How stable are the urine samples stored in your freezer? An LC-MS metabonomic study

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Introduction

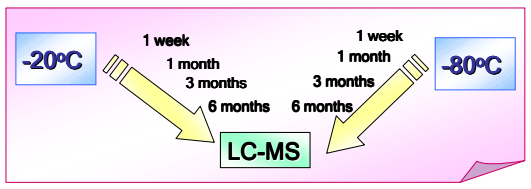
Metabolic phenotyping using LC-MS generates multivariate data that can be mined for biomarkers and become diagnostics tools for e.g. disease monitoring. Such exploration of biochemical pathways in disease requires that the methods used must be robust and differences between samples should reflect the underlying biochemistry of the disease and not artefacts arising as a result of sample handling and storage.The conditions under which the samples are stored / prepared might be expected to have a major impact on the quality of the data obtained in any such study of this type.

In this study the effect of storage conditions and treatment of urine samples collected from normal healthy male volunteers are examined to optimise sample collection for various periods of storage.

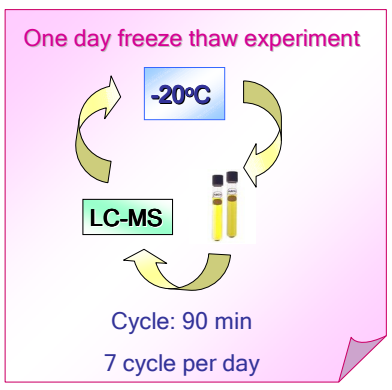
Stability at -20ºC and -80ºC were examined to see if the lower temperature offered any advantages. Moreover the effect of one or more freeze thaw cycles on samples stability was also examined.

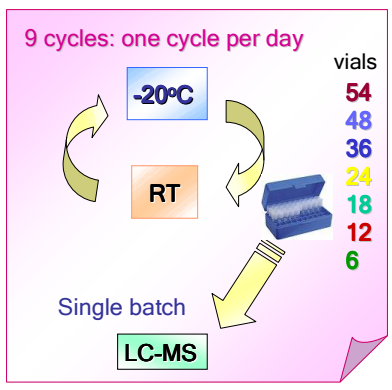
Study overview

Sample storage stability at -20 and -80ºC : Urine samples from 6 healthy adult males were divided into 9 aliquots. One set was analysed 1/2 hour after the collection, the rest being stored at either -20 °C or -80 °C analysed after 1 week , 1, 3 and 6 months.



Freeze-Thaw stability: Urine samples, stored at -20ºC, underwent two types of freeze thaw cycles: In one case, six tubes were thawed and frozen 7 times within a day and samples were immediately analysed. In the second case (diagram on the right) the samples were aliquoted in autosampler vials that underwent from 1 to 9 freeze – thaw cycles. Every set of aliquots was taken from the freezer and allowed to thaw at room temperature (ca. 3 h) before refreezing. All sample subsets were analysed as a single batch.





Experimental

Sample preparation: Urine was 1) centrifuged at 13,000 rpm 30 min, 2) diluted 1:4 v:v with 0.1 % aqueous formic acid and 3) injected on LC-MS

LC-MS QTRAP® 4000 analysis (AB|MDS Sciex):
Symmetry C18, 3.5um (2.1x100 mm) column at 40 (±0.2)oC, gradient acetonitrile (B) over 0.1% formic acid in water (A), (0-0.5 min) 100 % A, (0.5-4 min) 80% A, (4-8 min) 5% A, (8-9) 5% A. flow rate 400 uL/min. Injection 10 uL
(+),(-) ESI TurboIonSpray® at 350°C, ±4500 V separately. Curtain Gas 20 psi, Auxiliary Gas 40 psi. Profile mode: 12 min EMS 100 to 850 m/z (1000 amu/s), Ion Trap: Dynamic Fill Time mode.

ACQUITY UPLC™ Q-TOF Micromass® analysis (Waters):
Acquity BEH C18, 1.7um (2.1x100 mm) column at 45 ºC, Gradient acetonitrile (B) over 0.1% formic acid in water (A), (0-0.5 min) 100 % A, (0.5-8 min) 100% A, (8-9 min) 0% A. flow rate 400 uL/min. Injection 10 uL.
Ion spray 3500 V, Desolvation Temp 300°C, Ion source 120°C, Cone gas 10L/hr, Acceleration (V) 200.0, Reflectron (V) 1780.0, Lteff 1080.80 and Veff 5630.00. Scan from 80 to 850 m/z, scan speed 0.32 sec

Data analysis : MarkerViewTM software 1.1.0.1, Marker Lynx, Simca P 11 (Umetrics).

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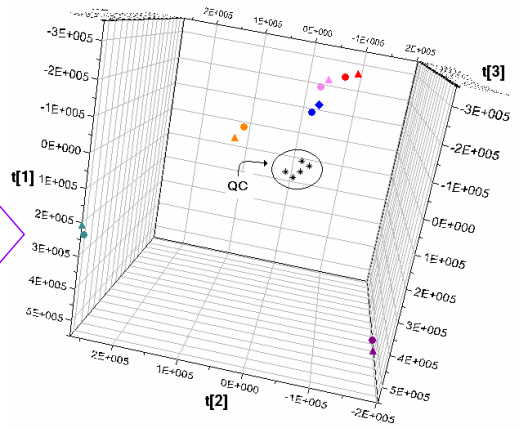
Results

Sample Stability

Samples from 6 male subjects were analysed after storage for up to 6 months at -20 and -80ºC: PCA analysis of the data obtained from analysis of these samples, at either -20 °C or -80ºC, showed the samples from each subject clustered closely together, irrespective of the storage conditions, implying that the samples were unchanged over a storage period up to 6 month.

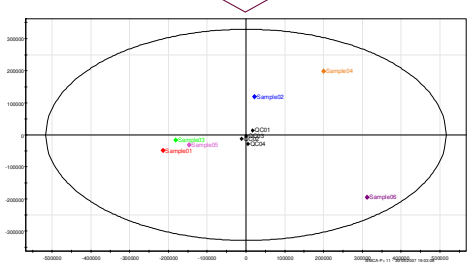
PCA of the 6 same samples analysed after one month storage at **-80ºC** (solid triangles) And **-20ºC** (solid circles).



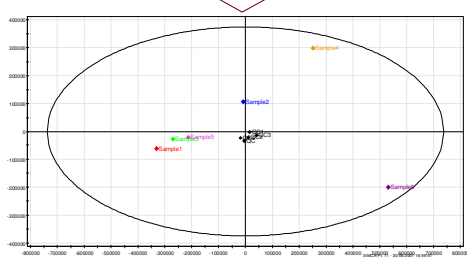
The QC samples, showing the analytical variability are indicated by the "star " symbols

For these periods of storage when samples are compared with the pre-storage data the PCA plots are similar: samples are plotted relatively to each other in the same positions before and after storage (either at -20 or -80ºC)

Pre-storage (1/2h after collection) analysis

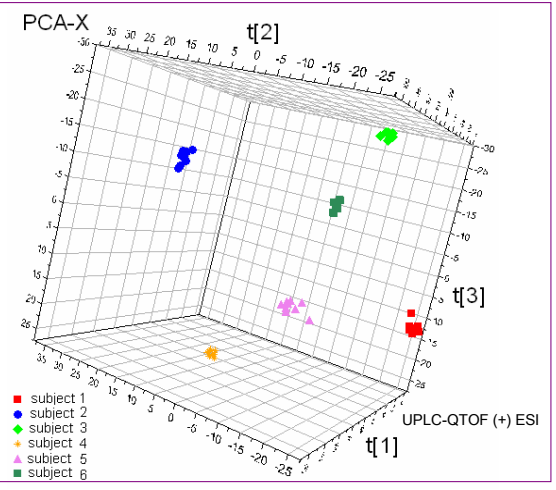


After 1 month storage at -20ºC analysis



Freeze-Thaw stability

Analysis of the data obtained from samples that had undergone from 1 to 9 freeze-thaw cycles showed that, by PCA, **the number of freeze thaws did not affect how they clustered in the scores plot**. This suggests that, for urine samples such as these, the overall stability of the samples with respect to freeze-thaw seems acceptable.



Conclusions

These data suggest that storage of urine samples at -20ºC for at least 6 months is acceptable. Similar results for sample stability have recently been reported [2] for ^{1}H NMR-based analysis which showed sample stability for human urine at -25ºC for 26 weeks. No differences are found between -20 and -80ºC storage conditions.

All of the results with respect to stability described above apply to the "bulk properties" of the sample as determined by LC-MS and subsequent PCA. This represents a pragmatic tool for assessing stability and can be criticised as representing a rather blunt instrument. Indeed there may well be individual components that are subject to degradation under the conditions described above that are **not** highlighted by this approach. Clearly, once a particular analyte, or set of analytes, is detected that may be a candidate(s) biomarker(s) a more targeted assessment of stability must be undertaken.

References

1. FDA Guidance for Industry, Bioanalytical method Validation, Food and Drug Administration, centre for Drug valuation and Research (CDER), May 2001.
2. M. Lauridsen, S. Hansen, et al Anal Chem 79,1181-1186, 2007
3. H. Gika, G. Theodoridis, J. Wingate, I. Wilson, J. Prot. Research (*accepted)*