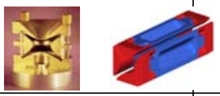
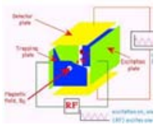
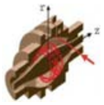
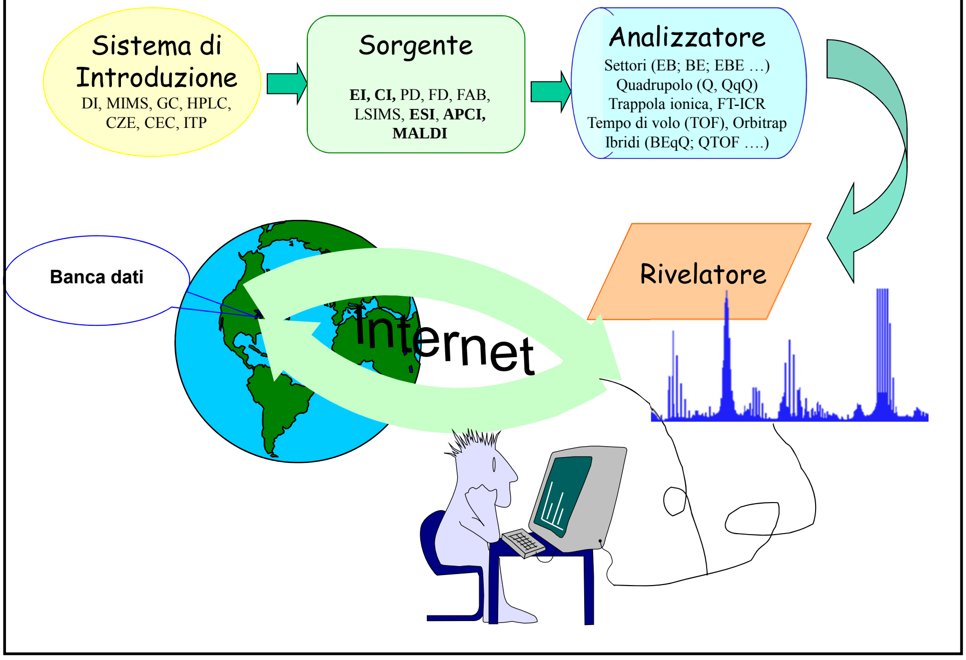
Separazione degli ioni nel tempo

Analizzatore	Forza	Separazione in base a	Range *m/z*	Risoluzione	Massa accurata
Trappola ionica (3D e 2D) 	Campo elettrico + RF	Frequenza delle orbite	<6.000	<500	no
Cella a risonanza ciclotronica (FT-ICR) 	Campo elettrico + RF + campo magnetico	Frequenza delle orbite	>10.000	> 100.000	< 1 ppm
Orbitrap 	Campo elettrico	Frequenza delle oscillazioni armoniche	<6000	100.000	2-5 ppm



Sistema di Introduzione
DI, MIMS, GC, HPLC, CZE, CEC, ITP

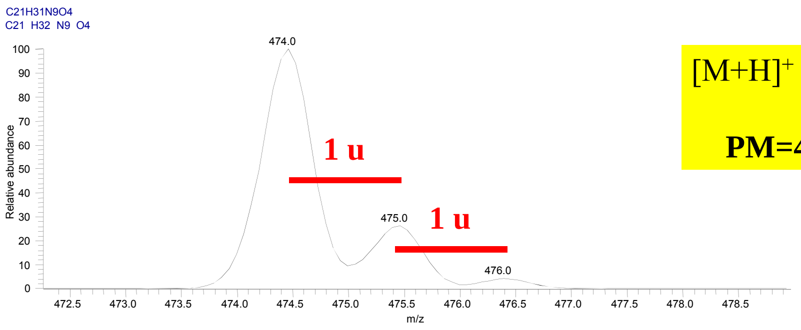
Sorgente
EI, CI, PD, FD, FAB, LSIMS, **ESI**, **APCI, MALDI**

Analizzatore
Settori (EB; BE; EBE …)
Quadrupolo (Q, QqQ)
Trappola ionica, FT-ICR
Tempo di volo (TOF), Orbitrap
Ibridi (BEqQ; QTOF ….)

Rivelatore

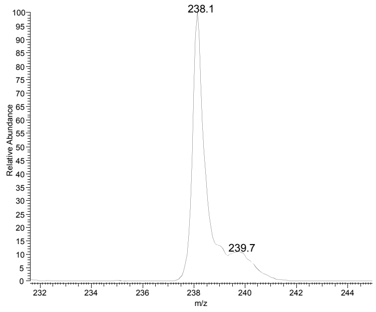
Banca dati

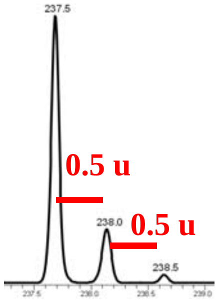
Internet

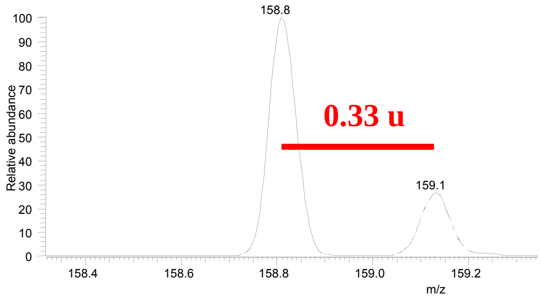


$[M+H]^+ = 474$

PM=473

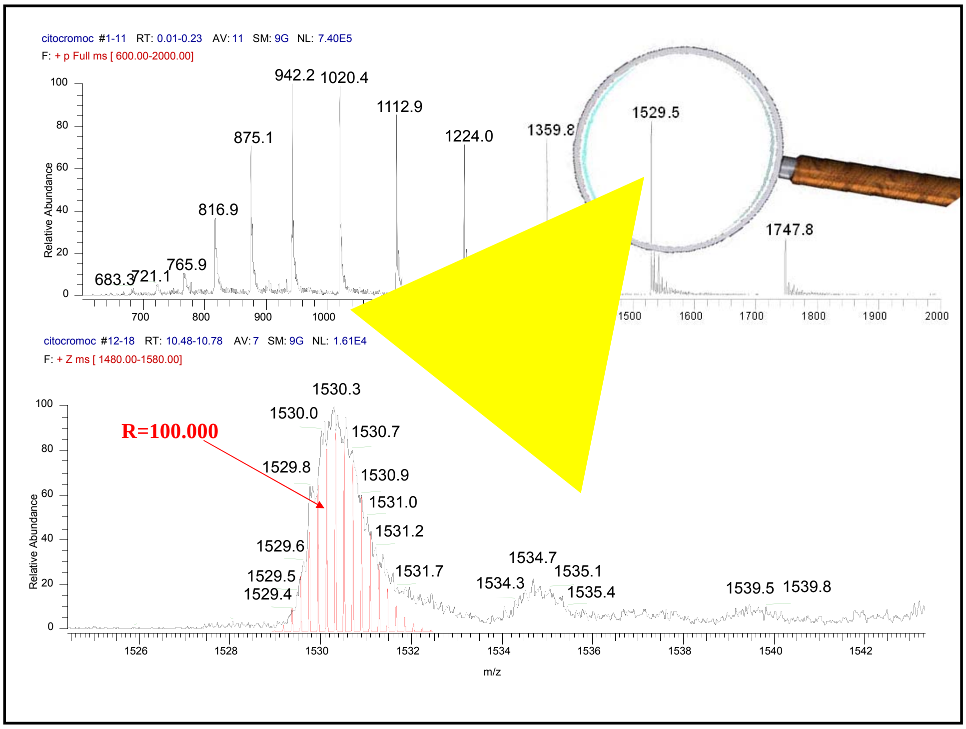


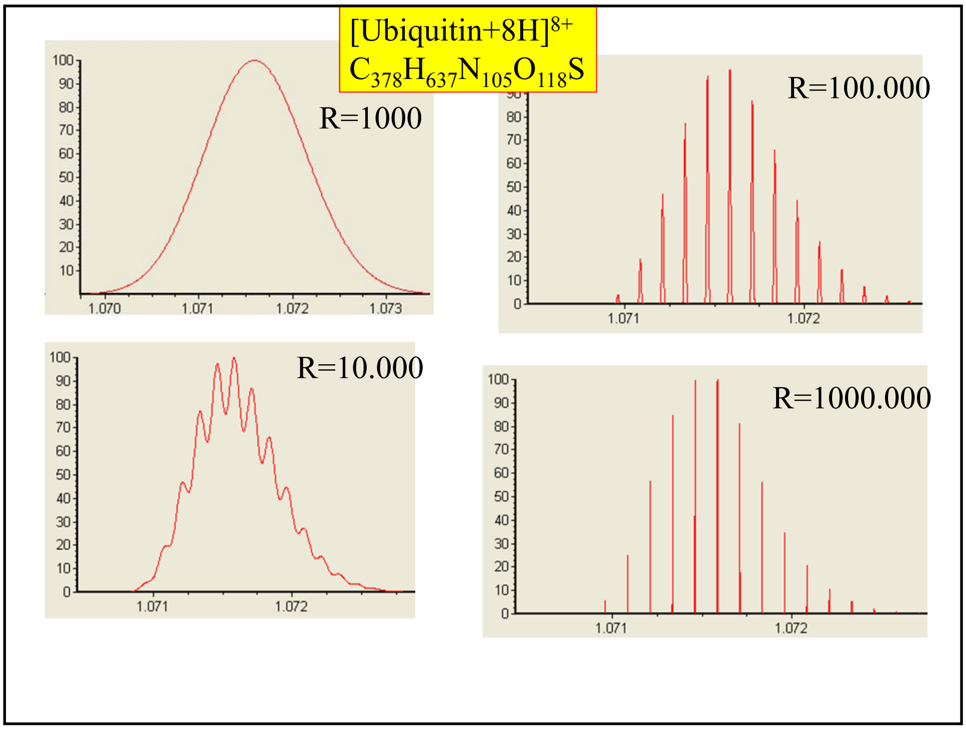




Separazione tra i picchi isotopici: Δm=1/*z*

Ione *z* = +8 Δm = 1/8 = 0.125





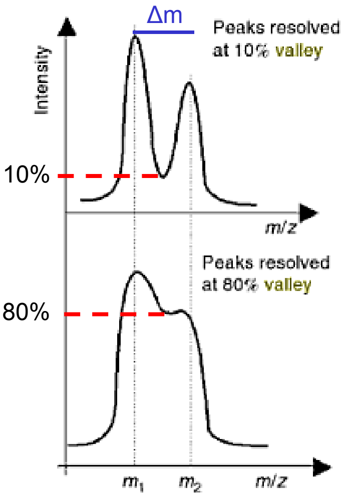
$[\text{Ubiquitin}+8H]^{8+}$

$C_{378}H_{637}N_{105}O_{118}S$

Resolution, resolving power:

$$R = \frac{m}{\Delta m}$$

RESOLUTION



Valley definition

Let two peaks of equal height in a mass spectrum at masses *m* and *m* – Δ*m* be separated by a valley which at its lowest point is just x % of the height of either peak.

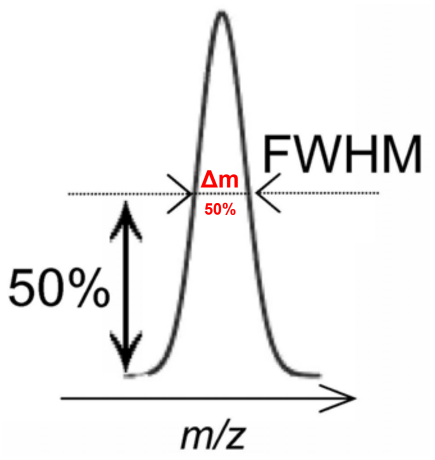
For similar peaks at a mass exceeding *m*, let the height of the valley at its lowest point be more (by any amount) than x % of either peak height.
Then the resolution (x % valley definition) is *m*/Δ*m*. It is usually a function of *m*. The ratio *m*/Δ*m* should be given for a number of values of *m*.

Double sectors, FT-ICR: 10% valley

Q, IT, TOF: 50% valley

IUPAC: http://goldbook.iupac.org/R05318.html

RESOLUTION

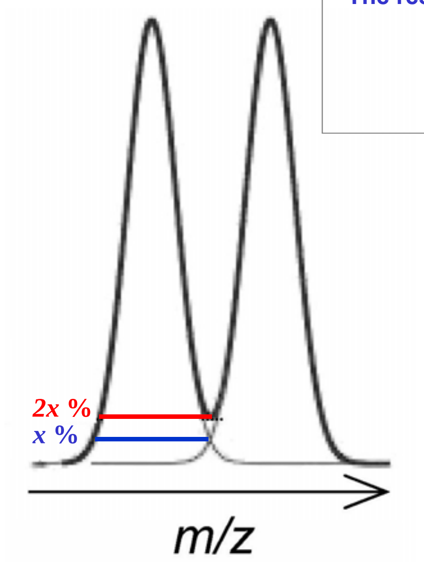


Resolution at full width at half maximum (FWHM).

Peak width definition

For a single peak made up of singly charged ions at mass *m* in a mass spectrum, the resolution may be expressed as *m*/Δ*m*, where Δ*m* is the width of the peak at a height which is a specified fraction of the maximum peak height.

$$R = \frac{m}{\Delta m_{50\%}}$$



The resolution full width at x% of the peak height

=

resolution at 2x% for the valley

In fact the bottom of the valley is the sum of the intensities at the peak cross

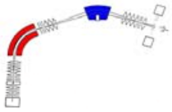
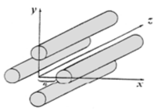
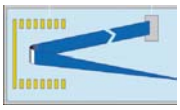
Resolving power

The ability to distinguish between ions differing in the quotient mass/charge by a small increment.

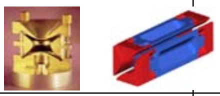
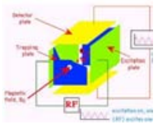
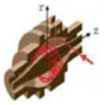
It may be characterized by giving the peak width, measured in mass units, expressed as a function of mass, for at least two points on the peak, specifically at fifty percent and at five percent of the maximum peak height.

$$RP = \frac{m}{\Delta m}$$

Separazione degli ioni nello spazio

Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
Doppio fuoco (EB, BE) 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000	10.000	< 1ppm
Quadrupolo 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000	Unitaria (0.2 u FWHM)	No
Time of flight 		Velocità	>100.000	>10.000	2-5 ppm

Separazione degli ioni nel tempo

Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
Trappola ionica (quadrupolare e lineare) 	Campo elettrico + RF	Frequenza delle orbite	4.000	<500	no
Cella a risonanza ciclotronica (FT-ICR) 	Campo elettrico + RF + campo magnetico	Frequenza delle orbite	>10.000	> 100.000	< 1 ppm
Orbitrap 	Campo elettrico	Frequenza delle oscillazioni armoniche	4.000	100.000	<1-3 ppm

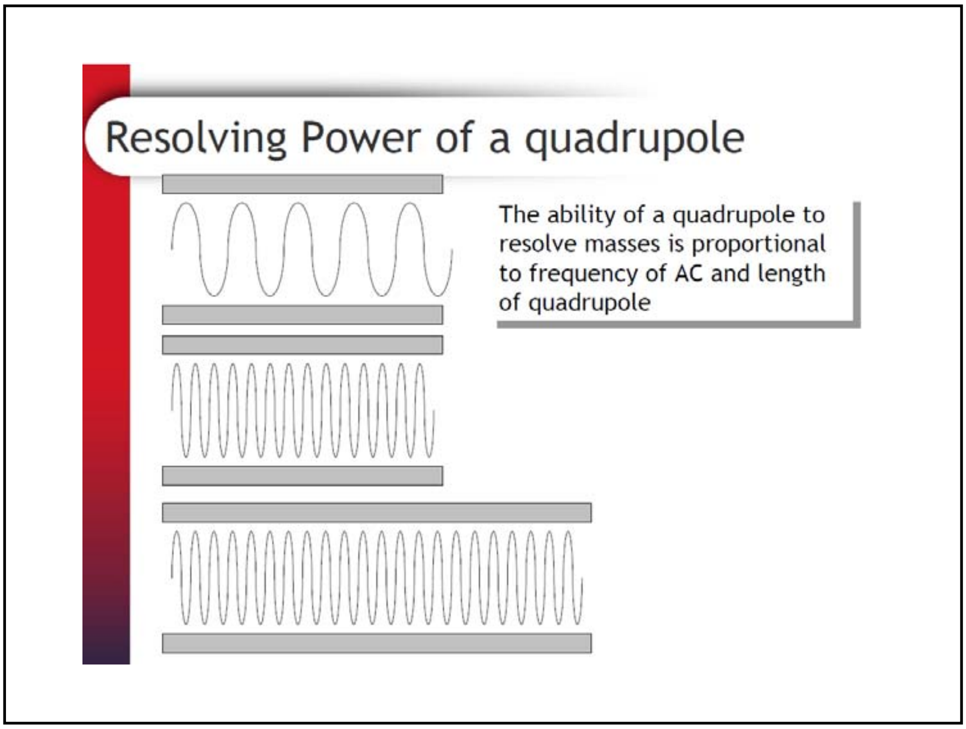
Resolving powerS:

1) <u>Quadrupole and Ion Traps</u>

Constant peak width
Variable resolution
FWHM Definition

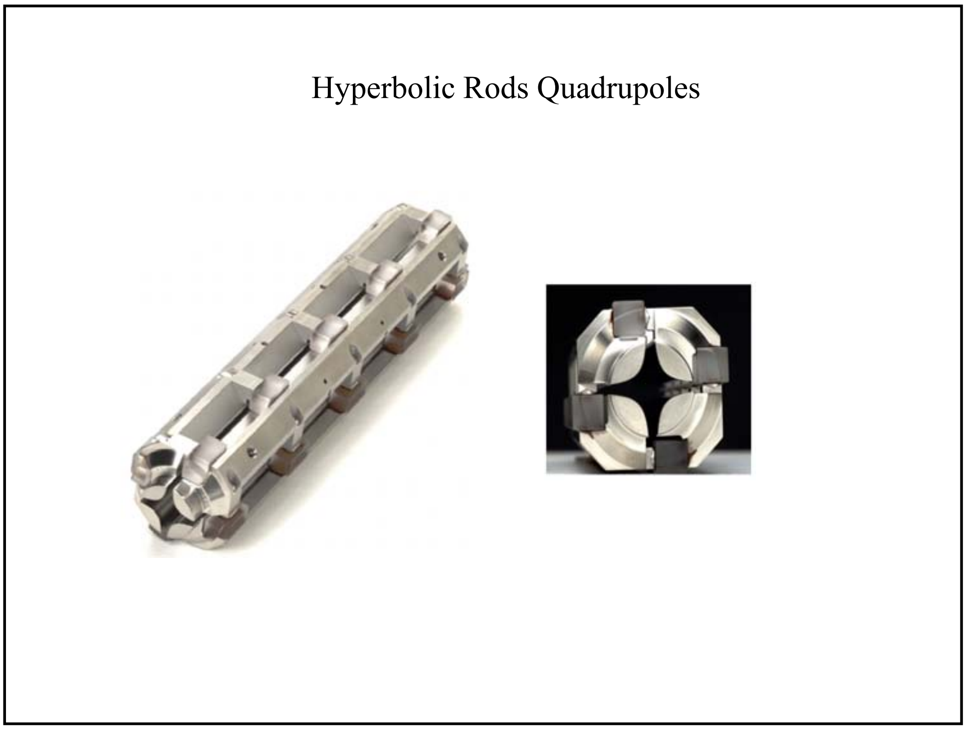
FWHM 0.5u at *m/z* 40 → Res. 40/0.5=**80**, but also

FWHM 0.5u at *m/z* 1000 → Res. 1000/0.5=**2000**

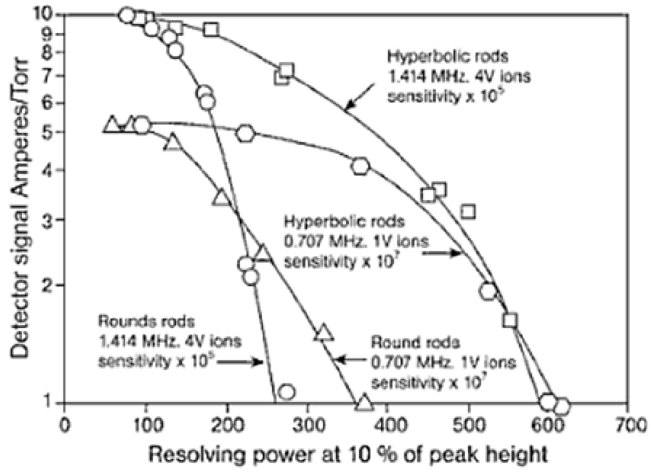


Resolving Power of a quadrupole

The ability of a quadrupole to resolve masses is proportional to frequency of AC and length of quadrupole

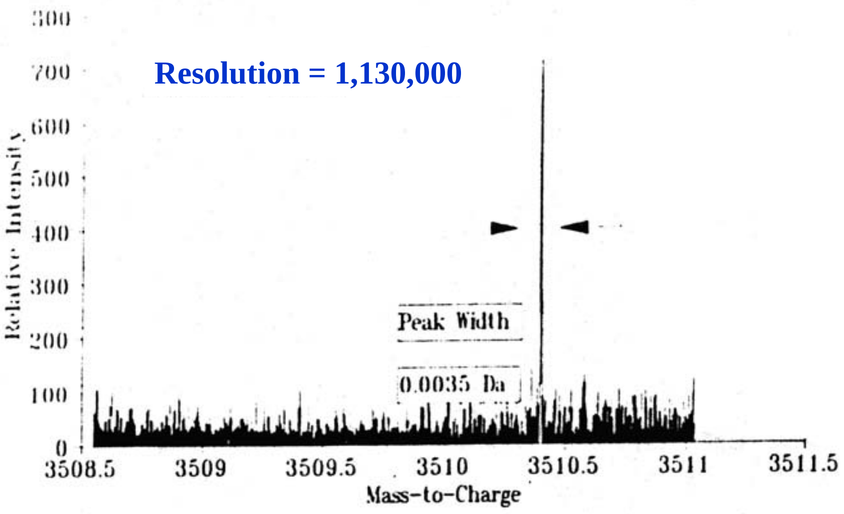


Hyperbolic Rods Quadrupoles



Ion transmission of round and hyperbolic quadrupole systems (resolving power ag. ion transmission), first documented investigation by Brubaker 1968 (two curves left: round rods; two curves right: hyperbolic rods).

3D-Ion Trap



Resolution = 1,130,000

The CsI cluster ion at m/z 3510 showing mass resolution in excess of 10^6

R. E. March, *Int. J. Mass Spectrom. Ion Processes* **118**/**119**, 71 (1992)

Resolving powerS:

2) TOF

$$m / \Delta m = t / 2\ \Delta t$$

Δt = FWHM of the peak measured

Resolving powerS:

2) TOF and sector analysers

Constant Resolution with *m/z*
10% Valley Definition for BE
FWHM definition for TOF

Res_{FWHM}=20,000 at *m/z* 40 → FWHM=40/20,000=0.002 u

but also

Res_{FWHM}=20,000 at *m/z* 4000 → FWHM=4,000/20,000=0.2 u

Resolving powerS:

3) FTICR:

$$R_{FWHM} = \frac{\upsilon_c \cdot T}{2} \qquad \text{and} \qquad \upsilon_c = \frac{q \cdot B}{2 \cdot \pi \cdot m}$$

T = transient duration (detection time)

υ_c = cyclotron frequency

Resolving powerS:

3) FTICR:

at constant detection time:

resolving power inversely proportional to *m/z*.

Thus if

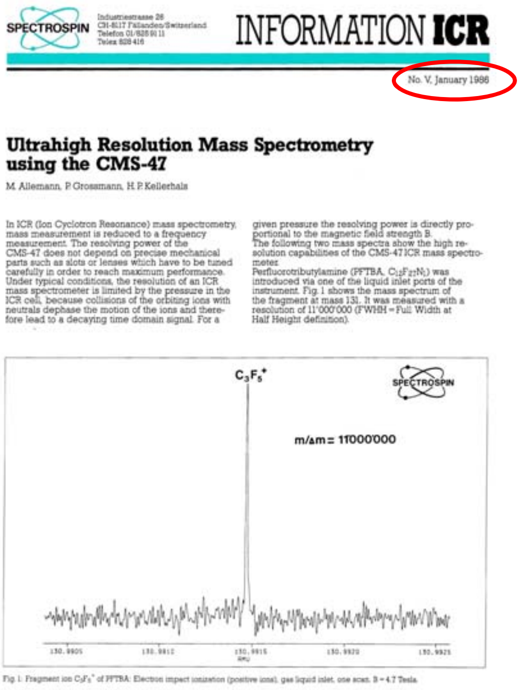
R=$\mathbf{1\times10^{7}}$ at *m/z* 100, it will be $\mathbf{1\times10^{6}}$ at *m/z* 1000

http://www.warwick.ac.uk/staff/M.P.Barrow/

A new ultra-high resolution FT-ICR cell with dynamic harmonization

extreme mass resolution greater than 10 million

Nikolaev EN, Boldin IA, Jertz R, Baykut G., *J Am Soc Mass Spectrom.* 2011, **22**:1125-33



SPECTROSPIN
Industriestrasse 26
CH-8117 Fällanden/Switzerland
Telefon 01/825 91 11
Telex 828 416

INFORMATION **ICR**

No. V, January 1986

Ultrahigh Resolution Mass Spectrometry using the CMS-47

M. Allemann, P. Grossmann, H. P. Kellerhals

In ICR (Ion Cyclotron Resonance) mass spectrometry, mass measurement is reduced to a frequency measurement. The resolving power of the CMS-47 does not depend on precise mechanical parts such as slots or lenses which have to be tuned carefully in order to reach maximum performance. Under typical conditions, the resolution of an ICR mass spectrometer is limited by the pressure in the ICR cell, because collisions of the orbiting ions with neutrals dephase the motion of the ions and therefore lead to a decaying time domain signal. For a given pressure the resolving power is directly proportional to the magnetic field strength B.

The following two mass spectra show the high resolution capabilities of the CMS-47 ICR mass spectrometer.

Perfluorotributylamine (PFTBA, $C_{12}F_{27}N_1$) was introduced via one of the liquid inlet ports of the instrument. Fig. 1 shows the mass spectrum of the fragment at mass 131. It was measured with a resolution of 11'000'000 (FWHH = Full Width at Half Height definition).

Fig. 1: Fragment ion $C_3F_5^+$ of PFTBA: Electron impact ionization (positive ions), gas liquid inlet, one scan, B = 4.7 Tesla

Resolving powerS:

4) Orbitrap:

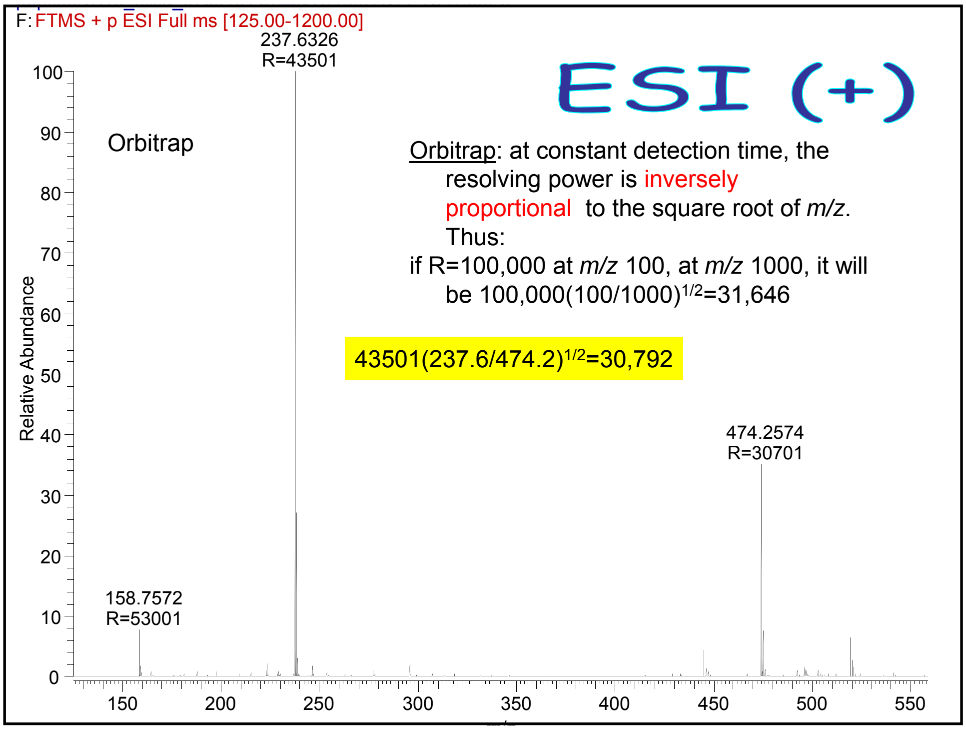
at constant detection time:

resolving power inversely proportional to $\sqrt{m/z}$

Thus if

R=**100,000** at *m/z* 100,

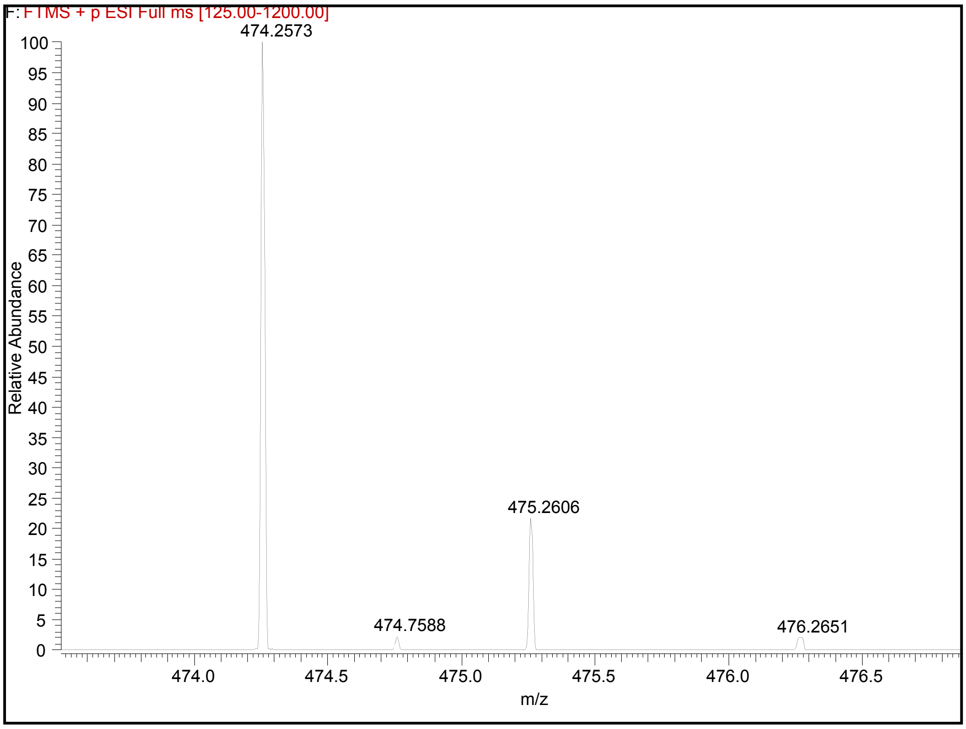
at *m/z* 1000 it will be R=100,000(100/1000)$^{1/2}$=**31,646**

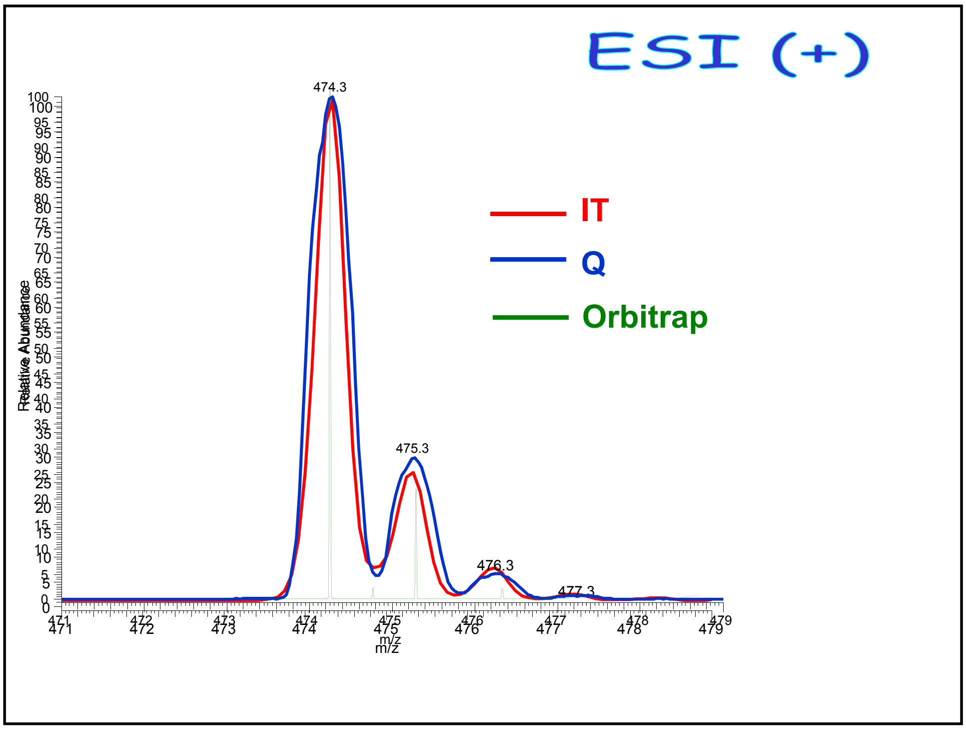


ESI (+)

Orbitrap: at constant detection time, the resolving power is inversely proportional to the square root of *m/z*.
Thus:
if R=100,000 at *m/z* 100, at *m/z* 1000, it will be 100,000(100/1000)$^{1/2}$=31,646

43501(237.6/474.2)$^{1/2}$=30,792


F: FTMS + p ESI Full ms [125.00-1200.00]
474.2573
475.2606
474.7588
476.2651
Relative Abundance
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
474.0
474.5
475.0
475.5
476.0
476.5
m/z


ESI (+)

IT
Q
Orbitrap
474.3
475.3
476.3
477.3
Relative Abundance
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
471
472
473
474
475
476
477
478
479
m/z

Resolving powerS:

1) Quadrupole and Ion Trap: constant peak width:

FWHM 0.5u at *m/z* 40 → Res. 40/0.5=80, but also
FWHM 0.5u at *m/z* 1000 → Res. 1000/0.5=2000

2) TOF and sector analysers have a resolution that remains constant when the mass increases

Res_{FWHM}=20,000 at *m/z* 40 → FWHM=40/20,000=0.002 u

but also

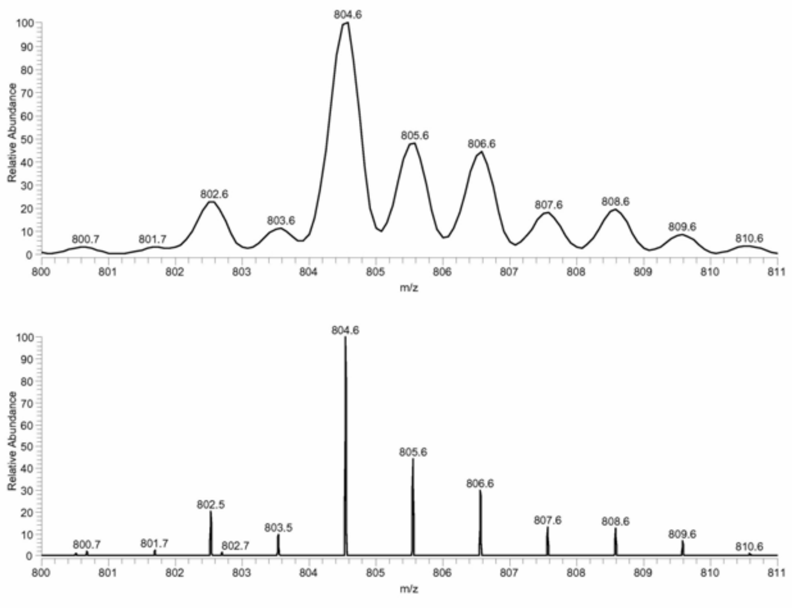
Res_{FWHM}=20,000 at *m/z* 4000 → FWHM=4,000/20,000=0.2 u

3) FTICR: at constant detection time, the resolving power is inversely proportional to *m/z*. Thus:

if $R=1\times10^7$ at *m/z* 100, it will be 1×10^6 at *m/z* 1000

4) Orbitrap: at constant detection time, the resolving power is inversely proportional to the square root of *m/z*. Thus:

if R=100,000 at *m/z* 100, at *m/z* 1000, it will be $100{,}000(100/1000)^{1/2}=31{,}646$

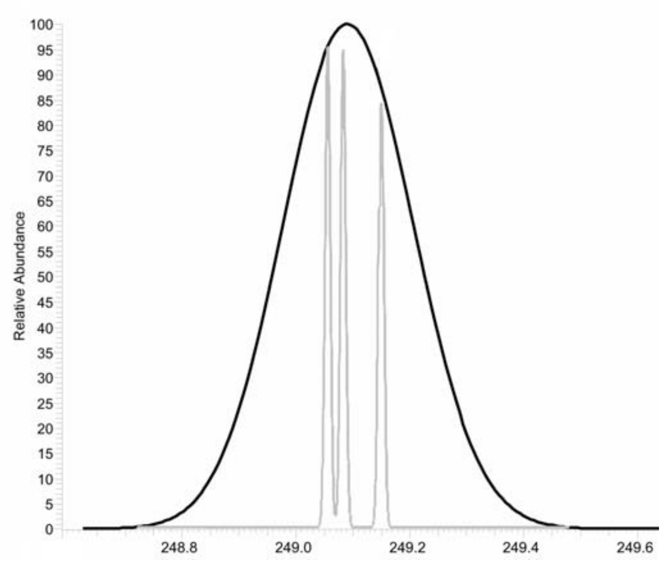


Mass spectra recorded at different resolutions: mass spectrum obtained by a 2D-ion trap low resolution (top) and by Orbitrap at resolving power 50,000 (bottom)

ISOBARIC IONS

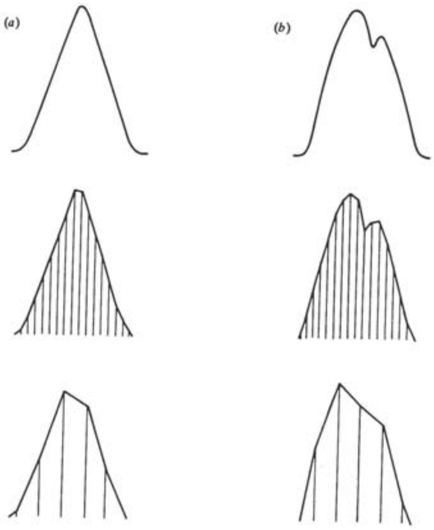
Ions having the same nominal mass, but different chemical compositions

i.e. CO and N_2



Mass spectra recorded at different resolutions: mass spectrum of a mixture of three isobaric species $[C_{19}H_7N]^+$, $[C_{20}H_9]^+$, $[C_{13}H_{19}N_3O_2]^+$ obtained at low resolution (black line) and at resolving power 50,000 (grey line). It is noteworthy that at low resolution the three peaks are completely unresolved.

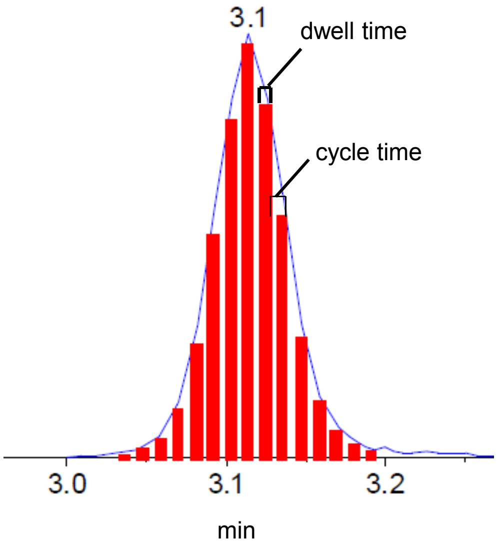
Effect of sampling rate on peak definition



Analogue signal

15 samplings

5 samplings



Quale risoluzione si deve usare?

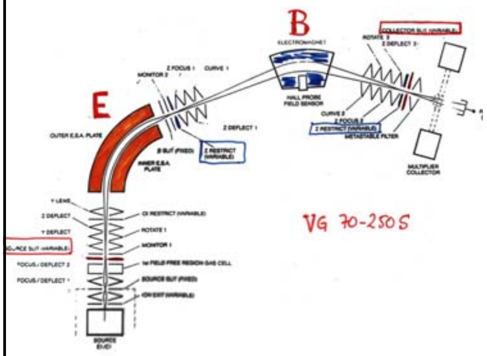
Melius abundare quam deficere ?

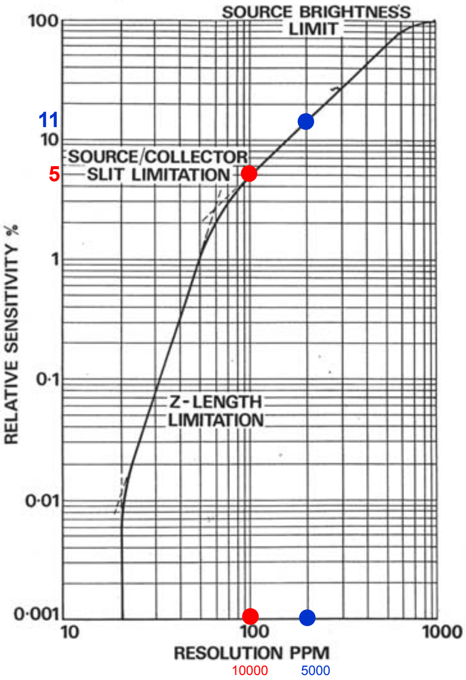
NO !!!!

Risoluzione e ….

1. Sensibilità
2. Specificità
3. Velocità di scansione

1. Risoluzione *vs* sensibilità



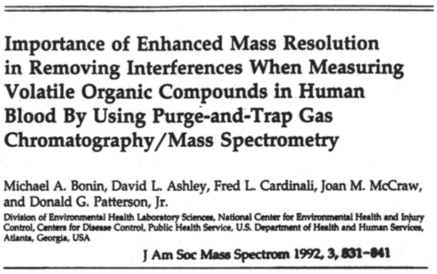


Negli strumenti a settori, l'aumento di risoluzione porta a una drastica diminuzione di sensibilità ⇒
effetti sulla misura della massa accurata

$$R\ ppm = \frac{10^6\ \Delta m}{m}$$

2. Risoluzione *vs* specificità

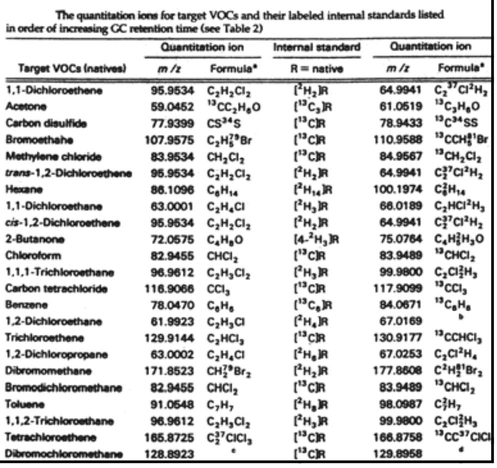
L'alta risoluzione aumenta la specificità eliminando specie isobariche interferenti



Importance of Enhanced Mass Resolution in Removing Interferences When Measuring Volatile Organic Compounds in Human Blood By Using Purge-and-Trap Gas Chromatography/Mass Spectrometry

Michael A. Bonin, David L. Ashley, Fred L. Cardinali, Joan M. McCraw, and Donald G. Patterson, Jr.
Division of Environmental Health Laboratory Sciences, National Center for Environmental Health and Injury Control, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia, USA

J Am Soc Mass Spectrom 1992, 3, 831–841

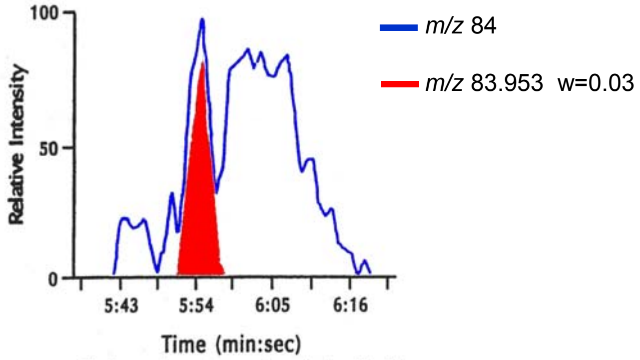


The quantitation ions for target VOCs and their labeled internal standards listed in order of increasing GC retention time (see Table 2)

	Quantitation ion		Internal standard	Quantitation ion	
Target VOCs (natives)	m/z	Formula^a	R = native	m/z	Formula^a
1,1-Dichloroethene	95.9534	$C_2H_2Cl_2$	$[^2H_2]R$	64.9941	$C_2{}^{37}Cl^2H_2$
Acetone	59.0452	$^{13}CC_2H_6O$	$[^{13}C_3]R$	61.0519	$^{13}C_3H_6O$
Carbon disulfide	77.9399	$CS^{34}S$	$[^{13}C]R$	78.9433	$^{13}C^{34}SS$
Bromoethahe	107.9575	$C_2H_5^{79}Br$	$[^{13}C]R$	110.9588	$^{13}CCH_5^{81}Br$
Methylene chloride	83.9534	CH_2Cl_2	$[^{13}C]R$	84.9567	$^{13}CH_2Cl_2$
trans-1,2-Dichloroethene	95.9534	$C_2H_2Cl_2$	$[^2H_2]R$	64.9941	$C_2^{37}Cl^2H_2$
Hexane	86.1096	C_6H_{14}	$[^2H_{14}]R$	100.1974	$C_6^2H_{14}$
1,1-Dichloroethane	63.0001	C_2H_4Cl	$[^2H_3]R$	66.0189	$C_2HCl^2H_3$
cis-1,2-Dichloroethene	95.9534	$C_2H_2Cl_2$	$[^2H_2]R$	64.9941	$C_2^{37}Cl^2H_2$
2-Butanone	72.0575	C_4H_8O	$[4\text{-}^2H_3]R$	75.0764	$C_4H_5^2H_3O$
Chloroform	82.9455	$CHCl_2$	$[^{13}C]R$	83.9489	$^{13}CHCl_2$
1,1,1-Trichloroethane	96.9612	$C_2H_3Cl_2$	$[^2H_3]R$	99.9800	$C_2Cl_2^2H_3$
Carbon tetrachloride	116.9066	CCl_3	$[^{13}C]R$	117.9099	$^{13}CCl_3$
Benzene	78.0470	C_6H_6	$[^{13}C_6]R$	84.0671	$^{13}C_6H_6$
1,2-Dichloroethane	61.9923	C_2H_3Cl	$[^2H_4]R$	67.0169	^{b}
Trichloroethene	129.9144	C_2HCl_3	$[^{13}C]R$	130.9177	$^{13}CCHCl_3$
1,2-Dichloropropane	63.0002	C_2H_4Cl	$[^2H_6]R$	67.0253	$C_2Cl^2H_4$
Dibromomethane	171.8523	$CH_2^{79}Br_2$	$[^2H_2]R$	177.8608	$C^2H_2^{81}Br_2$
Bromodichloromethane	82.9455	$CHCl_2$	$[^{13}C]R$	83.9489	$^{13}CHCl_2$
Toluene	91.0548	C_7H_7	$[^2H_8]R$	98.0987	$C_7^2H_7$
1,1,2-Trichloroethane	96.9612	$C_2H_3Cl_2$	$[^2H_3]R$	99.9800	$C_2Cl_2^2H_3$
Tetrachloroethene	165.8725	$C_2^{37}ClCl_3$	$[^{13}C]R$	166.8758	$^{13}CC^{37}ClCl$
Dibromochloromethane	128.8923	^{c}	$[^{13}C]R$	129.8958	^{d}

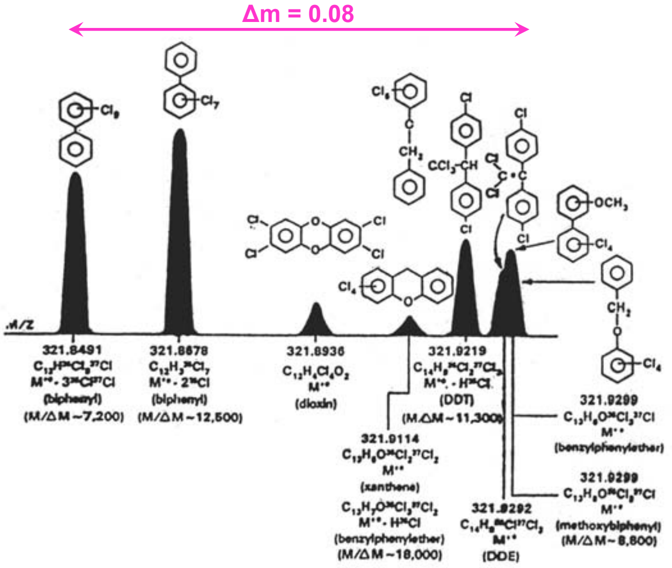
2. Risoluzione *vs* specificità

L'alta risoluzione aumenta la specificità eliminando specie isobariche interferenti



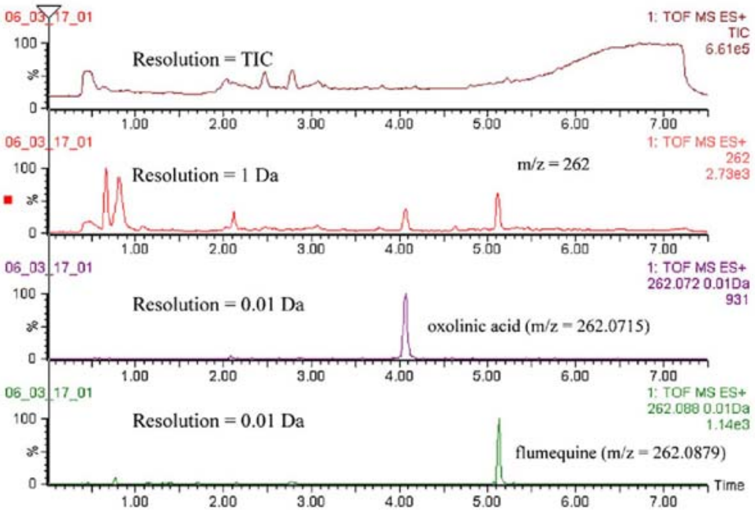
Single-mass chromatograms for methylene chloride from a blood sample (9.59 g): (a) nominal mass curve for *m/z* 84; (b) accurate mass curve for $CH_2Cl_2^+$ (*m/z* 83.953 with a window of 0.03).

Summary of some PCDDs mass spectral interferences of environmental origin



PCDDs=polychlorinated dibenzo-*p*-dioxins

Multiresidue screening of veterinary drugs in urine by HPLC-MS TOF



A. Kaufmann et al., *Anal. Chim. Acta* ***586****, 13–21* (2007)

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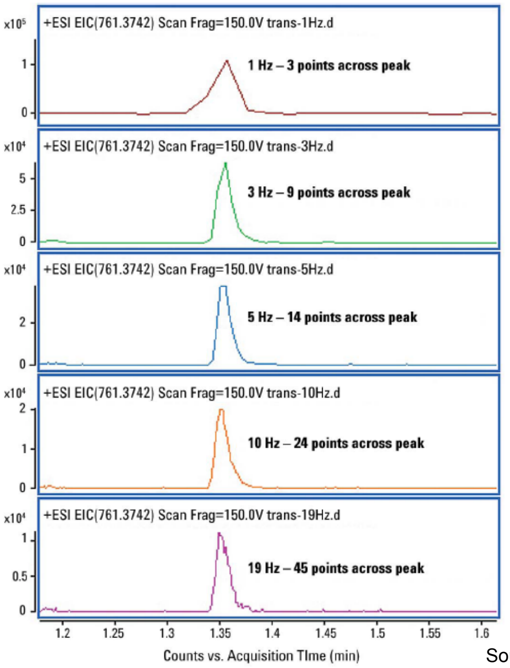
3. Risoluzione
vs
velocità di scansione

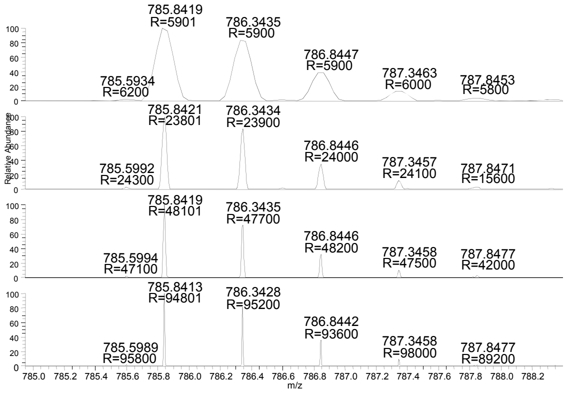


Source: Agilent

L'aumento di risoluzione richiede *in genere* una diminuzione della velocità di scansione

Strumenti a settori: da 1 sec/dec a 10 sec/dec

Orbitrap: Resolving Power vs Cycle Time



RP 7500
0.2 s

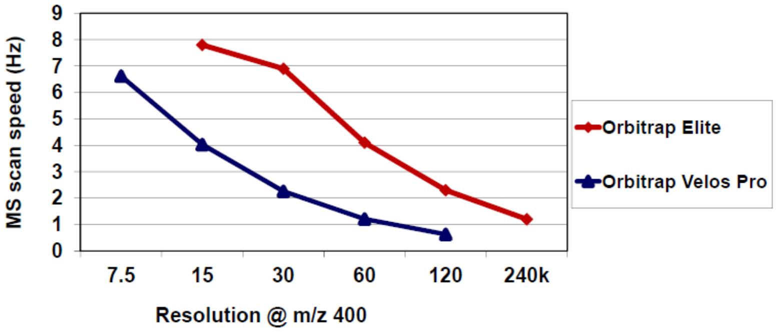
RP 30000
0.5 s

RP 60000
0.9 s

RP 100000
1.6 s

(courtesy by Thermo)

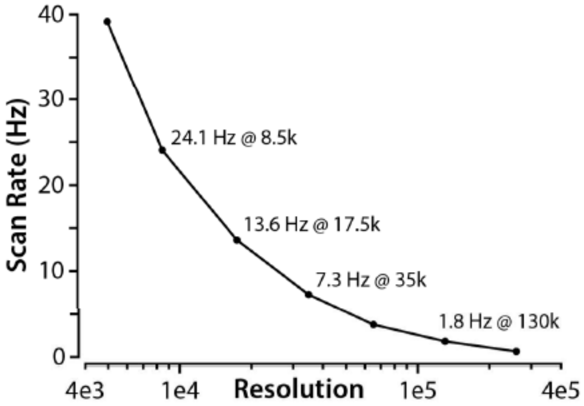
MS Scan Speed at Different Resolutions



- **240,000 at 1 Hz: Exploring new possibilities**
- **60,000 at 4 Hz: Exploiting the faster acquisition rate**
- **15,000 at 8 Hz: Increasing HCD scan speed**

ThermoFisher SCIENTIFIC

GC/Quadrupole-Orbitrap



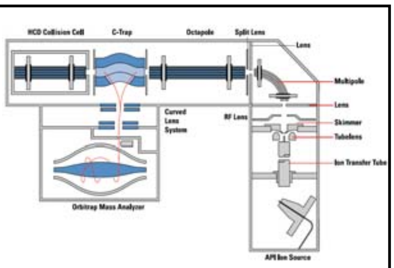
Scan rate (Hz) as a function of mass analysis resolution.

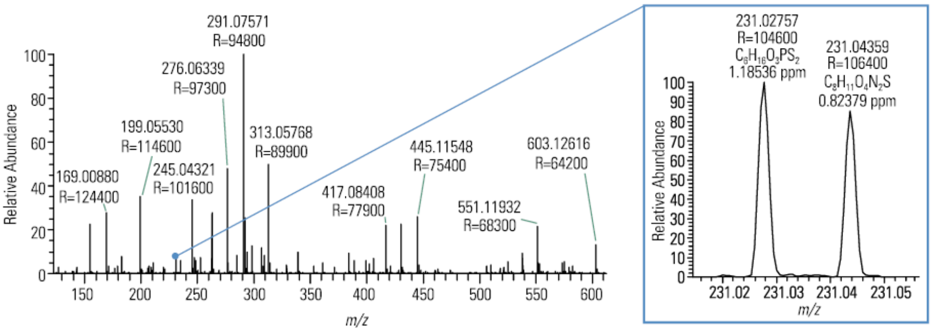
<u>Orbitrap Exactive</u>: Resolving Power vs Cycle Time

Scan rate = 10Hz RP 10,000 at *m/z* 200

Scan rate = 1Hz RP 100,000 at *m/z* 200

(Source Thermo)





Full scan spectrum of a pesticide mixture demonstrating a resolving power of up to 100,000.

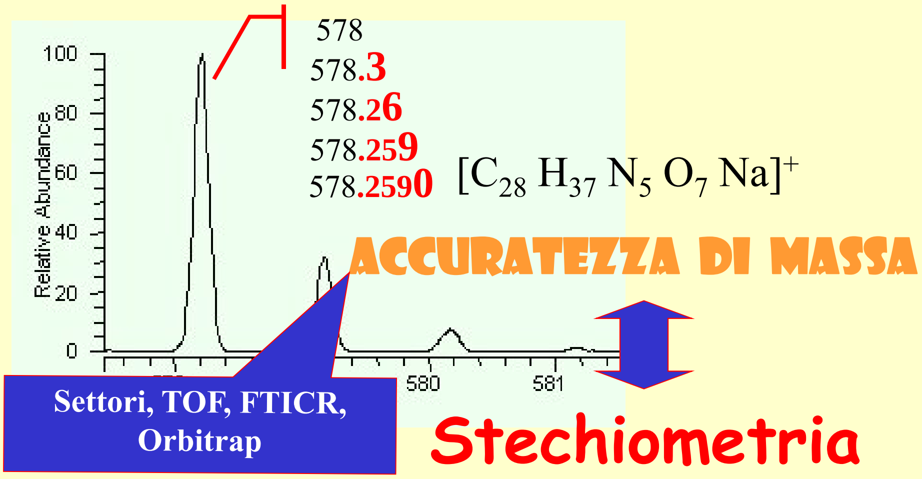
L’alta risoluzione è requisito indispensabile per la

misura della massa accurata

Analisi qualitativa → massa accurata → formula bruta

Analisi quantitativa → eliminazione interferenti
(MS: SIM; MS^n)

Accuratezza nella misura del valore *m/z*



578
578.**3**
578.2**6**
578.25**9**
578.259**0**

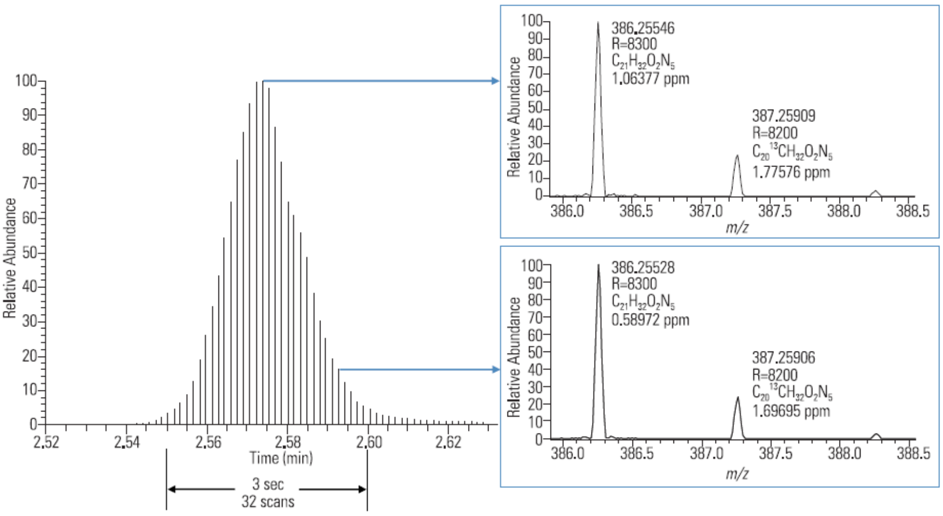
$[C_{28}H_{37}N_5O_7Na]^+$

ACCURATEZZA DI MASSA

Settori, TOF, FTICR, Orbitrap

Stechiometria

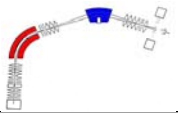
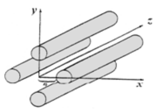
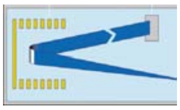
Orbitrap Exactive



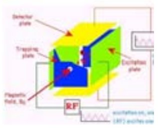
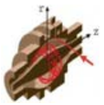
LC peak and mass spectra of Buspirone acquired at a scan rate of 10 scans per second.

(courtesy by Thermo)

Separazione degli ioni nello spazio

Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
Doppio fuoco (EB, BE) 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000	10.000	< 1ppm
Quadrupolo 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000	Unitaria (0.2 u FWHM)	No
Time of flight 		Velocità	>100.000	>10.000	2-5 ppm

Separazione degli ioni nel tempo

Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
Trappola ionica (quadrupolare e lineare) 	Campo elettrico + RF	Frequenza delle orbite	4.000	<500	no
Cella a risonanza ciclotronica (FT-ICR) 	Campo elettrico + RF + campo magnetico	Frequenza delle orbite	>10.000	> 100.000	< 1 ppm
Orbitrap 	Campo elettrico	Frequenza delle oscillazioni armoniche	4.000	100.000	< 1-3 ppm

---

Spettrometria di **massa** ……..

Quale massa?

Exact mass

The **calculated mass** of an ion or molecule containing a single isotope of each atom

Accurate mass

An **experimentally determined mass** of an ion that is used to determine an elemental formula

Mass Spec Terms Project: http://mass-spec.lsu.edu/msterms/index.php/Category:Mass_spectrometry_terms

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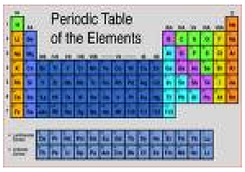
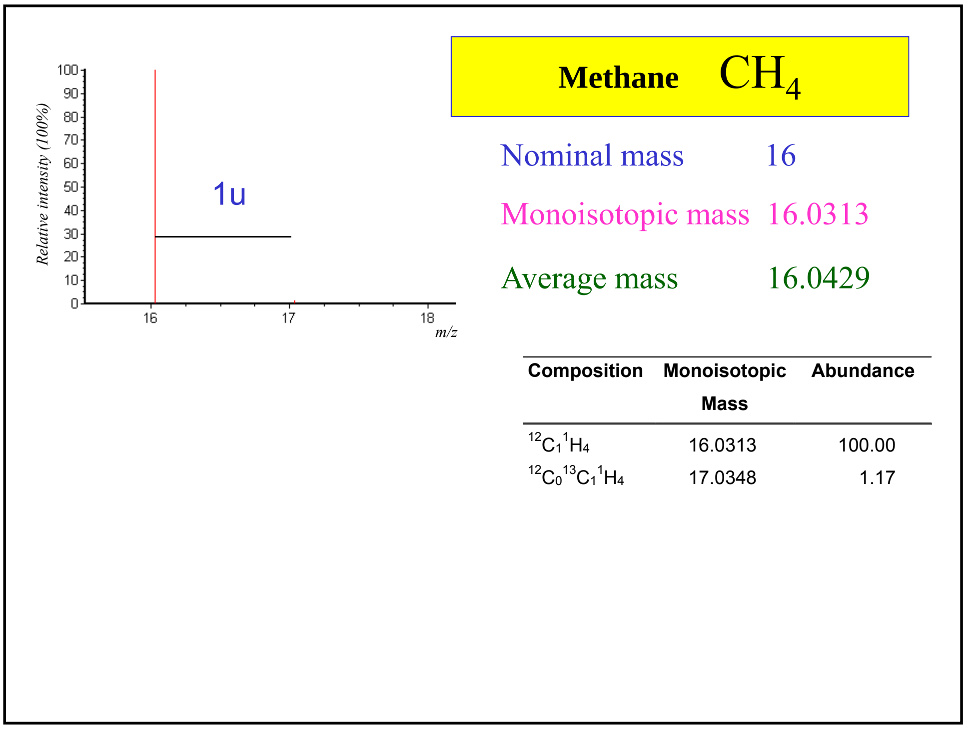
Nominal Mass – The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value.*i.e.* H=1, C=12, N=14, ….

Monoisotopic Mass – The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element.
i.e. H=1,007825, C=12,000000, N=14,00307

Average Mass – The mass of an ion or molecule calculated using the average mass of each element weighted for its natural isotopic abundance. *i.e.* centroid of the distribution





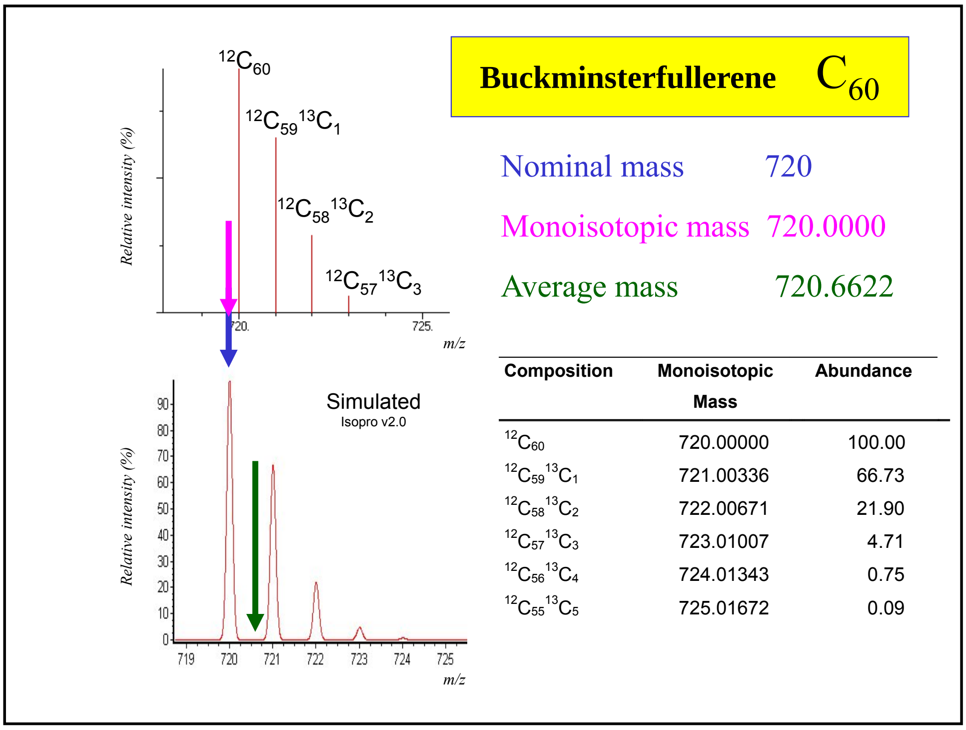
Methane CH_4

Nominal mass 16

Monoisotopic mass 16.0313

Average mass 16.0429

Composition	Monoisotopic Mass	Abundance
$^{12}C_1{}^1H_4$	16.0313	100.00
$^{12}C_0{}^{13}C_1{}^1H_4$	17.0348	1.17



Buckminsterfullerene C_{60}

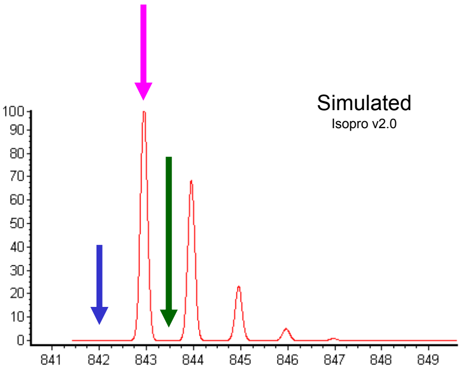
Nominal mass 720

Monoisotopic mass 720.0000

Average mass 720.6622

Composition	Monoisotopic Mass	Abundance
$^{12}C_{60}$	720.00000	100.00
$^{12}C_{59}{}^{13}C_1$	721.00336	66.73
$^{12}C_{58}{}^{13}C_2$	722.00671	21.90
$^{12}C_{57}{}^{13}C_3$	723.01007	4.71
$^{12}C_{56}{}^{13}C_4$	724.01343	0.75
$^{12}C_{55}{}^{13}C_5$	725.01672	0.09

Hexacontane $C_{60}H_{122}$



Nominal mass 842

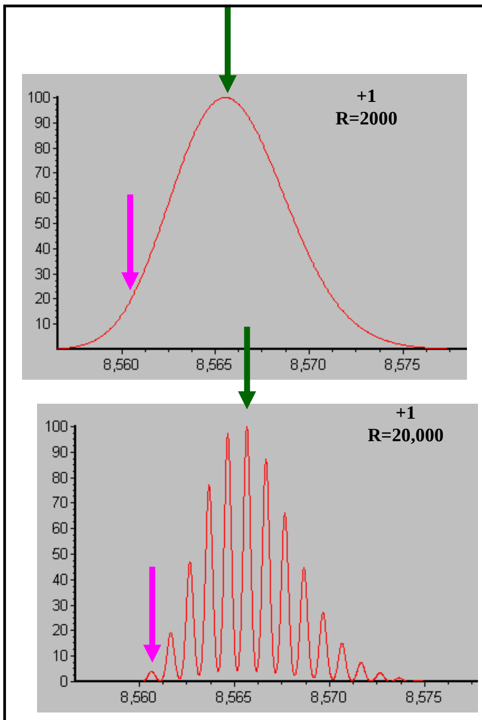
Monoisotopic mass 842.95465

Average mass 843.5363

Composition	Monoisotopic Mass	Abundance
$^{12}C_{60}{}^{1}H_{122}$	842.95465	100.00
$^{12}C_{59}{}^{13}C_{1}{}^{1}H_{122}$	843.95801	69.34
$^{12}C_{60}{}^{1}H_{121}{}^{2}H_{1}$	843.96093	0.62
$^{12}C_{58}{}^{13}C_{2}{}^{1}H_{122}$	844.96136	23.40
$^{12}C_{59}{}^{13}C_{1}{}^{1}H_{121}{}^{2}H_{1}$	844.96429	0.41
$^{12}C_{57}{}^{13}C_{3}{}^{1}H_{122}$	845.96472	1.59
$^{12}C_{58}{}^{13}C_{2}{}^{1}H_{121}{}^{2}H_{1}$	845.96764	0.14
$^{12}C_{59}{}^{13}C_{1}{}^{1}H_{120}{}^{2}H_{2}$	845.97056	0.004
$^{12}C_{56}{}^{13}C_{4}{}^{1}H_{122}$	846.96807	0.25

……………

$[\text{Ubiquitin+H}]^{+}$ $C_{378}H_{630}N_{105}O_{118}S$



Nominal mass 8,556

Monoisotopic mass 8,560.6230

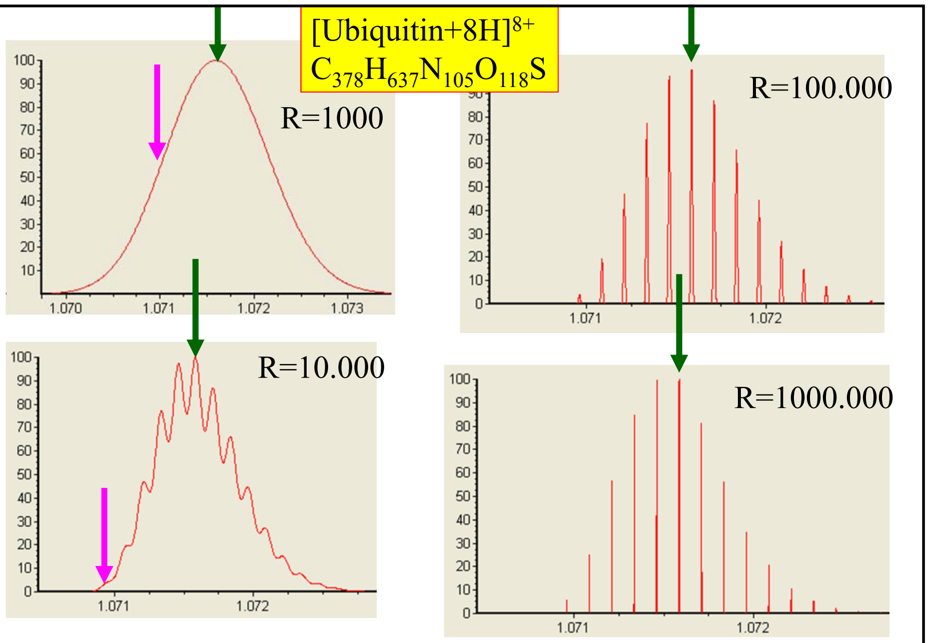
Average mass 8,565.8799

Risoluzione

massa

e …..

ioni multicarica

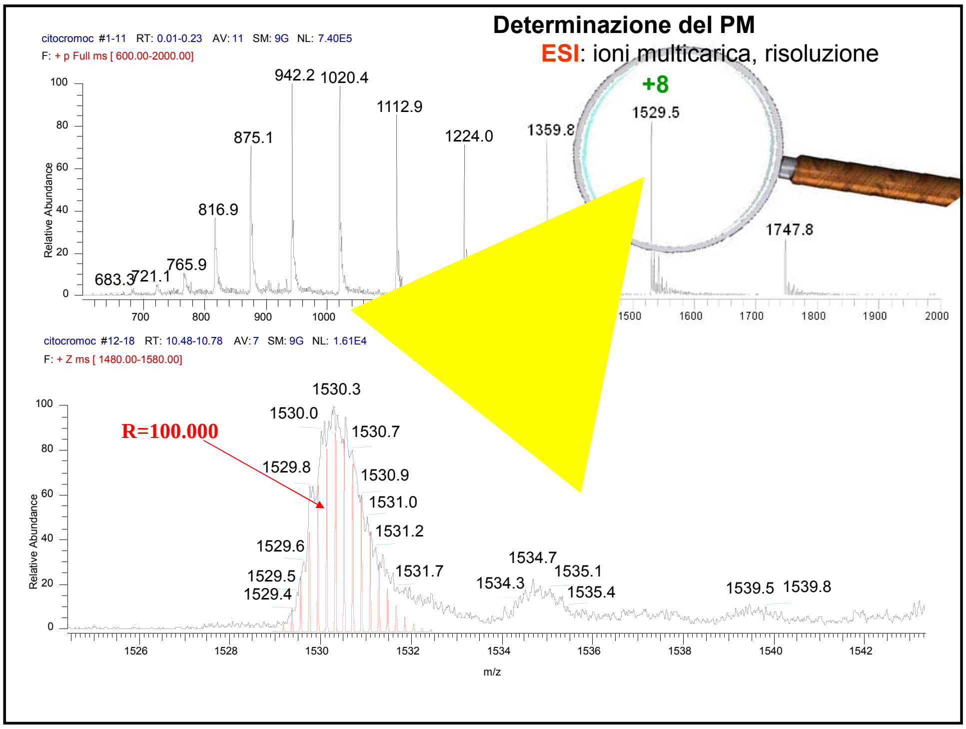


[Ubiquitin+8H]$^{8+}$
$C_{378}H_{637}N_{105}O_{118}S$

Monoisotopic mass 1070,9597 Average mass 1071,6169

Determinazione del PM

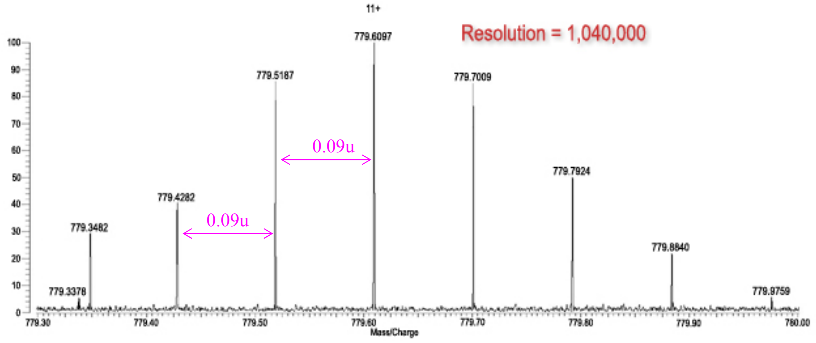
ESI: ioni multicarica, risoluzione



FT-ICR

ESI (+) of Ubiquitin

1,040,000 Resolution at *m/z* 779

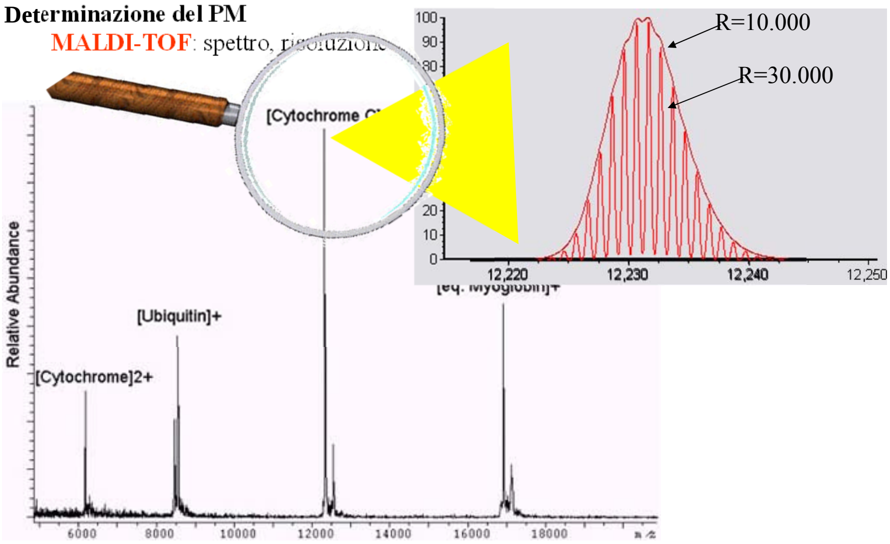


Conclusion:

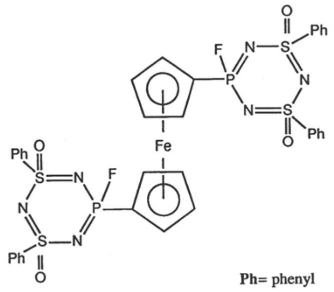
- At low mass (< 1000 u) the local maximum in an isotopic display will be close to the **monoisotopic mass.**
- At masses above 2000 u the local maximum will shift to be closer to the **average mass** of the compound.

Determinazione del PM

MALDI-TOF: spettro, risoluzione



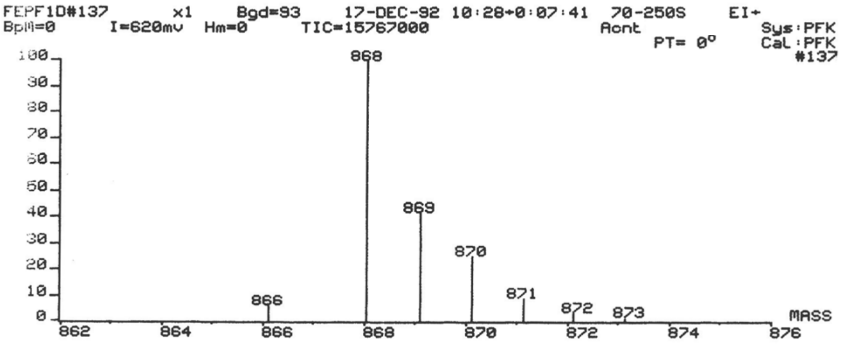
MALDI TOF mass spectrum of a mixture of ubiquitin, cytochrome C and equine myoglobin using 2,5-dihydroxybenzoic acid (DHB) as the matrix.



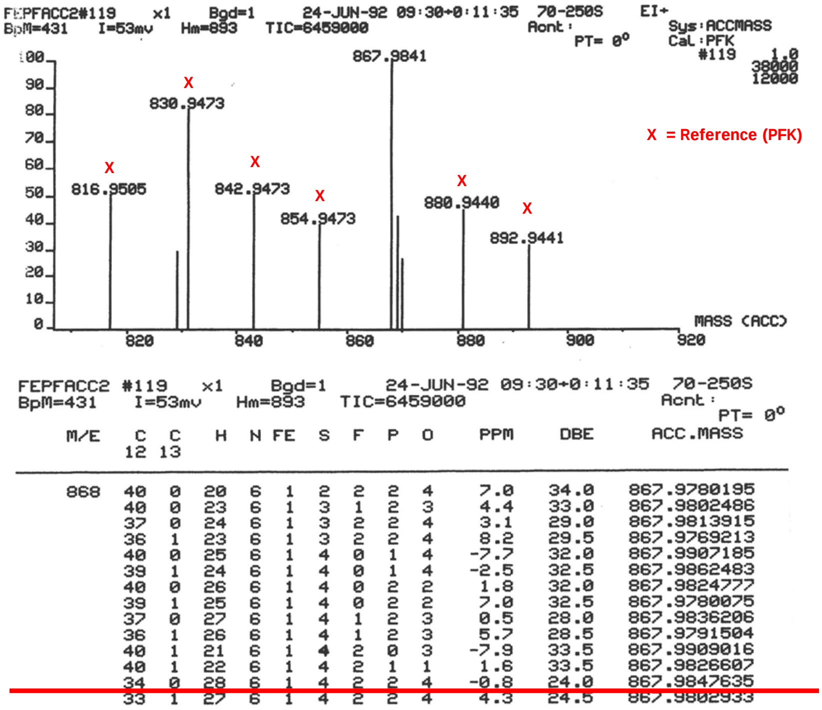
Ph= phenyl

VG 70-250S
m/z 900-200
10 sec/dec
Res=8.000 (10% valley)

$C_{34}H_{28}N_6O_4FeS_4P_2F_2$



```
FEPF1D#137        x1      Bgd=93      17-DEC-92 10:28+0:07:41   70-250S     EI+
BpM=0        I=620mv  Hm=0        TIC=15767000                  Acnt                  Sys:PFK
                                                                          PT= 0°      Cal:PFK
                                                                                        #137
```



```
FEPFACC2#119     x1      Bgd=1        24-JUN-92 09:30+0:11:35   70-250S     EI+
BpM=431     I=53mv    Hm=893    TIC=6459000                    Acnt:            Sys:ACCMASS
                                                                        PT= 0°      Cal:PFK
                                                                                     #119     1.0
                                                                                            38000
                                                                                            12000
```

X = Reference (PFK)

```
FEPFACC2 #119    x1      Bgd=1        24-JUN-92 09:30+0:11:35   70-250S
BpM=431     I=53mv    Hm=893    TIC=6459000                    Acnt:
                                                                        PT= 0°
     M/E    C   C     H   N  FE   S   F   P   O      PPM      DBE       ACC.MASS
           12  13
  ---------------------------------------------------------------------------------
     868   40   0    20   6   1   2   2   2   4      7.0     34.0     867.9780195
           40   0    23   6   1   3   1   2   3      4.4     33.0     867.9802486
           37   0    24   6   1   3   2   2   4      3.1     29.0     867.9813915
           36   1    23   6   1   3   2   2   4      8.2     29.5     867.9769213
           40   0    25   6   1   4   0   1   4     -7.7     32.0     867.9907185
           39   1    24   6   1   4   0   1   4     -2.5     32.5     867.9862483
           40   0    26   6   1   4   0   2   2      1.8     32.0     867.9824777
           39   1    25   6   1   4   0   2   2      7.0     32.5     867.9780075
           37   0    27   6   1   4   1   2   3      0.5     28.0     867.9836206
           36   1    26   6   1   4   1   2   3      5.7     28.5     867.9791504
           40   1    21   6   1   4   2   0   3     -7.9     33.5     867.9909016
           40   1    22   6   1   4   2   1   1      1.6     33.5     867.9826607
           34   0    28   6   1   4   2   2   4     -0.8     24.0     867.9847635
           33   1    27   6   1   4   2   2   4      4.3     24.5     867.9802933
```

C 0÷100
H 1÷100
N 0÷8
Fe 0÷1
S 0÷6
F 0÷4
P 0÷4
O 0÷6

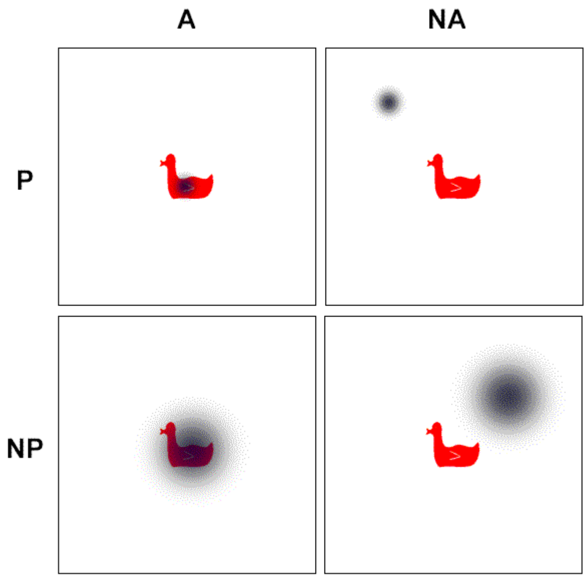
Misura della massa accurata

ERRORE:

mmu: *m/z* misurato – *m/z* calcolato

ppm:

$$\frac{m/z \text{ misurato} - m/z \text{ calcolato}}{m/z} \times 10^{6}$$



For a discrete mass spectrum (as in most current instruments),

mass precision is given by

$$\text{Mass precision} = c(\text{S/N})\sqrt{\text{number of data points per peak width}}$$

in which *c* is a constant (of order unity), determined by the peak shape and spectral baseline noise, independent of signal. It follows that:

1. high mass-measurement precision requires the highest possible S/N *and* smallest digital point spacing;
2. mass-measurement precision for low-magnitude peaks is necessarily lower than for high magnitude peaks;
3. it is possible to predict the precision that would be obtained from many measurements on the basis of the S/N and discrete sampling for a single measurement;

A. G. Marshall, C. L. Hendrickson, *Ann. Rev. Anal. Chem.* 579–599 (2008).

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mass accuracy:

Mass calibration :

consists of fitting the observed mass measurements to the accurate masses of two or more different ions.

- internal : the reference masses are for ions of known elemental composition in the same mass spectrum as the analyte

- external : reference masses from a mass spectrum of another analyte acquired under similar conditions.

Internal calibration is typically at least twice as accurate as external calibration.

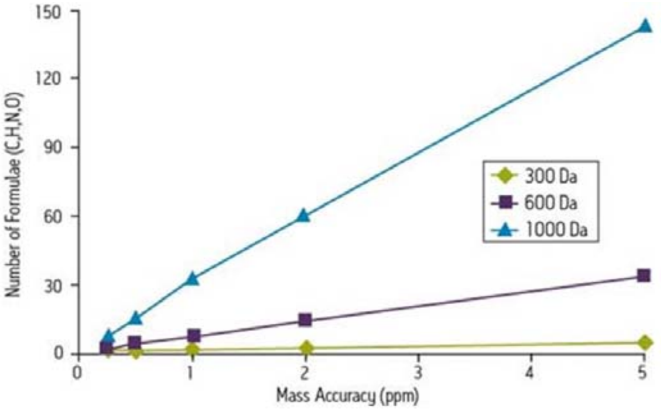
Lock mass

Systematic errors

Repeatability—this is the short-term precision of multiple replicate experimental measurements made under similar conditions, i.e., the same instrument, operator and over a limited time, normally the same day.

Reproducibility—refers to differences among experimental measurements made under different circumstances i.e., a measurement of the same quantity made by different operators, even different instruments and often with a significant time difference between groups of measurements.

All'aumentare del valore di *m/z* è richiesta un'accuratezza maggiore per un'identificazione non ambigua



T.L. Quenzer, J.M. Robinson, B. Bolanios, E. Milgram, M.J. Greig, Automated accurate mass analysis using FTICR mass spectrometry, Proceedings of the 50th ASMS Conference, Orlando, FL, 2002.

All’aumentare del valore di *m/z* è richiesta un’accuratezza maggiore per un’identificazione non ambigua

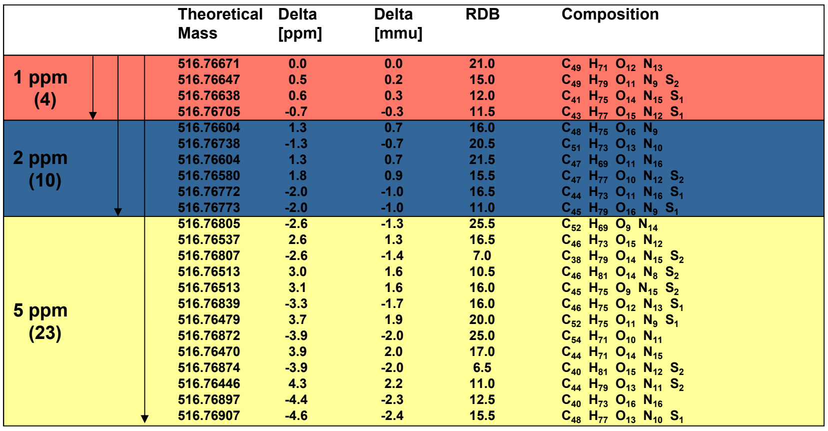
Requirements for unambiguous characterization

(*J. Am. Soc. Mass Spectrom.* author’s guidelines (March 2004))

For C, H, O, N compositions ($C_{0\div100}$, $H_{3\div74}$, $O_{0\div4}$ and $N_{0\div4}$):

at ***m/z* 118** needs only an error not exceeding **34 ppm** to be unambiguous;

at ***m/z* 750** requires precision better than **0.018 ppm** to eliminate “all extraneous possibilities”

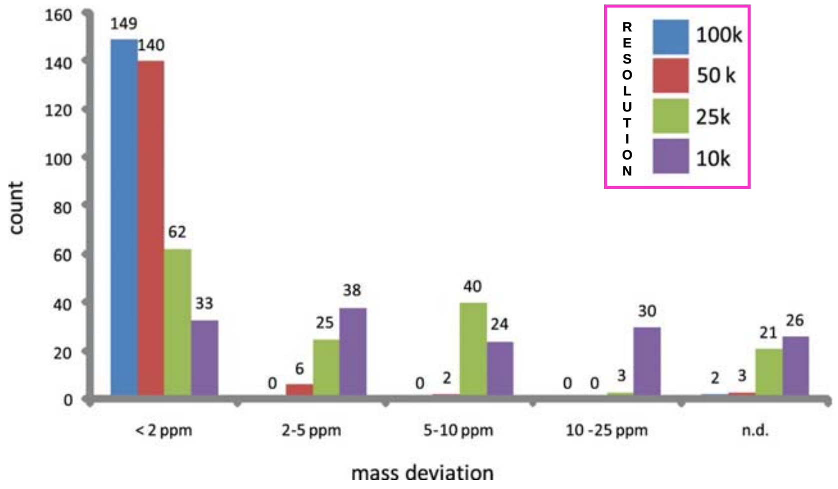


	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	516.76671	0.0	0.0	21.0	$C_{49}H_{71}O_{12}N_{13}$
	516.76647	0.5	0.2	15.0	$C_{49}H_{79}O_{11}N_{9}S_{2}$
	516.76638	0.6	0.3	12.0	$C_{41}H_{75}O_{14}N_{15}S_{1}$
	516.76705	-0.7	-0.3	11.5	$C_{43}H_{77}O_{15}N_{12}S_{1}$
2 ppm (10)	516.76604	1.3	0.7	16.0	$C_{48}H_{75}O_{16}N_{9}$
	516.76738	-1.3	-0.7	20.5	$C_{51}H_{73}O_{13}N_{10}$
	516.76604	1.3	0.7	21.5	$C_{47}H_{69}O_{11}N_{16}$
	516.76580	1.8	0.9	15.5	$C_{47}H_{77}O_{10}N_{12}S_{2}$
	516.76772	-2.0	-1.0	16.5	$C_{44}H_{73}O_{11}N_{16}S_{1}$
	516.76773	-2.0	-1.0	11.0	$C_{45}H_{79}O_{16}N_{9}S_{1}$
5 ppm (23)	516.76805	-2.6	-1.3	25.5	$C_{52}H_{69}O_{9}N_{14}$
	516.76537	2.6	1.3	16.5	$C_{46}H_{73}O_{15}N_{12}$
	516.76807	-2.6	-1.4	7.0	$C_{38}H_{79}O_{14}N_{15}S_{2}$
	516.76513	3.0	1.6	10.5	$C_{46}H_{81}O_{14}N_{8}S_{2}$
	516.76513	3.1	1.6	16.0	$C_{45}H_{75}O_{9}N_{15}S_{2}$
	516.76839	-3.3	-1.7	16.0	$C_{46}H_{75}O_{12}N_{13}S_{1}$
	516.76479	3.7	1.9	20.0	$C_{52}H_{75}O_{11}N_{9}S_{1}$
	516.76872	-3.9	-2.0	25.0	$C_{54}H_{71}O_{10}N_{11}$
	516.76470	3.9	2.0	17.0	$C_{44}H_{71}O_{14}N_{15}$
	516.76874	-3.9	-2.0	6.5	$C_{40}H_{81}O_{15}N_{12}S_{2}$
	516.76446	4.3	2.2	11.0	$C_{44}H_{79}O_{13}N_{11}S_{2}$
	516.76897	-4.4	-2.3	12.5	$C_{40}H_{73}O_{16}N_{16}$
	516.76907	-4.6	-2.4	15.5	$C_{48}H_{77}O_{13}N_{10}S_{1}$

Average: $C_{4.9384}H_{7.7583}N_{1.3577}O_{1.4773}S_{0.0417}$

Limits set to: $C_{35} - C_{70}$; $H_{45} - H_{100}$; $N_{8} - N_{16}$; $O_{9} - O_{16}$; $S_{0} - S_{2}$

Samples of honey and animal feed spiked with 151 pesticides



M. Kellmann, H. Muenster, P. Zomer, H. Molb, *J. Am. Soc. Mass Spectrom.* **20**, 1464-1476 (2009)

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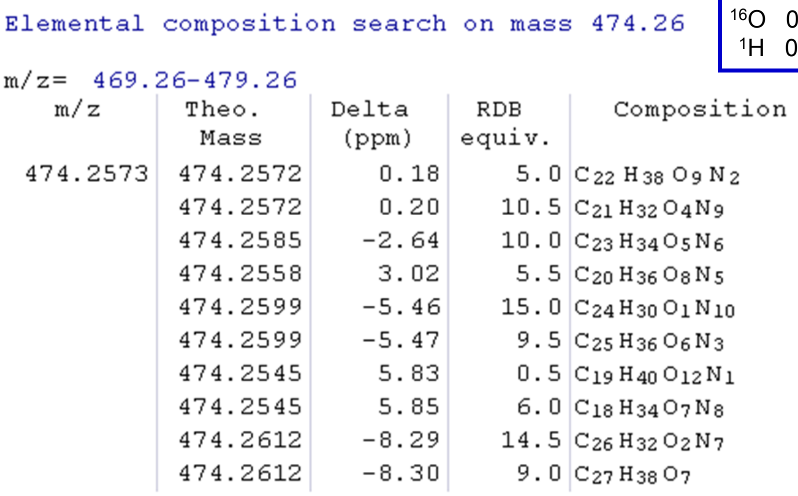
^{12}C 0÷30
^{13}C 0÷1
^{14}N 0÷10
^{16}O 0÷15
^{1}H 0÷60



```
Elemental composition search on mass 474.26

m/z= 469.26-479.26
   m/z       Theo.      Delta      RDB      Composition
              Mass      (ppm)     equiv.
 474.2573  474.2572      0.18       5.0   C22 H38 O9 N2
           474.2572      0.20      10.5   C21 H32 O4 N9
           474.2585     -2.64      10.0   C23 H34 O5 N6
           474.2558      3.02       5.5   C20 H36 O8 N5
           474.2599     -5.46      15.0   C24 H30 O1 N10
           474.2599     -5.47       9.5   C25 H36 O6 N3
           474.2545      5.83       0.5   C19 H40 O12 N1
           474.2545      5.85       6.0   C18 H34 O7 N8
           474.2612     -8.29      14.5   C26 H32 O2 N7
           474.2612     -8.30       9.0   C27 H38 O7
```

Restrizioni sui valori di massa accurata:

Non solo il valore di massa accurata, ma è necessario considerare anche:

Double bond/ring equivalents (RDB)

$$D = 1 + \frac{\left[\sum_{i}^{imax} Ni\,(Vi - 2) \right]}{2}$$

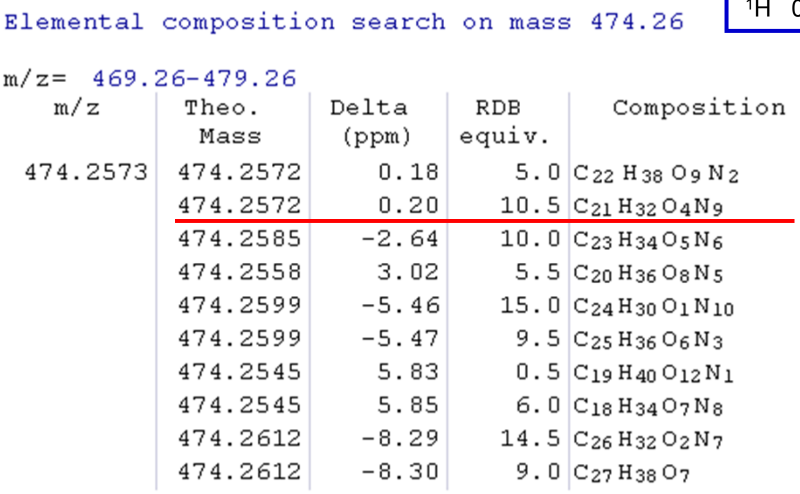
Ni is the number of atoms of element i, and Vi is the valence of atom i.

Odd-electron ions = RDB integer

Even-electron ions = RDB + 0.5

Ionization technique: ESI (+) ⟶ for $[M+H]^+$ RDB=x.5

^{12}C 0÷30
^{13}C 0÷1
^{14}N 0÷10
^{16}O 0÷15
^{1}H 0÷60



Elemental composition search on mass 474.26

m/z= 469.26-479.26

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
474.2573	474.2572	0.18	5.0	$C_{22}H_{38}O_9N_2$
	474.2572	0.20	10.5	$C_{21}H_{32}O_4N_9$
	474.2585	-2.64	10.0	$C_{23}H_{34}O_5N_6$
	474.2558	3.02	5.5	$C_{20}H_{36}O_8N_5$
	474.2599	-5.46	15.0	$C_{24}H_{30}O_1N_{10}$
	474.2599	-5.47	9.5	$C_{25}H_{36}O_6N_3$
	474.2545	5.83	0.5	$C_{19}H_{40}O_{12}N_1$
	474.2545	5.85	6.0	$C_{18}H_{34}O_7N_8$
	474.2612	-8.29	14.5	$C_{26}H_{32}O_2N_7$
	474.2612	-8.30	9.0	$C_{27}H_{38}O_7$

Restrizioni sui valori di massa accurata:

RDB

Cluster isotopico:

non si considera solo il valore di massa accurata dello ione molecolare/ protonato/deprotonato, ma anche quelli degli ioni M+1, M+2 insieme alla loro intensità relativa

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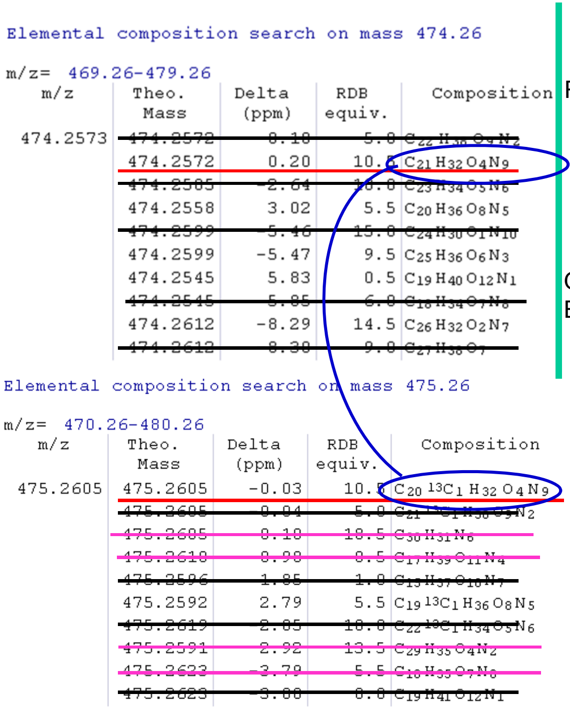


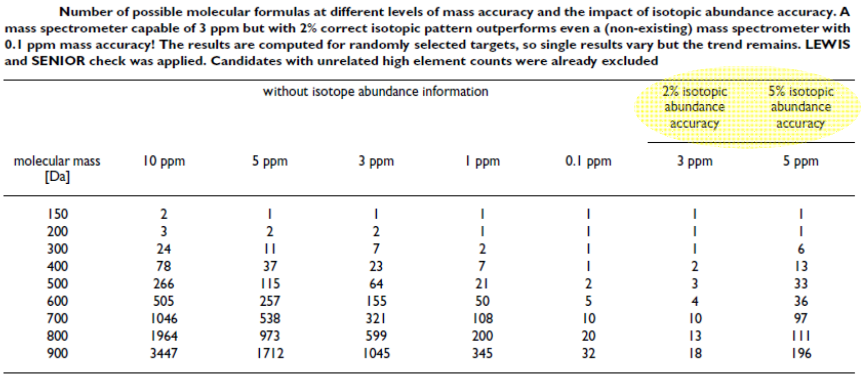
```
Elemental composition search on mass 474.26

m/z=  469.26-479.26
   m/z     Theo.     Delta    RDB     Composition
           Mass      (ppm)   equiv.
 474.2573 ~~474.2572    0.10     5.0  C22 H38 O4 N2~~
           474.2572    0.20    10.5  C21 H32 O4 N9
          ~~474.2585   -2.64    10.0  C23 H34 O5 N6~~
           474.2558    3.02     5.5  C20 H36 O8 N5
          ~~474.2599   -5.46    15.0  C24 H30 O1 N10~~
           474.2599   -5.47     9.5  C25 H36 O6 N3
           474.2545    5.83     0.5  C19 H40 O12 N1
          ~~474.2545    5.85     6.0  C18 H34 O7 N8~~
           474.2612   -8.29    14.5  C26 H32 O2 N7
          ~~474.2612   -8.30     9.0  C27 H38 O7~~

Elemental composition search on mass 475.26

m/z=  470.26-480.26
   m/z     Theo.     Delta    RDB     Composition
           Mass      (ppm)   equiv.
 475.2605  475.2605   -0.03    10.5  C20 13C1 H32 O4 N9
          ~~475.2605   -0.04     5.0  C21 13C1 H38 O5 N2~~
          ~~475.2605    0.10    18.5  C30 H31 N6~~
          ~~475.2610    0.98     8.5  C17 H39 O11 N4~~
          ~~475.2596    1.85     1.0  C13 H37 O10 N7~~
           475.2592    2.79     5.5  C19 13C1 H36 O8 N5
          ~~475.2619   -2.85    10.0  C22 13C1 H34 O5 N6~~
          ~~475.2591    2.92    13.5  C29 H35 O4 N2~~
          ~~475.2623   -3.79     5.5  C18 H35 O7 N8~~
          ~~475.2623   -3.80     0.0  C19 H41 O12 N1~~
```

RDB=double bond/ring equivalents

$$D = 1 + \frac{\left[\sum_{i}^{imax} N_i (V_i - 2)\right]}{2}$$

Odd-electron ions = RDB integer
Even-electron ions= RDB x.5

^{12}C 0÷30
^{13}C 0÷1
^{14}N 0÷10
^{16}O 0÷15
^{1}H 0÷60



Number of possible molecular formulas at different levels of mass accuracy and the impact of isotopic abundance accuracy. A mass spectrometer capable of 3 ppm but with 2% correct isotopic pattern outperforms even a (non-existing) mass spectrometer with 0.1 ppm mass accuracy! The results are computed for randomly selected targets, so single results vary but the trend remains. LEWIS and SENIOR check was applied. Candidates with unrelated high element counts were already excluded

	without isotope abundance information					2% isotopic abundance accuracy	5% isotopic abundance accuracy
molecular mass [Da]	10 ppm	5 ppm	3 ppm	1 ppm	0.1 ppm	3 ppm	5 ppm
150	2	1	1	1	1	1	1
200	3	2	2	1	1	1	1
300	24	11	7	2	1	1	6
400	78	37	23	7	1	2	13
500	266	115	64	21	2	3	33
600	505	257	155	50	5	4	36
700	1046	538	321	108	10	10	97
800	1964	973	599	200	20	13	111
900	3447	1712	1045	345	32	18	196

T. Kind, O. Fiehn, *BMC Bioinformatics,* **7**, 234 (2006)

Restrizioni sui valori di massa accurata ottenuti:

Ioni frammento:

non si considera solo il valore di massa accurata dello ione molecolare/ protonato/deprotonato, ma anche quelli degli ioni frammento.

p.es. $[C_{10}H_{20}N_2]^+$ non può avere come ione frammento $[C_9H_{18}O]^+$

MALDI-FTICR Imaging Mass Spectrometry of Drugs and Metabolites in Tissue

High mass-resolution makes it possible to measure many isobaric peptide ions from tissue

For many analytes of interest in a small-molecule imaging experiment, the specificity of having an elemental composition will be sufficient to confirm identity.

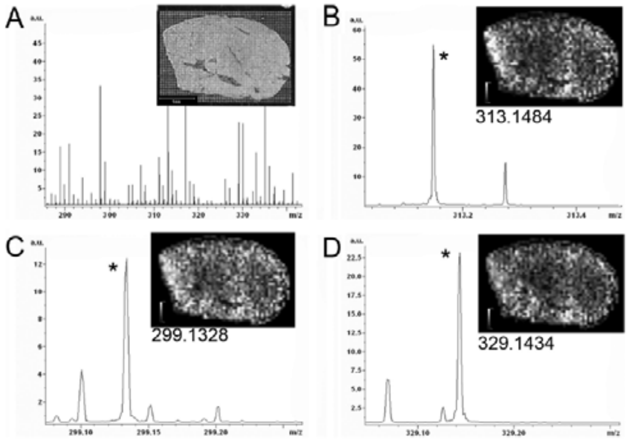
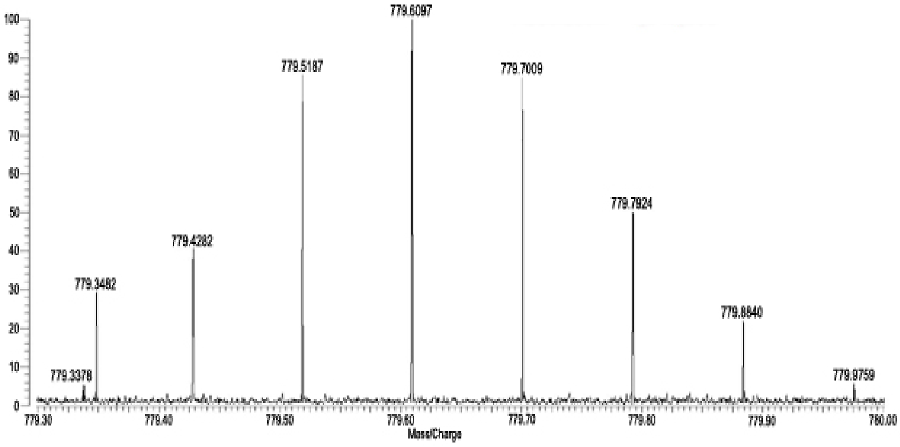


Figure 1. FTICR images from a liver of a rat dosed with 8 mg/kg olanzapine (2 h postdose). (A) Average of all spectra collected from matrix spots on the tissue (inset image). The *m/z* region of the average spectrum in the regions of protonated molecule of (B) olanzapine, (C) desmethyl metabolite, and (D) hydroxymethyl metabolite. Ion images of each marked peak is inset to each spectrum.

D. S. Cornett, S. L. Frappier, R. M. Caprioli, *Anal. Chem.* **2008,** *80,* 5648–5653

Esercizio N. 1

Determinare il peso molecolare



Spettrometria di massa in alta risoluzione (HRMS):

Introduzione diretta, infusione

GC-HRMS

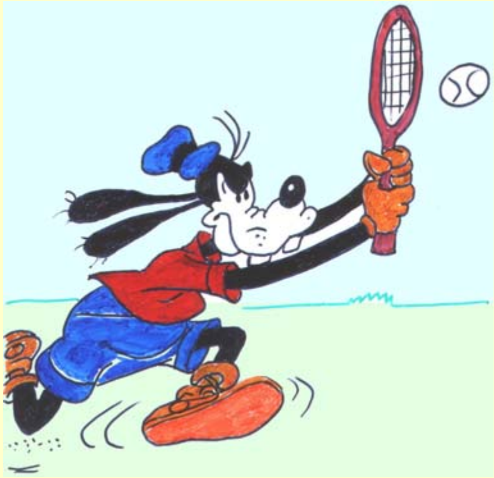
GC-HRMS/MS, GC-HRMS^n

HPLC-HRMS

HPLC-HRMS/MS, HPLC-HRMS^n

………………..

Metodi di desorbimento (ESI, MALDI)



Ionizzazione

SOFT

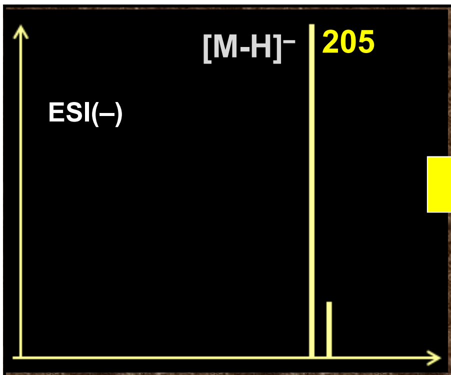
✓ **Frammenti assenti** → **informazione strutturale?**

Spettrometria di massa

Spettro di massa ⟶ Peso molecolare

HR + massa accurata ⟶ Stechiometria

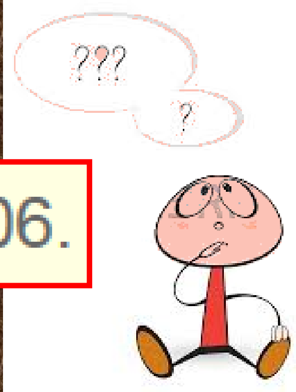
? ⟵ Informazioni strutturali



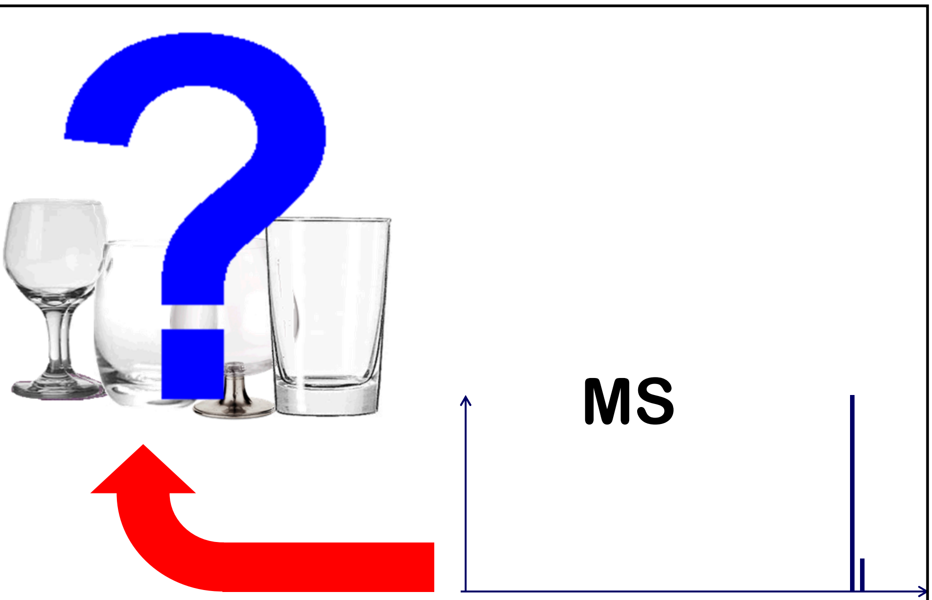
Quale è il tuo PM?

CHI SEI ?

PM=206

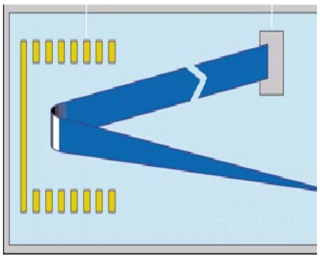


total of 96906.



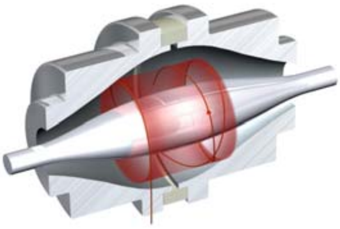
È possibile aumentare la specificità?

Spettrometria di massa **in alta risoluzione**



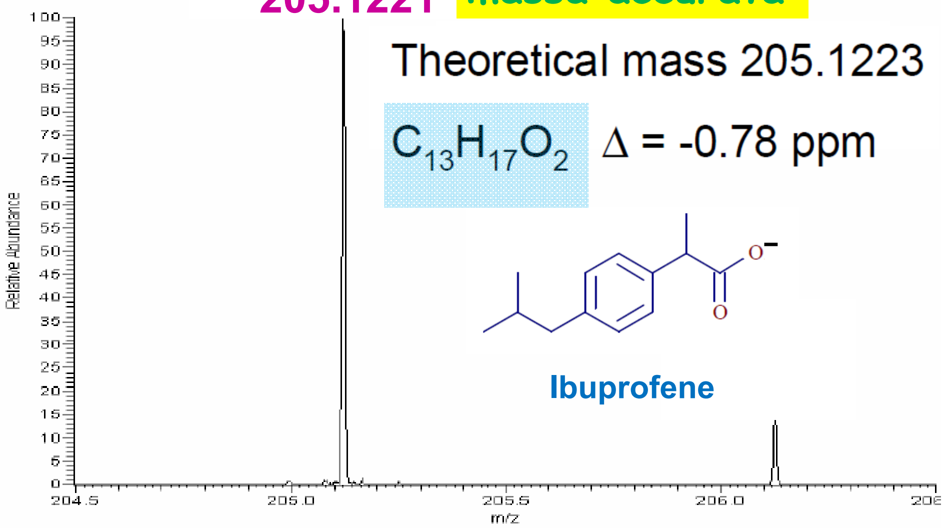
Tempo di volo (TOF)

Orbitrap



Spettrometria di massa in alta risoluzione

specificità



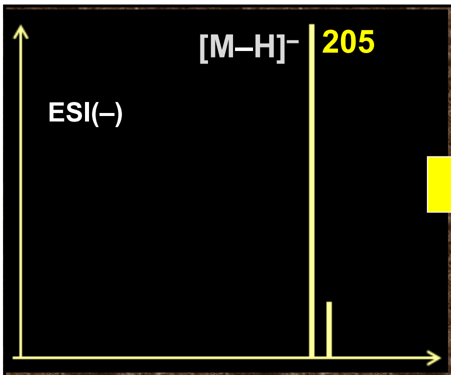
205.1221

massa accurata

Theoretical mass 205.1223

$C_{13}H_{17}O_2$ Δ = -0.78 ppm

Ibuprofene

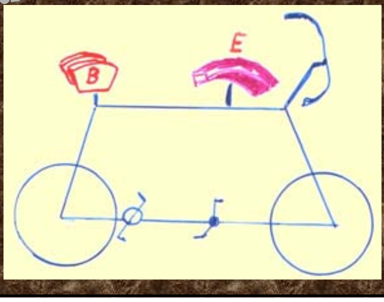


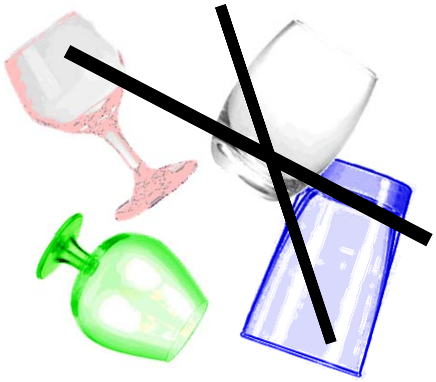
Quale è il tuo PM? **206 !!**
Qual è la tua formula bruta?
$C_{13}H_{17}O_2$

CHI SEI ?

Qual è la tua struttura?
Quali sono i tuoi frammenti?

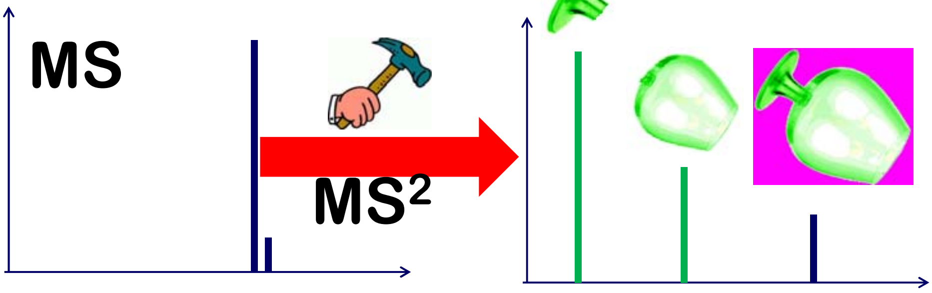
Spettrometria di massa tandem





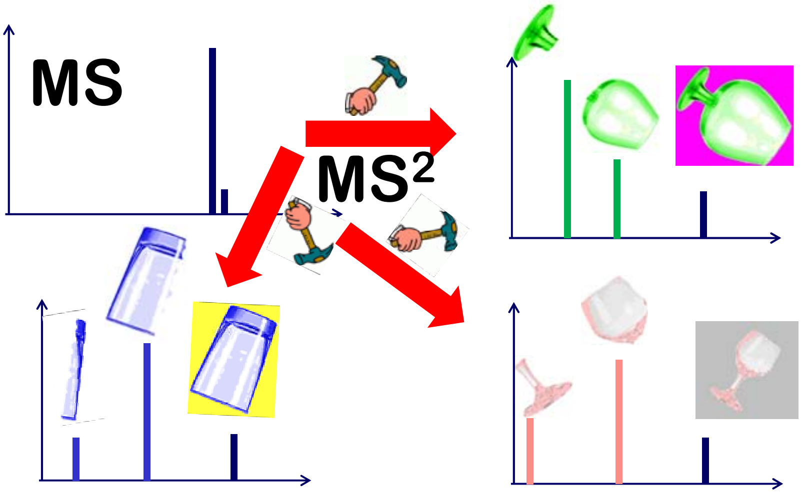
Spettrometria di massa tandem

specificità



Spettrometria di massa tandem

specificità



Spettrometria di massa
tandem

MS^2 MS/MS

~~Tandem massa~~

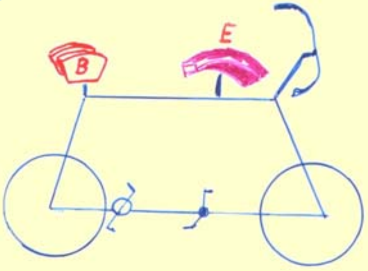
~~Tandem max~~

~~Abcgftrd max~~

Metodi di desorbimento (ESI, MALDI)

- ✓ **Informazioni sullo ione molecolare**
- ✓ **Frammenti assenti** ⟶ informazione strutturale?

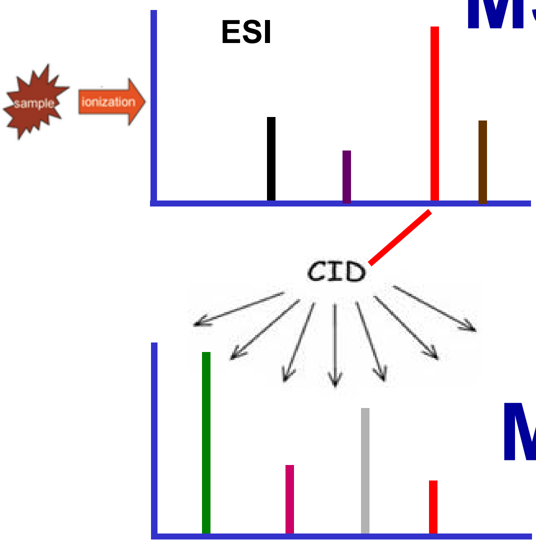
Spettrometria di massa tandem



✓ **Spettrometria di massa tandem**

La spettrometria di massa tandem impiega **due o più stadi** di analisi di massa con lo scopo di esaminare **selettivamente** le frammentazioni di una famiglia di ioni

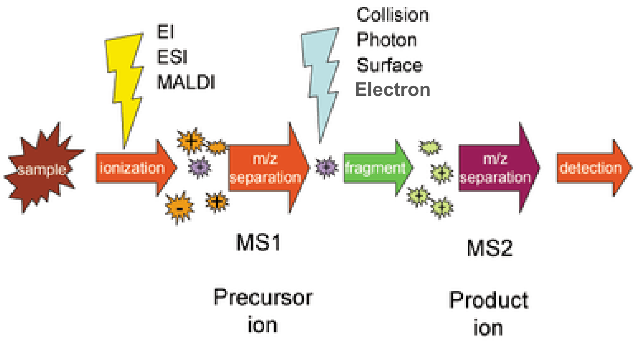
MS/MS **MS^2** .... **MS^n**



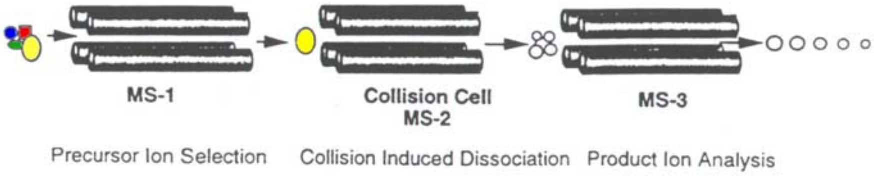
MS: determinazione PM

isolamento
collisione con un gas
analisi degli ioni frammento

MS^2: determinazione della struttura

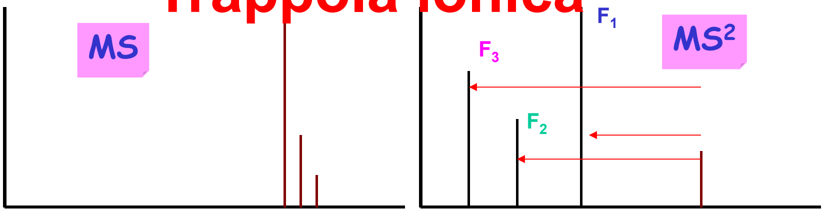


✓ **Spettrometria di massa** **tandem**



Q	**q**	**Q**
Q	**q**	**TOF**

Trappola ionica



2274 *Journal of the American Chemical Society / 100:7 / March 29, 1978*

Selected Ion Fragmentation with a Tandem Quadrupole Mass Spectrometer

R. A. Yost, C. G. Enke*
Department of Chemistry, Michigan State University
East Lansing, Michigan 48824

Received November 16, 1977

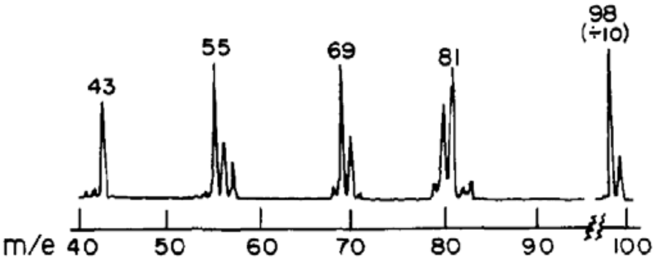


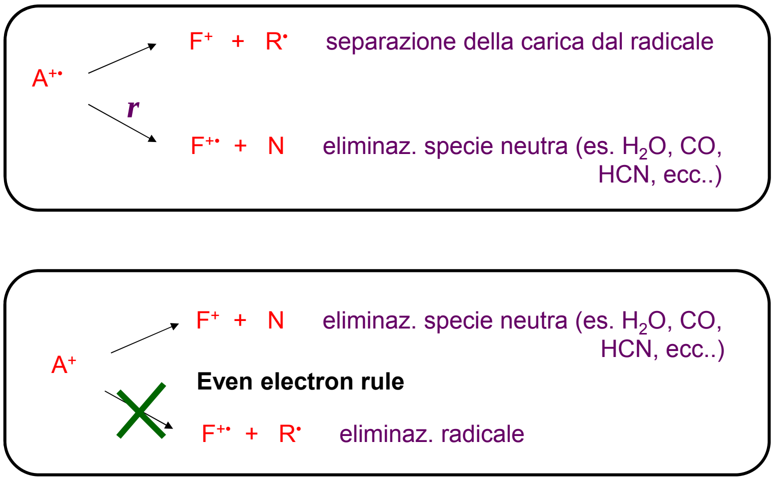
Figure 1. CID spectrum of the parent ion (m/e 98) of cyclohexanone present as 5% of a mixture.

Il triplo quadrupolo: trent'anni di successi !

Attualità, strumentazione, applicazioni, prospettive

Milano, 18 dicembre 2008

Reazioni di decomposizione



$A^{+\bullet}$ → F^{+} + $R^{\bullet}$ separazione della carica dal radicale

$A^{+\bullet}$ $\xrightarrow{r}$ $F^{+\bullet}$ + N eliminaz. specie neutra (es. H_2O, CO, HCN, ecc..)

A^{+} → F^{+} + N eliminaz. specie neutra (es. H_2O, CO, HCN, ecc..)

Even electron rule

A^{+} → $F^{+\bullet}$ + $R^{\bullet}$ eliminaz. radicale

Decomposizioni spontanee:

decomposizioni metastabili

Decomposizioni indotte da:

collisioni con un gas (Collision induced dissociation (CID) collision activated dissociation (CAD))

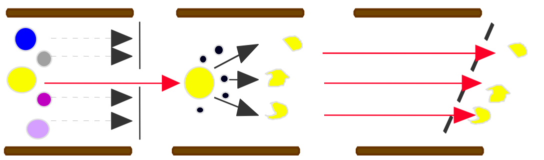
interazioni con elettroni (ECD, ETD)

interazioni con superfici (SID)

interazioni con fotoni (IRMPD, ion spectroscopy)

MS/MS

Product Ion Scan



Scelta dello Ione

Precursore

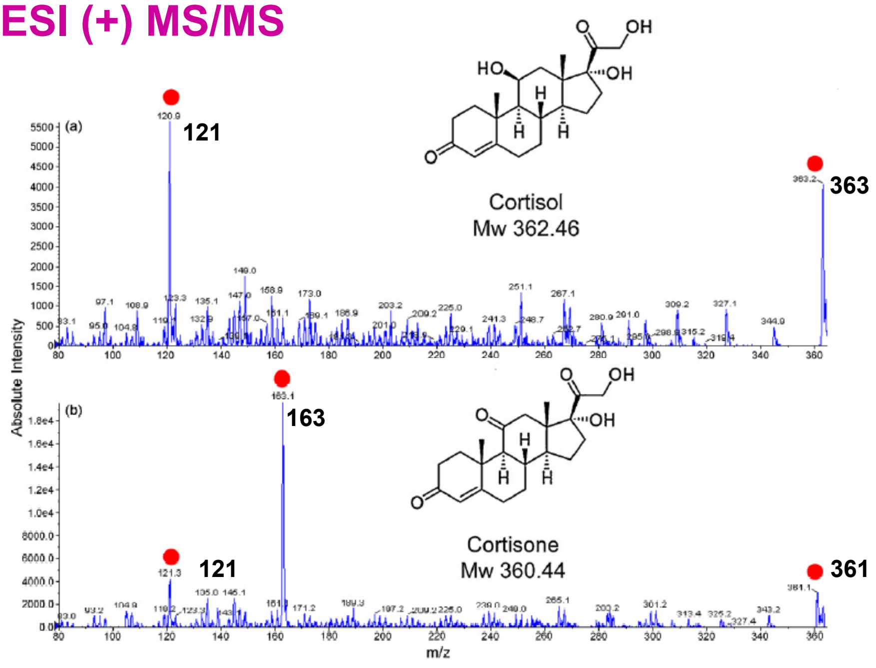
Frammentazione
CID

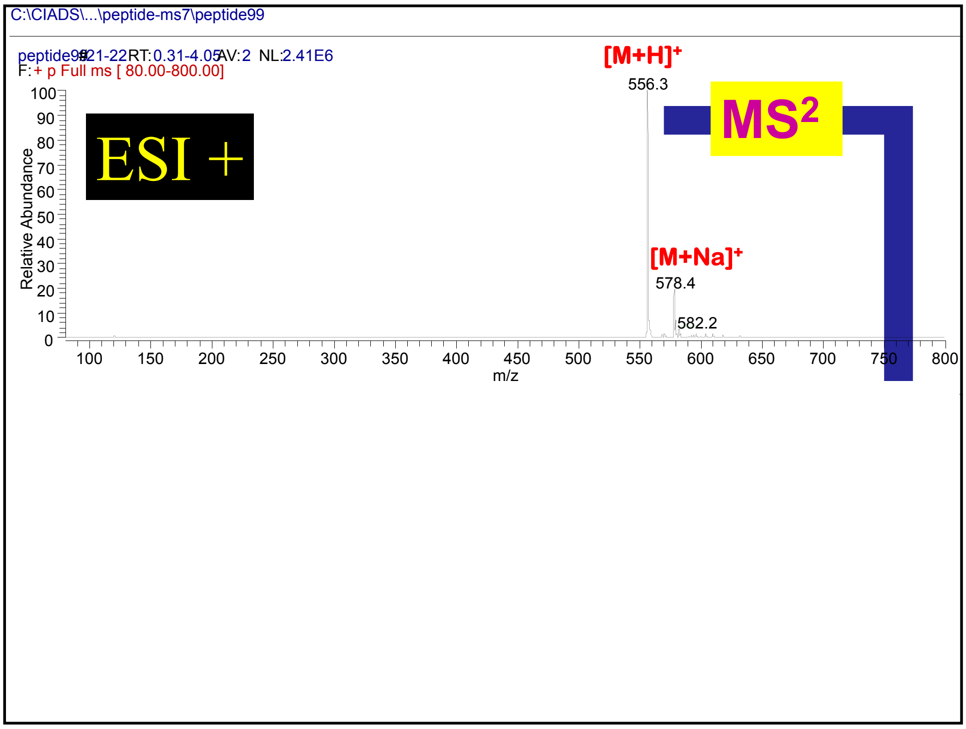
Spettro dei prodotti di
Frammentazione

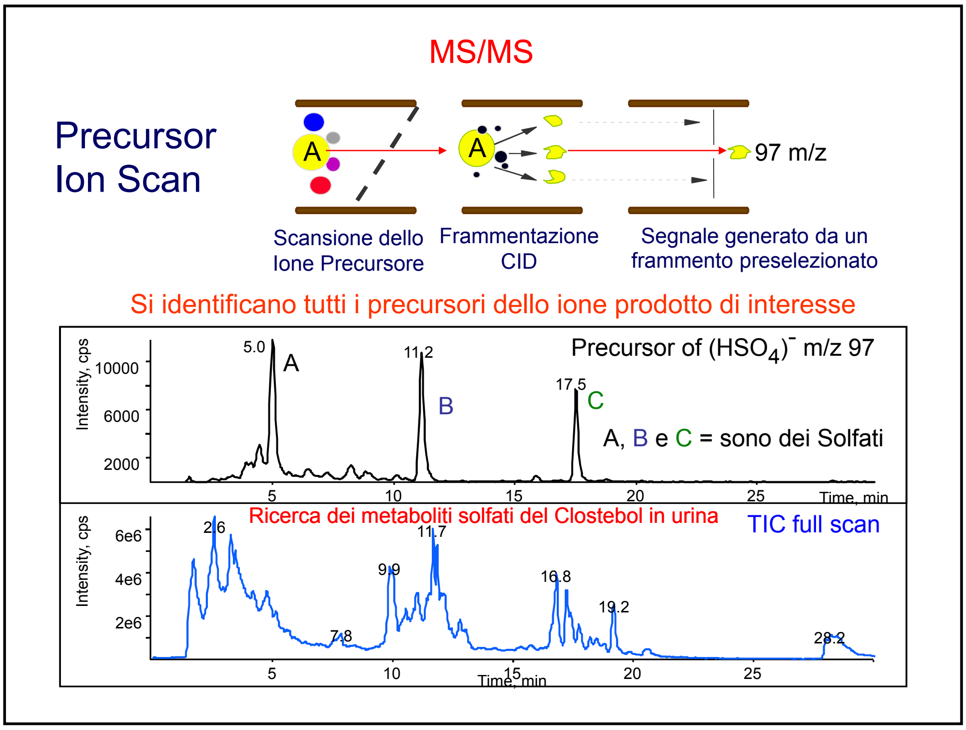
Si identificano tutti i prodotti dello ione precursore selezionato

---

ESI (+) MS/MS







MS/MS

Precursor Ion Scan

Scansione dello Ione Precursore

Frammentazione CID

Segnale generato da un frammento preselezionato

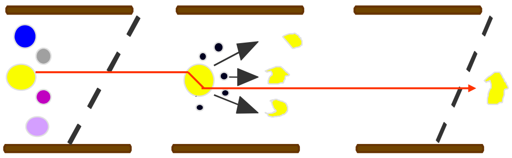
Si identificano tutti i precursori dello ione prodotto di interesse

A, B e C = sono dei Solfati

Ricerca dei metaboliti solfati del Clostebol in urina

MS/MS

Neutral Loss Scan



Scansione di Q1 e Q3 con ΔM costante

Frammentazione CID

Segnale generato da frammenti con predefinita differenza di massa dal precursore

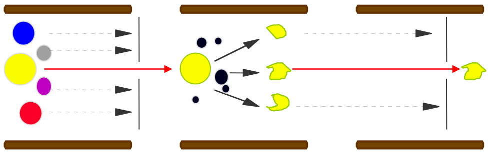
Si identifica la perdita di un frammento neutro da parte di un certo numero di precursori

---

MS/MS

SRM

MRM



Scelta dello Ione Precursore

Frammentazione CID

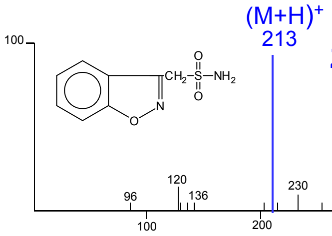
Analisi dello ione prodotto (SRM) o degli ioni prodotti (MRM)

Si monitora una transizione specifica (SRM)
o più transizioni (MRM)
sia con scansioni di ioni prodotto, di ioni precursori
o neutral loss

Identificazione rapida di metaboliti di farmaci

①

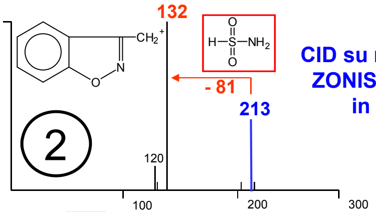
Scegliere il sistema di ionizzazione in cui $M^{+\bullet}$, $[M+H]^+$ o $[M-H]^-$ è il picco base e dà poca frammentazione



ZONISAMIDE in CI⁺
Antiepilettico

TIPI DI SCANSIONI IN MS/MS

-Collidere lo ione molecolare protonato **(213)** per ottenere lo spettro degli ioni prodotto

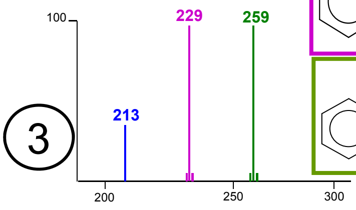


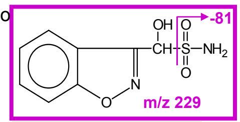
CID su m/z 213
ZONISAMIDE
in CI⁺

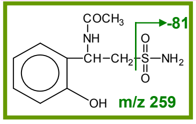
SUL CAMPIONE BIOLOGICO ESTRATTO DA PLASMA O DA URINE IDROLIZZATE

-Spettro **PERDITA NEUTRA** selezionando la perdita del frammento **neutro (- 81)** gruppo sulfonamidico
Gli ioni presenti sono possibili **METABOLITI**

SPETTRO
NEUTRAL LOSS
(-81)

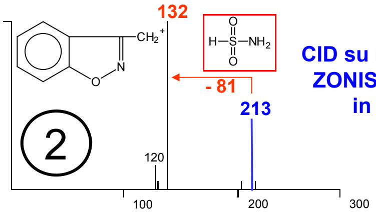






TIPI DI SCANSIONI IN MS/MS

-Collidere lo ione molecolare protonato **(213)** per ottenere lo spettro degli ioni prodotto

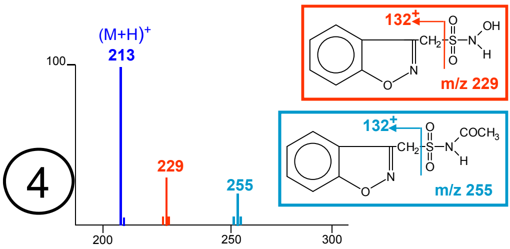


CID su m/z 213
ZONISAMIDE
in CI^+

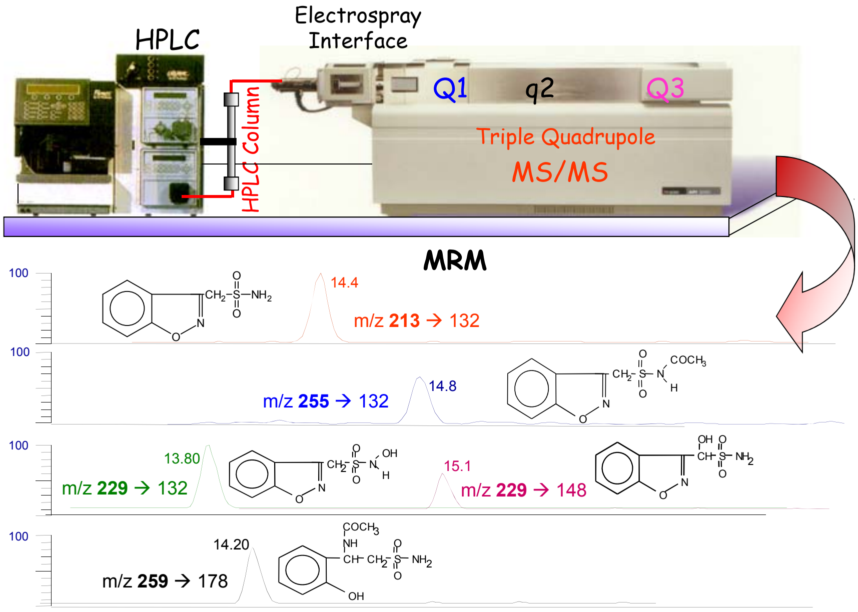
SUL CAMPIONE BIOLOGICO ESTRATTO DA PLASMA O DA URINE IDROLIZZATE

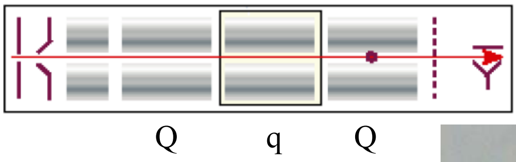
-Spettro **DEGLI IONI PRECURSORI** selezionando lo ione **(132)** di cui si ottengono tutti i precursori
Gli ioni presenti sono altri possibili **METABOLITI**

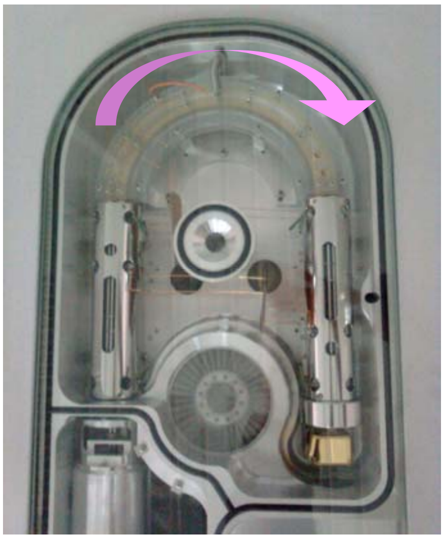
SPETTRO PRECURSORI DELLO IONE (132)



LC-ESI-MS/MS

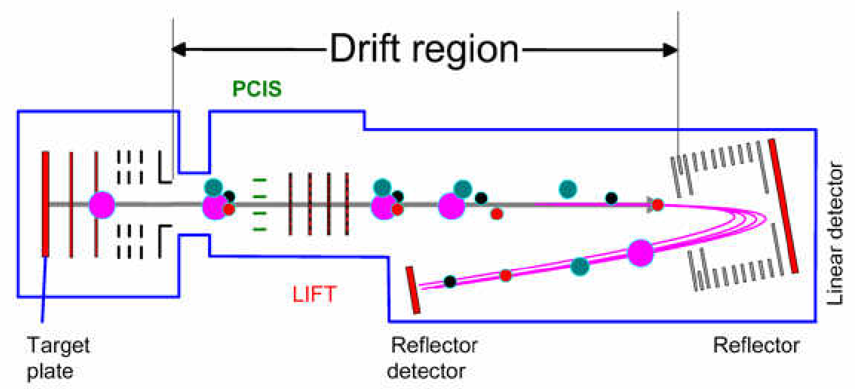


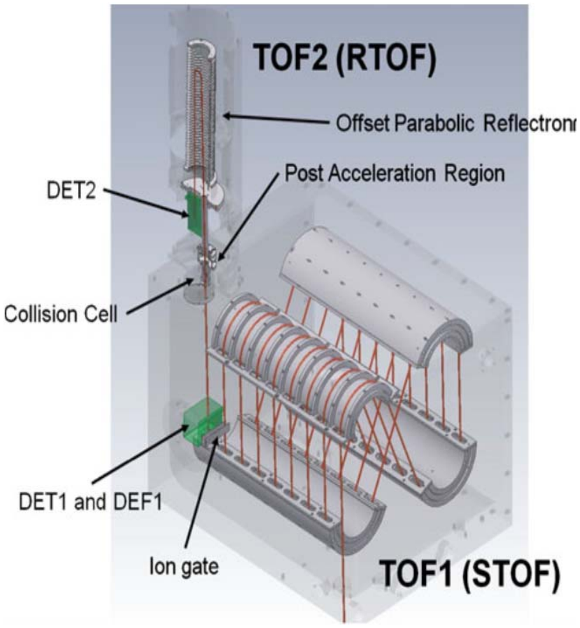




QqQ con cella di collisione a 180°

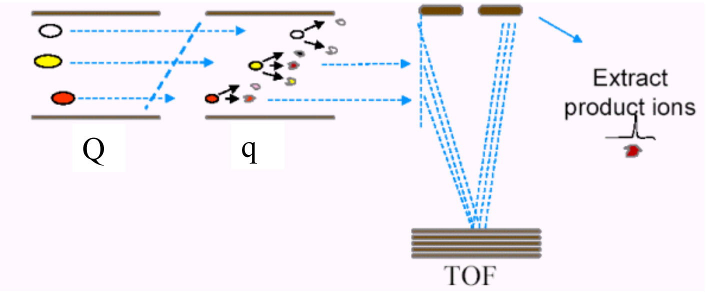
TOF-TOF



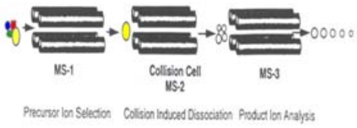


MS/MS
Analizzatori ibridi …..

QqTOF

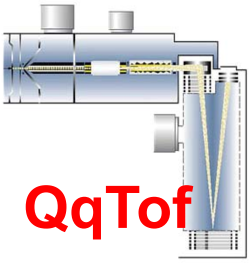


MS/MS nello spazio

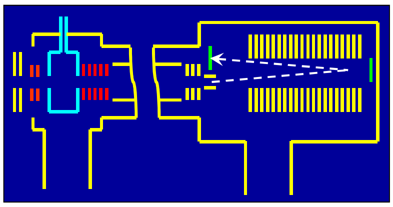


QqQ

BE



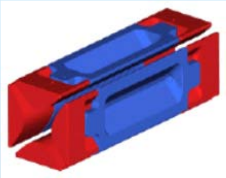
QqTof



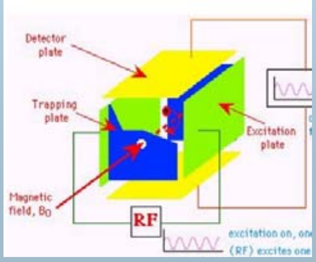
Tof-Tof

MS/MS nel tempo

3D



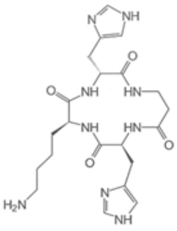
2D



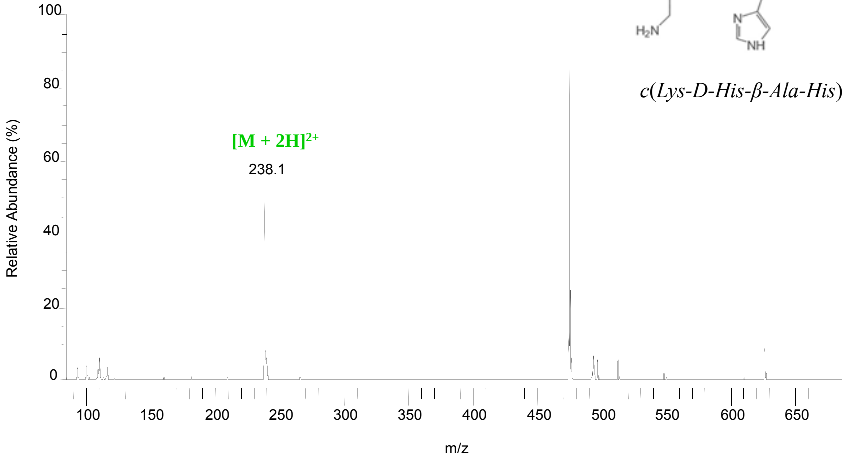
Cella a risonanza ciclotronica

Animazione IT 3D e 2D

ESI (+)

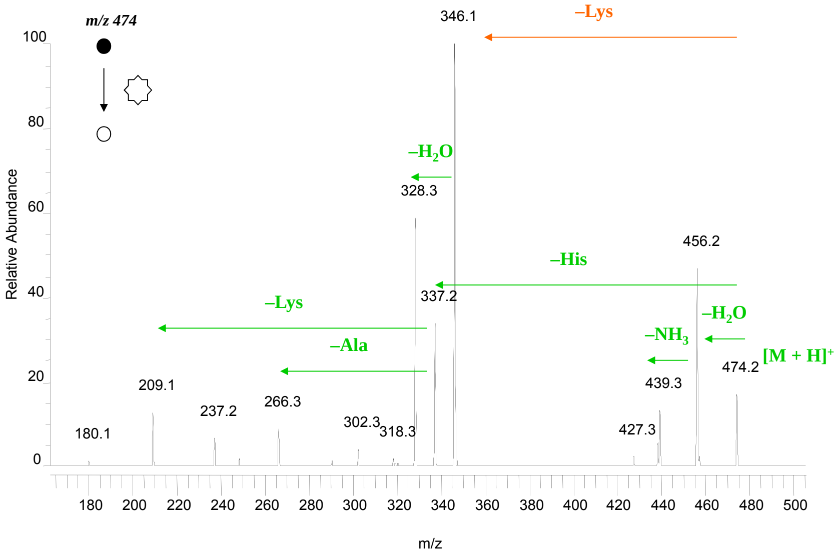


c(*Lys-D-His-β-Ala-His*)

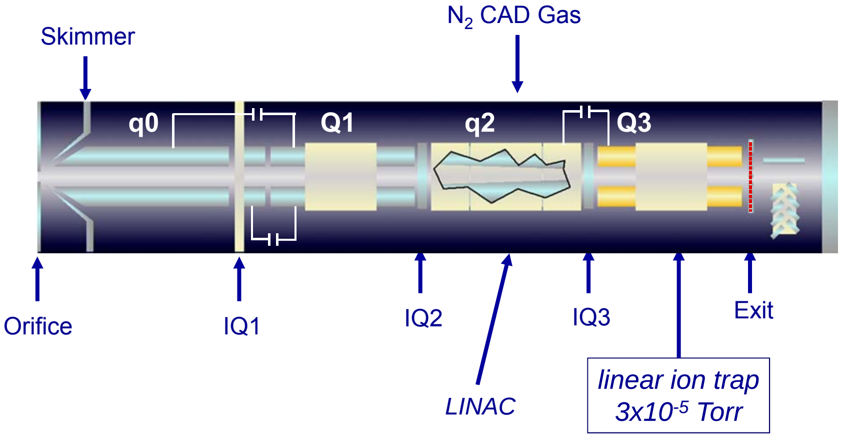


G. Giorgi et al., SCI2006, Firenze, 10-15 settembre 2006

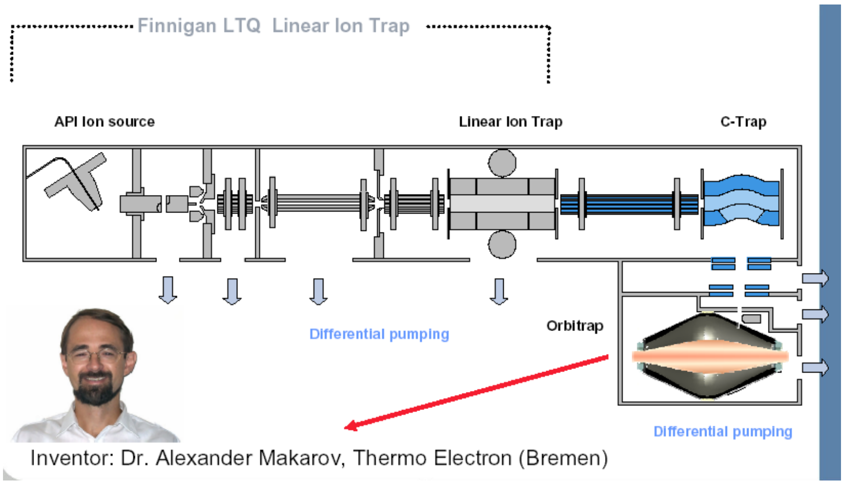
MS^2 dello ione a m/z 474



Hybrid Instrument with Q3 Linear Ion Trap MS



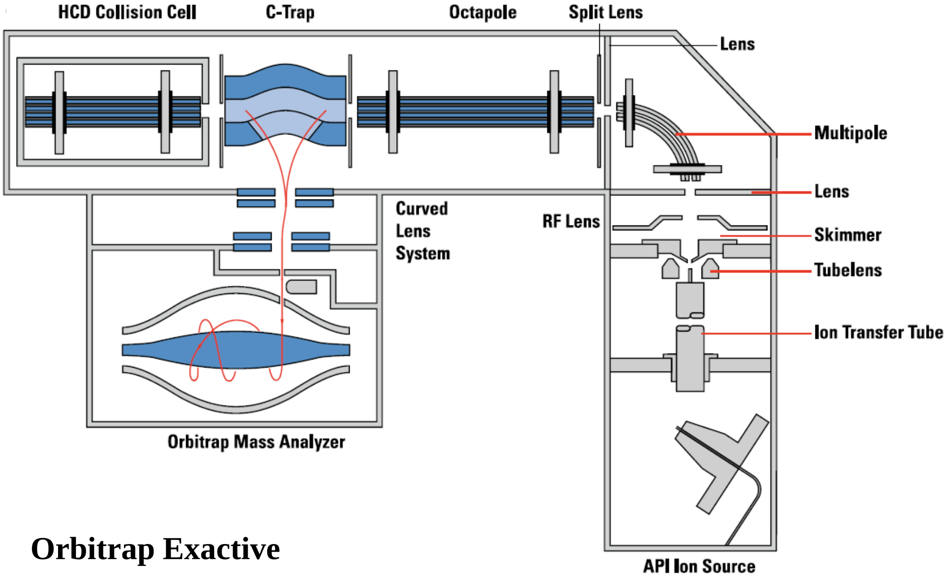
Linear Ion Trap – Orbitrap Hybrid



Inventor: Dr. Alexander Makarov, Thermo Electron (Bremen)

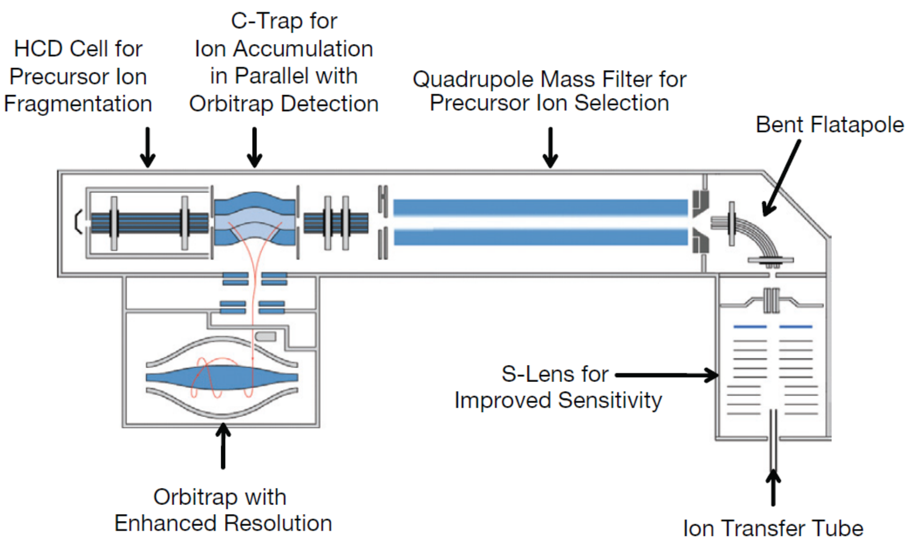
All Ion Fragmentation

Higher energy collisional dissociation (HCD)



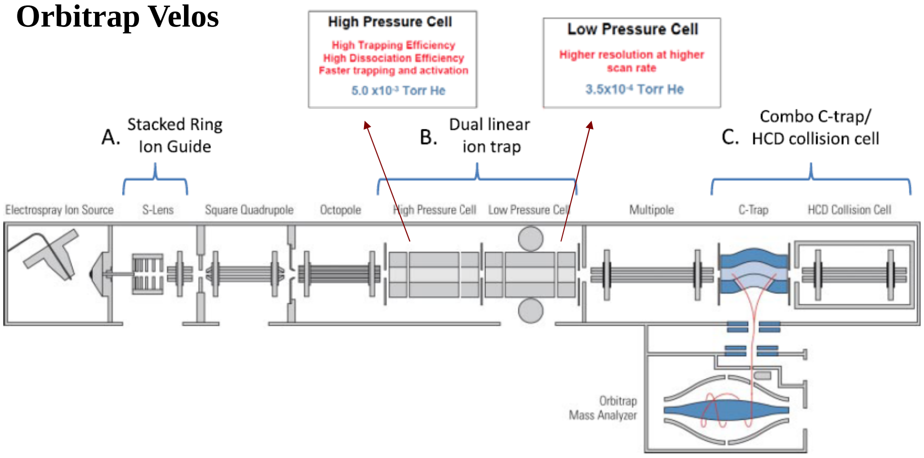
Orbitrap Exactive

HCD energy **20-60 V**.

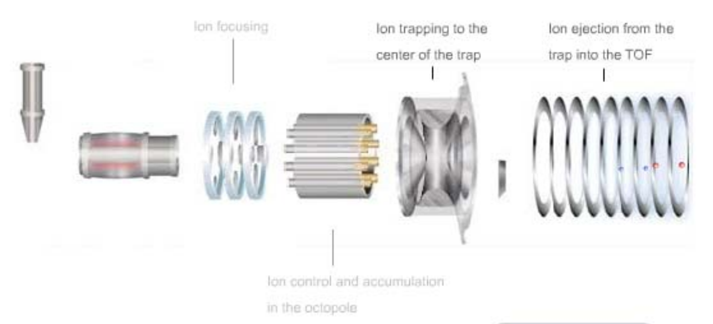


Orbitrap Q Exactive

Orbitrap Velos



MS/MS IT-TOF ibrido



http://www.shimadzu.com/an/lcms/lcmsittof/qn504200000063pm-att/oh80jt000000afsr.swf

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Compressed Ion Introduction (CII™)