What You Cant See Can Hurt You

(or what you get when you ask someone to “think outside the box“!)

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With thanks to Liz thomas

Acknowledgements

- In Particular
- Eleni Gika, Filippos Michopoulos, Lindsay Lai and Georgios Theodoridis
- Timothy Sangster, Tony Edge, Hilary Major, Rob Plumb, and many others…..

Outline

- What you already know (TYAK)
- More things you already know (MTYAK)
- Some things that you might not know (TYMNK)
- Ways of looking at things that you may already know without fully appreciating them!

Advantages of LC-MS (TYAK)

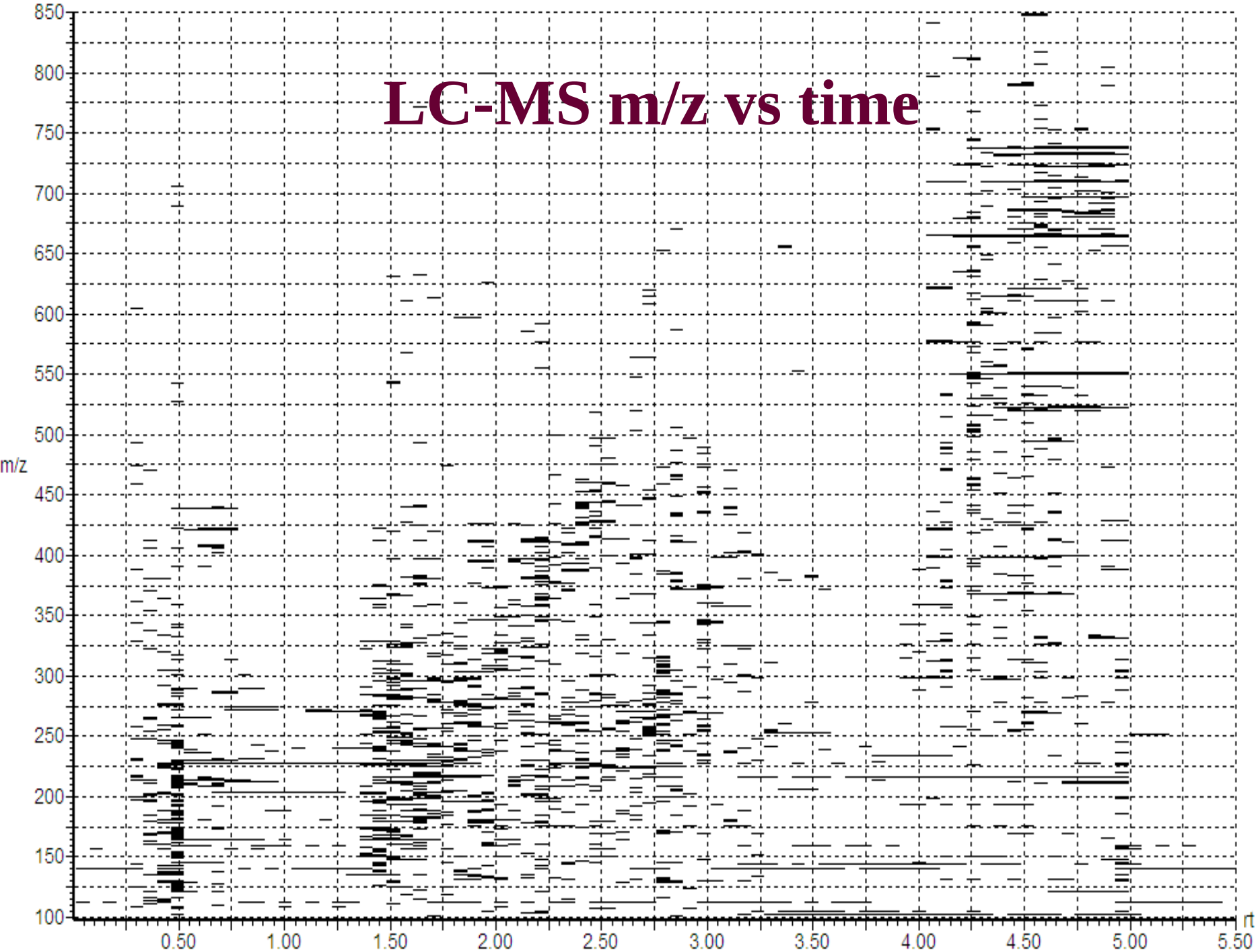
- Sensitive.
- Specific.
- Accurate.
- Reproducible.
- Capability for identification
- Widely available – it is the current bioanalytical methodology

Disadvantages of LC-MS (MTYAK)

- Unstable.
- Irreproducible.
- Temperamental.
- Not always robust.
- Subject to ion suppression/enhancement
- Only really quantitative with an isotpic internal standard

Lessons from metabonomics (TYMNK)

- **Method requirements are *a bit frightening*, very broad analyte classes and wide concentration ranges.**
- **How do you develop "valid", sensitive, specific methods for metabolic profiling when the analytes are unknown in advance?**
- **How do you demonstrate precision and reproducibility when you don't know what you are measuring?**
- **How do you get it repeatable + stable over long Periods?**
- ….


LC-MS m/z vs time
m/z
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
5.50
rt

Obvious sources of variation

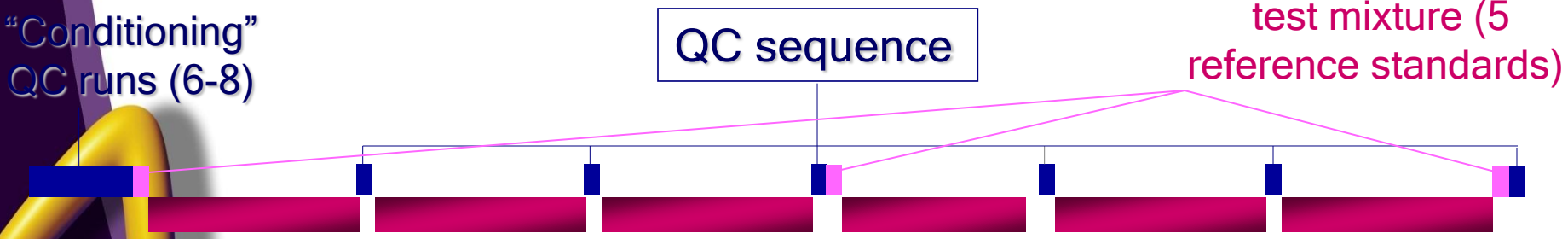
- Changes in chromatographic properties (retention, peak shape, resolution, selectivity).
- Changes in mass accuracy
- Changes in sensitivity/response.

method “validation”

- Biological QC made from aliquots of samples.
- Ca. 100 test URINE samples
- Run a number of QCs first to check the system and then every 10 samples
- IF THE METHOD IS PERFECT THE QCs SHOULD ALL BE THE SAME

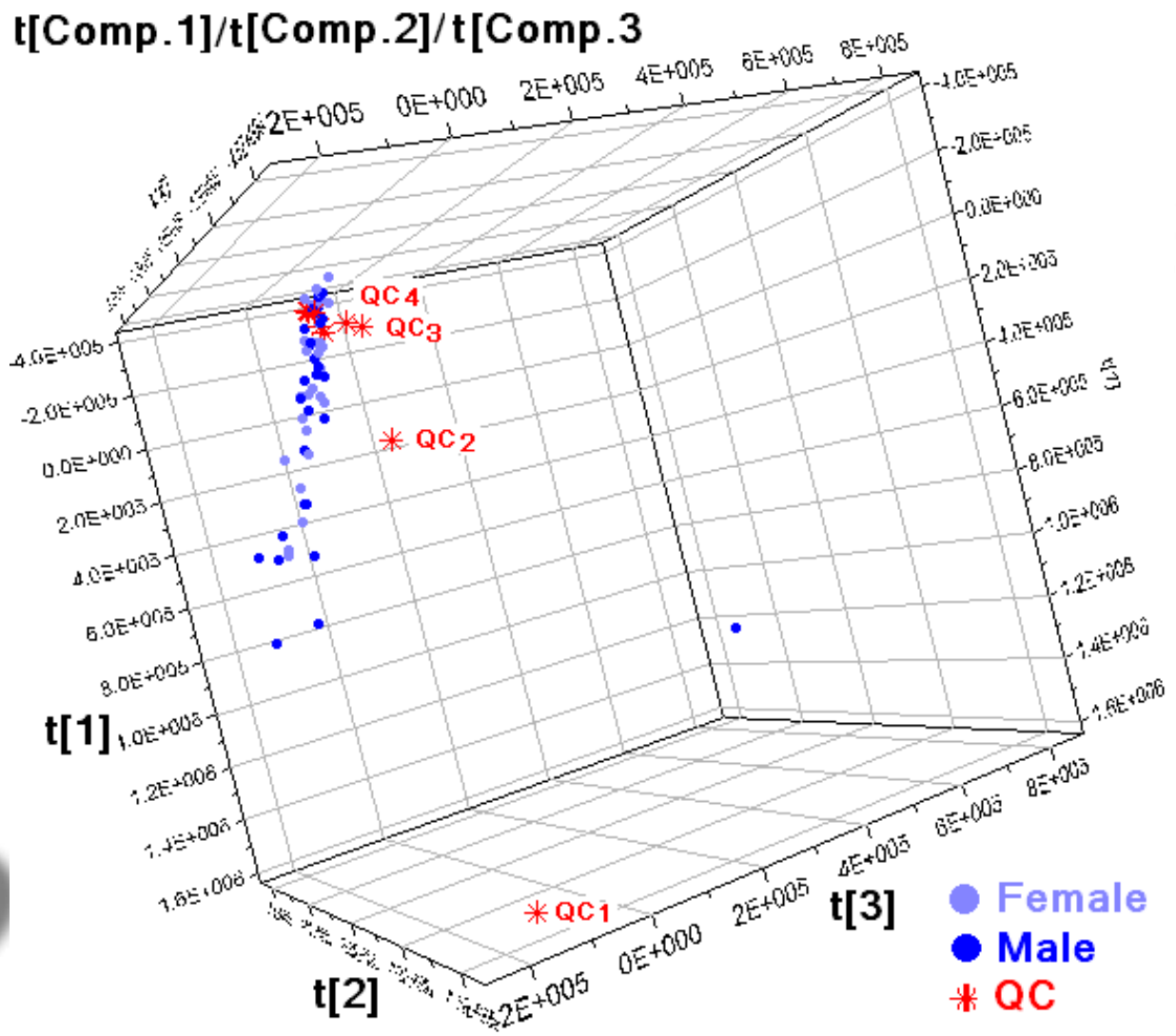
QC approach : Pooled urine Quality Control injection sequence

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

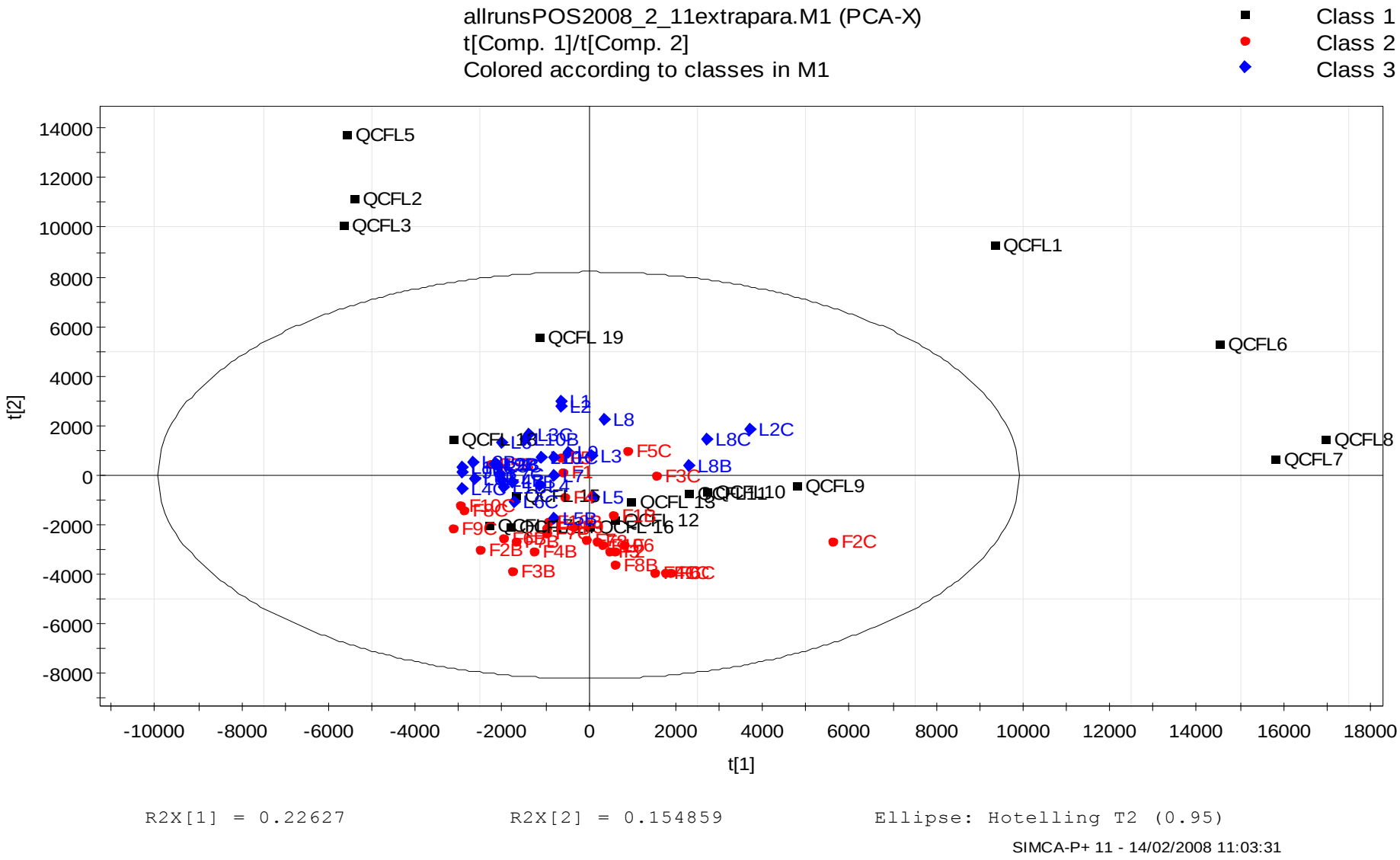


H. Gika et. al. J. Proteome Res. 2007

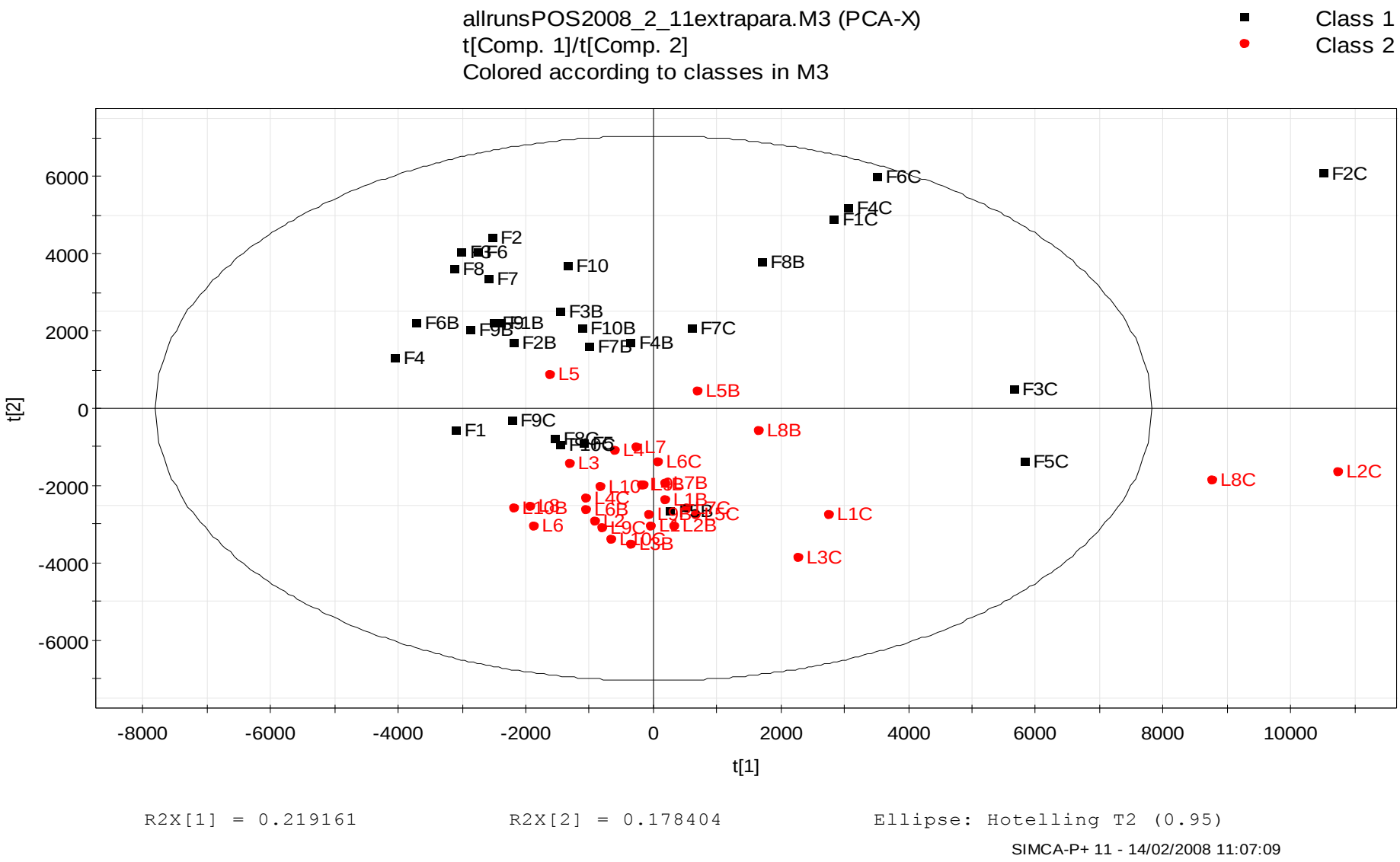
System conditioning



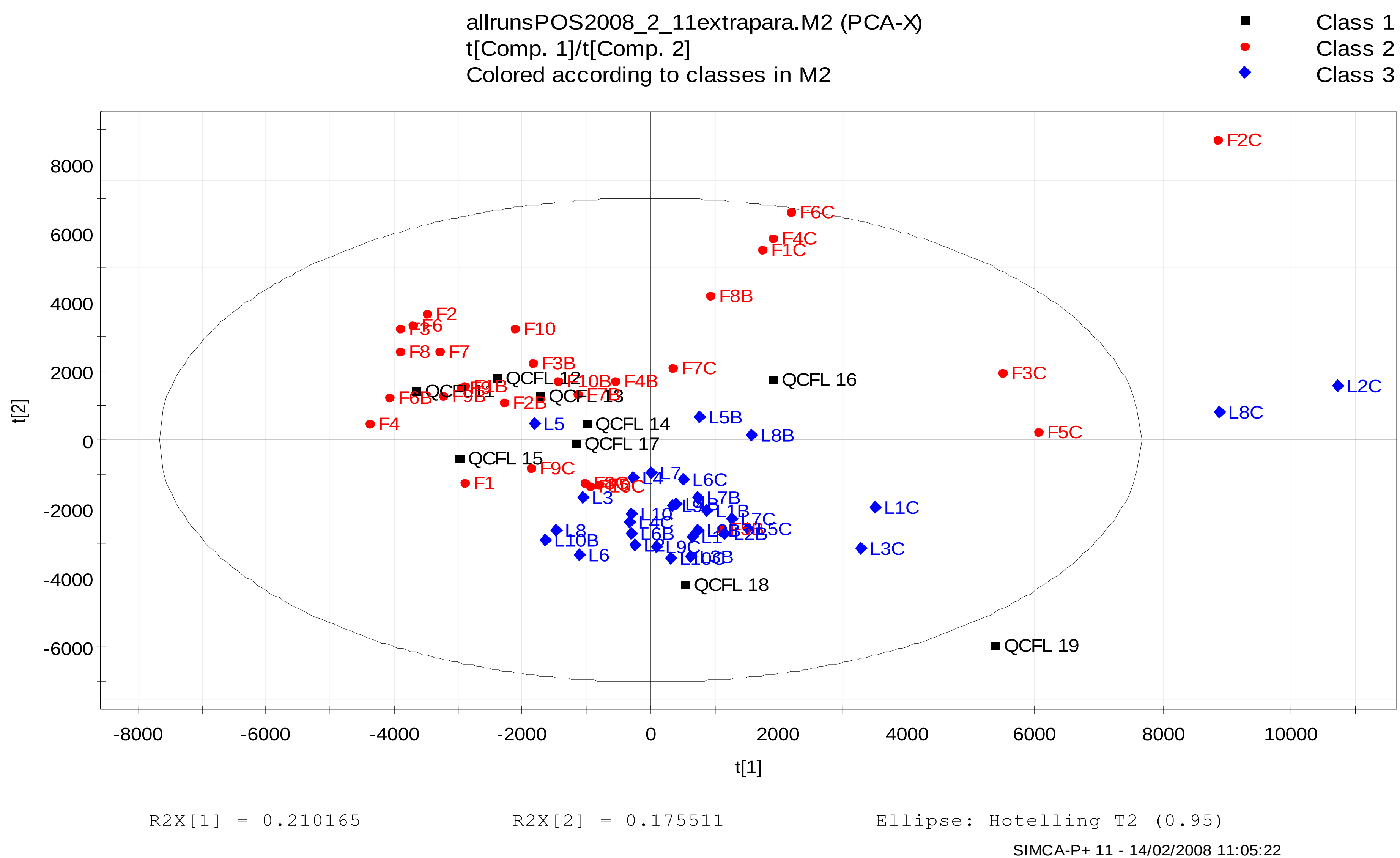
Conditioning injections + QCs



Plasma extracts: 2.1 mm column



QCs

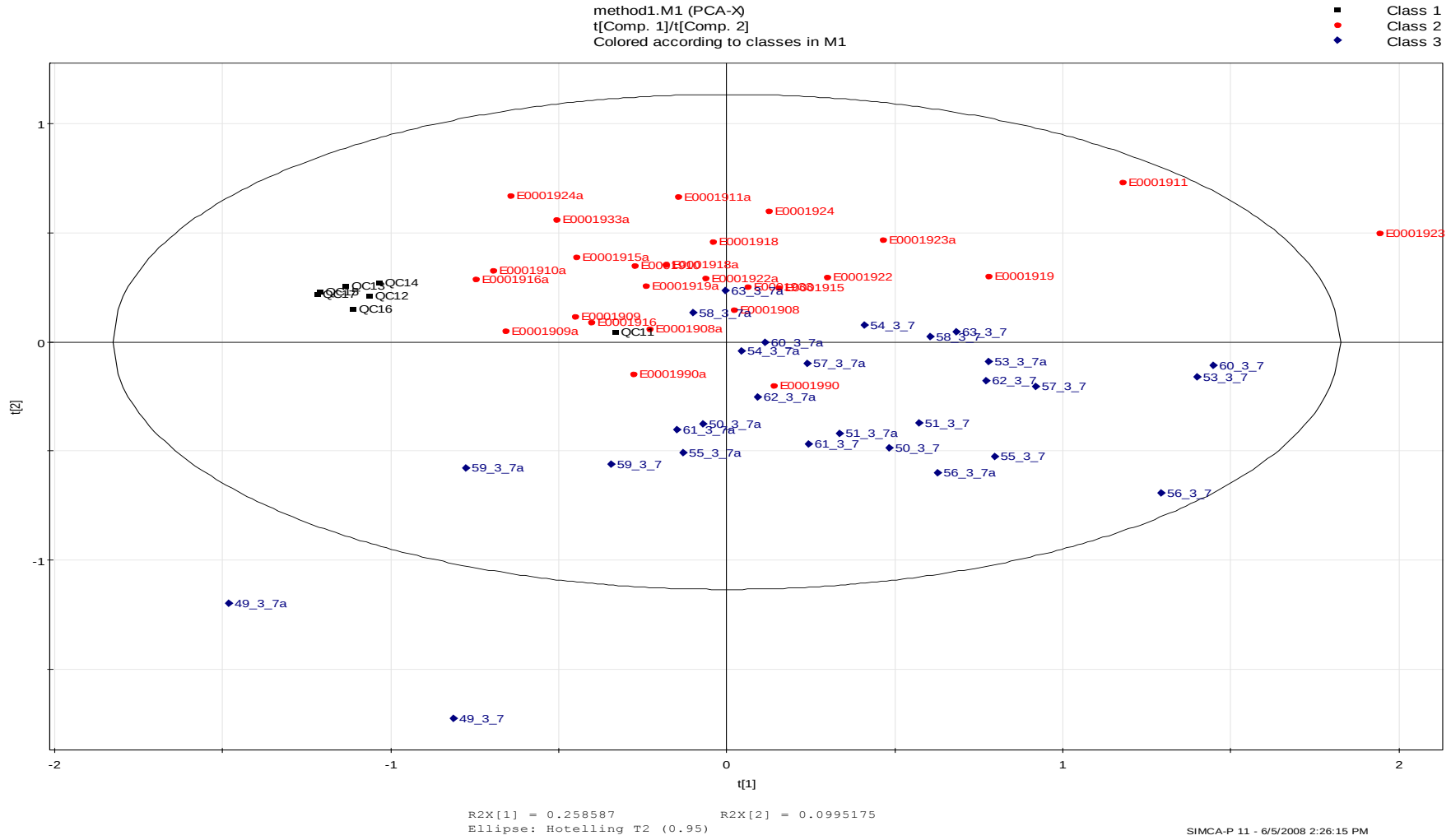


allrunsPOS2008_2_11extrapara.M2 (PCA-X)
t[Comp. 1]/t[Comp. 2]
Colored according to classes in M2
Class 1
Class 2
Class 3
t[2]
t[1]
QCFL 12
QCFL 13
QCFL 14
QCFL 15
QCFL 16
QCFL 17
QCFL 18
QCFL 19
F2C
F6C
F4C
F1C
F8B
F3C
F5C
F7C
L2C
L8C
L5B
L8B
L1C
L3C
L5
L6C
L3
L6
R2X[1] = 0.210165
R2X[2] = 0.175511
Ellipse: Hotelling T2 (0.95)
SIMCA-P+ 11 - 14/02/2008 11:05:22

Equilibration/column conditioning

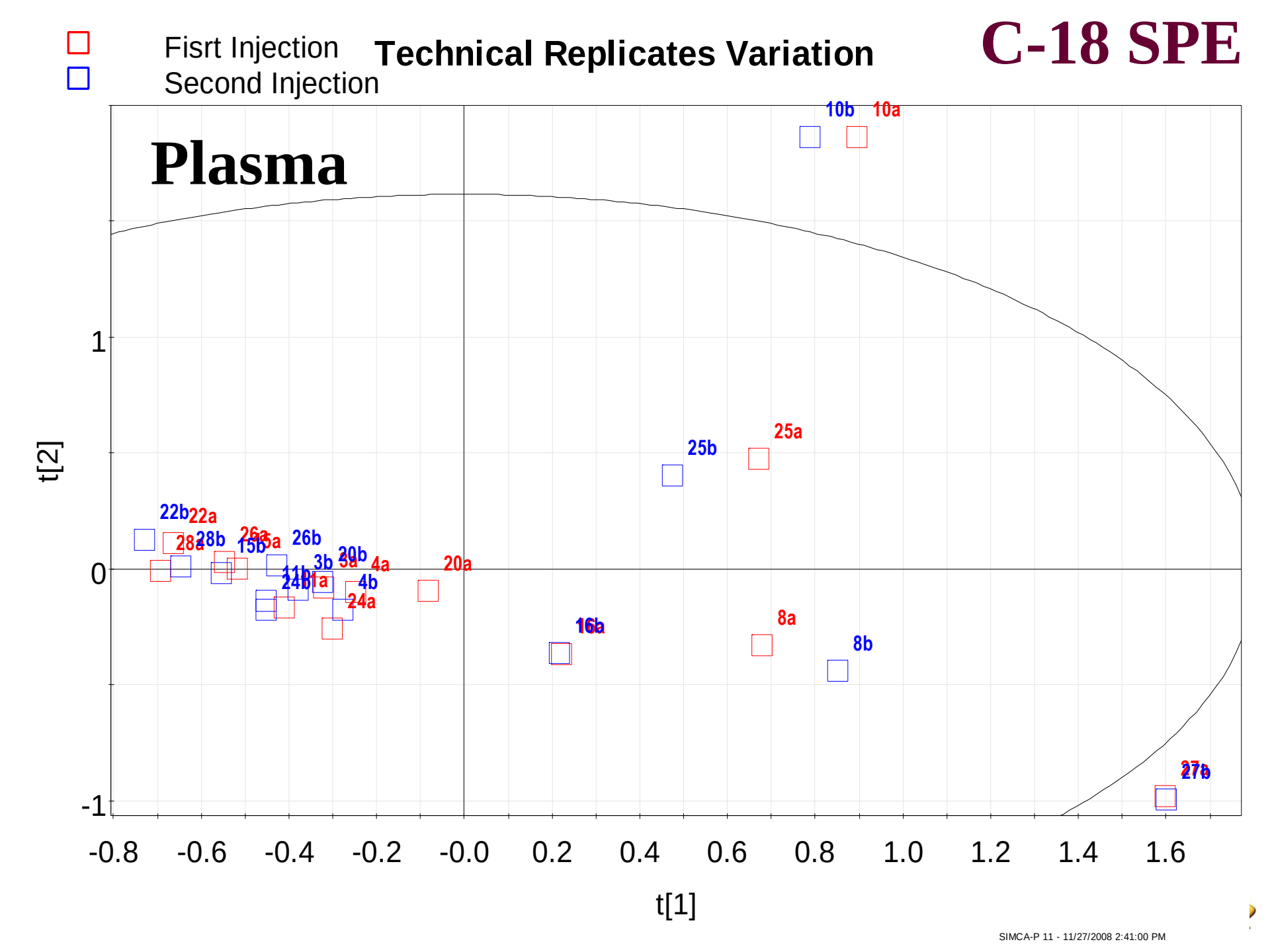
- For plasma we seem to need a lot of column conditioning
- If we need 10 injection on a 15 min run that's nearly 3 hours before we START to acquire data
- Can we do it faster? Larger injections and short runs

10 Injections of 20 ul Then Start

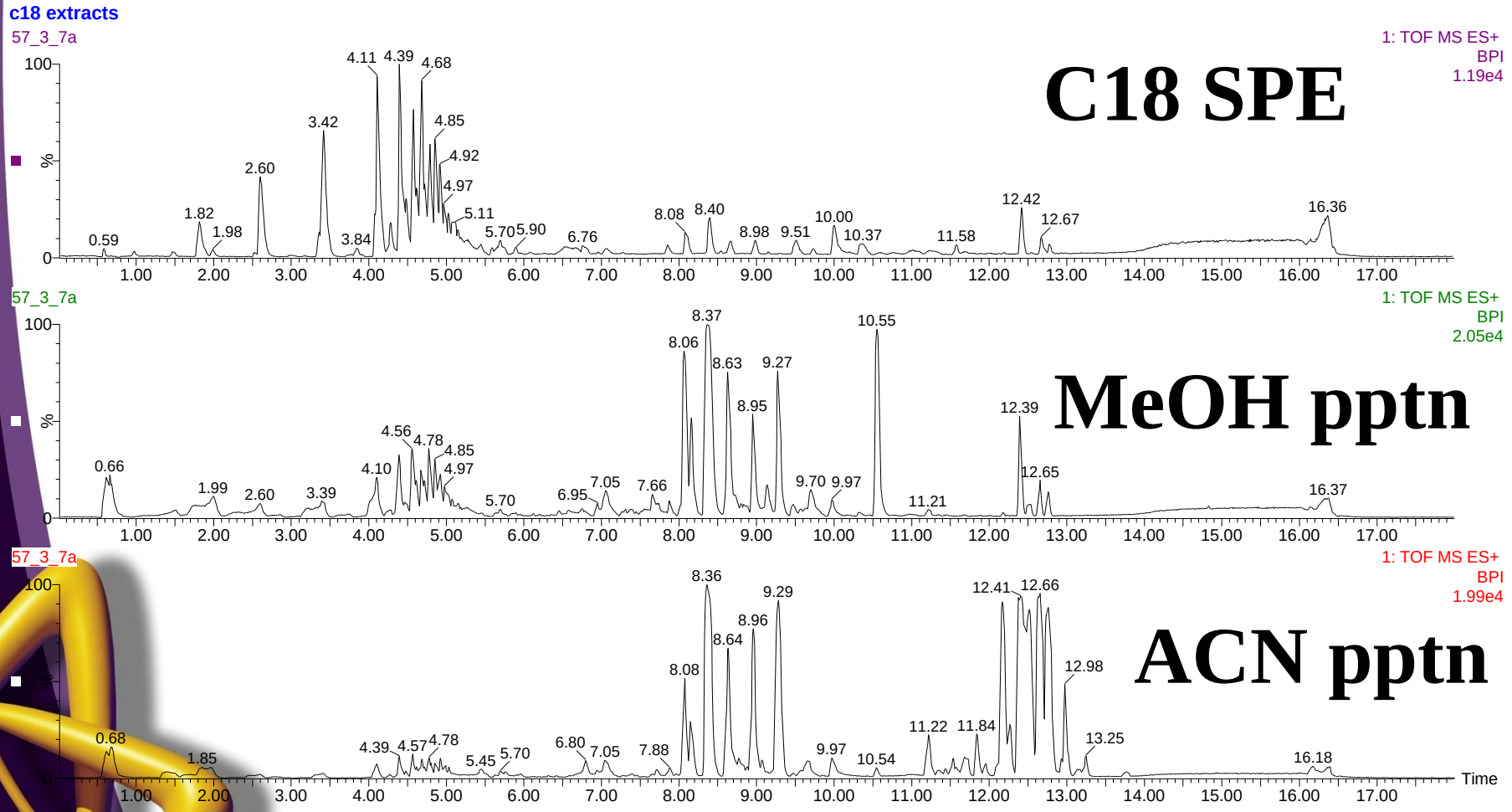


Does Sample Prep Make a difference?

- What I've shown you for plasma was based on solvent precipitation
- What do samples that have been subject to SPE show with respect to repeatability?


Fisrt Injection
Second Injection
Technical Replicates Variation
C-18 SPE
Plasma
10b
10a
25b
25a
22b
22a
28b
28a
26a
15b
15a
26b
20b
3b
3a
4a
20a
11b
24b
1a
4b
24a
16b
16a
8a
8b
27b
27a
1
0
-1
t[2]
-0.8
-0.6
-0.4
-0.2
-0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
t[1]
SIMCA-P 11 - 11/27/2008 2:41:00 PM

Plasma Sample Preparation



The sample prep defines what you see

So What Is Going On?

- Changes for the “conditioning” injections are due to changes in chromatography
- The cleaner the sample the long it can take to “condition”, and for retention times to stabilise
- Working hypothesis A – sample components building up on the column making a new stationary phase
- Working hypothesis B – it’s the phospholipids!

Consequences for Bioanalysis?

- If you have an isotopic internal standard there may be none for quantification
- But you might get retention time drift
- Once you have “conditioned” the column the ion source had better watch out!
- Samples from disease states may not replicate well the control plasma that you used for method development.

Conclusions

- **LC-MS is wonderfully selective in what it shows you, don't be fooled.**
- **The sample changes the chromatography, and also, eventually the MS response.**
- **Is it a problem? ……**