

massaspectrometrie

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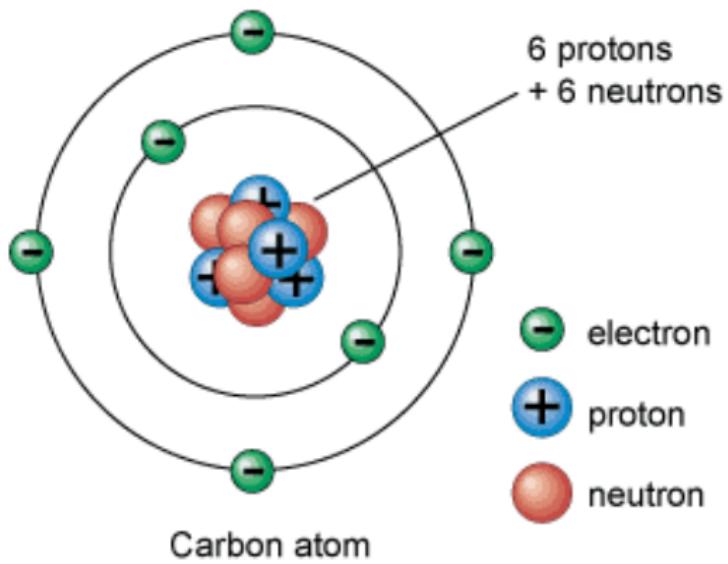
link

<http://mass.rega.kuleuven.be/industry>

massa van een atoom

- ▶ elektronen
- ▶ protonen
- ▶ neutronen

massa van een atoom



massa van een atoom

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

massa van een atoom

- ▶ Neutron = $1.6749286 \times 10^{-27}$ kg (939.56563 MeV)
- ▶ Proton = $1.6726231 \times 10^{-27}$ kg (938.27231 MeV)
- ▶ Elektron = $9.1093897 \times 10^{-31}$ 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

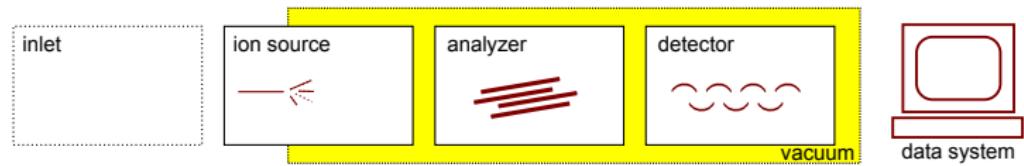
stappen in kwantitatieve analyse

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

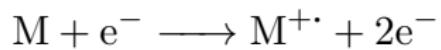
analyse met een massaspectrometer

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

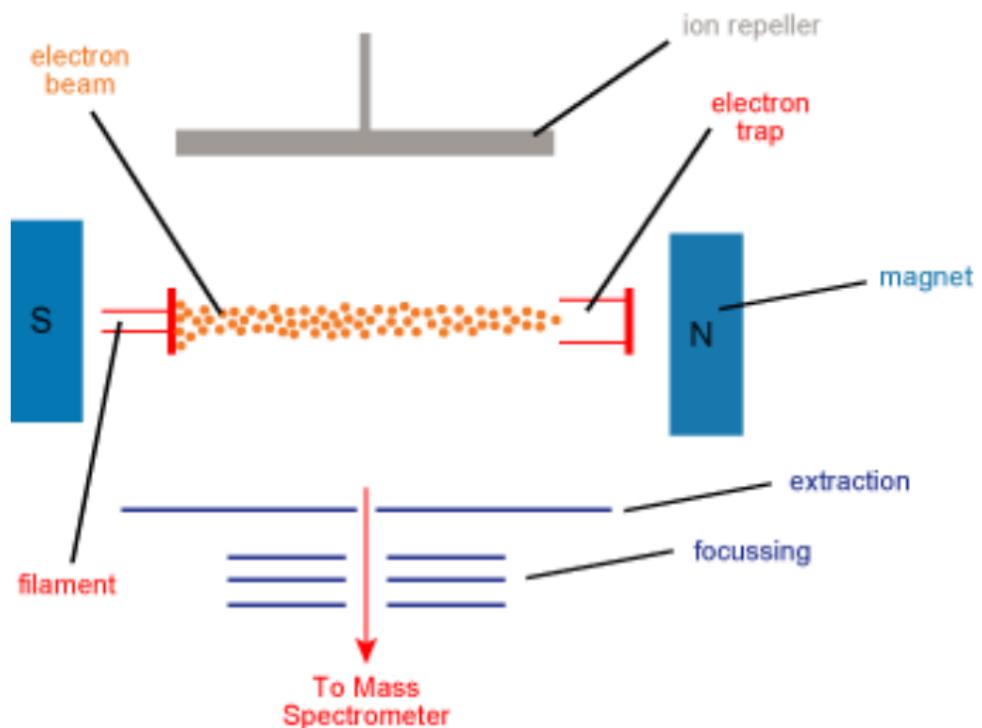
ionisatie



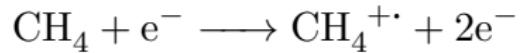
ionisatie - EI



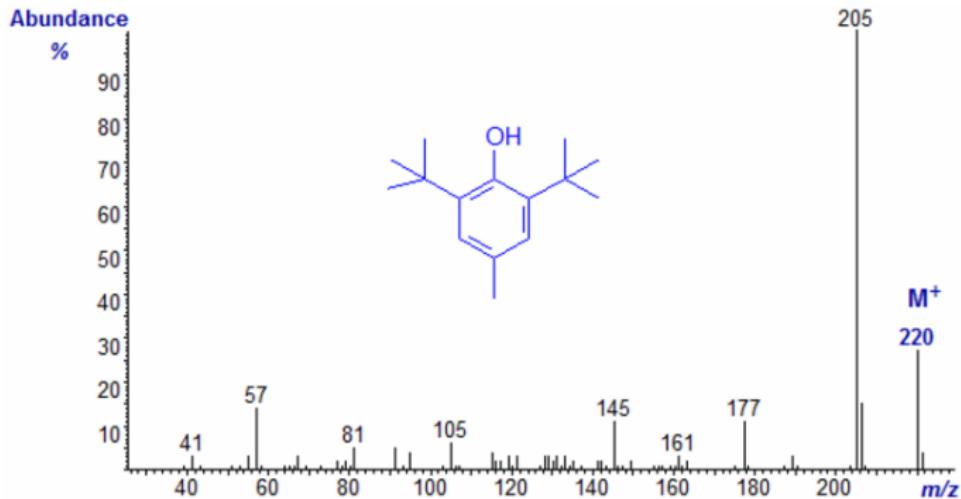
ionisatie - EI



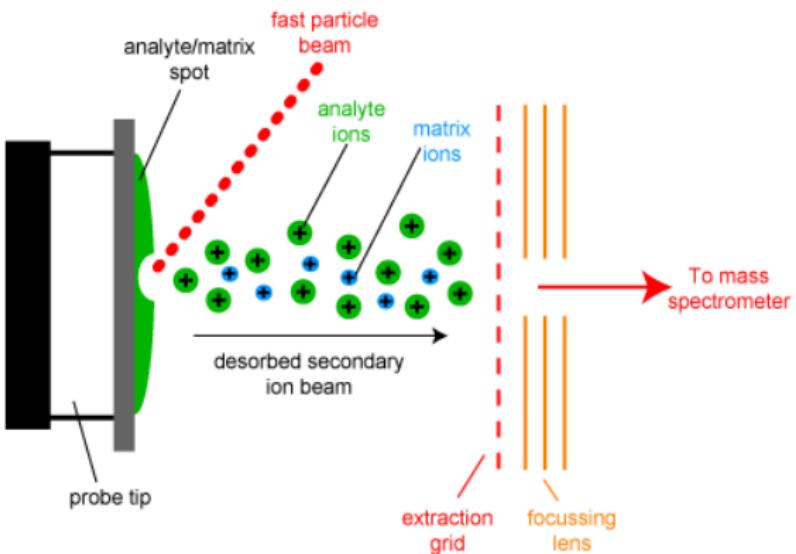
ionisatie - Cl



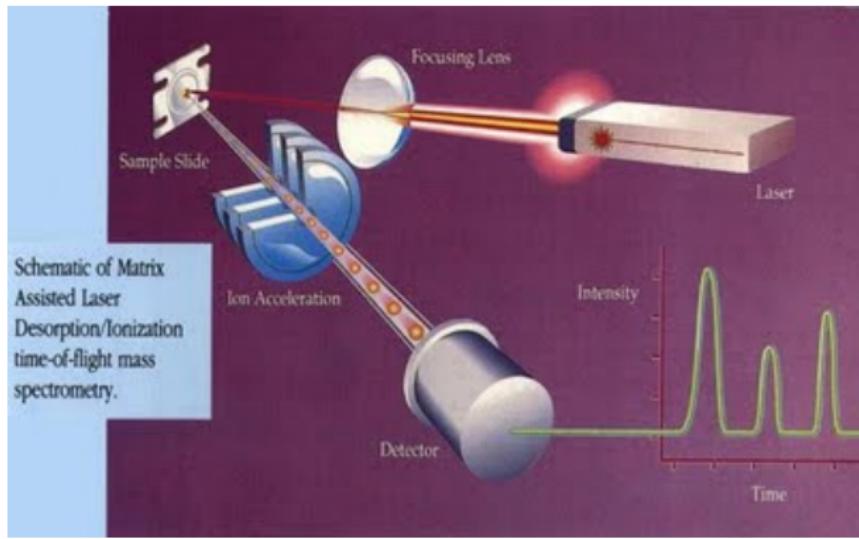
ionisatie - EI/CI spectrum



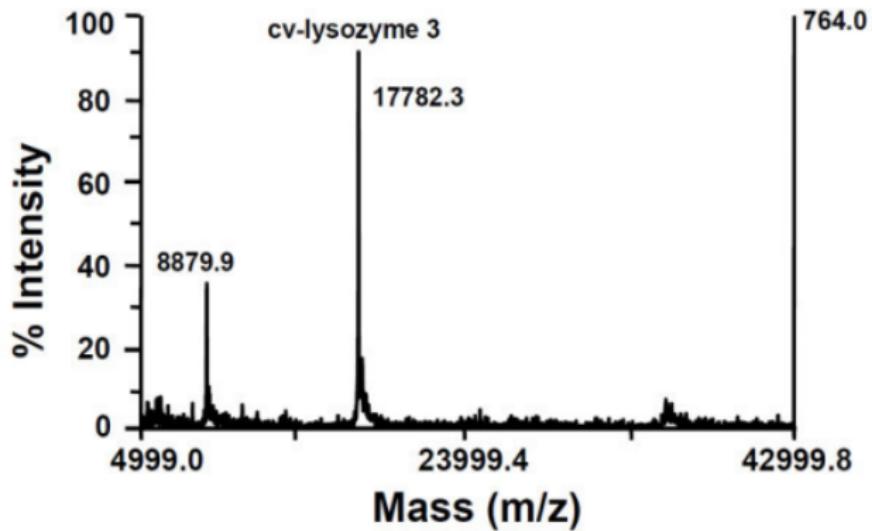
ionisatie - FAB



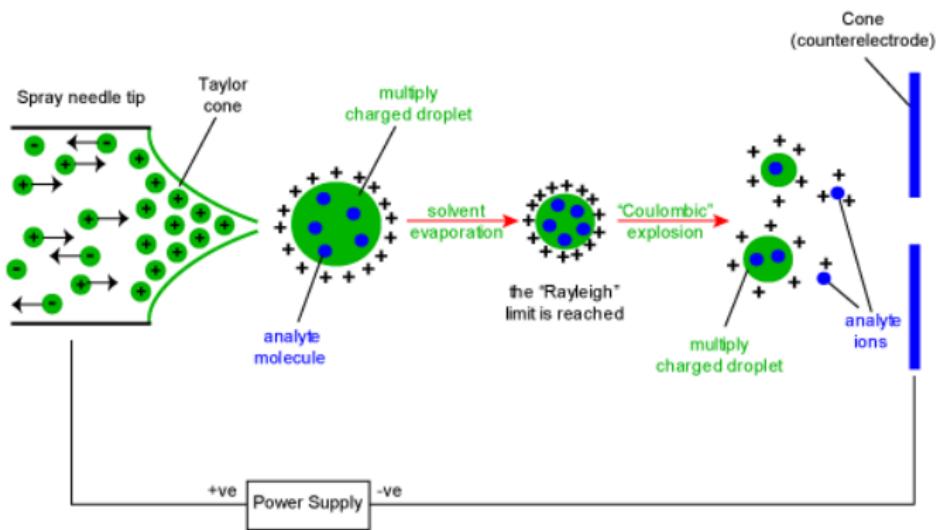
ionisatie - MALDI



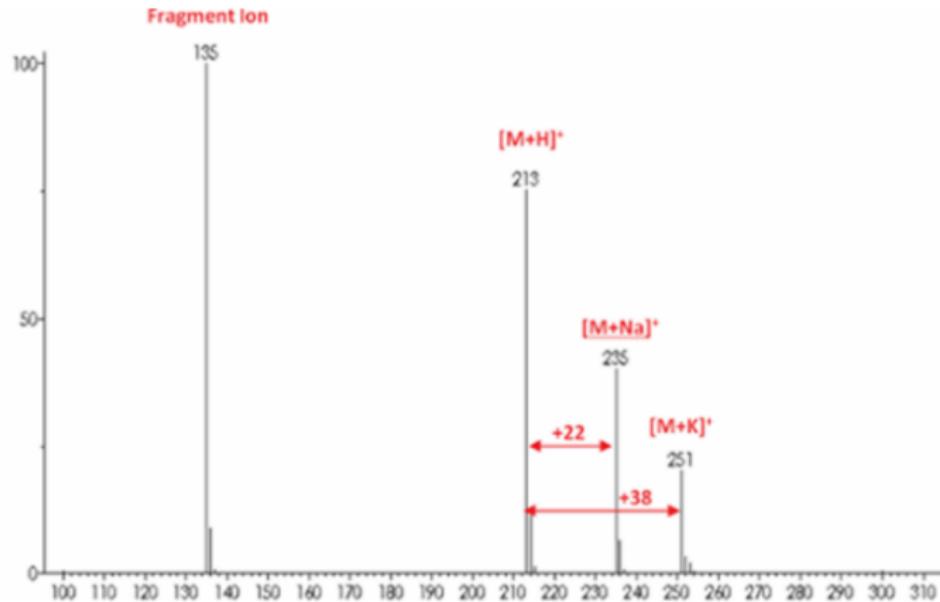
ionisatie - MALDI spectrum



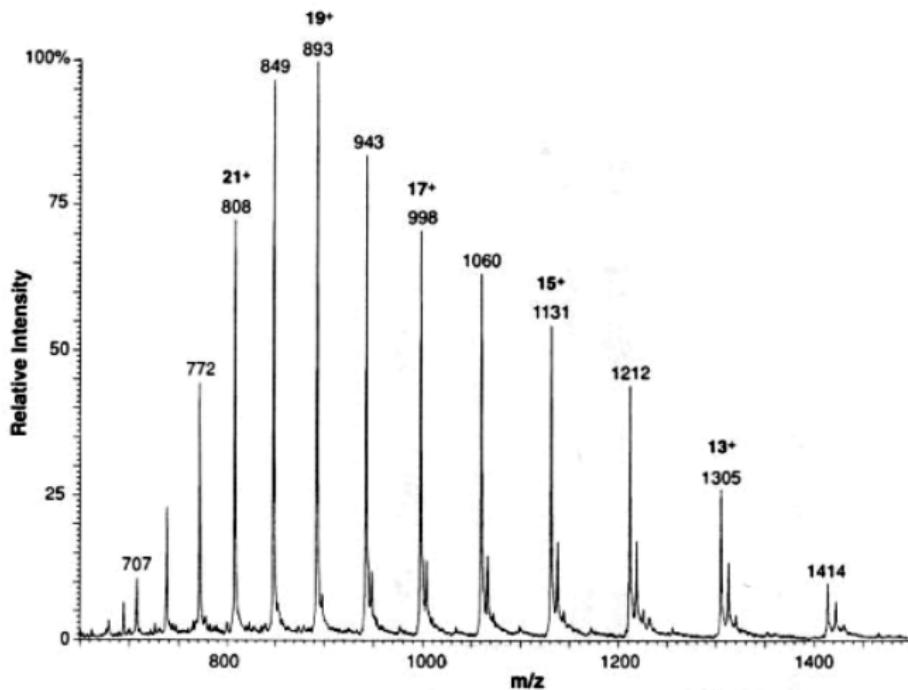
ionisatie - elektrospray



ionisatie - ESI spectrum



ionisatie - elektrospray



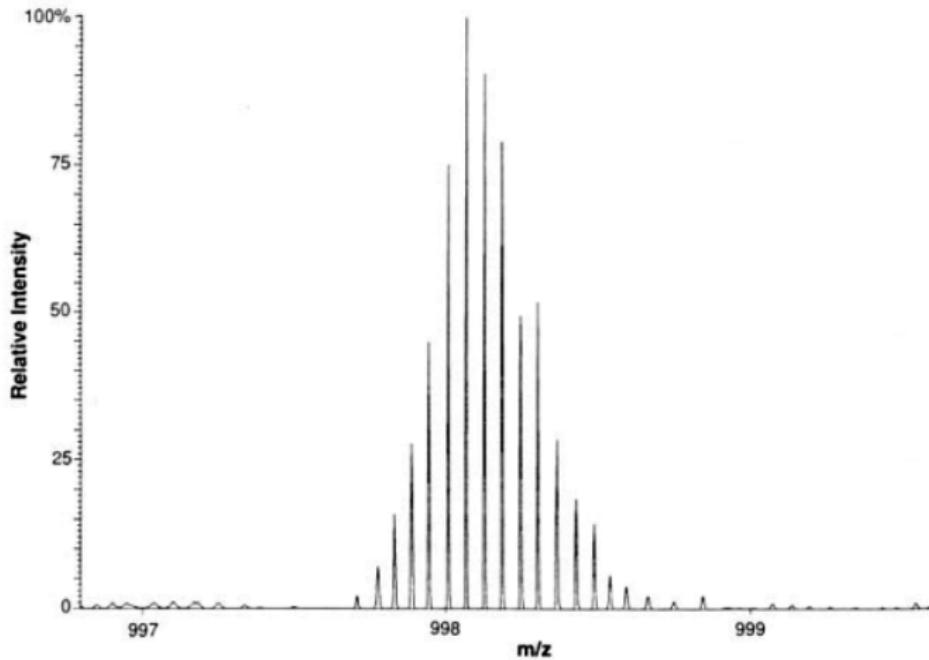
ionisatie - elektrospray

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

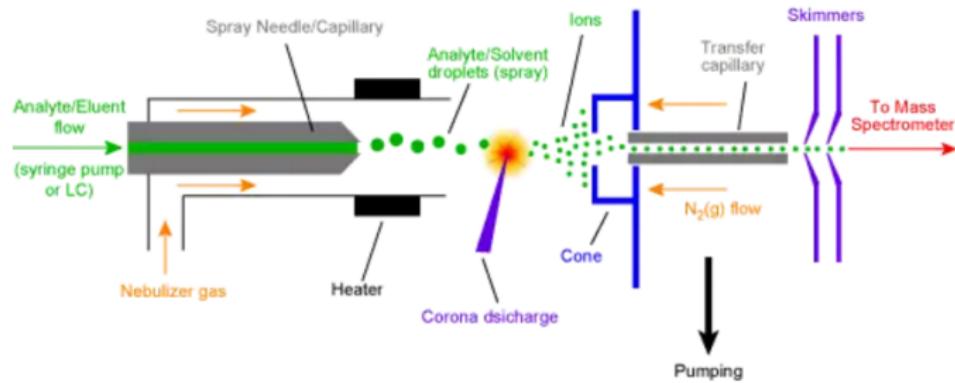
$$M = n_1(m_1 - mA) = n_2(m_2 - mA)$$

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

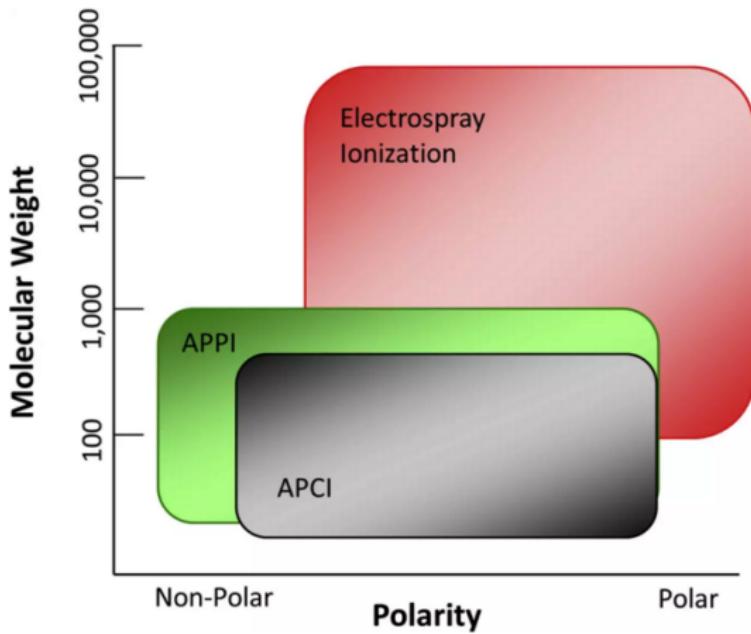
ionisatie - elektrospray



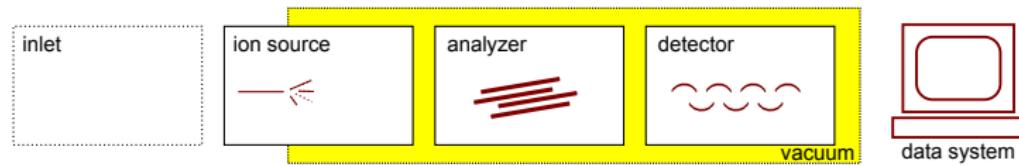
ionisatie - APCI



ionisatie



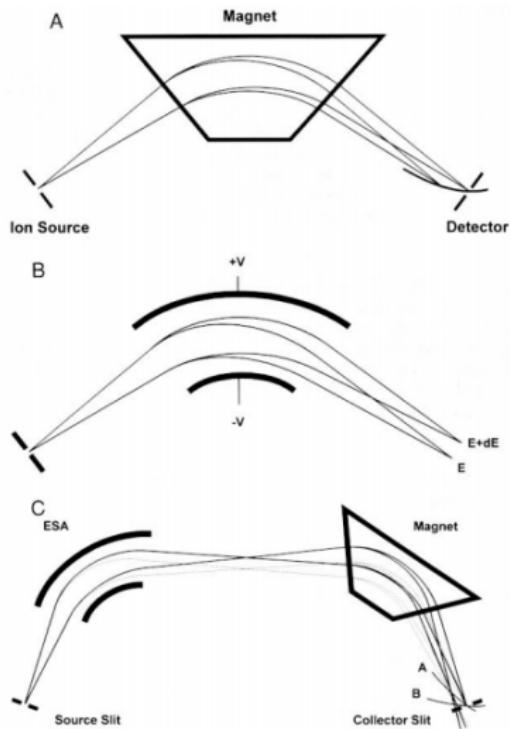
massa analysatoren



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



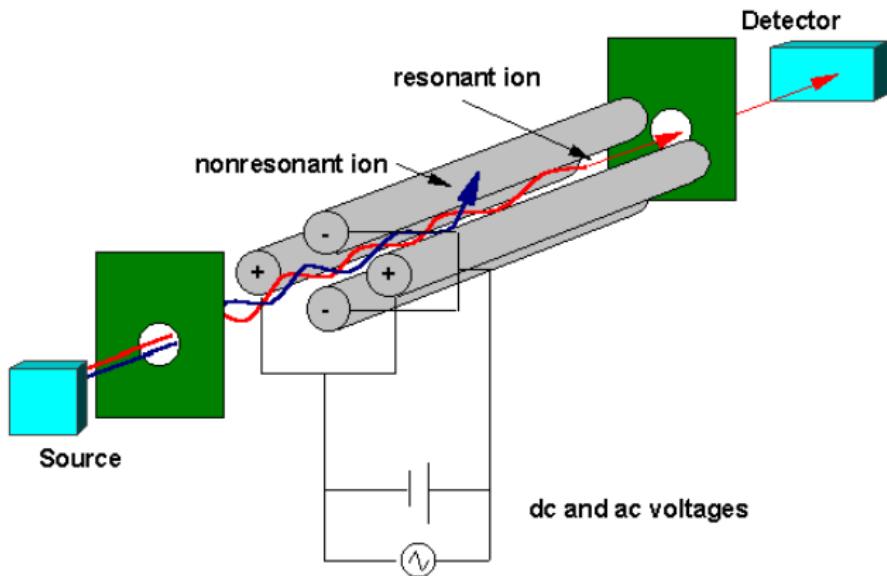
massa analysatoren

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

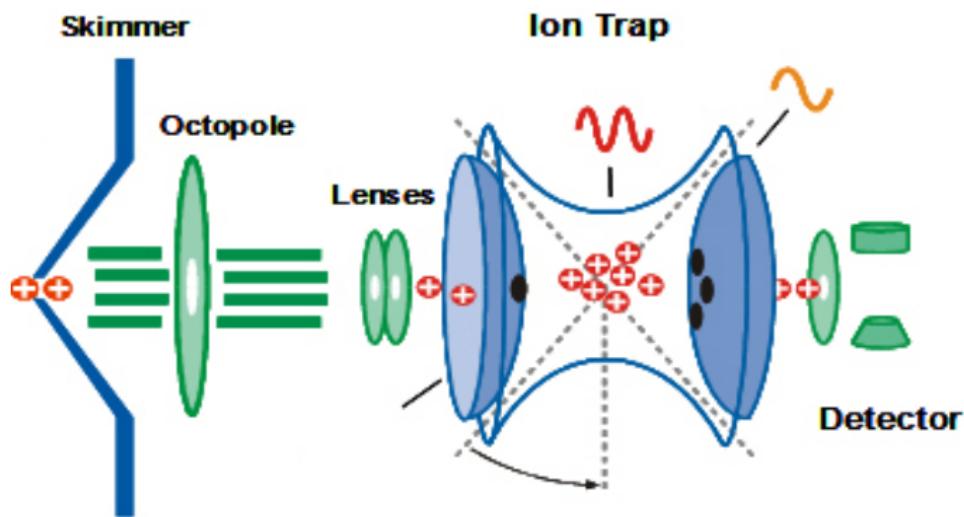
$$F = qvB = mv^2/r$$

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

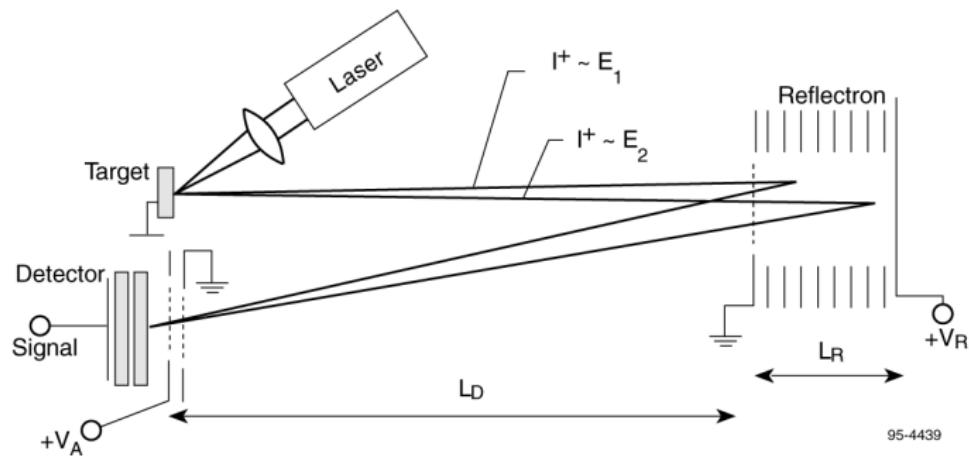
massa analysatoren - quadrupool



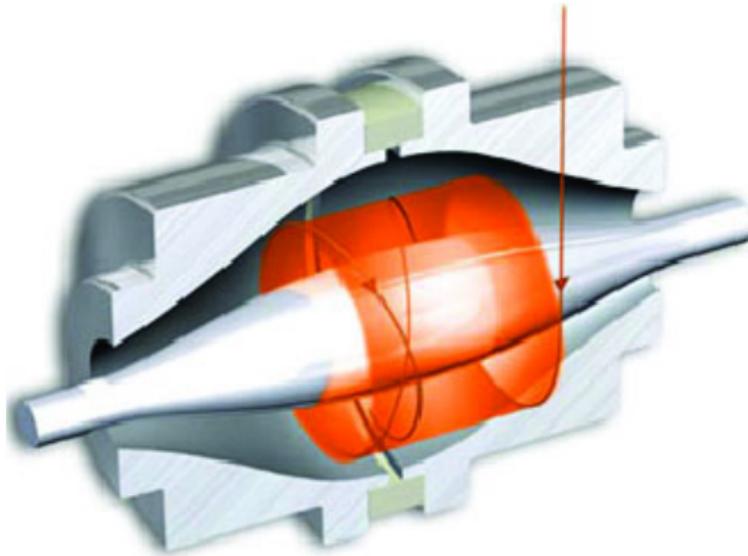
massa analysatoren - ion trap



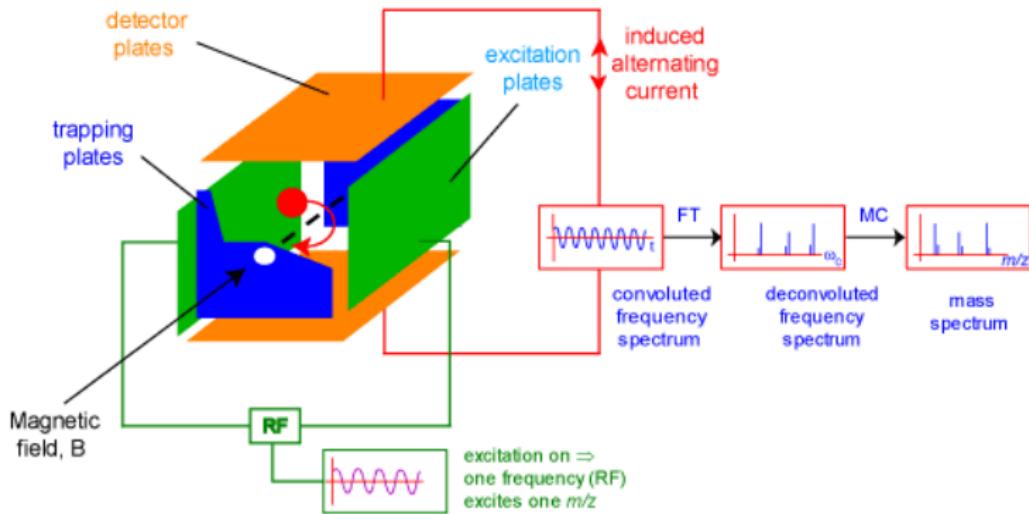
massa analysatoren - time of flight



massa analysatoren - orbitrap



massa analysatoren - FT-ICR



waarom LC/MS

voordelen:

- ▶ selectiviteit
- ▶ snelheid
- ▶ gevoeligheid

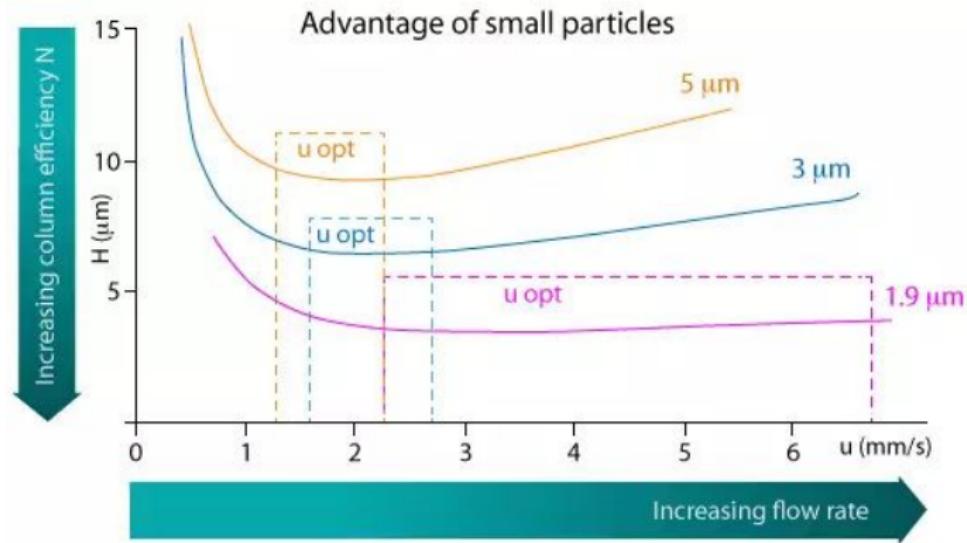
nadelen:

- ▶ kostprijs
- ▶ complexiteit
- ▶ dynamische range
- ▶ overdreven selectiviteit

HPLC methode optimaliseren

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

debiet - partikelgrootte



debiet - kolomdiameter

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

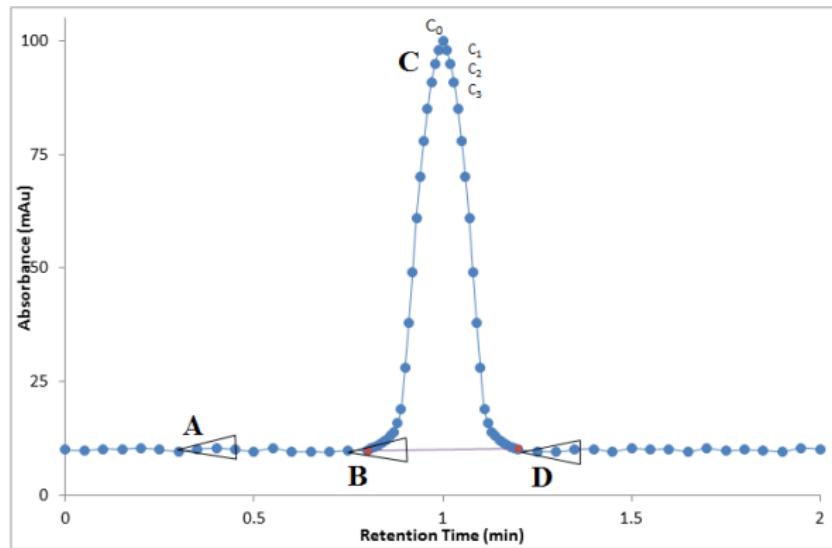
debiet - kolomdiameter

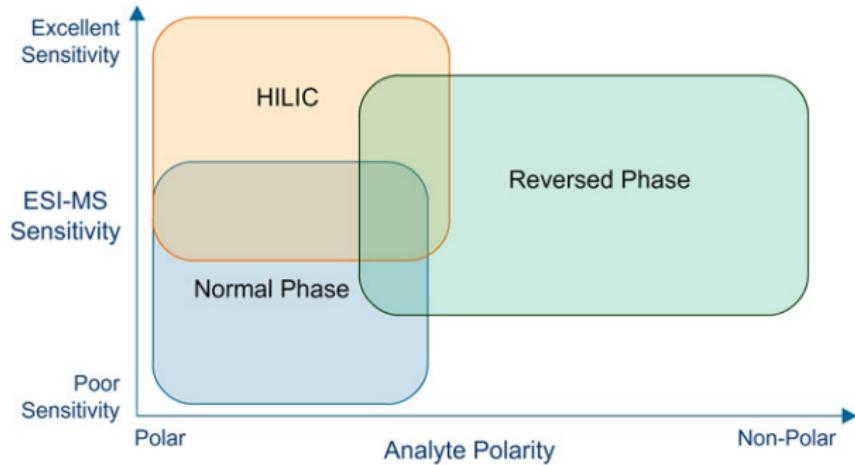
Column i.d. (μm)	Flow Rate ($\mu\text{L/min}$)	Classification
4600	1000	Analytical
2100	208	Narrowbore
1000	47	Microbore
500	12	Microbore
320	5	Microcolumn
250	3	Microcolumn
50	0.12	Nanolumn/ Packed Capillary

debiet - kolomdiameter

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

piekbreedte - datapoints





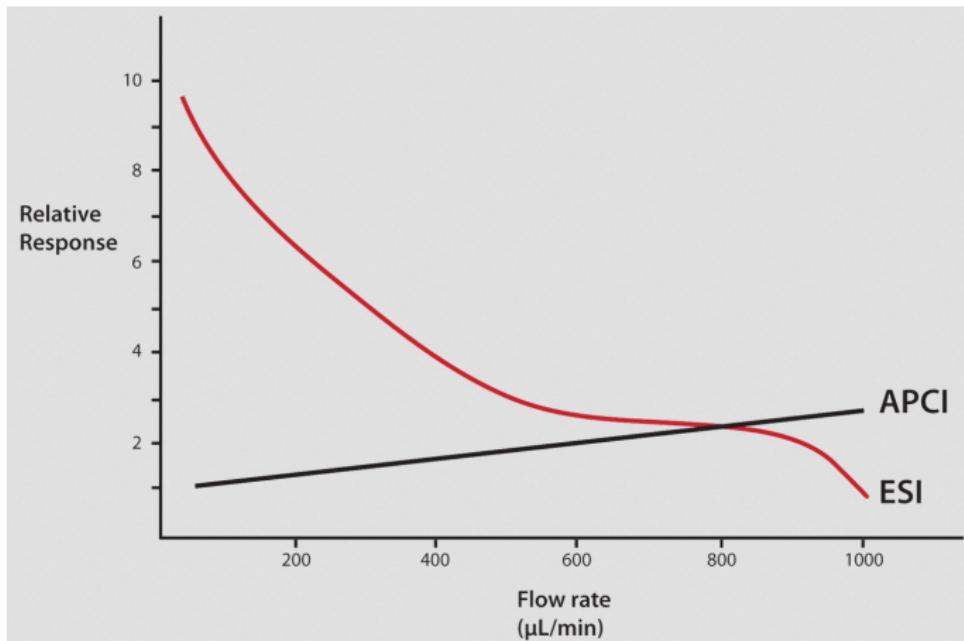
buffers

- 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 quadrupole

- ▶ toestellen met 1 analysator
- ▶ tandem massaspectrometers

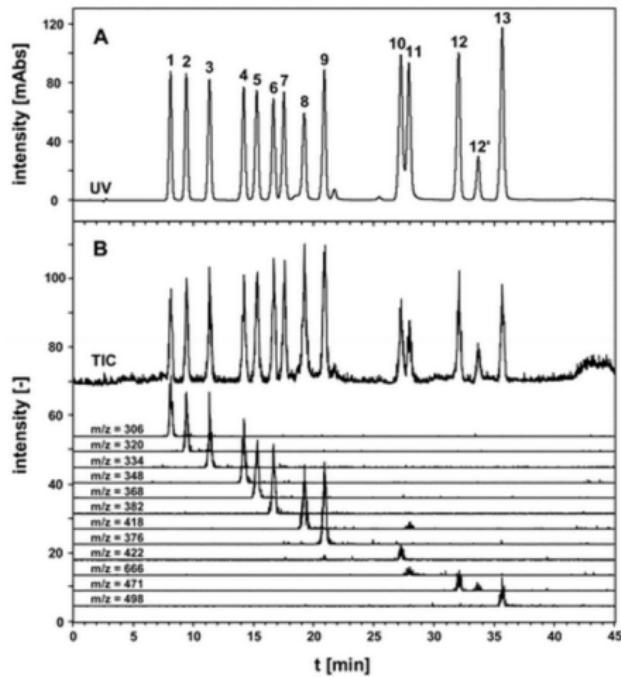
scannen of niet scannen

- ▶ 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)

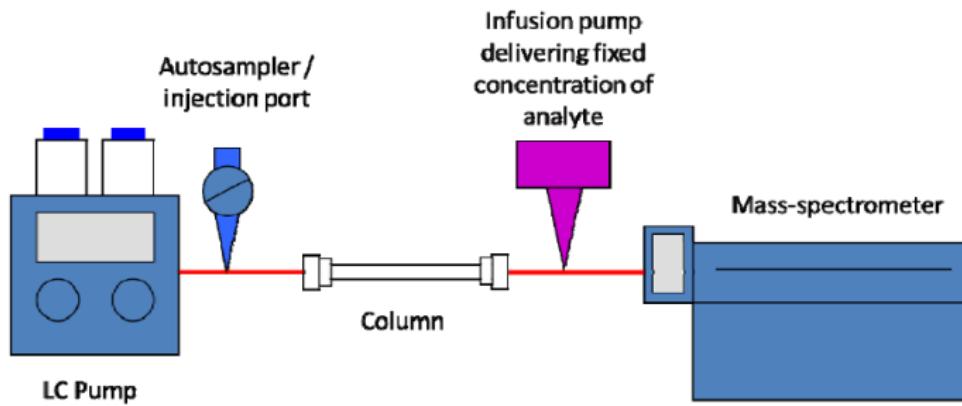
weergeven van resultaten



matrix suppression

- ▶ constant infusion
- ▶ spiked matrix

matrix suppression

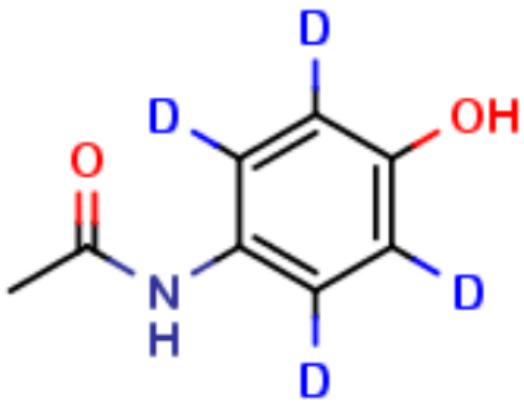


gebruik van stabiele isotopen

inwendige standaard

- ▶ zelfde eigenschappen
- ▶ zelfde retentietijd
- ▶ verschillende massa

gebruik van stabiele isotopen

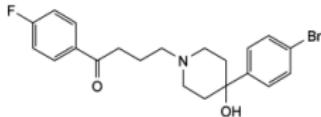


paracetamol-D4

voorbeeld

BROMPERIDOL

Bromperidolum



C₂₁H₂₃BrFNO₂
[10457-90-6]

M_r 420.3

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: $l = 0.1 \text{ m}$, $\varnothing = 4.0 \text{ mm}$;
- stationary phase: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 μm).

Mobile phase:

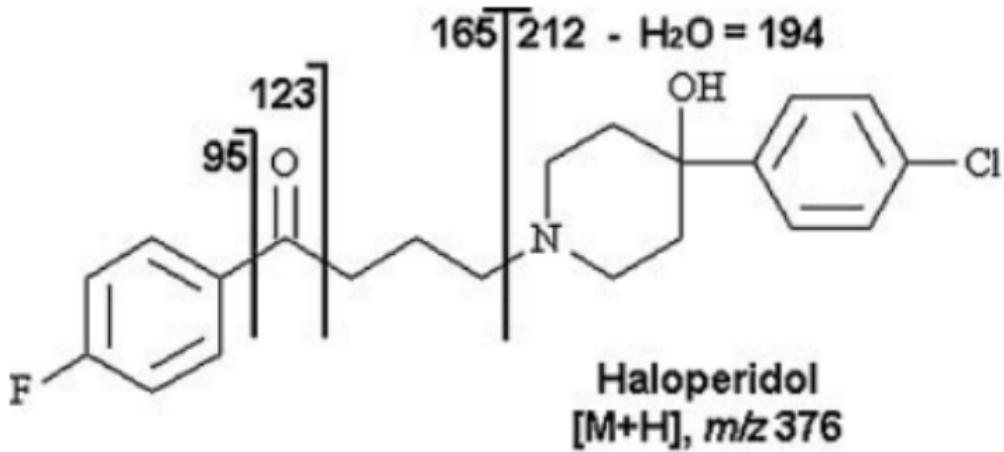
- mobile phase A: 17 g/L solution of *tetrabutylammonium hydrogen sulfate R*;
- mobile phase B: *acetonitrile R*;

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

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 230 nm.

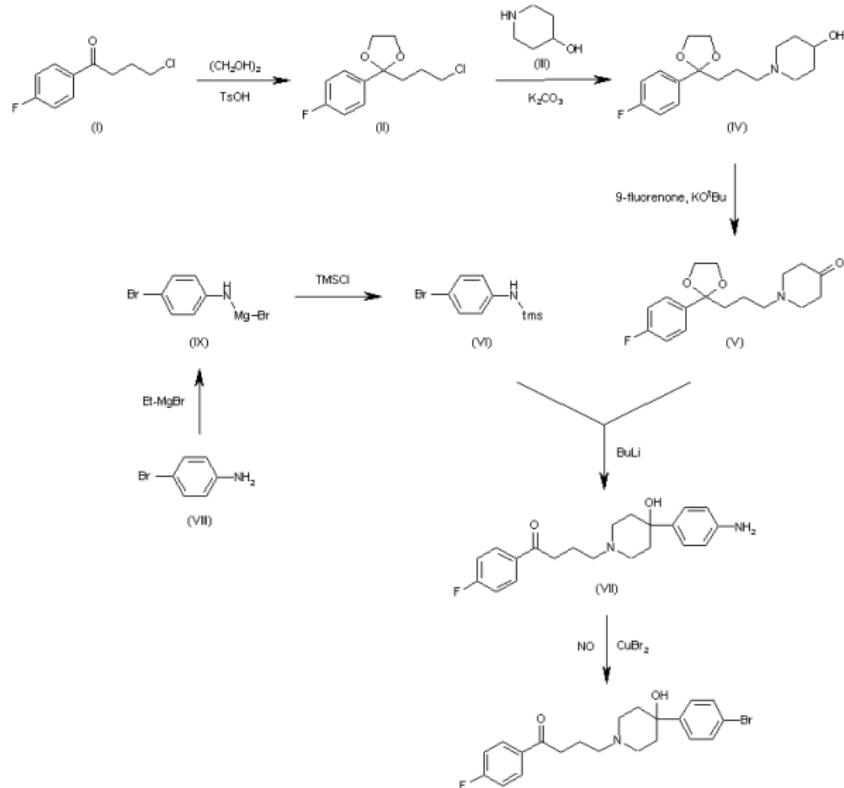
voorbeeld



voorbeeld

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

voorbeeld



voorbeeld

4-Bromoaniline-2,3,5,6-d₄

p-Bromoaniline; 1-Amino-2-bromobenzene

98 atom % D

BrC₆D₄NH₂

