Comparison of UPLC-MS vs LC-MS in sample fingerprinting for metabonomics research. Is there a real niche for UPLC?

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Introduction

The use of LC-MS in global metabolite profiling (metabonomics / metabolomics) is hampered by experimental variability and inconsistency. The ruggedness and robustness of NMR is not a characteristic of the LC-MS technology we have at hand. Both MS and LC are multri-parametric, multi-dynamic technologies. Hence the combination of the two, although now the work horse of the pharmaceutical industry, is by no means as reproducible as NMR.

Major parameters that affect the integrity of a metabonomic study are identified in the stabilisation of the MS (mostly its ion source) and the reproducibility of the LC separation. Although normalisation of the MS signal can be applied (and is in fact implemented in all metabolomic software), retention time drift is much more difficult to address.

UPLC has been introduced as an advanced Liquid Chromatographic Technology that utilises smaller particle sizes and thus provides higher separation efficiency.

We therefore decided to compare HPLC and UPLC as the liquid separation part in an LC-MS metabonomic study.

Our **scope** was to investigate:

- if any real advantage is offered by UPLC?
- which technology provides more features (variables in PCA)?
- which system is more reproducible?
- the utility of the combination of UPLC with different MS machines (QTOF, QTRAP, triple quad, ORBI TRAP)

Experimental Design

Urine was chosen as the specimen of choice. Two sample sets

1) Samples from 30 healthy male & 30 female volunteers were analysed sequentially in HPLC-MS and UPLC MS in one-go.

2) A *diseased* population (57 individuals) provided urine along with the same number of control individuals with matching gender, age, living environment ethnicity, BMI index. The 114 samples (plus 16 QCs) were analysed in one-go.

A Waters UPLC Acquity system and a Perkin Elmer LC solvent delivery system were coupled to a AB Sciex QTRAP 4000.

A "quality control" (QC) sample ("pooled" from all the studied urine samples) was injected in the beginning of the run (5 injections) and then every ten injections to provide a representative "mean" sample.

A standard "academic" mixture containing 5 endogenous metabolites was used to check the initial "system suitability" (injected in the beginning/ middle and end of study).

Run duration 17 -26 hours.

Non-targeted MS analysis: full scan (100-850 amu)

Experimental

Samples: Mid-stream urine samples: 30 healthy male & 30 female volunteers. 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

ACQUITY UPLC Acquity BEH C18, 1.7um (2.1x100 mm) column at 45 °C, 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.

LC/ESI-MS analysis : Symmetry C18, 3.5um (2.1x100 mm) column at 40 (±0.2)°C, gradient acetonitrile (B) over 0.1% formic acid in water (A), flow rate (0-0.5 min) 100 % A, (0.5-4 min) 80% A, (4-8 min) 5% A, (8-9) 5% A. 400 uL/min. Injection 10 uL

QTRAP® 4000 analysis (AB|MDS Sciex):
(+),(-) 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.
UPLC was also combined with an ORBITRAP (Thermo), a 365 triple quadrupole (AB Sciex) and a QTOF (Waters)

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

Results

Both LC and UPLC provide **powerful tools** for global metabolite profiling accumulating massive data sets. Full scan Mass Chromatograms (TICs in QTRAP) exhibit very complex visualisation patterns.

UPLC provides sharper peaks
and better separation efficiency

N calculated as **5.55 ($t^2/w_{1/2}^2$)**
Selected ions from the QC sample

	QTRAP				QTOF	
amu	LC		UPLC		UPLC	
	t_R	N	t_R	N	t_R	N
180	5.15	5749	3.61	86384	3.03	58551
180	1.4	8014	1.16	33982	1.03	26909
220	4.22	23393	3.58	24393	3.41	86486
155	1.6	9716	1.15	62254	1.14	60605

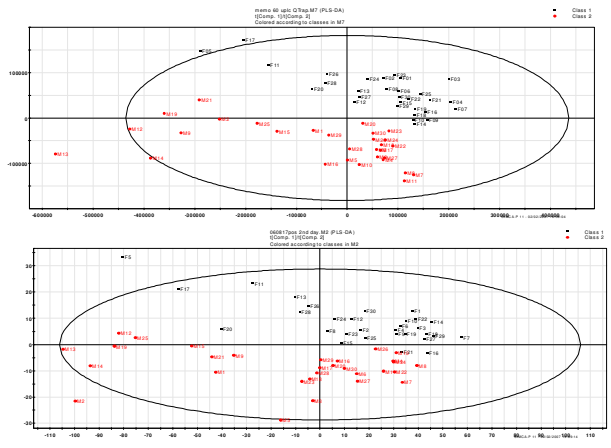
PEAK PICKING-ALLIGNMENT

For the healthy individuals sample set, using the same parameters for peak picking-alignment, the LC run produced **3341** variables whereas the UPLC gave **2846** variables.

For the diseased individual sample set the UPLC provided **8358** variables whereas the LC gave **3436** variables.

From the 8358 variables in the UPLC-MS dataset 7154 peaks (86%) were found in all 114 subjects; 7874 peaks (94%) were found for at least 85% subjects

Results

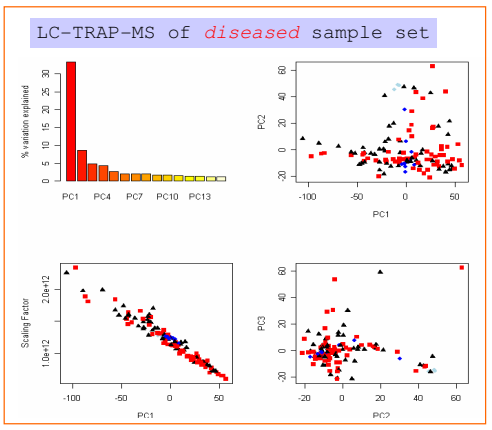


QTRAP Data

PCA plots of the 60 healthy individuals sample set reveal that the same samples lay out of the herd in both instrumental set-ups.

PLS-DA of the UPLC data set reveal a more eloquent separation of the two gender groups compared to the LC-MS data set.

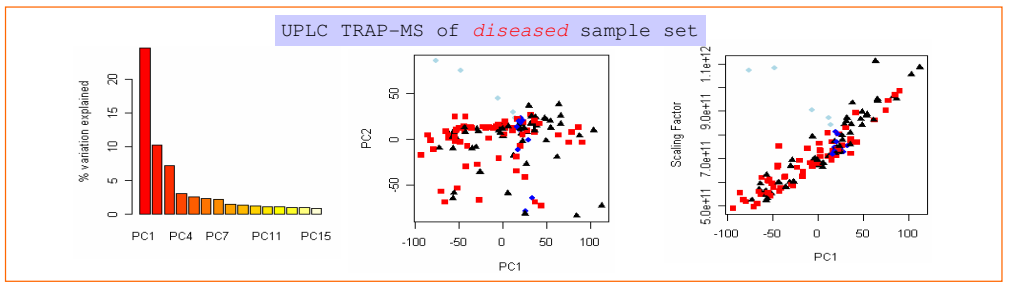
Loadings of the two data sets can not be compared as they differ and can not be incorporated in one data set.



For both instrument configurations (LC-QTRAP & UPLC-QTRAP) PC1 represents a major source of variability (ca 30%). This is well correlated to the scaling factor.

PCA does not reveal separation of diseased from healthy in either LC or UPLC data sets.

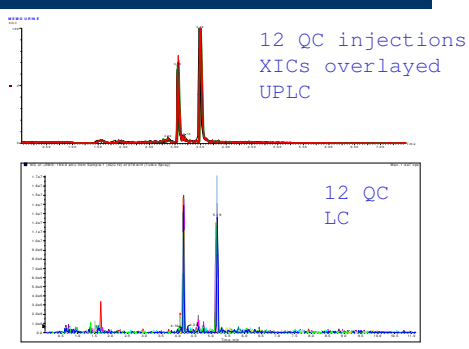
Separation of the two groups is seen in PLS-DA (data not shown).
Again UPLC provides somewhat superior group clustering.



Retention Time Stability

Retention time variation and drift are a primary concern in metabolomics because these can not be addressed by most software tools. In real life studies of hundreds of samples we are tempted to choose between high-throughput and optimised LC separation.

UPLC provided superior separation and retention time reproducibility.



Characteristic peaks as detected in

UPLC-MS				LC-MS			
t_R average				t_R average			
3.05	3.49	1.05	2.21	4.52	1.6	0.87	1.39
RSD(%) (n=10)				RSD(%) (n=10)			
0.45	**0.39**	**0.57**	**1.78**	**2.32**	**3.10**	**2.17**	**3.97**

Synopsis

- UPLC provided advantages over HPLC for global metabolite profiling. The higher N number, the higher peak capacity and the highest separation quality resulted in LC-MS datasets of higher fidelity.
- Narrow peaks (3-8 sec) are very useful for rapid MS analysers such as the TOF. They are **not** so useful in fact may prove detrimental for information rich high-end MS machines (Orbi-TRAP) which require more time for data accumulation (data not shown).
- Retention times are more reproducible thus providing more meaningful peak tables after peak alignment.
- The highest number of variables does **not** necessarily provide a real advantage. We have seen group clustering remaining "intact" even after eliminating more than 50% of the found variables.

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