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Application of LC/MS(MS) to metabonomics studies

Robert Plumb, 8th Oct 2002 CPSA2002

Outline of presentation

- *Metabonomics*
- *Advantages and disadvantages of LC/MS vs NMR*
- *LC/MS methodology*
- *Data analysis a test case*
- *Identification of biomarkers*
- *What do we do with polar compounds*
- *Conclusions and Acknolwdgements*

Understanding of the Concept

- *Study of endogenous metabolites rather than xenobiotics*
- *Primary aim is the identification and quantitation of small molecules in a biological system*
- *Down-regulation and Up-regulation of these metabolites are indicative of biological insults due to disease, toxicity, genetic modification or environmental factors*
- *Knowledge of these metabolites and possible biomarkers, may be used for diagnosis and screening*

Metabonomics and Metabolomics

Metabonomics

"Quantitative measurement of time-related multiparametric metabolic responses of multicellular systems to pathophysiological stimuli or genetic modification".

Metabolomics

"The measurement of metabolite concentrations and fluxes in isolated (and usually identical) cell systems or cell complexes".

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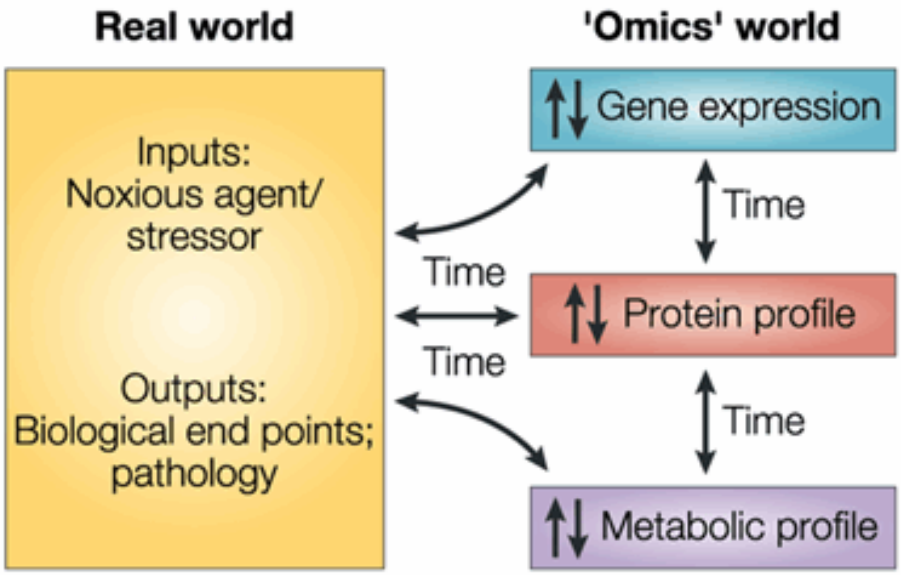
Real World vs the "Omics" World



Nature Reviews 2002 Vol1. 153-161

"Metabolic changes are real world end points, whilst gene expressions indicate the potential for an end-point change"

(J Nicholson Nature 2002)

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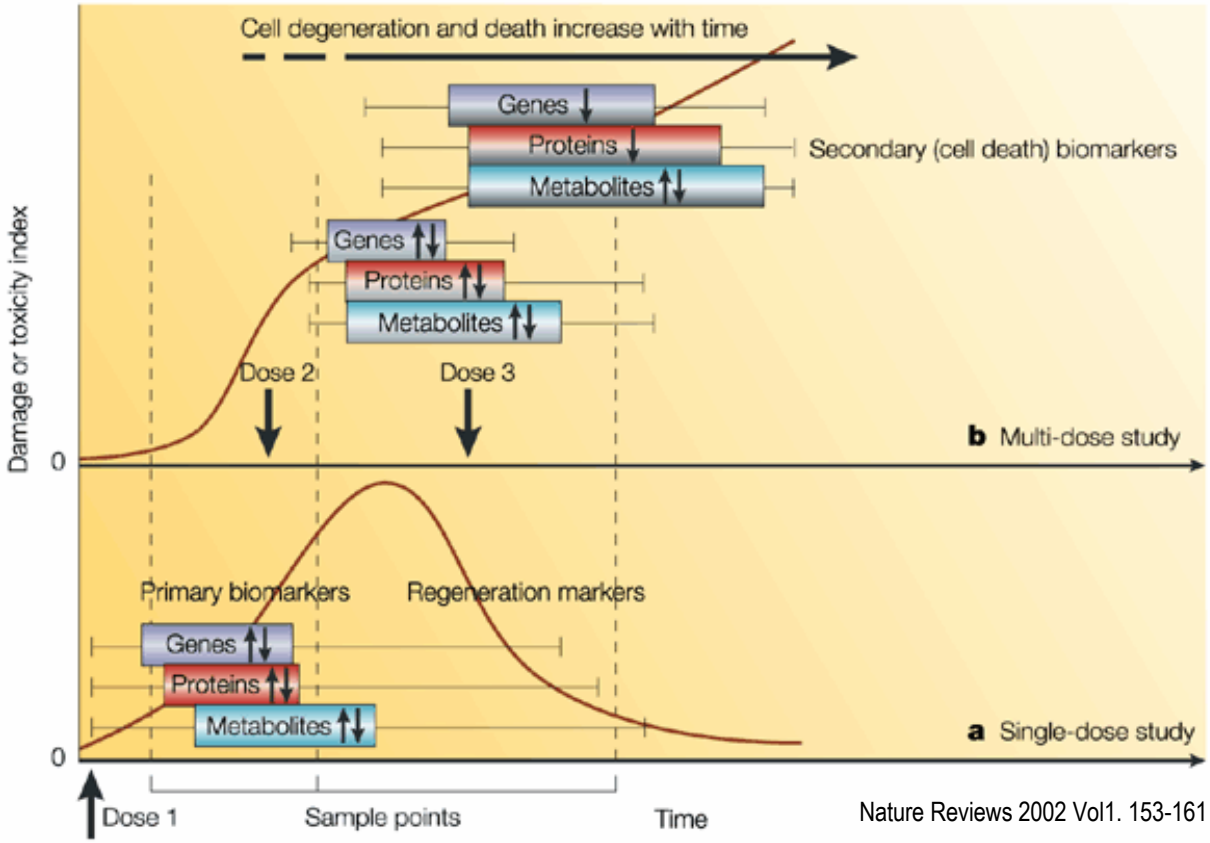
Effect of Time in Toxicological Studies



Nature Reviews 2002 Vol1. 153-161

The "Omics" response varies as cell degeneration increases

(J Nicholson Nature 2002)

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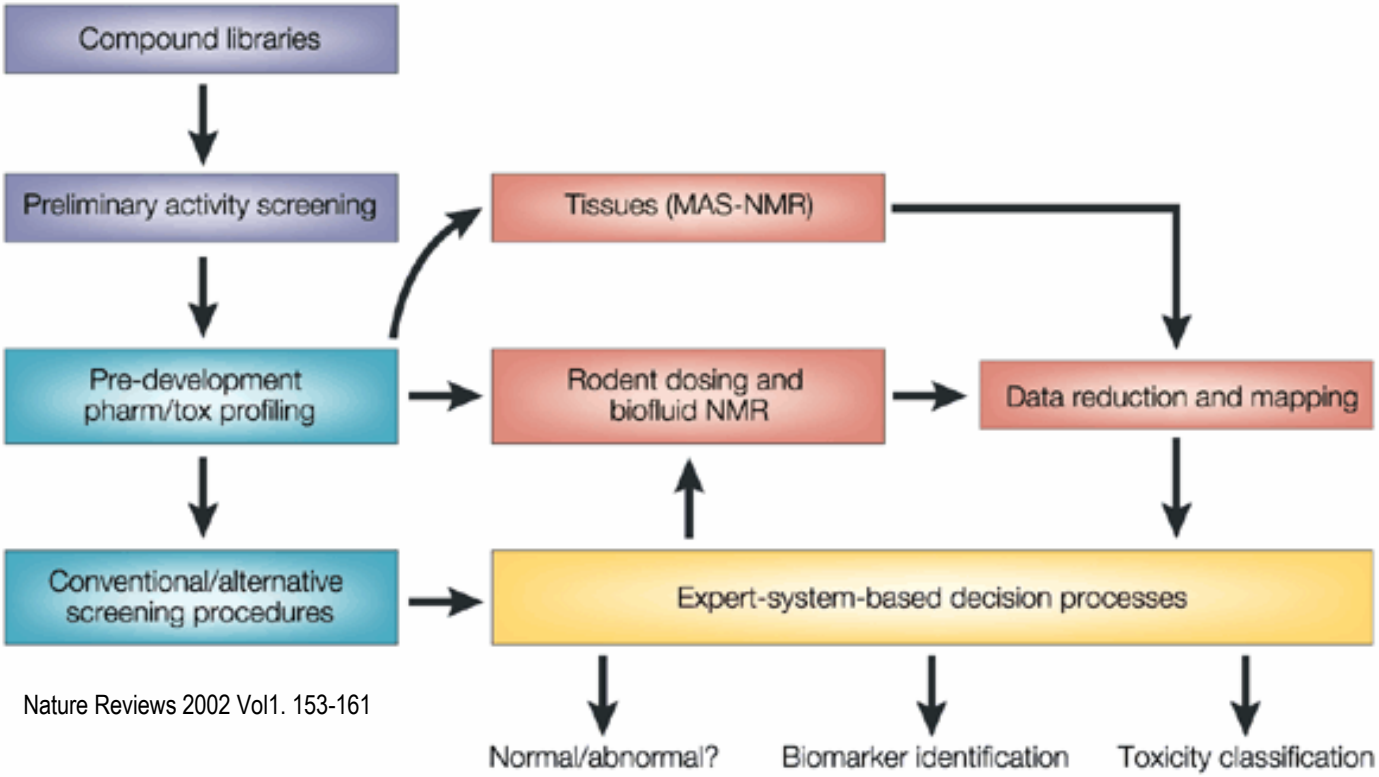
Metabonomic Integration for Toxicity in DMPK



Nature Reviews 2002 Vol1. 153-161

"Integration of data types will also pave the way to understanding the relationship between gene function and metabolic control in health and disease"

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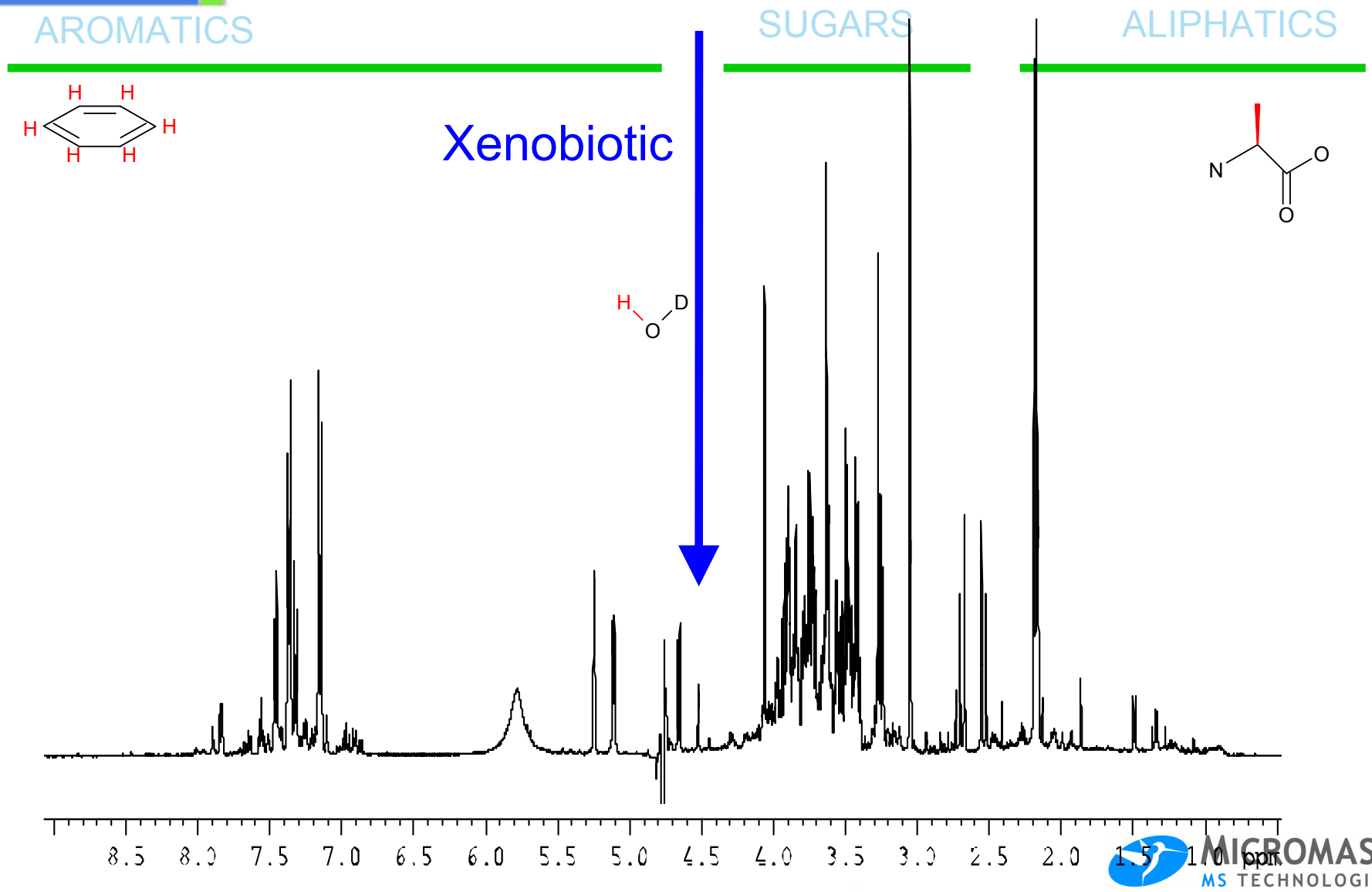
Advantages of LC/MS over NMR



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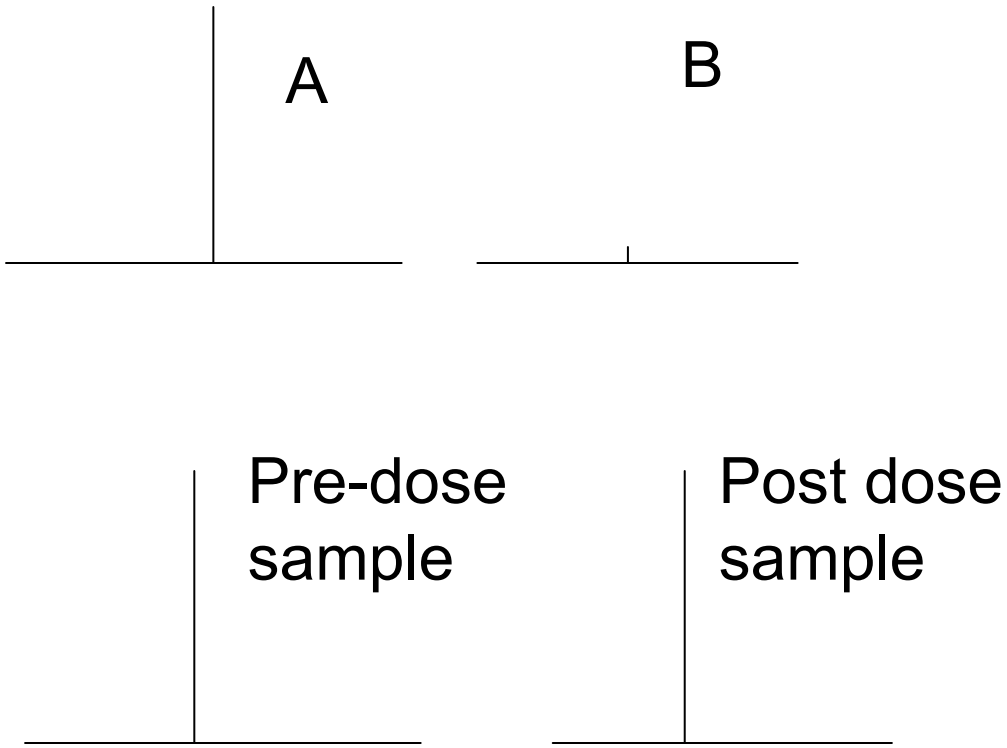
Advantages of LC/MS over NMR contd…

- *When removing xenobiotics NMR data is lost. This can be replaced with archive control data.*
- *But what if there was a biomarker is in this region that had been up/down regulated ?*
- *This also takes time and assumes that you have an archive of data for every dosing vehicle combination, DMSO, PEG, Tween 80 etc..*

Overlaid data

- *Scenario:-*
  - *Small abundance component appears in the same region of the NMR spectra as a large concentration component.*
  - *The peak height ratio is 1:10*
  - *Natural variation in large component is +/- 15%*
  - *After dosing B is 50% down regulated.*
- *Result:-*
  - *Variation in B never observed*



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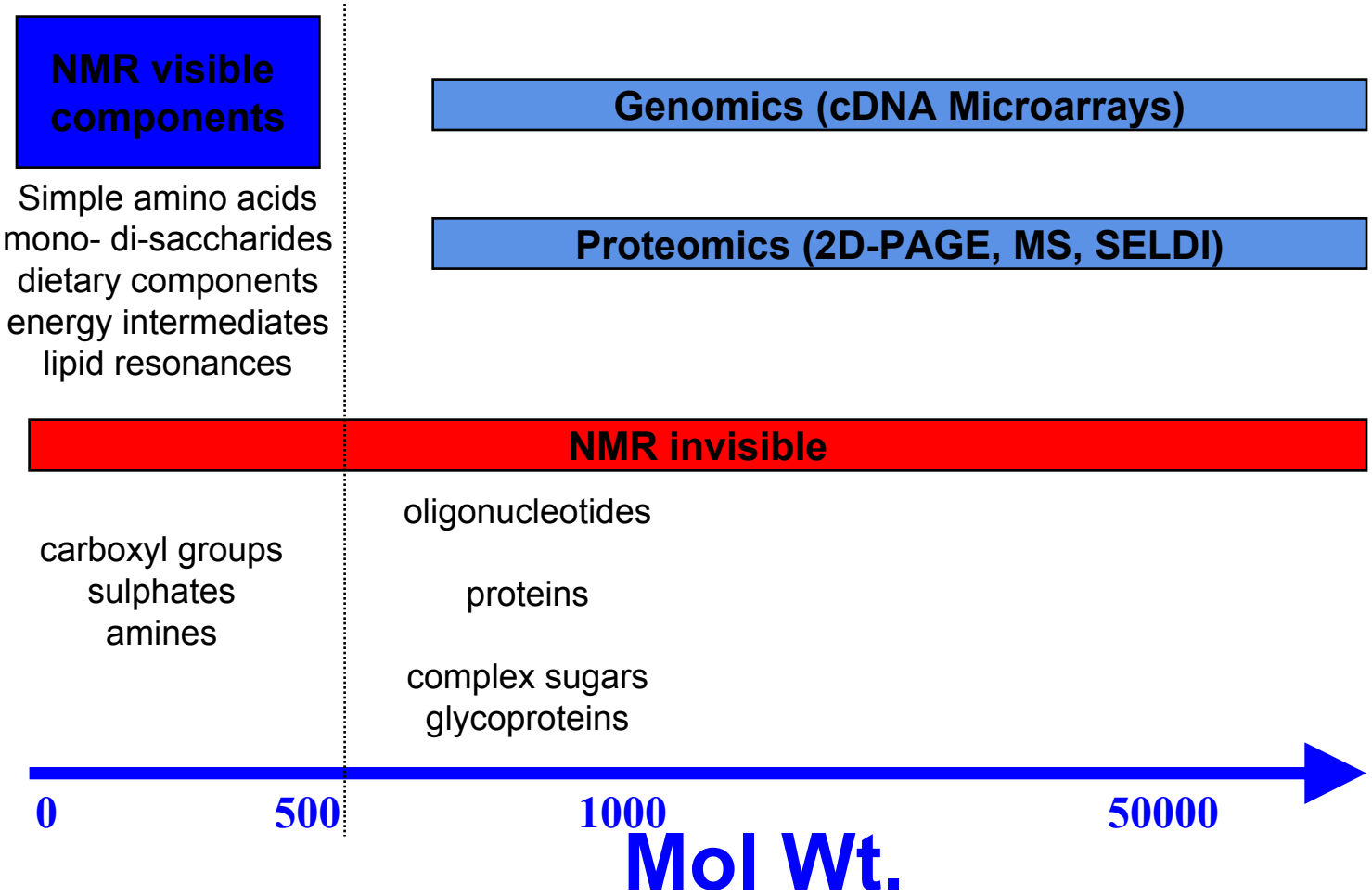
NMR is the Current Method of Choice, but with Limitations.



Provided by John Haselden (GSK)

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Sample Set to LC/MS

- *20 rat urine samples*
- *Two time points: 0-8 hr and 8-24hr*
- *3 x 2 controls*
- *3 compounds, 3 samples per time point, 2 time points*
- *Simple Sample Prep: Samples centrifuged then diluted 1:4 with distilled water prior to analysis by LC/MS*

LC/MS methodology

- *Chromatography:*
  - *Column: Waters XTerra® C18 100 x 2mm 3.5µm*
  - *Eluent: Reverse Phase*
  - *Flow Rate: 600 uL/min (slower for LC/MS/MS)*
- *Mass spectrometry*
  - *Quattro micro™ or Q-Tof micro™*
  - *Scan range 100-1000m/z*
  - *Data collected from 0-10mins (30 minutes for LC/MS/MS)*

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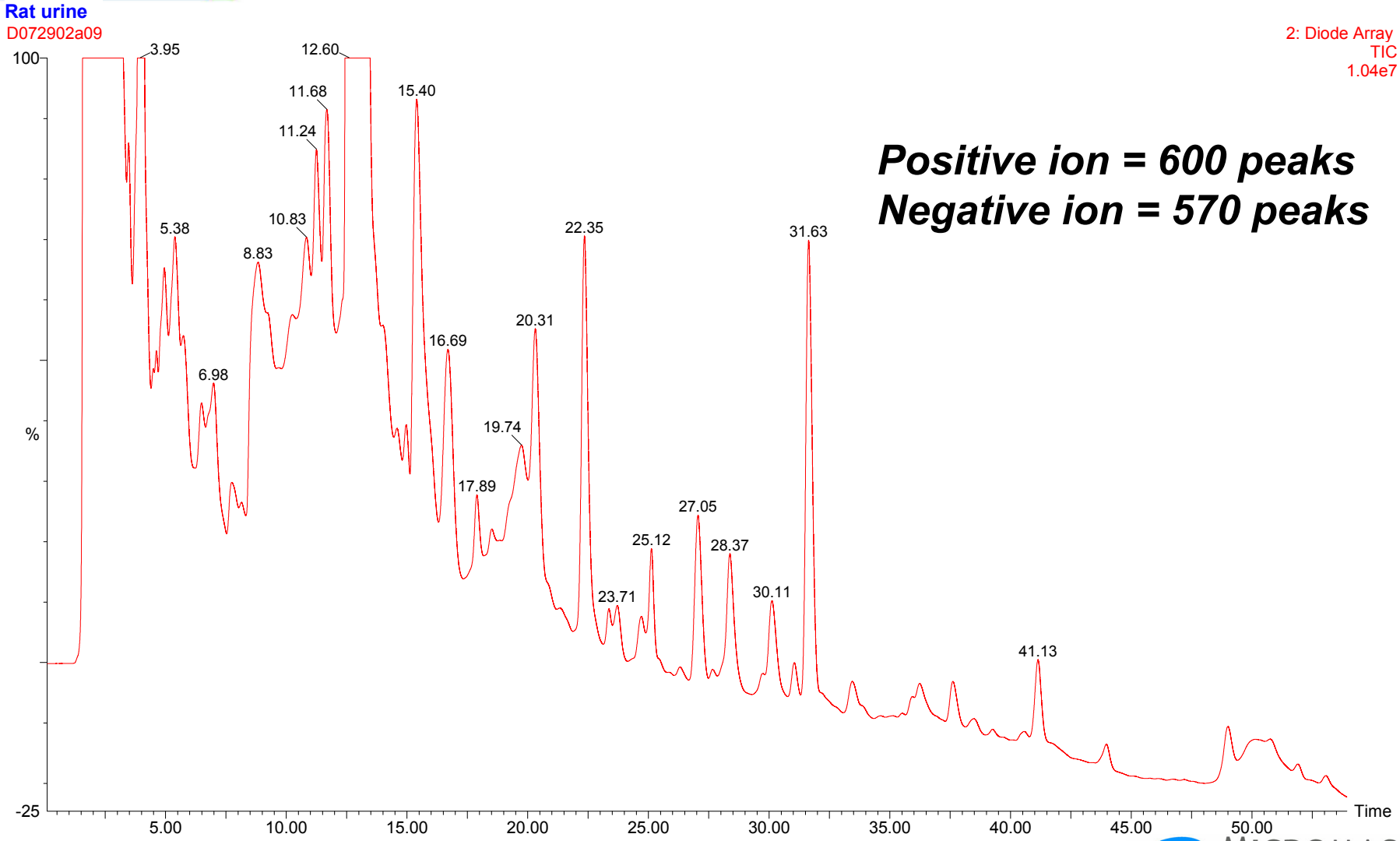
Typical urine 60 minute chromatogram



Positive ion = 600 peaks
Negative ion = 570 peaks

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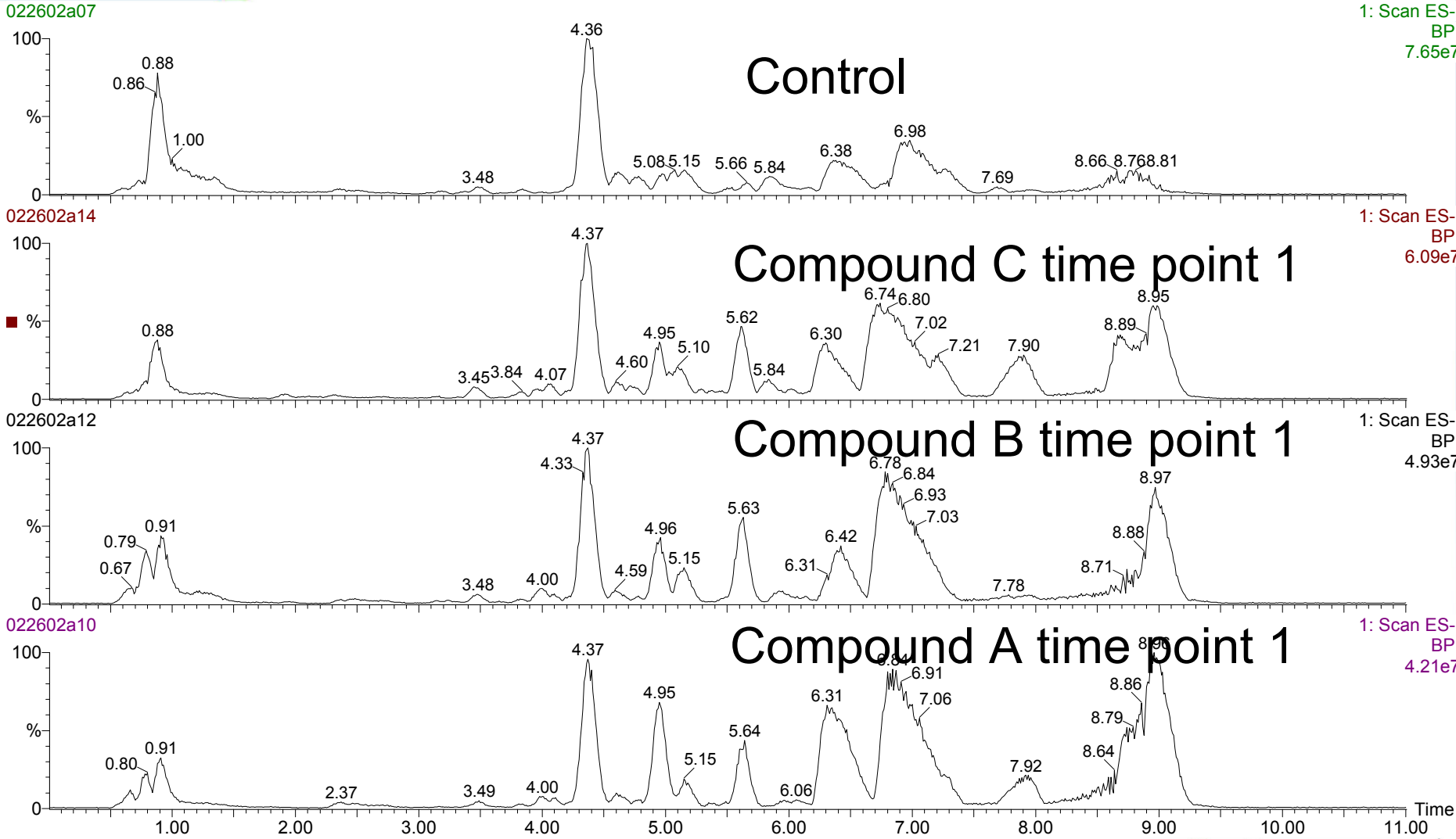
LC/MS TIC data: Qualitative Differences



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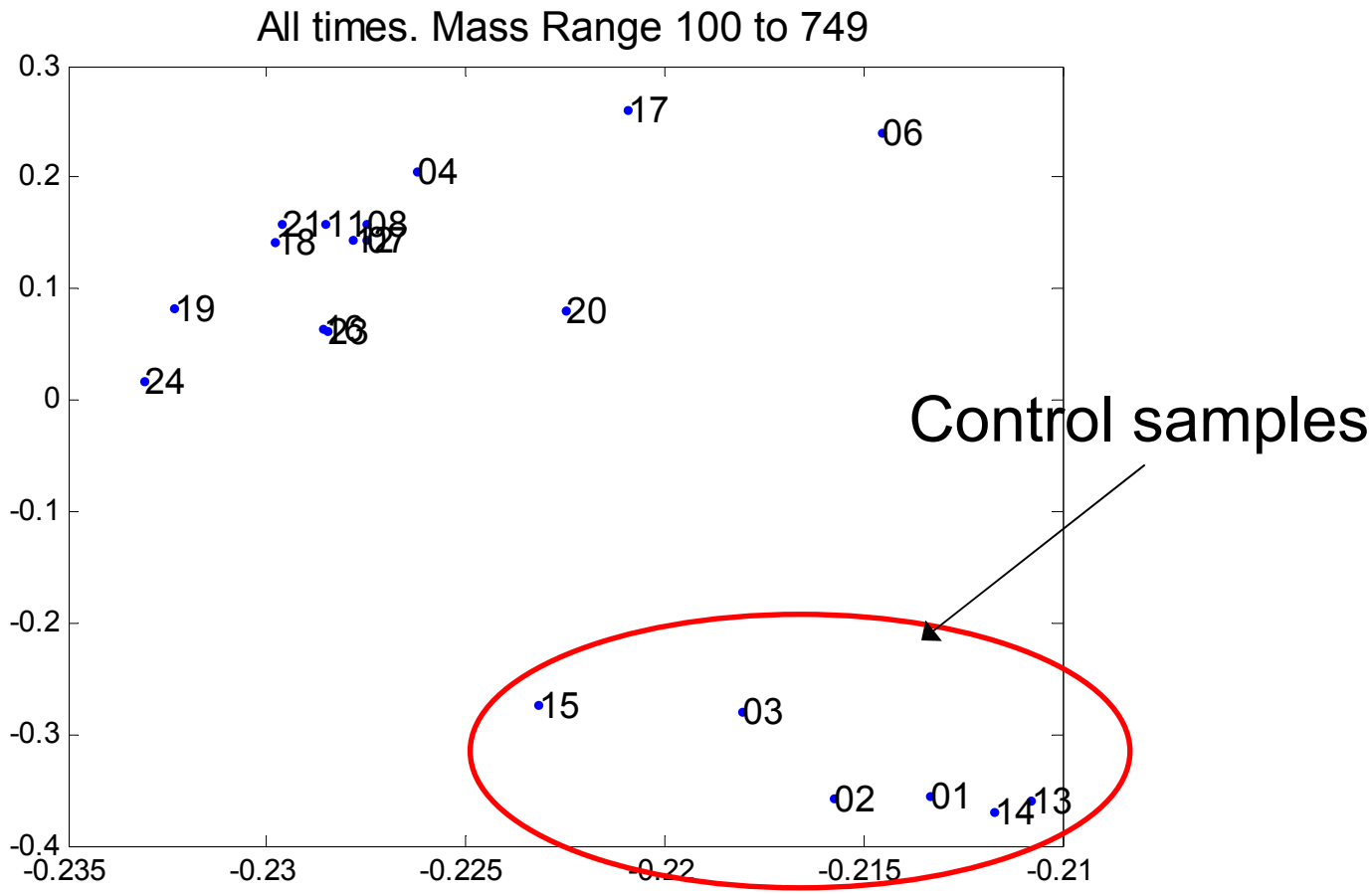
An Easier Way to Analyze the Data?

- *BioMarkers can be found by analyzing the Mass Spectra one-by-one. However this is cumbersome.*

- *Principal Component Analysis is a Multi-variant analysis technique that provides a Global View of the Data! (Potentially this could be faster).*

PCA for the whole data set

MassLynx -> Excel -> MatLab (PCA)



Data from March 2002

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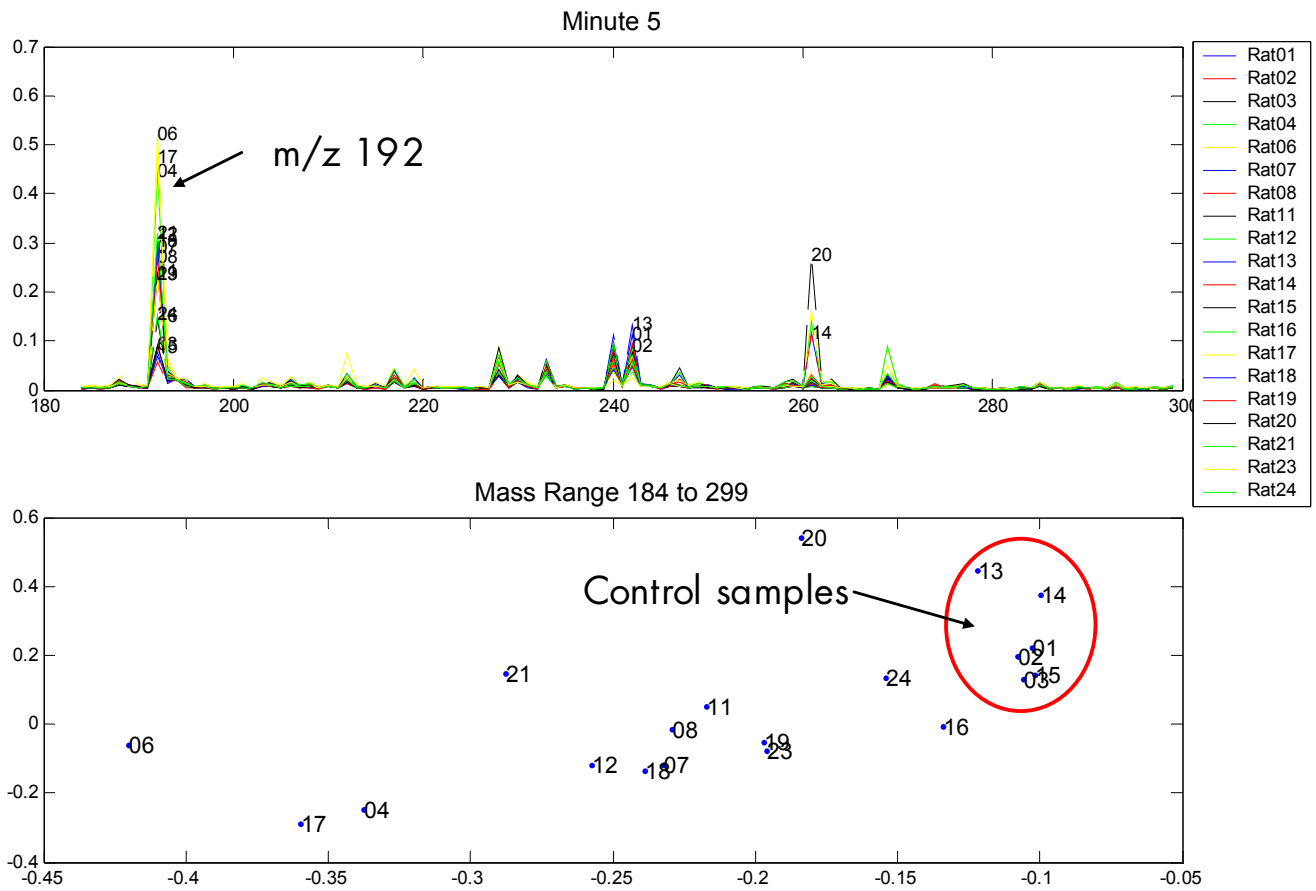
PCA investigation from Minute 5 of Chromatogram



Note: LC Retention Information is Utilized!

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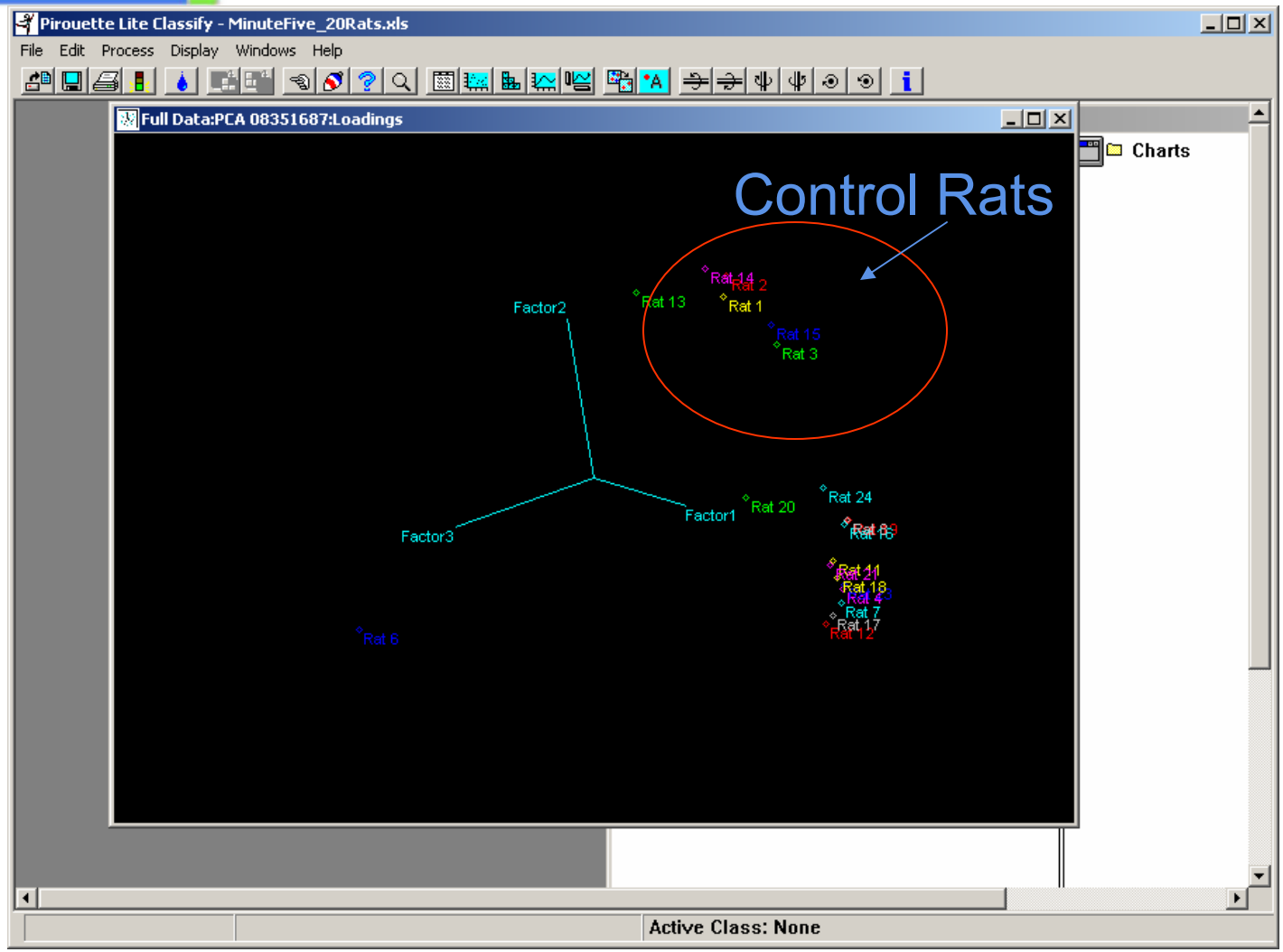
Pirouette Loadings Plot: Minute 5



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Ions identified as responsible for PCA separation

Compound dosed	Analyte m/z value	Change
A	283	10 fold increase
A	461	5 fold increase
A	187	10 fold increase
B	338	2 fold reduction
B	283	10 fold increase
B	461	10 fold increase
B	187	10 fold increase
C	283	20 fold increase
C	187	30 fold increase
A,B,C	192	3 fold increase

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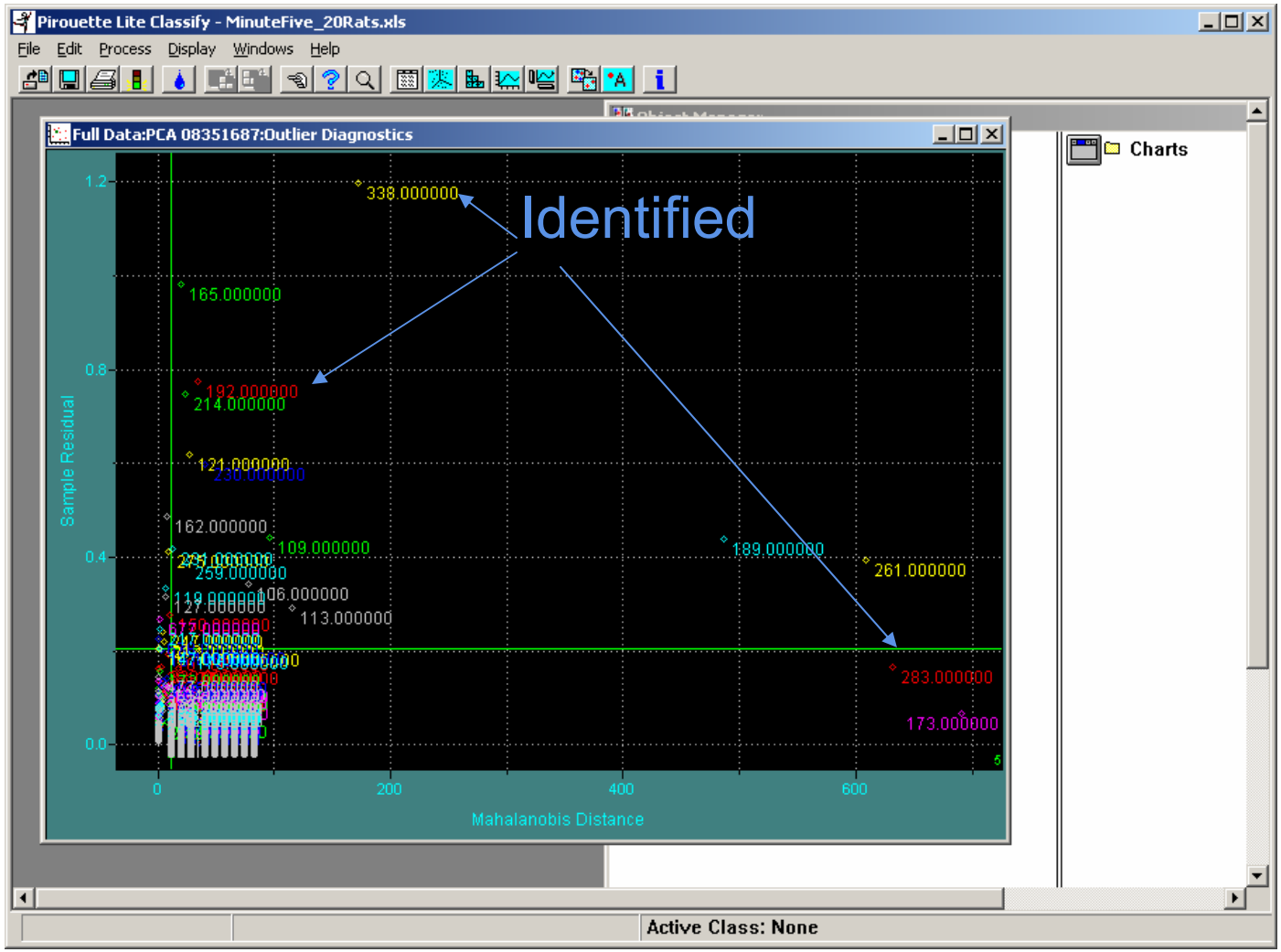
BioMarkers Indicated!



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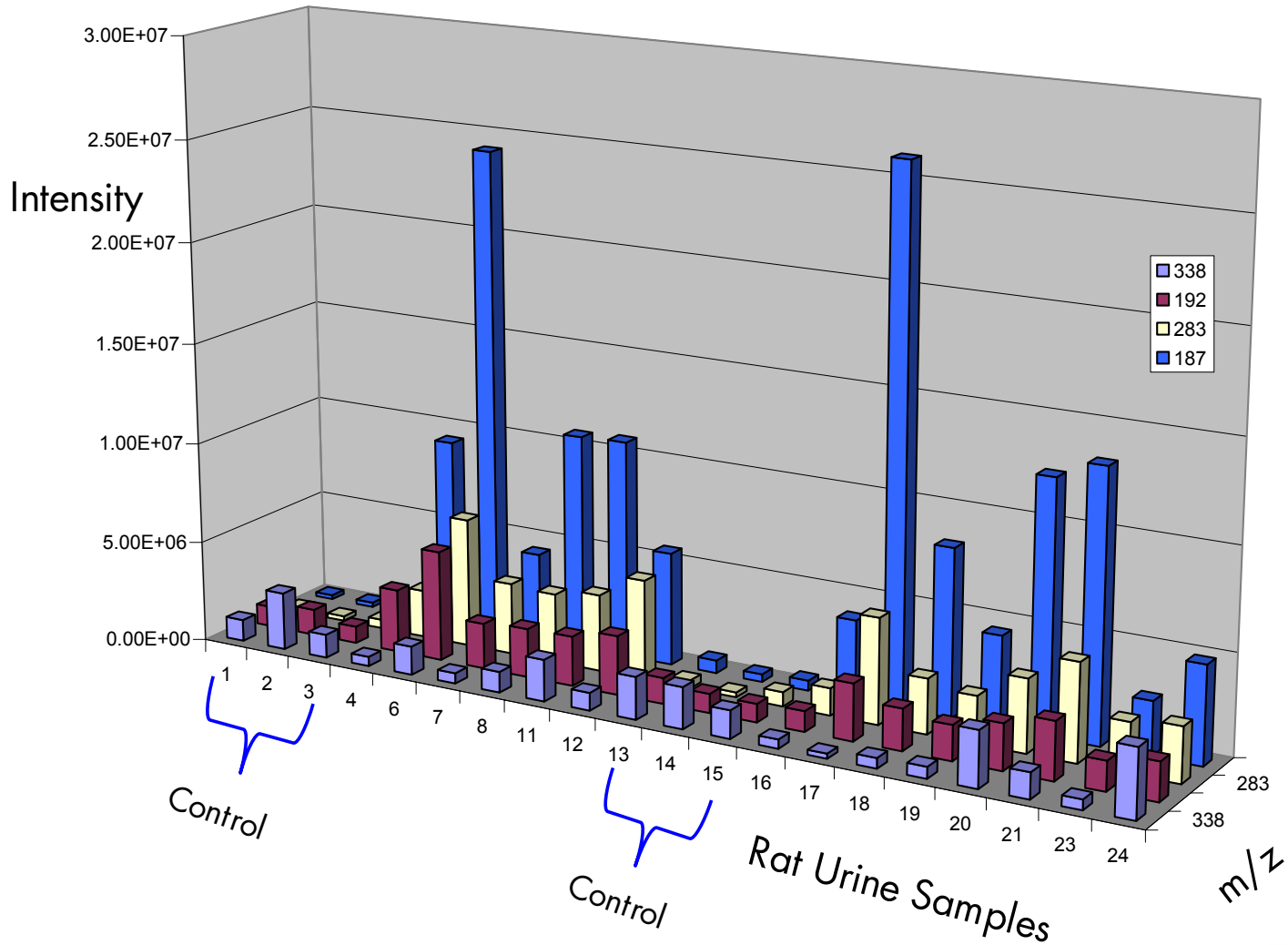
Ion intensity comparison of PCA identified ions (m/z 187, 192, 283, 338) in all 20 rat urine samples.



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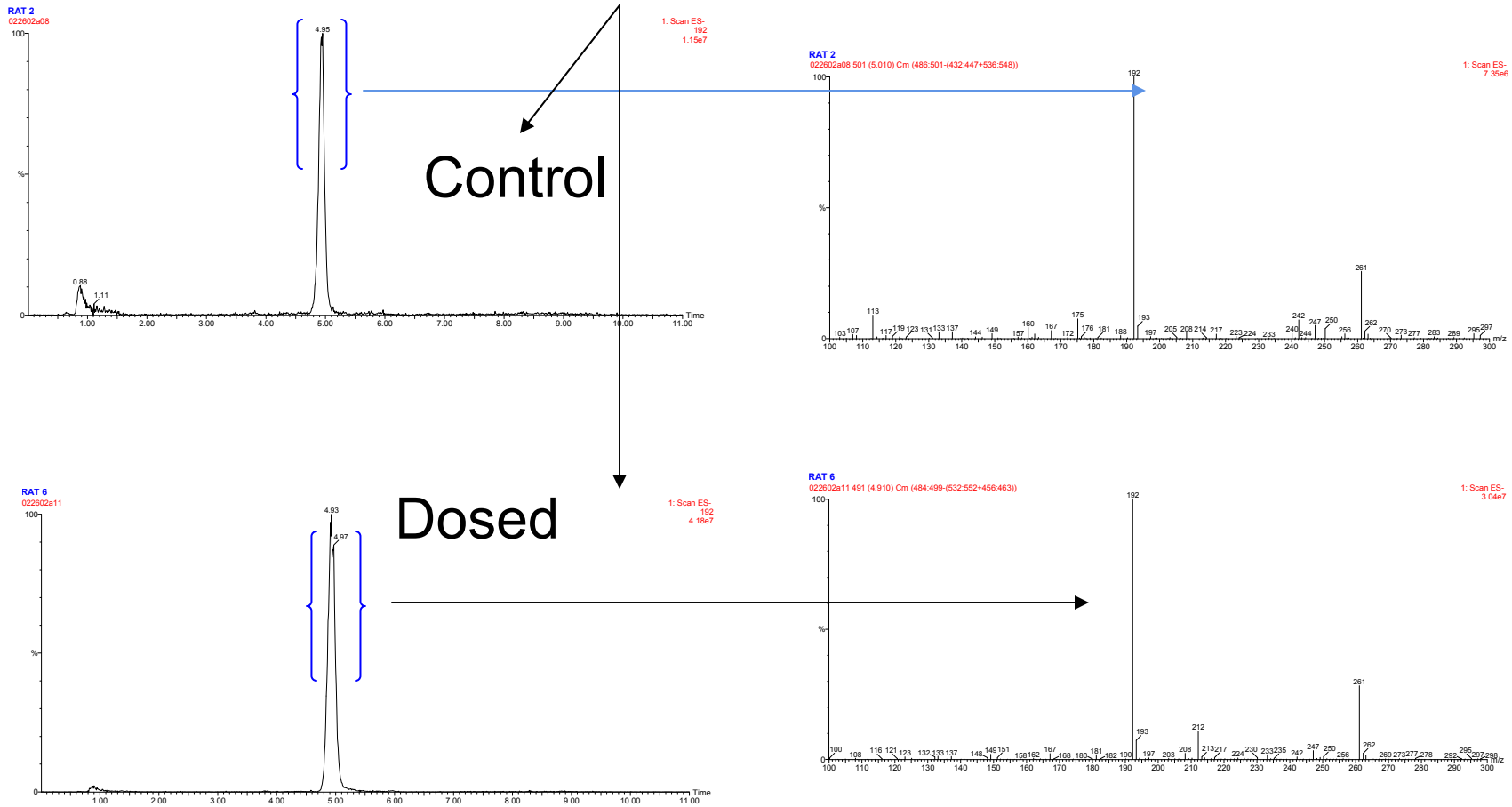
LC/MS analysis of 192 ion

Endogenous Metabolites



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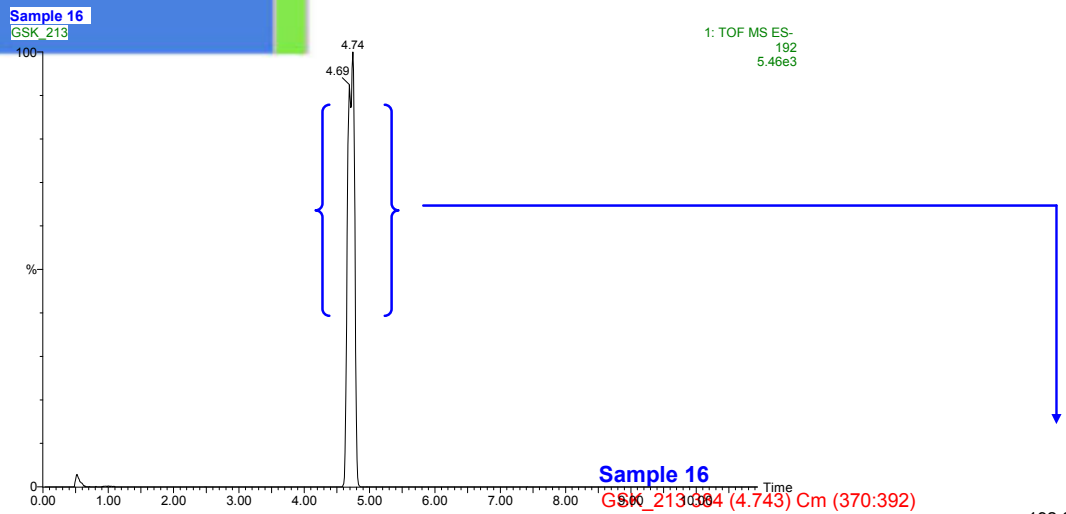
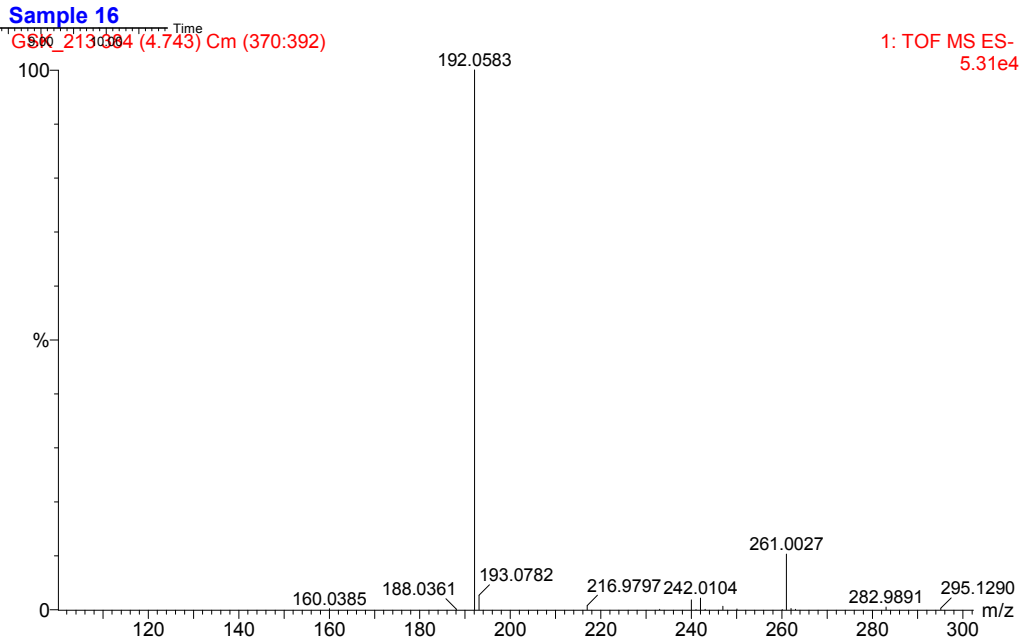
LC/MS of 192 ion using a Q-Tof



Accurate mass information obtained for the 192 ion was used to determine the elemental composition $C_{10}H_{10}NO_3$.



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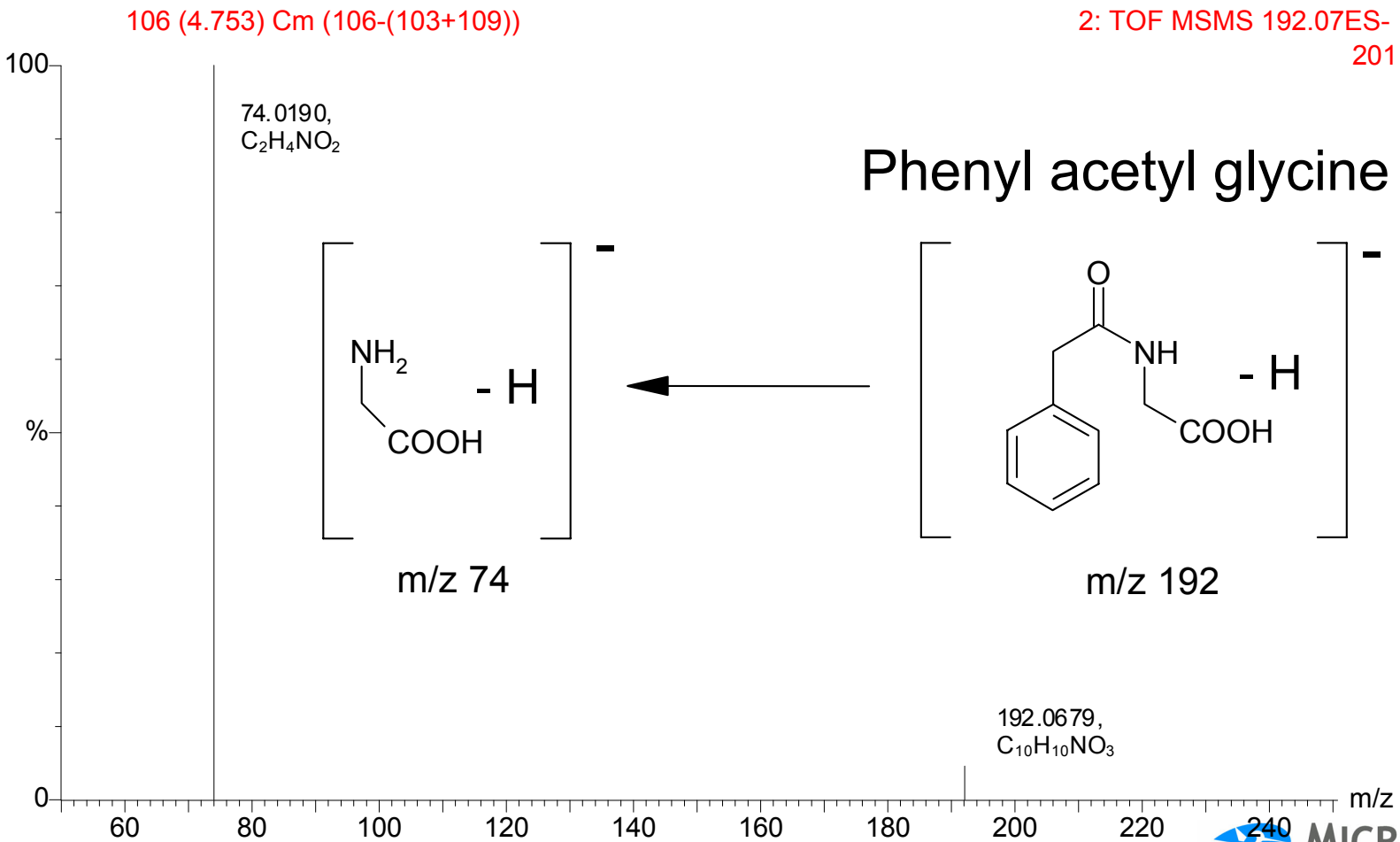
LC/MS/MS of 192 ion



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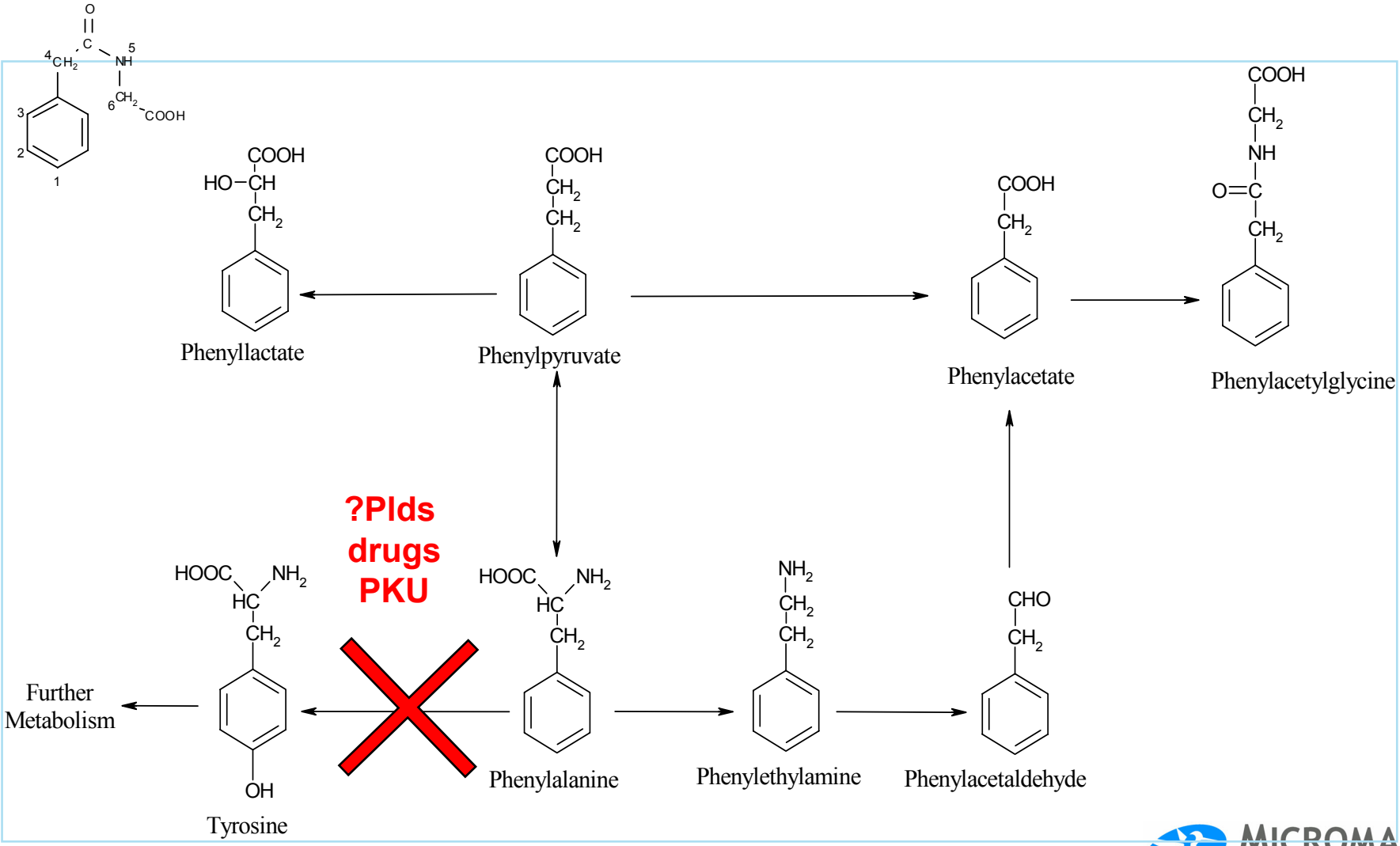
Phenylacetylglycine (PAG) -biochemical pathways of formation



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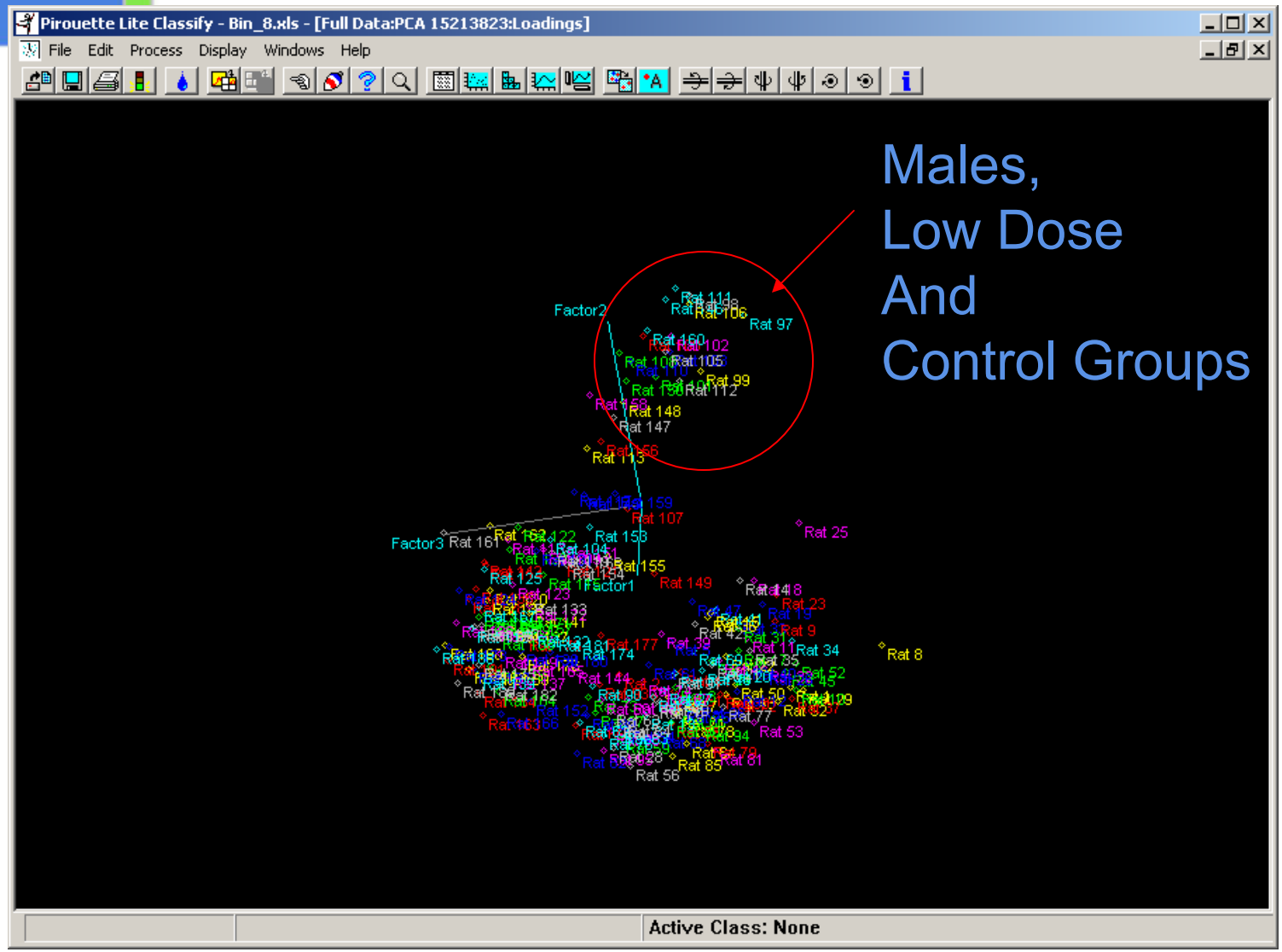
Larger Study: 192 Samples



Males,
Low Dose
And
Control Groups

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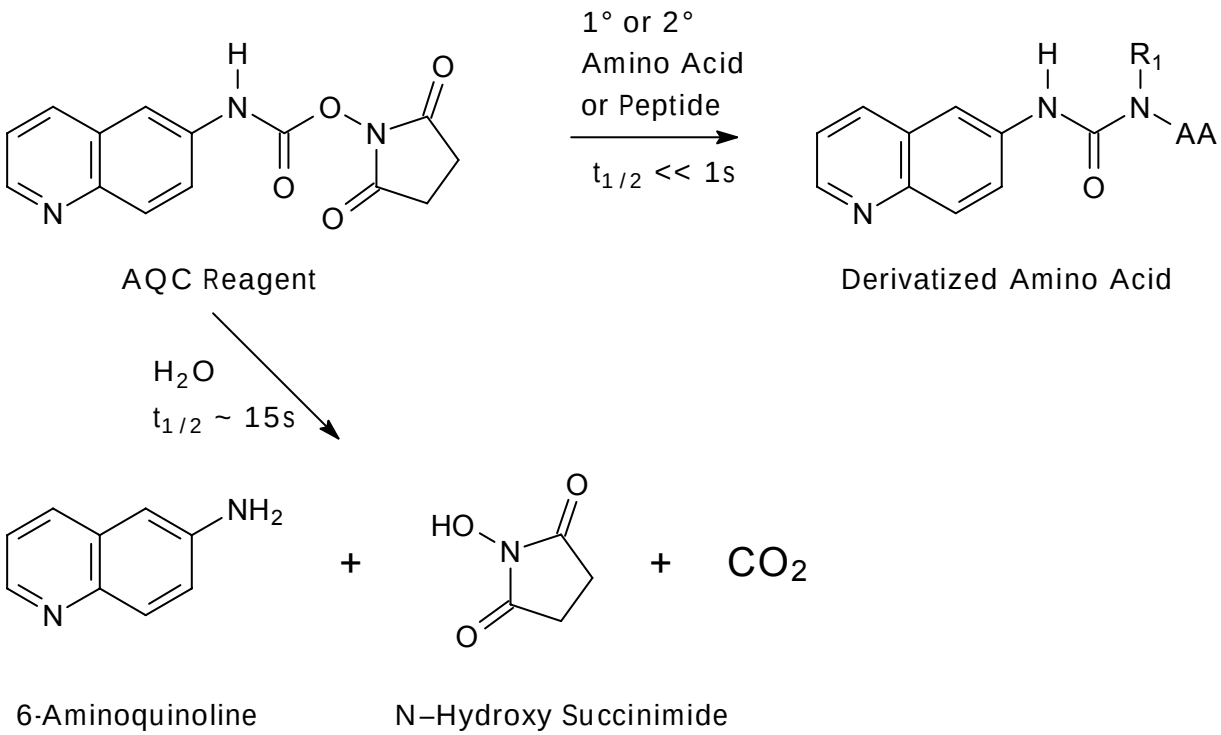
Polar compounds

- *These are typically very difficult to analyse by reversed-phase LC/MS, eluting at the solvent front and suffering ion suppression.*
- *Using alternative LC methodologies such as HILIC would mean that more than one analytical run is required.*
- *This would not necessarily ensure that the polar compound is detected.*
- *These compounds can be important biomarkers of toxicity, e.g. amino acids.*

Amino acid using AccQ-Tag™

- *Amino acids are unretained on reversed-phase LC systems.*
- *Potential solution to use Waters AccQ-Tag with MS detection.*
- *Sample prep:- evaporate sample add buffer add derivatizing reagent.*
- *Chromatograph by gradient LC/MS/MS, injection volume 2uL. Using MRM analysis for 20 amino acids*
- *Run times 10 mins per sample.*

AccQ-Tag™ chemistry



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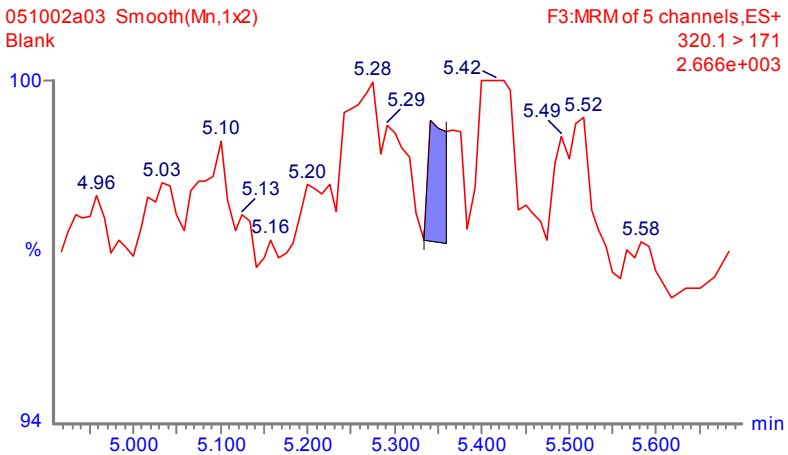
Derivitization of Methionine



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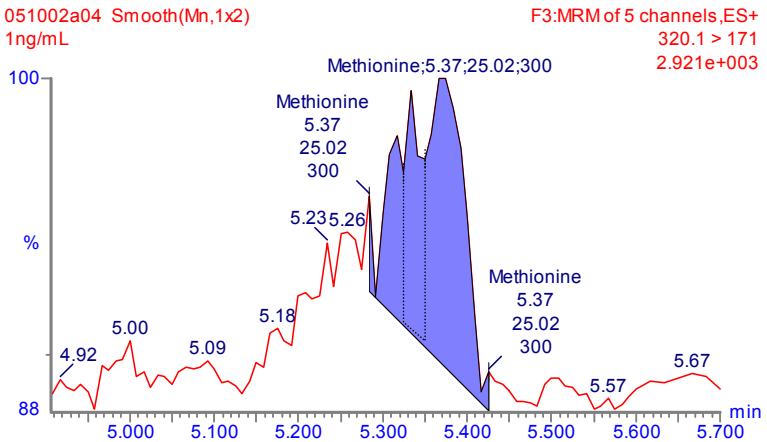
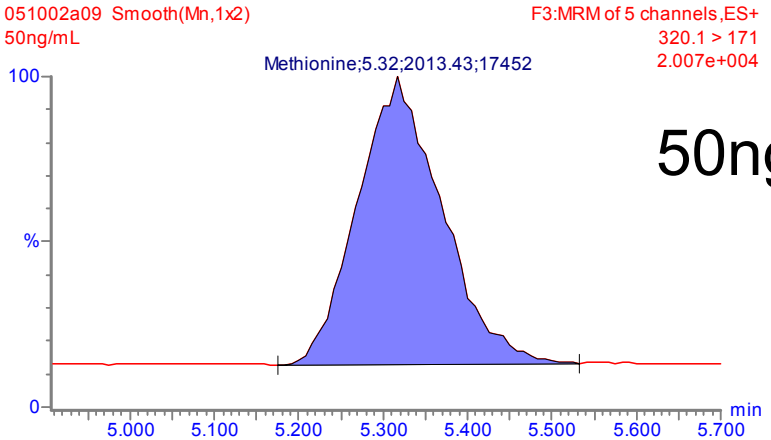


1ng/mL



50ng/mL std

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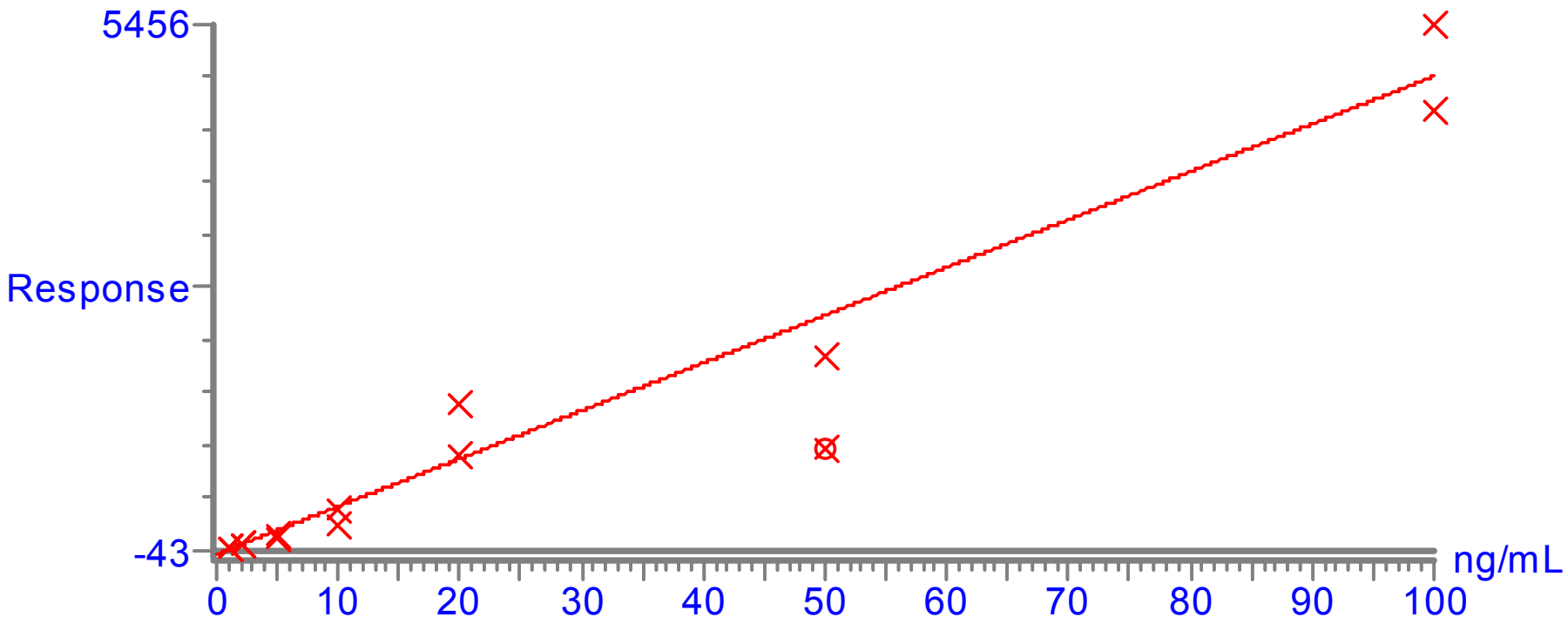
Calibration line

Compound name: Methionine
Coefficient of Determination: 0.976096
Calibration curve: 49.6451 * x + -42.9708
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



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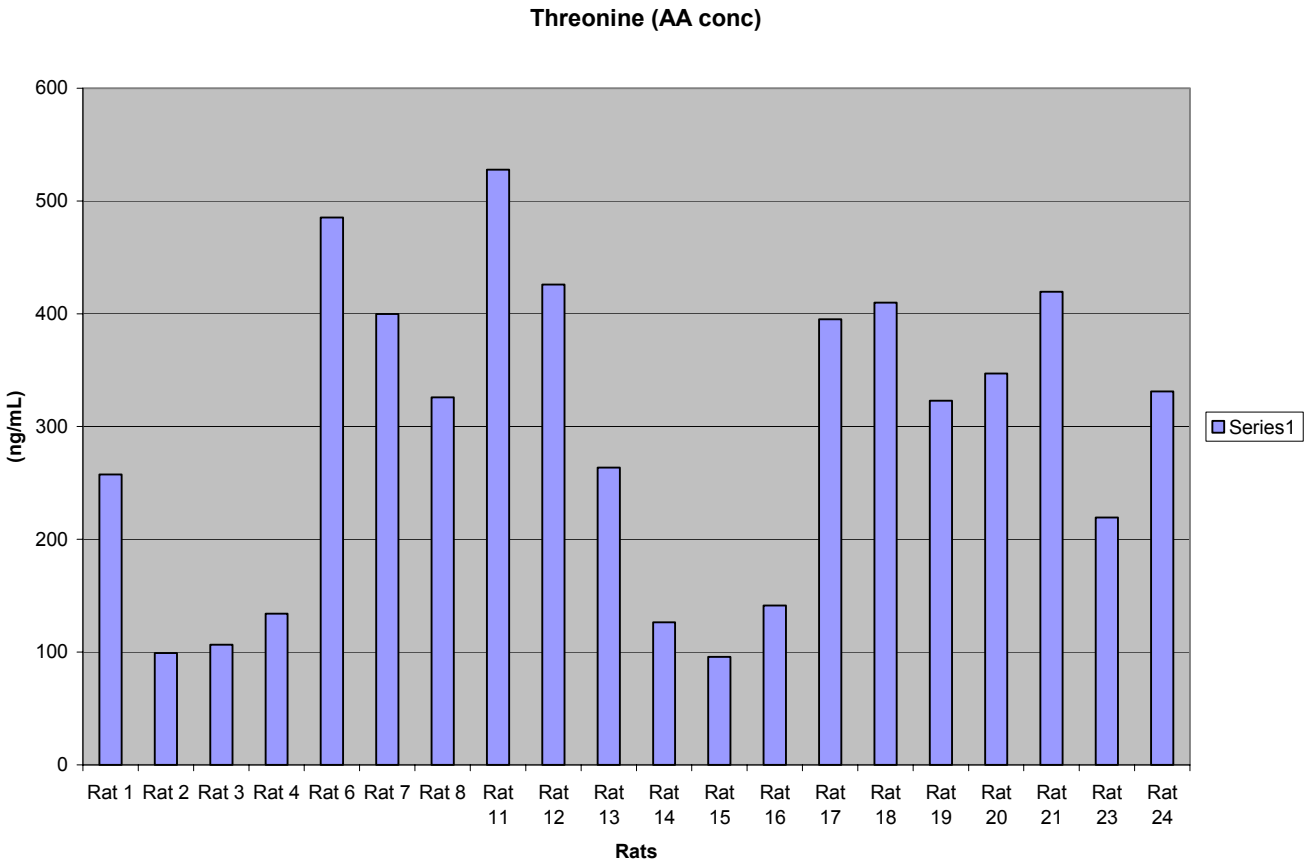
Threonine data



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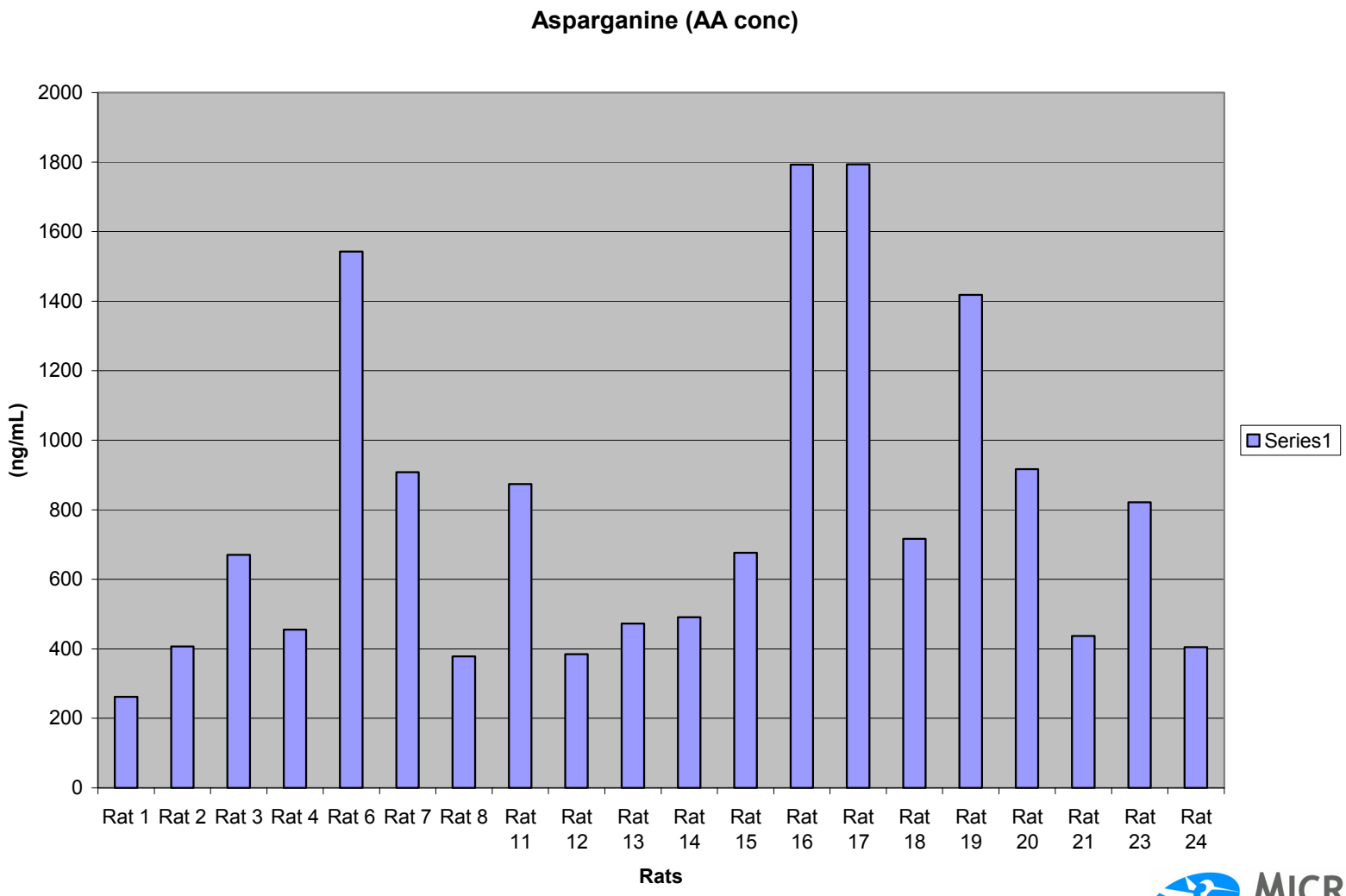
Asparganine



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Amino acid summary

Amino Acids	Retention Window (min)	Max Conc (ng/mL)	M/Z
Glycine	3.25-3.9	820	246.1
Serine	3.1-3.85	340	276.1
Glutamine	3.5-4.0	2200	317.1
Histidine	3.5-4.0	810	326.1
Alanine	3.7-4.25	1450	260.1
Proline	4.125-4.5	1630	286.1
Threonine	3.5-4.125	540	290.1
Taurine	3.5-4.25	64000	296.1
Valine	4.85-5.25	325	288.1
Methionine	5.125-5.65	1800	320.1
Asparganine	5.125-5.5	1800	321.1
Tyrosine	4.75-5.25	270	352.1
Cystine	4.75-5.25	50	581.3
Leucine	5.5-6.0	460	302.1
Phenylalanine	6.0-6.5	850	336.2
Tryptophan	6.0-6.5	1150	375.1
Lysine	5.75-6.25	1650	487.2
Homocysteine	5.75-6.25	1.6	609.3

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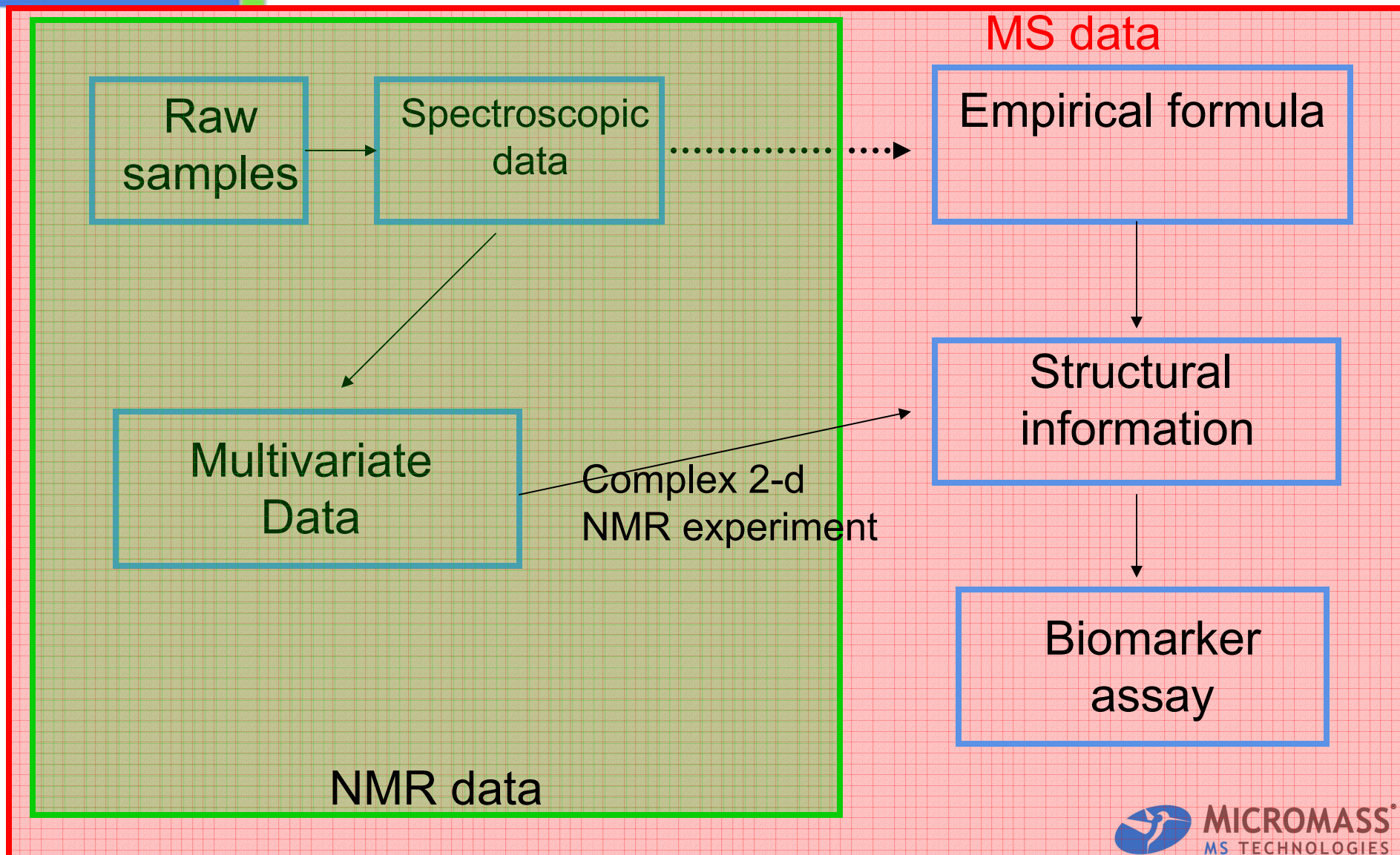
Data flow


MS data
Raw samples
Spectroscopic data
Empirical formula
Structural information
Multivariate Data
Complex 2-d NMR experiment
Biomarker assay
NMR data

Conclusions

- *With a relatively simple reversed-phase LC/MS system it was possible to separate the control from dosed samples.*
- *m/z value of analytes responsible for the PCA separation were identified.*
- *Analytes responsible for PCA separation were identified by LC/MS/MS and TOF MS.*
- *AccQ-Tag™ was successfully employed to analyse amino acid in urine.*

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Acknowledgements

- *John Haselden, Safety Assessment, GlaxoSmithKline, Ware, UK,*
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- *Hilary Major, Micromass, Manchester, UK.*

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