Basics of mass spectrometry

Stephen Barnes, PhD

Department of Pharmacology & Toxicology and Mass Spectrometry Shared Facility, UAB

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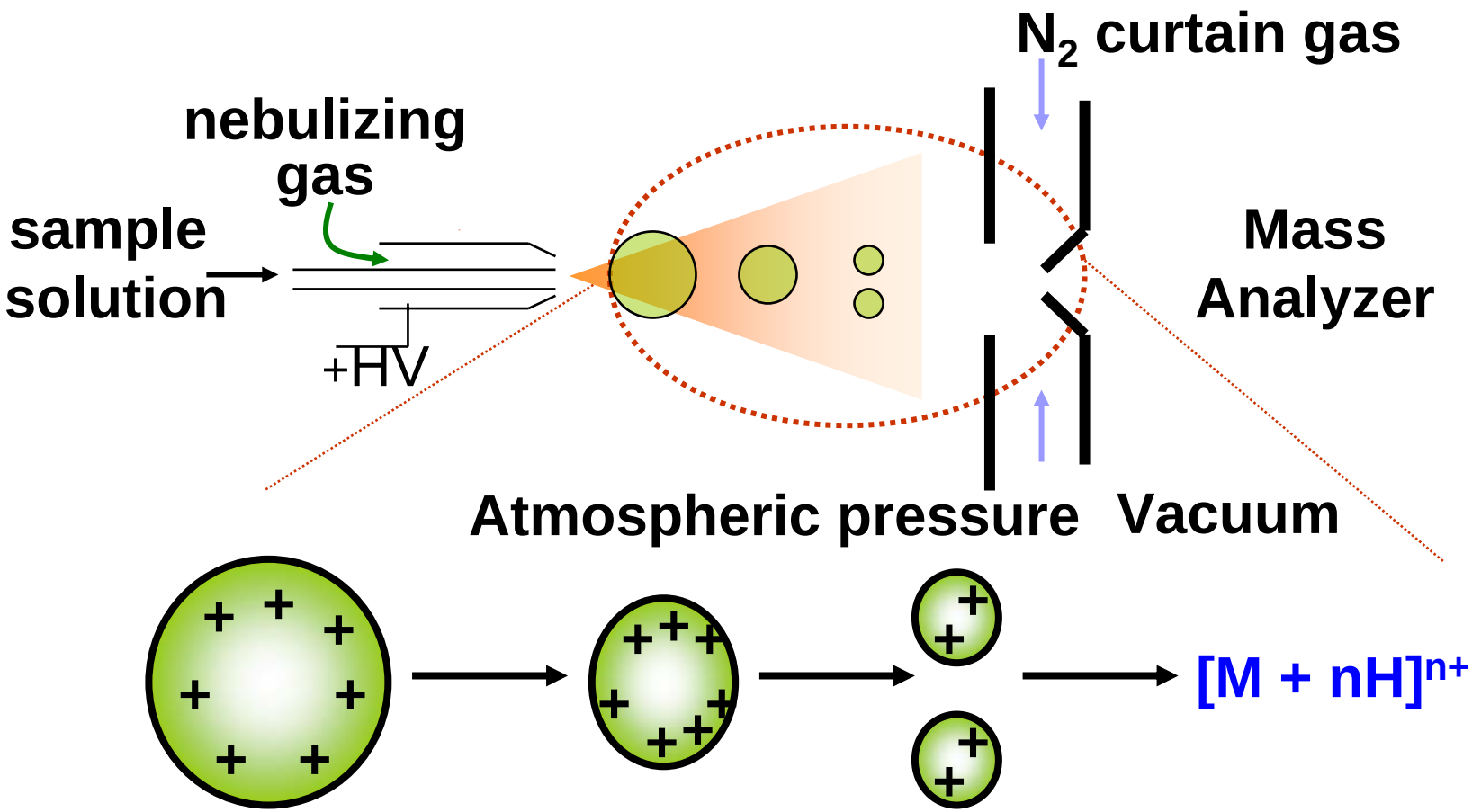
Synopsis

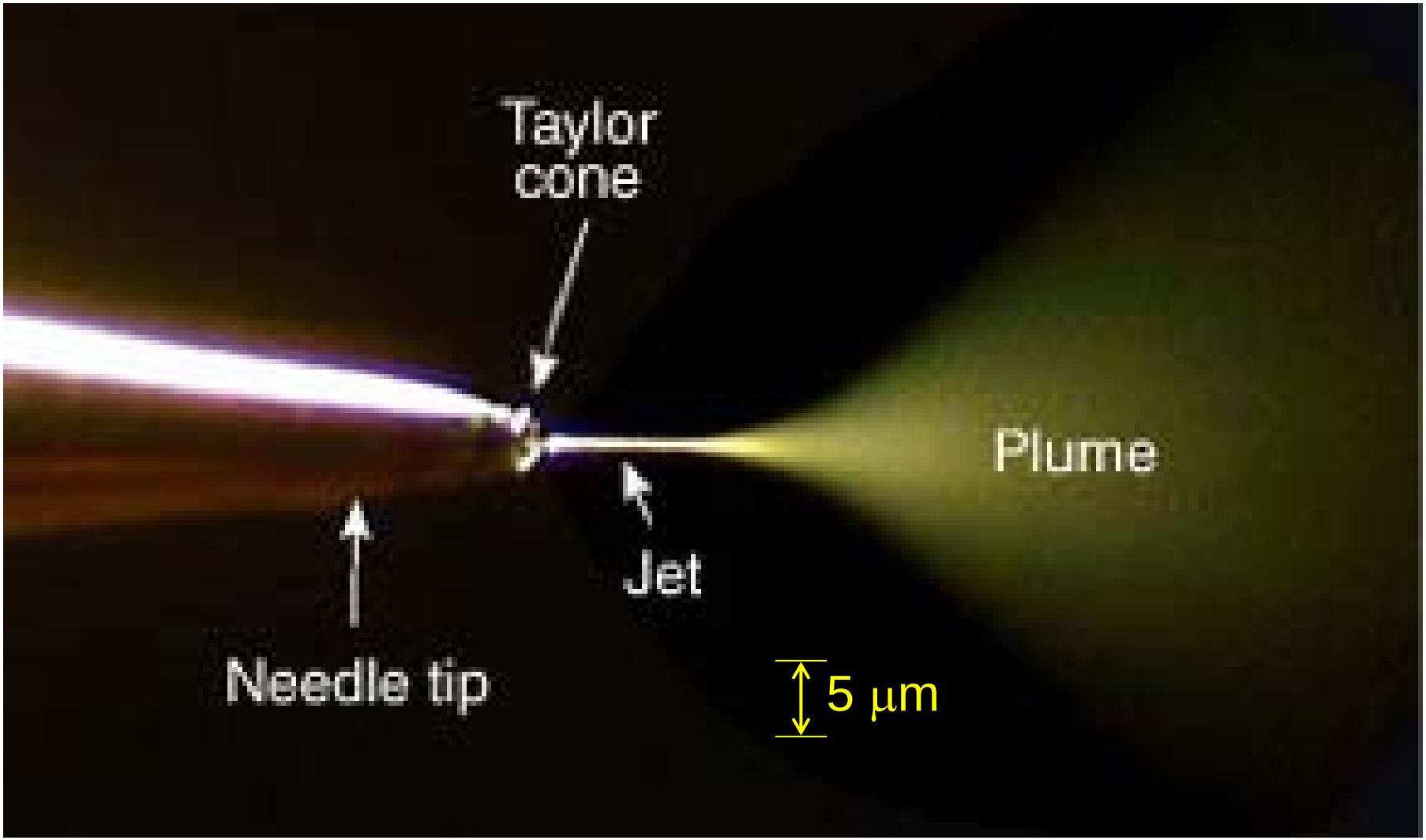
- **Transferring analytes from the liquid phase to the gas phase following LC analysis without damaging them**
  - **Electrospray ionization**
  - **Atmospheric pressure chemical ionization**
  - **Negative and positive ionization**
- **Tandem mass spectrometry**
  - **Daughter, parent and MRM**
- **Ion traps**

Electrospray Ionization (ESI)



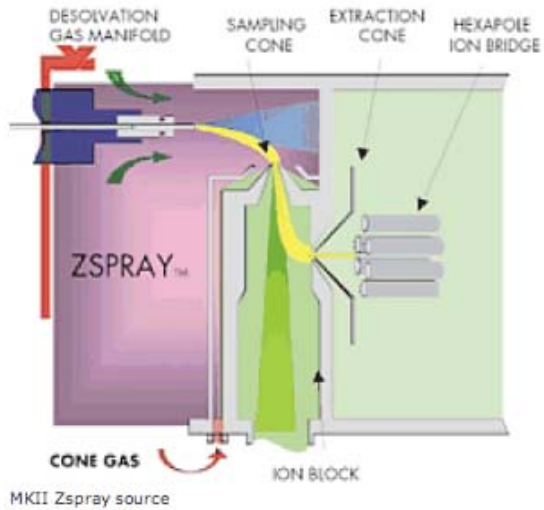
1. Solvent evaporation
2. Coulombic repulsion

NanoElectrospray



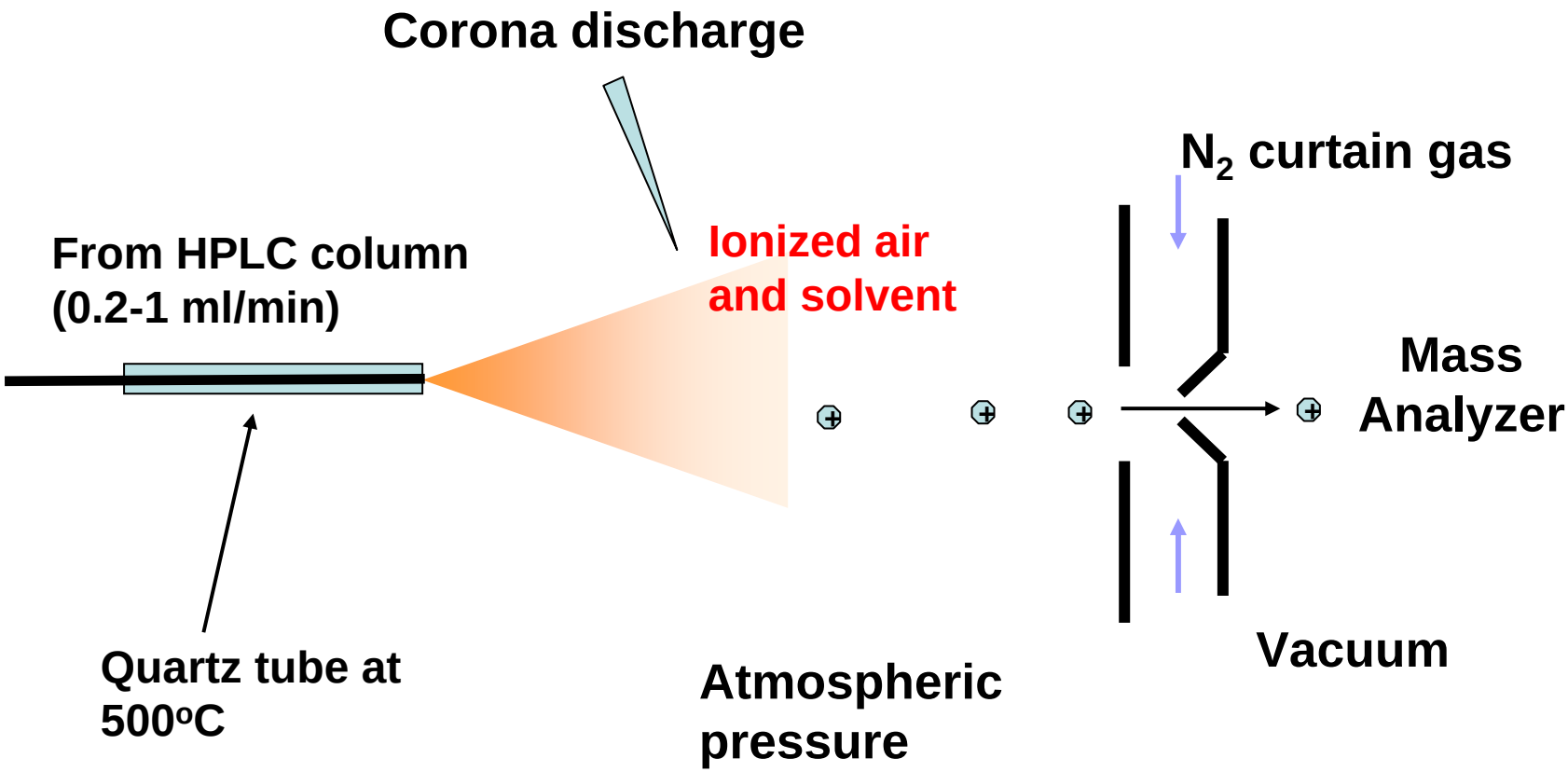
Improvements in electrospray ionization

- **The use of a Z-shaped interface to decrease noise**

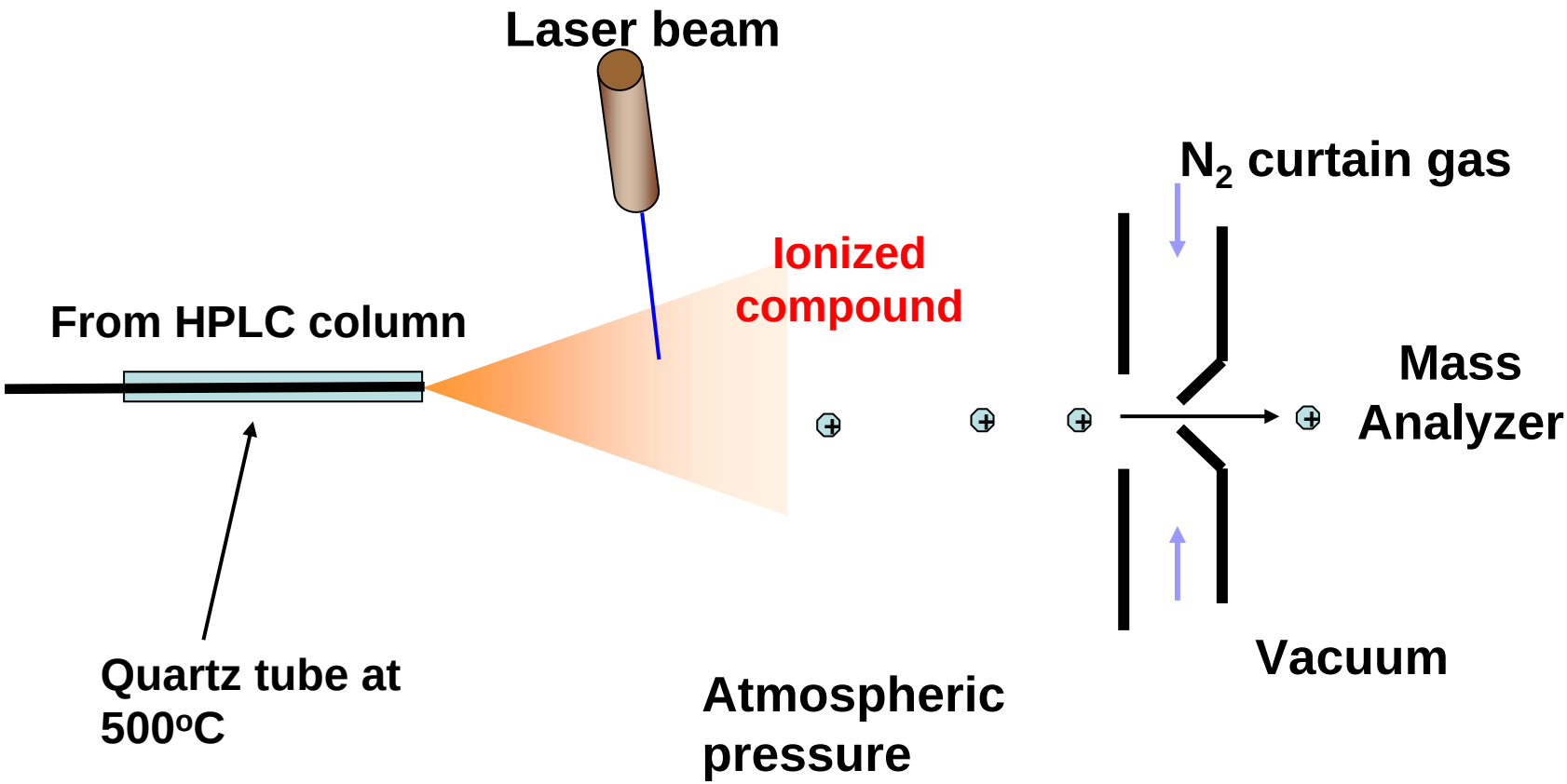


- **The use of directed heat on the spray to allow the use of more aqueous solutions and higher flow rates - Turbo-ionspray**

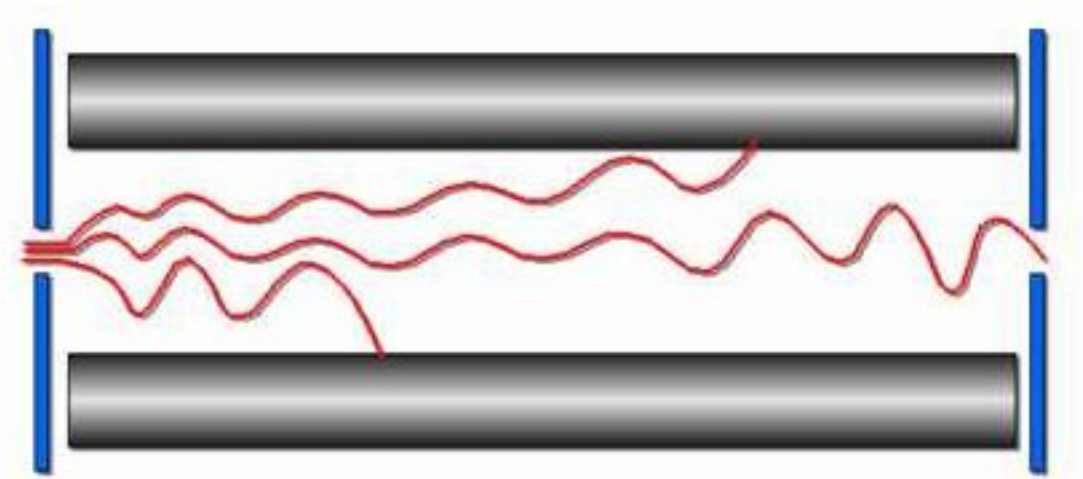
HN-APCI interface

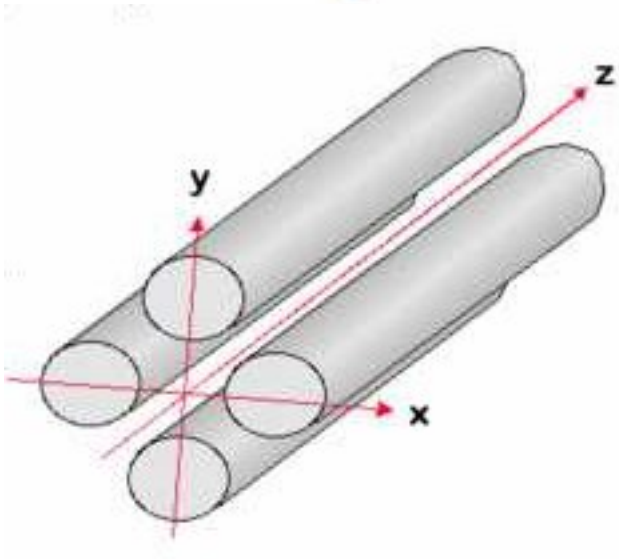


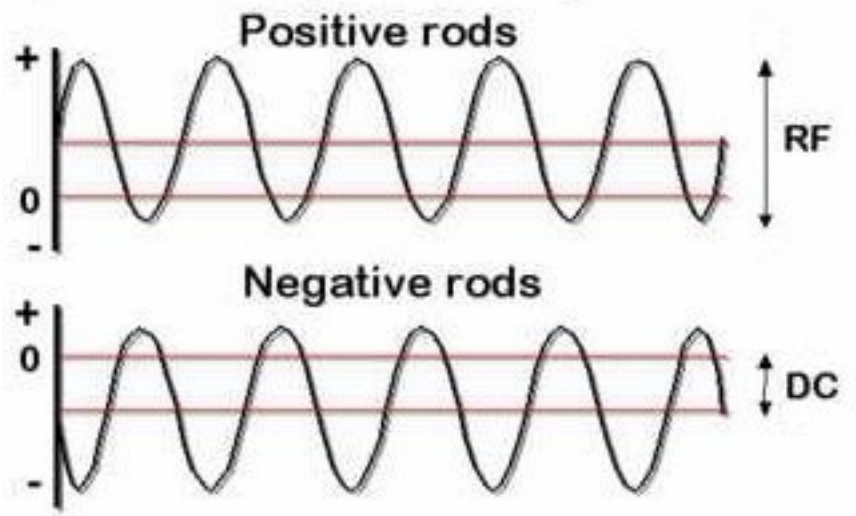
HN-APPI interface



Elements of a quadrupole analyzer

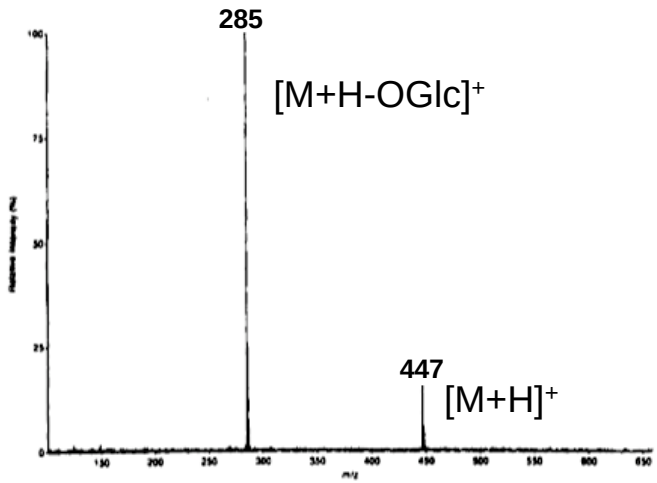






The mass spectrum

- **The mass spectrum is obtained by changing the potential and RF on the rods of the quadrupole - this is calibrated with known compounds to establish the mass-to-charge (*m/z*) ratio and is the x-axis.**
- **The y-axis is represented by the intensity of ions reaching the detector at a given *m/z* value**
- **The spectrum can consist of positive $[M+H]^+$ or negative $[M-H]^-$ molecular ions**

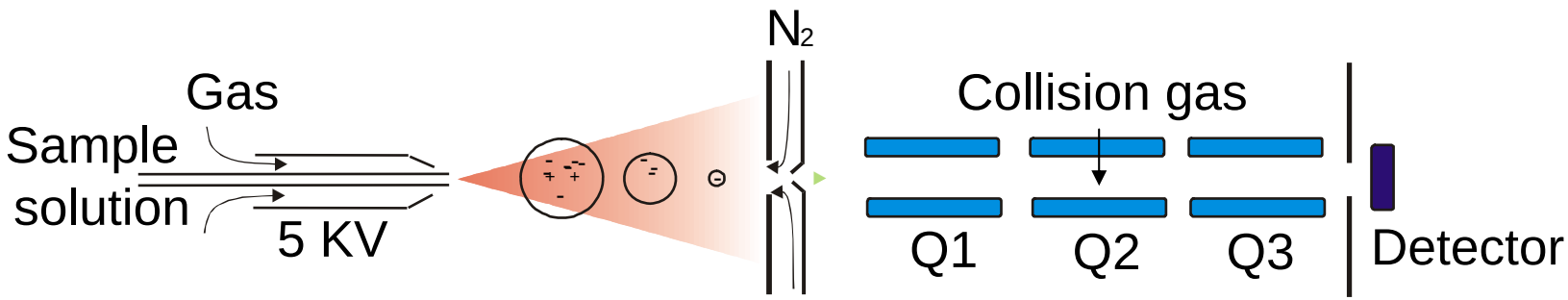


The *m/z* 285 ion results from collision events in the interface

Ions observed in mass spectrum

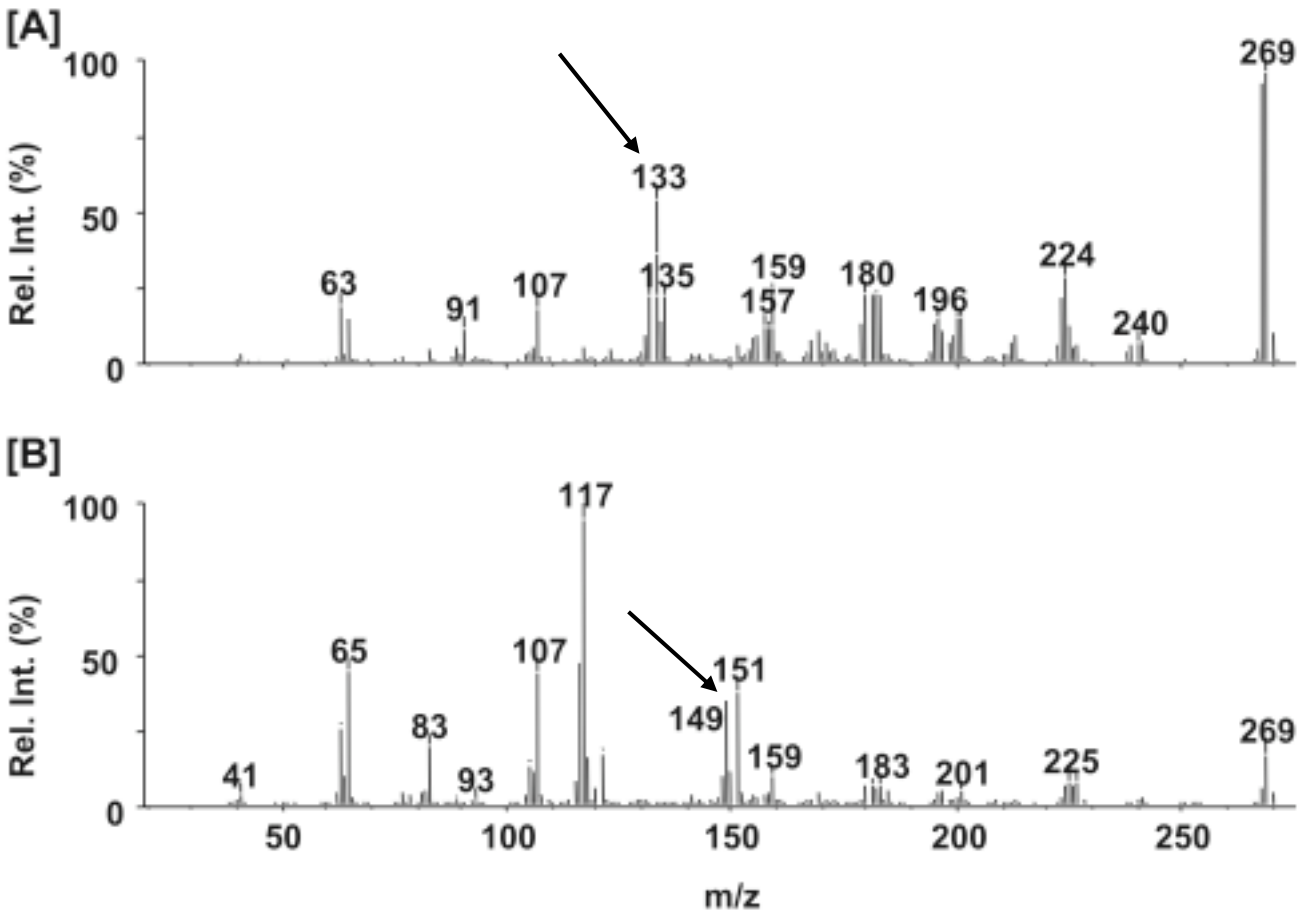
- Nominally should be $[M+H]^+$ or $[M-H]^-$
- Loss of easily cleavable group (i.e, -162 for O-Glc) can occur in the interface before full vacuum can be achieved
- Ions in the solvent can form clusters with the analyte that do not dissociate
  - Negative mode $[M-H+Cl]^-$
  - Positive mode $[M+H+Na]^+$
- Avoid Na salts and phosphate in sample and running solvent
- TFA causes ion inhibition - use HCOOH

Tandem mass spectrometry on a triple quadrupole instrument

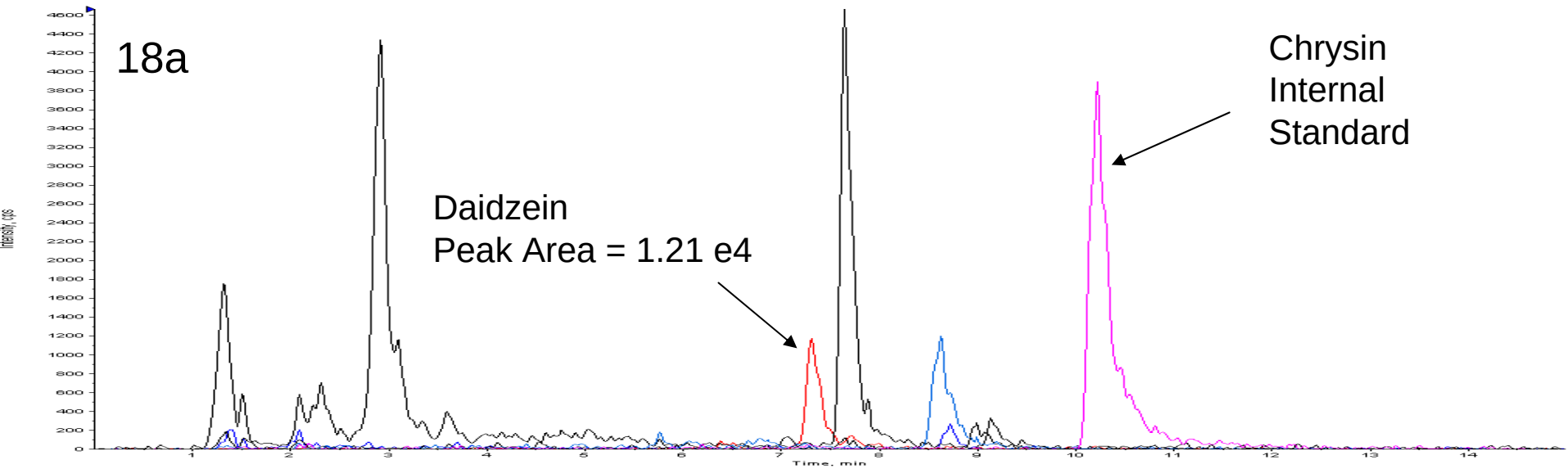


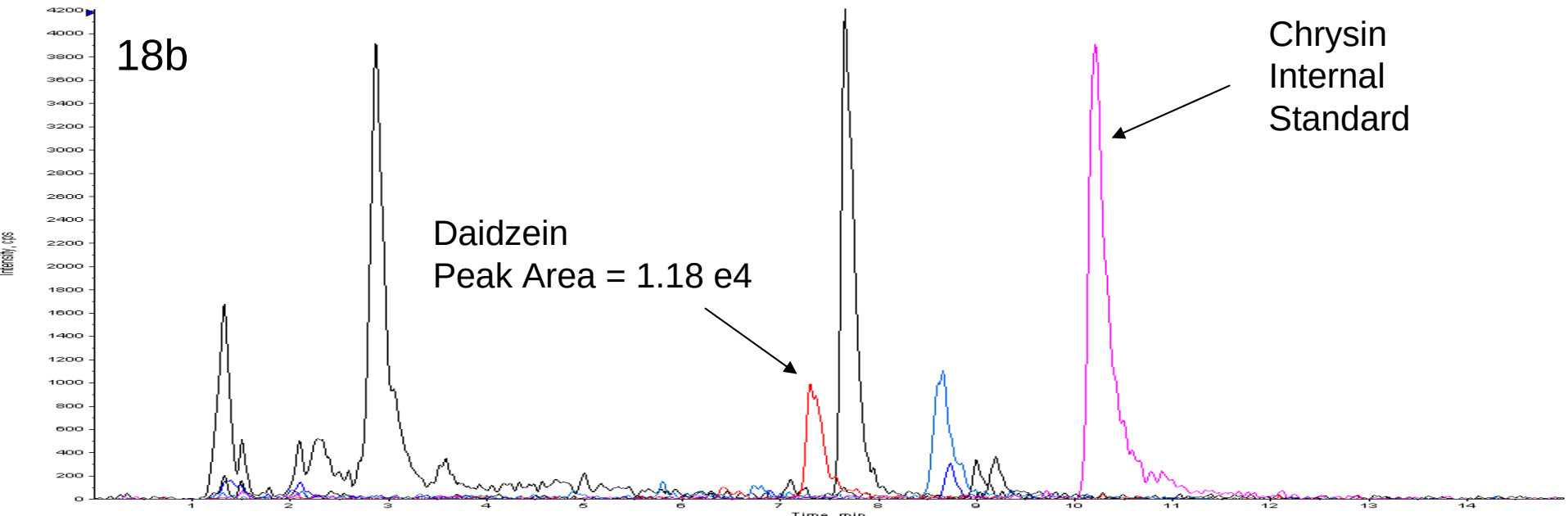
- **Daughter ion spectra - fragments of a parent ion**
- **Parent ion spectra - parents giving rise to a common fragment ion**
- **Multiple reaction ion scanning - ion intensity after selecting for a particular parent ion/daughter ion combination**

Daughter ion tandem mass spectra of genistein [A] and apigenin [B]



LC-MRM analysis of urinary isoflavones on triple quadrupole - individual isoflavone “channels” are color-coded





Ion Traps

The ion trap is an energy well - ions with sufficient energy to enter the trap are retained by an energy barrier on the exit side of the trap. The advantage of the ion trap is that it accumulates selected ions prior to their analysis giving it high initial sensitivity (detection limit of approx. 20 fmol). Ions are fragmented by collision with helium gas and their daughter ions analyzed within the trap. Selected daughter ions can undergo further fragmentation, thus allowing MS^n. This is important for structural experiments. The ion trap has a high efficiency of transfer of fragment ions to the next stage of fragmentation (unlike the triple quadrupole instrument).

Ion traps at UAB

AbI-Sciex API 4000Qtrap

LTQ

FT-ICR

Hybrid instrument

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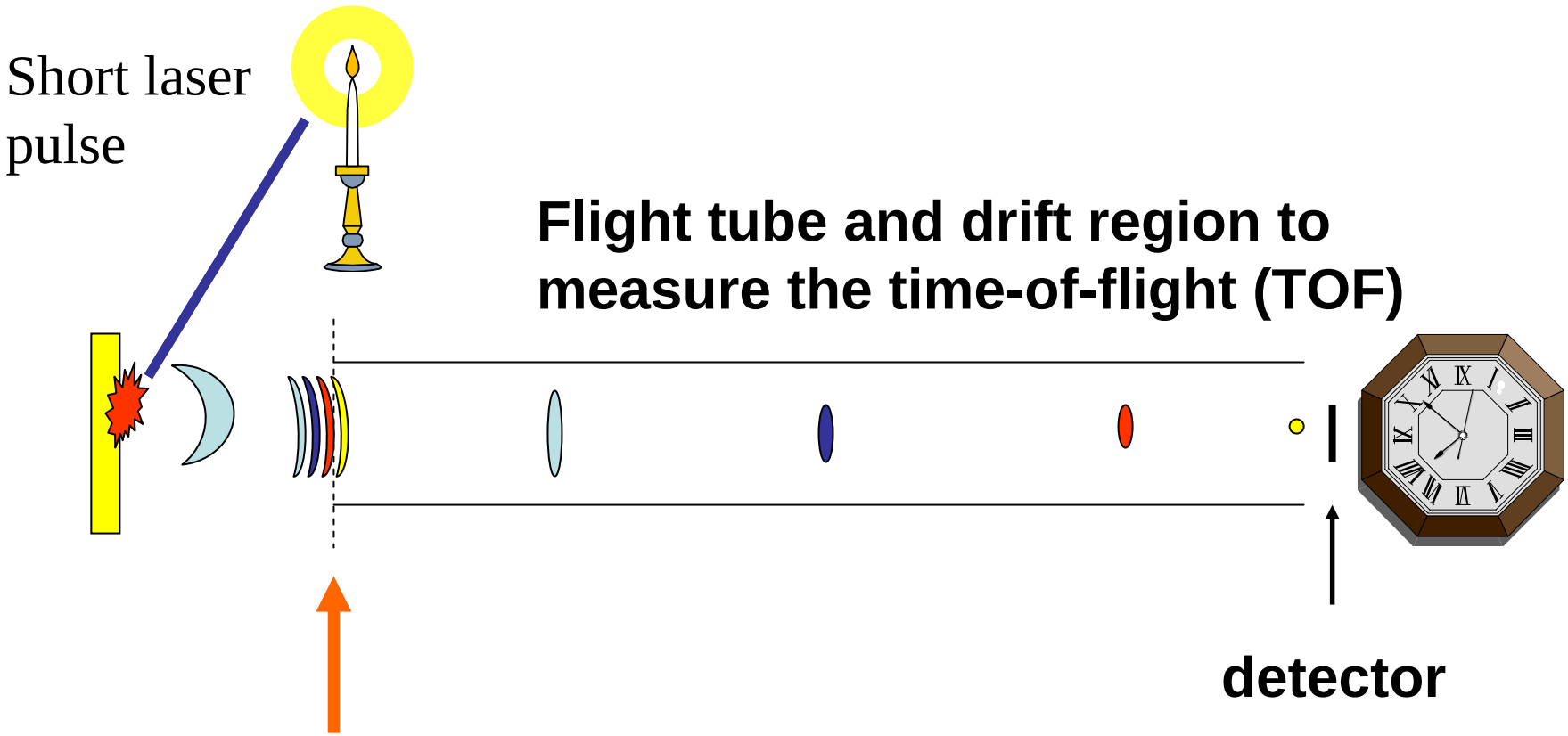
Matrix-Assisted Laser Desorption Ionization (MALDI)



Bibliography

- Fenn, J.B. Electrospray ionization mass spectrometry. How it all began. *Journal of Biomolecular Techniques*, 13: 101-118, 2002.

- Wang, C-C., Prasain, J., and Barnes, S. Methods used in the analysis of phytoestrogens*. Journal of Chromatography*, 777: 3-28, 2002.

- Prasain, J., Wang, C-C., and Barnes, S. Mass spectrometric methods for the determination of flavonoids in biological samples. *Free Radical Biology and Medicine*, 37: 1324-1350, 2004