

# massaspectrometrie

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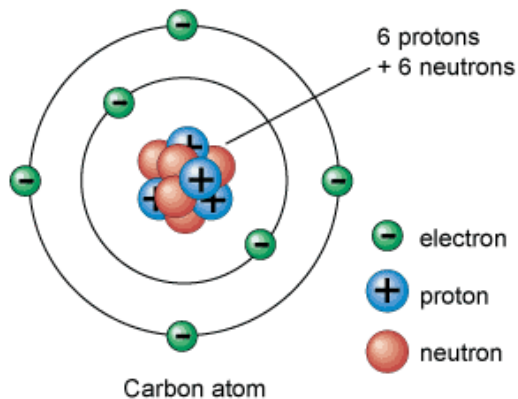
2023

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

# massa van een atoom

- ▶ elektronen
- ▶ protonen
- ▶ neutronen

# massa van een atoom



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

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

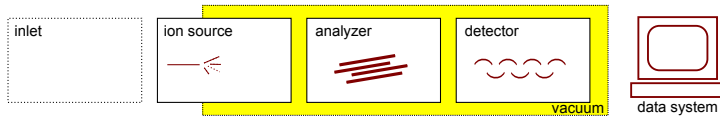
gemiddelde atomaire massa = som van de gewogen isotoopmassa's

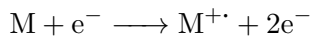
# stappen in kwantitatieve analyse

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

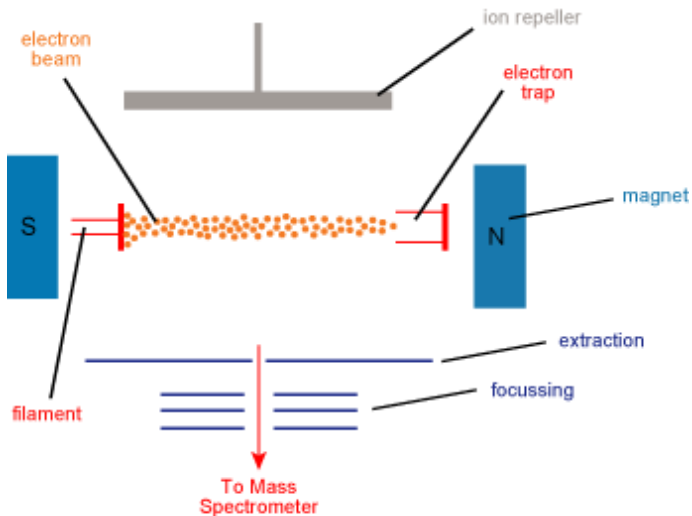
# analyse met een massaspectrometer

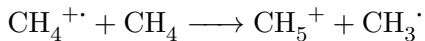
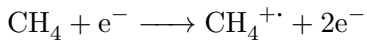
- ▶ inbrengen staal
- ▶ ionisatie
- ▶ massa analyse
- ▶ ion detectie
- ▶ data verwerking



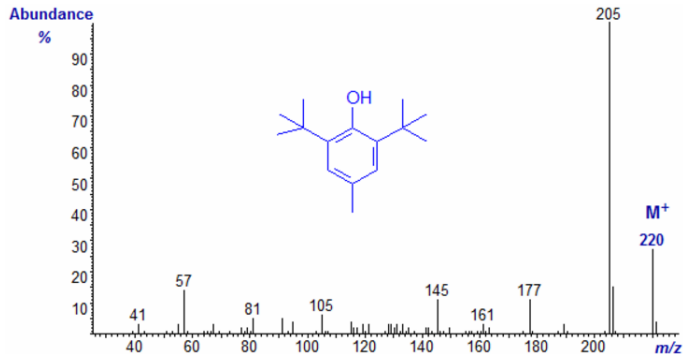


# ionisatie - EI



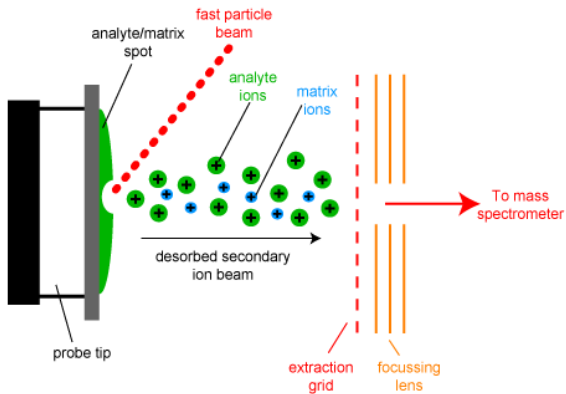


# ionisatie - EI/CI spectrum

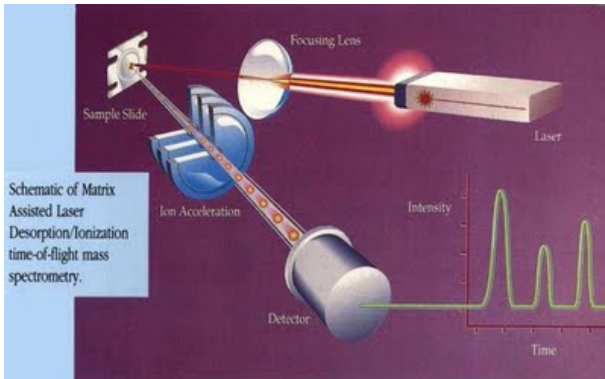




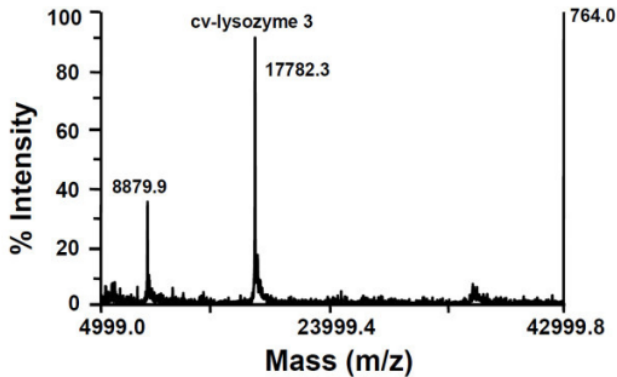
# ionisatie - FAB



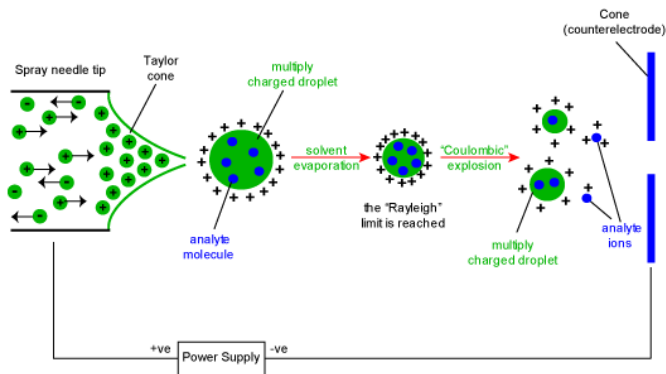
# ionisatie - MALDI



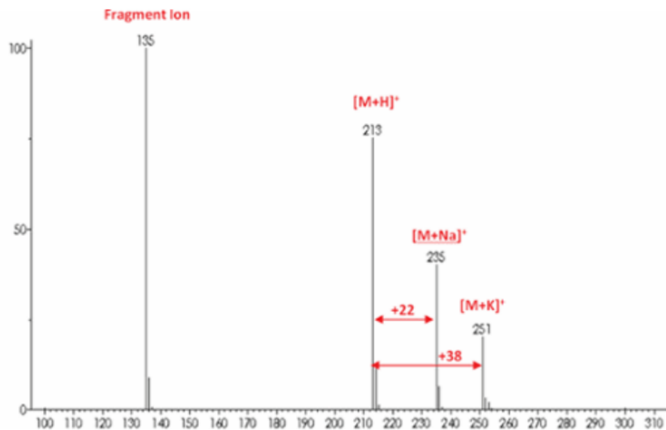
# ionisatie - MALDI spectrum



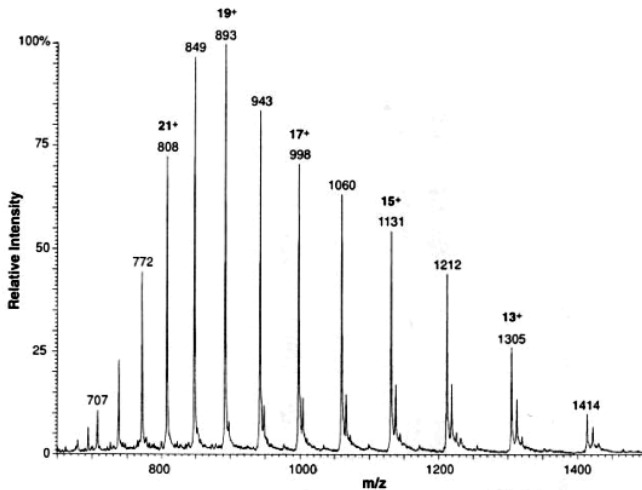
# ionisatie - elektrospay



# ionisatie - ESI spectrum



# ionisatie - elektropray

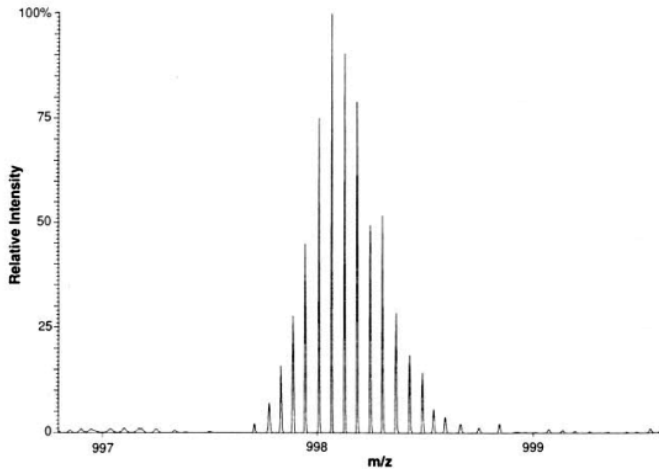


Indien  $m_1 < m_2$  en  $n_2 = n_1 - 1$  dan is

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

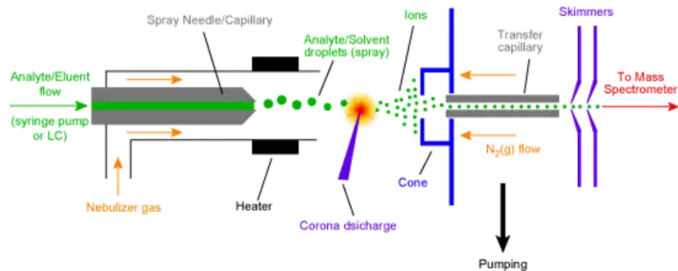
$$n_2 = (m_1 - m_A)/(m_2 - m_1)$$

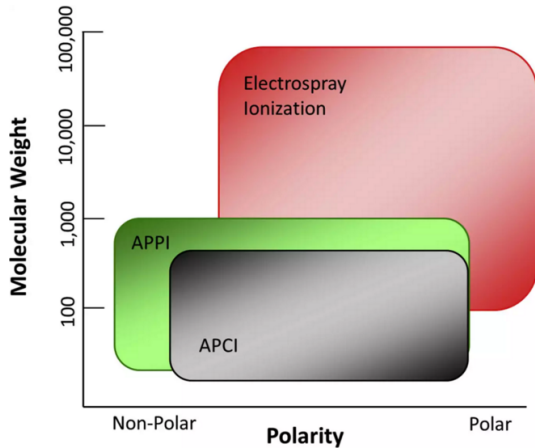
# ionisatie - elektropray



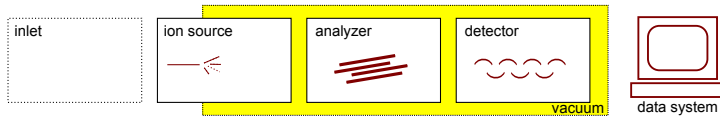


# ionisatie - APCI



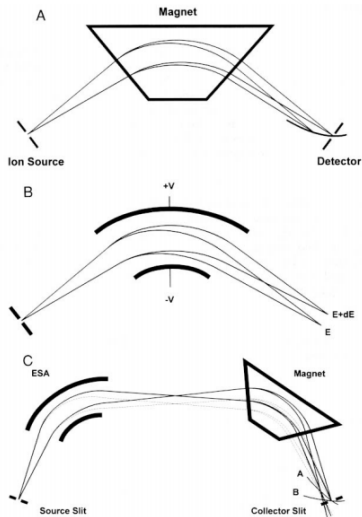


# massa analysatoren



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

# massa analysatoren - sector instrument

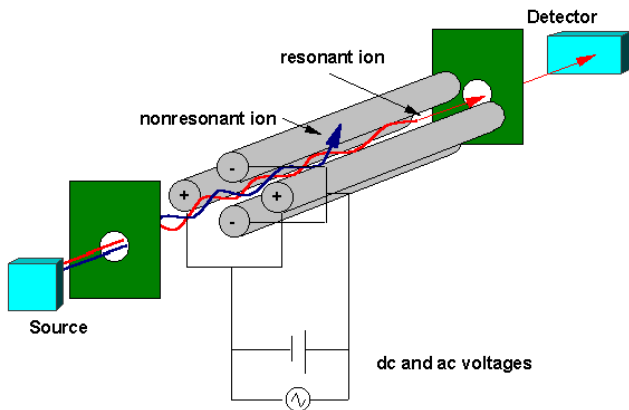


$$E_{kin} = qV_{acc} = mv^2/2$$

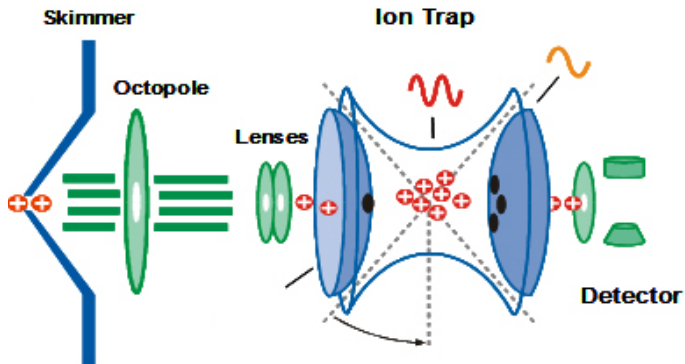
$$F = qvB = mv^2/r$$

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

# massa analysatoren - quadrupool

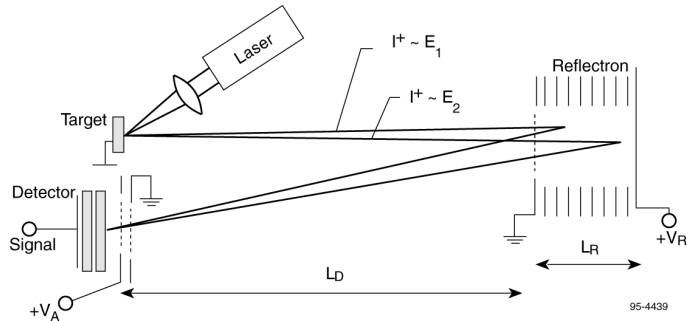


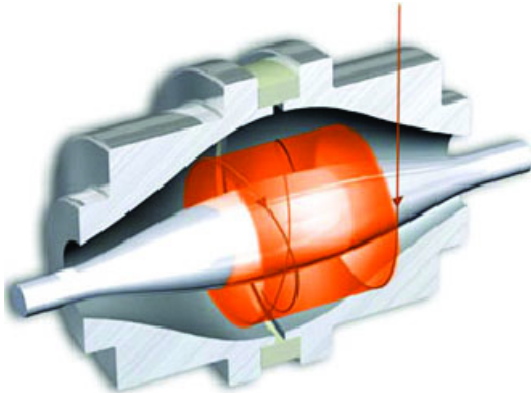
# massa analysatoren - ion trap



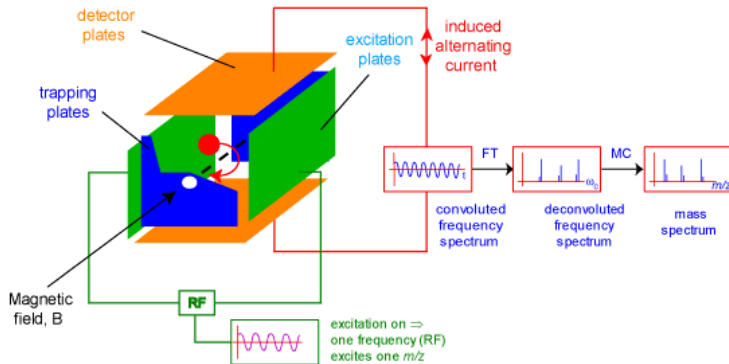


# massa analysatoren - time of flight





# massa analysatoren - FT-ICR



voordelen:

- ▶ selectiviteit
- ▶ snelheid
- ▶ gevoeligheid

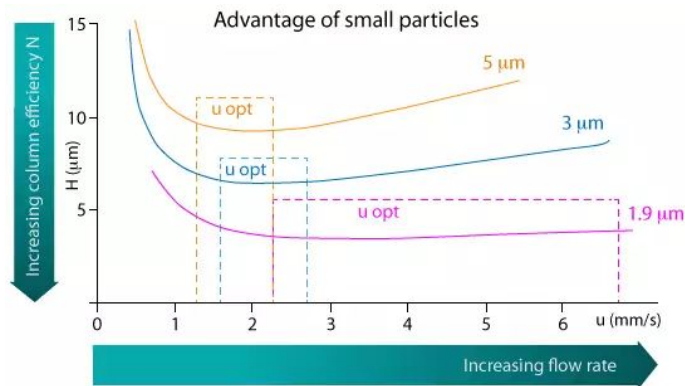
nadelen:

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

# HPLC methode optimaliseren

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

# debiet - partikelgrootte



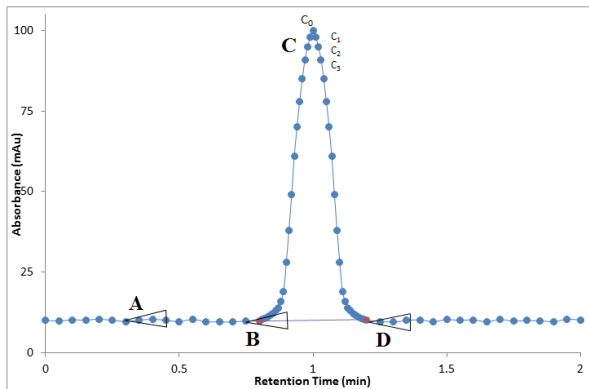
$$F_{C2} = \left( \frac{d_{c2}}{d_{c1}} \right)^2 \times F_{C1}$$

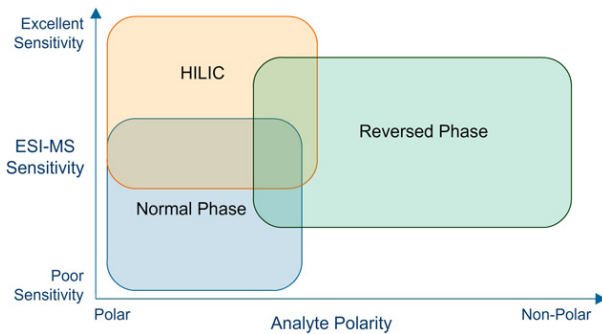
<b>Column i.d. (<math>\mu\text{m}</math>)</b>	<b>Flow Rate (<math>\mu\text{L}/\text{min}</math>)</b>	<b>Classification</b>
4600	1000	Analytical
2100	208	Narrowbore
1000	47	Microbore
500	12	Microbore
320	5	Microcolumn
250	3	Microcolumn
50	0.12	Nanocolumn/ Packed Capillary



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

# piekbreedte - datapoints



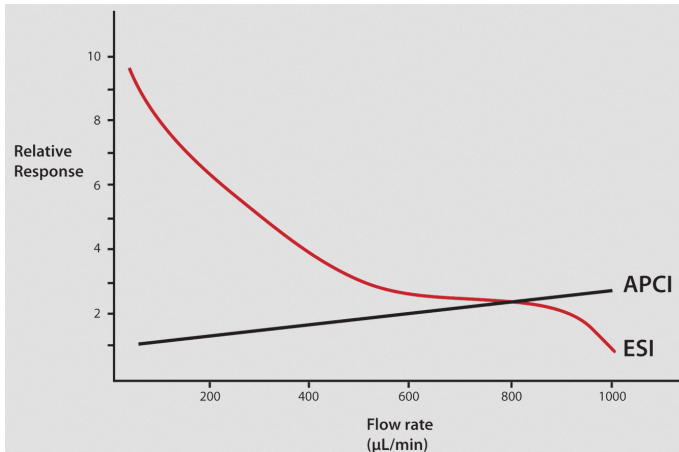


- Buffer concentrations below 25 mM (best below 10 mM)
- Poor compatibility with non-volatile buffers
  - Deposit buildup
  - Metal ion buffers interfere with ionization
- Acidic mobile phases generally favor positive mode ionization
  - 0.1% - 1% formic acid, 0.1% - 1% acetic acid, 0.05% - 0.2% TFA
  - 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
- ▶ elektropray: 1uL - 500uL/min
- ▶ APCI: 100uL - 2000uL/min

# single of triple quadrupole

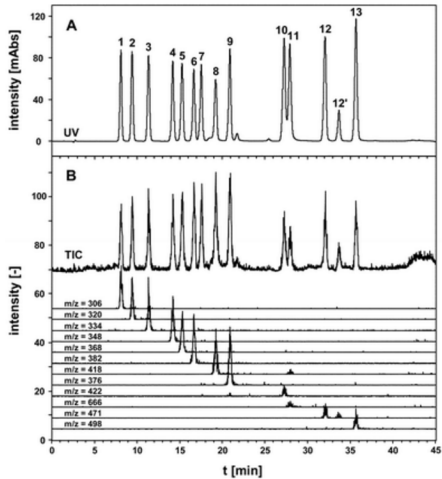
- ▶ toestellen met 1 analysator
- ▶ tandem massaspectrometers



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

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

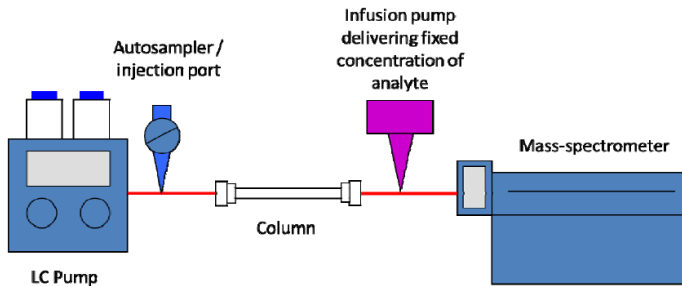
# weergeven van resultaten



# matrix suppression

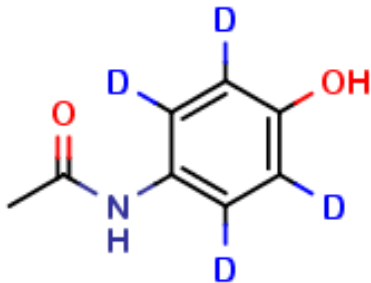
- ▶ constant infusion
- ▶ spiked matrix

# matrix suppression



inwendige standaard

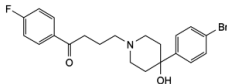
- ▶ zelfde eigenschappen
- ▶ zelfde retentietijd
- ▶ verschillende massa



paracetamol-D4

## BROMPERIDOL

Bromperidolum



$C_{21}H_{23}BrFO_2$   
[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:  $l = 0.1$  m,  $\varnothing = 4.0$  mm;
- stationary phase: base-deactivated octadecylsilyl silica gel for chromatography R (3  $\mu$ m).

*Mobile phase:*

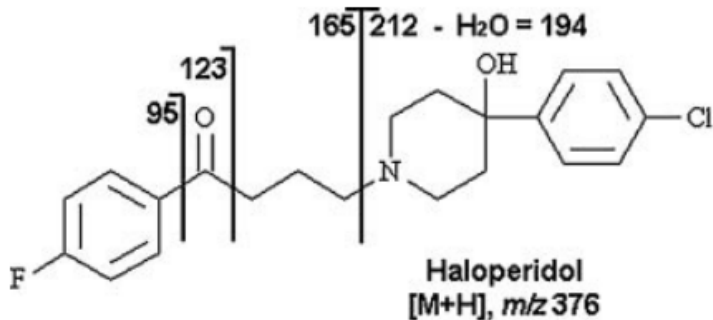
- mobile phase A: 17 g/L solution of tetrabutylammonium hydrogen sulfate R;
- mobile phase B: acetonitrile R;

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.

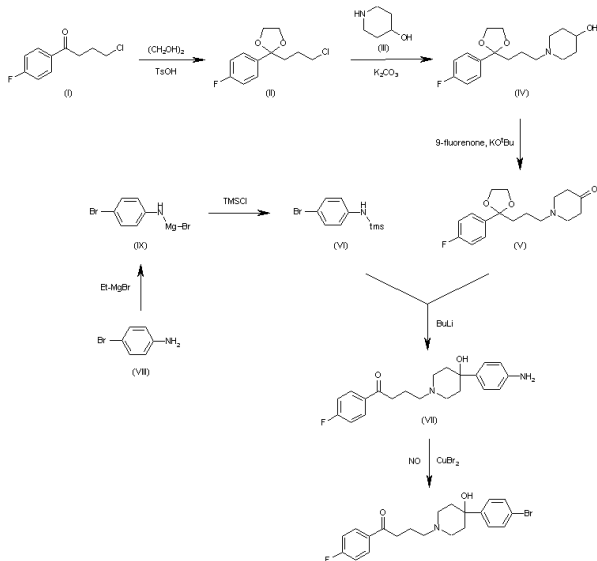
*Detection:* spectrophotometer at 230 nm.





Component	Mobderion (m/z)	Cone Voltage (V)	Fragmention (m/z)	Botsingsenergie (eV)	Retentietijd (min)	Tijdsinterval (min)
Amisulpride	370,2	30	149,0	*	2,04	1,90 - 2,40
			214,0	50		
Aripiprazol	448,2	30	98,1	*	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	*	4,52	4,20 - 4,70
			165,1	30		
Clozapine	327,1	50	84,0	*	4,07	3,80 - 4,30
			192,0	40		
Clozapine-d4	333,2	30	88,1	30	4,03	3,80 - 4,30
Desmethylozapine	313,2	30	227,0	*	3,91	3,70 - 4,10
			270,1	30		

# voorbeeld



4-Bromoaniline-2,3,5,6-d<sub>4</sub>

*p*-Bromoaniline; 1-Amino-2-bromobenzene

98 atom % D

BrC<sub>6</sub>D<sub>4</sub>NH<sub>2</sub>

