WP 537 A comparative study on the identification of plasma lipids using collision-induced dissociation and electron-activated dissociation

Kiran Maan[1], Dipankar Malakar[1], Cagakan Ozbalci[2]

[1]Sciex, Bangalore, India, [2]Sciex, United Kingdom

ABSTRACT

Lipidomics profiling is widely being used for untargeted and targeted analysis. The diversity of lipids makes comprehensive profiling and identification of lipid species challenging. There is an ongoing need for accurate lipid identification. For fragmentation, collision-induced dissociation (CID) and electron-activated dissociation (EAD) are currently available. EAD, as addition to standard CID, delivers unique fragment ions that are required for deep lipid characterization. Good number of hits were obtained using SimLipid and MS-DIAL in both CID and EAD fragmentation but with some unique identification. **Novel Aspect:** Utilization of both CID and EAD for better identification and characterization along with use of multiple software (MS-DIAL and SimLipid).

INTRODUCTION

Lipids are important class of biomolecules playing role in many critical biological functions. In recent years, the lipidomics domain has expanded dramatically. This has shown that lipids have many biological functions in addition to being structural components and energy storage, such as working as signaling molecules. Lipids are heterogenous in terms of fatty acyl/alkyl, sphingosine, or isoprene moieties as hydrophobic part[1]. Lipidomics workflow involves various crucial steps starting from sample preparation to data processing along with lipid identification. Despite the fact that lipid species commonly fit into classes with fundamental subgroups and configurations, the diversity of lipid molecules is tremendous. The diversity of lipids makes comprehensive profiling and identification of lipid species challenging. Liquid chromatography-mass spectrometry (LC-MS) is one of the most extensively used platforms for untargeted lipidomic profiling due to its high sensitivity and resolution along with structure elucidation using MS/MS. Currently, collision-induced dissociation (CID) and electron-activated dissociation (EAD) are available for fragmentation in MS. In comparison to conventional CID, EAD provides unique fragment ions that are essential for complete lipid characterization[2]. Electron based fragmentation can provide important information about the structure.

MATERIALS AND METHODS

Sample Preparation:
Human plasma (25 µL) was extracted using a liquid-liquid extraction method by utilizing a mixture of methanol, dichloromethane, and water in glass tubes. The upper lipid extract was dried using a nitrogen evaporator and was further reconstituted in 200 µL ethanol.

UHPLC and MS Conditions:
A ExionLC system was utilized for separating lipid species through Phenomenex Luna Omega PS C18 (100 x 2.1 mm, 1.6 µm) column operating at 40 ° C. For gradient elution, mobile phase A consisted of water: acetonitrile (2:3 v/v) and mobile phase B consisted of 2-propanol: acetonitrile (4:1 v/v) (both mobile phase containing 10 mM ammonium formate with 0.1% formic acid) at a flow rate of 0.2 mL/min. The injection volume was set to 10 µL. Herein, a highly sensitive QTOF instrument (ZenoTOF 7600 system) was utilized for both CID and EAD fragmentation using a data-dependent acquisition (DDA) scan mode for untargeted lipidomics. Among the various software programs available, SimLipid and MS-DIAL software were used for identification of lipids in both fragmentation modes for comparison.

RESULTS

- An untargeted chromatographic method was developed on Luna Omega PS C18 for lipidome coverage.
- One dataset was created using CID fragmentation with collision energy (CE) of 35 V and collision energy spread (CES) of 15 V in positive ionization mode.
- Other dataset on the same sample was created using EAD fragmentation with electron energy of 15 eV.
- **Fig. 1** shows potentiality of CID and EAD to produce different fragmentation pattern.
- SimLipid and MS-DIAL software were used for lipid identification.

MS-DIAL

- Lipid identification based on CID fragmentation was performed using MS-DIAL (v.4.9.221218) whereas MSDIAL-v.5.1.221218 version was used for EAD fragmentation which can employ EAD (EIEIO) lipid database in addition to conventional CID database.
- All lipid classes were selected in positive ionization mode and matching was performed by using $[M+H]^+$ or $[M+NH_4]^+$ adduct forms.
- Minimum intensity threshold was set to 1000 amplitude and identification cut off score set to 80% to reduce the number of the false positive results.

SimLipid

- Lipid database searching was also performed using SimLipid software.
- SimLipid compares the exact masses of lipid precursor and product ions to those to an in-silico database.
- Tolerance for MS was set to 10 ppm and MS-MS identification was set at 50 mDa.
- $[M+H]^+$ or $[M+NH_4]^+$ adduct forms were selected for identification.

All the identified lipid by CID and EAD fragmentation were compared by Venn diagram (Fig. 2) whereas lipid classes were compared by using bar graph (Fig. 3)

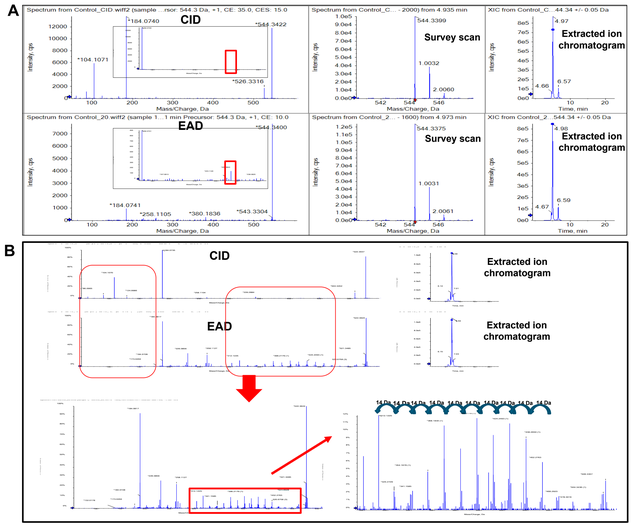

Fig 1. Comparison between CID and EAD MS/MS spectrum.
A) Fragment m/z of 184.07 is common to sphingomyelin (SM) and phosphatidylcholine (PC). EAD showed m/z of 226.08 which is unique to PC; B) Unique peaks are observed in both CID and EAD. The mass region is expanded further to show fragmentation of the alkyl chain showing 14 Da m/z difference.

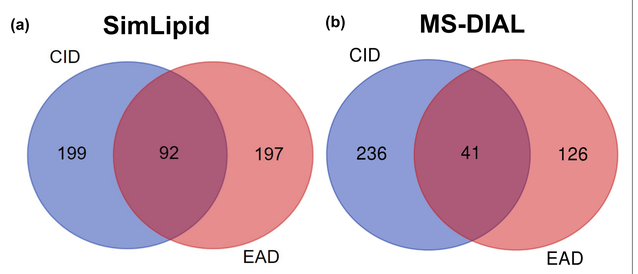

Fig. 2 Comparison between CID and EAD based identified lipids using (a) SimLipid and (b) MS-DIAL software

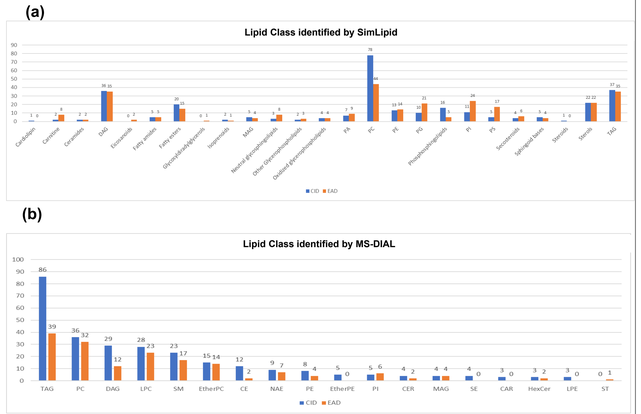

Fig. 3 Lipid Classes identified by (a) SimLipid and (b) MS-DIAL based on CID and EAD fragmentation.
Abbreviations: DAG: Di(acyl|alkyl)glycerols, MAG: Mono(acyl|alkyl)glycerols, PA: Glycerophosphates, PC: Glycerophosphocholines, PE: Glycerophosphoethanolamines, PG:Glycerophosphoglycerols, PI:Glycerophosphoinositols, PS: Glycerophosphoserines, TAG: Tri(acyl|alkyl)glycerols, LPC: lyso phosphatidylcholines, SM: Sphingomyelins, CE: Cholesterol esters, CER: Ceramides, SE, CAR: Carnitine, LPE: lysophosphatidylethanolamine, ST: Sterols, inc. bile acids

- Different fragmentation patterns are generated as a result of CID and EAD. Fragments produced from EAD were able to reveal a 14 Da difference, indicating the presence of a successive CH2 group in the analyte.
- EAD allows for alternate fragmentation, which provides higher degrees of structural information.
- Identification using SimLipid software showed that out of the total lipids being identified using either CID or EAD fragmentation, approximately 1/3 were common in both the fragmentation pattern.
- On the other hand, 2/3 were unique to type of fragmentation source being used (Fig. 2a).
- When lipid classes were compared between different fragmentation modes, CID was found to be better at detecting PC and phosphosphingolipids (Fig. 3a).
- On the other hand, compared to CID fragmentation, EAD provided better identification for carnitine, neutral glycosphingolipids, PG, PI, and PS lipid classes (Fig. 3a).
- Identification using MS-DIAL software showed higher lipids in CID compared to SimLipid which is reverse in case of EAD fragmentation.
- TAG, DAG and Cholesterol ester were identified more in CID compared to EAD using MS-DIAL. MS-DIAL uses LipidBlast library.
- Overall, both the software showed few common and distinct lipids.
- MS-DIAL 5.22 is integrated with EAD specific database, which provides information about the lipid isomers such as Sn-1, Sn-2, and double bond locations in the acyl chains.
- When it came to overall identification, both software programs identified a relatively comparable number, but MS-DIAL showed more identification by CID fragmentation whereas SimLipid identified comparable lipids by both fragmentation method.
- It is also observed that EAD generated a diverse set of chain fragments that originated from the precursor ion, with successive loss of CH2 from the fatty acid backbone compared to CID.
- Overall, the use of both fragmentation sources will provide more comprehensive coverage for lipid identification and its confirmation.

CONCLUSIONS

- Various unique fragments were observed in both CID and EAD fragmentation.
- EAD based fragmentation provided information about the chain length.
- It is recommended to use both CID and EAD for fragmentation to have more confidence for the identified lipid due to tremendous diversity of lipids.
- Fragmentation based on EAD have the potential to address shortcomings of CID extending the MS/MS capabilities for molecular structural elucidation.
- Similarly, identification using SimLipid and MS-DIAL will also be beneficial for authentic lipid identification.
- MS-DIAL can provide automated near complete identification of lipid by using its EAD MS/MS library.

CONFLICT OF INTEREST DISCLOSURE

The authors declare no competing financial interest.

REFERENCES

1. Züllig, T., Trötzmüller, M., & Köfeler, H. C. (2020). Lipidomics from sample preparation to data analysis: a primer. Analytical and bioanalytical chemistry, 412, 2191-2209.

2. Pearson, M., Hunter, C., & Baba, T. (2021). Complete structural elucidation of lipids in a single experiment using electron activated dissociation (EAD). SCIEX technical note.

TRADEMARKS/LICENSING