Electrospray and Atmospheric Pressure Chemical Ionization Quadrupole MS for Small Molecules

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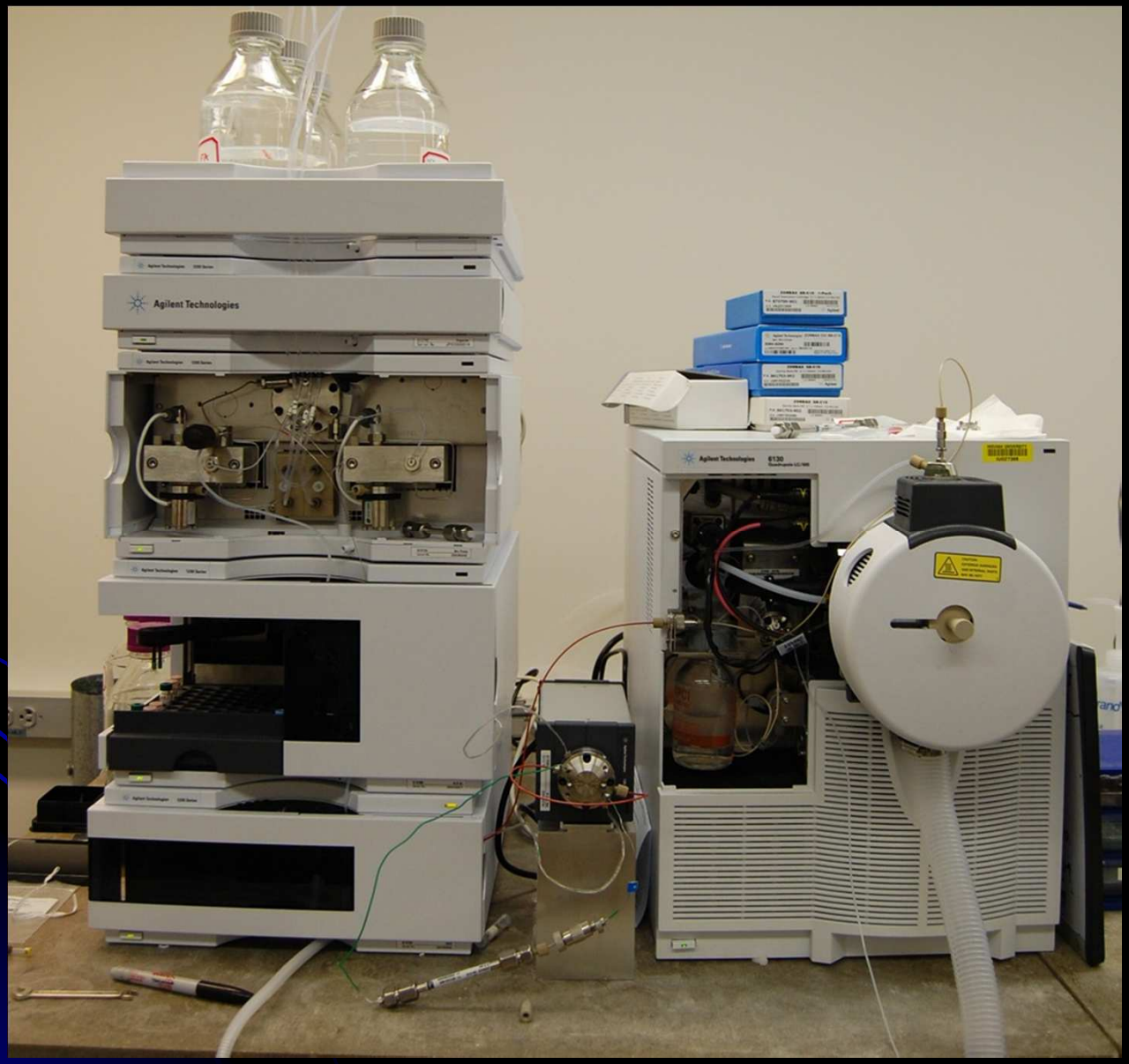
LC-MS Picture



General Sample Guidelines

- **Purify analyte if possible**
  - Analyte should be 1-100 mg/L in concentration
    - Colored samples should be translucent
  - ***Sample must have no particulates or precipitates***
- **Use only volatile solvents/buffers**
  - MeOH, H_2O, acetone, CH_3CN, THF, $CHCl_3$, etc.
  - HOAc, HCOOH, NH_4OAc, NH_3, etc. (weak, volatile)
  - Ionic strength < 20 mM is best (e.g. 0.1% v/v HOAc)
  - *1 – 5 µM NaOAc for acid/base labile samples*
- **Need at least 50 µL for loop injection**
- **If you need non-polar solvent for APCI, see Dr. Karty first**

Agilent 6130 System Description

- Inlet is an Agilent 1200 HPLC System
  - Binary gradient (14 solvent bottles, only 2 can be used in an analysis (A1 or A2 with B1 or B2, not A1 and A2)
    - 50-2,500 uL/min flow rates
  - Autosampler 0.5-100 µL injection volume
- 6-column selection valve (loop or 1 of 5 HPLC columns)
  - C_{18} reversed phase is default column (H_2O-CH_3OH solvents)
- Agilent 1200 DAD detector
  - 190-800 nm UV-VIS detector
  - Can record entire UV-VIS spectrum throughout a run
- Electrospray Ionization-Atmospheric Pressure Chemical Ionization/Quadrupole (ESI/APCI-Q) mass spectrometer
  - 50 – 3,000 m/z range
  - Can make ions by ESI, APCI, or both
  - Can alternate positive and negative ion modes during a run

Air Sensitive MS

- Source on 6130 MS is flushed constantly with gas from a liquid nitrogen dewar
  - Enables walk-up air senstive ESI/APCI MS
- A syringe pump with air-tight “Sample-Lock” syringe can be used for air-sensitive analyses
  - Requires special training from Dr. Karty
  - A separate syringe is needed for each sample
- A “dummy” sample must still be placed in the autosampler
- Remember to keep concentrations around 20 mg/L
  - Colored solutions should be translucent

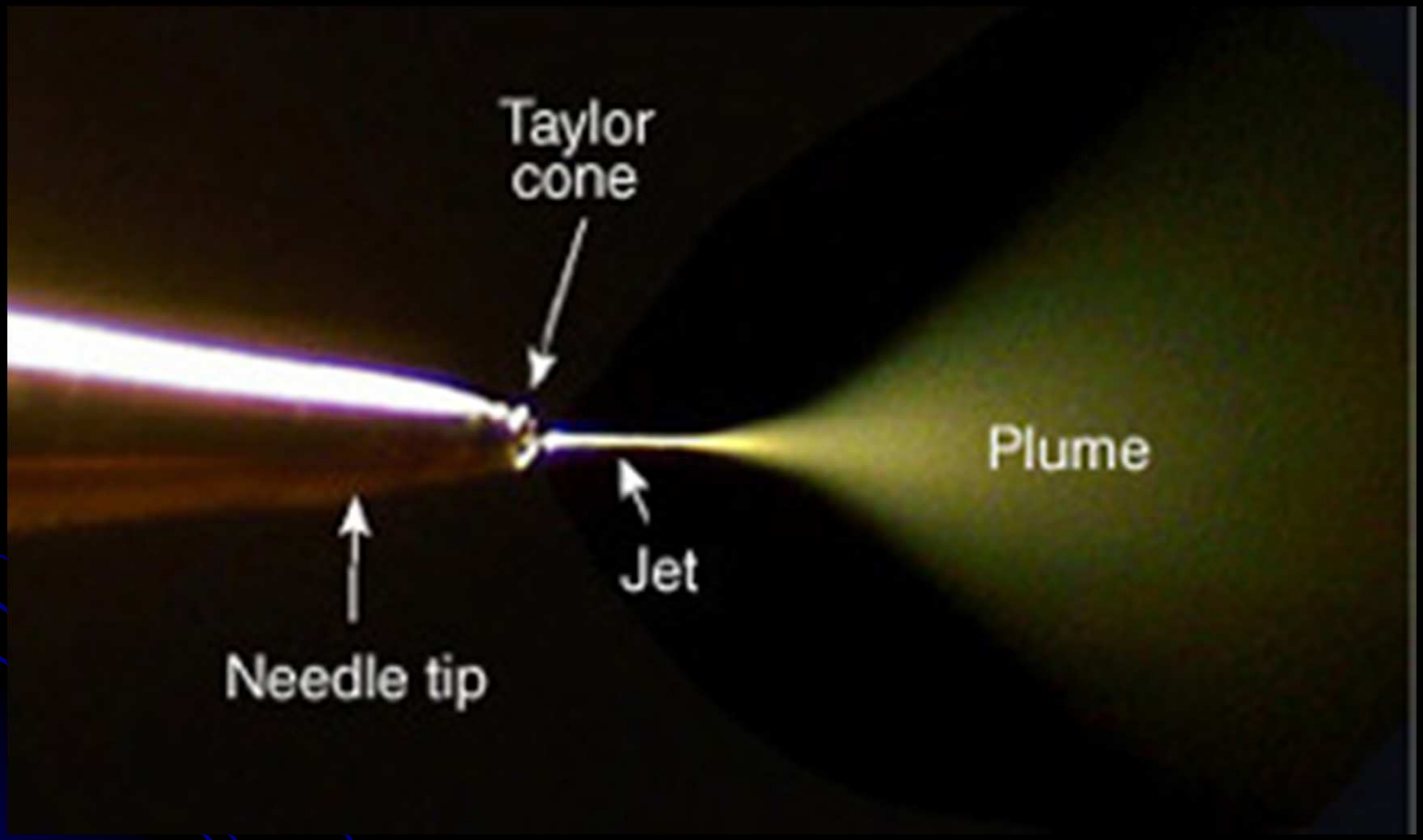
HPLC-MS vs. Loop Injection

- MSF uses the 1200 autosampler for nearly all samples
  - Must inject something, even if syringe pump is used
- Sample can go through an empty loop
  - Solution must contain only sample and volatile components
  - Allows 2.5 minute analysis time
- Sample can also be separated by HPLC
  - Up to 5 columns are available
  - Currently only C_{18} reversed phase available for general use

Electrospray Ionization (ESI)

- Dilute solution of analyte (1 mg/L) infused through a fine needle in a high electric field
- Very small, highly charged droplets are created
- Solvent evaporates, droplets split and/or ions ejected to lower charge/area ratio
  - Warm nebulizing gas accelerates drying
  - Basic sites are eventually protonated $(M+H)^+$
  - Ketones, esters, and alcohols can sodiate $(M+Na)^+$
  - Acidic sites can be deprotonated $(M-H)^-$
  - Chlorinated solvents can add a chloride $(M+Cl)^-$
- Free ions are directed into the vacuum chamber

ESI Picture



http://newobjective.com/images/electro/spraytip_bw.jpg

Characteristics of ESI Ions

- ESI is a thermal process (1 atm in source)
  - Little fragmentation due to ionization (cf EI)
- Solution-phase ions are often preserved
  - e.g. organometallic salts
- ESI ions are generated by ion transfer
  - $(M+H)^+$, $(M+Na)^+$, $(M-H)^-$, or $(M+Cl)^-$, rarely $M^{+\bullet}$ or $M^{-\bullet}$
- ESI often generates multiply charged ions
  - $(M+2H)^{2+}$ or $(M+10H)^{10+}$
  - *Most ions are 500-1500 m/z*
  - ESI spectrum x-axis must be mass/charge (m/z or Th, not amu or Da)

Advantages of ESI

- Gentle ionization process
  - High chance of observing intact molecular ion
  - Very labile analytes can be ionized
  - Non-covalent complexes can be studied by ESI
- Molecule need not be volatile
  - Proteins/peptides easily analyzed by ESI
  - Salts can be analyzed by ESI
- Easily coupled with HPLC
- Both positive and negative ions can be generated by the same source

ESI Disadvantages

- Analyte must have an acidic or basic site
  - Hydrocarbons and steroids not readily ionized by ESI
- Analyte must be soluble in polar, volatile solvent
- ESI is less efficient than other sources
  - Most ions don’t make it into the vacuum system
- ESI is very sensitive to contaminants
  - Solvent clusters can dominate spectra
- Distribution of multiple charge states can make spectra of mixtures hard to interpret
  - e.g. polymer mass spectra

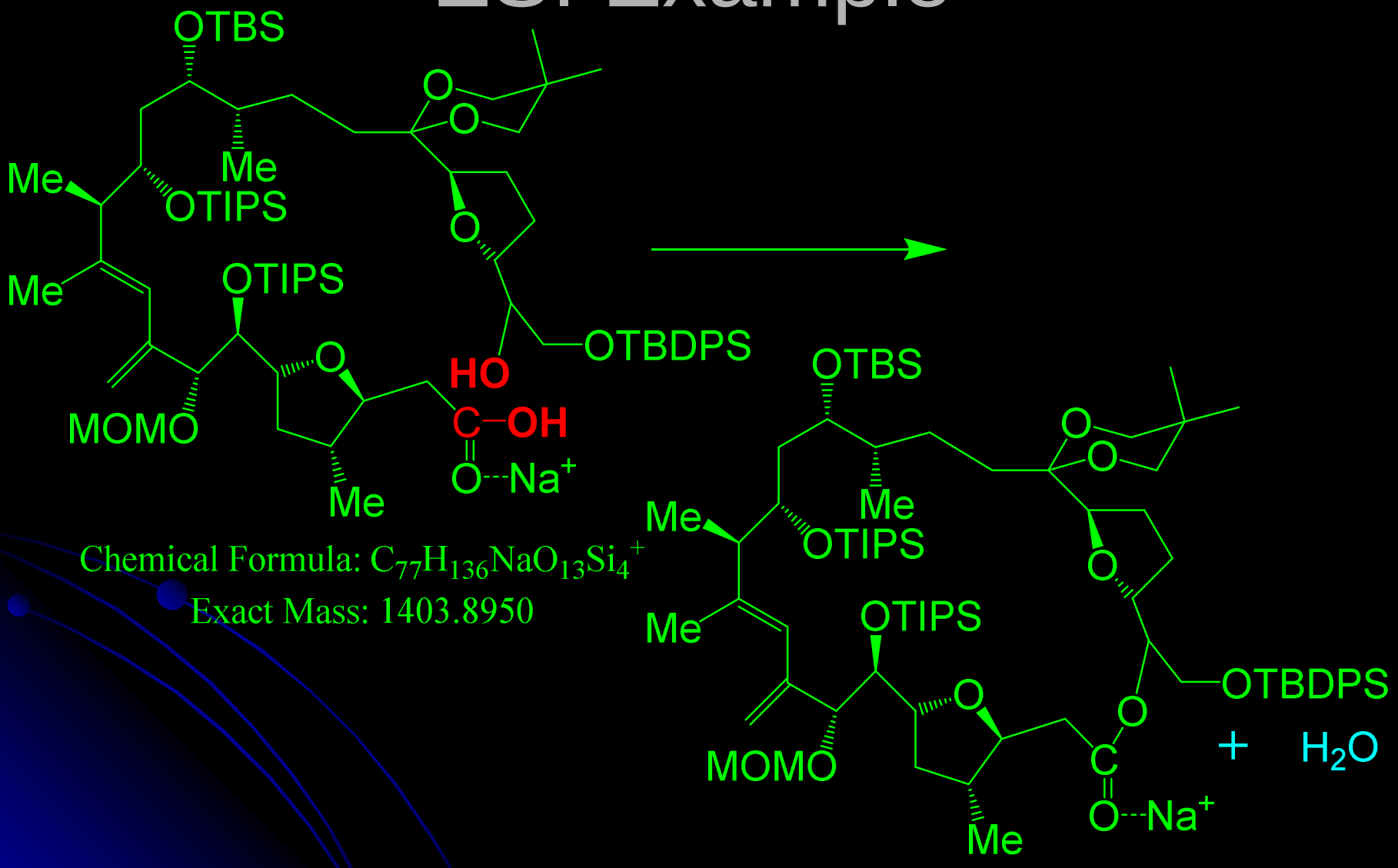
What Samples Can Be Analyzed by ESI-QMS?

- Non-volatile organic molecules
  - Should be soluble in polar, volatile solvents
  - Molecule must be ionizable (MS detects ions)
    - R-NH_2, R-CO_2H, R-HSO_3, R-OH, R-H_2PO_3 work best for ESI
    - Aromatics, ketones, protected heteroatoms work well by APCI
- Organometallic complexes
  - Organometallic salts work especially well
  - Source is flooded with N_2 gas from a dewar
- HPLC-MS
  - With appropriate mobile phases

What Samples Are Inappropriate for ESI-QMS analysis?

- Samples in non-polar or non-volatile solvent:
  - Hexane, benzene, CH_2Cl_2, DMSO, etc.
  - Run these in APCI or mixed mode
- Buffer systems incompatible with ESI
  - 6M urea, 10% glycerol, 0.1 M NaH_2PO_4, TBAF, (involatile)
  - Strong acid/base solutions (e.g. 10 mM HCl or NaOH too conductive)
  - detergents
- Molecules that have no ionizable groups

ESI Example



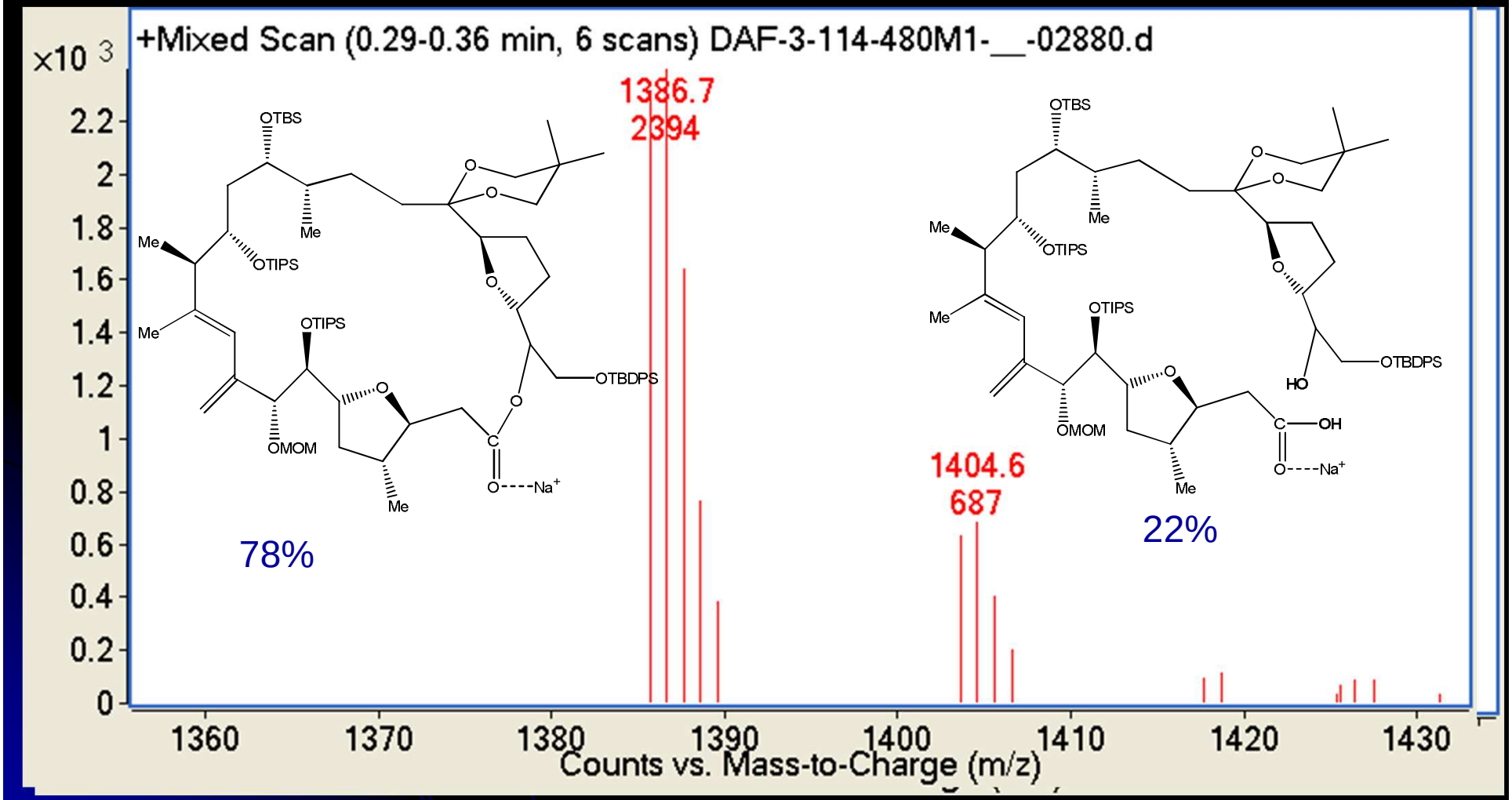
Chemical Formula: $C_{77}H_{136}NaO_{13}Si_4^+$

Exact Mass: 1403.8950

Chemical Formula: $C_{77}H_{134}NaO_{12}Si_4^+$

Exact Mass: 1385.8845

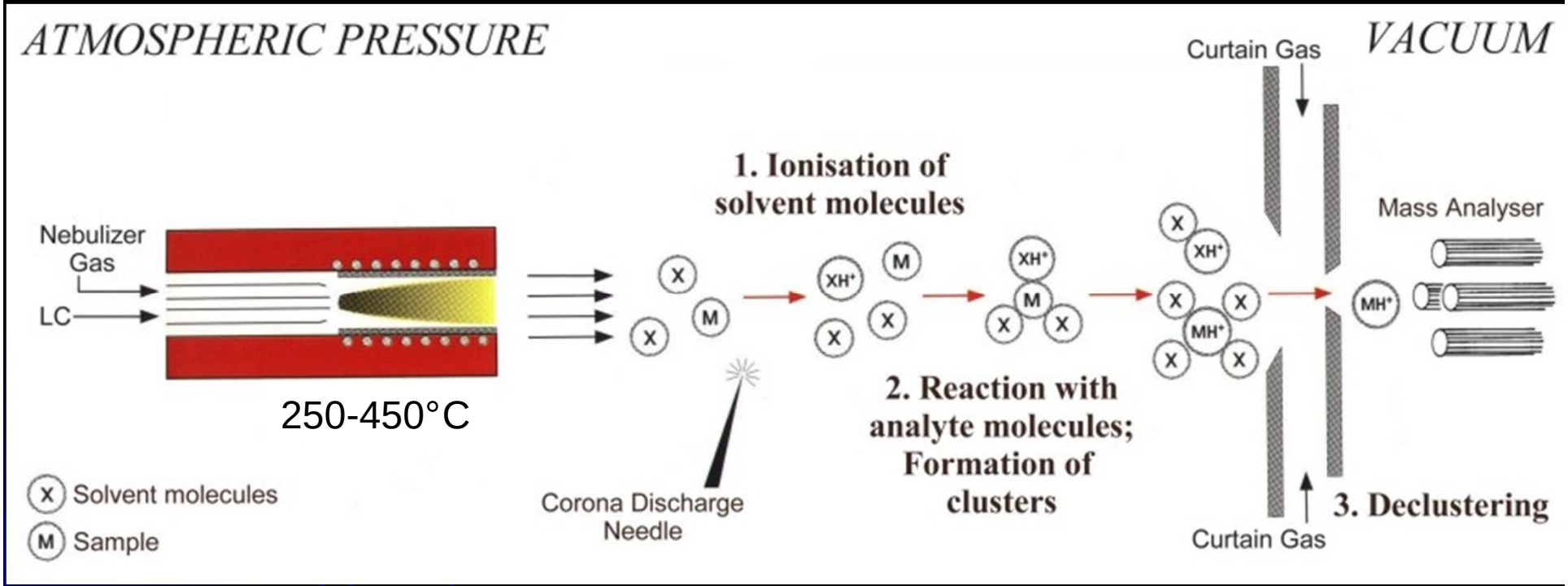
Data from 613//1200



Atmospheric Pressure Chemical Ionization (APCI)

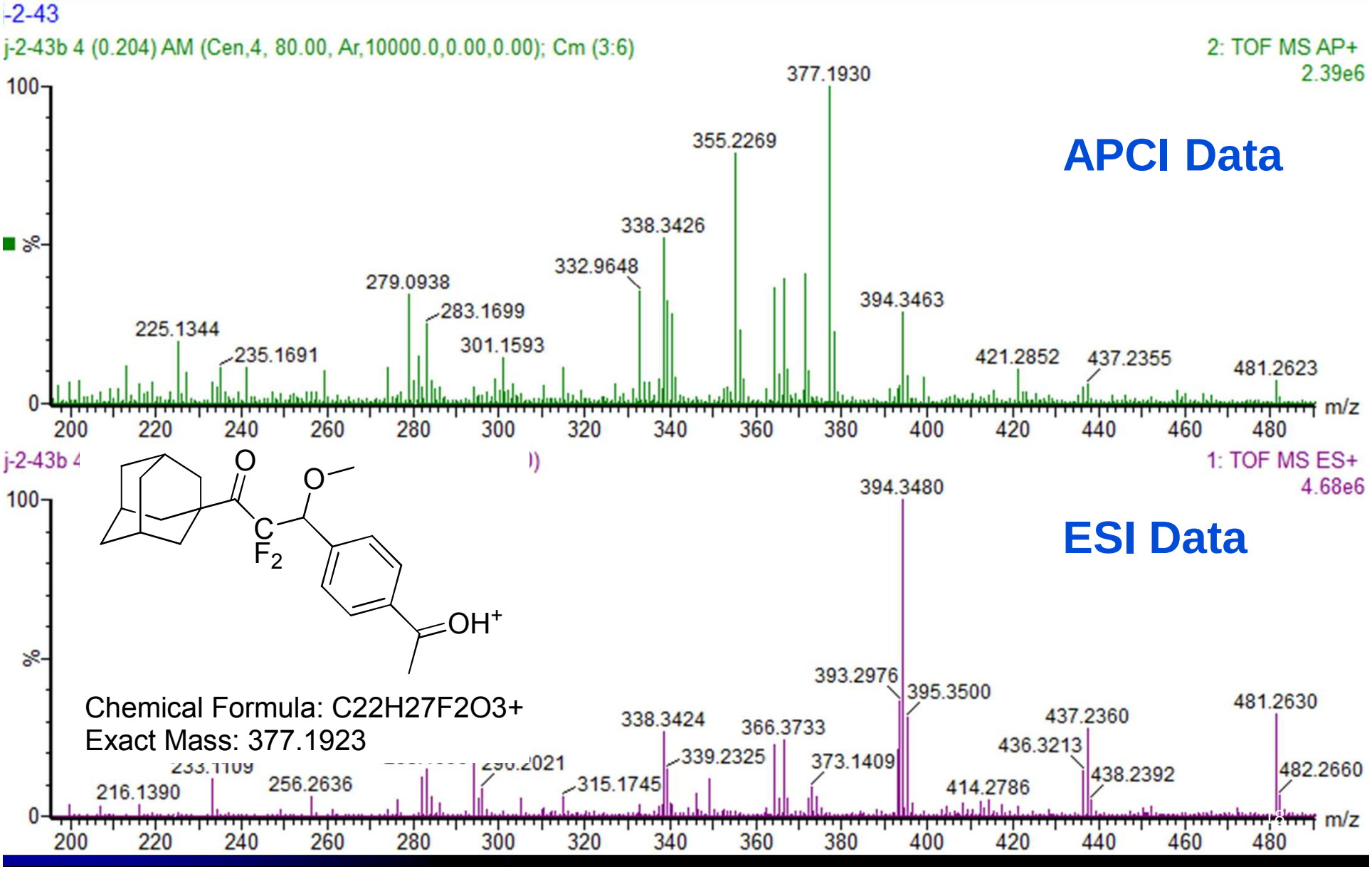
- APCI uses a corona discharge to generate acidic solvent cations from a vapor
  - Ionizing reagent often $CHCl_3^+$ or CH_3OH^+
- These solvent cations can protonate hydrophobic species not amenable to ESI
  - APCI can be done from hexane or THF
  - Often used to study lipids and steroids
  - In MSF, completely protected macrocycles are routinely studied by APCI
- APCI is a little harsher than ESI
  - Labile molecules might fragment

APCI Diagram

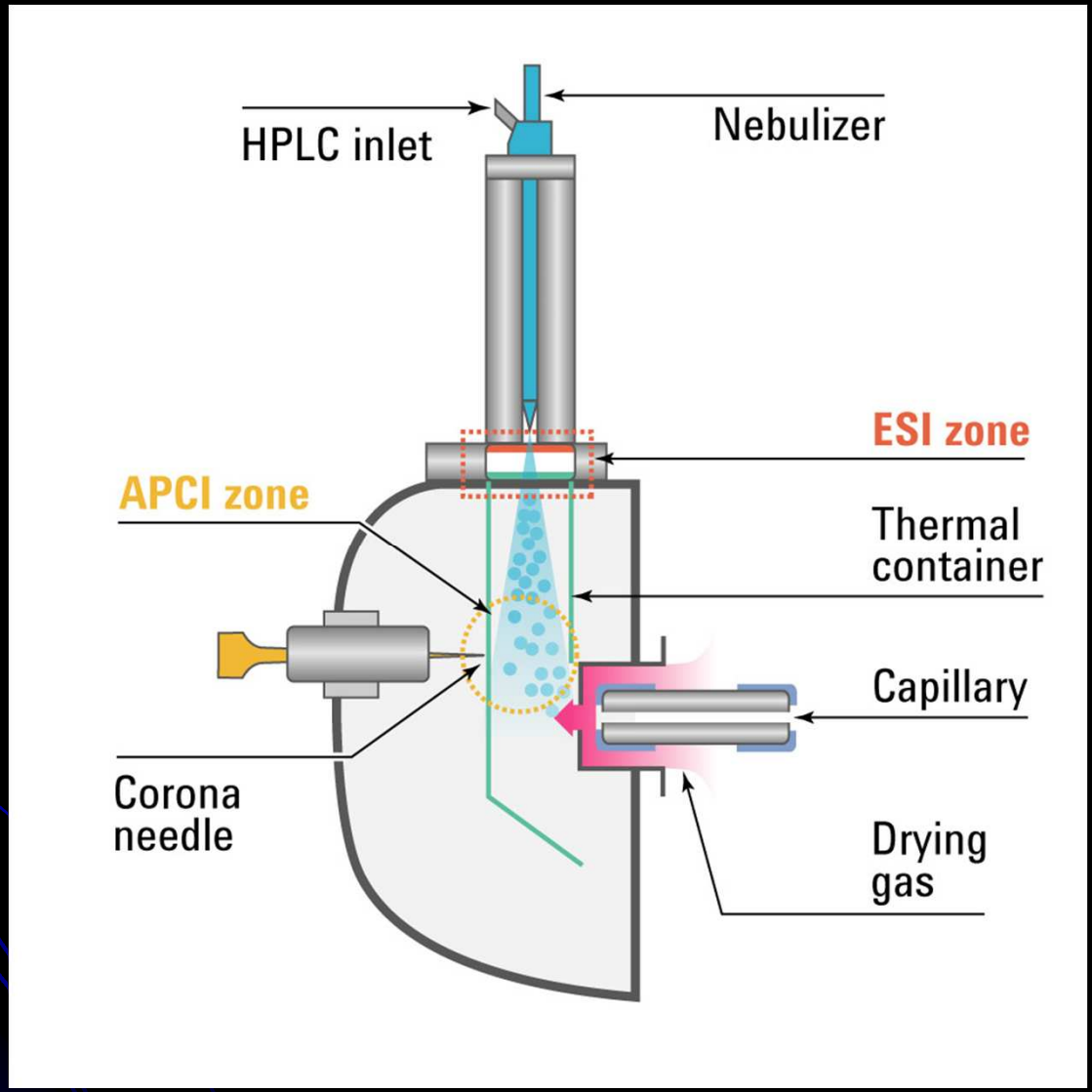


http://imaisd.usc.es/riaidt/masas/imagenes/apci1.jpg

APCI Example



Agilent 6130 Multi-mode Source



http://www.chem.agilent.com/Library/Images1/MMS_schematic_300dpi_039393.jpg

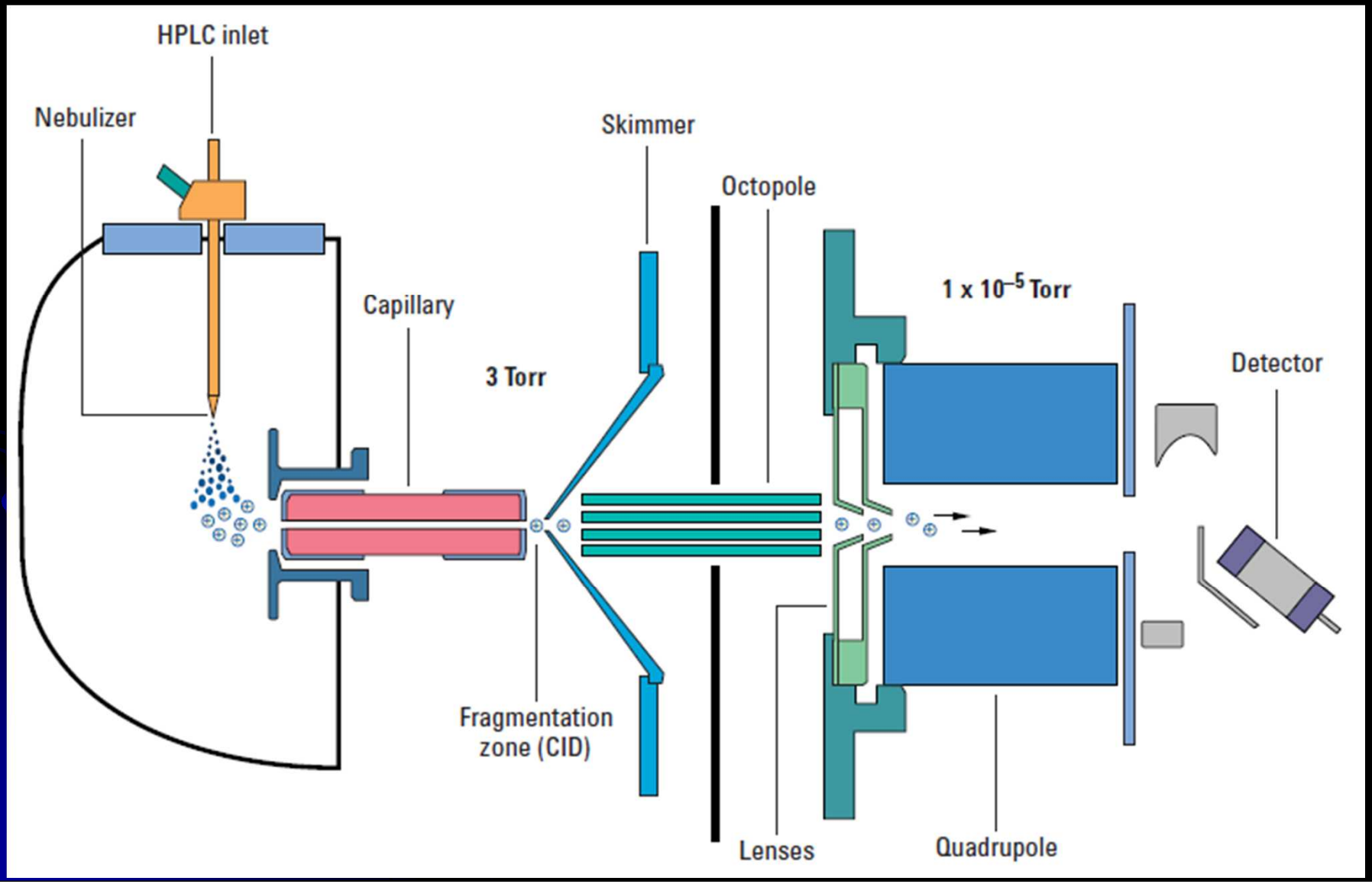
Cluster and Background Ions

- The low m/z (<300) region of the mass spectrum is often dominated by solvent cluster ions
  - These are distinguished by low mass defects due to Na^+ and K^+ incorporation
  - Masses are XXX.0 or XXX.9
- Compounds from previous samples can coat the source leading to persistent “background” ions
  - e.g. 242.1 for tetrabutylammonium
  - Might see large ion from previous person’s run
- To confirm an ion is “background” try injecting a blank of ESI solution with your solvent system

About the Fragmentor…

- There is a region in the source where ion-nitrogen collisions can cause fragmentation
  - This occurs between the end of the heated capillary and the first skimmer cone
- The voltage difference between capillary and skimmer is called the “Fragmentor” voltage
- Fragmentor voltage has 2 helpful main effects
  - It increases the number of ions that make it to the quad
  - It can knock non-covalent attached neutrals
- However, a high fragmentor value can cause labile groups to dissociate
  - Phospho-diesters can be rather vulnerable to this

Source Diagram



A Word About Quantification

- LC-MS is a quantitative technique
- Use only 1 m/z when quantifying a compound
- Intensity is proportional to concentration
  - $I \alpha [X]$
  - α is unique to each compound
  - **The more two compounds differ chemically, the more careful one must be when comparing their intensities**
- Ideally a calibration curve is constructed using multiple solutions of pure analyte at varying concentrations
- A calibration lecture will be given next month if there is sufficient interest

Hands-on Training

- Training starts soon, contact Jon or Angie
- Groups of no more than three
- One hour or so to complete
- No charge for first session
- After training, students must demonstrate competency by running their own samples prior to being granted after-hours access