Effectiveness of ion fragmentation techniques for high throughput lipid analysis

Xiaoli Gao[1], Keqi Tang[1], Qibin Zhang[1], Rosey K. Chu[2], Da Meng[3], Jianying Zhou[1], Ronald J. Moore[1], Richard D. Smith[1], Michael G. Katze[4,5], and Thomas O. Metz[1]

[1]Biological Sciences Division, [2]Environmental Molecular Sciences Laboratory, and [3]Computational Sciences & Mathematics Division, Pacific Northwest National Laboratory, Richland, WA 99352
[4]Department of Microbiology, School of Medicine and [5]Washington National Primate Research Center, University of Washington, Seattle, WA 98195

Pacific Northwest NATIONAL LABORATORY

Overview

- **Fragmentation efficiencies for lipid molecular species fragmented using CID and HCD were calculated as the abundance of signature ions / abundance of precursor ions x 100 %**
- **Normalized collision energies were optimized for CID and HCD in positive and negative electrospray ionization modes.**
- **Evaluation of CID and HCD ion fragmentation techniques highlights their effectiveness for high throughput lipid analysis.**

Introduction

- Lipidomics – the comprehensive identification and quantitation of lipids within biological systems – relies on accurate and efficient structural characterization of detected molecular species.
- In a previous study[1], we observed different fragmentation patterns among different classes of lipids depending on dissociation method.
- Misidentification of lipids may occur due to low fragmentation efficiencies caused by non-optimal dissociation techniques or collision energies.
- Techniques for enhancing peptide identifications have been well-investigated; however, there are relatively few reported investigations of using different ion fragmentation techniques to enhance lipid fragmentation and therefore identification.
- In this study, we investigated the effectiveness of collision induced dissociation (CID) and higher energy collisional dissociation (HCD) for high throughput lipid analysis, using 47 lipid species from 5 lipid classes.

Methods

- Solutions of 5 µM lipid standards in isopropanol/water (1:1, v/v) containing 10 mM ammonium acetate were infused into a LTQ-Orbitrap Velos operating in alternating (CID or HCD) data-dependent MS/MS mode
- Normalized collision energies for both CID and HCD were varied from 5-60% at 5% intervals for positive ion mode and from 10-100% at 10% intervals for negative ion mode, and the intensities of fragment ions from five microscans were averaged.
- A target mass list for the 47 lipid standards was incorporated into the instrument method, and these ions were isolated for MS/MS.
- Both CID and HCD were set with a maximum charge state of 2+ and an isolation width of 2 *m/z* units. An activation Q value of 0.18 was used for CID.
- Fragmentation efficiencies for lipid molecular species were calculated as the abundance of signature ions / abundance of precursor ions x 100 %

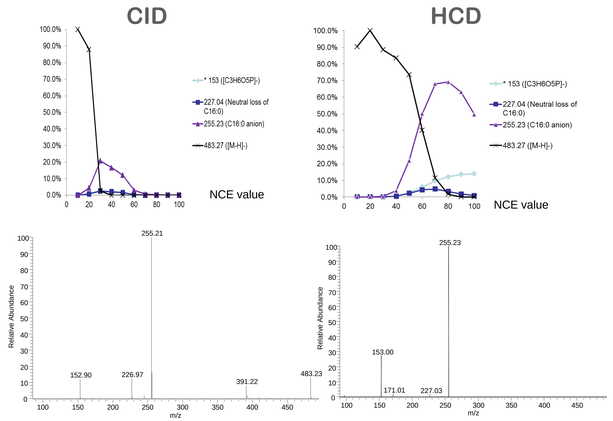


Figure 2. Fragmentation profile (NCE 0-100%) and MS/MS spectra under CID (NCE 30%) and HCD (NCE 100%) of 16:0 LPG.

Results

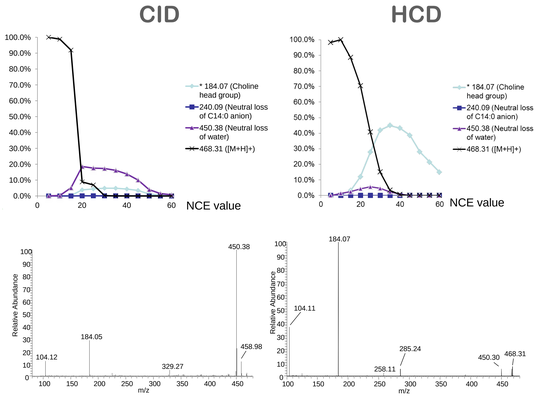


Figure 1. Fragmentation profile (NCE 0-60%) and MS/MS spectra under CID (NCE 30%) and HCD (NCE 35%) of 14:0 LPC.

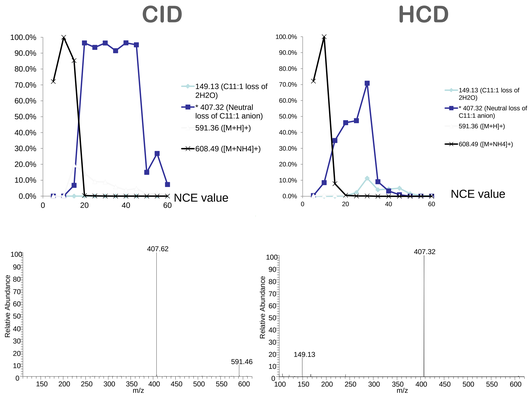
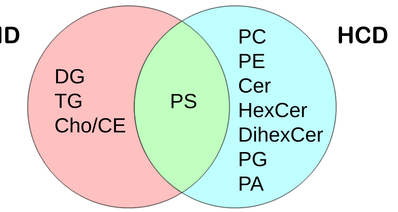


Figure 3. Fragmentation profile (NCE 0-60%) and MS/MS spectra under CID (NCE 40%) and HCD (NCE 30%) of 11:1/11:1/11:1 TG.

Table 1. Optimized NCEs and corresponding fragmentation efficiencies using CID and HCD. Shown are the lipid species with their detected precursor and product ions ([M+H]+ and [M+NH4]+ in positive ion or [M-H]- in negative ion ESI modes).

Species	[M+H]+ Precursor>Product	[M+NH4]+ Precursor>Product	CID NCE (%)	Efficiency (%)	HCD NCE (%)	Efficiency (%)	[M-H]- Precursor>Product	CID NCE (%)	Efficiency (%)	HCD NCE (%)	Efficiency (%)
14:0 LPC	468.31>184.07		30	4.9	35	44.9					
18:1 LPC	522.36>184.07		35	7.1	35	64.8					
26:0 LPC	636.5>184.07		35	5.1	35	33.9					
14:0/14:0 PC	678.51>184.07		25	18.2	30	79.7					
18:1/18:1 PC	786.6>184.07		30	10.7	30	77.8					
14:0 LPE	426.26>285.24		35	12.9	20	16.8	424.25>196.04	30	0.86	70	11.2
18:1 LPE	480.31>339.29		25	18.5	25	35.1	478.29>196.04	35	0.65	70	10.7
18:0 LPE	482.32>341.30		35	3.3	30	9.5	480.31>196.04	35	0.81	70	10.1
12:0/12:0 PE	580.40>439.38		25	33.1	30	15.9	578.38>196.04	NA	NA	80	2.4
18:0/18:1 PE	746.57>605.55		20	34.3	20	23.6	744.55>196.04	30	0.02	70	1.9
18:0/18:0 PE	748.59>607.57		30	42.2	15	67.8	746.57>196.04	40	0.03	70	2.4
16:0 LPS	498.28>313.27		40	25.2	25	18.4	496.27>409.24	30	91.7	50	74.6
18:1 LPS	524.3>339.29		35	15.1	25	21.6	522.28>435.25	40	95.8	50	70.1
18:0 LPS	526.31>341.29		40	16.6	25	19.9	524.30>437.27	40	89.9	50	64.9
12:0/12:0 PS	624.39>439.38		35	85.8	20	39.9	622.37>535.34	40	84.0	50	78.5
18:0/18:1 PS	790.56>605.55		30	89.3	20	25.6	788.54>701.51	30	92.2	50	58.0
18:0/18:0 PS	792.58>607.56		20	80.9	20	13.9	790.56>703.52	30	90.8	50	44.3
14:0 LPG							455.24>153	30	3.7	90	28.5
16:0 LPG							483.27>153	30	2.4	100	13.9
18:0 LPG							511.30>153	30	2.4	100	14.5
14:0/14:0 PG							665.44>153	30	0.88	100	7.8
22:6/22:6 PG							865.50>153	NA	NA	80	5.1
17:0/17:0 PG							749.53>153	30	0.22	70	6.1
12:0/12:0 DG		474.43>257.21	30	11.3	30	0.60					
24:1/24:1 DG		806.76>423.38	20	4.9	25	0.29					
11:1/11:1/11:1 TG		608.49>407.32	40	96.4	20	69.6					
24:1/24:1/24:1 TG		1155.1>771.72	40	38.2	20	18.4					
19:0 CE		684.67>369.35	15	2.4	NA	NA					
24:1 CE		752.73>369.35	20	23.2	NA	NA					
d18:1/17:0 SM	717.59>184.07		30	20.4	25	78.9					
d18:1/18:1 SM	729.59>184.07		25	22.0	30	66.3					
d18:1/12:0 SM	647.51>184.07		25	45.7	30	80.1					
d18:1/17:0 Cer	552.54>264.27		45	0.18	35	1.0					
d18:1/22:0 Cer	622.61>264.27		45	0.14	30	0.94					
d18:1/24:0 Cer	650.65>264.27		50	0.09	40	0.19					
d18:1/12:0 Hex Cer	644.51>264.27		45	0.08	40	3.8					
d18:1/16:0 Hex Cer	700.57>264.27		45	0.15	40	5.0					
d18:1/24:1 Hex Cer	810.68>264.27		60	0.09	35	1.8					
d18:1/12:0 Dihex Cer	806.56>264.27		35	0.67	40	4.4					
d18:1/16:0 Dihex Cer	862.63>264.27		35	0.70	35	1.3					
d18:1/24:0 Dihex Cer	974.75>264.27		40	0.33	30	0.85					
13:0 LPA							367.19>153	50	1.1	60	1.8
14:0 LPA							381.21>153	30	61.8	50	77.8
18:1 LPA							435.26>153	30	47.1	50	83.6
14:0/14:0 PA							591.41>153	NA	NA	80	14.3
16:0/16:0 PA							647.47>153	40	1.5	60	37.3
								40	1.1	60	44.5
17:0/17:0 PA							675.50>153				

Utility of CID and HCD for identifying different lipids classes



Conclusions

- Normalized collision energies of 30-40% in CID produced MS/MS spectra useful for identifying all lipid species evaluated in this study in both positive and negative ion modes. For HCD, relatively low normalized collision energies of 15-35% produced the best data for lipid identification in the positive ion mode, and a high (≥40%) normalized collision energy gave better results in the negative ion mode.
- In positive ion mode, fragmentation efficiencies are higher with CID than with HCD for diradylglycerols, triradylglycerols, and cholesterol esters, while the efficiencies are higher with HCD for glycerophos-phocholines, sphingomyelins, and ceramides.
- Overall, different lipid species are preferentially identified using either CID or HCD. Therefore, a combination of the two dissociation methods is recommended to provide comprehensive lipid identifications in complex samples.

Acknowledgements

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Reference

1. Gao X, Zhang Q, Meng D, Isaac G, Zhao R, Fillmore TL, Chu RK, Zhou J, Tang K, Hu Z, Moore RJ, Smith RD, Katze MG, Metz TO. *Anal Bioanal Chem* 402:2923-2933 (2012).

CONTACT: Thomas O. Metz, Ph.D.
Biological Sciences Division, K8-98
Pacific Northwest National Laboratory
E-mail: thomas.metz@pnnl.gov

Xiaoli Gao, Ph.D.
E-mail: gaox@uthscsa.edu