What is a semiquantitative non-targeted method?

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Outline

This presentation applies primarily to MS & LC/MS

- **What is a semi-quantitative non-targeted method?**
- **Some definitions – where does a semi-quantitative targeted method fit into analysis?**
- **Characteristics of a non-targeted semi-quantitative method**
- **How do we ensure that untargeted semi-quantitative methods deliver "actionable" data?**
- **Examples based on metabolic phenotyping**

Definitions – Quantitative Methods

- Quantitative methods provide absolute concentrations and are usually targeted to known analytes
- Are validated, over defined ranges, for a wide range of "figures of merit"
- These include accuracy, precision, LOD, LOQ, ULOQ, stability etc.

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Qualitative Methods

- Qualitative targeted methods usually targeted to known analytes but are there to detect but not quantify them
- Fit for purpose give yes/no answers
- Can be validated in terms of LOD, stability & robustness and specificity are very important

Semi-quantitative Targeted-methods

- Semi-quantitative methods are usually targeted to known analytes but do not provide absolute concentrations
- They are often used over a defined concentration range
- They can be subject to some validation for e.g., LOD, linear range etc.
- Often designed to "trigger" a specific analysis

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Semi-quantitative Un-targeted methods

- Semi-quantitative untargeted methods are not targeted to particular analytes but are aimed a characterizing sample
- Often "Class-based" & generic methods are used
- Analytes of interest are not known before analysis
- They do not provide absolute concentrations but relative ones
- Results usually expressed as fold changes
- limited validation for e.g., precision, "linear range" etc.
- These assays are usually used in "discovery mode"

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Semi-quantitative Methods Examples: "Omics" Methods Transcriptomics, Proteomics & Metabolomics

- In "discovery" mode transcriptomics, proteomics and metabolomics methods provide semi-quantitative "fold change" data.
- But, in order to be useful these semi-quantitative untargeted methods must provide reproducible results that are good enough to identify potential biomarkers.

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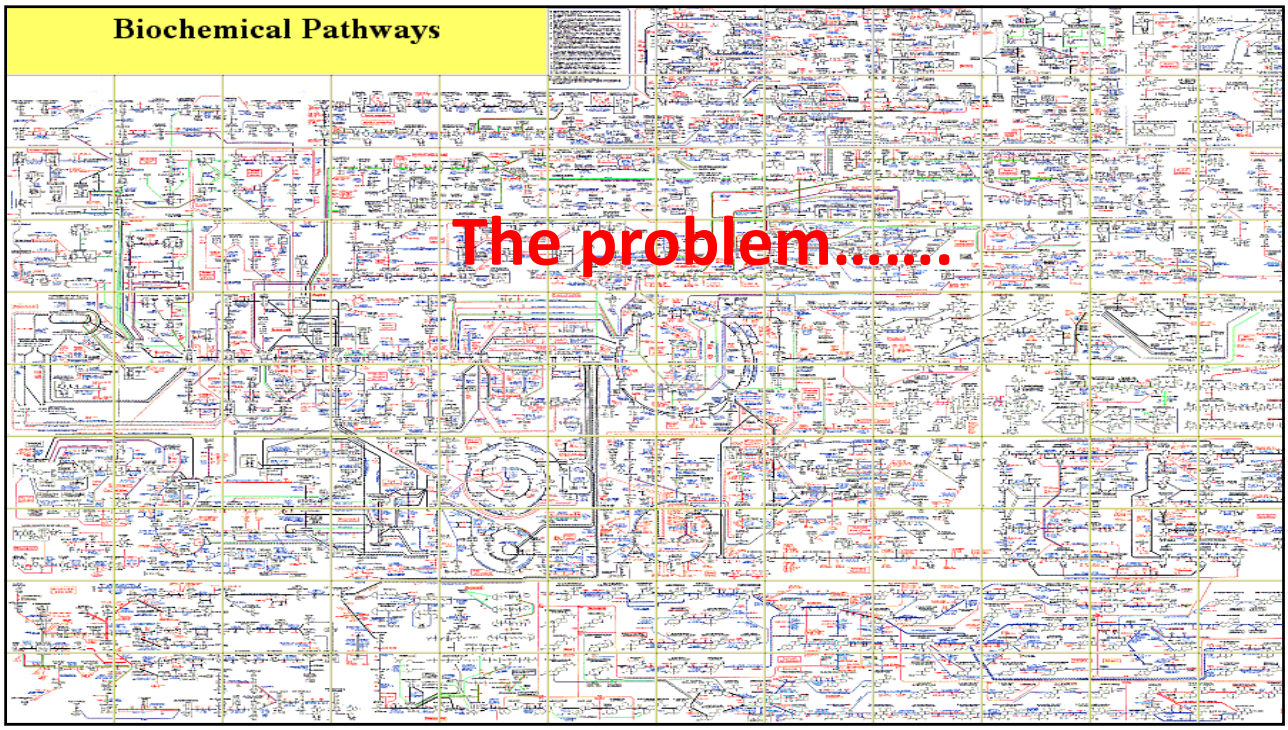
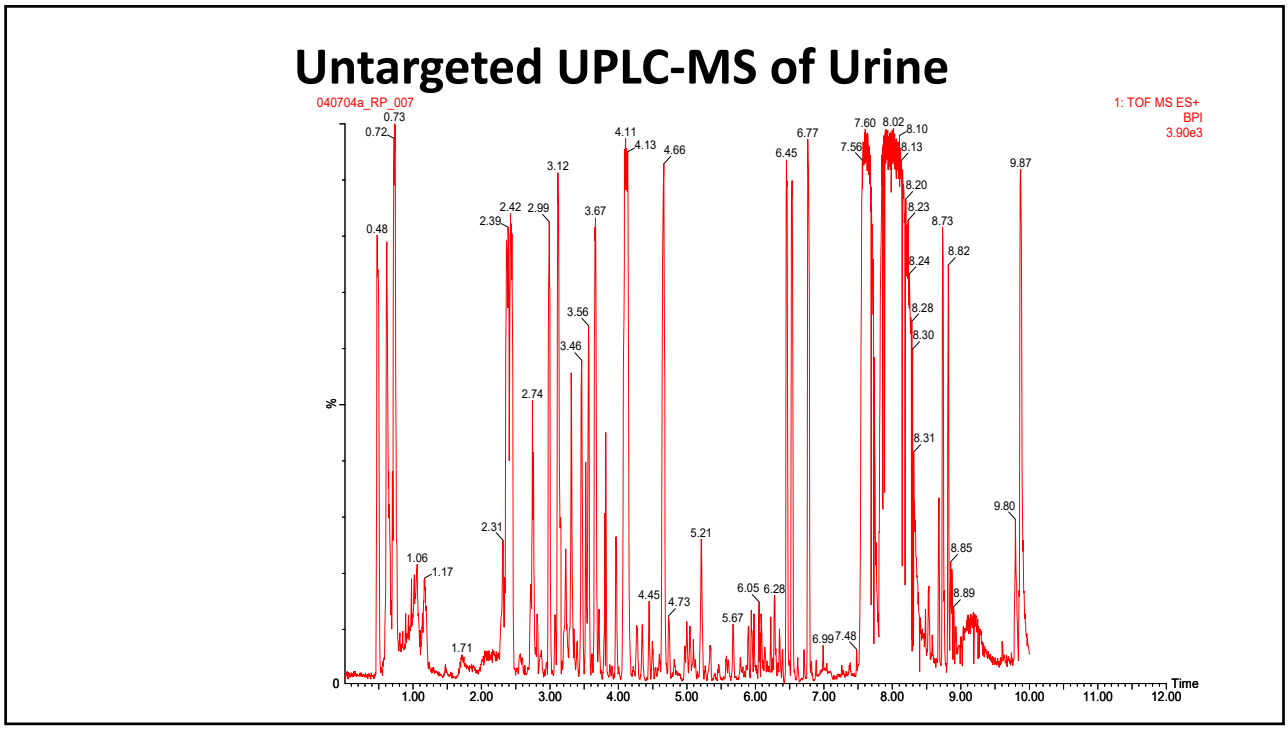
An Example: Untargeted Metabolomics

- Metabonomics/metabolomics (metabolic phenotyping) – untargeted analysis in its purest form.
- Intended to be unbiased, hypothesis free, but hypothesis generating.
- The aim is to find metabolites, or patterns of metabolites, that define a phenotype, in specific areas of basic biology, disease, toxicity etc.
- Ideally these "biomarkers" should be mechanistic and specific for the condition under investigation
- In the absence of knowing what they are the quantitative measurement is not an absolute concentration but a relative amount or fold change


Biochemical Pathways
The problem……


Untargeted UPLC-MS of Urine
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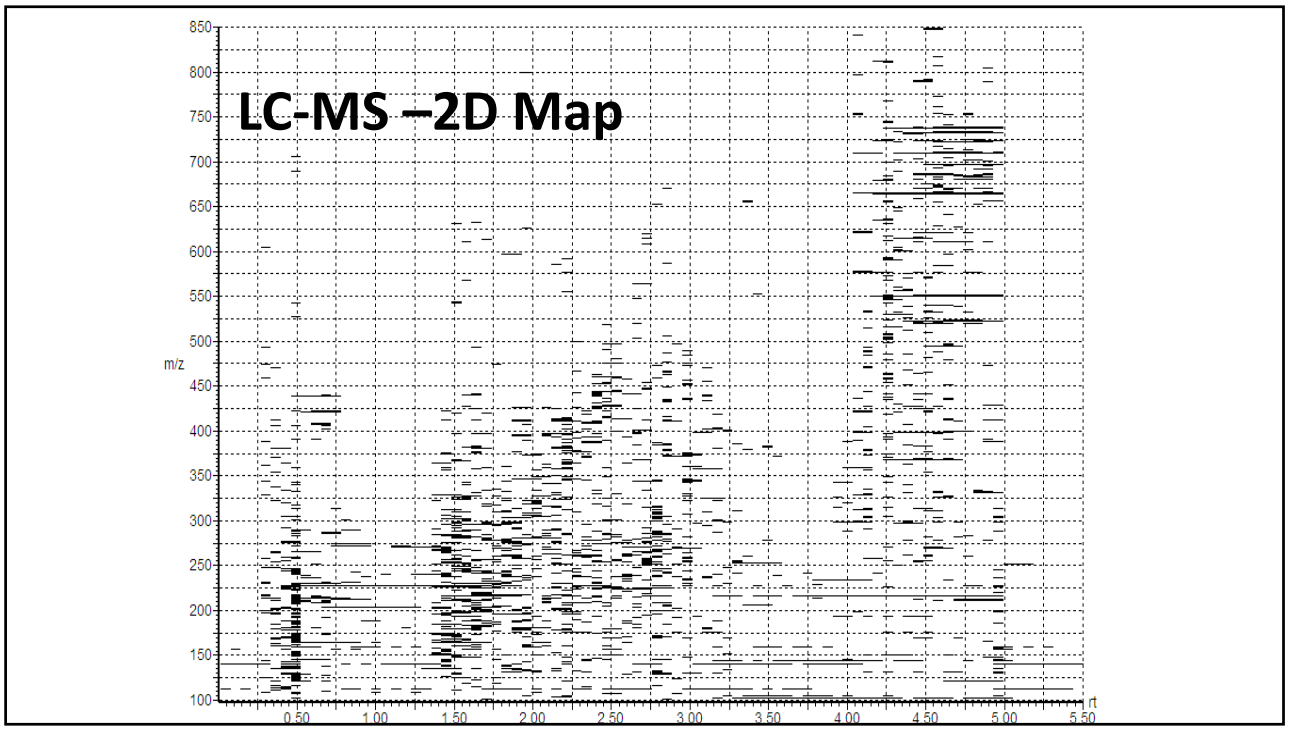


Sources of variability in LC/MS–based methods

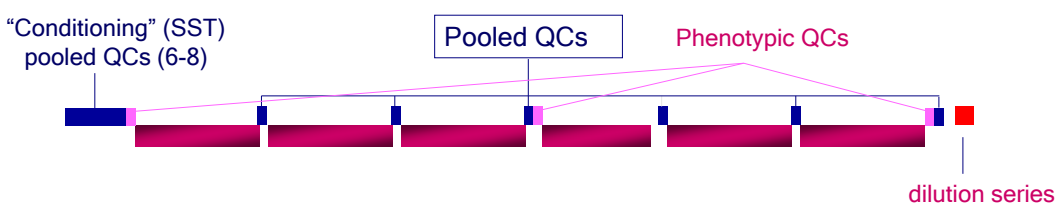
- Changes in chromatographic properties (retention, peak shape, resolution, selectivity).
- Changes in mass accuracy.
- Changes in sensitivity with time.

Showing Precision and Stability via Quality Control

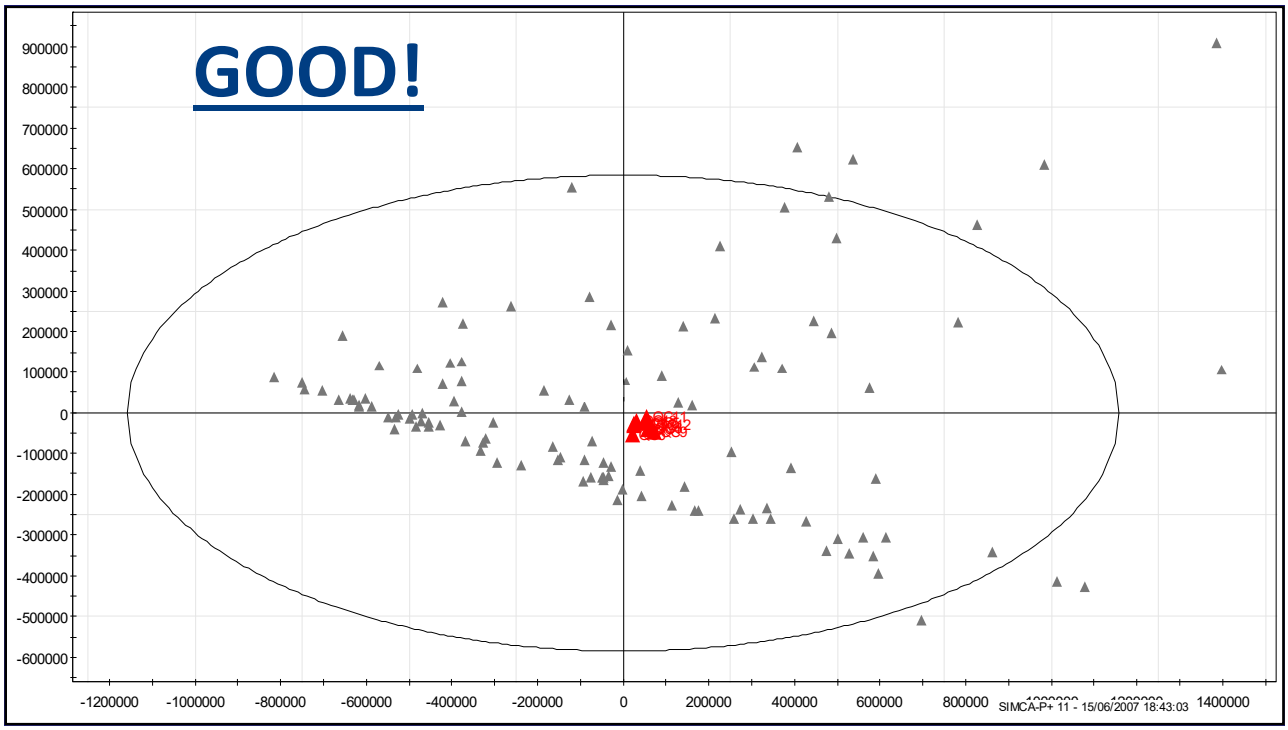
- **How do you formulate a QC policy when you don't know what you want to measure?**
- **One approach is to use a standard/representative BIOLOGICAL sample, ideally by taking an aliquot of each sample to make a pooled sample.**
- **This pooled QC can also be used as a system suitability test, and via a "dilution series, to show the linear range of the system**
- **For long term studies may also want to use a reference sample/QC for inter-batch correction**

QC approach : Pooled urine Quality Control injection sequence

Sample sequence; random sequence of (e. g. male and female) urine samples in blocks of 5/10 samples



H. Gika et. al. J. Proteome Res. 2007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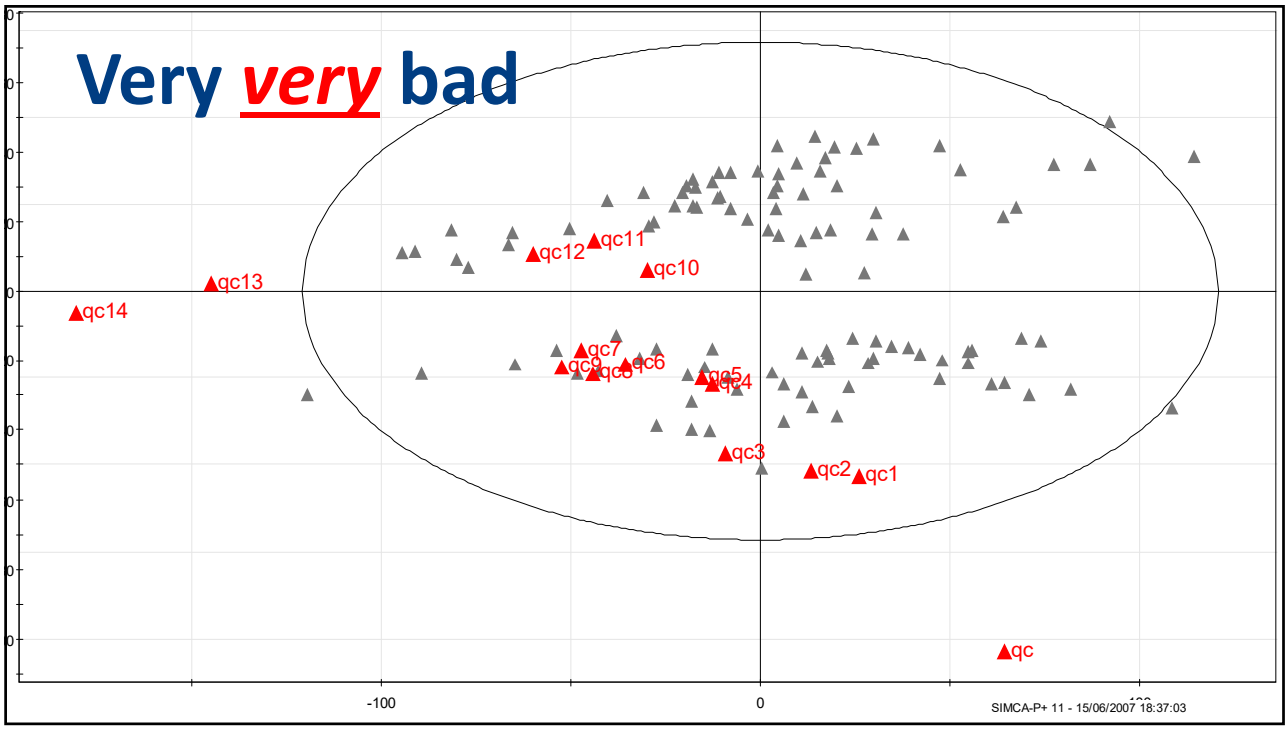
Very very bad
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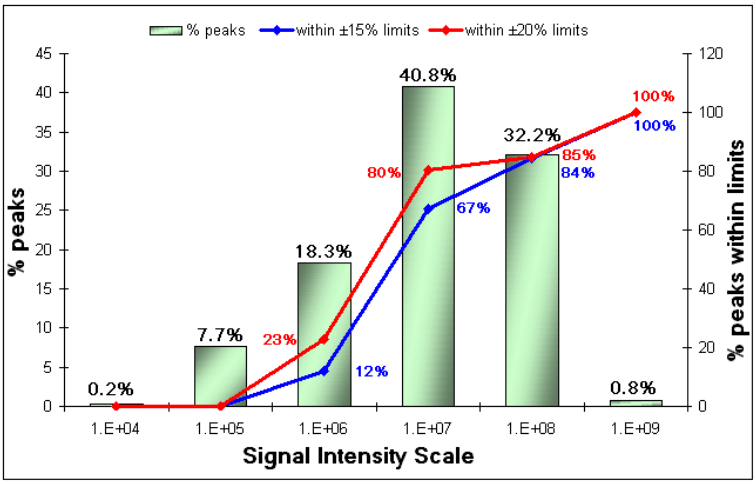
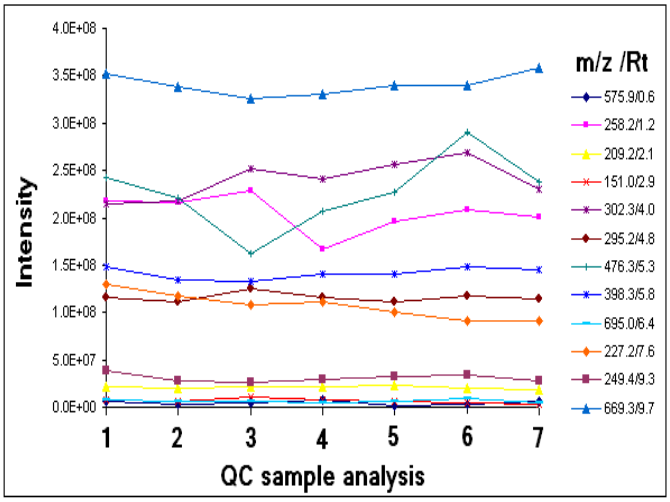
Intensity vs reproducibility



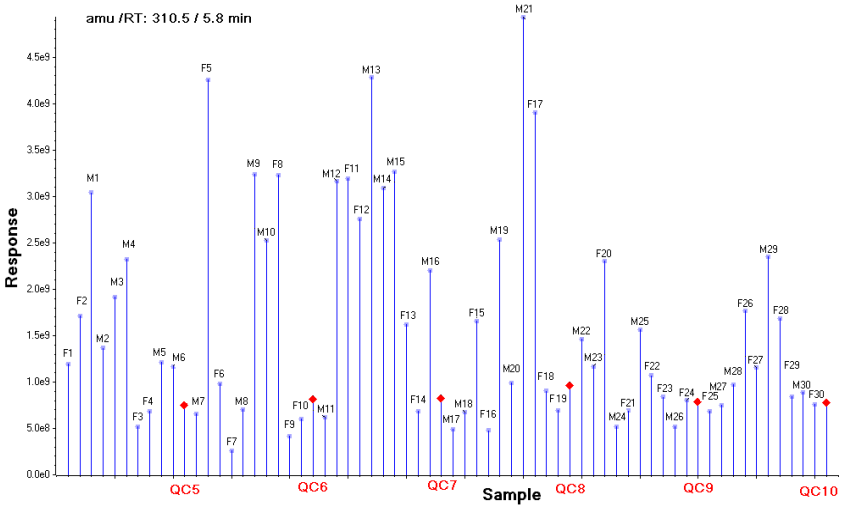
Gika H.G, et al *J. Proteome Res*. **6** (2007) 3291

Variablity of Response with time……

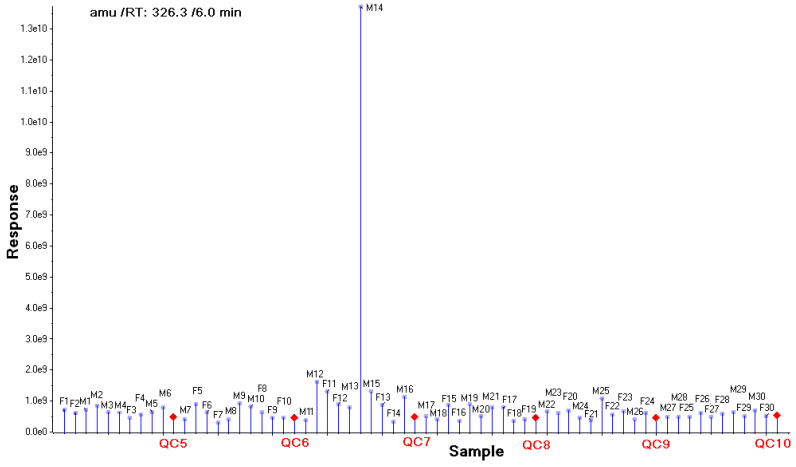


Gika H.G, et al *J. Proteome Res*. **6** (2007) 3291

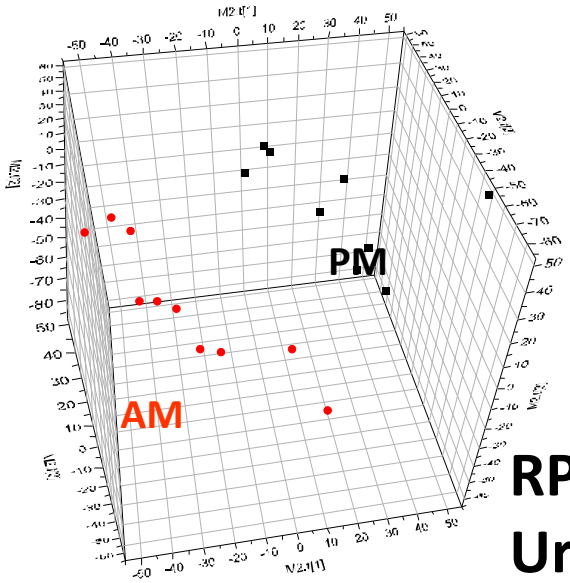
QCs for Data Assessment (I)



QCs for Data Assessment (2)

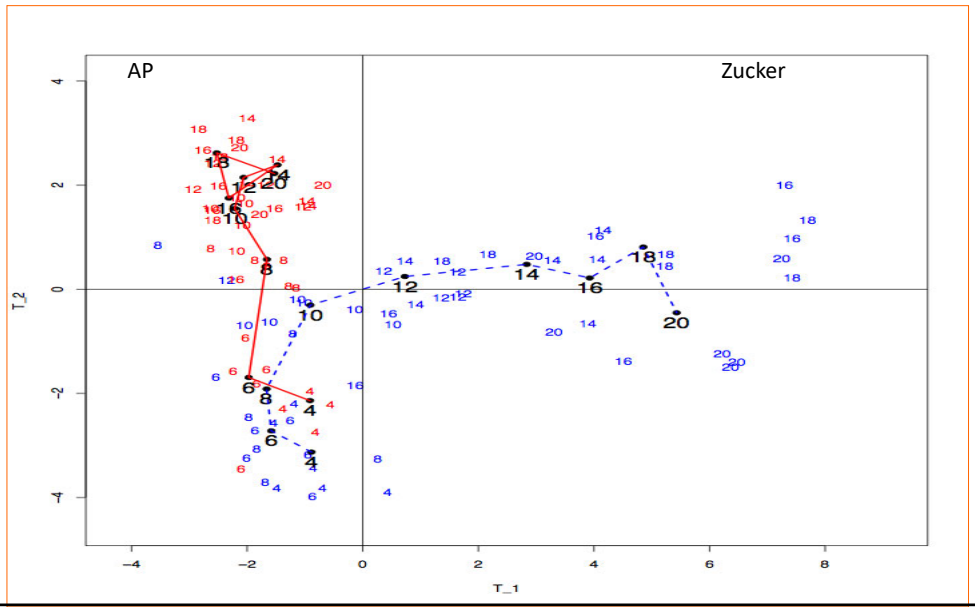


AM vs PM in the Rat

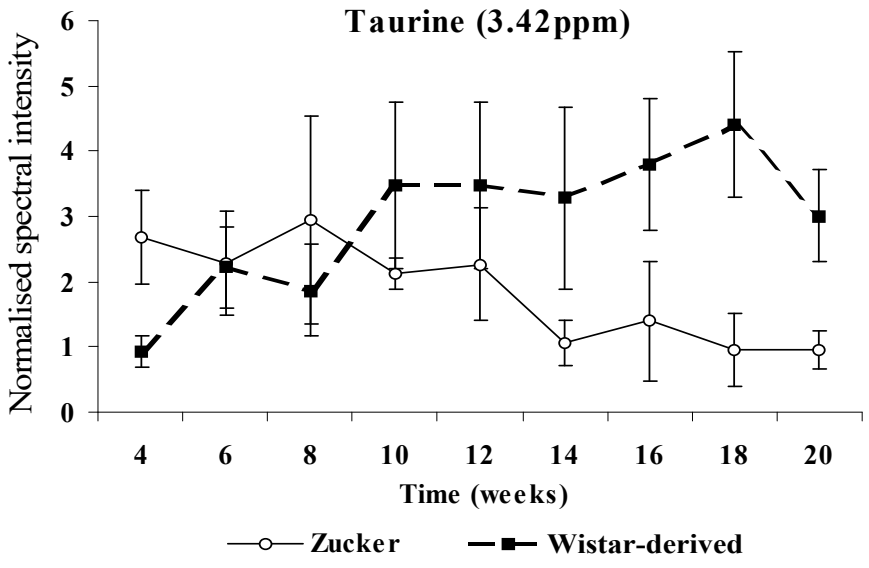


RPLC-MS of Urine + PCA

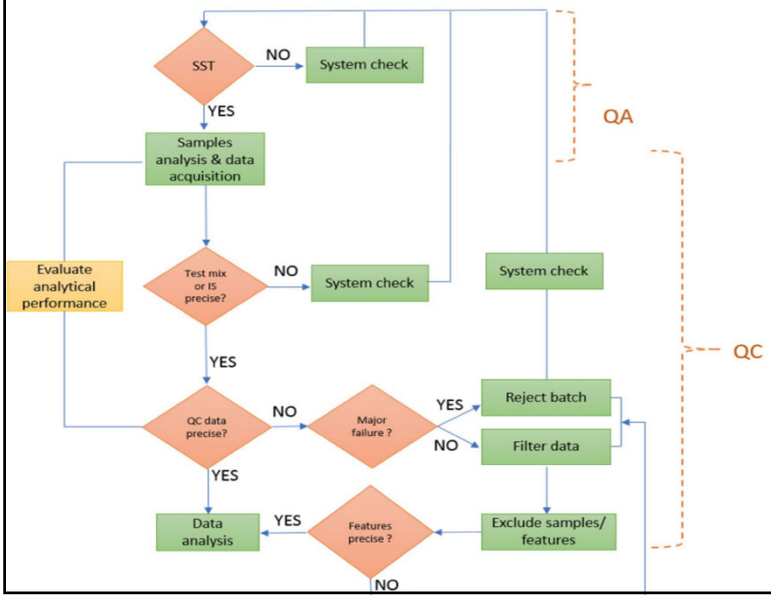
The trajectory of disease



Normal vs Zucker (fa/fa) obese Rats



Using QCs in untargeted semi-quantitative assays



Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management Kirwan *et al*, Metabolomics, 18, 70 (2022) https://doi.org/10.1007/s11306-022-01926-3
OPEN ACCESS

Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. Broadhurst, et al. Metabolomics 14, 72 (2018). https://doi.org/10.1007/s11306-018-1367-3
OPEN ACCESS

Conclusions

- **Untargeted semi-quantitative methods can provide repeatable and robust fold change data to identify potential biomarkers**
- **Instrument performance, analyte precision, repeatability, robustness can be demonstrated, even when the identity of the analyte has yet to be determined, via pooled (and other) QCs**
- **Having identified the biomarkers develop quantitative targeted methods**

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