massaspectrometrie

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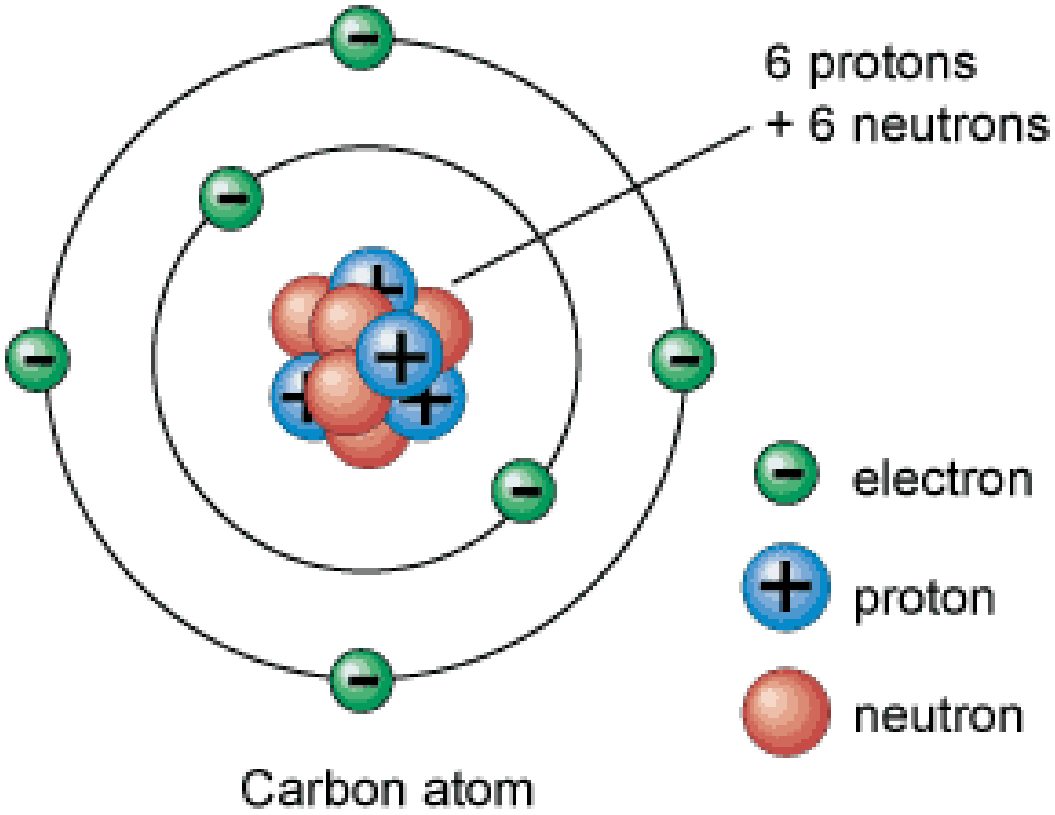
link

http://mstoolbox.github.io/manama

massa van een atoom

- elektronen
- protonen
- neutronen

massa van een atoom



massa van een atoom

$$1\ \mathrm{u} = 1\ \mathrm{Da} = \mathrm{m}(^{12}\mathrm{C})/12$$

massa van een atoom

- Neutron = 1.6749286*10^{-27} kg (939.56563 MeV)
- Proton = 1.6726231*10^{-27} kg (938.27231 MeV)
- Elektron = 9.1093897*10^{-31} kg (0.51099906 MeV)

- Neutron = 1.0087
- Proton = 1.0073
- Elektron = 0.0005449

massa van een molecule

- nominale massa
- gemiddelde massa
- accurate massa

massa van een molecule - nominale massa

nominale massa = som van de nominale atomaire massa's

massa van een molecule - gemiddelde massa

gemiddelde massa = som van de gemiddelde atomaire massa's

gemiddelde atomaire massa = som van de gewogen isotoopmassa's

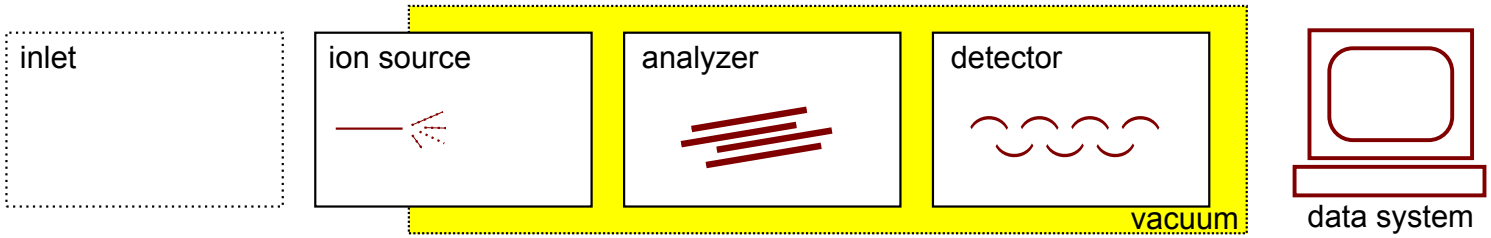
stappen in kwantitatieve analyse

- collectie staal
- stalen voor calibratie en kwaliteitscontrole
- staalvoorbereiding
- analyse
- rapportering

analyse met een massaspectrometer

- inbrengen staal
- ionisatie
- massa analyse
- ion detectie
- data verwerking

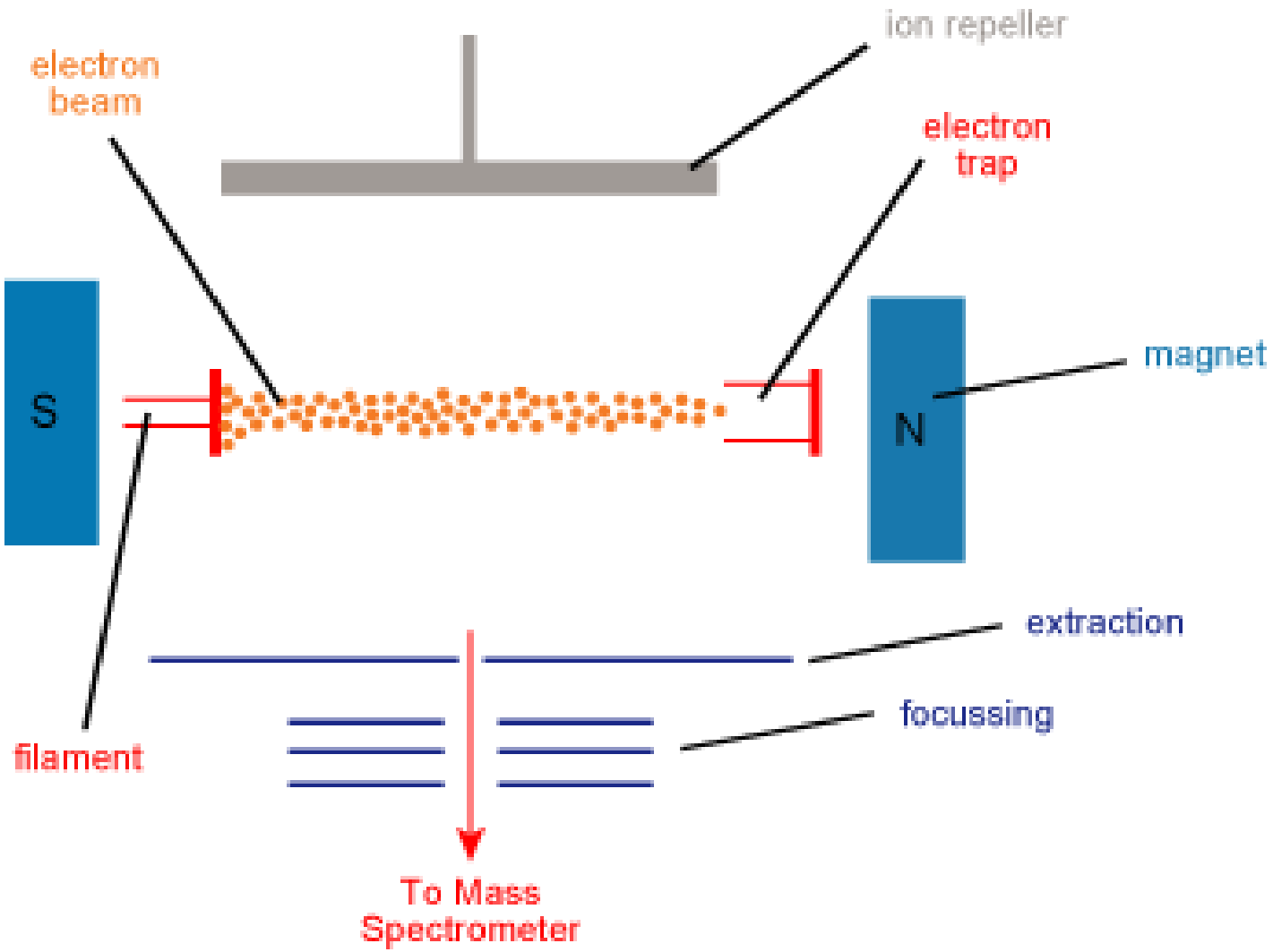
ionisatie



ionisatie - EI

$$\mathrm{M} + \mathrm{e}^- \longrightarrow \mathrm{M}^{+\cdot} + 2\mathrm{e}^-$$

ionisatie - EI

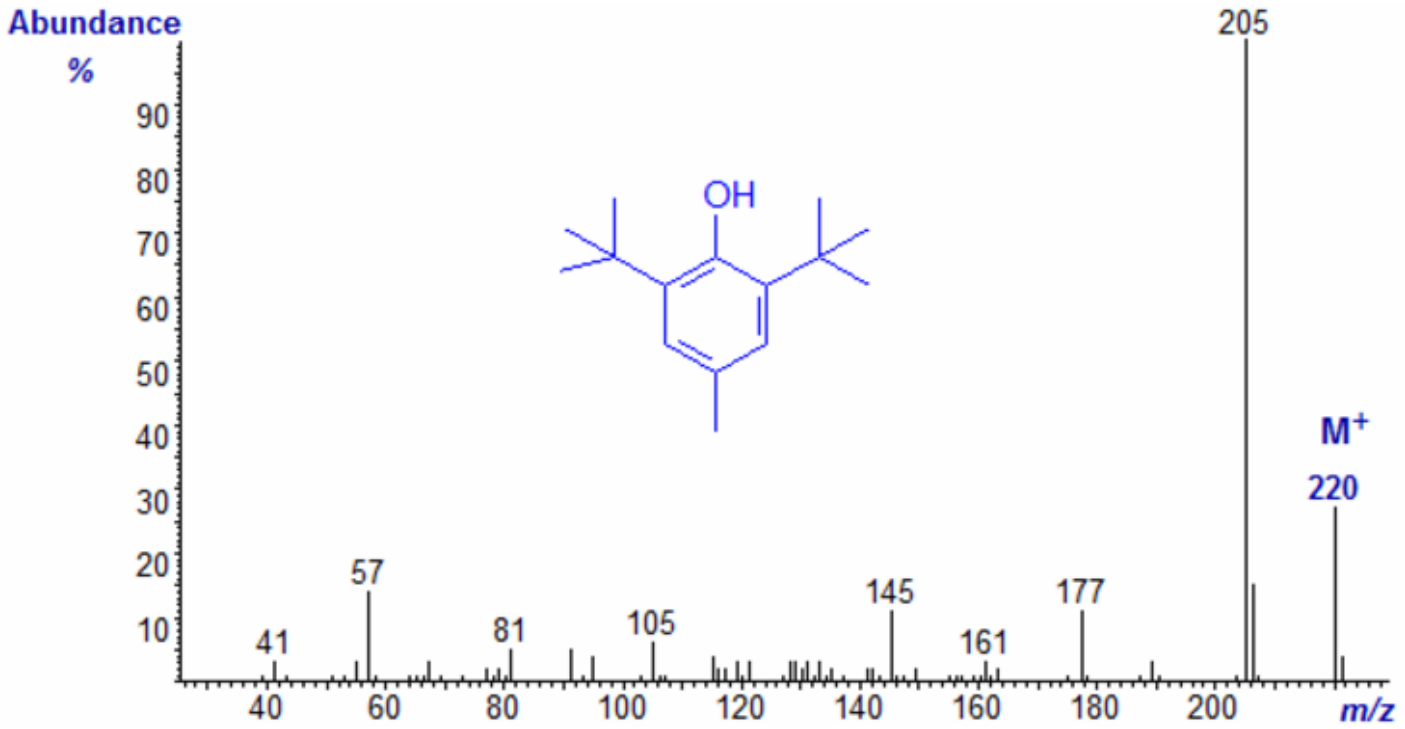


ionisatie - CI

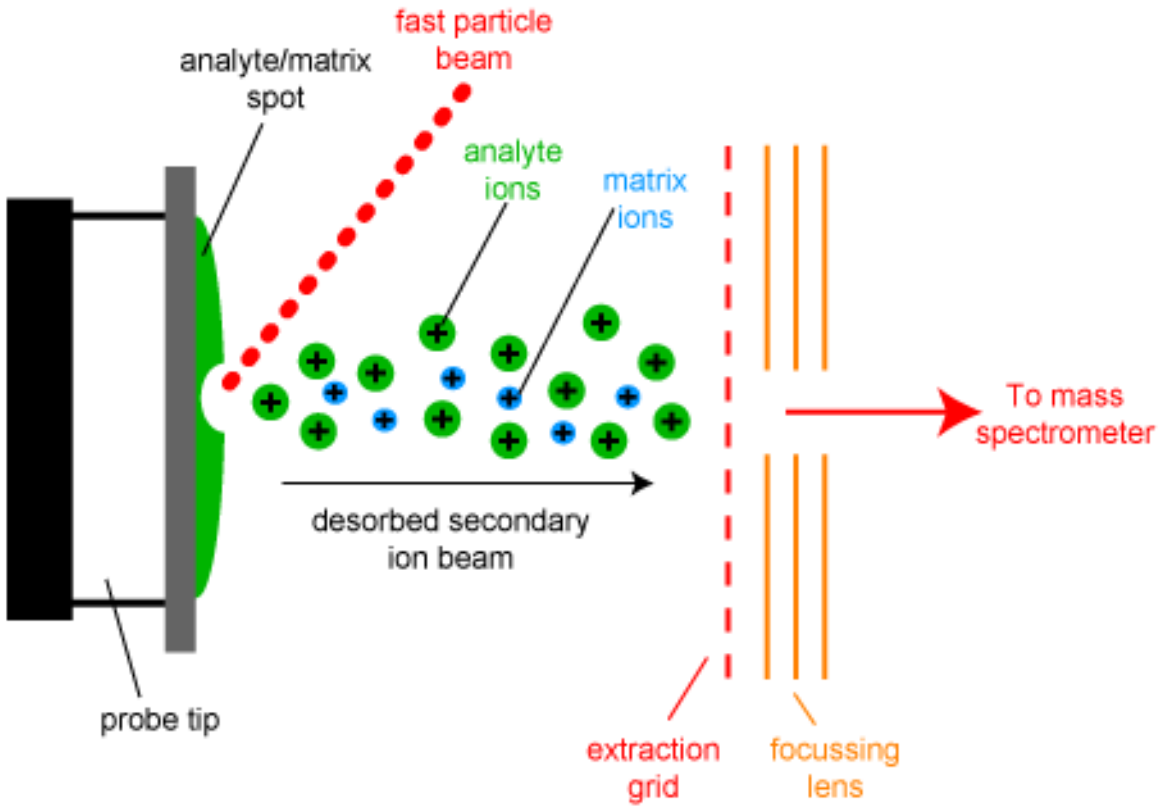
$$CH_4 + e^- \longrightarrow {CH_4}^{+\cdot} + 2e^-$$

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

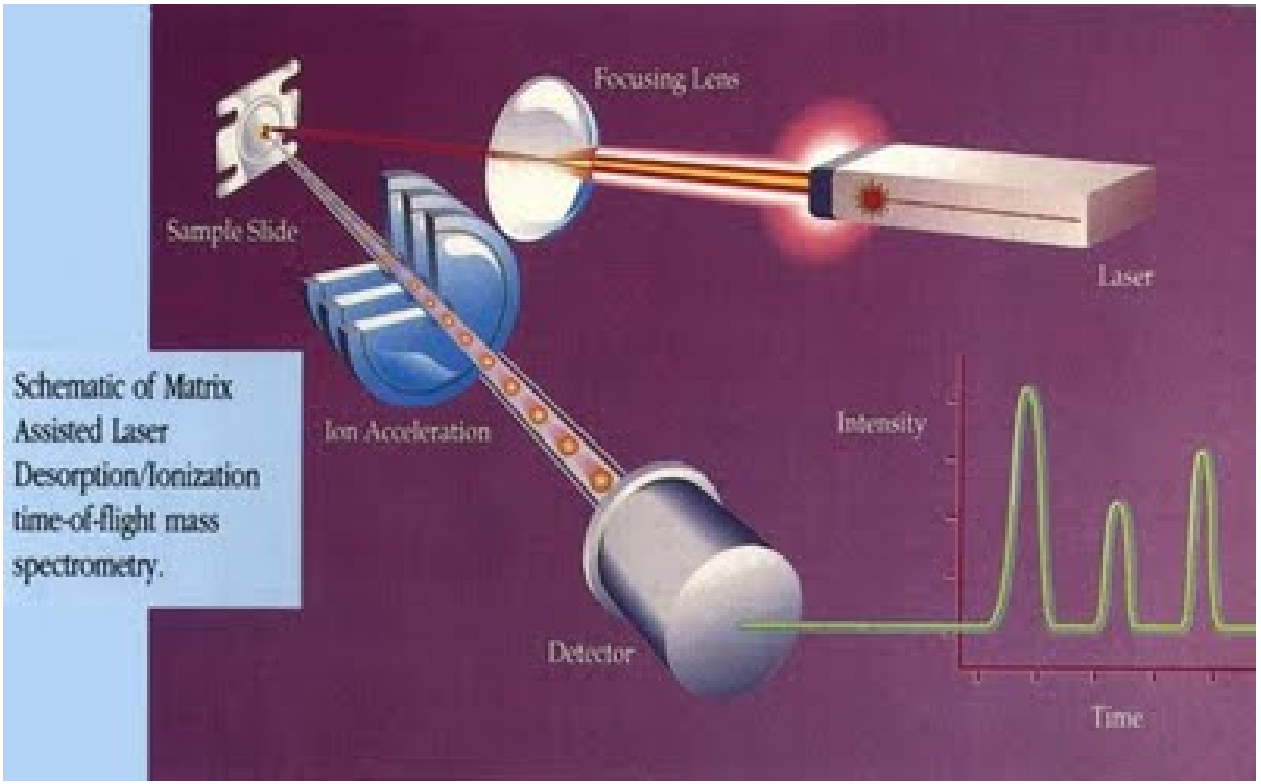
ionisatie - EI/CI spectrum



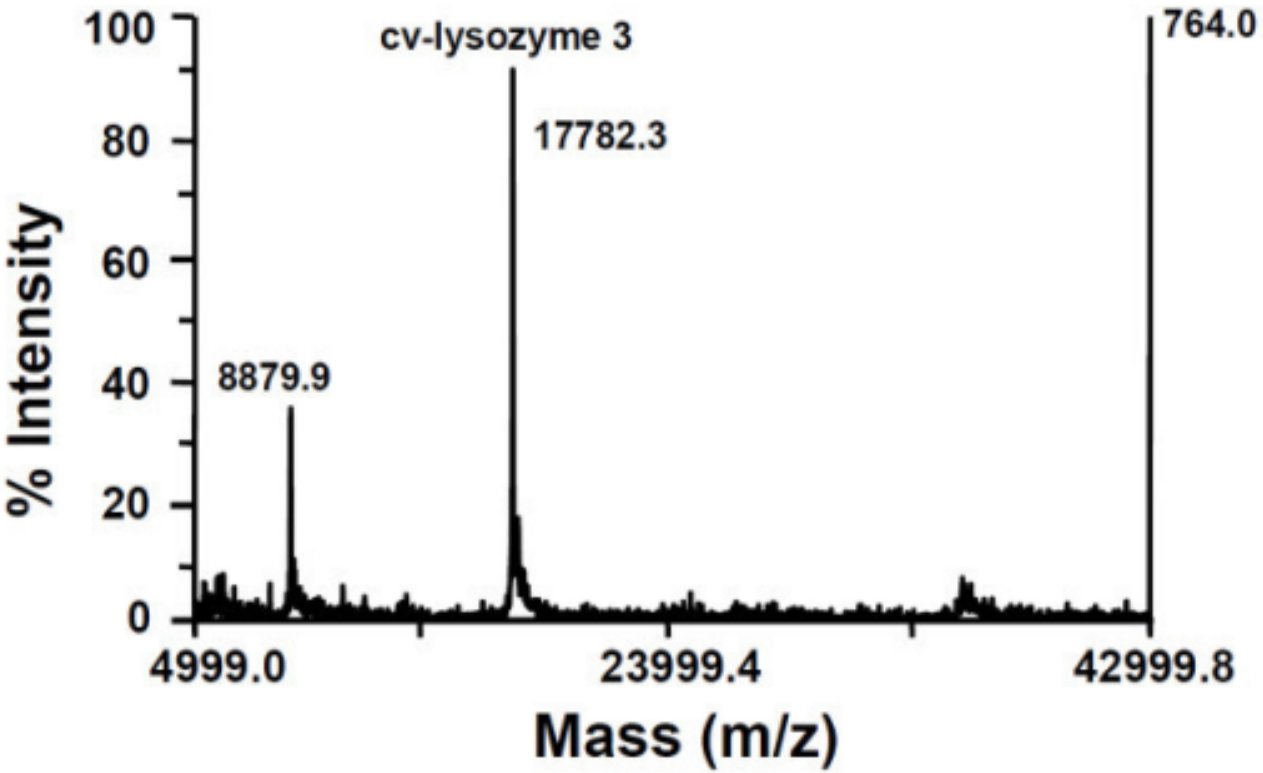
ionisatie - FAB



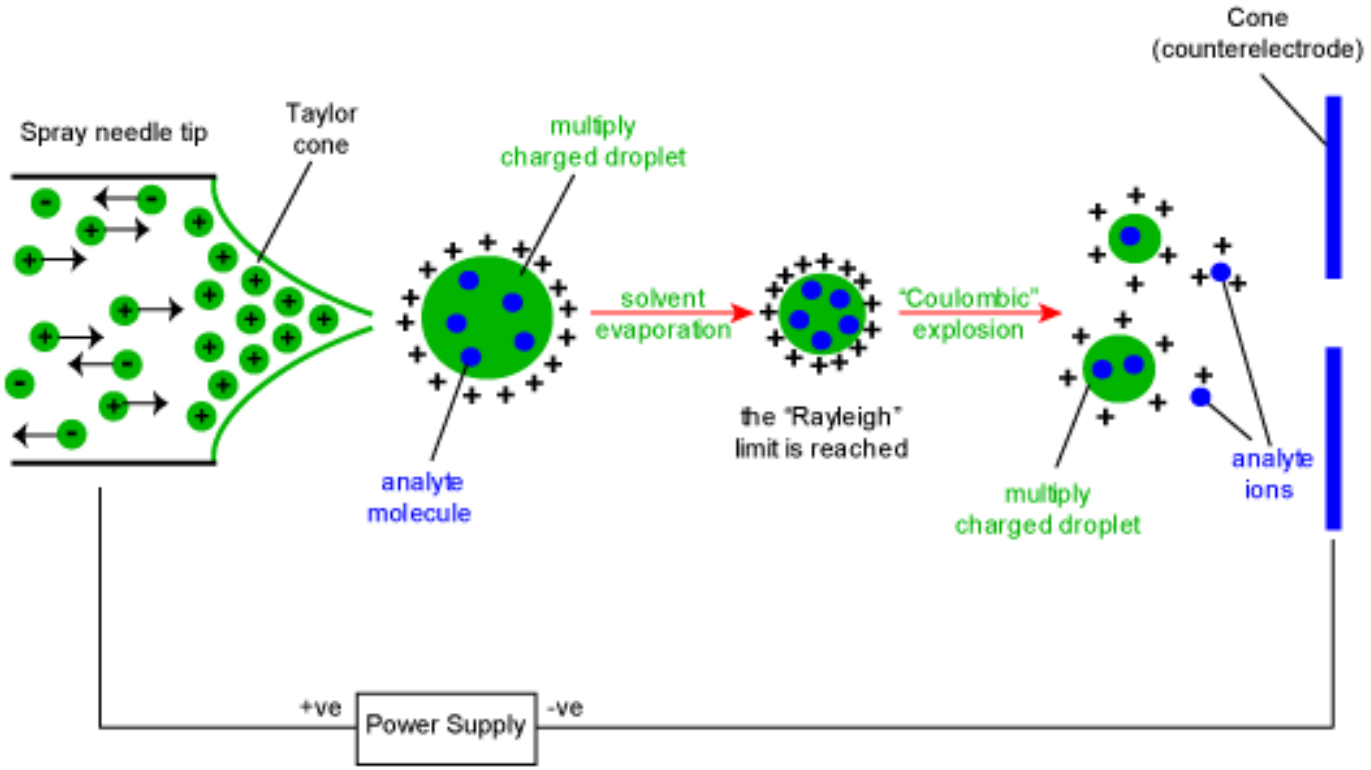
ionisatie - MALDI



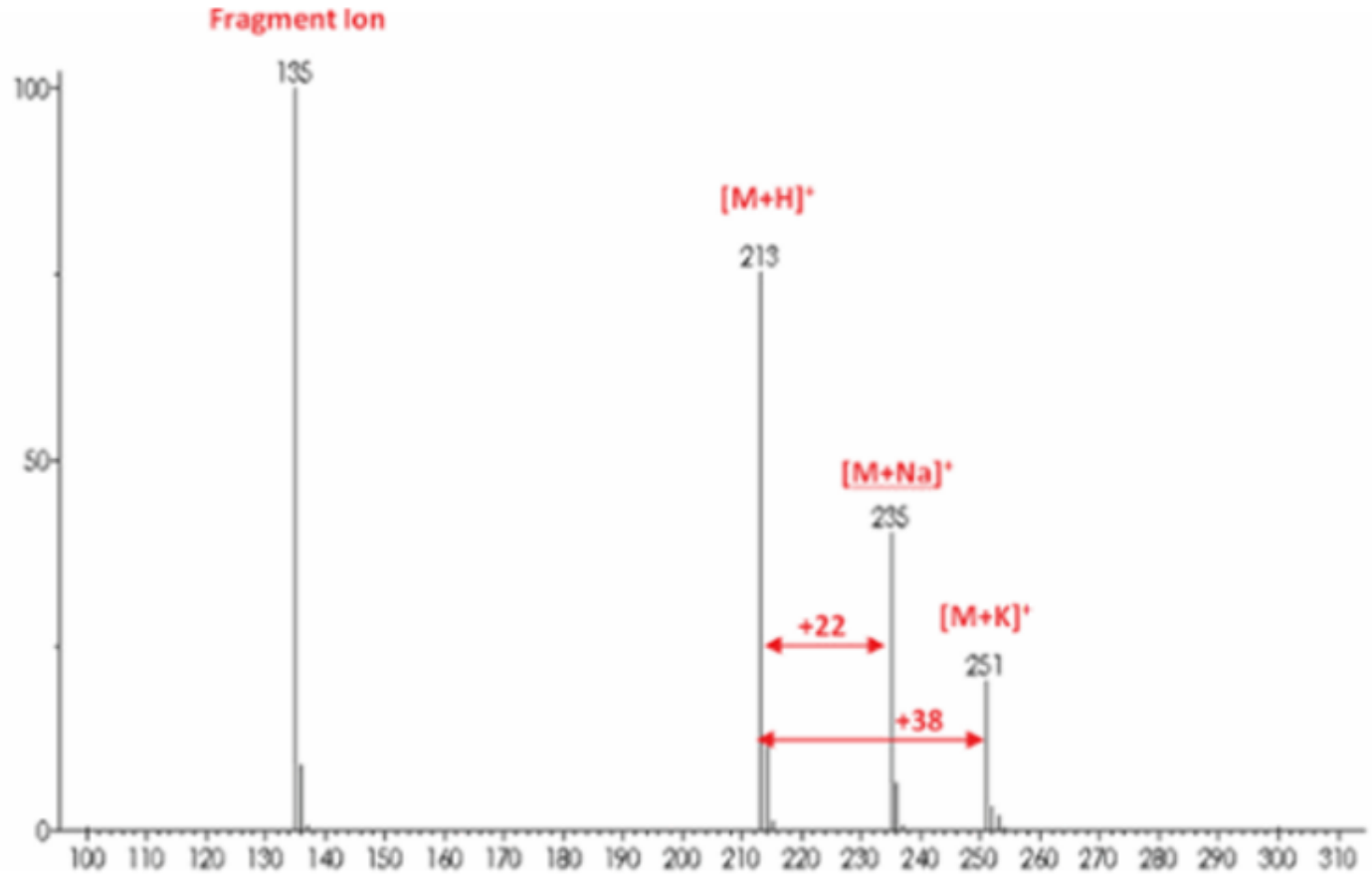
ionisatie - MALDI spectrum



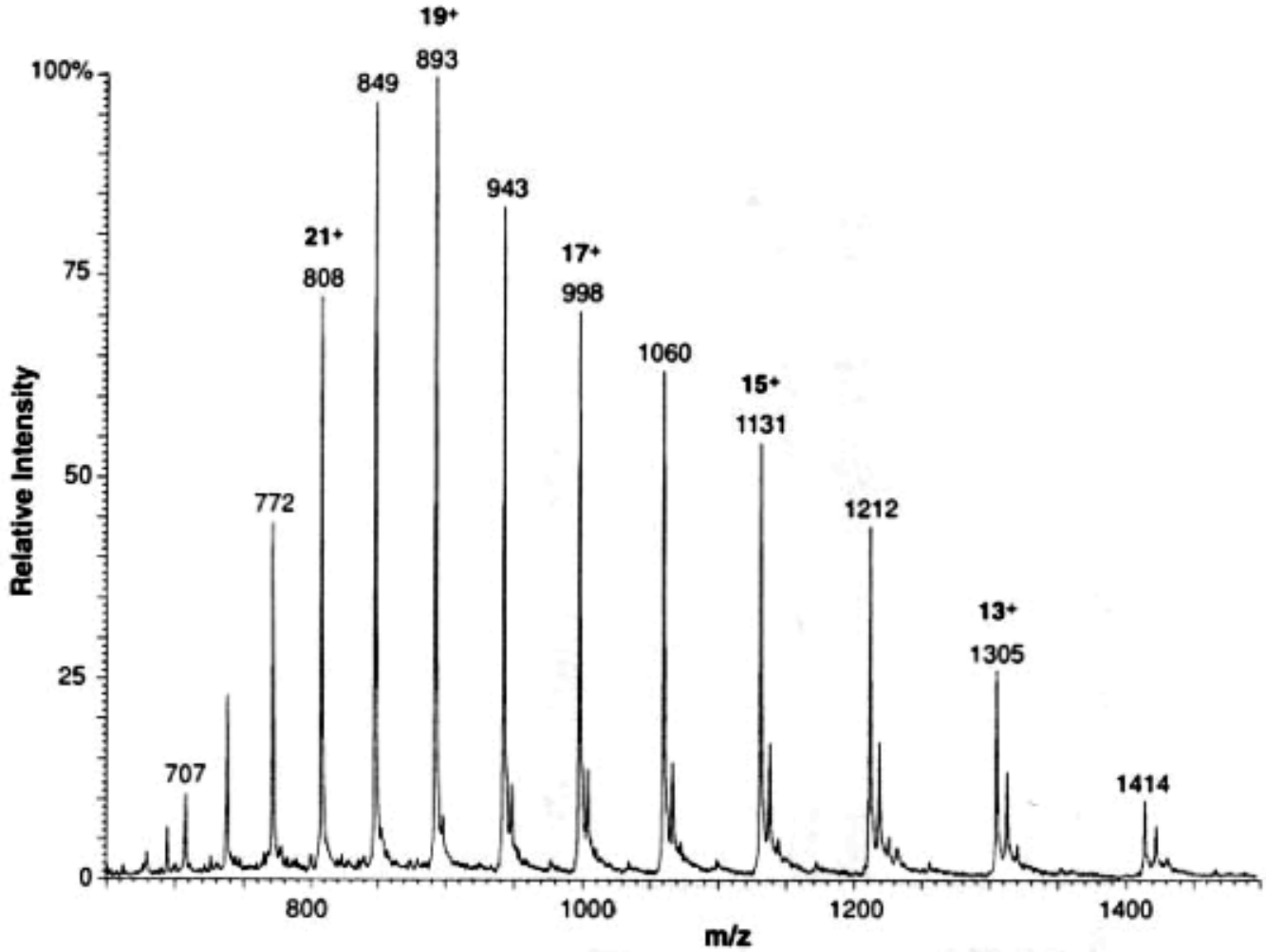
ionisatie - elektrospray



ionisatie - ESI spectrum



ionisatie - elektrospray



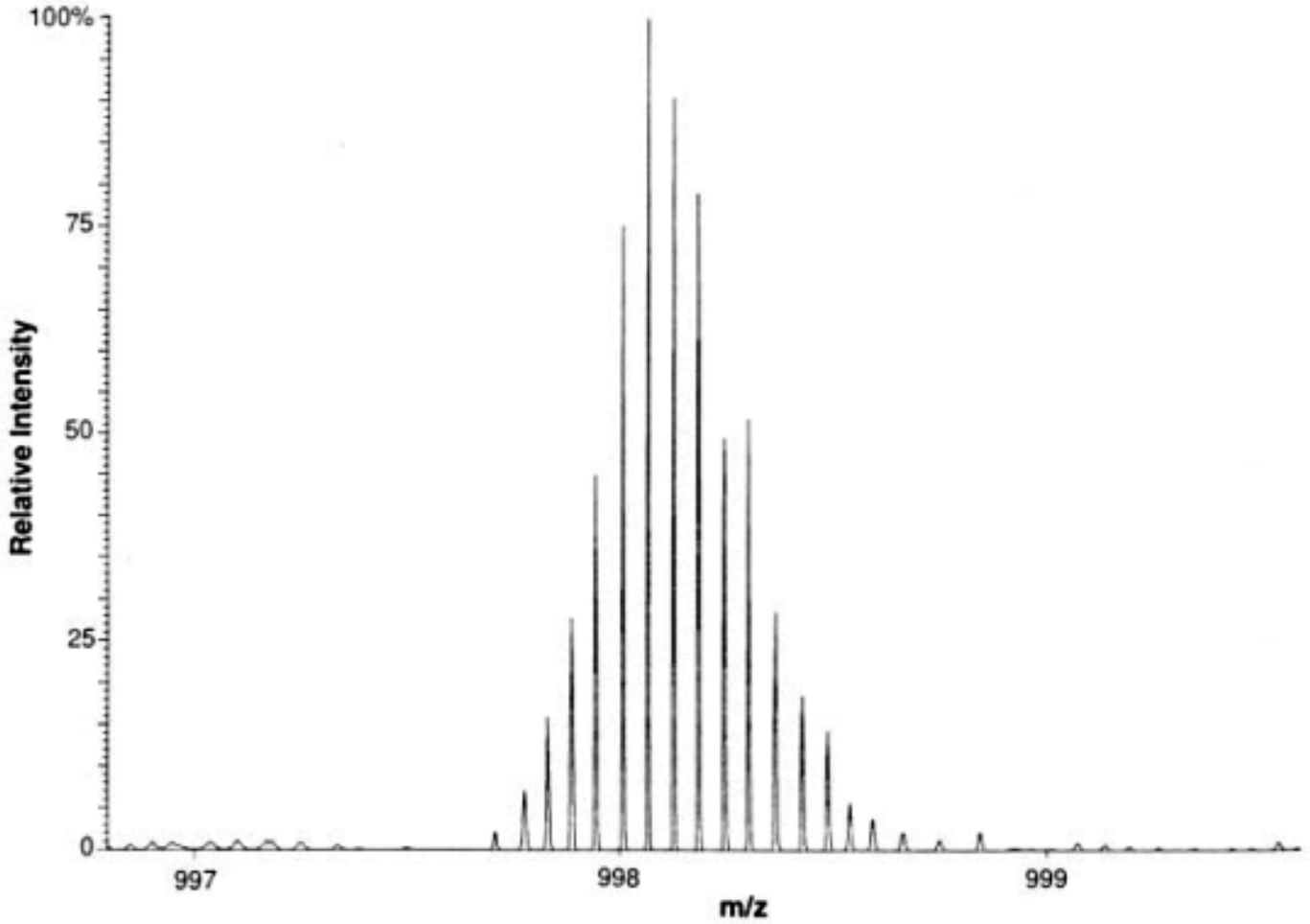
ionisatie - elektrospray

Indien $m_1 < m_2$ en $n_2 = n_1 - 1$ dan is

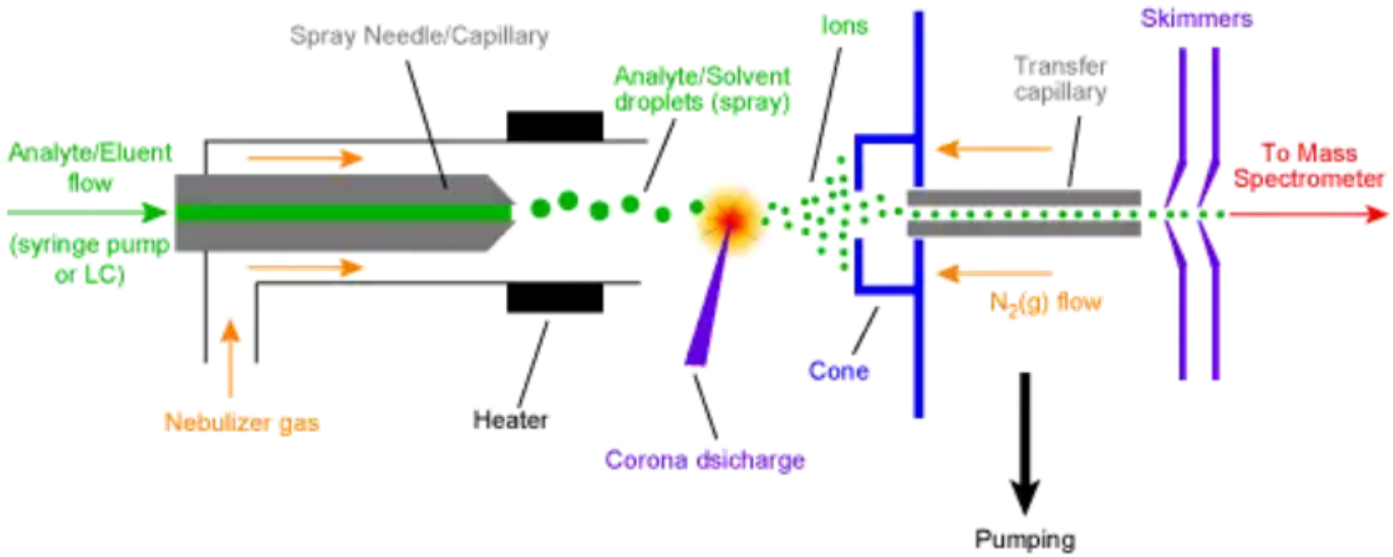
$$M = n_1(m_1 - mA) = n_2(m_2 - mA)$$

$$n_2 = (m_1 - m_A)/(m_2 - m_1)$$

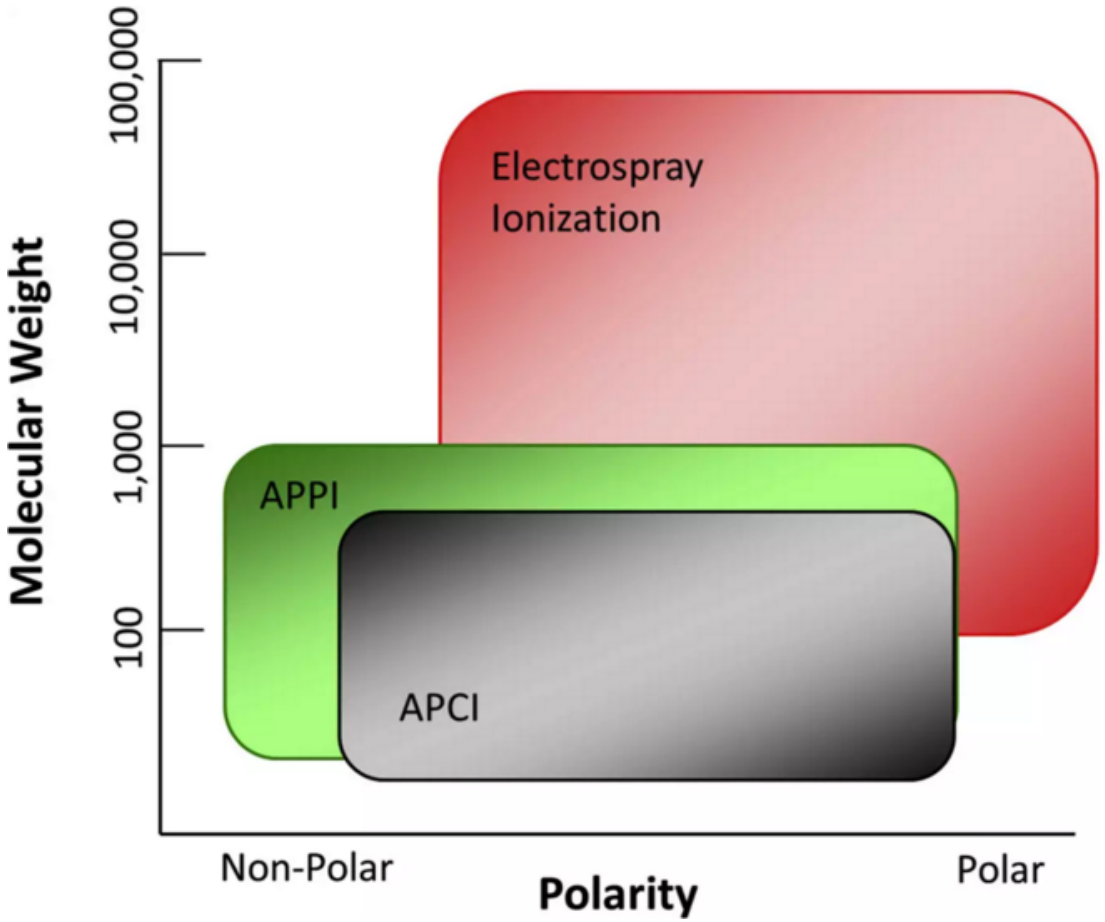
ionisatie - elektrospray



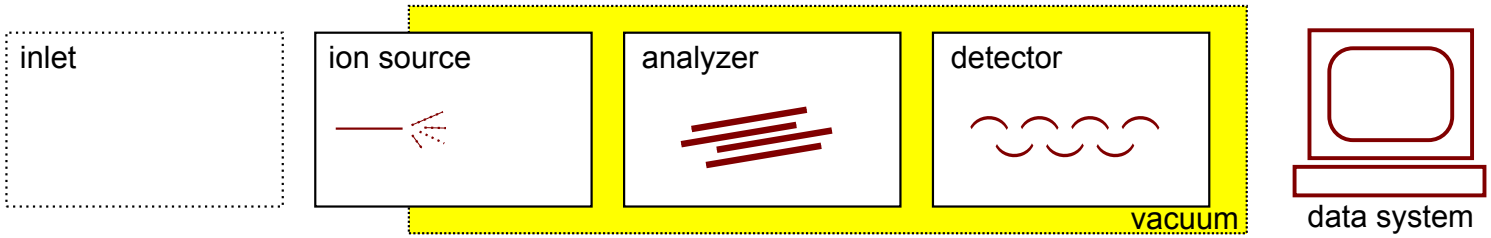
ionisatie - APCI



ionisatie


Molecular Weight
100,000
10,000
1,000
100
Electrospray
Ionization
APPI
APCI
Non-Polar
Polarity
Polar

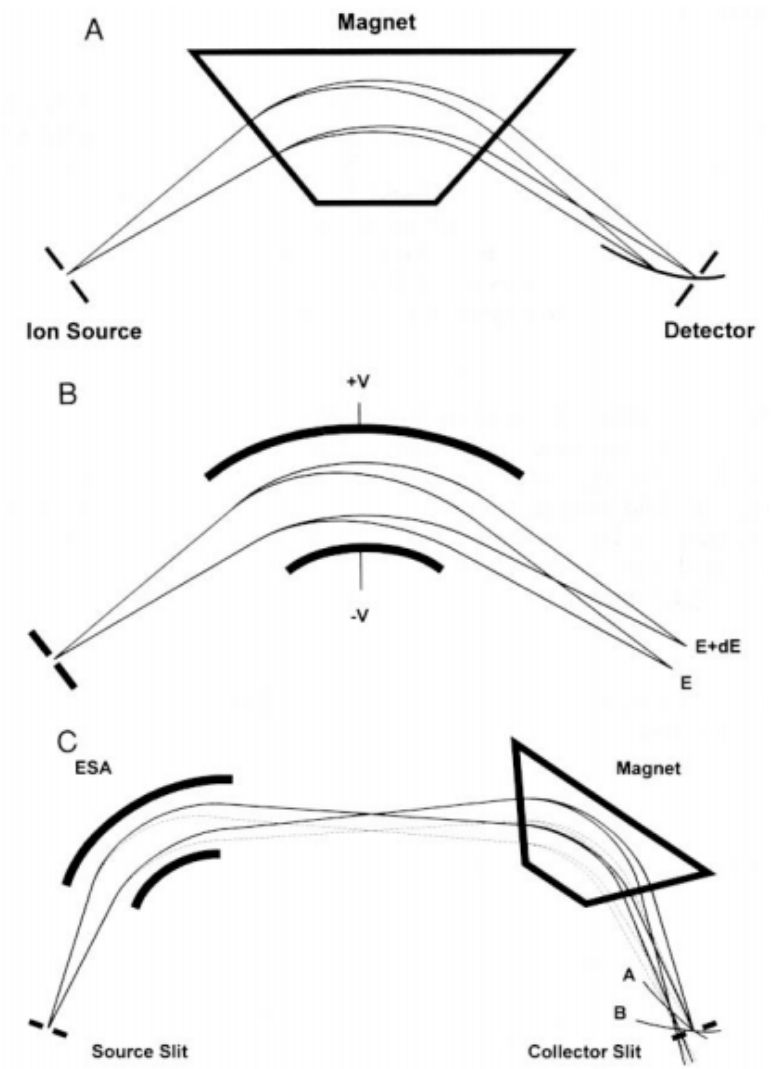
massa analysatoren



massa analysatoren

- meetgebied (*mass range*, maximum *m/z* waarde)
- gevoeligheid (*sensitivity*)
- accuraatheid (*accuracy*, uitgedrukt in *parts per million*, ppm)
- oplossend vermogen (*resolution*)

massa analysatoren - sector instrument



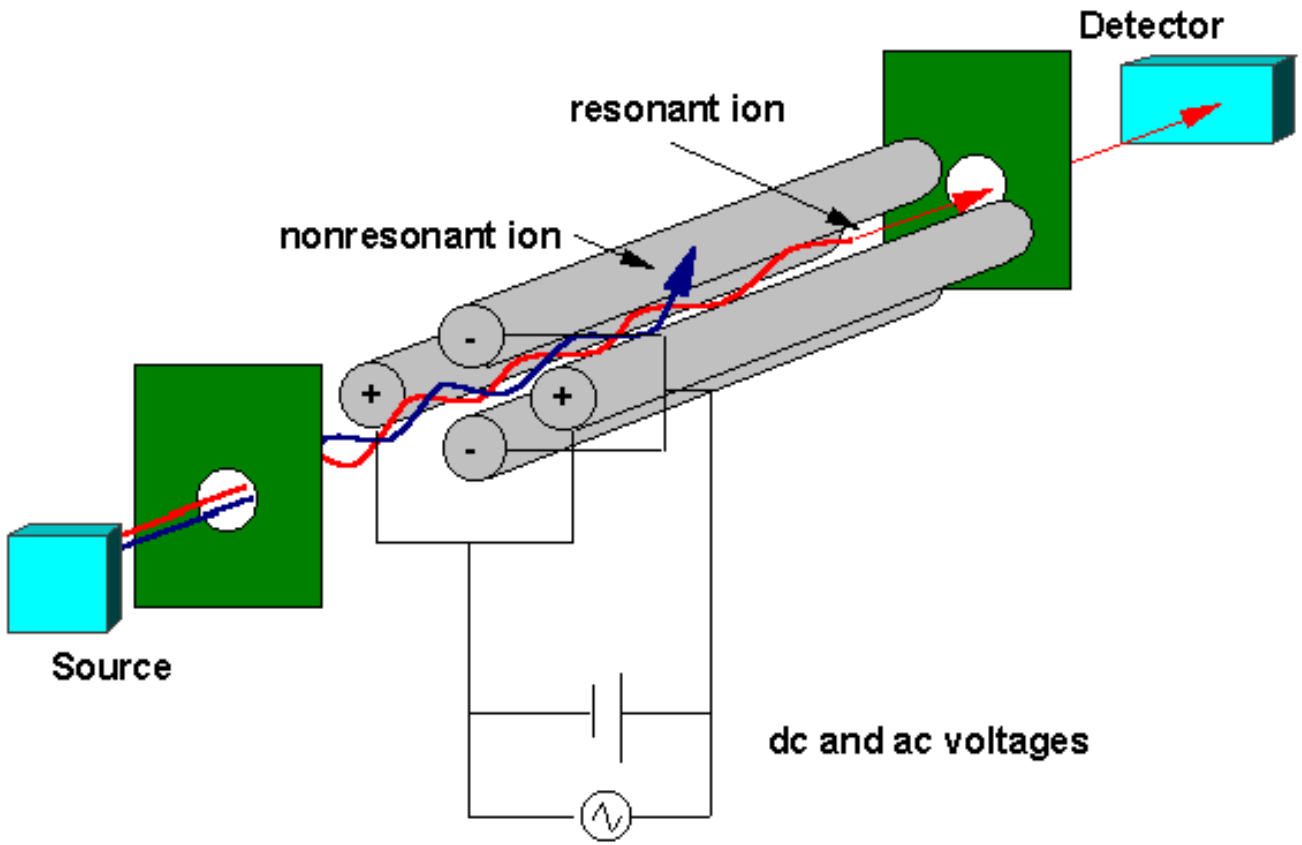
massa analysatoren

$$E_{kin} = qV_{acc} = mv^2/2$$

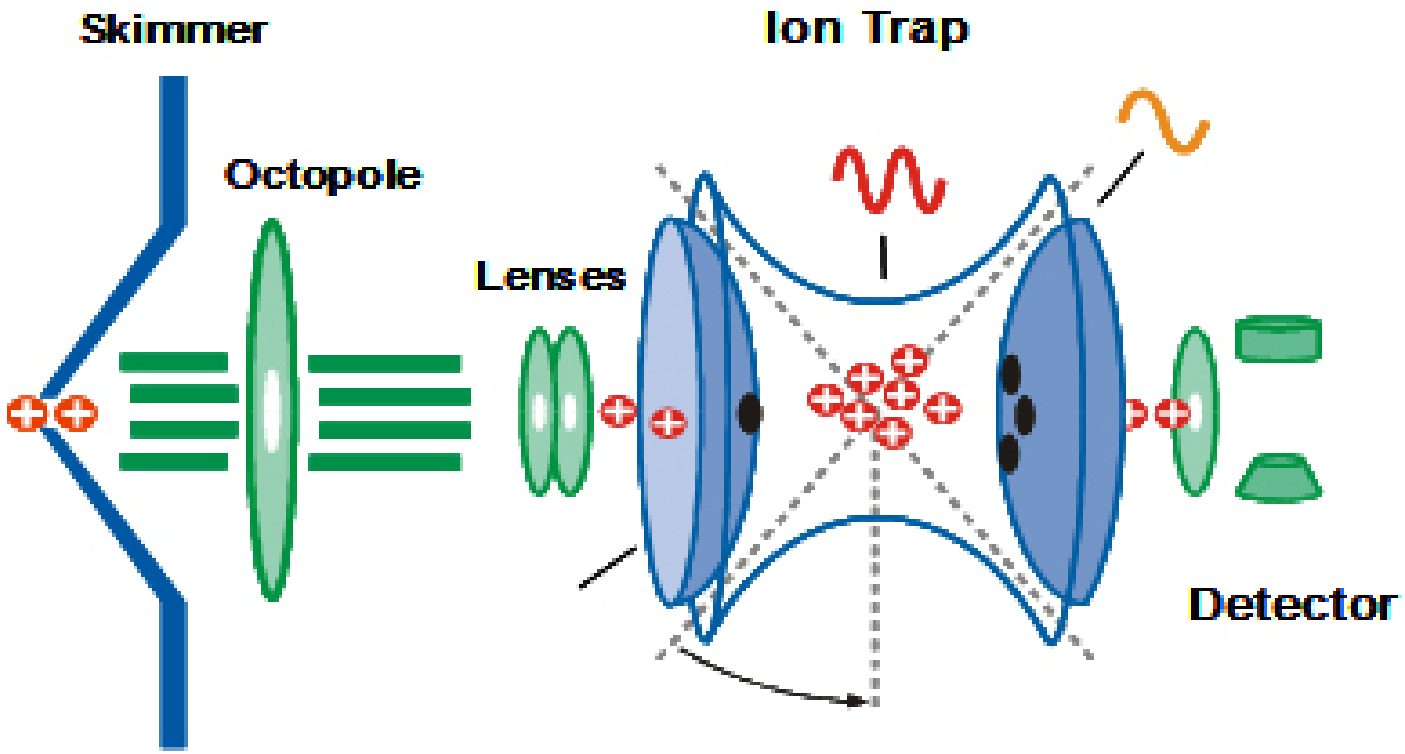
$$F = qvB = mv^2/r$$

$$m/z = r^2B^2e/2V_{acc}$$

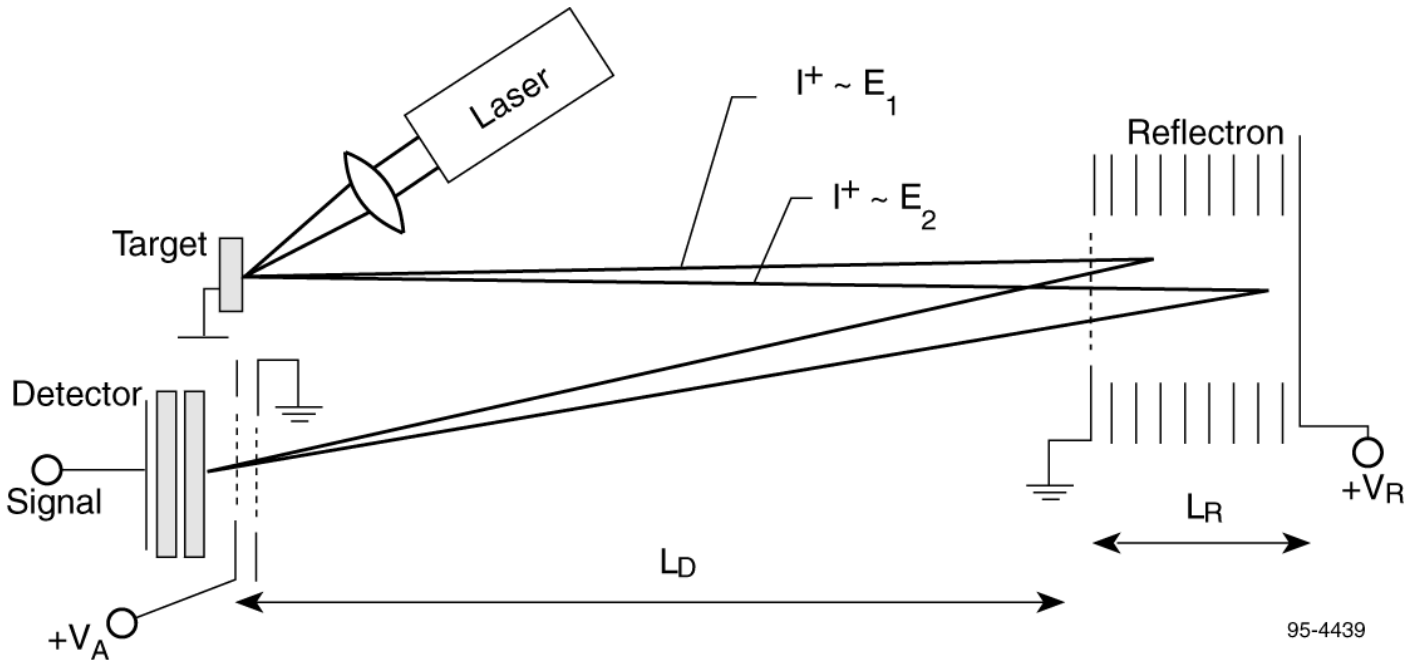
massa analysatoren - quadrupool



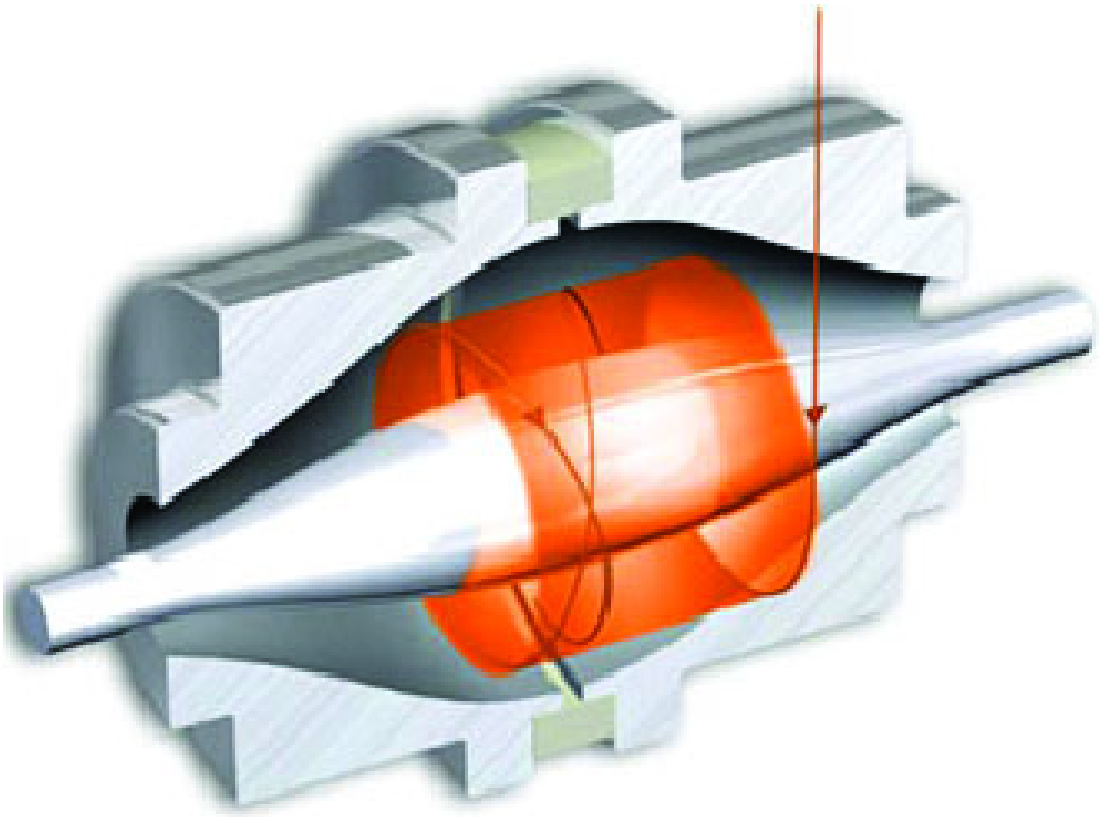
massa analysatoren - ion trap



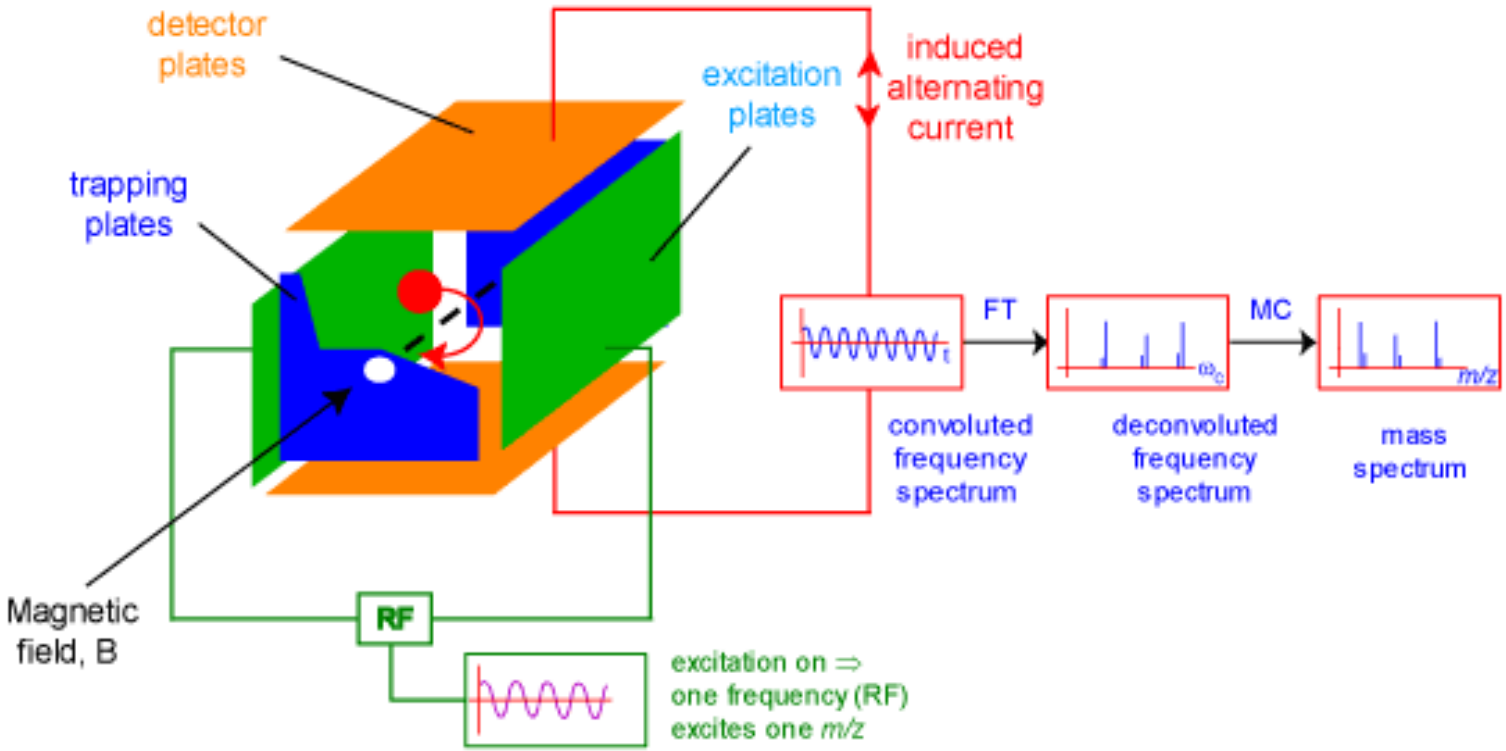
massa analysatoren - time of flight



massa analysatoren - orbitrap



massa analysatoren - FT-ICR



waarom LC/MS

voordelen:

- selectiviteit
- snelheid
- gevoeligheid

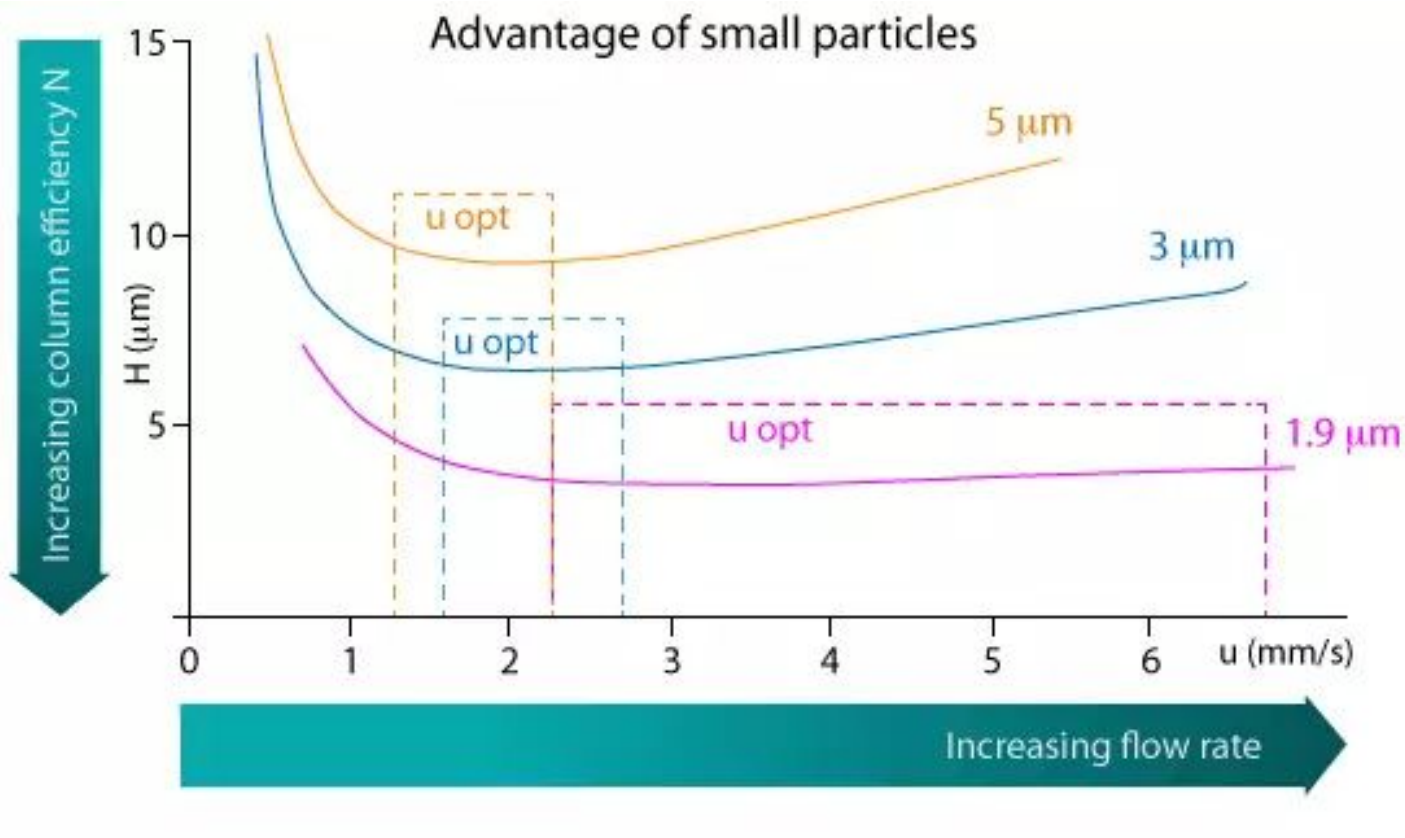
nadelen:

- kostprijs
- complexiteit
- dynamische range
- overdreven selectiviteit

HPLC methode optimaliseren

- debiet - partikelgrootte
- debiet - kolom diameter
- piekbreedte - datapoints
- kolomtype
- mobiele fase - buffers

debiet - partikelgrootte



debiet - kolomdiameter

$$F_{C2} = \left(\frac{d_{c2}}{d_{c1}}\right)^2 \times F_{C1}$$

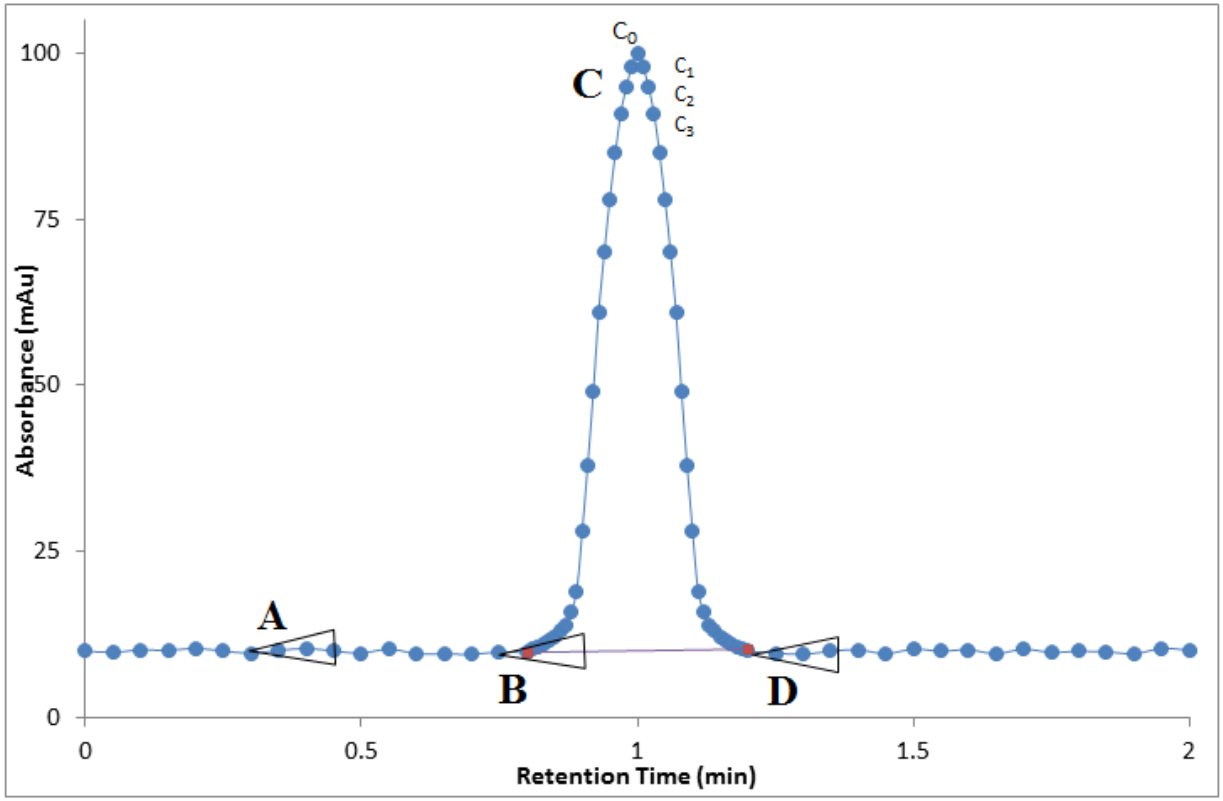
debiet - kolomdiameter

Column i.d. (µm)	Flow Rate (µL/min)	Classification
4600	1000	Analytical
2100	208	Narrowbore
1000	47	Microbore
500	12	Microbore
320	5	Microcolumn
250	3	Microcolumn
50	0.12	Nanocolumn/ Packed Capillary

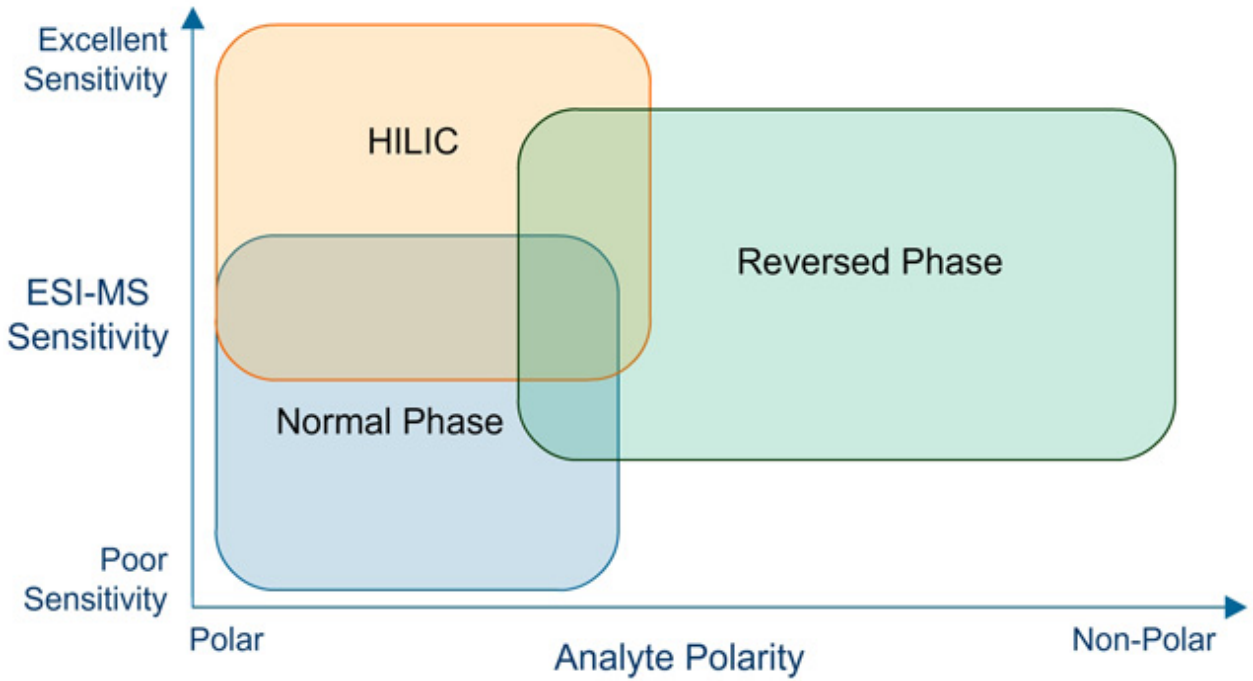
debiet - kolomdiameter

- kleinere kolomdiameter = hogere druk
- kleinere kolomdiameter = lager solventverbruik

piekbreedte - datapoints



kolomtype


Excellent Sensitivity
ESI-MS Sensitivity
Poor Sensitivity
HILIC
Reversed Phase
Normal Phase
Polar
Analyte Polarity
Non-Polar

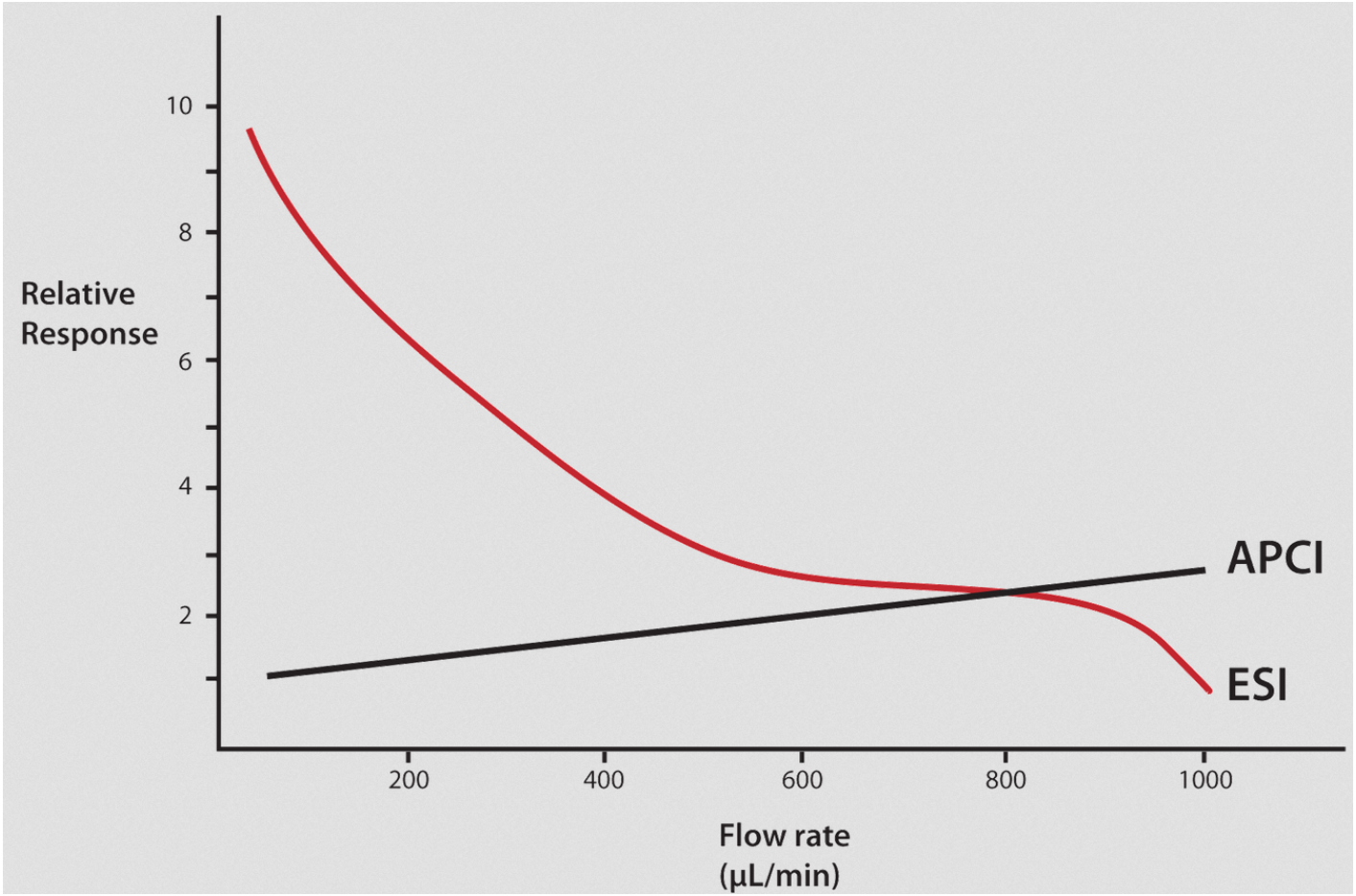
buffers

- Buffer concentrations below 25 mM (best below 10 mM)
- Poor compatibility with non-volatile buffers
  - Deposit buildup
  - Metal ion buffers interfere with ionization
- Acidic mobile phases generally favor positive mode ionization
  - 0.1% - 1% formic acid, 0.1% - 1% acetic acid, 0.05% - 0.2% TFA
  - Ammonium salts (ammonium formate and ammonium acetate) favor formation of ammonium adducts
  - TFA causes ion suppression
  - Use TFA "fix" – post column addition of acetic or propionic acid
- Basic mobile phases generally favor negative mode ionization
  - Ammonium hydroxide, triethylamine, diethylamine, piperidine, ammonium bicarbonate
- pH 1 to 2 units away from the pKa of the analytes

MS parameters kiezen

- keuze ionisatiemethode
- instrument tuning
- selectie van scan functie
- MS/MS settings
- kation adducten vermijden
- matrix suppression
- gebruik van stabiele isotopen

MS parameters kiezen



MS parameters kiezen

- nanospray: 100 - 1000 nL/min
- elektrospray: 1uL - 500uL/min
- APCI: 100uL - 2000uL/min

single of triple quadrupool

- toestellen met 1 analysator
- tandem massaspectrometers

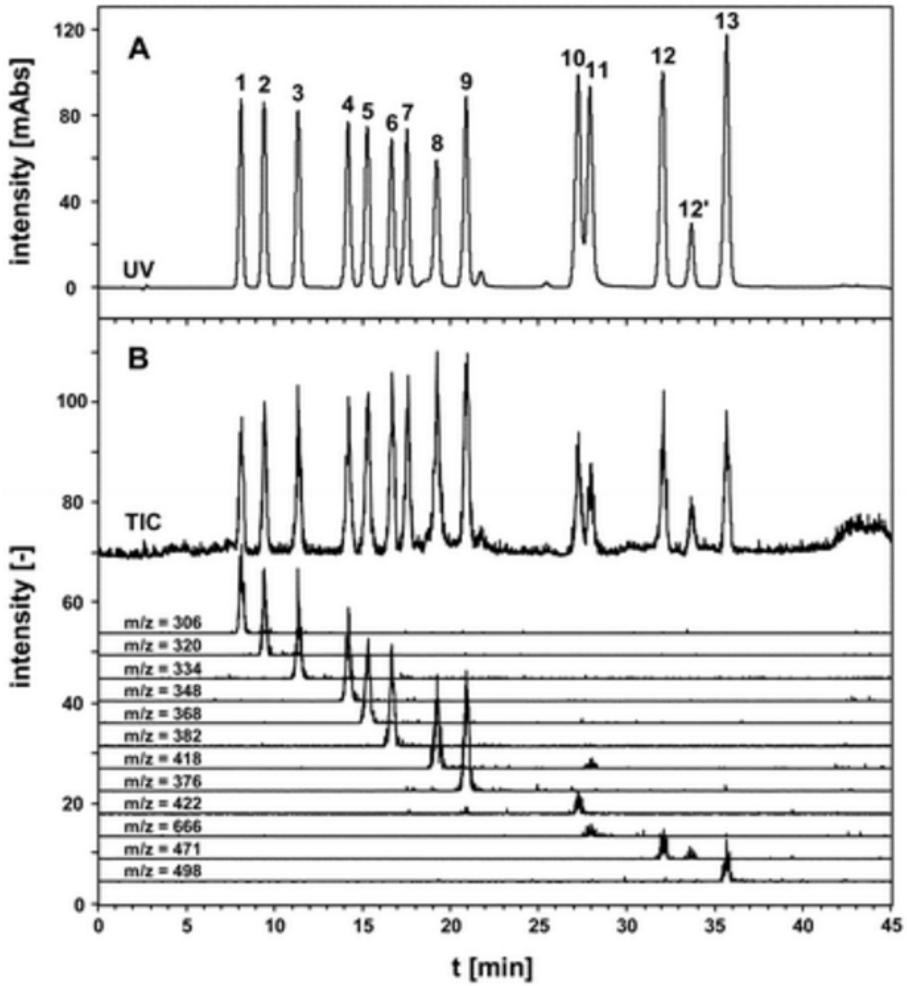
scannen of niet scannen

- scannen = full scan
- selected ion recording (SIM)
- multiple reaction monitoring (SRM, MRM)

weergeven van resultaten

- total ion current (TIC)
- extracted ion chromatogram (XIC)
- base peak chromatogram (BPC)

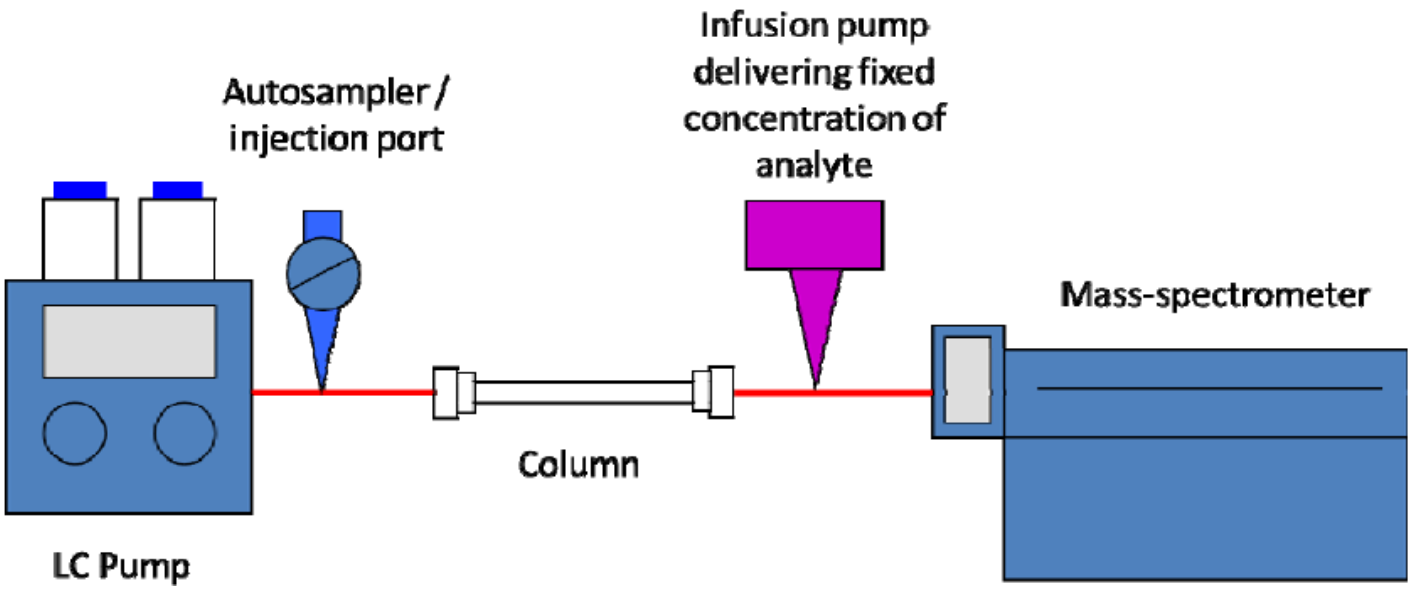
weergeven van resultaten



matrix suppression

- constant infusion
- spiked matrix

matrix suppression

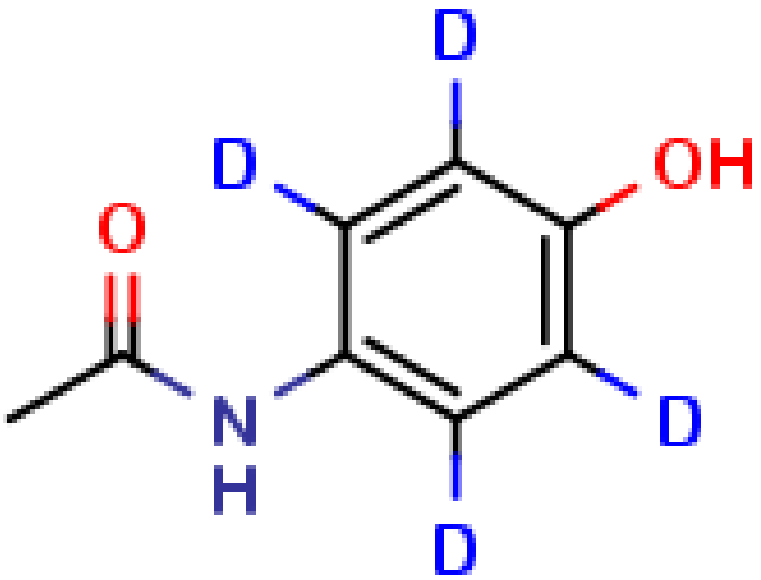


gebruik van stabiele isotopen

inwendige standaard

- zelfde eigenschappen
- zelfde retentietijd
- verschillende massa

gebruik van stabiele isotopen

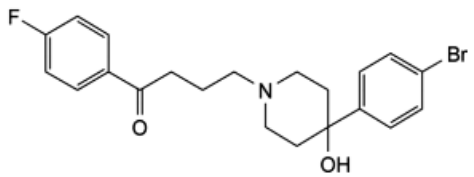


paracetamol-D4

voorbeeld

BROMPERIDOL

Bromperidolum



$C_{21}H_{23}BrFNO_2$ M_r 420.3
[10457-90-6]

DEFINITION

4-[4-(4-Bromophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one.

Content: 99.0 per cent to 101.0 per cent (dried substance).

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 mL with the same solvent.

Reference solution (a). Dissolve 2.5 mg of *bromperidol CRS* and 5.0 mg of *haloperidol CRS* in *methanol R* and dilute to 50.0 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of the test solution to 100.0 mL with *methanol R*. Dilute 1.0 mL of this solution to 10.0 mL with *methanol R*.

Column:

- *size*: *l* = 0.1 m, Ø = 4.0 mm;
- *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

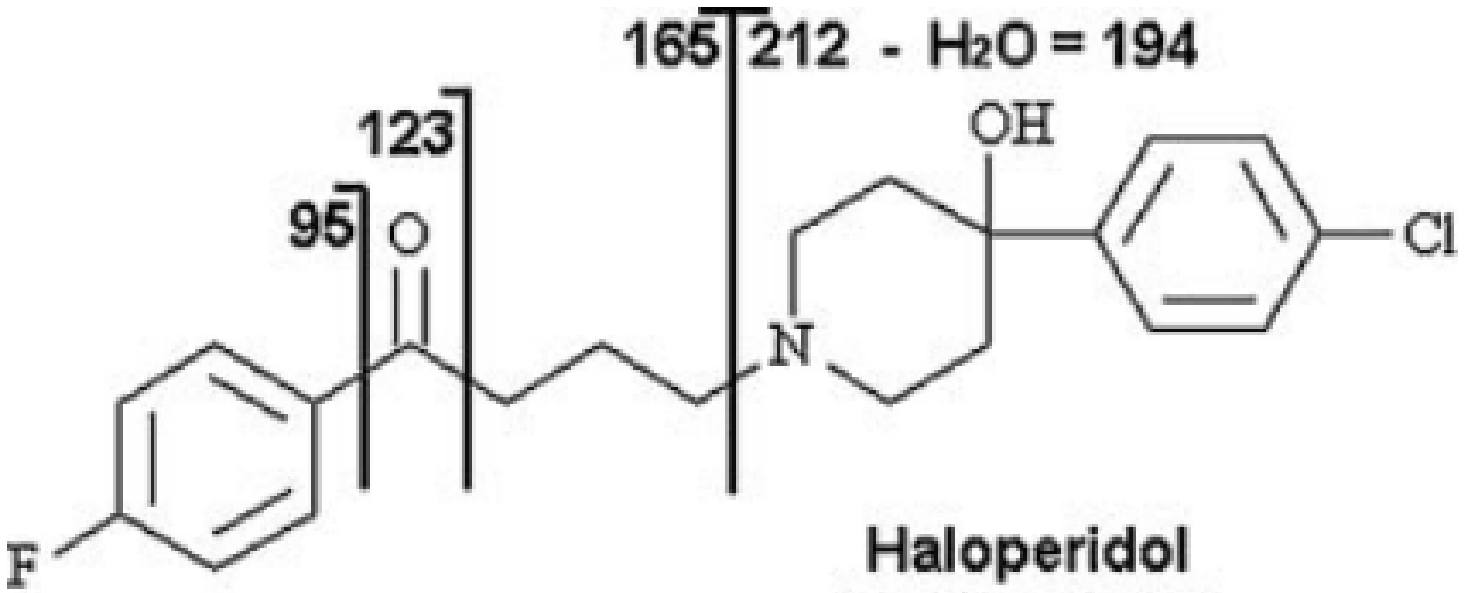
- *mobile phase A*: 17 g/L solution of *tetrabutylammonium hydrogen sulfate R*;
- *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 15	90 → 50	10 → 50
15 - 20	50	50
20 - 25	90	10

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 230 nm.

voorbeeld

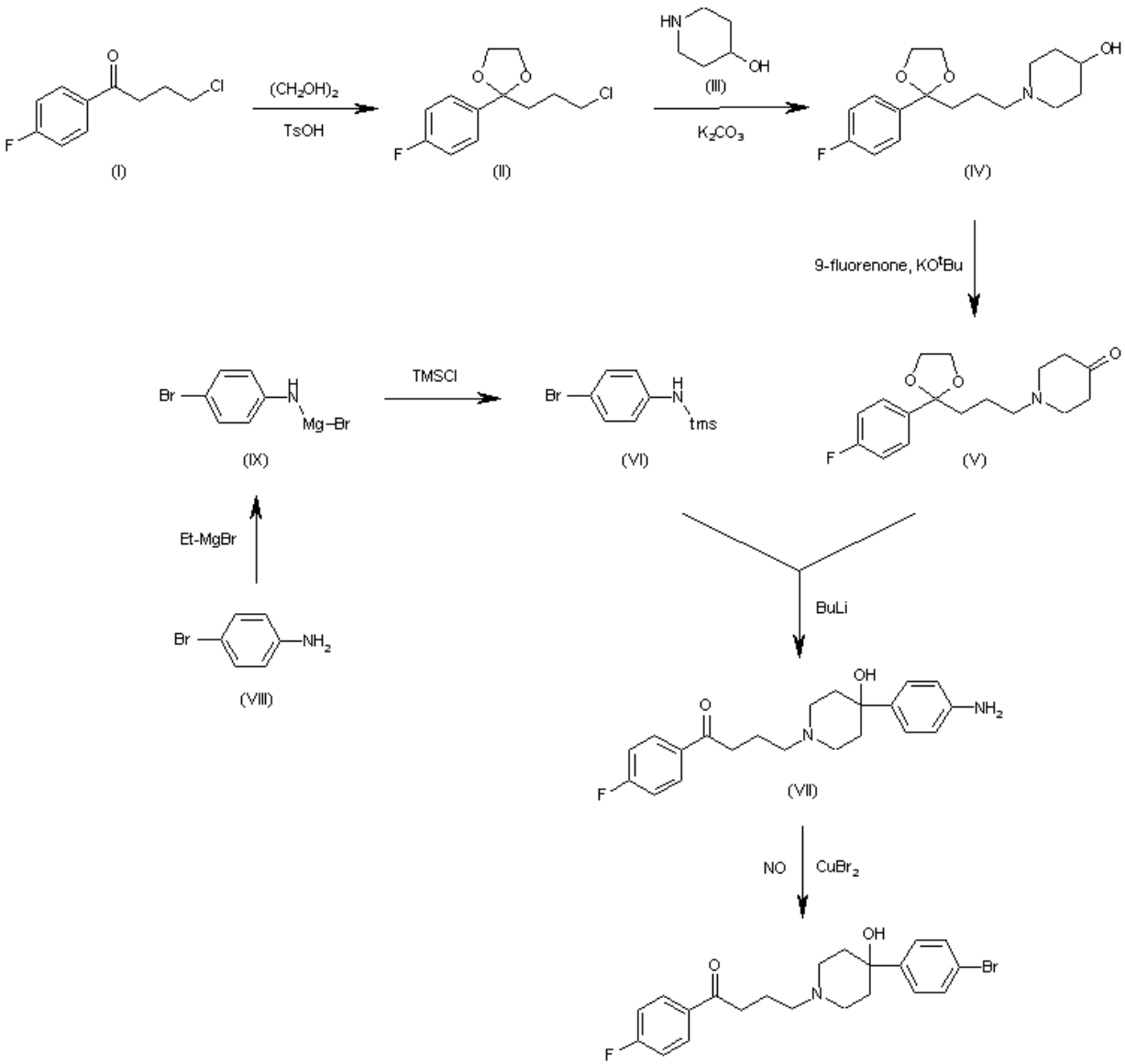


Haloperidol
[M+H], *m/z* 376

voorbeeld

Component	Moederion (m/z)	Cone Voltage (V)	Fragmention (m/z)		Botsingsenergie (eV)	Retentietijd (min)	Tijdsinterval (min)
Amisulpride	370,2	30	149,0	*	50	2,04	1,90 - 2,40
			214,0		50		
Aripiprazol	448,2	30	98,1	*	50	4,94	4,80 - 5,20
			285,1		40		
Aripiprazol-d8	456,2	35	293,1		45	4,92	4,75 - 5,20
Bromperidol	420,1	35	123,0	*	60	4,52	4,20 - 4,70
			165,1		30		
Clozapine	327,1	50	84,0	*	30	4,07	3,80 - 4,30
			192,0		40		
Clozapine-d4	333,2	30	88,1		30	4,03	3,80 - 4,30
Desmethylclozapine	313,2	30	227,0	*	40	3,91	3,70 - 4,10
			270,1		30		

voorbeeld


(I)
(CH2OH)2
TsOH
(II)
HN
OH
(III)
K2CO3
(IV)
9-fluorenone, KOtBu
(V)
Br
N
H
Mg–Br
(IX)
TMSCl
Br
N
H
tms
(VI)
Et-MgBr
Br
NH2
(VIII)
BuLi
OH
NH2
(VII)
NO
CuBr2
OH
Br

voorbeeld

4-Bromoaniline-2,3,5,6-d_4

p-Bromoaniline; 1-Amino-2-bromobenzene

98 atom % D

$BrC_6D_4NH_2$