



Purdue-UAB Botanicals Center for Age-Related Disease

Getting ready to do mass spectrometry

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**Purdue-UAB Botanical Center Workshop 2002
Mass Spectrometry Methods in Botanicals Research**



Objectives

- **What is electrospray ionization (ESI)?**
- **What is heated nebulizer-atmospheric pressure chemical ionization (HN-APCI)?**
- **Conditions for extraction and analysis by mass spectrometry**



What is mass spectrometry?

- Carried out on charged ions in the gas phase
- These ions move in an electric or magnetic field according to their mass-to-charge ratio (m/z)
- To ensure that the motion of the ions is unaffected by collisions with molecules in air, the analysis is carried out in a hard vacuum (better than 10^{-6} Torr)



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The challenge

How to transfer biological molecules from the liquid or solid phase to the gas phase without damaging them?

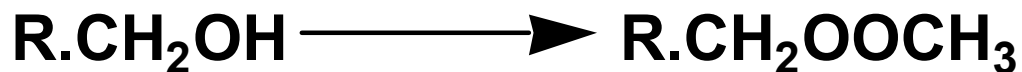
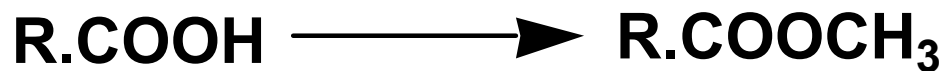


Evaporating molecules

- Forces that control volatility are molecular weight and polarity (difficult to break up a charged or hydrogen bond network)
- For gas chromatography, the solution was to derivatize the polar groups
- Not suitable for delicate or large molecules



Typical derivatives for GC





Solutions for liquid-to-gas phase transfer of complex, underivatized biomolecules

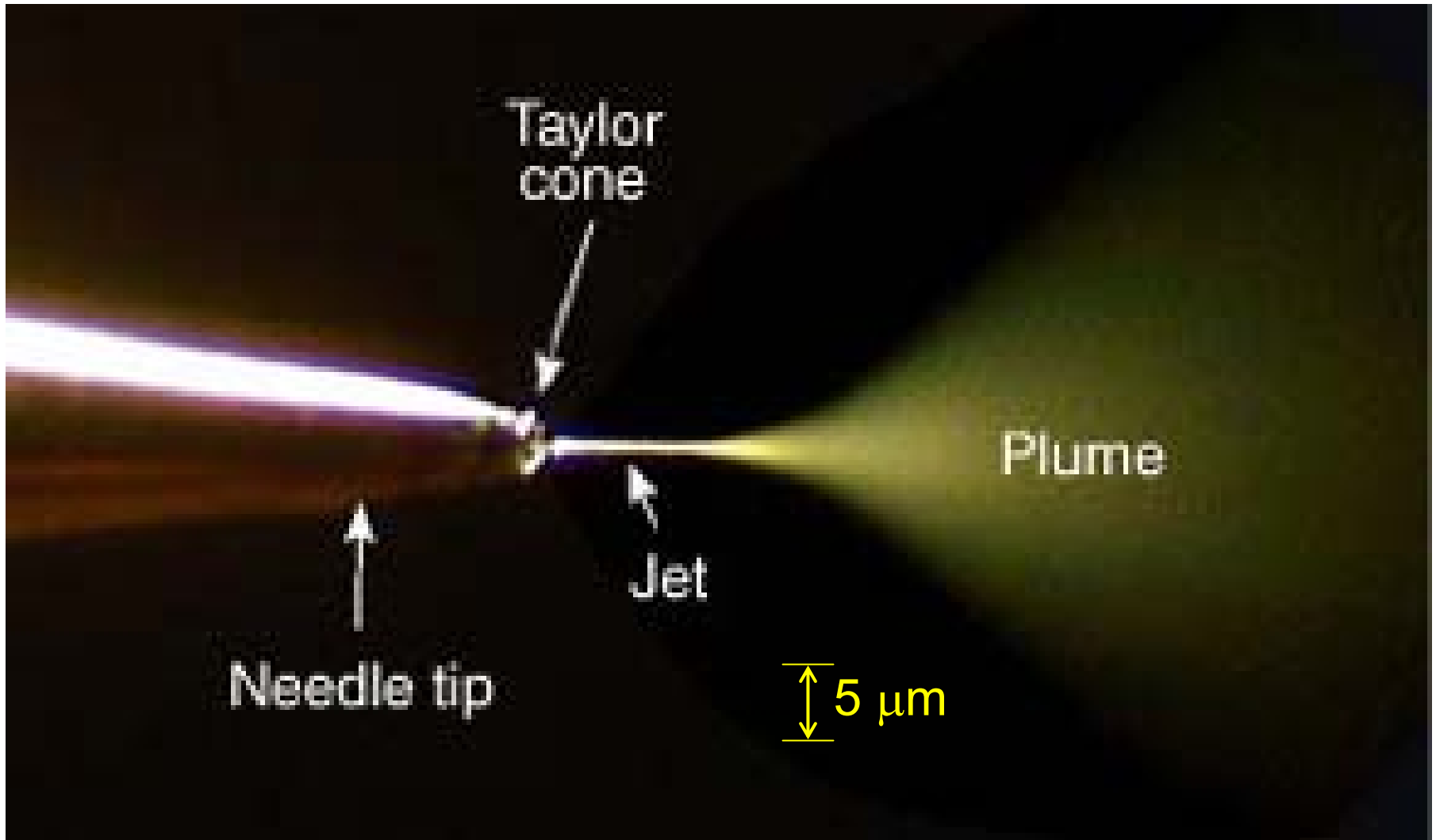
- **Thermospray (not in use any more)**
- **Electrospray ionization (Fenn)**
- **Heated nebulizer atmospheric pressure chemical ionization**



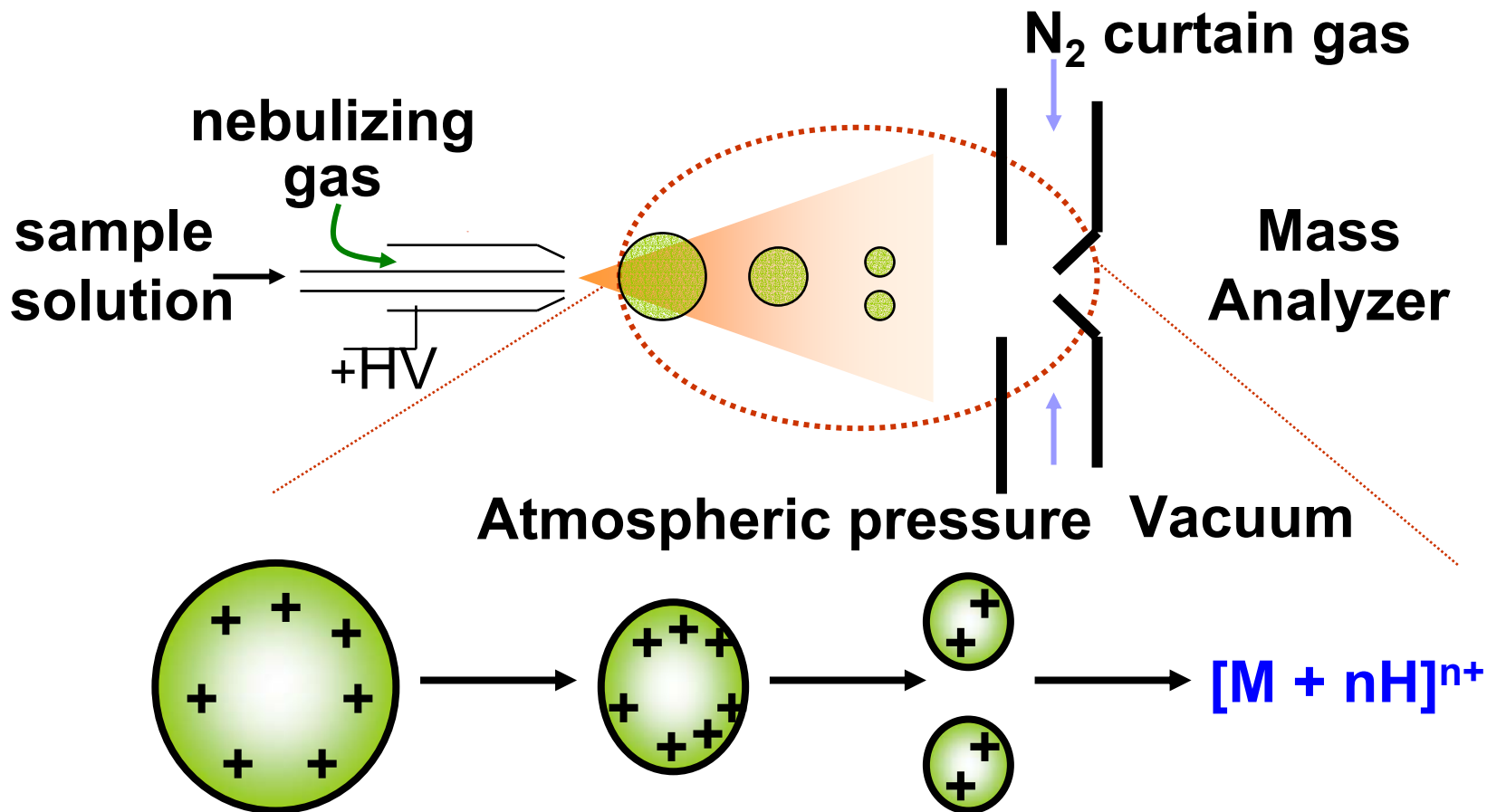
Electrospray ionization

- **Creation of a spray of finely divided, charged droplets**
- **Rapid evaporation at atmospheric pressure, resulting in the droplets remaining cold**
- **Coulombic repulsion of droplets in a jet and then formation of a Taylor cone where solutes are ejected into the gas phase**

NanoElectrospray



Electrospray Ionization (ESI)



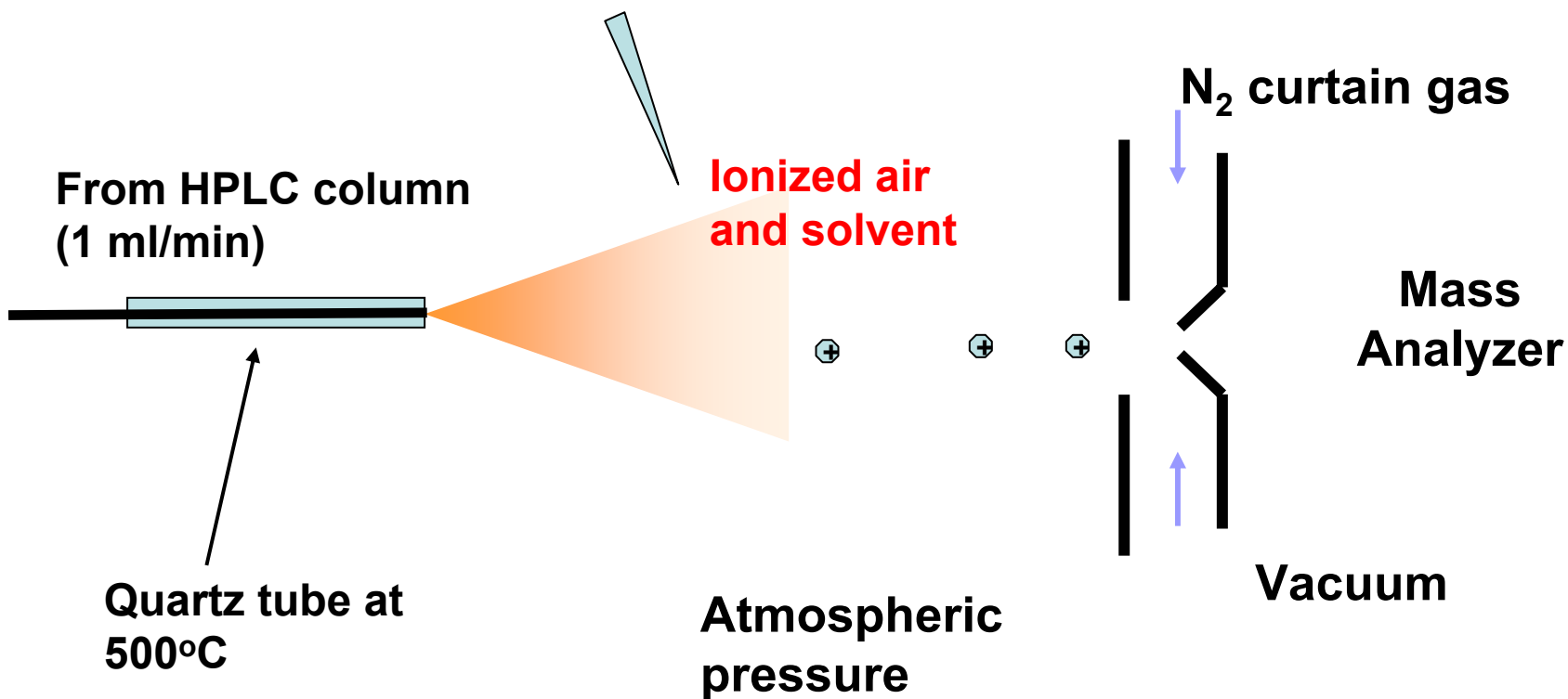
1. Solvent evaporation
2. Coulombic repulsion



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HN-APCI interface

Corona discharge



Use of HN-APCI-MS

- **This method can accommodate mobile phase flow rates up to 1 ml/min, even when it is mostly water**
- **Useful for compounds that are not naturally ionizable**
- **Some problems with compounds that are thermally labile**
- **Possible advantages with electron-capturing derivatives**

The Kudzu as a supplement



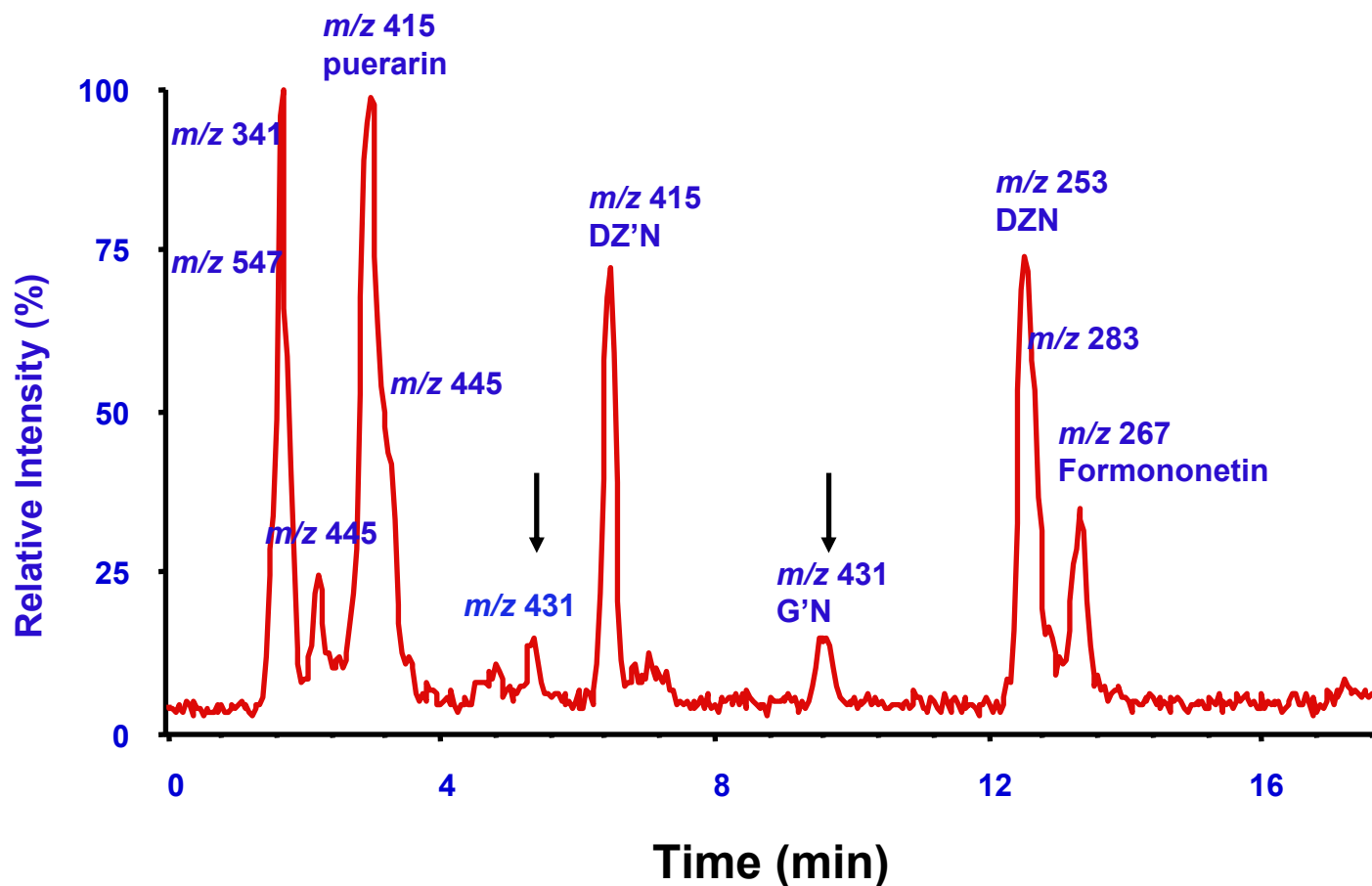
Buried in kudzu



The purple flower of *Pueraria lobata*

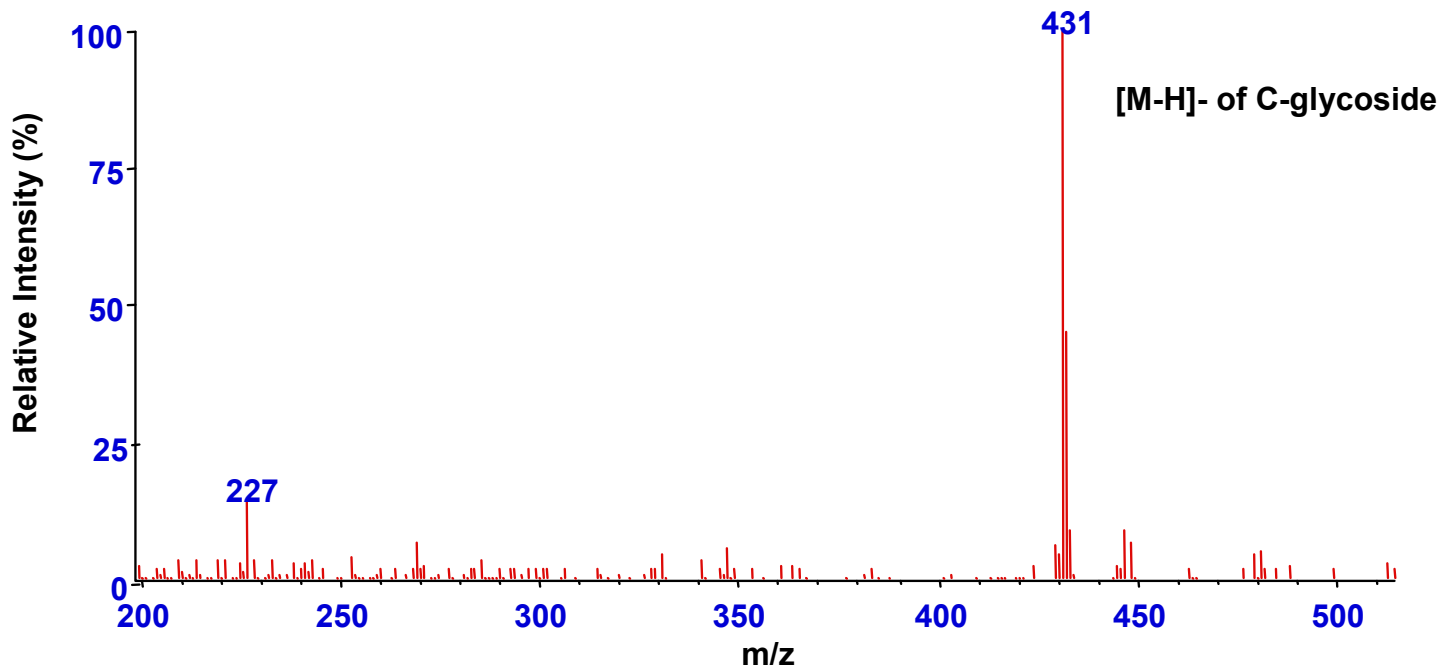


Linking of LC analysis to ESI-MS



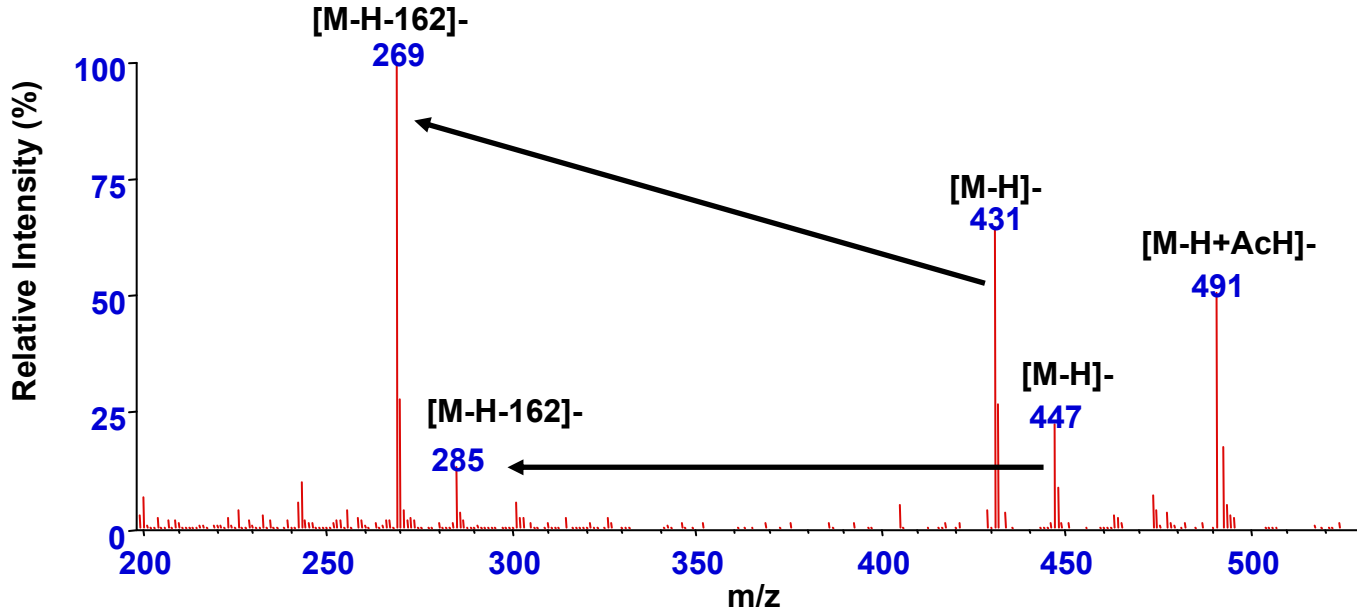
Ionization of polyphenols

Unconjugated or C-glycosides of polyphenols form simple molecular ions, $[M+H]^+$ or $[M-H]^-$, by gaining or losing a proton



Ionization of polyphenols

Conjugated polyphenols may also form molecular ions - however, depending on the energy given to the ion prior to analysis, the molecular ion may largely decompose to give the parent polyphenol ion, as well as other fragment ions





Extracting compounds for MS analysis - what can it tolerate?

In ESI-MS, only the solutes being studied should be ionized and preferably be in one charge state

- Na⁺, K⁺ and other metal ions are a problem**
- Cannot use polyionic buffers for LC mobile phase, i.e., no phosphate**



Sample extraction- polyphenolics

MeOH or EtOH (50-100%) in water (for glycoside, glucuronide conjugates) - extraction



Ethyl acetate or diethyl ether (for aglucones) - solvent partition



Solid-phase cartridges - can require organic counter ion for hydrophilics





HPLC mobile phases for ESI-MS

- **Solvents for reverse-phase LC**
 - MeCN, MeOH, EtOH, n-Propanol, iso-propanol
- **Aqueous phase**
 - 0.1- 0.5% acetic or formic acid
 - 2 - 25 mM ammonium acetate or formate