HIGH THROUGHPUT LIPIDOMICS USING ION MOBILITY ENABLED RAPID LC-MS PROFILING SHOWS PROMISE FOR THE ANALYSIS OF HUMAN PLASMA SAMPLES OBTAINED FROM BREAST CANCER PATIENTS

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INTRODUCTION

Lipids encompass a large group of compounds of various classes, each exhibiting a range of physiochemical properties and biological function. Changes in expression and metabolism of lipids has been linked to numerous diseases including diabetes[1] and various cancers[2]. In order to screen the lipid phenotype of large batches of samples obtained from clinical trials and biobanks requires a high throughput analytical assay in order to analyse thousands of potential samples.

Conventional discovery LC-MS lipidomic assays have sample acquisition times of >15 minutes per sample. Using these methodologies to analyse larger cohorts of samples from biobanks can lead to weeks of expensive analysis putting pressure on laboratory resources.

Reducing a column's internal diameter, column length and scaling down mobile phase flow rates and gradients can dramatically reduce the overall acquisition time with minimal impact on chromatographic performance. Here we describe the development and application of a high throughput discovery lipidomic assay.

METHODS

Sample preparation

- Human plasma samples from breast cancer patients (n=20) and normal control subjects (n=6).
- Pooled QC sample prepared from each study sample (50 µL).
- Lipids extracted from 100 µL of sample with 400 µL of IPA.
- Extracts then incubated at 2—8 °C for 2 hours.
- Extracts centrifuged to remove proteins.
- Supernatant removed for analysis.

MS conditions

- Waters Synapt G2-Si with IMS enabled
- Positive ESI, sensitivity mode, 50—1200 m/z
- Capillary voltage: 0.5 kV, Sampling cone: 30 V
- Source temp: 120 °C, desolvation temp: 500 °C#
- Desolvation gas: 800 L/hr, cone gas: 50 L/hr.
- IMS wave velocity: 600 m/s, wave height: 40 V

LC conditions

- Column: Waters BEH C8, 1.0 x 50 mm (1.7 µM)
- Mobile phase:
  - A) H_2O:IPA:MeCN w/ $NH_4CH_3CO_2$ & CH_3COOH
  - B) IPA:MeCN w/ $NH_4CH_3CO_2$ & CH_3COOH

Table 1. Gradient composition and injection volumes for both the conventional and rapid lipid profiling assay.

Conventional method		
Injection Vol (µL)		2.0
Time (min)	Flow rate (mL/min)	%B
Initial	0.6	1.0
0.10	0.6	1.0
2.00	0.6	30.0
11.50	0.6	90.0
12.00	0.6	99.9
12.50	0.6	99.9
12.75	0.6	1.0
13.25	0.6	1.0

Rapid lipid method		
Injection Vol (µL)		0.2
Time (min)	Flow rate (mL/min)	%B
Initial	0.25	1.0
0.05	0.25	1.0
0.50	0.25	30.0
2.80	0.25	90.0
3.00	0.25	99.9
3.15	0.25	99.9
3.25	0.25	1.0
3.70	0.25	1.0

Data processing

- All data were processed using Progenesis QI (Nonlinear Dynamics, UK).
- Raw data were aligned, peak picked and normalized to all compounds.
- Detected ions underwent adduct deconvolution to determine neutral mass values.
- Statistical analysis was performed using EZinfo (Umertrics, SWE).
- Group separation was visualized by principle component analysis (PCA).
- Significant features were determined through orthogonal partial least squared discriminate analysis (OPLS-DA) and S-plots of the features.
- LipidMaps and Waters IROA CCS databases were used to provide compound identifications.

RESULTS

Rapid LC methodology

- Scaled chromatography reduced sample acquisition times from 13.2 mins to 3.7 mins and solvent consumption by 75 %.
- Column I.D. was reduced by a factor of 4 whilst the flow rate reduced by only 2.4, enabling equivalent column volumes and increased linear velocity.
- Chromatographic separation of lipid classes was maintained through the scaling process (Figure 1).

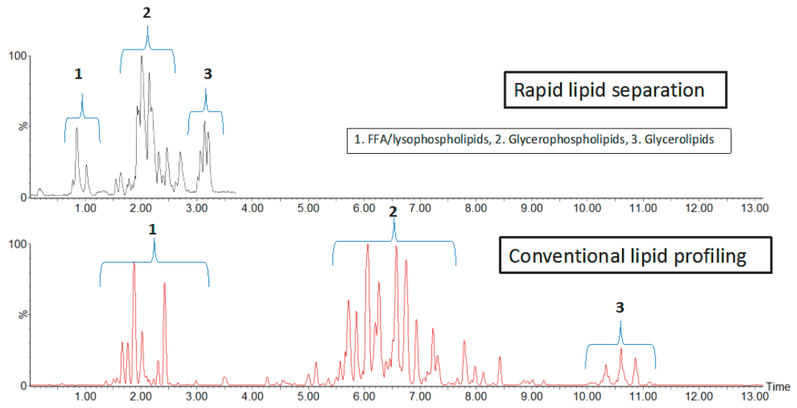

Figure 1. Comparison of a conventional discovery lipid assay with the scaled down 1.0 mm rapid lipid separation from human plasma.

Ion mobility spectrometry

- Due to the reduction in chromatographic resolution, incorporating ion mobility (IM) into the workflow, provided additional separation for co-eluting ions.
- The collision cross sectional (CCS) measurements generated increased specificity with lipid classes forming distinct groups according to CCS value (Figure 2).

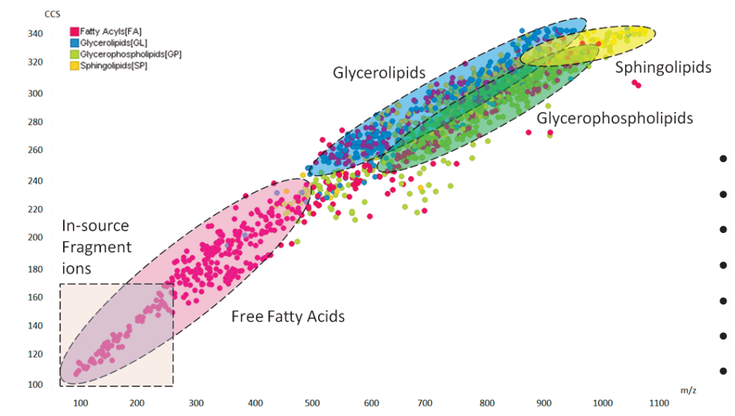

Figure 2. Trend plot of identified plasma features by measured m/z and CCS value, with the various lipid classes highlighted.

- With IMS enabled the spectral quality of co-eluting features was improved (Figure 3).
- Using conventional data independent acquisition modes, fragment ions from co-eluting species can be miss assigned.
- The ion mobility separation prior to CID improved the assignment of fragment to precursor ions.
- This in turn improved the spectral matching when database searching.

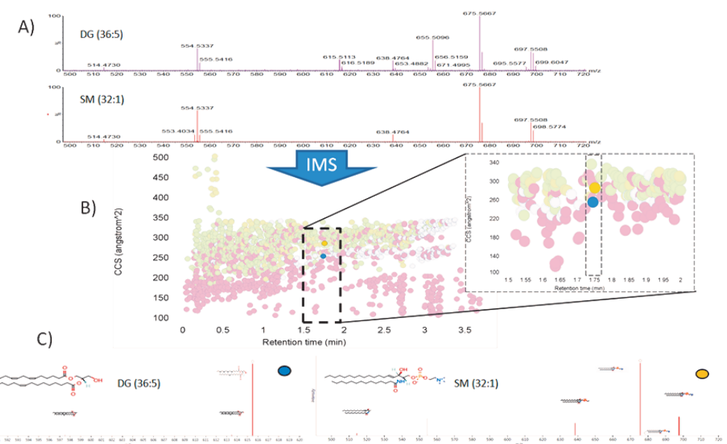

Figure 3. A) High collision energy fragment ion spectra without IMS of two co-eluting lipids; B) Plot of identified features by retention time and CCS with the co-eluting features highlighted and separated by CCS value; C) Improved fragment ion spectra following IMS and thereby database identifications.

Feature Identification

- Ions that had a CV in the QC samples >30 % were filtered out and the remaining underwent statistical analysis.
- Following OPLS-DA (Figure 4A), significant features were determined by S-plot (Figure 4B).
- 5 features were determined to be up regulated in the breast cancer samples with another 10 lipids exhibiting down regulation.
- These features were firstly considered for database searching though lipid maps, with additional database searches conducted against the IROA CCS database.

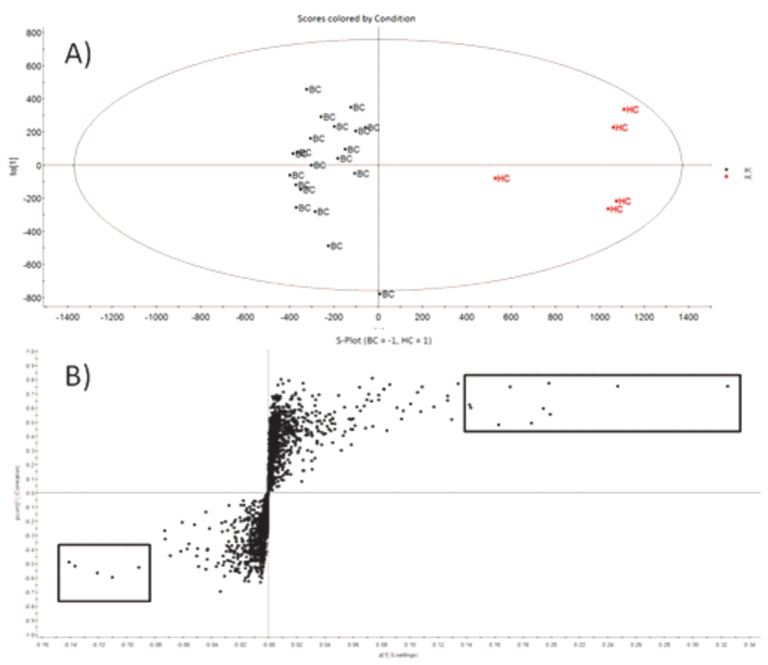

Figure 4. A) OPLS-DA comparison of breast cancer samples and healthy controls; B) Resulting S-plot of significant features.

- Of the lipids identified, a number of PCs and TAGs were under expressed for breast cancer patients (Table 2).
- The degrease in PCs can be indicative of an increase in phospholipase A2 activity, previously reported by Yunping *et al* [3] to be linked to breast cancer.
- Five PS were also noted to be increased in breast cancer patients.
- In the literature, PS has been noted as a potential biomarker for cancer, corresponding to the results shown here[4].

Table 2. List of up regulated (green) and down regulated (red) features in breast cancer patients and potential identifications following database searching.

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

CONCLUSION

- **A rapid discovery lipidomic method has been developed and applied in the assessment of plasma breast cancer samples.**
- **The reduction in acquisition time demonstrated potential for larger cohort studies to be acquired in a matter of days rather than weeks.**
- **The addition of IMS improved the spectral quality of fragmentation spectra.**
- **Generation of CCS measurements augmented the database searches and increased ID confidence.**
- **Significant lipids which were identified corresponded to those reported in the literature.**

References

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