Robust and High Throughput Lipid Profiling of dry blood spot samples using Automated FIA MS/MS^{All} technique

Kuldeep Ravivanshi[a], Narinder Sahni[b], Akanksha Singh[c], Dipankar Malakar[c], Baljit Ubhi, Manoj Pillai[c]

[a]Lipomic Healthcare Pvt. Ltd., New Delhi, INDIA ; [b]Jawaharlal Nehru University, New Delhi, INDIA; [c]SCIEX, Redwood City, CA, USA; [d]SCIEX, 121, Udyog Vihar, Gurugram 122015, Haryana, INDIA

INTRODUCTION

Lipidomics is opening new ways to clinical research though it is in a nascent stage. One of the major challenges in the study of lipids and other biological molecules is the analysis time. We have developed a high throughput and robust method on TripleTOF® 5600 system using Dried Blood Spot (DBS) samples during clinical research, so as to simplify overall procedure and shorten the assay time. Majority of the literature so far is based on using either plasma or serum considered as gold-standard matrix but both these media require a phlebotomist, functional laboratory and storage at very low temperatures - 40°C to -70°C. Advantage of using DBS over the plasma/serum makes it ideal for screening studies.

MATERIALS AND METHODS

Sample Preparation:

90 dried blood spot samples were used for the analysis. Extraction of lipids was carried out as described by Koulman *et al* with some modifications. 3.1 mm DBS punches were taken, to which 100uL water was added followed by 250ul of Methanol and 500 ul of Methyl tert-butyl ether (MTBE). After 10 minutes, the mixture was centrifuged for another 10 minutes. 200ul of upper organic layer was transferred to 2 ml glass vial and dried under nitrogen. It was then reconstituted in 25 ul of MTBE and 90ul of 7.5mM of ammonium acetate in IPA:MeOH 2:1.

HPLC Conditions:

The lipid extracts were analyzed using Shimadzu LC XR system coupled to the TripleTOF® 5600 system. Only one pump was used for delivering the mobile phase at a gradient flow rate. The mobile phase used was methanol with 0.1% formic acid and 5mM ammonium acetate and initially delivered at a flow rate of 10 µl/min. The lipid samples injection volume was 50 µl. This was initially pumped for 9 mins at 10 µl/min for data acquisition for a period of 3 minutes from 5 minutes to 8 minutes gradient. The flow rate was then increased to 100 µl/min again for washing and equilibration. The total LC run time was 12 minutes.

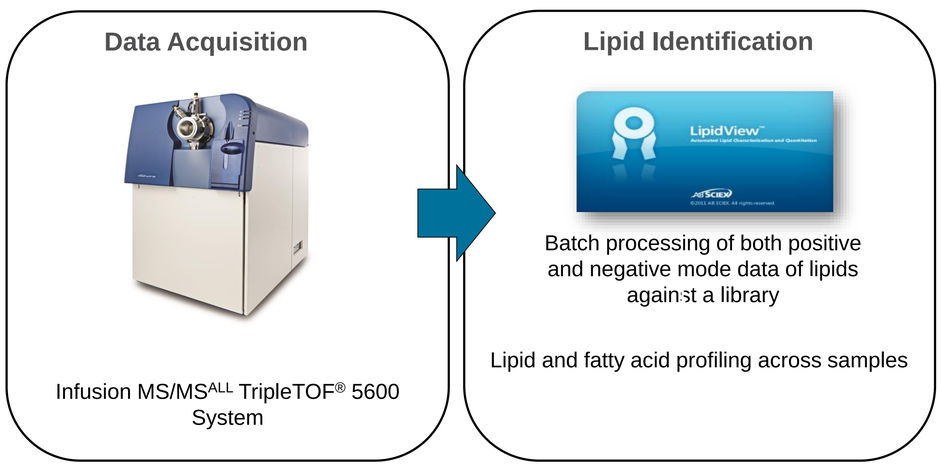


Fig 1. Worklow for lipid analysis

Mass Spectrometric Conditions:

The TripleTOF® 5600 system was used to analyse the lipid extracts using the MS/MS^{ALL} workflow. Analysis was done in both the positive ionization and negative ioniation mode. Samples were analysed using the Infusion MS/MS^{ALL} workflow for complete lipidome coverage. The MS/MS^{ALL} workflow experiments performed the data independent acquisition and consisted of a TOF MS scan from m/z 200-1200 followed by a sequential acquisition of 1001 MS/MS spectra acquired from m/z 200 to 1200, with a step size of 1 Da.

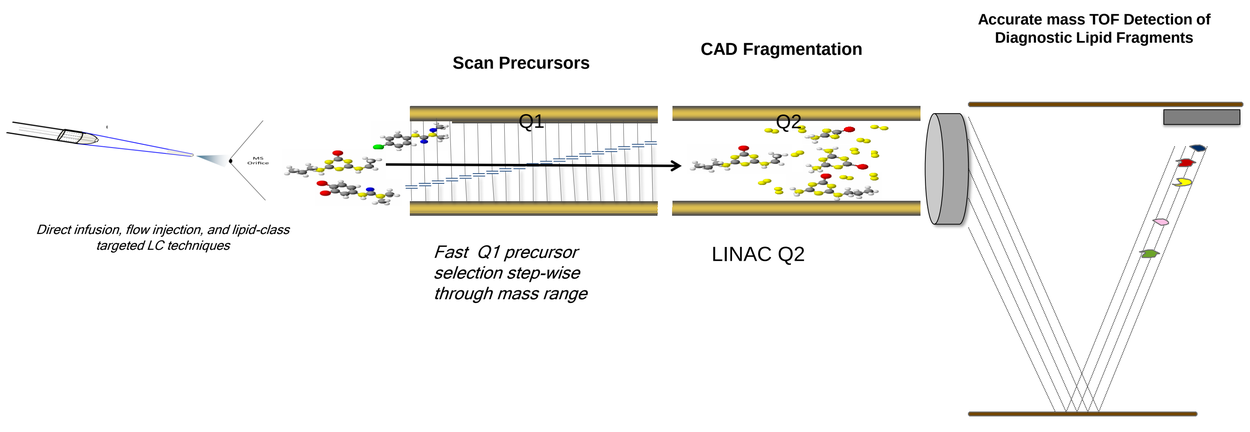


Fig 2. MS/MS^{ALL} Workflow for Information Independent Data Collection: Product ions acquired from every precursor within a specified mass range

Key Features of Automated Infusion Workflow coupled with Data Independent Acquisition

- Infusion MS/MS^{ALL} workflow acquires product ion spectra of all precursors within a specified mass range, providing a digital record of your sample.
- Complete digitization of your sample allows for retrospective data mining, removing the need to re-run samples in the future as your research needs change.
- Minimal set up is required to automate infusion using an HPLC system and a flow injection strategy.
- Automated infusion gives improved accuracy and %CV compared to manual infusion by a syringe pump.
- Identification and semi-quantitation from a discovery experiment using LipidView™ and MarkerView™ Software.

RESULTS

The lipid identification was performed by the LipidView™ software 1.2. Batch processing for lipid identification was performed for all the replicates samples. In this shotgun approach we had been successfully able to get a coverage of about 1027 lipids in all detected classes. Lipid classes like Triacylglyceride (TAG), N-acyl PE (NAPE), Cholesteryl Ester (CE), Phosphatidylcholine (PC), Phosphatidylinositol (PI) were found to be abundantly in the DBS samples using present method with promising reproducibility in terms of inter and Intra-day assay. The developed method is fast and robust enough that may prove to be helpful in large epidemiology study cohorts.

A number of Lipids from classes Cholesteryl Ester (CE), Phosphatidylcholine (PC), Triacylglyceride (TAG), Lyso-Phosphatidylcholine (LPC), N-acyl PE (NAPE), Phosphatidylinositol (PI) and Monogalactosyl DAG (MGDG) were found to be present in majority of samples. Lipids from classes Phosphatidylglycerol (PG), Phosphatidyletanolamine (PE) and Glycidol Ester (GlcdE) were also present in low abundance. % CV obtained for replicate testing was found to be as low as 2.7 for high abundant lipid classes and close to 8% for medium abundant lipid classes and less than 25% for very low abundant lipid classes. Details mentioned in figure 5.

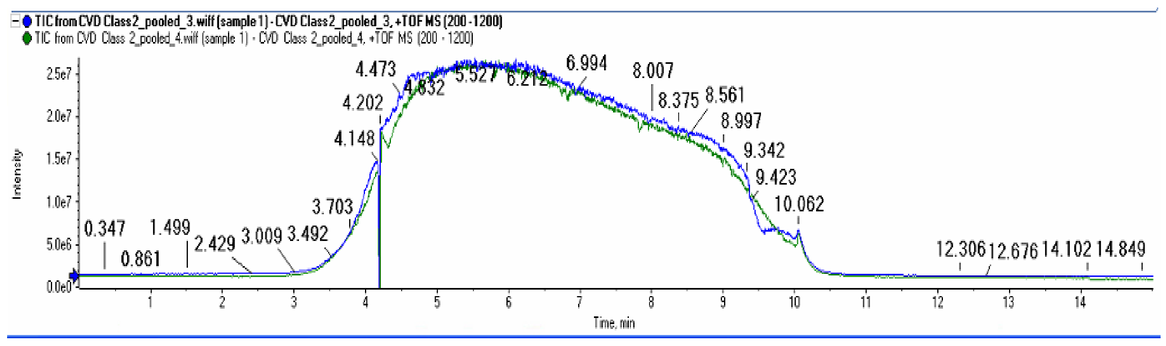


Fig. 3. Elution Profile

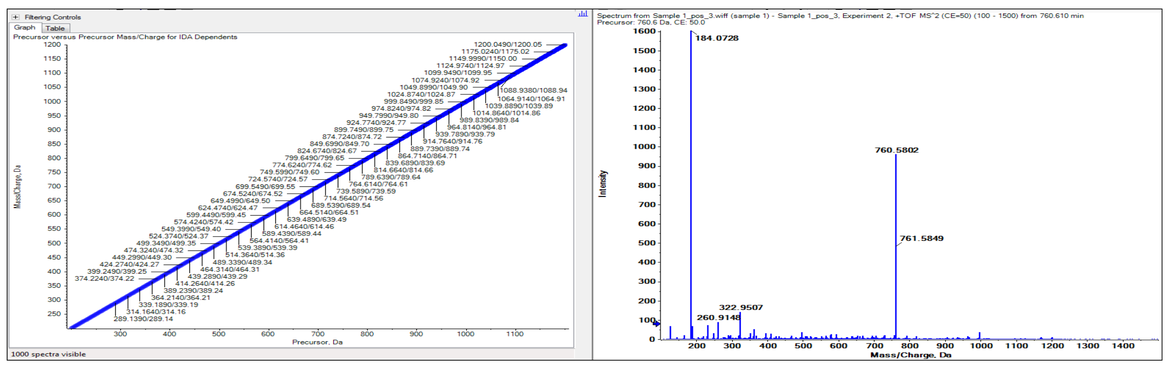
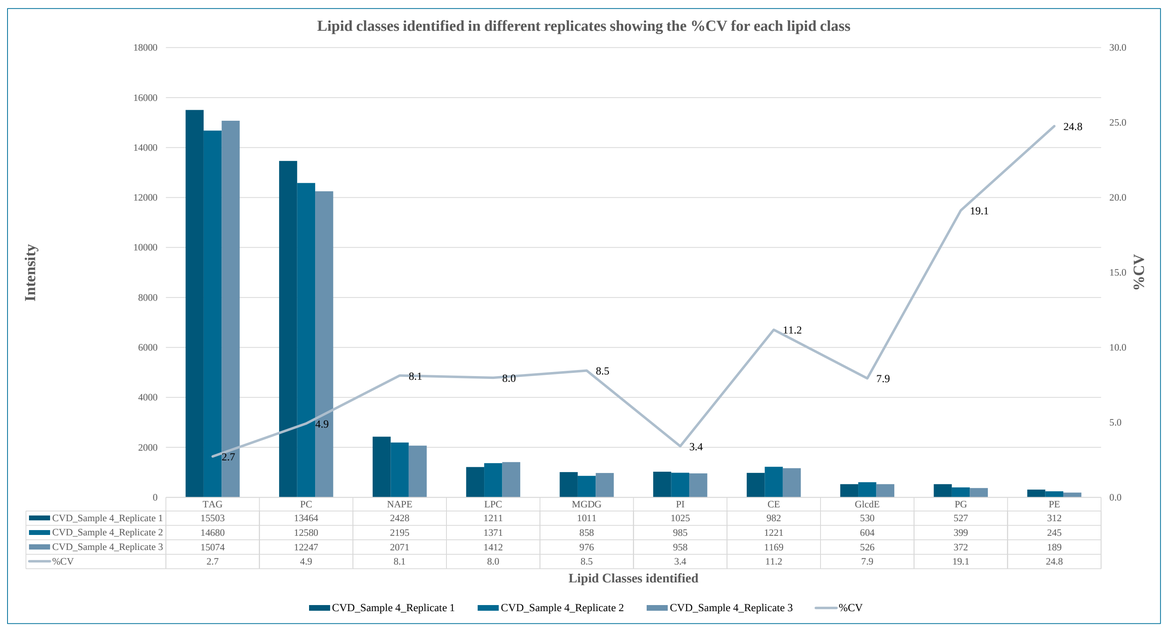


Fig. 4. FIA MSMS^{ALL} Workflow for Shotgun Lipidomics



	TAG	PC	NAPE	LPC	MGDG	PI	CE	GlcdE	PG	PE
CVD_Sample 4_Replicate 1	15503	13464	2428	1211	1011	1025	982	530	527	312
CVD_Sample 4_Replicate 2	14680	12580	2195	1371	858	985	1221	604	399	245
CVD_Sample 4_Replicate 3	15074	12247	2071	1412	976	958	1169	526	372	189
%CV	2.7	4.9	8.1	8.0	8.5	3.4	11.2	7.9	19.1	24.8

Fig. 5. Different replicates injections showing %CV among lipid classes identified in high, medium and low abundance

CONCLUSIONS

The Infusion MS/MS^{ALL} workflow on the TripleTOF® 5600 system is a novel data independent acquisition strategy for qualitative and quantitative molecular characterization of complex lipid samples. A high-throughput quantitative profiling for lipids can be carried out when coupled to the automated flow injection strategy described in this work. The simple workflow enables easy data acquisition and post acquisition data analysis for lipid identification.

REFERENCES

Kang *et al.*, "Evaluation of Dried Blood Spots as Sample Matrix for GC/MS based Metabolic Profiling", *Anal Chem.,* **2011**, 83, 4314-4318.9

Koulman *et al.*, "The development and validation of a fast and robust dried blood spot based lipid profiling method to Study infant metabolism", *Metabolomics,* February 2014.

TRADEMARKS/LICENSING

Document number: RUO-MKT-10-5907-A