# Non-targeted Lipidomic Analysis by Direct Infusion Mass Spectrometry

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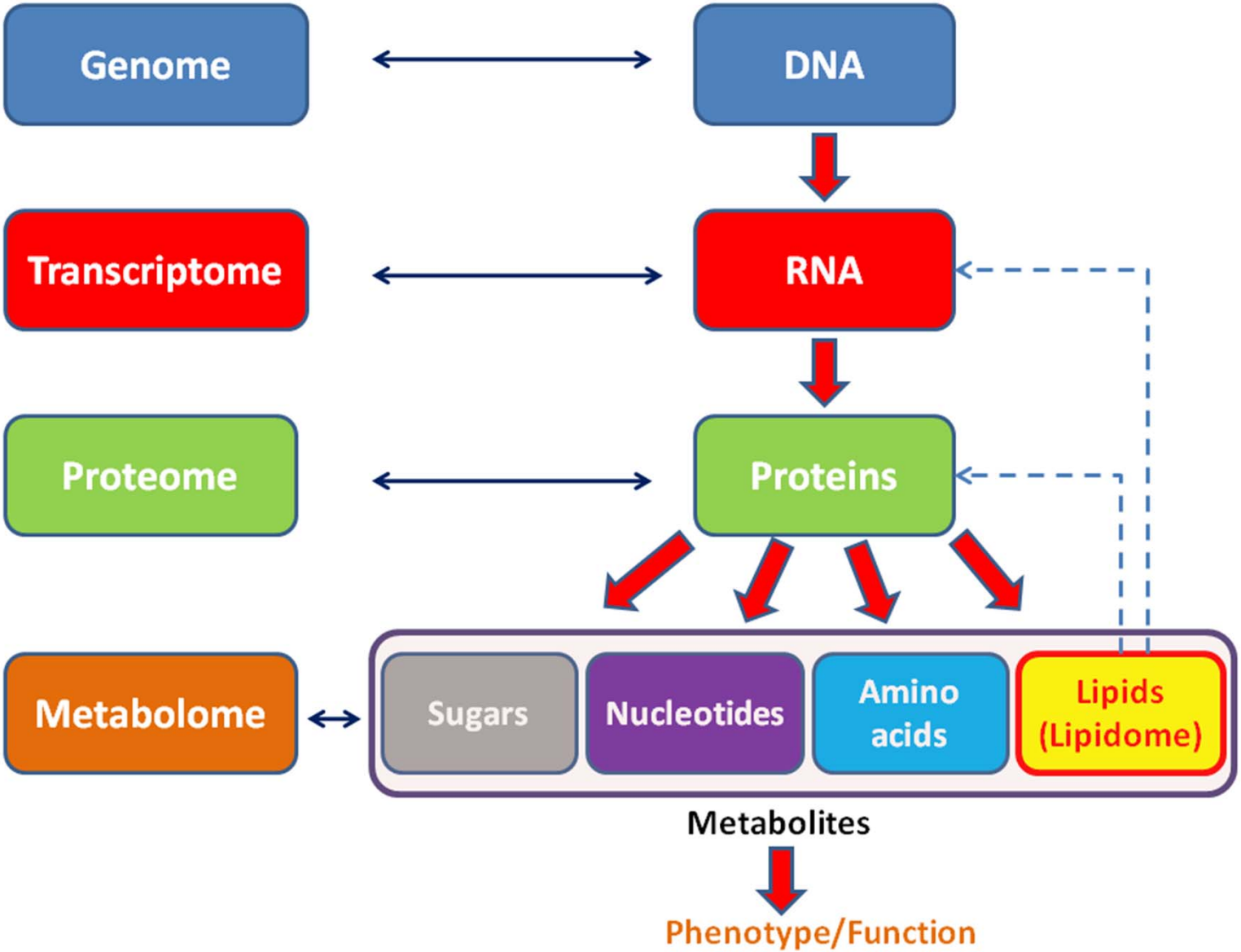
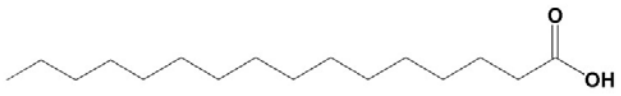
# Lipidomics: A subset of Metabolomics

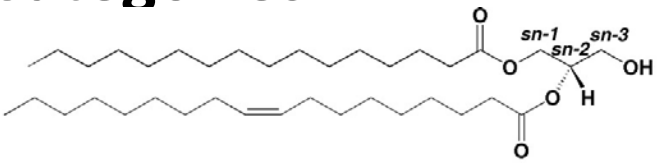



*https://en.wikipedia.org/wiki/Lipidomics*

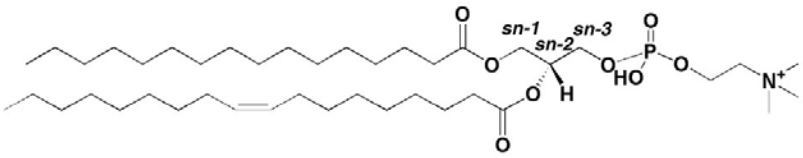
# Lipids: Eight Categories



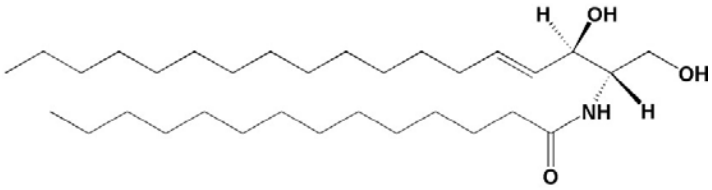
(a) Fatty Acyls: hexadecanoic acid



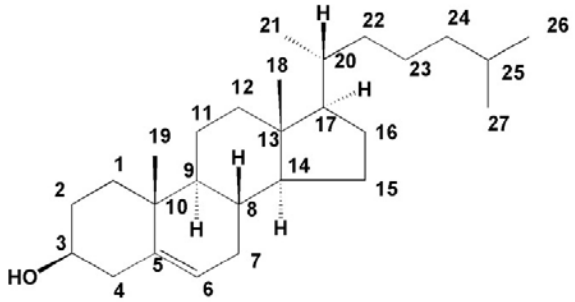
(b) Glycerolipids: 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycerol



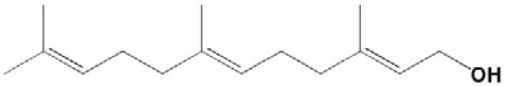
(c) Glycerophospholipids: 1-hexadecanoyl-2-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine



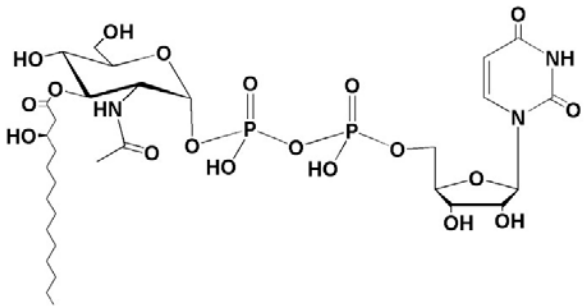
(d) Sphingolipids: N-(tetradecanoyl)-sphing-4-enine



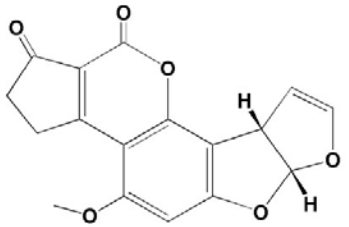
(e) Sterol Lipids: cholest-5-en-3β-ol



(f) Prenol Lipids: 2E,6E-farnesol



(g) Saccharolipids: UDP-3-O-(3R-hydroxy-tetradecanoyl)-αD-N-acetylglucosamine



(h) Polyketides: aflatoxin B1

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

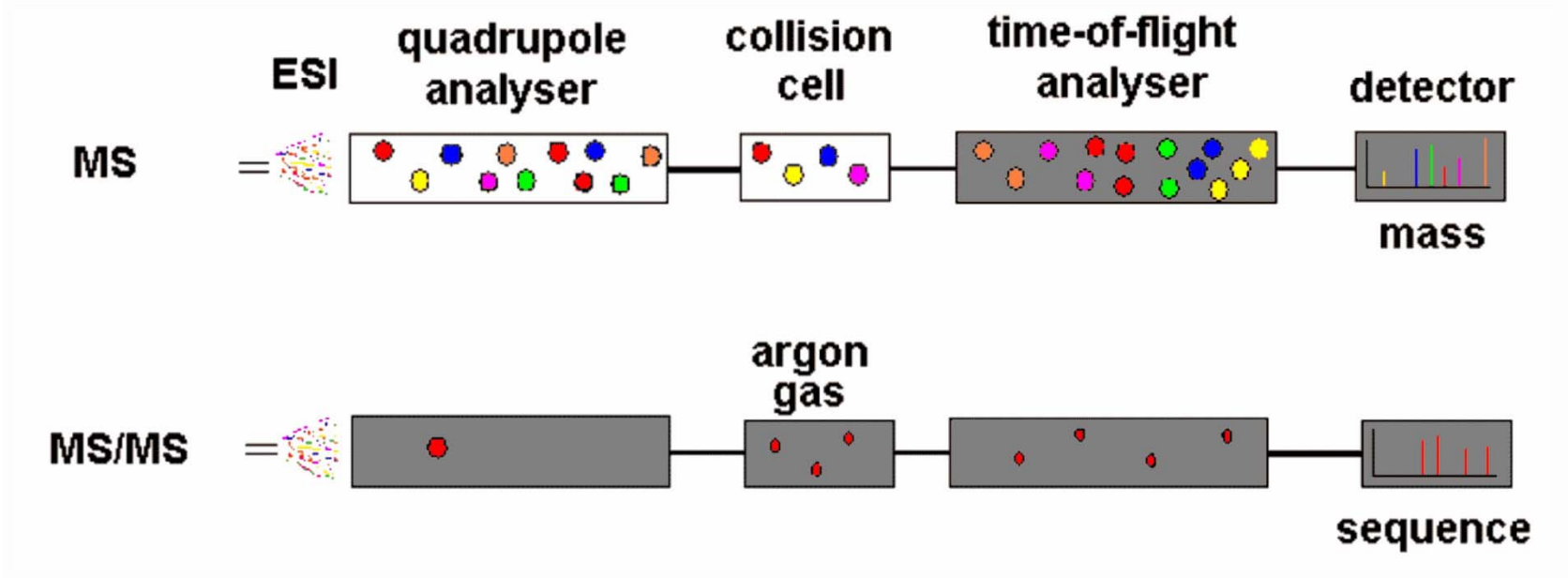
# Mass Spectrometric Approaches for Lipidomic Analysis

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

# 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
    - Overlapping

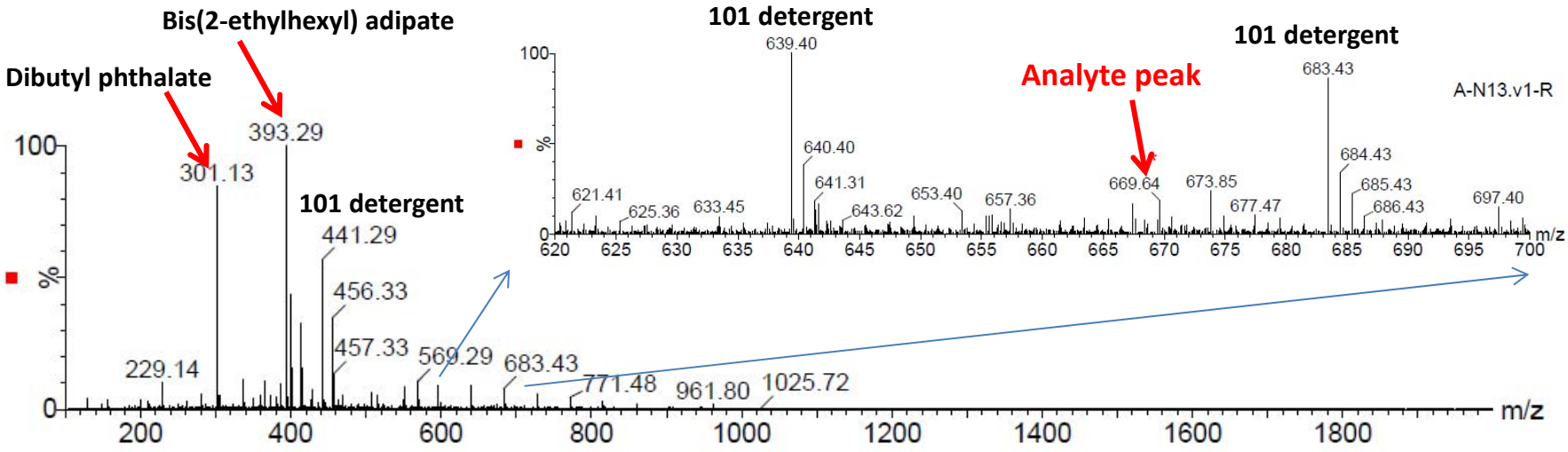
# Electrospray-Q-TOF Mass Spectrometer



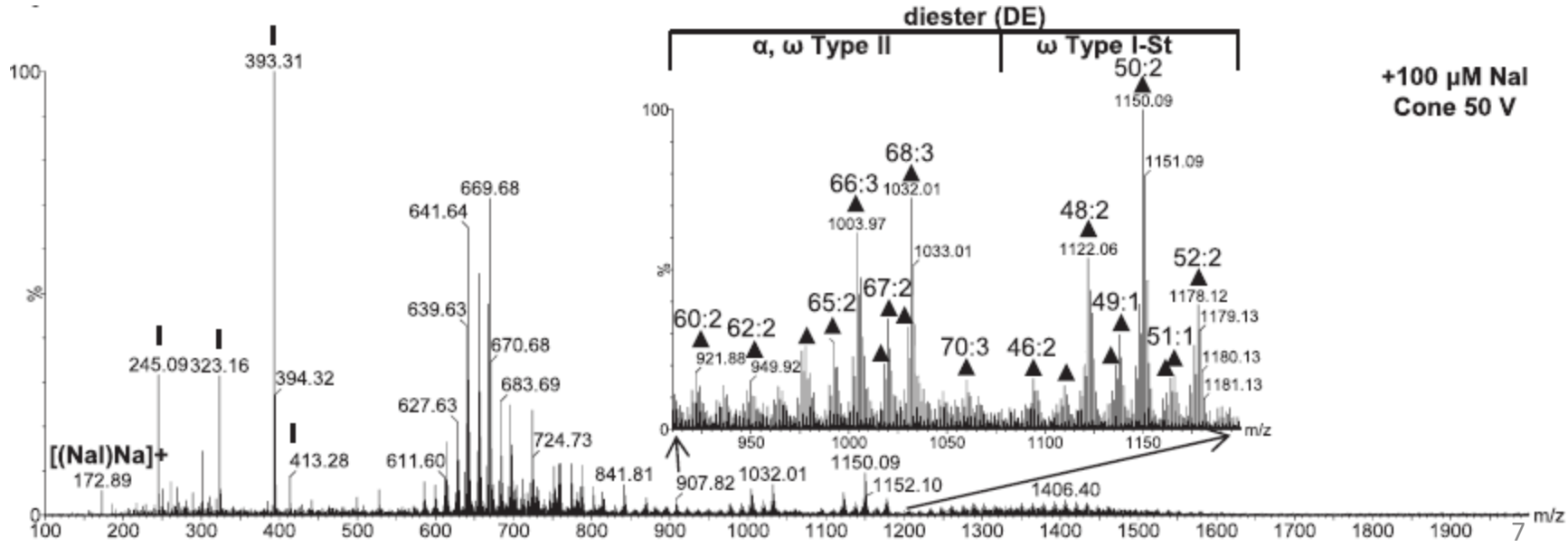

*http://www.astbury.leeds.ac.uk/facil/MStut/mstutorial.htm*

# Non-targeted Mass Spectrometry Analysis of Lipids

## Before optimization




## After optimization



# Optimize Instrument Condition

- Electrospray probe position
- Desolvation temperature
- Flow rate
- MS profile
- Other parameters
    - heating gas, nebulization gas, cone voltage/decluster voltage

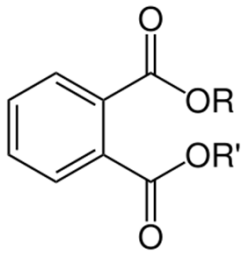
# Interference Peaks in Mass Spectrometric Analysis

- 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

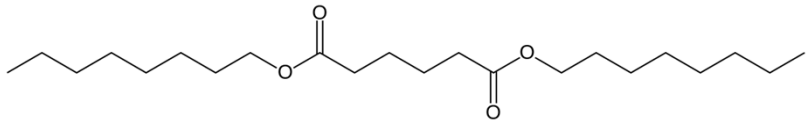
# Common Contaminants in Samples

- Plasticizers:

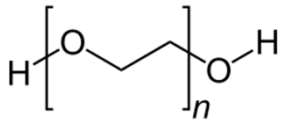
  phthalates




  esters of aliphatic dicarboxylic acids

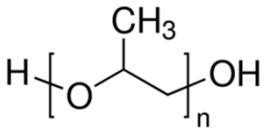



- Polymers:



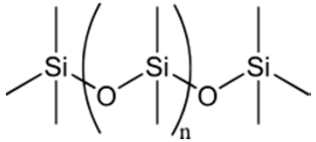

44.03 Da

PEG




58.04 Da

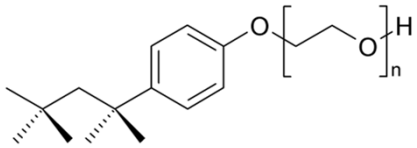
PPG




74.02 Da

silicone rubber

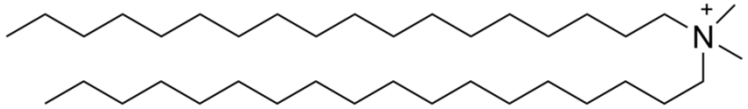
- Detergents:




44.03 Da

Triton X-100

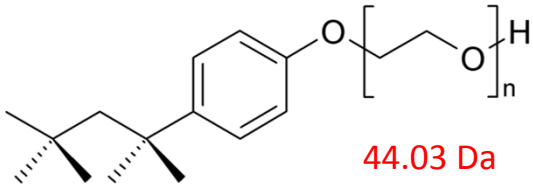
- Ingredients in cosmetics and hair conditioners




Distearyldimethylammonium chloride

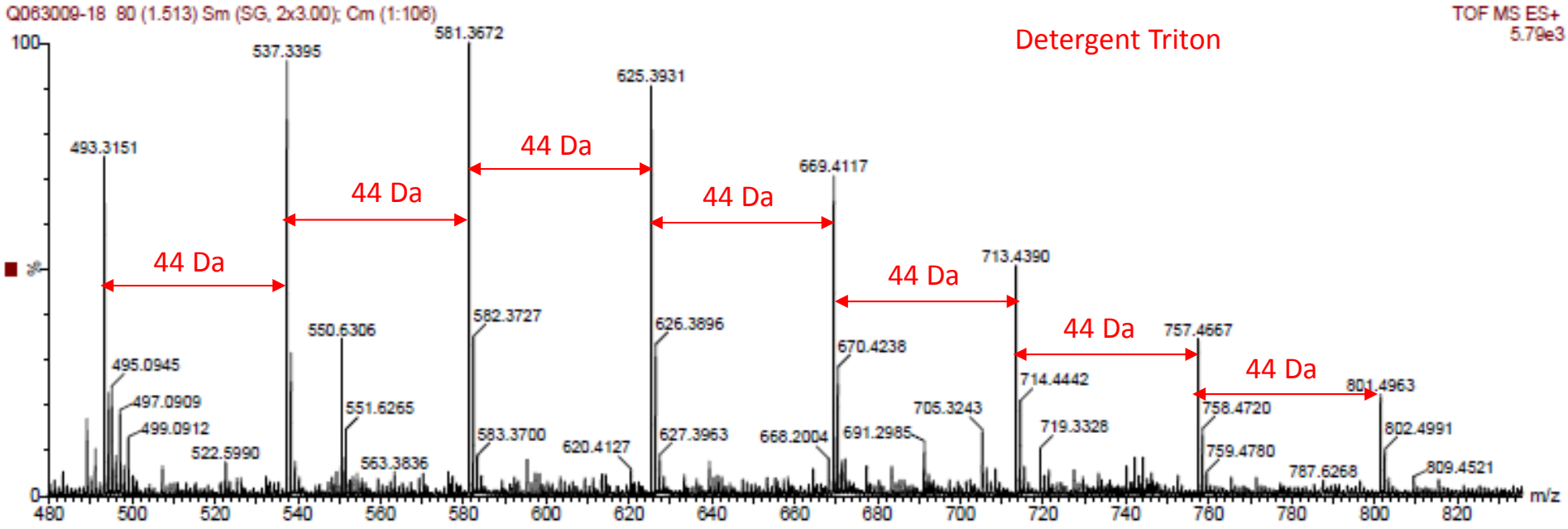
*m/z* 550.63

# Contamination from Detergent

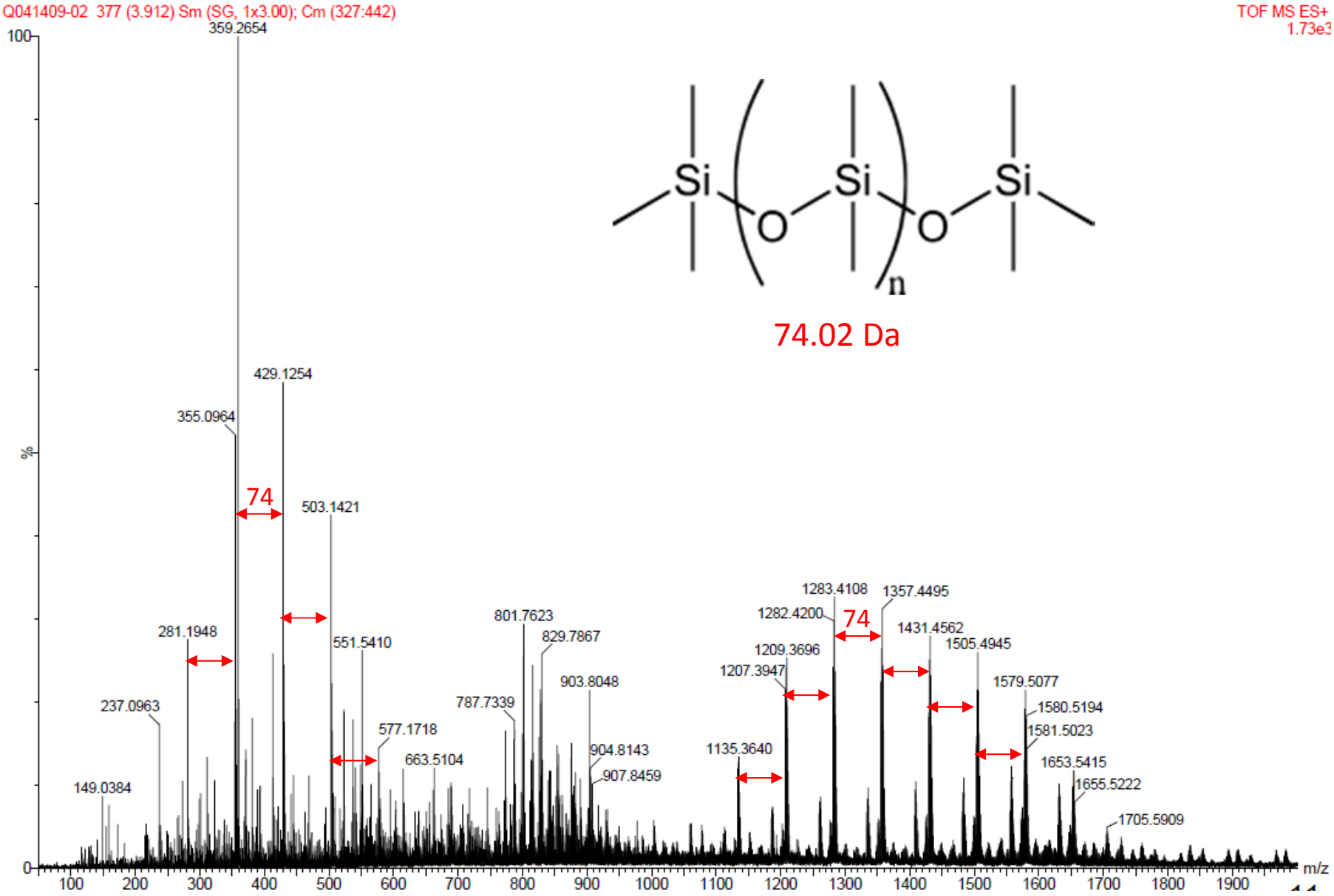



44.03 Da

Detergent Triton



# Contamination from Silicone Rubber in LC/MS



# Multiple adduct formation

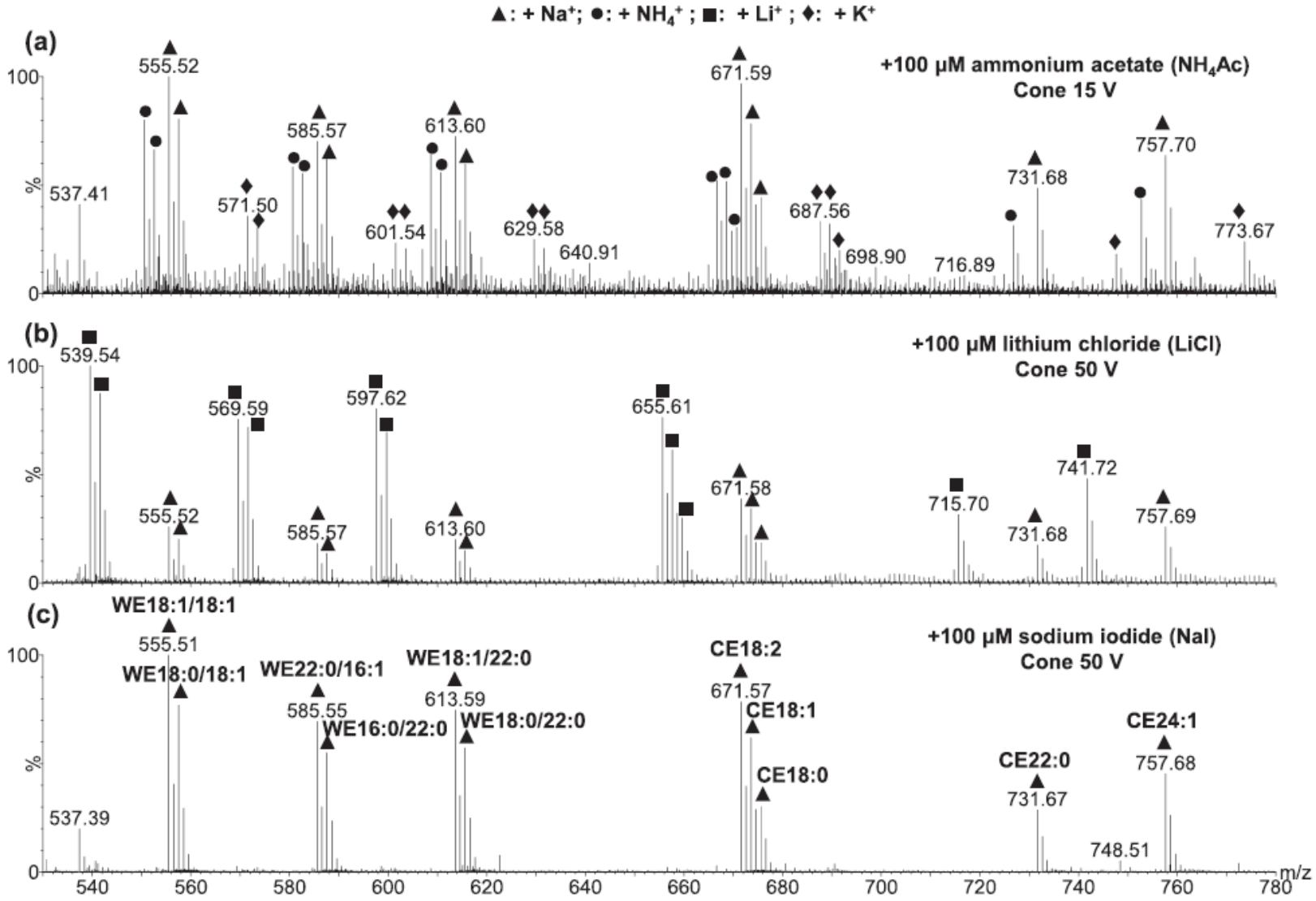
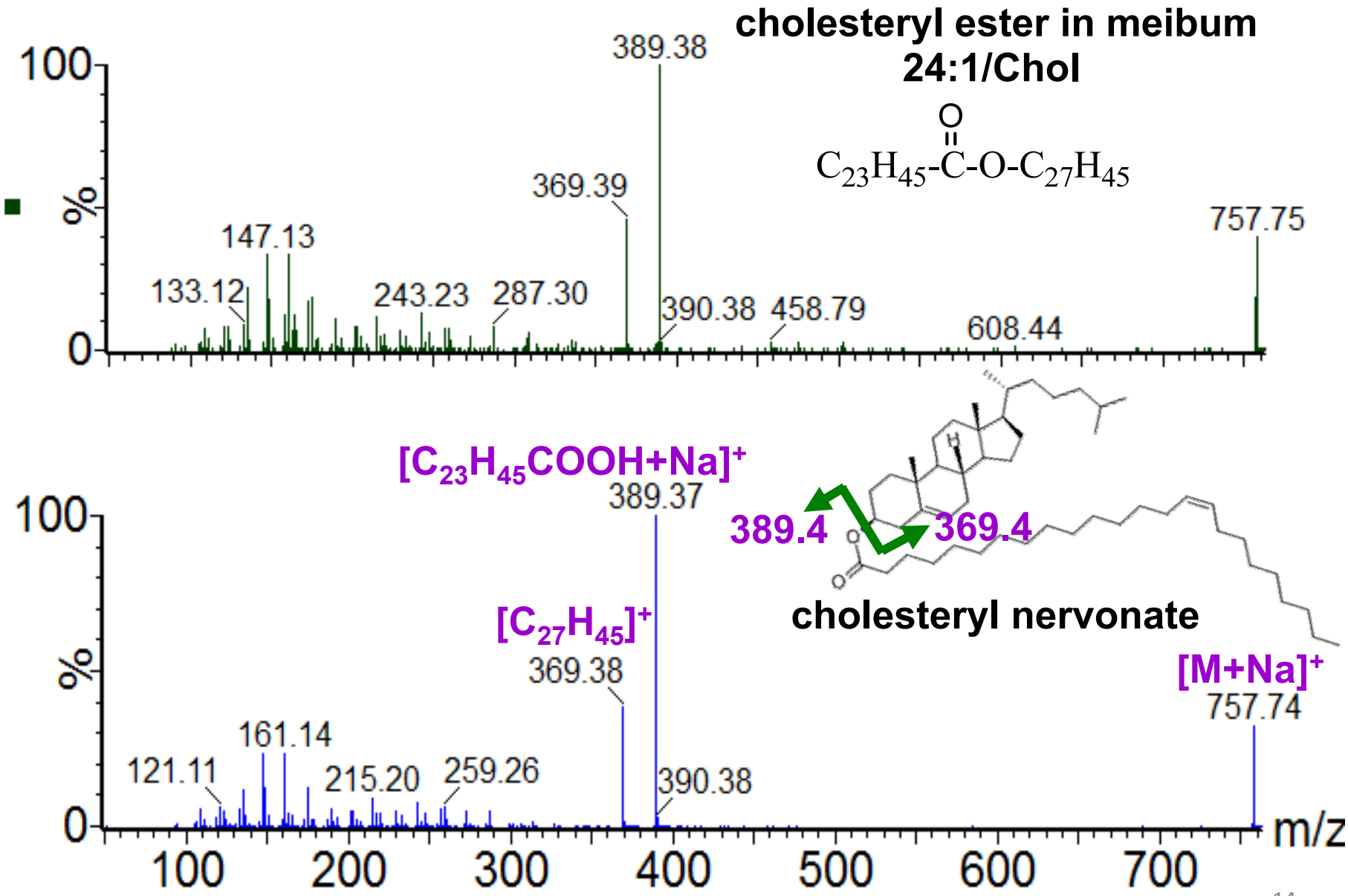



FIGURE 2. Electrospray ionization mass spectra of 11 equimolar WE and CE standards (100 nM each, 1.1 µM total) using 100 µM of the following additives: (a) ammonium acetate, (b) lithium chloride, and (c) sodium iodide. The sample solution was in a mixture of chloroform and methanol (1:14, vol/vol). The 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.

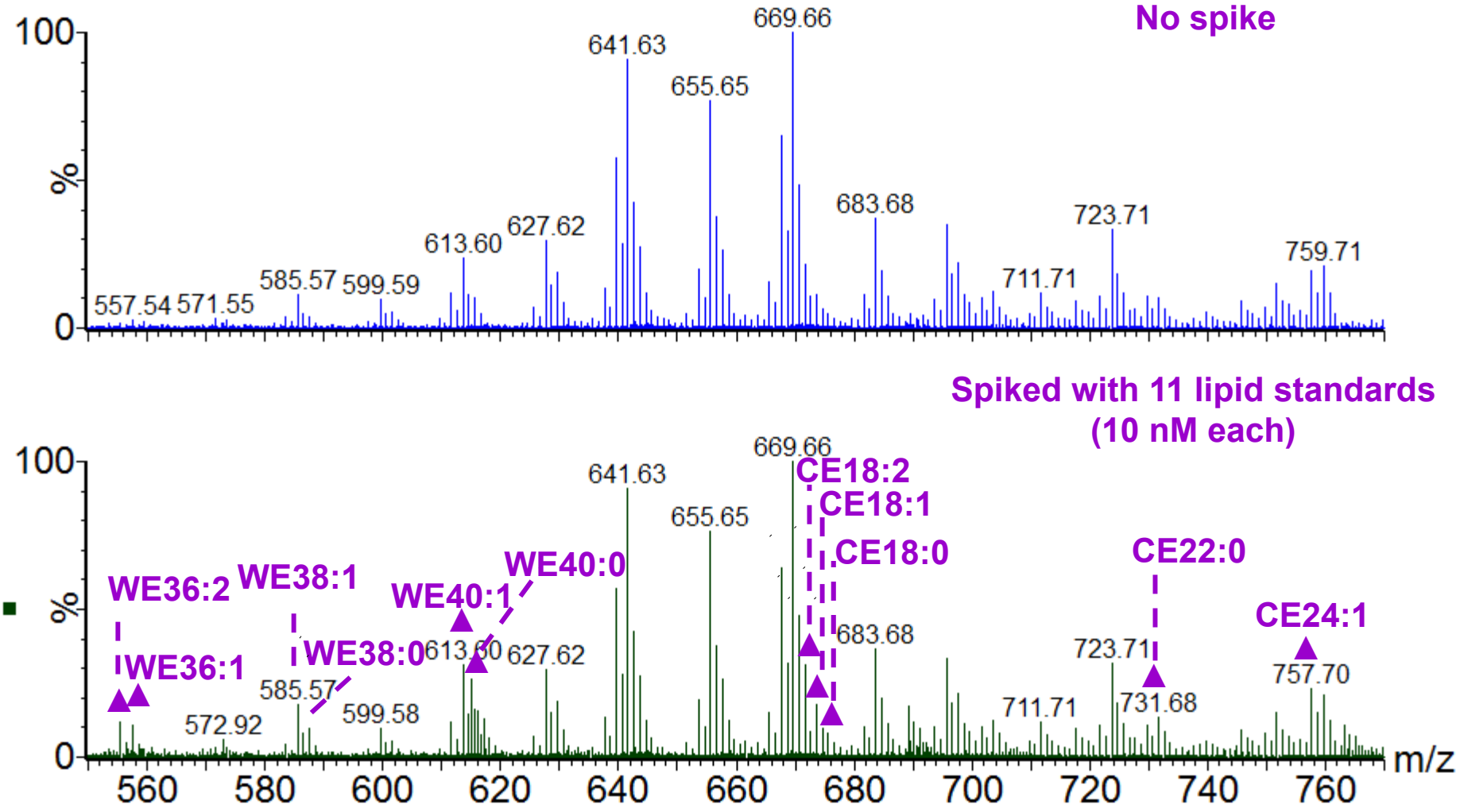
*Chen JZ, et. al, IOVS, 2013, 54: 5730-5753.*

# Identification of lipids by MS/MS



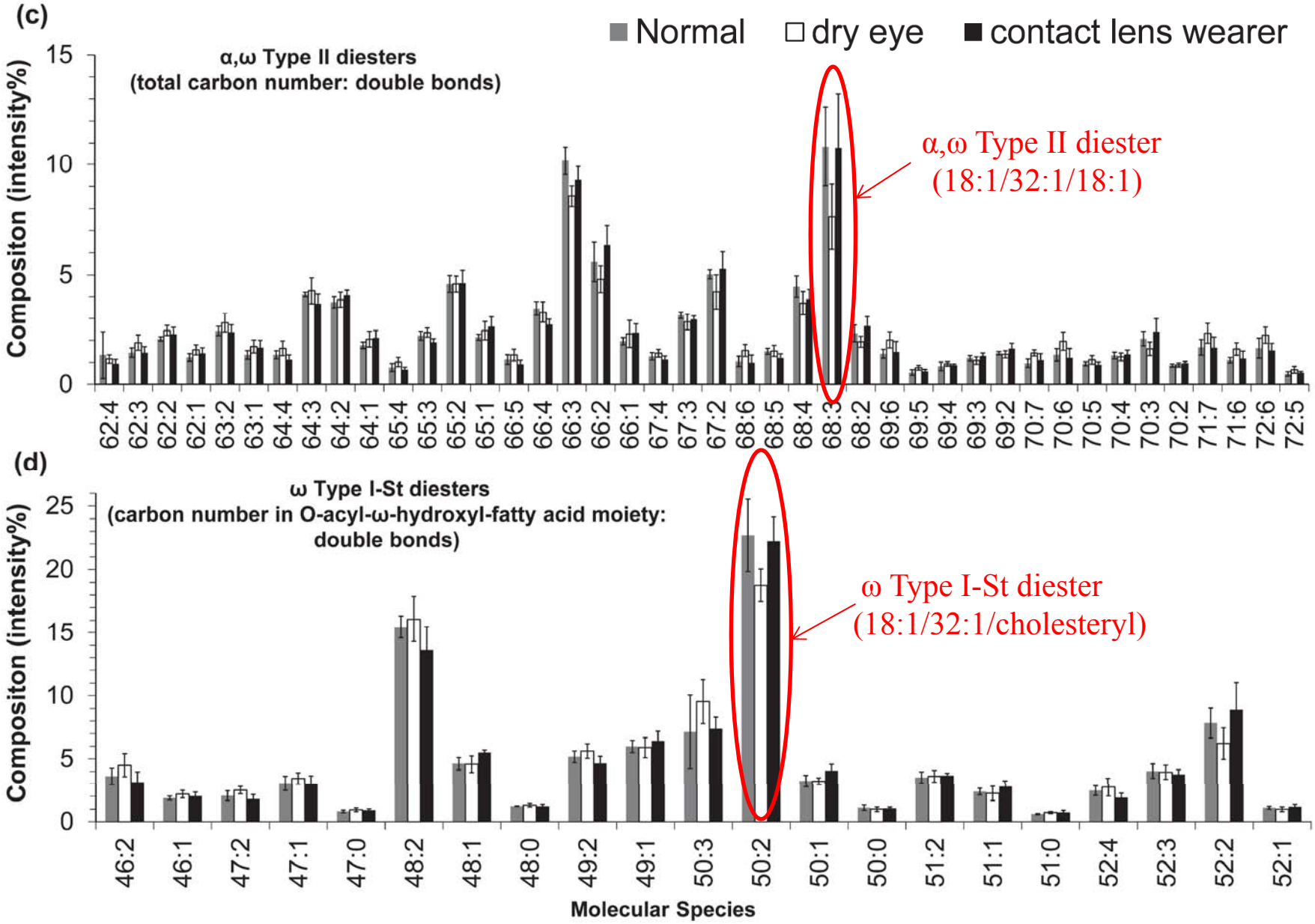

*Chen J. et al., IOVS, 2010, 51(12), 6220-6231*

# Quantification of lipids by MS




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

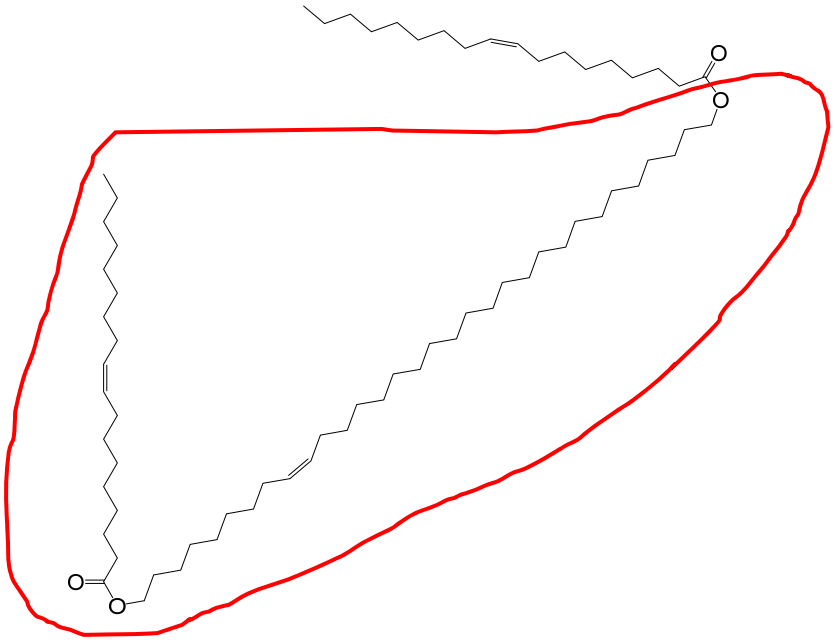
# Manual Comparison of Relative Peak Intensities



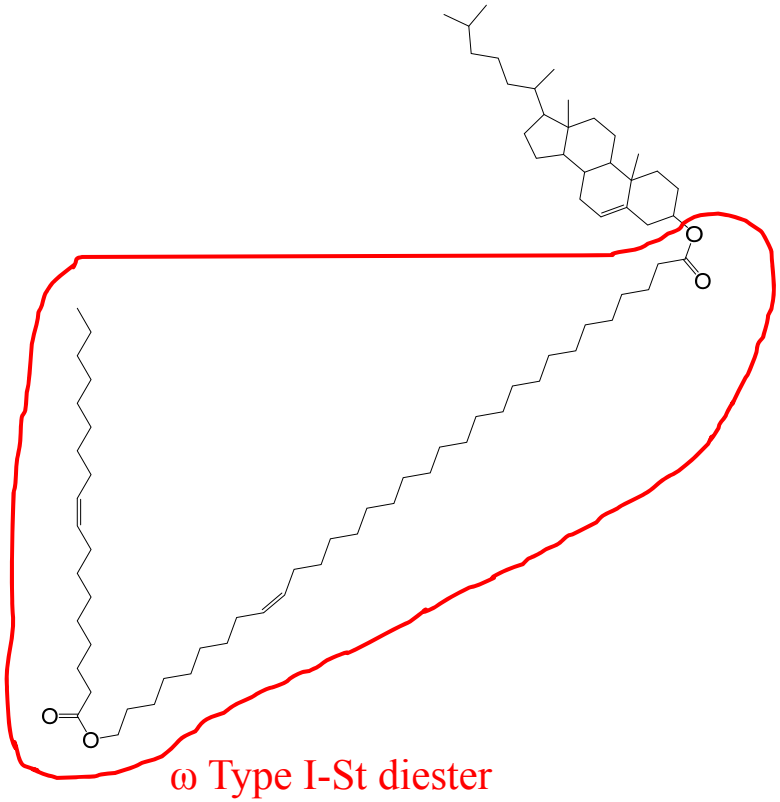



*Chen J. et al., IOVS, 2013, 54(8), 5730-5753*

# Diesters down-regulated in Dry Eye Meibum



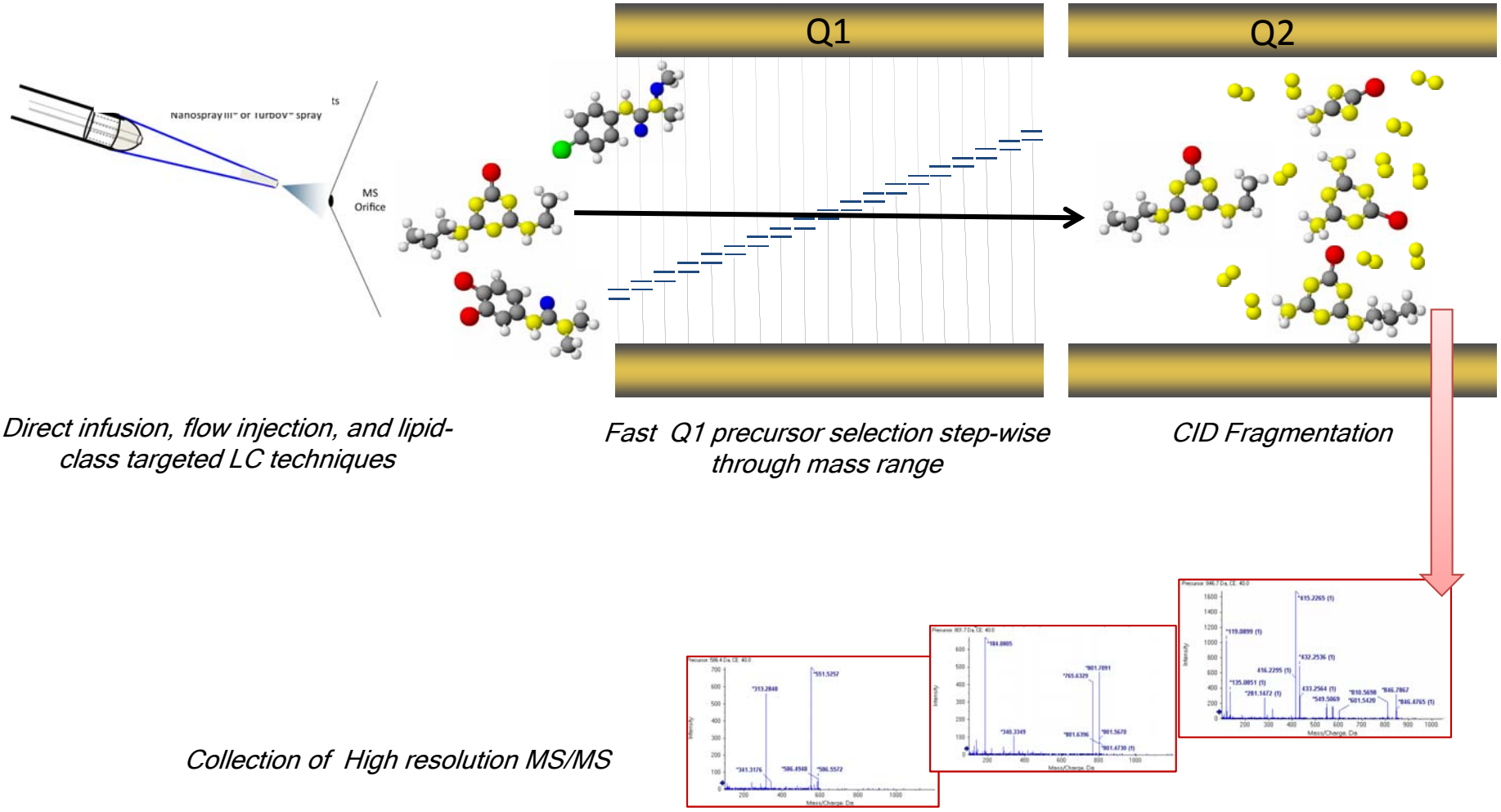
α,ω Type II diester
(18:1/32:1/18:1)



ω Type I-St diester
(18:1/32:1/cholesteryl)

*Chen J. et al., IOVS, 2013, 54(8), 5730-5753*

# SWATH: Full MS/MS Archive of Every Compound in 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*

# 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