Non-targeted Lipidomic Analysis by Direct Infusion Mass Spectrometry

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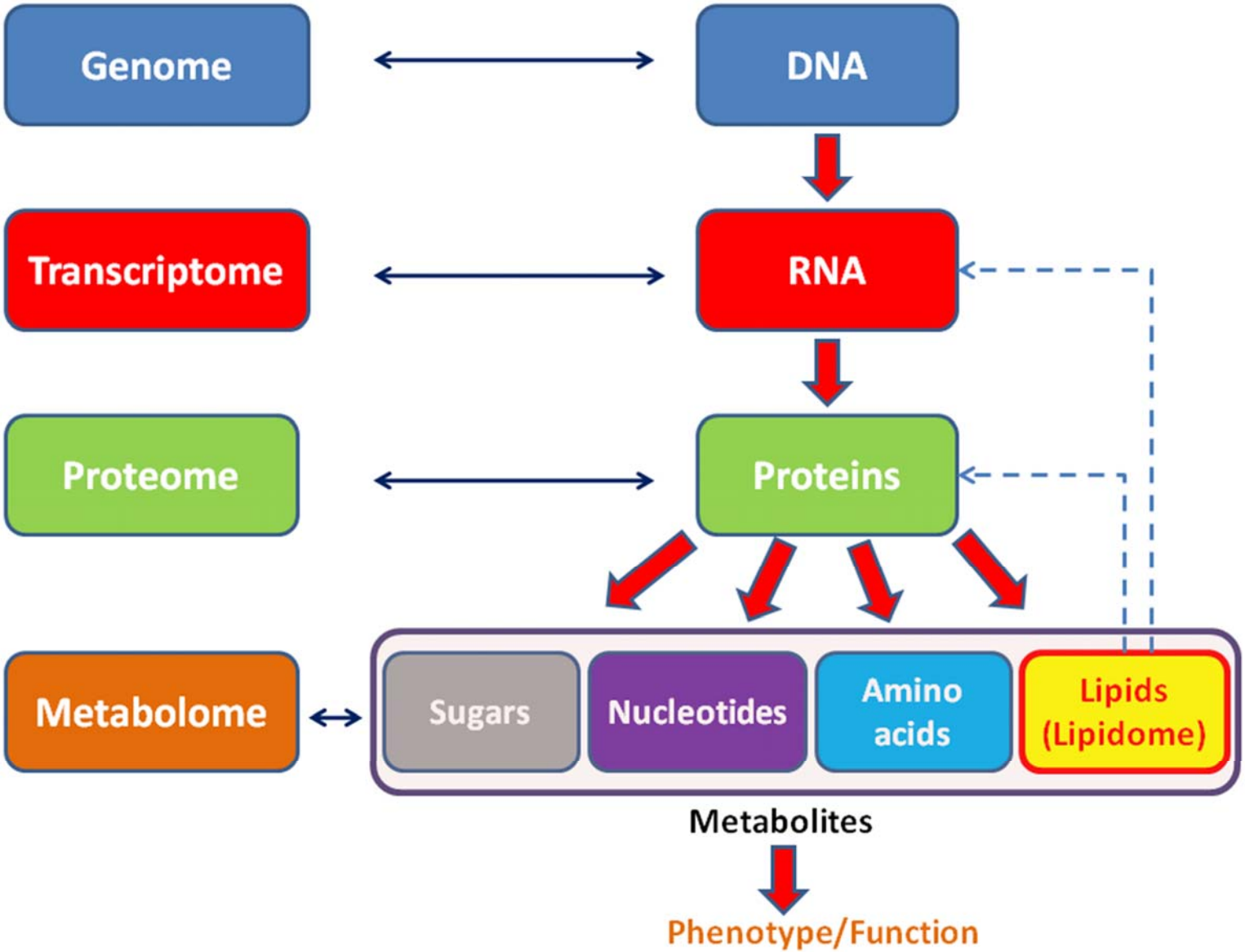
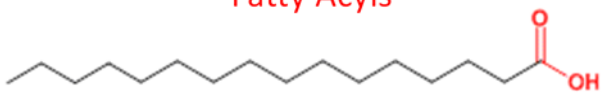
Lipidome: A subset of Metabolome



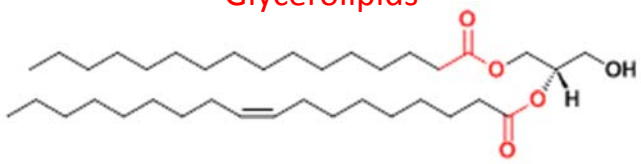
https://en.wikipedia.org/wiki/Lipidomics

Eight Categories of Lipids

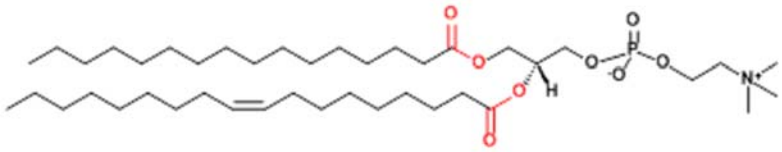
Fatty Acyls



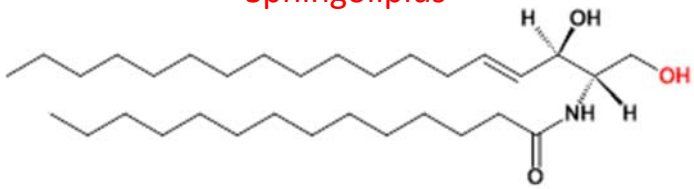
Glycerolipids



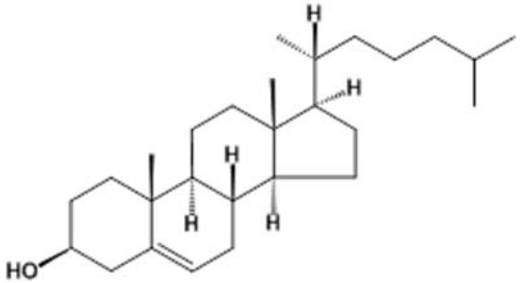
Glycerophospholipids



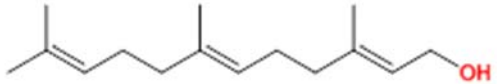
Sphingolipids



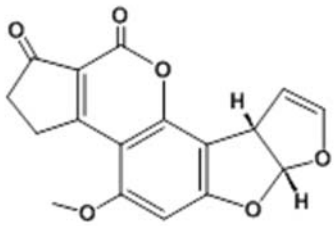
Sterol lipids



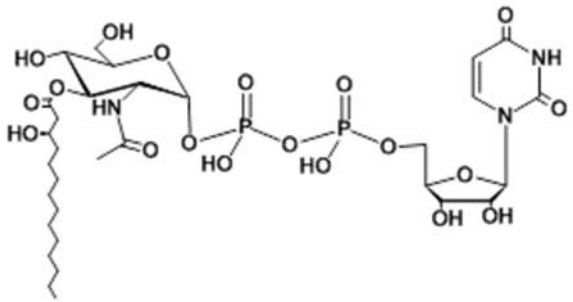
Prenol lipids



Polyketides



Saccharolipids



Fahy E. et. al., J. Lipid Res. 2005, 839-861

Mass Spectrometric Analysis of Lipids

	Lipids of Interest	Mass Spectrometric Analysis
Targeted	one or several specific lipid species	multiple reaction monitoring (MRM)
	one specific lipid class/subclass	• precursor ion scan • Neutral loss scan
Non-targeted	all lipid classes	• SWATH (Sequential Window Acquisition of all Theoretical fragment-ion spectra) • MS/MS (Identification) combined with high resolution MS (Quantification)

Non-targeted Lipidomic Analysis

- Advantages
  - Comprehensive
  - Rapid
  - Big picture
- Challenges
  - Relatively low sensitivity
    - Full scan, neutral lipids, more severe interference peaks
  - Complexed data analysis
    - More severe interference peaks, multiple adduct forms, peak overlapping

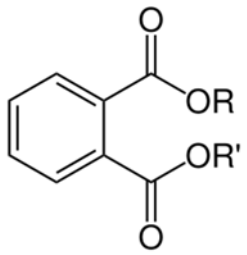
Interference Peaks in Mass Spectrometric Analysis

- Contaminants from plastics (additive, polymer)
- Multiple adducts formation: H^+, NH_4^+, Na^+, K^+
- Non-covalent adduct formation
  - homo/hetero lipid dimers;
  - between lipid and impurity
- In-source dissociation
- Solvent degradation: $CHCl_3 \rightarrow HCl$
- Carryover: previous runs, glassware, calibrants

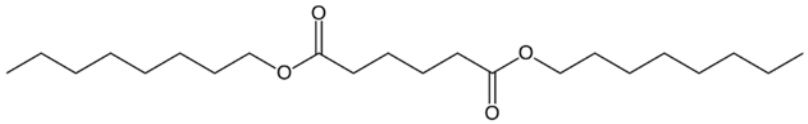
Common Contaminants in Samples

- Plasticizers:

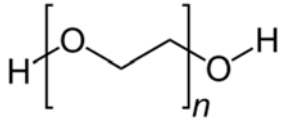
  phthalates



  esters of aliphatic dicarboxylic acids

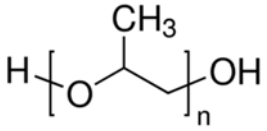


- Polymers:



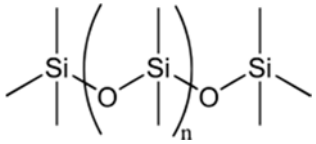
44.03 Da

PEG



58.04 Da

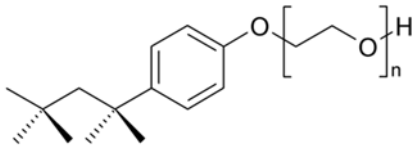
PPG



74.02 Da

silicone rubber

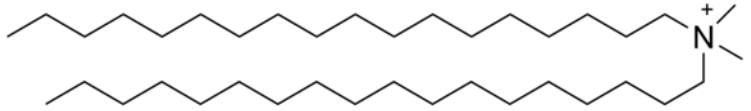
- Detergents:



44.03 Da

Triton X-100

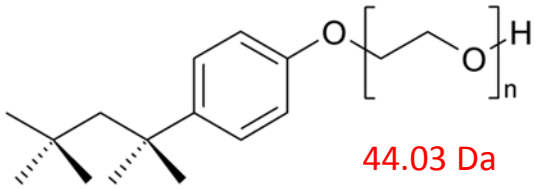
- Ingredients in cosmetics and hair conditioners



Distearyldimethylammonium chloride

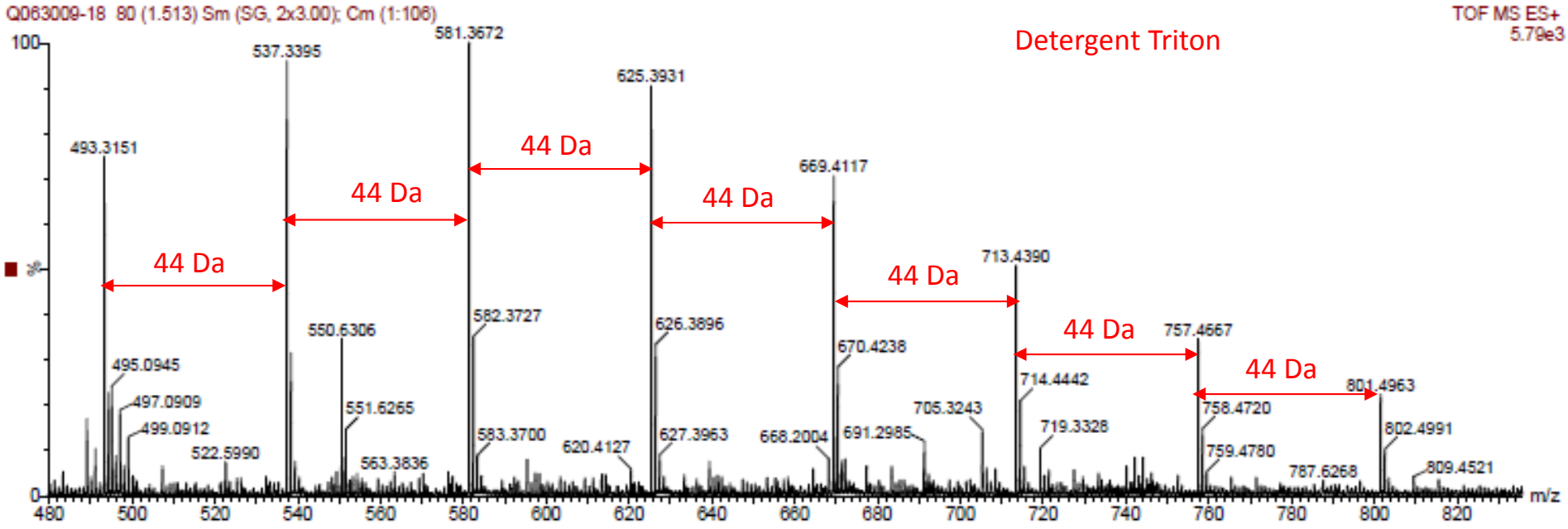
m/z 550.63

Contamination from Detergent

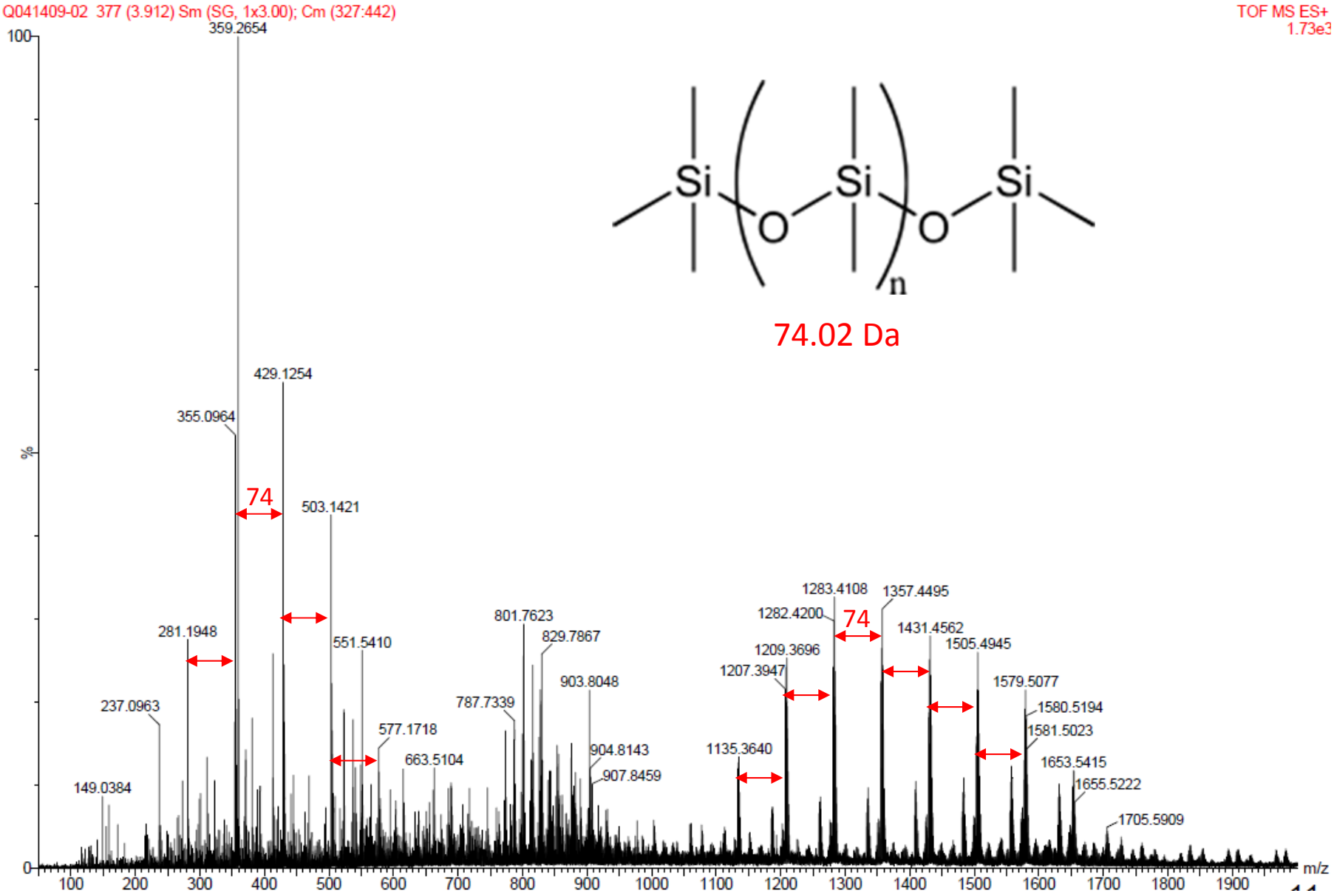


44.03 Da

Detergent Triton



Contamination from Silicone Rubber in LC/MS



Approaches for Non-targeted Lipidomic Analysis

- Minimize interference from common contaminants
  - Do not use plastics
  - Rinse thoroughly
- Tuning instruments to maximize signal of lipids of interest
  - Electrospray probe position
  - Desolvation temperature
  - Flow rate
  - MS profile
  - Other parameters
  - heating gas, nebulization gas, cone voltage/decluster voltage
- Convert multiple adduct forms to one dominant form

Multiple adduct formation

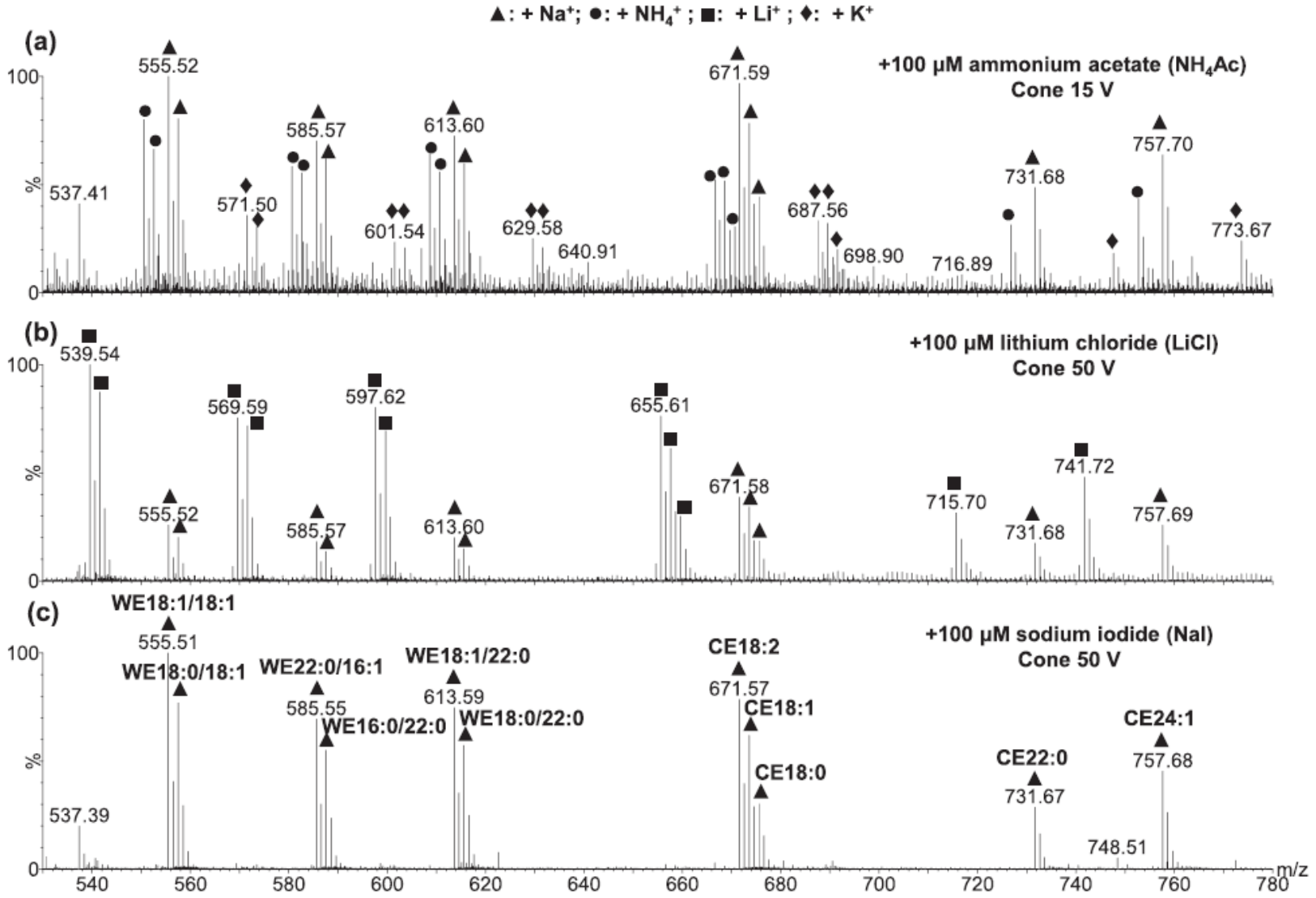
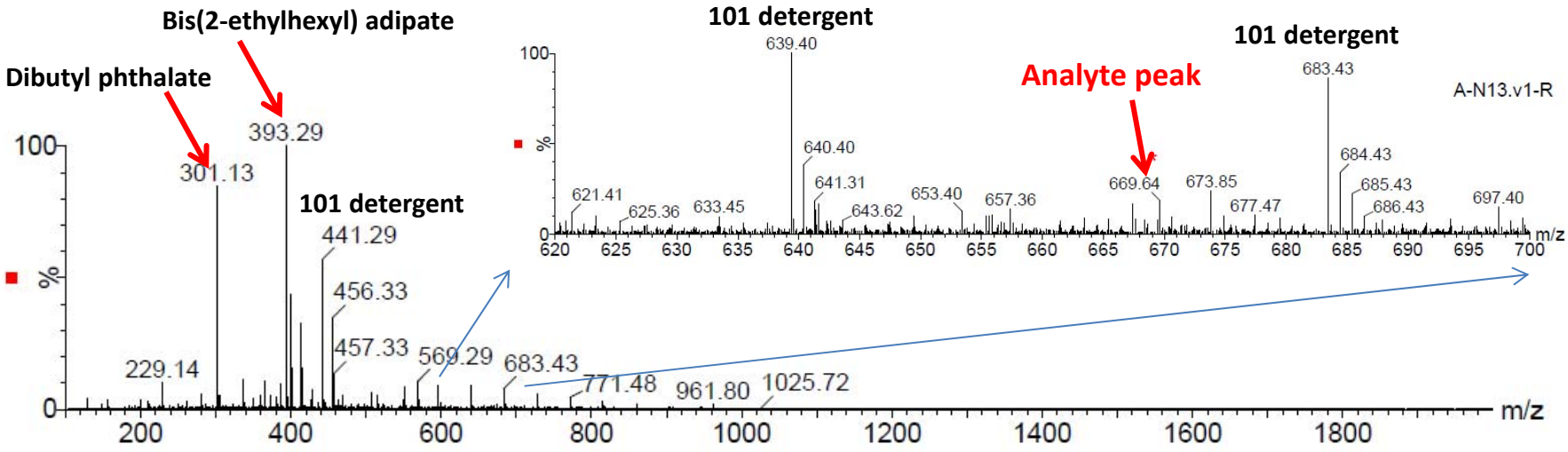


FIGURE 2. Electrospray ionization mass spectra of 11 equimolar WE and CE standards (100 nM each, 1.1 µM total) using 100 µM of the following additives: (a) ammonium acetate, (b) lithium chloride, and (c) sodium iodide. The sample solution was in a mixture of chloroform and methanol (1:14, vol/vol). The flow rate was 40 µL/min, the desolvation temperature was 250°C, and the acquisition time was 1 minute. For clarity, only the peaks in (c) were labeled.

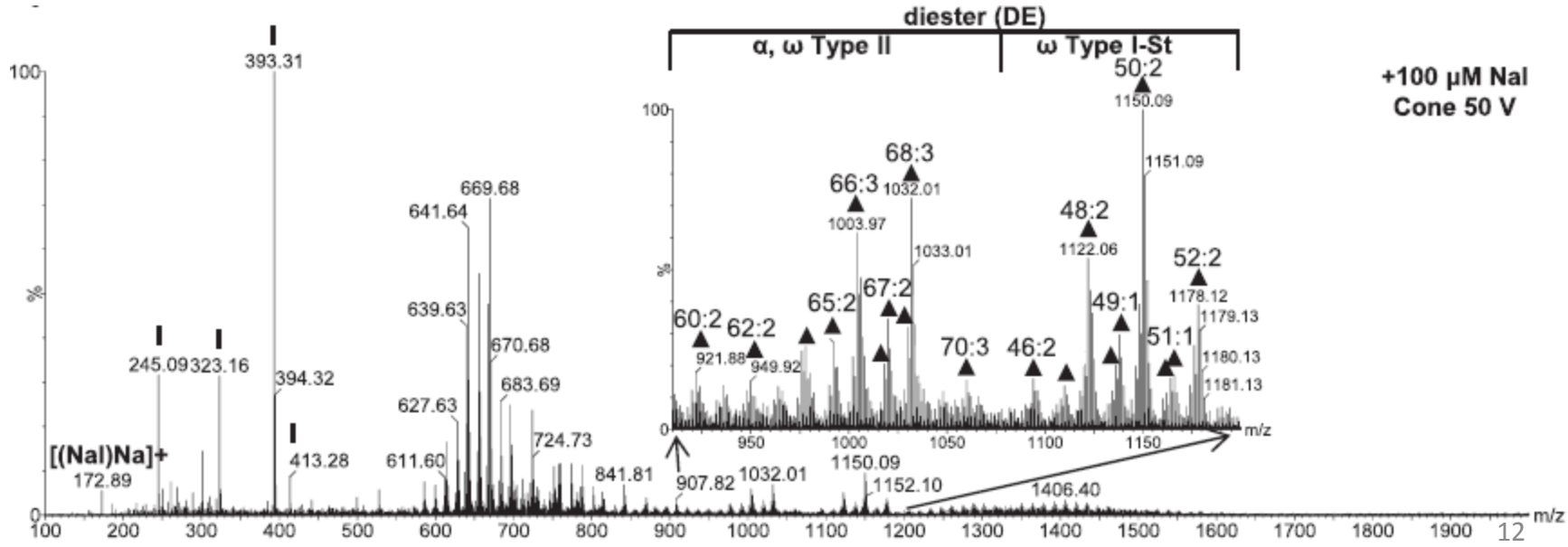
Chen JZ, et. al, IOVS, 2013, 54: 5730-5753.

Non-targeted Mass Spectrometry Analysis of Lipids

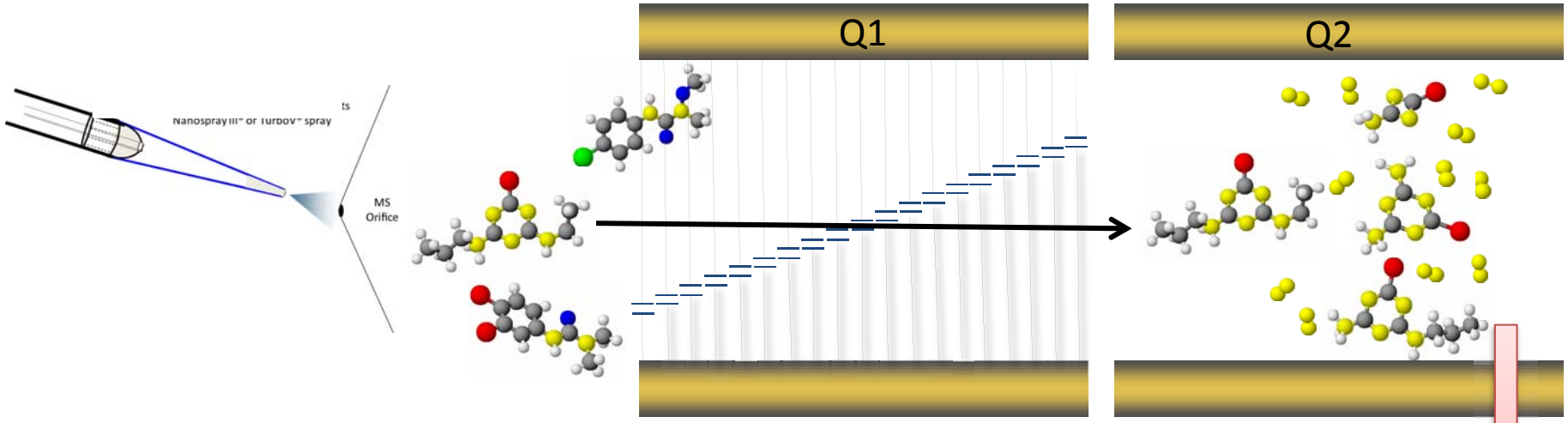
Before optimization



After optimization



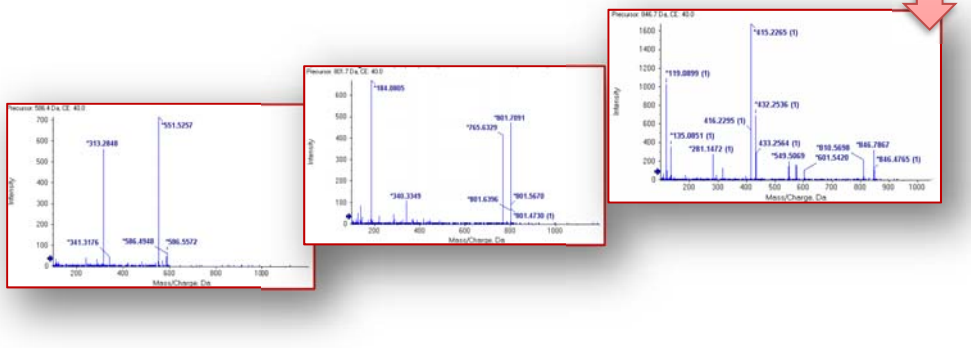
MS/MS^{all} Analysis of All Lipid Peaks in a Sample



Direct infusion, flow injection, and lipid-class targeted LC techniques

Fast Q1 precursor selection step-wise through mass range

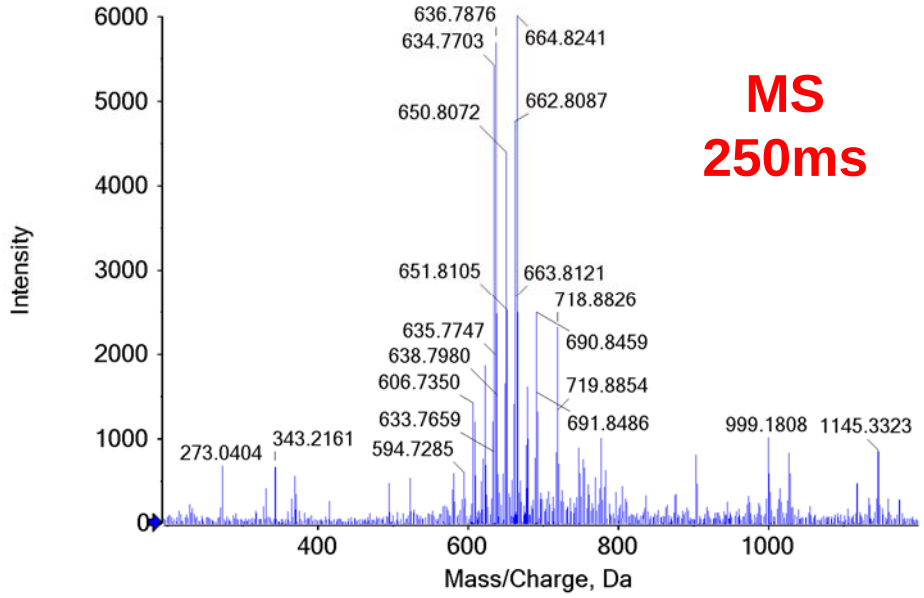
CID Fragmentation

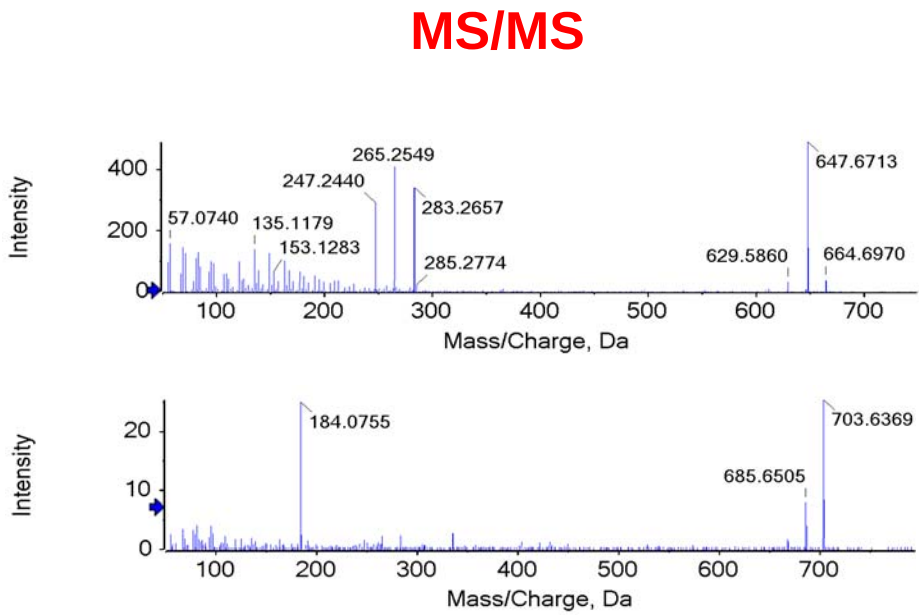


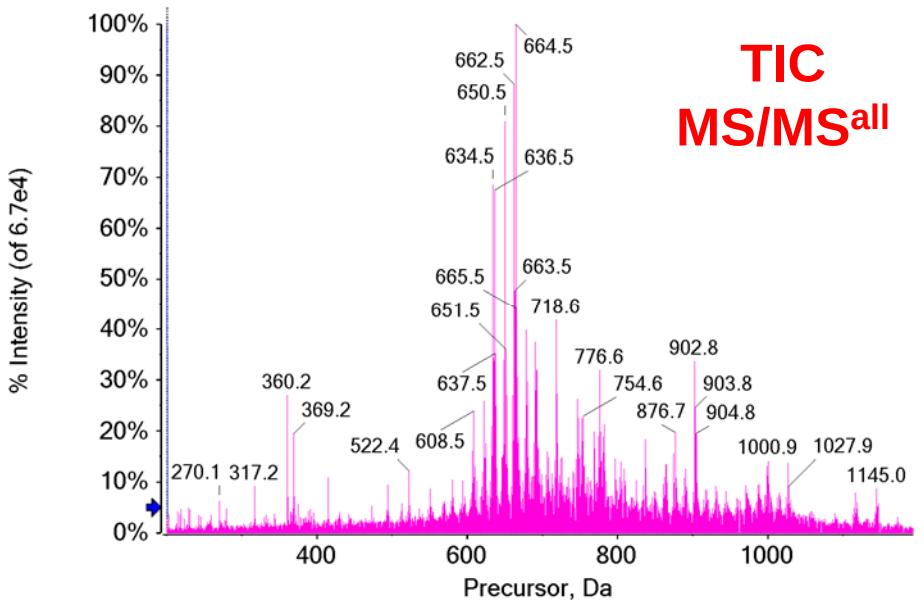
Collection of High resolution MS/MS

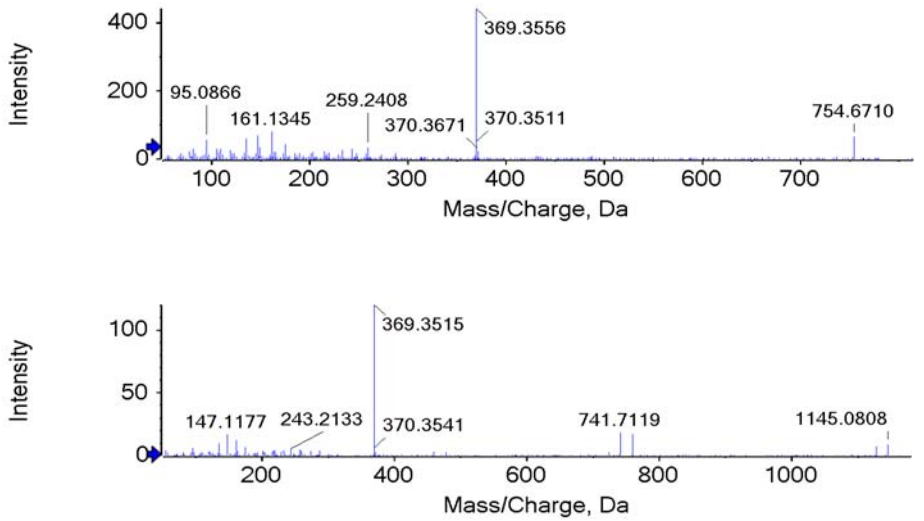
https://www.uab.edu/proteomics/metabolomics/symposium/symoposium_dec2012.php

SWATH: Full MS/MS Archive of Every Compound in a Sample

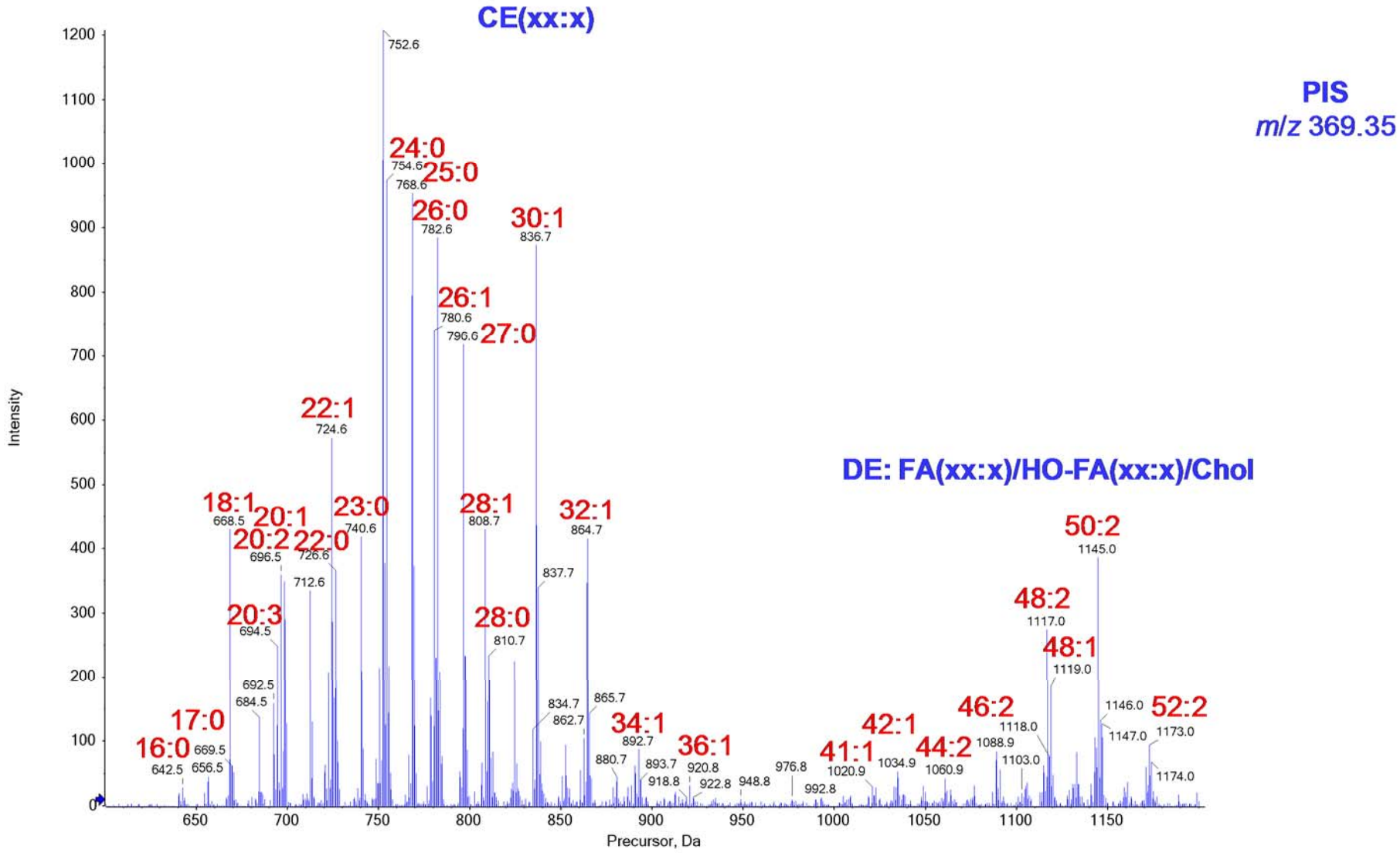




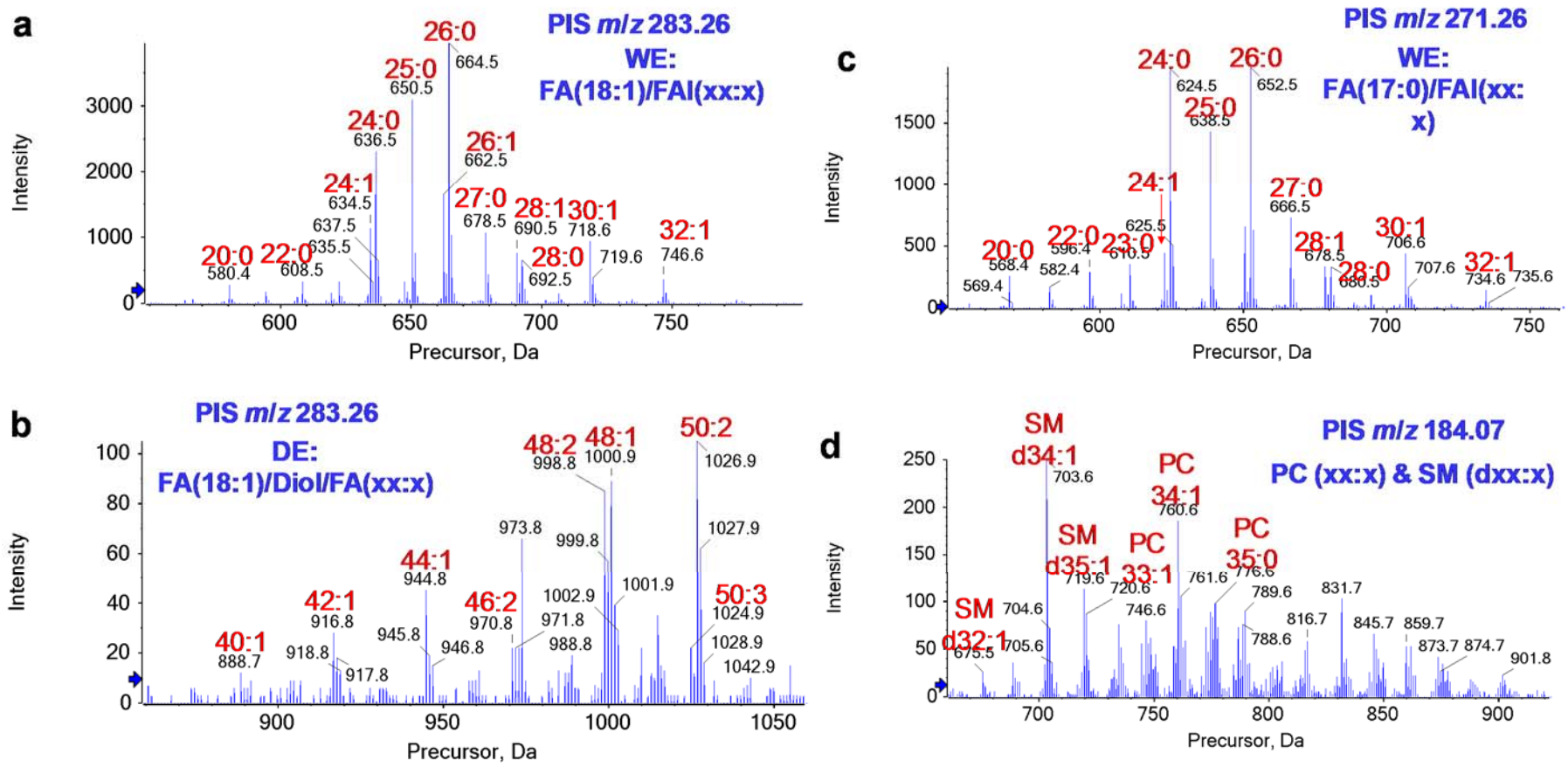




Pseudo Precursor Ion Scanning Extracted from MS/MS^{all} Analysis



Pseudo Precursor Ion Scanning Extracted from MS/MS^{all} Analysis



Summary

- Minimizing contaminants/interference peaks is particularly important for non-targeted lipidomic analysis.
- It is important to confirm the identity of lipids by MS/MS before quantifying lipids by MS.
- SWATH appears to be a promising method for non-targeted lipidomic analysis.

References on Contaminants

1. Keller BO, Jie Suib, Alex B. Youngc, Randy M. Whittal, *Interferences and contaminants encounterd in modern mass spectrometry*, Analytica Chimica Acta, 2008, 627: 71-81

2. Ende M, Spiteller G, *Contaminants in mass spectrometry,* Mass Spectrometry Review, 1982, 1: 29-62

3. http://www.waters.com/webassets/cms/support/docs/715001307d_cntrl_cntm.pdf

4. http://www.abrf.org/index.cfm/list.msg/66994

Useful websites for lipid analysis

1. http://lipidlibrary.aocs.org/
2. http://www.cyberlipid.org/
3. http://www.lipidmaps.org/
4. http://lipidlibrary.aocs.org/news/links.html