Published in final edited form as:

*Biochim Biophys Acta*. 2017 August ; 1862(8): 804–807. doi:10.1016/j.bbalip.2017.02.012.

# Lipidomics for Precision Medicine and Metabolism: A Personal View

**Xianlin Han, Ph.D.**

Center for Metabolic Origins of Disease, Sanford Burnham Prebys Medical Discovery Institute, 6400 Sanger Road, Orlando, Florida 32827, USA, Telephone: (407) 745-2139, Fax: (407) 745-2016

## Abstract

This essay is to provide my personal view of lipidomics for precision medicine and lipid metabolism on its experimental procedures, inherent limitations, assessment of cross-platform reproducibility, available resource, and future challenges.

## Introduction

Lipidomics, a subdiscipline of metabolomics, is referred as to the studies of cellular lipids in a large scale based on the principles and technologies of analytical chemistry. It emerged in early 2000 [1–3] after numerous previous studies showed the importance of individual lipid species in cellular functions (see review [4] for references), and the complication of lipid metabolism and the differences between lipids and water-soluble metabolites were recognized. Lipidomics has rapidly evolved since then, largely due to the advances in mass spectrometry (MS). Its great power for lipid metabolism and precision medicine has been well demonstrated [5–11].

As one of the pioneers in lipidomics [1], the author has broad expertise in elucidating the underlying mechanisms responsible for the altered lipid metabolism, trafficking and homeostasis manifest under a variety of patho(physio)logical conditions in mammalian systems. Investigation of these underlying molecular mechanisms greatly facilitates understanding the pathogeneses of the diseases. As one of the inventors of shotgun lipidomics (SL) [4, 12], the author has extensive experience in analysis of a broad range of lipid classes, subclasses and individual molecular species after direct infusion using nominal and high mass accuracy/resolution MS. The author also had nearly ten-year experience in separation and analysis of lipids using various chromatographic techniques (e.g., TLC, GC, and HPLC) [13] prior to the lipidomics era.



Conflict of Interest

The authors declare that there is no conflict of interest.

### Key References


**a.** X. Han, K. Yang, R.W. Gross, Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses, *Mass Spectrom. Rev.* 31 (2012) 134–178.

**b.** M. Wang, C. Wang, R.H. Han, X. Han, Novel advances in shotgun lipidomics for biology and medicine, *Prog. Lipid Res.* 61 (2016) 83–108.

**c.** X. Han, Lipidomics for studying metabolism, *Nat. Rev. Endocrinol.* 12 (2016) 668–679.

**d.** X. Han, Lipidomics: Comprehensive mass spectrometry of lipids, John Wiley & Sons, Inc., Hoboken, New Jersey, 2016.

**e.** M. Wang, C. Wang, X. Han, Selection of internal standards for accurate quantification of complex lipid species in biological extracts by electrospray ionization mass spectrometry-What, how and why? Mass Spectrom. Rev. (2016) DOI: 10.1002/mas.21492.


## Your experience/opinion on experimental procedures

Sample collection from biological sources is one of the important issues in lipidomics. Since only a very small amount of sample is needed for lipidomics analysis, it is a big waste if more than needed is taken. However, when only a small amount of sample is taken, representation of the sample to the entire source material should be addressed. For example, blood vessels/capillaries, epidermis, fat pads, and other non-homogeneously distributed tissues always exist in mammalian organs. A small piece of sample randomly taken from such an organ unlikely represents the entire organ. It is advised to make the organ into a fine powder at the temperature of liquid $N_2$ by a homogenizer prior to taking a small amount of sample. For a study focusing on a particular organ section, careful dissection is essential. For example, the brain is highly complicated and contains a variety of very different cell populations in different regions, thus leading to the presence of different lipid compositions [14]. Criteria for determining sample representation must be established prior to analysis [15, 16]

Lipid extraction is another key step in lipidomics and has been extensively discussed [6]. For any methods utilizing external standard(s), extraction recovery of lipid species should be determined during method validation. For any methods utilizing internal standards (IS), extraction recovery is less critical since the added IS can compensate the incomplete extraction to a great degree. SL can be affected by the presence of inorganic ions in lipid extracts (e.g., due to resultant ionization instability and ion suppression), whereas these effects are reduced by pre-separation (e.g., liquid/liquid partitioning, solid phase extraction) or chromatographic separation in LC-MS. A suitable type of salt should be used during lipid extraction to enhance the phase separation and extraction efficiency. It is better to add the salt that matches with the adducts preferred for analysis of lipids by MS. Detergents are problematic for lipid analysis and should be avoided in sample preparation when possible. Although acidic conditions are favorable in effective extraction of anionic lipids, they can lead to degradation of acid-labile plasmalogens. We found that a low-concentration of LiCl

solution is preferable for lipid extraction since its weakly acidic condition improves the extraction efficiency of anionic lipids without inducing plasmalogen degradation, and the lithium adduct of lipid species yield unique and informative fragmentation patterns after collision-induced dissociation [17], which are very useful for some SL platforms [6, 18, 19].

It is advised to introduce IS at the earliest step of lipid extraction for quantification in lipidomics [20]. The reasons include (1) minimizing the effects of incomplete extraction, (2) eliminating differential ionization efficiencies between lipid classes and subclasses, (3) normalizing MS data for comparison between sample groups, (4) reducing any fluctuation of MS analysis from unexpected variations of any experimental conditions, among others. The topics on what, how, and why to select IS have recently been extensively discussed [21].

Selection of an approach (shotgun *vs.* LC-based platform) for lipid analysis after sample preparation depends on personal preference, experience, the target of analysis, and throughput. A general consensus is that SL (particularly the platforms based on high accuracy/high resolution mass spectrometers) is more suitable for high throughput analysis of lipids and LC-based approaches are preferable for analyzing low abundance lipids. Of course, LC-based platforms for high throughput analysis also exist [22, 23] and SL has been applied for analysis of low abundance lipid classes [24].

In addition to adding IS in sample preparation, analyzing a lipid solution at a concentration circumventing lipid aggregation is also critical to accurate quantification [25]. Aggregated lipids cannot be ionized efficiently, and their ionization is molecular species dependent. Specifically, the species containing shorter acyl chains generally display higher ionization response factors than those containing longer ones [26]. Solvent can also affect lipid aggregation. For instance, the concentration (pmol/μL) where lipids start forming aggregates in the solvent system of $CHCl_3$/MeOH (v/v) is ~100 in 2:1, 50 in 1:1, and 10 in 1:2. Presence of non-polar lipids (e.g., triacylglycerol, cholesterol and its esters) in a lipid solution could substantially lower this concentration. The effects of lipid aggregation on quantification by LC-based lipidomics have not yet been broadly recognized. Due to column enrichment, a species eluted from a column is substantially concentrated where lipid aggregation could happen. Moreover, the mobile phase used in reversed-phase LC is typically more polar than in normal-phase LC. The lipid aggregation formed in LC-based lipidomics potentially affects their response factors and consequently their quantification. Thus, stable isotope-labeled IS are always ideal for accurate quantification in LC-based lipidomics.

## What are the inherent limitations of MS-based lipidomics?

First, unlike the analysis with optical devices, which follows the Lambert-Beer law, a relationship between the counts of a molecular ion as determined by MS and its concentration in a solution is more complicated. The ion intensity of an analyte determined by ESI-MS could be affected by many factors [21]. They range from sample preparation (e.g., the existence of inorganic contaminants), ionization conditions (e.g., flow rate, ion source temperature, and ionization voltage), tuning conditions (e.g., different solvent(s) used in experiments from tuning solution), instrumentation (e.g., analyzer and detector), solvent

gradient shift, etc. Minor changes of these factors could lead to significant changes in ion peak intensities. This inherent limitation of MS makes it difficult for scientists to virtually repeat a measurement of absolute ion counts for an analyte in a biological sample from time to time, from one instrument to another, or from one laboratory to the others. Therefore, addition of IS for relative comparison and normalization becomes essential for accurate quantification of lipid species in MS-based lipidomics. It should be recognized that as advances in MS instruments to higher sensitivity, the influences of these factors on determining ion intensities by MS become more and more evident.

Another limitation of MS-based lipidomics is that MS is unable to accurately analyze aggregated lipids. In addition to the non-efficient and species-dependent ionization as discussed above, aggregated lipids can possess very different polarity, geometry, size, etc. from lipid monomers, and thus lead to very different ionization process. Also, the aggregates can contain a different number of lipid species and form adducts with a variety of small ions. Thus, these aggregates can possess a different distribution of *m/z*, some of which may be out of the mass-detection range and not be realized. This limitation has to be resolved by adapting low concentrations of lipid solutions circumventing lipid aggregation.

Formation of multiple adducts of an individual lipid species is another possible limitation. The formed adducts depend on the availability of small ions as well as the concentrations and affinities of these ions. This limitation makes the lipid analysis extra complicated. The researchers should make efforts to minimize this limitation through introduction of an appropriate modifier. If no modifier is introduced, adducts associated with NaCl are often predominantly present because NaCl is ubiquitously present.

Although ESI and MALDI belong to soft ionization techniques, they generate fragment ions to a certain degree during ionization. These ion source-generated ions may be “identical” to the ions of endogenous species. This limitation complicates the lipid analysis, often leading to inaccurate results and incorrect conclusions. Some examples are that phosphatidylserines fragment to phosphatidic acids, triacylglycerols result in diacylglycerols, and small anionic adducts of phosphatidylcholines yield dimethyl phosphatidylethanolamines. This limitation should be eliminated through optimizing ionization conditions or closely monitored through addition of endogenously absent standards.

## Are there any other limitations?

The advantages of SL include the analysis conducted under constant lipid concentration and its high throughput compared to LC-MS approaches. The constant concentration guarantees that all individual lipid species of a polar class are ionized under an identical condition, leading to virtually identical ionization response factors after correction for differential $^{13}C$ isotope distributions [4]. The major limitations of SL include (1) the difficulties to analyze the lipid classes present in low abundance (e.g., (oxy)sterols, steroid hormones, and bile acids) due to ion suppression of abundant or readily ionizable lipid classes, despite some efforts have been made [27, 28]; and (2) being unable to determine the isomers possessing identical fragmentation patterns. However, the former could be solved through appropriate derivatization and the latter could be tackled through application of ion mobility technology.

The advantage of LC-based approaches is the power of separation science. The major limitations include its relative slow throughput and dynamic ion suppression. The dynamic ion suppression results from the constantly changing lipid concentrations. Using isotope labeled IS or adding extra numbers of IS representing differential lipid species may resolve, at least partially, this limitation [21].

## What is your assessment of cross-platform reproducibility?

In current lipidomics practice, there is a lack of comparison of results between platforms. In fact, it is hard to assess the cross-platform reproducibility. This difficulty is largely due to the lack of standardization in lipidomics. Such standardization includes the measurement of lipid amounts vs. composition, the normalizers (e.g., protein content, RNA level, wet weight, phosphorus content, etc.), and the IS including their numbers, levels, and structures.

In my opinion, the researchers should measure the amounts of individual lipid species as possible since their composition can be easily derived from the measured amounts and their mass levels can provide additional information for studying lipid metabolism and precision medicine. Protein content might be the best normalizer for analyzing lipidomes of tissue samples as previously described [6]. The topic on how to accurately quantify lipid species using IS has been extensively discussed [21].

Prior to making standardization of lipidomics for cross-platform comparison, it is important to validate a method in its development stage, particularly, by employing alternative approaches. For example, the data obtained by SL could be validated by LC-MS analysis and *vice versa*. Other alternative approaches, including NMR, GC-MS, or other chromatographic analysis, could also be employed for this purpose under certain situations. The bottom line is that the obtained data should make sense at the biological levels and agree with each other as a ballpark estimate.

## Databases and data analysis tools

This topic has been extensively described in Chapter 5 (Bioinformatics in Lipidomics) of my monograph [6].

## Current limitations and future challenges

Despite tremendous advances made in recent years, a few areas of progress on the technology are still desirable. Accurate quantification of lipid species is still under debate [21]. Quantitative analysis of signaling lipids to include chiral isomers for eicosanoids, phospholipids carrying modified fatty acids, a variety of sphingoids, and numerous intermediate metabolites, etc. are still not fully achievable. Coverage for the entire lipidome is still a dream. Definitive unraveling of the biochemical mechanisms responsible for a disease is still rare. Bioinformatics for lipidomics is largely limited to the levels of lipid classes currently.

Increase in coverage of lipid classes and molecular species is a key factor in lipidomics to better understand the inter-relationship between these classes and species within a metabolic

pathway or between the metabolic networks and to enhance the power for precision medicine. Derivatization appears very useful for development of targeted approaches to increase coverage for both SL and LC-based approaches [24]. Further increases in the coverage of lipid classes and individual species using an automated, high throughput manner in any platform remain demanding.

Bioinformatics for interpretation of lipidomics data based on mass spectral simulation or dynamic modeling is very useful and powerful [29, 30]. Similar modeling or novel approaches for analysis of a more comprehensive network or ideally for the entire lipidome are highly desired. As advances in lipidomics, interweaving of this discipline with other omics becomes demanding, which is critical for precision medicine. The reconstruction of lipid metabolic pathways require novel strategies for pathway mapping of lipid data at the molecular species levels instead of at lipid class/subclass levels [31].

Studies on assessing enzymatic activities, lipid turnover kinetics, and the effects of individual enzyme activation on lipid homeostasis in a metabolic pathway and/or network are missing. This type of work apparently requires the platforms possessing sensitive, high throughput, and broad coverage capabilities. More complex studies in the fluxomics scale to reveal the reaction rates in lipid metabolism are also needed.

Precision medicine, according to the NIH, is an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person. In practice, it depends on the availability of molecular profiling tests by omics including lipidomics. However, lipidomics for precision medicine is still at its infant stage. In addition to those outlined above, many aspects of lipidomics for precision medicine (e.g., standardized sample preparation, method validation, inter-laboratory comparison, and bioinformatic integration with other omics) have the limitations to serve the purpose and, therefore, also greatly challenge us.

Finally, as advances in instrumentation, detection sensitivity of lipids is greatly progressed, which is comparably for the analysis of single cells [32]. It can be foreseen that single cell lipidomics is achievable in the near future.

## Recommended lipidomics resources

The websites lipidlibrary.aocs.org/ and www.lipidhome.co.uk/constantly update the publications including review papers that utilize modern MS methods for lipidomics. The Lipidomics Gateway site (http://www.lipidmaps.org/data/databases.html) maintained by LIPID MAPS, which provides a wealth of information about lipid database and analysis protocols, could be consulted. Useful references could be found from a few recent special issues on lipidomics: Biochimica et Biophysica Acta, Vol. 1811(11), 2011; Analytical Chemistry, Virtual Issue: Lipidomics, pubs.acs.org/page/vi/2014/Lipidomics.html; Analytical and Bioanalytical Chemistry, Vol. 407 (17), 2015. Recently edited books and monographs on the areas of lipid analysis and lipidomics [6, 33, 34] should be the useful resources.

## Concluding remarks

Since the emerging of lipidomics discipline in 2003 [1, 2], the field has already demonstrated the power for “visualizing” and understanding the changes of hundreds to thousands of individual species [35–37], thereby potentially allowing us to map the entire metabolic pathways of lipid classes, subclasses, and individual species of the system of interest. The accomplishments in lipidomics are due to not only the technique development, but also the nature of lipidomics for lipid metabolism and precision medicine. It can be foreseen that the discipline will resolve the challenges outlined above and leads to a revolution of lipid metabolism and precision medicine in the future.

## Acknowledgments

This work was partially supported by National Institute of General Medical Sciences Grant R01 GM105724 and intramural institutional research funds.

## References

1. Han X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. J Lipid Res. 2003; 44:1071–1079. [PubMed: 12671038]
2. Lagarde M, Geloen A, Record M, Vance D, Spener F. Lipidomics is emerging, Biochim. Biophys Acta. 2003; 1634:61.
3. Lee SH, Williams MV, DuBois RN, Blair IA. Targeted lipidomics using electron capture atmospheric pressure chemical ionization mass spectrometry. Rapid Commun Mass Spectrom. 2003; 17:2168–2176. [PubMed: 14515314]
4. Han X, Gross RW. Shotgun lipidomics: Electrospray ionization mass spectrometric analysis and quantitation of the cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev. 2005; 24:367–412. [PubMed: 15389848]
5. Han X. Lipidomics for studying metabolism. Nat Rev Endocrinol. 2016; 12:668–679. [PubMed: 27469345]
6. Han, X. Lipidomics: Comprehensive mass spectrometry of lipids. John Wiley & Sons, Inc; Hoboken, New Jersey: 2016.
7. Yang K, Han X. Lipidomics: Techniques, Applications, and Outcomes Related to Biomedical Sciences. Trends Biochem Sci. 2016; 41:954–969. [PubMed: 27663237]
8. Nguyen A, Rudge SA, Zhang Q, Wakelam MJ. Using lipidomics analysis to determine signalling and metabolic changes in cells. Curr Opin Biotechnol. 2016; 43:96–103. [PubMed: 27816901]
9. Hyotylainen T, Oresic M. Bioanalytical techniques in nontargeted clinical lipidomics. Bioanalysis. 2016; 8:351–364. [PubMed: 26856187]
10. Rolim AE, Henrique-Araujo R, Ferraz EG, de Araujo Alves Dultra FK, Fernandez LG. Lipidomics in the study of lipid metabolism: Current perspectives in the omic sciences. Gene. 2015; 554:131–139. [PubMed: 25445283]
11. Cajka T, Fiehn O. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. Trends Analyt Chem. 2014; 61:192–206.
12. Wang M, Huang Y, Han X. Accurate mass searching of individual lipid species candidates from high-resolution mass spectra for shotgun lipidomics. Rapid Commun Mass Spectrom. 2014; 28:2201–2210. [PubMed: 25178724]
13. Han X, Zupan LA, Hazen SL, Gross RW. Semisynthesis and purification of homogeneous plasmenylcholine molecular species. Anal Biochem. 1992; 200:119–124. [PubMed: 1317681]
14. Han X. Neurolipidomics: challenges and developments. Front Biosci. 2007; 12:2601–2615. [PubMed: 17127266]

15. Han X, Holtzman DM, McKeel DW Jr. Plasmalogen deficiency in early Alzheimer's disease subjects and in animal models: molecular characterization using electrospray ionization mass spectrometry. J Neurochem. 2001; 77:1168–1180. [PubMed: 11359882]
16. Han X. Multi-dimensional mass spectrometry-based shotgun lipidomics and the altered lipids at the mild cognitive impairment stage of Alzheimer's disease. Biochim Biophys Acta. 2010; 1801:774–783. [PubMed: 20117236]
17. Hsu FF, Turk J. Electrospray ionization with low-energy collisionally activated dissociation tandem mass spectrometry of glycerophospholipids: mechanisms of fragmentation and structural characterization. J Chromatogr B. 2009; 877:2673–2695.
18. Han X, Yang K, Gross RW. Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses. Mass Spectrom Rev. 2012; 31:134–178. [PubMed: 21755525]
19. Han X, Gross RW. Shotgun lipidomics: multi-dimensional mass spectrometric analysis of cellular lipidomes. Expert Rev Proteomics. 2005; 2:253–264. [PubMed: 15892569]
20. Wang C, Palavicini JP, Wang M, Chen L, Yang K, Crawford PA, Han X. Comprehensive and Quantitative Analysis of Polyphosphoinositide Species by Shotgun Lipidomics Revealed Their Alterations in db/db Mouse Brain. Anal Chem. 2016; 88:12137–12144. [PubMed: 28193056]
21. Wang M, Wang C, Han X. Selection of internal standards for accurate quantification of complex lipid species in biological extracts by electrospray ionization mass spectrometry-What, how and why? Mass Spectrom Rev. 2016; doi: 10.1002/mas.21492
22. Shui G, Bendt AK, Pethe K, Dick T, Wenk MR. Sensitive profiling of chemically diverse bioactive lipids. J Lipid Res. 2007; 48:1976–1984. [PubMed: 17565170]
23. Fauland A, Kofeler H, Trotzmuller M, Knopf A, Hartler J, Eberl A, Chitraju C, Lankmayr E, Spener F. A comprehensive method for lipid profiling by liquid chromatography-ion cyclotron resonance mass spectrometry. J Lipid Res. 2011; 52:2314–2322. [PubMed: 21960706]
24. Wang M, Wang C, Han RH, Han X. Novel advances in shotgun lipidomics for biology and medicine. Prog Lipid Res. 2016; 61:83–108. [PubMed: 26703190]
25. Yang K, Han X. Accurate quantification of lipid species by electrospray ionization mass spectrometry - Meets a key challenge in lipidomics. Metabolites. 2011; 1:21–40. [PubMed: 22905337]
26. Koivusalo M, Haimi P, Heikinheimo L, Kostiainen R, Somerharju P. Quantitative determination of phospholipid compositions by ESI-MS: effects of acyl chain length, unsaturation, and lipid concentration on instrument response. J Lipid Res. 2001; 42:663–672. [PubMed: 11290839]
27. Jiang X, Ory DS, Han X. Characterization of oxysterols by electrospray ionization tandem mass spectrometry after one-step derivatization with dimethylglycine. Rapid Commun Mass Spectrom. 2007; 21:141–152. [PubMed: 17154356]
28. Wang M, Han RH, Han X. Fatty acidomics: Global analysis of lipid species containing a carboxyl group with a charge-remote fragmentation-assisted approach. Anal Chem. 2013; 85:9312–9320. [PubMed: 23971716]
29. Kiebish MA, Bell R, Yang K, Phan T, Zhao Z, Ames W, Seyfried TN, Gross RW, Chuang JH, Han X. Dynamic simulation of cardiolipin remodeling: greasing the wheels for an interpretative approach to lipidomics. J Lipid Res. 2010; 51:2153–2170. [PubMed: 20410019]
30. Han RH, Wang M, Fang X, Han X. Simulation of triacylglycerol ion profiles: bioinformatics for interpretation of triacylglycerol biosynthesis. J Lipid Res. 2013; 54:1023–1032. [PubMed: 23365150]
31. Hyotylainen T, Oresic M. Systems biology strategies to study lipidomes in health and disease. Prog Lipid Res. 2014; 55:43–60. [PubMed: 24995945]
32. Ellis SR, Ferris CJ, Gilmore KJ, Mitchell TW, Blanksby SJ, M. in het Panhuis. Direct Lipid Profiling of Single Cells from Inkjet Printed Microarrays. Anal Chem. 2012; 84:9679–9683. [PubMed: 23116365]
33. Ekroos, K. Lipidomics: Technologies and Applications. John Wiley & Sons; Weiheim, Germany: 2013. p. 356
34. Murphy, RC. Tandem Mass Spectrometry of Lipids: Molecular Analysis of Complex Lipids. RSC Publishing; 2014.

35. Yang K, Cheng H, Gross RW, Han X. Automated lipid identification and quantification by multi-dimensional mass spectrometry-based shotgun lipidomics. Anal Chem. 2009; 81:4356–4368. [PubMed: 19408941]

36. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res. 2010; 51:3299–3305. [PubMed: 20671299]

37. Almeida R, Pauling JK, Sokol E, Hannibal-Bach HK, Ejsing CS. Comprehensive lipidome analysis by shotgun lipidomics on a hybrid quadrupole-orbitrap-linear ion trap mass spectrometer. J Am Soc Mass Spectrom. 2015; 26:133–148. [PubMed: 25391725]

## Highlights

A commentary on the experimental procedures, inherent limitations, assessment of cross-platform reproducibility, available resource, and future challenges of lipidomics.