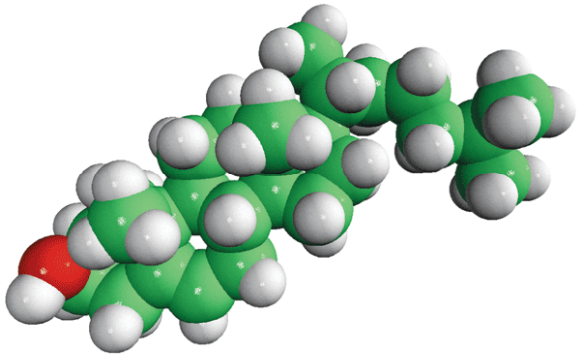
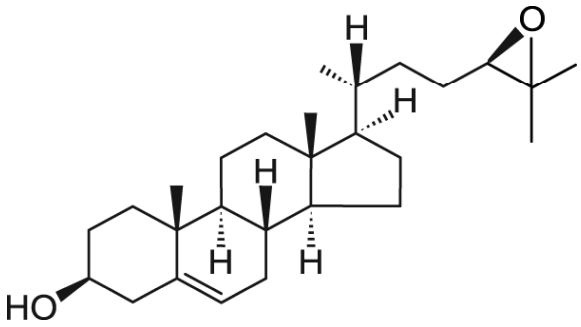
LIPID MAPS Lipidomics Workshop

April 28, 2007

www.lipidmaps.org

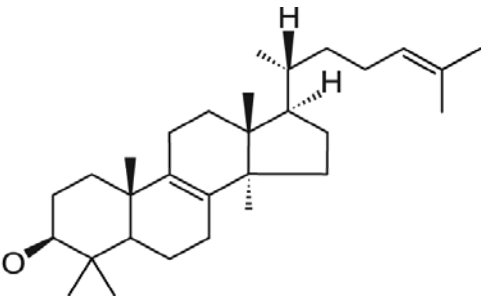




Sterols

Jeff McDonald

Molecular Genetics
The University of Texas Southwestern Medical Center
At Dallas

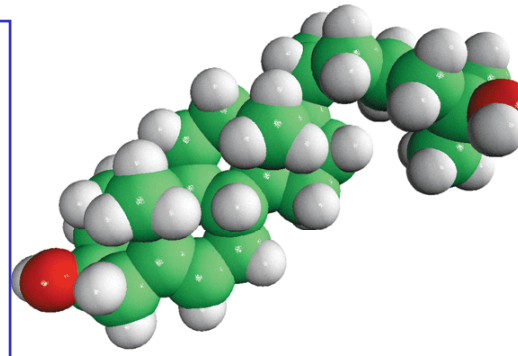


Other LIPID MAPS Sterol Core members:

David Russell

Bonne Thompson, David Bauman, Erin McCrum

NIH GM-069338

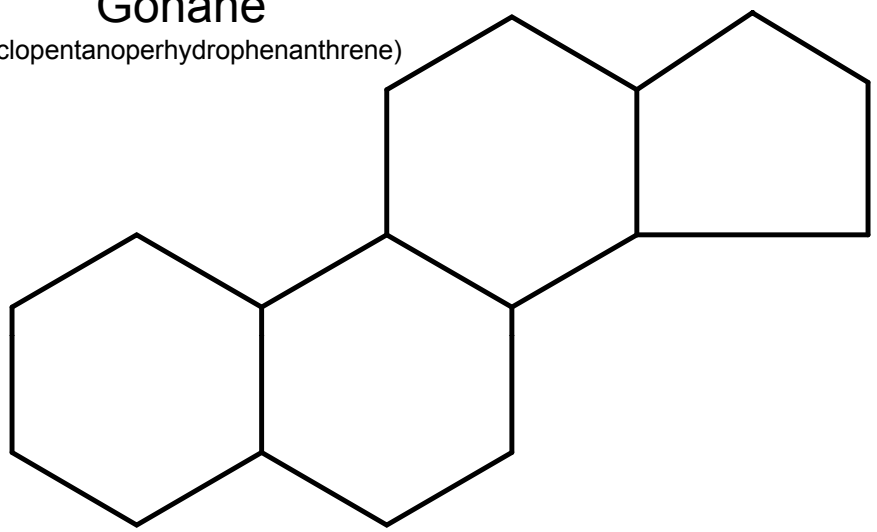


Outline:

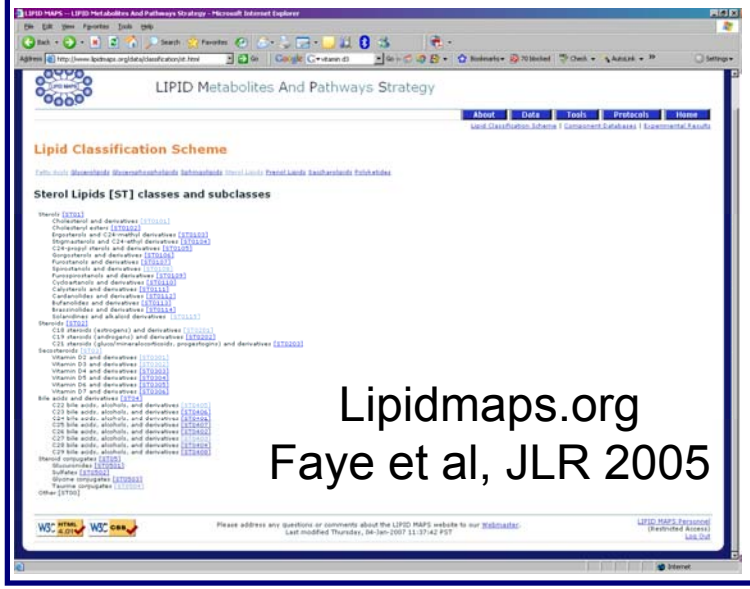
A. Brief introduction to the lipid class: nomenclature & range of compounds to analyze
B. Sample preparation issues: solvents, chromatography, recovery, reproducibility
C. Compound identification: HPLC MS/MS (MRM); GC/MS
D. Quantitation: Internal standards, etc.
E. Data analysis/visualization: LIMS, Website, Marker View, other.
F. Comparison of Lipid MAPS methods with others in the literature
G. Remaining challenges and opportunities
H. Discoveries from Sterol analysis thus far

Nomenclature and Range of Compounds to Analyze

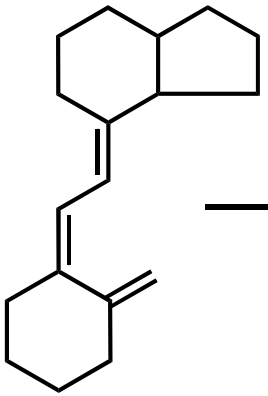
Gonane
(cyclopentanoperhydrophenanthrene)



- Sterols → (Cholesterol)
- Steroids → (Testosterone)
- Bile Acids → (Cholic acid)
- Steroid Conjugates → (Cholesterol sulfate)

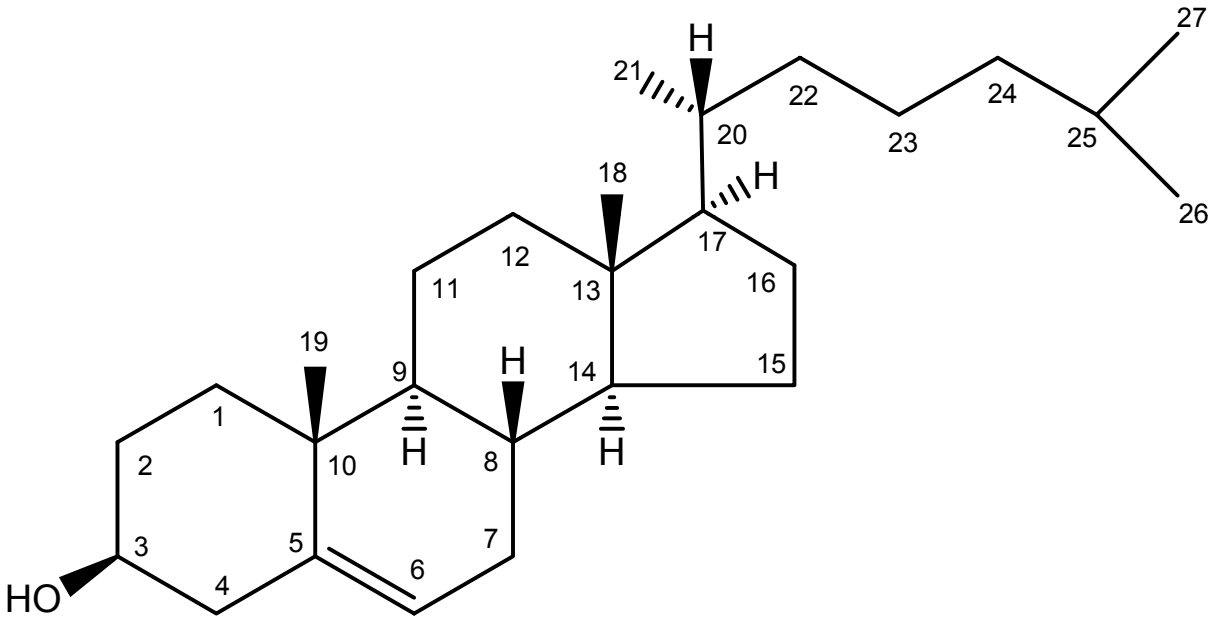


Lipidmaps.org
Faye et al, JLR 2005

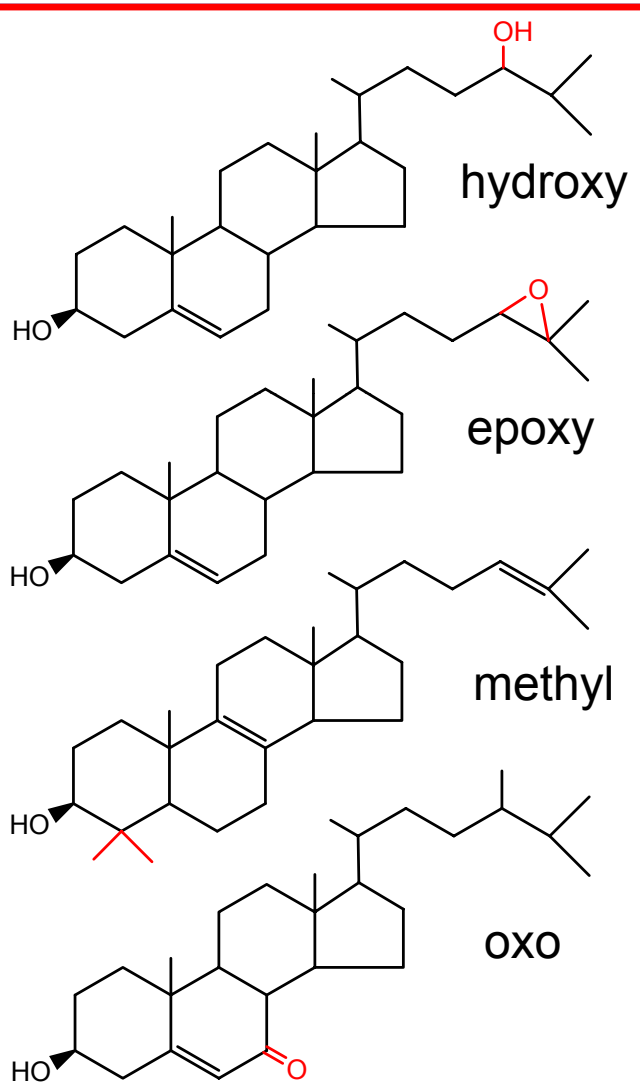


- Secosteroids → (Vitamin D3)

Nomenclature and Range of Compounds to Analyze

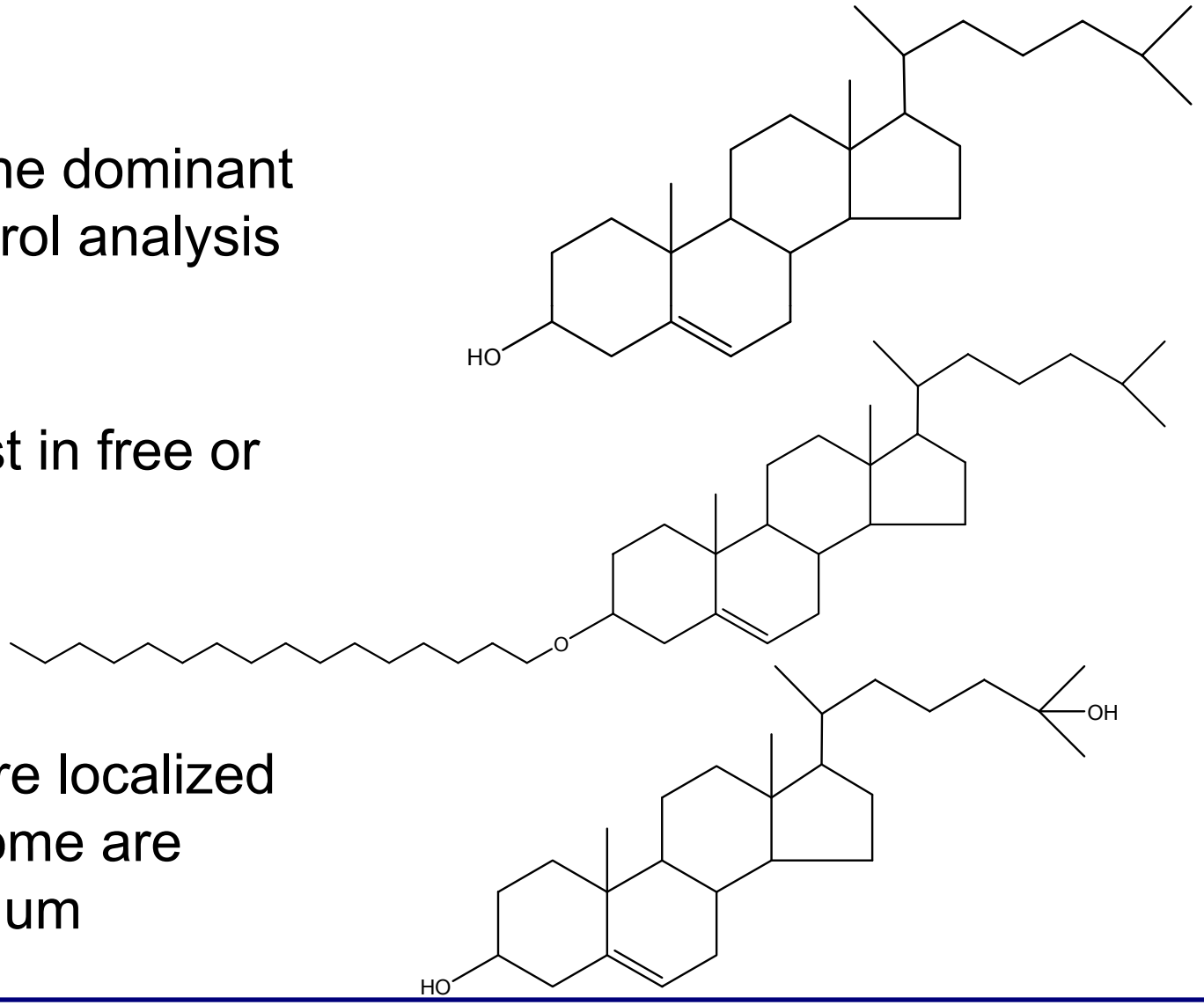


cholest-5-en-3b-ol
Cholesterol

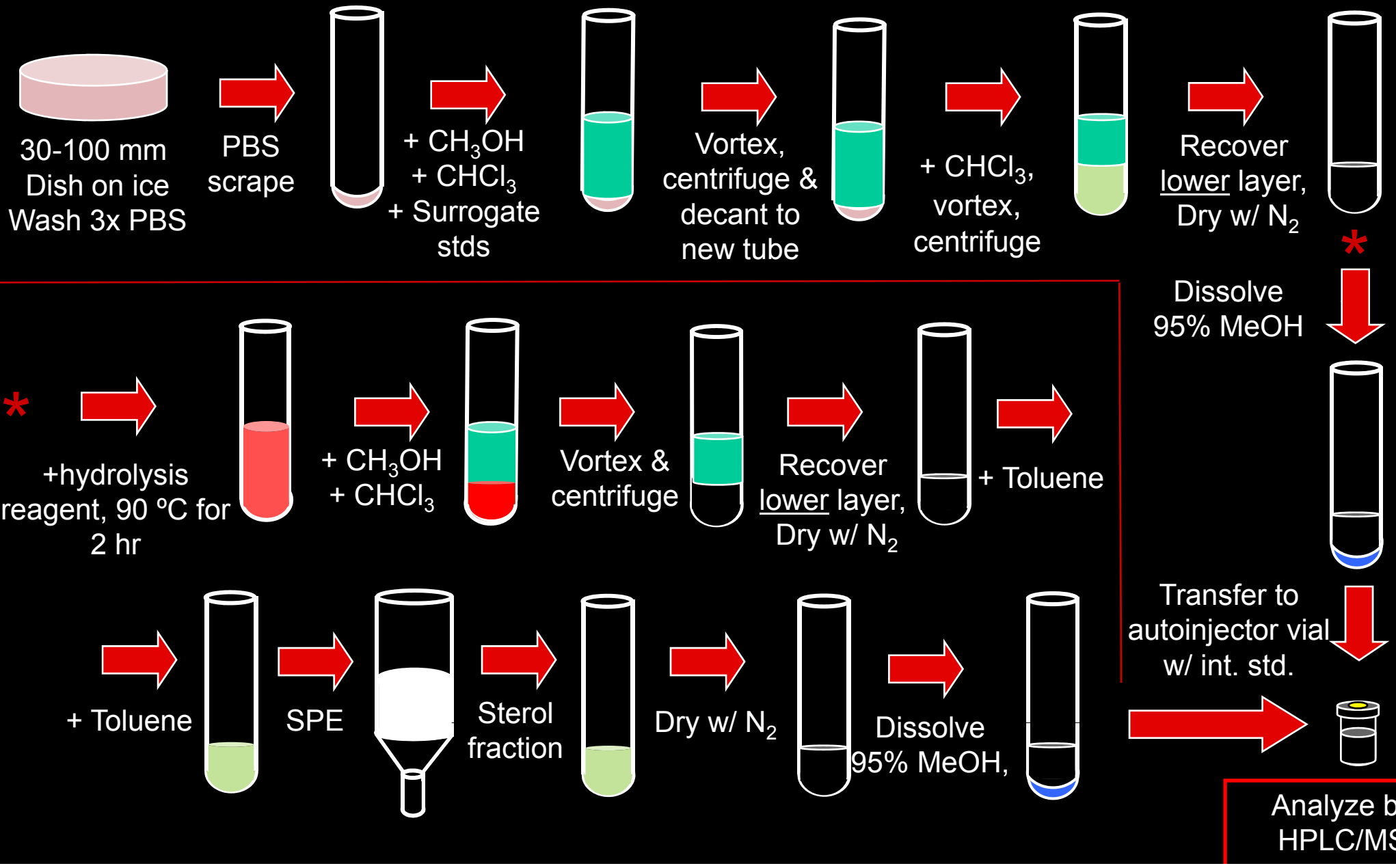


Nomenclature and Range of Compounds to Analyze

- Cholesterol is the dominant species in a sterol analysis
- Sterols can exist in free or esterified forms
- Many sterols are localized in cells only; some are located in medium



Sample Preparation: Extraction of Sterols



Sample preparation issues: solvents, chromatography, recovery, reproducibility

- Only saponify and perform SPE if necessary
  - SPE increases oxidation of cholesterol
  - Introduces “plastics” into samples
  - Increases time and effort
  - Columns may be used to process complex samples

- Extraction efficiency for basic extraction ~75%
- Lower phase is “organic” phase
- Dry solvents are necessary

See McDonald et al. In *Methods In Enzymology* (forthcoming) for detailed description of extraction methods

Compound identification: HPLC MS/MS; GC/MS

Reverse Phase HPLC

- Phenomenex Luna C_{18} (250 × 2 mm; 3 µm)
- A: 85% MeOH

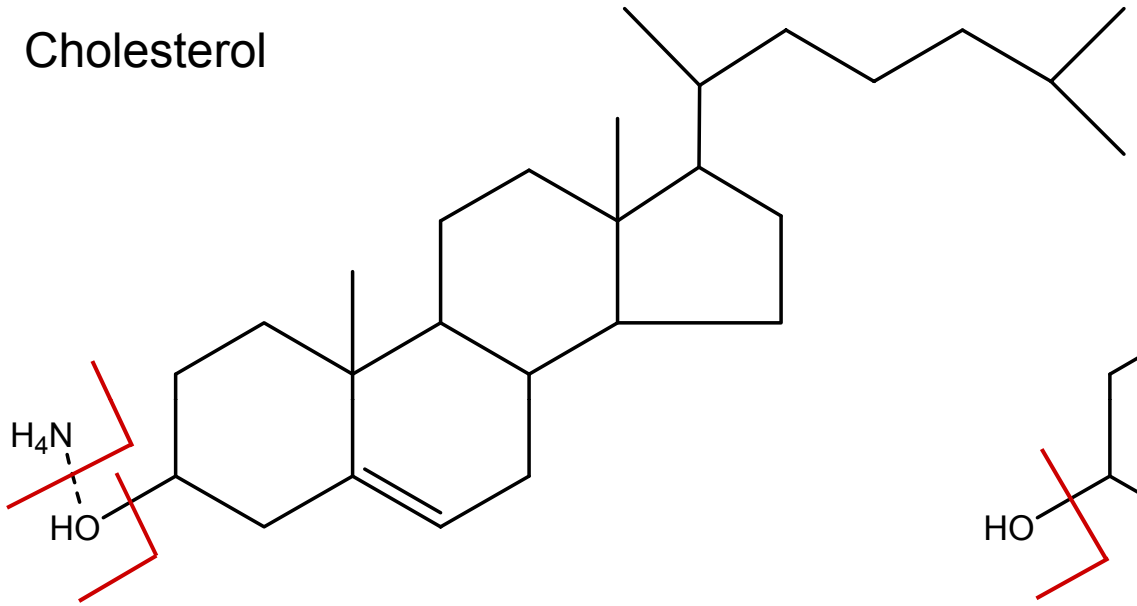
  B: 100% MeOH

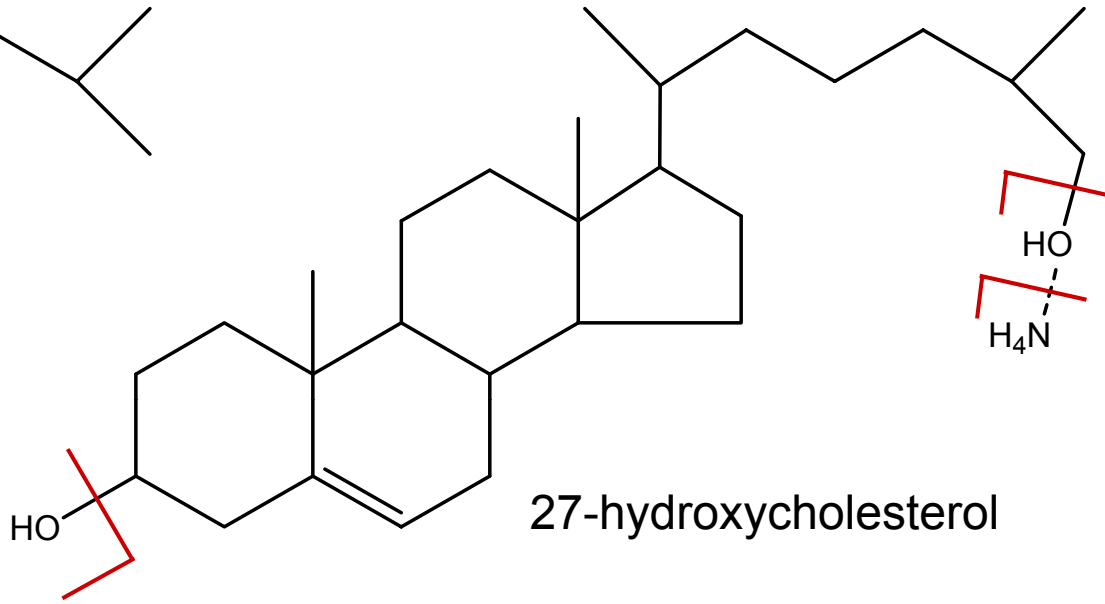
  (both with 5mM NH_4acetate)
- 30 ºC column temp
- 0.25 mL/min, 1 min 100%A to 100%B 15 min, 100%B 10 min, 100%A 5 min

Mass Spectrometry

- Applied Biosystems 4000QTrap
- TurboV Electrospray Ion Source
- Positive (+) mode
- Multiple Reaction Monitoring
- Optimized collision energy, declustering potential

Compound identification: HPLC MS/MS; GC/MS





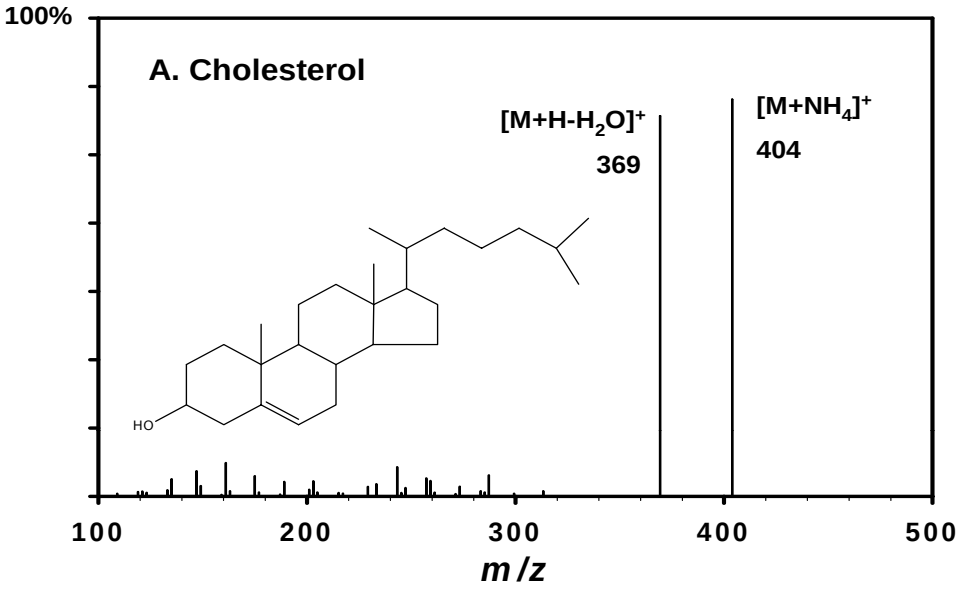
27-hydroxycholesterol

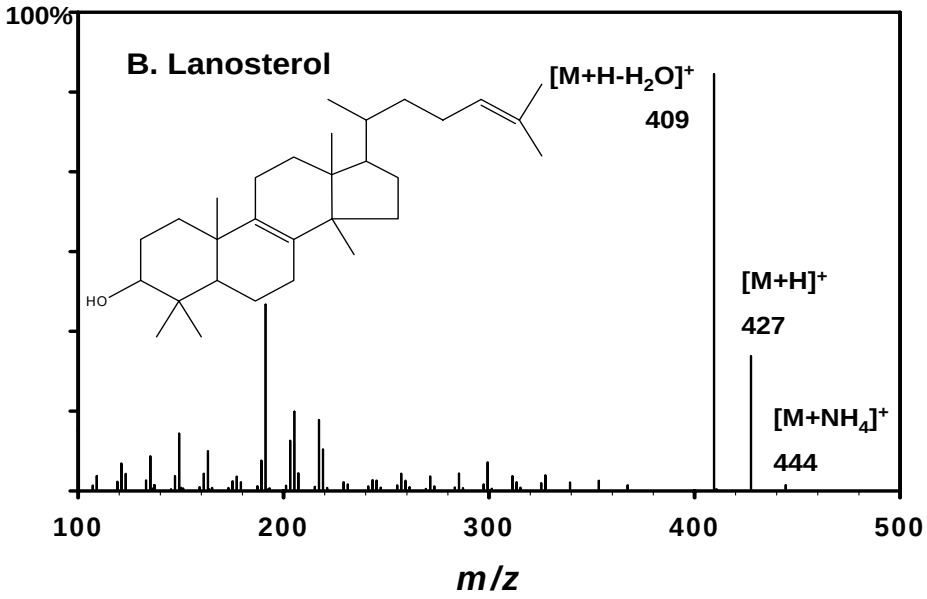
Common Ions Observed by Scan (in-source decay) and Product Ion

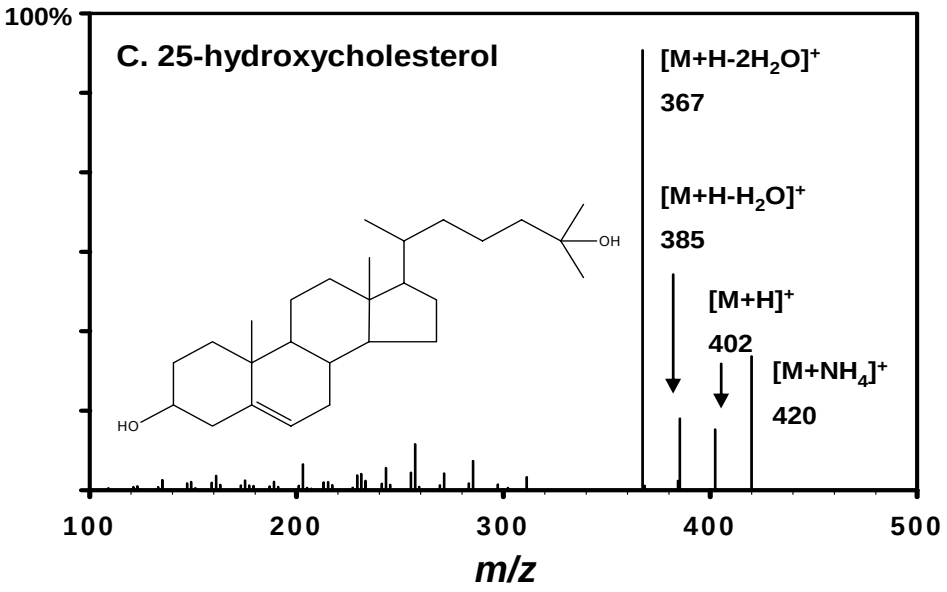
$[M+NH_4]^+$ $[M+NH_4-NH_3]^+$ (M+H) $[M+H-H_20]^+$ $[M+NH_4-H_20]^+$?	$[M+NH_4]^+$ $[M+NH_4-NH_3]^+$ (M+H) $[M+H-H_20]^+$ $[M+H-2H_20]^+$ $[M+NH4-H20]^+$?

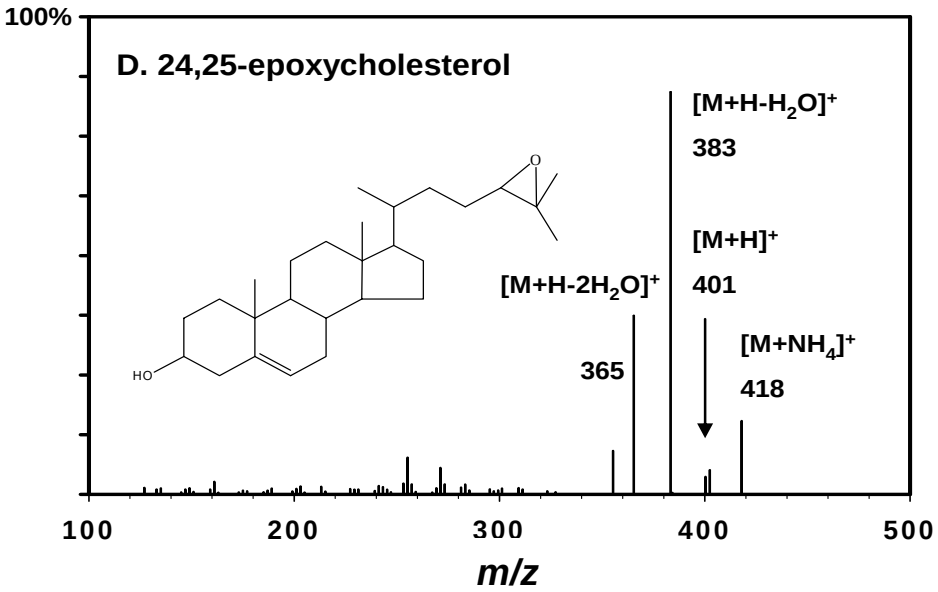
No abundant disassociation products beyond loss of waters

Compound identification: HPLC MS/MS (MRM); GC/MS

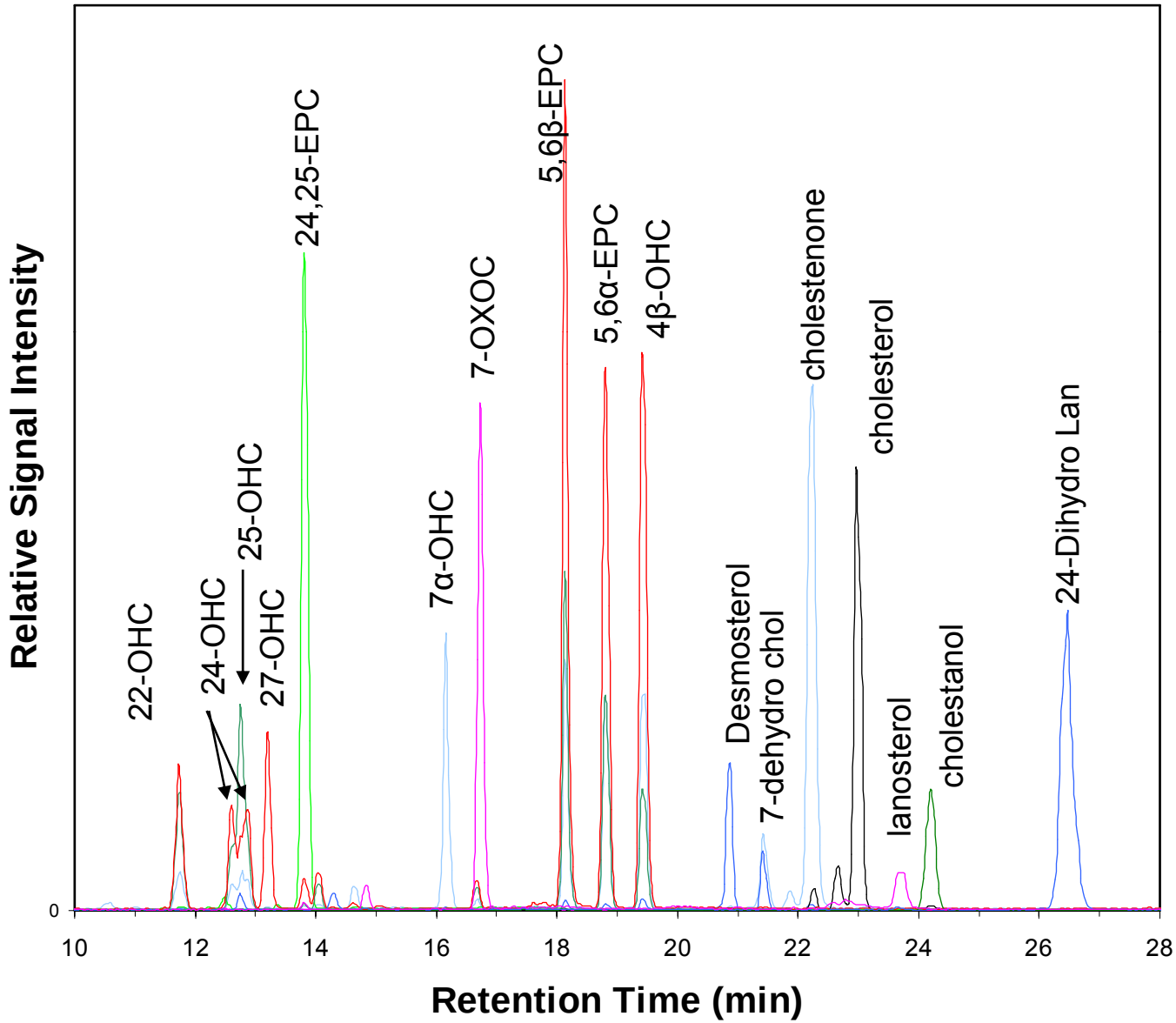








Compound identification: HPLC MS/MS (MRM); GC/MS

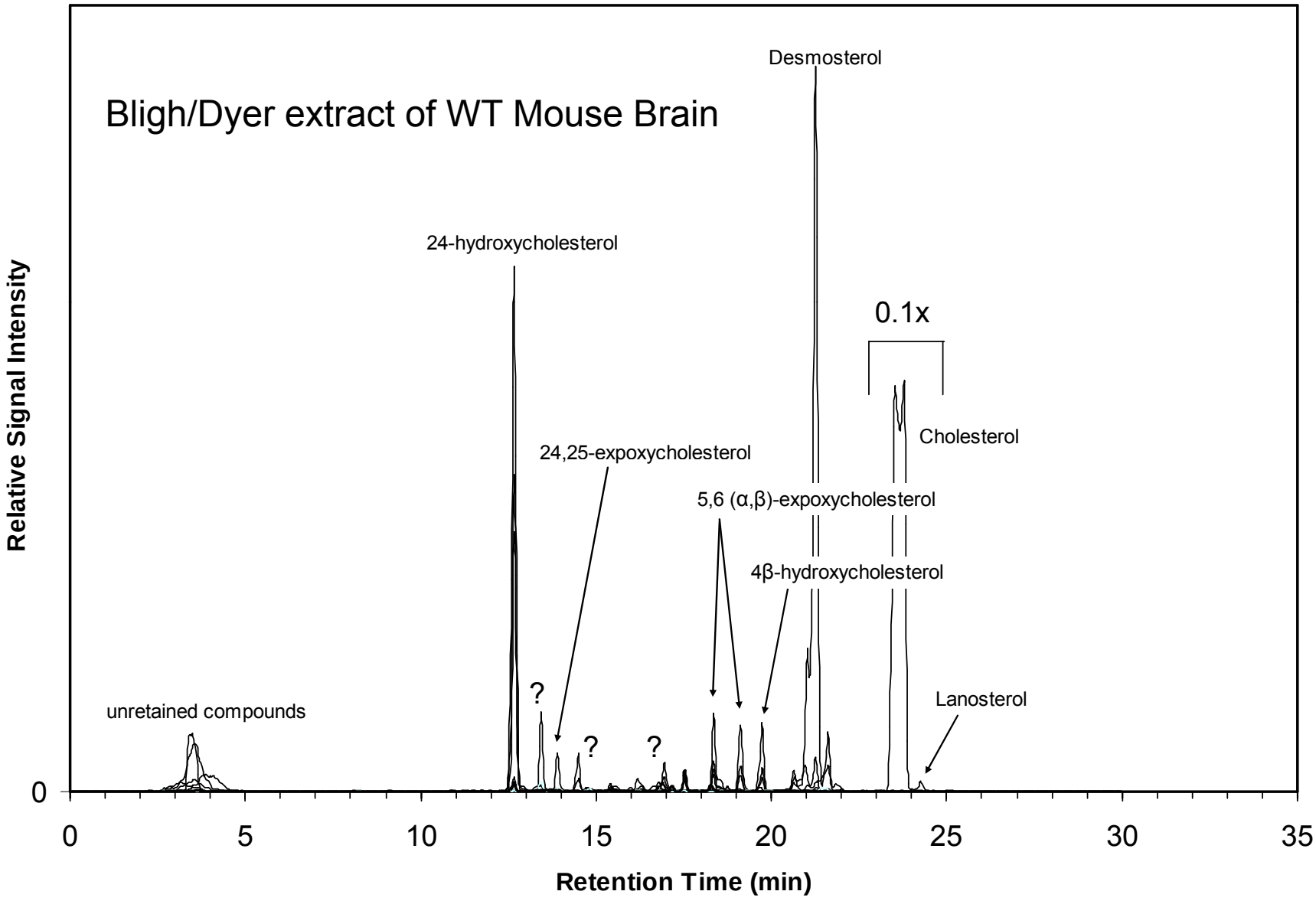


- Multiple Reaction Monitoring
- Most sterols well-resolved
- Peak widths between ~15 and 20 s @ FWHM

LOD

- 5-100 fmol on-column for oxysterols
- 200-2000 fmol on-column for sterols

Compound identification: HPLC MS/MS (MRM); GC/MS



Quantitation: Internal standards, etc.

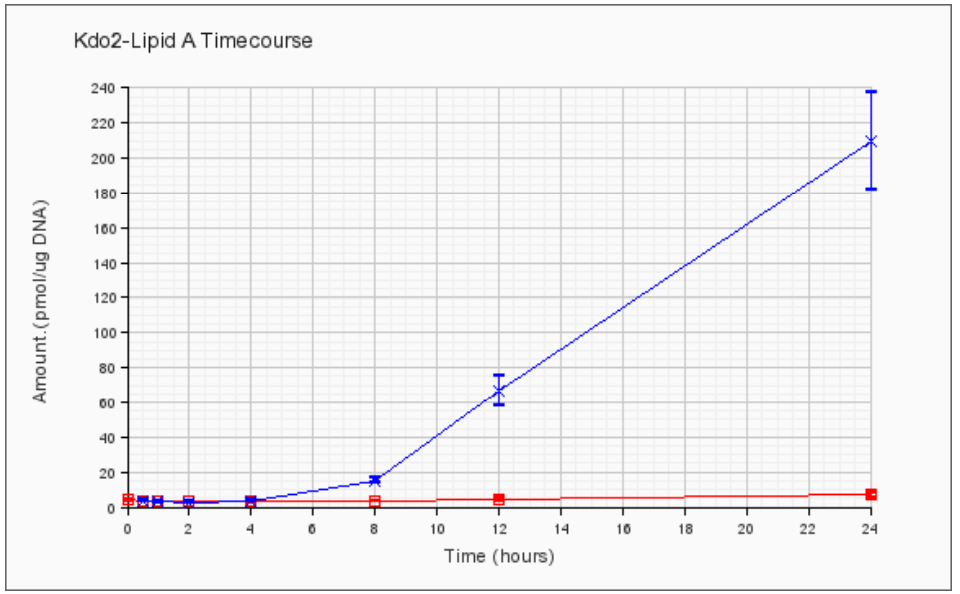
COMMON NAME	MW	SOURCE
22*R*-hydroxycholesterol	402.7	Sigma Aldrich
24-hydroxycholesterol(*R*+*S*)	402.7	Avanti Polar Lipids
25-hydroxycholesterol	402.7	Avanti Polar Lipids
27-hydroxycholesterol-(25*R*)	402.7	Avanti Polar Lipids
24,25-epoxycholesterol	400.6	Avanti Polar Lipids
7α-hydroxycholesterol	402.7	Avanti Polar Lipids
7-ketocholesterol	400.6	Avanti Polar Lipids
5,6β-epoxycholesterol	402.7	Avanti Polar Lipids
5,6α-epoxycholesterol	402.7	Avanti Polar Lipids
4β-hydroxycholesterol	402.7	Avanti Polar Lipids
Zymosterol	384.7	*
Desmosterol	384.7	Avanti Polar Lipids
7-dehydrocholesterol	384.7	Sigma Aldrich
Cholestenone	384.7	Avanti Polar Lipids
Lathosterol	386.7	Steraloids
Cholesterol	386.7	Avanti Polar Lipids
Lanosterol	426.7	Steraloids
Cholestanol	388.7	Avanti Polar Lipids
24-dihydrolanosterol	428.7	Avanti Polar Lipids

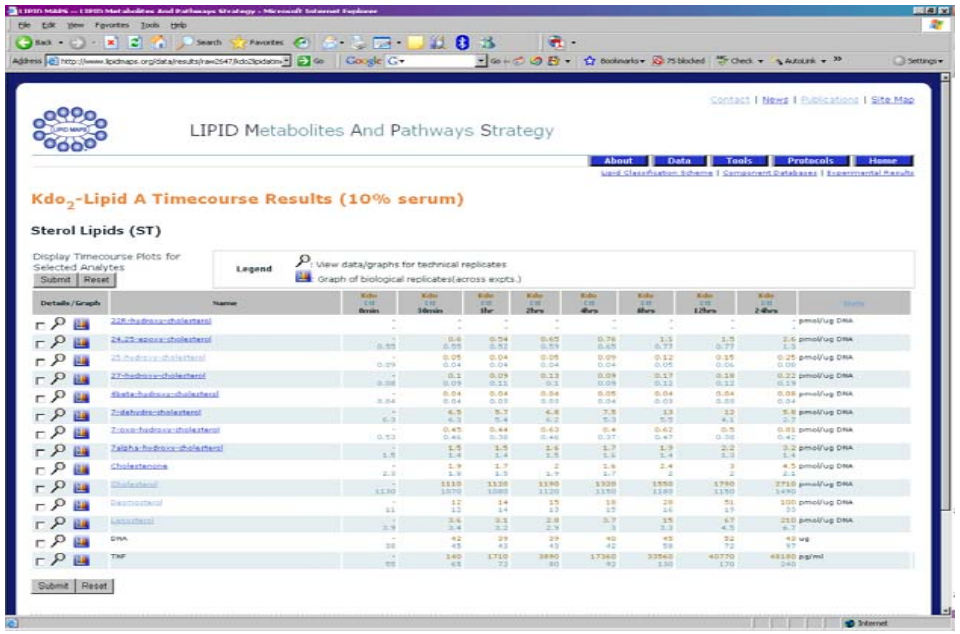
* = not available at this time

- **BOLD** = deuterated analog available from Avanti
- Avanti Polar Lipid Standards
  - Extensive characterization
    - NMR, LC, MS
    - On-going stability
- Additional deuterated and primary standards in progress
- www.avantilipids.com

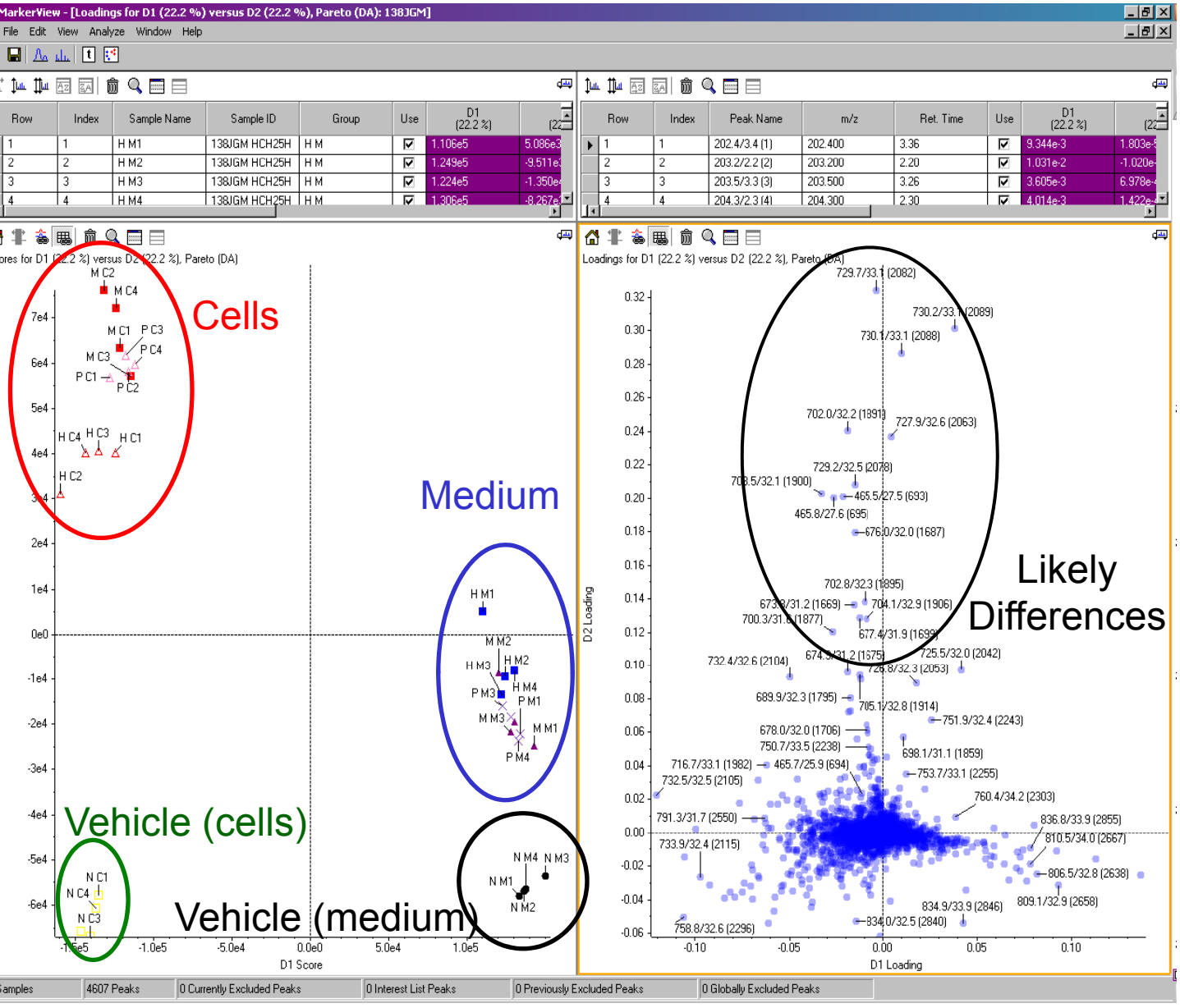
Data analysis/visualization: LIMS, Website, Marker View, other

- Quantitation by isotope dilution with known standards
- Data normalized to mass of DNA
- Time course data for all sterols measured located at:
  - lipidmaps.org





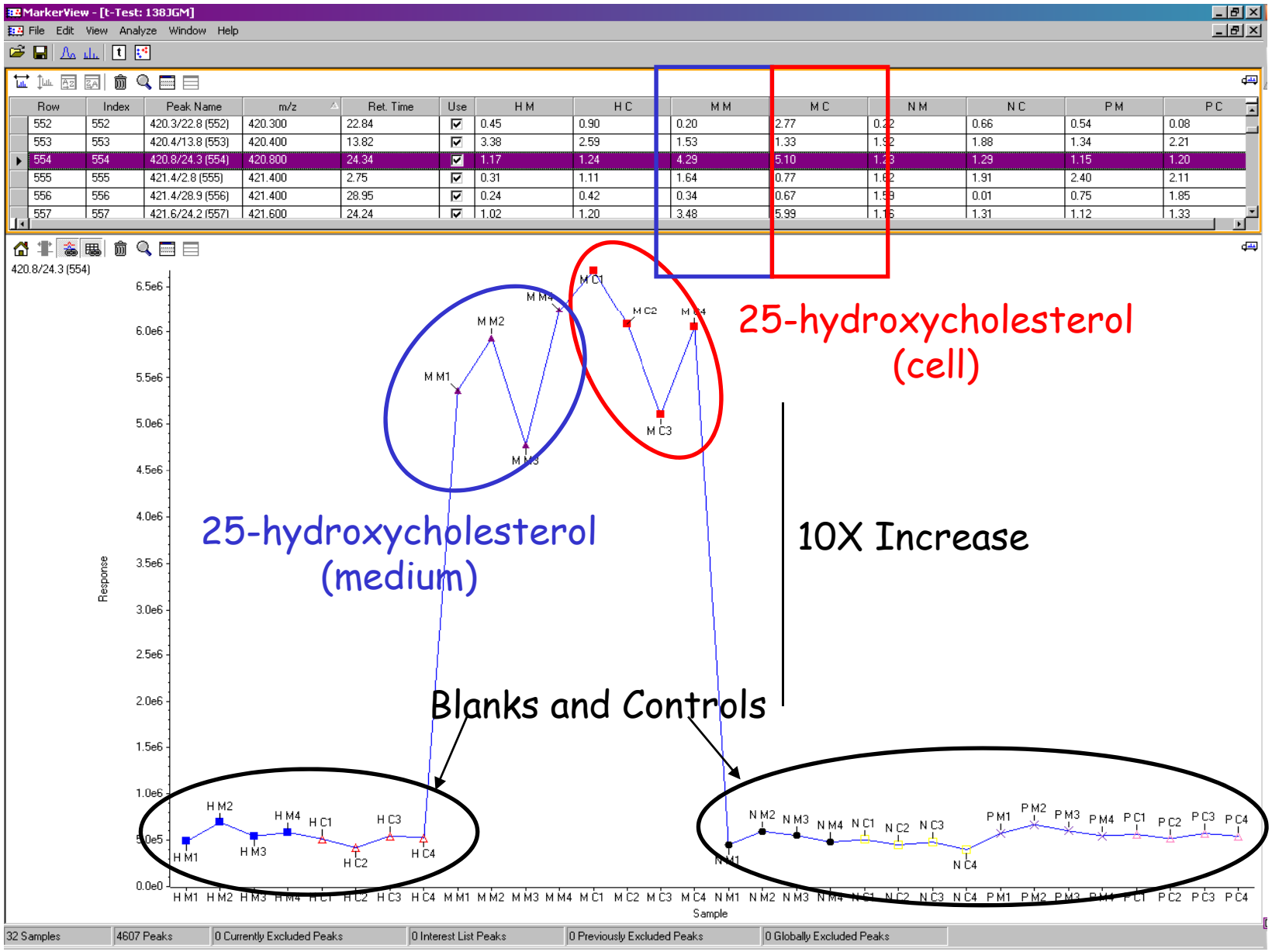
Data analysis/visualization: LIMS, Website, Marker View, other



Multivariate analysis

- Principal component and statistical analysis of LC/MS data for changes
- Compare samples and ask “what’s different?”
- MarkerView, Metabolynx, MZMine, XCMS
  - Cravatt et al
- Unbiased, global profiling
- Products/substrates of (orphan) enzymes
- Identifying potential unknowns

Data analysis/visualization: LIMS, Website, Marker View, other



Comparison of Lipid MAPS methods with others in the literature

Gas-Chromatographic Mass Spectrometry (GC-MS)

Pros

- Cost
- Superior chromatographic resolution
- Fragmentation
- Compound databases
- Established methods
- Good signal intensities

Cons

- Rigorous sample clean-up
- Samples require derivatization
- Limited injection volume
- Run time
- Generally limited to electron impact (EI), single quadrupole MS

Sterol analysis ideally utilizes both LC-MS and GC-MS

Remaining challenges and opportunities

Identification of Novel Sterols

- No precursor, product, or neutral loss scans for native sterols
  - Derivitize
    - Griffiths, Sjövall, and company
- GC/MS (EI) lacks sensitivity and flexibility
- Supplement with labeled (^{2}H, ^{13}C) substrates and monitor mass shift
  - Acetate, mevalonate
  - Collect fractions, analyze with Triversa NanoMate (nanospray)
- Use predicted MRM pairs
  - ~75 MRM pairs covers most logical sterols
- Continue to increase compound library by acquiring standards

Discoveries from sterol analysis thus far