Rapid Identification of Lipids in Human RBC membrane using Direct Infusion MS/MS^{all} and Online Information Dependent Analysis Methods

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ABSTRACT

The rapid advances in mass spectrometry technology have dramatically accelerated the development of lipidomics research. The most common approaches for lipid identification and quantitation are infusion shotgun lipidomics and liquid chromatography coupled with high resolution mass spectrometry, although the two methods both have strengths and weaknesses. The combination of both methods would be more effective in rapid identification of lipids. We present here a rapid and comprehensive assay for lipid profiling in human RBC membrane using high resolution mass spectrometry system. The combined method enabled the identification of more than 300 lipid compounds from 15 lipid classes.

INTRODUCTION

High resolution mass spectrometry (HRMS) with powerful identification and quantitation ability has became an essential tool in lipidomics research. Infusion shotgun MS/MS^{all} and LC/MS method were two predominant methods on HRMS. Infusion MS/MS^{all} workflow can provide the quick screening of lipid species and LC/MS is useful to identify very low level lipid species for lipids deeper coverage. The combination of both methods would be more effective in rapid identification of lipids.

Results from lipidomics profiling provides insights into the roles of lipids in cellular networks and is being used to identify prognostic or diagnostic markers of disease progression. However, rapid identification and interpretation of lipids has became a very big obstacle during data processing. LipidView™ (AB SCIEX) software has many built-in features to help rapid identification of lipid species, for example, a lipid database composed of over 28000 individual lipid species (over 60 classes) and characteristic fragment ions from 600 lipid species, and batch processing for multiple samples by respective method. Lipid bioinformatics could also be carried out through a seamless link from LipidView™ to MarkerView™ (AB SCIEX) software with multivariate analysis function.

MATERIALS AND METHODS

Sample Preparation:
Lipids were extracted from RBC cell membrane using the Bligh & Dyer's method. Extract was dissolved in 200μL of chloroform/method (1:2), including 10mM ammonium acetate, before submitting for MS analyses on a TripleTOF® 5600+ system (AB SCIEX).

Infusion MS/MS^{all} workflow using TripleTOF® 5600+ system:
For infusion MS/MS^{all}, sample was introduced using NanoESI ion source at the flow rate of 500 nL/min. MS/MS data was collected from m/z 200 to 1200 at 1 Da step. (Figure 2).

LC/MS Experiment using TripleTOF® 5600+ with Shimadzu UFLC System:
LC-MS/MS experiment was carried out using a Shimadzu UFLC system with ESI ion source on a Kinetex C_{18} (100 x 2.1mm, 2.6μm) column. The mobile phase consisting of acetonitrile/ methanol/water(1:1:1, v/v)+5mM ammonium acetate (A) and Isopropanol+5mM ammonium acetate (B) ran at the flow rate of 300 μL/min by a 30 min gradient. MS/MS data for the top ten precursor ions were collected using information dependent acquisition (IDA) workflow at the mass range of m/z 200-1200Da.

Data Processing:
Data acquired using Infusion MS/MS^{all} and LC/MS experiments were processed in batch mode using AB SCIEX LipidView™ software for automated identification and quantitation of lipid species.

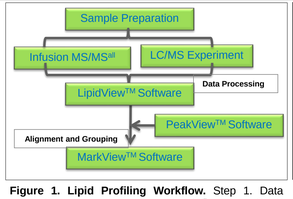

Figure 1. Lipid Profiling Workflow. Step 1. Data acquisition using infusion MS/MS^{all} and LC/MS methods. Step 2. Lipid molecular species identification and quantitation using LipidView™ software. Step 3. Grouping difference Compare using MarkerView™ software.

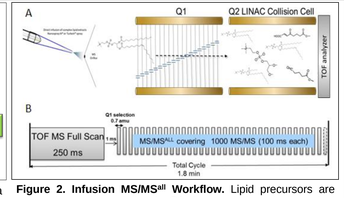

Figure 2. Infusion MS/MS^{all} Workflow. Lipid precursors are selected at unit resolution in Q1, fragmented, and all product ions are detected simultaneously (A). Using infusion MS/MS^{all} method, which is automated collection of TOF MS and TOF MS/MS of all lipids via step-wise sampling of all precursor ions selected at unit resolution (B). This acquisition requires 1.8 min to generate a comprehensive dataset.

RESULTS

RBC membrane samples were analyzed by AB SCIEX TripleTOF® 5600+ systems using both infusion MS/MS^{all} and LC/MS IDA methods. Rapid identification of lipid species and structures elucidation as well as quantitation were performed by LipidView™. Based on the built-in database of MS and MS/MS fragments, the powerful software can remarkably improve the data analysis efficiency and lipid species could be easily confirmed by means of the high mass accuracy MS and MS/MS spectra (Figure 3 and 4).

Direct infusion MS/MS^{all} method can automatically collect high resolution TOF MS and TOF MS/MS of all lipids via step-wise sampling of all precursor ions selected at unit resolution. By MS/MS^{all} approach, 14 lipid classes were quickly identified including PC, PE, PG, TAG, etc. (Figure 5 and 6).

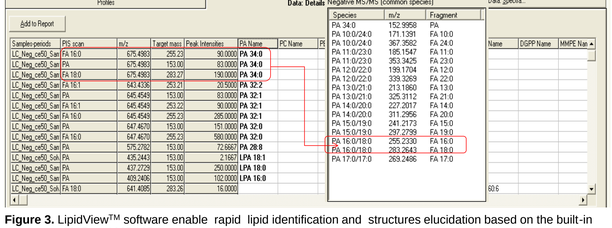

Figure 3. LipidView™ software enable rapid lipid identification and structures elucidation based on the built-in database of MS and MS/MS fragments with high mass accuracy

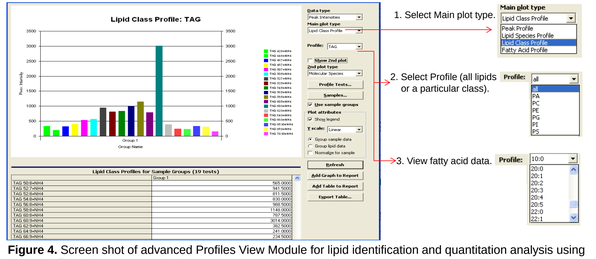

Figure 4. Screen shot of advanced Profiles View Module for lipid identification and quantitation analysis using LipidView™ software.

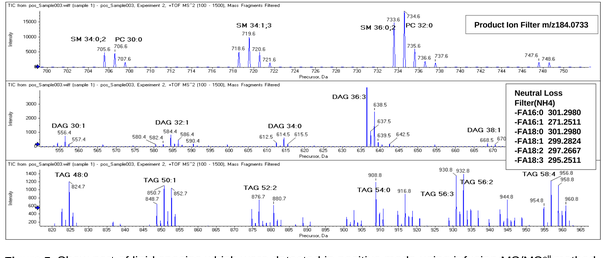

Figure 5. Show part of lipid species which were detected in positive mode using infusion MS/MS^{all} method.

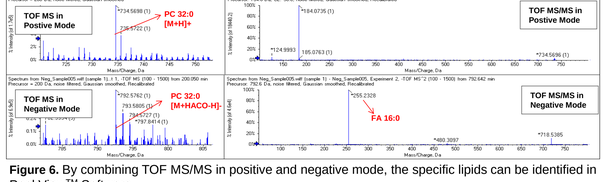

Figure 6. By combining TOF MS/MS in positive and negative mode, the specific lipids can be identified in PeakView™ Software.

In LC/MS IDA method, dynamic background subtraction (DBS) was applied to ensure the acquisition of high quality TOF MS/MS spectra (Figure 7). By means of IDA workflow, enough and effective data for the confirmation of lipids can be acquired by only one injection. In addition, separation of lipids by classes resulted in less ion suppression, higher ionization yield and increased sensitivity for minor components so that low level lipid classes such as PA, MMPE etc. could be detected and identified, which are usually not readily found by infusion MS/MS^{all} approach (Figure 8). A total of 323 lipid species from 15 lipid classes were identified and confirmed by the combination of chromatographic retention time, MS and MS/MS spectra with high mass accuracy. The relative quantitation of individual lipid species in RBC membranes can be simultaneously obtained.

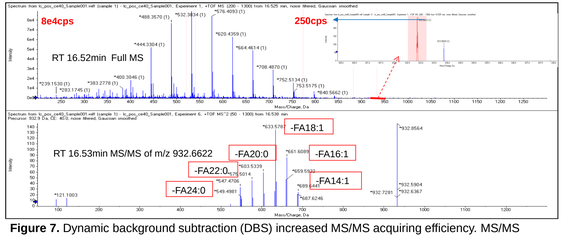

Figure 7. Dynamic background subtraction (DBS) increased MS/MS acquiring efficiency. MS/MS Spectrum of TAG 56:2(m/z932.6622) collected using DBS in the presence of high background conditions.

Lipid Name	Formular	Type	Error(ppm)	Fragment
MMPE 32:0	C38H76O8PN	[M-H]-	-0.8	FA 16:0
MMPE 34:2	C40H76O8PN	[M-H]-	0.3	FA16:1,FA18:1
MMPE 34:4	C44H86O8NP	[M-H]-	0.7	FA 18:1 FA 18:0
MMPE 36:1	C42H82O8PN	[M-H]-	1.2	FA 18:1 FA 16:0
MMPE 36:4	C41H76O7PN	[M-H]-	-0.3	FA 20:4 FA 20:4-CO2
MMPE 38:1	C44H86O8PN	[M-H]-	1	FA 18:1 FA 18:0
PA 32:0	C35H69O8P	[M-H]-	-0.7	PA 153 FA 16:0
PA 32:1	C35H67O8P	[M-H]-	-1.1	PA 153 FA 16:0 FA16:1 FA 18:1 FA 14:0
PA 34:0	C37H73O8P	[M-H]-	0.5	PA153, FA18:0,FA16:0
PA 34:1	C37H71O8P	[M-H]-	-0.8	PA 153 FA 18:1 FA 16:0
PA 34:2	C37H69O8P	[M-H]-	0.1	PA 153 FA 18:2 FA 16:0
PA 36:1	C39H75O8P	[M-H]-	-1	PA 153 FA 18:0 FA 18:1
PA 36:2	C39H73O8P	[M-H]-	0.1	PA 153 FA 18:0 FA 18:2
PA 36:3	C39H71O8P	[M-H]-	-0.7	PA 153 FA 18:1 FA 18 :2
PA 36:4	C39H69O8P	[M-H]-	-1.2	PA 153 FA 20:4 FA 16:0
PA 38:3	C41H75O8P	[M-H]-	-0.5	PA 153 FA 20:3 FA 18:0
PA 38:4	C41H73O8P	[M-H]-	-0.2	PA 153 FA 20:4 FA 18:0

Figure 8. Some lipid species at low level can be identified by LC/MS Method, but can't be found by Infusion MS/MS^{all}

CONCLUSIONS

Infusion MS/MS^{all} workflow provides the rapid lipid screening and LC/MS IDA approach is more useful for identification and quantitation of isobaric and isomeric lipid species at low level for deeper coverage. The combination of these two methods together with LipidView™ software could dramatically improve not only the number of lipids identified but also the efficiency of data interpretation.

TRADEMARKS/LICENSING