MASS SPECTROMETRY (MS)

Alternative names (connection with GC, LC, CZE):

Mass spectrometric (selective) detector (MSD)

Spectrometry

- a method based on the interaction of radiation and matter

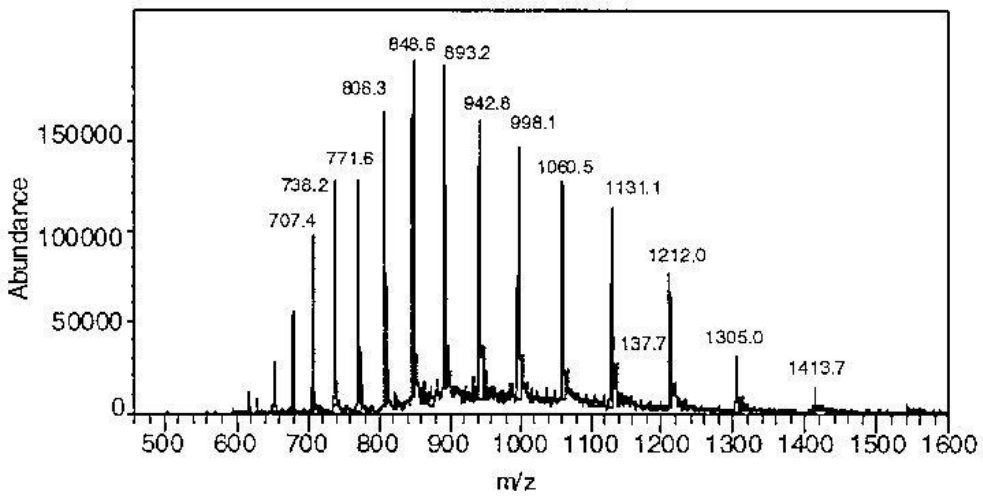
Mass spectrometry - a method based on the formation of ions at the interaction of electric field and analytes, which are then analyzed (separated, filtered) according to the *m/z* value using electric/magnetic fields

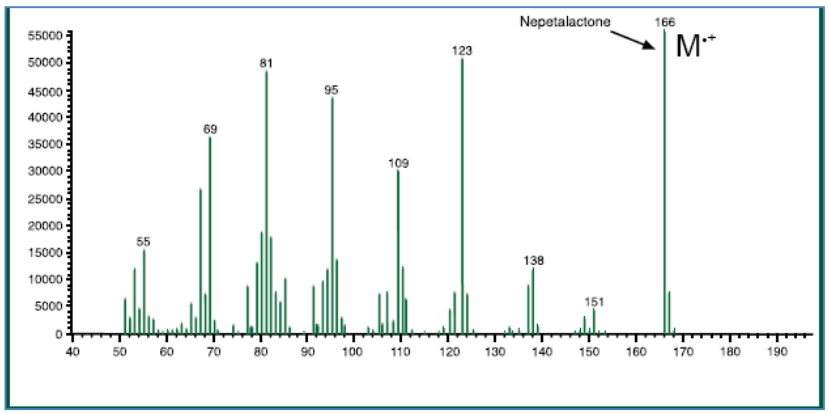
MS principle and measurement outputs

No.	MS part	Process	Parameters
1.	Ion source	Ionization → ion formation	Ion type: [+] or [-] Single/multiple charge M; fragments; adducts
2.	Mass analyzer (Mass filter)	Mass analysis (ion filtration) according to *m/z* value	Range, accuracy, resolution, speed
3.	Ion detector	Ion abundance measurement	Sensitivity, lifetime
4.	PC with software	Mass spectrum record/analysis	Automatic algorithms, spectral library

Mass spectrum: formats

Graphic – profile or centroid





Tabular

m/z	Intensity / Abundance	Relative intensity (%)
57	560	3.72
58	800	5.31
59	550	3.65
125	2532	16.81
126	2935	19.48
127	2580	17.13
128	2100	13.94
134	5785	38.40
135	15065	100.00
136	6256	41.53
137	10258	68.09
138	4618	30.65

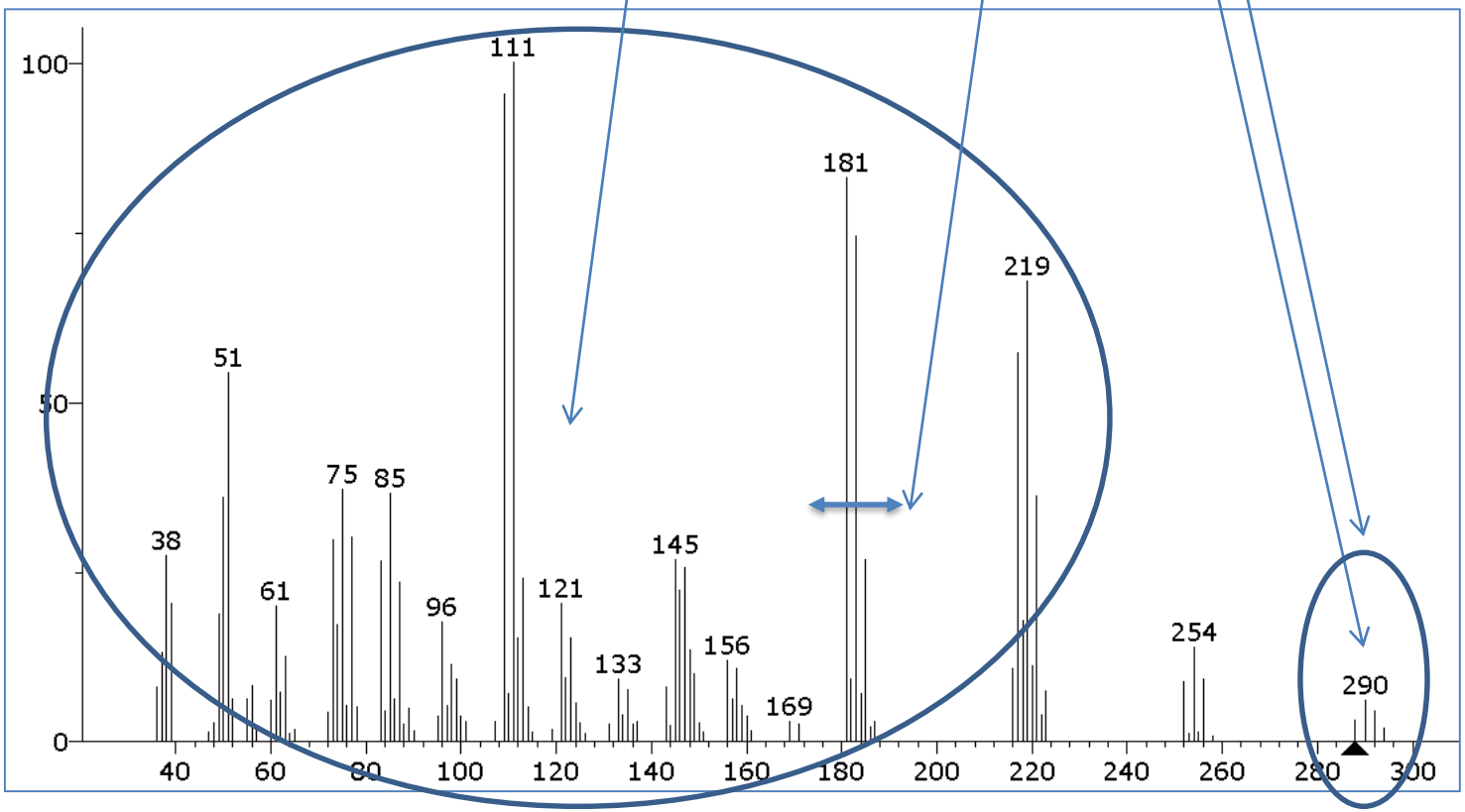
Mass spectrum - contained information

Molecular weight - (pseudo) molecular ion

Elemental composition - exact mass, isotope cluster

Charge number - distance of ions in cluster

Structure – fragmentation



MS application options

Identification of compounds

Molecular weight / elemental composition / summary formula

- almost absolutely at the sufficiently high resolution

Structure identification

- depending on the ionization type
- possibility of the fragmentation

Identification by spectra comparison

- identification of compounds based on the formation and ratio of ion abundances with certain *m/z* values
- application of spectral libraries

Quantification of compounds

Universal

- LOD varies according to the ability of compounds to provide under given conditions ions

Historical development of MS - summary

From a cathode ray tube to a modern mass spectrometer

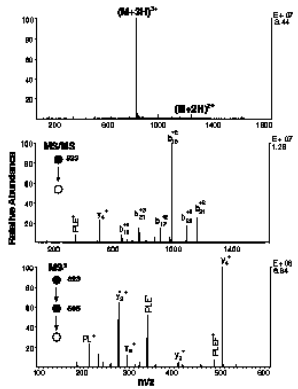
Combination of ion mobility and MS
***2006**

J.B. Fenn (1984)

LC-MS(ESI)
***1984**

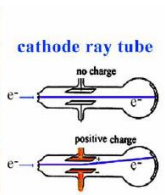
GC-MS
***1956**

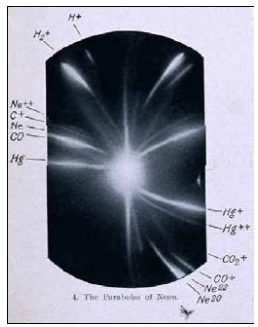
TOF-MS
***1946**



J.J. Thomson (1899)

1899 - 1920





F.W. Aston (1920)

Development of spectra recording

Formation and types of ions

- **formation by various mechanisms - type of mechanism fundamentally affects the resulting spectrum**

- **positively or negatively charged ions are formed**
  (always only one type at a time - sometimes switchable)
  - POSITIVE and NEGATIVE ionization is distinguished

- **ions formed according to the original structure:**
  a) molecular: $[M]^{+\cdot}$ or $[M]^{-}$
  b) pseudomolecular and adduct: $[M+H]^{+}$, $[M-H]^{-}$, $M+CH_4]^{+}$
  c) fragment: e.g. $[M-CH_3]^{+\cdot}$

Electron Ionization (EI)

Principle: electron is lost from electroneutral molecule

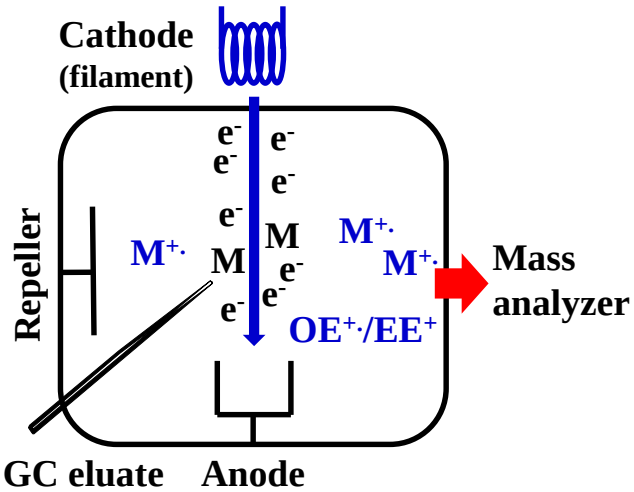
A) 'Impact' of electrons (Electron Impact - EI) ***or another theory*** **→**

B) by releasing of the valence electron captured by the electron stream

- ⊕ ions are formed:

$M + e^- (70\ eV) \rightarrow M^{+\cdot} + 2e^-$

→ a radical cation with odd number of electrons is formed



- in case of subsequent fragmentation there are two possible mechanisms:

$M^{+\cdot} \rightarrow OE^{+\cdot} + N$ ***or*** $M^{+\cdot} \rightarrow EE^+ + R^\cdot$

→ a fragment radical cation with an odd number of electrons and a neutral loss *or* a fragment cation with an even number of electrons and a radical is formed

Electron Ionization (EI)

Characterization:

'hard' ionization ⇒ spectrum rich in fragment ions
⇒ molecular ions - sometimes are not stable
(can be very low or not present in spectrum)

Application of EI can be used for determination of:

- **molecular weight / elementary composition / summary formula**
  - **based on the exact mass**
  - **based on isotope cluster of molecular ion**
- **partially/completely identify the structure based on fragmentation**
- **to perform identification using spectral library**
  - **contains EI spectra measured at standard ionization energy 70 eV**
- **charge of ions with multiple charges**
  - **based on differences in *m/z* values of cluster ions**

Application: GC-MS

Chemical ionization (CI)

Principle: ionization by reaction gas (CH_4)

- reagent gas (ratio to analytes approx. 10^4 : 1) undergoes to EI ionization and its ionized forms then transfer charge to analytes

- ⊕ ions formation (Positive CI - PCI):

$CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2e^-$

$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3\cdot$

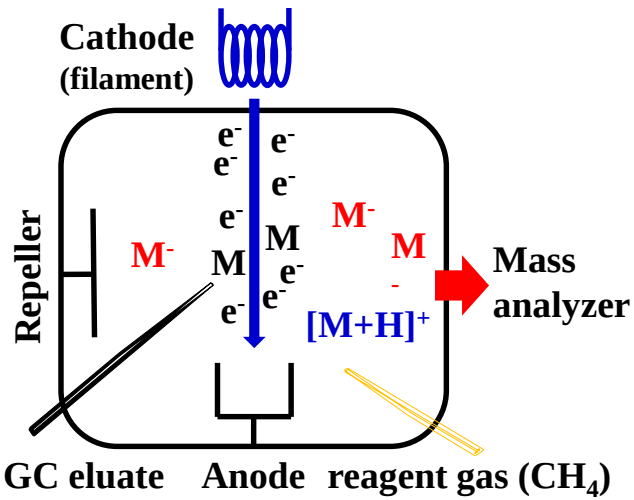
→ reaction ion is formed

$M + CH_5^+ \rightarrow [M + H]^+ + CH_4$

→ protonated molecule is formed

- ⊖ ions formation (Negative CI - NCI):

$M + e^- \rightarrow M^{-\cdot}$; $[M - H]^-$



Chemical ionization (CI)

Characterization:

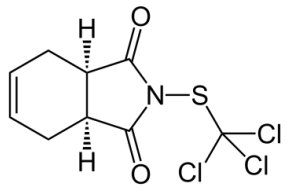
'soft' ionization ⇒ predominant molecular ions (PCI)
⇒ fragment ions are formed minimally (PCI)

Application of CI can be used for determination of:

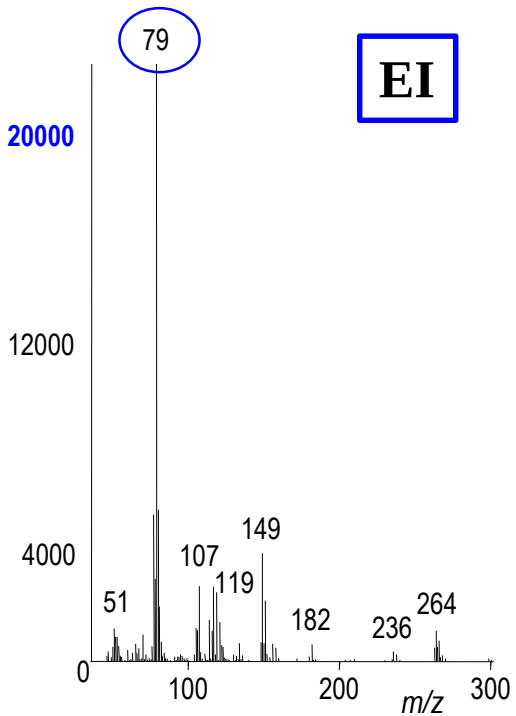
- molecular weight / elementary composition / summary formula
  - based on the exact mass
  - based on isotope cluster of molecular ion
- charge of ions with multiple charges
  - based on differences in m/z values of cluster ions

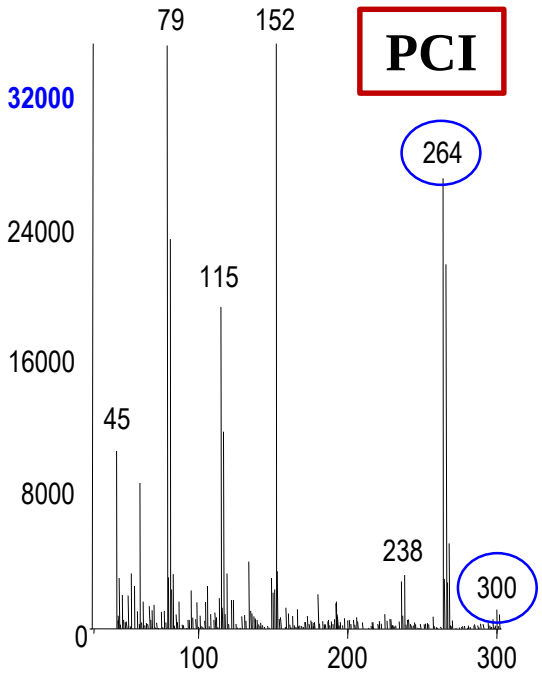
Application: GC-MS

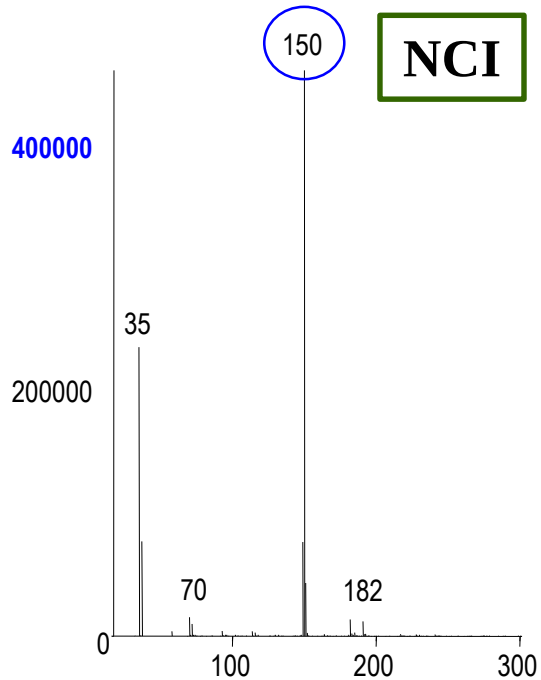
Comparison of ionization techniques



Captan - M_w 299

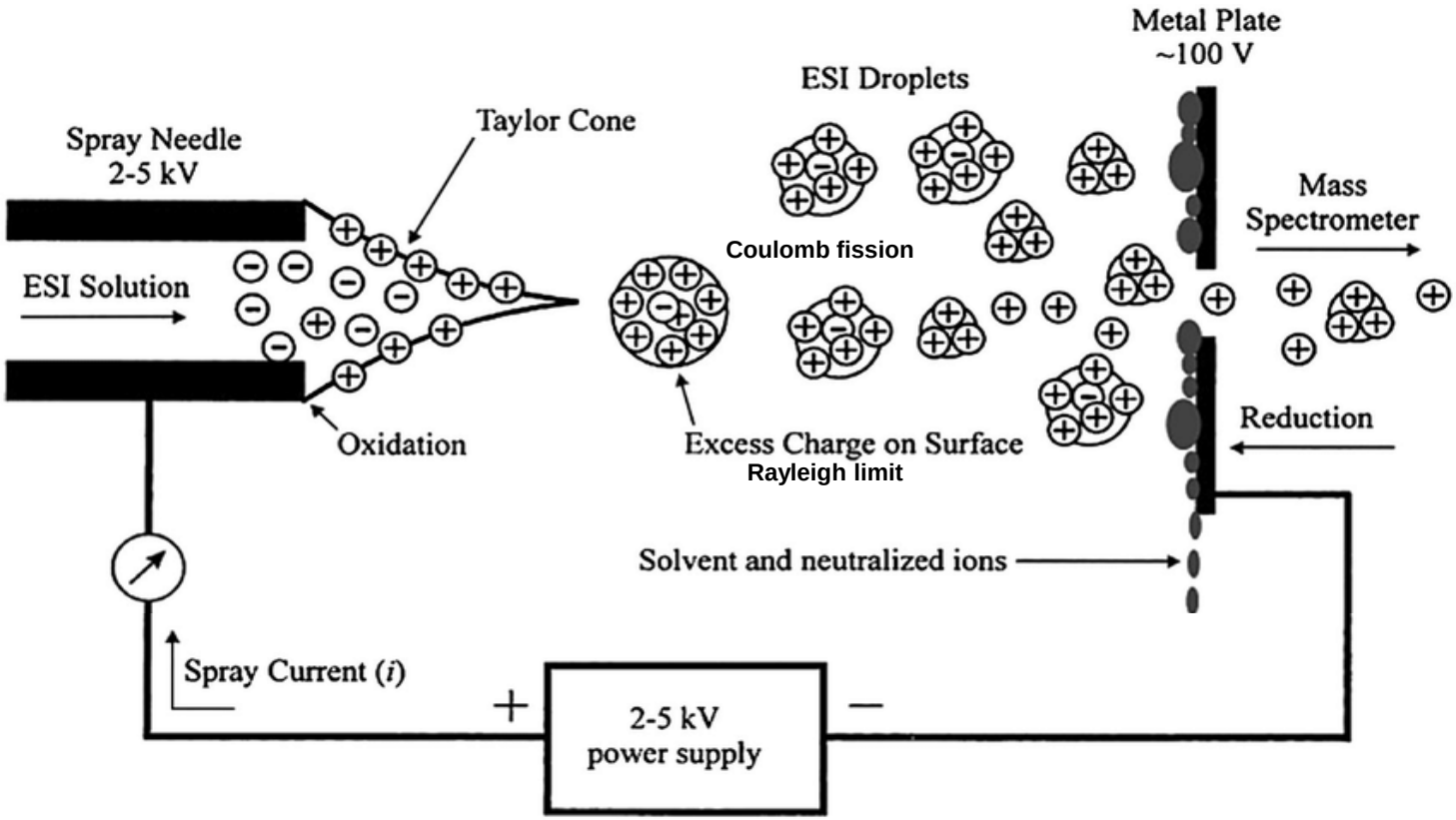






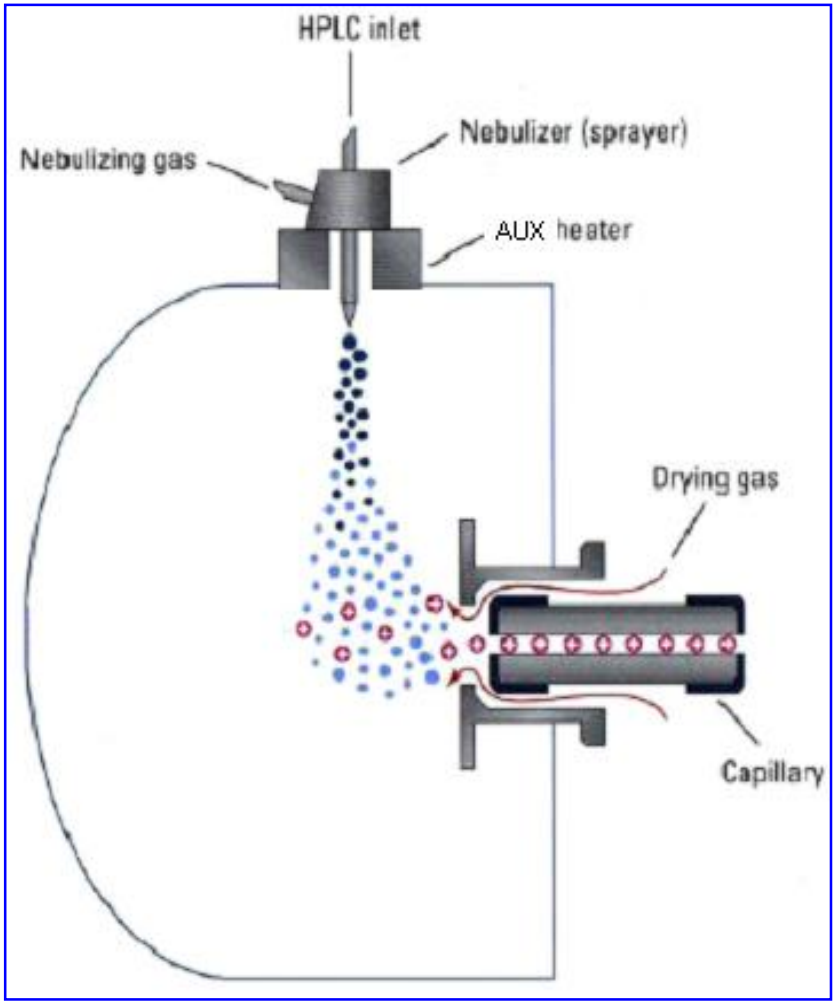
Electrospray ionization – ESI (Electrospray – ESP)

Principle: high voltage ionization (≈ 2-5 kV) with simultaneous nebulization



Cech et al., Mass Spectrometry Review, 20, 362, 2001

Electrospray ionization – ESI (Electrospray – ESP)



(FIGURE: Agilent Technologies)

$[M+H]^+$
$[M+NH_4]^+$
$[M-H]^-$
$[M+CH_3COO]^-$
$[M \pm zH]^{z\pm}$

$t_{N2} \approx 50 - 400$ °C

Voltage: 2 - 8 kV

MP flow rate 0.001 - 1 ml/min

Volatile additives / buffers:
ammonium acetate / formate
acetic/formic acid

Non-volatile additives / buffers:
phosphate buffers

Electrospray ionization – ESI (Electrospray – ESP)

Characterization:

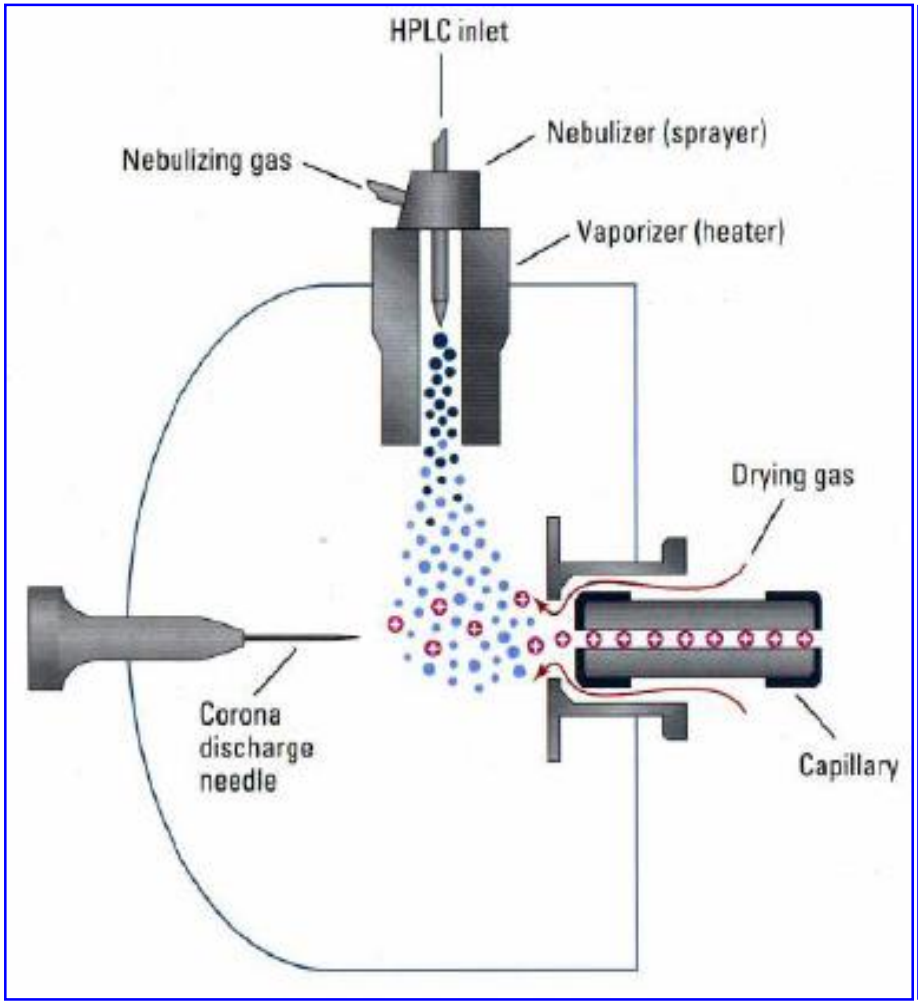
‘soft‘ ionization ⇒ spectra contain mostly molecular or pseudomolecular ions
⇒ multiple charged ions may be formed
⇒ large molecules are ionized (biopolymers)

Application of ESI can be used for determination of:

- molecular weight / elementary composition / summary formula
  - based on the exact mass
  - based on isotope cluster of molecular ion
- charge of ions with multiple charges
  - based on differences in m/z values of cluster ions

Application: LC-MS

Atmospheric Pressure Chemical Ionization - APCI



(FIGURE: Agilent Technologies)

$[M+H]^+$

$[M+NH_4]^+$

$[M-H]^-$

$[M+CH_3COO]^-$

~~$([M \pm zH]^{z\pm})$~~

t ≈ 400 – 650 °C

Discharge voltage: 2 - 8 kV

MP flow rate 0.2 - 2 ml/min

Volatile additives / buffers:
ammonium acetate / formate
acetic/formic acid

Non-volatile additives / buffers:
phosphate buffers

Atmospheric Pressure Chemical Ionization - APCI

Principle: discharge needle ionization (≈ 5 kV) with simultaneous nebulization at high temperature (400 - 650 ° C)

Formation of adduct ions by means of ions formed from MP additives

Ions of 'small' molecules are formed

Characterization:

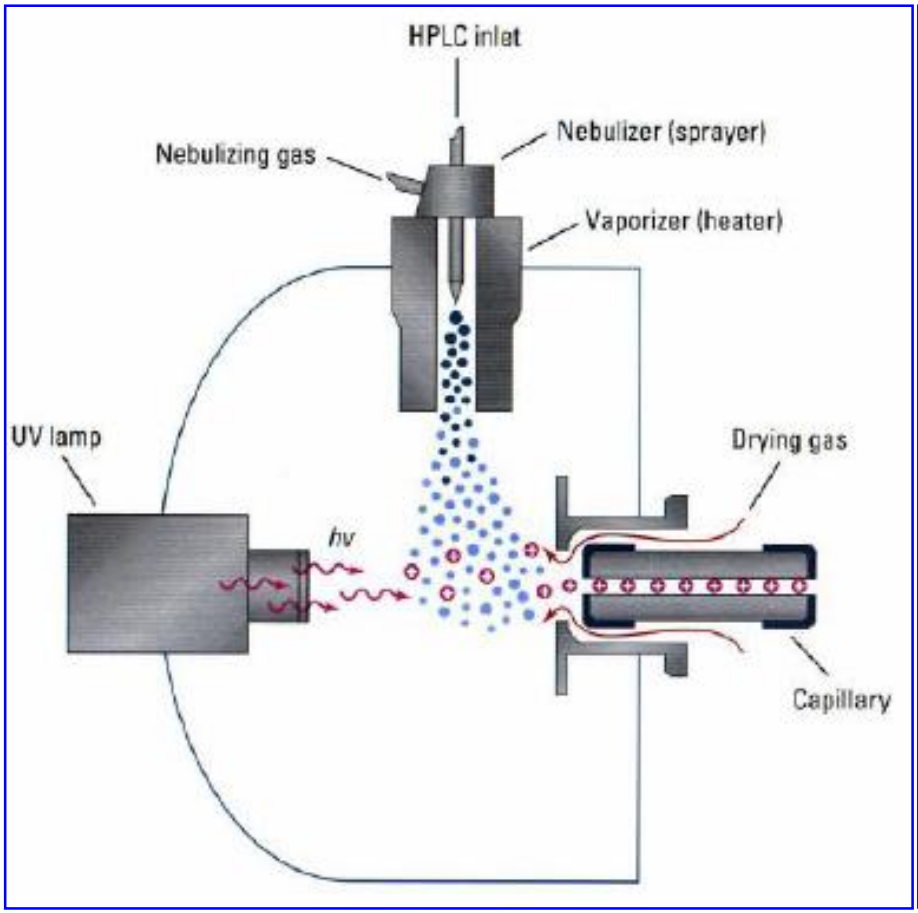
'soft' ionization ⇒ spectra contain mostly molecular or pseudomolecular ions

Application of APCI can be used for determination of:

- **molecular weight / elementary composition / summary formula**
  - **based on the exact mass**
  - **based on isotope cluster of molecular ion**
- **charge of ions with multiple charges**
  - **based on differences in m/z values of cluster ions**

Application: LC-MS

Atmospheric Pressure Photoionization - APPI



(FIGURE: Agilent Technologies)

$[M+H]^+$

$[M-H]^-$

t ≈ 400 – 650 °C

UV radiation ≈ 10 eV

MP flow rate 0.2 - 2 ml/min

Dopant: toluene (↑ sensitivity)

Atmospheric Pressure Photoionization - APPI

Principle: ionization by UV radiation (≈ 10 eV) with simultaneous nebulization at high temperature (400 - 650 ° C)

Selective ionization of suitable analytes occurs

Characterization:

'soft' ionization ⇒ spectra contain mostly molecular or pseudomolecular ions

⇒ suitable for non(semi)-polar and labile compounds

Application of APPI can be used for determination of:

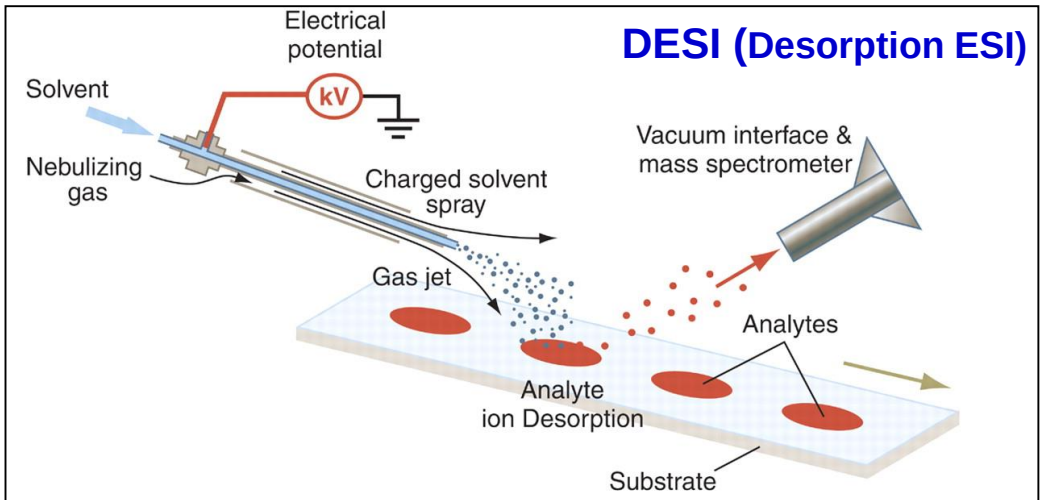
- **molecular weight / elementary composition / summary formula**
  - **based on the exact mass**
  - **based on isotope cluster of molecular ion**
- **charge of ions with multiple charges**
  - **based on differences in m/z values of cluster ions**

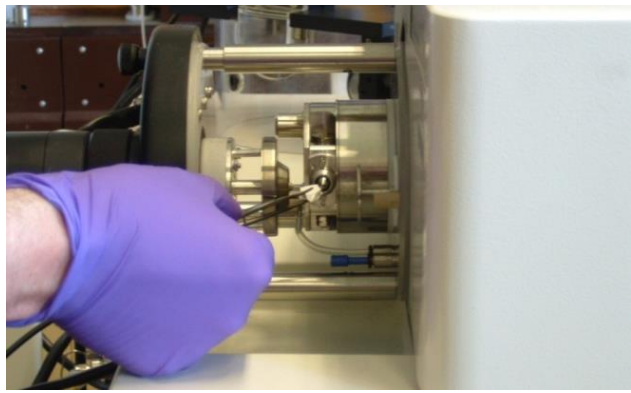
Application: LC-MS

Direct ionization techniques

Principle: by means of ionizing stream of liquid or gas on the sample

A mixed spectrum of all present compounds is created - to ensure the representativeness of results, it is necessary to analyze homogeneous samples or extracts





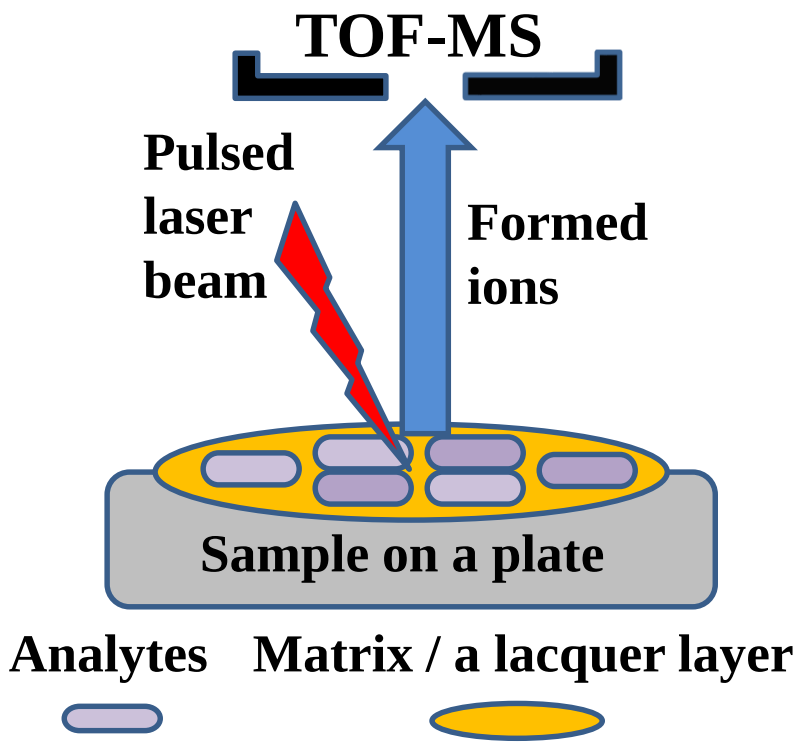
R. G. Cooks, Z. Ouyang, Z. Takats and J. M. Wiseman, Science, 2006, 311, 1566–1570

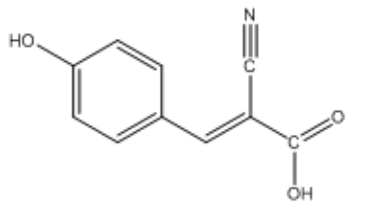
Direct ionization techniques

Principle: by means of laser beam on the sample in presence of absorbing matrix

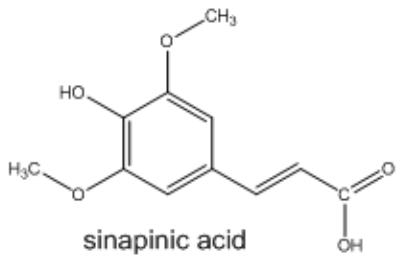
A mixed spectrum of all present compounds is created - to ensure the representativeness of results, it is necessary to analyze homogeneous samples or extracts

MALDI (Matrix Assisted Laser Desorption Ionization)

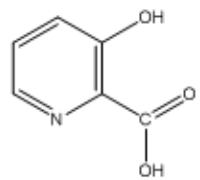




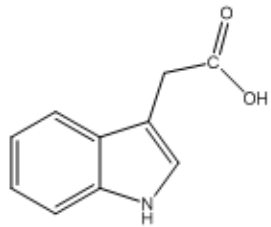
a-cyano-4-hydroxycinnamic acid
CCA $C_{10}H_7NO_3$
peptides and small molecules



sinapinic acid
SA $C_{11}H_{12}O_5$
proteins



3-hydroxypicolinic acid
HPA $C_6H_5NO_3$
oligonucleotides



3-indoleacrylic acid
IAA $C_{10}H_9NO_2$
organic polymers

Comparison of applicability of ionization techniques