METABOLOMICS

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Stanford Mass Spectrometry
Users' Meeting
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Stanford University
MASS SPECTROMETRY

Types of Experiments in Metabolomics

targeted		non-targeted
quantitative	semi-quantitative	
• Number of analyzed metabolites is limited by the number of available ***standards*** • ***Absolute quantitation*** of metabolites (nM, mg/mL)	•Number of analyzed metabolites is limited by the number of available ***library spectra*** • ***Relative quantitation*** of metabolites (fold)	•Number of analyzed metabolites is limited by capacity of ***analytical instrumentation*** • ***Relative quantitation*** of metabolites (fold)

Profiling of Amino Acids: Overview of Mass Spectrometric Methods

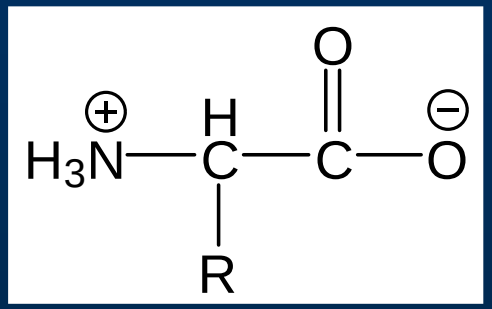
Advantages:

- Low detection limits especially important if sample size is limited or for rare AAs (sub nM LODs)
- High precision if isotopically labeled internal standards are used
- High selectivity of hyphenated LC-MS and GC-MS method

? Can derivatization be omitted?

Profiling of Amino Acids: Derivatization in Mass Spectrometry

Reasons to keep using derivatization:



- **Chromatography**

  Non-derivatized AAs are not volatile for GC

  For LC, non-derivatized AAs ideally require ion exchange or ion pairing LC, which are not compatible with the most common liquid interfaces (ESI and APCI)

- **Detection**

  Loss of ampholytic properties improves ionization

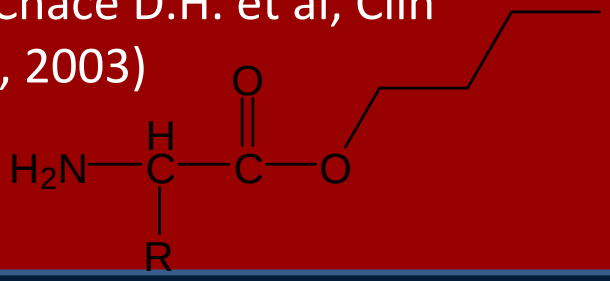
  Increase in molecular mass of analytes after derivatization elevates them over the background chemical noise

Derivatization of Amino Acids

LC-MS

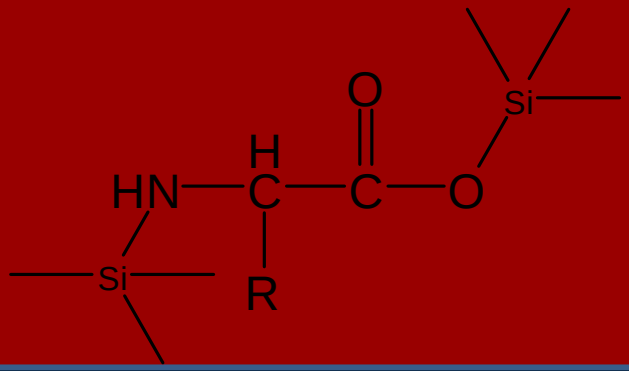
0) <u>no derivatization</u>
Ion pairing (Piraud M et all, RCMS, 2005) or HILIC

1) <u>n-butylation</u>
acidified butanol, for 15 min at 65°C (Chace D.H. et al, Clin Chem, 2003)



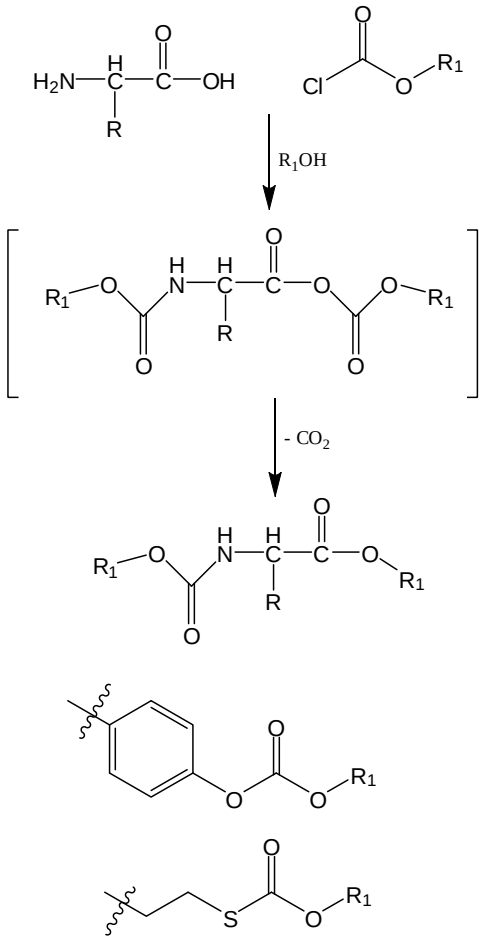
GC-MS

<u>silylation</u>
Ideally, 2 h at 150 °C
Some protocols use 15-30 min at 37-60 °C (1 TMS per NH2)



Alkyl Chloroformates

Derivatization of Amino Acids: Alkyl Chloroformates



- 1 min reaction at room temperature

- Chloroformates react with amino-, carboxygroups as well as thiols (Cys) and phenols (Tyr)

- Applicable both for routine GC-MS detection (*Kaspar H et all, J Chrom B, 2008*) and LC-MS trace analysis

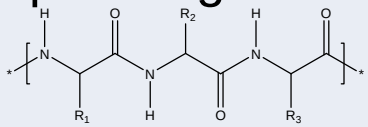
Profiling of Amino Acids: Applications

2-2:30 pm: Mass spectrometric profiling of airway fluid reveals peripheral metabolic defect in cystic fibrosis patients.
Rabin Tirouvanziam, *Dept. of Pediatrics*

TALK CANCELED

2-2:30 pm: Integrative Genomics: Towards Understanding Our Drugs and Diseases.
Gary Peltz, *Dept. of Anesthesia*

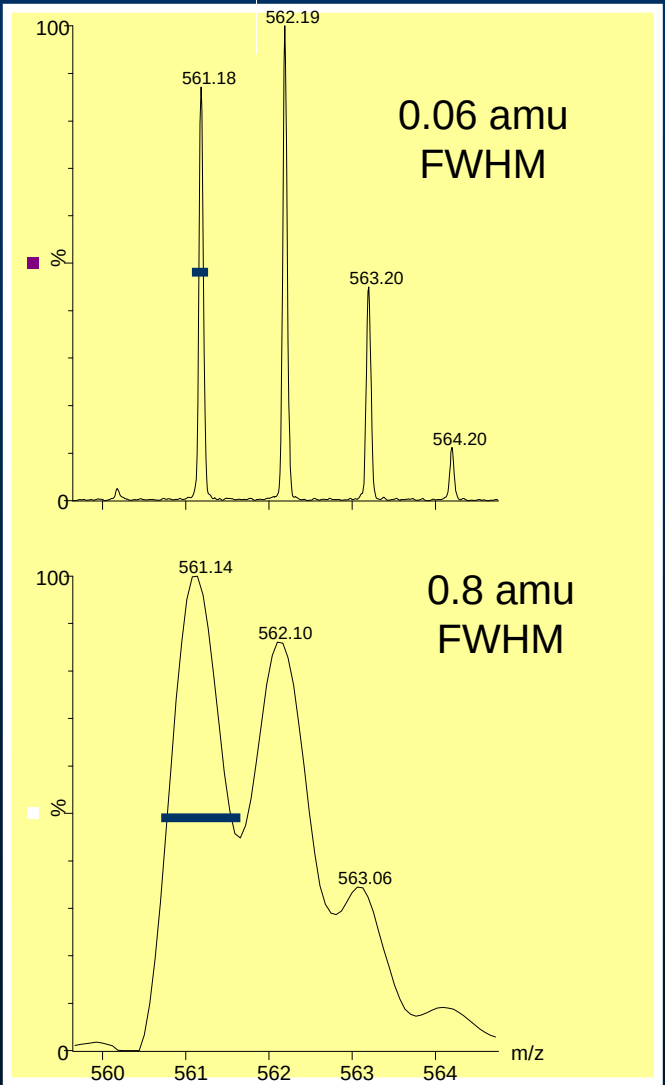
Identification in Metabolomics

	Proteomics	Metabolomics
Identification	**Well established** sequencing  PTMs still a challenge	**Under development** Huge diversity of structures, NMR often required Moderate success with mass spectral libraries

- **Peptide** structure is sequential, MS/MS experiments are usually sufficient (ion traps).

- Typical MS/MS does not break all bonds in **metabolites**; *accurate mass measurements* provide more information than MS/MS. Ideally both experiments are required.

High Resolution



High Resolution: R = 561/0.06 ~ 9,000

TOF: 7,000-50,000
Orbitrap: 10^4-10^5
FT ICR: 10^5-10^6

Nominal Mass Resolution (<1000)
R = 561/0.8 ~ 700

Accurate mass measurement is possible without high resolution. High resolution improves precision of mass measurement.

Determination of Elemental Composition from Accurate Mass

^{1}H	1.0078 u
^{12}C	12.0000 u
^{14}N	14.0031 u
^{16}O	15.9949 u

What is 28 u?

N_2 (2 x 14 u), CO (12 u + 16 u) or C_2H_4 (2 x 12 u + 4 x 1 u)?

What is 28.0313 u? [high accuracy]

C_2H_4 (2 x 12.0000 u + 4 x 1.0078 u)

Kind T. et al, BMC Bioinformatics, 2007

Mass Defect

^{1}H ($p+e^{-}$)	1.0078 u
^{12}C	12.0000 u
^{14}N	14.0031 u
^{16}O	15.9949 u
n	1.0087 u

Carbon-12: 6 protons, 6 neutron and 6 electrons

6 x 1.0078 u + 6 x 1.0087 u = 12.0990 u

Mass Defect = 12.0990 u – 12.0000 u = 0.0990 u

$$E = mc^2$$

0.1 u = 93 MeV

Mass Excess

^{12}C	12.0000 u
^{14}N	14.0031 u
^{16}O	15.9949 u

Mass Excess defined by $\Delta = m_a - Am_u$,
***where m_a is the mass* of the atom,**
A the number of nucleons,
***and m_u the unified* atomic mass constant (m_u = *1 u*).**
(CRC handbook of chemistry and physics)

Sample Preparation

1. Protein Precipitation or Filtration (3 kDa filters)

- *Prevents column clogging*
- *Removes signal from small proteins*

Want E.J. et al, Anal Chem, 2006
Polson C. et al, J Chrom B, 2003

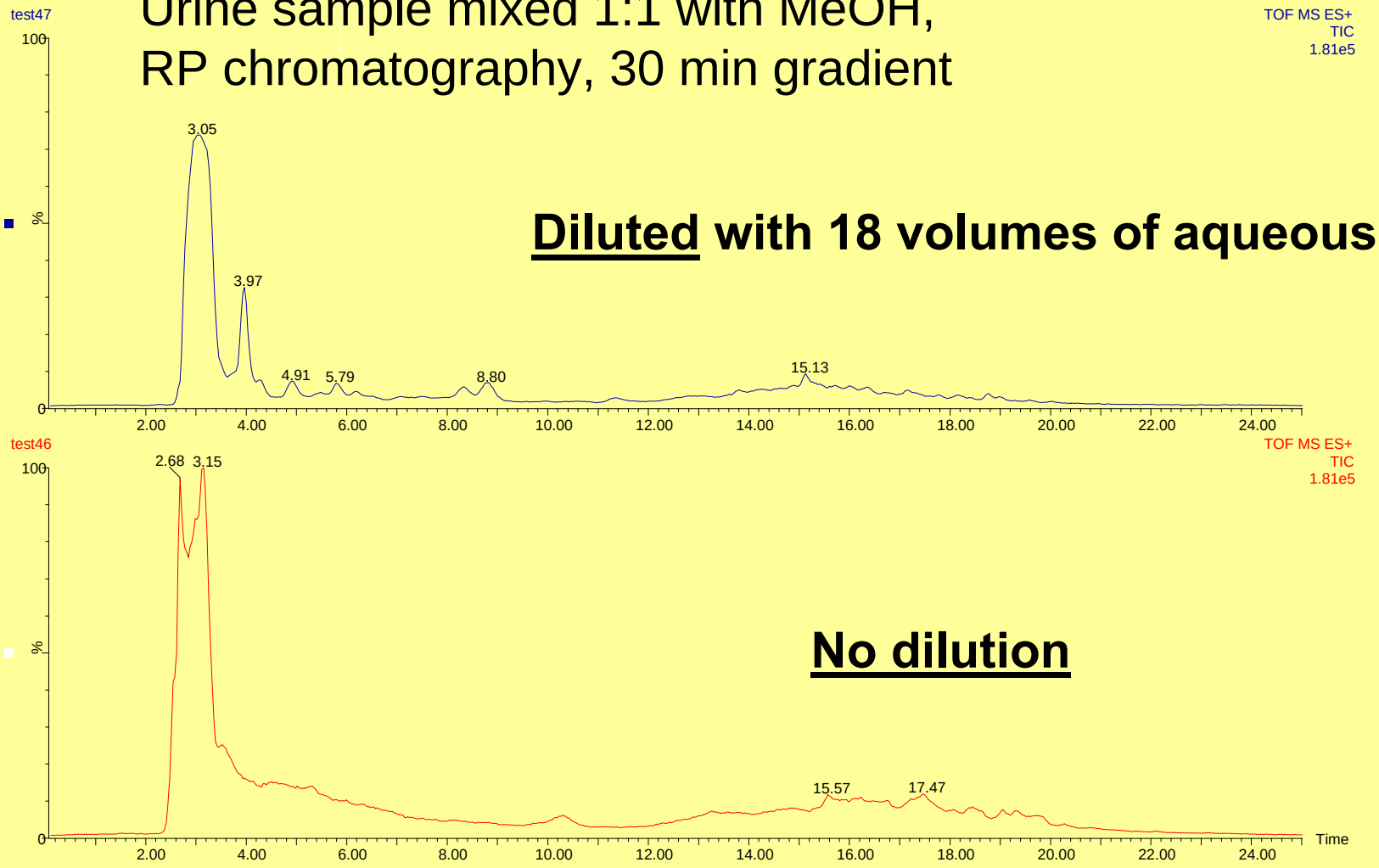
2. Dilute and Shoot

- *Makes sample less hydrophobic for reverse phase chromatography*
- *Prevents column saturation*
- *Decreases ion suppression (electrospray)*
- *Shifts signal to linear regime of detector*

Effect of Sample Dilution (TIC)

same scale of Y-axis

Urine sample mixed 1:1 with MeOH,
RP chromatography, 30 min gradient

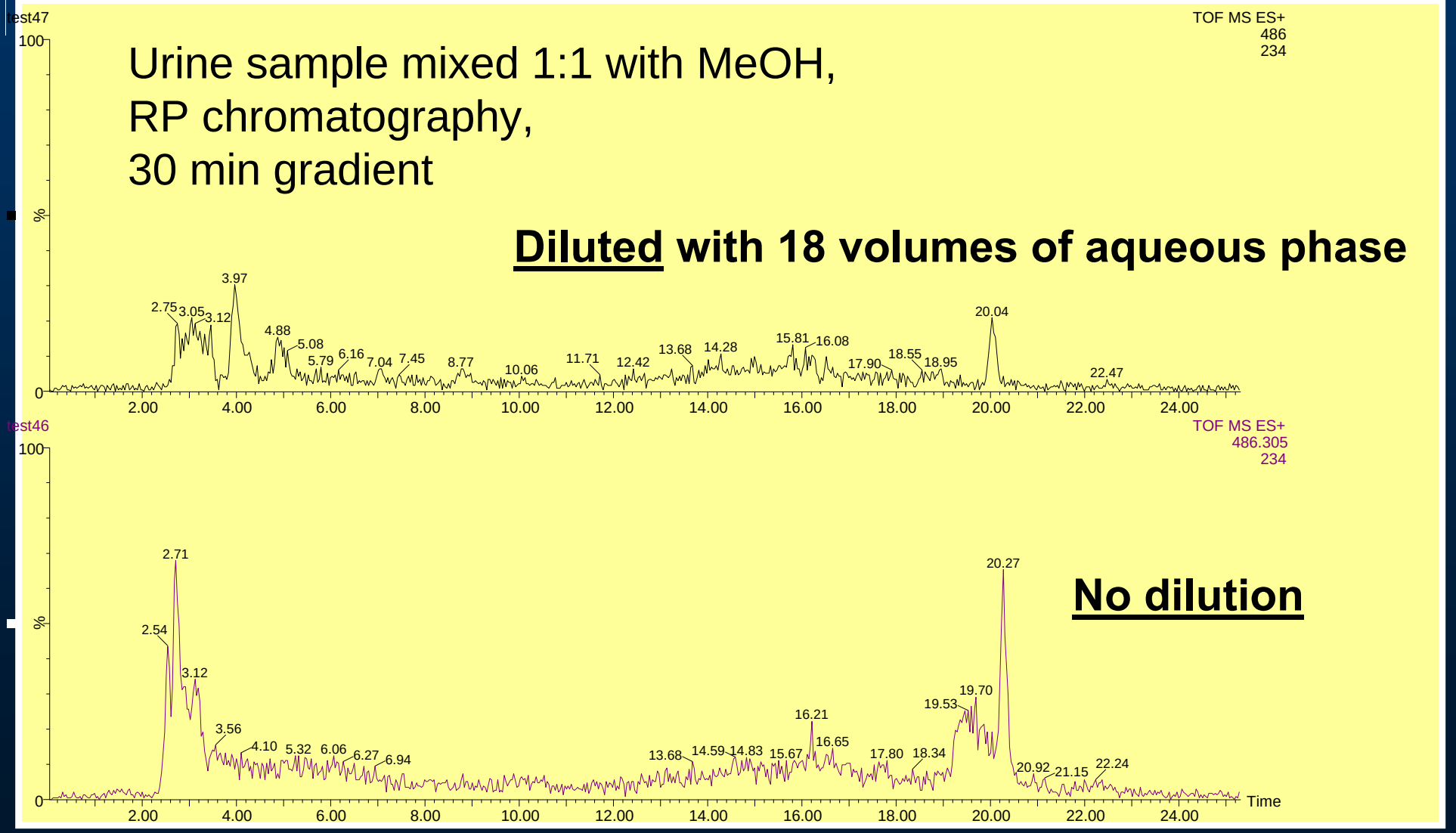


Diluted with 18 volumes of aqueous phase

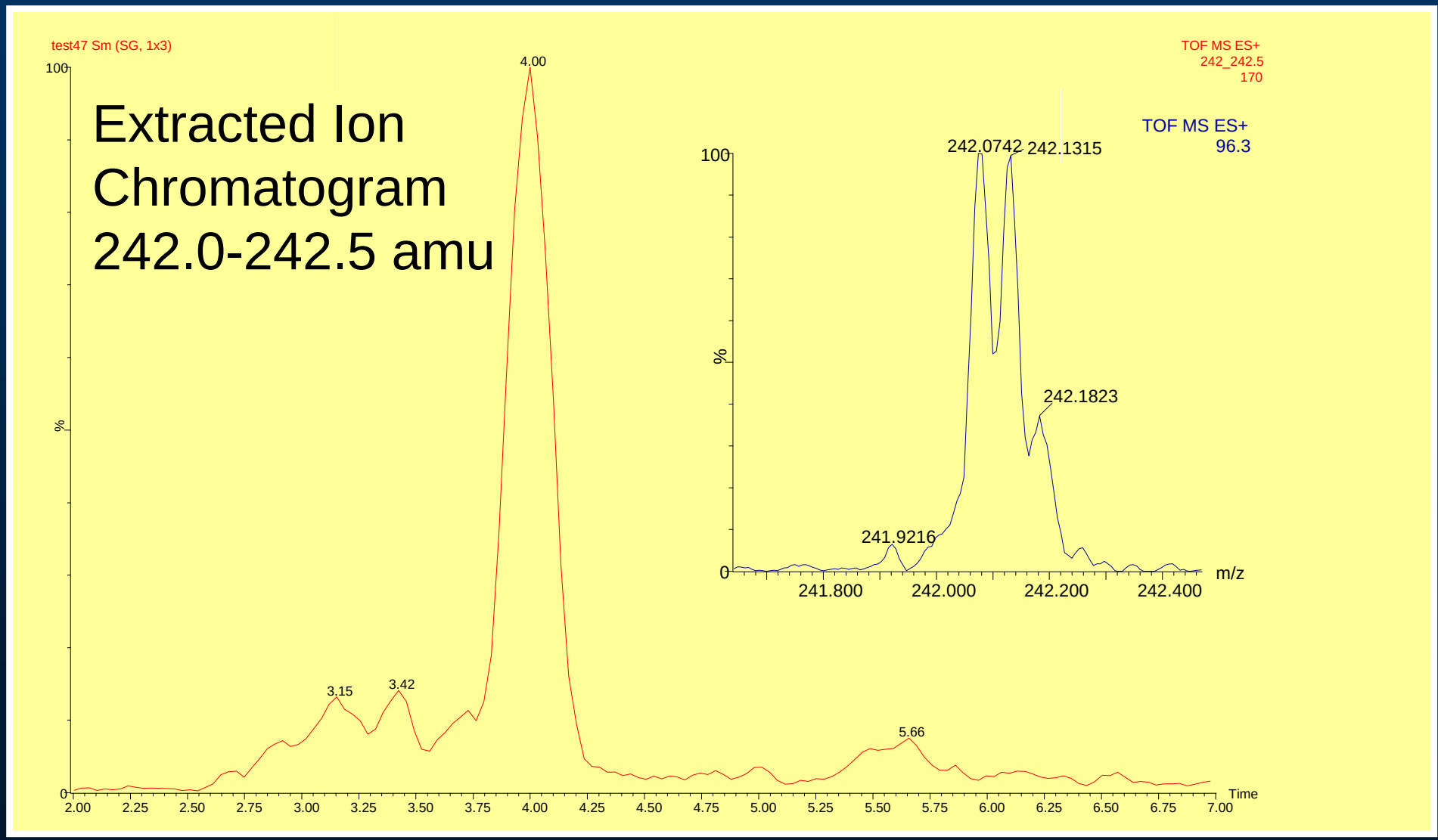
No dilution

Effect of Sample Dilution (EIC)

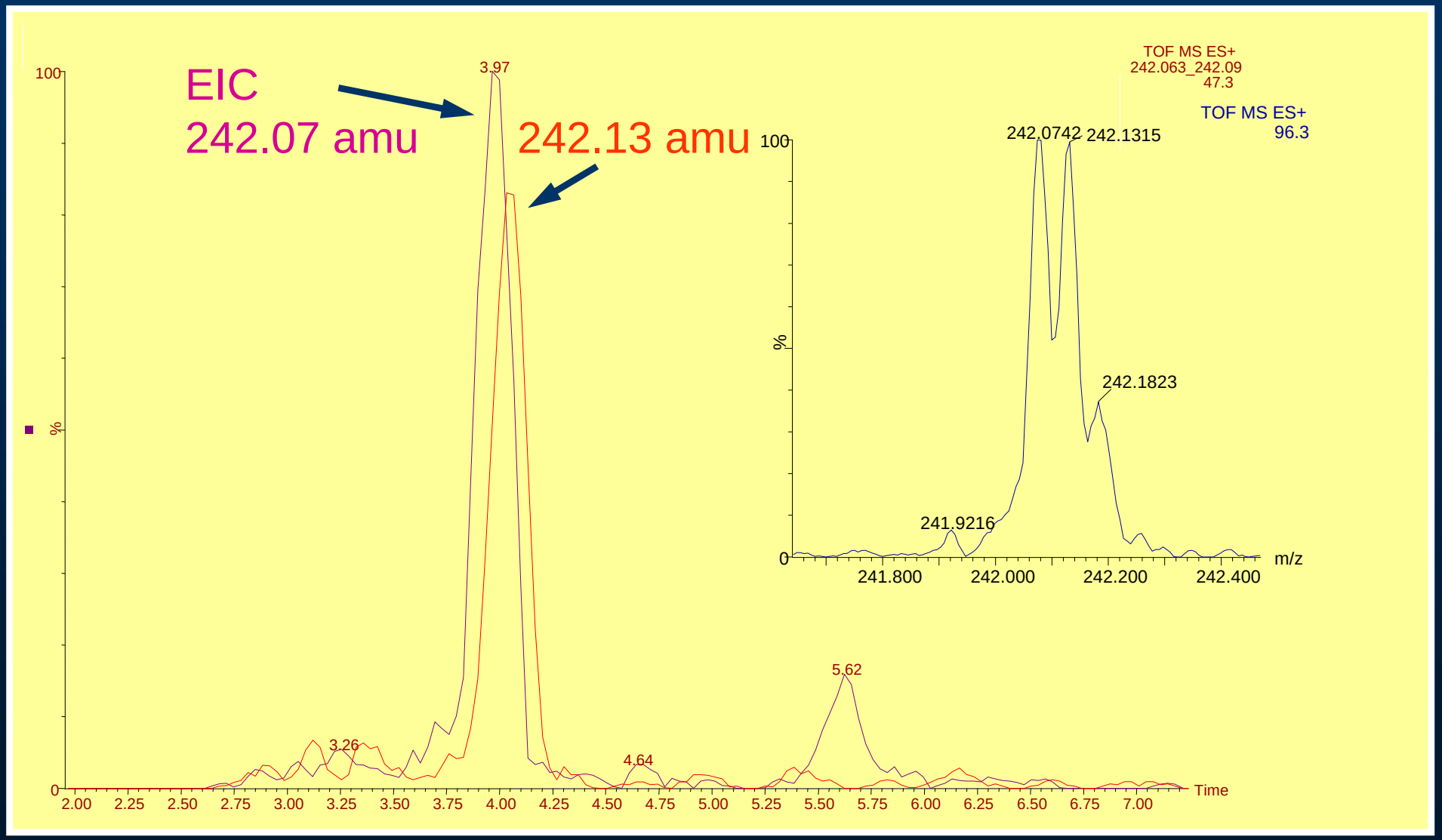
same scale of Y-axis



Advantage of High Resolution MS



Advantage of High Resolution MS



Advantages of High Resolution MS

1. **Provides additional means of distinguishing compounds, especially if chromatographic separation is not sufficient**

2. **Determines putative elemental composition of unknowns**

Combination of Separation Modes and Ionization Techniques

Separation modes:

Reversed phase and HILIC

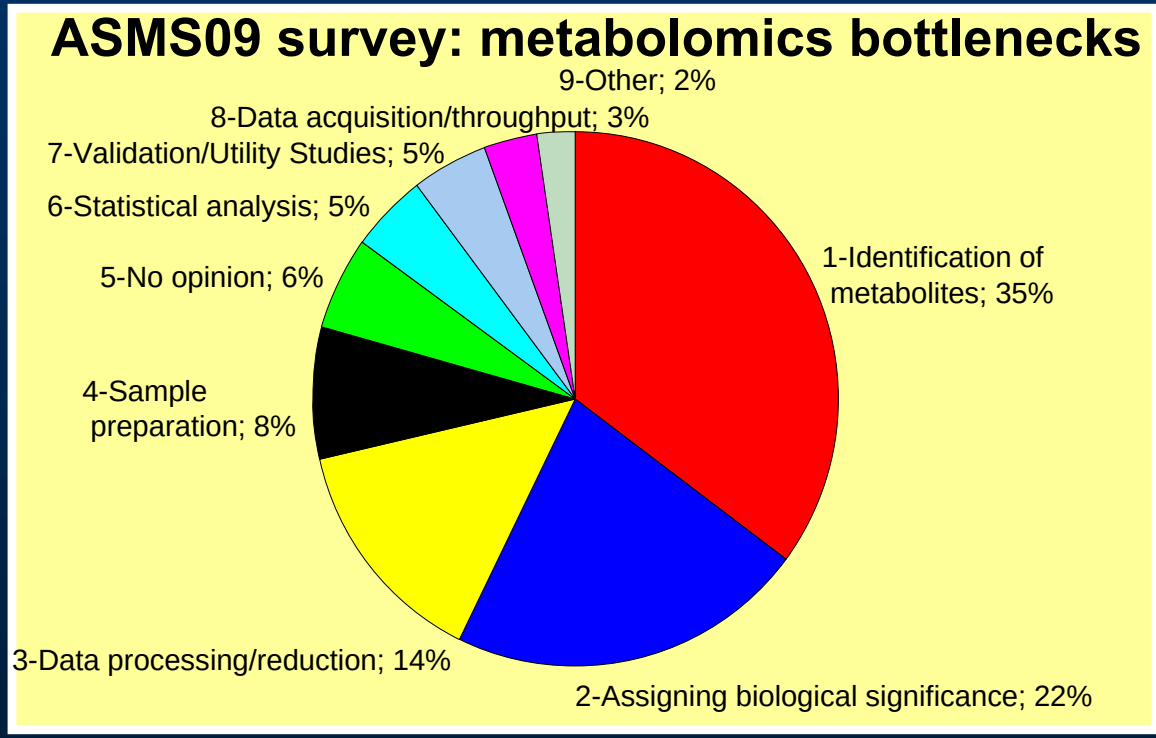
Ionization modes:

ESI and APCI/APPI

Ionization polarities:

+ and -

Nordstrom A. et al, Anal Chem, 2008. Kind T. et al, Anal Biochem, 2007



Although modern MS is capable of fast polarity switching and implements combined ion sources, there are always some data quality trade-offs for using universal modes

Typically, there is no need to save time by increasing throughput, because most of the project time will be spent on data analysis

Data output: XCMS

XCMS: http://masspec.scripps.edu/xcms/xcms.php

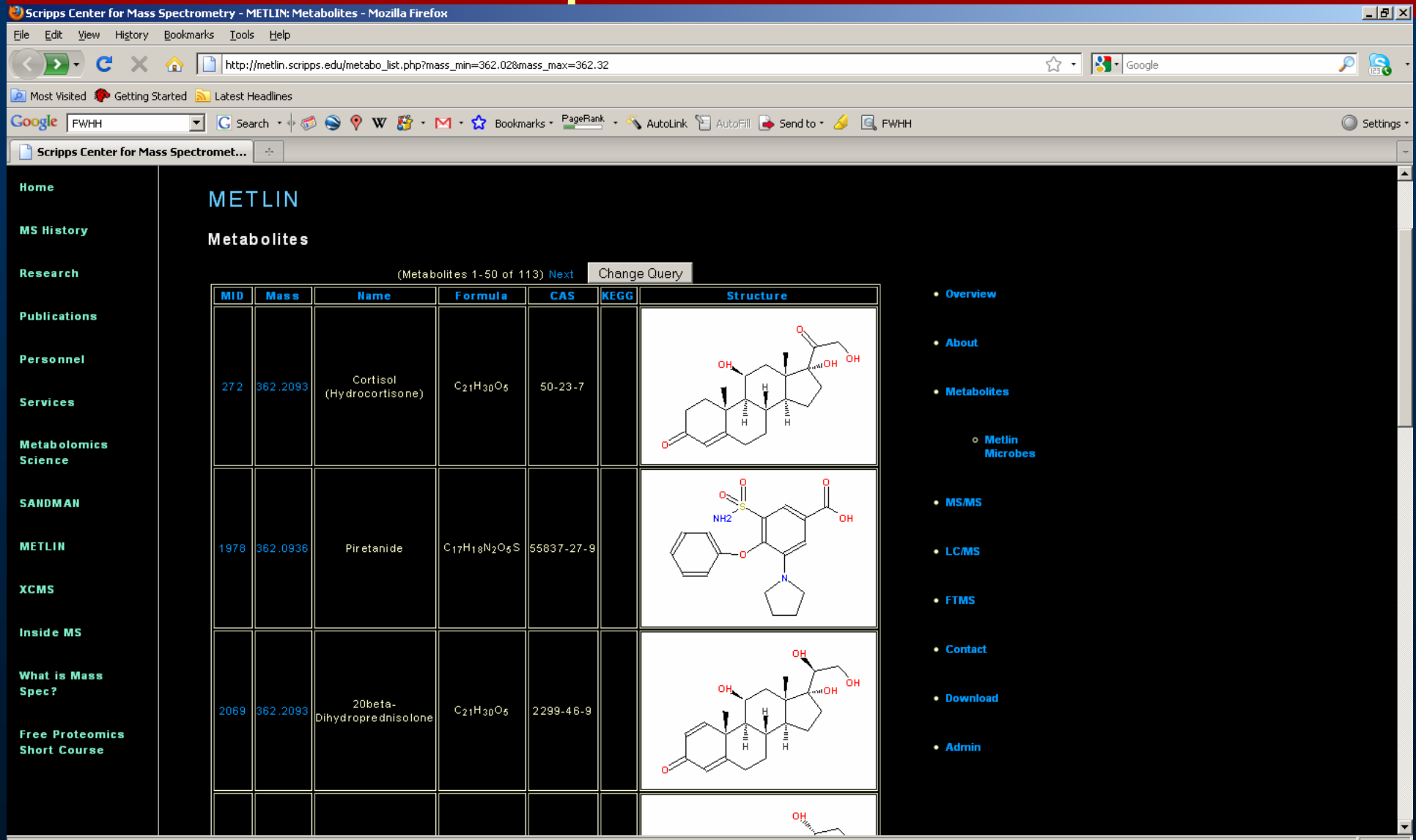
Starting this summer, includes support of true high resolution data and MS/MS data (XCMS^2)

Microsoft Excel format

Statistics			Accurate Mass			Retention Time			Groups		
fold	tstat	pvalue	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	npeaks	KO	WT
4.921	9.407	0.000814	363.1651	363.1604	363.1671	1062.014	1061.567	1063.066	3	3	0

http://metlin.scripps.edu/metabo_list.php?mass_min=362.02&mass_max=362.32

Data output: METLIN



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