

# COMBINING A SUITE OF RAPID PROFILING LC-MS/MS METHODS AND ION MOBILITY WORKFLOWS TO INVESTIGATE THE METABOLOME OF PROSTATE CANCER PATIENTS.

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## INTRODUCTION

Analysis of large cohorts of samples from a population is essential in identifying statistically significant markers of diseases like prostate cancer (1). Current diagnosis of prostate cancer is determined using a prostate specific antigen test (PSA), but lack of specificity of PSA to prostate cancer can lead to miss diagnosis (2).

Untargeted metabolomics and lipidomics are useful in determining unknown markers linked to specific conditions, but to fully profile a series of samples requires analysis using several complimentary methods. Therefore, conventional chromatographic separation methods can put a lot of pressure on instrument and personnel resources in addition to suffering from instrumental variation over the course of the analysis (3).

Here we describe the use of a suite of reproducible, complimentary rapid LC-MS/MS metabolic profiling methods, in the analysis of a cohort of serum samples from prostate cancer patients and controls.

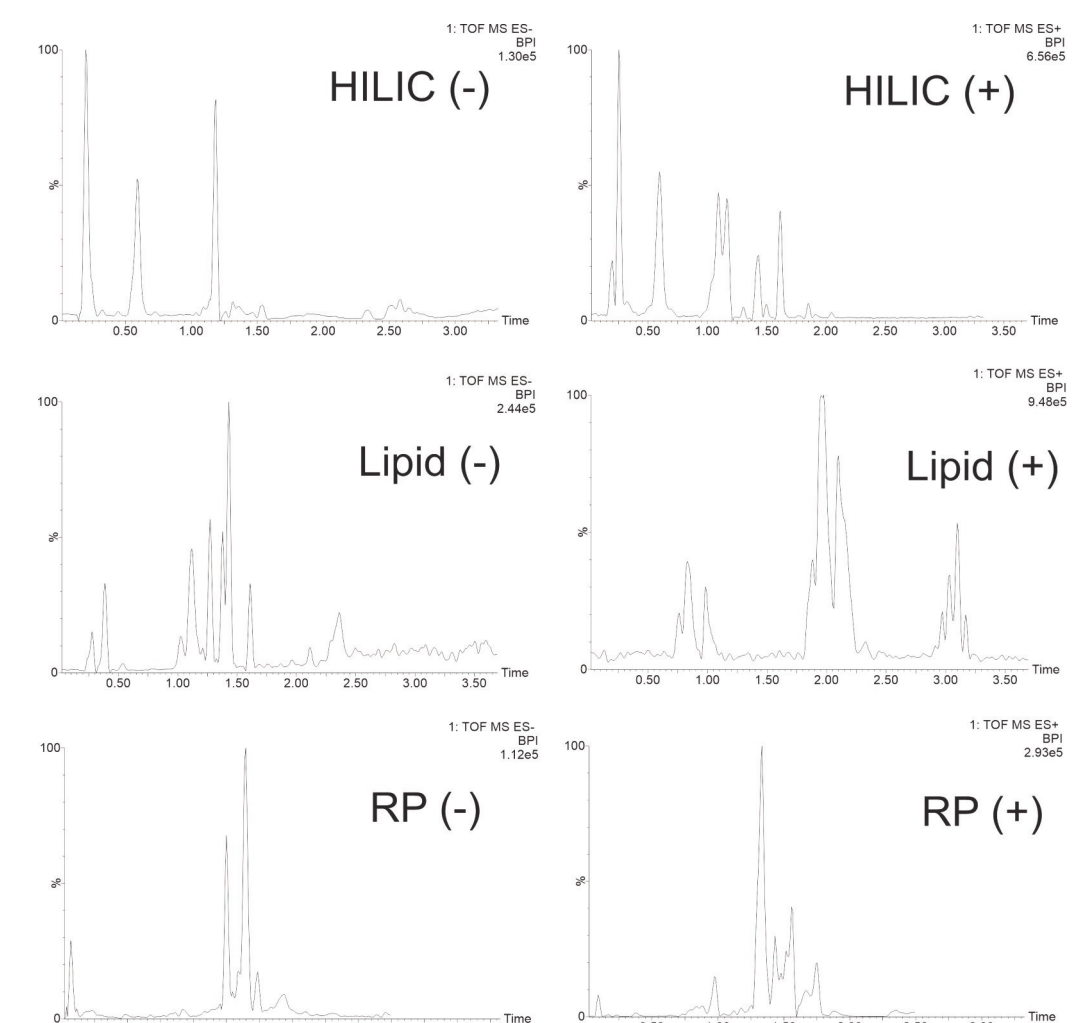


Figure 1. Example chromatograms for each rapid profiling method in positive and negative ESI mode.

## METHODS

### Rapid LC-MS/MS methods

The three rapid analytical methods employed used reversed-phase and a HILIC separation for polar metabolites and a reversed-phase separation for lipid profiling. Each method has previously been employed individually (4,5,6) and utilised analytical columns with a reduced internal diameter (1 mm). Furthermore, to reduce post column dispersion, a reduced flow ESI probe and reduced diameter peek tubing was also used. Each chromatographic separation was achieved in < 4 mins (Fig.1). Due to the reduction in run time, and the more compressed chromatography, in order to increase the method peak capacity, all acquisition was performed using ion mobility calibrated for collisional cross section (CCS).

The reversed-phase lipid separation was achieved using a Waters™ BEH™ C8 column with the small molecule separations using the Waters BEH amide and HSS™ T3 columns for HILIC and reversed-phase separation respectively.

### Sample Preparation

For lipid analysis, each serum sample underwent protein precipitation using cold IPA. The samples were incubated for 2 hours at 2-8 °C prior to centrifuging at 13,000 rpm for 10 minutes. The supernatant was then analysed using the rapid lipid method.

For HILIC analysis, sample underwent protein precipitation using acetonitrile. The samples were incubated for 2 hours at 2-8 °C prior to centrifuging at 13,000 rpm for 10 minutes. The supernatant was then analysed using the rapid HILIC method.

For small molecule reversed-phase analysis, sample underwent protein precipitation using acetonitrile. The samples were incubated for 2 hours at 2-8 °C prior to centrifuging at 13,000 rpm for 10 minutes. The supernatant was then evaporated until dryness and reconstituted in water prior to analysis.

### MS acquisition

All data was acquired on a Waters Synapt™ XS (Waters Corp, UK) using the HDMS<sup>E</sup> data independent acquisition mode over the mass

range of 50—1200 m/z. The mass spectrometer was calibrated using sodium formate and the ion mobility T-wave was calibrated for CCS using the Major Mix.

## RESULTS

The serum samples (n=350) were acquired in triplicate with pooled quality controls (QC) samples and phenotypic pools for each group of diseased, benign cases and controls. In total, 1120 injections were acquired for each method and ESI polarity.

Compared to conventional analytical methods (~10—15 mins), the suite of rapid profiling methods enabled

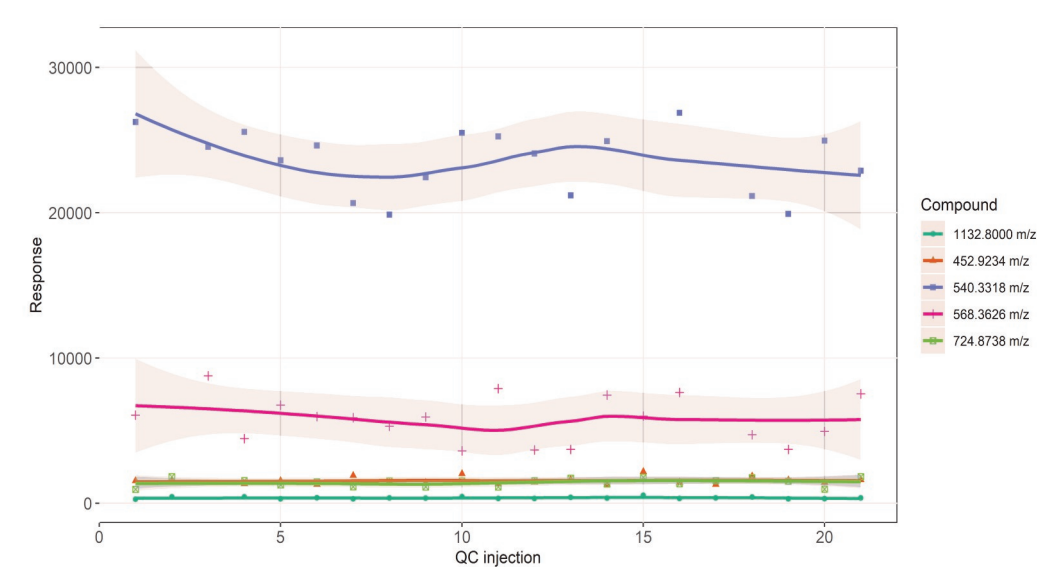


Figure 2. Representative scatter plot of example compound response across the pooled QC samples for the HILIC negative analysis.

a reduction in acquisition time of the batch of samples by 2/3, reducing the burden on instrument and analyst logistics.

In order to assess the quality of each rapid profiling method, a selection of endogenous compounds (n=5) for each method were identified in the pooled QC sample, to determine the reproducibility and mass accuracy of the analysis. The data was processed using Progenesis Q1™ software (Waters Corp. UK) where the data was aligned, peak picked and normalised.

The response for each compound across the QC injections were plotted to demonstrate the consistency of the MS detection. Figure 2 highlights the response of the 5 compounds selected from the HILIC negative mode analysis and shows a stable response across the 29 QC injections throughout the batch of 1120 injections. All compound ions investigated for all

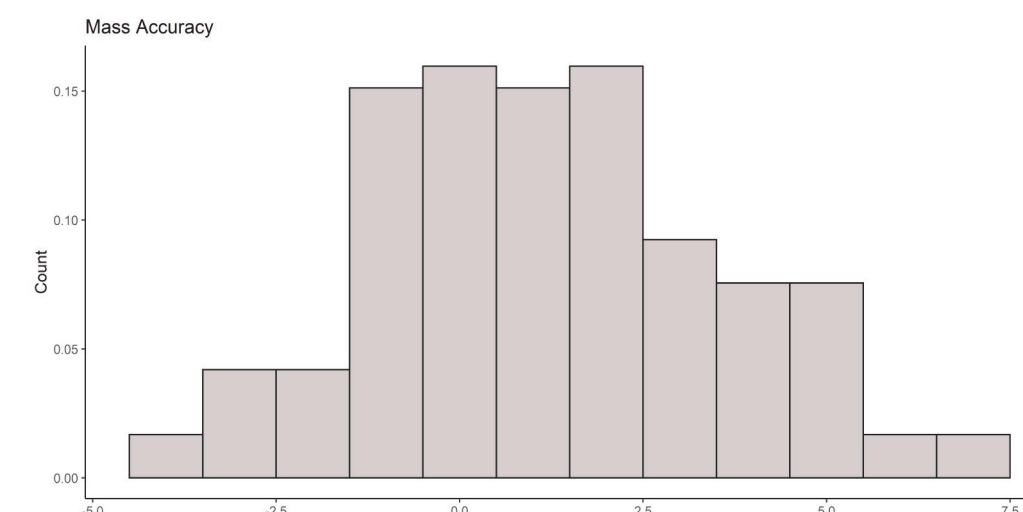


Figure 3. Representative histogram of mass accuracy for the lipid negative analysis.

Table 1. summary table of endogenous compound reproducibility across 29 QC injections from each of the rapid LC-MS/MS methods.

Compound	IR (min)	IR Std Dev	IR % CV	Average Response	Response Std Dev	Response %CV	Average Mass Error	Mass Error Std Dev	
HILIC Negative									
391.2953 m/z	0.25	0.00	7	2.67	166.15	22.02	13.25	-0.15	2.25
566.3459 m/z	1.18	0.00	6	0.54	2842.17	303.71	10.69	1.43	2.61
568.3624 m/z	1.17	0.00	6	0.51	3361.55	306.19	9.11	1.11	2.40
540.3322 m/z	1.19	0.00	9	0.80	10514.71	1171.18	11.14	0.51	2.67
271.0695 m/z	1.48	0.01	6	0.64	128.24	14.56	11.35	-0.51	2.74
284.2940 m/z	0.21	0.00	6	2.62	241.82	59.01	24.40	-0.69	3.41
HILIC Positive									
496.3403 m/z	1.19	0.01	5	1.26	49725.86	5244.06	10.55	4.83	2.08
524.3718 m/z	1.17	0.01	8	1.55	17807.14	2232.71	12.54	0.65	2.54
834.6005 m/z	0.55	0.04	3	7.78	13877.93	1048.06	7.55	0.16	2.03
184.0739 m/z	1.12	0.02	2	1.92	326.43	75.93	23.26	-0.64	2.36
540.3319 m/z	1.35	0.12	0	8.90	21944.50	5279.72	24.06	0.83	2.42
283.2943 m/z	1.82	0.13	5	7.43	6439.66	1009.50	15.68	3.20	2.21
Lipid Negative									
476.2791 m/z	1.17	0.10	0	8.57	1097.42	139.69	12.73	1.62	1.78
769.0104 m/z	0.35	0.04	6	13.05	419.52	111.98	26.69	0.75	2.08
365.0229 m/z	0.35	0.04	9	13.93	201.90	53.89	26.69	0.18	2.08
758.5692 m/z	2.20	0.19	0	8.64	512189.04	75463.13	14.73	4.20	1.96
Lipid Positive									
496.3400 m/z	1.00	0.14	3	14.33	147336.50	17371.75	11.79	5.11	2.25
520.3380 m/z	0.93	0.14	0	15.11	81419.49	10340.42	12.70	6.13	2.16
282.2798 m/z	1.14	0.14	6	12.76	40194.07	7377.55	18.35	4.80	2.07
822.7538 m/z	3.17	0.14	4	4.54	17779.69	2127.93	11.97	2.24	2.17
Per-Negative									
540.3318 m/z	1.27	0.03	7	2.92	23574.88	2215.08	9.40	0.58	2.21
568.3626 m/z	1.35	0.01	6	1.16	5793.07	1588.69	27.42	1.64	2.13
452.8234 m/z	0.08	0.00	5	6.11	1658.34	256.03	16.43	-0.89	2.58
724.8738 m/z	0.08	0.00	4	5.26	1447.46	278.57	19.25	-0.19	2.15
1132.8000 m/z	0.08	0.00	4	5.26	372.38	67.98	18.26	0.51	1.89
444.4046 m/z	1.20	0.14	7	12.27	1049.68	291.91	27.81	0.46	2.44
Per-Positive									
564.3600 m/z	0.89	0.08	8	9.88	579.77	187.67	32.37	-0.60	2.97
768.4788 m/z	1.00	0.01	5	1.54	469.41	120.57	25.69	1.21	3.32
393.2098 m/z	0.76	0.02	0	2.69	258.98	61.03	23.57	-0.74	4.30
608.3861 m/z	0.85	0.01	8	2.15	463.74	100.05	21.58	-0.62	2.46

acquisition modes had a %CV of < 30 % across the whole batch as summarised in table 1.

In addition to response, each compounds variation in mass accuracy (Fig. 3) and retention time (Fig.4) were also investigated across the QC injections and additionally summarised in Table 1. Out of the 30 compounds selected, 28 compounds have an average Mass error of less than 5 ppm, while all were below 7 ppm.

Retention time variation for the extracted compound ions deviated less than 8 seconds over the batch for all compounds bar one which had a variation of 11 seconds over the run.

Following database searches using HMDB and METLIN libraries, in addition to in-house CCS libraries for lipids and small molecules, a series of pharmaceuticals were identified including paracetamol, Ibuprofen in addition to the antihypertensive drug, deserpidine. For the endogenous compounds tentatively identified from the search, the coverage was assessed by grouping by class. Figure 5 highlights the class coverage of compounds from the HILIC negative analysis showing 1/3 of compounds being lipids and lipid like compounds. The next largest class of compounds were benzenoids at 20 % including many pharmaceuticals. Organoheterocyclic compounds like pyrimidine derivatives represented 16 % of the coverage, while organic oxygen compounds (e.g. carbohydrates, sugars) encapsulated 13 % of compounds.

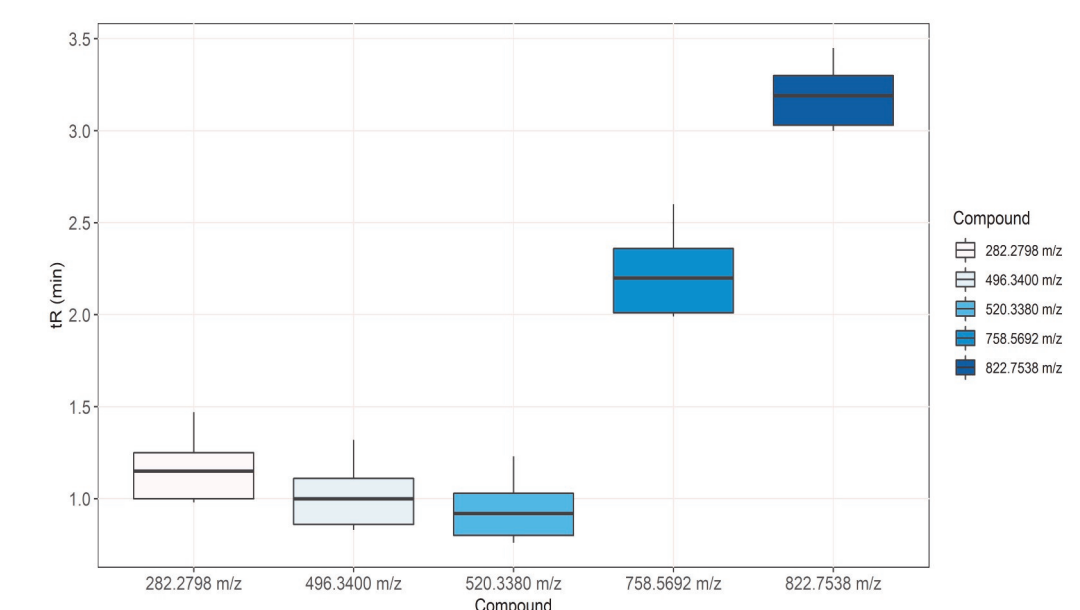


Figure 4. Boxplot of retention time variation for 5 endogenous compounds identified in the rapid lipid positive QC samples.

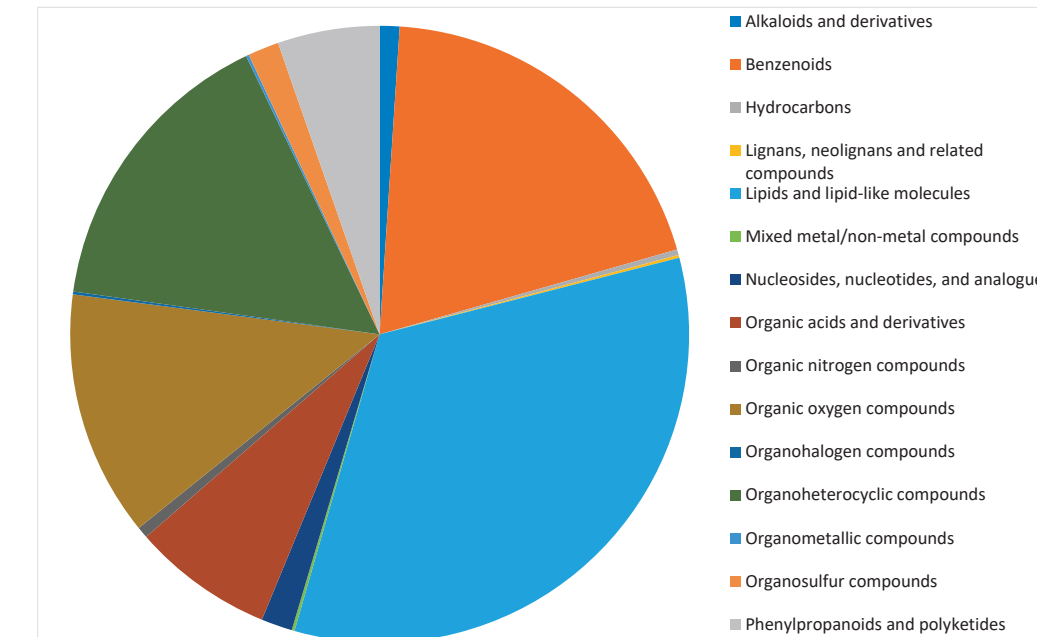


Figure 5. Pie chart of tentative compound class coverage following database searches for the HILIC negative analysis.

## CONCLUSION

- Untargeted metabolomic research for biomarker discovery requires accurate reproducible methods.
- The rapid suite of LC-MS/MS methods enabled serum profiling of 350 samples in a 1/3 of the time of conventional methods (totalling 6,720 injections).
- The methods were reproducible with stable analyte response, retention time and mass accuracy over a large batch of samples.
- The complimentary suite of methods enabled analysis of a broad selection metabolites across varying compound classes.
- These methods provide a reliable way of screening large cohorts of samples for disease research.

### References

1. Armitage EG, Barbas C. Metabolomics in cancer biomarker discovery: current trends and future perspectives. J Pharm Biomed Anal. 2014;87:1-11.
2. Cuzick J, Thorat MA, Andriole G, Brawley OW, Brown PH, Culig Z, et al. Prevention and early detection of prostate cancer. The Lancet Oncology. 2014;15(11):e484-e92.
3. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc. 2011;6(7):1060-83.
4. Gray N, Adesina-Georgiadis K, Chekmeneva E, Plumb RS, Wilson ID, Nicholson JK. Development of a Rapid Microbore Metabolic Profiling Ultra-performance Liquid Chromatography-Mass Spectrometry Approach for High-Throughput Phenotyping Studies. Anal Chem. 2016;88(11):5742-51.
5. King AM, Mullin LG, Wilson ID, Coen M, Rainville PD, Plumb RS, et al. Development of a rapid profiling method for the analysis of polar analytes in urine using HILIC-MS and ion mobility enabled HILIC-MS. Metabolomics. 2019;15(2).
6. King AM, Trenlove RD, Mullin LG, Rainville PD, Isaac G, Plumb RS, et al. Rapid profiling method for the analysis of lipids in human plasma using ion mobility enabled-reversed phase-ultra high performance liquid chromatography/mass spectrometry. J Chromatogr A. 2020;1611:460597.

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