UNM Chemistry and Chemical Biology Dept.

Group training materials

Mass Spectrometry Facility (MSF)

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Every analytical measurement uses the same building blocks.

1. Sample preparation

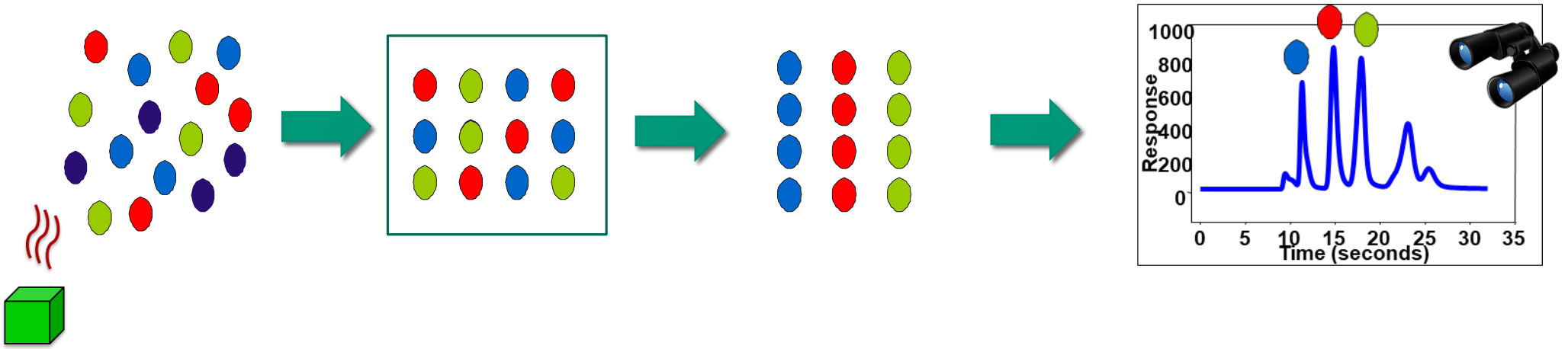
Extraction Digestion
Purge & trap Cryo-focus
Preconcentration
Thermal extraction
Derivatization

2. Separation

Gas, Liquid or Ion chromatography
thermal
permeation

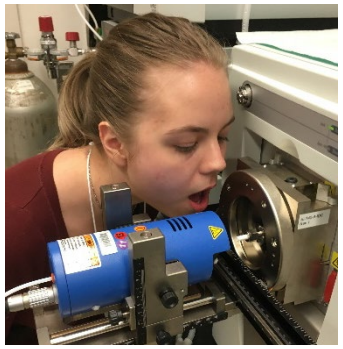
3. Detection

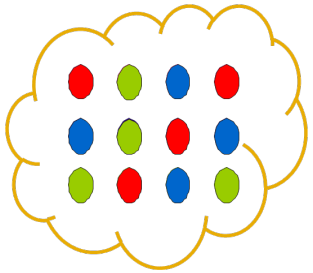
Electrochemical
Spectroscopy (emission, UV/VIS)
Mass spectrometry (Q, QQQ, ITMS^n)
FID, TCD, FPD, PDID, ECD, NMR

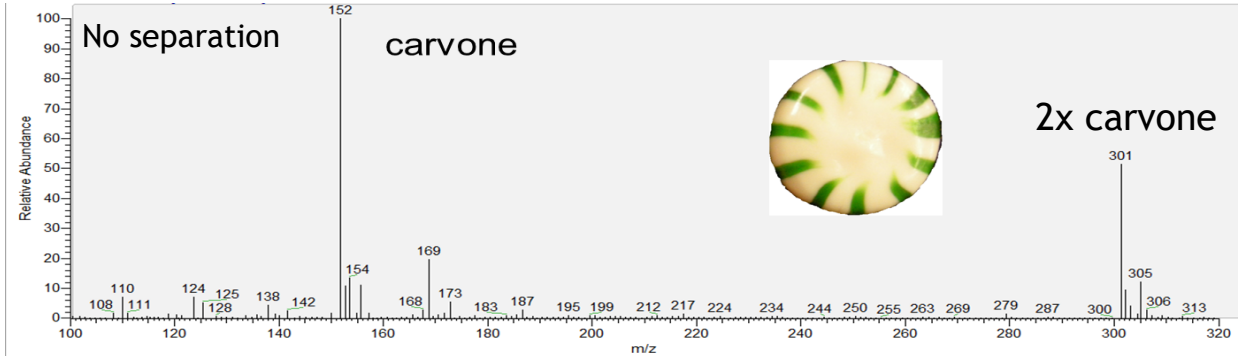


Many tradeoffs! Mix and match, group, or use multiple methods for desired results!

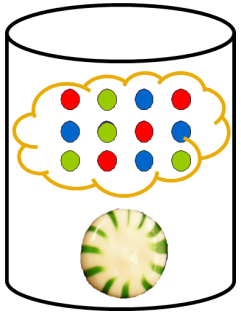
Step 1: Sample Prep. There are lots of ways…

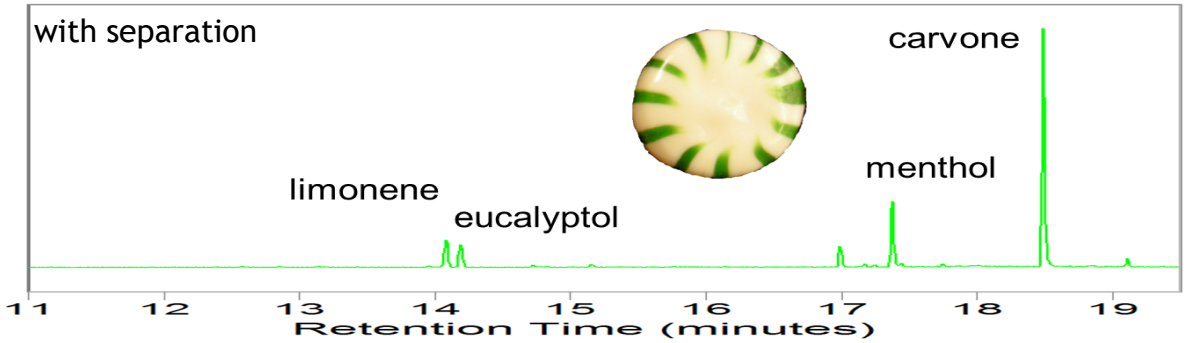




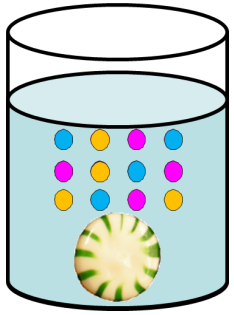


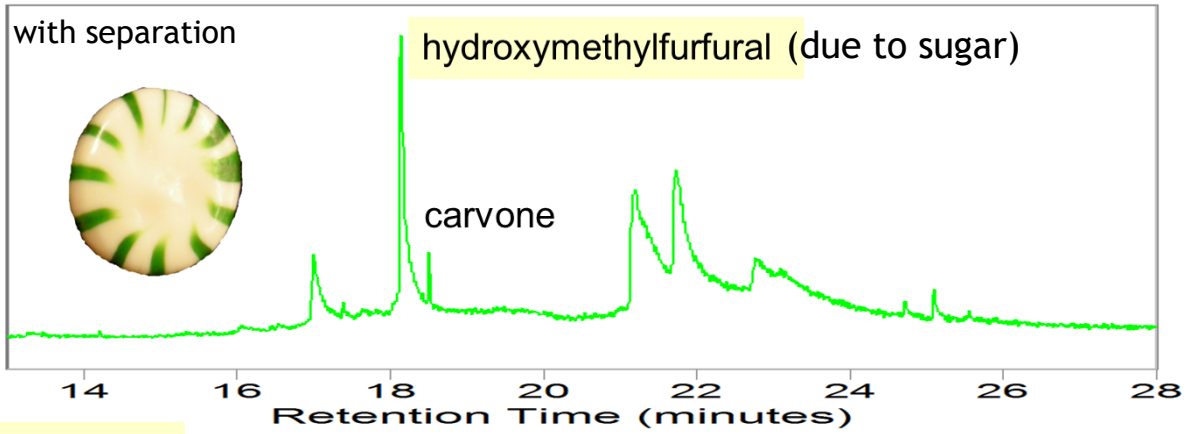
vapor





liquid



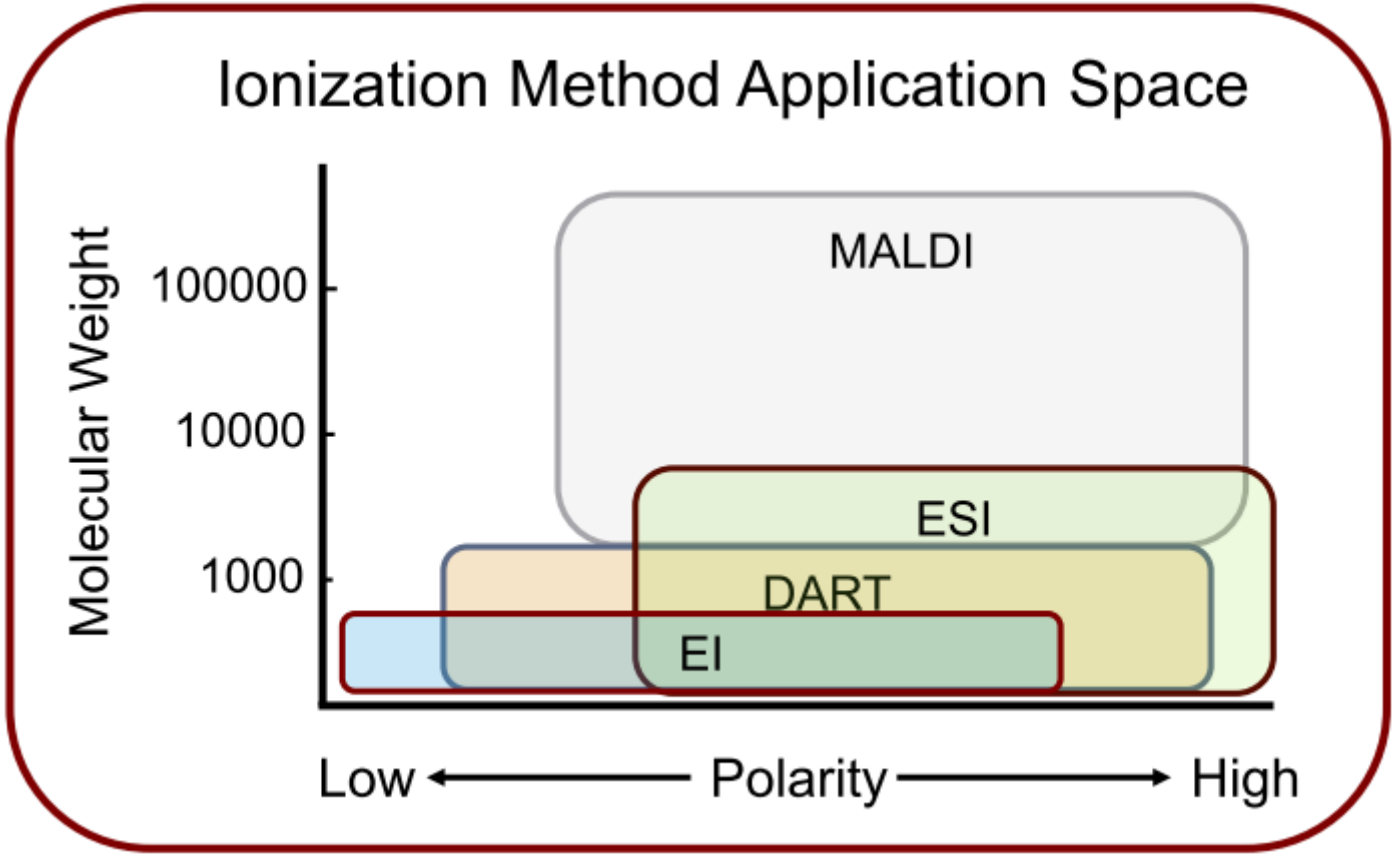


The method of sample prep affects what you detect!

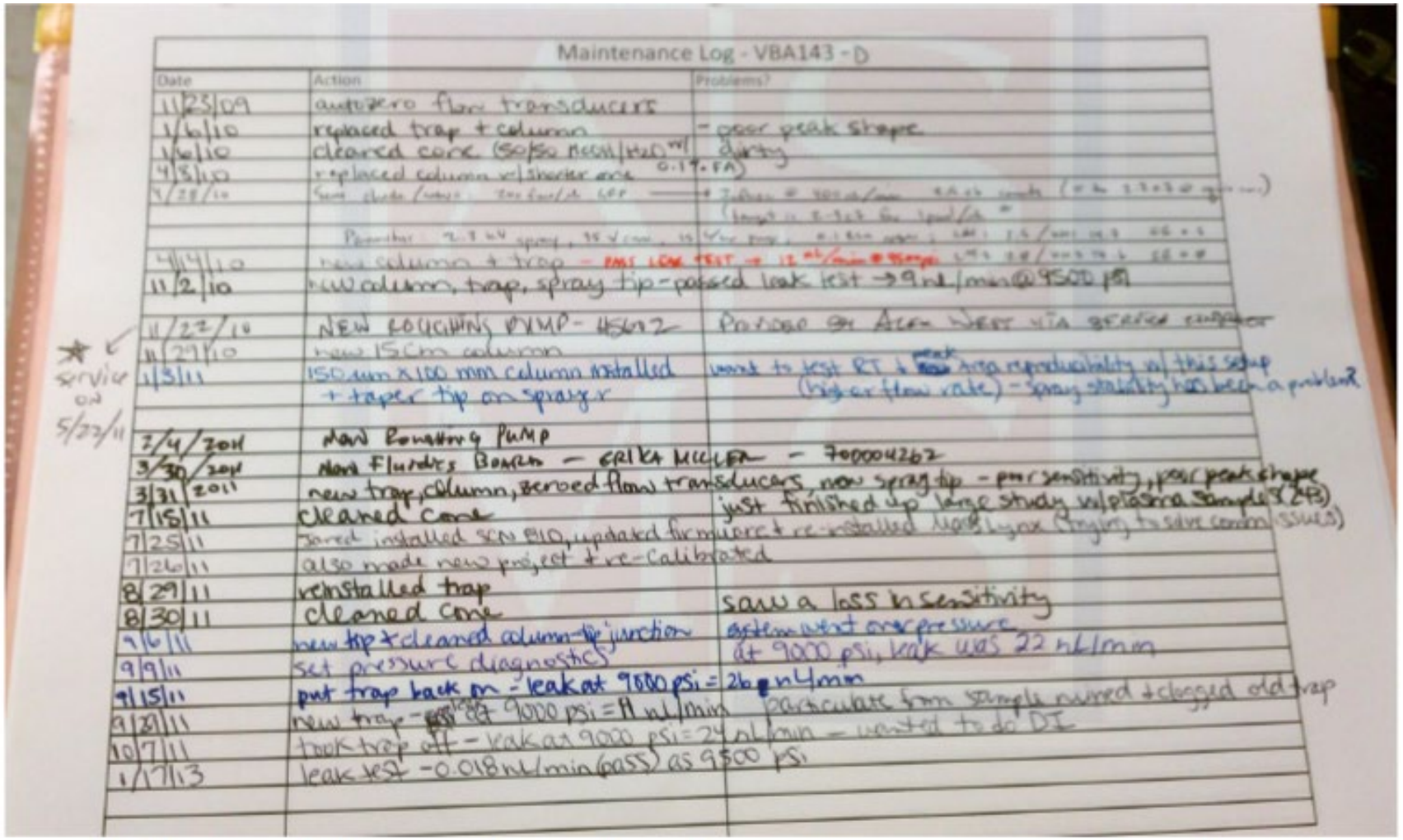
More Detection and how to choose

Flow chart

App space from 2020-ms-basics2



Two words about log books – have one!



It doesn't need to be fancy, or even electronic.

Choosing flow injection analysis (FIA) versus liquid chromatography

Reasons to choose FIA

- Solubility - if your sample is NOT SOLUBLE in chromatography solvents

Reasons to choose LC

- Contaminants, background species
- Multiple reaction products

Step 2: Separation. There are lots of ways

Solubility (NH_4NO_3 vs. DMNB)

Molecular weight (DNT vs. TNT)

Reactivity

Vapor pressure (TATP vs. HMX)

Filtration (TNT on particles)

Detector

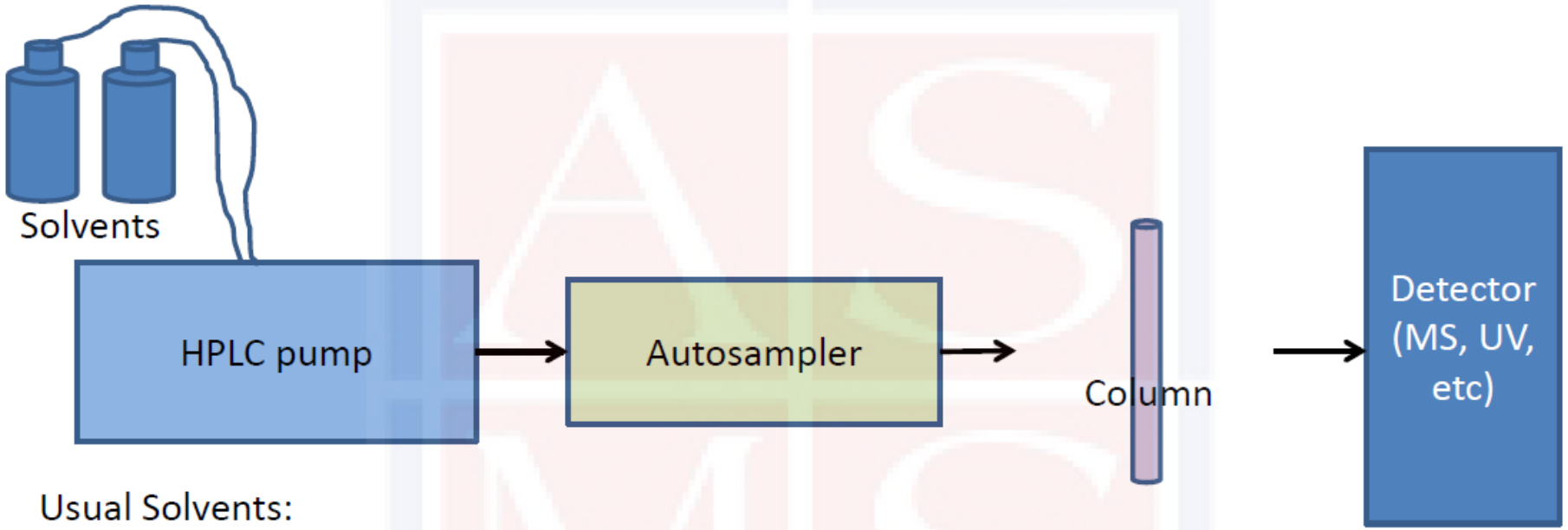
(+)

(-)

(- or +)

Your detector can be a separation mode!

HPLC/UPLC general layout

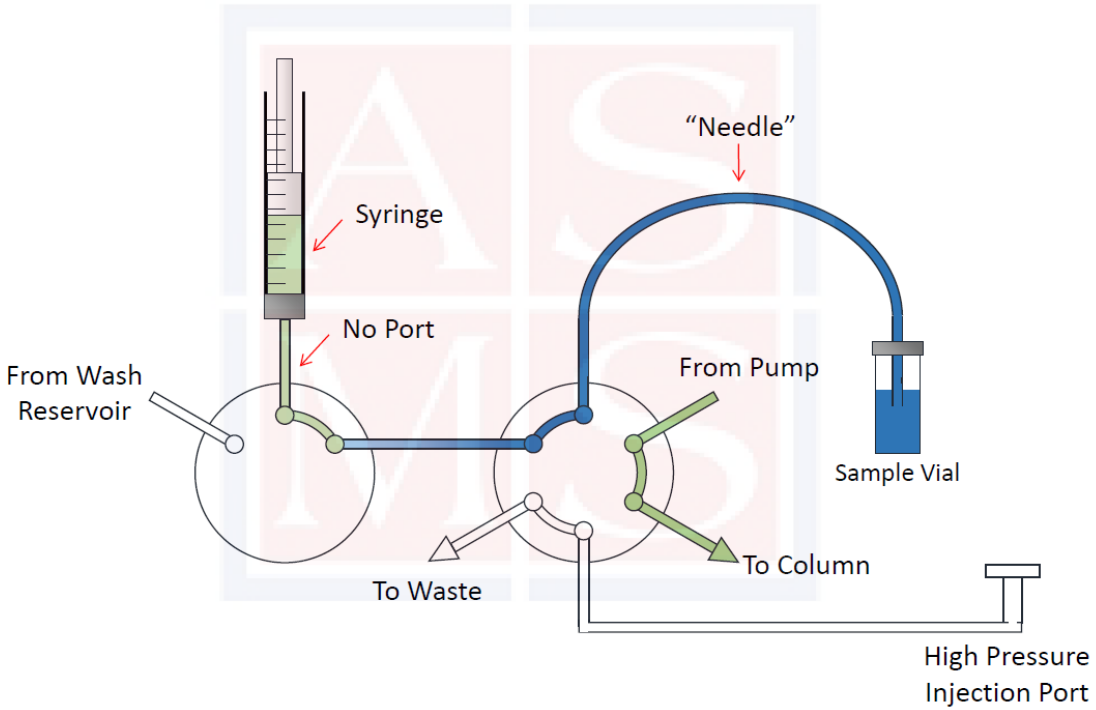


Usual Solvents:

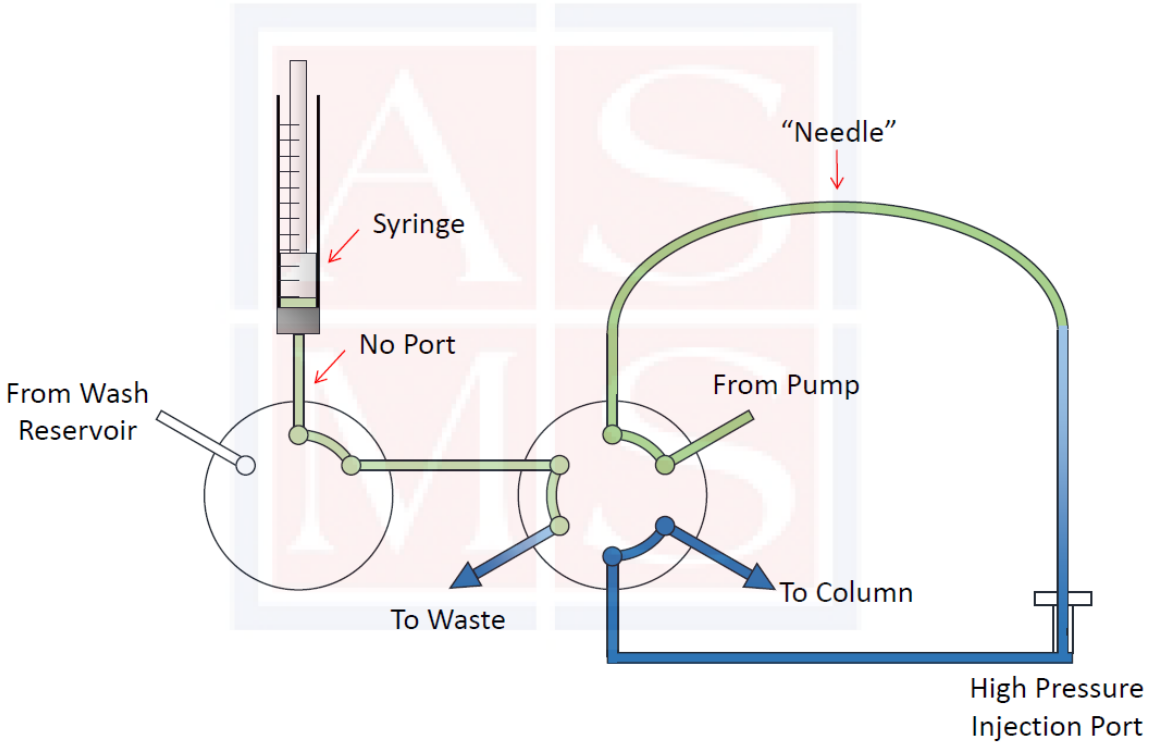
- Water
  - HPLC grade, deionized, filtered
- Miscible organics
  - Acetonitrile, methanol
- Ion pairing agents
  - Acids: Formic acid, acetic acid
  - Bases: Triethylamine
  - Buffers: ammonium formate, ammonium acetate
- Filter and degas

Waters ‘flow through needle’ sample introduction.

Load sample



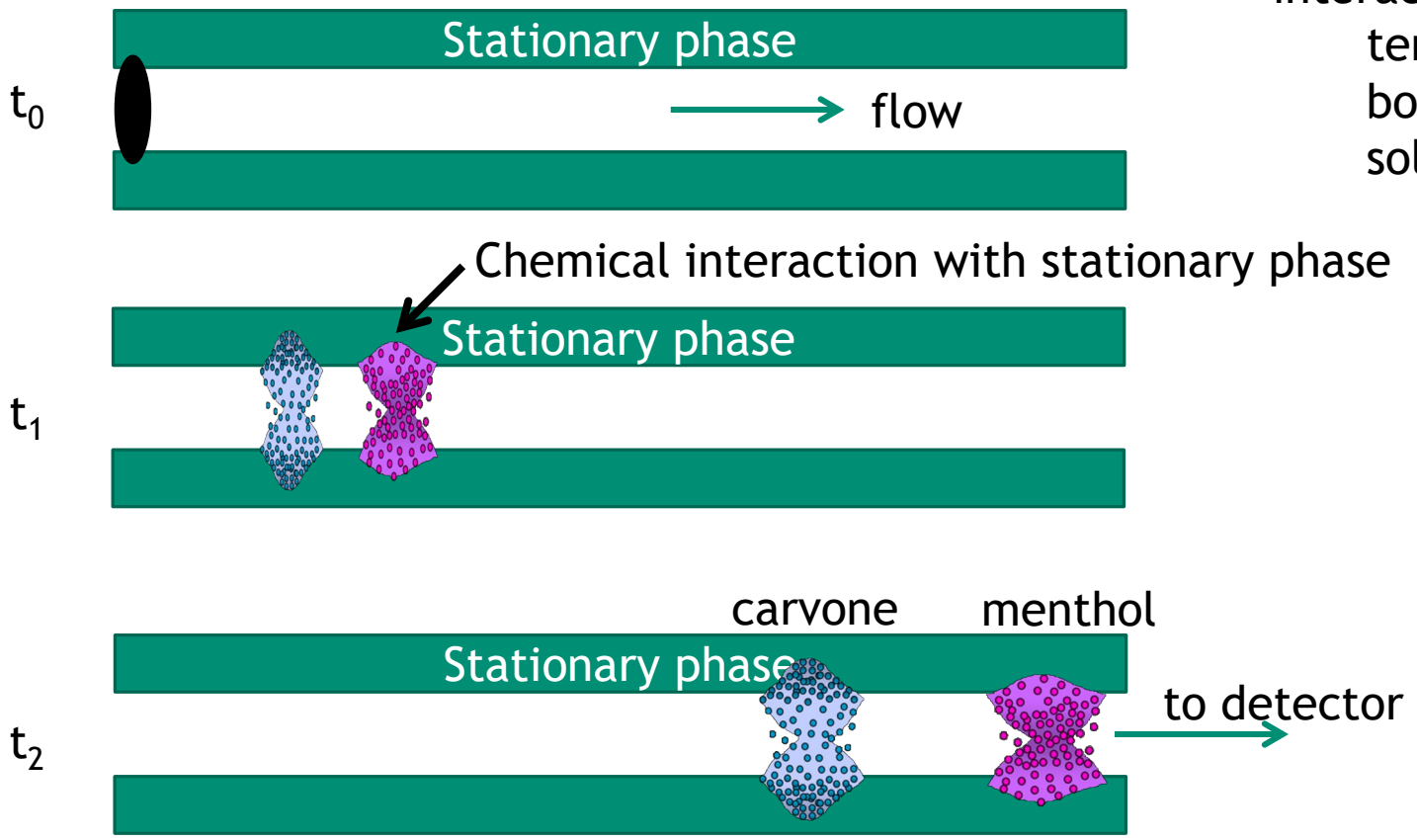
Inject sample



ASMS troubleshooting class: Sue Abbatiello, Tom Blau, Will Thompson

Step 2: Separation. Most are based on chemical interactions…

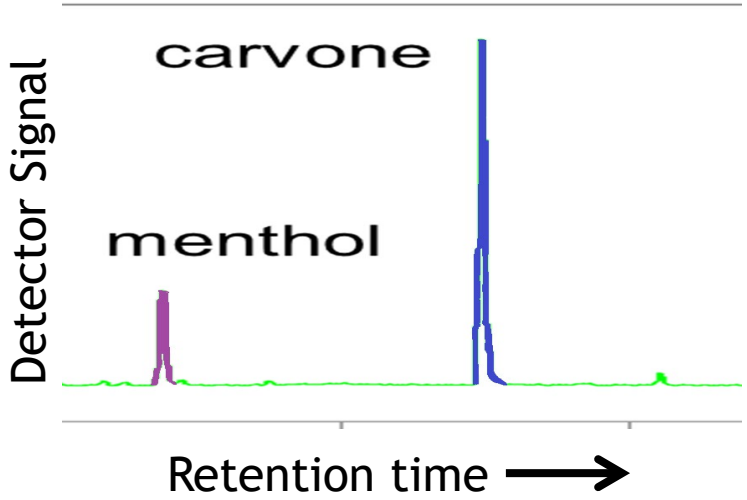
Start: all molecules together



Liquid and gas chromatography use chemical interactions and other properties:

- temperature
- boiling point
- solubility, pH, and more

Finish: groups are separate



Quality and Basic Analytical Factors – Great Reference.

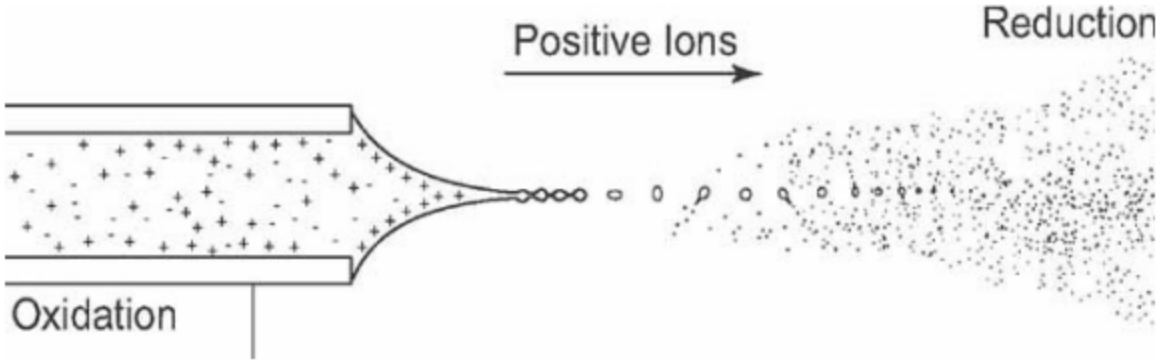
Good mass spectrometry and its place in good science

Mark W. Duncan[a,b,c]*

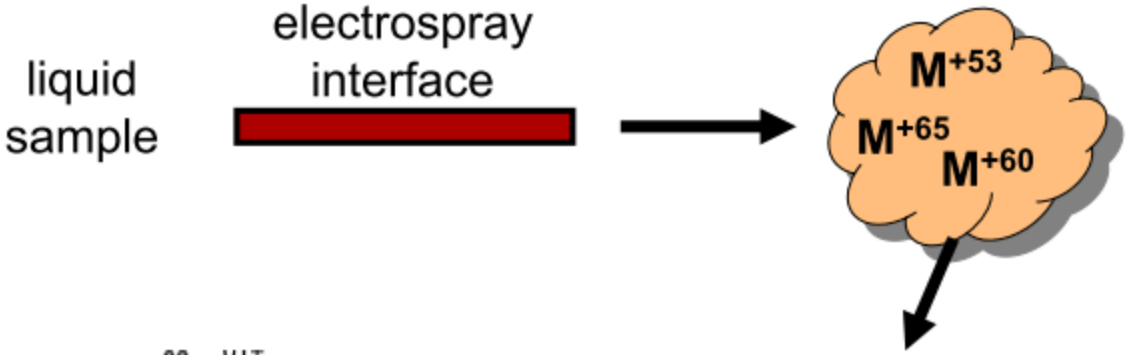
The mass spectrometry community has expanded as instruments became more powerful, user-friendly, affordable and readily available. This opens up opportunities for novice users to perform high impact research, using highly advanced instrumentation. This introductory tutorial is targeted at the novice user working in a research setting. It aims to offer the benefit of other people's experiences and to help newcomers avoid known pitfalls and problematic issues. It discusses some of the essential features of sound analytical chemistry and highlights the need to use validated analytical methods that provide high quality results along with a measure of their uncertainty. Examples are used to illustrate potential pitfalls and their consequences.

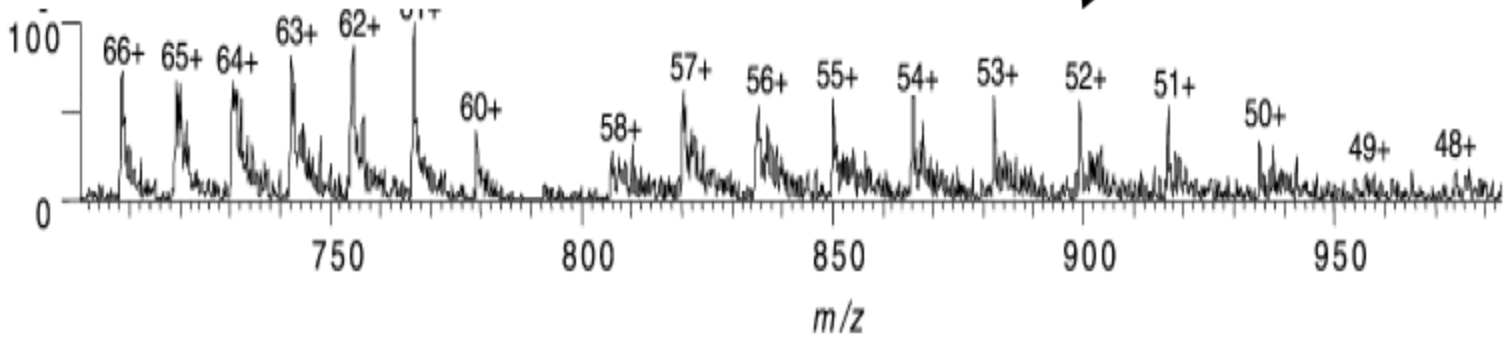
Keywords: proteomics; quantification; precision; accuracy; certainty; fitness-for-purpose

Electrospray Ionization – ESI – Nobel Prize 2002



$[M+H]^+$ typical





Spectrum reference: J. Murrell et al. / International Journal of Mass Spectrometry 227 (2003) 223–234

What kind of ions are created by ESI?

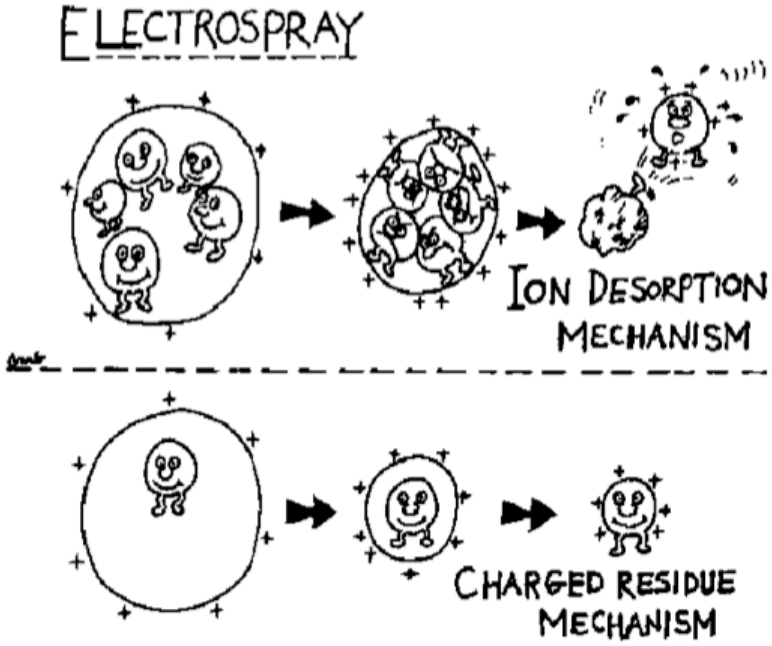
- Types of positive ions:
  - $[M+H]^+$
  - $[M+NH_4]^+$
  - $[M+Na]^+$
  - $[M+K]^+$
  - All of the above plus solvent
- Dimer ions
  - $[2M+H]^+$
  - $[2M+X]^+$ (X = Na, K, NH_4)

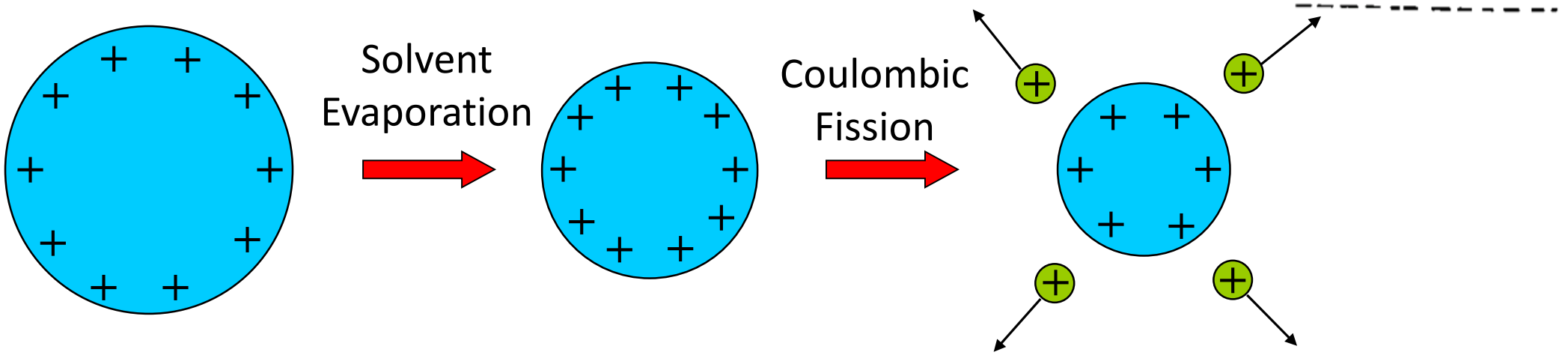
- Types of negative ions:
  - $[M-H]^-$
  - $[M+formate]^-$
  - $[M+acetate]^-$
  - $[M+Cl]^-$
  - All of the above plus solvent
- Dimer ions
  - $[2M-H]^-$
  - $[2M+Y]^-$ (Y = formate, acetate, Cl)

ESI models

Ion evaporation model: Droplet reduces in size and ions evaporate from droplet surface. Surface active ions have advantage.

Charged residue model: Droplets continue to lose solvent molecules until the charge residues are left behind.





Competition for charge

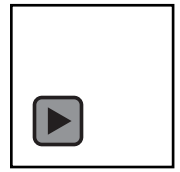
ESI introduces more molecules (excess solvent + analytes) than available charge (e.g. excess H^+)

All molecules compete for the charge (to be ionized)

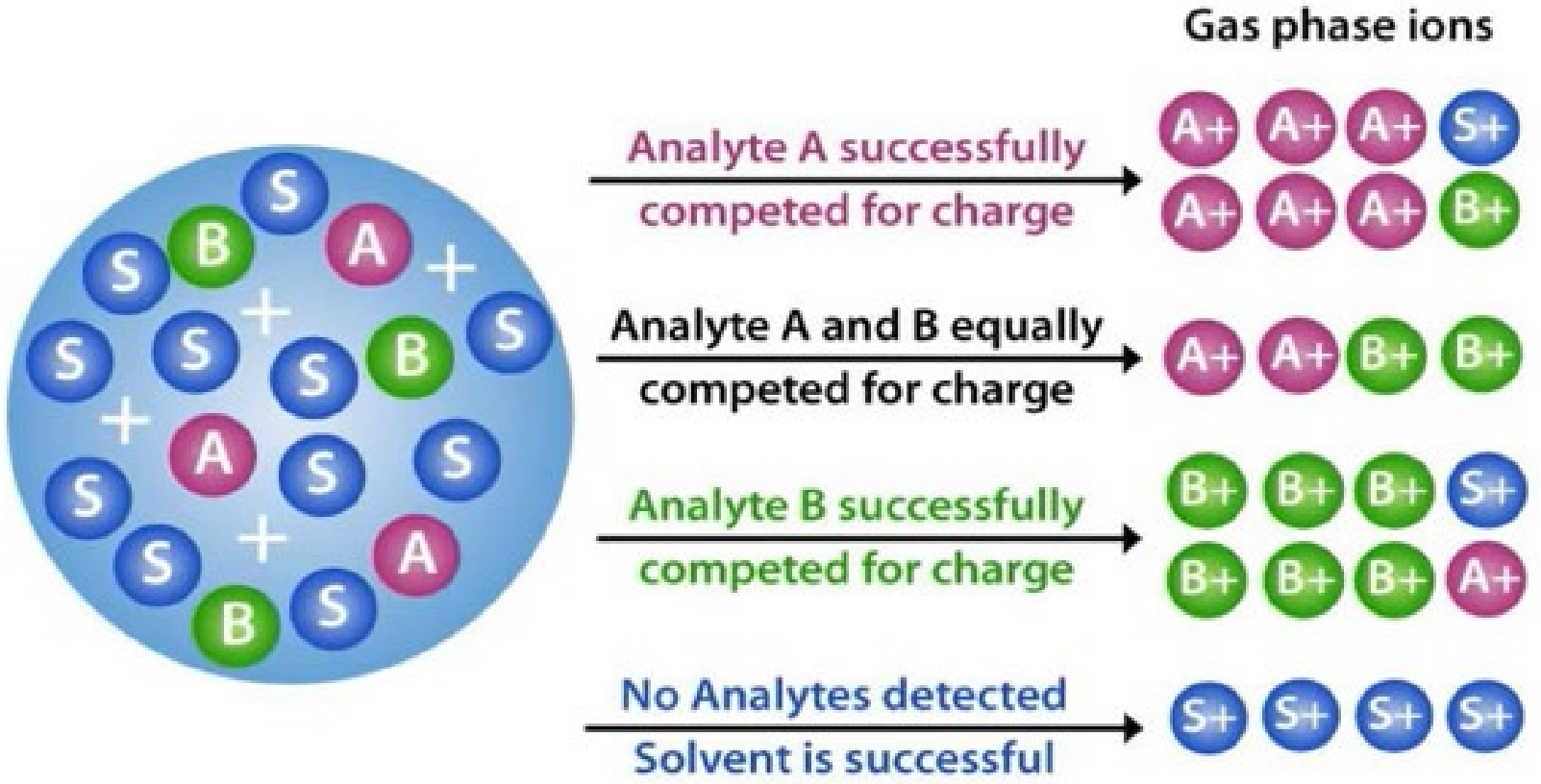
In ESI, molecules with high surface activity (relatively low solubility and high hydrophobicity) are more likely to be ionized

Molecules must have higher affinity for charge (e.g. H^+) than solvent molecules to be ionized with high efficiency; (more basic than solvent)

Molecules that evaporate faster than solvent usually do not ionize well (e.g. trimethylamine, b.p. 89 ^oC)



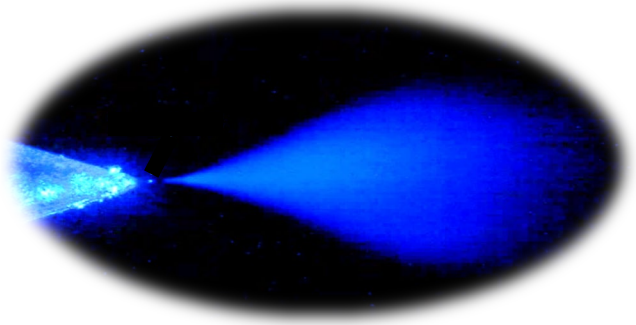
ESI – competition for charge between analytes, matrix, and solvent



In practical terms, this can be viewed as ion suppression

Reference: [(652) Getting The Most Out Of Your LCMSMS Separations and Method Development - YouTube]

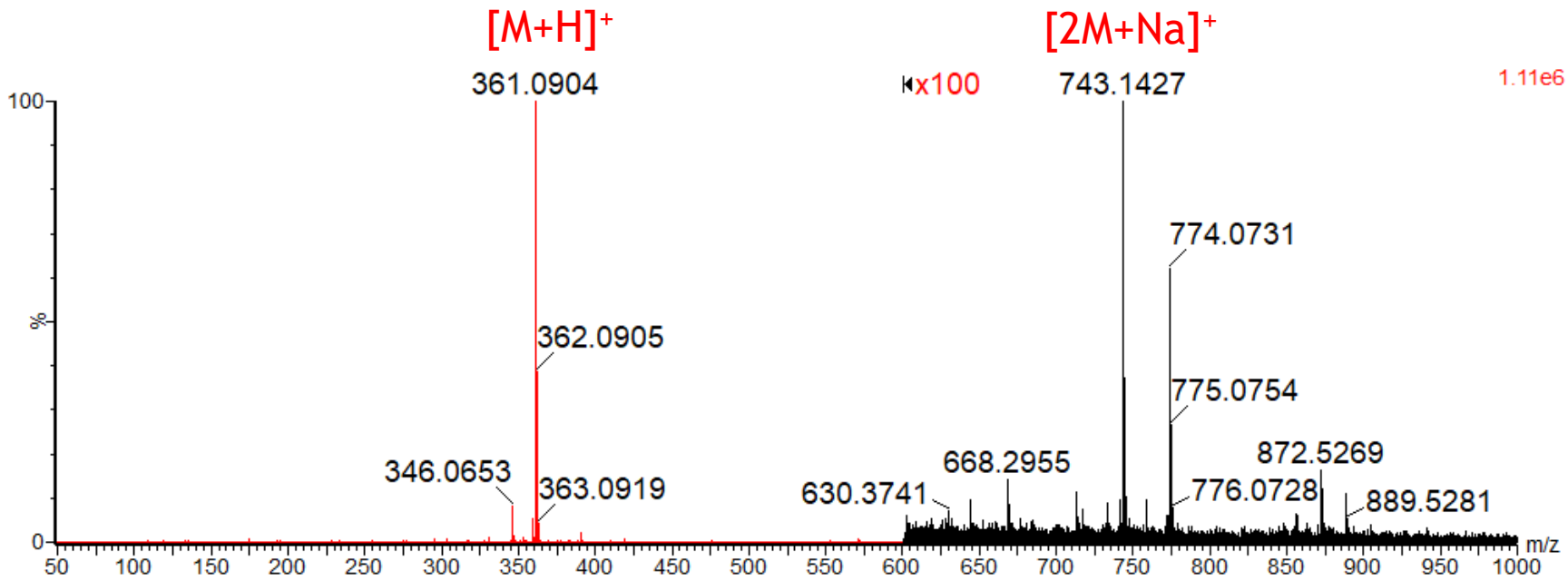
Multiply-charged peptide created by electrospray ionization



Noncovalent dimer ions

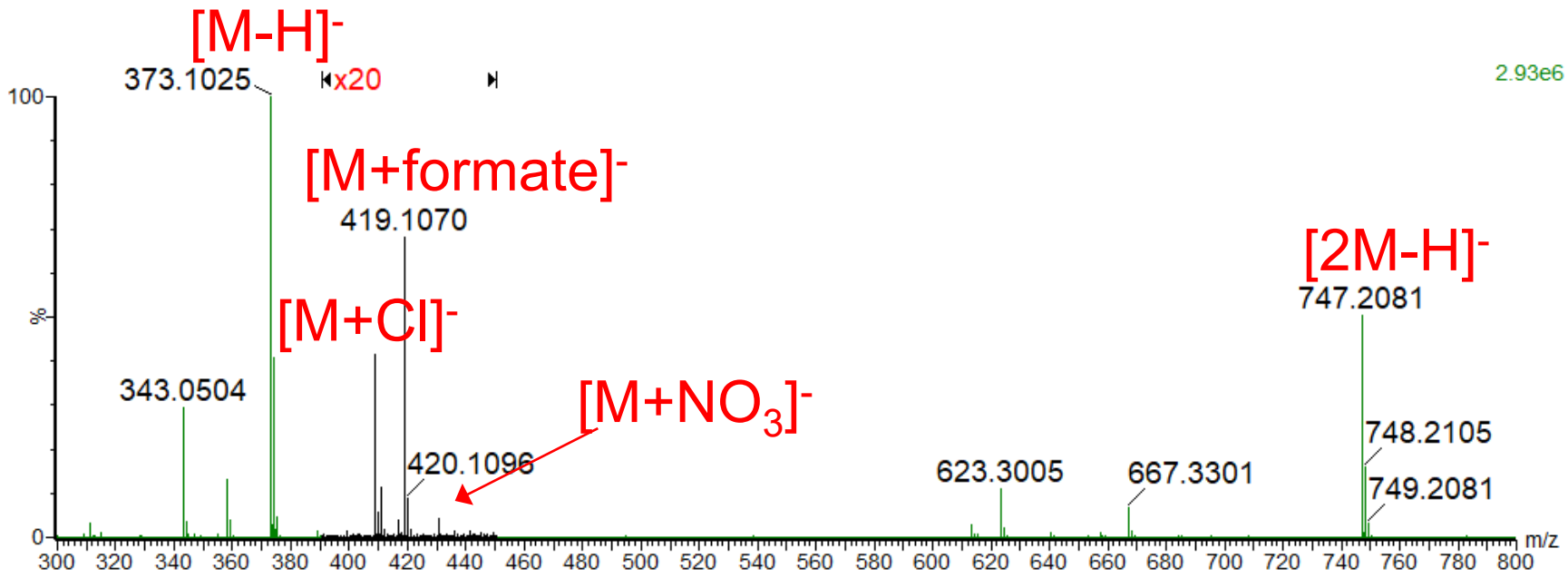
Electrospray ionization is so gentle that noncovalent associations are often preserved.

Noncovalent dimer ions may form, particularly when analyte concentrations are high

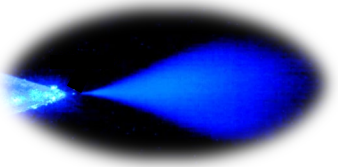


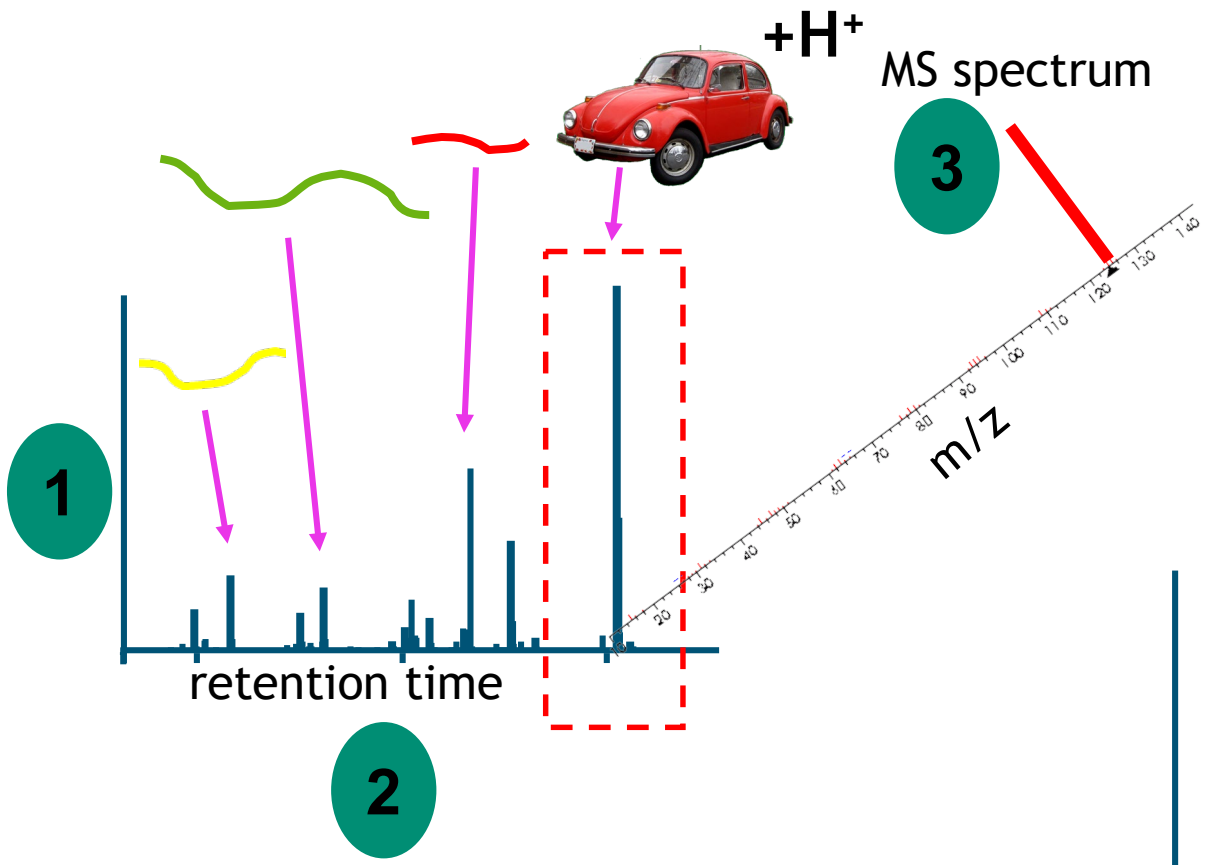
Negative mode ESI

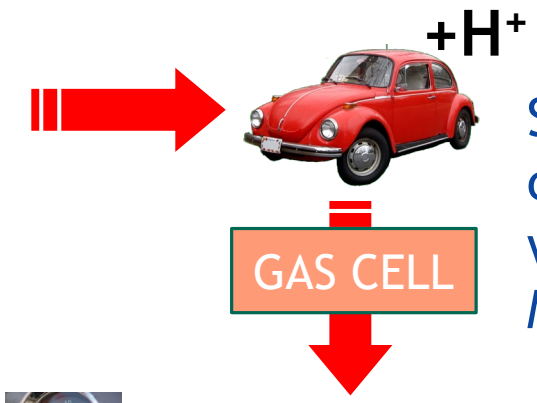
- Acidic compounds usually form $[M-H]^-$
- Compounds may form adducts with anions (and other coeluting substances



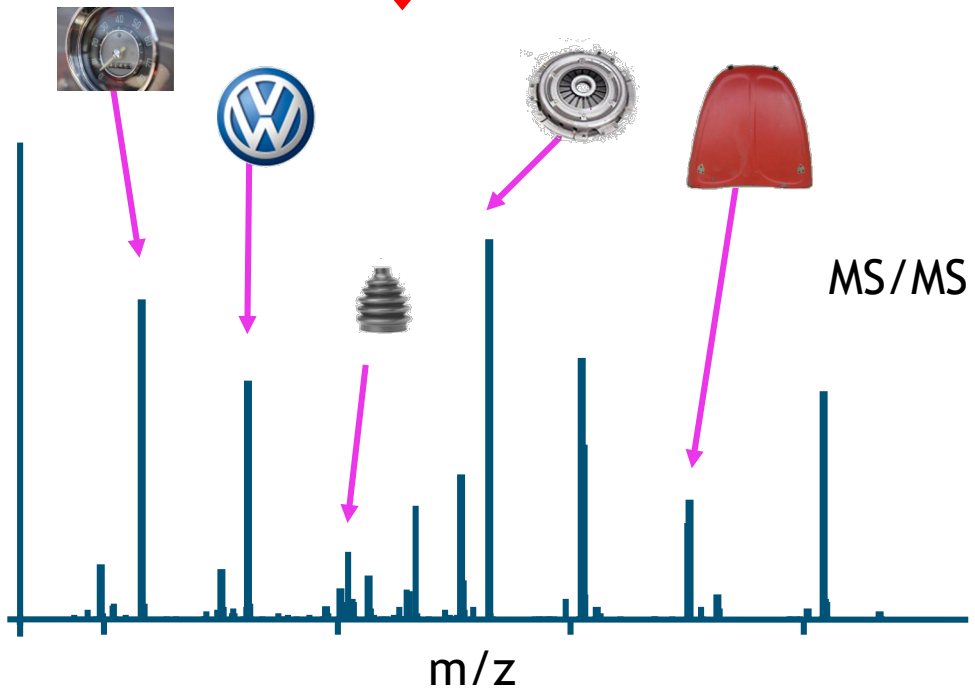
How do we get a fingerprint from ESI data? Tandem Mass Spectrometry (MS/MS)!







Select, collide with gas to create fragments – measure with a second MS – thus MS/MS

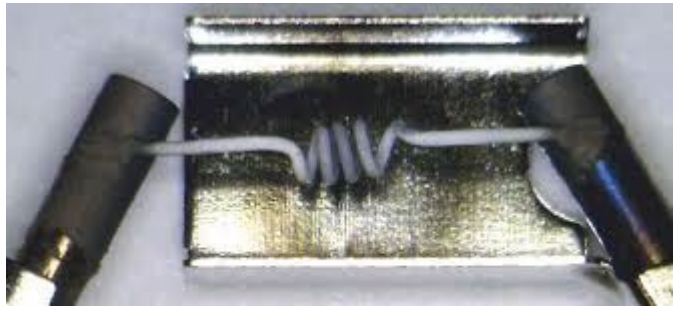


So we can make a fingerprints even from low energy electrospray ion!

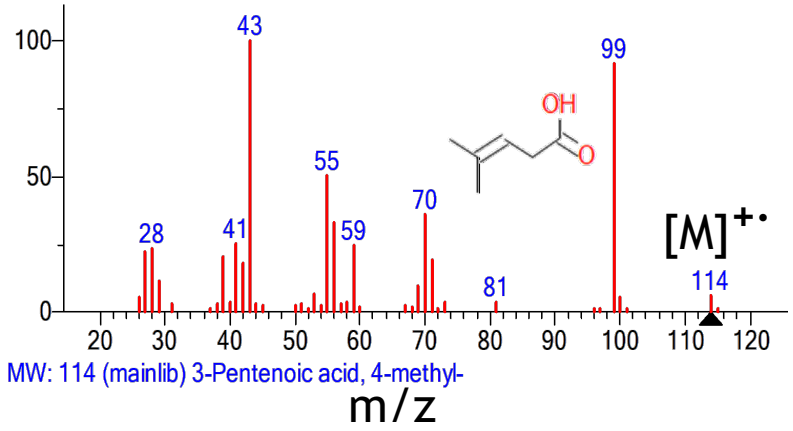
ESI: electrospray ionization

How do we make charged species? Two examples…

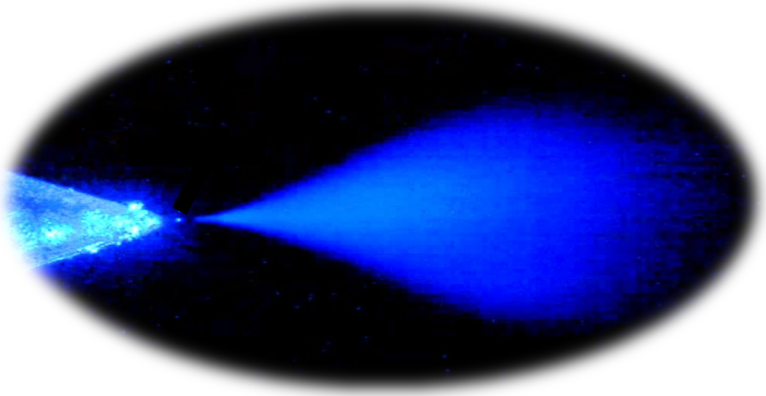
- Electron ionization (EI)
  - Hot filament emits electrons
  - High energy
  - Reproducible
  - Al Nier 1930s



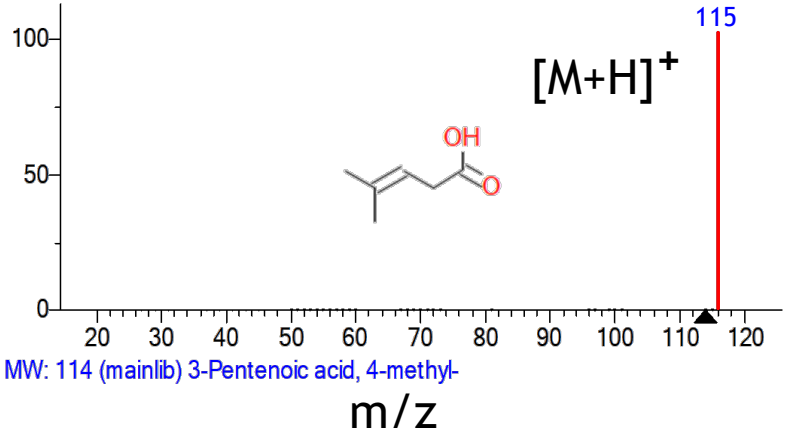
For Gas Chromatography (GC)



- Electrospray (ESI)
  - Solvent creates charged species
  - Low energy
  - Nobel prize 2002 – ionization for large (>500 Da) species
  - John Fenn ~1985



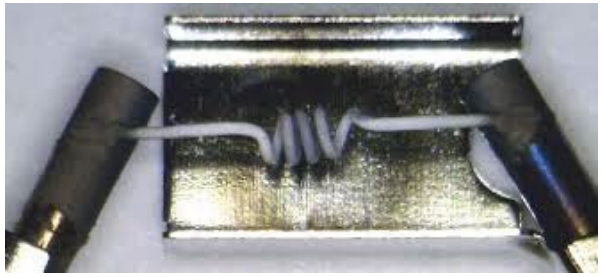
For Liquid Chromatography (LC)

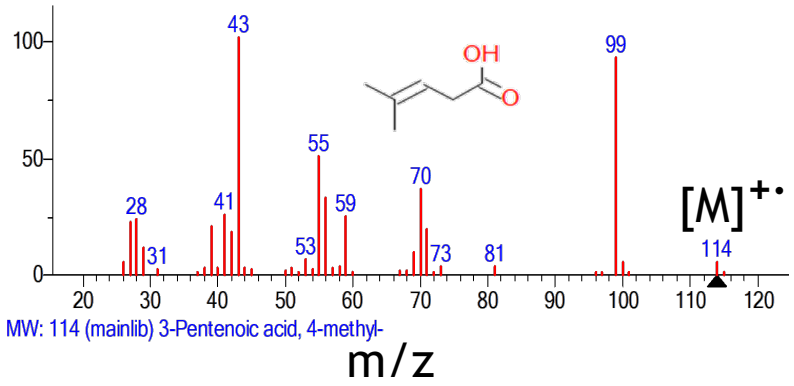


These are just 2 of many methods that can be used.

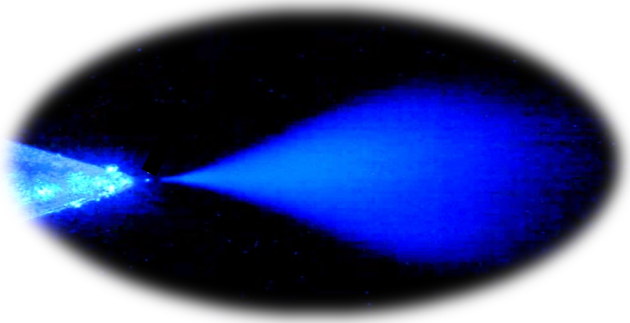
What instruments does the MSF have?

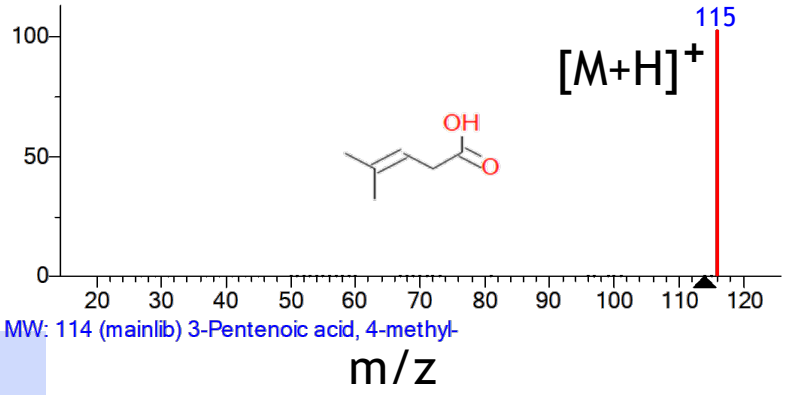
GC/MS



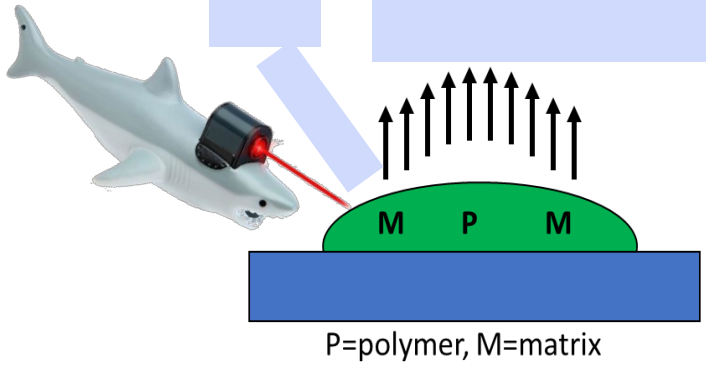


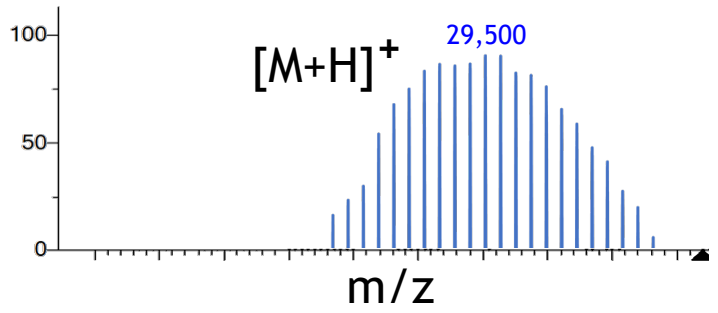
GC
-or-
LC/MS





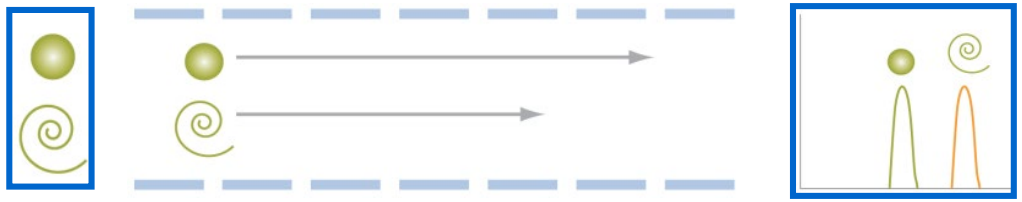
MALDI/MS



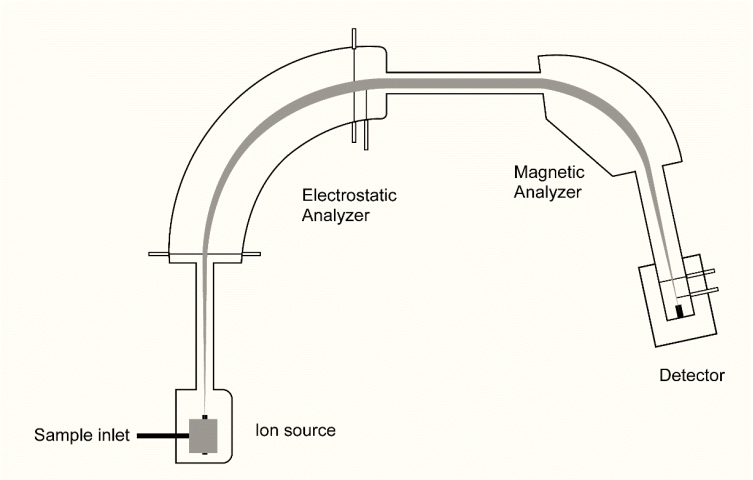


m/z separation (types of analyzers)

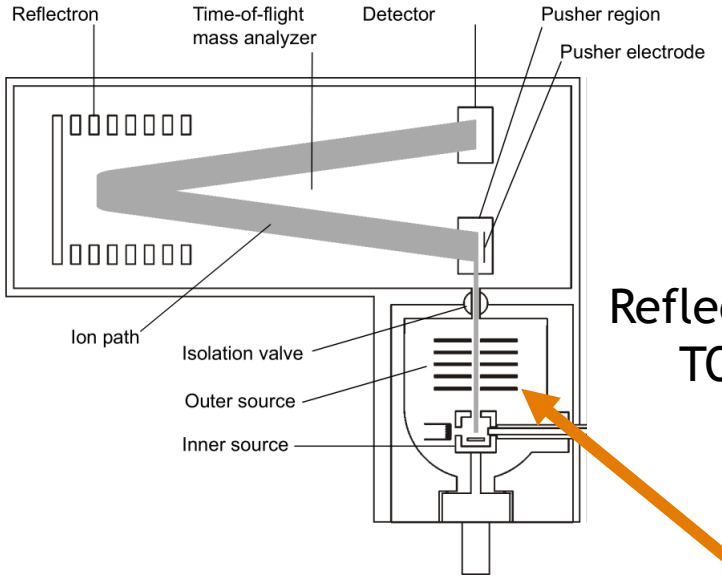
Quadrupole mass filter



Time-of-flight



Dual electric / magnetic sector



Reflectron TOF

Hybrid TOFs have a quadrupole here

Some Nomenclature about ions

Exact mass: calculated

Accurate mass: measured

Nominal mass: integer value

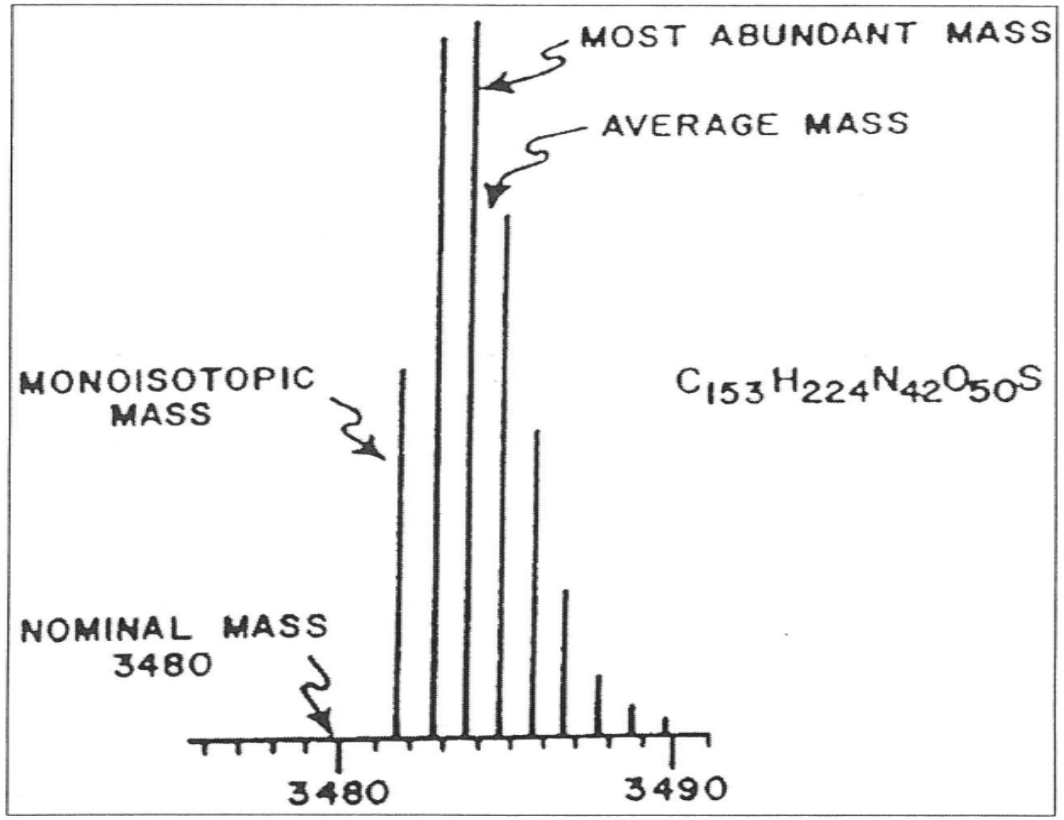
Monoisotopic mass: exact mass calculated using most abundant isotopes

Mass defect: Difference between nominal mass and monoisotopic mass

Most abundant m/z in spectrum: due to combined probabilities of isotopes and molecular formula

Mass Accuracy (mmeas-mcalc)/mmeas
- Typically expressed in parts-per-million (ppm)

Continuum vs. centroid
- "raw" and "manipulated"



For more:
K.K. Murray, R.K. Boyd, M.N. Eberlin, G.J. Langley, L. Li, Y. Naito, Definitions of Terms Relating to Mass Spectrometry (Iupac Recommendations 2013), in *Pure and Applied Chemistry*, vol. 85, pp. 1515-609, 2013.

Figure

From: O. David Sparkman "Mass Spec Desk Reference" Global View Publishing, Pittsburgh, PA, 2000. Page 37.

More Nomenclature

1. EI: electron ionization (not electron impact)
2. Precursor ion
3. Product ion
4. n th generation product ion
5. MRM: multiple reaction monitoring
6. m/z mass-to-charge ratio (preferred in labeling spectra to Da or amu)

K.K. Murray, R.K. Boyd, M.N. Eberlin, G.J. Langley, L. Li, Y. Naito, Definitions of Terms Relating to Mass Spectrometry (Iupac Recommendations 2013), in Pure and Applied Chemistry, vol. 85, pp. 1515-609, 2013.

Resolution: definition and calculation

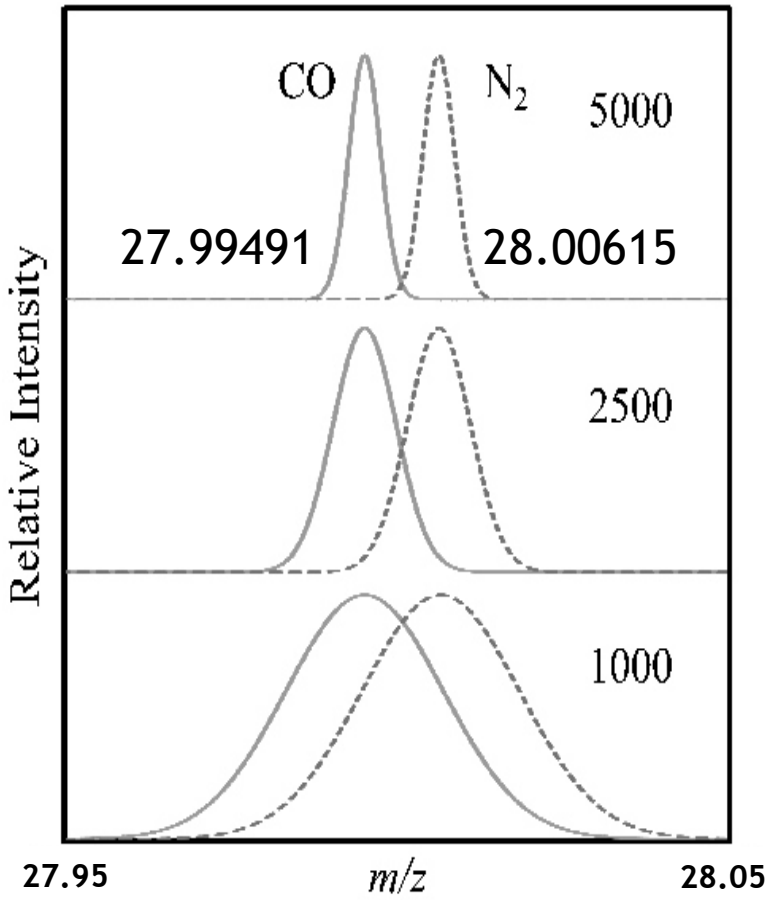
Varies by instrument type

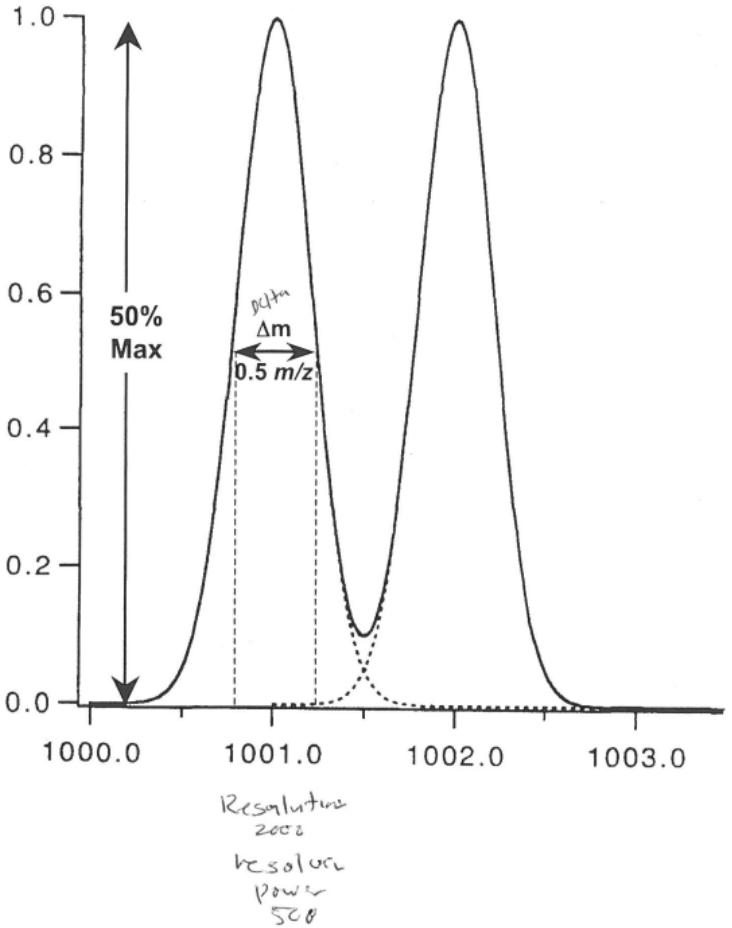
Can be adjusted on some instruments

For TOF can be a function of m/z

- Thus specified as ‘a resolution of 5000 at m/z 28’

R_{FWHM}= m/Δm=1000/0.5=2000





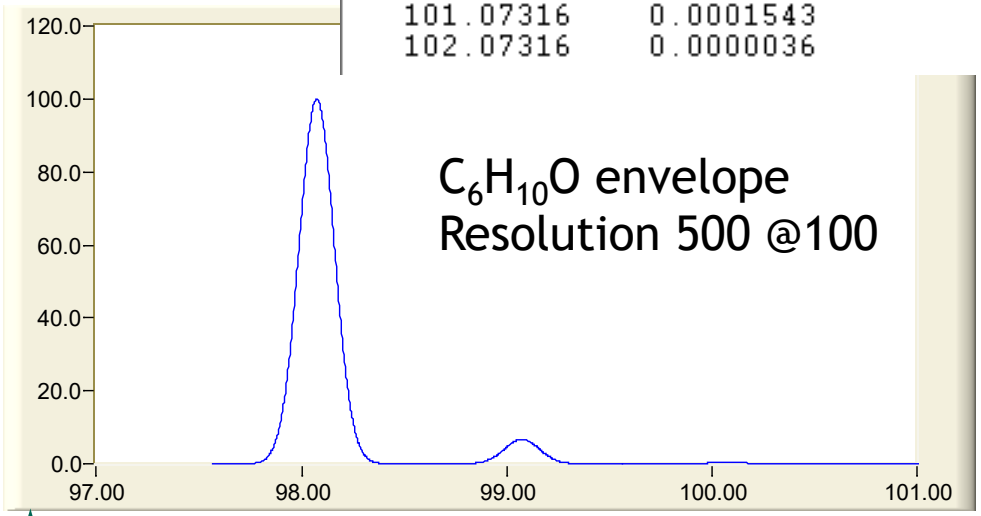
Always define how YOU are calculating resolution

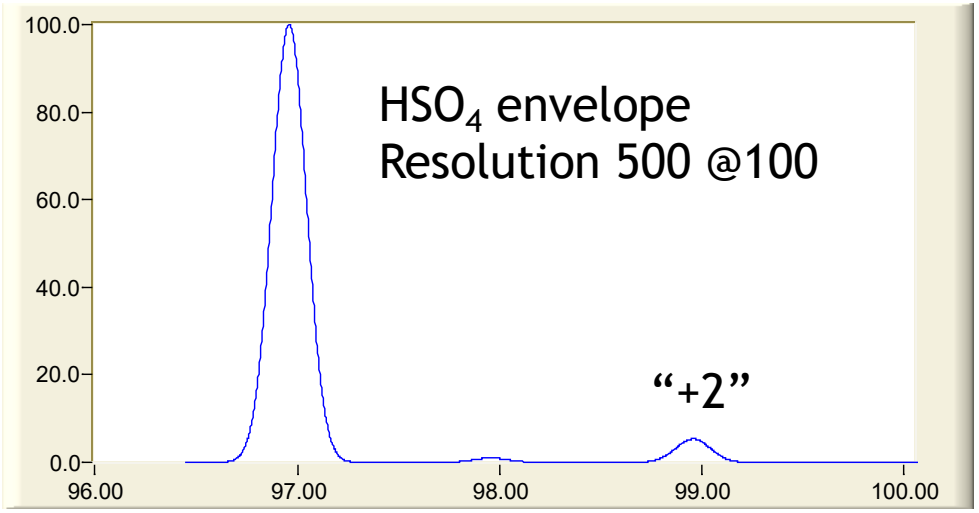
Isotope usefulness

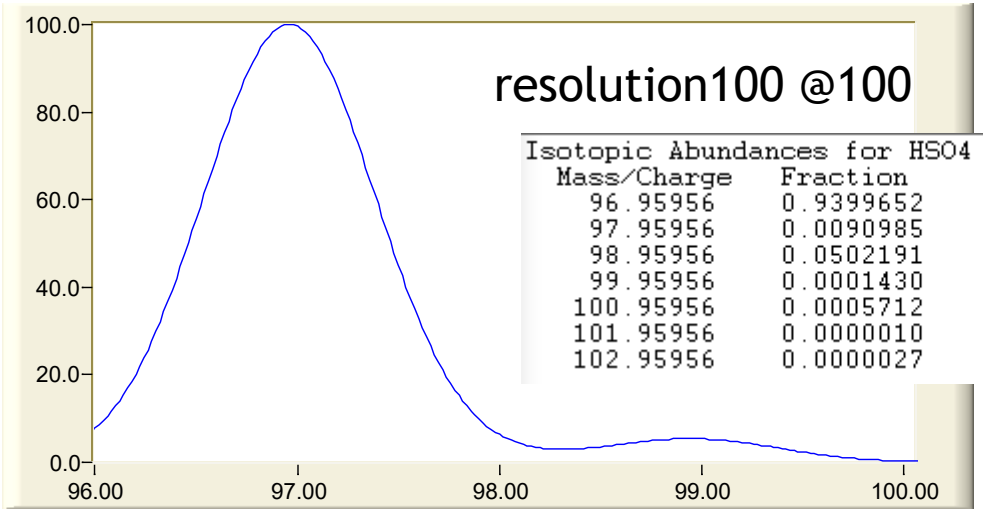
- Elements to watch for
  - "+1" - C
  - "+2" – Cl, Br, S, Si
  - Lots of metals have unique patterns!

Isotopic Abundances for C6H10O

Mass/Charge	Fraction	Intensity
98.07316	0.9338129	100.00
99.07316	0.0623558	6.68
100.07316	0.0036740	0.39
101.07316	0.0001543	0.02
102.07316	0.0000036	0.00







Isotopic Abundances for HSO4

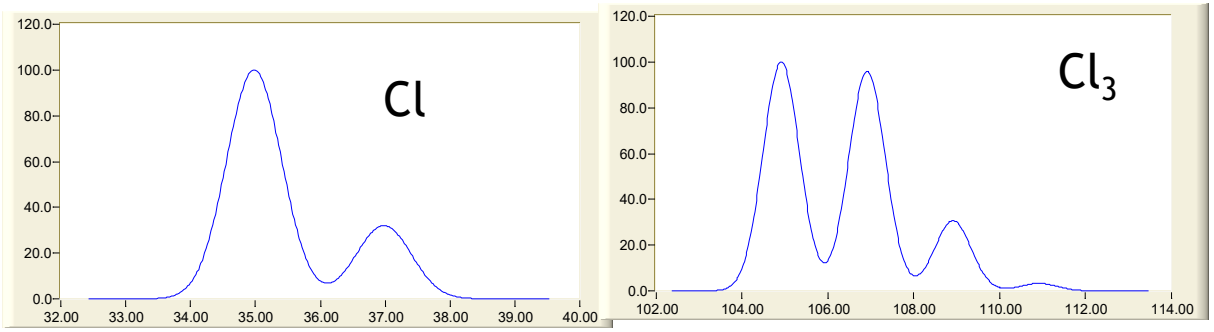
Mass/Charge	Fraction	Intensity
96.95956	0.9399652	100.00
97.95956	0.0090985	0.97
98.95956	0.0502191	5.34
99.95956	0.0001430	0.02
100.95956	0.0005712	0.06
101.95956	0.0000010	0.00
102.95956	0.0000027	0.00

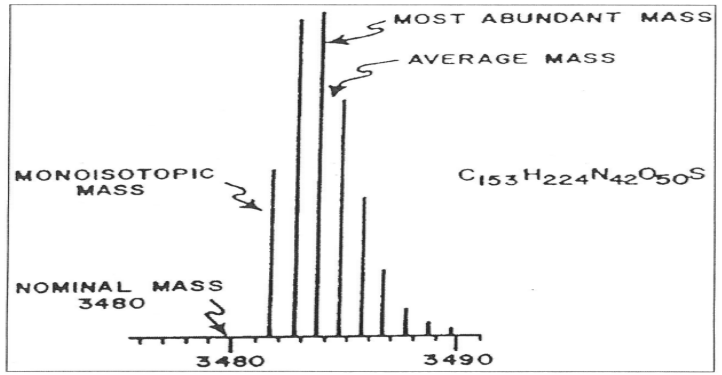
Your resolution matters!

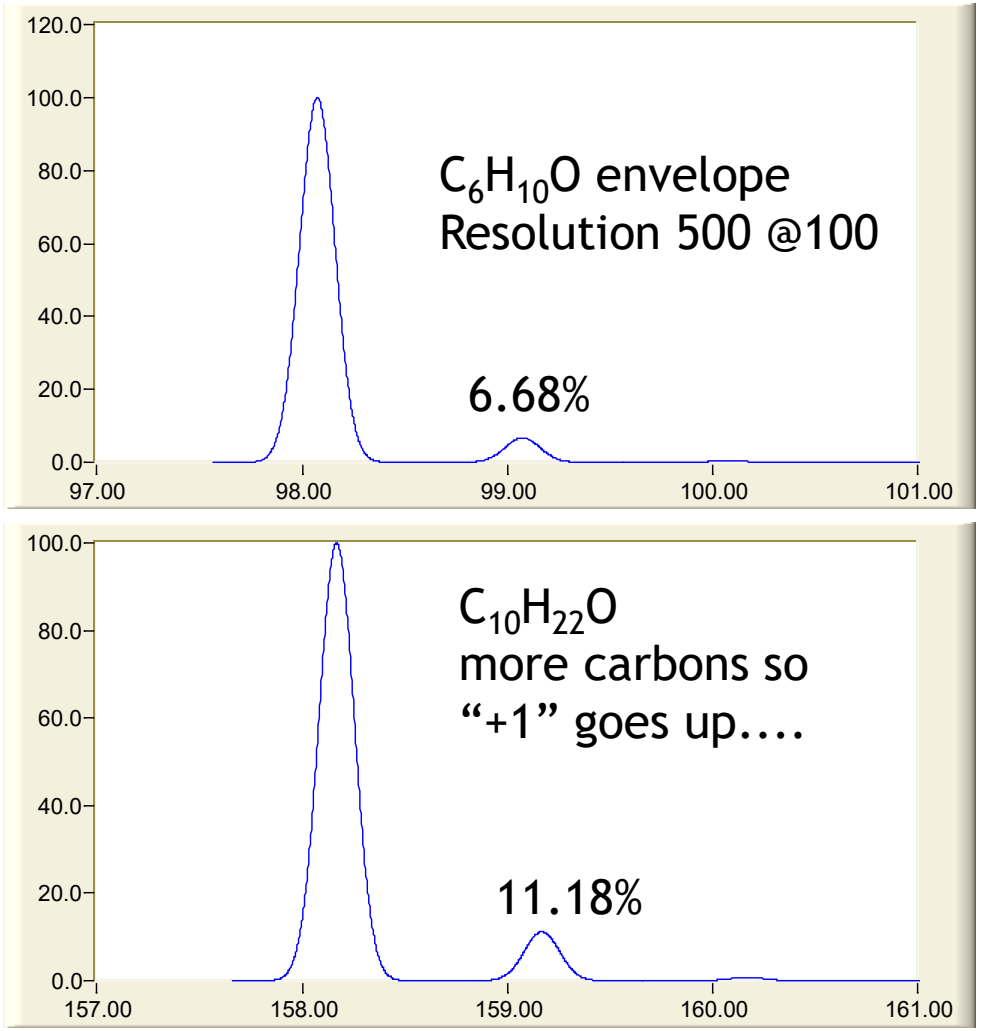
Freeware: https://omics.pnl.gov/software/molecular-weight-calculator

More useful isotopes

The patterns change predictably







Tips for getting the best data

- If colleague requests method or instrument, find out why!
- Do they have expertise? Prior work?
- If they ‘read a paper’ – get it and read it!
- Use accurate reporting and language
- Try to educate customer on what else is possible
- Back up the data!
- Try to always include method blanks, instrument blanks
- Know your instrument!

So, sometimes you need higher confidence! MS data has many layers!

YES!

…..but you'll need multiple instruments and maybe even a known reference compound!

Here's what we use most often:

Retention time (RT)

Fragmentation patterns

Library

Reference compound

Usually verified by NMR, UV/VIS, etc.

MORE INFO

Isotopes

MS/MS fragmentation map

Exact mass

Alternate Separations

Fragmentation model

Odd / Even

Rings / DB

Alternate Sample Prep

Quality Meas.

Internal stds.
Isotopic labels
Blanks, etc.

In Conclusion…

Analytical Messages

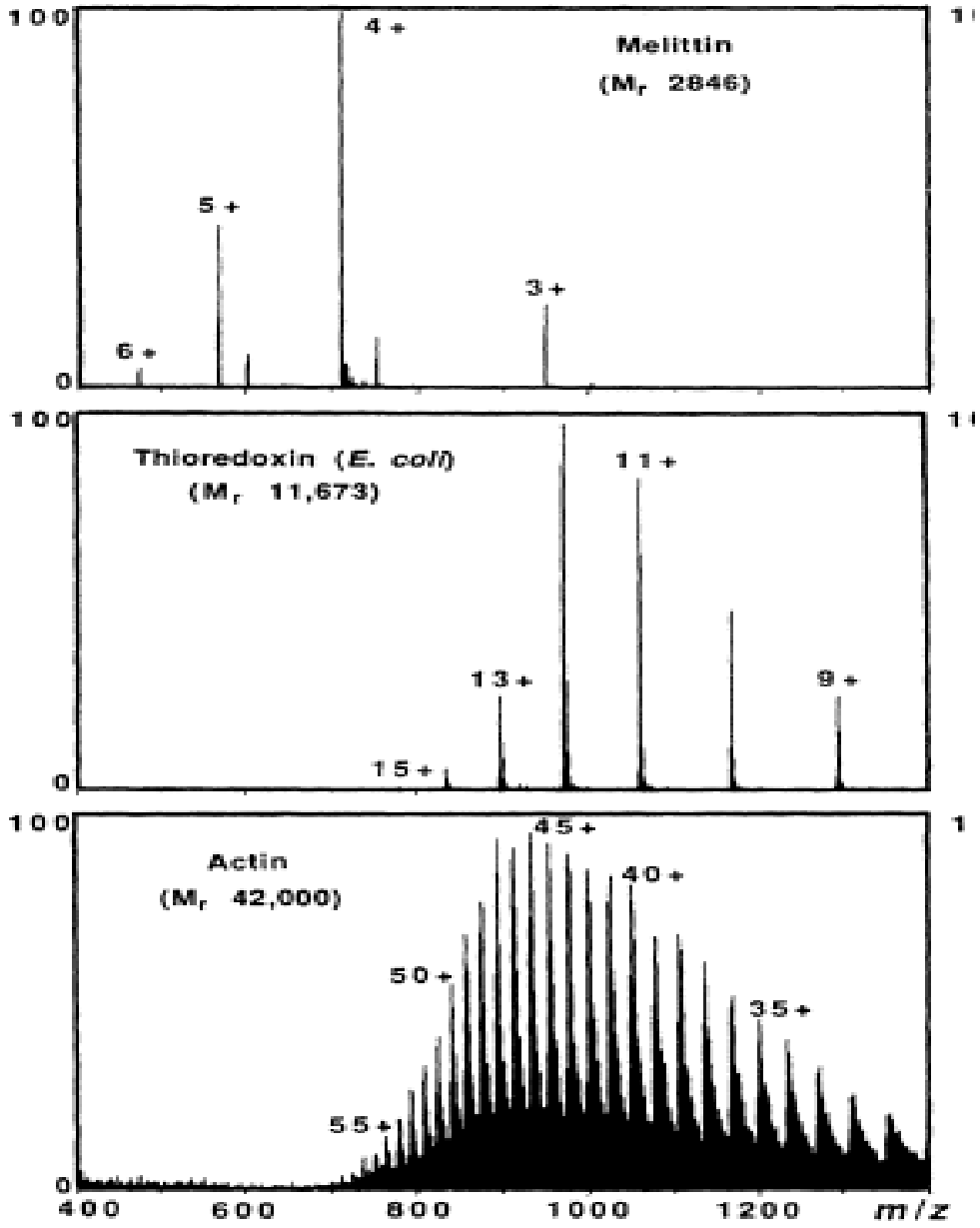
- No single technique can measure the universe of molecules
- Tradeoffs: limits of detection, analysis time, interferences, cost
- Mix and match methods of collection, separation, and detection to achieve measurement goals
- True identification is a multi-step process (effort should match data objectives)
- Carefully identify THE QUESTION – what does customer need to know?

What skills do we use every day?

- Math
- Lab notebooks
- Reporting
- Summarizing to appropriate audience (boss, customer, colleague)

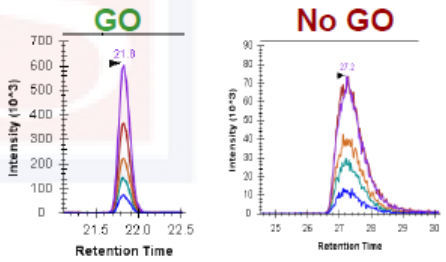
Additional ESI examples

- Proteins usually exist in solution as multiply-charged forms
- Multiple charging allows for detection of large proteins at relatively low *m/z* values
- Deconvolution software is capable of consolidating this information into identifying the mass of the protein



What is system suitability?

- "System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated". (FDA)
- "The checking of a system, before or during analysis of unknowns, to ensure system performance" (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use [ICH])
- **Simply put: Analysis of a known sample to assess system performance**
  - Helps to identify when the system is not working



Define Pass/Fail Criteria

Is System Suitability the same as "QC"

- No!
- "QC" = Quality Control of your method
  - Takes into account sample prep
  - L, M, H QC standards used to show the entire workflow gives "expected" responses
- QC is method specific!!
- System suitability is designed for long-term system evaluation

When should you run system suitability tests?

- All the time!
  - Before unknown samples
  - Periodically (daily to weekly)
  - Before and After:
    - instrument repair/maintenance
    - changes to hardware/plumbing
    - Before and after changes to software
  - Anytime you think your system is not performing optimally
  - When you think your system IS performing optimally, for longitudinal comparison

Evaluation of system suitability test

Use pre-define QUANtititative acceptance limits

ASMS troubleshooting class: Sue Abbatiello, Tom Blau, Will Thompson