

# **LC/MS: The Techniques of Electrospray, APCI and APPI**

*Understanding and Optimizing to Develop successful LC/MS Methods*

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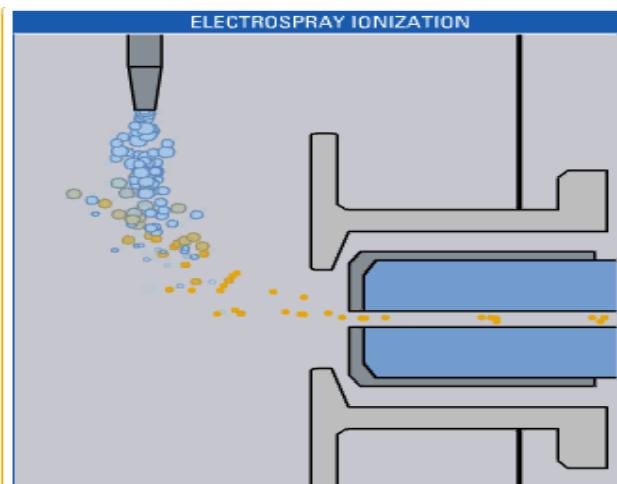
## **Topics that will be covered-general outline**

- Introduction to HPLC Separations and Mass Analyzers
- Understanding ESI, APCI and APPI ionization Mechanisms, Operation and Performance in real matrices
- Coupling ESI, APCI and APPI –MS to LC separations- optimization of conditions to produce the best sensitivity with minimal matrix effects
- Interpretation of Mass Spectra Generated by API-LC/MS/MS
- Quantitative LC/MS/MS
- New Techniques: Chip Based Systems and direct analysis approaches
- Problem Set

# Understanding API-MS Processes

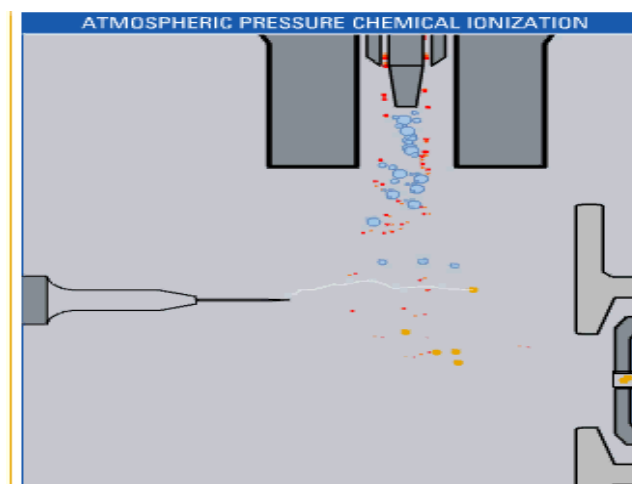
## ELECTROSPRAY:

Ionization process which uses electrical fields to generate charged droplets and subsequent analyte ions by ion evaporation for ms analysis.



## PNEUMATICALLY ASSISTED ELECTROSPRAY:

Same as electrospray (above) except the initial droplet formation is the result of pneumatic nebulization.

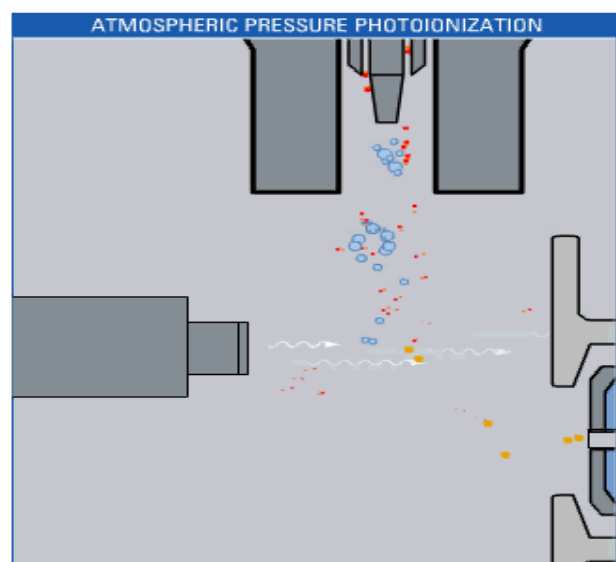


## APCI:

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

## Atmospheric Pressure Photoionization (APPI):

Krypton lamp producing ultraviolet light ionizes gas phase analytes or dopants with subsequent gas-phase reactions.



# Factors that Effect ESI APCI and APPI Sensitivity

Discussion on the effect various parameters have on ionization resulting in changes in sensitivity and specificity in API-LC/MS

- Ionization in solution (pH and charged complexes)
- Nebulization and Desolvation
- Solvent choice
- Additive Choice and Concentration
- Flowrate
- Adduct formation
- Ion suppression from matrix
- LC peak width and resolution

Applicability of the API techniques to parameters listed below:

	APPI	APCI	ESI	MALDI		APPI	APCI	ESI	MALDI
<b>Compound Class</b>					<b>Volatility / Thermal Stability</b>				
Proteins/peptides			Best	Good	non-volatile			Best	Good
Natural products	Chance	Good	Best	Chance	thermally unstable			Best	Good
Forensics	Chance	Good	Good	Chance	volatile and stable	Good	Good	Chance	
Pharmaceuticals	Chance	Good	Best	Chance	<b>LC separation</b>				
Environmental	Good	Good	Good		reverse phase	Chance	Good	Good	
Polymers			Chance	Best	normal phase	Good	Best	Good	
Carbohydrates			Good	Best	size exclusion	Chance	Chance	Best	
DNA			Good	Best	ion paired	Chance	Chance	Good	
Organic Chemistry	Chance	Good	Good	Chance	<b>Flowrate</b>				
Biochemistry	Chance	Chance	Good	Good	1 ml/min	Good	Good	Chance	
<b>Functional Groups</b>					0.1 - 0.4 ml/min	Good	Good	Good	
Acid/Basic	Chance	Chance	Best	Good	5 - 20 ul/min	Chance	Chance	Best	Chance
Alcohols/Carbonyls	Good	Good	Chance	Chance	less than 0.1 ul			Best	Good
PAHs	Best	Chance							

Best Chance of Success  
 Good Chance of Success  
 Chance of Success  
 Not Applicable

# Tricks and tips for API LC/MS/MS

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**Helpful hints and examples that highlight ways to improve ionization, compound specificity, sample throughput and ways to address the analysis of difficult compounds for LC/MS.**

## **Topics covered include:**

- Post column addition
- Post column splits
- Optimization of API source parameters
- Tips on achieving better LC separations and better resolution
- The addition of cation or anion species to improve sensitivity or MS/MS specificity
- Reducing the formation of adduct ions
- The effect of retention time on API-LC/MS/MS performance.
- Key MS/MS parameters that need optimization.
- Negative vs. positive ion detection
- Other modes of chromatography (HILIC, CE size exclusion, normal phase, ion exchange)

# Interpretation of CID Mass Spectra generated by LC/MS/MS

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- **Variability of Spectra**

The fragmentation patterns of CID spectra are affected by several variables. The most significant of these are type and pressure of collision gas, energy of the ions, instrument configuration, and charge state. There is no standard set of conditions under which all CID experiments are performed and as a result the CID spectra generated on any given compound will be different. Manual interpretation of CID spectra appears to be the only choice for identifying unknowns.

- **Fragmentation Mechanisms**

CID fragmentation occurs through unimolecular decomposition of the internally excited even-electron ion. The basic energetic considerations that are used for odd-electron interpretations (i.e. from EI) still apply to even-electron interpretation; **the stability of the product ion formed, stability of the leaving group and bond liability are important driving forces.**

**Keep in mind that the decomposition pathways overwhelmingly favor formation of another even-electron ion and even electron leaving group.**

Radical losses can be observed in resonance stabilized conjugated systems (aromatic compounds) when the energy to break a resonance system exceeds the energy to form a radical loss

**There are several fragmentation mechanisms common to even-electron ions:**

- Single bond cleavage with charge migration
- Cyclization with charge migration
- Multiple cleavages
  - with charge migration
  - with charge retention
  - ring fragmentations
- Rearrangements
  - hydrogen rearrangements
- Homolytic cleavages
  - charge site remote fragmentation

# Understanding Quantitative LC/MS/MS- Factors to consider in developing an assay

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- **Sample Preparation**

- Clean up sample
- Concentrate sample
- Final solution in weak LC solvent (on-column concentration)

- **LC Separation**

- Sharp peaks (maximize peak concentration, 1-3  $\mu\text{m}$  particles)
- Fast (short columns)
- Minimize additives that cause ion suppression

- **API Source**

- Acid/base chemistry
- Optimize capillary exit/cone/orifice voltage
- All ion current in one  $m/z$  ion vs. adducts
- +/- ion detection
- Post column addition
- Optimize all lenses for ions of interest
- Divide chromatogram into segments

- **MS Analyzer**

- Tandem MS
- Optimize product ion formation
- High resolution
- SIM or MRM
- Optimize dwell times to get sufficient points across peak
- Divide MS acquisition into segments