Introduction to Mass Spectrometry

Ionization Sources, Ion Trajectory, and Method Development

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GULF COAST CONFERENCE 2012
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WORKSHOPS | BOOTH | POSTERS | GENERAL SESSION PRESENTATIONS

Agenda

- Introduction to mass spectrometry
  - Benefits of using mass spectrometry
  - Ionization techniques
  - Mass Analyzer
- Parameters for the Agilent 6100 Mass Spectrometers
- Tuning the mass spectrometer
- Optimizing MSD Analyses

Myths of LC/MS

"LC/MS is not routine"

"LC/MS is not sensitive"

"LC/MS is not quantitative"

"LC/MS is not cost-effective"

Benefits of LC/MS

For the chromatographer

- Complements existing LC detectors
- Does not depend on particular functional group
- Can be used as a mass-specific detector
- Provides both qualitative and quantitative information

For the mass spectrometrist

- Can analyze compounds not amenable to GC (large, polar, thermally labile)
- Allows direct coupling of LC separation; produces better information faster than "offline" LC/MS
- Automates probe analysis via flow injection

Interfacing HPLC to MS

HPLC

High pressure liquid phase separation

produces high gas load

no mass range limitation

can use inorganic buffers

MS

high vacuum required

tolerates limited gas load

elevated temperatures

depends on m/z and analyzer

prefers volatile buffers

Atmospheric Pressure Ionization Mass Spectrometry (API-MS)

API-MS is a detection method for samples in the liquid phase (HPLC, FIA, Infusion). The sample is desolvated, ionized, analyzed by mass/charge ratio and detected.

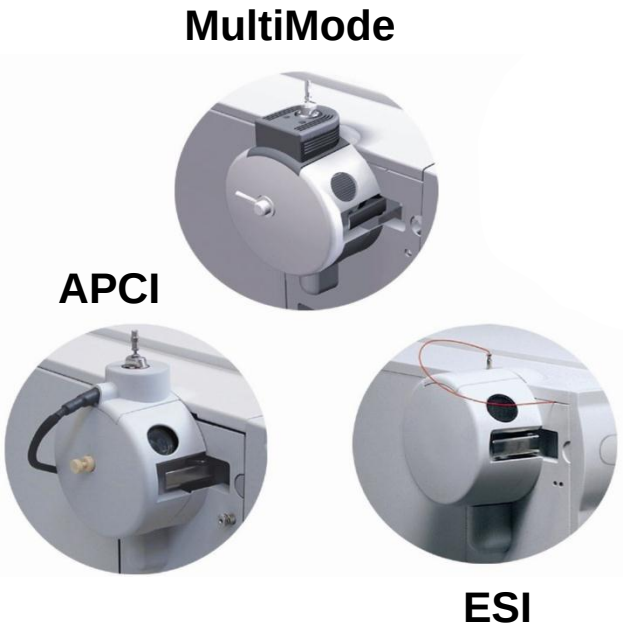
- Compatible with a broad range of compounds
- fg - pg sensitivity
- Qualitative information (MW up to 100,000 daltons or more with 0.02% accuracy)

SQ Ion Sources

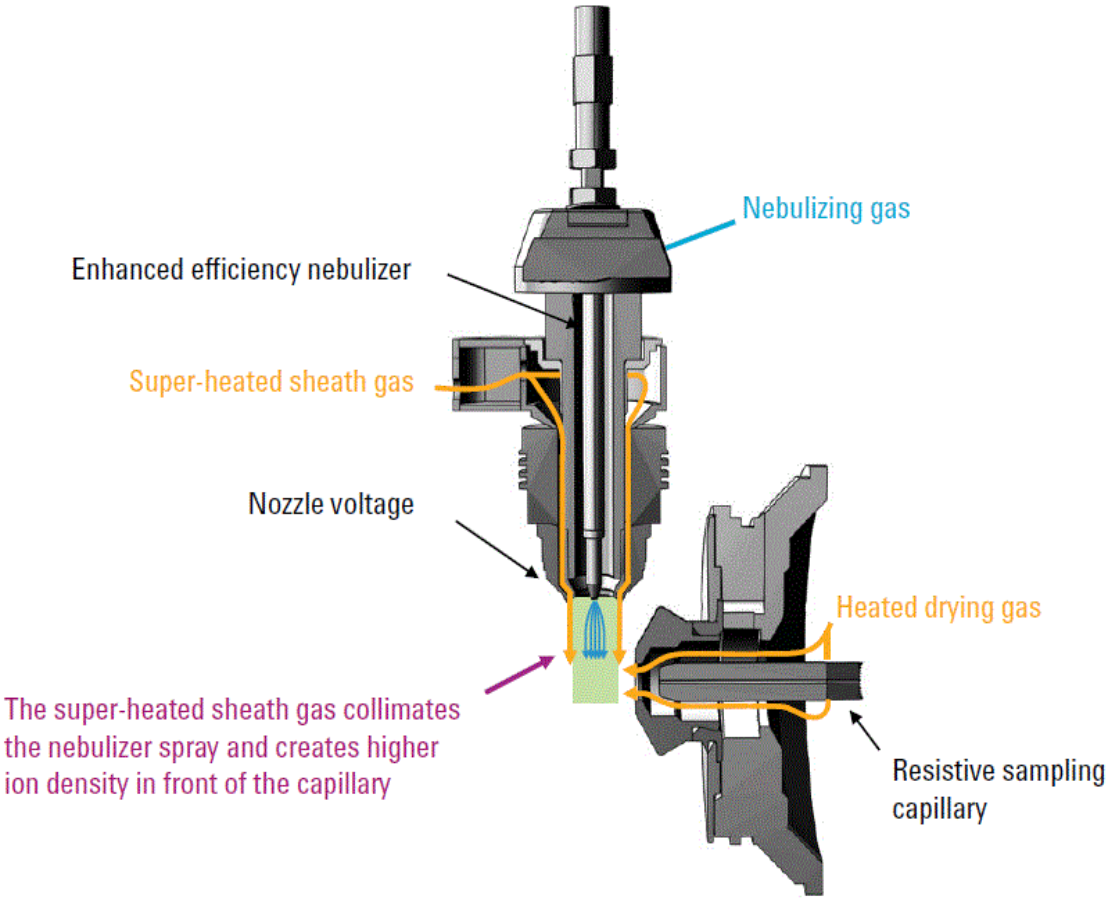
Electrospray:
Ionization process which uses an electrical field to generate charged droplets and subsequent analyte ions by ion evaporation for MS analysis. Nebulization is usually pneumatically assisted.

Atmospheric Pressure Chemical Ionization (APCI): Gas phase chemical ionization (CI) process where the solvent acts as the CI reagent gas to ionize the sample.

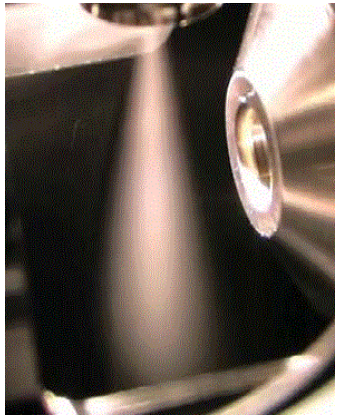
Multimode: Operates in ESI-only, APCI-only, or mixed mode (ESI+APCI).



MSD Methodology – 6150 Single Quad AJS Source

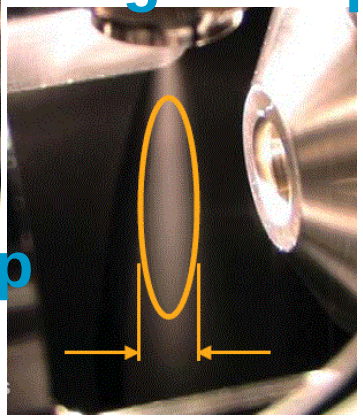


Vcap	3000
Fragmentor	110
Drying Gas	12 L/min
Nebulizer Gas	60 psi
Gas Temp	300°C
Polarity	Dual
Nozzle Voltage	1500
AJS Gas Flow	11 L/min, 1L/min
AJS Gas Temp	300°C, 100°C



Low Temp

High Temp



http://www.chem.agilent.com/Library/technicaloverviews/Public/5990-3494en_lo%20CMS.pdf

Thermal energy is focused to the nebulizer spray

Thermal focusing produces the most efficient desolvation and ion generation possible

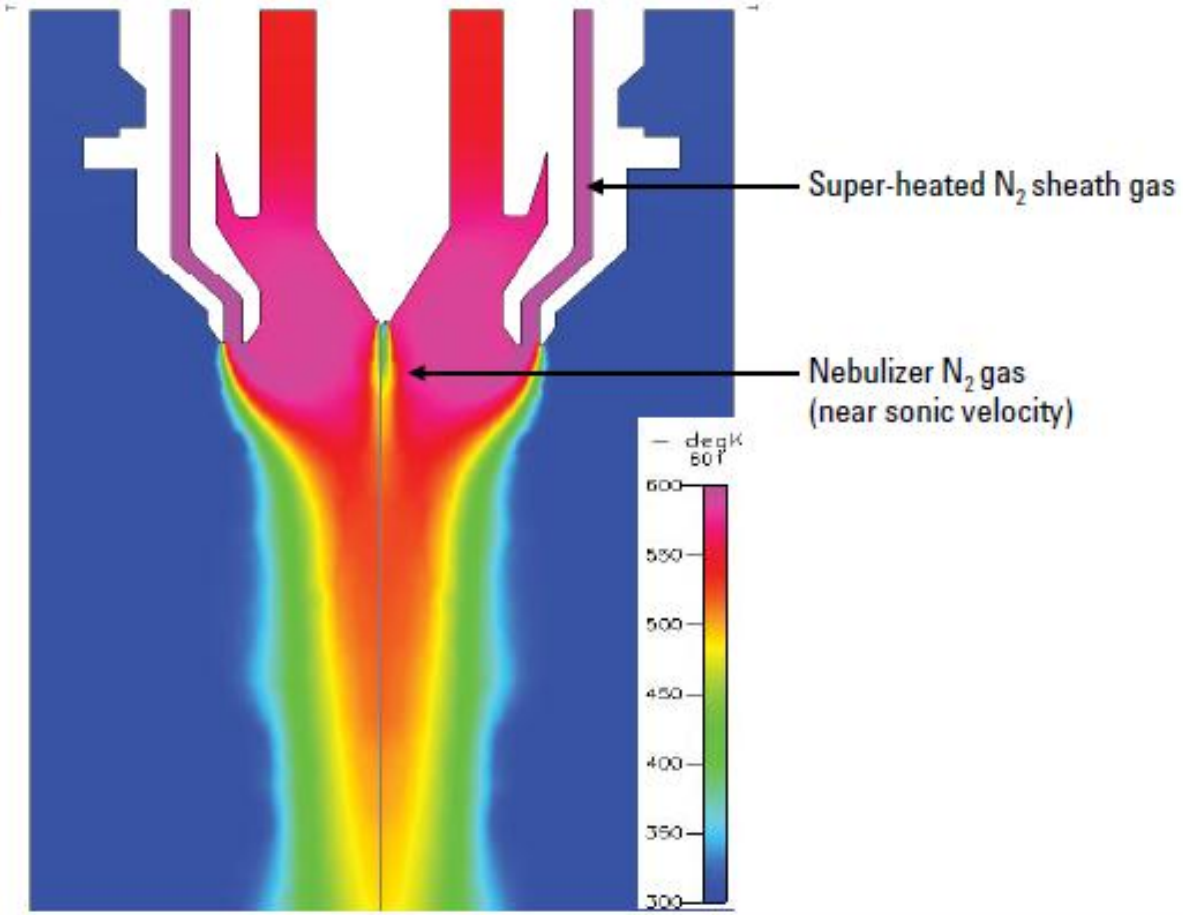
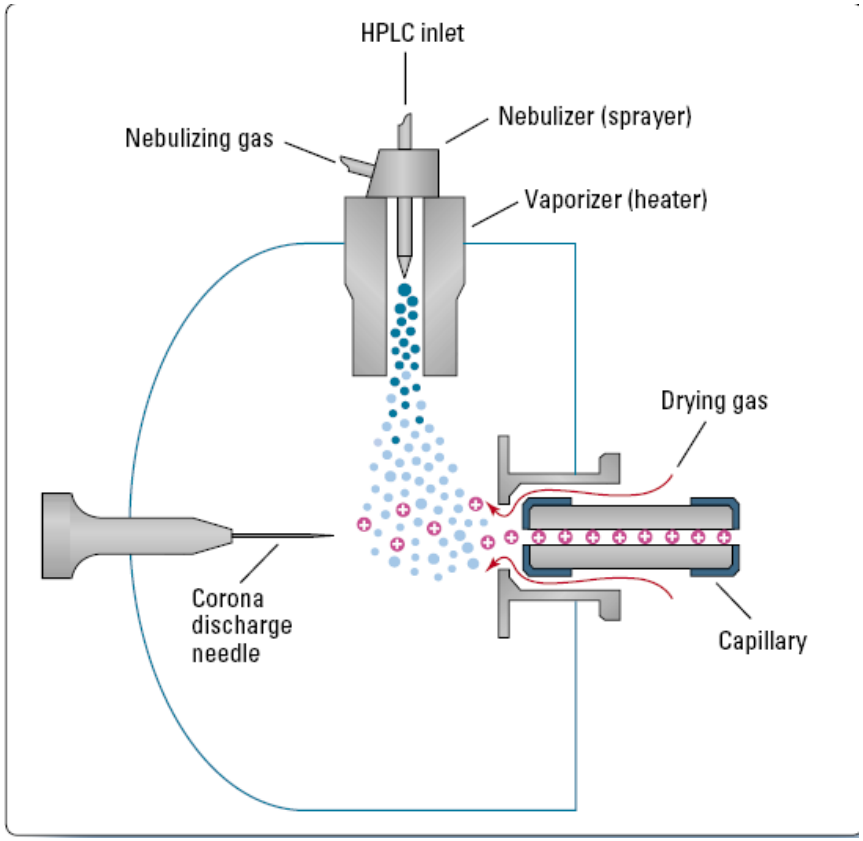


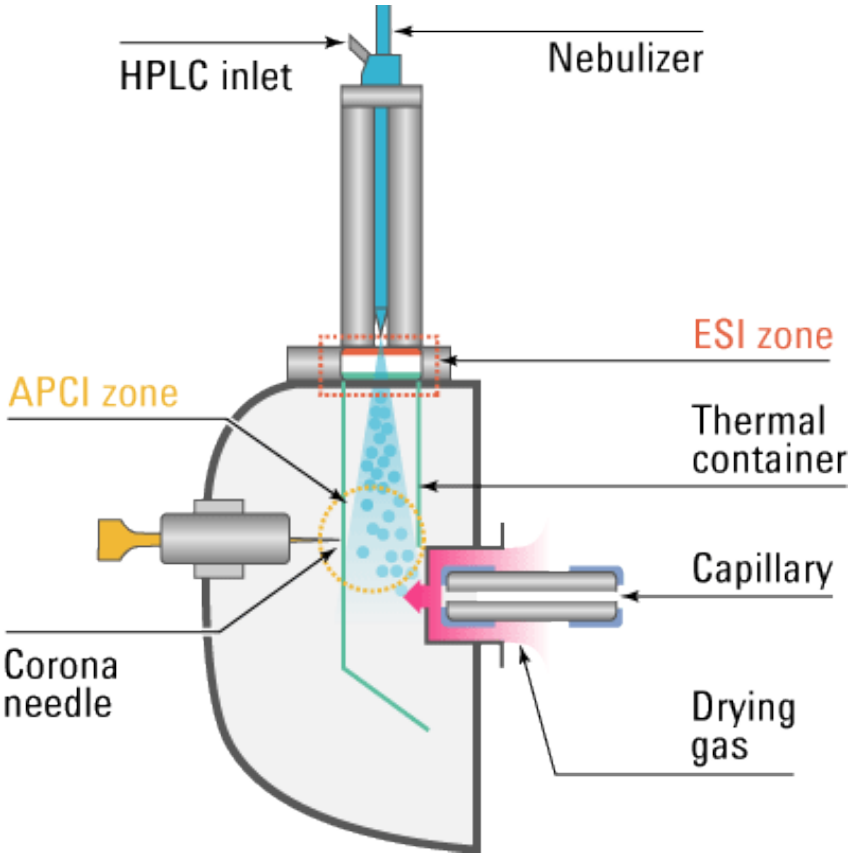
Figure 2. Simulation showing the thermal profile of the Agilent Jet Stream technology. Note the creation of a thermal confinement zone by introduction of a super-heated N_2 sheath gas.

MSD Methodology
– 6150 Single Quad APCi Source



Vcap	3000
Fragmentor	110
Drying Gas	12 L/min
Nebulizer Gas	60 psi
Gas Temp	300°C
Polarity	Dual
Corona	7, 15
Vaporizer	450°C

MSD Methodology
– 6150 Single Quad Multi-Mode Source



Vcap	2000
Vcharge	1500/2000
Fragmentor	110
Drying Gas	12 L/min
Nebulizer Gas	60 psi
Gas Temp	300°C
Polarity	Dual
Corona	4, 10
Vaporizer	250°C

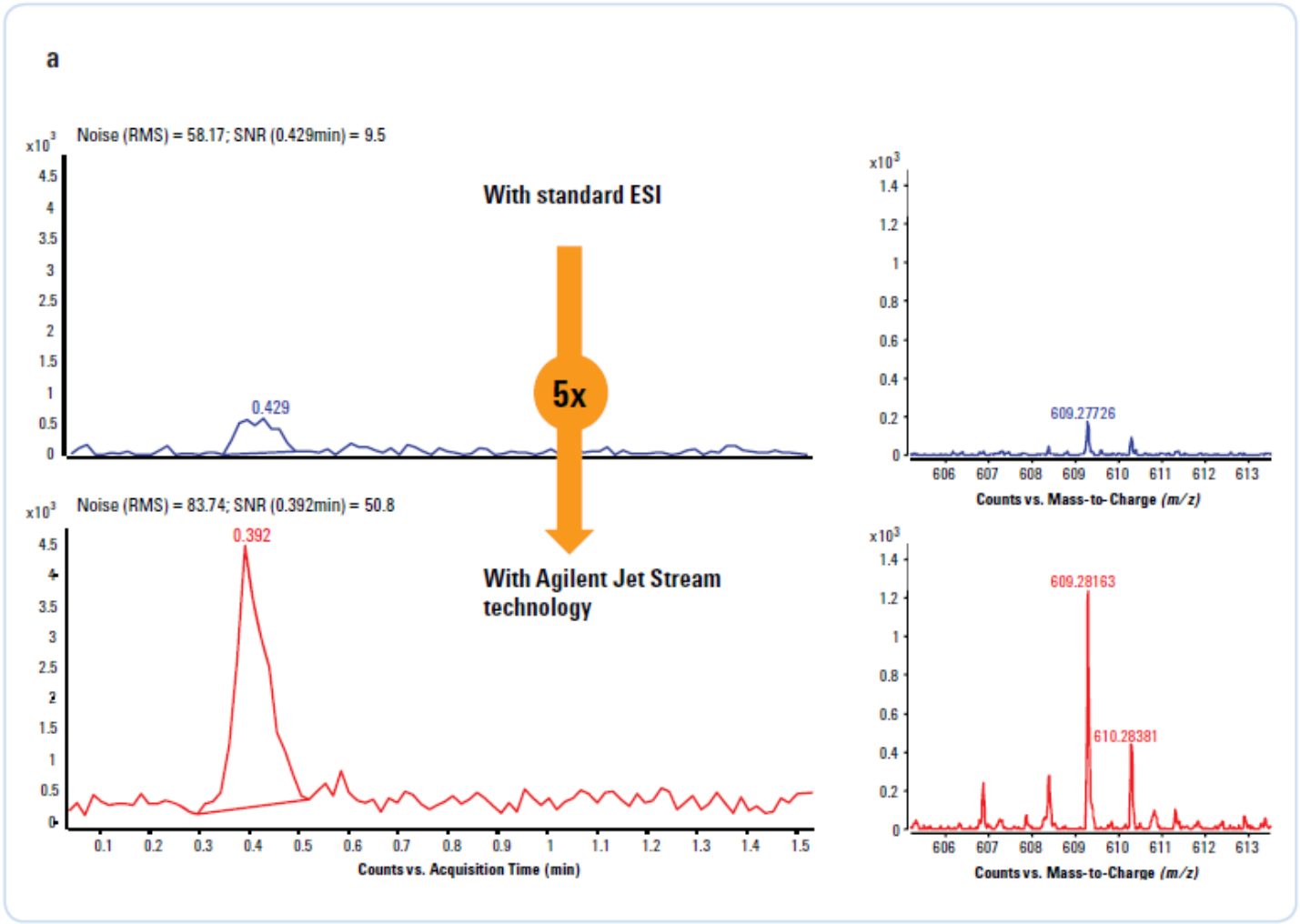


Figure 4a. Comparison of the MS spectra of a 1 pg sample of the drug reserpine obtained using conventional Agilent ESI source and Agilent Jet Stream technology on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. A 5-fold gain in signal intensity is observed with Agilent Jet Stream technology. LC conditions: Agilent 1200 LC system. Column: 2.1 x 30 mm Zorbax SB-C18, 3.5 µm; flow rate: 0.4 mL/min of 75:25 methanol/water containing 0.1% (v/v) formic acid and 5 mM ammonium formate. Agilent Jet Stream technology conditions: sheath gas temperature: 350° C; sheath gas flow: 12 L/min.

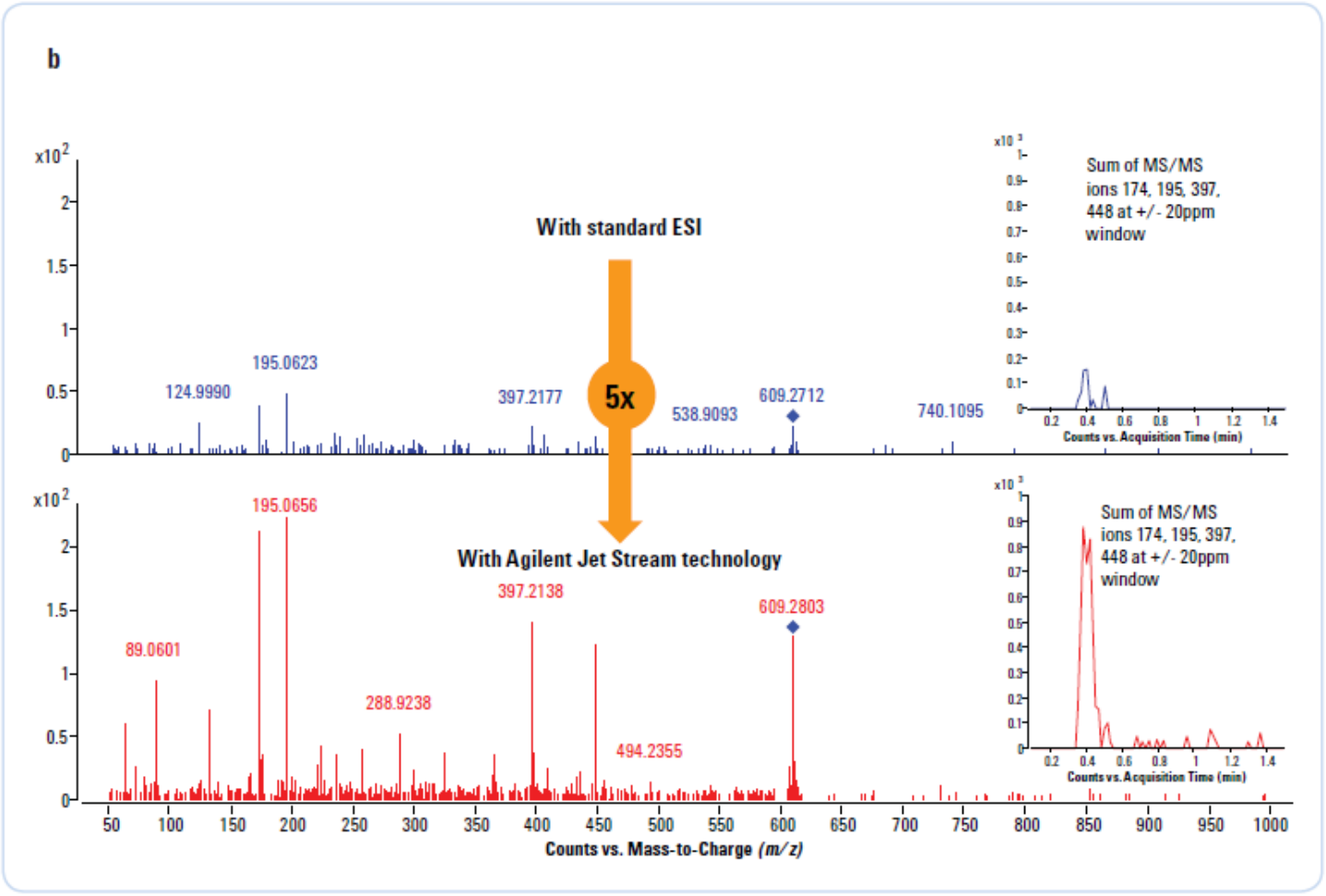


Figure 4b. Comparison of the MS/MS spectra of a 1 pg sample of the drug reserpine obtained using conventional Agilent ESI source and Agilent Jet Stream technology on an Agilent 6530 Q-TOF LC/MS system. A 5-fold gain in signal intensity is observed with Agilent Jet Stream technology. Conditions: same as for figure 4a.

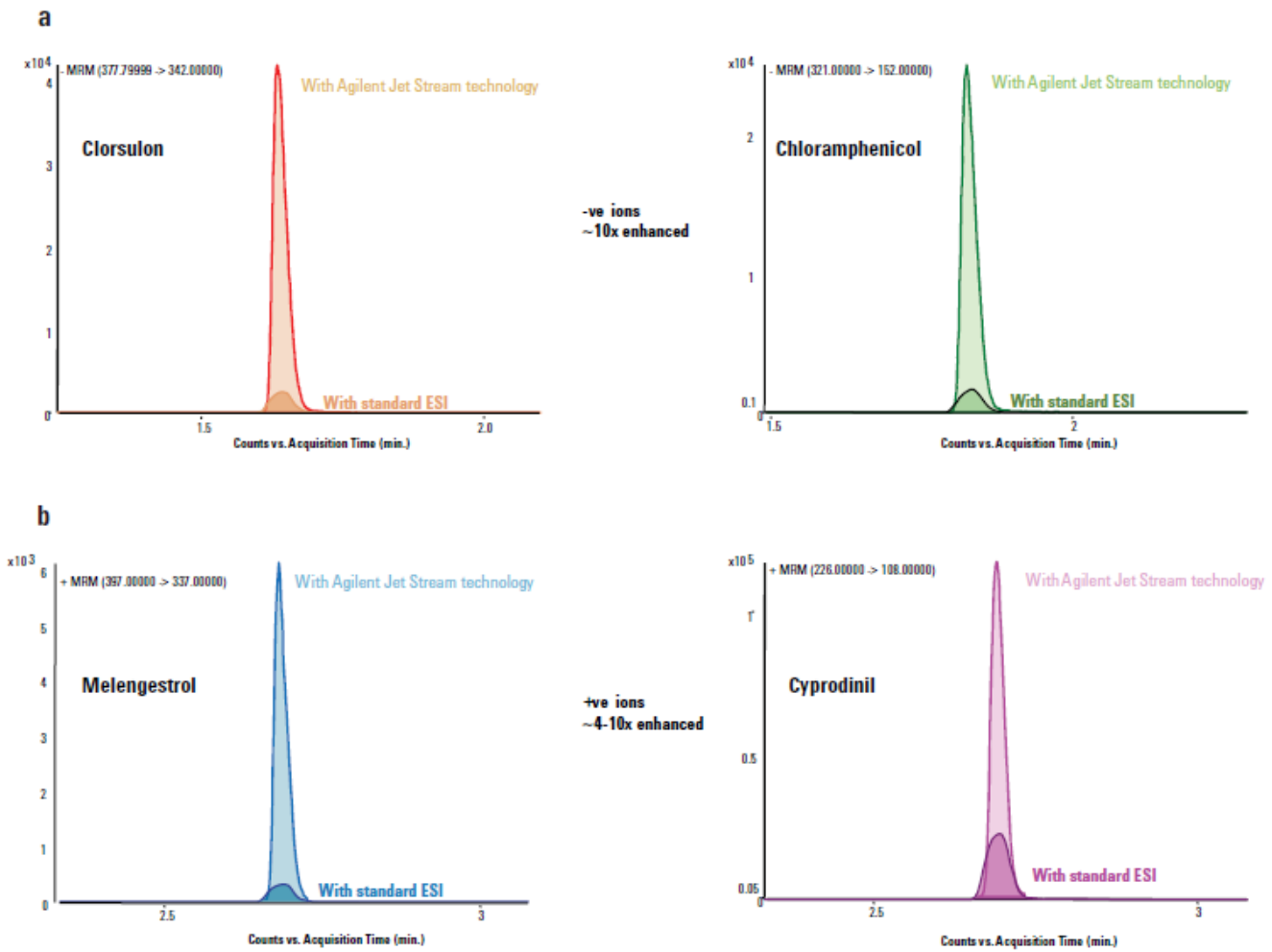
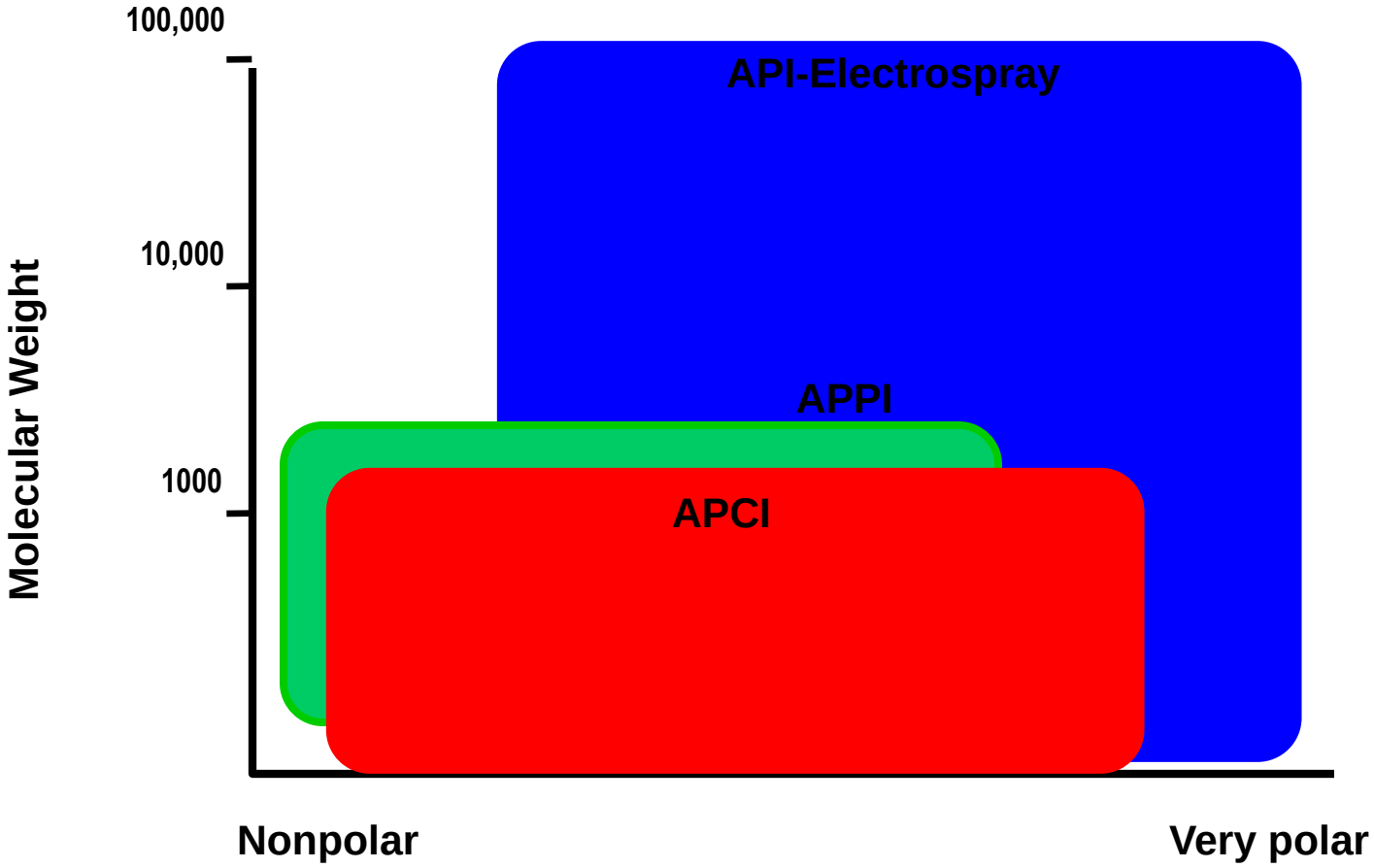


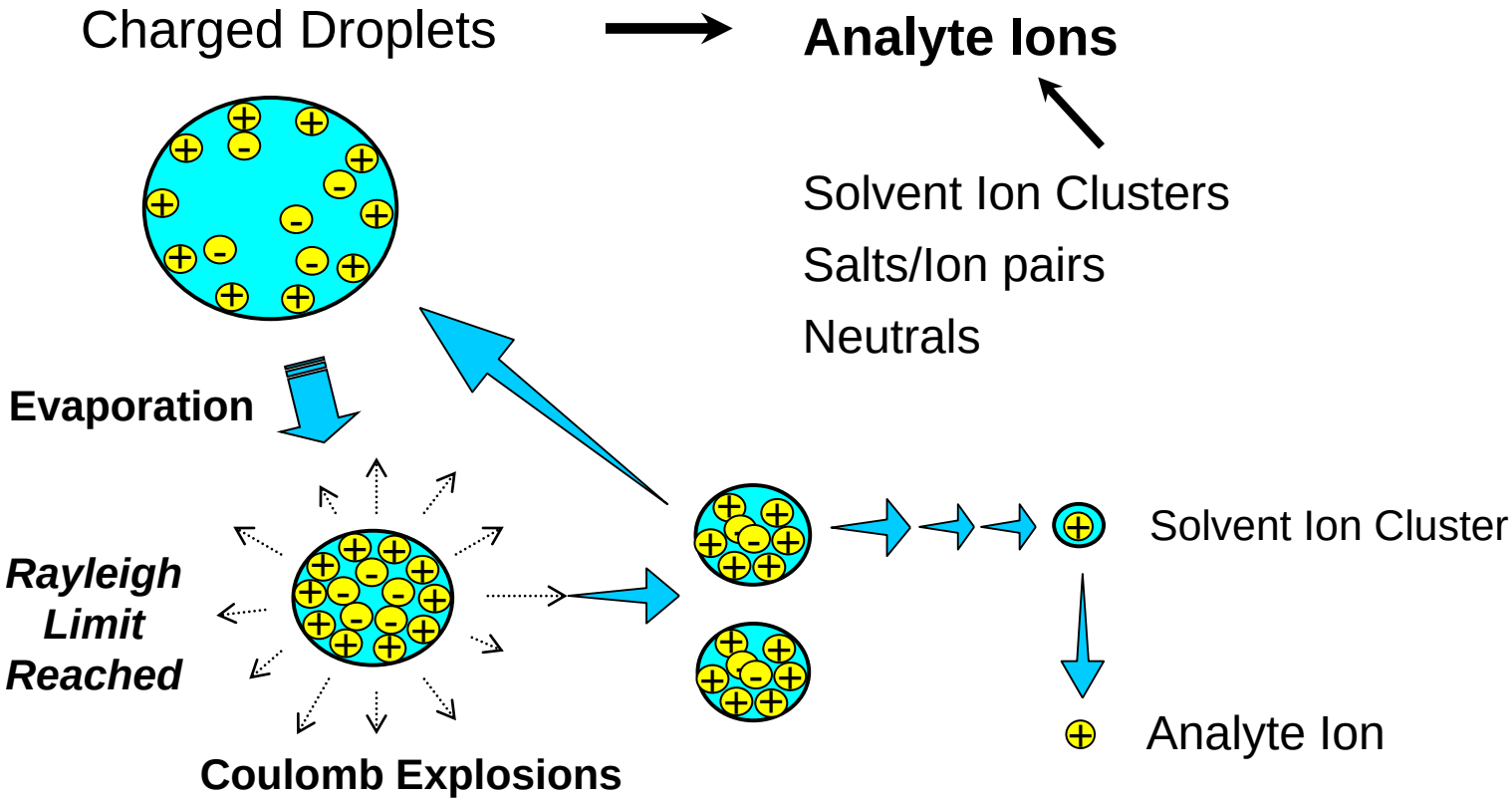
Figure 7. Pharmaceuticals spiked into potable water analyzed in **(a)** negative ion mode and **(b)** positive ion mode. Compared to conventional ESI (lower traces in each of the four graphs), Agilent Jet Stream technology enabled sensitivity improvements of approximately 10-fold in negative ion mode and between 4-to-10-fold in positive ion mode. Injected volume was 5 µL of a 50 ppb solution. LC Conditions: Agilent 1200 LC system. Column: 2.1 x 50 mm Zorbax Eclipse Plus C-18, flow rate: 0.5 mL/min, gradient: A=water, B= methanol, 5% B to 90% B. Agilent Jet Stream technology conditions: sheath gas temperature: 380° C, sheath flow: 11 L/min.

Relative Applicability of LC/MS Ionization Techniques

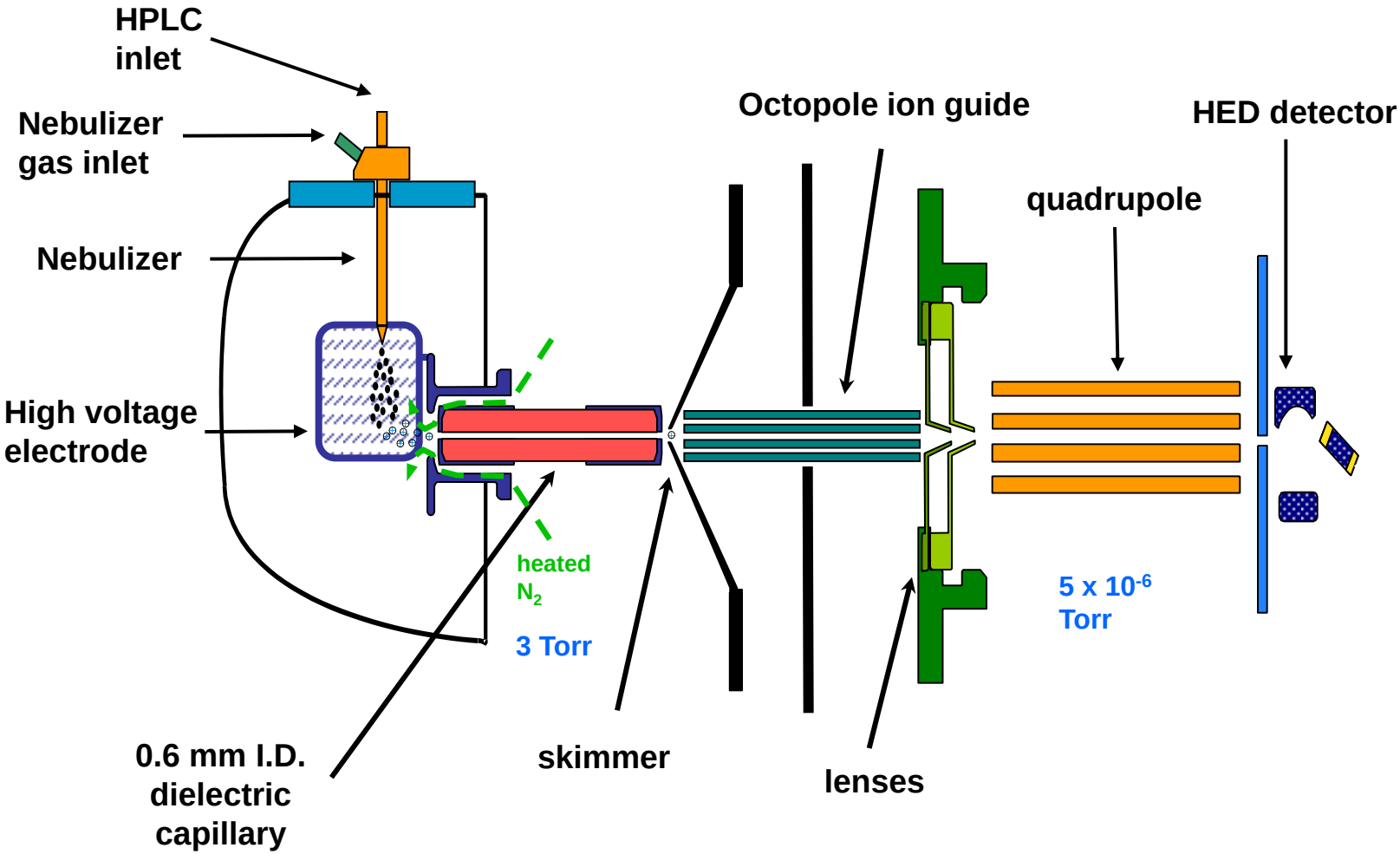


Electrospray Ionization

ionization process which uses an electrical field to generate charged droplets and subsequent analyte ions by ion evaporation for MS analysis. Nebulization is usually pneumatically assisted.



LC/MSD - ESI


HPLC inlet
Nebulizer gas inlet
Nebulizer
High voltage electrode
heated N_2
3 Torr
0.6 mm I.D. dielectric capillary
skimmer
Octopole ion guide
lenses
quadrupole
HED detector
5 x 10^{-6} Torr

Applications of Electrospray Technique

- Electrospray can be used for high and low molecular weight ionizable solutes.
- Compounds which are ions in solution - catecholamines, sulfate conjugates, quaternary amines
- Samples that multiply charge in solution (i.e. peptides, proteins, oligonucleotides)
- Samples that contain heteroatoms: carbamates, benzodiazepines
- Compounds which can accept a charge by induction
- Avoid samples with extremely non-polar samples, where charge induction is inefficient

Factors Affecting Electrospray Ionization

Ion Source Setup

- Inner needle position
- Drying gas –flow and temp.
- Nebulizer pressure

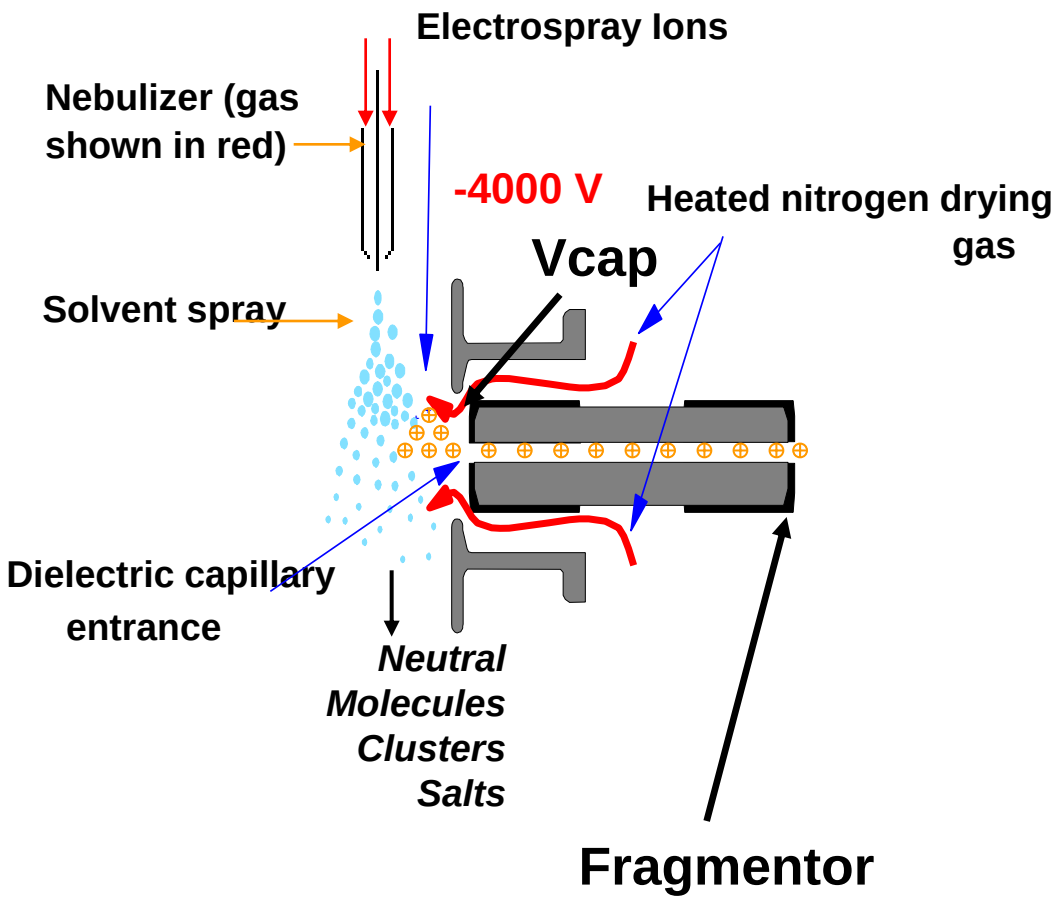
High Voltage Electrodes

- Capillary and Chamber voltage settings
- Condition of Capillary and Chamber high voltage
- Condition of insulators

Solution Chemistry

- Flow rate
- Sample pK_a
- Solution pH
- Solution conductivity

Typical Electrospray Source Settings



Nebulizer Pressure

<200 µL/min	10-20 psig
200-400 µL/min	20-30 psig
400-800 µL/min	30-45 psig
>800 µL/min	45-60 psig

Drying Gas Flow (6-10 LPM)

- high water needs higher flow
- if too low, spikes in spectra from droplets
- when in doubt, use excess

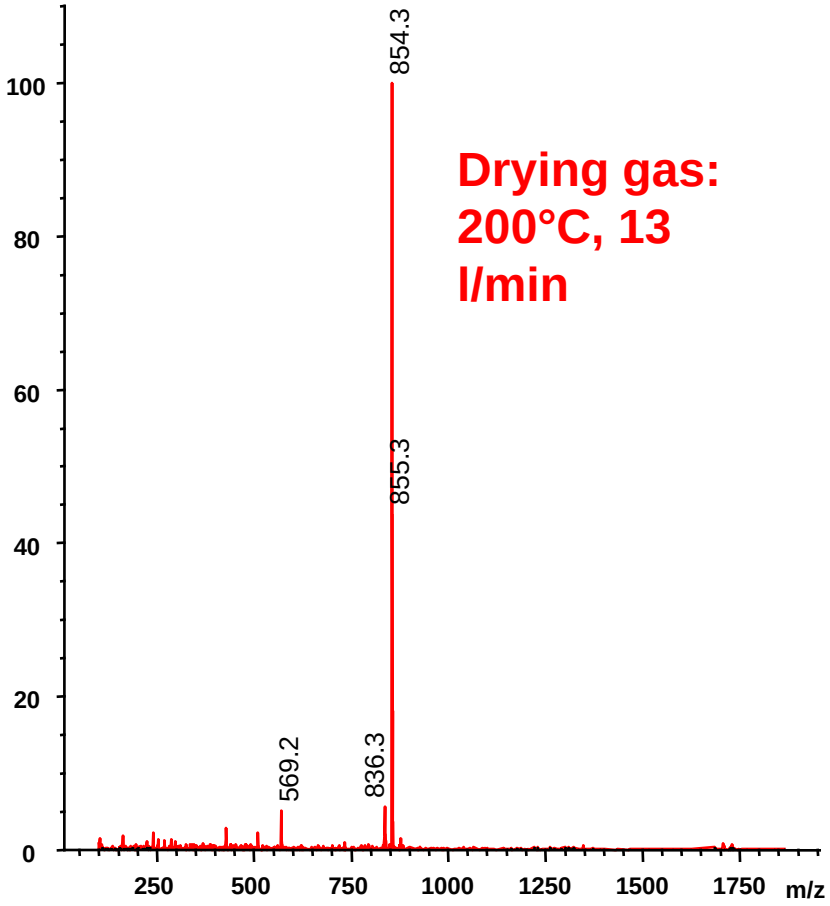
Drying Gas Temperature

- higher for low vapor pressure solvents
- start with 300 - 350°C

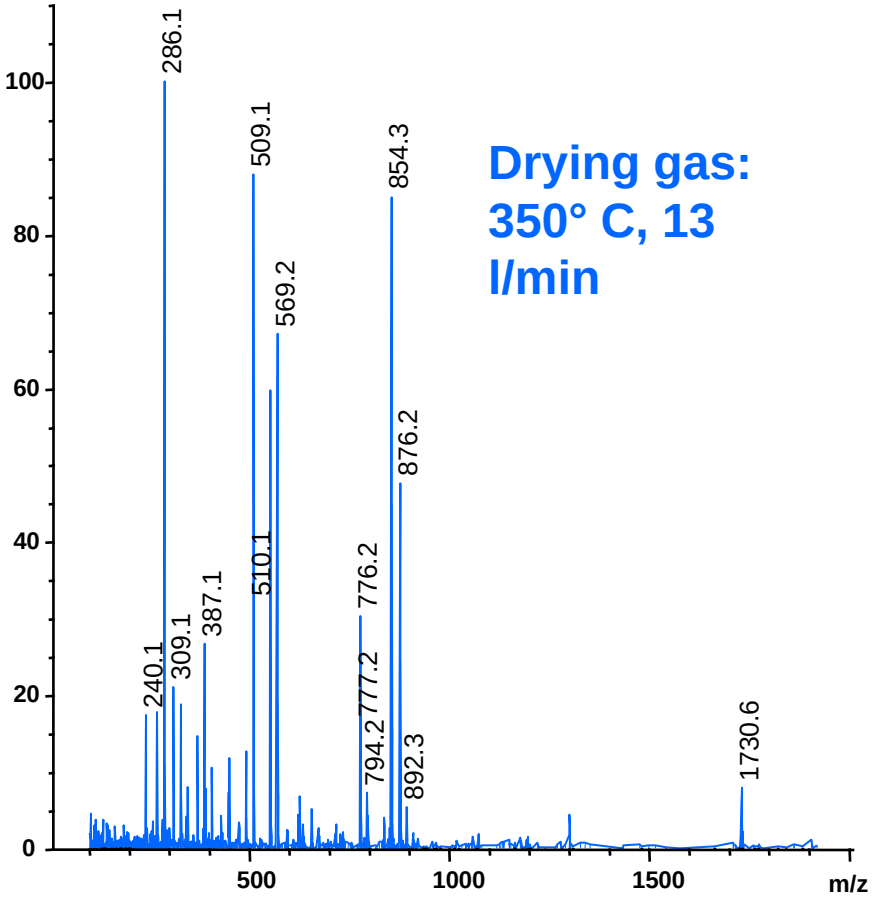
Vcap

- optimize with FIA (2000-6000)
- start with 3000 V
- in negative mode, look for high chamber current or blue glow (indicates corona): reduce Vcap if this happens

An Unusual Effect of Drying Gas Temperature



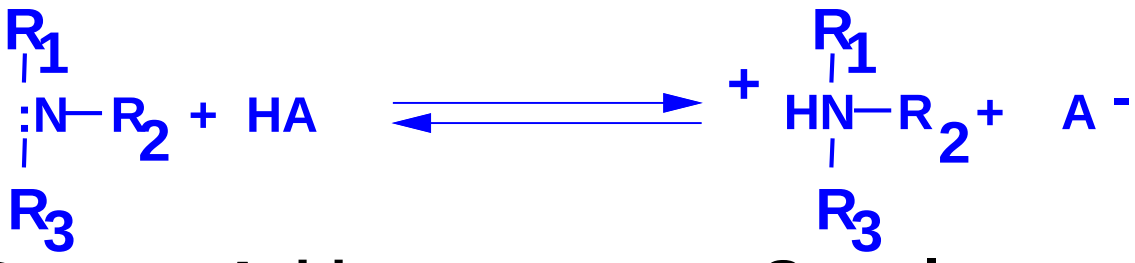
Drying gas: 200°C, 13 l/min



Drying gas: 350° C, 13 l/min

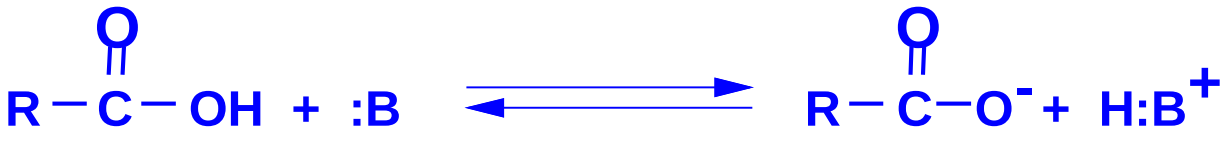
Solution Chemistry

Positive Ion Mode (pH <7)



Base **Acid** **Sample**
$[M + H]^+$

Negative Ion Mode (pH >7)



Acid **Base** **Sample**
$[M - H]^-$

Electrospray requires preformed ions in solution

Mobile Phase Polarity and Buffer Selection for ESI

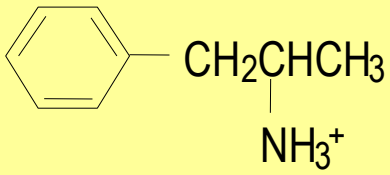
Positive ion detection of basic analytes

Buffer choices (10 mM or less)

- Acetate pKa 4.8
- Propionic acid pKa 4.8
- Formate pKa 3.8
- TFA highly acidic

Typical analytes – amines, amides, antibiotics

Amphetamine



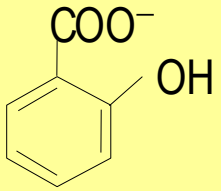
Negative ion detection of acidic analytes

Buffer choices (10 mM or less)

- Ammonia pKa 9.2
- Diethylamine pKa 10.5
- Triethylamine pKa 10.7
- Piperidine pKa 11.1

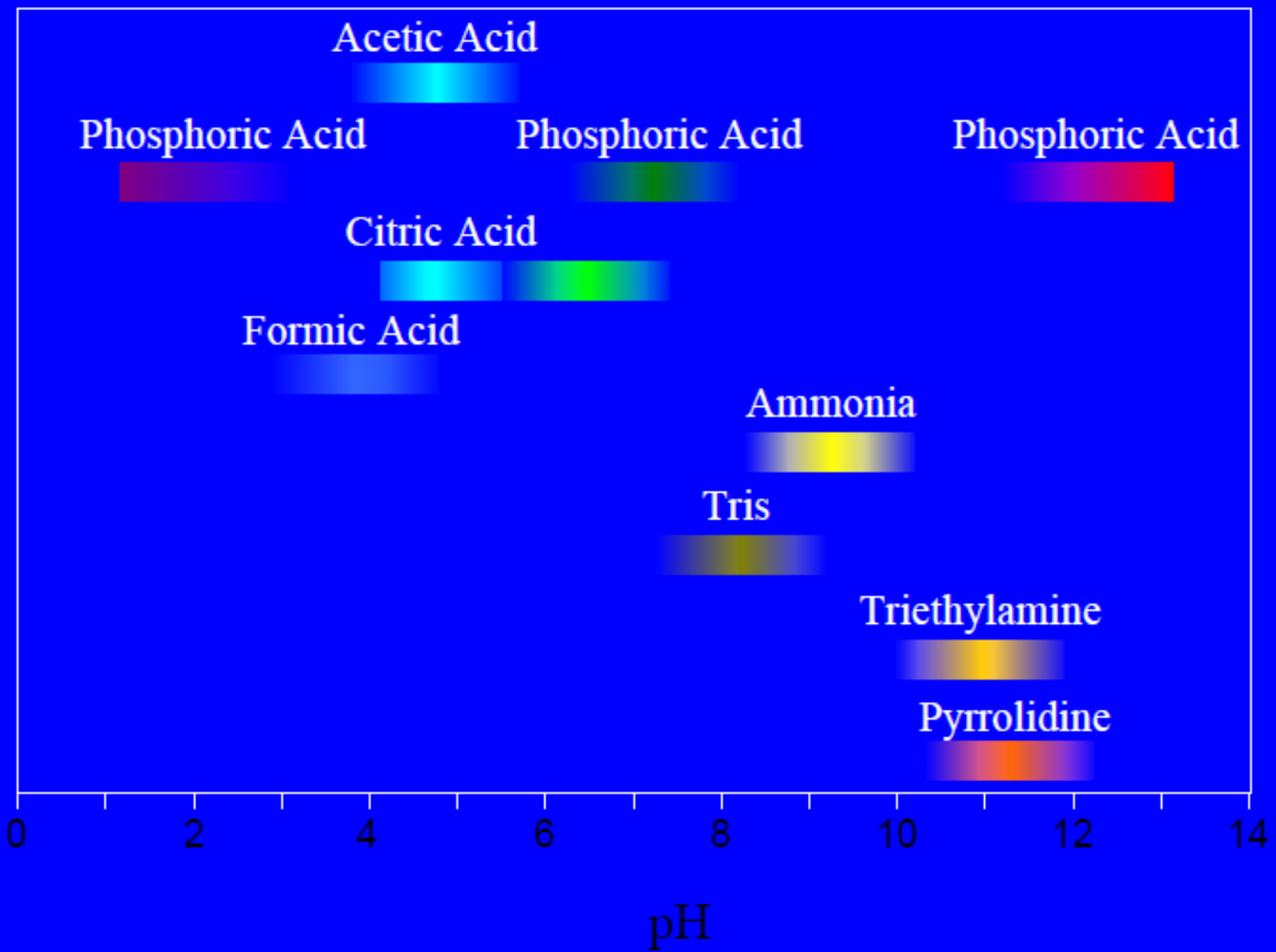
Typical analytes – acids, hydroxyls, phosphates, sulfates

Salicylic Acid



Keep pH 1 –2 pH units above, below pKa of analytes.
Avoid using salts and detergents

Common pH Buffers



Cationization in Electrospray

Neutral molecules which have any propensity for hydrogen bonding will form adduct ions with ammonium or alkali metal ions

Examples:
- **menthol**
- **carbohydrates**

Add a buffer of ammonium acetate or sodium acetate to facilitate ionization.

Key Chromatographic Points in Use of Electrospray

- Mobile phase can provide charged analytes
  - Mobile phase pH is critical
  - Know the pKa values of sample components
  - Analyze acids, bases – anything with a charge
- Operates over a wide flow rate range – 1 μL/min up to 1 mL/min (with Agilent LC/MS)
- Accommodates columns from nano/capillary (proteins and peptides) up to analytical 4.6 mm id with smaller id's usually preferred for best sensitivity
- Compatible with reversed-phase solvents
- Reversed phase column selection for some charged analytes may be difficult due to limited retention

Summary: Electrospray LC/MS

Advantages

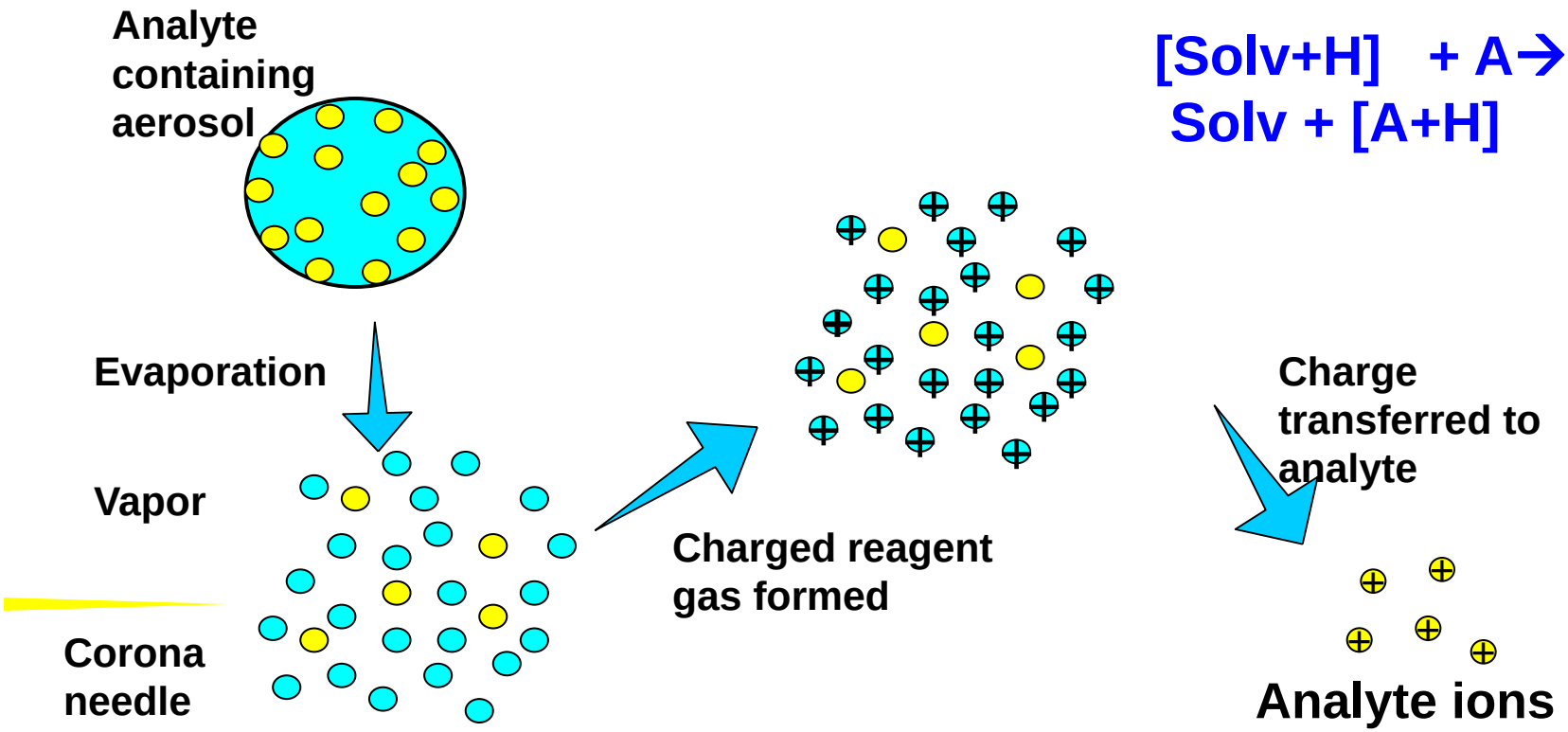
- Softest ionization available
- LC/MS interface with best sensitivity
- Extends mass range for multiply charged analytes
- Works with a wide range of medium to high polarity compounds
- Low maintenance

Disadvantages

- Solution chemistry influences ionization process
- Works less well with nonpolar analytes
- Adduct ions (other than M+H) possible with some analytes
- Some sensitivity loss at higher flow rates (~1 ml/min)

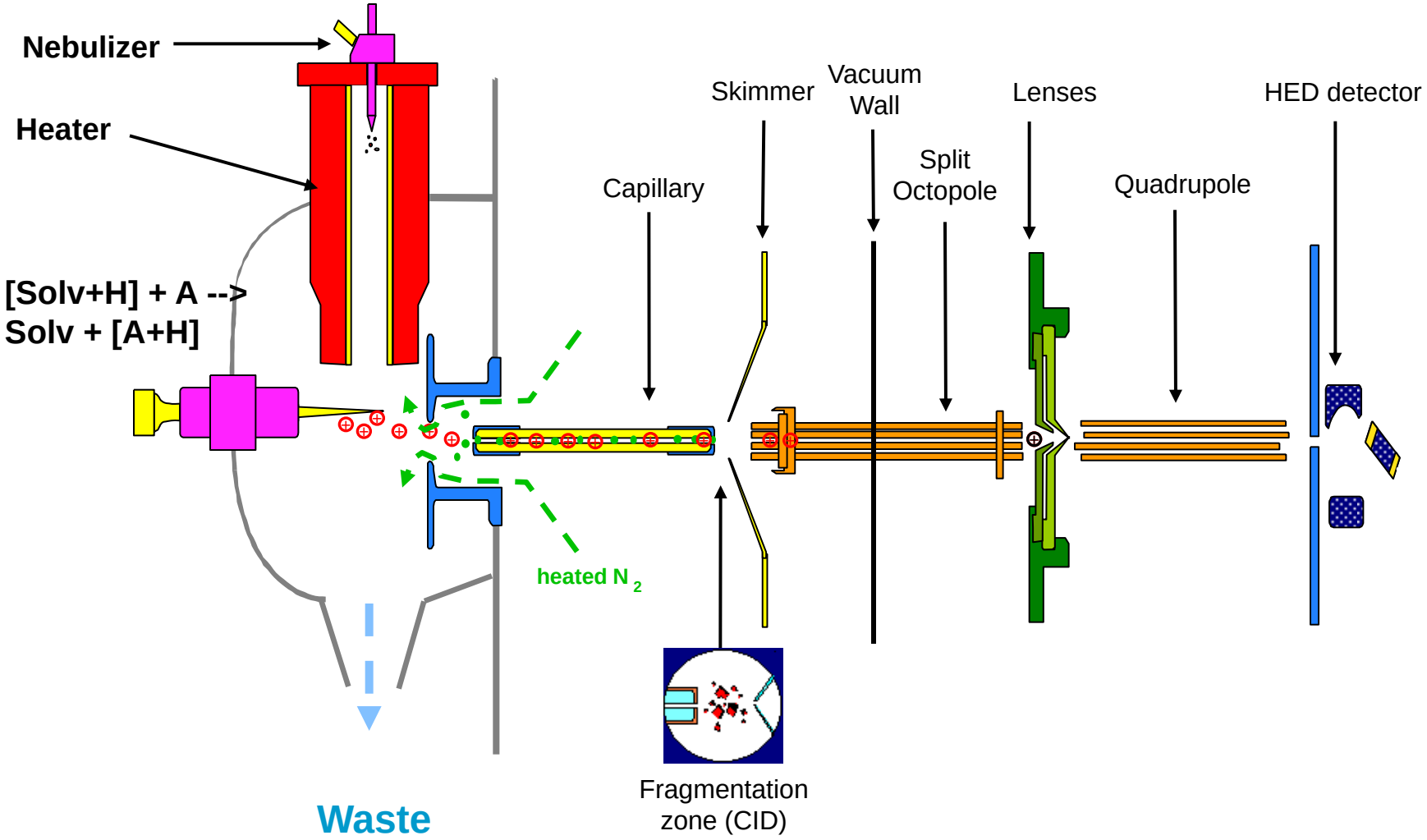
Atmospheric Pressure Chemical Ionization (APCI)

gas phase chemical ionization (CI) process where the solvent acts as the CI reagent gas to ionize the sample

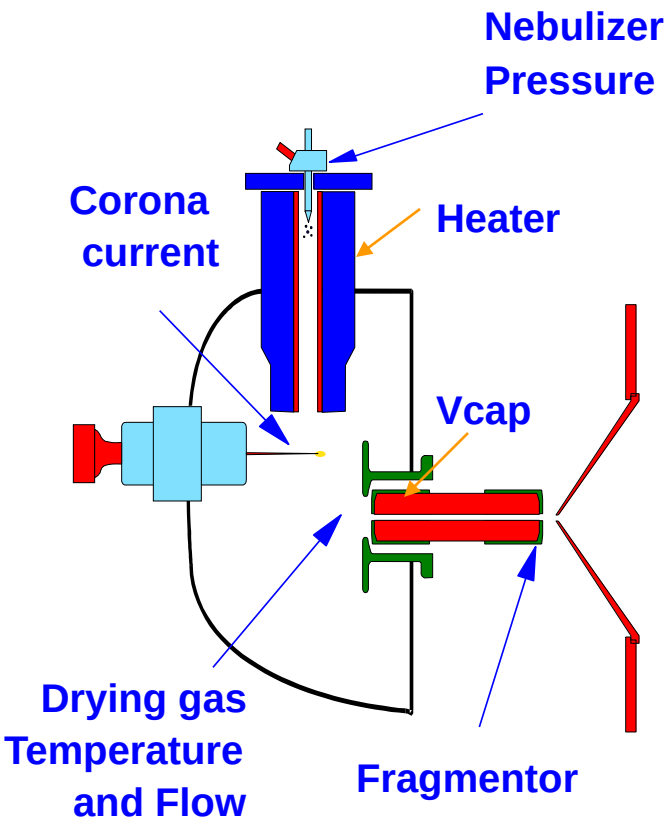


LC/MSD - APCI

Vaporize in gas phase and ionize the gas with a discharge



APCI Spray Chamber Settings



HPLC Flow Rate >500µL/min

Nebulizer pressure

- 60 psig

Drying Gas Temperautre

- start with 350° C

Drying gas flow

- 4 L/min

Vaporizer temperature

- optimize with FIA*

Vcap

- optimize with FIA (2000-6000)
- start with 2500 V

Corona current

- optimize with FIA
- start with 25 µA (neg) or 4 µA (pos)

APCI Considerations

Samples

- Compounds of intermediate MW and polarity: PAHs, PCBs, fatty acids, phthalates.
- Compounds that don't contain acidic or basic sites (e.g. hydrocarbons, alcohols, aldehydes, ketones, and esters
- samples containing heteroatoms: ureas, benzodiazepines, carbamates
- samples that exhibit a poor electrospray response

Solution Chemistry Parameters

- less sensitive to solution chemistry effects than ES
- tolerates higher flow rates than ES
- accommodates some solvents not compatible with ES

Samples to Avoid

- thermally labile compounds due to vaporization process
- charged in solution
- biomolecules because they are rarely volatile

LC/MS Solvent Selection and Guidelines for Successful APCI

- Select more volatile solvents
- Select protic solvents (MeOH) for positive ion mode when possible
- Select solvents that readily capture an electron for negative ion mode
- Ammonium salts in the mobile phase can cause ammonium adducts to form

Summary: APCI

Advantages

- Complementary to API-Electrospray for less polar analytes
- Good sensitivity for compounds of intermediate MW and polarity
- Less sensitive to solution chemistry effects than API-ES
- Tolerates higher flow rates without decrease in sensitivity
  - up to 1.5 mL/min
- can use with 2.1 – 4.6 mm id columns
- reversed-phase buffered mobile phases up to 100 mM and selected normal phase solvents to accommodate nonpolar analytes.

Disadvantages

- Less useful for thermally labile compounds
- Requires some compound volatility

General Comparison – ESI vs APCI

ESI

Ionization: Pre-formed analyte ions transferred to gas phase

Mobile Phase Issues:

- Organic Solvent:

  little effect on ionization

- pH: key to pre-formed ions

- Buffer Concentration: < 25 mM

- Flow Rate: < 0.5 ml/min

APCI

Ionization: Charge exchange of gas phase neutral analytes

Mobile Phase Issues:

- Organic Solvent:

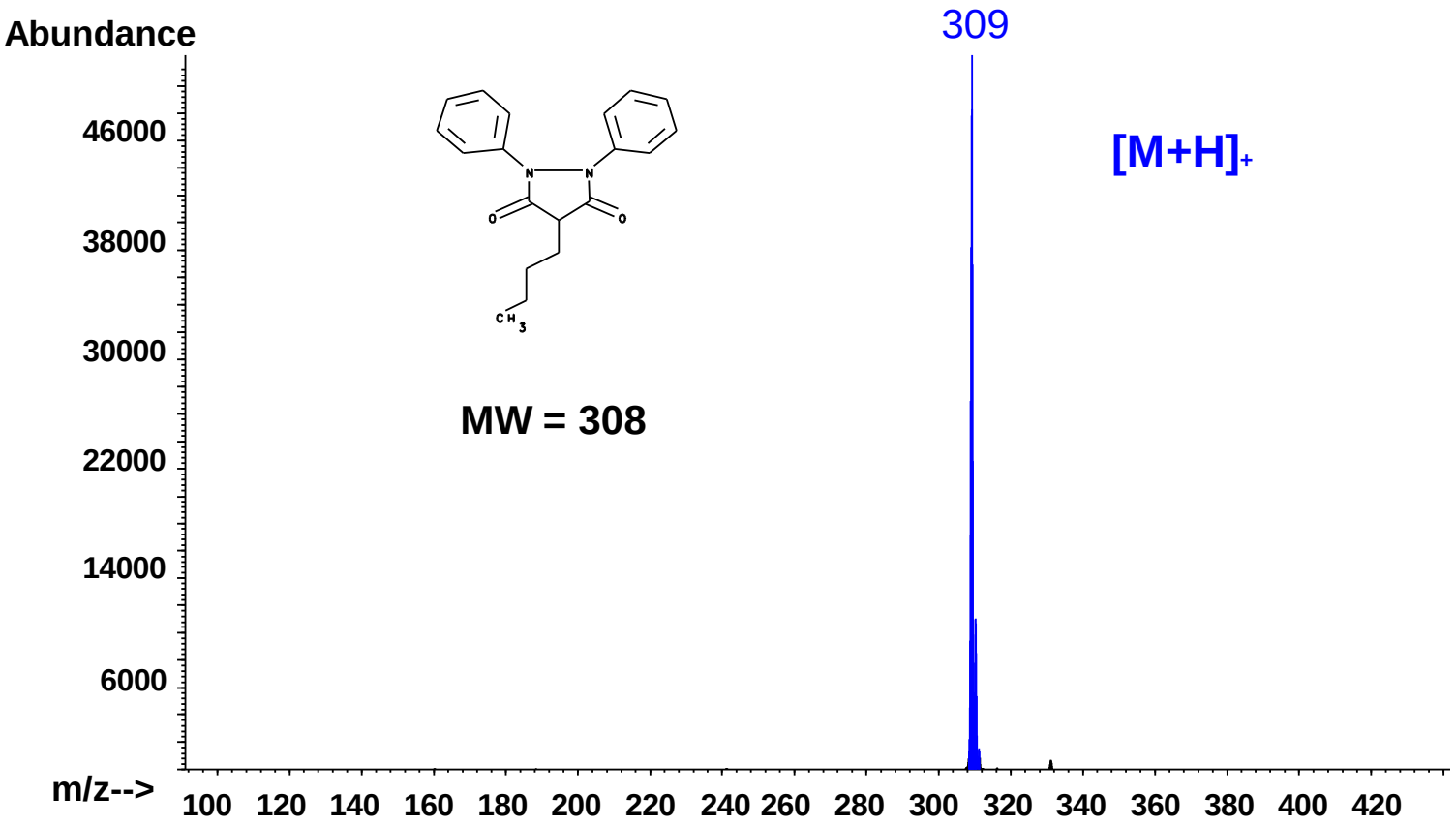
  MeOH usually best

- pH: neutral analytes

- Buffer Concentration: < 100 mM

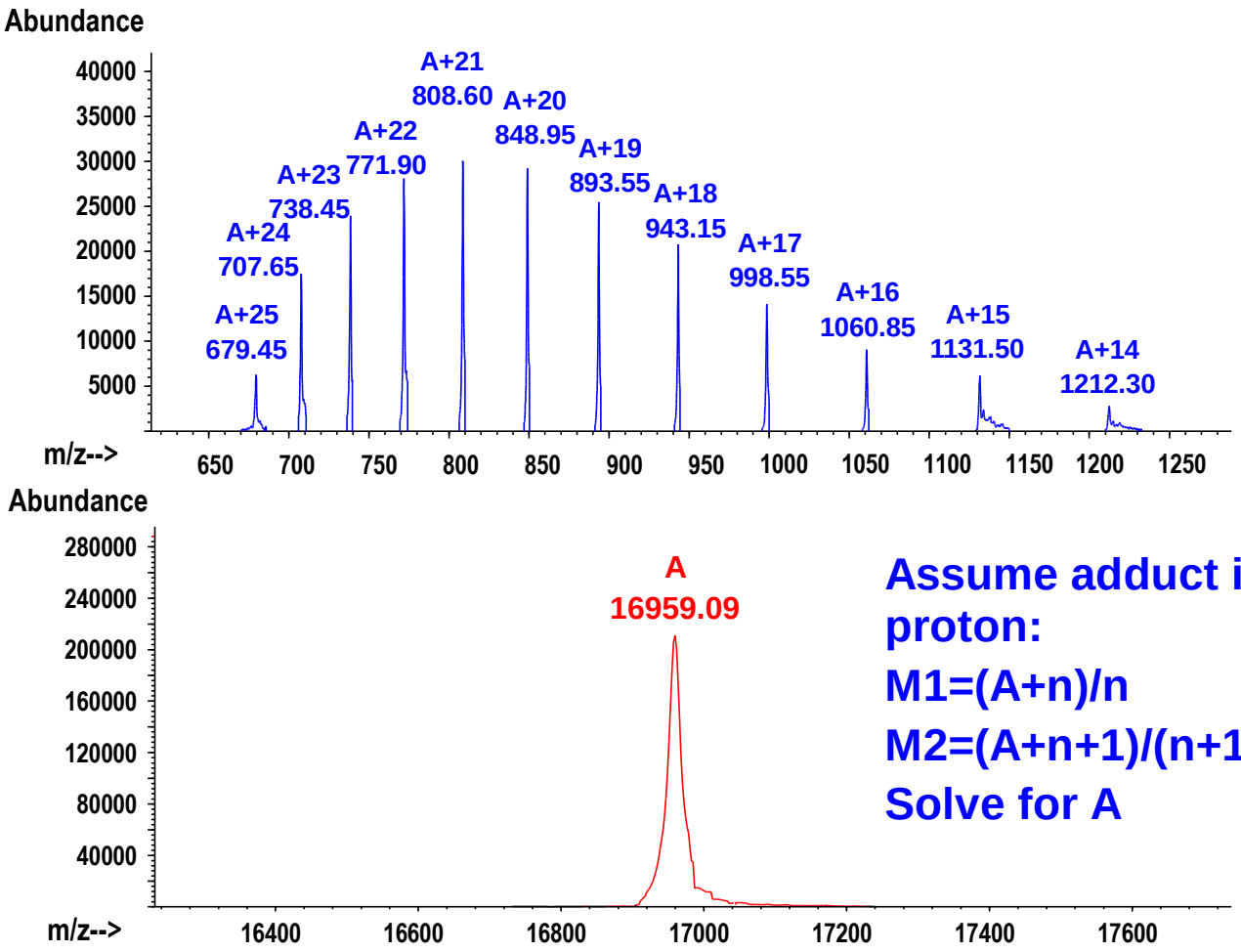
- Flow Rate: > 0.5 ml/min

What Kind of Data Do You Obtain?



API-ES spectrum of Phenylbutazone

What Kind of Data Do You Obtain?



Assume adduct is a proton:

M1=(A+n)/n

M2=(A+n+1)/(n+1)

Solve for A

API-ES spectrum of Myoglobin

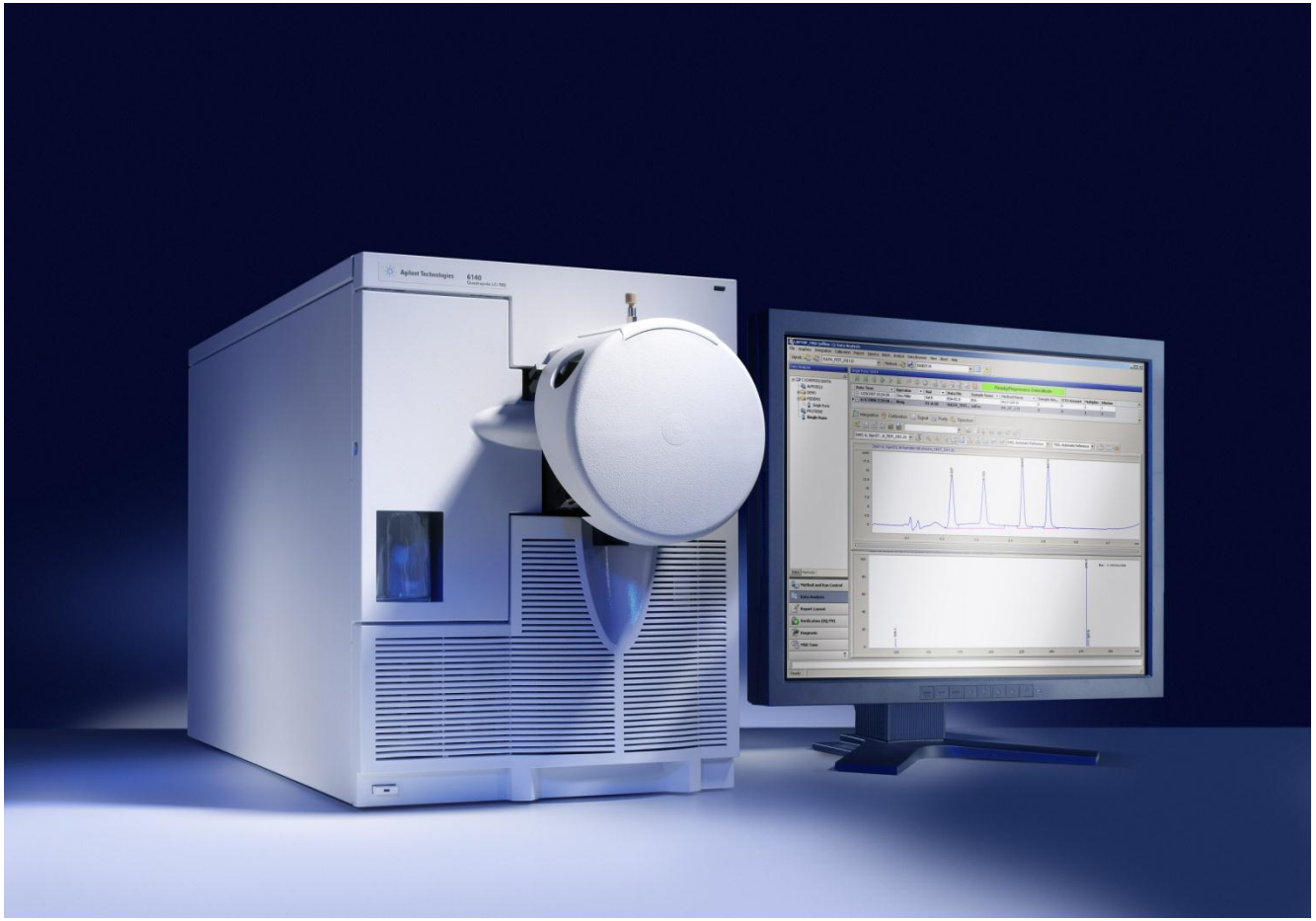
Comparison of Electrospray and APCI

- **Sensitivity**
  - If a sample can be ionized by both techniques, electrospray is generally more sensitive and has less background noise

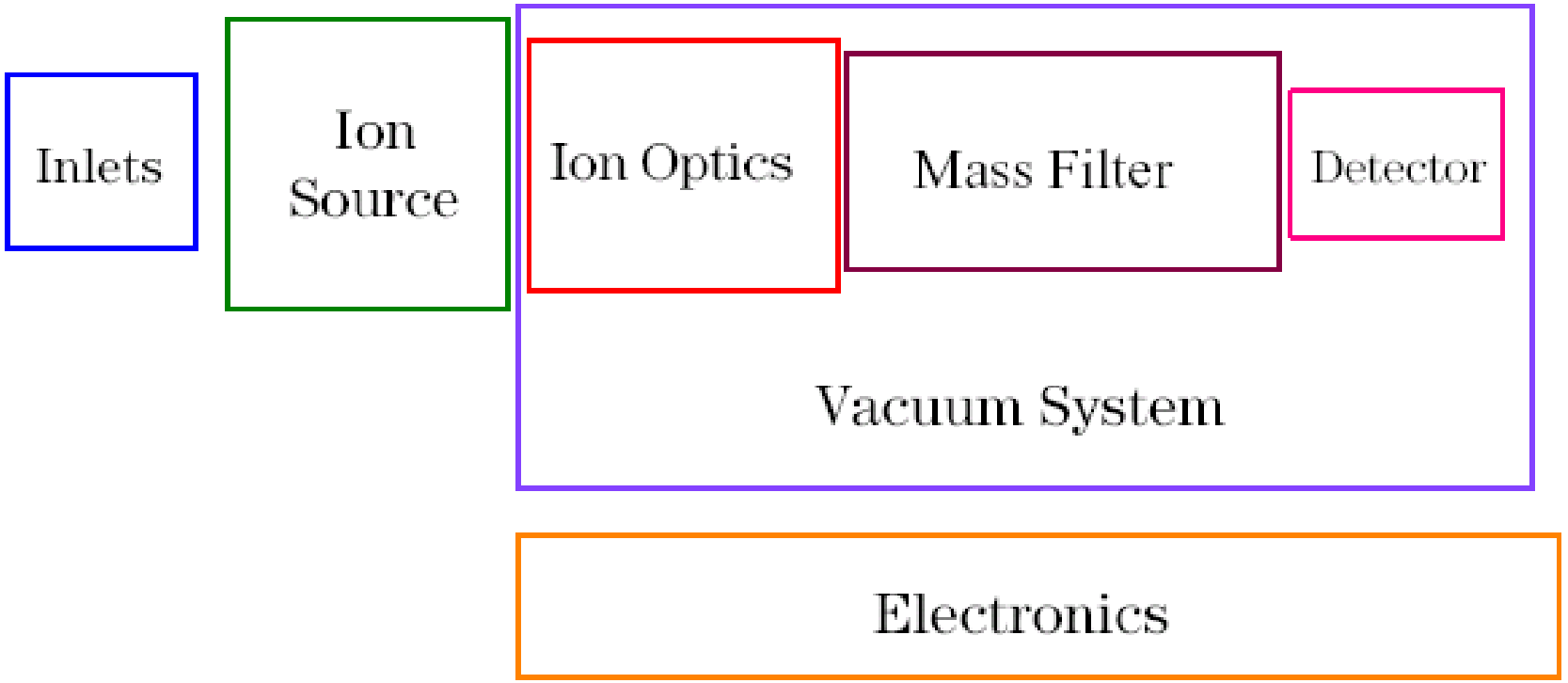
- **Matrix and Mobile Phase Effects**
  - Electrospray is more sensitive to sample and solvent matrix than APCI (i.e. signal suppression)
  - Electrospray requires a lower concentration of very volatile buffers relative to APCI
  - Choice of organic solvent strongly affects ionization in APCI
  - ESI is concentration sensitive & APCI is mass sensitive detector

- **Flow Rates**
  - Electrospray works well at low flow rates (<100 µL/min) while APCI does not
  - APCI is more sensitive and has less noise than electrospray at high flow rates (>750 µL/min)

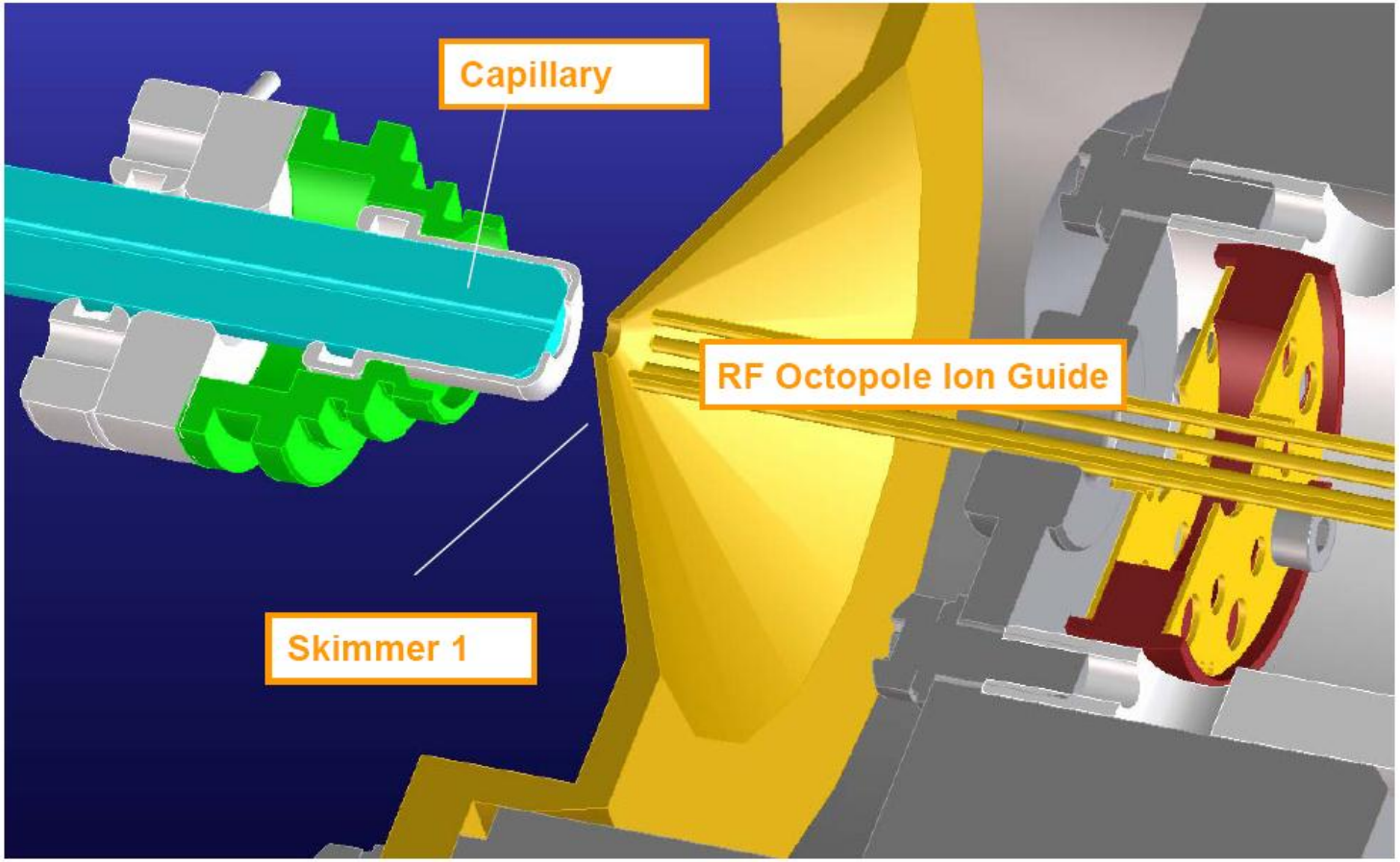
6100 Series LCMS Quadrupole



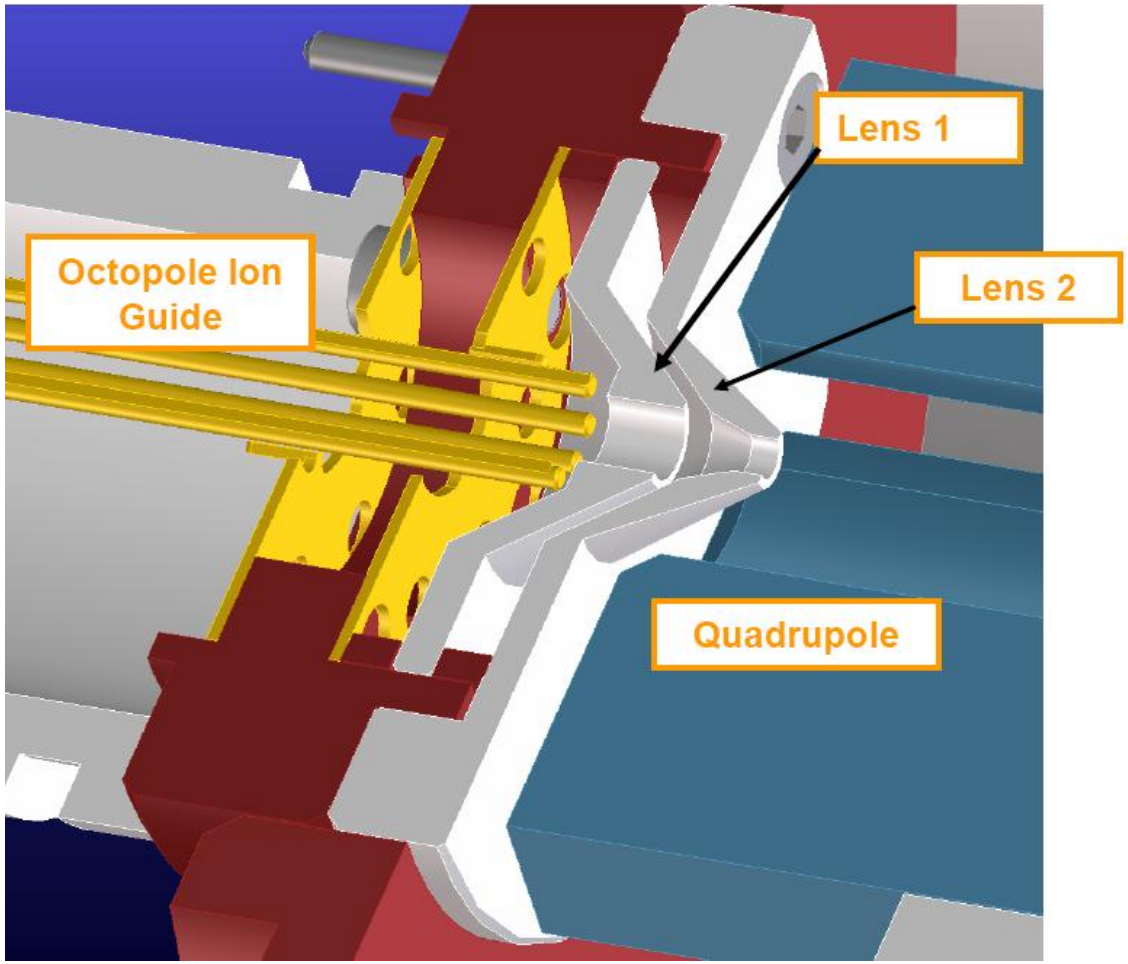
Block diagram of MSD



Ion Optics: Octopole Rf Ion Guide



Octopole Ion Guide and Lenses 1 & 2



Mass Analyzer Terminology

m/z - mass-to-charge ratio

- mass of an ion (Daltons or u) divided by the number of charges on the ion

SIM - selected ion monitoring

- selecting a particular ion or ions to monitor which improves sensitivity

Scan -

- monitoring a range of m/z ions

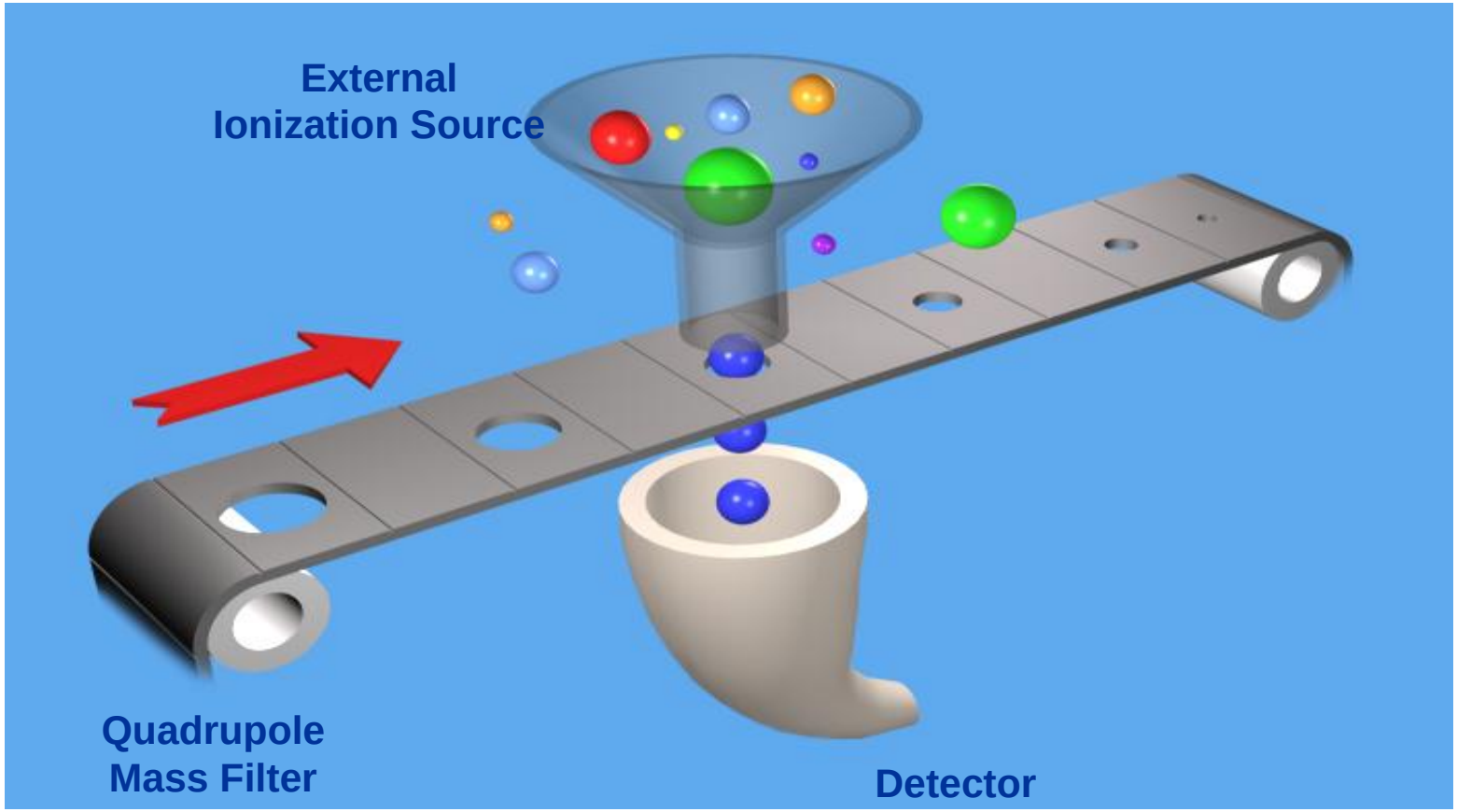
TIC - total ion chromatogram

- the total signal (current) generated by all ions monitored

EIC - extracted ion chromatogram

- the signal over a limited m/z range (traditionally, 1 m/z with a -0.3/ +0.7 window)

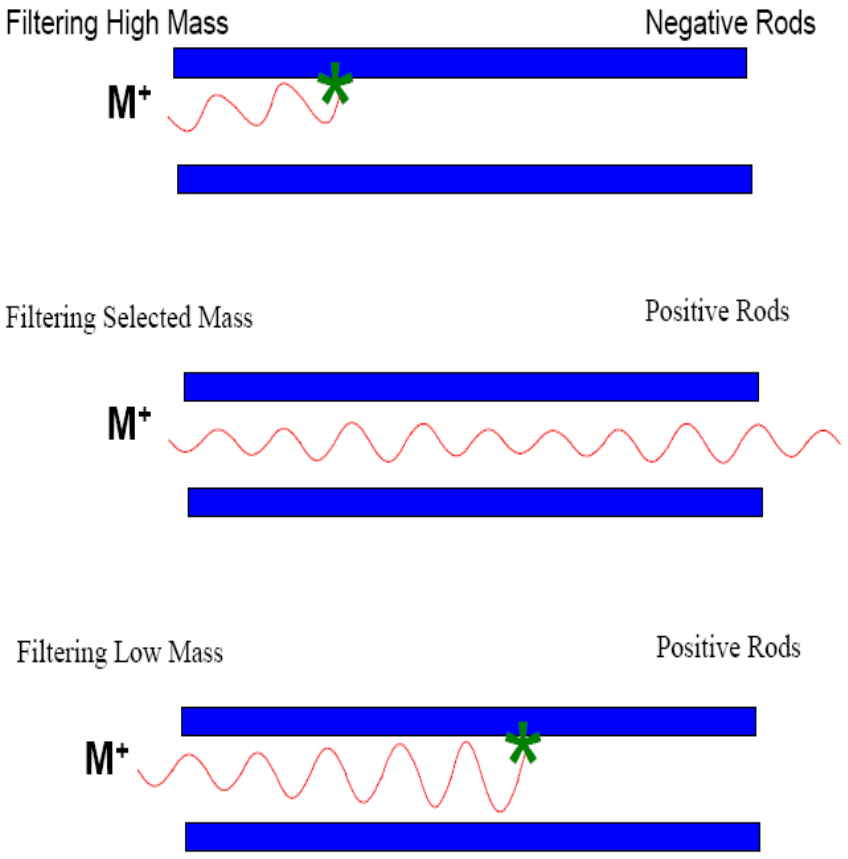
Model of a Single Quadrupole Mass Spectrometer



Quadrupole Mass Analyzer Operation

- **Quadrupole determines which ions make it to the detector by setting up oscillating electric fields:**
  - RF and DC voltages are applied to quad.
  - Opposite pairs of rods are connected:
    - *one pair - positive DC potential with RF signal.*
    - *one pair - negative DC potential with RF signal shifted 180 degrees out of phase from the first pair.*
    - *RF superimposed on DC potentials causing ions to oscillate between the rods.*
  - The m/z ratio of an ion successfully passing through quad is proportional to the amplitude of RF.

So, how exactly does a quadrupole mass analyzer work?

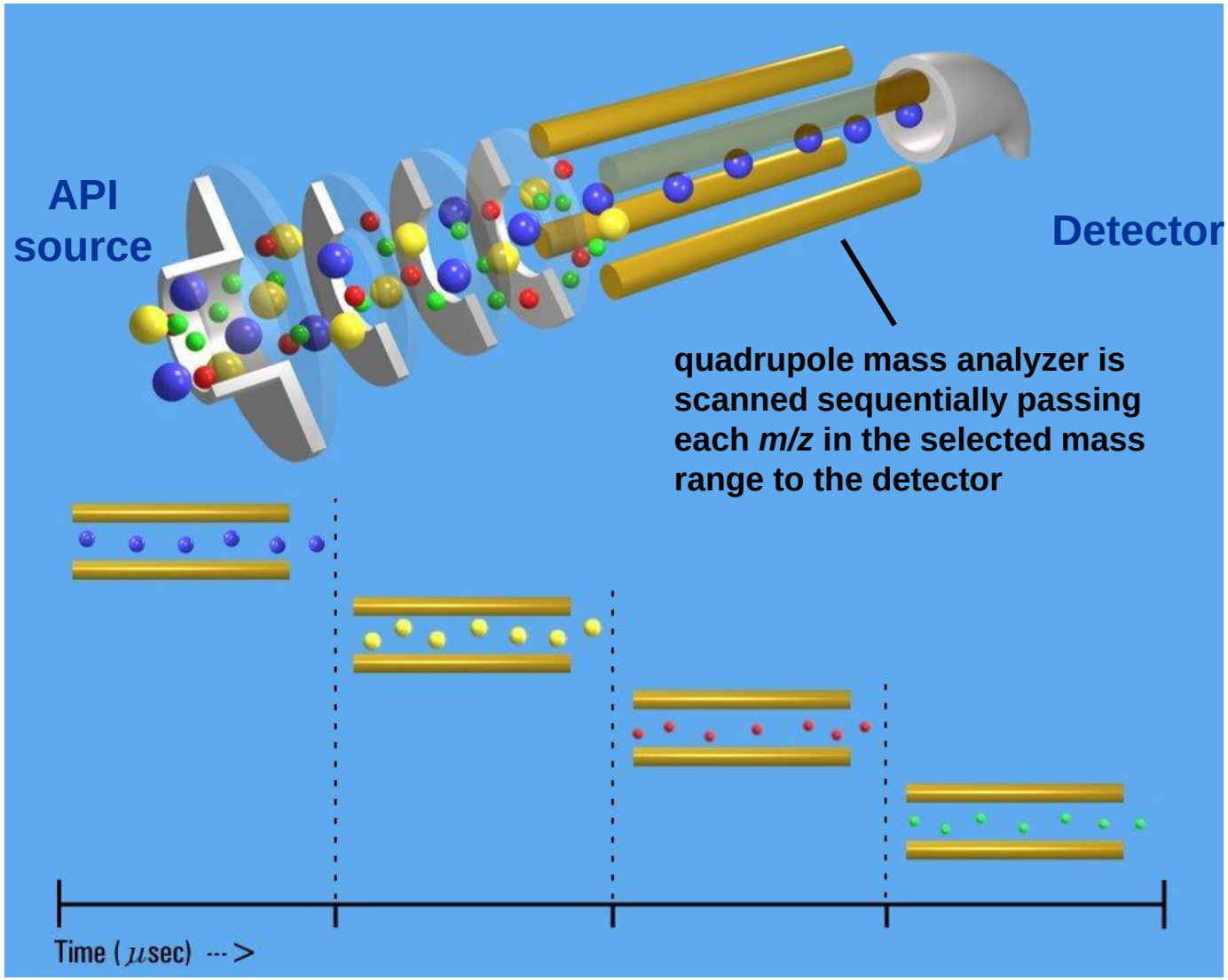


- Mathieu equation – high level equation indicating stable region of analytes

- Use Mathieu Equation to determine Rf and DC potentials for m/z to be in phase

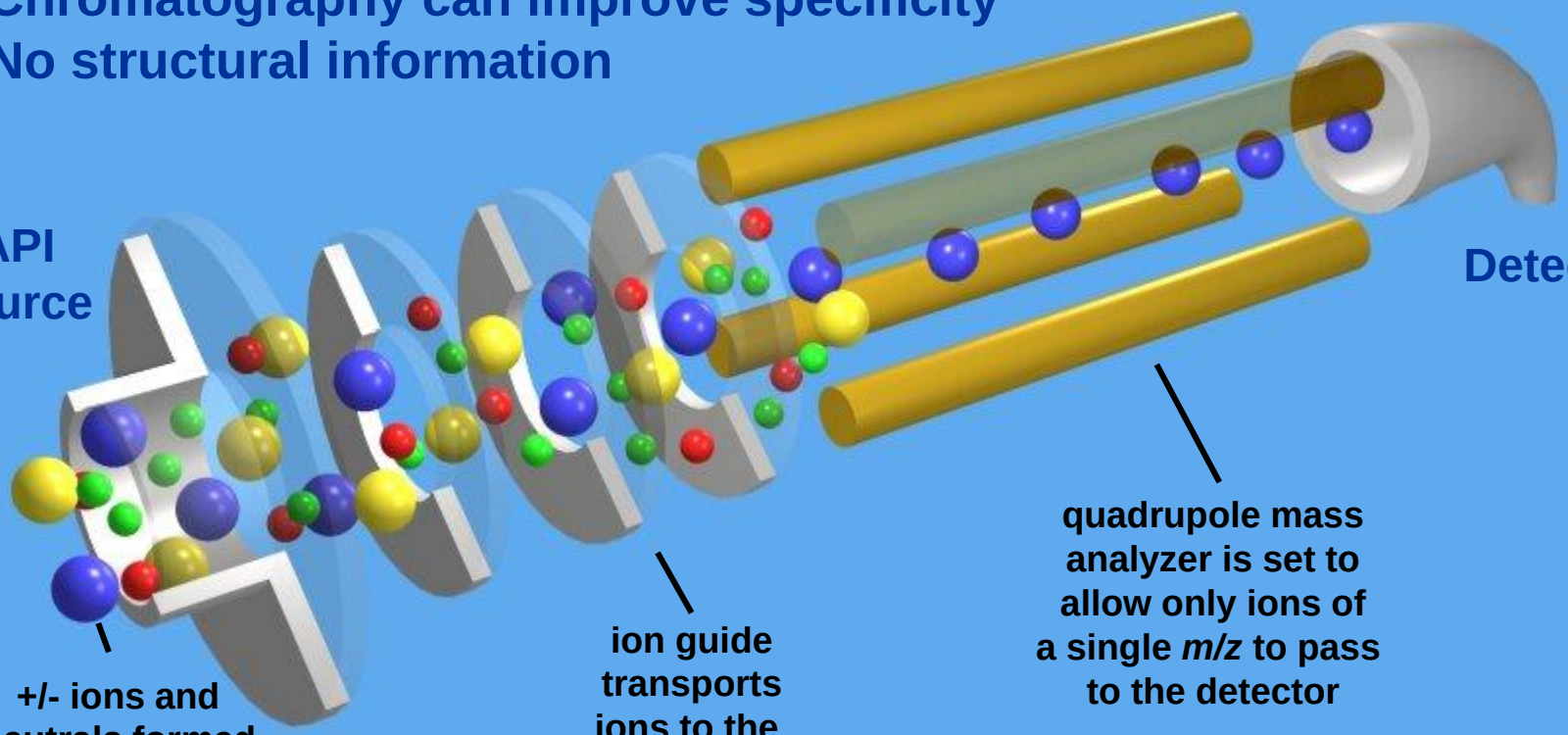
- Analytes not in phase will collide with quadrupole

Single Quadrupole: Full Scan MS

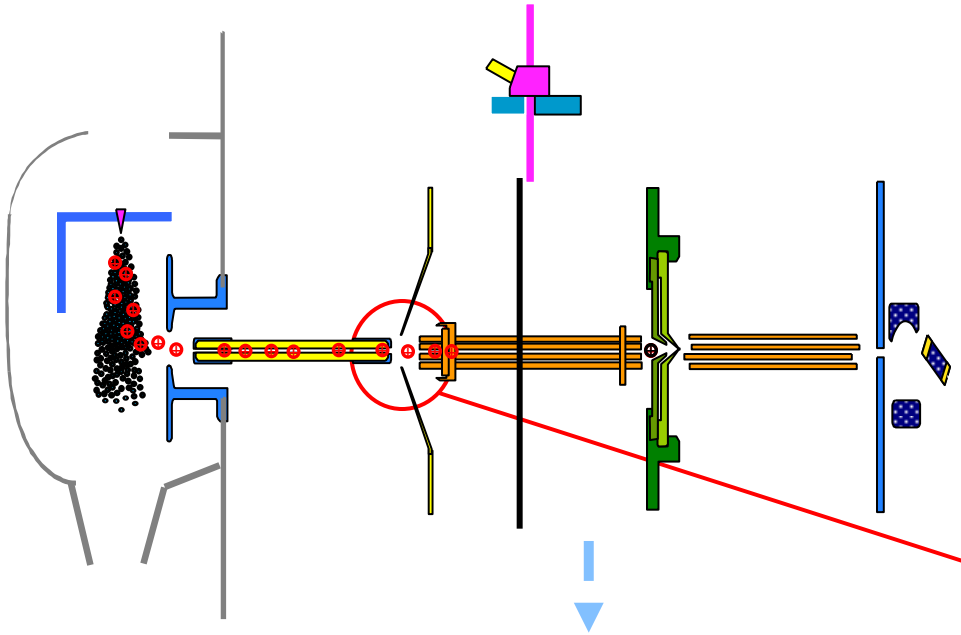


Single Quadrupole: SIM

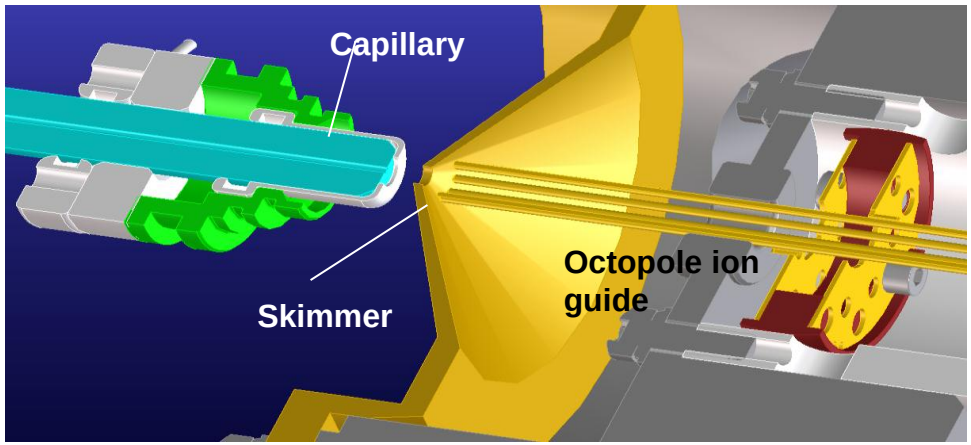
- Best sensitivity for quantitation
- Provide good selectivity
- Chromatography can improve specificity
- No structural information

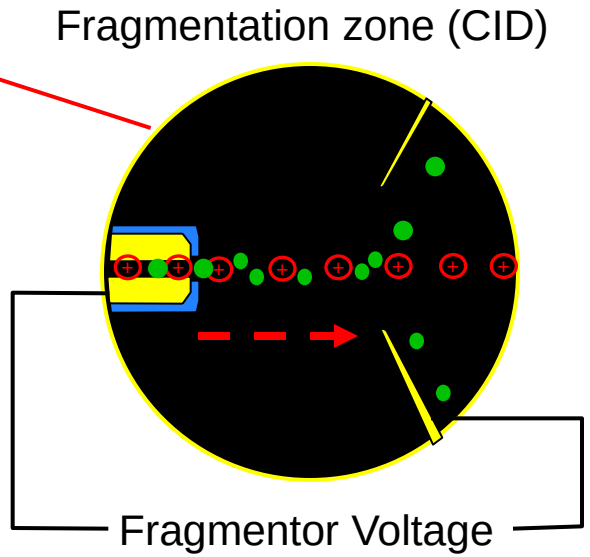


Collision Induced Dissociation



- Offers a broad range of energy that can produce significant fragmentation
- Works well with LC separation
- Only fragmentation mechanism available on a single quad
- Limited use on mixtures without separation
- Can't easily follow fragmentation pathways
- Limited utility when metal (i.e. sodium) adducts dominate





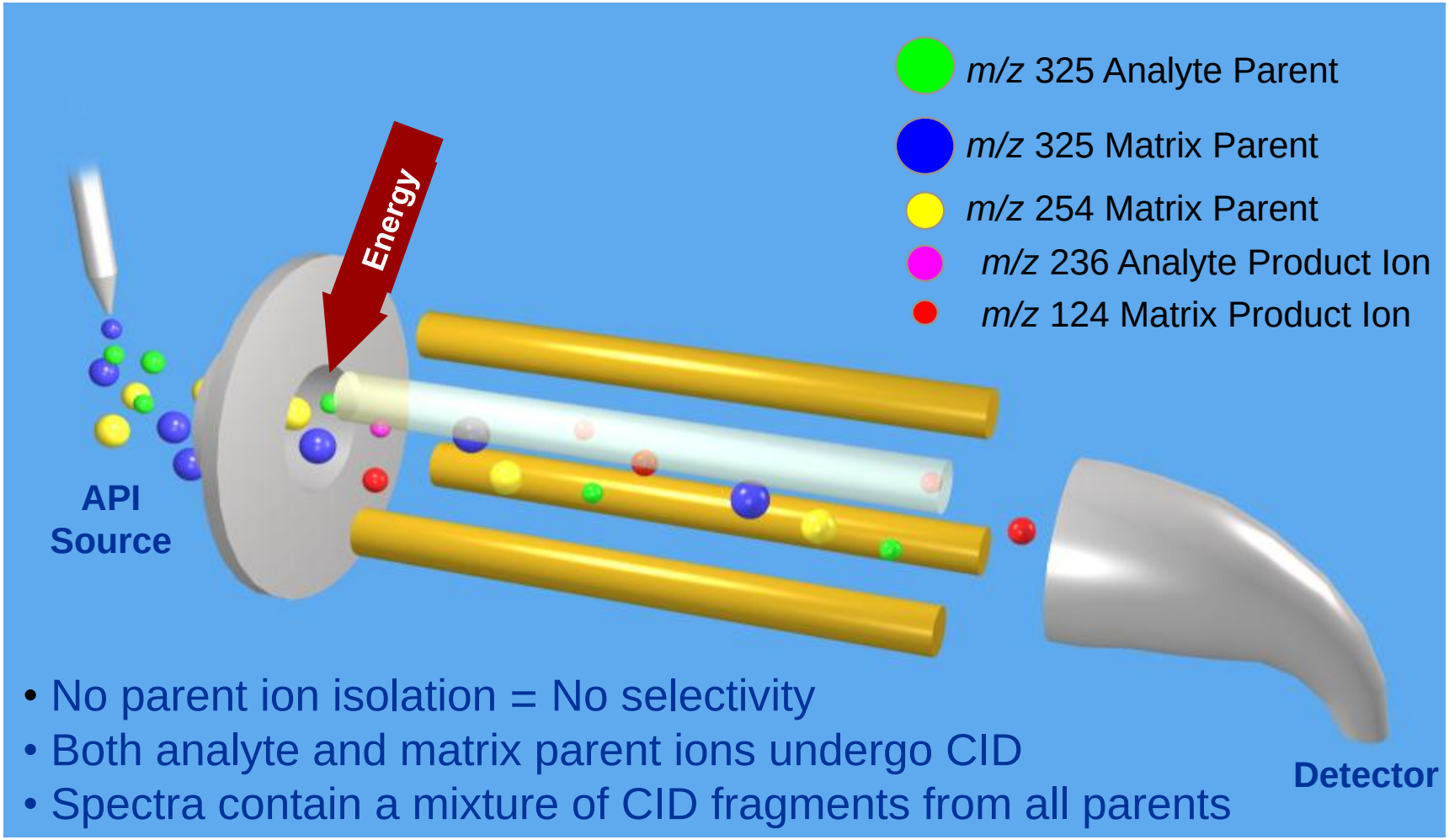
Collision Induced Dissociation (CID)

- In the API process, Quasi-molecular ions are formed with an even number of electrons.
- Fragmentation can be obtained by application of CID. The following fragmentation process can be observed:

$$ABCD^+ \longrightarrow ABC^+ + D \text{ (neutral fragment)}$$

- The charge is retained on the fragment with higher proton affinity.

In-Source CID



- No parent ion isolation = No selectivity
- Both analyte and matrix parent ions undergo CID
- Spectra contain a mixture of CID fragments from all parents

Common CID Losses

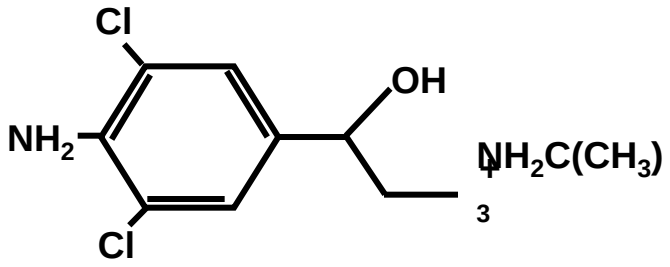
Even Losses

$(M+X)^+$- 2	hydrogen molecule	$(M+X)^+$- H_2
$(M+X)^+$- 18	water	$(M+X)^+$- H_2O
$(M+X)^+$- 20	hydrogen fluoride	$(M+X)^+$- HF
$(M+X)^+$- 28	CO or ethylene	$(M+X)^+$- CO or $(M+X)^+$- C_2H_2
$(M+X)^+$- 30	formaldehyde	$(M+X)^+$- H_2CO
$(M+X)^+$- 31	methylamine	$(M+X)^+$- CH_3NH_2
$(M+X)^+$- 32	ethanol	$(M+X)^+$- CH_3CO_2H
$(M+X)^+$- 36	hydrogen chloride	$(M+X)^+$- HCl
$(M+X)^+$- 44	carbon dioxide	$(M+X)^+$- CO_2
$(M+X)^+$- 46	nitrogen dioxide	$(M+X)^+$-NO_2
$(M+X)^+$- 60	acetic acid	$(M+X)^+$- CH_3CO_2H
$(M+X)^+$- 90	Silanol	$(M+X)^+$- HO-Si-$(CH_3)_3$

CID Fragmentation of Clenbuterol

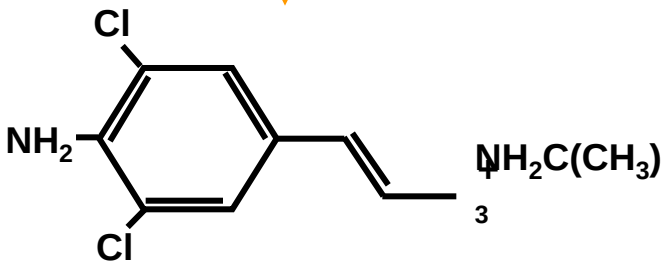
m/z

277



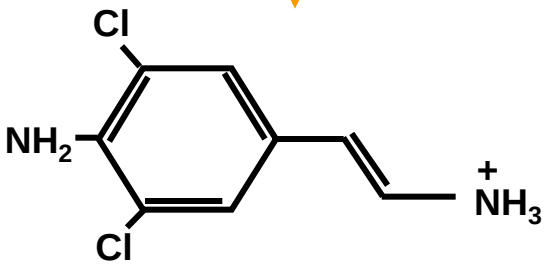
-18 $-H_2O$

259



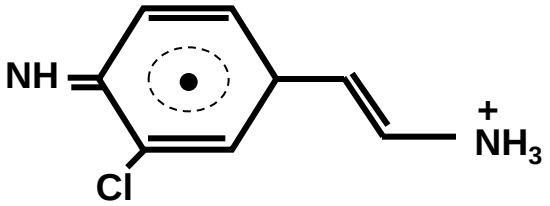
-56 -2-methylpropene

203



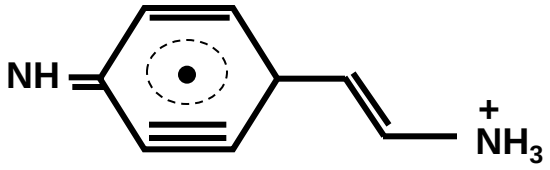
m/z

-35 -Cl



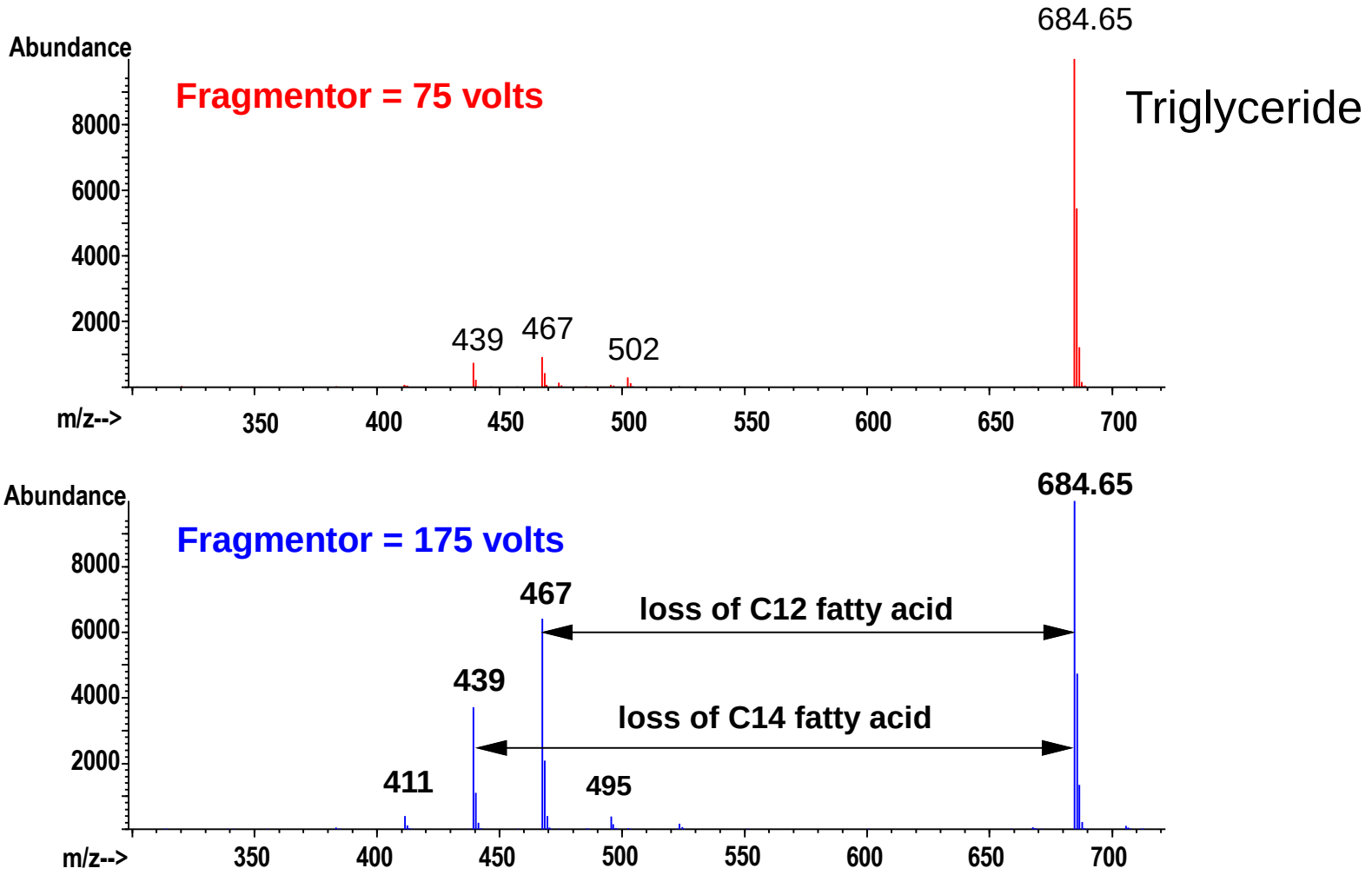
168

-36 -HCl



132

Small Molecule In-Source CID



Fragmentor Voltage

6120

- Ion abundance is fragmentor voltage setting dependent.
  - Dynamic ramping allows you to maximize both parent and fragment ions in same scan.

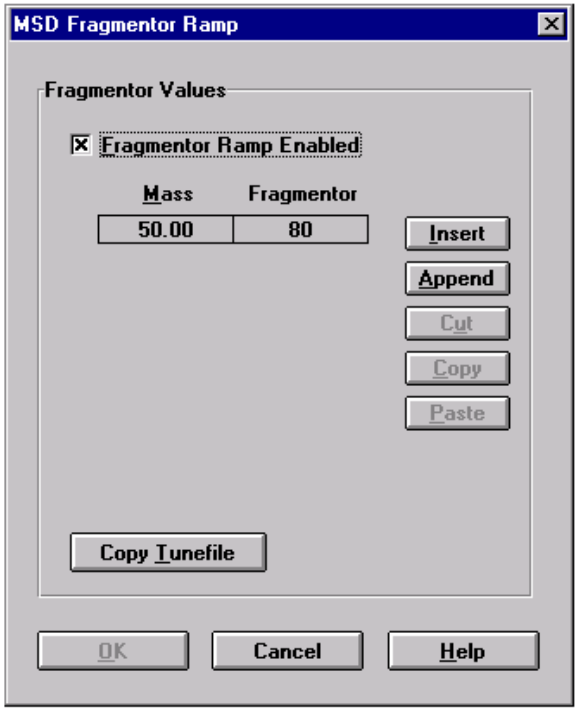
6130 and 6150

- Ion abundance is less fragmentor voltage dependent
  - A typical value is 100 V.
  - Dynamic ramping of the fragmentor offers little advantage.
  - Fragmentation (CID) requires higher fragmentor voltages.

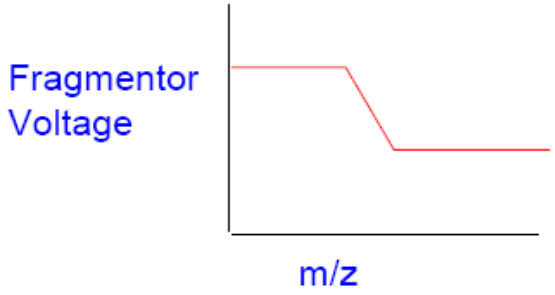
Therefore:

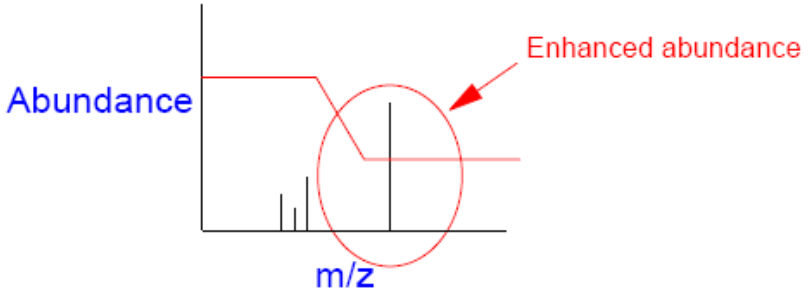
- Generic methods are possible
- Better survival of $[M+H]^+$ ions for fragile compounds such as carbamates

Ramp fragmentor voltage

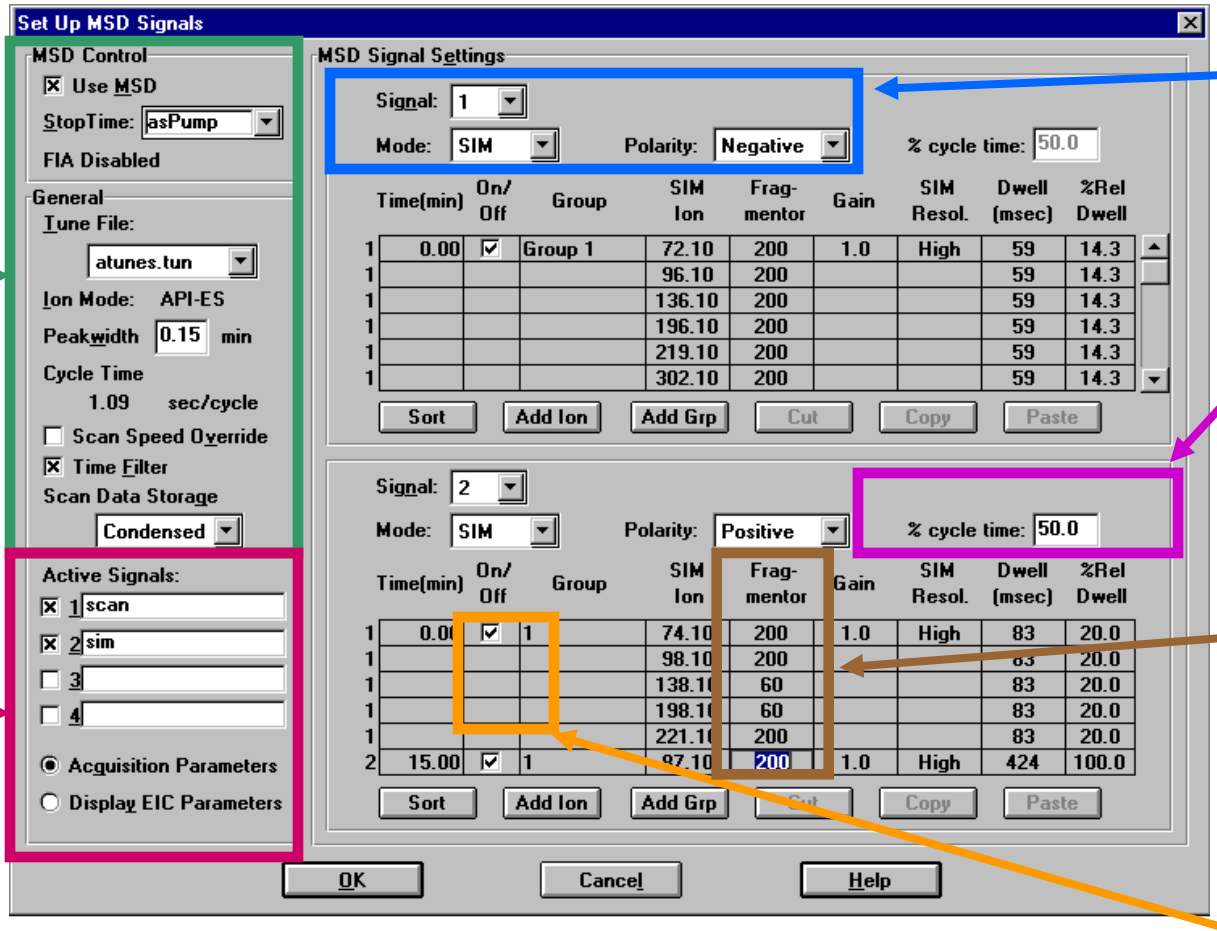


Ramping the Fragmentor is the process of changing the Fragmentor voltage for specified masses during an MSD data acquisition scan.





Acquire Multiple MS Signals: Basics



Same settings as current LC/MSD

Acquire up to 4 signals with optional signal description

Mode and polarity set for each signal

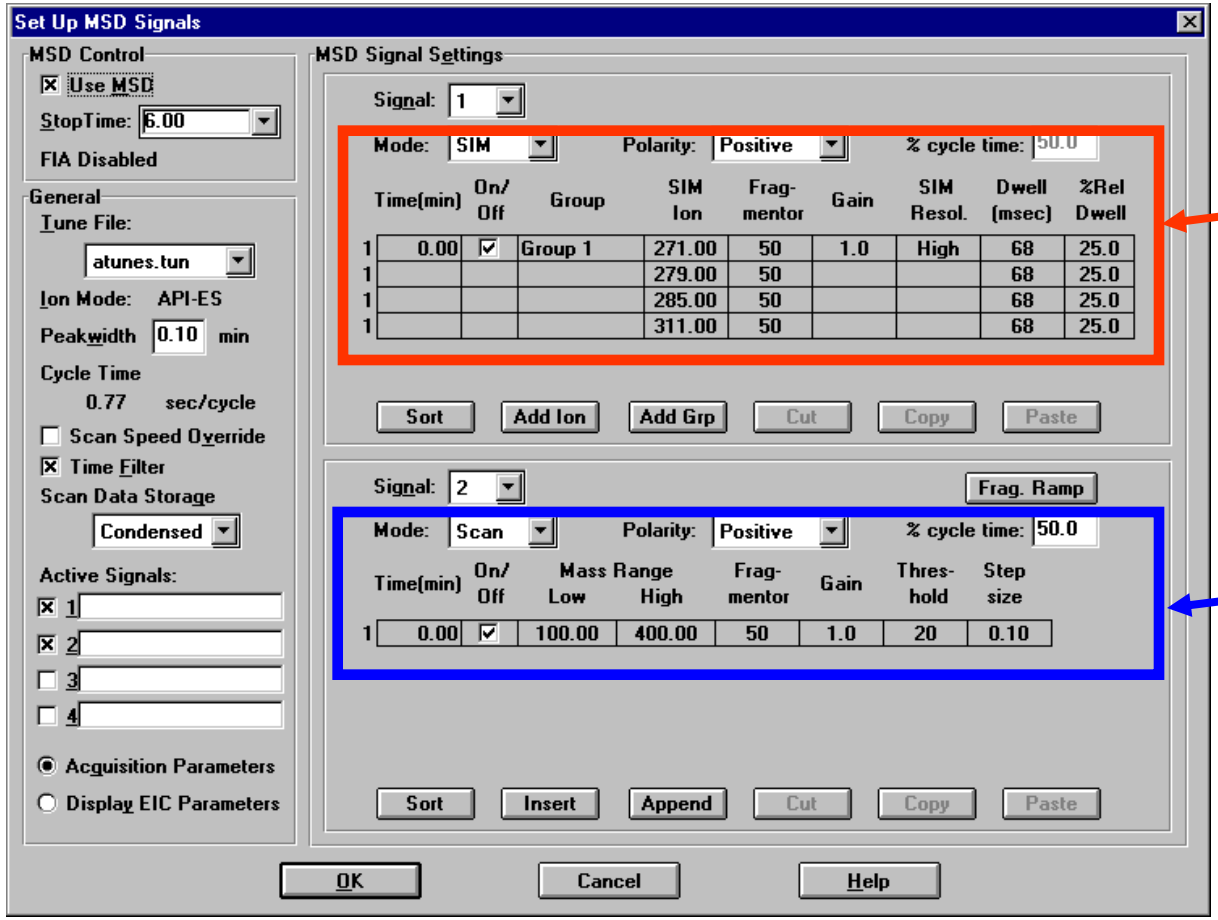
Specify the % of time spent collecting each signal

Specify an optimal fragmentor value for each SIM ion

Turn each signal on/off as desired

Acquire Multiple MS Signals:

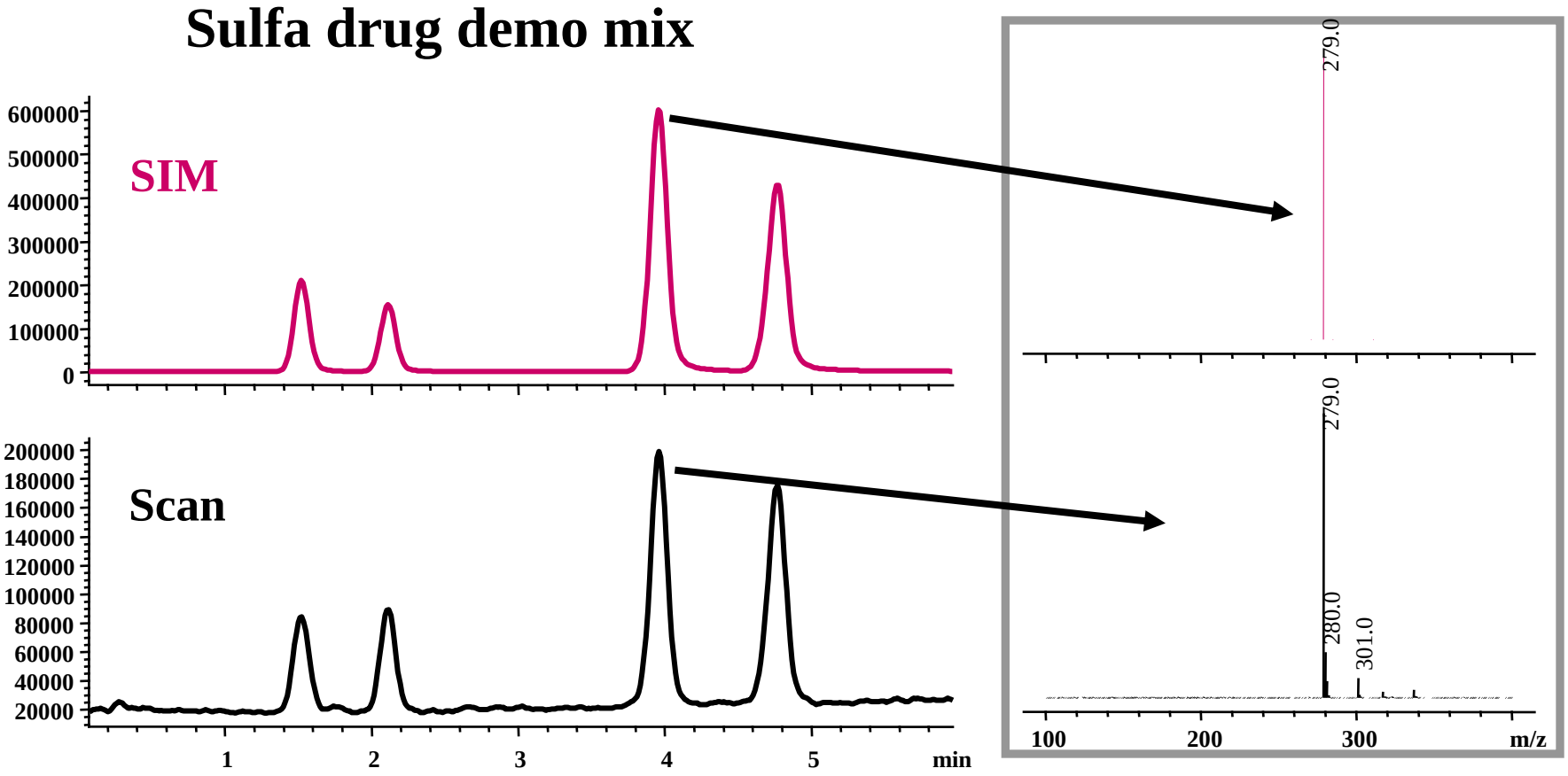
SIM/Scan



SIM signal for quantification of target compounds

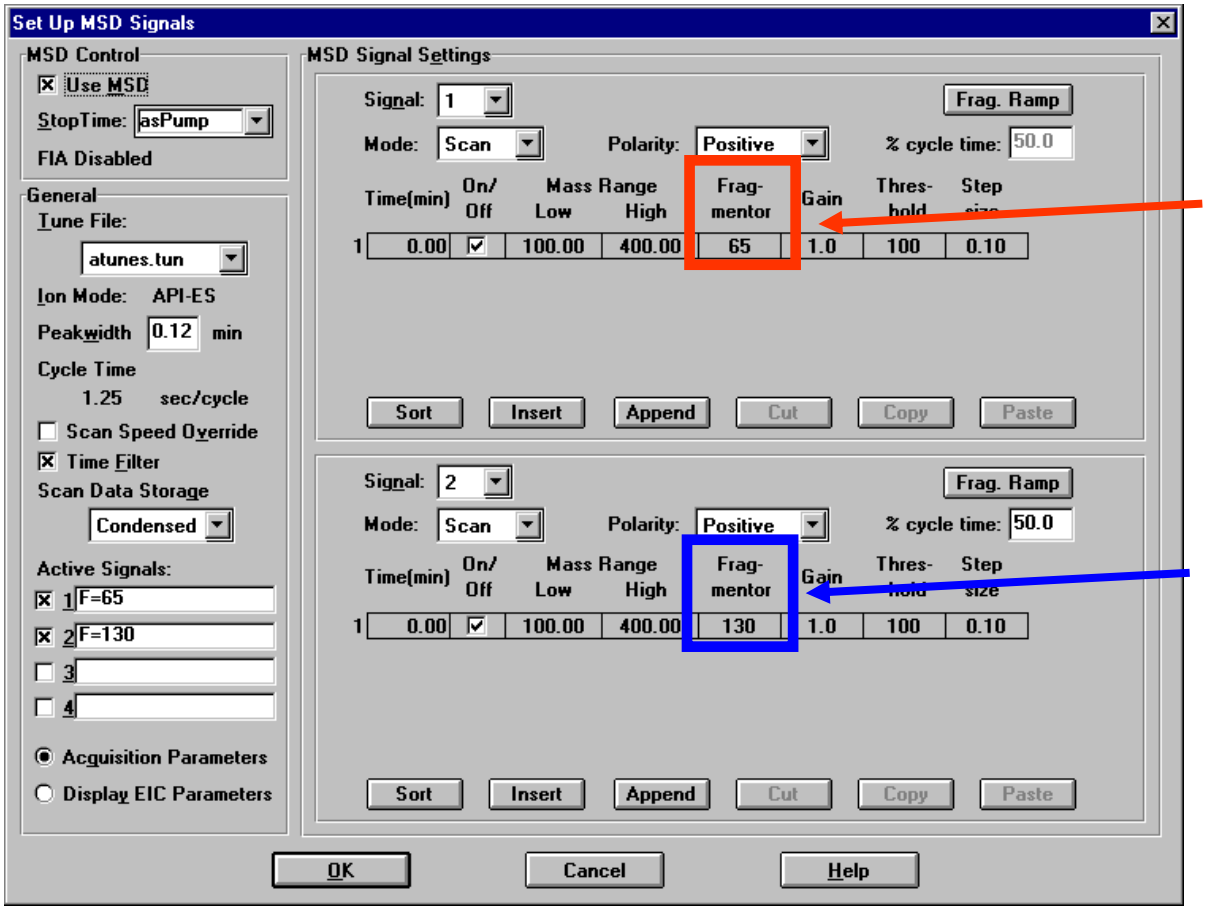
Scan signal for screening for unknowns or for structural information (CID)

Acquire Multiple MS Signals: SIM/Scan



Acquire Multiple MS Signals:

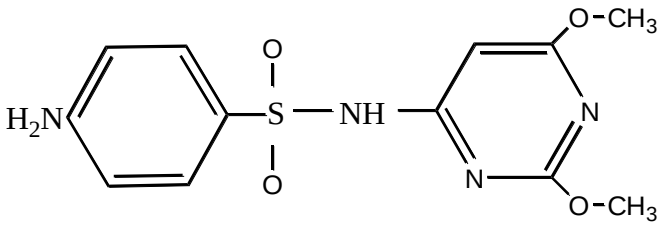
High/Low Fragmentor



Low fragmentor value will produce predominantly the pseudomolecular ion for easy confirmation of MW

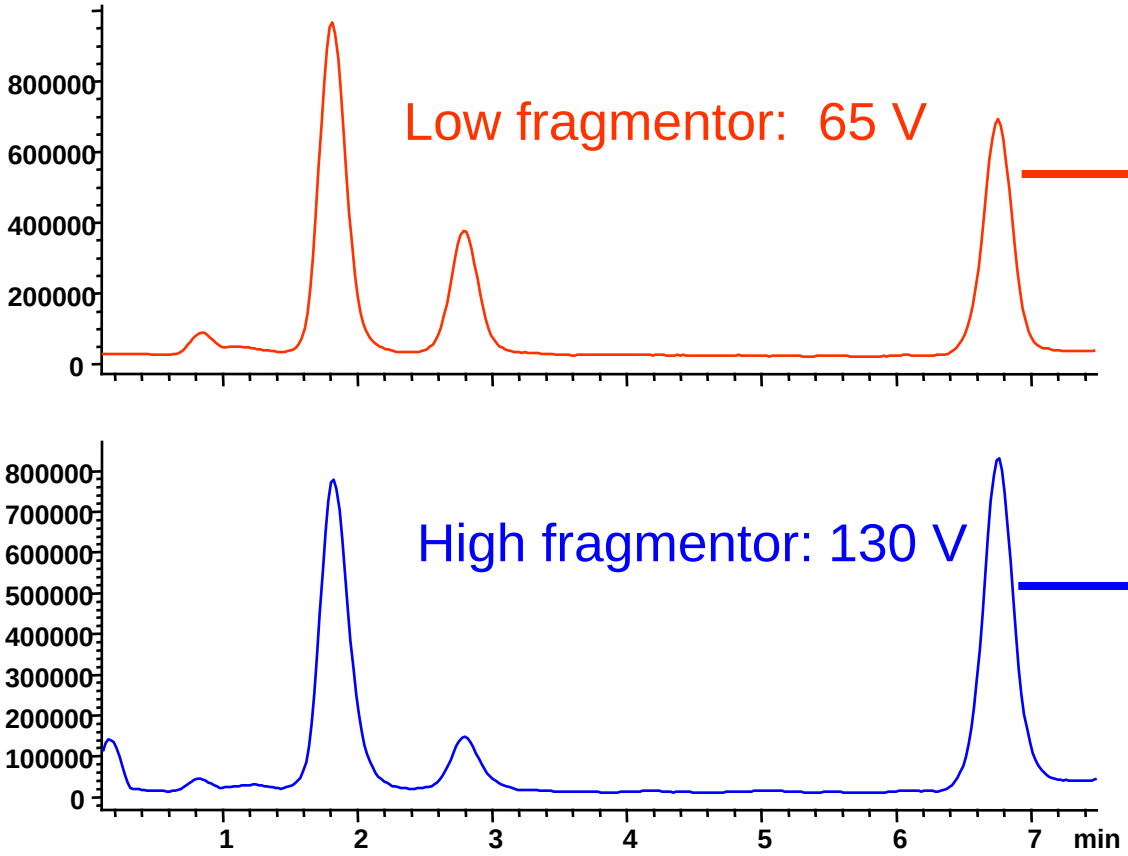
High fragmentor value increases fragmentation for additional structural information

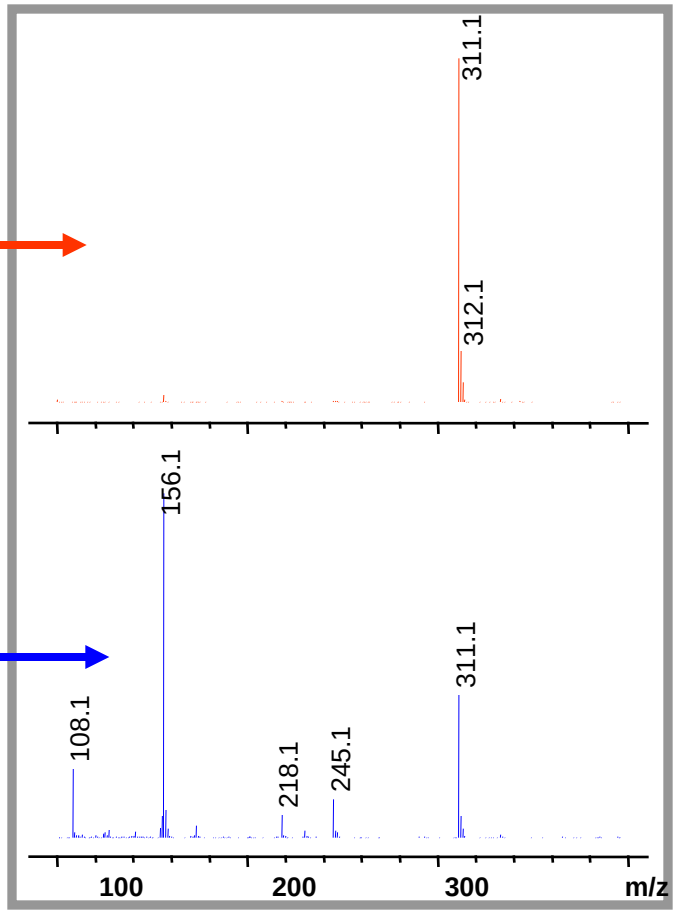
Acquire Multiple MS Signals:



High/Low Fragmentor

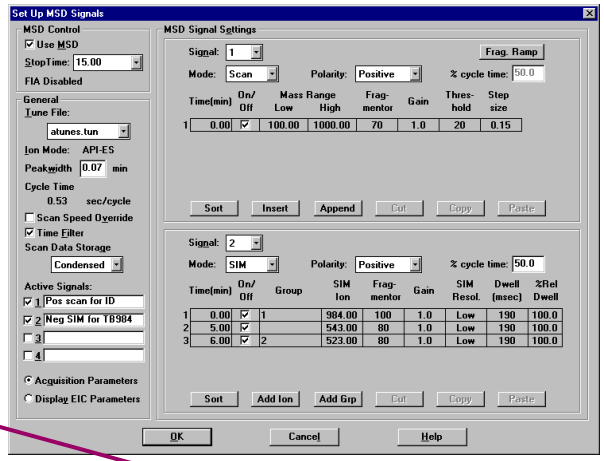
Sulfa drug demo mix



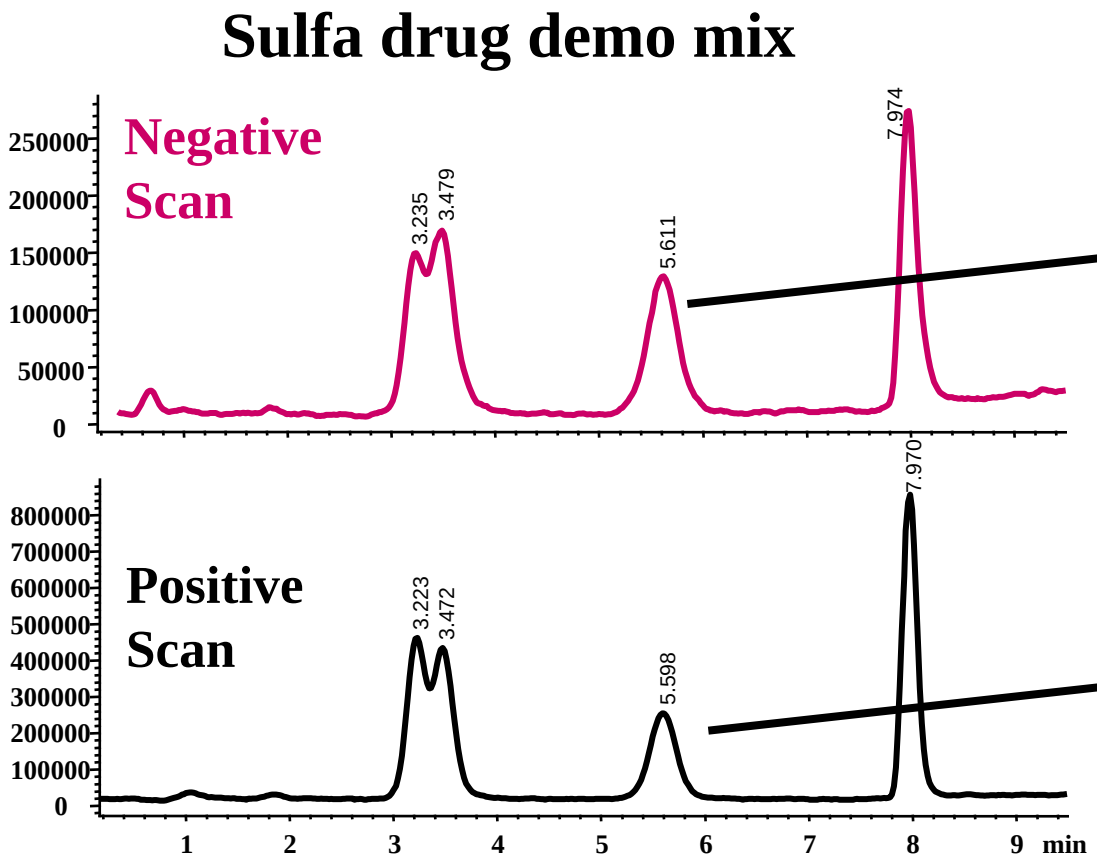


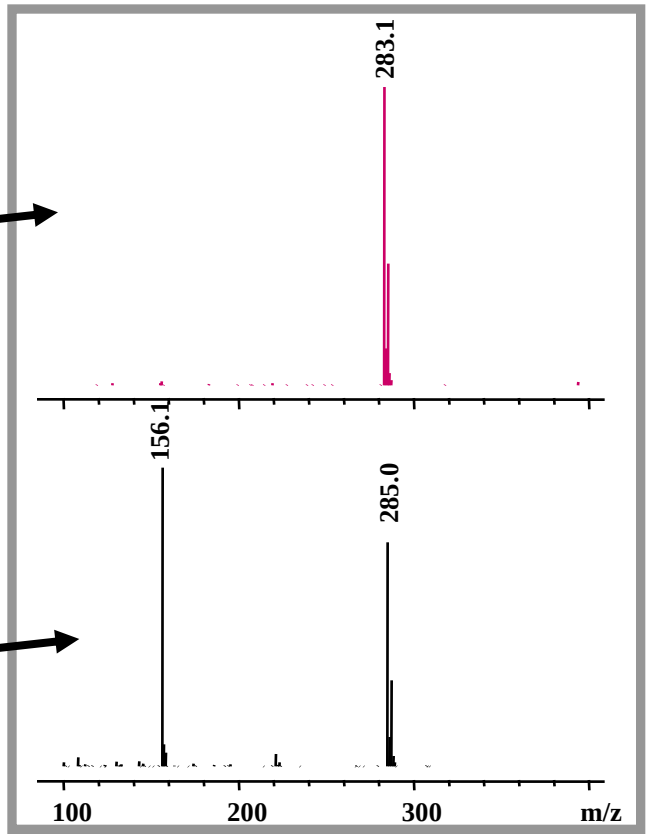
Agilent 6100 LC/MSD SL
Acquire Multiple MS Signals

Time sliced - Up To 4 Signals per Run



Acquire Multiple MS Signals:
Positive/Negative Mode





Multi-signal Analysis

Define up to four signals which will execute in a cyclical fashion.

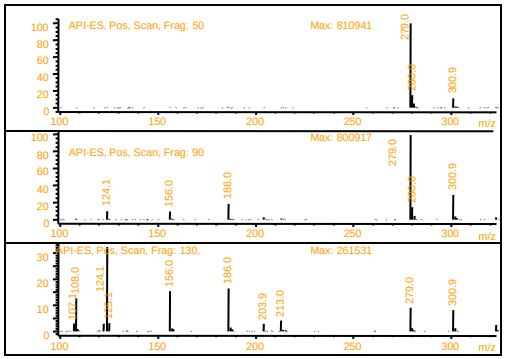
Change:

- Polarity
- Fragmentor Voltage
- Mass range and other mass spec acquisition parameters (EMV)
- Alternating SIM and Scan

A single injection provides the same amount of data as multiple injections

Trade-Offs

- Faster scan speed
- Shorter dwell time for SIM
- Increased Noise



MS Parameter Setup: Summary

General Approach

Nebulizer pressure, drying gas temperature and drying gas flow rate are determined by HPLC flow rate

Compound Dependent Parameters

ESI: Capillary Voltage (Vcap), and Fragmentor

APCI: Vaporizer temperature, Vcap, Corona current, and Fragmentor

Qualitative Mass Spectral Analysis

Mass Measurement

Average mass

- mass of an ion calculated from a given empirical formula using the atomic weight for each element. Atomic weight is an average of the isotopes for an element (C = 12.1115).

Monoisotopic mass

- mass of an ion calculated from a given empirical formula using the exact mass of the most abundant stable isotope for each element (C = 12.000000)

Mass Defect

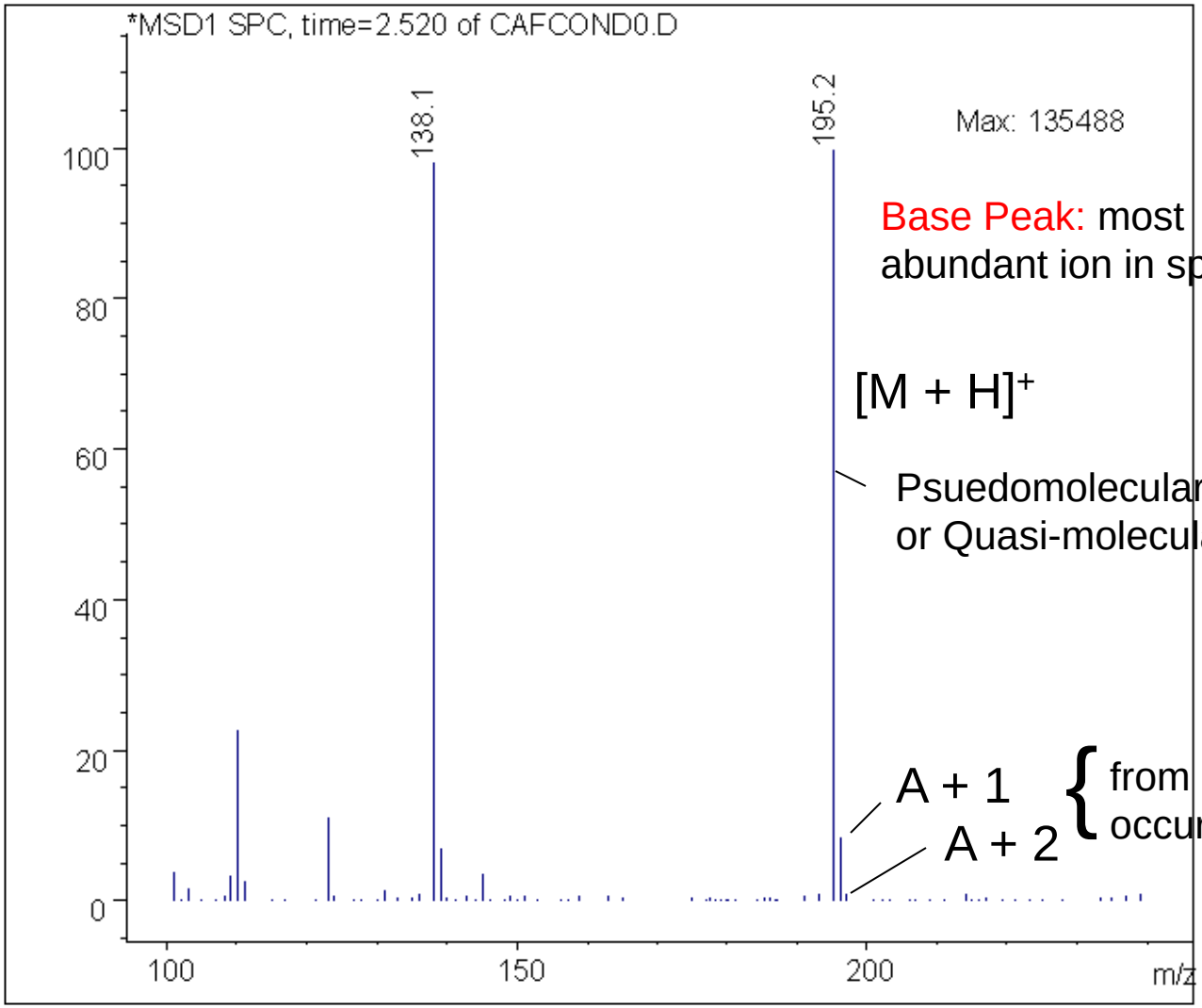
- the difference between the mass of an ion and the integer mass (protons +neutrons) -
  Cl 34.9689 - 35 = -0.0311
  H 1.0078 - 1 = +0.0078

Resolution – differing definitions

- resolving power of a mass spectrometer ($M/\Delta M$ where M is the m/z value of a singly-charged ion and ΔM is the difference between M and the next highest distinguishable m/z)
- Resolution (FWHM): $M/\Delta M$ where M is the m/z value of a peak in the spectrum, and ΔM is the Full Width at Half Maximum of this peak

Mass Spectra

abundance



Base Peak: most abundant ion in spectrum

$[M + H]^+$

Psuedomolecular ion or Quasi-molecular ion

A + 1

A + 2

{ from naturally occurring isotopes

Atomic Definitions

Atom	Mass	Rel. Abund.	Mass	Rel. Abund.	Mass	Rel. Abund.	Class
Hydrogen	1.0078	100					A
Carbon	12.0000	100	13.0034	1.1			A+1
Nitrogen	14.0031	100	15.0001	0.37			A+1
Oxygen	15.9949	100			17.9992	0.2	A+2
Fluorine	18.9984	100					A
Sodium	22.9898	100					A
Silicon	27.9769	100	28.9865	5.1	29.9738	3.4	A+2
Phosphorous	30.9738	100					A
Sulfur	31.9720	100	32.9715	0.8	33.9679	4.4	A+2
Chlorine	34.9989	100			36.9659	32.5	A+2
Potassium	38.9637	100		0.01	40.9618	7.2	A+2
Bromine	78.9183	100			80.9163	98	A+2
Iodine	126.9045	100					A

Optimizing MSD Analyses

Adapting Existing LC Methods to LC/API-MS

Replace non volatile buffers with volatile buffers at a concentration of <10 mM for ESI or <100 mM for APCI

- Substitute phosphates and borates with ammonium acetate, ammonium formate, TFA
- If a non-volatile buffer must be used, select a buffer with only the anionic or cationic part is non-volatile (i.e. ammonium phosphate and keep concentration very low) and keep column id and flow rate low (2.1 or 1.0 mm id)

Keep the pH the same as in the original separation with volatile additives – formic acid, acetic acid, TFA, ammonium hydroxide

Use volatile ion-pair reagents only when needed – heptaflurobutyric acid (HFBA) and tributylamine (TBA)

MS Friendly Modifiers

Acetic, formic acid

Avoid TFA or keep below 1 mM

- Use TFA “fix” – post column addition of acetic or propionic acid

Ammonium acetate, formate

- < 10 mM for ESI
- < 100 mM for APCI

pH should be appropriate for desired ion polarity

- Won’t see negative ions with formic acid, TFA. Raise pH or use post column addition of base

Summary

• Single quadrupole mass analyzer offers a sensitive detection of both polar and nonpolar compounds

•Chromatography conditions have a significant impact on mass spectrometric analysis

• Important considerations need to be taken for both ESI and APCI ionization

• Single quadrupole mass analyzer has significant mass resolving power for qualitative mass spectral analysis

• Autotune performs tuning and calibration ensuring optimal mass spectrometric performance