

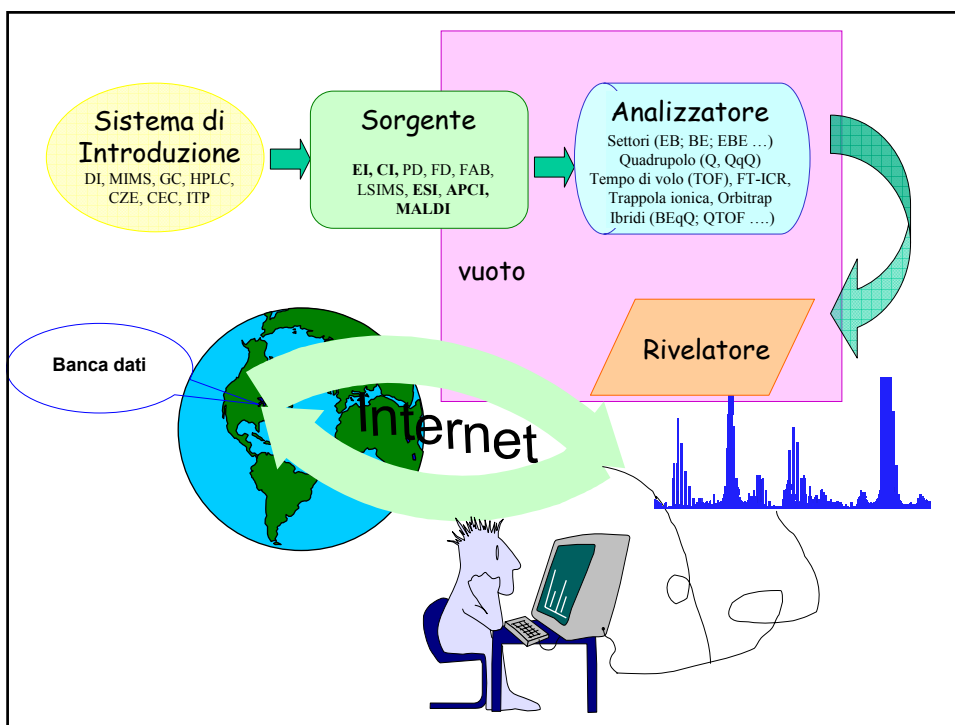
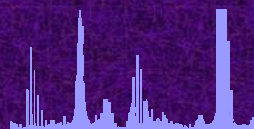
# Basi teoriche e definizioni della spettrometria di massa in alta risoluzione

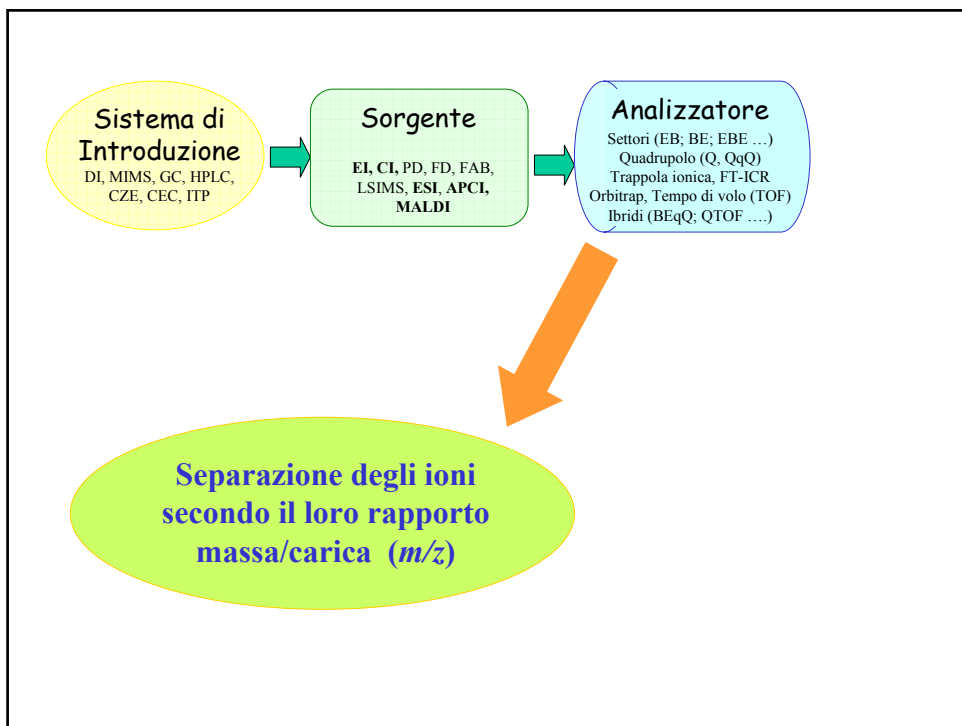


**Gianluca Giorgi**

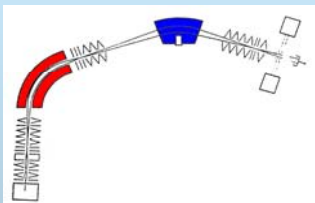
Università di Siena  
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53100 Siena

e-mail: gianluca.giorgi@unisi.it



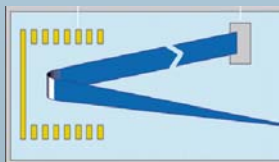
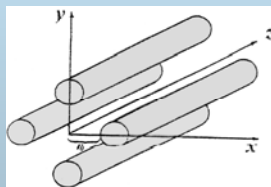


# Separazione degli ioni ..... nello spazio



**Settori:** elettrostatico e magnetico

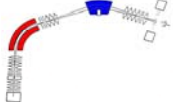
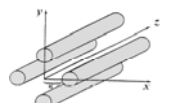
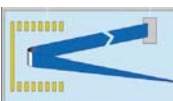
**Quadrupolo**



**Tempo di volo**

## Separazione degli ioni nello spazio



Analizzatore	Forza	Separazione in base a	Range m/z
<b>Doppio fuoco (EB, BE)</b> 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000
<b>Quadrupolo</b> 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000
<b>Time of flight</b> 		Velocità	>100.000

# Separazione degli ioni

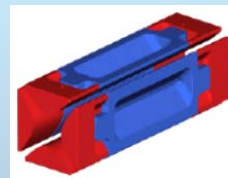
..... nel tempo



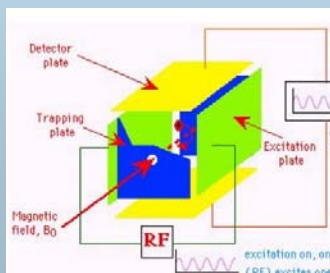
## Separazione degli ioni ..... nel tempo



**Trappola ionica 3D**

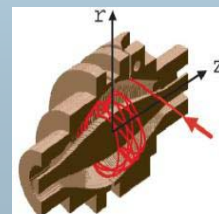


**2D**


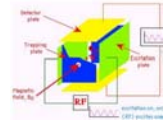
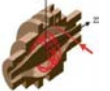


**Cella a  
risonanza  
ciclotronica**

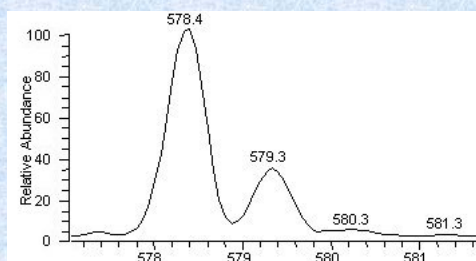
**Orbitrap**



## Separazione degli ioni nel tempo

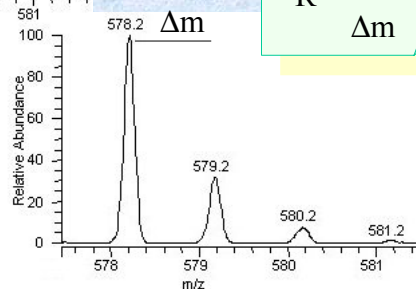
Analizzatore	Forza	Separazione in base a	Range m/z
<b>Trappola ionica</b> (quadrupolare e lineare) 	Campo elettrico + RF	Frequenza delle orbite	4.000
<b>Cella a risonanza ciclotronica (FT-ICR)</b> 	Campo elettrico + RF + campo magnetico	Frequenza delle orbite	>10.000
<b>Orbitrap</b> 	Campo elettrico	Frequenza delle oscillazioni armoniche	4.000

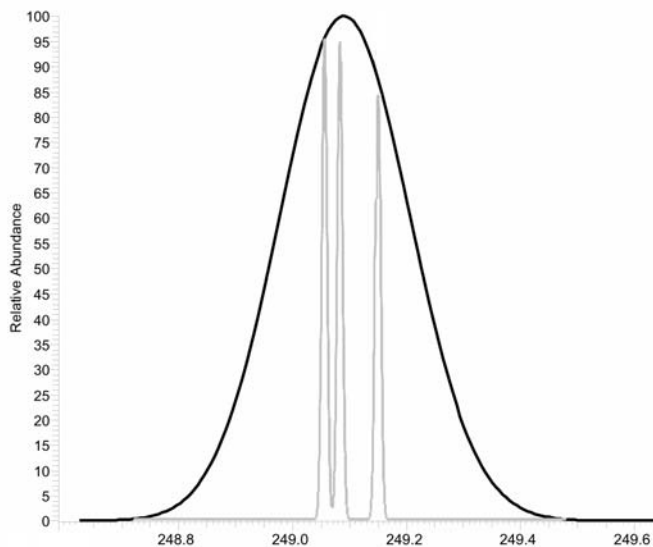
## Proprietà degli analizzatori:



### RISOLUZIONE

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





Mass spectra recorded at different resolutions: mass spectrum of a mixture of three isobaric species  $[\text{C}_{19}\text{H}_7\text{N}]^+$ ,  $[\text{C}_{20}\text{H}_9]^+$ ,  $[\text{C}_{13}\text{H}_{19}\text{N}_3\text{O}_2]^+$  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.

## Resolution, resolving power:



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

# RISOLUZIONE

## Valley definition

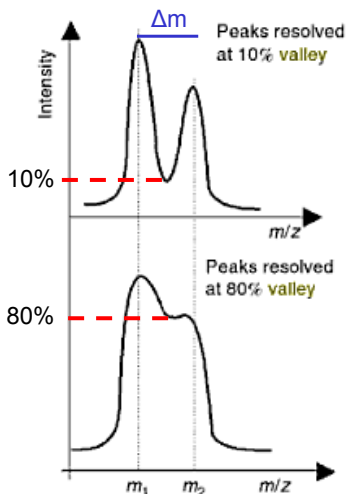
Let two peaks of equal height in a mass spectrum at masses  $m$  and  $m - \Delta m$  be separated by a valley which at its lowest point is just  $x$  % of the height of either peak.

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  $m/\Delta m$ . It is usually a function of  $m$ . The ratio  $m/\Delta m$  should be given for a number of values of  $m$ .

Double sectors, FT-ICR: 10% valley

Q, IT, TOF: 50% valley

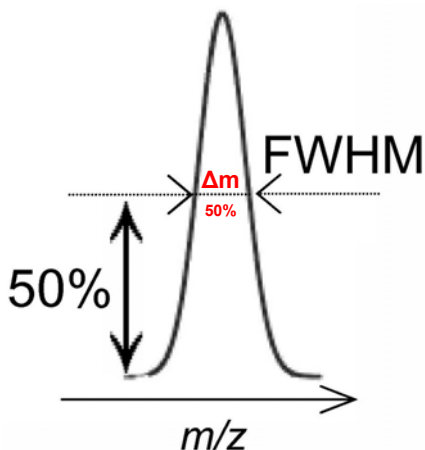


IUPAC: <http://goldbook.iupac.org/R05318.html>

# RISOLUZIONE

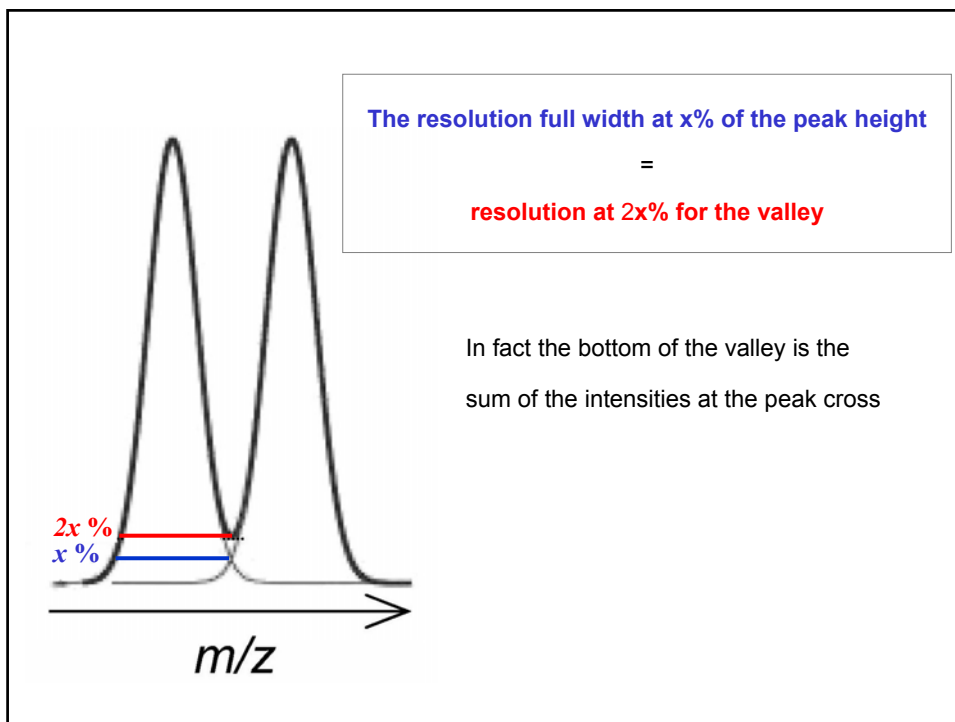
## Peak width definition

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



Resolution at full width at half maximum (FWHM).


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



## Resolving power:

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

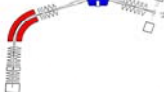
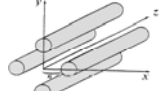
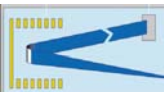
It may be characterized by giving the peak width, measured in mass units, expressed as a function of mass, for at least two points on the peak, specifically at fifty percent and at five percent of the maximum peak height.

PROBLEM TERM
Resolving power (mass spectrometry)

<small>The term Resolving power (mass spectrometry) is listed as a problem term due to obsolescence, misuse, conflict with another term, trademark, commercial use, or other reason.</small>



## Separazione degli ioni nello spazio



Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione
<b>Doppio fuoco (EB, BE)</b> 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000	<b>10.000</b>  <b>Valley</b>
<b>Quadrupolo</b> 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000	<b>Unitaria</b>  <b>FWHM</b>
<b>Time of flight</b> 		Velocità	>100.000	<b>&gt;10.000</b>  <b>FWHM</b>

## Separazione degli ioni nel tempo

Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione
<b>Trappola ionica (quadrupolare e lineare)</b>  	Campo elettrico + RF	Frequenza delle orbite	4.000	<b>&lt; 500</b>  <b>FWHM</b>
<b>Cella a risonanza ciclotronica (FT-ICR)</b> 	Campo elettrico + RF + campo magnetico	Frequenza delle orbite	>10.000	<b>&gt; 500.000</b>  <b>FWHM</b>
<b>Orbitrap</b> 	Campo elettrico	Frequenza delle oscillazioni armoniche	4.000	<b>&gt; 100.000</b>  <b>FWHM</b>

## Resolving power **S**:

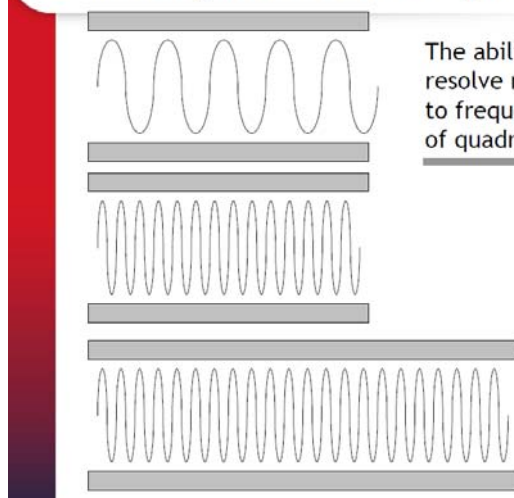
### 1) Quadrupole and Ion Traps

Constant peak width  
Variable resolution  
FWHM Definition

FWHM 0.5u at  $m/z$  40  $\rightarrow$  Res.  $40/0.5=80$ , but also

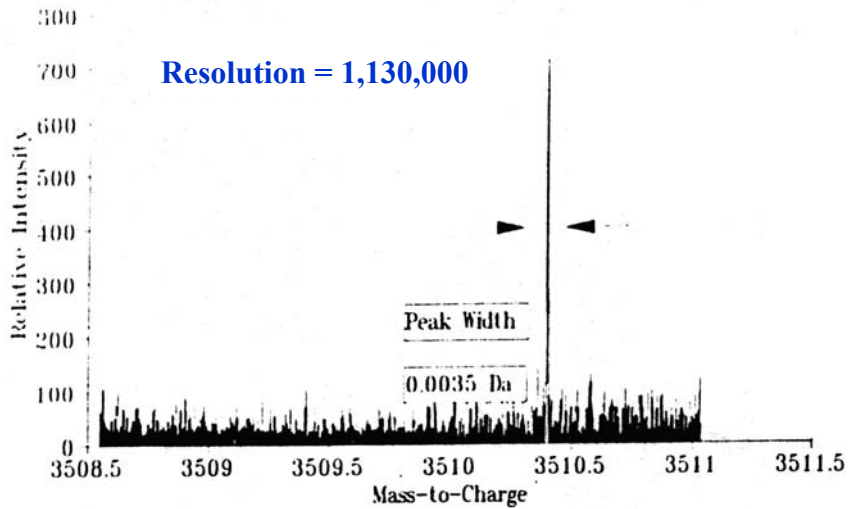
FWHM 0.5u at  $m/z$  1000  $\rightarrow$  Res.  $1000/0.5=2000$

### Resolving Power of a quadrupole



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

## 3D-Ion Trap



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 power $S$ :

### 2) TOF

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

$$\Delta m = \text{FWHM}$$

## Resolving power **S**:

### 2) TOF and sector analysers

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

$$\text{Res}_{\text{FWHM}} = 20,000 \text{ at } m/z \ 40 \rightarrow \text{FWHM} = 40/20,000 = 0.002 \text{ u}$$

but also

$$\text{Res}_{\text{FWHM}} = 20,000 \text{ at } m/z \ 4000 \rightarrow \text{FWHM} = 4,000/20,000 = 0.2 \text{ u}$$

## Resolving power **S**:

### 3) FTICR:

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

$T$  = transient duration (detection time)

$\nu_c$  = cyclotron frequency

# Resolving power $S$ :

## 3) FTICR:

at constant detection time:  
resolving power inversely  
proportional to  $m/z$ .

Thus if

$R=1 \times 10^7$  at  $m/z$  100, it will be  $1 \times 10^6$  at  $m/z$  1000

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

**SPECTROSPIN**  
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CH-8117 Fällanden (Zürich)  
Telefon 01 828 16 11  
Telex 829 416

**INFORMATION ICR**

No. V, January 1996

## 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 slits 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. Perfluorobutylamine (PFBA,  $C_4F_9N$ ) 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 (FWHM ~ Full Width at Half Height definition).

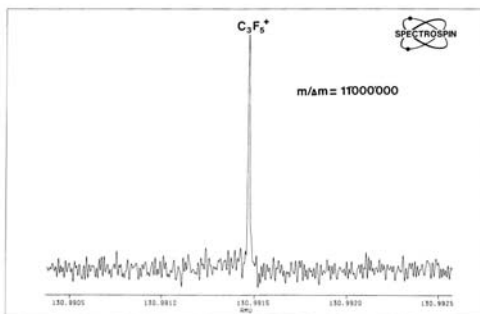


Fig. 1: Fragment ion  $C_4F_8^+$  of PFBA: Electron impact ionization (positive ions), gas liquid inlet, ion scan,  $B = 4.7$  Tesla.

## Resolving power $S$ :

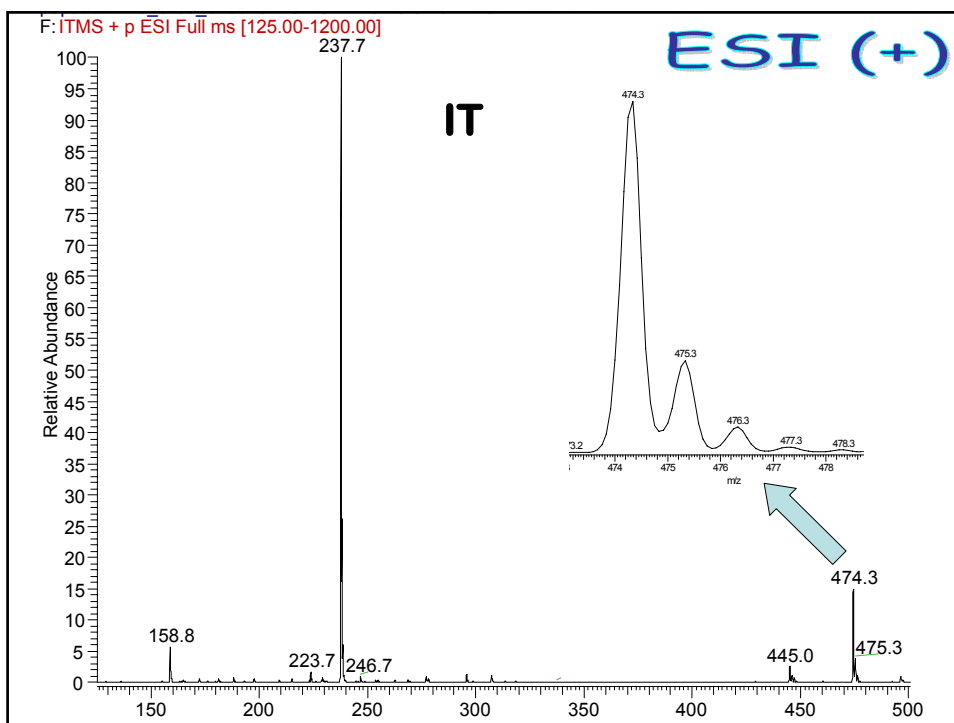
### 4) Orbitrap:

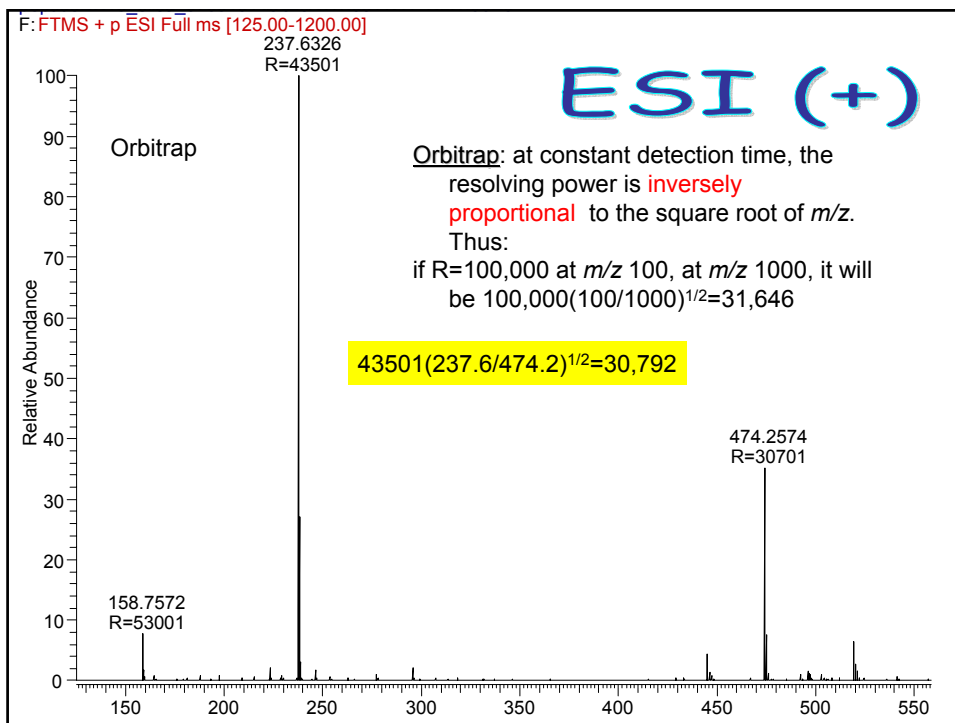
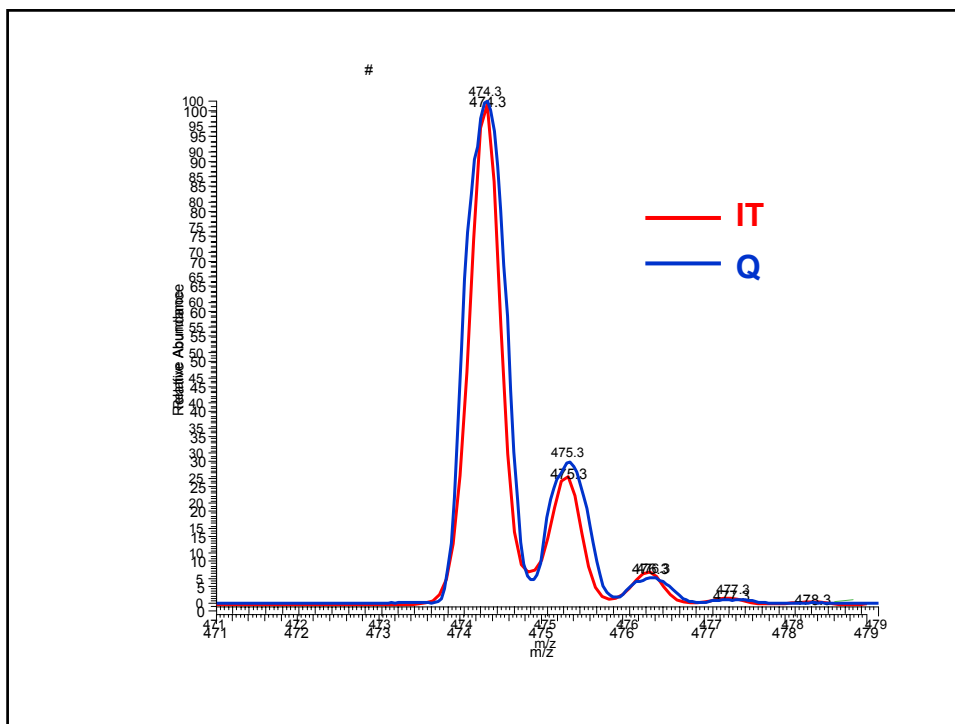
at constant detection time:  
resolving power inversely  
proportional to  $\sqrt{m/z}$

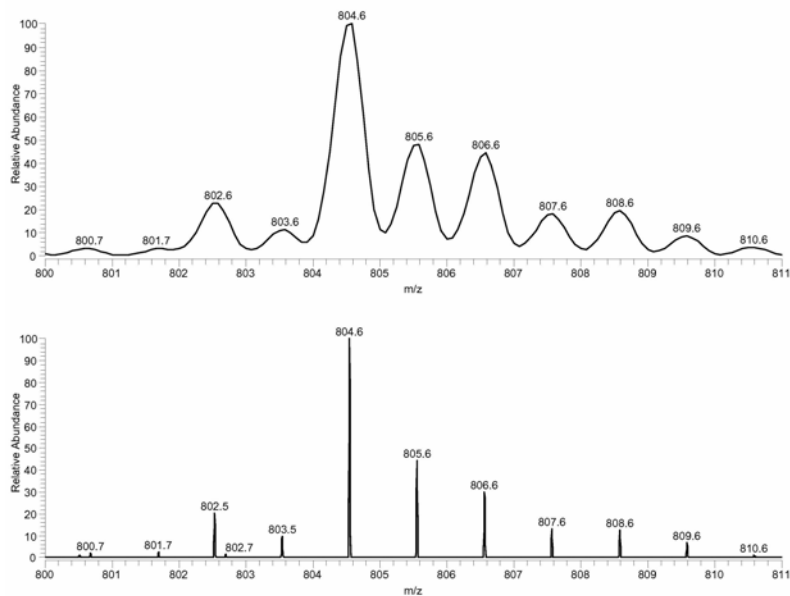
Thus if

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

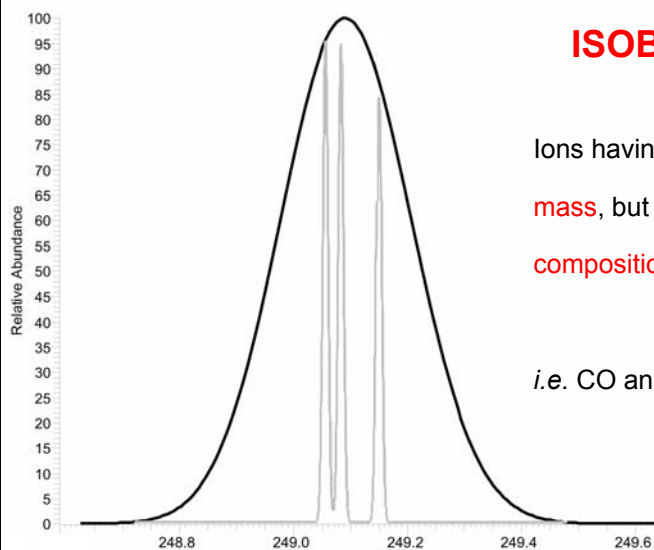
at  $m/z$  1000 it will be  $R=100,000(100/1000)^{1/2}=31,646$







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<sub>2</sub>

Mass spectra recorded at different resolutions: mass spectrum of a mixture of three isobaric species [C<sub>19</sub>H<sub>7</sub>N]<sup>+</sup>, [C<sub>20</sub>H<sub>9</sub>]<sup>+</sup>, [C<sub>13</sub>H<sub>19</sub>N<sub>3</sub>O<sub>2</sub>]<sup>+</sup> 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.



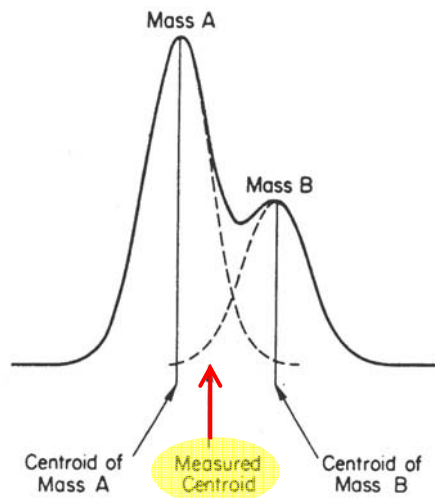
Quale risoluzione si deve usare?

*Melius abundare quam deficere ?*

**NO !!!!**

Quanta risoluzione serve?

Effect of inadequate mass resolution on mass measurement accuracy



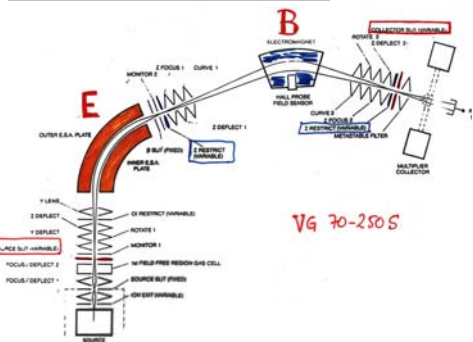
## Risoluzione e ....


## 1. Sensibilità

## 2. Specificità

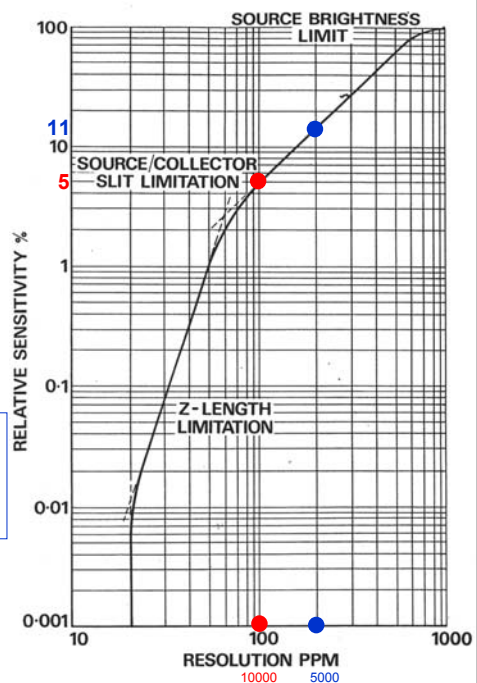
### 3. Velocità di scansione

## 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

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



## 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

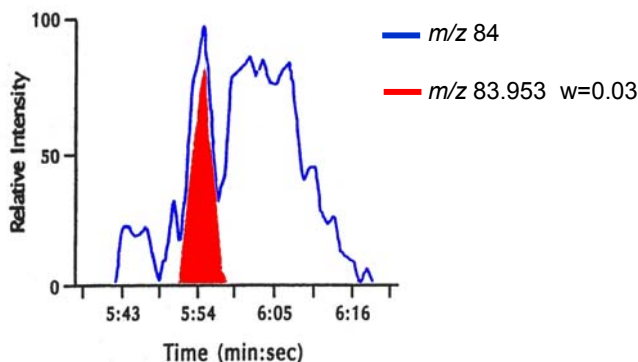
J Am Soc Mass Spectrom 1992, 3, 831-841

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

Target VOCs (natives)	Quantitation ion		Internal standard	Quantitation ion	
	m/z	Formula*		m/z	Formula*
1,1-Dichloroethene	95.9534	C <sub>2</sub> H <sub>2</sub> Cl <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	64.9941	C <sub>2</sub> <sup>37</sup> Cl <sup>37</sup> H <sub>2</sub>
Acetone	59.0452	<sup>13</sup> CC <sub>3</sub> H <sub>6</sub> O	[ <sup>13</sup> C <sub>2</sub> ]R	61.0519	<sup>13</sup> C <sub>3</sub> H <sub>6</sub> O
Carbon disulfide	77.9399	CS <sub>2</sub> <sup>34</sup> S	[ <sup>13</sup> C]R	78.9433	<sup>13</sup> C <sup>34</sup> SS
Bromoethane	107.9575	C <sub>2</sub> H <sub>5</sub> <sup>79</sup> Br	[ <sup>12</sup> C]R	110.9588	<sup>12</sup> CCH <sub>2</sub> <sup>79</sup> Br
Methylene chloride	83.9534	CH <sub>2</sub> Cl <sub>2</sub>	[ <sup>12</sup> C]R	84.9567	<sup>12</sup> CH <sub>2</sub> Cl <sub>2</sub>
trans-1,2-Dichloroethene	95.9534	C <sub>2</sub> H <sub>2</sub> Cl <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	64.9941	C <sub>2</sub> <sup>37</sup> Cl <sup>37</sup> H <sub>2</sub>
Hexane	86.1096	C <sub>6</sub> H <sub>14</sub>	[ <sup>12</sup> H <sub>14</sub> ]R	100.1974	C <sub>6</sub> <sup>14</sup> H <sub>14</sub>
1,1-Dichloroethane	63.0001	C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	66.0189	C <sub>2</sub> HCl <sup>37</sup> H <sub>2</sub>
cis-1,2-Dichloroethene	95.9534	C <sub>2</sub> H <sub>2</sub> Cl <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	64.9941	C <sub>2</sub> <sup>37</sup> Cl <sup>37</sup> H <sub>2</sub>
2-Butanone	72.0675	C <sub>4</sub> H <sub>8</sub> O	[4- <sup>2</sup> H <sub>2</sub> ]R	75.0764	C <sub>4</sub> H <sub>8</sub> O
Chloroform	82.9455	CHCl <sub>3</sub>	[ <sup>13</sup> C]R	83.9489	<sup>13</sup> CHCl <sub>3</sub>
1,1,1-Trichloroethane	96.9612	C <sub>2</sub> H <sub>3</sub> Cl <sub>3</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	99.9800	C <sub>2</sub> Cl <sup>37</sup> H <sub>3</sub>
Carbon tetrachloride	116.9066	CCl <sub>4</sub>	[ <sup>13</sup> C]R	117.9099	<sup>13</sup> CCl <sub>4</sub>
Benzene	78.0470	C <sub>6</sub> H <sub>6</sub>	[ <sup>13</sup> C <sub>6</sub> ]R	84.0671	<sup>13</sup> C <sub>6</sub> H <sub>6</sub>
1,2-Dichloroethane	61.9923	C <sub>2</sub> H <sub>4</sub> Cl <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	67.0169	<sup>12</sup> C <sub>2</sub> H <sub>4</sub>
Trichloroethene	129.9144	C <sub>2</sub> HCl <sub>3</sub>	[ <sup>13</sup> C]R	130.9177	<sup>13</sup> CCHCl <sub>3</sub>
1,2-Dichloropropane	63.0002	C <sub>3</sub> H <sub>4</sub> Cl <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	67.0253	C <sub>3</sub> CH <sup>37</sup> H <sub>4</sub>
Dibromomethane	171.8523	CH <sub>2</sub> <sup>79</sup> Br <sub>2</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	177.8608	C <sup>79</sup> H <sup>79</sup> Br <sub>2</sub>
Bromodichloromethane	82.9455	CHCl <sub>2</sub>	[ <sup>13</sup> C]R	83.9489	<sup>13</sup> CHCl <sub>2</sub>
Toluene	91.0648	C <sub>7</sub> H <sub>8</sub>	[ <sup>12</sup> H <sub>8</sub> ]R	98.0987	C <sup>13</sup> H <sub>7</sub>
1,1,2-Trichloroethane	96.9612	C <sub>2</sub> H <sub>3</sub> Cl <sub>3</sub>	[ <sup>12</sup> H <sub>2</sub> ]R	99.9800	C <sub>2</sub> Cl <sup>37</sup> H <sub>3</sub>
Tetrachloroethene	185.8725	C <sub>2</sub> <sup>37</sup> Cl <sub>4</sub>	[ <sup>13</sup> C]R	166.8758	<sup>13</sup> CC <sup>37</sup> Cl <sub>3</sub>
Dibromochloromethane	128.8923	*	[ <sup>13</sup> C]R	129.8958	*

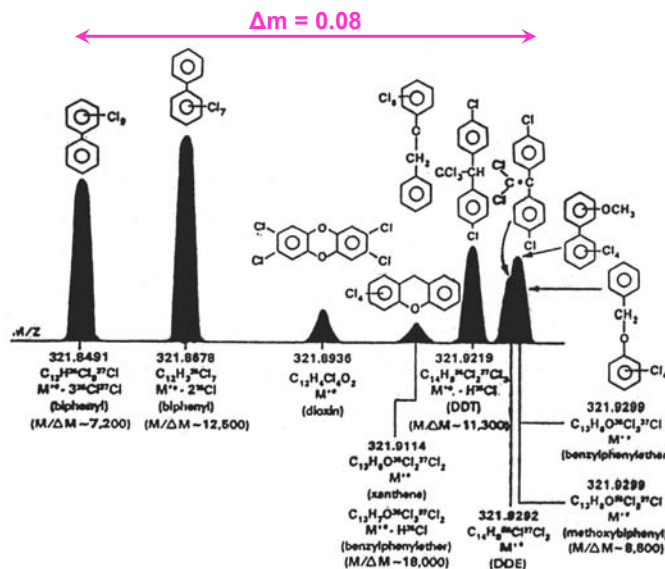
## 2. Risoluzione vs specificità

L'alta risoluzione aumenta la specificità eliminando specie isobariche interferenti



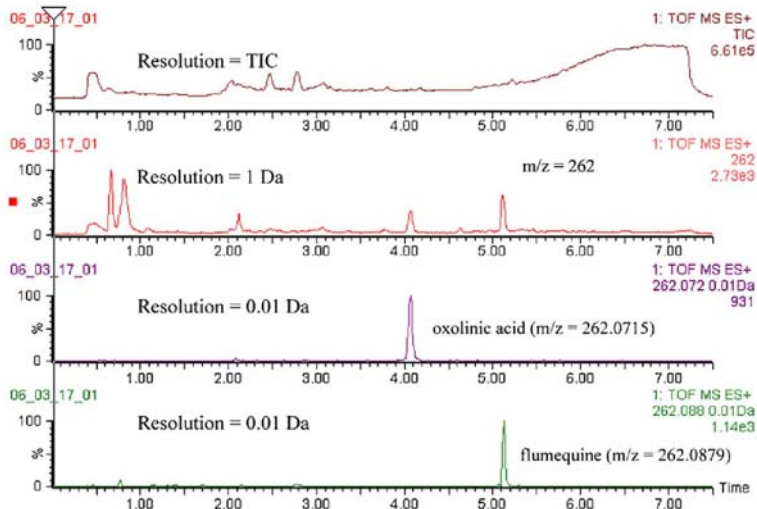
Single-mass chromatograms for methylene chloride from a blood sample (9.59 g): (a) nominal mass curve for m/z 84; (b) accurate mass curve for CH<sub>2</sub>Cl<sub>2</sub><sup>+</sup> (m/z 83.953 with a window of 0.03).

## Summary of some PCDDs mass spectral interferences of environmental origin

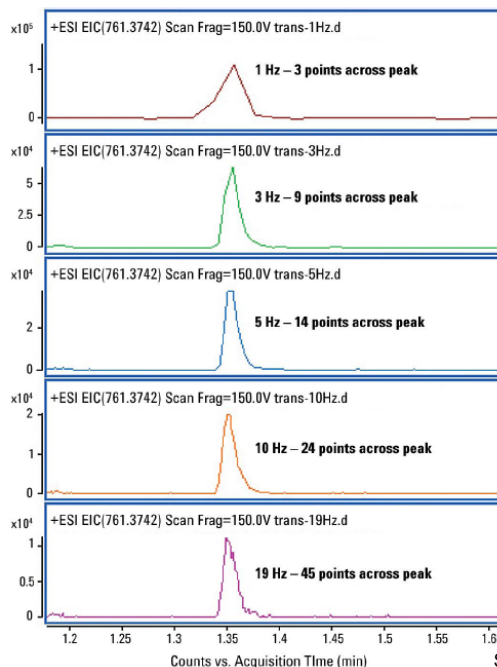


PCDDs=polychlorinated dibenzo-p-dioxins

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



### 3. Risoluzione vs velocità di scansione

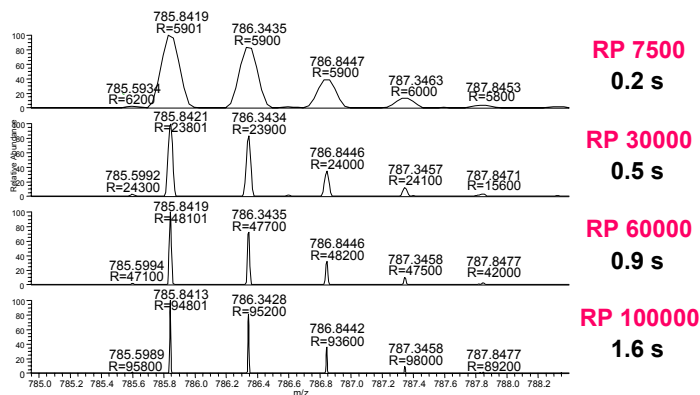


Source: Agilent

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

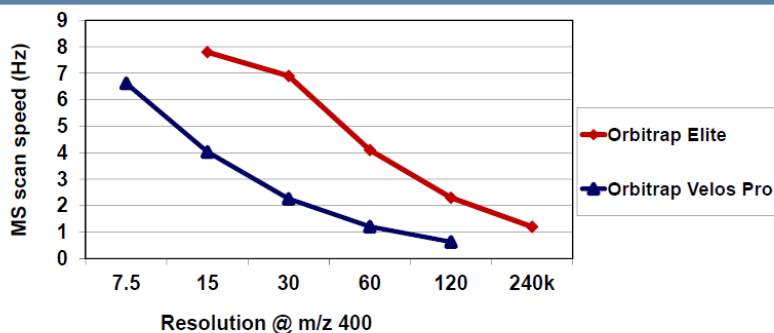
Strumenti a settori: da 1 sec/dec a 10 sec/dec

#### Orbitrap: Resolving Power vs Cycle Time



(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

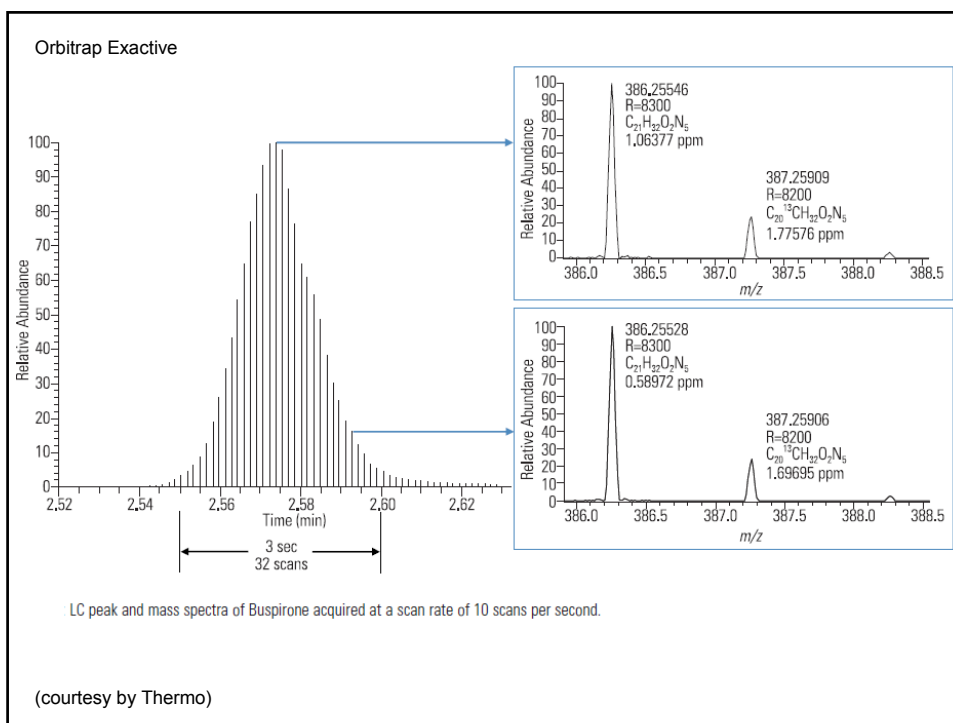
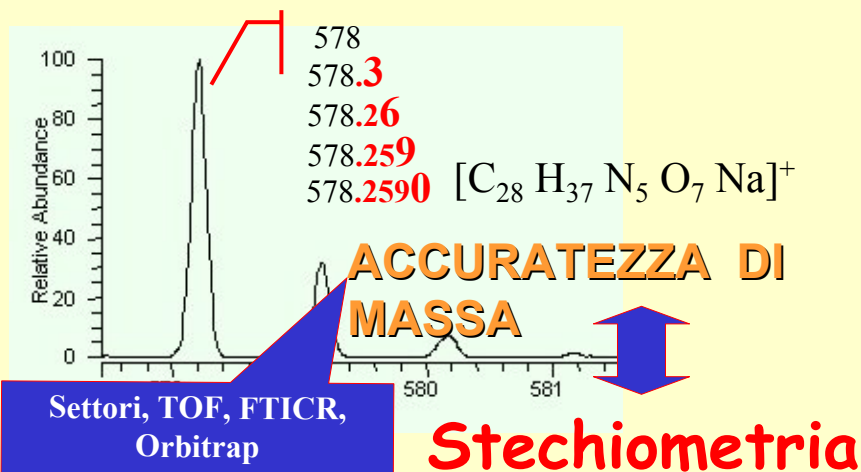
**L'alta risoluzione è requisito indispensabile  
per la**

**misura della massa accurata**

**Analisi qualitativa** → massa accurata → formula bruta

**Analisi quantitativa** → eliminazione interferenti  
(MS: SIM; MS<sup>n</sup>)

## Accuratezza nella misura del valore $m/z$



## Separazione degli ioni nello spazio



Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
<b>Doppio fuoco (EB, BE)</b> 	Campo magnetico + campo elettrico	Momento degli ioni + energia cinetica	10.000	10.000	< 1 ppm
<b>Quadrupolo</b> 	Campo elettrico e radiofrequenza	Stabilità/instabilità	2.000-4.000	Unitaria (0.2 u FWHM)	No
<b>Time of flight</b> 		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
<b>Trappola ionica (quadrupolare e lineare)</b> 	Campo elettrico + RF	Frequenza delle orbite	4.000	<500	no
<b>Cella a risonanza ciclotronica (FT-ICR)</b> 	Campo elettrico + RF + campo magnetico	Frequenza delle orbite	>10.000	> 100.000	< 1 ppm
<b>Orbitrap</b> 	Campo elettrico	Frequenza delle oscillazioni armoniche	4.000	100.000	< 1-3 ppm



### 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](http://mass-spec.lsu.edu/msterms/index.php/Category:Mass_spectrometry_terms)

Spettrometria di **massa** .....

**Quale** massa?

The unit of measurement used in MS is the

## unified atomic mass unit (u)

defined as 1/12 the mass of a  $^{12}\text{C}$  atom (1 u equals  $1.66054029 \times 10^{-27}$  kg).

In other words, the mass of a  $^{12}\text{C}$  atom is exactly 12 u.

This selection is simply a convention and, interestingly, before 1961,  $^{16}\text{O}$  was used for the same purpose.

Two important terms are *nominal mass* and *monoisotopic mass*. The nominal mass of a molecule is defined as the sum of the integer masses of the most abundant isotopes in a molecule. For example, let us consider molecular nitrogen,  $\text{N}_2$ , and ethene,  $\text{C}_2\text{H}_4$ . These molecules have different empirical formulae; however, their associated nominal masses are the same:  $\text{N}_2$ ,  $2 \times 14 = 28$  u is equal to  $\text{C}_2\text{H}_4$ ,  $2 \times 12 + 4 \times 1 = 28$  u. Mass spectrometers with insufficient mass resolving power such as quadrupole or ion-trap MS will not be able to distinguish these two molecules after ionization. The two resulting ions also are said to be *isobaric ions*.

**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.

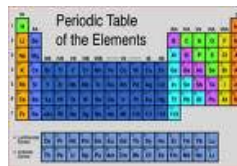
*i.e.* H=1, C=12, N=14, ....

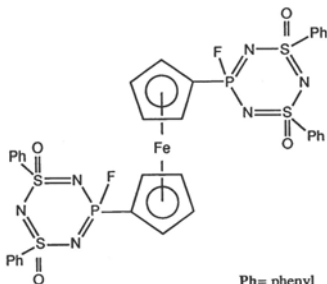
**Monoisotopic Mass** – The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element.

*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,

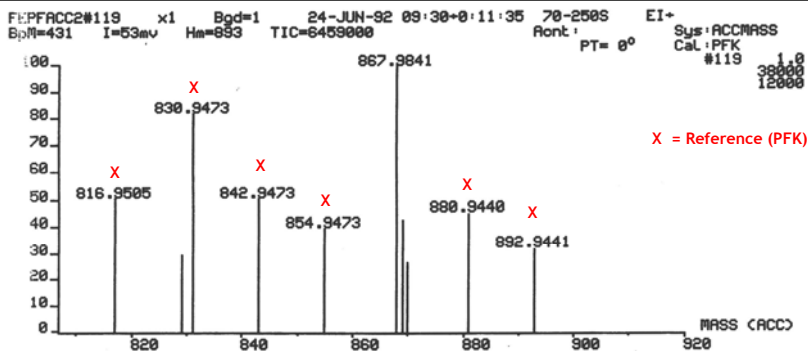
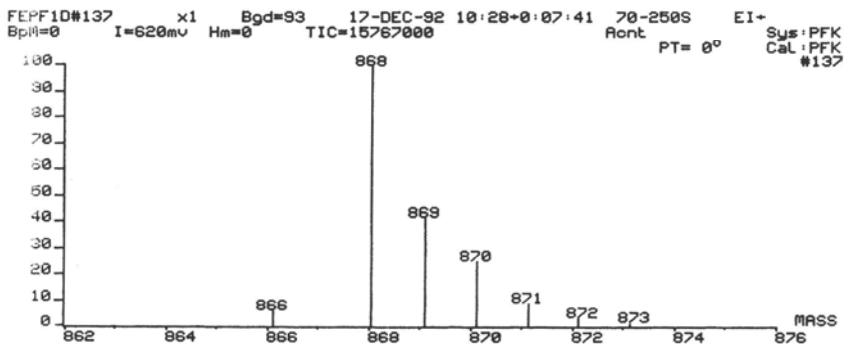
*i.e.* centroid of the distribution

A standard periodic table of elements, color-coded by groups. The title 'Periodic Table of the Elements' is at the top. The table shows elements from Hydrogen (1) to Oganesson (118) in the main body, and Lanthanides and Actinides in separate rows at the bottom.



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

$C_{34}H_{28}N_6O_4FeS_4P_2F_2$



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

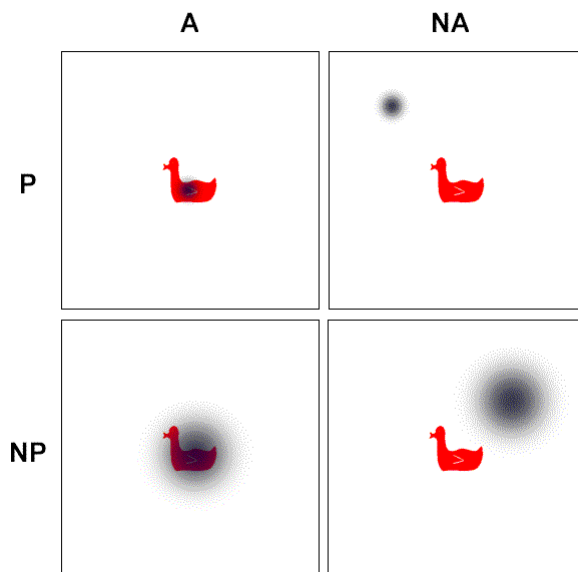
C 0+100  
H 1+100  
N 0+8  
Fe 0+1  
S 0+6  
F 0+4  
P 0+4  
O 0+6

## 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

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

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).

## **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

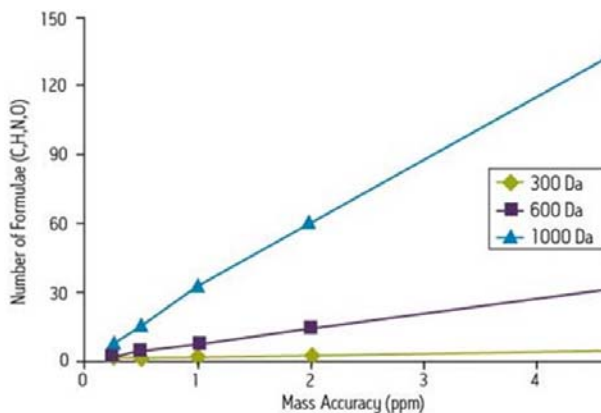
- **external** : reference masses from a mass spectrum of another analyte acquired under similar conditions.

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

Lock mass

Systematic errors

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



T.L. Quenzer, J.M. Robinson, B. Bolanos, 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 ( $C_{0+100}$ ,  $H_{3+74}$ ,  $O_{0+4}$  and  $N_{0+4}$ ):

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

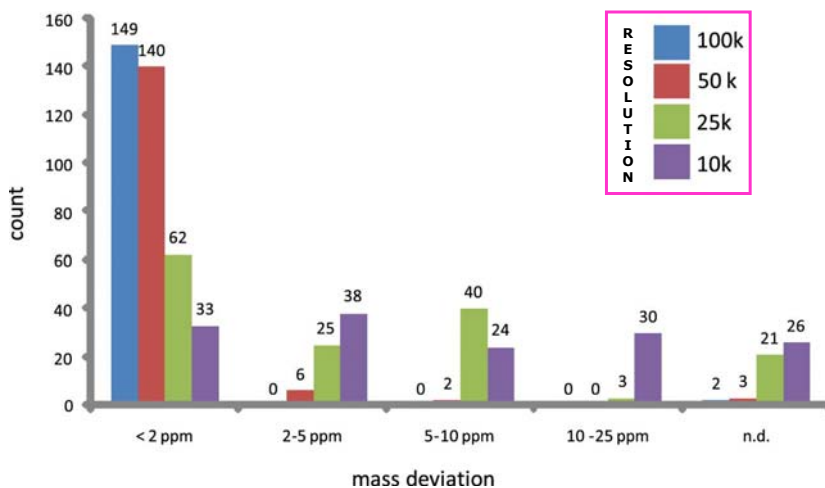
at  $m/z$  750 requires precision better than 0.018 ppm to eliminate "all extraneous possibilities"

		Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	↓	516.76671	0.0	0.0	21.0	C <sub>28</sub> H <sub>71</sub> O <sub>12</sub> N <sub>13</sub>
		516.76647	0.5	0.2	15.0	C <sub>28</sub> H <sub>79</sub> O <sub>11</sub> N <sub>9</sub> S <sub>2</sub>
		516.76638	0.6	0.3	12.0	C <sub>24</sub> H <sub>75</sub> O <sub>14</sub> N <sub>15</sub> S <sub>1</sub>
		516.76705	-0.7	-0.3	11.5	C <sub>28</sub> H <sub>77</sub> O <sub>15</sub> N <sub>12</sub> S <sub>1</sub>
2 ppm (10)	↓	516.76604	1.3	0.7	16.0	C <sub>28</sub> H <sub>75</sub> O <sub>16</sub> N <sub>9</sub>
		516.76738	-1.3	-0.7	20.5	C <sub>31</sub> H <sub>73</sub> O <sub>13</sub> N <sub>10</sub>
		516.76604	1.3	0.7	21.5	C <sub>27</sub> H <sub>69</sub> O <sub>11</sub> N <sub>16</sub>
		516.76580	1.8	0.9	15.5	C <sub>27</sub> H <sub>77</sub> O <sub>10</sub> N <sub>12</sub> S <sub>2</sub>
		516.76772	-2.0	-1.0	16.5	C <sub>24</sub> H <sub>73</sub> O <sub>11</sub> N <sub>16</sub> S <sub>1</sub>
5 ppm (23)	↓	516.76773	-2.0	-1.0	11.0	C <sub>28</sub> H <sub>75</sub> O <sub>16</sub> N <sub>9</sub> S <sub>1</sub>
		516.76805	-2.6	-1.3	25.5	C <sub>28</sub> H <sub>69</sub> O <sub>9</sub> N <sub>14</sub>
		516.76537	2.6	1.3	16.5	C <sub>28</sub> H <sub>73</sub> O <sub>15</sub> N <sub>12</sub>
		516.76807	-2.6	-1.4	7.0	C <sub>28</sub> H <sub>79</sub> O <sub>14</sub> N <sub>15</sub> S <sub>2</sub>
		516.76513	3.0	1.6	10.5	C <sub>28</sub> H <sub>81</sub> O <sub>14</sub> N <sub>9</sub> S <sub>2</sub>
		516.76513	3.1	1.6	16.0	C <sub>28</sub> H <sub>78</sub> O <sub>9</sub> N <sub>15</sub> S <sub>2</sub>
		516.76839	-3.3	-1.7	16.0	C <sub>28</sub> H <sub>78</sub> O <sub>12</sub> N <sub>13</sub> S <sub>1</sub>
		516.76479	3.7	1.9	20.0	C <sub>28</sub> H <sub>78</sub> O <sub>11</sub> N <sub>13</sub> S <sub>1</sub>
		516.76872	-3.9	-2.0	25.0	C <sub>24</sub> H <sub>71</sub> O <sub>10</sub> N <sub>11</sub>
		516.76470	3.9	2.0	17.0	C <sub>24</sub> H <sub>71</sub> O <sub>14</sub> N <sub>15</sub>
		516.76874	-3.9	-2.0	6.5	C <sub>20</sub> H <sub>81</sub> O <sub>15</sub> N <sub>12</sub> S <sub>2</sub>
		516.76446	4.3	2.2	11.0	C <sub>24</sub> H <sub>79</sub> O <sub>13</sub> N <sub>11</sub> S <sub>2</sub>
		516.76897	-4.4	-2.3	12.5	C <sub>20</sub> H <sub>73</sub> O <sub>16</sub> N <sub>16</sub>
		516.76907	-4.6	-2.4	15.5	C <sub>28</sub> H <sub>77</sub> O <sub>13</sub> N <sub>10</sub> S <sub>1</sub>

Average: C<sub>4.9384</sub> H<sub>7.7583</sub> N<sub>1.3577</sub> O<sub>1.4773</sub> S<sub>0.0417</sub>

Limits set to: C<sub>35</sub> - C<sub>70</sub>; H<sub>45</sub> - H<sub>100</sub>; N<sub>8</sub> - N<sub>16</sub>; O<sub>9</sub> - O<sub>16</sub>; S<sub>0</sub> - S<sub>2</sub>

Samples of honey and animal feed spiked with 151 pesticides



# Elemental composition search on mass 474.26

m/z= 469.26-479.26

<sup>12</sup> C	0÷30
<sup>13</sup> C	0÷1
<sup>14</sup> N	0÷10
<sup>16</sup> O	0÷15
<sup>1</sup> H	0÷60

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
474.2573	474.2572	0.18	5.0	C <sub>22</sub> H <sub>38</sub> O <sub>9</sub> N <sub>2</sub>
	474.2572	0.20	10.5	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub> N <sub>9</sub>
	474.2585	-2.64	10.0	C <sub>23</sub> H <sub>34</sub> O <sub>5</sub> N <sub>6</sub>
	474.2558	3.02	5.5	C <sub>20</sub> H <sub>36</sub> O <sub>8</sub> N <sub>5</sub>
	474.2599	-5.46	15.0	C <sub>24</sub> H <sub>30</sub> O <sub>1</sub> N <sub>10</sub>
	474.2599	-5.47	9.5	C <sub>25</sub> H <sub>36</sub> O <sub>6</sub> N <sub>3</sub>
	474.2545	5.83	0.5	C <sub>19</sub> H <sub>40</sub> O <sub>12</sub> N <sub>1</sub>
	474.2545	5.85	6.0	C <sub>18</sub> H <sub>34</sub> O <sub>7</sub> N <sub>8</sub>
	474.2612	-8.29	14.5	C <sub>26</sub> H <sub>32</sub> O <sub>2</sub> N <sub>7</sub>
	474.2612	-8.30	9.0	C <sub>27</sub> H <sub>38</sub> O <sub>7</sub>

## Restrizioni sui valori di massa accurata:

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

## Double bond/ring equivalents (RDB)

$$D = 1 + \frac{\sum_i^{i_{\max}} N_i (V_i - 2)}{2}$$

$N_i$  is the number of atoms of element  $i$ , and  $V_i$  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

$^{12}\text{C}$	0±30
$^{13}\text{C}$	0±1
$^{14}\text{N}$	0±10
$^{16}\text{O}$	0±15
$^1\text{H}$	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 <sub>22</sub> H <sub>38</sub> O <sub>9</sub> N <sub>2</sub>
	474.2572	0.20	10.5	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub> N <sub>9</sub>
	474.2585	-2.64	10.0	C <sub>23</sub> H <sub>34</sub> O <sub>5</sub> N <sub>6</sub>
	474.2558	3.02	5.5	C <sub>20</sub> H <sub>36</sub> O <sub>8</sub> N <sub>5</sub>
	474.2599	-5.46	15.0	C <sub>24</sub> H <sub>30</sub> O <sub>1</sub> N <sub>10</sub>
	474.2599	-5.47	9.5	C <sub>25</sub> H <sub>36</sub> O <sub>6</sub> N <sub>3</sub>
	474.2545	5.83	0.5	C <sub>19</sub> H <sub>40</sub> O <sub>12</sub> N <sub>1</sub>
	474.2545	5.85	6.0	C <sub>18</sub> H <sub>34</sub> O <sub>7</sub> N <sub>8</sub>
	474.2612	-8.29	14.5	C <sub>26</sub> H <sub>32</sub> O <sub>2</sub> N <sub>7</sub>
	474.2612	-8.30	9.0	C <sub>27</sub> H <sub>38</sub> O <sub>7</sub>

## 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

# 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	0.10	5.0	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub> N <sub>2</sub>
	474.2572	0.20	10.5	C <sub>21</sub> H <sub>32</sub> O <sub>4</sub> N <sub>3</sub>
	474.2565	-2.64	10.0	C <sub>23</sub> H <sub>34</sub> O <sub>5</sub> N <sub>6</sub>
	474.2558	3.02	5.5	C <sub>20</sub> H <sub>36</sub> O <sub>8</sub> N <sub>5</sub>
	474.2599	-5.46	15.0	C <sub>24</sub> H <sub>30</sub> O <sub>1</sub> N <sub>10</sub>
	474.2599	-5.47	9.5	C <sub>25</sub> H <sub>36</sub> O <sub>6</sub> N <sub>3</sub>
	474.2545	5.83	0.5	C <sub>19</sub> H <sub>40</sub> O <sub>12</sub> N <sub>1</sub>
	474.2545	5.85	6.0	C <sub>18</sub> H <sub>34</sub> O <sub>7</sub> N <sub>8</sub>
	474.2612	-8.29	14.5	C <sub>26</sub> H <sub>32</sub> O <sub>2</sub> N <sub>7</sub>
	474.2612	0.30	9.0	C <sub>27</sub> H <sub>36</sub> O <sub>7</sub>

RDB=double bond/ring equivalents

$$D = 1 + \frac{\sum_i^{imax} M_i (V_i - 2)}{2}$$

Odd-electron ions = RDB integer

Even-electron ions= RDB x.5

## Elemental composition search on mass 475.26

m/z= 470.26-480.26

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
475.2605	475.2605	-0.03	10.0	C <sub>20</sub> <sup>13</sup> C <sub>1</sub> H <sub>32</sub> O <sub>4</sub> N <sub>9</sub>
	475.2605	0.04	5.0	C <sub>21</sub> <sup>13</sup> C <sub>1</sub> H <sub>36</sub> O <sub>5</sub> N <sub>2</sub>
	475.2605	0.10	10.5	C <sub>30</sub> H <sub>31</sub> N <sub>6</sub>
	475.2610	0.98	0.5	C <sub>17</sub> H <sub>39</sub> O <sub>11</sub> N <sub>4</sub>
	475.2596	1.85	1.0	C <sub>15</sub> H <sub>37</sub> O <sub>16</sub> N <sub>7</sub>
	475.2592	2.79	5.5	C <sub>19</sub> <sup>13</sup> C <sub>1</sub> H <sub>36</sub> O <sub>8</sub> N <sub>5</sub>
	475.2619	-2.85	10.0	C <sub>22</sub> <sup>13</sup> C <sub>1</sub> H <sub>34</sub> O <sub>5</sub> N <sub>6</sub>
	475.2591	2.92	13.5	C <sub>29</sub> H <sub>35</sub> O <sub>4</sub> N <sub>2</sub>
	475.2623	-3.78	5.5	C <sub>18</sub> H <sub>35</sub> O <sub>9</sub> N <sub>8</sub>
	475.2623	-3.80	0.0	C <sub>19</sub> H <sub>41</sub> O <sub>12</sub> N <sub>1</sub>

<sup>12</sup> C	0÷30
<sup>13</sup> C	0÷1
<sup>14</sup> N	0÷10
<sup>16</sup> O	0÷15
<sup>1</sup> 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

molecular mass [Da]	without isotope abundance information						2% isotopic abundance accuracy	5% isotopic abundance accuracy
	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

## **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.  $[\text{C}_{10}\text{H}_{20}\text{N}_2]^+$  non può avere come ione frammento  $[\text{C}_9\text{H}_{18}\text{O}]^+$

## **Spettrometria di massa in alta risoluzione (HRMS):**

**Introduzione diretta, infusione**

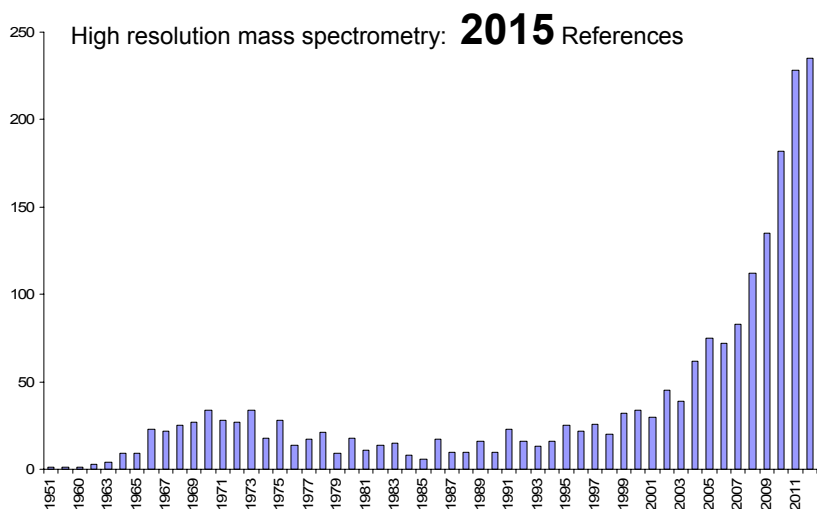
**GC-HRMS**

**GC-HRMS/MS, GC-HRMS<sup>n</sup>**

**HPLC-HRMS**

**HPLC-HRMS/MS, HPLC-HRMS<sup>n</sup>**

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