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Lipidomics by HILIC-Ion Mobility-Mass Spectrometry

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Abstract

Lipidomics is a rapidly growing field that enables the characterization of the entire lipidome either in cells, tissues, or an organism. Changes in lipid metabolism and homeostasis caused by different disease states or drug treatments can be probed by lipidomics experiments, which can aid our understanding of normal physiology and disease pathology at the molecular level. While current technologies using liquid chromatography coupled with high-resolution mass spectrometry has greatly increased the coverage of lipidome, there is still limitation in resolving the large number of lipid species with similar masses in a narrow mass window. We recently reported that two orthogonal separation techniques, hydrophilic interaction liquid chromatography (HILIC) and ion mobility (IM), enhance the resolution of lipid species based on headgroup polarity and gas-phase size and shape, respectively, for various classes of glycerolipids, glycolipids, phospholipids, and sphingolipids. Here we describe the application of our HILIC-IM-MS lipidomics protocol to the analysis of lipid extracts derived from either tissues or cells, to identify significant changes in the lipidome in response to an internal or external stimulus, such as exposure to environmental chemicals.

Keywords

hydrophilic interaction liquid chromatography; ion mobility; mass spectrometry; lipidomics; collision cross section

1. Introduction

In recent years, the advancement of singular and multi-disciplinary 'omics technologies have quickly taken storm as a systems biology approach is applied to biomedical research. They are highly informative experiments that can reveal molecular changes within some altered state of a system, such as a disease or a treatment condition [1]. The central biological processes of DNA transcription to mRNA, subsequent translation to protein, and eventual production of metabolites correspond to the studies of genomics, transcriptomics, proteomics, and metabolomics, respectively. Lipidomics falls under the category of metabolomics as the lipidome represents a major fraction of the human metabolome [2]. Specifically, lipidomics aims to systematically identify and quantify individual lipid species within cells, tissues, or an organism, as well as map out the complex interactions between lipids and other macromolecules. There is growing evidence that lipids are closely related to

the onset and progression of several diseases, including neurodegenerative, neurodevelopmental, cardiovascular, and metabolic disorders. Thus, lipidomics represents a unique niche to address specific questions about the role of altered lipid metabolism within disease etiologies, as well as advance biomarker identifications to aid diagnosis [3].

Lipids cover a diverse range of functions within the cell as structural components of membranes, energy reservoirs, and signaling molecules, which reflects their large chemical diversity. There are eight main lipid classes (FA, fatty acyls; GL, glycerolipids; GP, glycerophospholipids; SP, sphingolipids; ST, sterol lipids; PR, prenol lipids; SL, saccharolipids; PK, polyketides) and multiple subclasses [4, 5]. Within each subclass, further variations are possible from multiple types of head groups, backbones, fatty acyl chains, linkages (ester, ether, or vinyl ether), and other accessory modifications, like sugar molecules [4, 5]. This vast combinatorial space is estimated (by the LIPID MAPS database) to contain over 40,000 distinct lipids, which presents a major analytical challenge for lipidomics because most lipid species occupy a narrow mass range of 600 – 900 *m/z*. Modern mass spectrometry has greatly advanced the field of lipidomics and increased coverage of the lipidome over the past two decades, but some limitations remain.

Mass spectrometry-based lipidomics can be divided into two categories: shotgun [6, 7] and liquid chromatography (LC)-based experiments [8–10]. In shotgun lipidomics, samples are directly infused into the mass spectrometer without any prior chromatographic separation, aiming to maximize the throughput of analysis. However, this approach is unable to resolve isomeric and isobaric lipids and limits the detection of lower abundance lipids due to ion suppression. LC-based lipidomics involves LC separation, such as normal phase (NP), reverse phase (RP), or hydrophilic interaction liquid chromatography (HILIC), prior to ionization. RP separation utilizes nonpolar C18 columns with polar solvents, where lipids are separated based on hydrophobicity, resulting in separation by chain length and degree of unsaturation of fatty acyl chains. HILIC separation utilizes columns with polar stationary phase in combination with typical reverse phase solvents, such as acetonitrile and water, where lipids partition at the water/silica interface. HILIC separation is mostly dependent on the polarity of lipid head groups [11, 12]. Further separation, although to a lesser extent, occurs within each subclass by chain length and degree of unsaturation of fatty acyl chains.

Ion mobility (IM) is another technology that has gained popularity and shown advantages in lipidomics studies as it contributes to both separation and structural elucidation of analytes and can be coupled to LC separation without affecting throughput [13–16]. Ions are separated based on their size, shape, and charge as they are pushed through a drift tube by an electric field through an opposing flow of inert gas [16–18]. Drift times are related to a physicochemical property, called collisional cross section (CCS), which can be measured either directly on a drift tube-IM (DTIM) instrument using the Mason-Schamp equation [19, 20] or on a traveling wave-IM (TWIM) instrument through calibration [21–23]. Previous papers from our group have shown that in TWIM experiments, matched lipid calibrants produce more accurate and precise CCS values of lipid standards compared to calibration by calibrants from other classes of molecules, such as poly-DL-alanine, tetraalkylammonium compounds, and hexakis(fluoroalkoxy)phosphazines [21]. IM is a gas-phase technique that is orthogonal to LC and enables separation of lipid ions from other classes of

macromolecules due to their distinct IM profiles [24, 25]. Even within total lipids, individual lipid classes can be separated from each other, although complete resolution of certain species is still limited by instrumentation [15, 26–28]. Coupled with retention times (RT) and mass-to-charge (m/z) ratios, CCS values greatly increase the confidence in lipid identifications. Recently, the Zhu Lab reported the first platform for lipid identification using multidimensional RT- m/z -CCS data, LipidIMMS, which can utilize both RP-IM-MS and HILIC-IM-MS data [29].

Herein, we discuss the use of our HILIC-IM-MS protocol [14, 15] for the analysis of lipid species in the lipid extracts of biological samples using brain tissues and cells as examples. The workflow involves (1) sample preparation including tissue homogenization, lysis, and liquid phase extraction, (2) data acquisition on a Waters SYNAPT HDMS G2-Si Q-TOF ion mobility-mass spectrometer and (3) data analysis using Progenesis QI and EZ Info software. The data included in Figure 4 comes from a paper by members of our research group, investigating the lipidomic changes in a neuroblastoma cell line exposed to an environmental chemical, benzylalkonium chlorides (BACs), and related compounds known to disrupt cholesterol and lipid homeostasis [14]. Readers are encouraged to consult the original paper for the biological context.

2. Materials

General

Ammonium hydroxide (certified ACS reagent), ammonium acetate (Optima LC/MS grade), and sodium chloride (certified ACS crystalline) were purchased from Fisher Scientific. All mobile phase solvents (acetonitrile and water) and extraction reagents (chloroform, methanol, and methylene chloride) were Fisher Optima LC/MS grade. Lipid standards were purchased from Avanti Polar Lipids (Alabaster, AL) and Nu-Chek Prep (Elysian, MN). Phosphate buffer saline (PBS) was purchased from Gibco.

2.1 Sample Preparation

1. 1x phosphate buffer saline.
2. *DC*TM Protein Assay Kit II.

2.2 Folch Lipid Extraction

1. Folch solution: 2:1 (v/v) chloroform/methanol.
2. Sodium chloride solution: 0.9% (w/v) NaCl aqueous solution.

2.3 HILIC-MS

1. HILIC A mobile phase: 95% ACN, 5% H₂O, 5 mM ammonium acetate.
2. HILIC B mobile phase: 50% ACN, 50% H₂O, 5 mM ammonium acetate.

2.4 Lipid Standards

1. Lipid internal standard solution: prepare 1 mM stock solutions of lipid standards in chloroform. Prepare a 5 μ M mixture in HILIC A (see Table 1 for the list of lipid standards).
2. Lipid mixture solution: extracts purchased from Avanti Polar Lipids were prepared at 1 mM concentration in chloroform. Prepare a 10 μ M mixture in chloroform. For analysis, make a 1:2 dilution of the mixture in HILIC A (see Table 2 for the list of lipid extracts).
3. CCS calibrants: phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipid standards (Avanti Polar Lipids). Prepare 1 mM stock solutions of lipid standards. Prepare a mixture of PC 6:0–24:0 in methanol with 0.1% formic acid for positive mode analysis and PE 6:0–24:0 at 5–10 μ M in methanol with 50 μ M ammonium hydroxide for negative mode analysis. Prior to calibration, make a 1:4 dilution of the PC mixture and 1:2 dilution of the PE mixture in their respective solvents (see Table 3).

2.5 Supplies and Equipment

1. Analytical Column: HILIC Phenomenex Kinetex[®], 2.1 $\times$ 100 mm, 1.7 μ m.
2. Guard Cartridge: Phenomenex SecurityGuard[®] ULTRA HILIC Cartridge for 2.1mm ID UHPLC columns.
3. Guard Cartridge Holder: Phenomenex SecurityGuard[®] ULTRA Holder for 2.1–4.6 mm ID UHPLC columns.
4. Instrument: Waters Synapt[®] G2-Si HDMS equipped with an ESI source (Waters Corporation, Milford, MA, USA).
5. Inlet System: Waters[®] Acquity I-Class FTN UPLC with Autosampler (Waters Corporation, Milford, MA, USA).

3. Methods

Carry out procedures at room temperature.

3.1 Sample Preparation – Cultured Cells

1. Pellet a minimum of 10⁶ cells in Pyrex glass centrifuge tube at 104 x g (750 rpm) for 5 minutes at 4°C (Sorvall[®] Legend[®] X1R Centrifuge) and keep samples on ice (see Note 1).
2. Lyse in 300 μ L chilled 1x PBS using cold sonication for 30 minutes.
3. Conduct protein quantitation assay in a 96-well microplate (BioTek[®] Synergy[®] HTX Microplate Reader) (see Note 2).

¹Glass tubes and pipettes are recommended when working with chloroform to prevent leaching of plasticizers, typically eluting between 2–3 minutes, which will interfere with data analysis.

²Data should be normalized to protein weight or tissue weight, and internal standard (if used) post-data acquisition.

4. Store at -80°C or perform lipid extraction immediately.

3.1 Sample Preparation - Tissues

5. Measure tissue weight (*see* Note 2).
6. Add 4 mL Folch solution.
7. Disrupt and homogenize tissue for 30 seconds using a tissue homogenizer (Polytron[®] PT 1200 Kinematica).
8. Add 1 mL NaCl solution.
9. Continue to step 2 of “Folch Lipid Extraction”.

3.2 Folch Lipid Extraction

1. Add 4 mL Folch solution and 1 mL NaCl solution to cell lysates.
2. If including lipid internal standard, add to sample (*see* Note 3).
3. Vortex for 30 seconds.
4. Centrifuge at $1660 \times g$ (3000 rpm) for 5 minutes at 4°C .
5. Using a 9” glass Pasteur pipet with a mechanical pipet pump, pipette through the upper, aqueous phase and protein layer and transfer approximately 2 mL of the lower organic layer to a 10 mL glass tube.
6. Dry lipid extracts with a speed vacuum concentrator (Thermo Fisher Savant SpeedVac[®] SC210A).
7. Reconstitute in 300 μL CH_2Cl_2 for cells and tissue samples with weights below 100 mg. Reconstitute heavier tissue samples in 1 mL CH_2Cl_2 and transfer to HPLC vials with screw caps. Store at -80°C until HILIC-MS analysis.

3.3 Data Acquisition

1. To prepare samples for analysis, make a 1:4 dilution of lipid extracts in an LC glass autosampler vial. Adjust dilutions according to instrument signal intensity (*see* Note 4).
2. Prepare a pooled QC sample, combining an equal volume from each final sample into a separate LC autosampler vial.
3. Equilibrate LC column and instrument (*see* Table 4–6). The pressure should be around 4000 psi when the 0.5 mL/min flow rate is established.
4. Perform detector setup through Intellistart interface (*see* Note 5).

³. An example internal standard is d17:1/12:0 sphingo PE, N-lauroyl-D-erythro-sphingosyl phosphoethanolamine, catalog no. 860529. Please refer to the original paper (*see* ref. [12]) for more information on quantitative analysis using a single internal lipid standard.

⁴. Signal intensity of TIC should be on the order of 10^7 to ensure detection of lower concentration lipids. Preparing samples at two concentrations may be necessary to get the best results for both high and low abundance lipids.

⁵. LockSpray should be utilized (leucine-enkephalin; positive mode: m/z 566.2771, CCS 226.0; negative mode: m/z 564.2615, CCS 224.0) to correct shifts in masses and drift times throughout the run.

5. Mass calibration. Infuse sodium formate at 20 $\mu\text{L}/\text{min}$ and perform mass calibration through Intellistart interface for both positive and negative modes.
6. Switch instrument to Mobility TOF mode and allow the IM cell to equilibrate for an hour.
7. CCS calibration. Directly infuse PC and PE calibrant mixtures at 15 $\mu\text{L}/\text{min}$ and calibrate through Intellistart interface (see Figure 3).
8. LockSpray Source Setup to check calibration curve quality (checks that it gives the right CCS for LeuEnk based on the observed DT).
9. Inject the lipid extract mix (see Figures 1 and 2 for example chromatograms), standard mix and a blank sample.
10. Randomize the sample list and increment a QC sample every 10 injections.

3.4 Data Processing and Analysis (see Figure 4)

1. Import .raw files into Progenesis[®] QI (Nonlinear Dynamics) and process data in the chromatographic region between 0.4 and 8.5 minutes: data alignment to a reference QC sample, automatic peak detection of profile (continuum) data, and normalization to the external standard (such as tissue weight or protein weight).
2. Perform multivariate statistical analysis of the data (EZInfo).
3. If an internal standard was used, normalize the signals to the signal intensity of the appropriate internal standard in addition to external normalization.
4. Determine features that are significantly different between groups.
5. Identify features by matching retention time to the lipid classes, m/z to the METLIN, LIPID MAPS, and LipidBlast databases, and CCS values to LipidCCS/LipidIMMS [28, 29] and our own lipid CCS database [14, 15].

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References

1. Jurowski K, Kochan K, Walczak J, Baranska M, Piekoszewski W, and Buszewski B (2017) Analytical Techniques in Lipidomics: State of the Art, Crit Rev Anal Chem 47, 418–437. [PubMed: 28340309]
2. Wishart DS, Knox C, Guo AC, Eisner R, Young N, Gautam B, Hau DD, Psychogios N, Dong E, Bouatra S, Mandal R, Sinelnikov I, Xia J, Jia L, Cruz JA, Lim E, Sobsey CA, Shrivastava S, Huang P, Liu P, Fang L, Peng J, Fradette R, Cheng D, Tzur D, Clements M, Lewis A, De Souza A, Zuniga A, Dawe M, Xiong Y, Clive D, Greiner R, Nazzyrova A, Shaykhutdinov R, Li L, Vogel HJ, and Forsythe I (2009) HMDB: a knowledgebase for the human metabolome, Nucleic Acids Res 37, D603–610. [PubMed: 18953024]
3. Rustam YH, and Reid GE (2018) Analytical Challenges and Recent Advances in Mass Spectrometry Based Lipidomics, Anal. Chem 90, 374–397. [PubMed: 29166560]

4. Fahy E, Subramaniam S, Brown HA, Glass CK, Merrill AH Jr., Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL, and Dennis EA (2005) A comprehensive classification system for lipids, *J. Lipid Res* 46, 839–861. [PubMed: 15722563]
5. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, and Dennis EA (2009) Update of the LIPID MAPS comprehensive classification system for lipids, *J. Lipid Res* 50 Suppl, S9–14. [PubMed: 19098281]
6. Han X, and Gross RW (2005) Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples, *Mass Spectrom. Rev* 24, 367–412. [PubMed: 15389848]
7. Schwudke D, Liebisch G, Herzog R, Schmitz G, and Shevchenko A (2007) Shotgun lipidomics by tandem mass spectrometry under data-dependent acquisition control, *Methods Enzymol.* 433, 175–191. [PubMed: 17954235]
8. Quehenberger O, et al. (2010) Lipidomics reveals a remarkable diversity of lipids in human plasma, *J. Lipid Res* 51, 3299–3305. [PubMed: 20671299]
9. Merrill AH Jr., Sullards MC, Allegood JC, Kelly S, and Wang E (2005) Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry, *Methods* 36, 207–224. [PubMed: 15894491]
10. Ivanova PT, Milne SB, Byrne MO, Xiang Y, and Brown HA (2007) Glycerophospholipid identification and quantitation by electrospray ionization mass spectrometry, *Methods Enzymol.* 432, 21–57. [PubMed: 17954212]
11. Baker PR, Armando AM, Campbell JL, Quehenberger O, and Dennis EA (2014) Three-dimensional enhanced lipidomics analysis combining UPLC, differential ion mobility spectrometry, and mass spectrometric separation strategies, *J. Lipid Res*, Epub ahead of print. doi: 10.1194/jlr.D051581.
12. Cifkova E, Holcapek M, Lisa M, Ovcacikova M, Lycka A, Lynen F, and Sandra P (2012) Nontargeted quantitation of lipid classes using hydrophilic interaction liquid chromatography-electrospray ionization mass spectrometry with single internal standard and response factor approach, *Anal. Chem* 84, 10064–10070. [PubMed: 23072569]
13. Hinz C, Liggi S, and Griffin JL (2018) The potential of Ion Mobility Mass Spectrometry for high-throughput and high-resolution lipidomics, *Curr Opin Chem Biol* 42, 42–50. [PubMed: 29145156]
14. Hines K, Herron J, and Xu L (2017) Assessment of Altered Lipid Homeostasis by HILIC-Ion Mobility-Mass Spectrometry-Based Lipidomics, *J. Lipid Res* 58, 809–819. [PubMed: 28167702]
15. Hines KM, Waalkes A, Penewit K, Holmes EA, Salipante SJ, Werth BJ, and Xu L (2017) Characterization of the Mechanisms of Daptomycin Resistance among Gram-Positive Bacterial Pathogens by Multidimensional Lipidomics, *mSphere* 2, e00492–00417. [PubMed: 29242835]
16. Kliman M, May JC, and McLean JA (2011) Lipid analysis and lipidomics by structurally selective ion mobility-mass spectrometry, *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* 1811, 935–945.
17. Fenn LS, and McLean JA (2008) Biomolecular structural separations by ion mobility-mass spectrometry, *Anal. Bioanal. Chem.* 391, 905–909. [PubMed: 18320175]
18. McLean JA, Ruotolo BT, Gillig KJ, and Russell DH (2005) Ion mobility-mass spectrometry: a new paradigm for proteomics, *Int. J. Mass Spectrom* 240, 301–315.
19. Mason EA, and Schamp HW Jr (1958) Mobility of gaseous ions in weak electric fields, *Ann. Phys* 4, 233–270.
20. Mason EA, and McDaniel EW (1988) *Transport properties of ions in gases*, Wiley, New York.
21. Hines KM, May JC, McLean JA, and Xu L (2016) Evaluation of Collision Cross Section Calibrants for Structural Analysis of Lipids by Traveling Wave Ion Mobility-Mass Spectrometry, *Anal. Chem* 88, 7329–7336. [PubMed: 27321977]
22. Ruotolo BT, Benesch JL, Sandercock AM, Hyung SJ, and Robinson CV (2008) Ion mobility-mass spectrometry analysis of large protein complexes, *Nat. Protoc* 3, 1139–1152. [PubMed: 18600219]
23. Forsythe JG, Petrov AS, Walker CA, Allen SJ, Pellissier JS, Bush MF, Hud NV, and Fernandez FM (2015) Collision cross section calibrants for negative ion mode traveling wave ion mobility-mass spectrometry, *Analyst* 140, 6853–6861. [PubMed: 26148962]

24. Fenn LS, Kliman M, Mahsut A, Zhao SR, and McLean JA (2009) Characterizing ion mobility-mass spectrometry conformation space for the analysis of complex biological samples, *Anal. Bioanal. Chem* 394, 235–244. [PubMed: 19247641]
25. May JC, Goodwin CR, Lareau NM, Leaptrot KL, Morris CB, Kurulugama RT, Mordehai A, Klein C, Barry W, Darland E, Overney G, Imatani K, Stafford GC, Fjeldsted JC, and McLean JA (2014) Conformational ordering of biomolecules in the gas phase: nitrogen collision cross sections measured on a prototype high resolution drift tube ion mobility-mass spectrometer, *Anal. Chem* 86, 2107–2116. [PubMed: 24446877]
26. Paglia G, Angel P, Williams JP, Richardson K, Olivos HJ, Thompson JW, Menikarachchi L, Lai S, Walsh C, Moseley A, Plumb RS, Grant DF, Palsson BO, Langridge J, Geromanos S, and Astarita G (2015) Ion mobility-derived collision cross section as an additional measure for lipid fingerprinting and identification, *Anal. Chem* 87, 1137–1144. [PubMed: 25495617]
27. Kyle JE, Zhang X, Weitz KK, Monroe ME, Ibrahim YM, Moore RJ, Cha J, Sun X, Lovelace ES, Wagoner J, Polyak SJ, Metz TO, Dey SK, Smith RD, Burnum-Johnson KE, and Baker ES (2016) Uncovering biologically significant lipid isomers with liquid chromatography, ion mobility spectrometry and mass spectrometry, *Analyst* 141, 1649–1659. [PubMed: 26734689]
28. Zhou Z, Tu J, Xiong X, Shen X, and Zhu ZJ (2017) LipidCCS: Prediction of Collision Cross-Section Values for Lipids with High Precision To Support Ion Mobility-Mass Spectrometry-Based Lipidomics, *Anal. Chem* 89, 9559–9566. [PubMed: 28764323]
29. Zhou Z, Shen X, Chen X, Tu J, Xiong X, and Zhu ZJ (2018) LipidIMMS Analyzer: Integrating multi-dimensional information to support lipid identification in ion mobility - mass spectrometry based lipidomics, *Bioinformatics*, In Press. doi: 10.1093/bioinformatics/bty1661.

Figure 1 –.

(A) Drift time versus m/z spectrum showing IM profile of lipids. (B) Chromatogram of the most intense lipids from individual lipid subclasses that are present in the mammalian lipid extract mixture. Ceramide (Cer) d36:1; Diacylglycerol (DG) 36:4; Hexosylceramide (HexCer) d42:2; Phosphatidylglycerol (PG) 34:1; Phosphatidylinositol (PI) 38:4; Phosphatidylethanolamine (PE) 36:1; LysoPE 18:0; Phosphatidylcholine (PC) 34:1; Sphingomyelin (SM) d36:1; LysoPC 16:0.

Figure 2 –.

(A) Chromatogram showing separation of phosphatidyl and plasmalogen PEs. M+H adducts shown. (B) Chromatogram showing separation of PGs by fatty acyl chain composition. From left to right: PG 38:5; PG 38:4; PG 36:3; PG 36:2; PG 34:3; PG 34:1; PG 32:1. M+Na adducts shown.

Figure 3 –.
 (A) Example calibration plots and (B) corresponding residual plots for CCS calibration in both positive and negative ionization modes using PC and PE calibrant mixtures, respectively.

Figure 4 –.
Schematic of HILIC-IM-MS data analysis workflow from MS data processing to lipid identification.

Table 1.

Lipid standards included in discrete lipid internal standards mixture.

Lipid common name	Systematic name	Catalog no.
DG 13:0/13:0	Tridecanoin glyceride	D-136 (Nu-Chek)
TG 15:0/15:0/15:0	Tripentadecanoin glyceride	T-145 (Nu-Chek)
Cer d18:1/17:0	N-heptadecanoyl-D-erythro-sphingosine	860517
PG 15:0/15:0	1,2-dipentadecanoyl-sn-glycero-3-phospho-(1'-rac-glycerol)	840446
PE 15:0/15:0	1,2-dipentadecanoyl-sn-glycero-3-phosphoethanolamine	850704
PC 15:0/15:0	1,2-dipentadecanoyl-sn-glycero-3-phosphocholine	850350
PA 12:0/12:0	1,2-dilauroyl-sn-glycero-3-phosphate	840635
PS 12:0/12:0	1,2-dilauroyl-sn-glycero-3-phospho-L-serine	840038
SM d18:1/17:0	N-heptadecanoyl-D-erythro-sphingosylphosphorylcholine	860585
C12 Sphingosyl PE	N-lauroyl-D-erythro-sphingosyl phosphoethanolamine	860529
Lyso PC 15:0/0:0	1-pentadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine	855576
Lyso PE 13:0/0:0	1-tridecanoyl-sn-glycero-3-phosphoethanolamine	856706

Table 2.

Lipid extracts included in lipid mixture.

Lipid class	Catalog no.
Ceramide	860052P
Cerebroside	131303P
Sphingomyelin	860062C
L- α -phosphatidic acid	840101C
L- α -phosphatidylcholine	840051C
L- α -lysophosphatidylcholine	830071P
L- α -phosphatidylethanolamine	840022C
L- α -phosphatidylglycerol	841138C
L- α -phosphatidylinositol	840042C
L- α -phosphatidylserine	840032C
Diacylglyceride	Nu-Chek Prep

Table 3.

PE and PC lipid species included in the lipid CCS calibration mixture.

Lipid common name	Systematic name	Catalog no.	Conc. (μM)
PE			
PE 6:0/6:0	1,2-dihexanoyl-sn-glycero-3-phosphoethanolamine	850697C	5
PE 8:0/8:0	1,2-dioctanoyl-sn-glycero-3-phosphoethanolamine	850699C	5
PE 10:0/10:0	1,2-didecanoyl-sn-glycero-3-phosphoethanolamine	850700C	5
PE 12:0/12:0	1,2-dilauroyl-sn-glycero-3-phosphoethanolamine	850702X	5
PE 14:0/14:0	1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine	850745X	5
PE 15:0/15:0	1,2-dipentadecanoyl-sn-glycero-3-phosphoethanolamine	850704X	10
PE 16:1/16:1	1,2-dipalmitoleoyl-sn-glycero-3-phosphoethanolamine	850706C	10
PE 16:0/16:0	1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine	850705X	10
PE 16:0/18:1	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine	850757C	10
PE 17:0/17:0	1,2-diheptadecanoyl-sn-glycero-3-phosphoethanolamine	830756X	10
PE 18:1/18:1	1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	850725C	10
PE 18:0/18:1	1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine	850758C	10
PE 18:0/18:0	1,2-distearoyl-sn-glycero-3-phosphoethanolamine	850715X	10
PE 20:4/20:4	1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine	850800C	10
PC			
PC 6:0/6:0	1,2-dihexanoyl-sn-glycero-3-phosphocholine	850305C	5
PC 8:0/8:0	1,2-dioctanoyl-sn-glycero-3-phosphocholine	850315C	5
PC 10:0/10:0	1,2-didecanoyl-sn-glycero-3-phosphocholine	850325C	5
PC 12:0/12:0	1,2-dilauroyl-sn-glycero-3-phosphocholine	850335C	5
PC 14:0/14:0	1,2-dimyristoyl-sn-glycero-3-phosphocholine	850345C	5
PC 16:0/16:0	1,2-dipalmitoyl-sn-glycero-3-phosphocholine	850355C	10
PC 18:0/18:0	1,2-distearoyl-sn-glycero-3-phosphocholine	850365C	10
PC 20:0/20:0	1,2-diarachidoyl-sn-glycero-3-phosphocholine	850368C	10
PC 22:0/22:0	1,2-dibehenoyl-sn-glycero-3-phosphocholine	850371C	10
PC 24:0/24:0	1,2-dilignoceroyl-sn-glycero-3-phosphocholine	850373C	10

Table 4.

HILIC-IM-MS parameters: LC conditions.

LC Method	
Injection volume	5 µL for positive mode, 10 µL for negative mode
Flow rate	0.5 mL/min
Column temperature	40°C
Sample temperature	6°C
Mobile phase gradient	0–1 min: Hold at 100% HILIC A 4 min: Hold at 90% HILIC A 7–8 min: Linear gradient to 70% HILIC A 9–12 min: Equilibrate at 100% HILIC A

Table 5.

MS source conditions.

Source Conditions	
Mode	Resolution
Mass range	50–1200 m/z
Capillary voltage	+2.5 kV for positive, –2.0 kV for negative
Sampling cone	40 V
Source offset	80 V
Source temperature	150°C
Desolvation temperature	500°C
Desolvation gas flow	1000 L/hr
Cone gas flow	100 L/hr
Nebulizer gas flow	5.0 bar

Table 6.

Ion mobility parameters.

IM/MS Conditions	
IMS wave velocity	500 m/s
IMS wave height	40.0 V
Trap wave velocity	311 m/z
Trap wave height	4.0 V
Trap collision Energy	4.0 eV
Transfer wave velocity	380 m/s
Transfer wave height	4.0 V
Transfer collision energy, function 1 (MS)	2.0 eV
Transfer collision energy, function 2 (MS ^E)	Low, 35 eV High, 45 eV
Trap Gas Flow	2 mL/min
Helium Cell Gas Flow	180 mL/min
IMS Gas Flow	90 mL/min