Metabolomics/lipidomics of dried blood spots using a novel blood microsampling device (hemaPEN®)

Trajan Scientific and Medical

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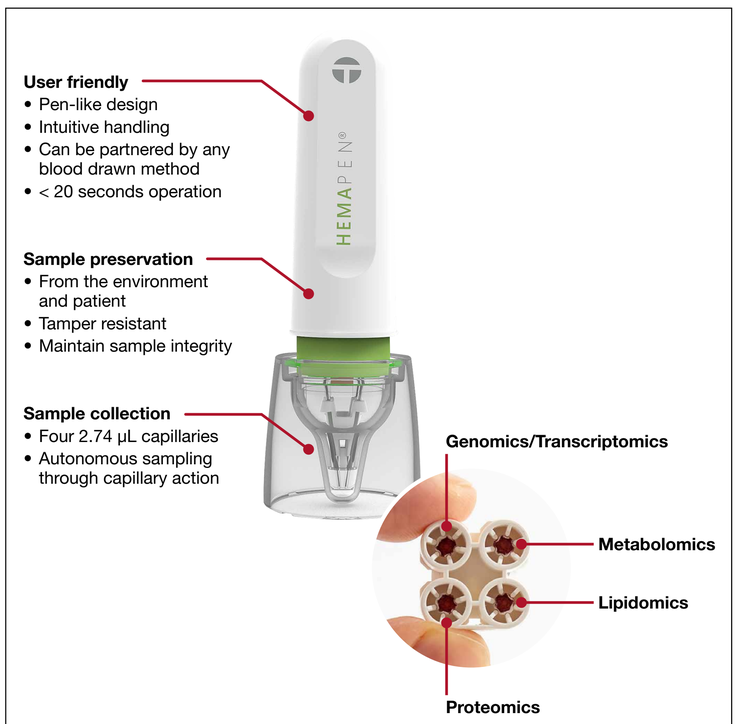

Figure 1. Four replicates from a single source of blood - ideal for multiomics studies.

Introduction

In this study we present metabolomic and lipidomic profiles of a blood microsample using a dried blood spot (DBS) workflow.

Key outcomes were:

- **Utility of a blood microsampling device that is designed to collect 4 x 2.74 µL technical replicates from a single source of capillary blood (Figure 1).**
- **A targeted metabolomics analysis of both biological and technical replicates using GC-MS (QQQ): 117 polar metabolites identified from a single 2.74 µL blood volume.**
- **An untargeted lipidomics analysis from a single 2.74 µL blood volume using LC-MS (Q-TOF) resulted in the identification of 375 lipids.**
- **No significant differences (p values <0.05, fold change >1.5) were observed for multiple analytes between biological and technical replicates.**
- **The potential to customize each storage well of the hemaPEN® for different 'omics' analysis makes it an attractive option for future longitudinal studies.**

Methods

Three biological (P02390, P20208, P20911) DBS samples (and 4 x 2.74 µL technical replicates (R1, R2, R3, R4)) were collected from a healthy donor by fingerstick capillary blood onto pre-punched grade 226™ paper (PerkinElmer Inc.) within the same timepoint (5 mins). The twelve samples were then transferred into a separate 1.5 mL Eppendorf tubes for extraction.

- **Metabolomics:** One hundred microliters of chilled methanol (with 13C-valine and 13C-sorbitol as internal standards at 5 µM) was used to extract polar metabolites using 1.5 mL Eppendorf tubes. Samples were then vortexed for 30 secs, thermomixed at 4°C for 10 mins and centrifuged at 15,000 rpm and 4°C for 5 mins. The supernatant was collected into an insert and placed in another 1.5 mL Eppendorf tube for drying in a vacuum concentrator. Once dried, the insert was then transferred to 2 mL GC vials and derivatized online using 20 µL methoxyamine hydrochloride (derivatization for one hour at 750 rpm and 37°C) and 20 µL BSTFA + 1% TMCS (derivatization for two hours at 750 rpm and 37°C). A 1 µL splitless injection was made into a Shimadzu GCMS-TQ8040 (QQQ) utilizing the Shimadzu Smart Metabolite Database (containing 475 MRM transitions), using a 30 m x 0.25 mm x 1 µm 5% phenyl polysilphenylene-siloxane column and providing a total analysis time of 37 mins. Data was processed and exported as a matrix using Shimadzu's Lab Solutions Software (GCMS solutions 4.20). Statistical analysis on the data matrix was performed using Metaboanalyst 4.0.

- **Lipidomics:** Lipidic components were extracted by adding 100 µL 1:1 butanol:methanol (PC 19:0/19:0 and PG 17:0/17:0 as internal standards at 5 µM) to 1.5 mL Eppendorf tubes. Samples were then vortexed for 30 secs, thermomixed at 20°C for 10 mins and centrifuged for 5 mins. The supernatant was collected in an insert and transferred to a 2 mL LC vial for analysis. A 2 µL injection was made into a Shimadzu LCMS 9030 (Q-TOF) using a C18 50 mm x 2.1 mm column at 40°C, flow rate 0.35 mL/min, mobile phase A: 60% acetonitrile, 40% H_2O + 10 mM ammonium formate, mobile phase B: 90% isopropanol, 10% acetonitrile + 10 mM ammonium formate, positive mode (data independent acquisition), mass range: 70-1200 *m/z*, total analysis time: 26 mins. The data was processed and analyzed using MS-DIAL 3.50.

Results

- **Metabolomics:** Overall 117 metabolites were detected/identified reproducibly (CVs(%) < 20%) from a single DBS sample (using 4 technical replicates). A range of metabolite classes were detected including: sugars, sugar alcohols, fatty acids, organic acids, amino acids, vitamins, glycolytic and TCA cycle intermediates (Table 1 and Figure 3). No significant differences (p-values <0.05, fold change >1.5) were observed between biological/technical replicates (log transformed and median normalized data) demonstrating the intra- and inter- volumetric precision of the hemaPEN device. A disease-based enrichment analysis was completed (Figure 2) on the 117 polar metabolites, which tests whether the metabolites detected are involved in specific diseases - towards the top (more metabolites involved) or bottom (less metabolites involved) of a ranked query metabolite list.
- **Lipidomics:** Untargeted lipidomics analysis provided a total of 375 identified lipids from one DBS sample, including a wide range of lipid species (Figures 4 and 5).

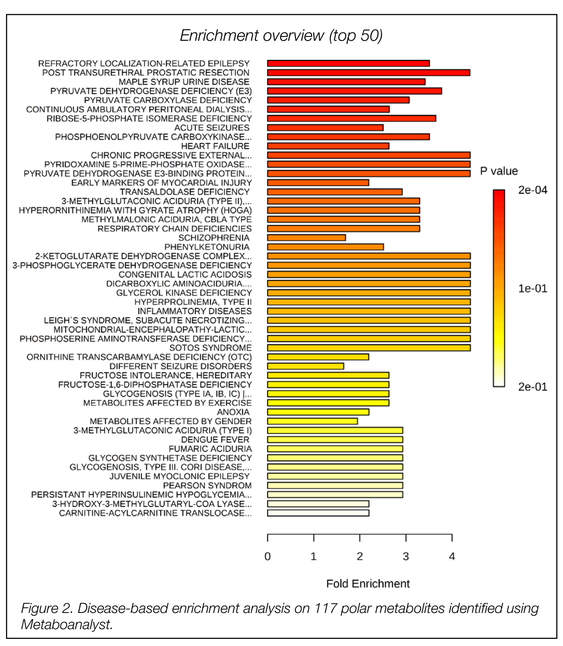

Figure 2. Disease-based enrichment analysis on 117 polar metabolites identified using Metaboanalyst.

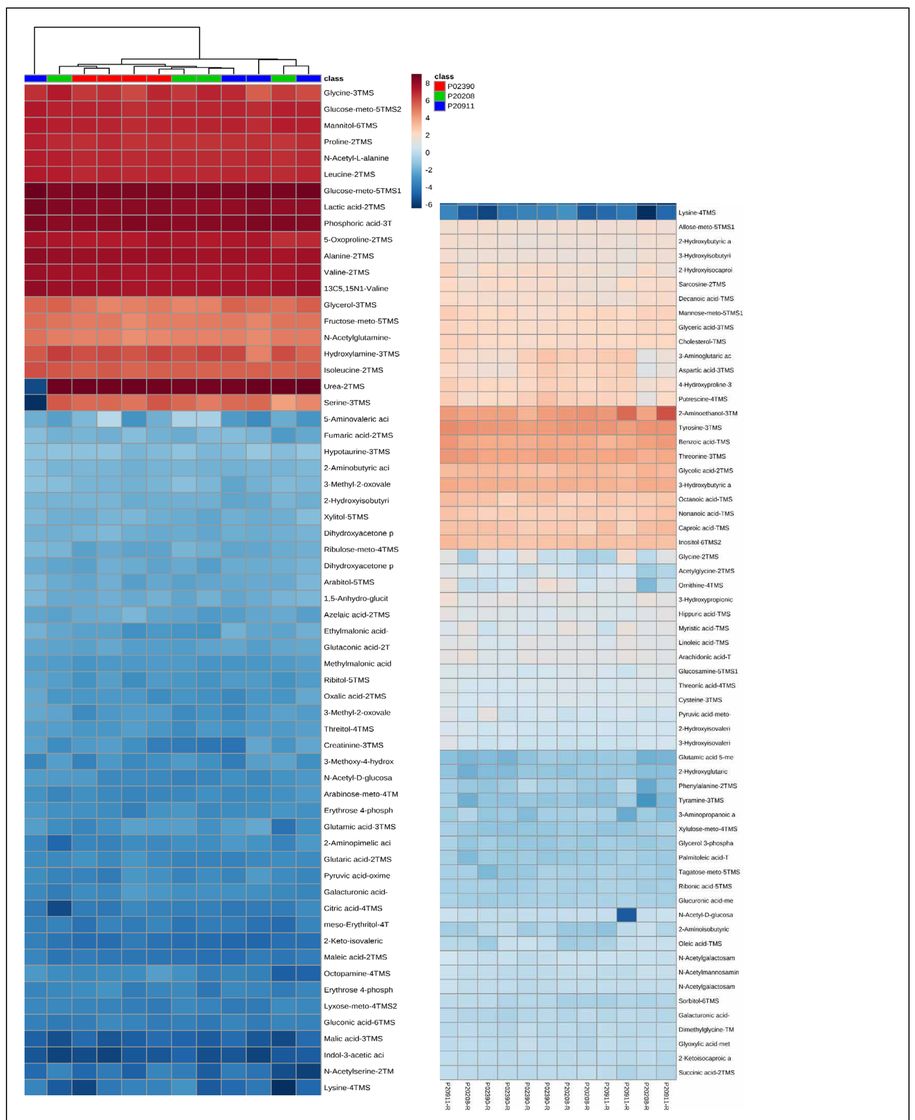

Figure 3. Two-way heat map displaying the normalized peak intensities for 117 metabolites detected from 3 biological replicates Red (P02390), Green (P20208) and Blue (P20911) and 4 technical replicates.

No.	Metabolite	No.	Metabolite
1	Urea-2TMS	61	N-Acetylmannosamine-meto-4TMS
2	Glucose-meto-5TMS	62	2-Ketoisocaproic acid-meto-TMS
3	Phosphoric acid-3TMS	63	Succinic acid-2TMS
4	Lactic acid-2TMS	64	Sorbitol-6TMS
5	Alanine-2TMS	65	Phenylalanine-2TMS
6	Valine-2TMS	66	Galacturonic acid-meto-5TMS
7	5-Oxoproline-2TMS	67	Glucuronic acid-meto-5TMS
8	Leucine-2TMS	68	Ribonic acid-5TMS
9	Mannitol-6TMS	69	2-Aminoisobutyric acid-2TMS
10	N-Acetyl-L-alanine-2TMS	70	Palmitoleic acid-TMS
11	Proline-2TMS	71	Tyramine-3TMS
12	Glycine-3TMS	72	Tagatose-meto-5TMS
13	Hydroxylamine-3TMS	73	Glycerol 3-phosphate-4TMS
14	Isoleucine-2TMS	74	2-Hydroxyglutaric acid-3TMS
15	Serine-3TMS	75	Xylulose-meto-4TMS
16	Glycerol-3TMS	76	Glutamic acid 5-methylester-2TMS
17	Fructose-meto-5TMS	77	Fumaric acid-2TMS
18	N-Acetylglutamine-3TMS	78	3-Aminopropanoic acid-3TMS
19	Tyrosine-3TMS	79	Xylitol-5TMS
20	Threonine-3TMS	80	Azelaic acid-2TMS
21	Benzoic acid-TMS	81	Hypotaurine-3TMS
22	3-Hydroxybutyric acid-2TMS	82	3-Methyl-2-oxovaleric acid-meto-TMS
23	2-Aminoethanol-3TMS	83	2-Aminobutyric acid-2TMS
24	Glycolic acid-2TMS	84	Dihydroxyacetone phosphate-meto-3TMS
25	Octanoic acid-TMS	85	2-Hydroxyisobutyric acid-2TMS
26	Inositol-6TMS	86	1,5-Anhydro-glucitol-4TMS
27	Caproic acid-TMS	87	Ribulose-meto-4TMS
28	Putrescine-4TMS	88	Glutamic acid-3TMS
29	3-Aminoglutaric acid-2TMS	89	Arabitol-5TMS
30	Nonanoic acid-TMS	90	Glutaconic acid-2TMS
31	Aspartic acid-3TMS	91	Threitol-4TMS
32	4-Hydroxyproline-3TMS	92	3-Methyl-2-oxovaleric acid-meto-TMS
33	Glyceric acid-3TMS	93	Galacturonic acid-meto-5TMS
34	2-Hydroxyisocaproic acid-2TMS	94	Oxalic acid-2TMS
35	Mannose-meto-5TMS	95	Methylmalonic acid-2TMS
36	Cholesterol-TMS	96	Pyruvic acid-oxime-2TMS
37	Decanoic acid-TMS	97	Glutaric acid-2TMS
38	Sarcosine-2TMS	98	5-Aminovaleric acid-3TMS
39	Allose-meto-5TMS	99	Ethylmalonic acid-2TMS
40	3-Hydroxyisobutyric acid-2TMS	100	Creatinine-3TMS
41	Glycine-2TMS	101	Ribitol-5TMS
42	2-Hydroxybutyric acid-2TMS	102	2-Aminopimelic acid-3TMS
43	Arachidonic acid-TMS	103	Octopamine-4TMS
44	Linoleic acid-TMS	104	N-Acetyl-D-glucosamine-meto-4TMS
45	Ornithine-4TMS	105	Citric acid-4TMS
46	3-Hydroxypropionic acid-2TMS	106	3-Methoxy-4-hydroxybenzoic acid-2TMS
47	Hippuric acid-TMS	107	Arabinose-meto-4TMS
48	Threonic acid-4TMS	108	Gluconic acid-6TMS
49	Myristic acid-TMS	109	Lysine-4TMS
50	Cysteine-3TMS	110	Lyxose-meto-4TMS
51	Glucosamine-5TMS	111	Erythrose 4-phosphate-meto-4TMS
52	Pyruvic acid-meto-TMS	112	2-Keto-isovaleric acid-meto-TMS
53	2-Hydroxyisovaleric acid-2TMS	113	meso-Erythritol-4TMS
54	Oleic acid-TMS	114	Maleic acid-2TMS
55	3-Hydroxyisovaleric acid-2TMS	115	N-Acetylserine-2TMS
56	Glyoxylic acid-meto-TMS	116	Malic acid-3TMS
57	Acetylglycine-2TMS	117	Indol-3-acetic acid-TMS
58	N-Acetyl-D-glucosamine-meto-5TMS		
59	Dimethylglycine-TMS		
60	N-Acetylgalactosamine-meto-4TMS		

Table 1. All 117 polar metabolites detected from a single DBS by GC-QQQ.

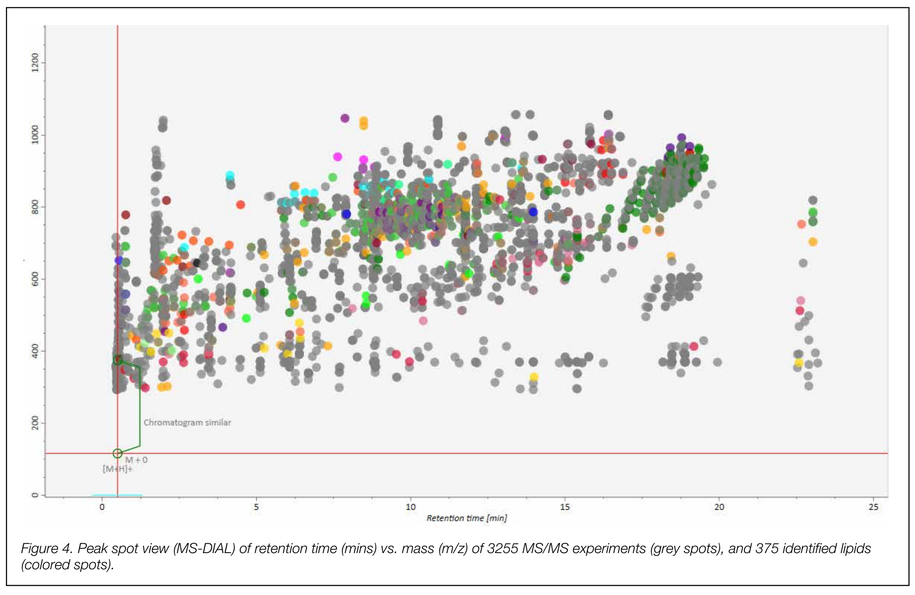

Figure 4. Peak spot view (MS-DIAL) of retention time (mins) vs. mass (m/z) of 3255 MS/MS experiments (grey spots), and 375 identified lipids (colored spots).

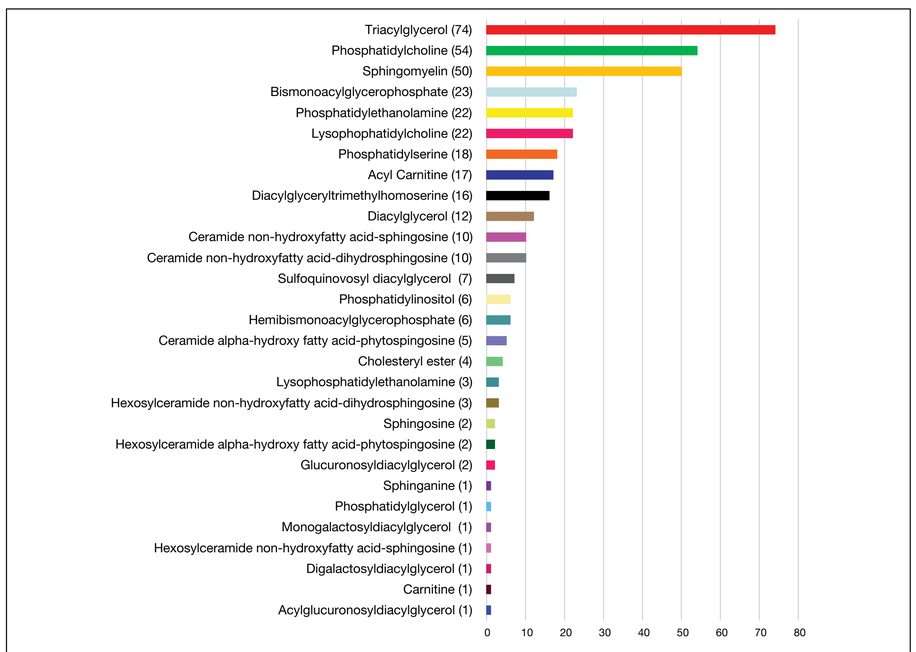

Figure 5. 29 different lipid classes comprised of 375 lipids identified from a single DBS.

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