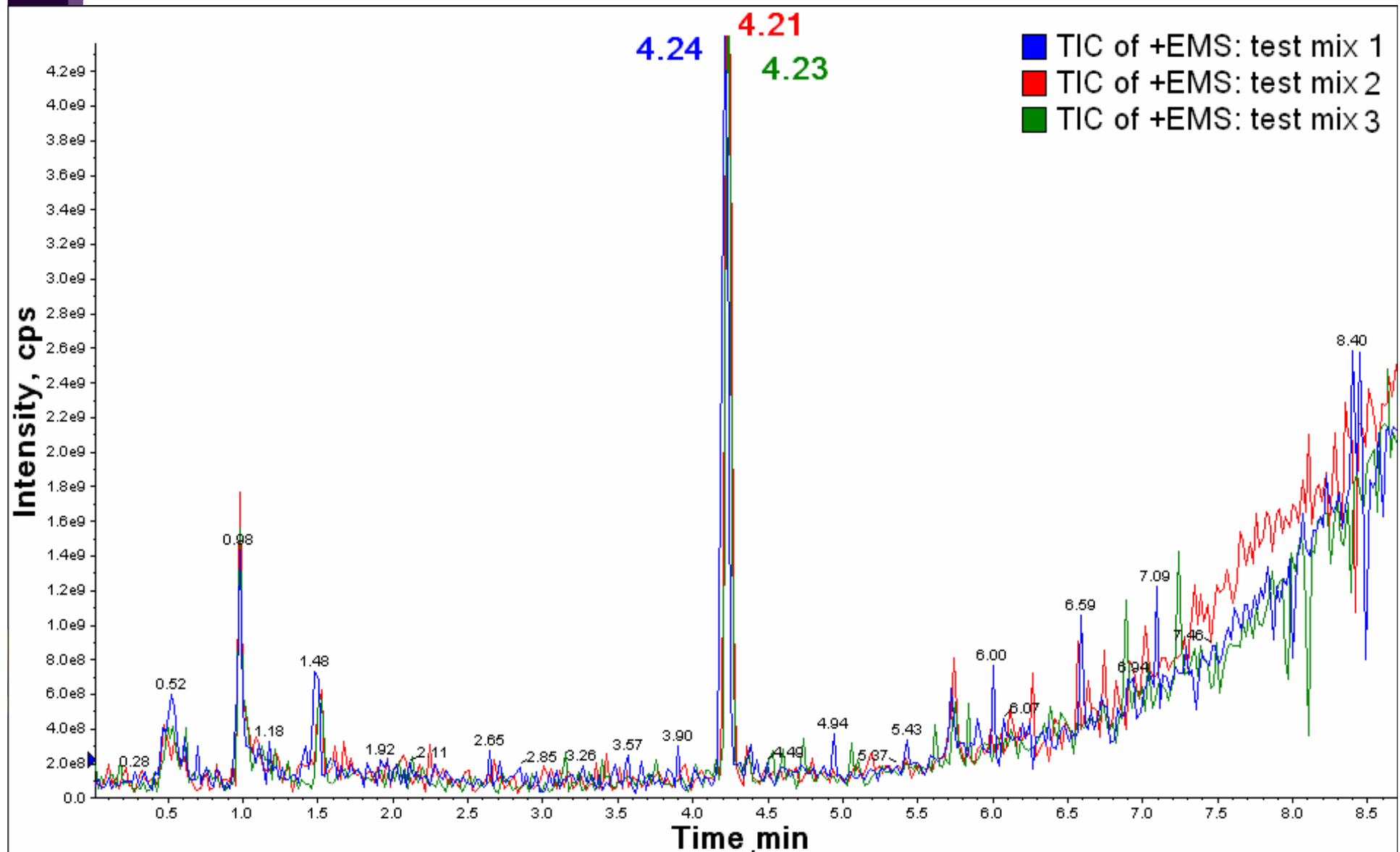
**Room for 1000 peaks in 60 min!**

# Method Development

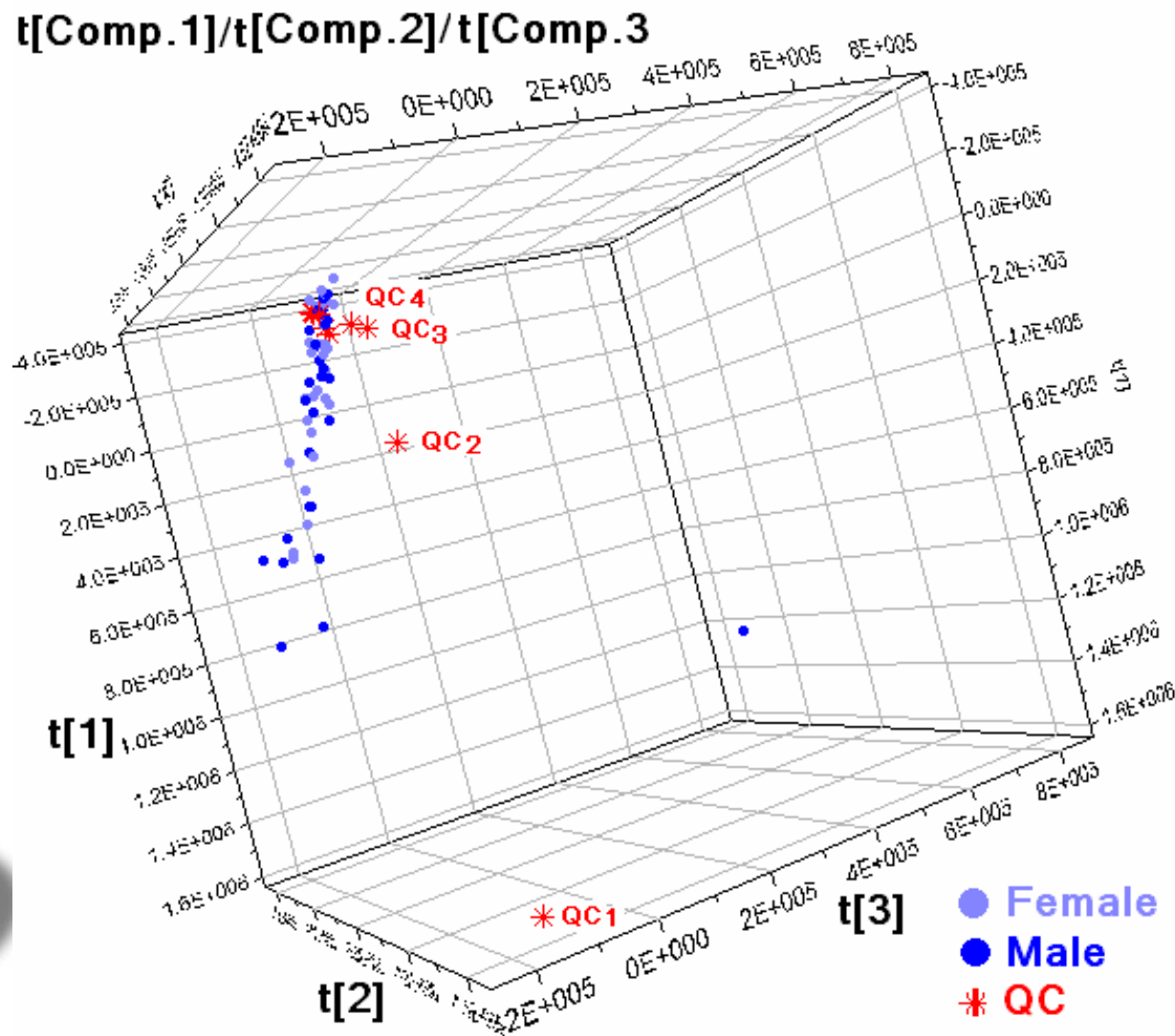
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- **NMR – minimal sample preparation, reproducible, essentially no method development needed.**
- **LC-MS - much less reproducible, more variables, more method development needed**
- **and once developed more effort needed to ensure valid data are produced**

# System unsuitability test mix!

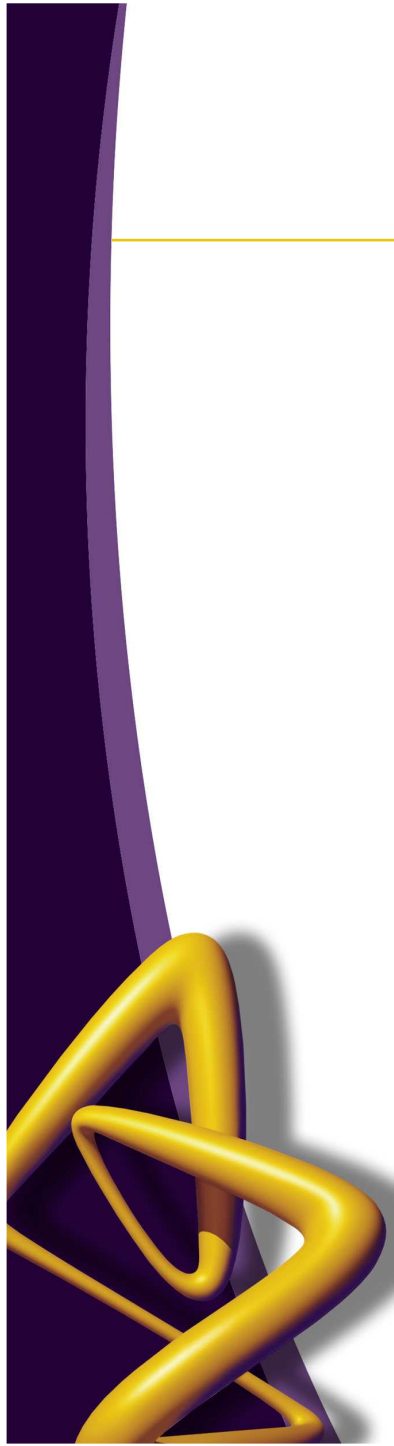
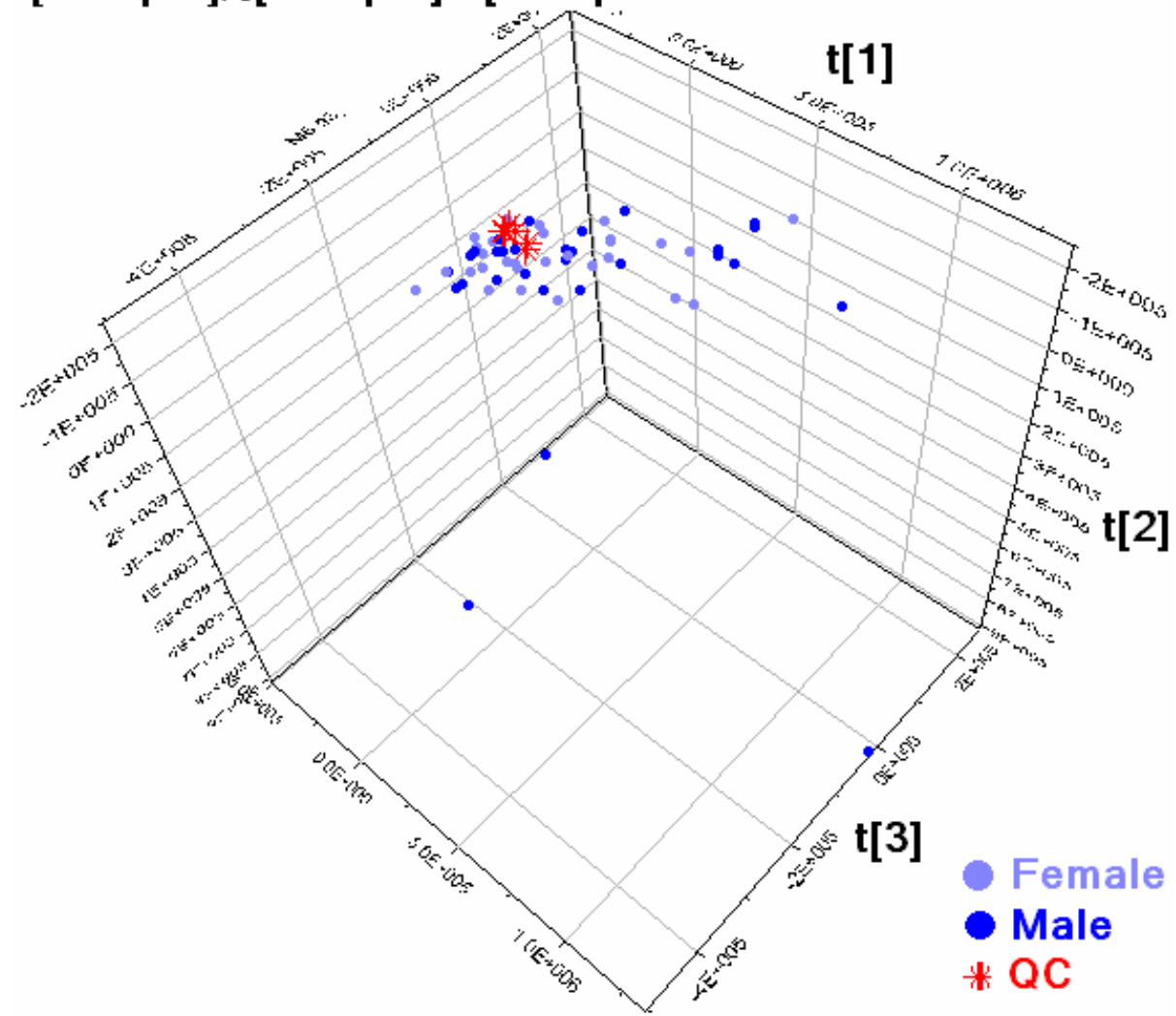


# System conditioning



# Conditioned

t[Comp.1]/t[Comp.2]/t[Comp.3]



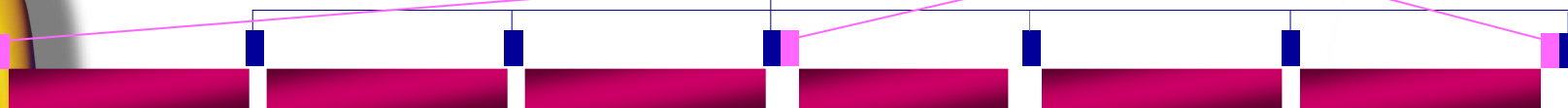
## QC approach : Pooled urine Quality Control injection sequence

Sample sequence; random sequence of (e. g. male and female) urine samples in blocks of 10 samples

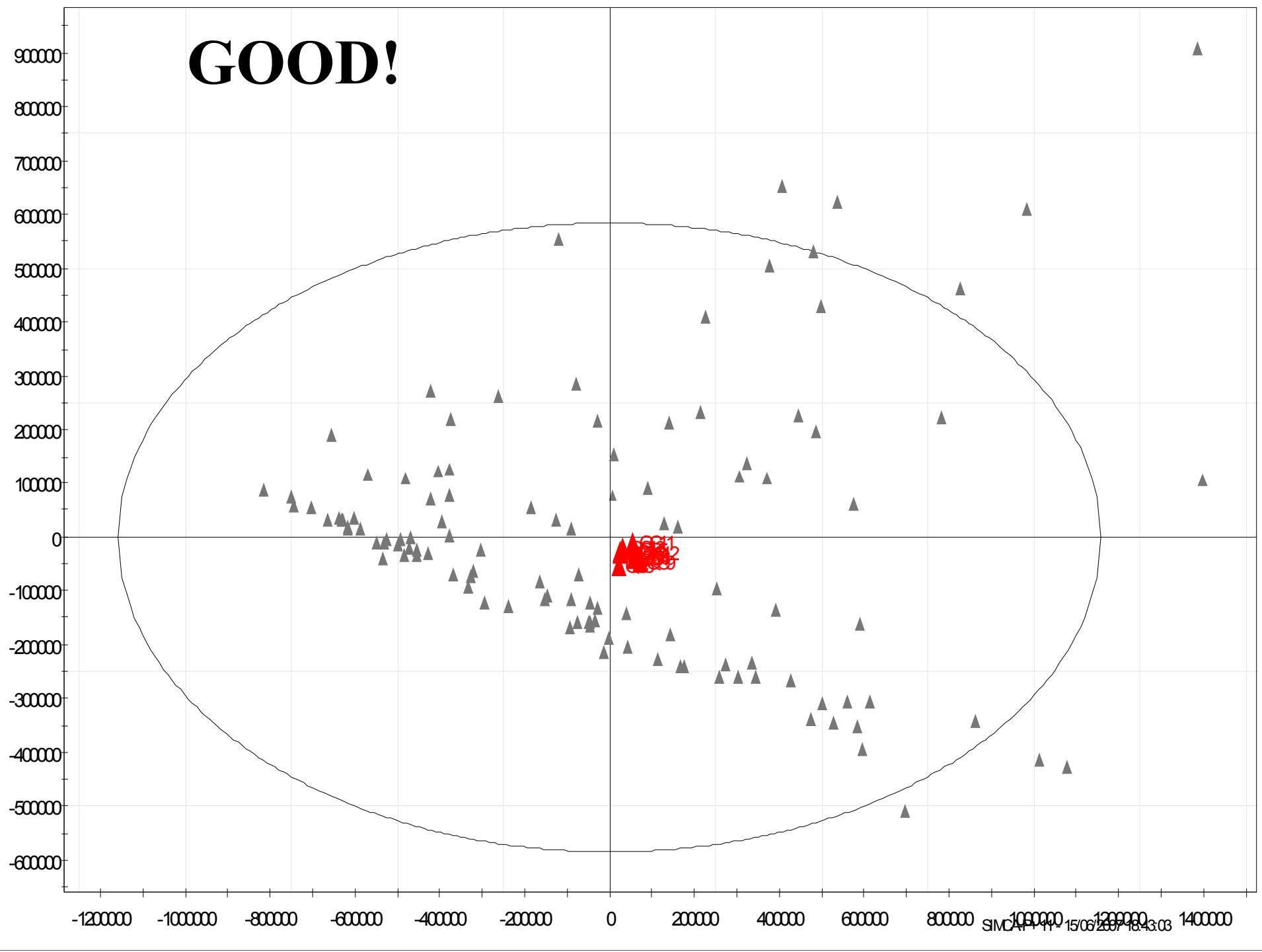
“Conditioning”  
QC runs (6-8)

QC sequence

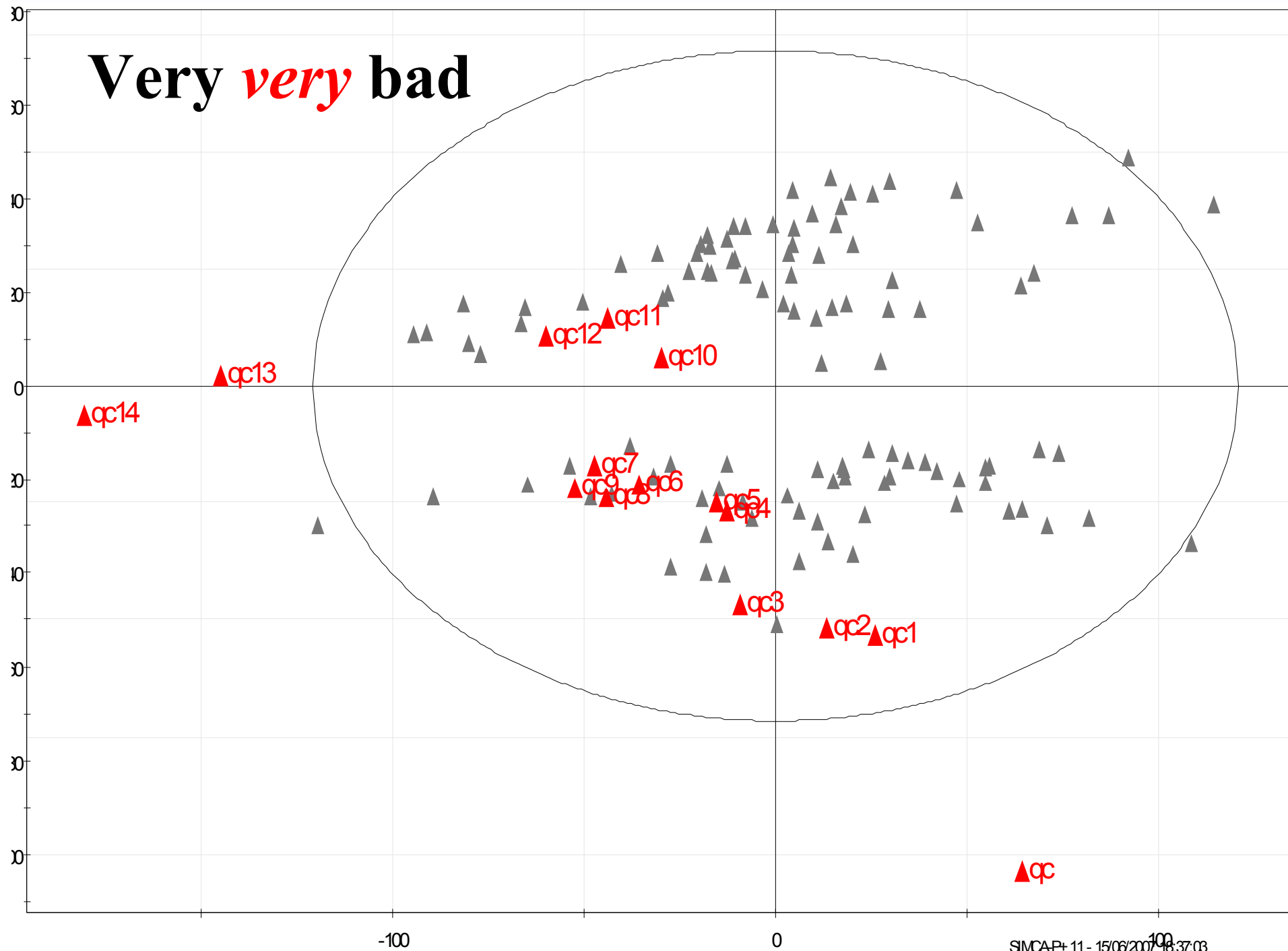
test mixture (5  
reference standards)



**GOOD!**

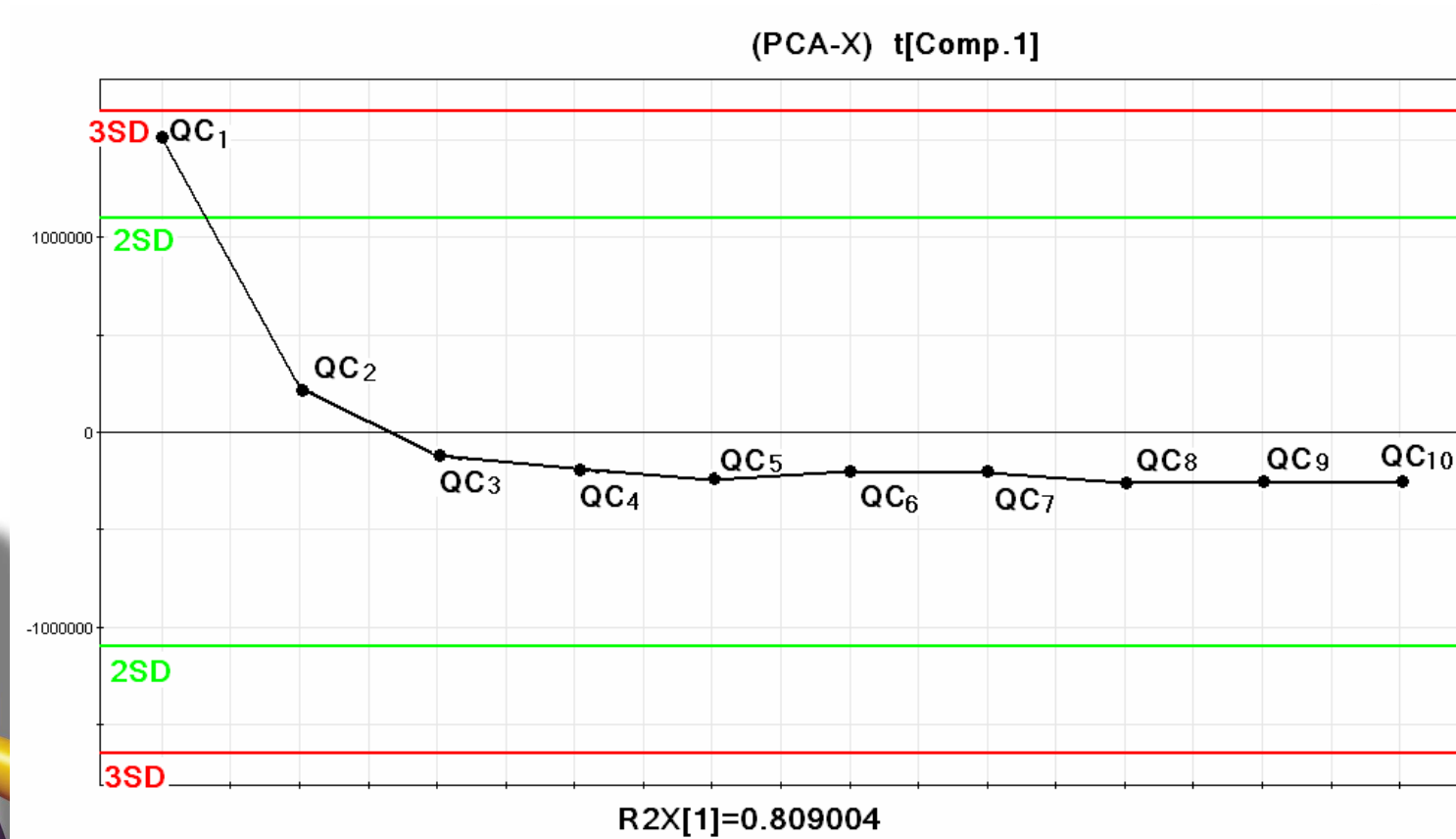


Very *very* bad





# The Control Chart

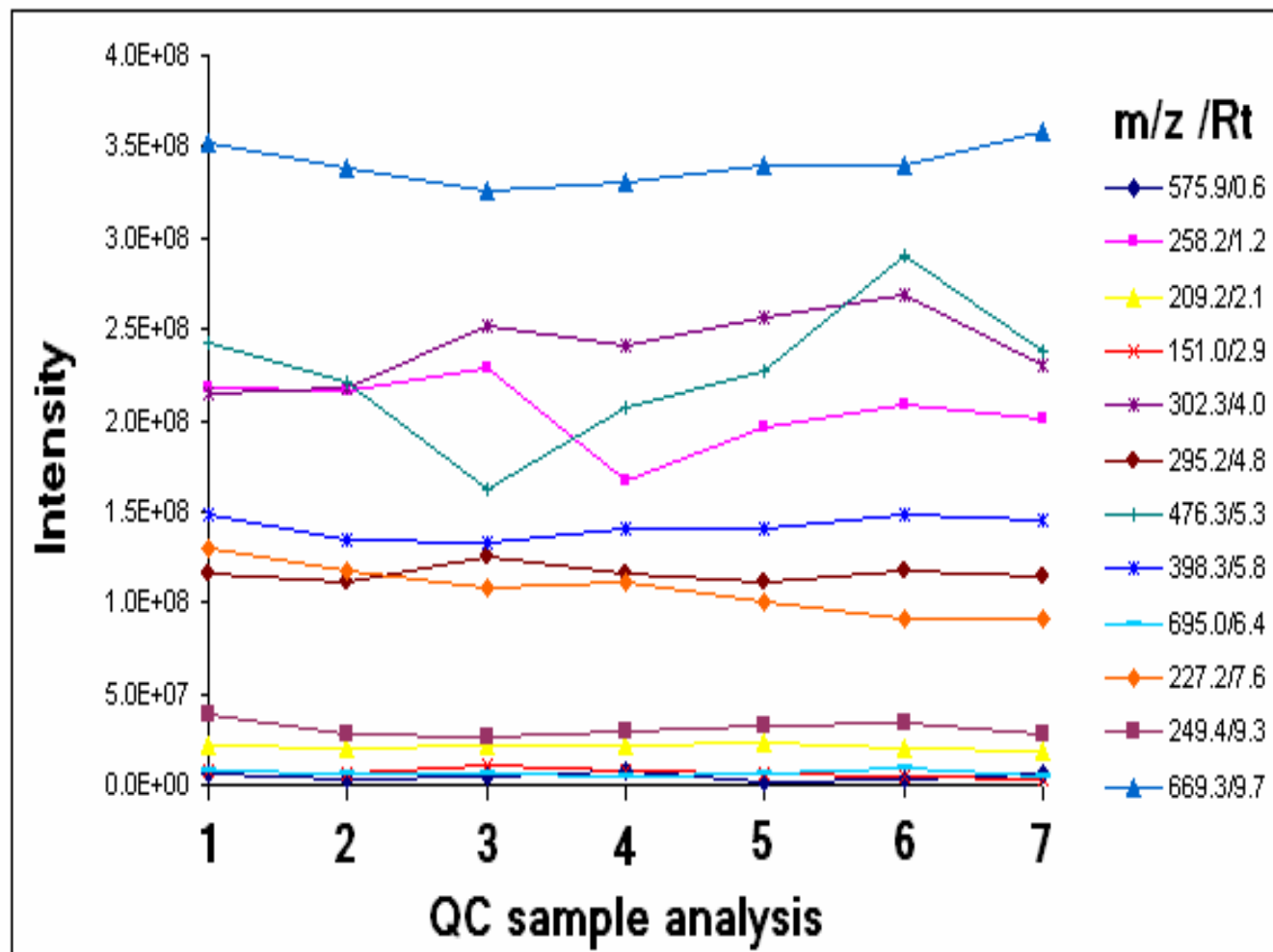


# Reproducibility of response



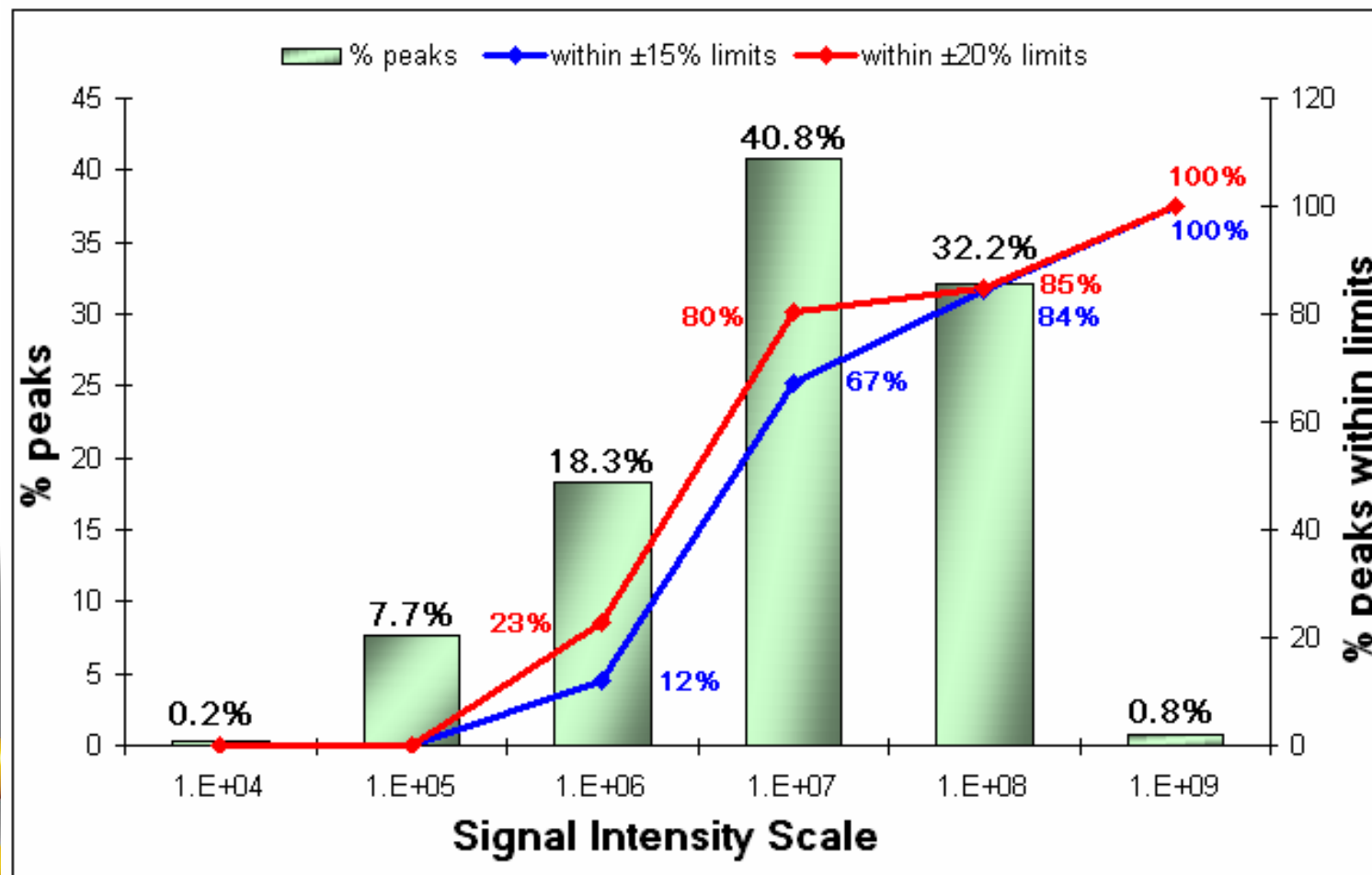
- **What are the factors?**
- **Are there any obvious trends?**
- **Would an internal standard(s) compensate?**
- **The QC samples can help you evaluate all of these possibilities**

# Response.....

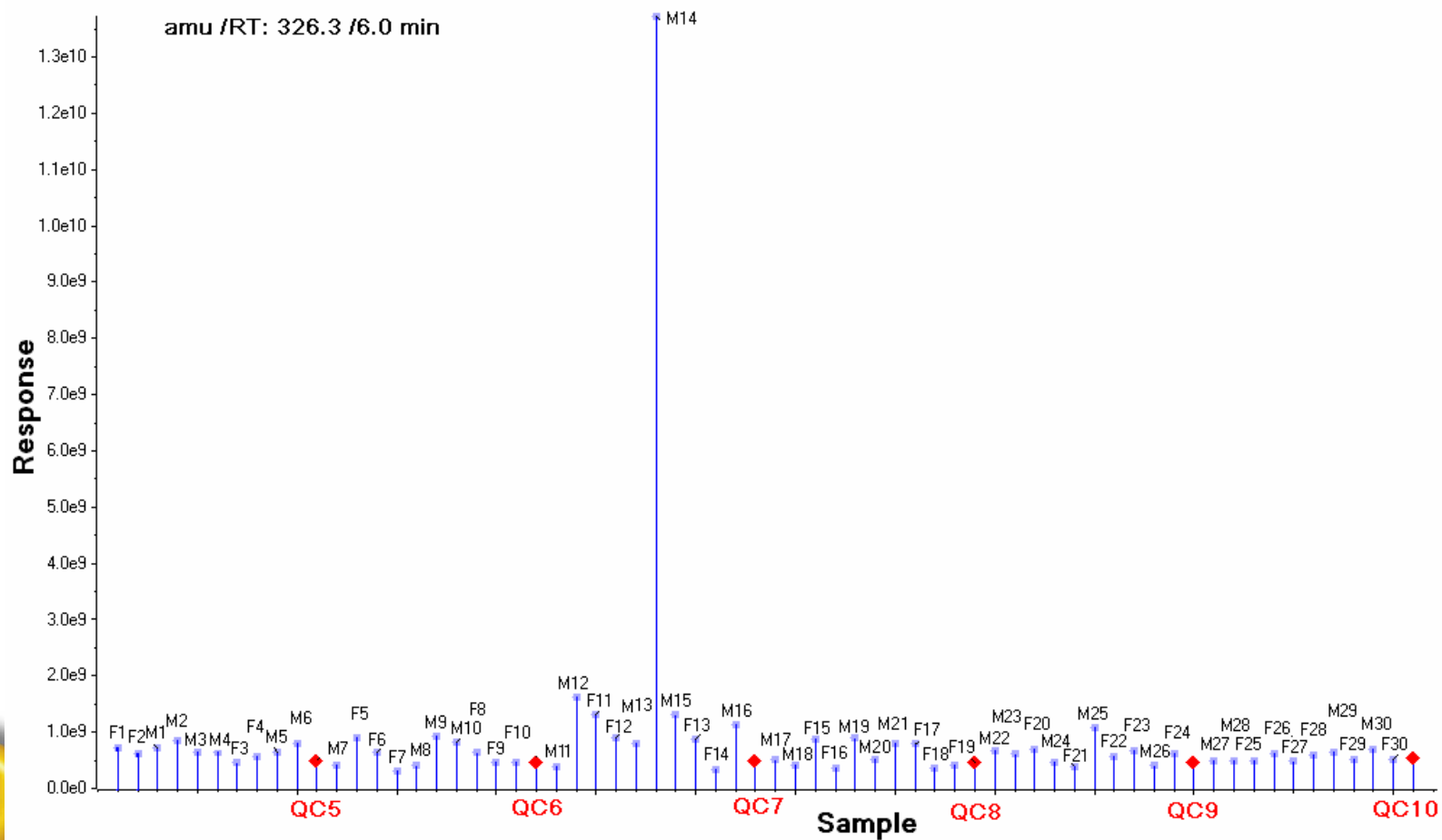


**And that's why internal standards don't work**

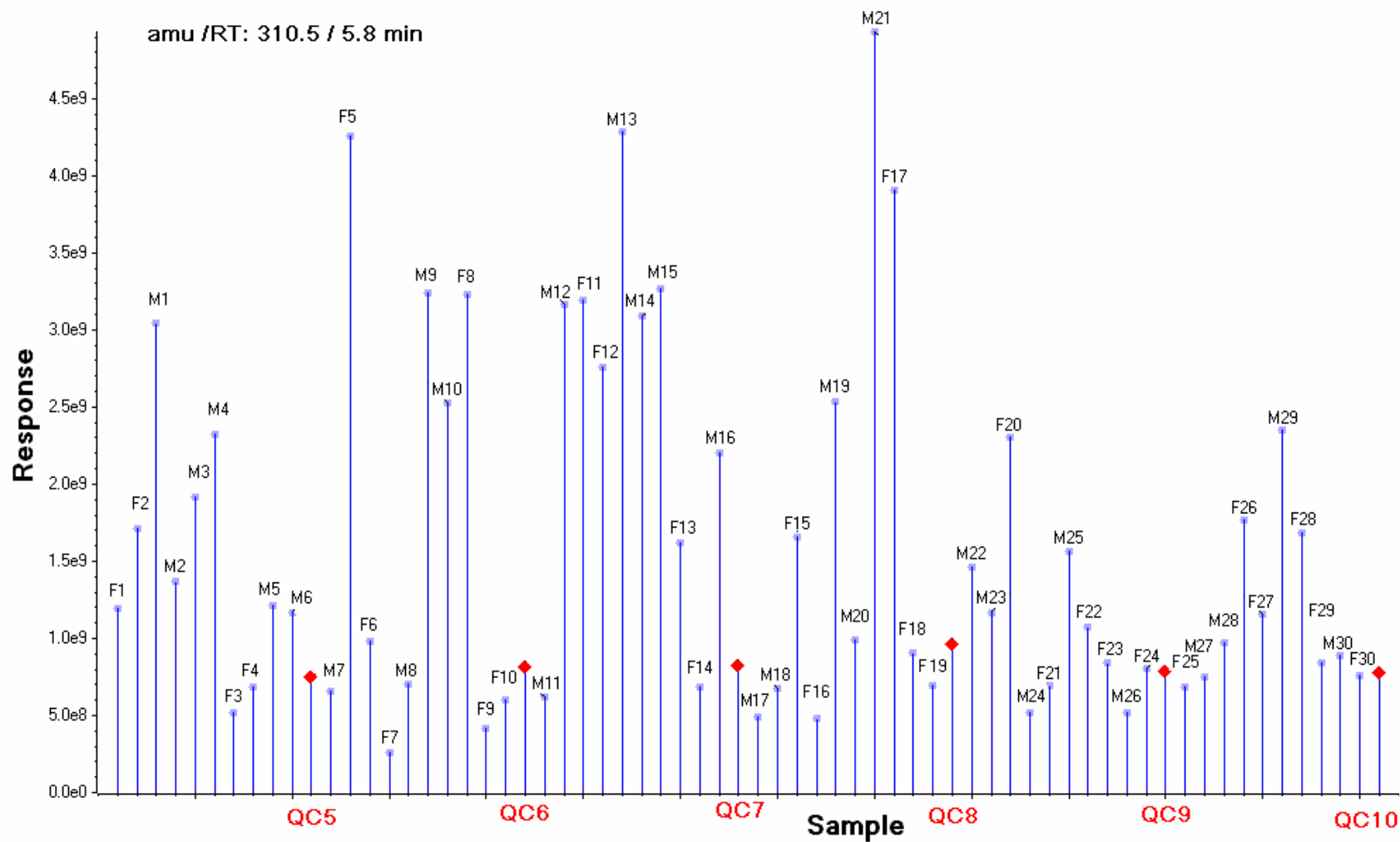
# Intensity vs reproducibility



# QCs for data assessment (1)



# QCs for data assessment (II)



# Applications to Disease Models

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- **A Type II diabetic rat model – the Zucker rat**
- **Tumours in the mouse**

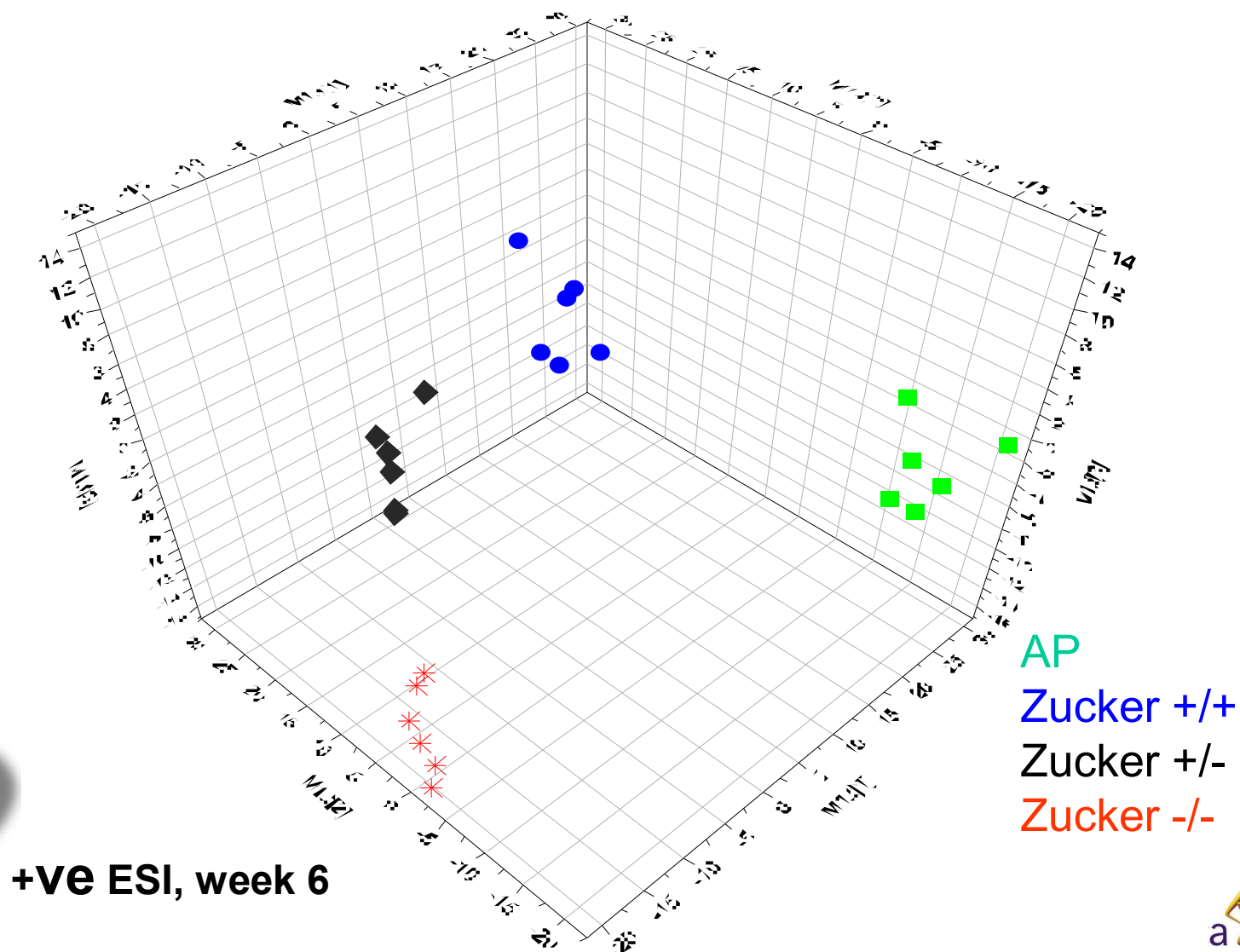
# The “Zucker” rat



- A model of adult onset diabetes
- Comes in 3 genetic “flavours” , -/-; +/- and +/+
- Experiment compared urinary HPLC-MS profiles of “control” vs Zuckers from 4 to 20 weeks.

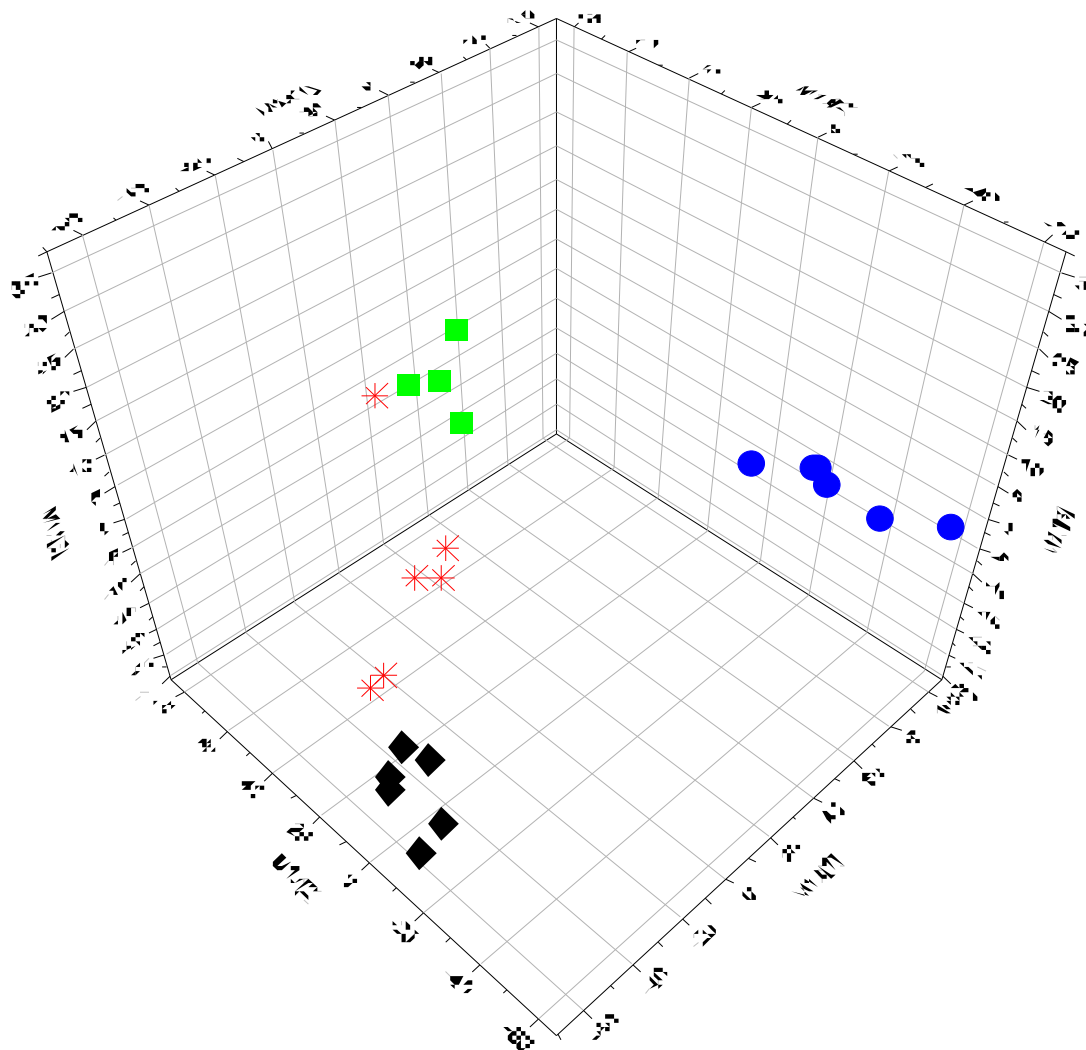


# HPLC-MS comparison by PLS DA



# HPLC-MS Comparison by PLS-DA

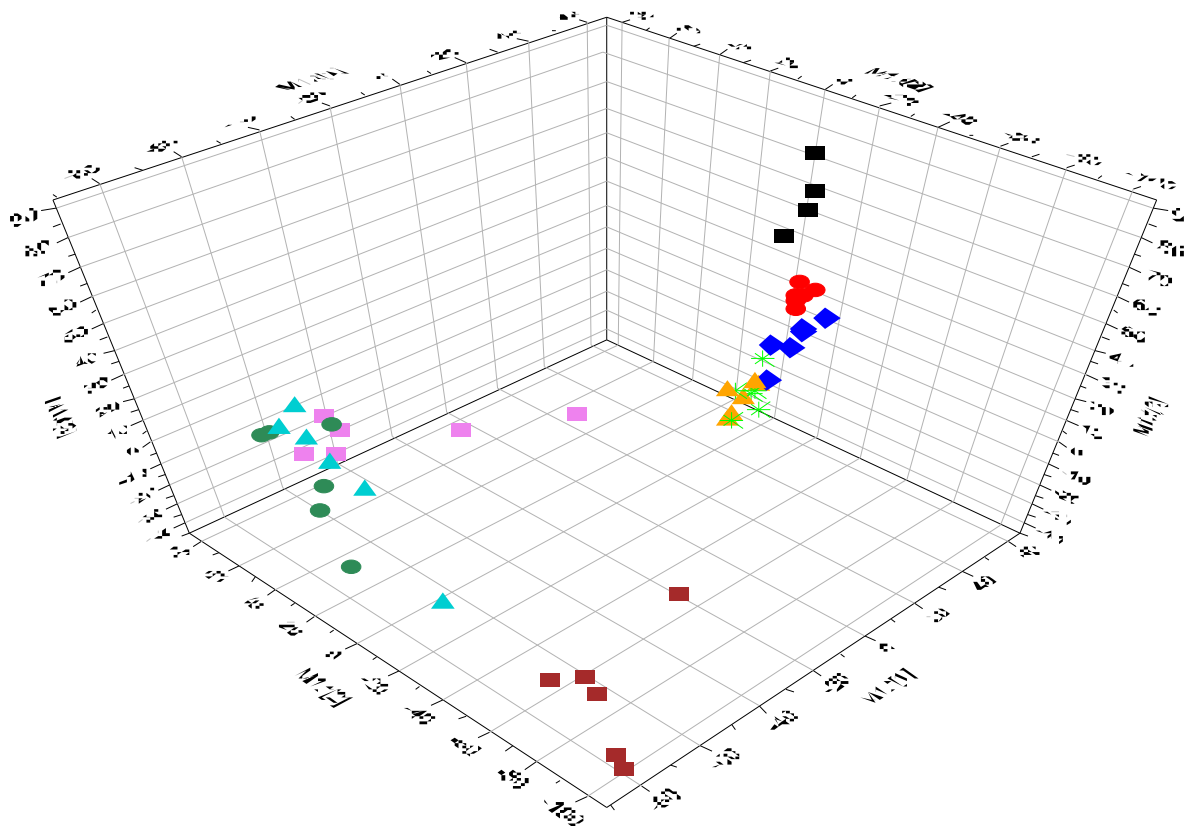
**+ve ESI**  
**week 20**



AP  
Zucker +/-  
Zucker +/-  
Zucker -/-

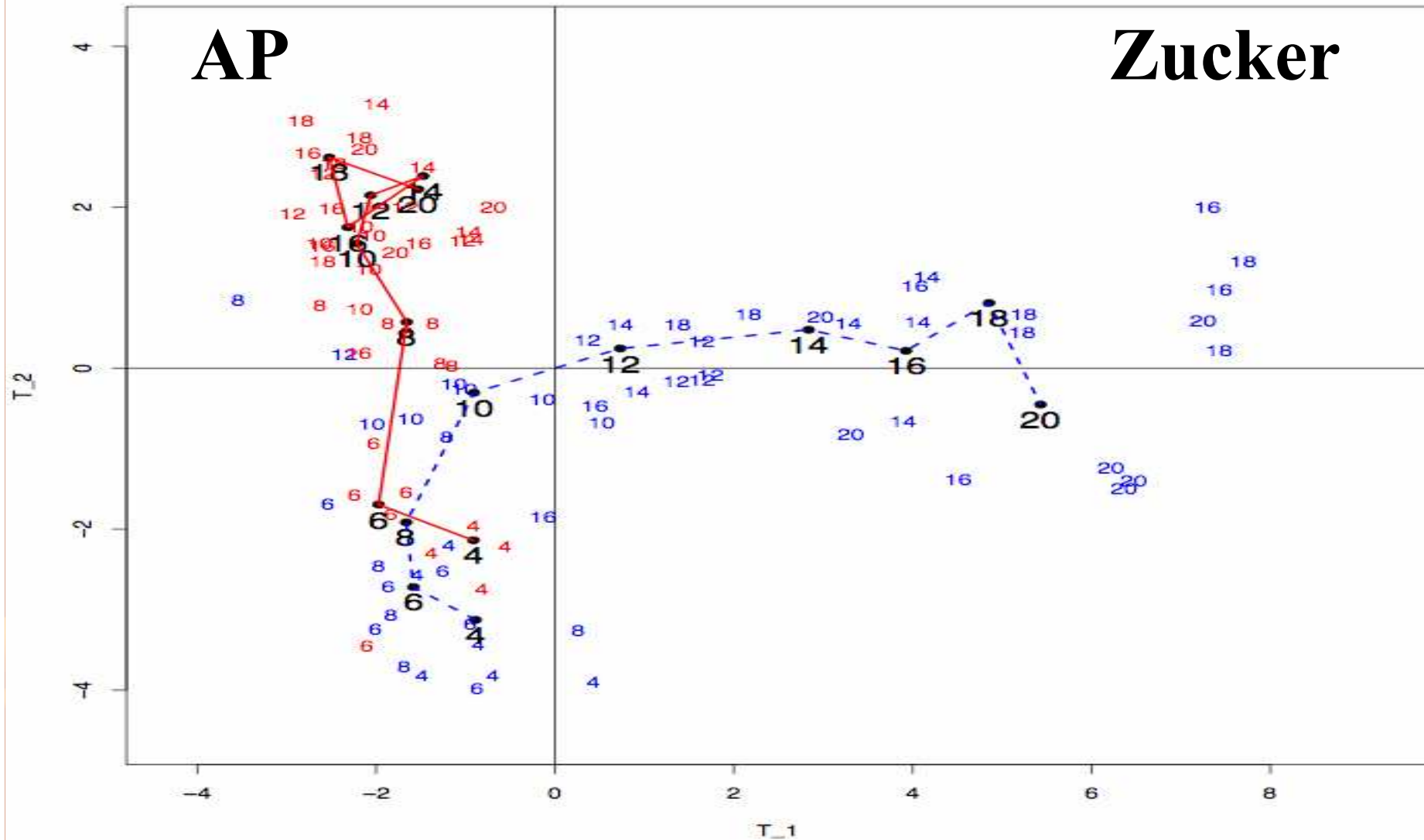
# Progression of +/+ rats through time

-ve ESI



Week 4  
Week 10  
Week 16  
Week 6  
Week 12  
Week 18  
Week 8  
Week 14  
Week 20

# The trajectory of disease



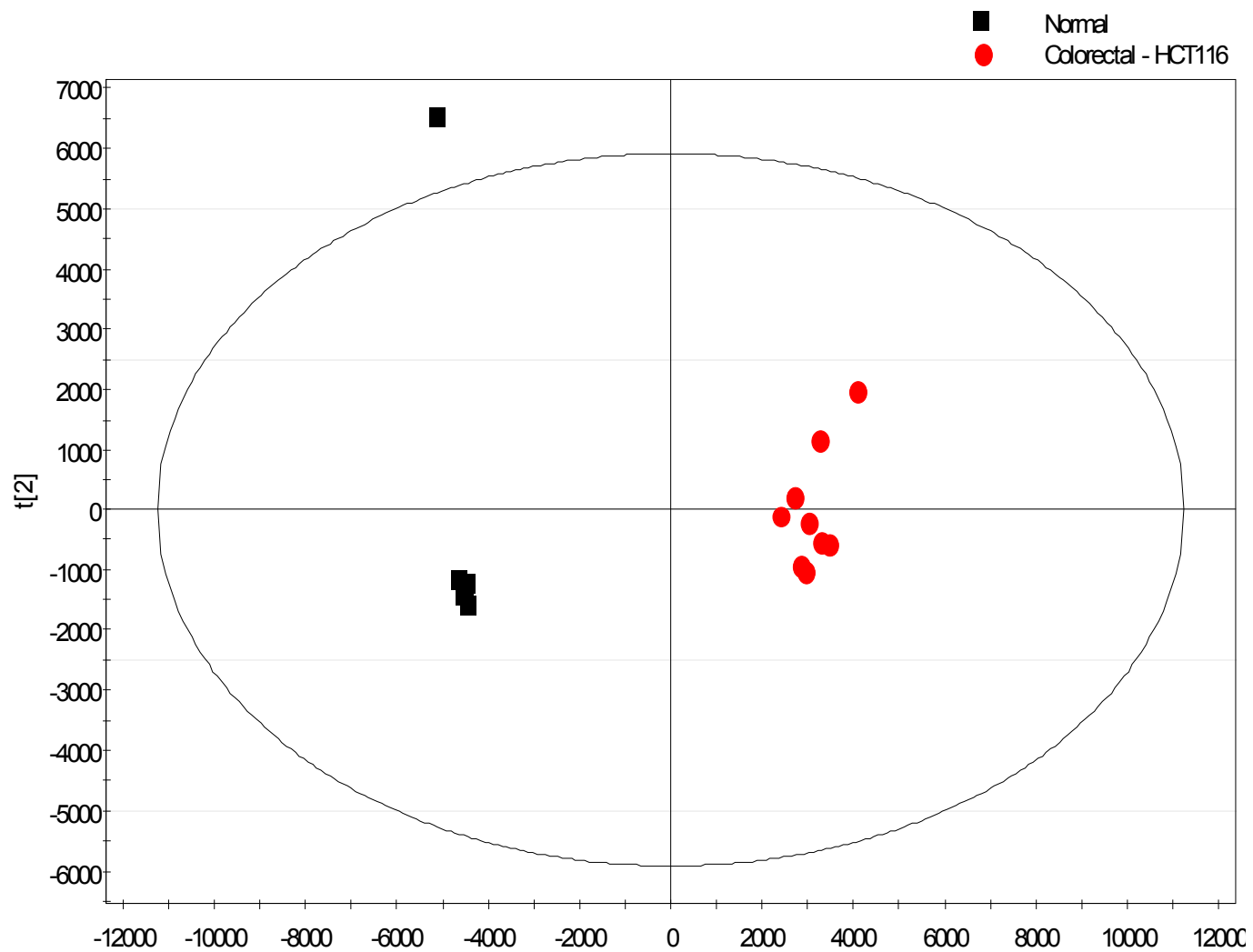
# Tumour models in mice

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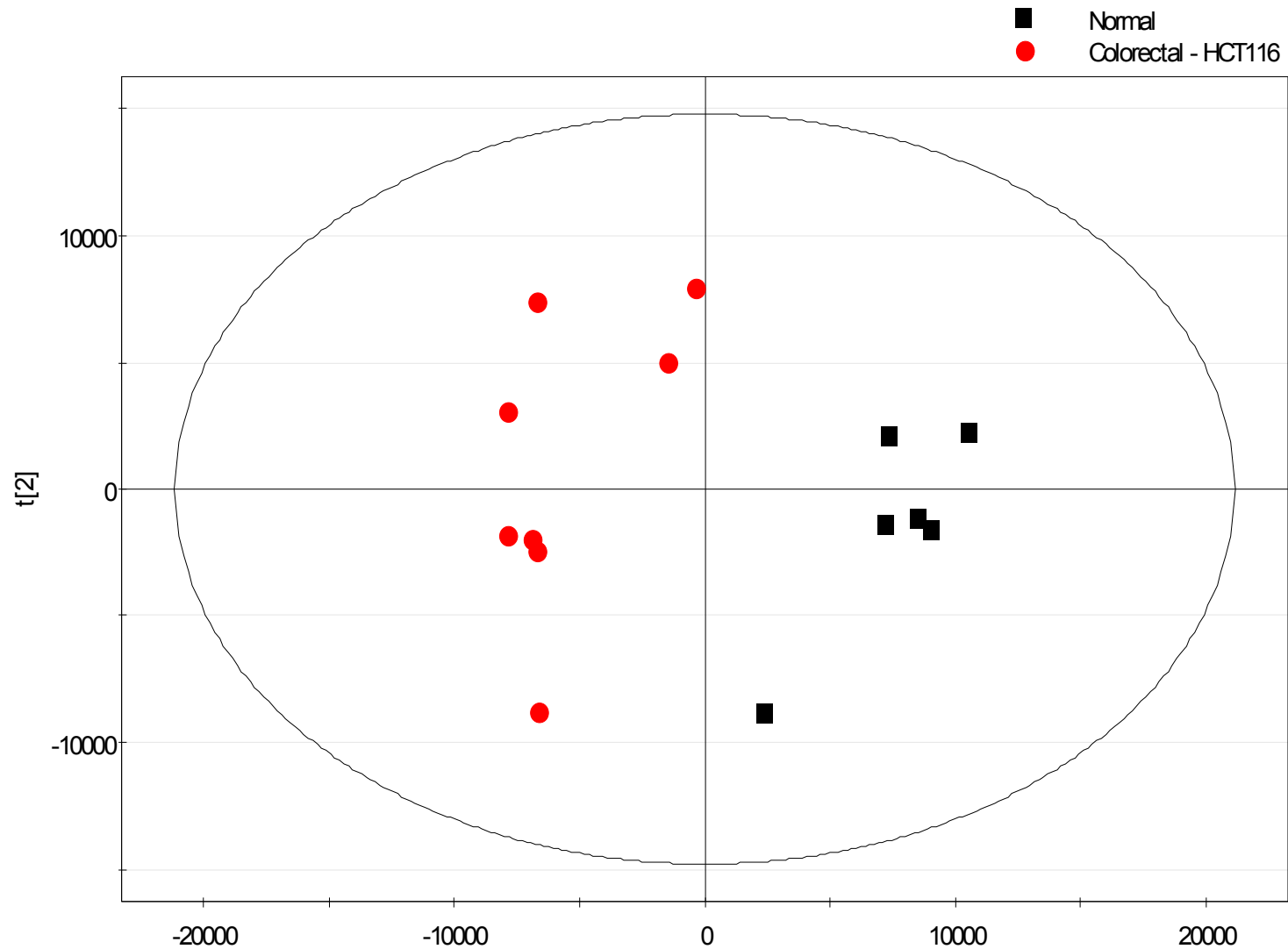


- **A range of tumours**
- **Plasma**
- **Urine**
- **Tumour samples**

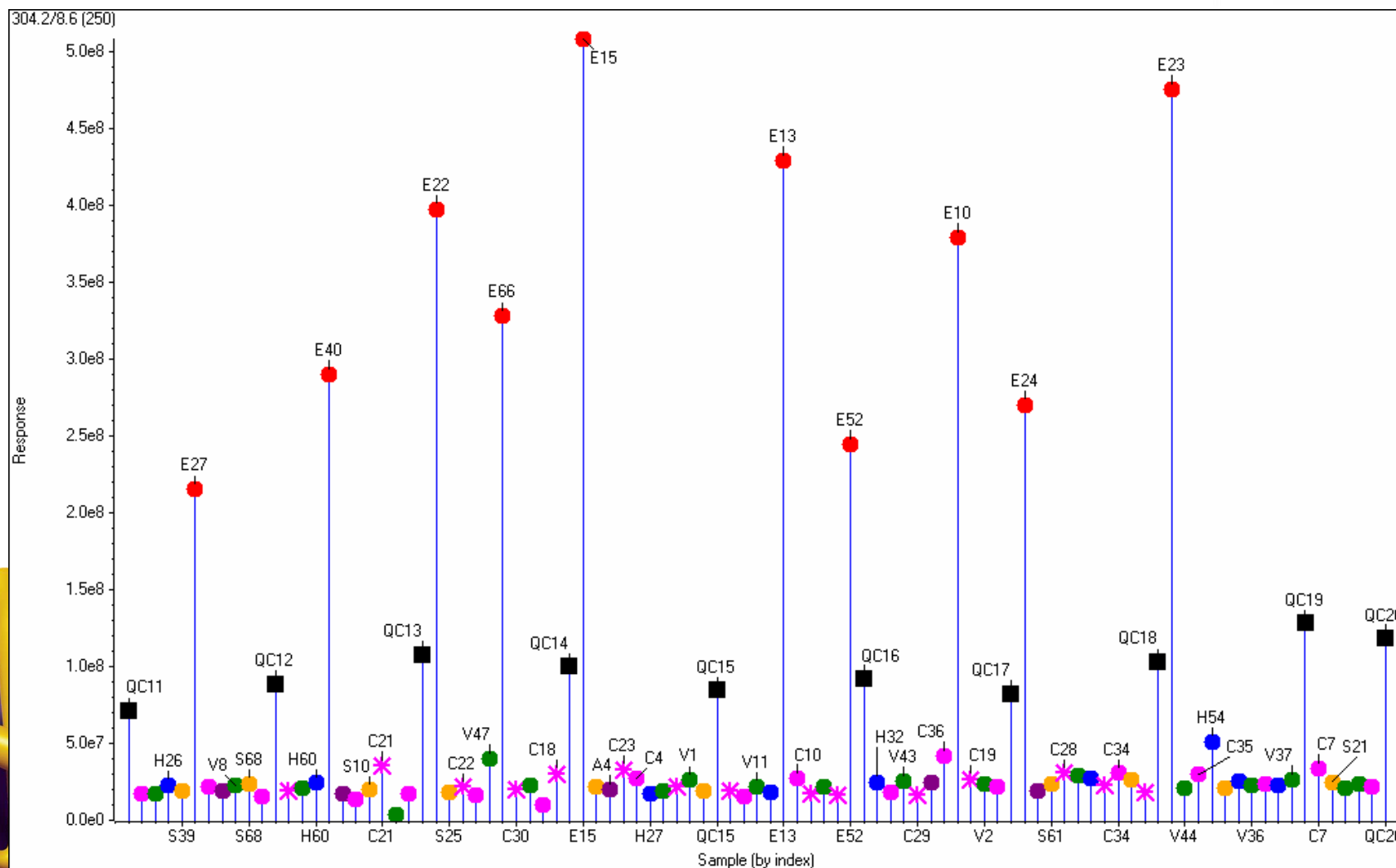
# Normal vs Tumour:Plasma



# Normal vs Tumour: Urine



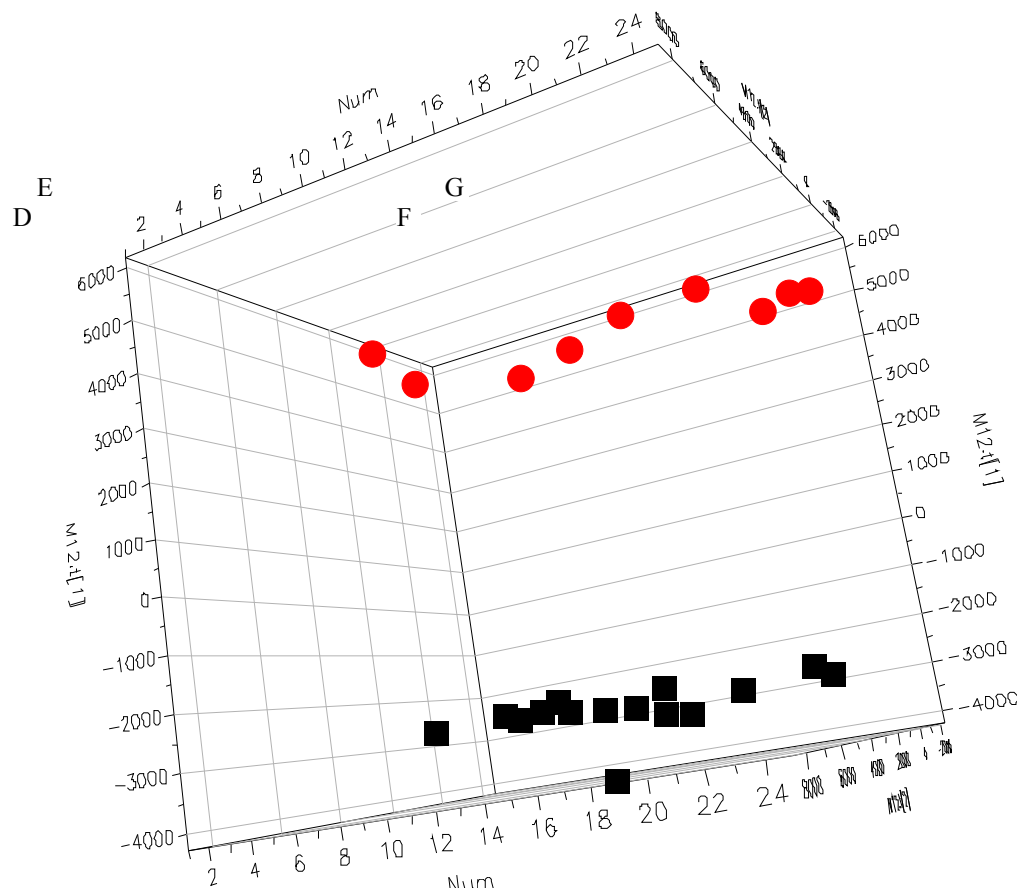
# A Plasma Marker of Colorectal Cancer





# Tumour vs Tumour: Plasma

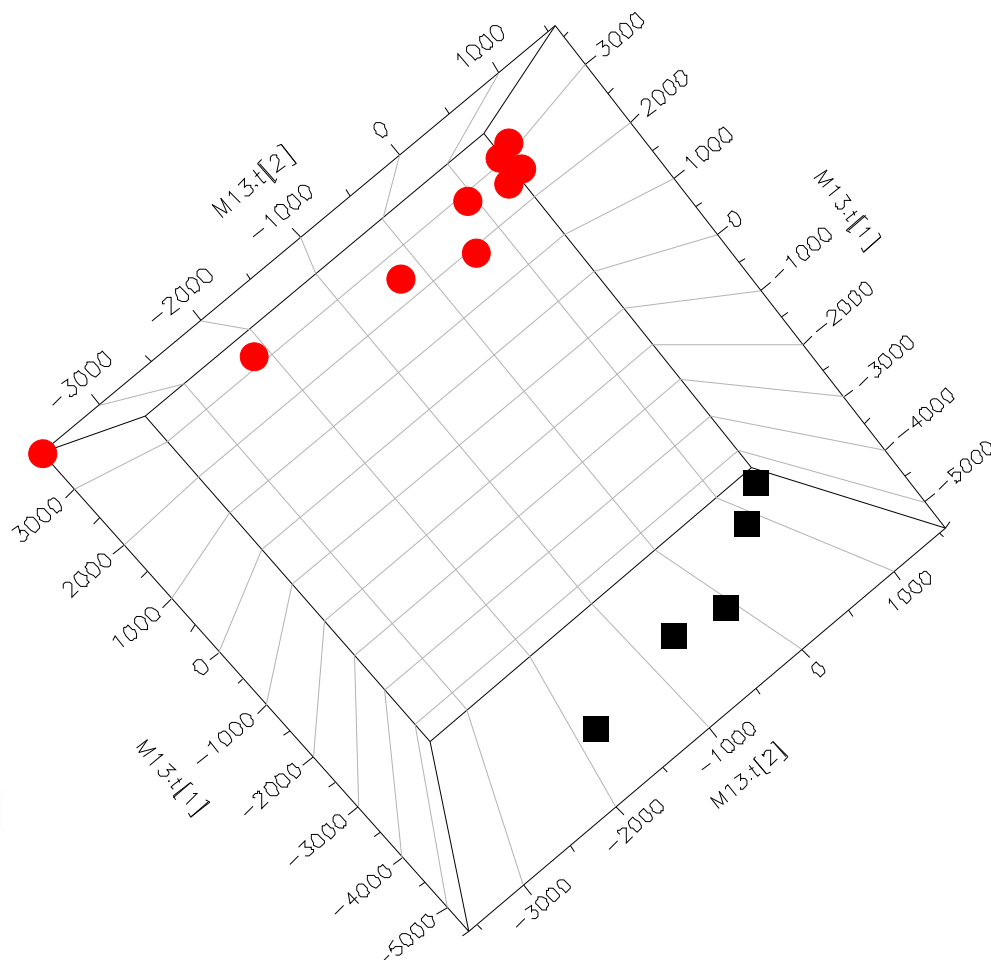
■ Non Small Cell Lung Cancer-CALU6      ● Colorectal-HCT116



Plasma  
A

# Tumour vs Tumour:Plasma

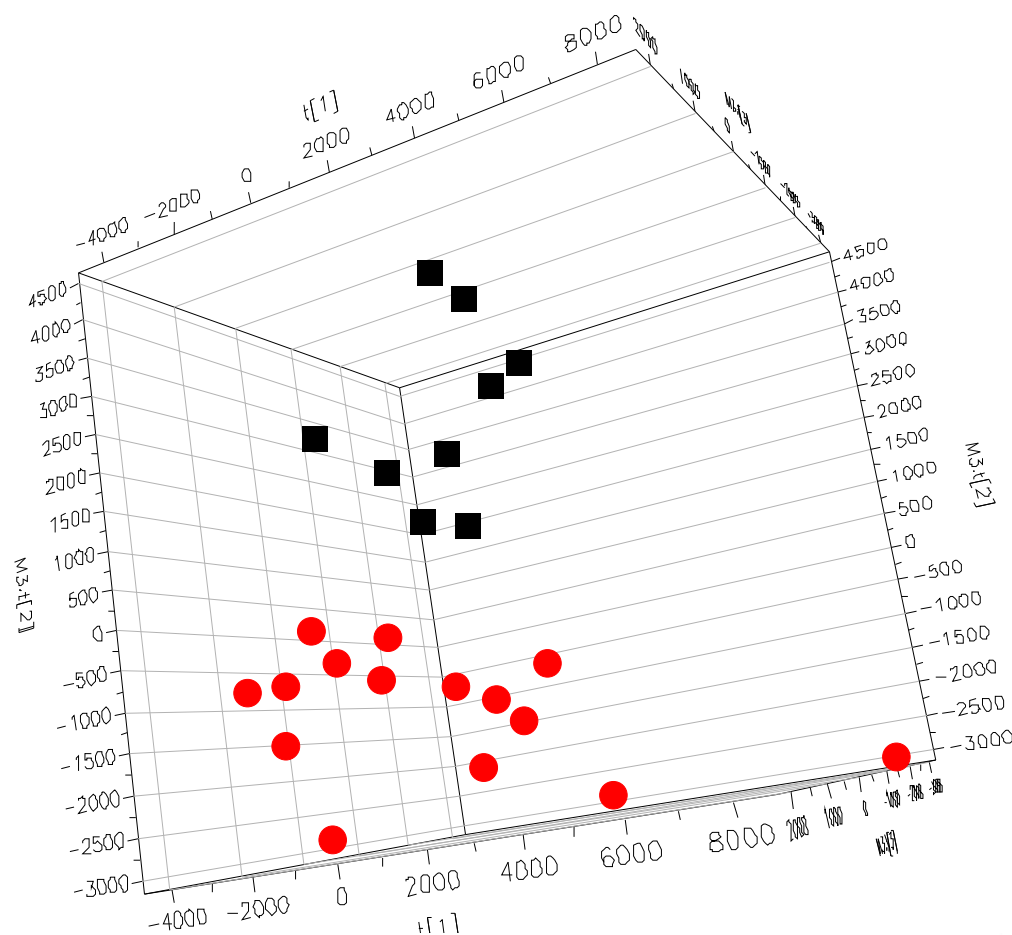
■ Skin-A431                      ● Colorectal-HCT116



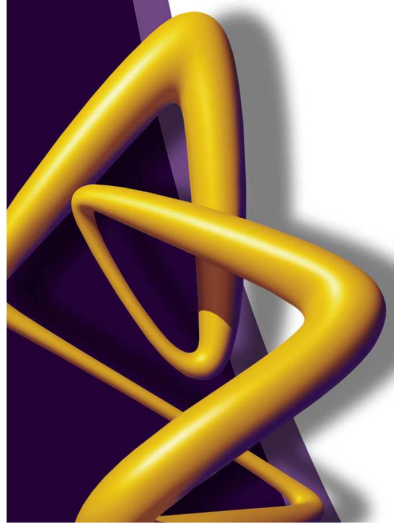
Plasma

# Tumour vs Tumour: Aqueous Extract

■ Colorectal-HCT116
 ● Non Small Cell Lung Cancer-CALU6



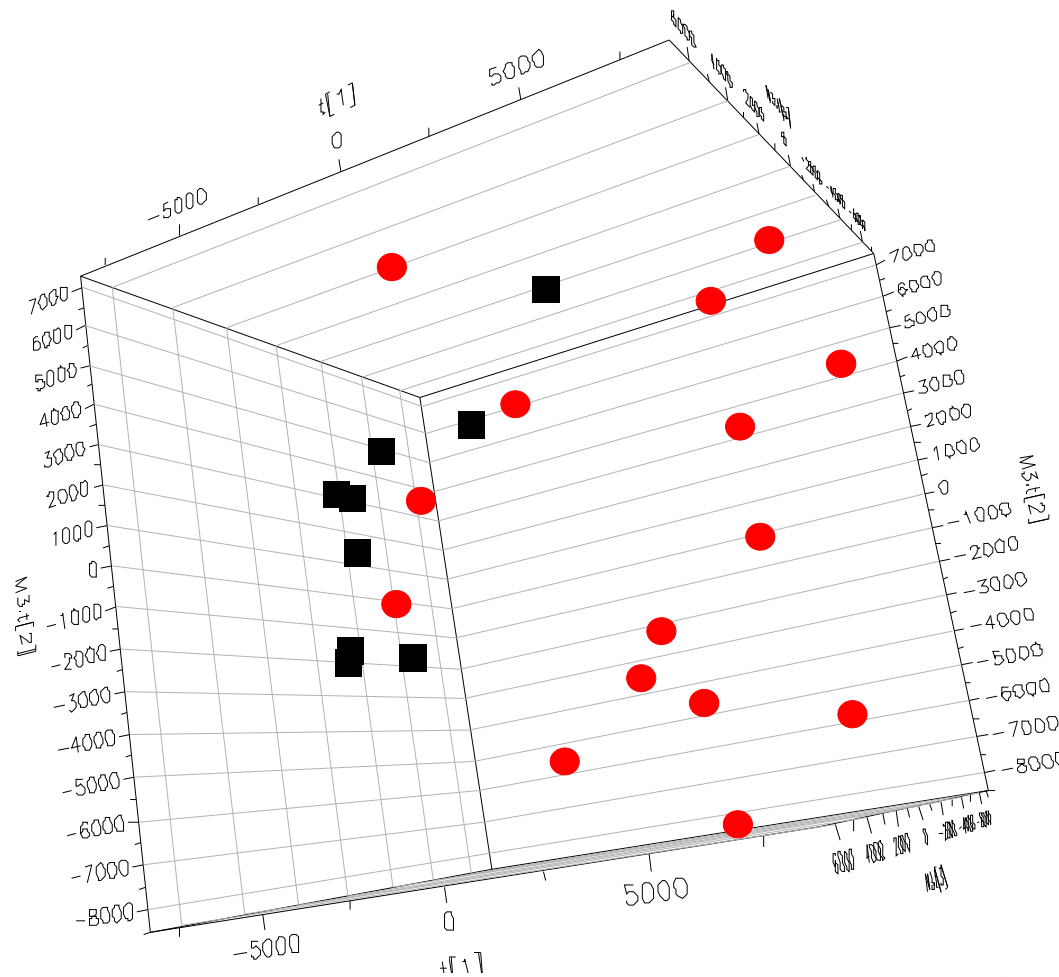
Uric D



# Tumour vs Tumour: Organic Extract

■ Colorectal-HCT116

● Non Small Cell Lung Cancer-CALU6



# Conclusions

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- **Global metabolic profiling (metabonomics/metabolomics) offers many opportunities for the discovery of biomarkers.**
- **NMR and LC-MS provide excellent platforms for obtaining such information**
- **Take great care with your methods!**