A RAPID ANALYTICAL PLATFORM FOR BIOFLUID PROFILING IN DISCOVERY METABOLOMICS AND LIPIDOMICS

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INTRODUCTION

- **Current analytical platforms for metabolomics have analysis times between 10—30 mins per sample[1].**
- **For large batches (>1000 samples), total run time can take weeks to complete and longer with additional assay panels[2].**
- **Long analytical runs can lead to an increase in data variability and "batch effects".**
- **Geometrically scaling down these methods can increase throughput, reduce mobile phase usage and decrease these batch effects.**
- **A suite of rapid profiling methods have been developed to address these issues and support a previously developed reverse phase (RP) method[3].**

HILIC-METABOLOMICS

The developed rapid HILIC method performed the separation over 2.33 min starting with 99% mobile phase B and reducing to 50%. Re-equilibration is essential in HILIC and an equilibration step (<1 min) was added at the end. The method exhibited a reduction in run time of ~60% when compared to the template method (10 min), producing an overall sample cycle time of 3.33 min.

A system suitability mix containing three target compounds was injected on both HILIC methods, and as shown in Fig. 1, the relative retention times of each was maintained. This demonstrated the scaling had no impact on the performance of the HILIC retention mechanism.

The RAMMP method was then applied to the analysis of a batch consisting of 134 sample injections of rat urine following treatment with tienilic acid. The batch took 7.5 hrs to complete as opposed to >20hrs.

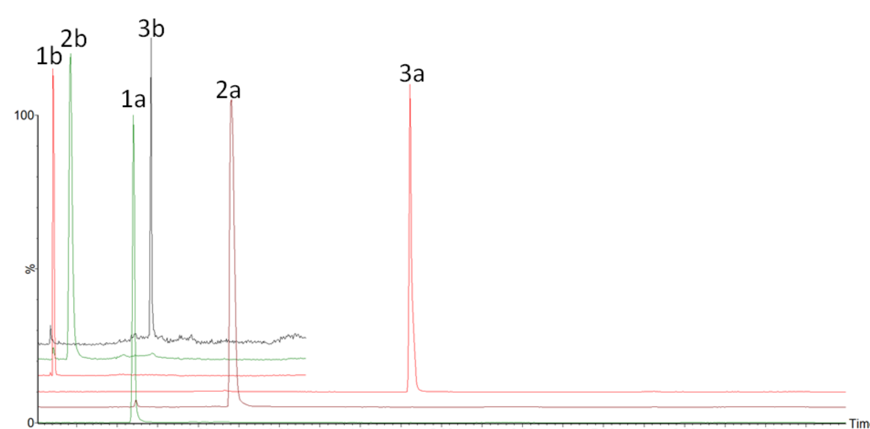


Figure 1. XIC chromatograms comparing retention time of three example compounds 1) sulfadimethoxine 2) sulfaguanidine 3) leucine enkephalin, analysed using a conventional HILIC method (a) and the rapid HILIC (b).

ION MOBILITY

- Due to the scaled down run time, the number of features detected reduced due to compound co-elution.
- Implementing ion mobility separation (IMS) increases the peak capacity of the assay and enables the number of detected features to double (Table 1).
- This is due to the IMS resolving co-eluting species based on different CCS measurements (Fig. 3).
- In combination with MS/MS database searches, searching measured collision cross section (CCS) values against the Waters CCS database improved identification confidence.

	Average peak width (s)	Peak capacity	Number of features detected
RAMMP HILIC	6.6	28	3007
RAMMP HILIC (w/IMS)	3.8	51	6711

Table 1. Comparison of peak capacity and feature detection for rapid HILIC with and without IMS

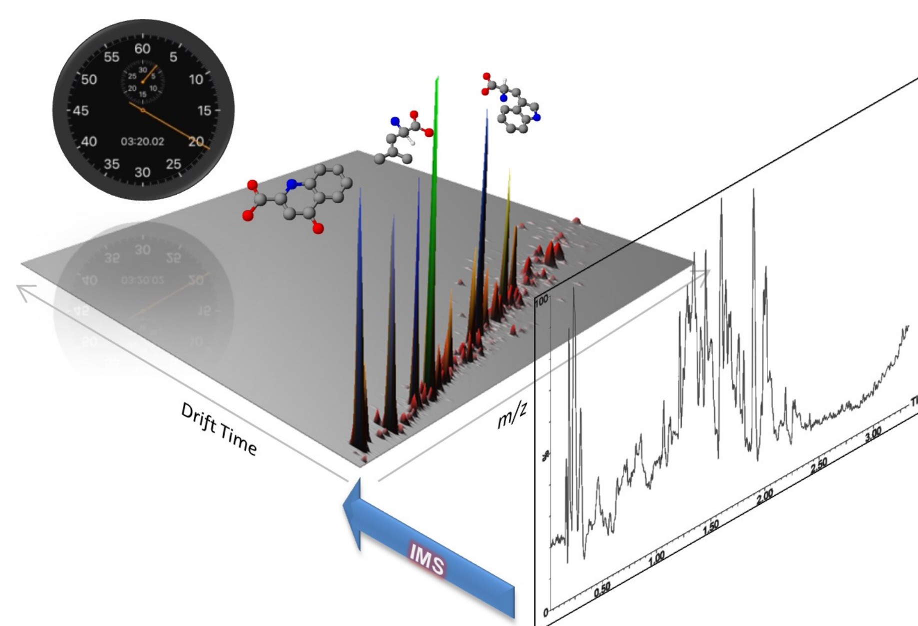
METHODS

Mass spectrometer:

- Waters Synapt G2-Si (Waters, UK).
- Positive and negative polarity.

UPLC:

- Waters Acquity I-class (Waters, MA, USA)
- 50 x 1.0 mm I.D. BEH Amide (HILIC) and BEH C8 (Lipid) columns.
- 0.2 µL injection volume.
- 0.2 & 0.25 mL/min flow rate (HILIC & lipid respectively).
- Column temperature of 50°C and 55°C (HILIC & lipid respectively).



Lipid identification	Neutral mass (Da)	m/z	Retention time (min)	CCS (Å²)	ΔCCS (Å²)	Peak width (min)	Anova (p)	q Value	Max Fold Change	Minimum CV%
TG(52:3)	856.75	874.79	3.14	334.1	-	0.20	5.5E-05	0.00166	1.5	6.03
TG(52:4)	-	872.77	3.06	331.4	-	0.23	1.0E-04	0.00201	1.8	4.85
TG(54:5)	880.75	898.78	3.07	337.6	-	0.18	1.5E-04	0.00237	2.6	5.53
PS (40:4)	839.57	822.56	1.62	304.4	-	0.30	2.1E-04	0.00274	2.0	4.38
TG(54:3)	-	902.82	3.22	341.1	-	0.24	3.4E-04	0.00386	2.0	5.54
DG(34:1)	-	577.52	3.2	267.1	-	0.18	3.7E-04	0.00404	1.8	2.97
TG(50:1)	832.75	850.79	3.19	332.5	-	0.16	3.9E-04	0.00408	1.7	5.15
PS(O-36:2)	773.56	774.56	1.55	295.8	-	0.31	5.0E-04	0.00452	1.9	3.16
PS(O-38:3)	799.57	782.57	1.62	299.7	-	0.28	6.5E-04	0.00529	1.8	3.09
PS(36:1)	789.55	790.56	1.54	298.7	-	0.41	2.3E-03	0.01119	2.9	2.21
PC(36:4)	781.56	782.57	2.01	306.6	-	0.28	6.6E-03	0.02300	1.5	5.15
PC (38:4)	809.59	810.60	2.19	312.2	7.2	0.37	8.3E-03	0.02592	1.6	4.06
PC (36:2)	785.60	786.60	2.19	304.6	4.6	0.20	1.9E-02	0.04109	1.3	4.69
PS (38:2)	815.57	816.57	1.63	306.3	-	0.41	2.2E-02	0.04428	1.5	4.56
PC(34:2)	757.57	758.57	2.02	296.9	-	0.24	2.7E-02	0.04926	1.3	4.84

Table 2. List of significant lipid species with potential database identifications with up regulated lipids highlighted in green and down regulated lipids in red.

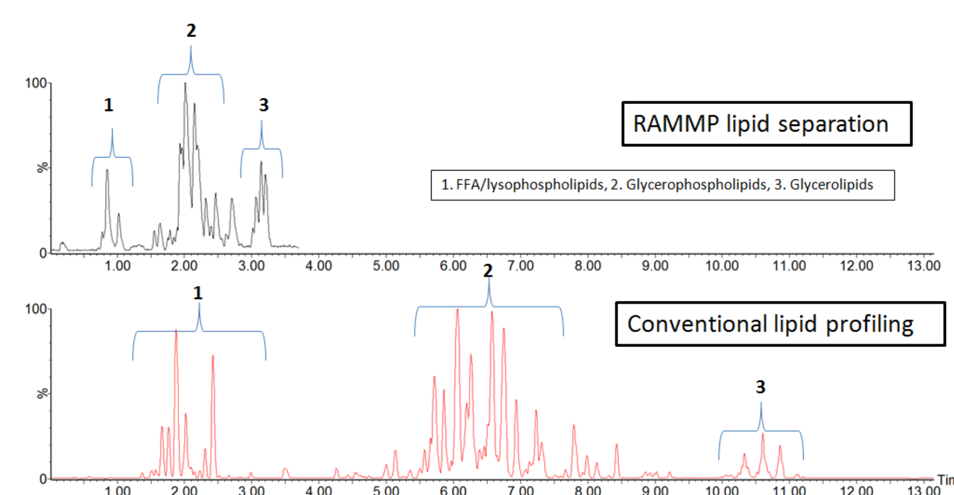


Figure 2. Example total ion current chromatograms of a pooled lipid extract QC sample run using a conventional lipid profiling assay and the rapid lipid assay.

Data processing:

- MassLynx (Waters, UK) - Data acquisition.
- Progenesis QI (Non-linear dynamics, UK) - Run alignment, peak picking, deconvolution and database searching.
- EzInfo (Umetrics, Ůmea, Sweden) - Data visualisation with principle component analysis (PCA), feature selection with OPLS-DA and S-plots.

Samples:

- Rat urine post treatment with Tienilic acid (HILIC).
- Human plasma samples from breast cancer patients (Lipid).
- QC samples created by pooling study samples.

RP-LIPID

The rapid lipid method developed resulted in a greater reduction of ~75 % in run time, with the overall run time at 3.70 mins (Fig.2). The conventional reversed phase lipid profiling method produces three regions in the chromatogram containing different lipid species. These separated regions are maintained in the condensed method assisting with confirmation of lipid class identity.

A series of plasma samples from healthy control and breast cancer patients were analysed using this rapid method. 15 lipids were determined to be significant, with 5 up regulated in the breast cancer samples, and the rest down regulated. Following database searching, 5 up regulated lipids were potentially identified as phosphotidylserine species (Table 2) which have previously been noted in the literature as potential breast cancer biomarkers[4].

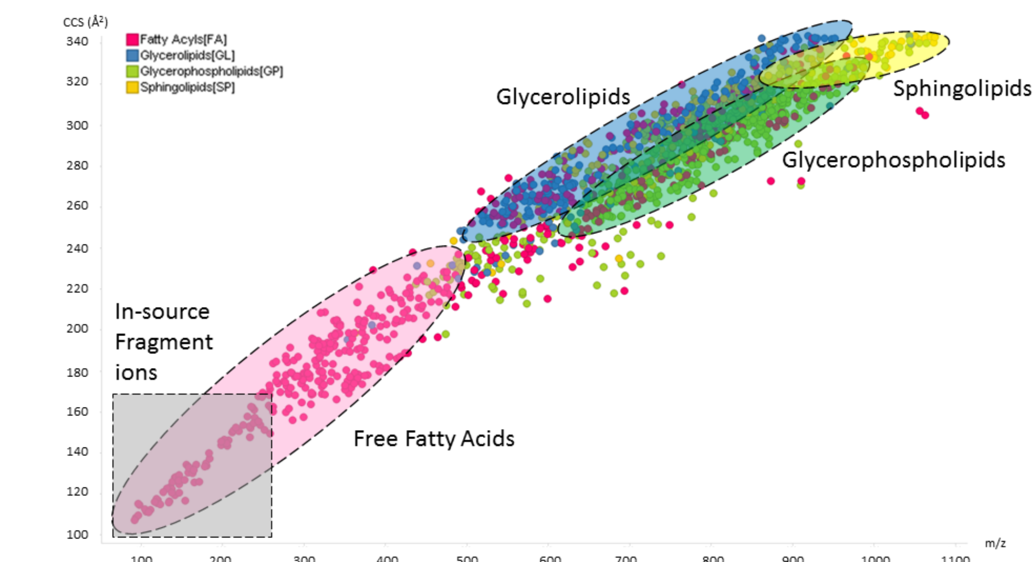


Figure 3. Plot of identified plasma features by measured m/z and CCS value. Different lipid classes are highlighted.

CONCLUSION

- **The developed, rapid 3 min profiling assays provide ~60—75% reduction in acquisition time.**
- **1000 samples can be acquired in under 3 days per assay, dramatically increasing sample throughput.**
- **Rapid profiling coupled with IMS provides an orthogonal dimension of separation to increase specificity and increase identification confidence.**
- **This rapid methodology is a powerful tool for large scale, high throughput metabolomic profiling studies.**

References

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4. Sharma, B., and S. S. Kanwar. 2017. Phosphatidylserine: A cancer cell targeting biomarker. *Semi Cancer Biol.*

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