Application of Dried Blood Spot Microsampling for High-Throughput Four-Dimensional Trapped Ion Mobility Spectrometry Lipidomics

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INTRODUCTION

Dried blood spot (DBS) microsamples offer a minimally invasive and cost-effective method for collecting and storing blood samples for metabolic phenotyping research[1] and high resolution lipidomic analyses using trapped ion mobility spectrometry (timsTOF Pro, Bruker) allow for further separation of molecular ions based on collisional cross-section (CCS)[2,3]. Currently, translation of DBS samples for high-resolution trapped ion mobility analysis of lipids is minimal. And sample stability at different temperatures remains to be elucidated before translation to de-centralised and remote sampling can be possible.

METHODS

Capillary blood was collected from 6 healthy individuals, pooled, and spotted (10 µL) onto a DBS collection matrix commonly employed in commercial microsampling devices (Whatman 903®). High-throughput extraction of DBS was performed using 80% IPA. Storage stability of triplicates were assessed at various temperatures (-80˚C, -20˚C, 4˚C, RT, 45˚C) for multiple timepoints (3-mth, 2-mth, 1-mth, 2-wk, 1-wk, 3-d, 2-d, 1-d, 12-h, 4-h). With 45˚C only assessed up to 1-wk. Data processing and *in silico* lipid annotation was performed using lipid-match algorithms (precursor *m/z*, isotopic pattern, characteristic fragments in MS/MS spectra) and predicted CCS values as an additional qualifier in MetaboScape 2024b.[4,5] (***Fig.1***).

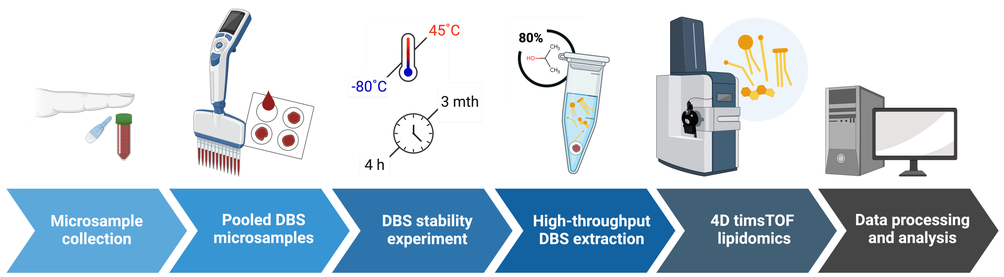

Fig. 1. **Microsample collection and analytical workflow for high-throughput DBS-based 4D timsTOF lipidomics**
Microsample collection employed BD "blue" lancets and lithium heparin (LH)-coated MiniCollect® blood tubes. Data processing was performed in MetaboScape 2024b, and figures were generated in R Studio.

RESULTS + DISCUSSION

A high-throughput 15-minute untargeted 4D-lipidomics method for DBS was developed using ultra-high-performance liquid chromatography (UHPLC, Waters) with timsTOF Pro equipped with a Vacuum Insulated Probe Heated ElectroSpray Ionization (VIP-HESI)[6] source. We previously identified 80% IPA in water to give the maximum annotation of reproducible lipids with <30% CV across a variety of DBS matrices[7] commonly employed in advanced microsampling devices (Whatman 903, Perkin-Elmer 226, and Ahlstrom 222). In the present work, 305 lipids (HESI+) and 237 lipids (HESI-) were annotated. Lipid species were from 22 subclasses across glycerolipids (DG, TG), glycerophospholipids (LPA, LPC, LPE, LPEO, LPG, LPI, LPS, PA, PC, PCO, PE, PEO, PGO, PI, PS), sterols (CE), sphingolipids (Cer, HexCer, SM), and fatty acyls (***Fig. 2***). With -80˚C as control, preliminary analysis demonstrates short-term storage stability of DBS up to 1-week, however, heat should be avoided (***Fig. 3***). These findings are consistent with recent research by Petrick et al. (2024)[8] with further characterisation of the short-term lipidomic changes (within two weeks) in DBS microsamples, across a greater number of lipids and subclasses.

CONCLUSION

We present a robust, high-throughput untargeted 4D-lipidomics workflow that enables comprehensive analysis of 22 lipid subclasses in DBS microsamples using timsTOF Pro. Our findings indicate that DBS microsamples maintain lipid stability under short-term storage conditions, up to 1-week, particularly at -20˚C and 4˚C. However, exposure to elevated temperatures, above room temperature, should be avoided. This workflow enhances the feasibility of large-scale, longitudinal studies using DBS, highlighting their potential for decentralized/remote sample collection.

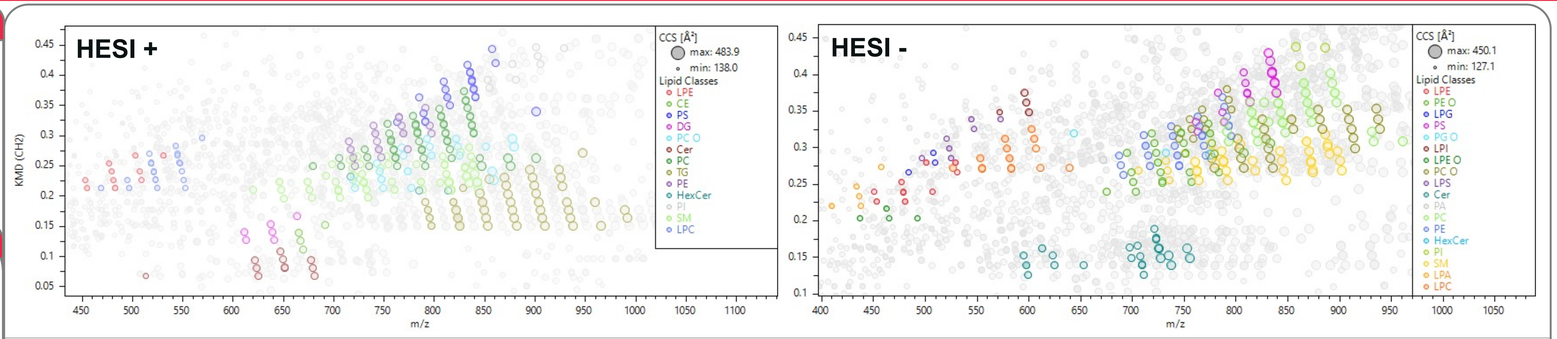

Fig. 2. **Kendrick mass-defect plot of lipid distribution for positive and negative heated electrospray ionization modes.**
Distribution of lipids from various classes from 10 µL DBS samples (Whatman 903®) in positive and negative heated electrospray ionization (HESI) modes. Unidentified features are overlayed (gray). KMD = Kendrick mass-defect (CH2), CCS = collisional cross section, *m/z* = mass to charge ratio. Lipids: CE = cholesteryl ester; CER = ceramide, DG = diacylglycerol, FA = fatty acyls, HCER = hexosylceramides, PA = phosphatidic acid, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PI = phosphatidylinositol, PS = phosphatidylserine, SM = sphingomyelins, TG = triacylglycerides, O – denotes alkyl ether linkage, L – denotes hydrolysed form.

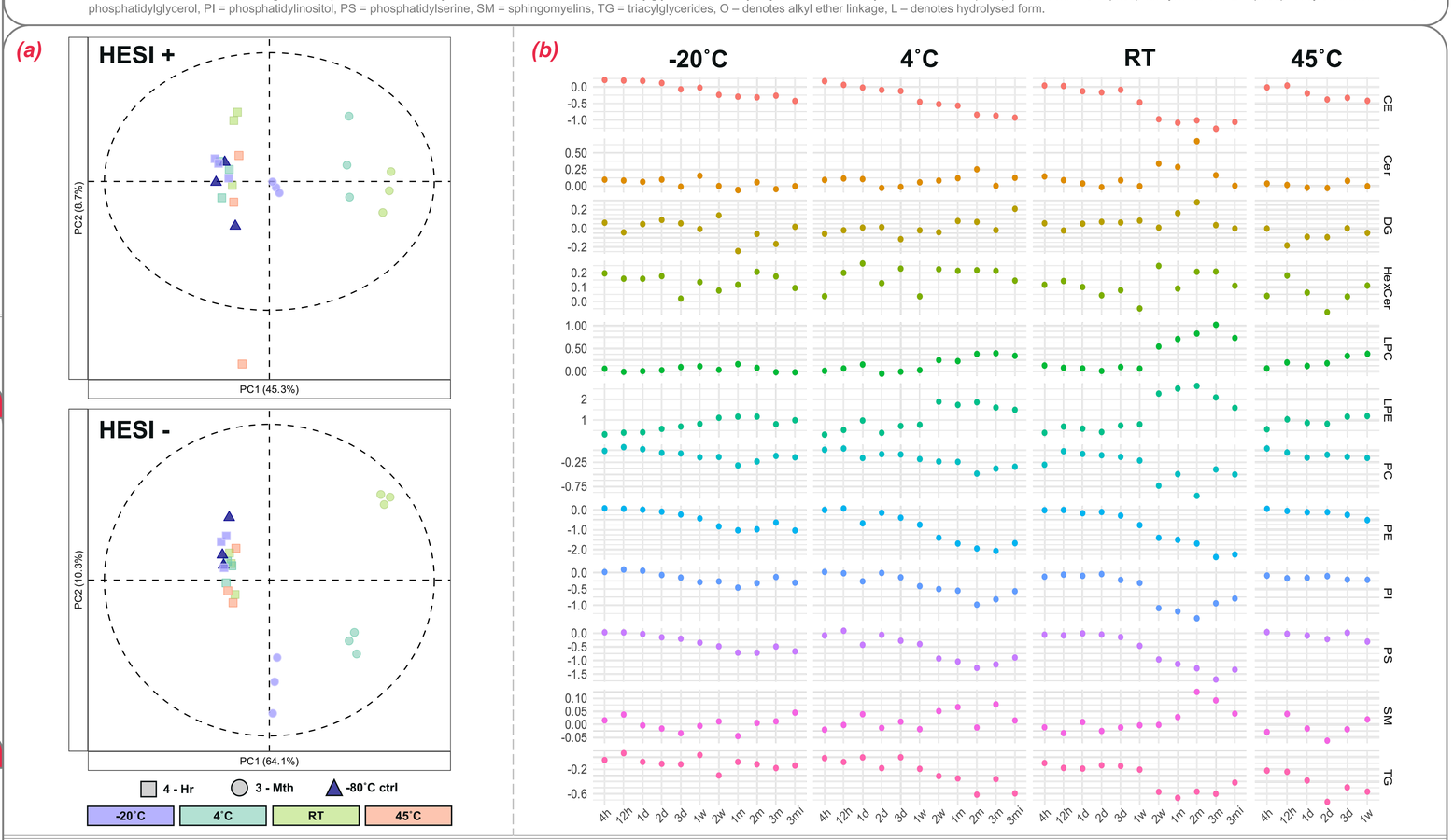

Fig. 3. **Stability of dried blood spot lipid profiles influenced by storage time and temperature.**
(a) Principal component analyses of lipids identified within triplicate DBS samples exposed to each storage temperature (<30% CV) at four hours (4 HOUR) and three months (3 MONTH) in HESI+/- modes. 4 HOUR HESI + = 305 lipids, 4 HOUR HESI- = 237 lipids, 3 MONTH HESI+ = 305 lipids, 3 MONTH HESI- = 235 lipids. ***(b)*** Time series plots of summed median intensities that were $\log_2$ normalised to the -80˚C control by temperature in HESI+ mode. Lipids: CE = cholesteryl ester; CER = ceramide, DG = diacylglycerol, HexCer = hexosylceramides, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PS = phosphatidylserine, SM = sphingomyelins, TG = triacylglycerides.

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