Selecting an LC/MS Interface

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LC/MS Interfaces

I. Background of LC/MS
   I. Historical Perspective
   II. Reasons for use
II. Interfaces
   I. Transport devices
   II. Particle Beam
   III. Thermospray
   IV. Atmospheric Pressure Interfaces

Historical Perspective

- Goldstein – 1886
  - Existence of positively charged particles
- Wein – 1898
  - Positively charged ions can be deflected in electrical and magnetic fields
- J.J. Thomson – 1913
  - Demonstrated isotopes of Neon
  - "Father of mass spectrometry"
- First GC-MS interface - 1960's

Historical Perspective

- First LC-MS interface developed - 1969
  - 1uL/min flow into an EI source
- Transport devices applied to LC/MS - 1970's
  - Loss of volatile components
  - Thermally-reactive compound losses
- Thermospray (TSI) gains popularity - 1983
  - 1.0 – 1.5 mL/min
  - Mobile phases consist primarily of an aqueous buffer

Historical Perspective

Atmospheric Pressure Interfaces (API)

- Early 1990's (commercialization)
- Now most common interface
- Electrospray (ESI)
  - Initial interfaces required lower flows (1-5 uL/min)
  - Able to produce multiply-charged molecules
- Atmospheric Pressure Chemical Ionization
  - Similar to Thermospray
  - "Solvent-mediated" ionization

Why Use Mass Spectrometry?

- Spectral resolution is possible
  - Chromatographic coelutions
- Compound identification from spectral data
  - Mass spectrum is very dependant on the ionization
  - Limited availability of LC/MS libraries
- High degree of specificity

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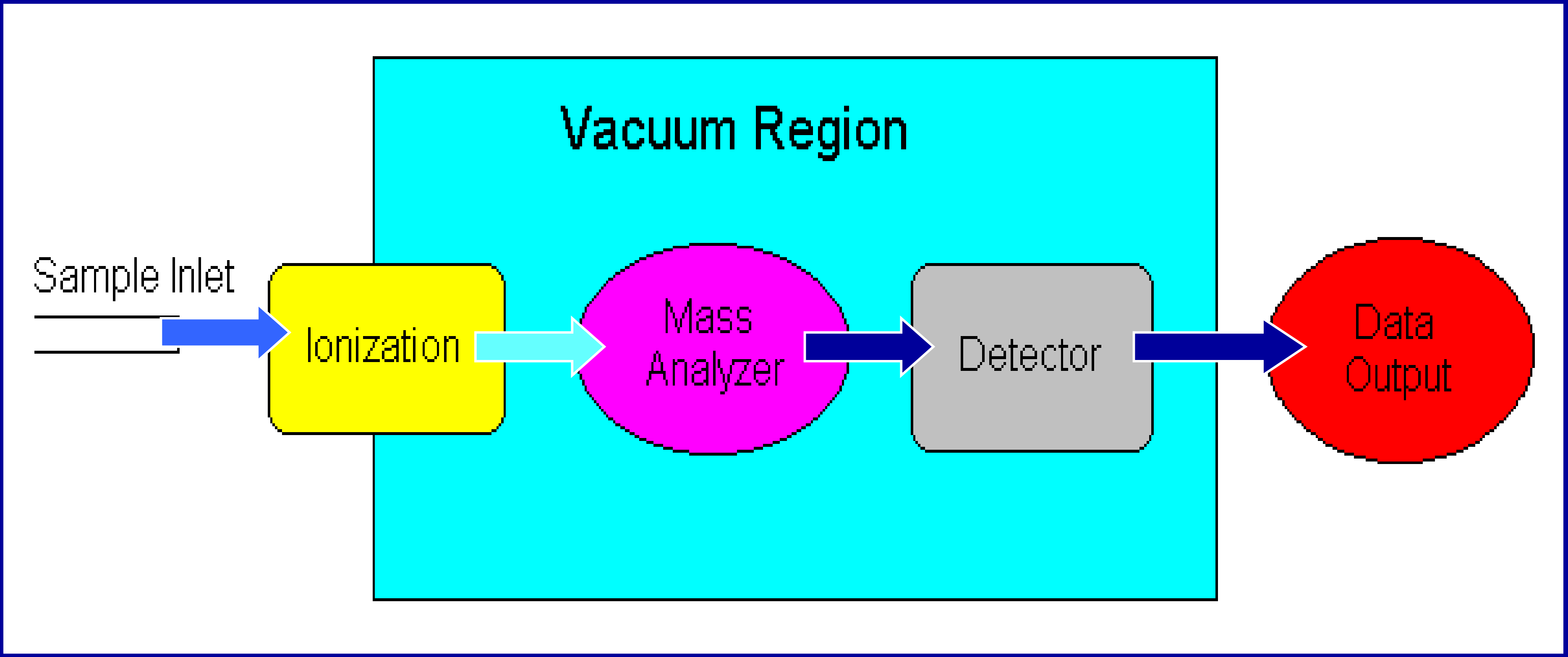
Components of an MS Detector



Ions Made

Ions Selected

Ions Detected

LC/MS Systems

Interfaces

- Transport Devices
- Particle Beam
- Thermospray
- Atmospheric Pressure
  - Electrospray
  - APCI
- Others

Analyzers

- Quadrupoles
- Ion Trap
- MS^n
- High Resolution
- Time-of-Flight (TOF)
- Others

Ion Sources

- Analytes must be charged (ions)
  - Needed to separate and detect
- Where ionization occurs
  - In the interface
  - In a separate ion source
- Types of ionization
  - “Hard” - considerable fragmentation
  - “Soft” - molecular ion is main product
- Ions can be positive or negative

Requirements of LC/MS Interfaces

- Elimination of the mobile phase
  - Most difficult step
  - Can use splitters
  - Volatilized solvent vapor removed under vacuum
- Often where ionization occurs
- Vacuum required by mass analyzers

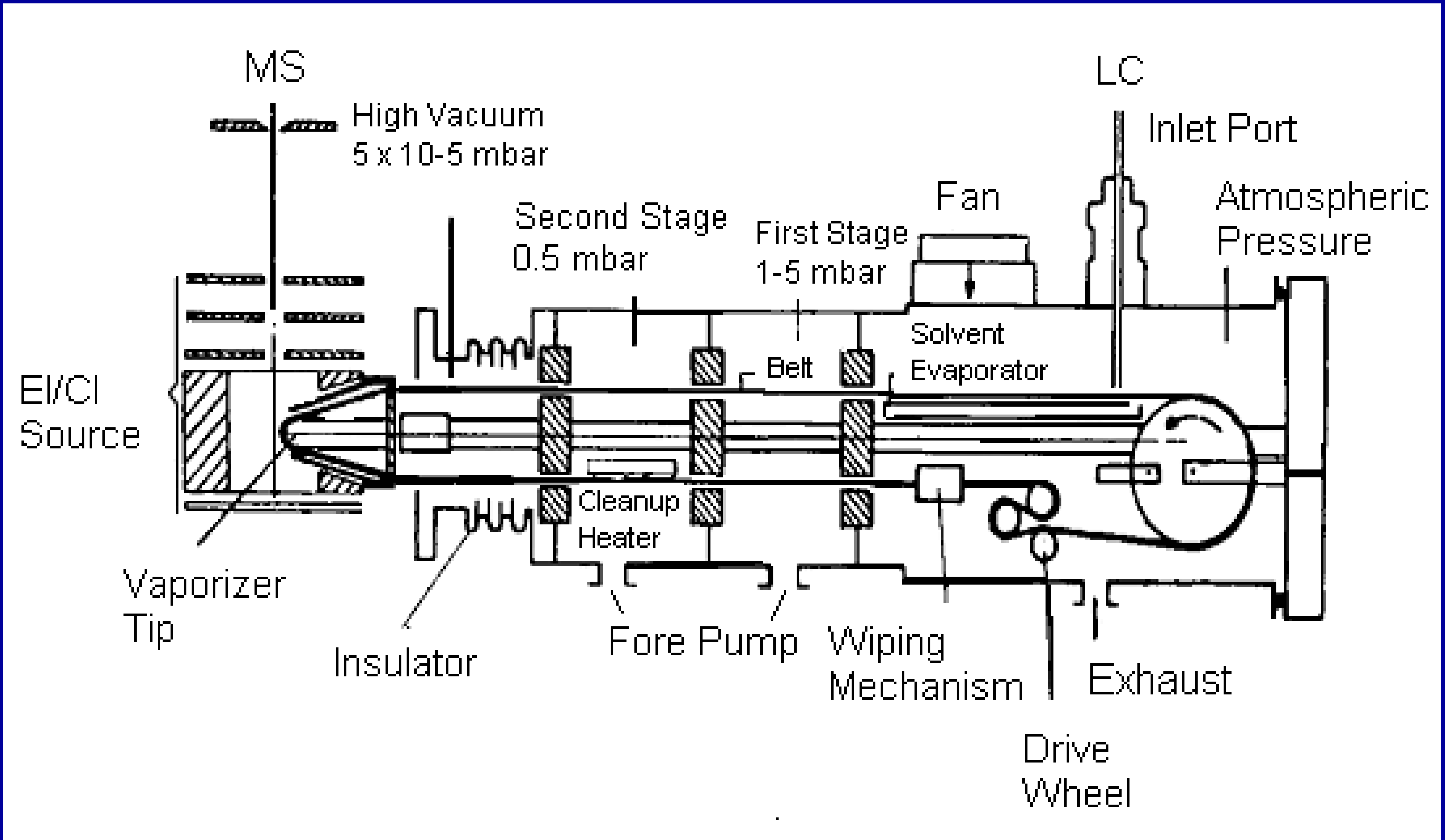
Challenges in Interfacing LC to MS

- Flow Rate Concerns
  - Differential pumping can only handle 2 μL/min of water
  - For maximum sensitivity, want to use all of the eluent
- Use of Buffers and Additives
  - Non-volatile buffers a concern
  - Some additives suppress ionization
- Wide Range of Analytes
  - Many are nonvolatile, thermally labile

Transport Devices

- One of the first commercial interfaces
- Sample deposited onto a moving belt or wire
- Sample passes through multiple vacuum zones
  - Solvent elimination
- Sample is desorbed into source using heat
  - Electron impact ionization
- Belt/wire cycles back

Moving Belt Interface

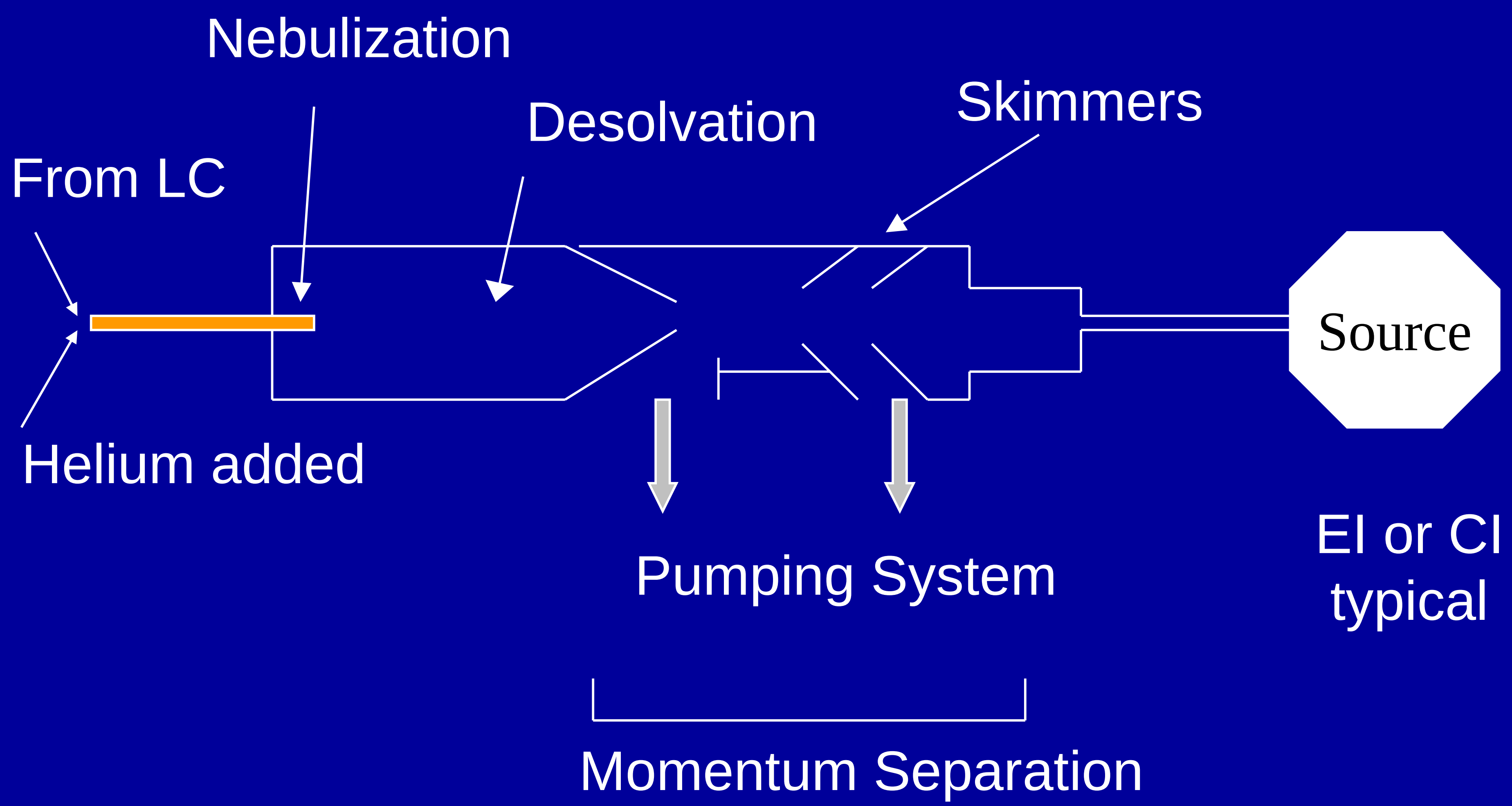


From: Niessen

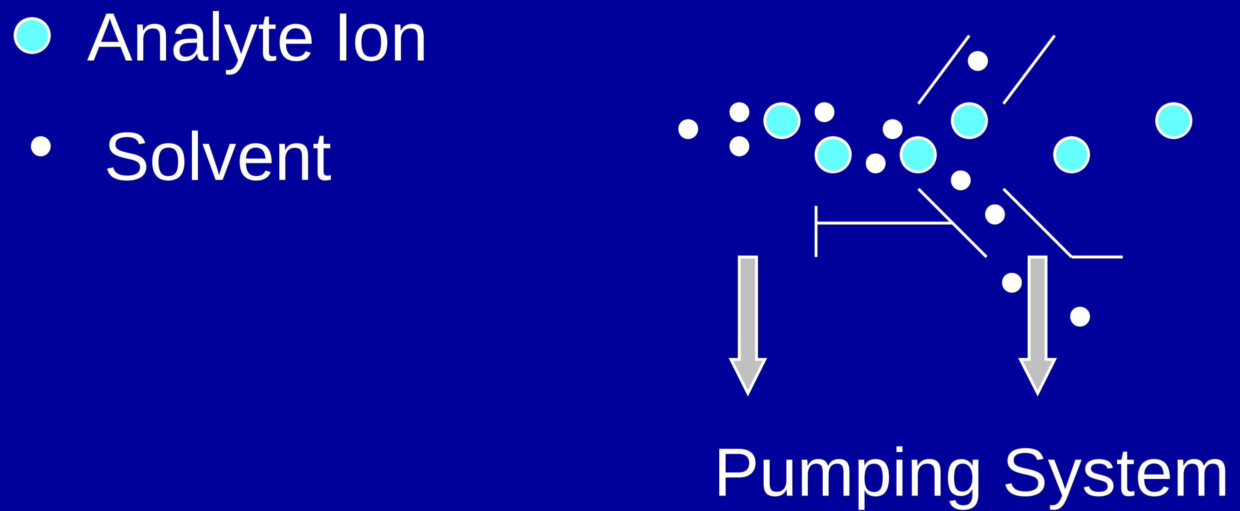
Particle Beam Interface

- Column effluent is nebulized
  - Pneumatic or thermospray nebulization
- Desolvation chamber is under a moderate vacuum
- A momentum separator is used for analyte enrichment
  - High MW compounds favored
- Analytes into the EI or CI source as small particles
  - Evaporative collisions with the walls

Particle Beam Interface



Analyte Enrichment in PB Interfaces



Analyte enrichment with a molecular beam approach

Heavier molecules are in the core of the vapor jet and are sampled through the skimmer

Electron Impact Ionization

- LC interfaces with the ion source
- Electrons are “boiled” from a hot wire (filament), and accelerated (70eV)
- As electrons pass neutral molecules, they may remove outer shell electrons

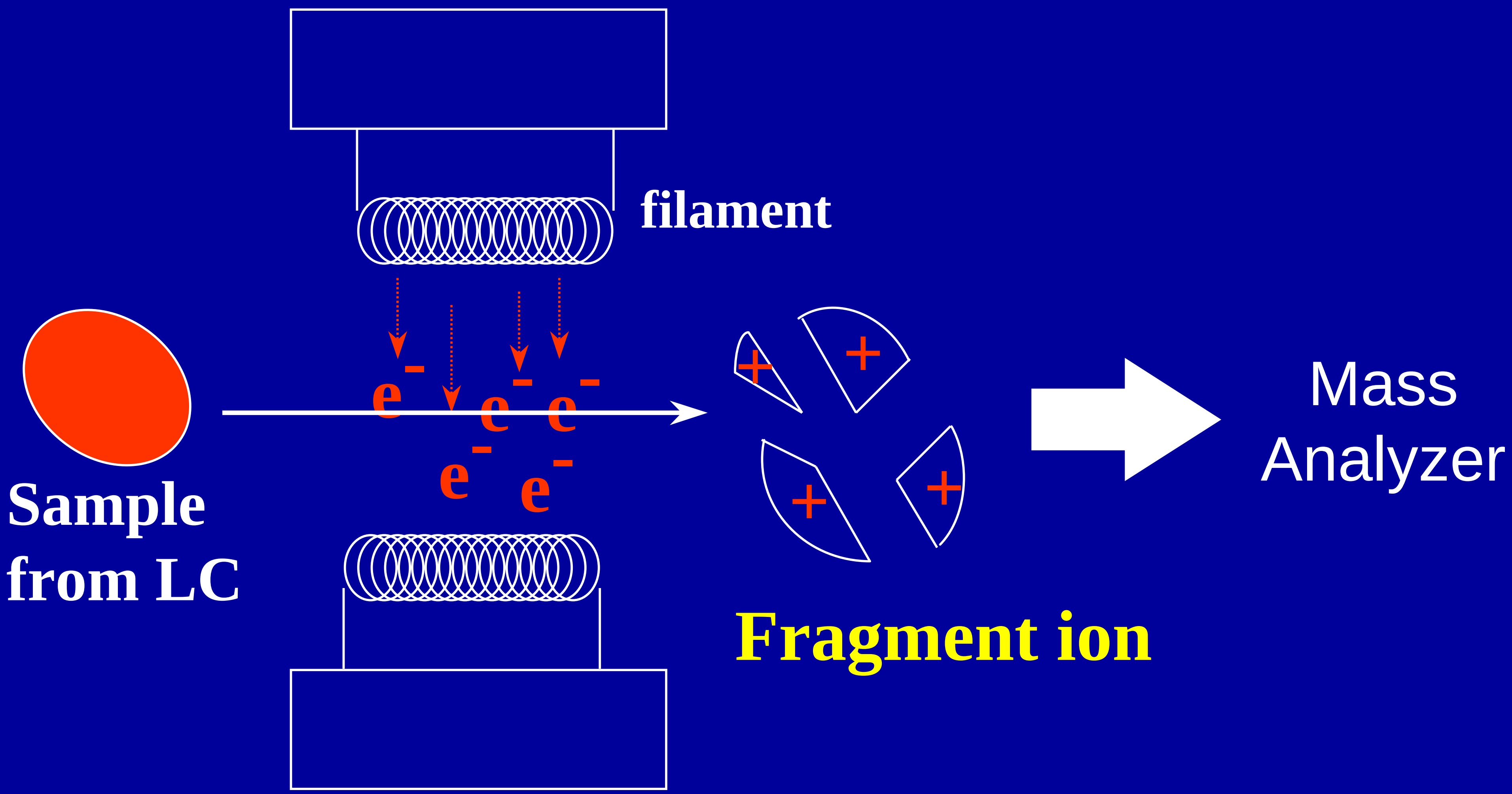
$$M + e^- \rightarrow M^{+\cdot} + 2e^-$$

$$M^{+\cdot} \rightarrow F^+ + N^\cdot$$

Electron Impact Ionization

- Produces positively charged ions
- Fragmentation is generally significant for most molecules – "hard ionization"
  - Masses of these fragments is the information used in interpretation
- Efficiency of ionization is $1/10^5$
  - Bulk of molecules are removed by vacuum pump – Use Traps

Electron Impact Ionization (EI)



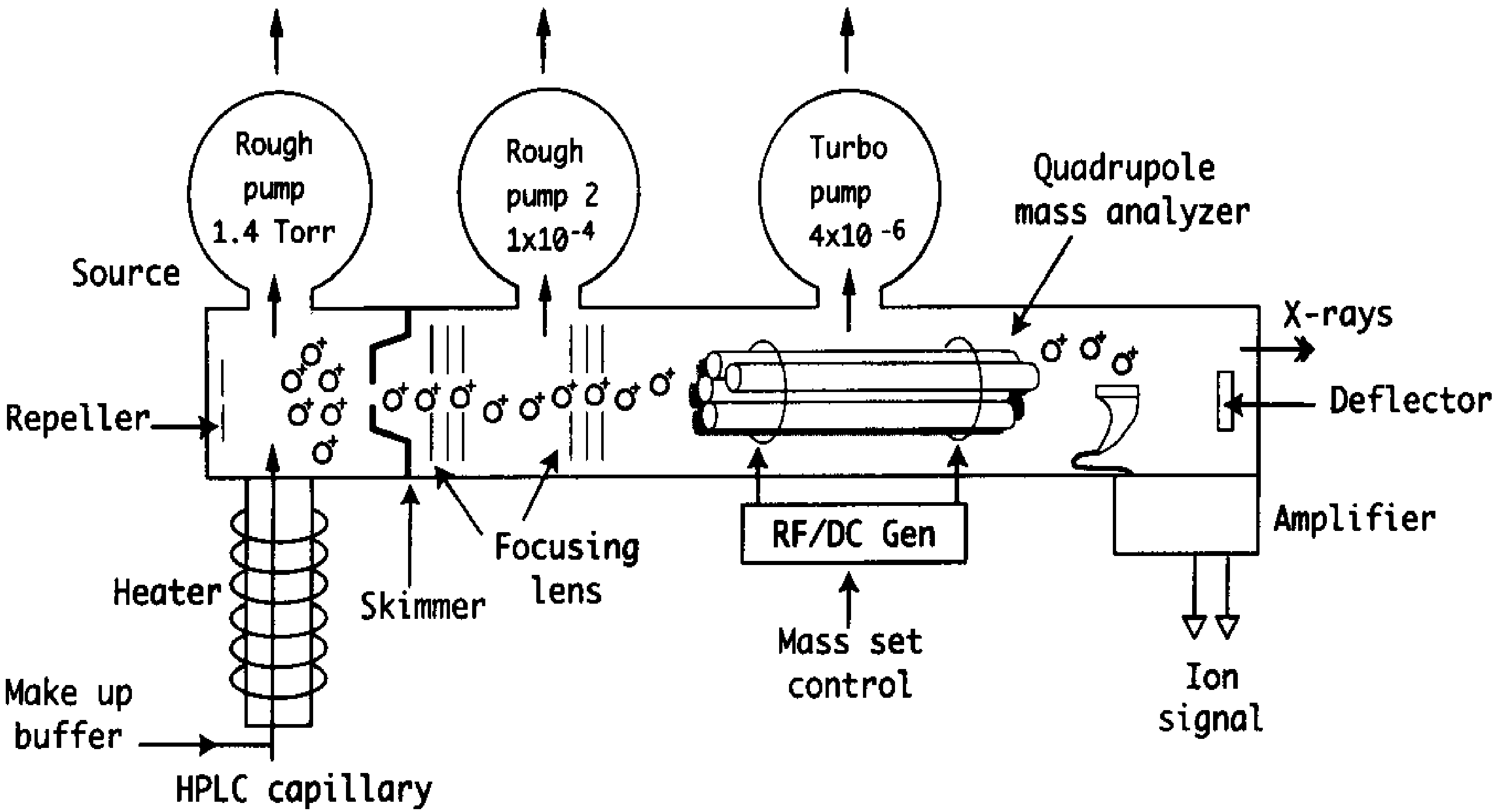
Thermospray Interface

- Nebulization of the eluent from a heated transfer tube
- Uses a “reagent gas”
  - Mobile phase buffer
  - Added buffer solution
  - Similar spectra to GC/MS CI
- Reagent gas is ionized
  - Volatilization
  - EI with high energy electrons
- Charge transfer to the analyte(s)

Thermospray Interface

- Effectively replaced transport systems
- Ionization in a medium pressure environment
  - Approx. 1000 Pa or 0.01 atm
- Inlet flows of 1 to 2 mL/min
  - Can use standard LC column flows
- Positive and negative ions are possible
- Temperature optimization is critical
  - Maintain gas phase

Thermospray LC/MS System



From: McMaster and McMaster

Thermospray Ionization

- Positive Ion Mode
  - $M + SH^+ \rightarrow MH^+ + S$ PA(M) > PA(S)
  - $M + SH^+ \rightarrow MHS^+$ PA(M) ≈ PA(S)

- Negative Ion Mode
  - $M + [S\text{-}H]^- \rightarrow [M\text{-}H]^- + S$ gpa(M) > gpa(S)
  - $M + A^- \rightarrow MA^-$ polar molecules

gpa = gas phase acidity

Typical Proton Affinities

Compound	Proton Affinity
Water	723
Methanol	773
Acetonitrile	797
Ethers, esters, ketones	630-670
PAHs	710-800
Carboxylic Acids	<800
Alcohols	750-840
Peptides	880-1000

From: Niessen

Thermospray Modes of Operation

- Solvent-Mediated CI
  - Typical mobile phase: MeOH or MeCN + 0.1M NH_4OAc
  - Ionization by ion evaporation (pre-formed ions) or ion-molecule reactions
- Discharge or Filament On Mode
  - High energy electrons (0.5-1 keV) ionize the reagent gas
  - Ion-molecule reactions

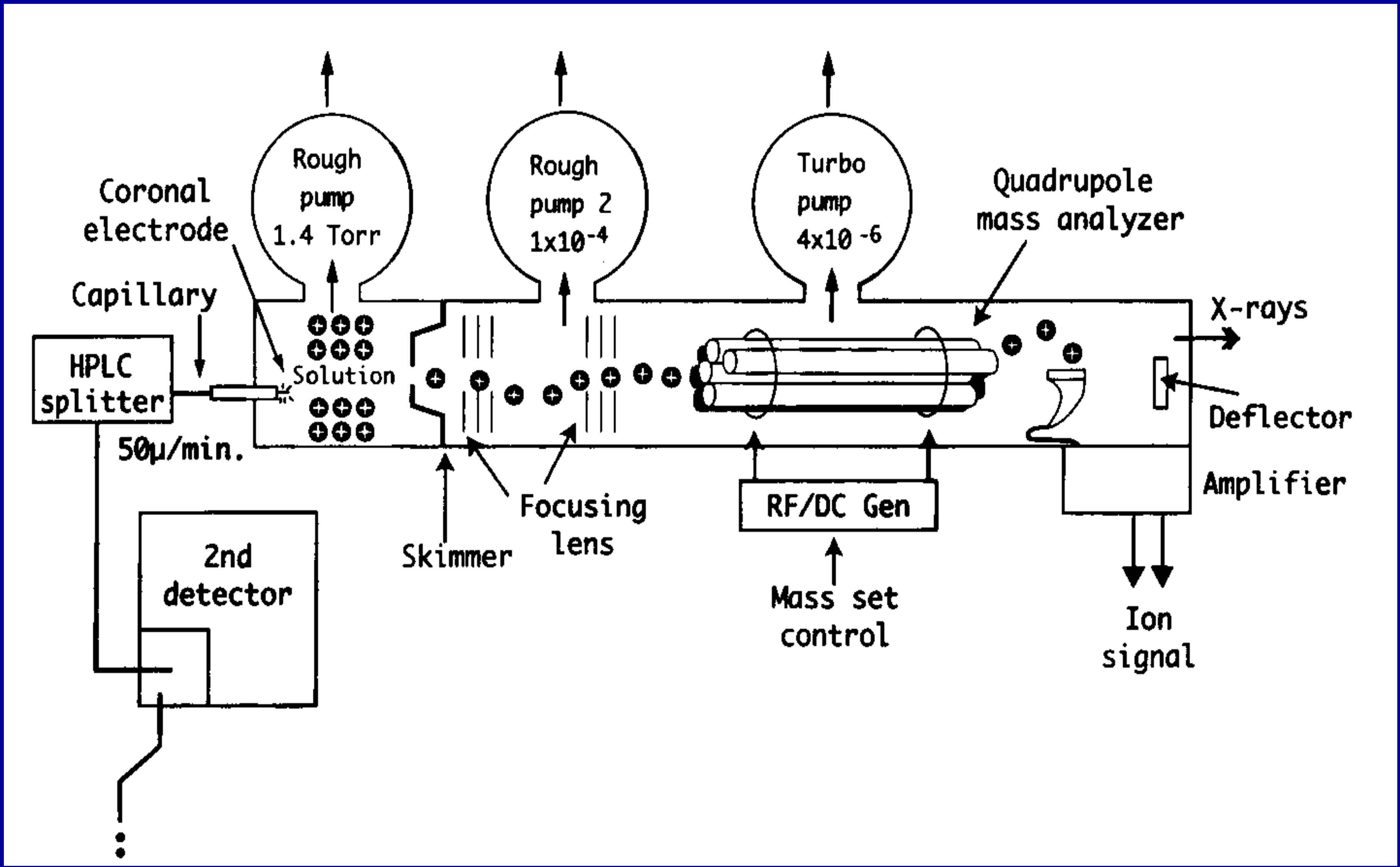
Electrospray Interface

- High electric potential applied to eluent from transfer capillary
  - Atmospheric pressure
  - Droplet formation
  - Ionization in the solution phase
- Orthogonal sampling of ions
  - Reduces contamination of the sampling orifice
  - Z-Spray devices

Electrospray Interface

- Higher flows now possible
  - Pneumatic + thermal nebulizers
- Applicable to volatile and nonvolatile analytes
  - Need to be ionizable
- Can create multiply-charged ions
  - Allows for analysis of large molecules

Electrospray LC/MS System



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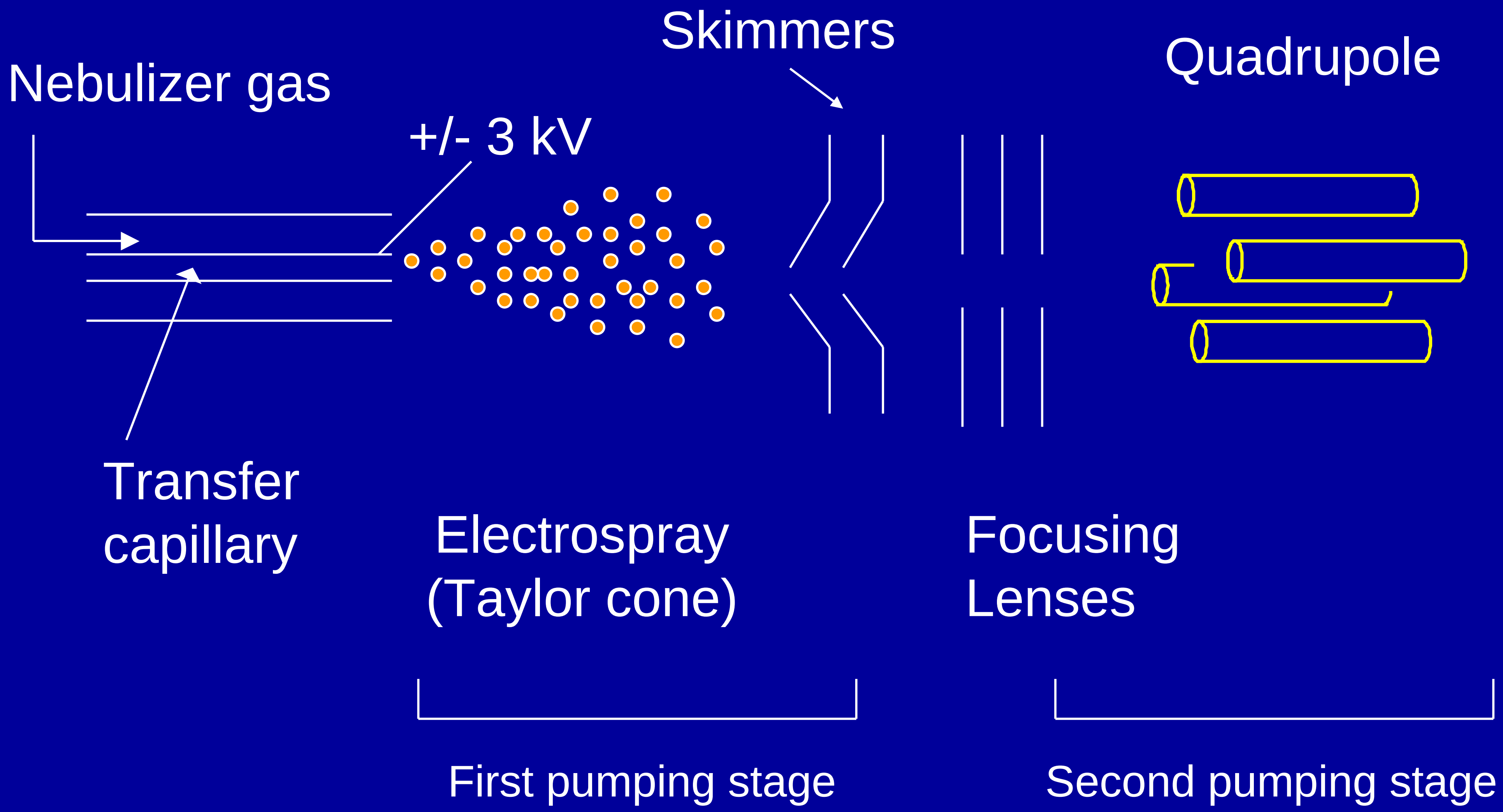
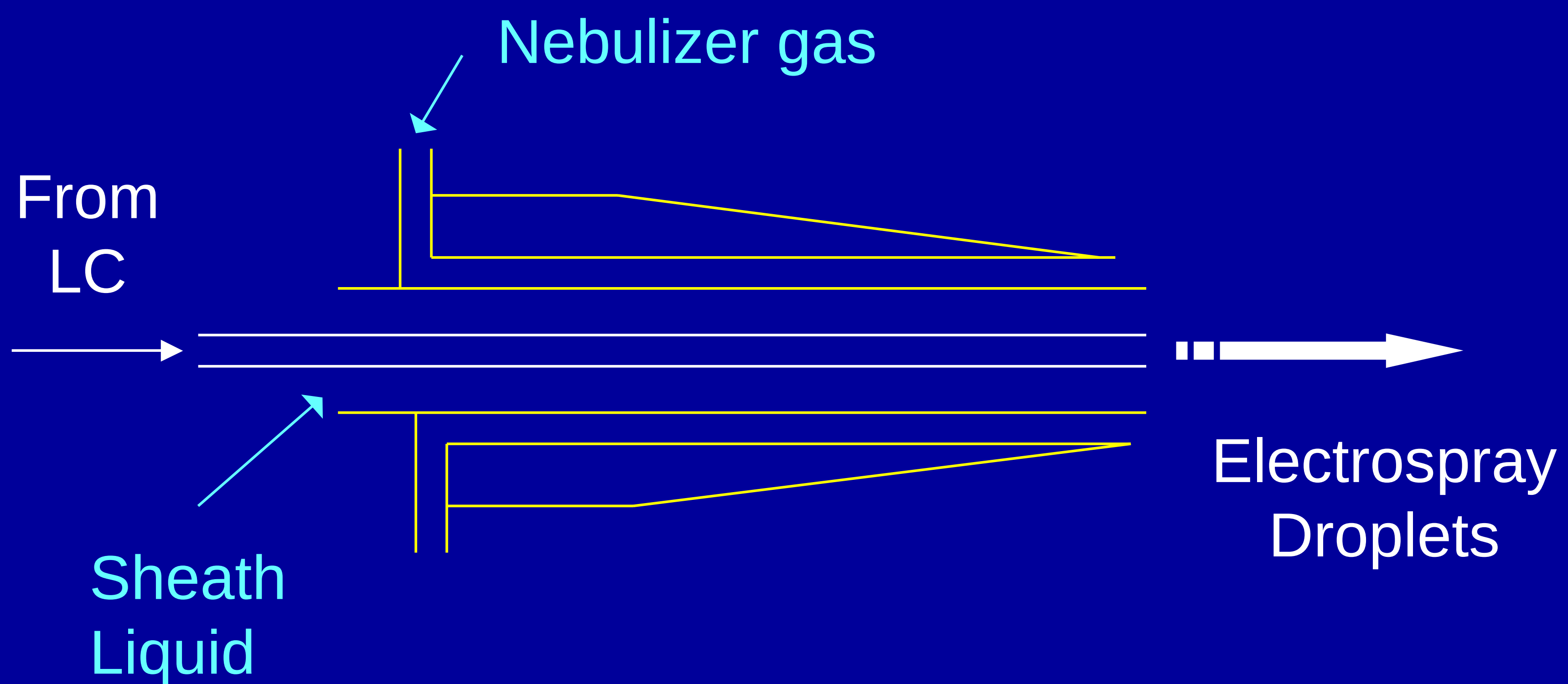
Electrospray Interface



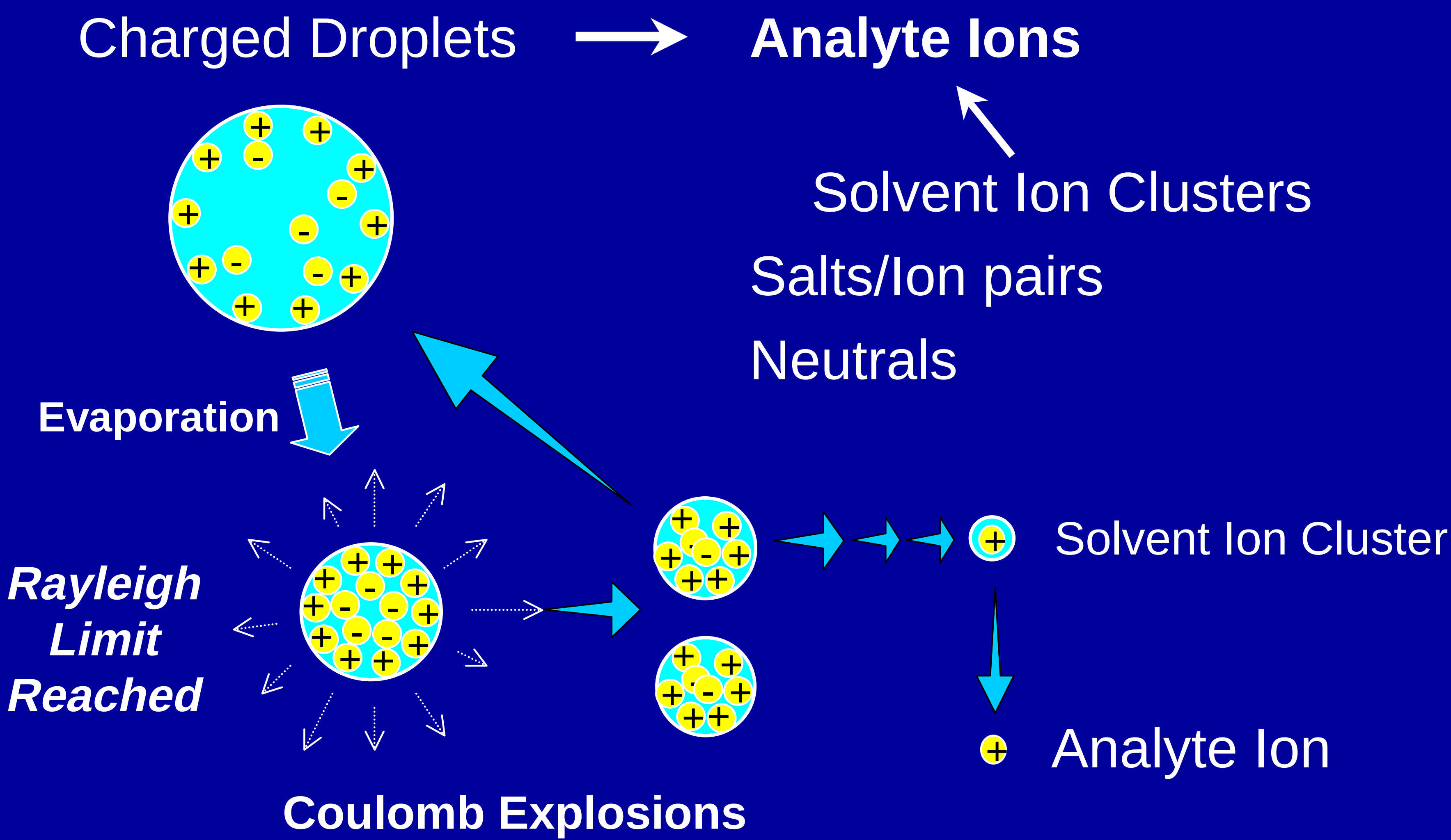
Electrospray Needle Design



Example of an electrospray needle design (coaxial flow)

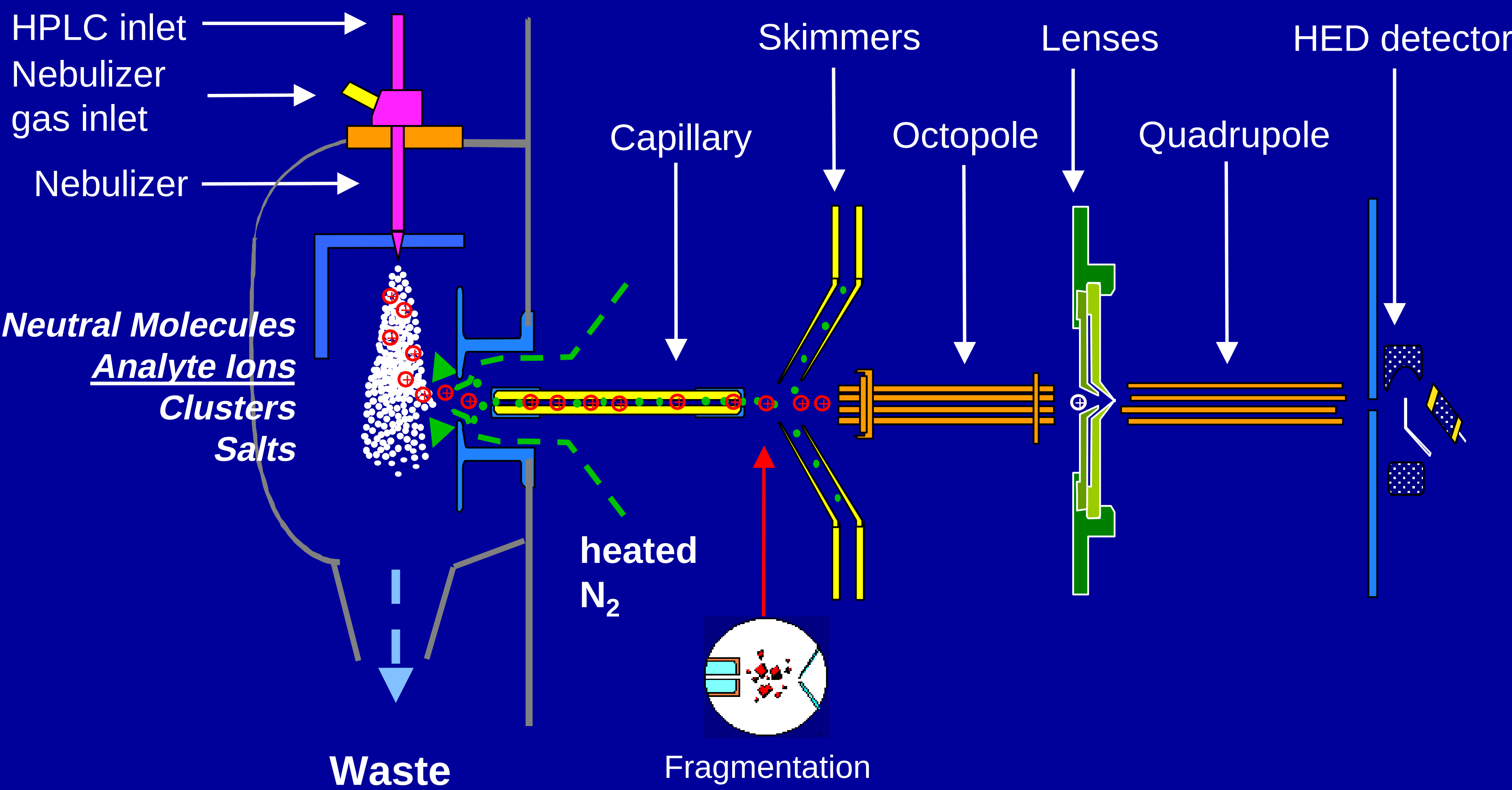
The nebulizer gas disrupts the liquid surface so that small droplets are formed and then dispersed by the gas

Theory of API Electrospray



Courtesy of Agilent Technologies

Agilent 1100 LC/MSD Electrospray



Courtesy of Agilent Technologies

Electrospray Interface

- Best vaporization with higher % organic and lower flow rates
- Cluster ion formation possible
  - Solvent clusters
  - Analyte/salt clusters
- Salts and sample impurities can affect the response
  - TFA causes signal suppression
  - TFA anion masks the analyte ion

APCI Interface

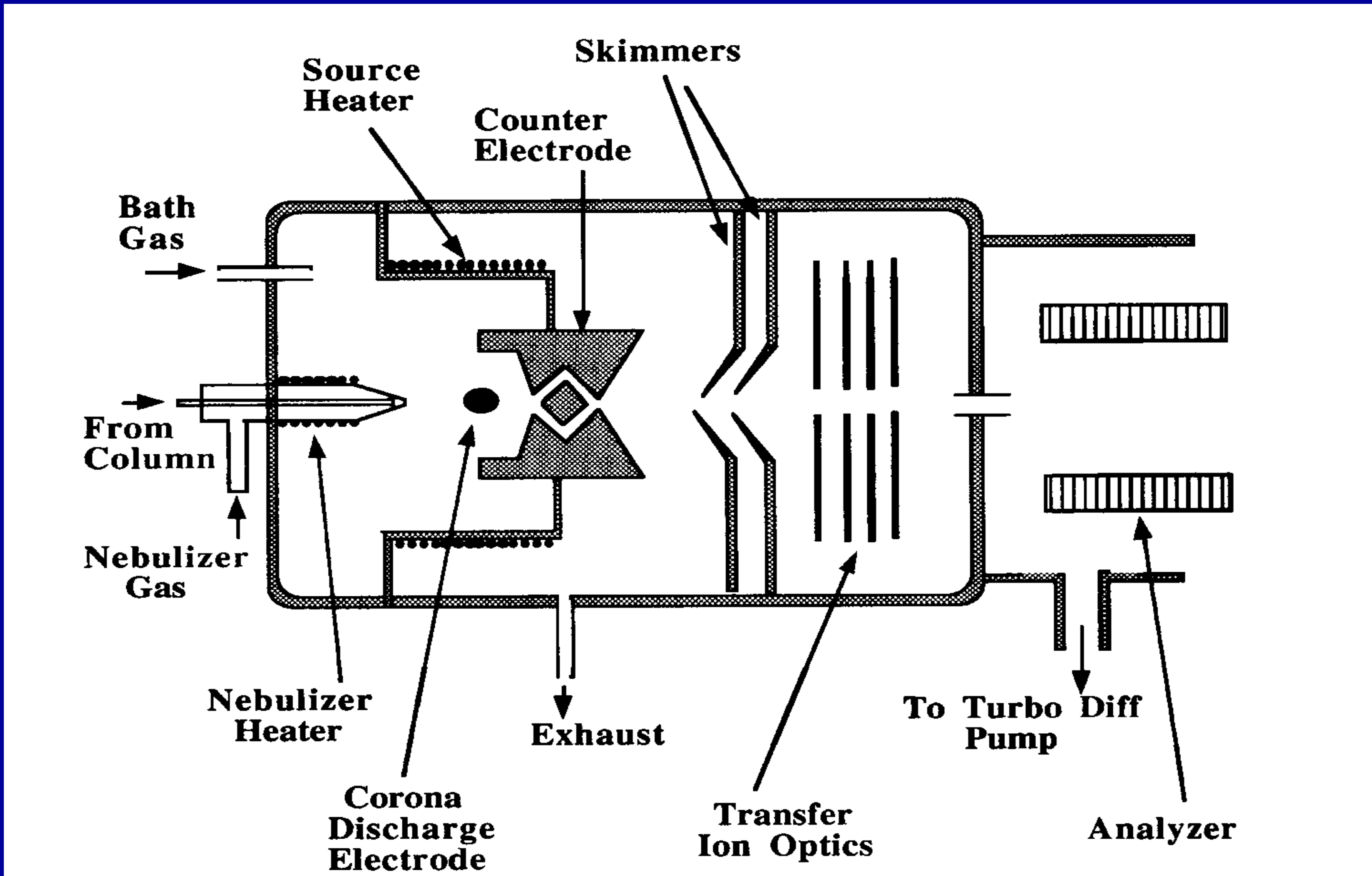
- Atmospheric Pressure Chemical Ionization
- Initially investigated in 1974
  - Popular in late 1980's
- Uses an API (ESI) source
- Column effluent nebulized into heated vaporizer tube
- Solvent vapor acts as a reagent gas
  - Charge transfer to the analytes
- Can be very sensitive

APCI Interface

- Results in a chemical ionization spectrum $[M+H]^+$ or $[M-H]^-$
- Products depend on equilibrium (concentration) conditions
- Analytes must have sufficient proton affinities
- May be simplest interface to operate
- Liquid flow rates of 0.2-2.0 mL/min

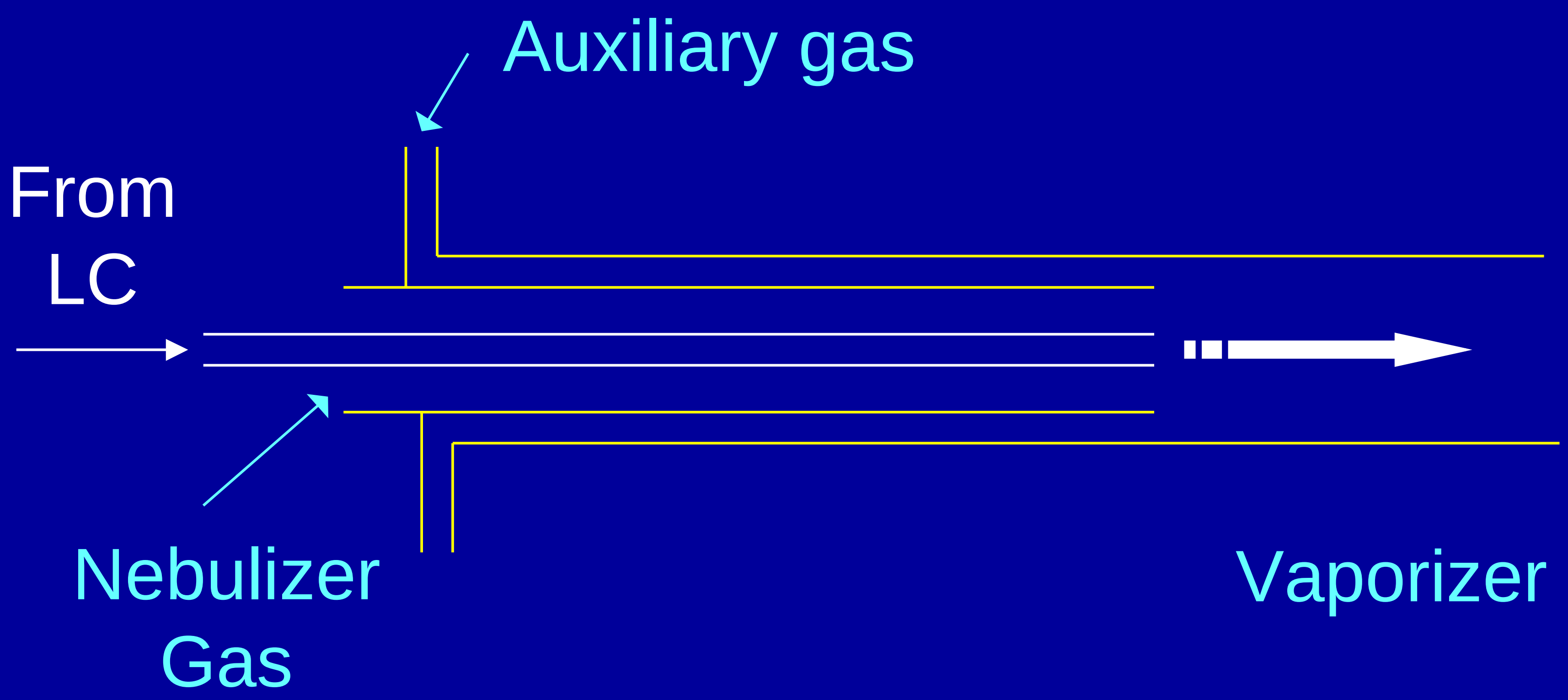
$[Solv+H]^+ + M \rightarrow Solv + [M+H]^+$

APCI Interface



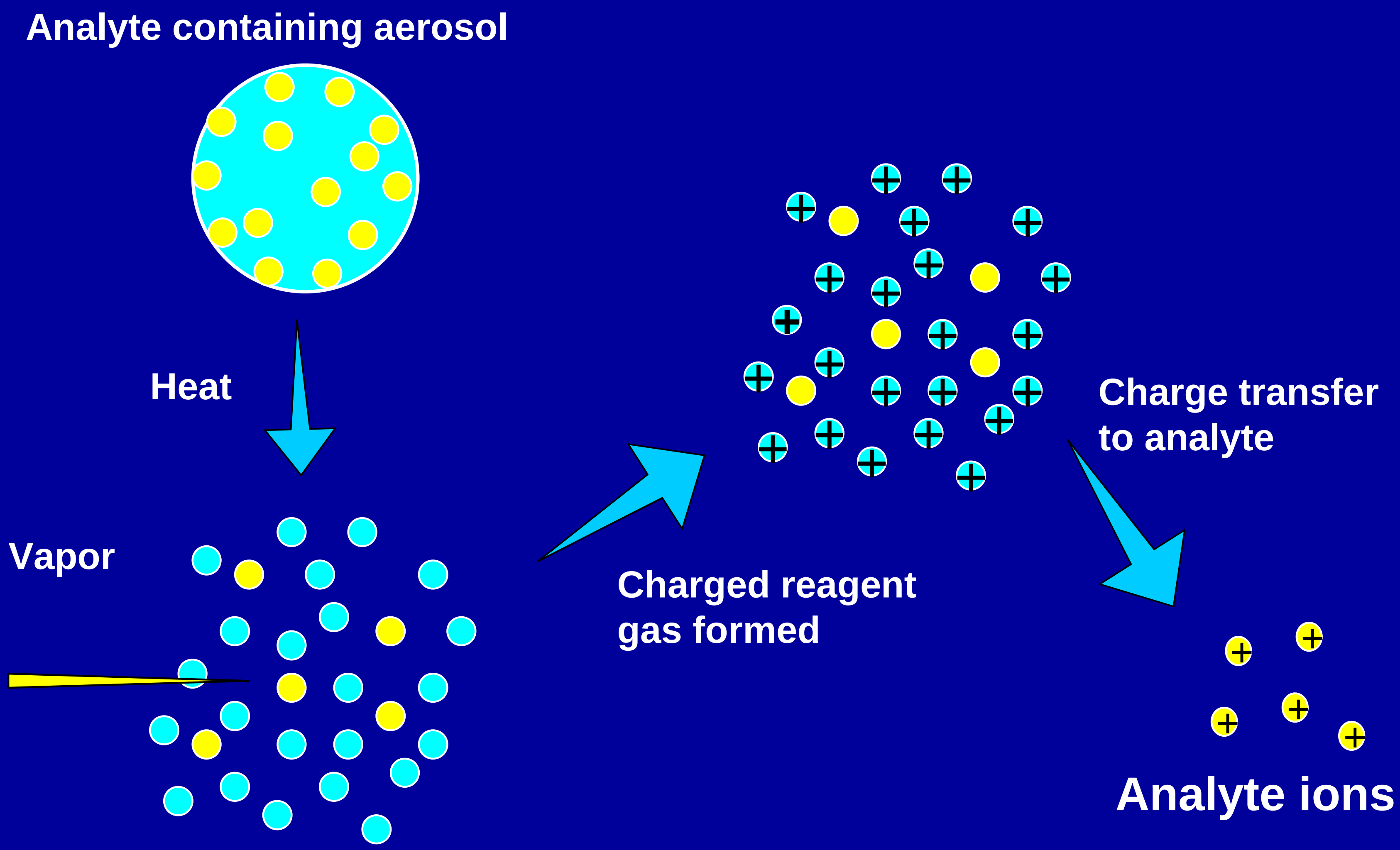
From: Scott

APCI Probe (Heated)



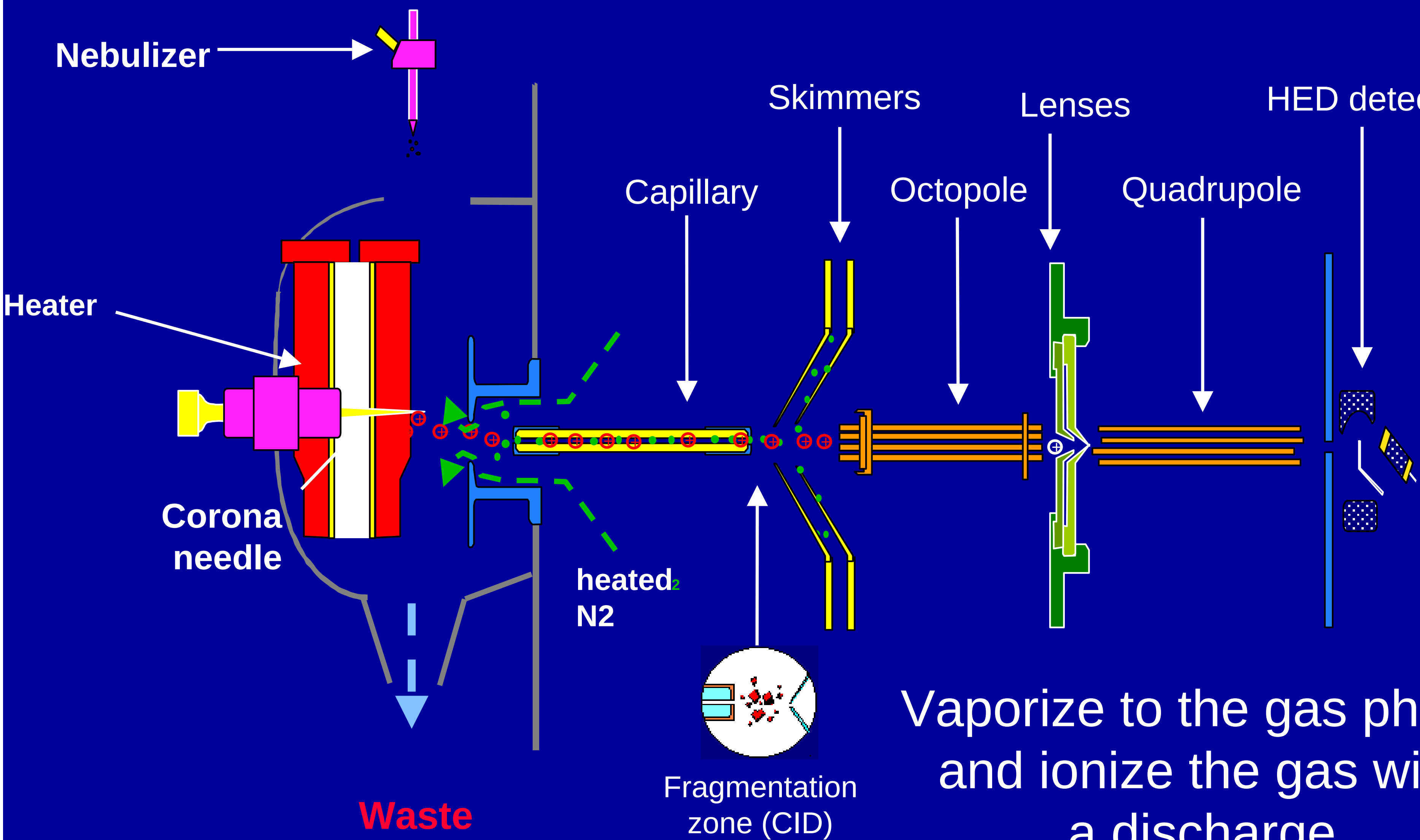
The vaporizer tube temperature is optimized for complete transfer to the vapor state

Theory of APCI



Courtesy of Agilent Technologies

Agilent 1100 LC/MSD - APCI

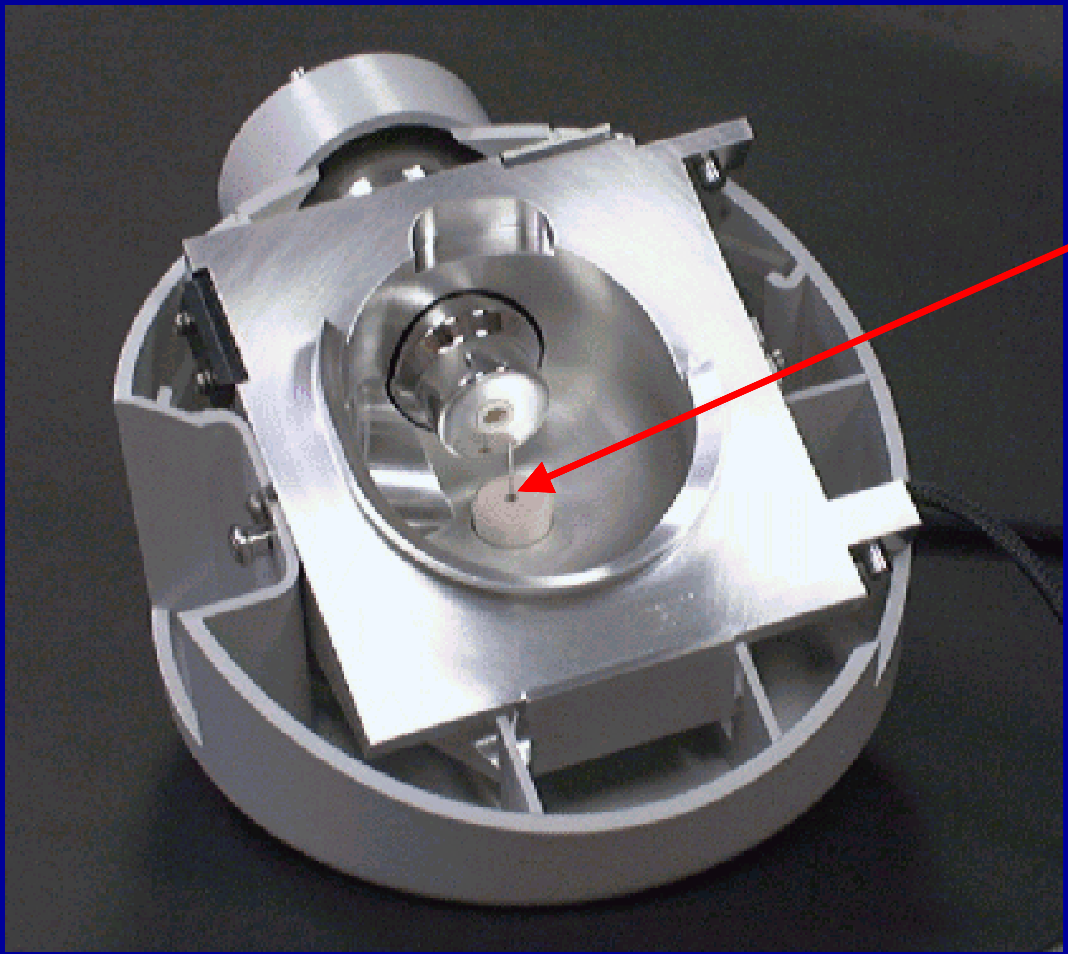


Vaporize to the gas phase and ionize the gas with a discharge

Courtesy of Agilent Technologies

Agilent 1100 LC/MSD

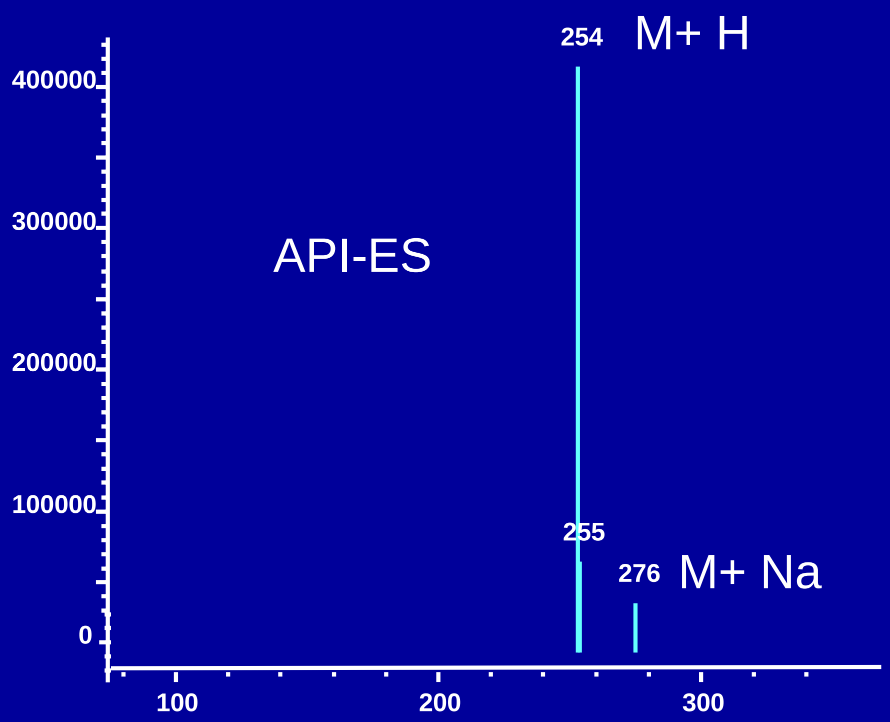
APCI Ion Source

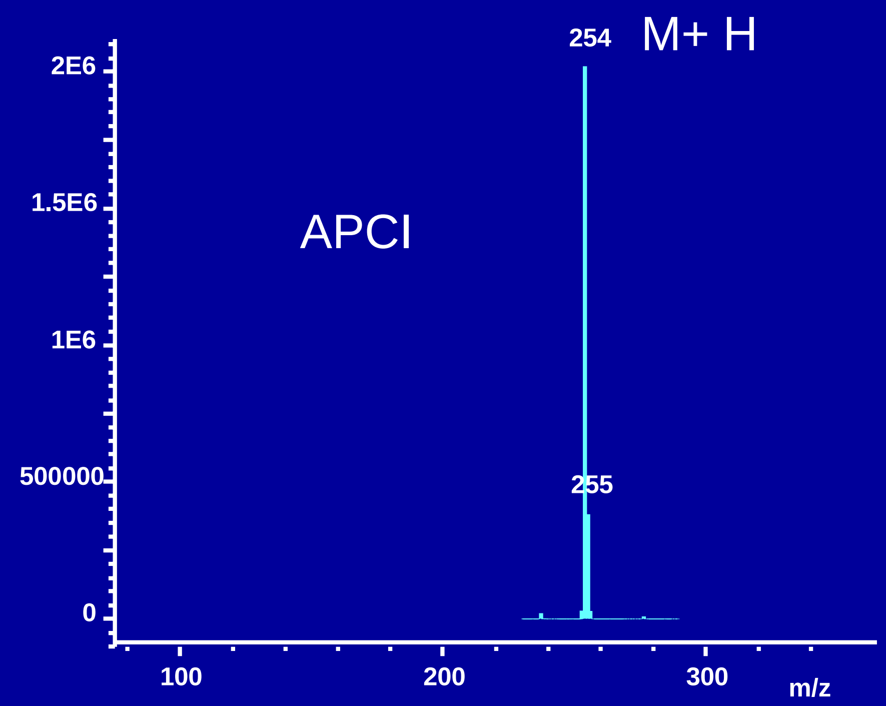


Vaporize into the gas phase and ionize the gas with a discharge

Courtesy of Agilent Technologies

API-ES vs. APCI for Triamterene





Comparison of the mass spectra typical of Electrospray vs. APCI

Optimizing Mobile Phases for API-MS

- Ion-Pair Agent Alternatives
  - Use highly bases-deactivated silica columns
  - Use low pH (3-4) to reduce tailing
  - Use columns that retain based on polar interactions (e.g. CN, IBD)
- Using Ion-Pair Reagents
  - Use low amounts (<0.02%)
  - Use post-column addition to negate the effect of the ion-pair agent

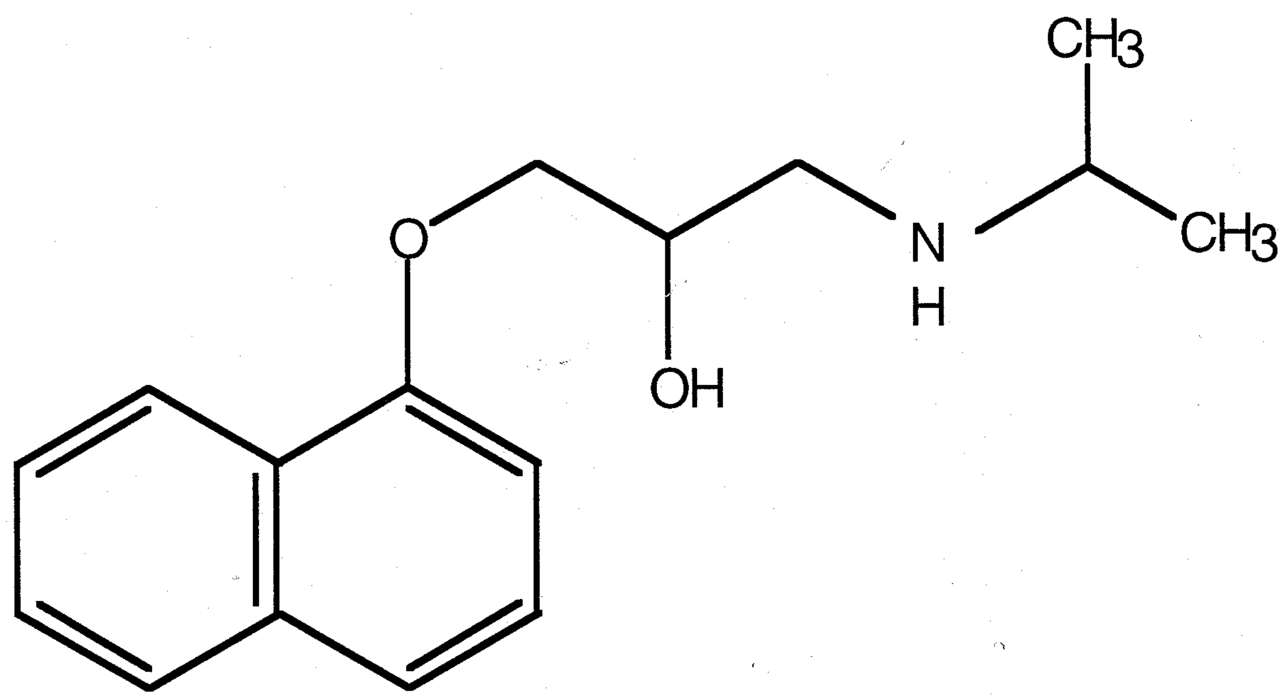
Sample Considerations for LC/MS

- The Analyte Must Have Ionizable Groups
  - Amines
  - Carboxylic Acids
  - Ketones, Aldehydes
- For Best Sensitivity, Work at a pH Where the Analyte is Ionized
  - Neutral to basic pH (7-9) for acids
  - Acidic pH (3-4) for bases

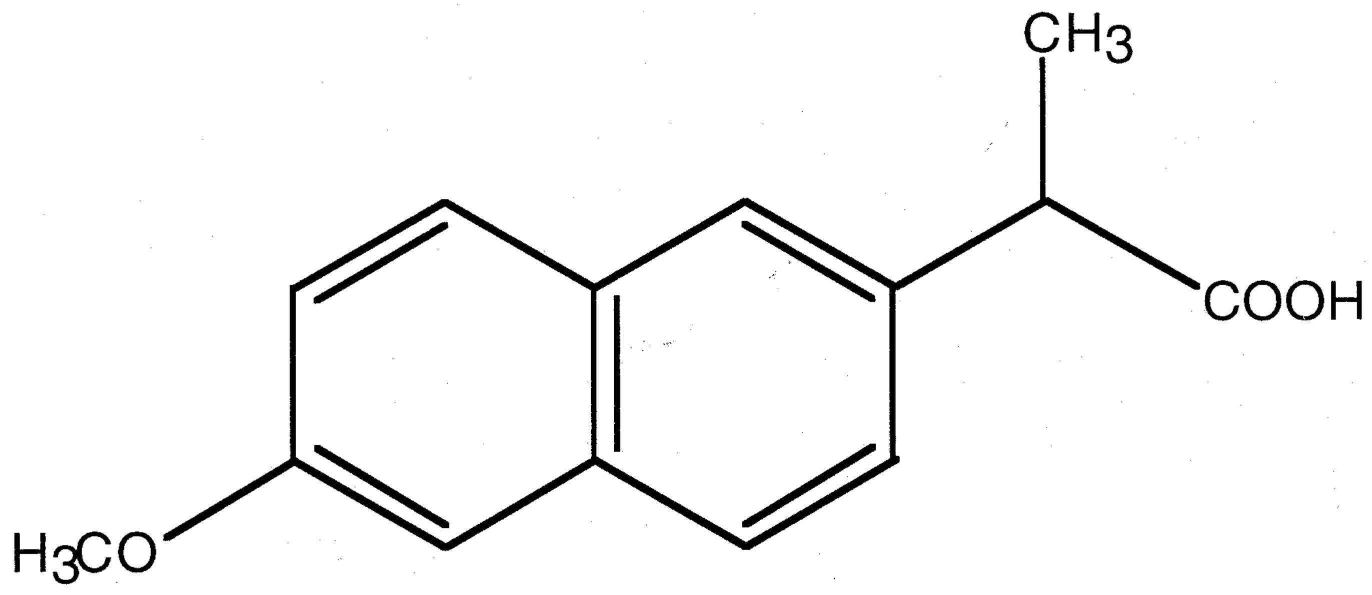
Sample Considerations

- Positive Ion Mode
  - Analyte = $(M+H)^+$
- Negative Ion Mode
  - Analyte = $(M-H)^-$

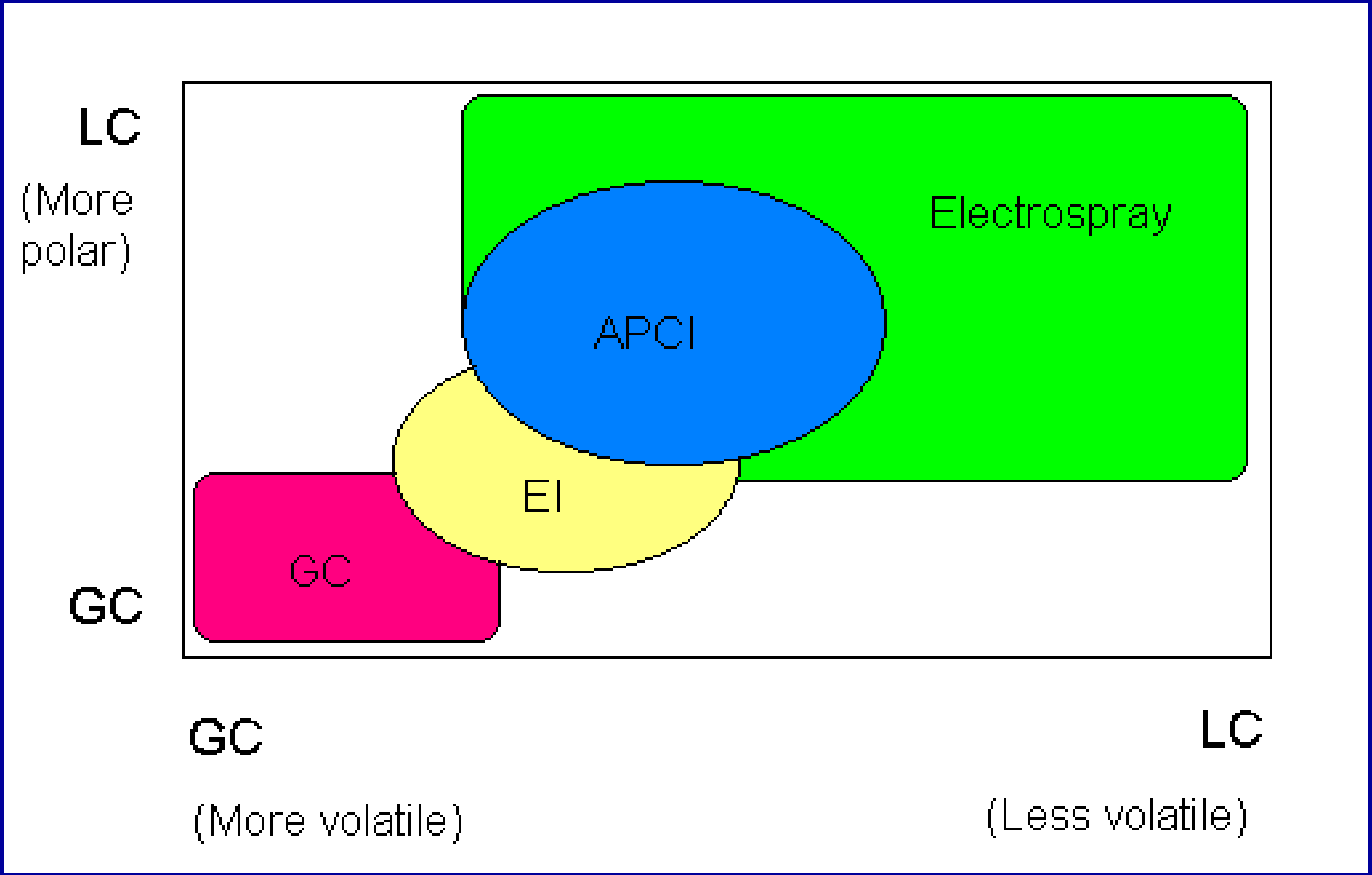
Basic Compound –
Sensitive in Positive Ion Mode



Acidic Compound – Sensitive in Negative Ion Mode



Selecting an Interface



“Advances in LC/MS”, Waters Corporation, Milford, MA.

Electron Ionization (EI)

- Analytes Suitable for EI
  - Small molecules with rings and double bonds
  - Compounds that would need derivatization for GC/MS
  - Pesticides, PAHs, natural products
- Compound Identifications
  - Fragmentation is possible
- Poor Detection Limits
- Will Not Tolerate Non-Volatile Buffers

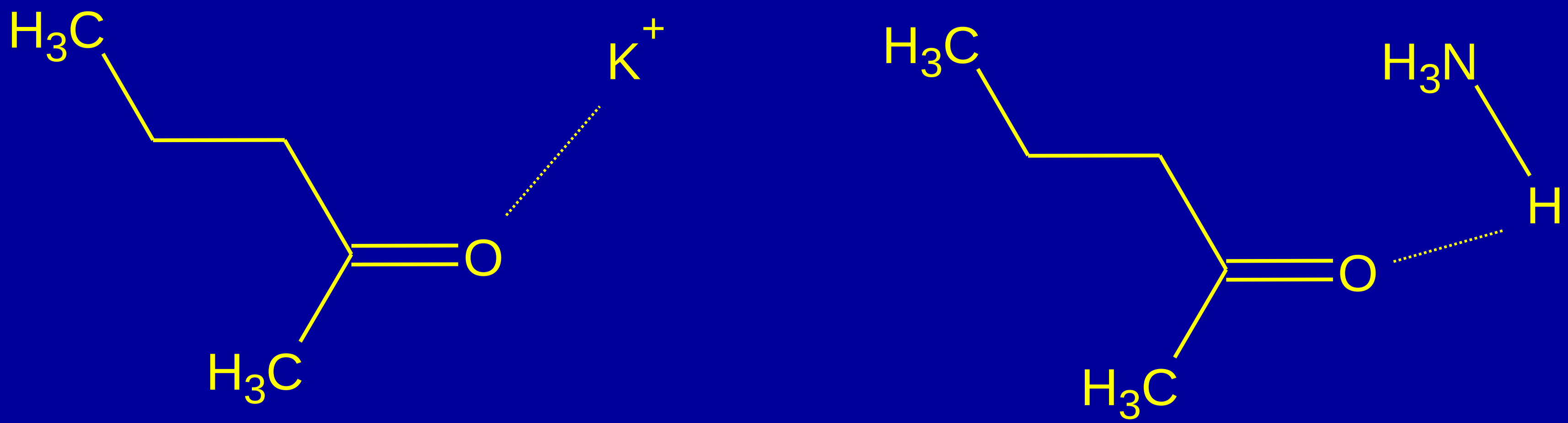
Comparison of API vs. EI

- Atmospheric Pressure Ionization (API)
  - MW confirmation
  - Good for fragile compounds
  - Able to fragment in the source
  - Low (ppb) LODs in SIM mode
- Electron Ionization (EI)
  - Ionization occurs in a vacuum
  - Standard libraries are available
  - Classical EI spectra (similar to GC/MS)
  - Higher LODs (ppm to high ppb)

Atmospheric Pressure Ionization (API)

- Electrospray Ionization (ESI) Uses Solution Phase Ionization
- Atmospheric Pressure Ionization (APCI) Uses Gas Phase Ionization
- Products are $[M+H]^+$ and $[M-H]^-$, adducts
- Suitable for Analyzing Drugs, Small Molecules, Dyes, Peptides
- Good Sensitivity
- Thermal Degradation is Possible (APCI)

Adduct Formation with API



Adducts can form between polar molecules and sample or solvent components. For example, adducts with Na+, K+, NH_4+, MeOH, MeCN, and H_2O are common.

Electrospray Ionization

- \+ Good molecular weight information, including high MW compounds
- \+ Can be used for volatiles, nonvolatiles, ionic/polar compounds
- \+ Good sensitivity
- – Need relatively low flow rates
- – Need to be able to form ions in solution
- – Limited structural information
- – Problems with high aqueous and buffer solutions

Atmospheric Pressure Chemical Ionization

- + Gives molecular weight information
- + Easy to use, rugged
- + Can use higher LC flow rates (up to 2 mL/min)
- – Thermal degradation can occur
- – Limited structural information
- – Not appropriate for higher MW (e.g., >1000 Da)

MALDI

- Matrix-assisted laser desorption/ionization
  - Sample is deposited on a target and co-crystallized with a solid matrix (dihydroxybenzoic acid)
  - Desorption/ionization occurs using a laser such as Nd-YAG (266nm, v^4)
  - Energy transferred to matrix, then analyte
  - Useful in excess of 200kDa (biomacromolecules)

Fast Atom Bombardment (FAB)

- Cf-FAB = Continuous Flow FAB
- Column eluent mixes with a matrix (glycerol)
- Eluent + matrix deposited on a target
- Analyte film hit with fast atoms or ions
- Only low flow rates used

Acknowledgements

Charlie Schmidt - Thermo Instruments

Agilent Technologies

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