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Enhanced mass spectrometric profiling of the human blood exposome using an optimised dispersive SPE protocol

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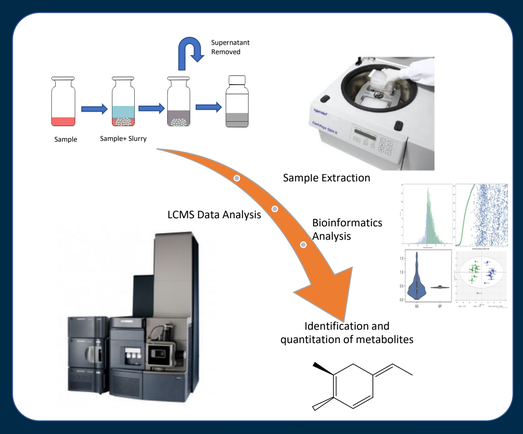
Introduction

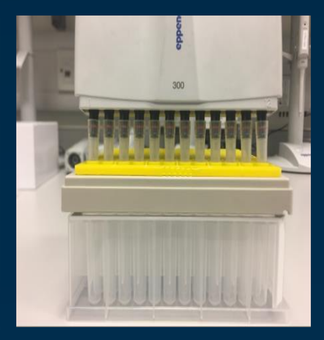
The untargeted nature of metabolomics allows measurement off biofluid chemistry related to both endogenous metabolism and host-environment exposures (i.e. the exposome). Comprehensive coverage of chemically diverse metabolites present in human blood products benefits from the use of multiple methods, each oriented toward a small molecule subset generally segregated by polarity and hydrophobicity. Whilst recent developments in LC-MS profiling methodologies have delivered numerous solutions for the analysis of polar molecules (e.g. via HILIC-MS) and complex lipids, the analysis of moderately hydrophobic and amphipathic molecules in blood products (including much of the exposome) by RPC methodology, is complicated by the suppressive effects of lipids on the ionisation of low molecular weight (LMW) metabolites. Efficient and inexpensive solutions are required for the separation of small molecules from the remaining sample matrix fit for large scale and high throughput applications



Methods

A lipid removal sample preparation technique was developed using a novel dispersive solid phase extraction (DSPE) technique. Factors optimised to facilitate lipid removal with minimal loss of other analytes included design of experiment (DOE) protocols for aspects of the DSPE sorbent specification, and the solvent composition used for extraction.

The feasibility and robustness of the extraction methodology was explored on an exemplar epidemiological plasma dataset (n=285) using well-characterised RP-UPLC-TOFMS phenotyping for high resolution detection of chemical species and data processing pipelines



Results – Method Development

Optimisation – DSPE

Sorbent

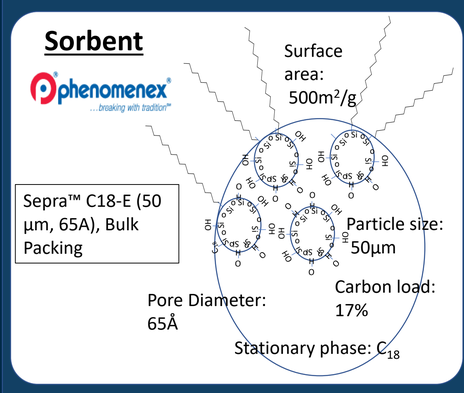
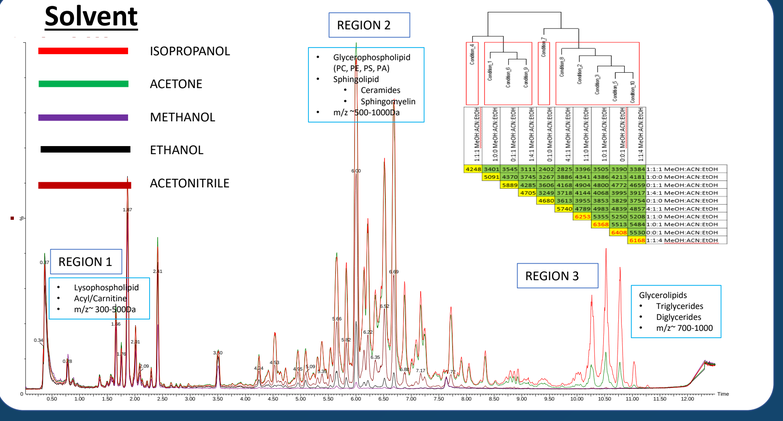


Figure 1. Small particle size will equate to a greater surface area thus allowing for a larger number of ligands (fatty acid chains) to attach to surface of the support material and thereby only targeting lipophilic species

Figure 2. 5 solvents were studied with methanol ,ethanol and Acetonitrile signifying adequate removal of lipophilic species associated with regions 2 & 3 and with minimal influence on chromatographic performance. The three solvents were tested individually and in combinations with one another and using clustering methods, were evaluated based on the number and quality of reproducible features detected. Methanol:Acetonitrile in a 1:1 (v/v) produced the highest number of features with low baseline & a significantly reduced signal of large asymmetric peaks. In addition no biphasic partitioning was observed.

Solvent



Sorbent + Solvent

MODDE	Expected Recovery Range (%)	RT bins (min)	Slurry conditions: Concentration and Slurry Volume											
			2mg/mL 600uL	20mg/mL 600uL	2mg/mL 100uL	20mg/mL 1000uL	2mg/mL 800uL	20mg/mL 800uL	11mg/mL 600uL	11mg/mL 1000uL	11mg/mL 800uL	11mg/mL 800uL	11mg/mL 800uL
Small Molecule Profiling	95-120	0-1min	99	104	106	106	95	103	101	108	101	102	105
		1-2min	100	107	108	110	93	108	103	114	104	105	108
		2-3min	99	105	108	109	93	107	102	113	104	104	110
		3-4min	96	111	108	111	90	110	101	113	108	105	112
		4-5min	96	110	107	107	88	111	99	110	104	100	109
		5-6min	93	108	103	111	89	123	98	130	104	107	119
		6-7min	93	106	107	105	84	104	97	109	95	100	109
		7-8min	97	110	112	123	86	117	101	125	106	109	118
	80-130	8-9min	95	112	109	133	80	118	101	129	102	113	123
		9-10min	94	113	117	146	77	124	95	128	97	121	127
Lipid Profiling	80-100	1-3min	101	70	106	90	103	81	83	94	84	89	88
	10-20	4-8min	85	3	86	15	78	7	7	22	12	17	17

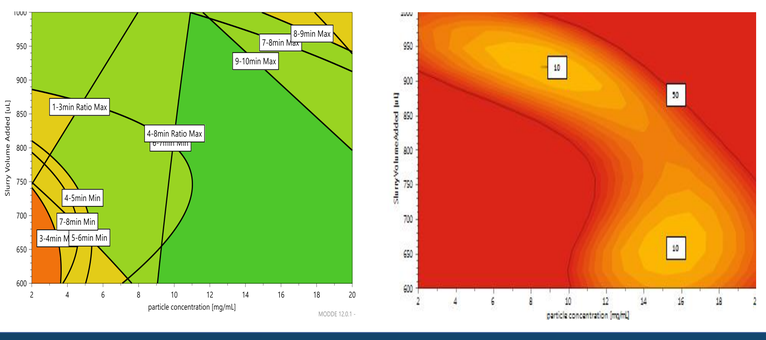


Figure 3. A DOE procedure was implemented in order to optimise the two factors; sorbent concentration (2-20mg/mL) and solvent volume (3X to 5X solvent volume). MODDE took into account these two factors and proposed a central composite Face design composing of 8 different conditions with 3 replicated centre points for a total of 11 experiments. Summation of peak intensities in 1 minute retention time bins (up to 10 minutes) were calculated for each optimisation condition, and the ratio taken with its retention time counterpart in a blood SHAM sample - and percentage calculated and labelled as the recovery. It is these recoveries that were used as the response measurements. An "analysis wizard" is utilised and is a guided workflow, bringing to the attention of the user, several performance indicator plots (shown is the contour and design space plots), required to evaluate and access the model.

Method Precision

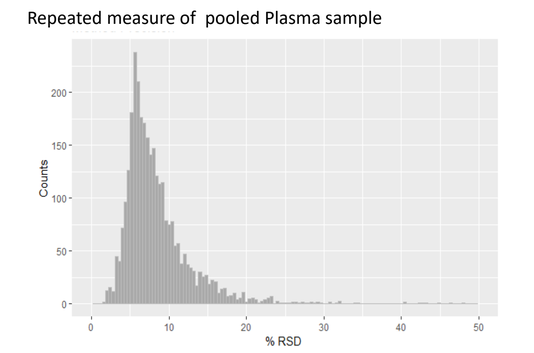
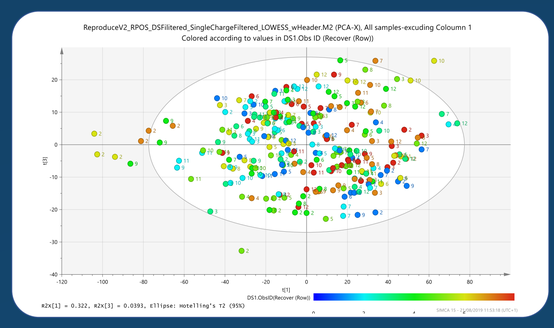
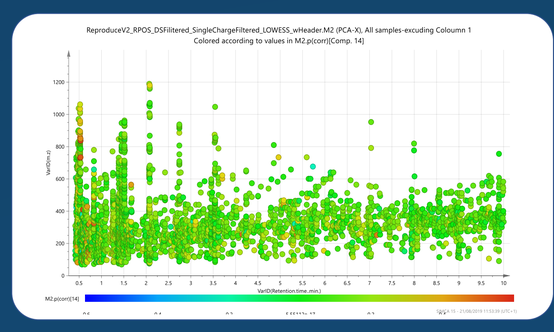


Figure 4. The precision of the sorbent weight (in a 96 well format suitable for high-throughput) and metabolic profiles was assessed.<10% sorbent weight was measured and Multiparametric data analysis of the UPLCMS results demonstrated good repeatability, Scores plots demonstrated no outlying samples and loadings were plotted on a Retention time Vs m/z axis showing no significant global change variations across all detected chemical features.





DSPE vs SPE

Light Blue: SPE Plate 1

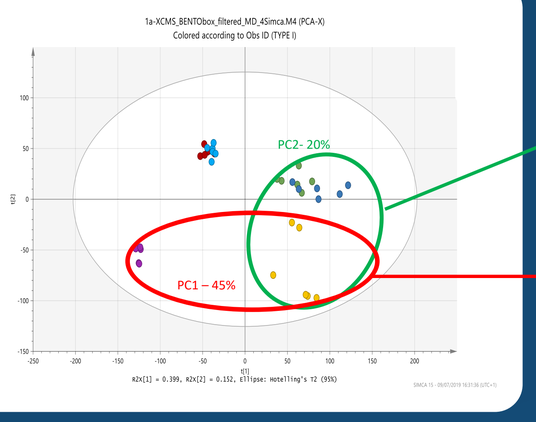
Purple: SPE Plate 2

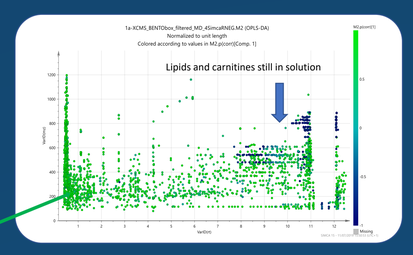
Maroon: SPE Plate 3

Green (Sepra DSPE)

Dark blue (Sepra SPE)

Yellow – Neat Plasma





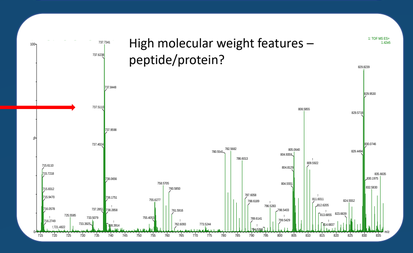
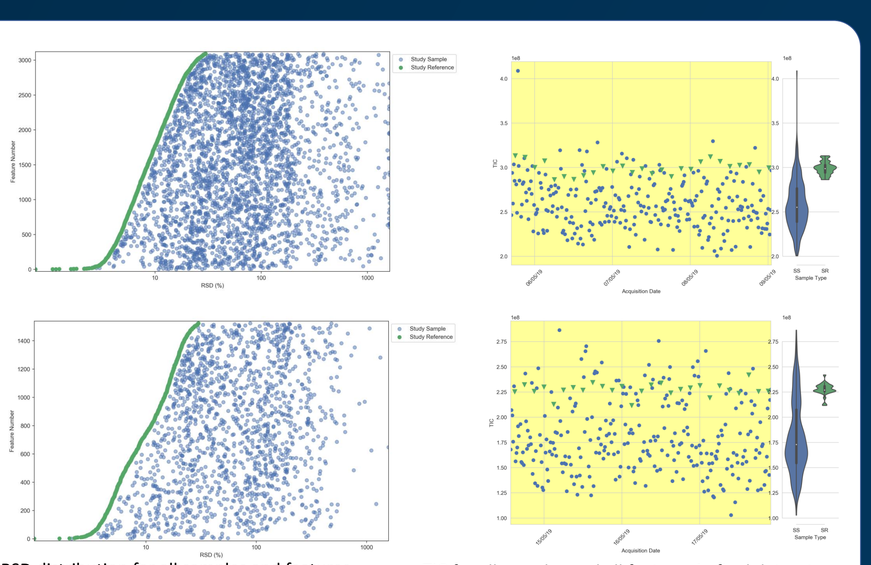


Figure 5. Comparison of the optimised DSPE method with known SPE methods/plate was conducted. For a fair comparison, Phenomenex also packed the Sepra C18 material in a 96 well SPE format and in the amount equivalent to the optimised dry weight. PC1 explained approximately 45% of the variation and was attributed to the high molecular weight metabolites present in neat plasma samples. PC2 explained approximately 20% of the variation and was attributed to the lipophilic metabolites still present

Results – Metabolic Profiling of human plasma samples RP-ESI (+) & RP-ESI (-)



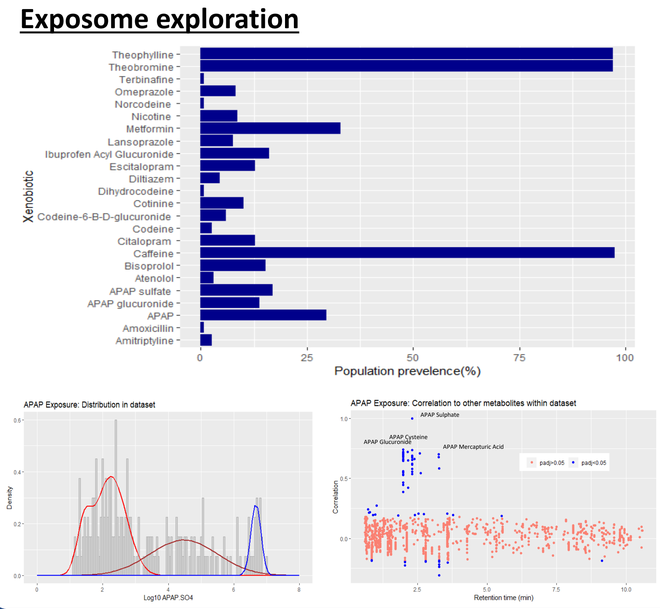
RSD distribution for all samples and features

TIC for all samples and all features in final dataset

Figure 6. UPLC-TOFMS data for both polarities on the exemplar study ,n=321, was processed using XCMS software resulting in 2837 detected metabolite features in ESI (+) and 1523 detected features in ESI(-). Repeated observation of reference features from pooled QC samples throughout the analytical batch demonstrated high precision with mean peak area RSD <10% with no post batch correction required.

Figure 7. To demonstrate the methods suitability to explore the exposome, known xenobiotics were annotated, and their population prevalence reported. Using Acetaminophen (APAP) as an example, its distribution was evaluated revealing a multimodal distribution. Gaussian mixture models were fitted converting the distributions to probability distribution functions, therefore dividing the data into different levels of exposure. Looking at samples in the different exposure groups, we looked for significant correlations of the main APAP LCMS feature to all other features – revealing known main metabolites of APAP as well as other features.

Exposome exploration



Annotated Metabolite coverage

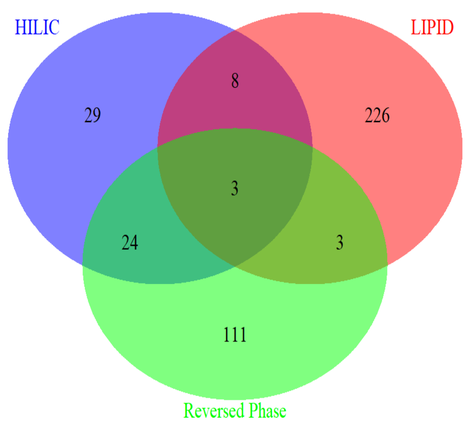


Figure 8. A Venn diagram representing the number of metabolites annotated in blood, by all NPC mass spectrometry profiling assays. Although the compound list is far more extensive, these are the metabolites which have passed all relevant QC metrics to make it into the final dataset

Conclusions

The optimised lipid removal method described enables improved characterisation of the human exposome using high-throughput metabolic phenotyping platform. DSPE provided a straightforward reproducible approach which enabled the use of uncompromised RPC-UPLC-TOFMS to complement the coverage provided by HILIC and lipid analyses. Additional advantages include reduced cost and increased robustness when compared to conventional solid-phase sample clean-up protocols.

Imperial College London

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***National Phenome Centre urinary profiling platform reference:**
Lewis MR et al. Development and Application of Ultra-Performance Liquid Chromatography-TOF MS for Precision Large Scale Urinary Metabolic Phenotyping, ANALYTICAL CHEMISTRY, 2016