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Monitoring The Toxicity of a Drug Candidate using LC/MS-TOF. A Metabonomics Approach

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Metabolic Profiling May 2003

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- Waters View of Metabonomics.
- Metabonomics Place in Drug Discovery.
- Why Multivariate Analysis.
- Advantages of LC/MS-TOF.
- LC/MS approach.
- Safety Assessment Study.
- Identifying Biomarkers.
- Conclusions.

- Nicholson definition:

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

- Simple view:

“Quantitative profiling of endogenous metabolites in biological fluids to identify toxic responses or disease state progression”.

- The major aim is to increase compound attrition in drug discovery.
- Reduce compound attrition in development and clinical analysis.
- Help explain reasons for toxicity.
- Produce expert system to identify route of toxicity.

- Complex data set.
- Simple sample to sample comparison not appropriate due to biological variation, phenotypes.
- Group clustering easily visualized.
- Time related data difficult to visualize using traditional comparison methods.
- Components responsible for clustering identified.

- High sensitivity.
- Large user base.
- Rapid analysis, with automation.
- TOF-MS gives high mass resolution.
- Use of LockSpray™ gives exact mass analysis.
- Easier biomarker identification, using exact mass.
- Easy method transfer to high throughput monitoring.

- Reversed phase chromatography.
- No sample prep employed.
- ESI-TOF-MS detection.
- Lock-mass employed to ensure correct mass measurement.

- 2.1mm x 10 cm Waters Symmetry C18 3.5 μ m.
- Reversed-Phase gradient elution.
- 0-95% 0.1% Formic acid (aq) : Acetonitrile over 10 mins at 600 μ L/min.
- Sample prep: Dilution 1:4 with distilled water.
- 10 μ L Injection
- Detection by full scan TOF MS positive and negative ion ESI.
- Lock mass luecine enkephelin 50fmol/ μ L, 50 μ L/min.

- **Positive Ion Electrospray**

- Capillary 3200V
- Sample Cone 40 V
- Extraction Cone 1.5 V
- Collision Energy 10 eV
- Source Temperature 100°C
- Desolvation Temperature 150°C
- Cone Gas Flow 50 L/hr
- Desolvation Gas Flow 450 L/hr
- Argon Collision Gas

- **Negative Ion Electrospray**

- As above for positive ion except
- Capillary 2600V

- **MS Acquisition Parameters – LockSpray Enabled**

- Scan Range m/z 50-1500
- Scan Time 1 sec
- Inter-scan Time 0.1 sec
- Mode centroid
- Lock mass frequency 5.0 sec
- Scans to average 10

- Peak detect raw data.
- Remove peaks from known compounds, xenobiotics dosing vehicle.
- Select samples for chemometric analysis.
- Perform PCA.
- Identify any xenobiotics missed and remove.
- Perform PCA again.
- Create trend plots for Biomarkers.

- Ion suppression can seriously effect data generation in LC/MS.
- Caused by competition between co-eluting species for ionization energy.
- Co-elution of salts can cause severe ion suppression.
- Urine is a very complex matrix containing high salt concentration.

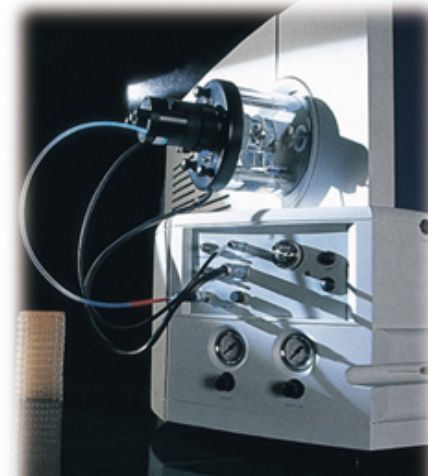
- Aim: Monitor ion suppression effects during urine LC/MS.
- Tee into LC stream infusion of standard mix solution.
- Make injection of urine sample.
- Monitor MS response for each m/z channel.
- Perform in both positive and negative ion mode.

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MS infusion urine LC/MS



Waters™ 2795
Alliance HT

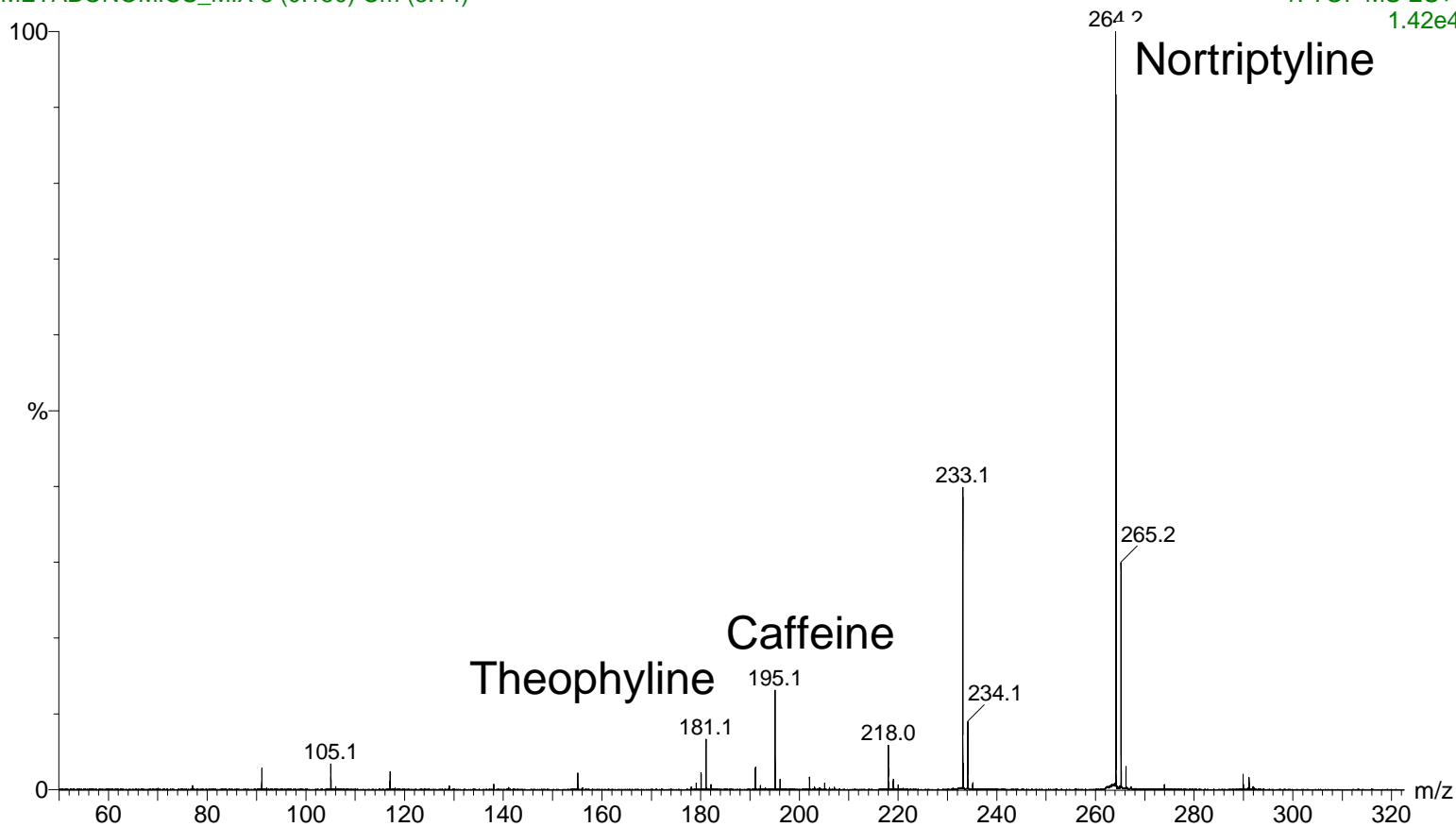


Q-Tof *micro*™

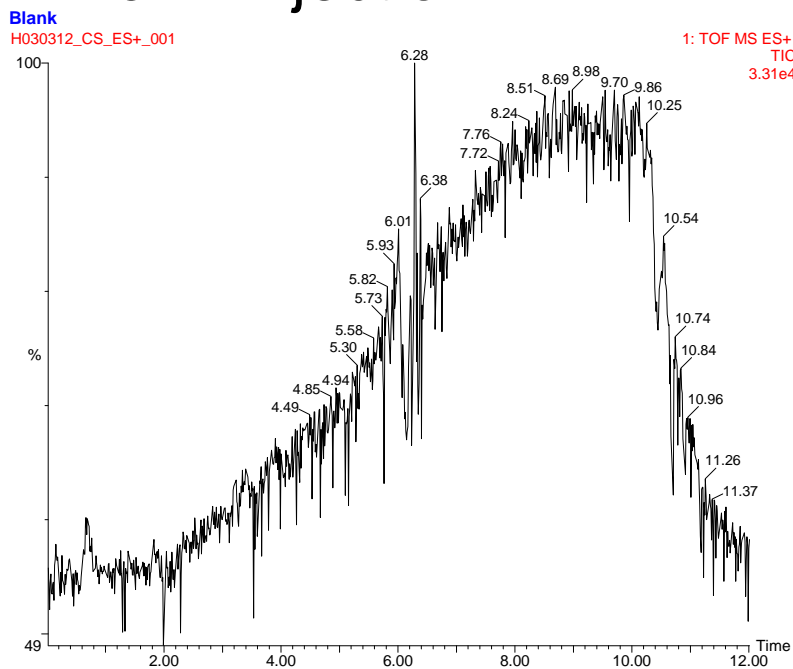
Standard pump

Metabonomics Mix Infusion

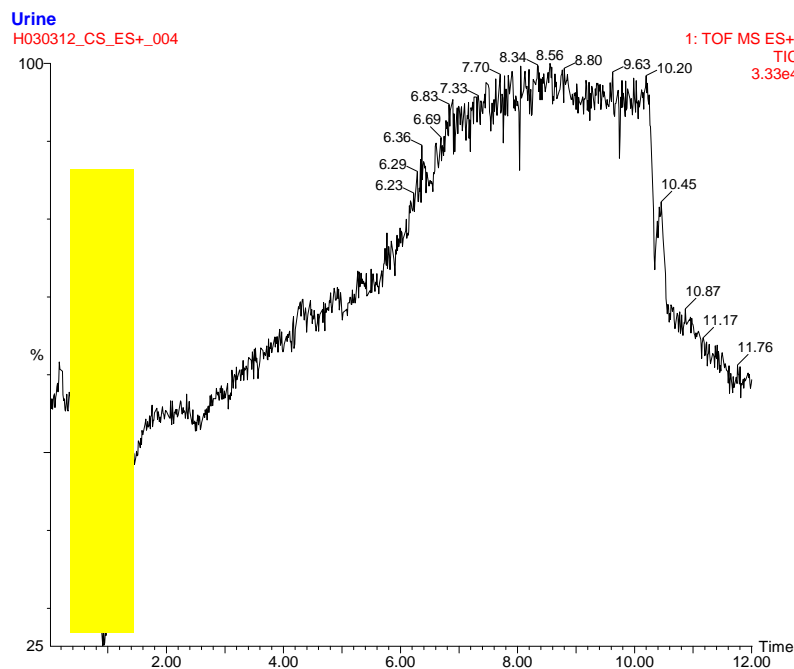
METABONOMICS_MIX 8 (0.150) Cm (8:14)

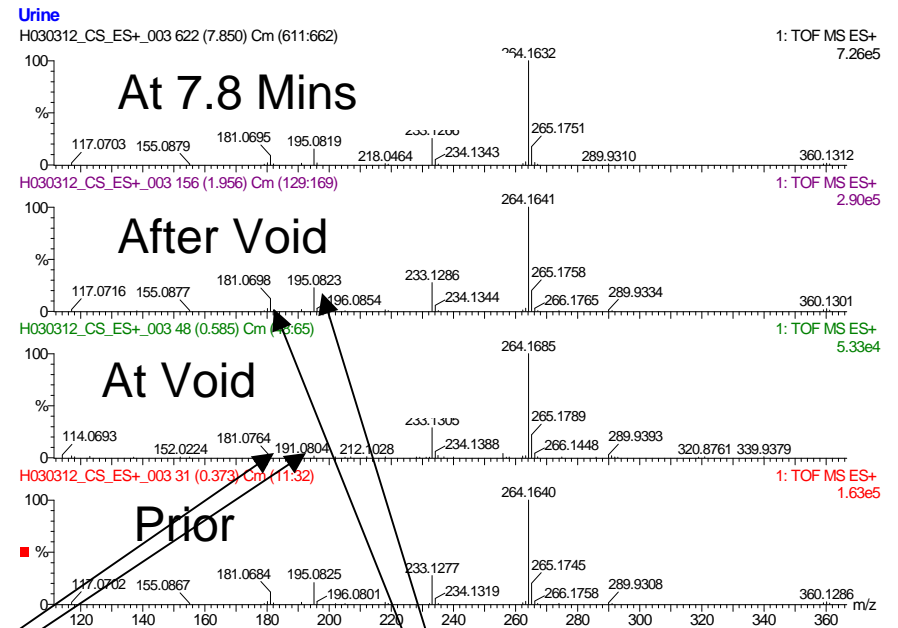
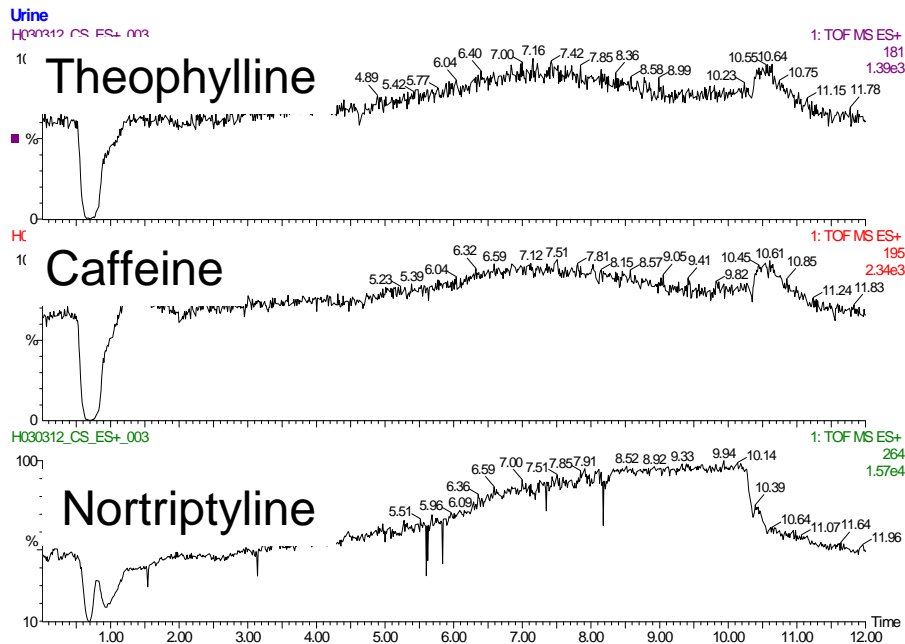
1: TOF MS ES+
1.42e4

Blank Injection



Urine Injection





Ions
Suppressed

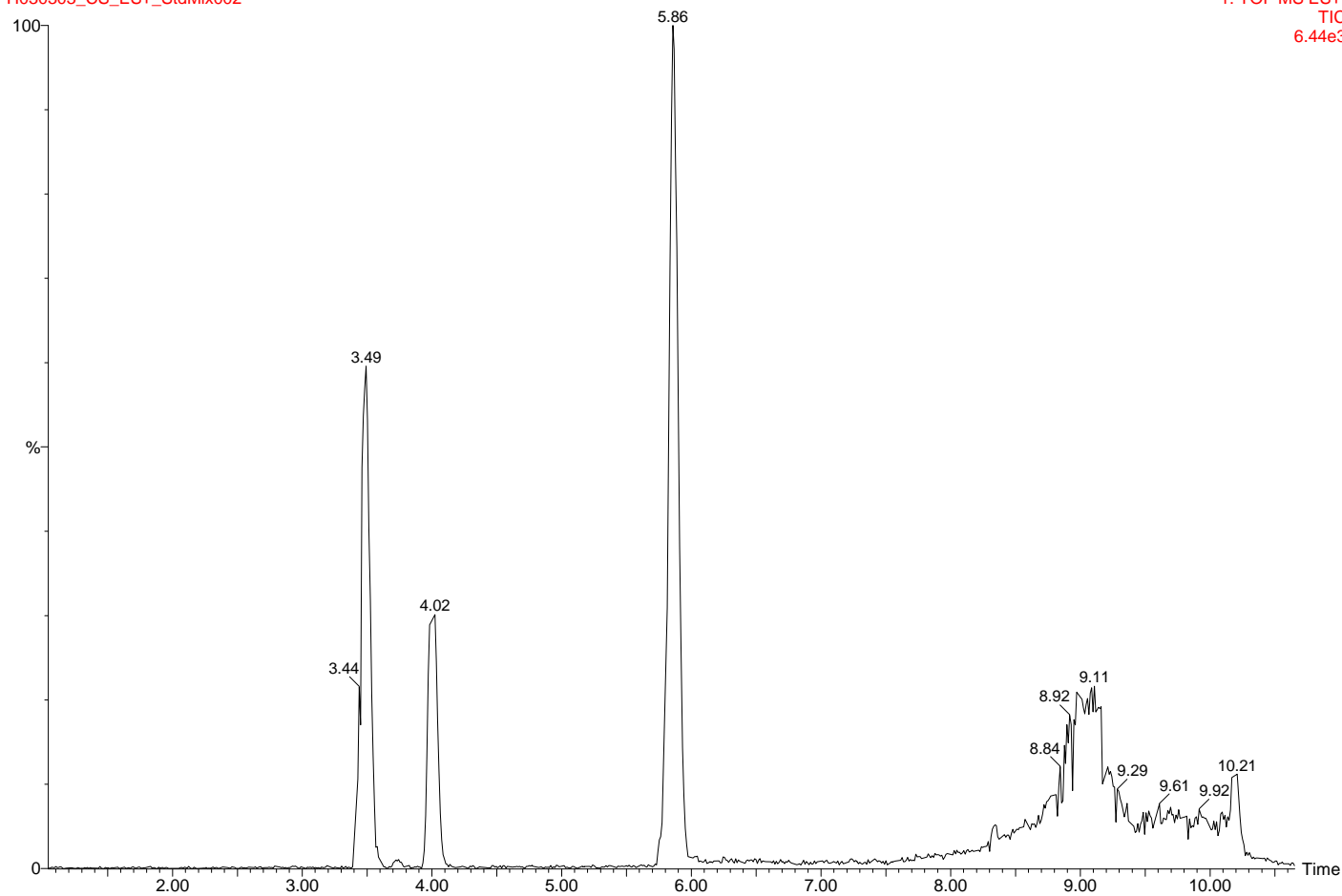
Response
restored

■ Study Design:-

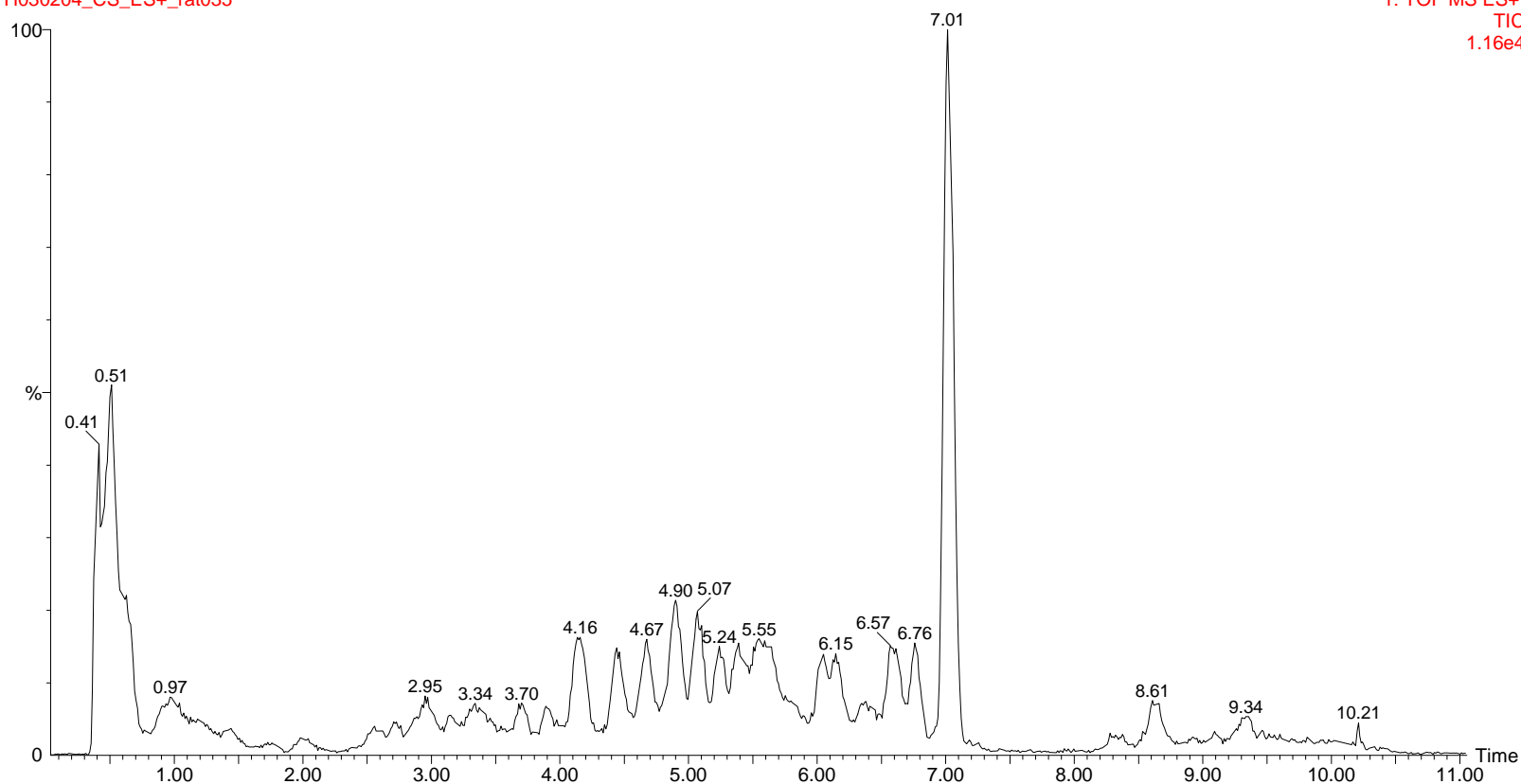
- 90 Day Safety Tolerability Study in Rats
- 3 Daily Dose levels, Control, 2mg/Kg, 18mg/Kg.
- Oral administration.
- Urine samples collected 0-8Hr and 8-24Hr
- Sample analysis D34 and Day 84

Metabonomics Test Mix
H030303_CS_ES+_StdMix002

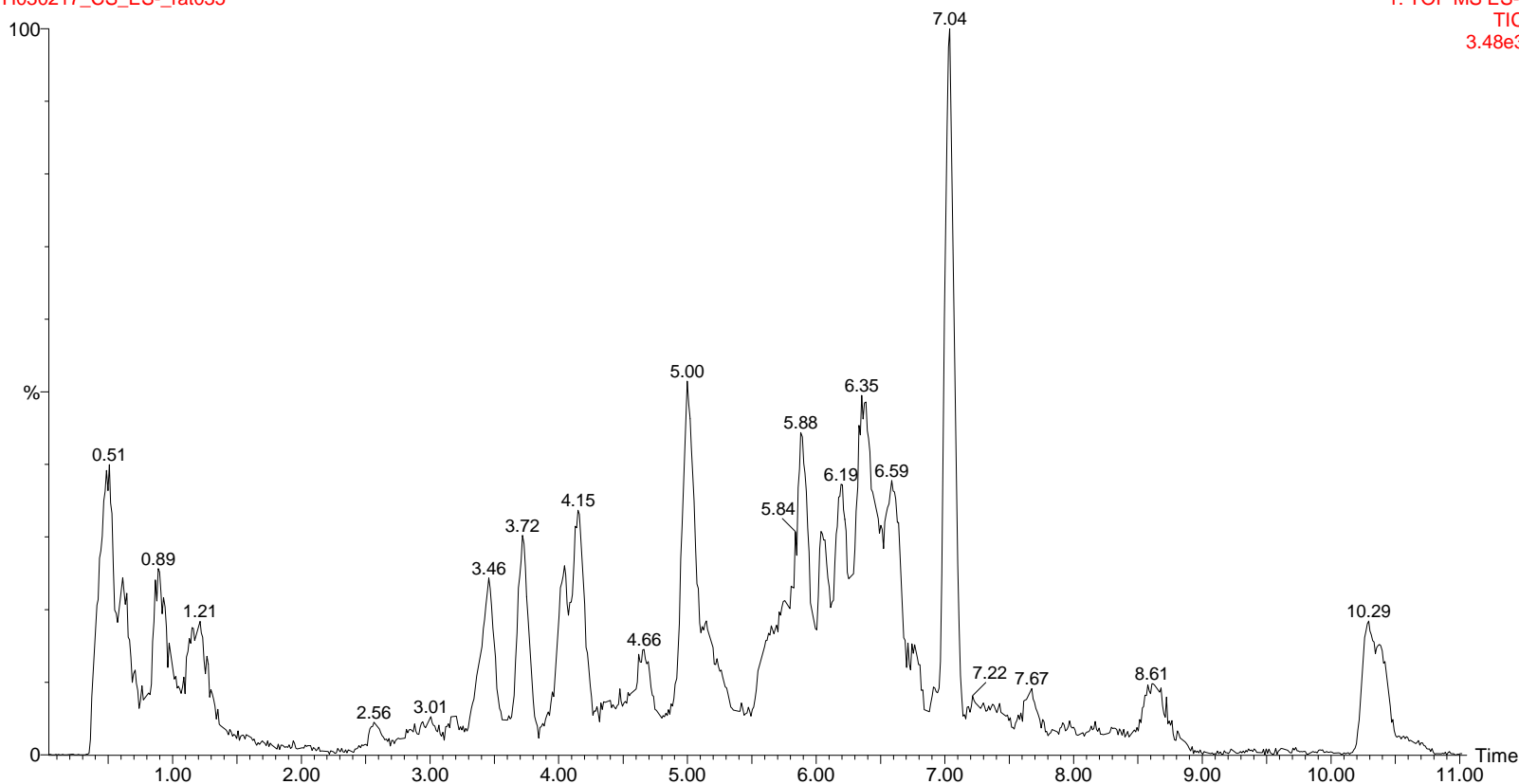
1: TOF MS ES+
TIC
6.44e3

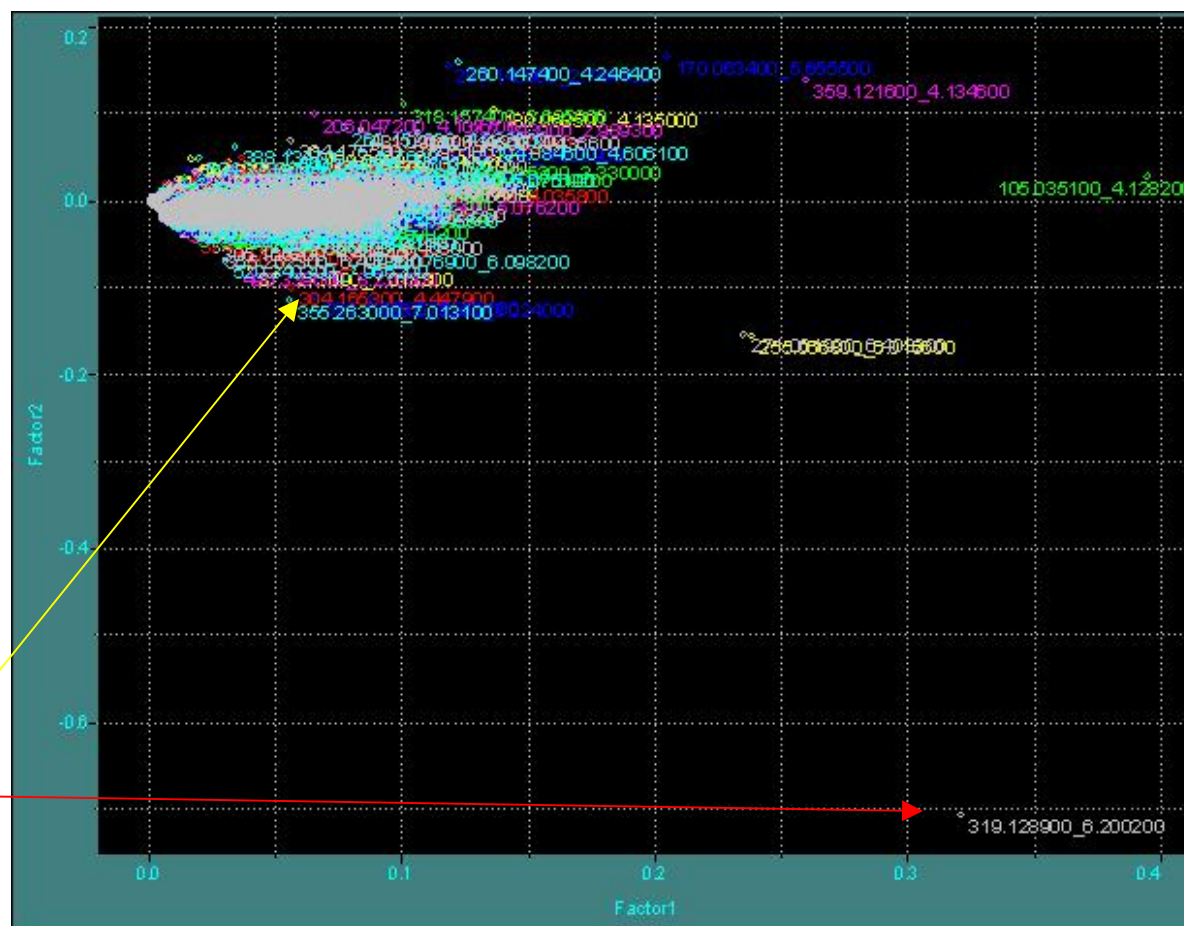


Female rat urine sample low dose day 34 8-24hr

GSK rat urine 35
H030204_CS_ES+_rat035

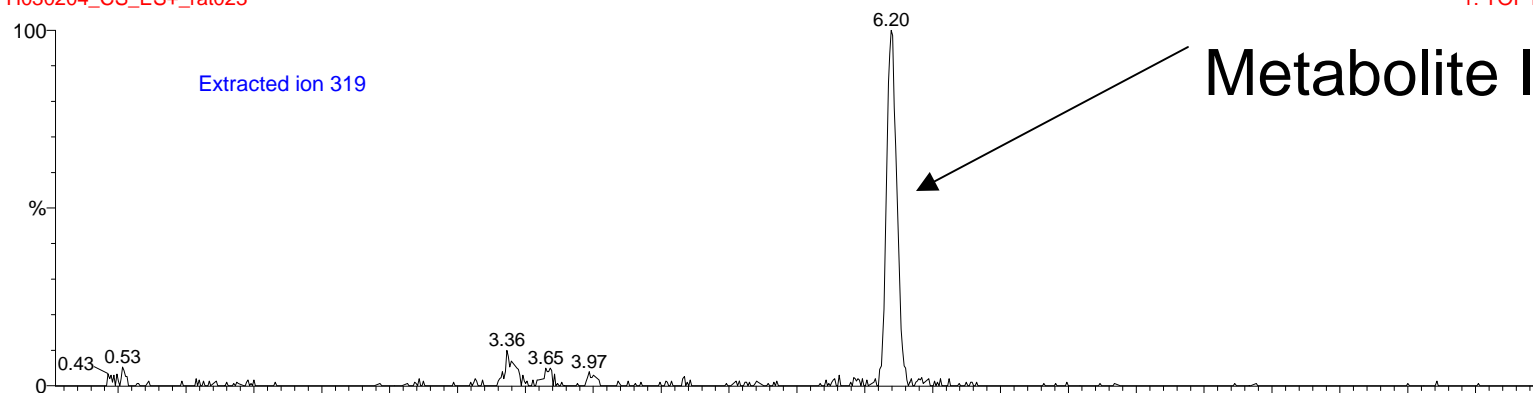
Female rat urine sample (low dose day 34 8-24hr)

GSK rat urine 35
H030217_CS_ES-_rat0351: TOF MS ES-
TIC
3.48e3



Drug
Metabolites

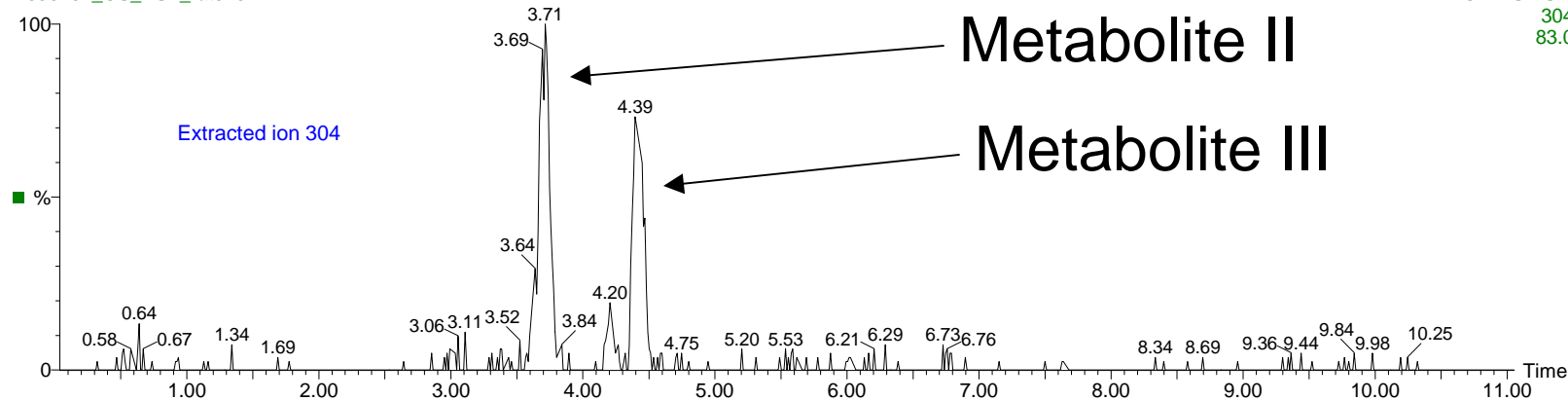
GSK rat urine 23
H030204_CS_ES+_rat023



1: TOF MS ES+
319
306

Metabolite I

H030204_CS_ES+_rat023



1: TOF MS ES+
304
83.0

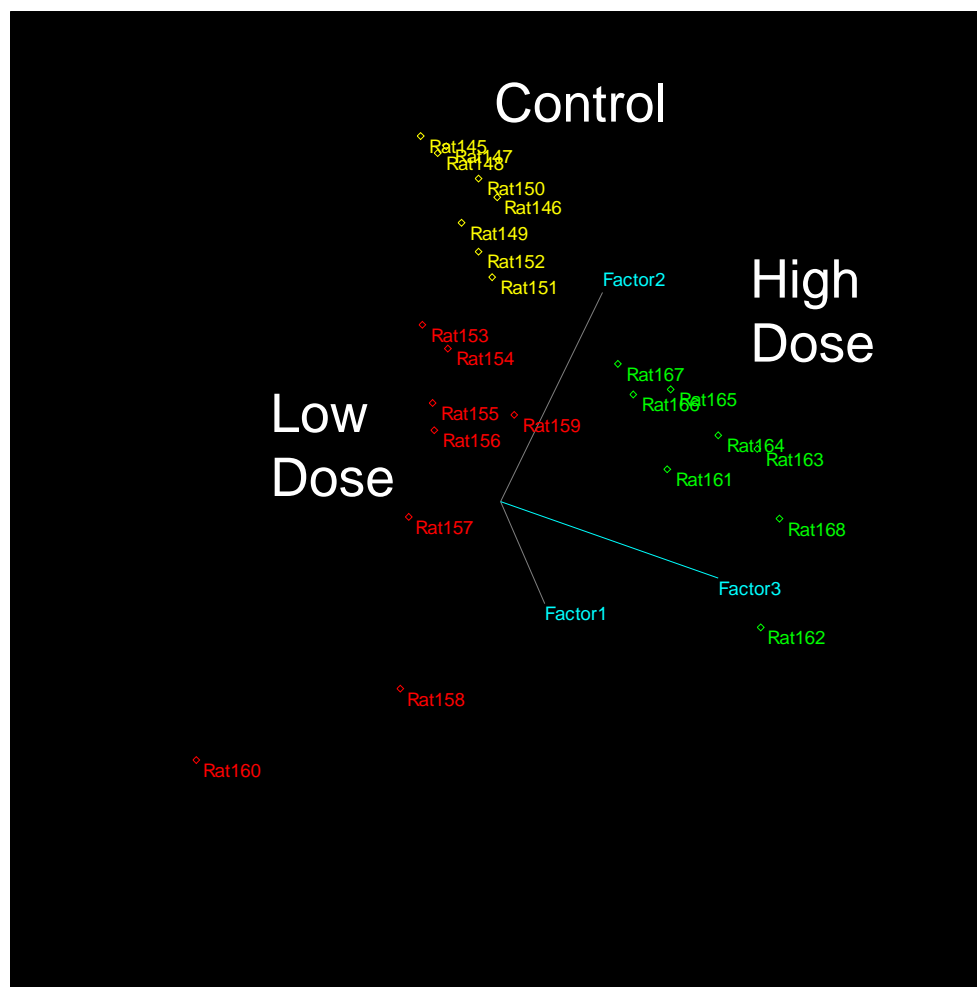
Metabolite II

Metabolite III

Control: Yellow

Low Dose: Red

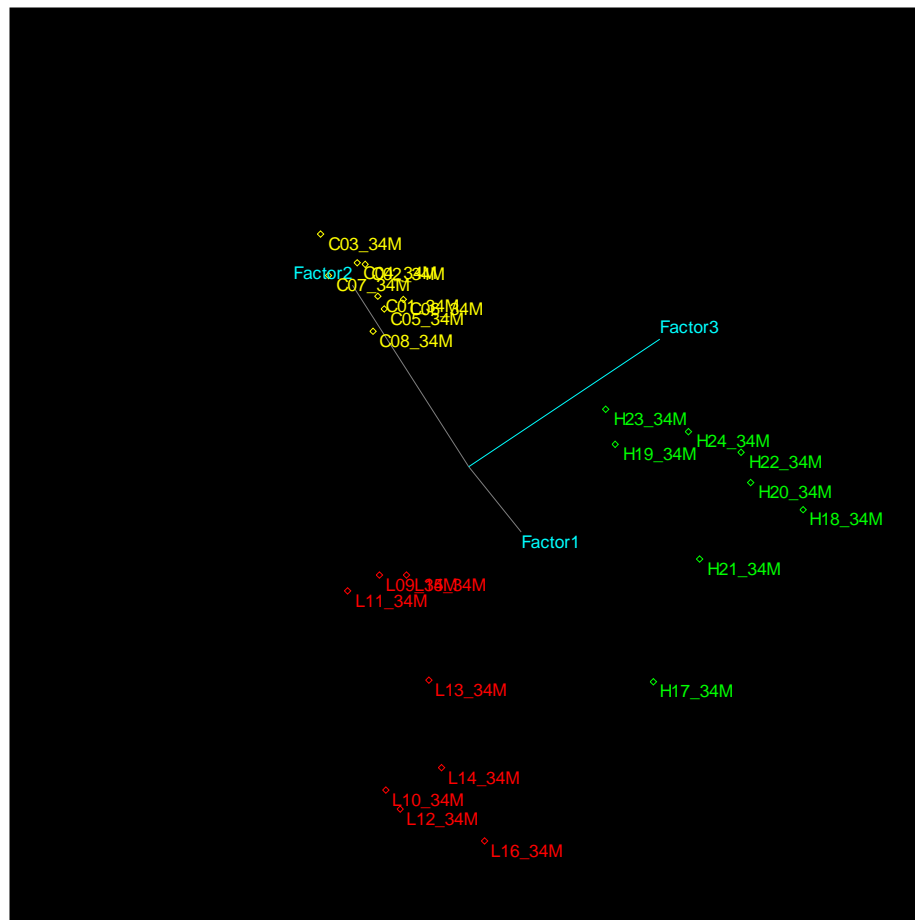
High Dose: Green



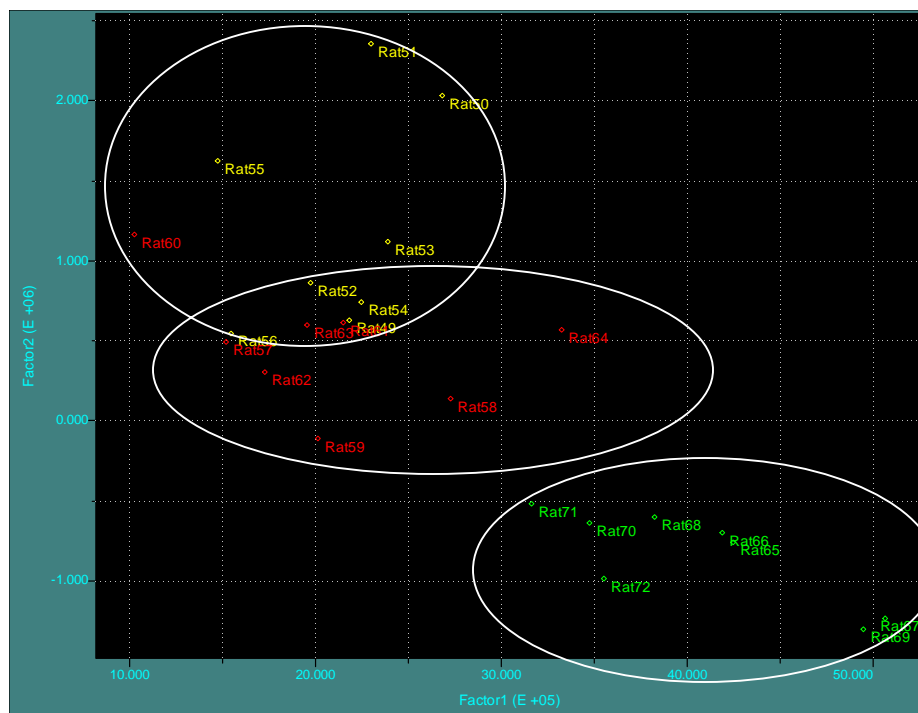
Control: Yellow

Low Dose: Red

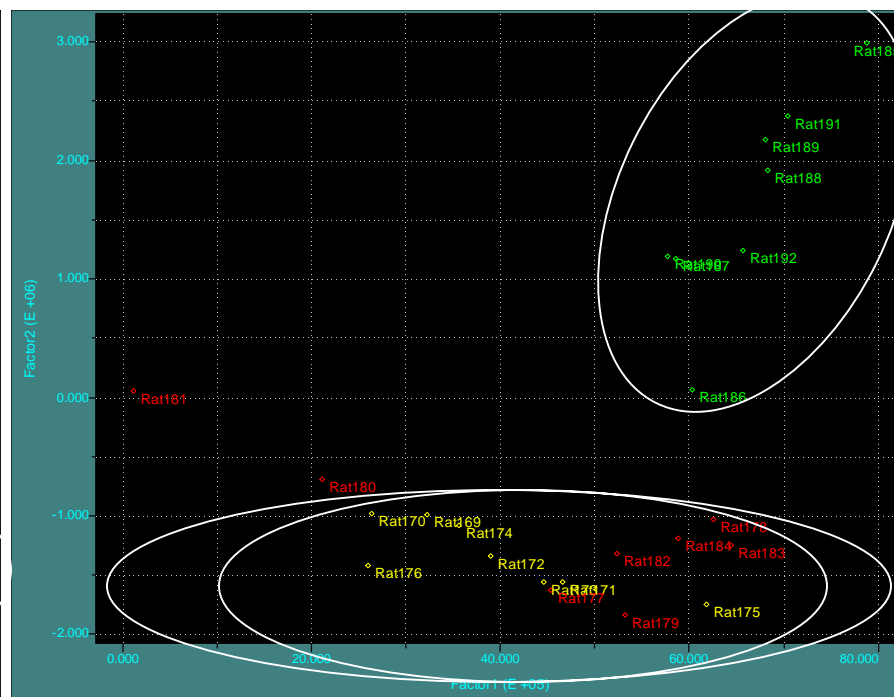
High Dose: Green



Male samples



Female samples

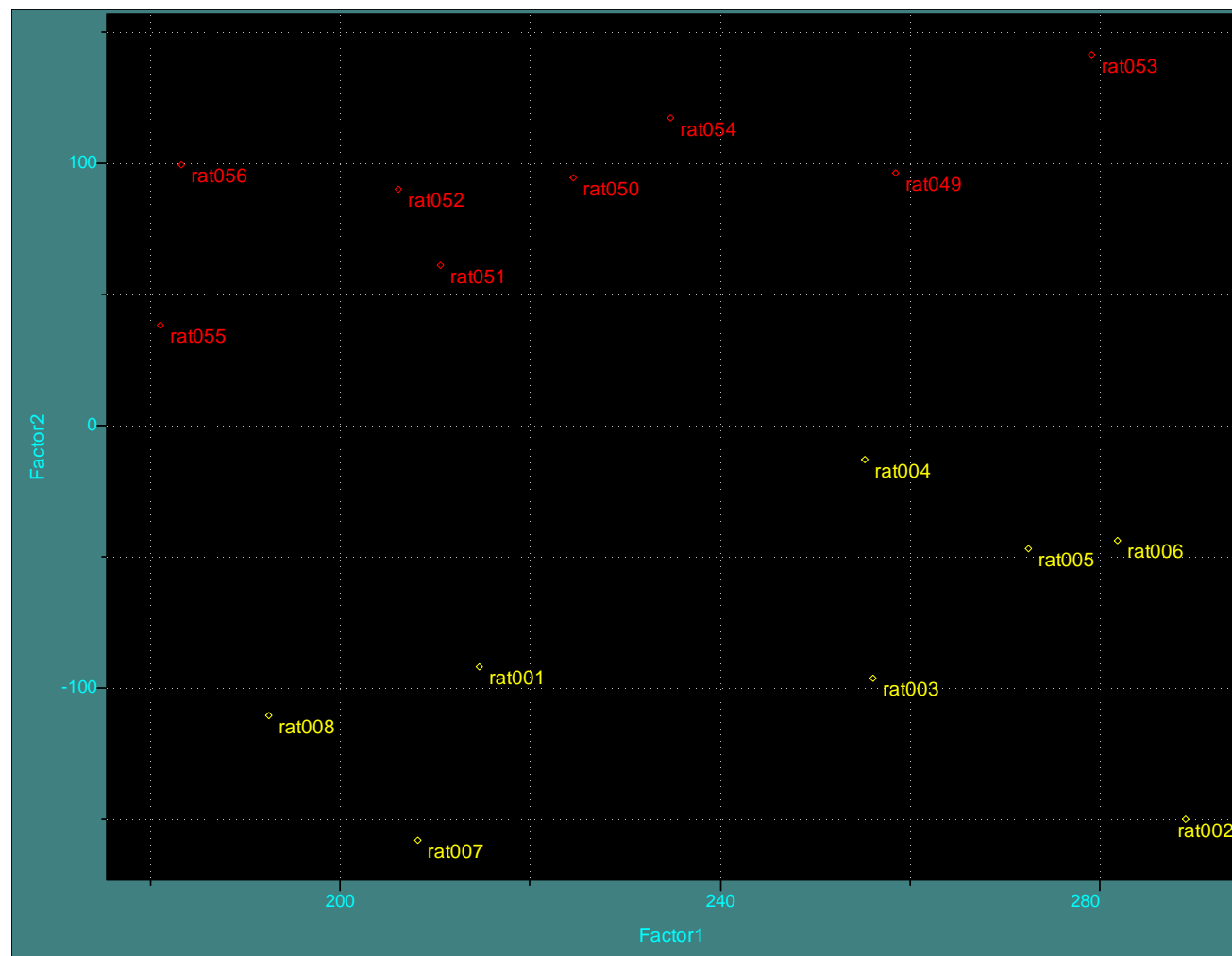


Control: Yellow
 Low Dose: Red
 High Dose: Green

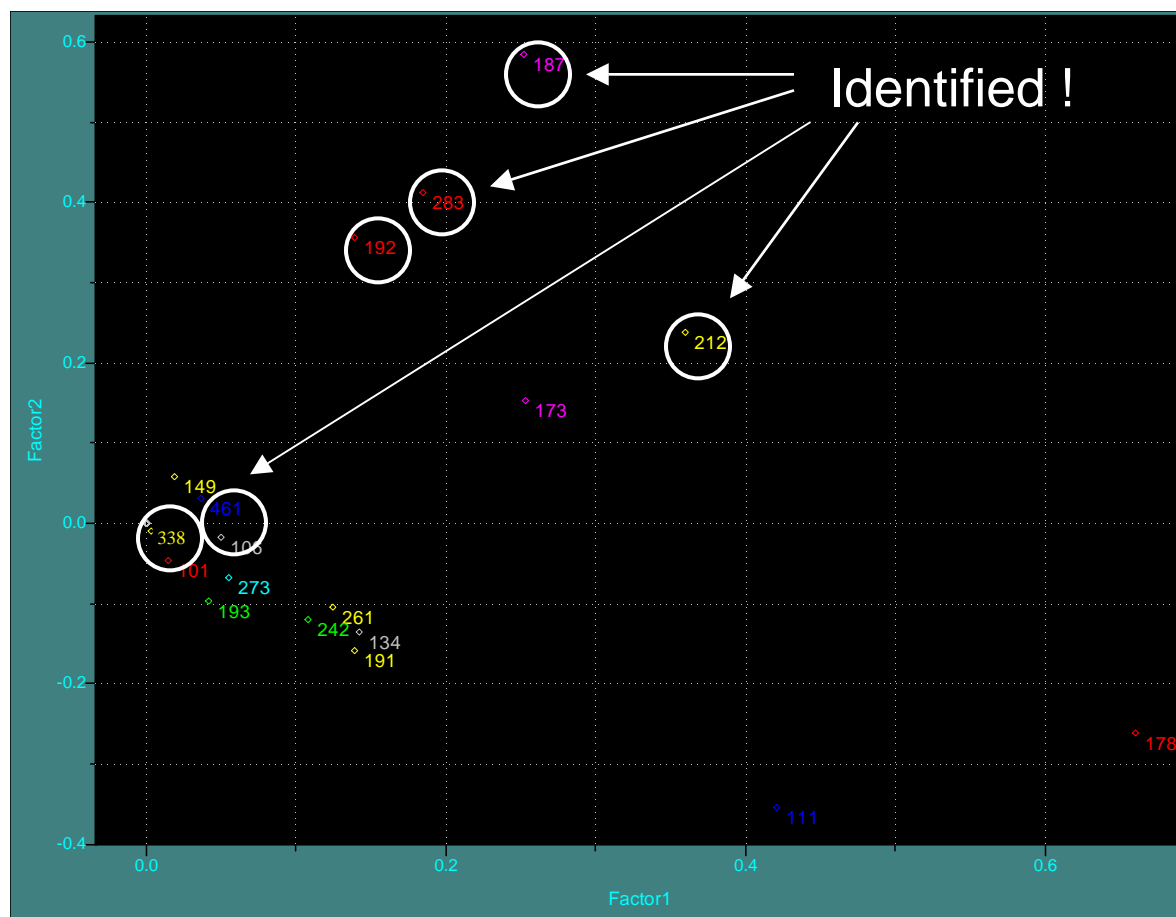
Male control positive ion samples day 34

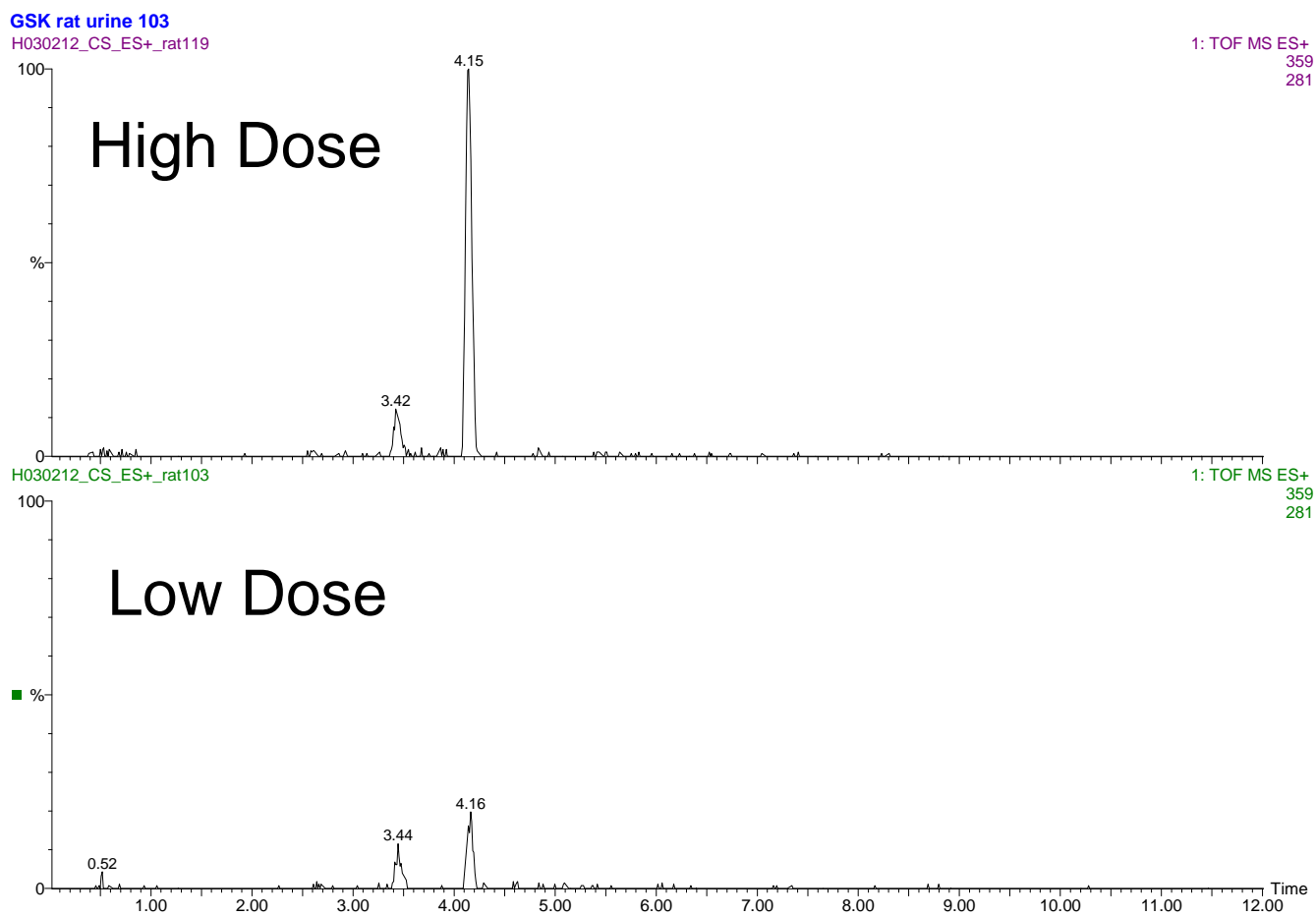
Red = day

Yellow = night

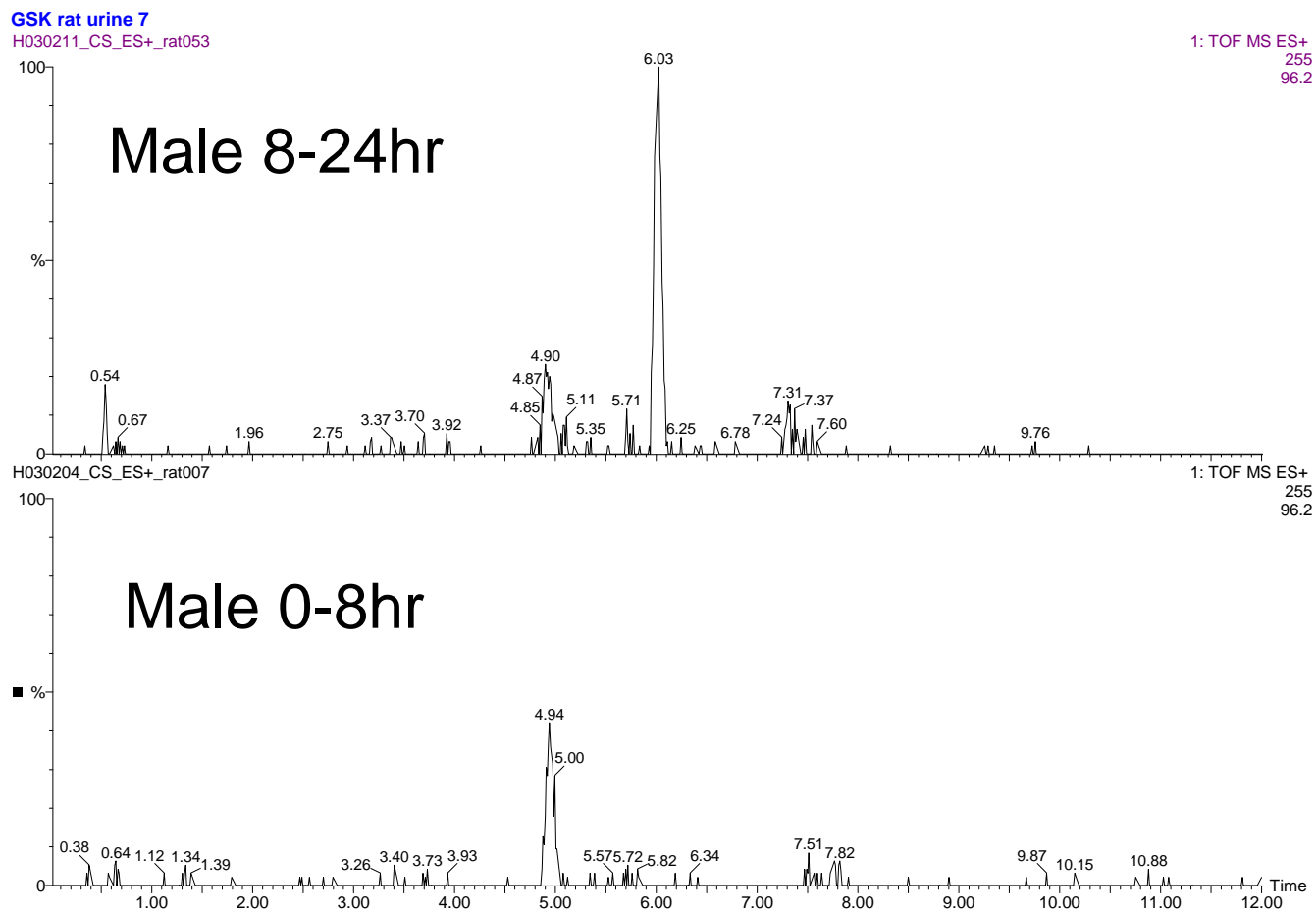


- Biomarker identification is simplified by LC/MS-TOF.
- LC/MS peaks of interest easily identified from the loadings plot.
- Extracted ion chromatograms from Dose and Control groups show if difference is true.
- Exact mass data allows rapid searching of potential biomarkers.
- LC/MS/MS analysis of ions of interest give fragment ion information.



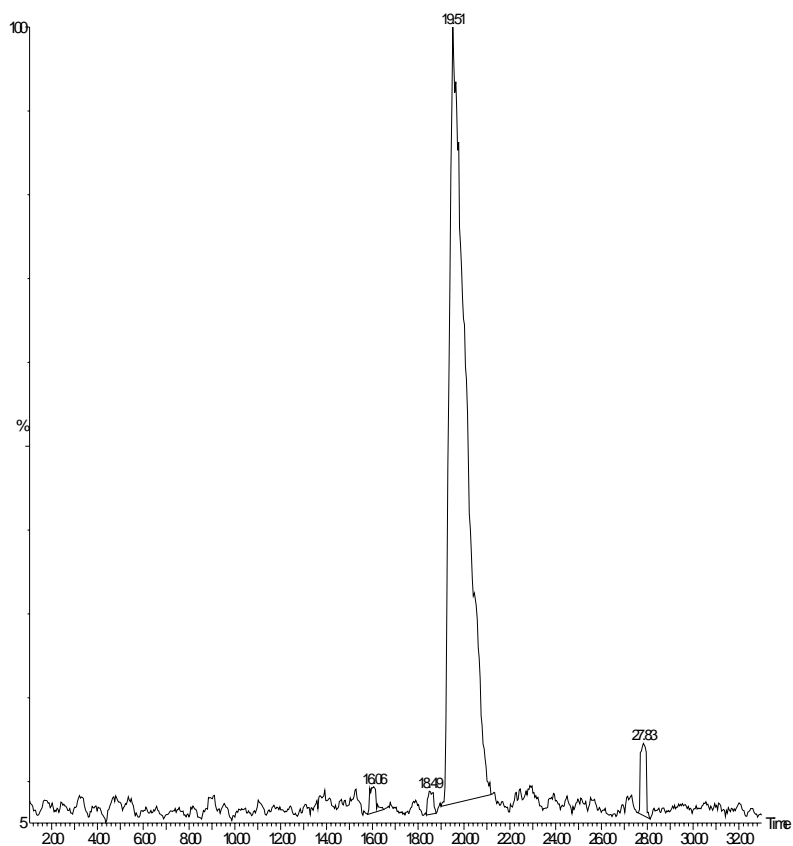
Extraction ion chromatogram of $m/z = 359$ positive ion for control
and high dose male rats day 84 8-24hr

Extracted ion chromatogram m/z = 255 for control male rats D34 0-8hr and 8-24hr



Negative ion product ion MS/MS of 212

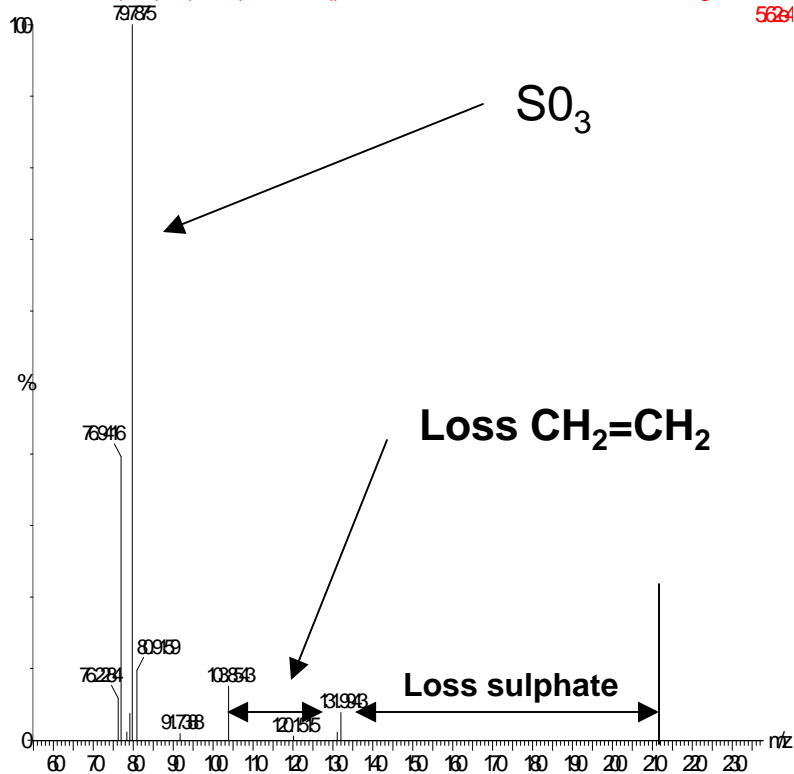
Rt 15



Rt 15

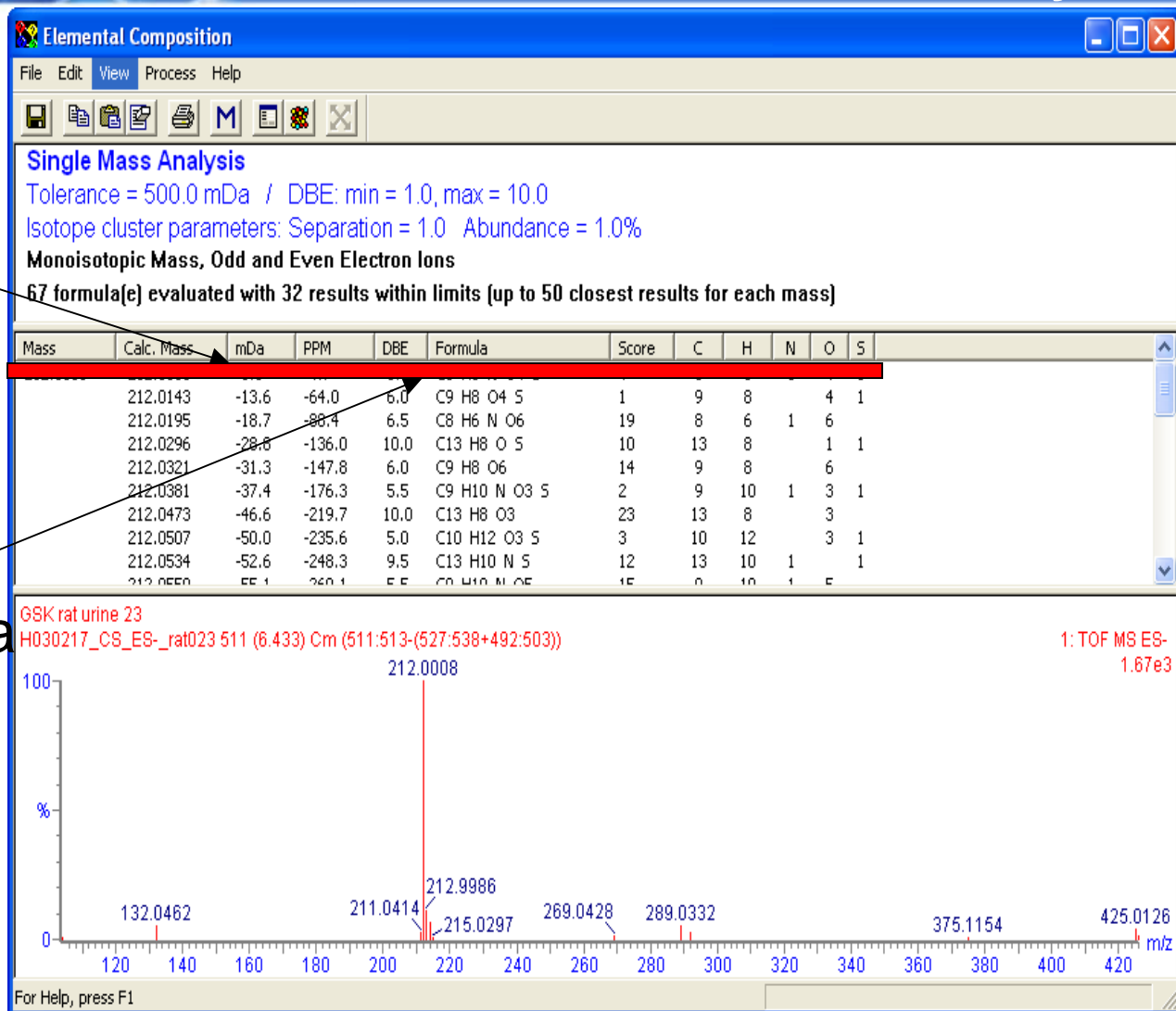
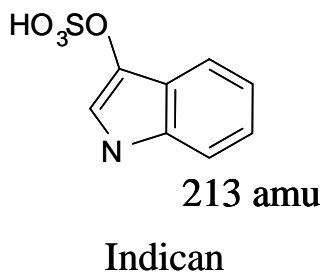
D11102AFR2437(1963) C1(45454(49534(37845))

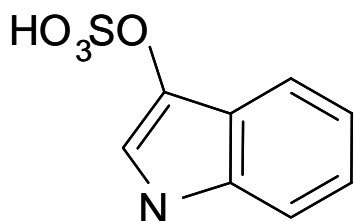
9 Da of 212S
5624



Mass Accuracy
1mDa, 4.7ppm

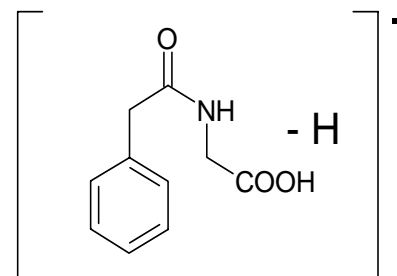
Predicted formula
[C₈H₆NO₄S]⁻





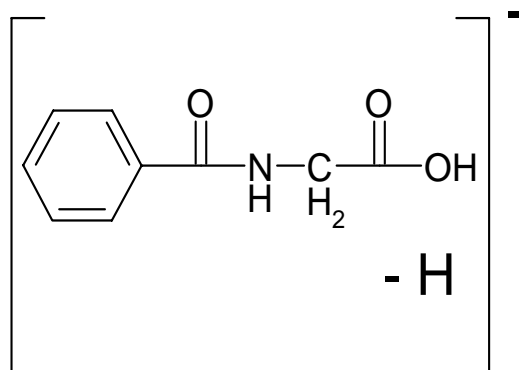
213 amu

Indican



m/z 192

Phenylacetyl glycine



Hippuric Acid

m/z 178

- LC/MS-Tof has great potential for an analytical engine for metabonomics.
- Chromatography has been optimized for throughput and peak capacity.
- Ion suppression should not be an issue with LC/MS-Tof.
- Dose related, gender and diurnal clustering observed in safety assessment study.
- Biomarkers identified by Exact Mass and MS/MS analysis.