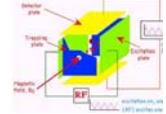
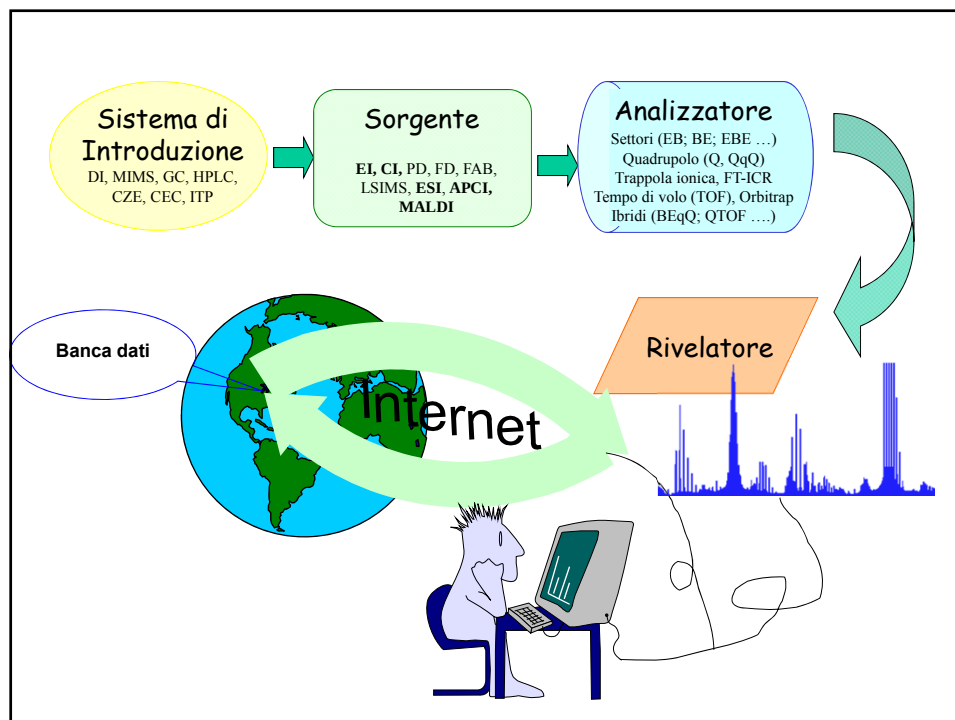
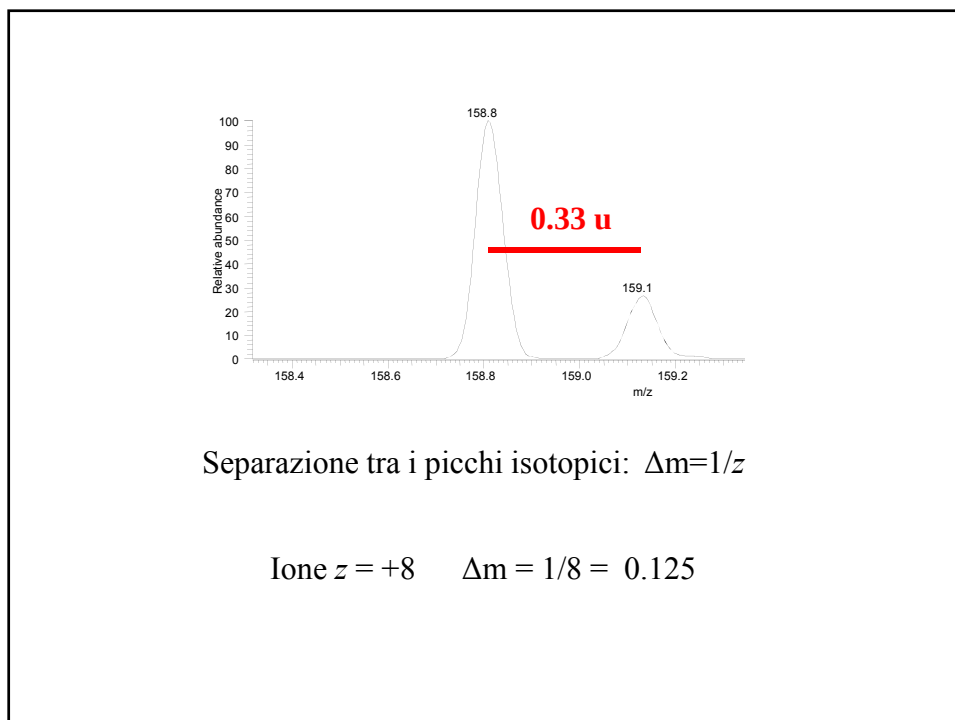
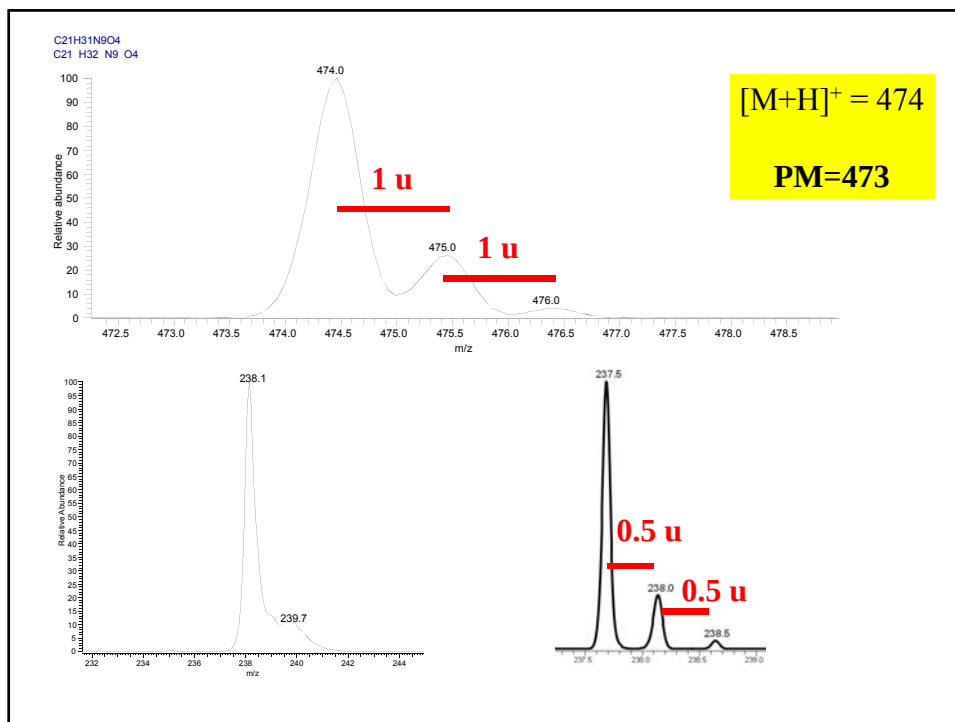
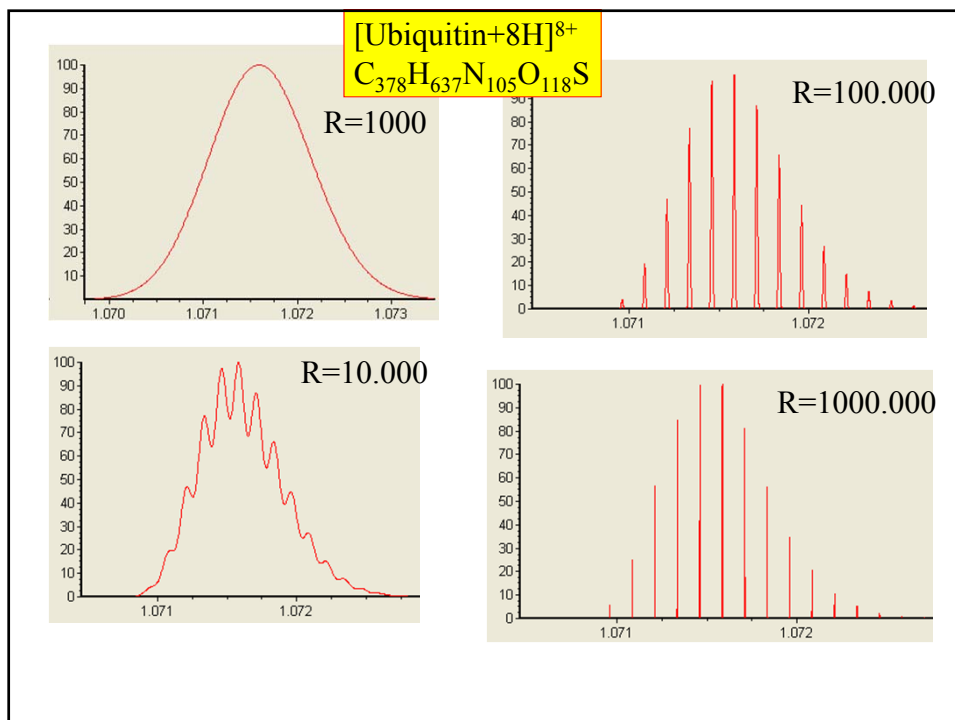
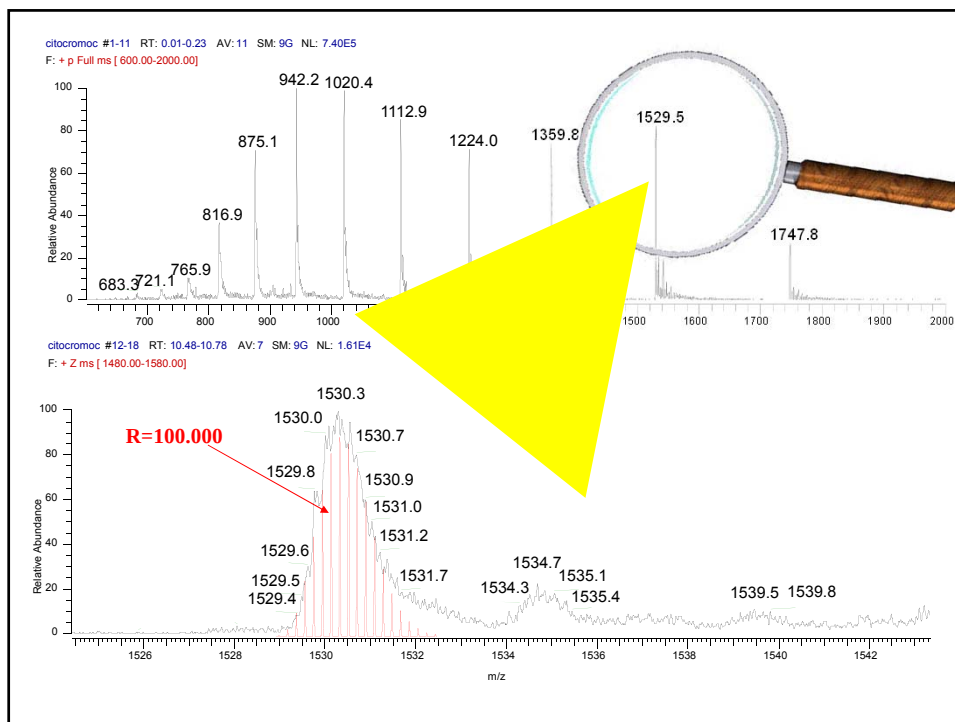


Separazione degli ioni nel tempo

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





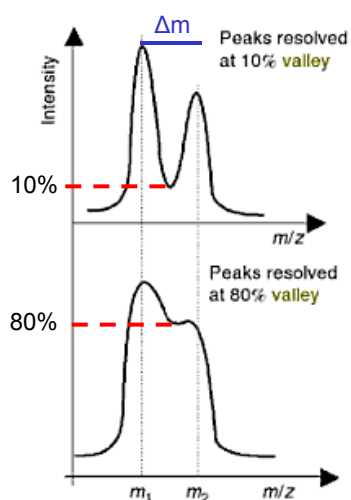


Resolution, resolving power:



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

RESOLUTION



Valley definition

Let two peaks of equal height in a mass spectrum at masses m and $m - \Delta m$ 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.

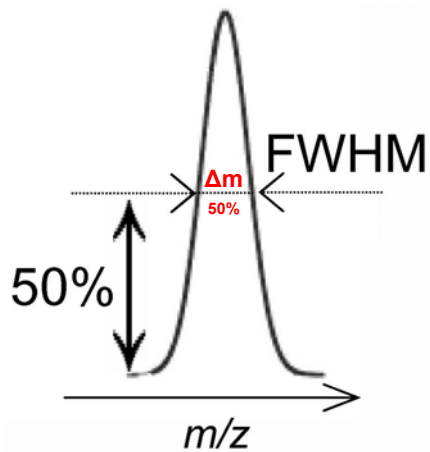
Then the resolution (x % valley definition) is $m/\Delta m$. It is usually a function of m . The ratio $m/\Delta m$ should be given for a number of values of m .

Double sectors, FT-ICR: 10% valley

Q, IT, TOF: 50% valley

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

RESOLUTION

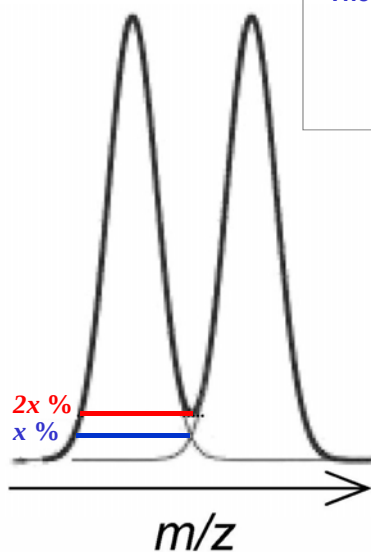


Resolution at full width at half maximum (FWHM).

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 $m/\Delta m$, where Δm 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

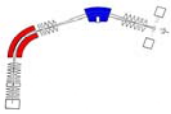
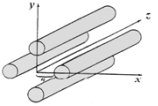
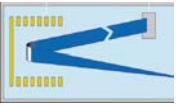
The ability to distinguish between ions differing in the quotient mass/charge by a small increment.

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.

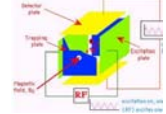
$$RP = \frac{m}{\Delta m}$$

Separazione degli ioni nello spazio



Analizzatore	Forza	Separazione in base a	Range m/z	Risoluzione	Massa accurata
Donnio fuoco (FB RF) 	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 power **S**:

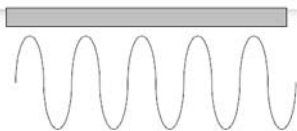
1) Quadrupole and Ion Traps

Constant peak width
Variable resolution
FWHM Definition

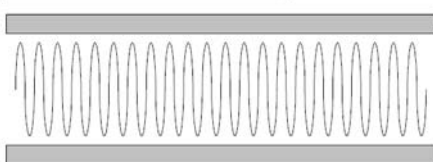
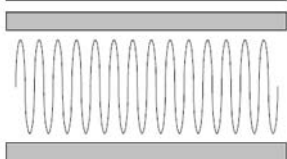
FWHM 0.5u at m/z 40 → Res. $40/0.5=80$, but also

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

Resolving Power of a quadrupole

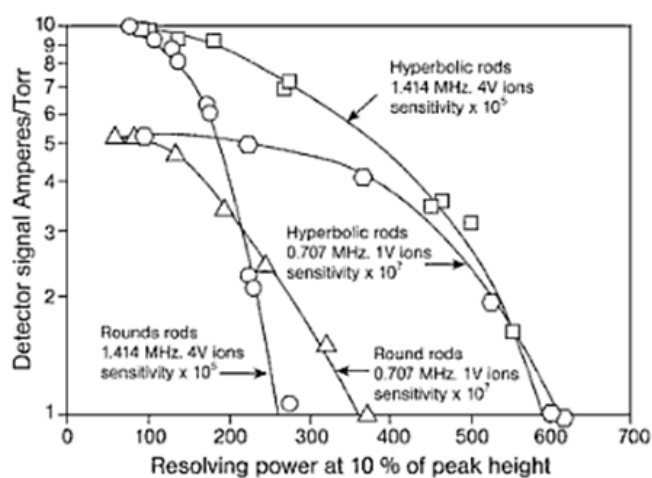


The ability of a quadrupole to resolve masses is proportional to frequency of AC and length of quadrupole



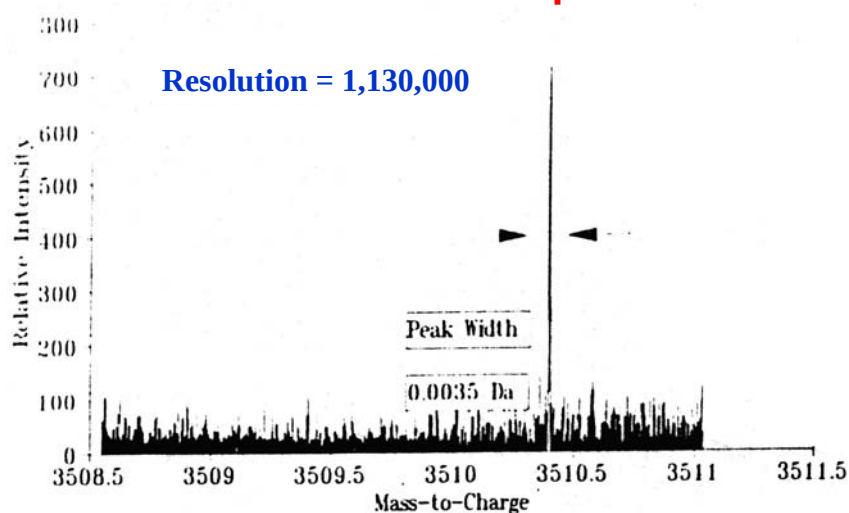
Hyperbolic Rods Quadrupoles





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



The CsI cluster ion at $m/z \approx 3510$ 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

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

Δt = FWHM of the peak measured

Resolving power **S**:

2) TOF and sector analysers

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

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

but also

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

Resolving power **S**:

3) FTICR:

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

T = transient duration (detection time)

ν_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 1×10^6 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
Industriemasse 28
CH-8117 Pfäfers (Switzerland)
Telefon 01 825 91 11
Telefax 01 825 418

INFORMATION ICR

No. V, January 1998

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 (PTBA, 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).

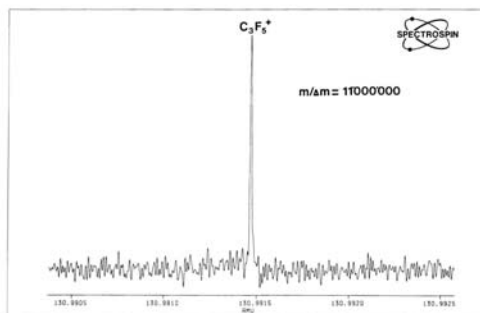


Fig. 1. Fragment ion $C_4F_9^+$ of PTBA. Electron impact ionization (positive ions), gas liquid inlet, one scan, $B = 4.7$ Tesla

Resolving power S :

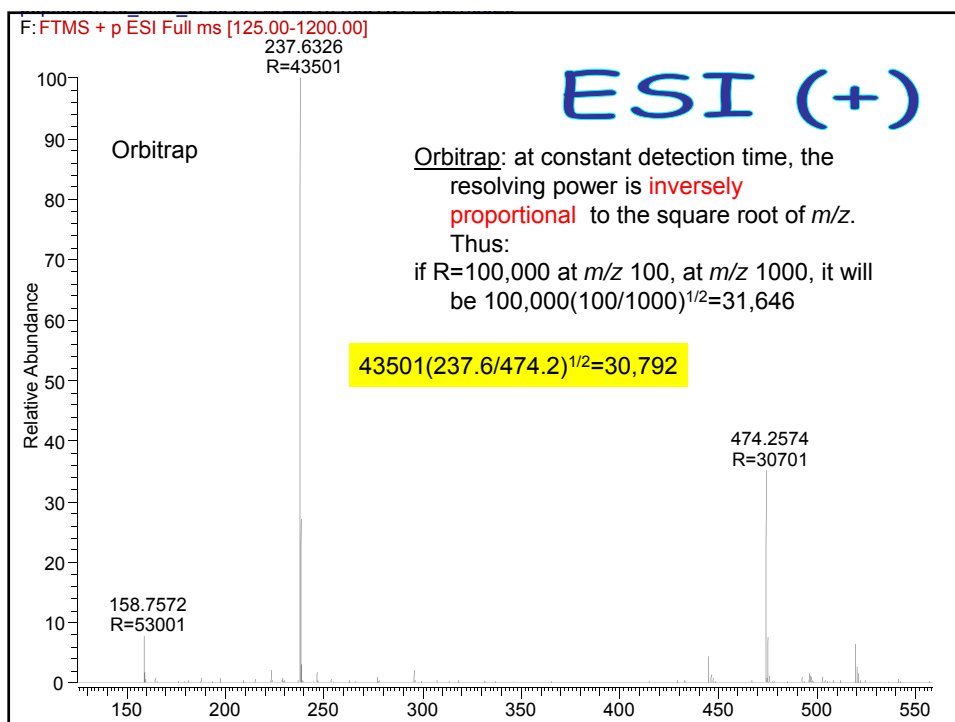
4) Orbitrap:

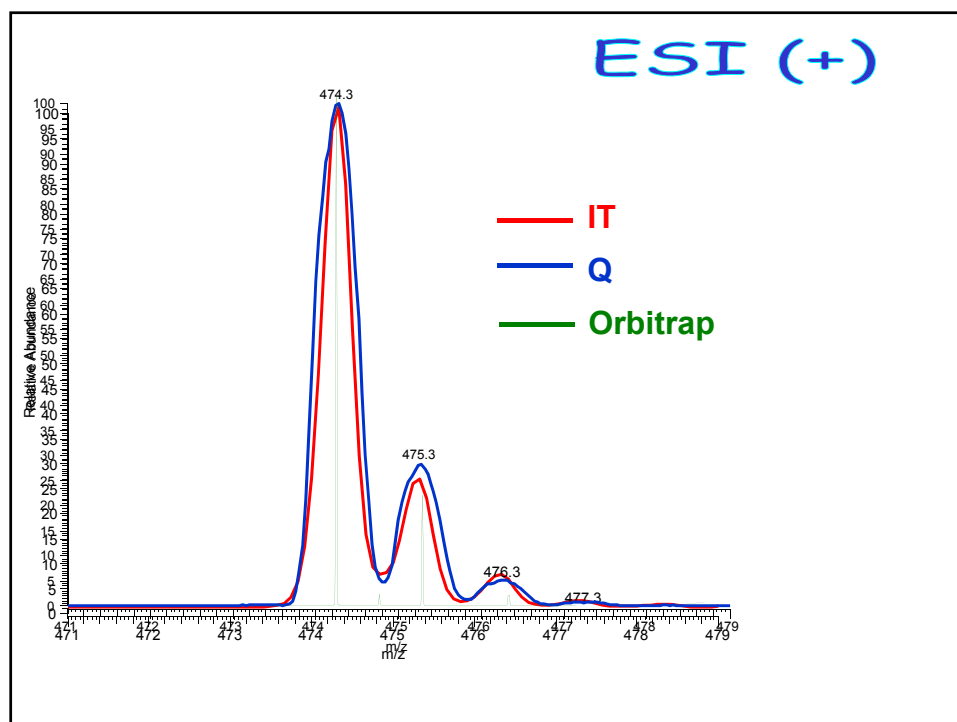
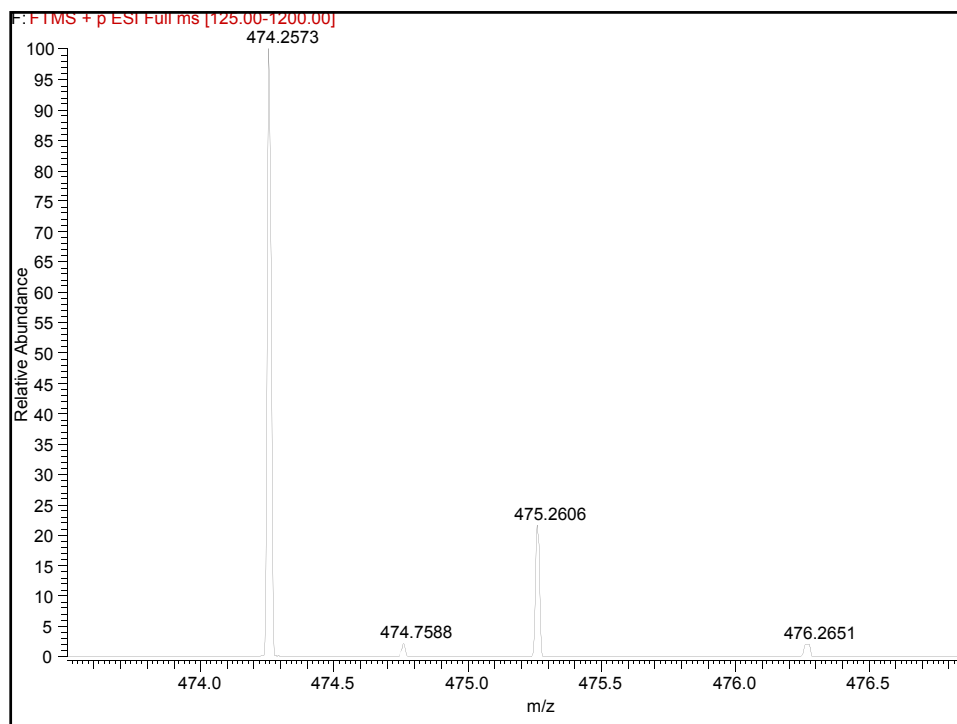
at constant detection time:
resolving power inversely
proportional to $\sqrt{m/z}$

Thus if

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

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





Resolving powerS:

- 1) Quadrupole and Ion Trap: **constant peak width**:

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

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

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

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

but also

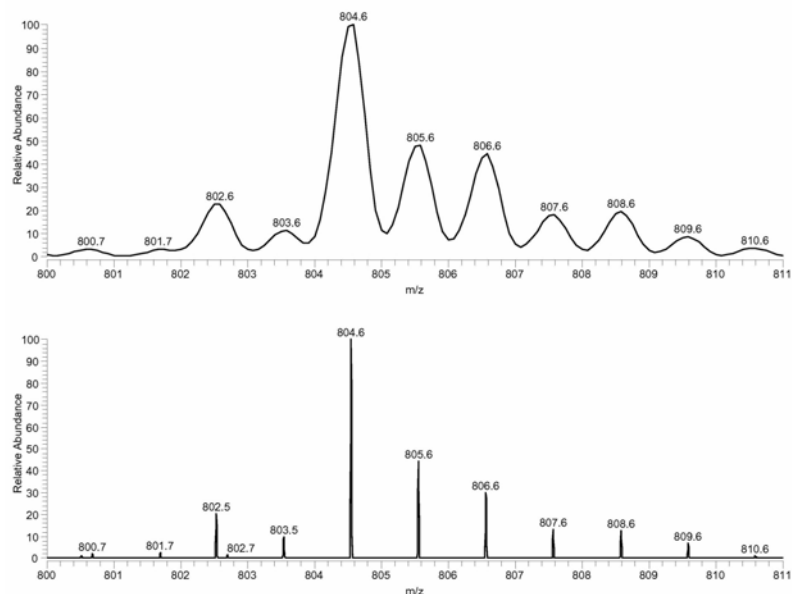
$\text{Res}_{\text{FWHM}}=20,000$ at m/z 4000 $\rightarrow$ FWHM $=4,000/20,000=0.2$ u

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

if $R=1 \times 10^7$ 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 $100,000(100/1000)^{1/2}=31,646$

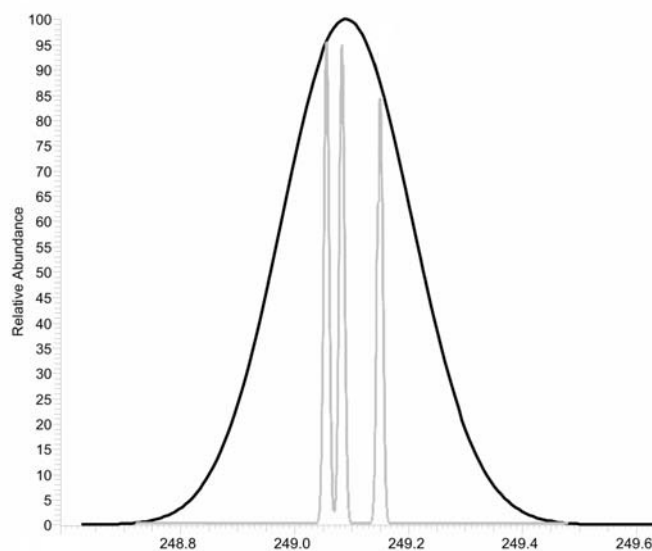


Mass spectra recorded at different resolutions: mass spectrum obtained by a 2D-ion trap low resolution (top) and by Orbitrap at resolving power 50,000 (bottom)

ISOBARIC IONS

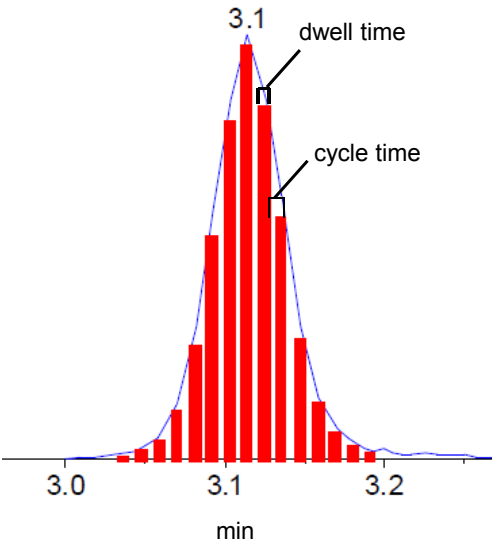
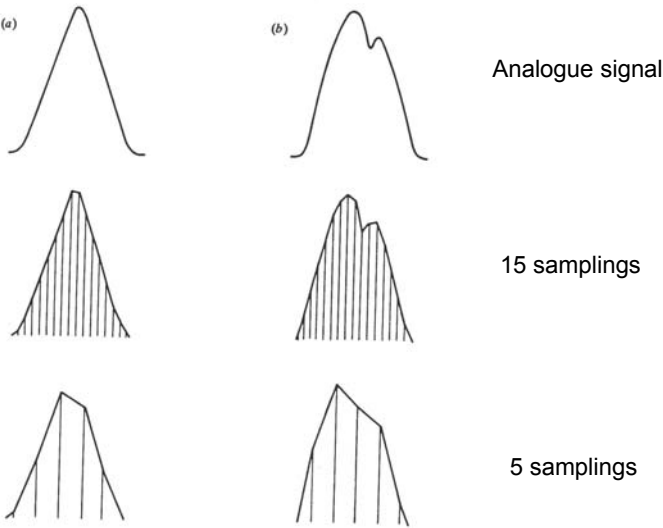
Ions having the same **nominal mass**, but different **chemical compositions**

i.e. CO and N₂



Mass spectra recorded at different resolutions: mass spectrum of a mixture of three isobaric species $[C_{10}H_7N]^+$, $[C_{20}H_0]^+$, $[C_{13}H_{19}N_3O_2]^+$ 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



Quale risoluzione si deve usare?

Melius abundare quam deficere ?

NO !!!!

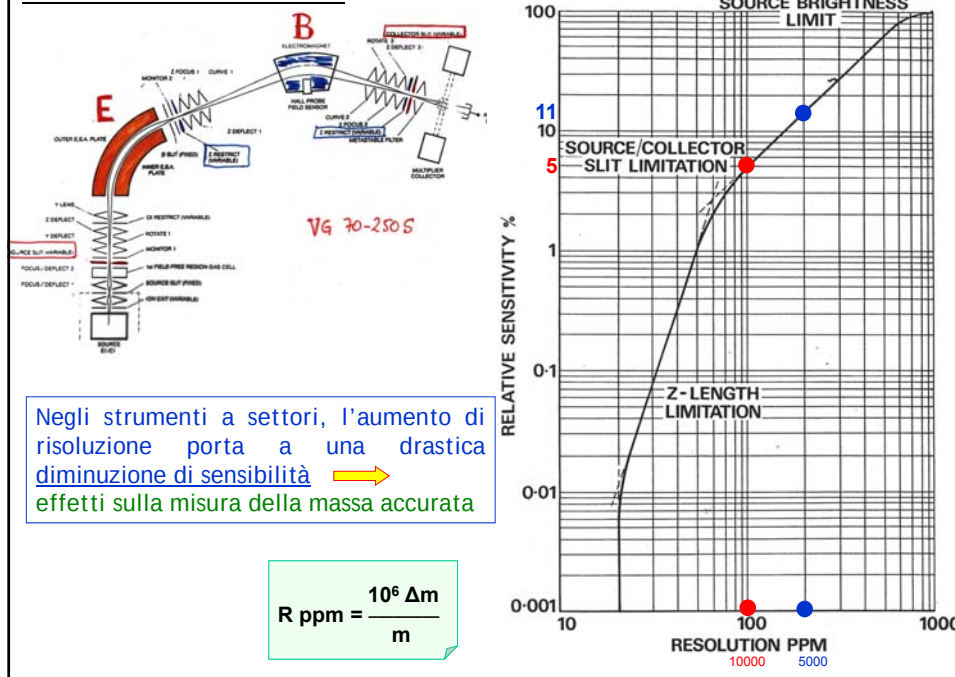
Risoluzione e

1. Sensibilità

2. Specificità

3. Velocità di scansione

1. Risoluzione vs sensibilità



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. Borin, 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

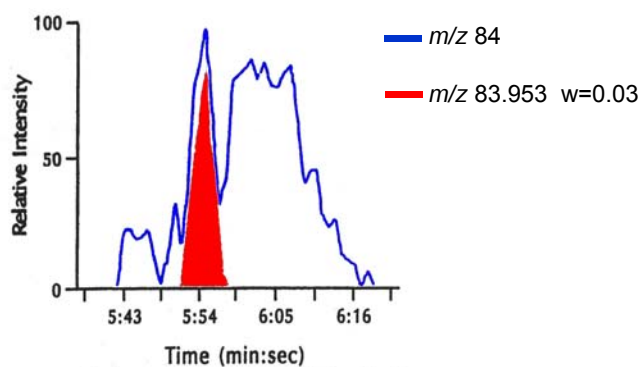
J Am Soc Mass Spectrom 1992, 3, 831-841

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

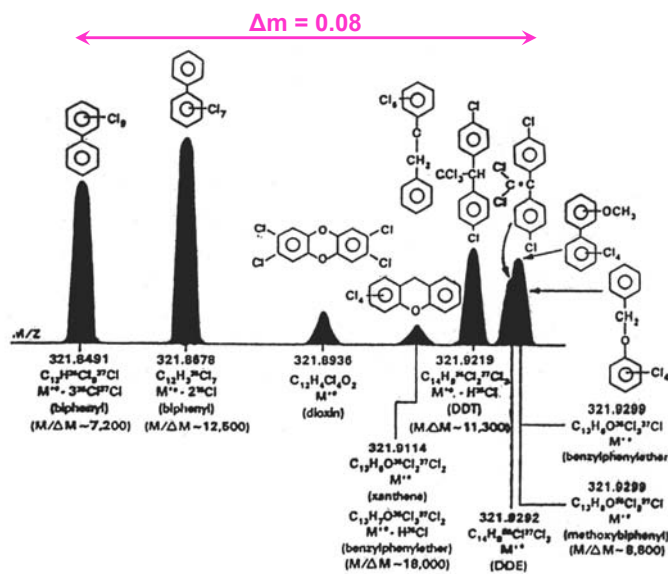
Target VOCs (natives)	Quantitation ion <i>m/z</i>	Formula ^a	Internal standard <i>R</i> = native	Quantitation ion <i>m/z</i>	Formula ^a
1,1-Dichloroethene	95.9534	C ₂ H ₂ Cl ₂	[² H ₂]R	64.9941	C ₂ ³⁷ Cl ² H ₂
Acetone	59.0452	¹³ CC ₃ H ₆ O	[¹³ C ₃]R	61.0519	¹³ C ₃ H ₆ O
Carbon disulfide	77.9399	CS ₂ ³⁴ S	[¹³ C]R	78.9433	¹³ C ³⁴ SS
Bromoethane	107.9575	C ₂ H ₅ ⁷⁹ Br	[¹³ C]R	110.9588	¹³ CCH ₂ ⁷⁹ Br
Methylene chloride	83.9534	CH ₂ Cl ₂	[¹³ C]R	84.9567	¹³ CH ₂ Cl ₂
<i>trans</i> -1,2-Dichloroethene	95.9534	C ₂ H ₂ Cl ₂	[² H ₂]R	64.9941	C ₂ ³⁷ Cl ² H ₂
Hexane	86.1096	C ₆ H ₁₄	[² H ₁₄]R	100.1974	C ₆ ¹⁴ H ₁₄
1,1-Dichloroethane	63.0001	C ₂ H ₄ Cl ₂	[² H ₂]R	66.0189	C ₂ HCl ² H ₂
<i>cis</i> -1,2-Dichloroethene	95.9534	C ₂ H ₂ Cl ₂	[² H ₂]R	64.9941	C ₂ ³⁷ Cl ² H ₂
2-Butanone	72.0575	C ₄ H ₈ O	[4- ² H ₂]R	75.0764	C ₄ H ₈ ¹³ H ₂ O
Chloroform	82.9455	CHCl ₃	[¹³ C]R	83.9489	¹³ CHCl ₃
1,1,1-Trichloroethane	96.9612	C ₂ H ₃ Cl ₃	[² H ₂]R	99.9800	C ₂ Cl ₃ ³⁷ H ₂
Carbon tetrachloride	116.9066	CCl ₄	[¹³ C]R	117.9099	¹³ CCl ₄
Benzene	78.0470	C ₆ H ₆	[¹³ C ₆]R	84.0671	¹³ C ₆ H ₆
1,2-Dichloroethane	61.9923	C ₂ H ₄ Cl ₂	[² H ₂]R	67.0169	¹³ C ₂ H ₄ Cl ₂
Trichloroethene	129.9144	C ₂ HCl ₃	[¹³ C]R	130.9177	¹³ CCHCl ₃
1,2-Dichloropropane	63.0002	C ₃ H ₄ Cl ₂	[² H ₂]R	67.0253	C ₃ Cl ² H ₄
Dibromomethane	171.8523	CH ₂ ⁷⁹ Br ₂	[² H ₂]R	177.8608	C ² H ₂ ⁷⁹ Br ₂
Bromodichloromethane	82.9455	CHCl ₂ Br	[¹³ C]R	83.9489	¹³ CHCl ₂ Br
Toluene	91.0648	C ₇ H ₈	[² H ₈]R	98.0987	C ₇ H ₈
1,1,1-Trichloroethane	96.9612	C ₂ H ₃ Cl ₃	[² H ₂]R	99.9800	C ₂ Cl ₃ ³⁷ H ₂
Tetrachloroethene	166.8725	C ₂ ³⁷ Cl ₄	[¹³ C]R	166.8758	¹³ CC ³⁷ Cl ₄
Dibromochloromethane	128.8923	C ₂ H ₂ Br ₂ Cl	[¹³ C]R	129.8958	¹³ C ₂ H ₂ Br ₂ Cl

2. Risoluzione vs specificità

L'alta risoluzione aumenta la specificità eliminando specie isobariche interferenti

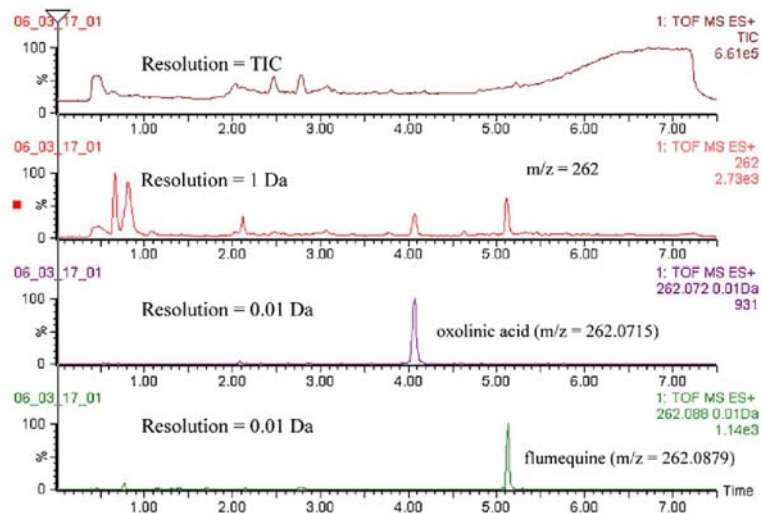


Summary of some PCDDs mass spectral interferences of environmental origin



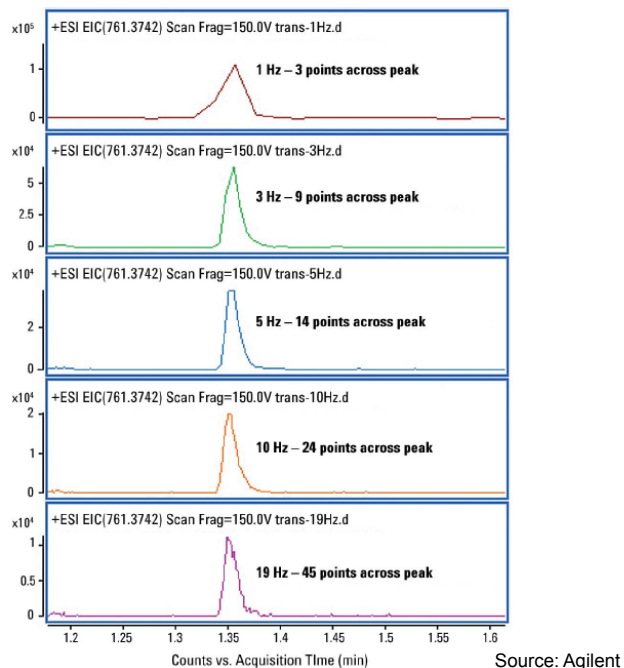
PCDDs=polychlorinated dibenzo-p-dioxins

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



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

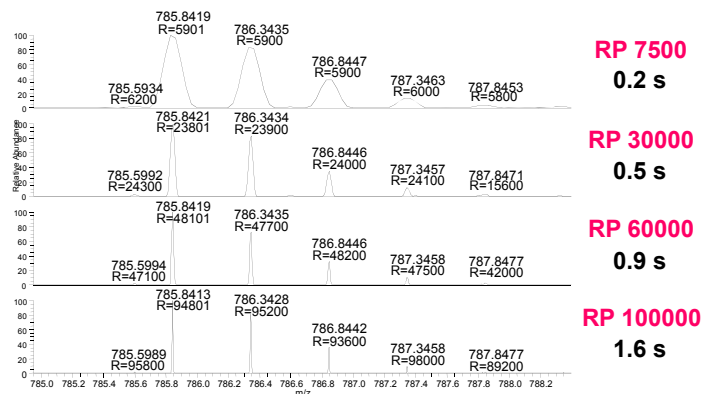
3. Risoluzione vs velocità di scansione



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

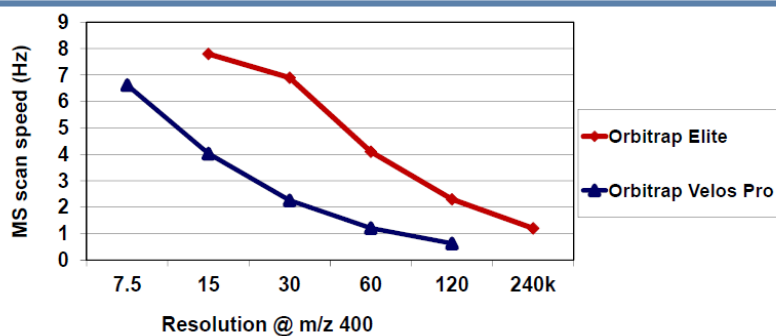
Strumenti a settori: da 1 sec/dec a 10 sec/dec

Orbitrap: Resolving Power vs Cycle Time



(courtesy by Thermo)

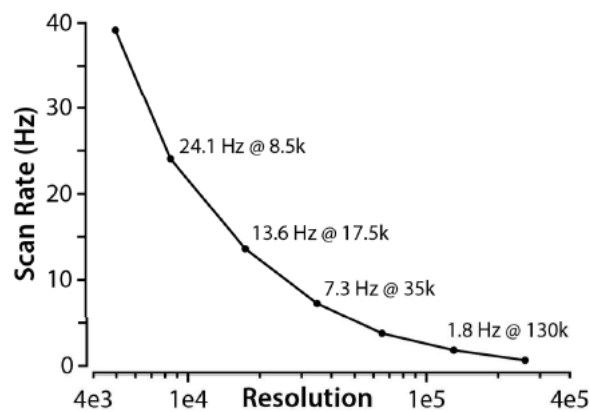
MS Scan Speed at Different Resolutions



- 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

GC/Quadrupole-Orbitrap



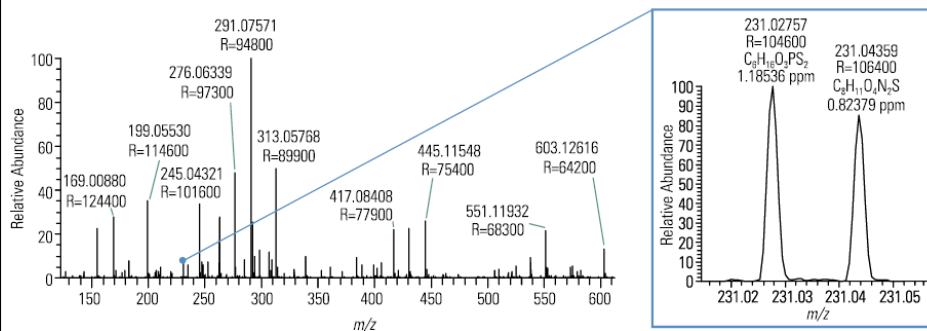
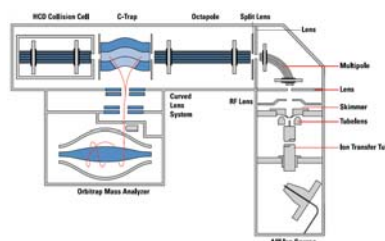
Scan rate (Hz) as a function of mass analysis resolution.

Orbitrap Exactive: 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.

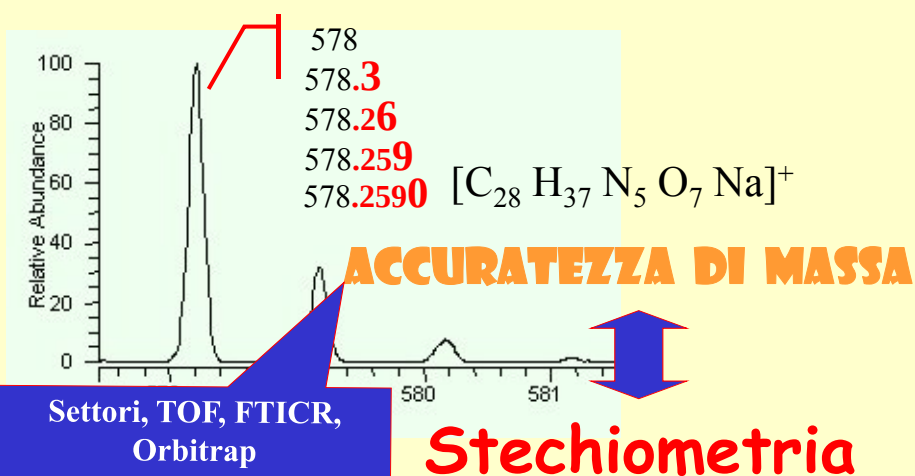
L'alta risoluzione è requisito indispensabile
per la

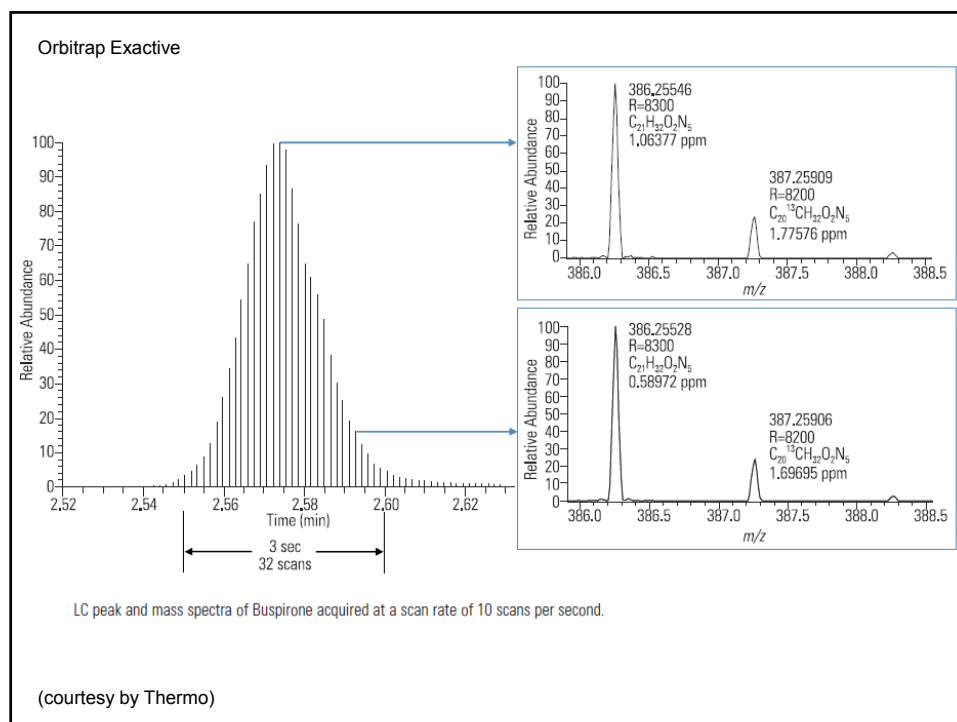
misura della massa accurata

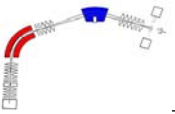
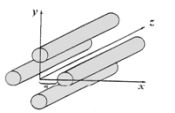
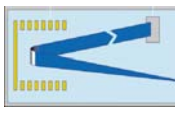
Analisi qualitativa → massa accurata → formula bruta

Analisi quantitativa → eliminazione interferenti
(MS: SIM; MSⁿ)

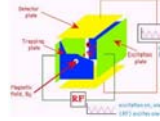
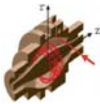
Accuratezza nella misura del valore m/z





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	< 1 ppm
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

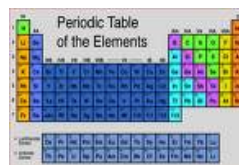
Mass Spec Terms Project: http://mass-spec.lsu.edu/msterms/index.php/Category:Mass_spectrometry_terms

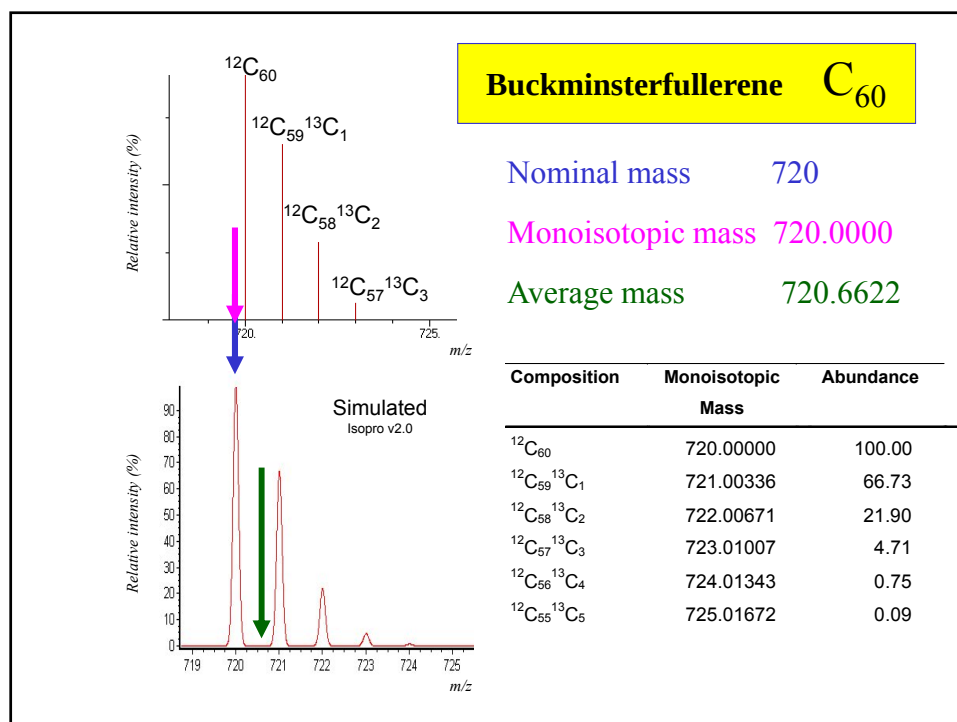
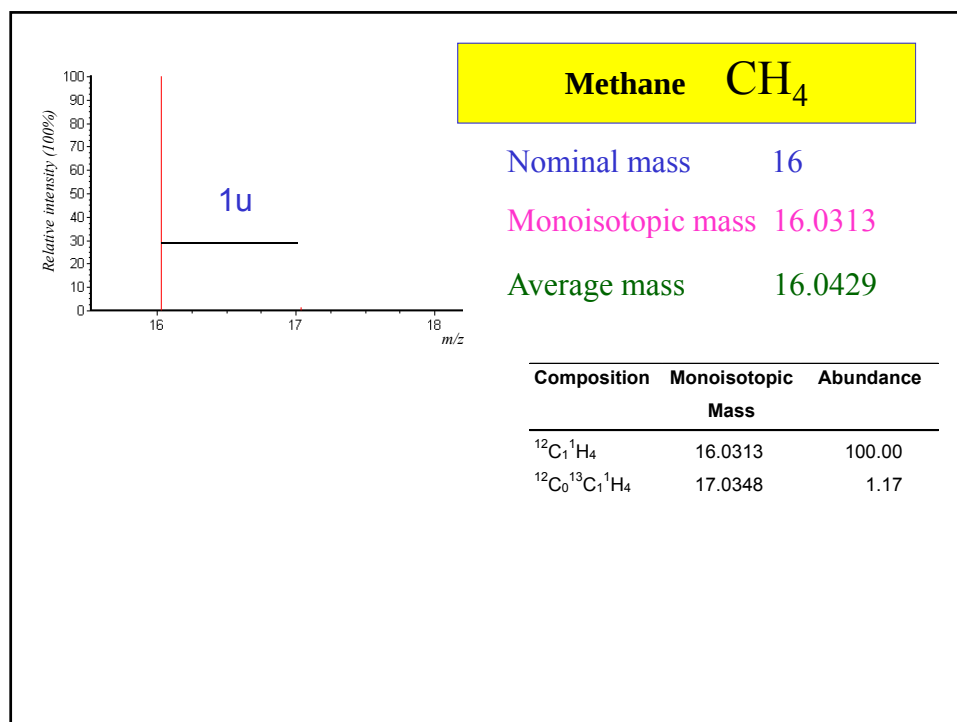
Nominal Mass – The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element rounded to the nearest integer value. *i.e.* H=1, C=12, N=14,

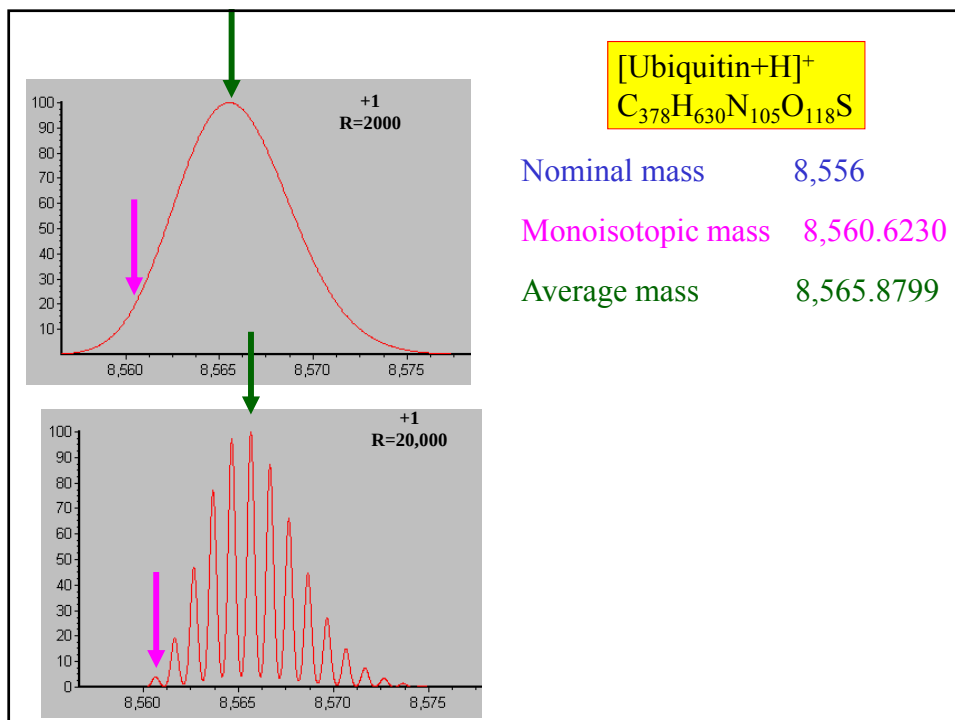
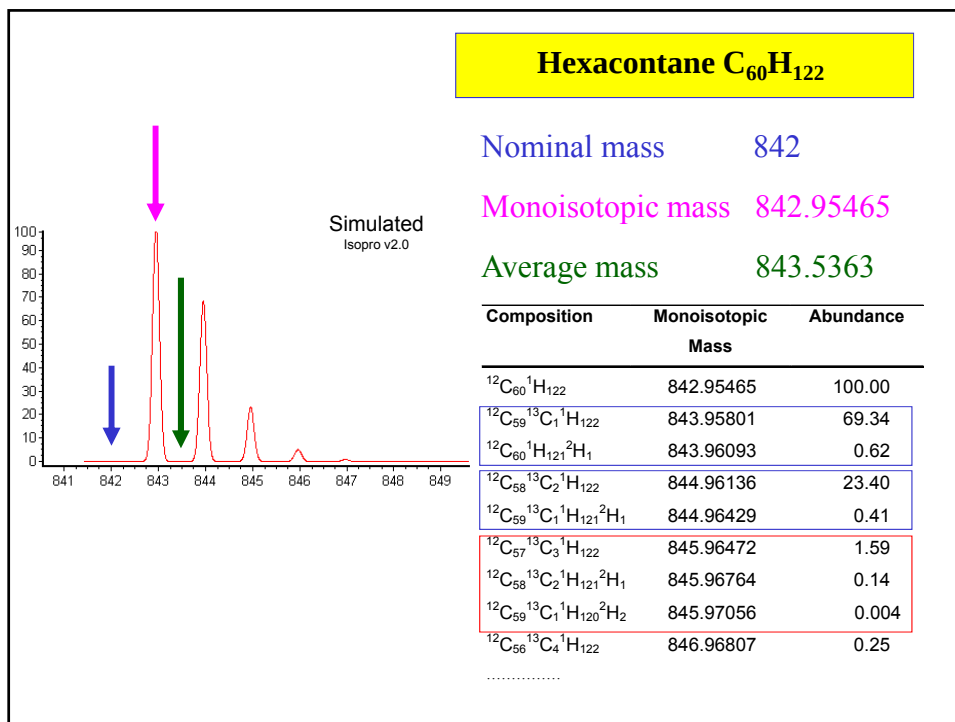
Monoisotopic Mass – The mass of an ion or molecule calculated using the mass of the most abundant isotope of each element.

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 includes element symbols, atomic numbers, and names. It is organized into rows (periods) and columns (groups).



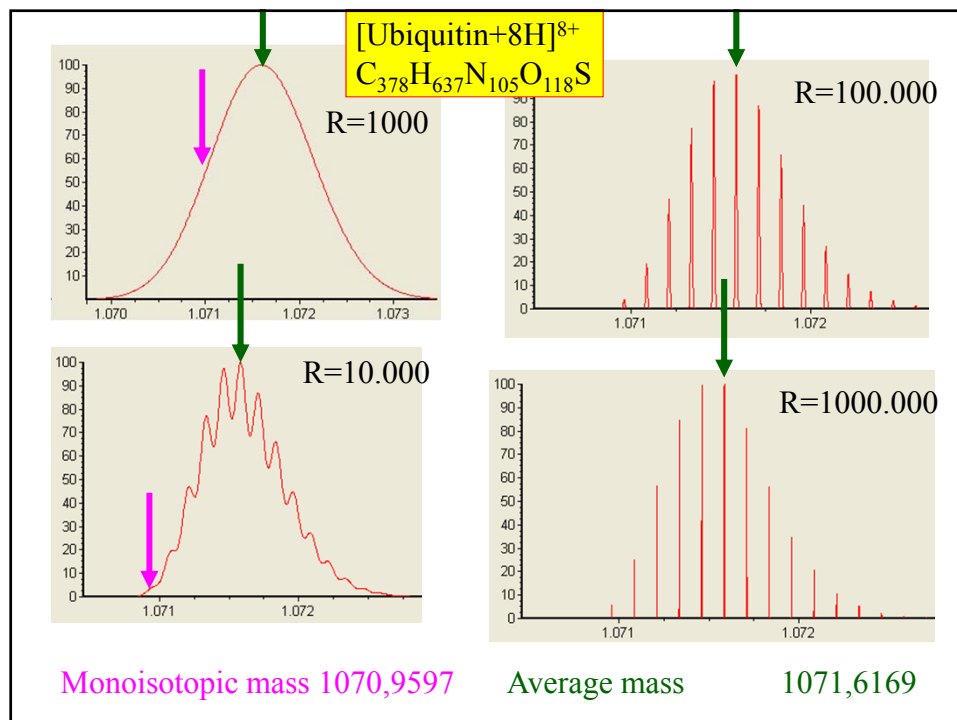


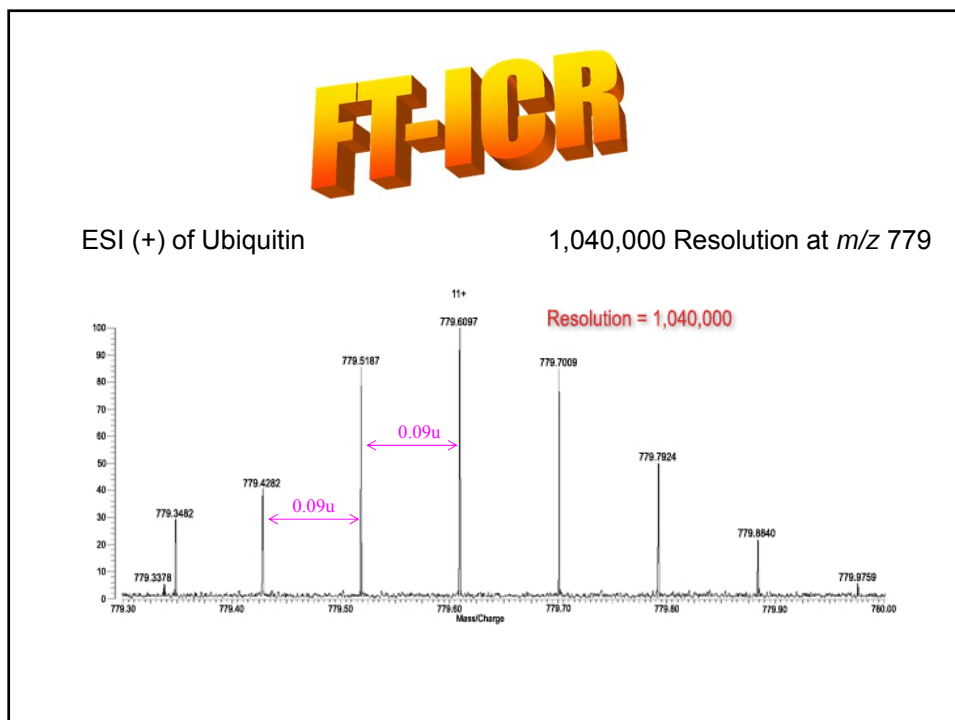
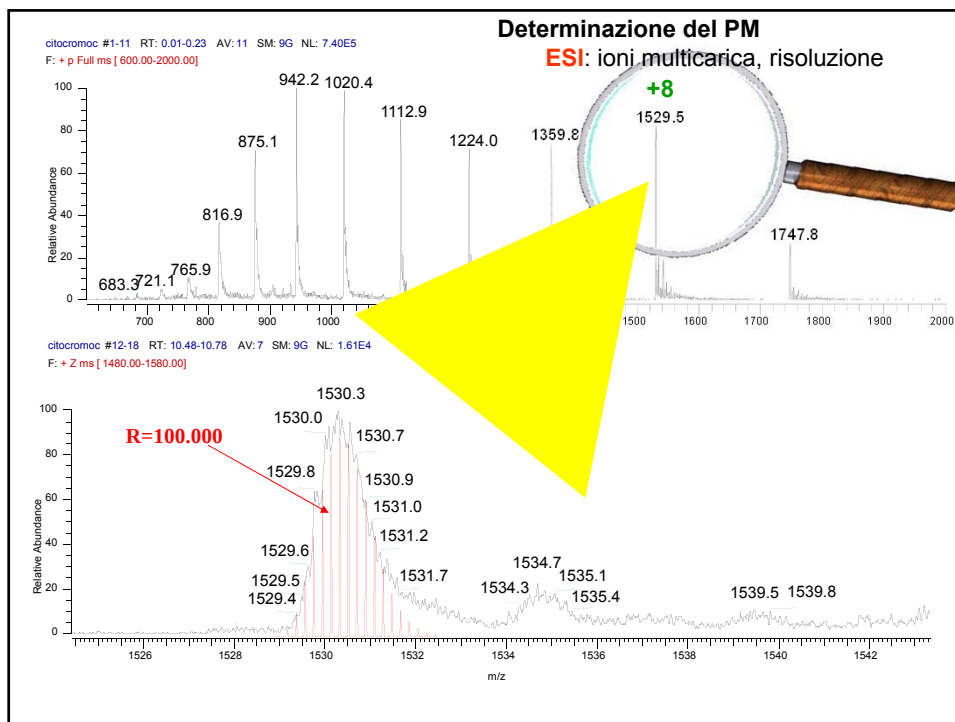
Risoluzione

massa

e

ioni multicarica



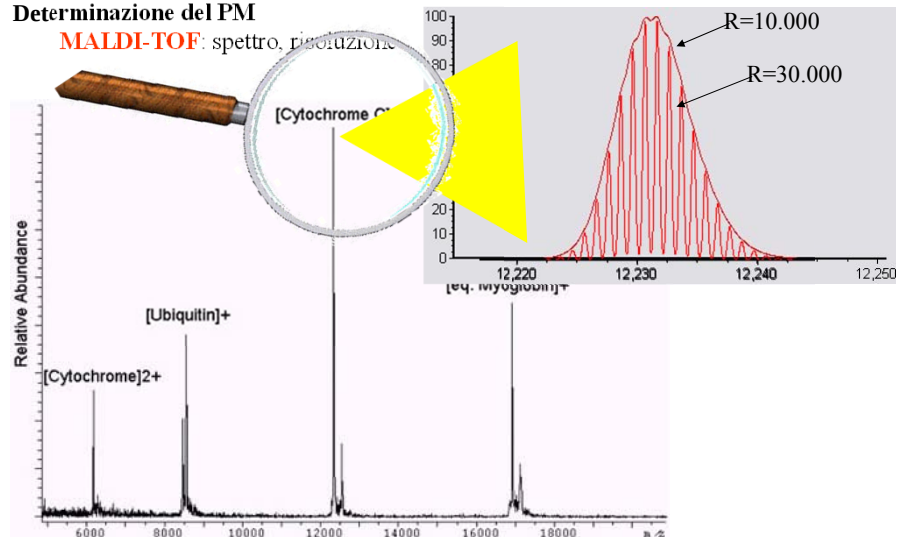


Conclusion:

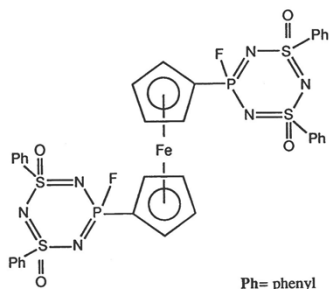
- At low mass (< 1000 u) the local maximum in an isotopic display will be close to the **monoisotopic mass**.
- At masses above 2000 u the local maximum will shift to be closer to the **average mass** of the compound.

Determinazione del PM

MALDI-TOF: spettro, risoluzione

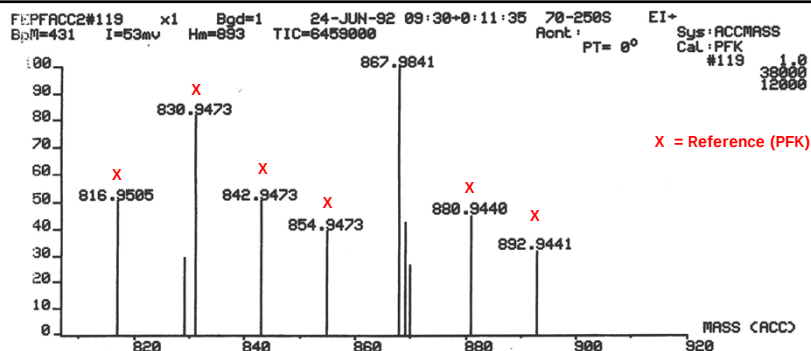
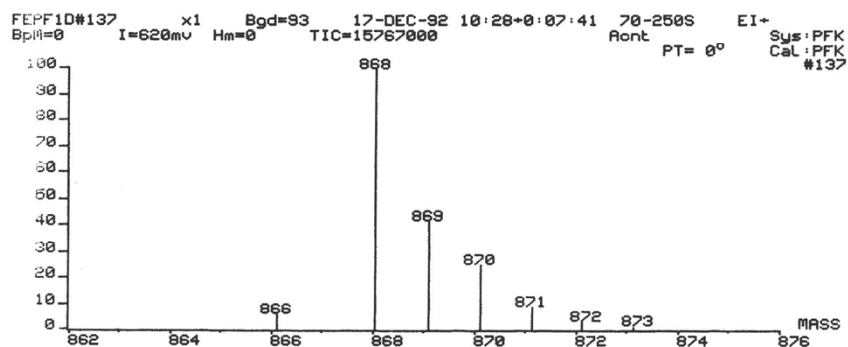


MALDI TOF mass spectrum of a mixture of ubiquitin, cytochrome C and equine myoglobin using 2,5-dihydroxybenzoic acid (DHB) as the matrix.



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

$C_{34}H_{28}N_6O_4FeS_4P_2F_2$



FEPPACC2 #119 x1 Bgd=1 24-JUN-92 09:30+0:11:35 70-250S
BpM=431 I=53mV Hm=893 TIC=6459000 Ront PT= 0° Sys: ACCMASS
Cal: PFK #119

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

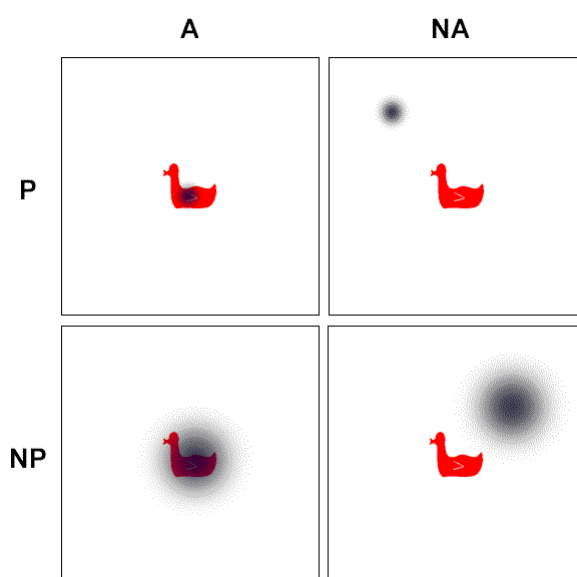
C 0+100
H 1+100
N 0+8
Fe 0+1
S 0+6
F 0+4
P 0+4
O 0+6

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.

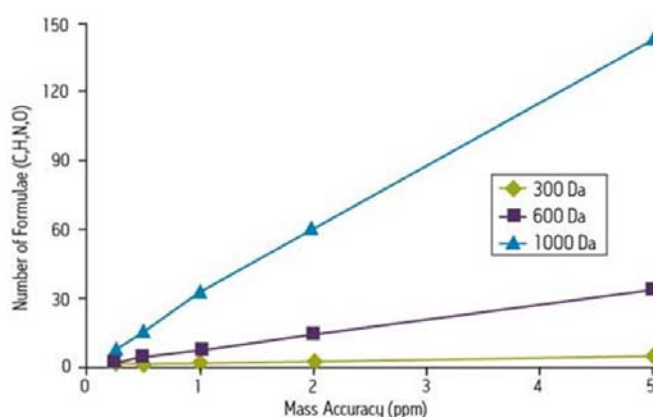
Lock mass

Systematic errors

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. 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;

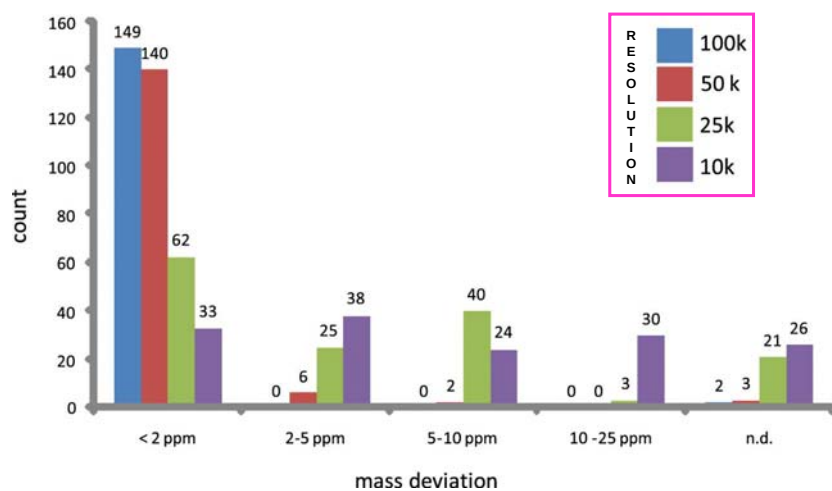
at m/z 750 requires precision better than 0.018 ppm to eliminate "all extraneous possibilities"

	Theoretical Mass	Delta [ppm]	Delta [mmu]	RDB	Composition
1 ppm (4)	516.76671	0.0	0.0	21.0	C ₄₈ H ₇₁ O ₁₂ N ₁₃
	516.76647	0.5	0.2	15.0	C ₄₈ 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 ₁₅
	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 ₁₅ S ₁
	516.76773	-2.0	-1.0	11.0	C ₄₆ H ₇₈ O ₁₆ N ₈ 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 ₄₆ 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 ₅₃ 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 ₄₆ 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 ₄₈ 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: C₃₅ - C₇₀; H₄₅ - H₁₀₀; N₈ - N₁₆; O₉ - O₁₆; S₀ - S₂

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)

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 ₉ N ₂
	474.2572	0.20	10.5	C ₂₁ H ₃₂ O ₄ N ₉
	474.2585	-2.64	10.0	C ₂₃ H ₃₄ O ₅ N ₆
	474.2558	3.02	5.5	C ₂₀ H ₃₆ O ₈ N ₅
	474.2599	-5.46	15.0	C ₂₄ H ₃₀ O ₁ N ₁₀
	474.2599	-5.47	9.5	C ₂₅ H ₃₆ O ₆ N 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 ₇ N ₈
	474.2612	-8.29	14.5	C ₂₆ H ₃₂ O ₂ N ₇
	474.2612	-8.30	9.0	C ₂₇ H ₃₈ O ₇

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

Restrizioni sui valori di massa accurata:

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

Double bond/ring equivalents (RDB)

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

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 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 ₉ N ₂
	474.2572	0.20	10.5	C ₂₁ H ₃₂ O ₄ N ₉
	474.2585	-2.64	10.0	C ₂₃ H ₃₄ O ₅ N ₆
	474.2558	3.02	5.5	C ₂₀ H ₃₆ O ₈ N ₅
	474.2599	-5.46	15.0	C ₂₄ H ₃₀ O ₁ N ₁₀
	474.2599	-5.47	9.5	C ₂₅ H ₃₆ O ₆ N 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 ₇ N ₈
	474.2612	-8.29	14.5	C ₂₆ H ₃₂ O ₂ N 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

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	C₂₂H₃₀O₄N₂
	474.2572	0.20	10.0	C₂₁H₃₂O₄N₃
	474.2565	-2.64	10.0	C₂₃H₃₄O₅N₆
	474.2558	3.02	5.5	C ₂₀ H ₃₆ O ₈ N ₅
	474.2553	-5.46	15.0	C₂₄H₃₀O₁N₁₀
	474.2599	-5.47	9.5	C ₂₅ H ₃₆ O ₆ N ₃
	474.2545	5.83	0.5	C ₁₉ H ₄₀ O ₁₂ N ₁
	474.2545	5.85	6.0	C₁₈H₃₄O₇N₆
	474.2612	-8.29	14.5	C ₂₆ H ₃₂ O ₂ N ₇
	474.2612	0.00	9.0	C₂₇H₃₈O₇

RDB=double bond/ring equivalents

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

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	C₂₀¹³C₁H₃₂O₄N₃
	475.2605	0.04	5.0	C₂₁H₃₄O₅N₂
	475.2605	0.10	10.5	C₃₀H₃₁N₆
	475.2610	0.90	0.5	C₁₇H₃₉O₁₁N₄
	475.2596	1.85	1.0	C₁₉H₃₇O₁₀N₇
	475.2592	2.79	5.5	C ₁₉ ¹³ C ₁ H ₃₆ O ₈ N ₅
	475.2619	2.85	10.0	C₂₂¹⁸C₁H₃₄O₅N₆
	475.2591	2.92	13.5	C₂₉H₃₅O₄N₂
	475.2623	3.70	5.5	C₁₈H₃₅O₇N₆
	475.2623	-3.00	0.0	C₁₉H₄₁O₁₂N₁

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

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	1
200	3	2	2	1	1	1	1	1
300	24	11	7	2	1	1	1	6
400	78	37	23	7	1	2	13	13
500	266	115	64	21	2	3	33	33
600	505	257	155	50	5	4	36	36
700	1046	538	321	108	10	10	97	97
800	1964	973	599	200	20	13	111	111
900	3447	1712	1045	345	32	18	196	196

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

Restrizioni sui valori di massa accurata ottenuti:

Ioni frammento:

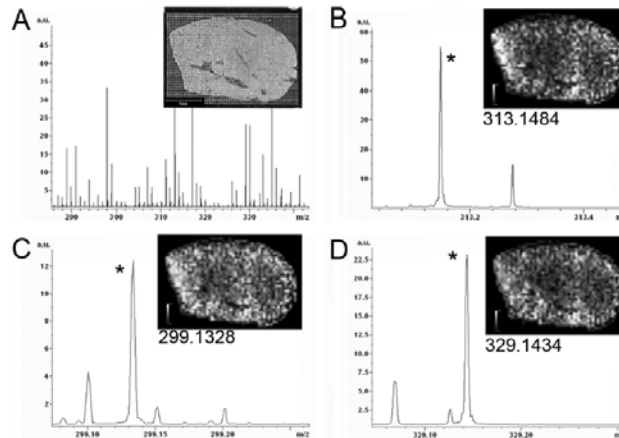
non si considera solo il valore di massa accurata dello ione molecolare/
protonato/deprotonato, ma anche quelli degli **ioni frammento**.

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.

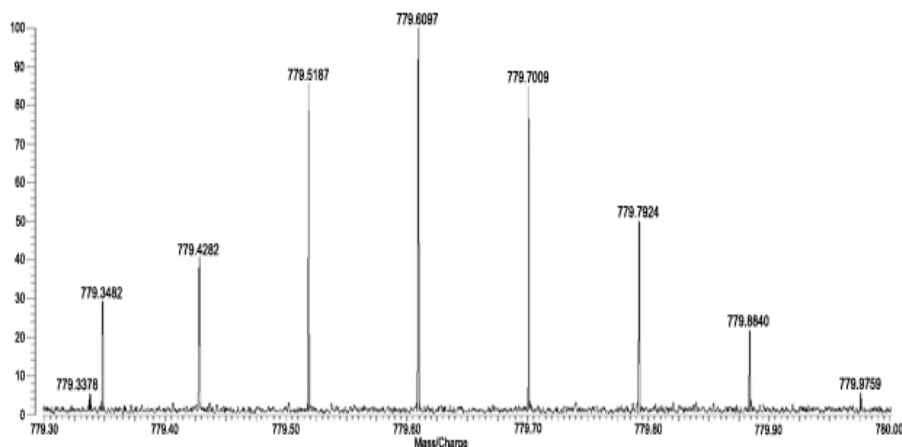


D. S. Cornett, S. L. Frappier,
R. M. Caprioli, *Anal. Chem.*
2008, 80, 5648–5653

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.

Esercizio N. 1

Determinare il peso molecolare



Spettrometria di massa in alta risoluzione (HRMS):

Introduzione diretta, infusione

GC-HRMS

GC-HRMS/MS, GC-HRMSⁿ

HPLC-HRMS

HPLC-HRMS/MS, HPLC-HRMSⁿ

.....

Metodi di desorbimento (ESI, MALDI)



Ionizzazione

SOFT

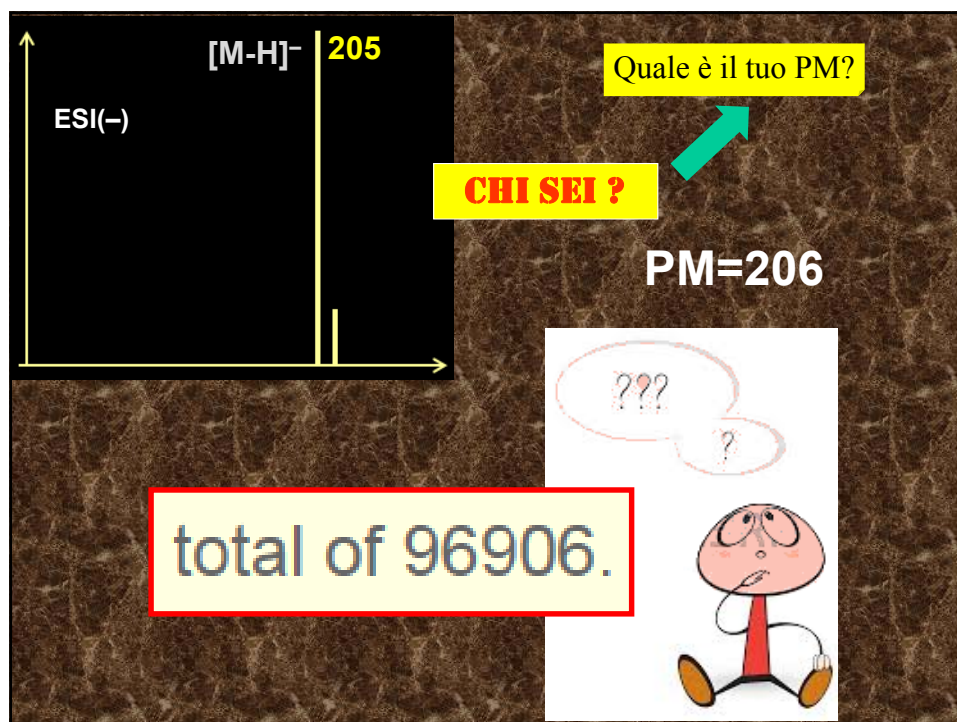
✓ Frammenti assenti → informazione strutturale?

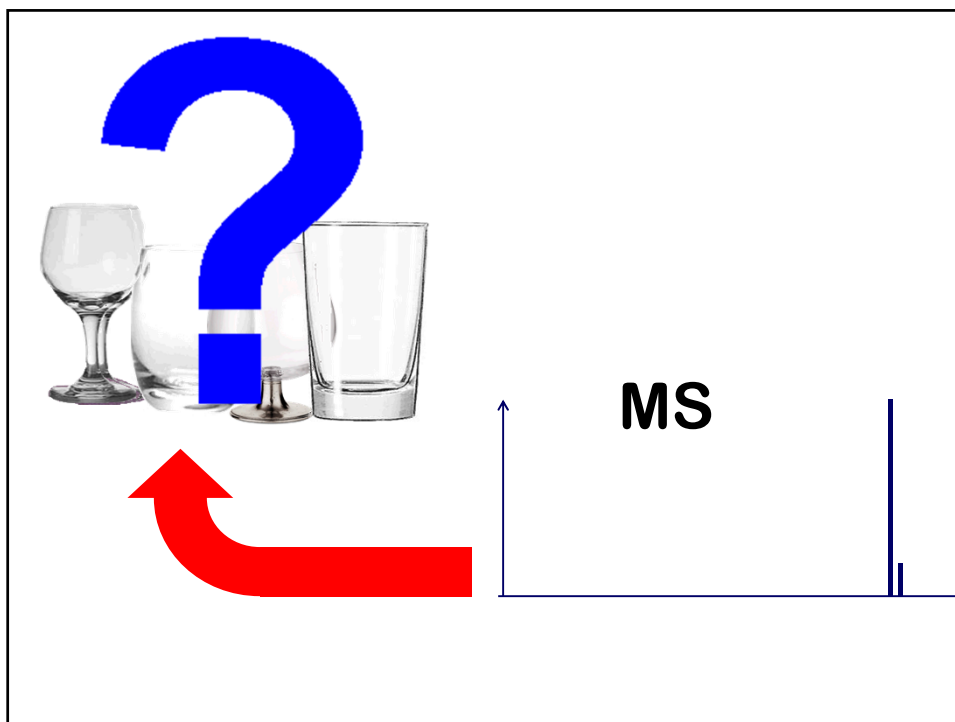
Spettrometria di massa

Spettro di massa $\longrightarrow$ Peso molecolare

HR + massa accurata $\longrightarrow$ Stechiometria

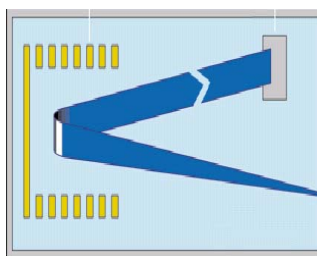
? $\longleftarrow$ Informazioni strutturali





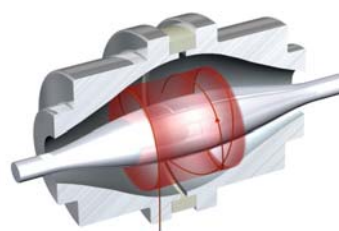
È possibile aumentare la specificità?

Spettrometria di massa **in alta risoluzione**



Tempo di volo (TOF)

Orbitrap

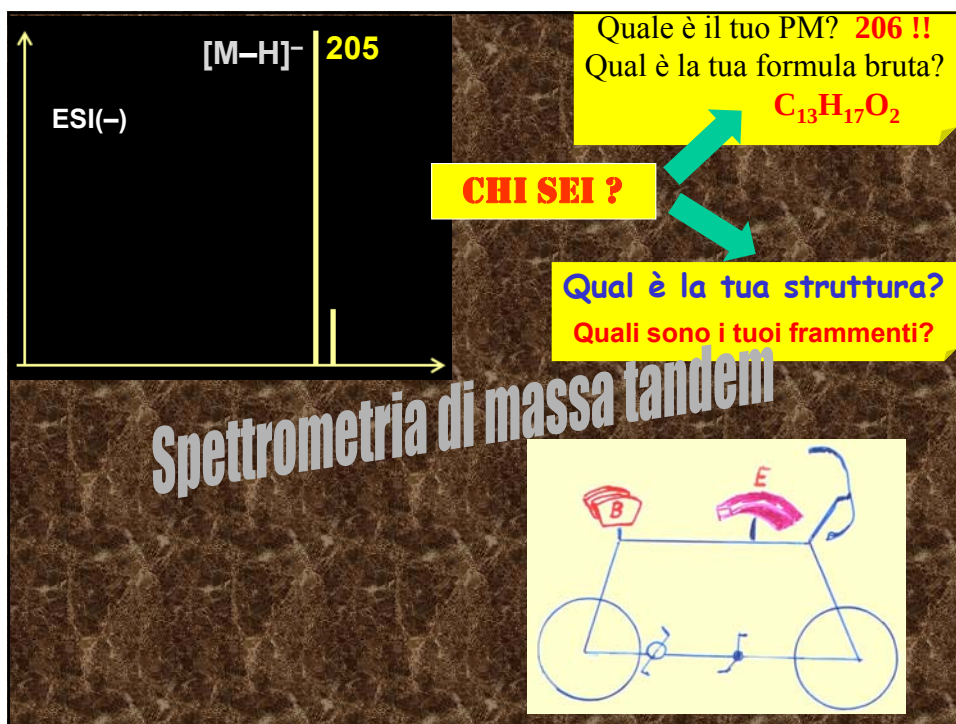
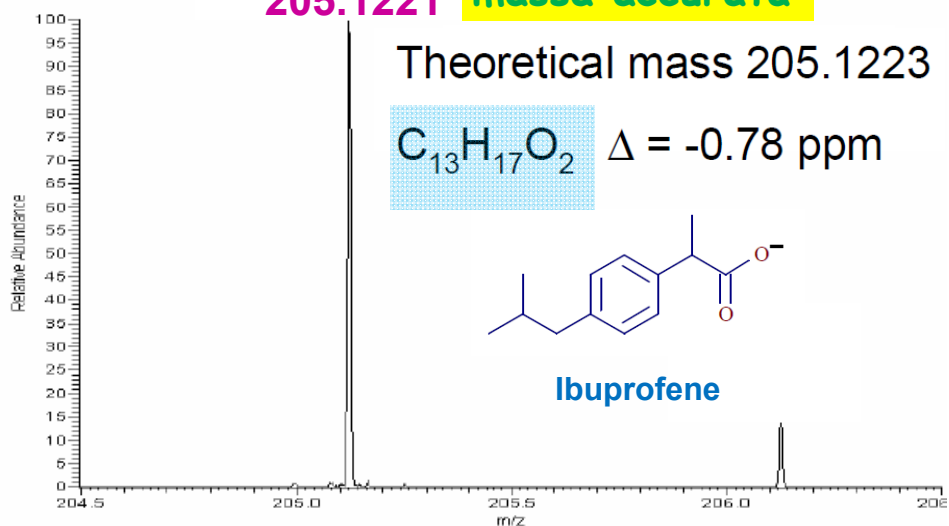


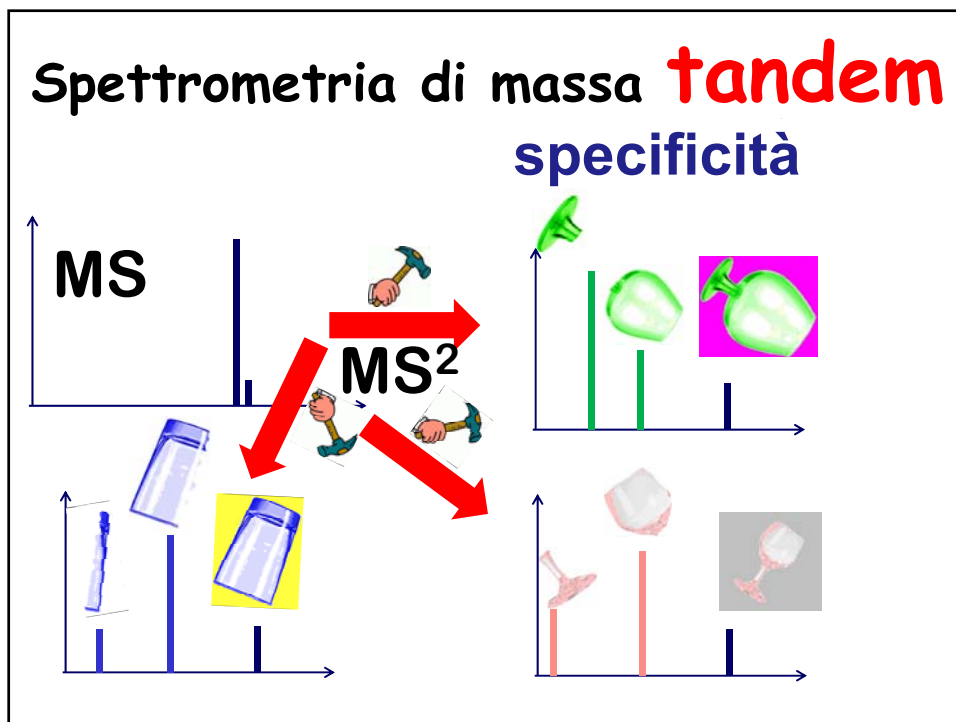
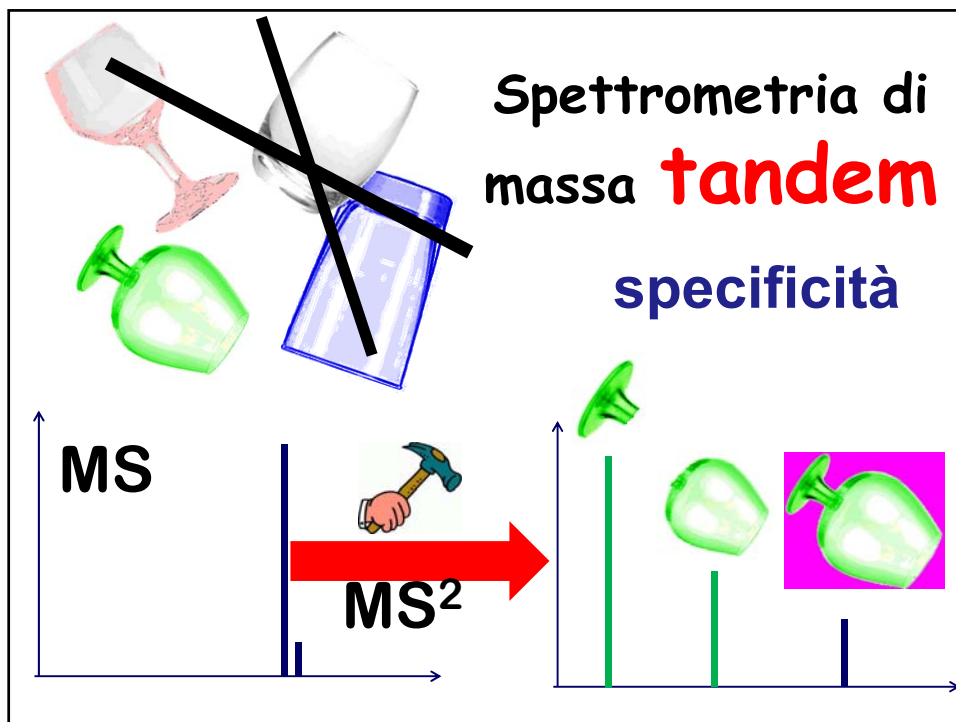
Spettrometria di massa in alta risoluzione specificità

205.1221 massa accurata

Theoretical mass 205.1223

$C_{13}H_{17}O_2$ $\Delta = -0.78$ ppm





Spettrometria di massa **tandem**

MS²

MS/MS

~~Tandem massa~~

~~Tandem max~~

~~Abcgftrd max~~



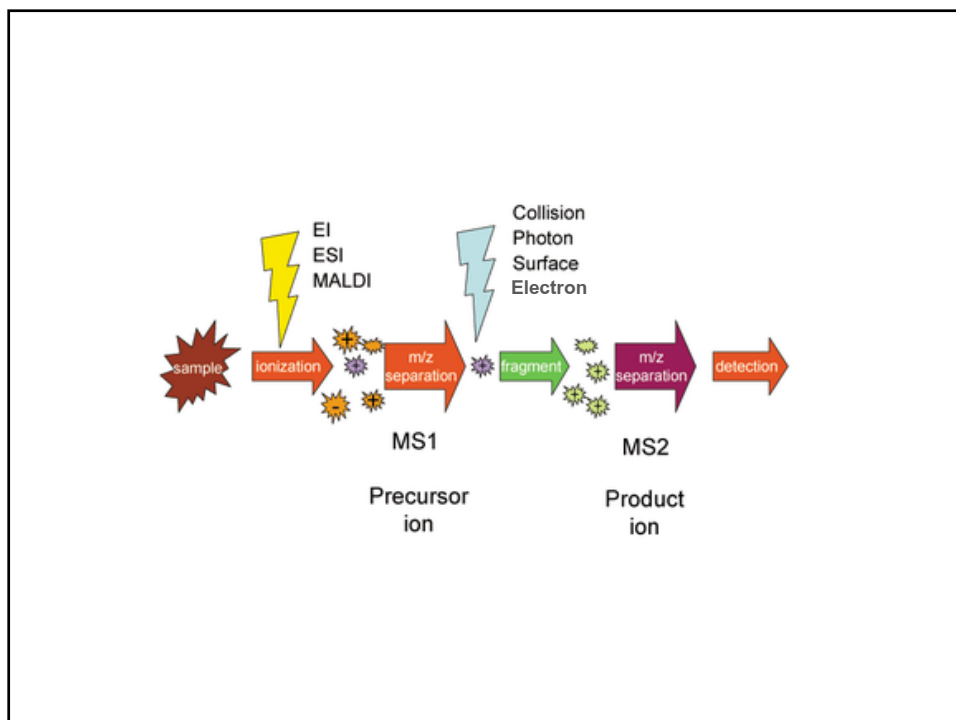
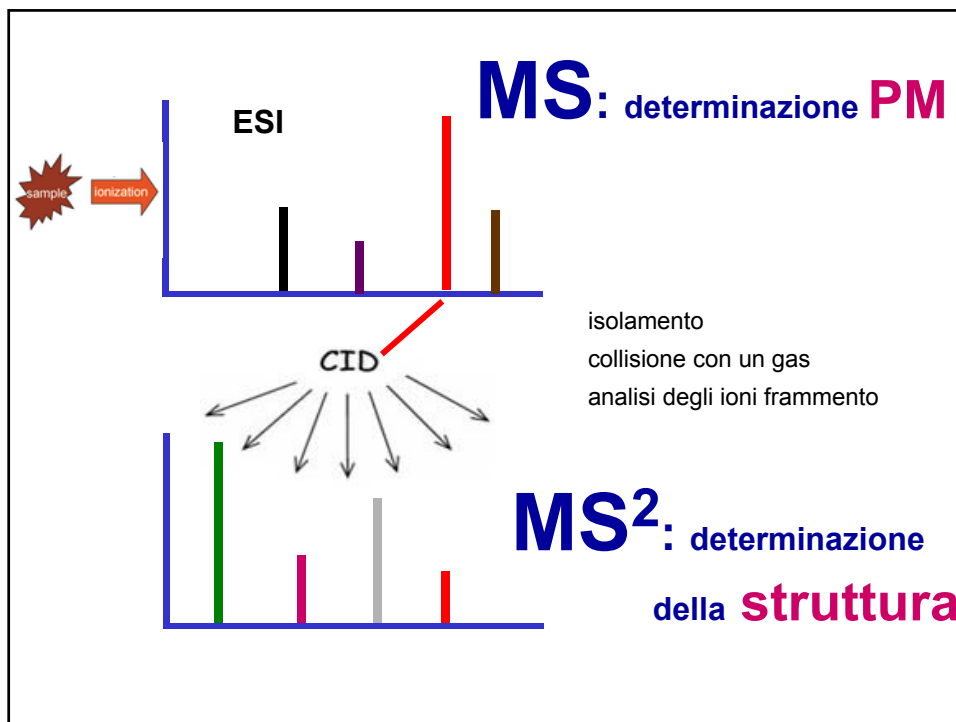
✓ Spettrometria di massa **tandem**

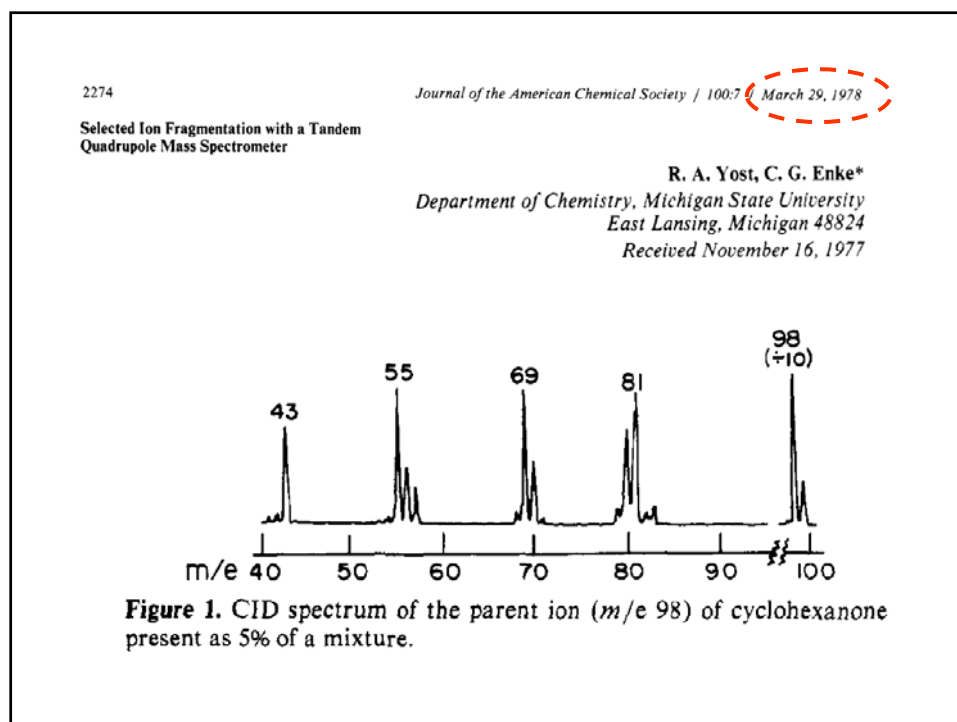
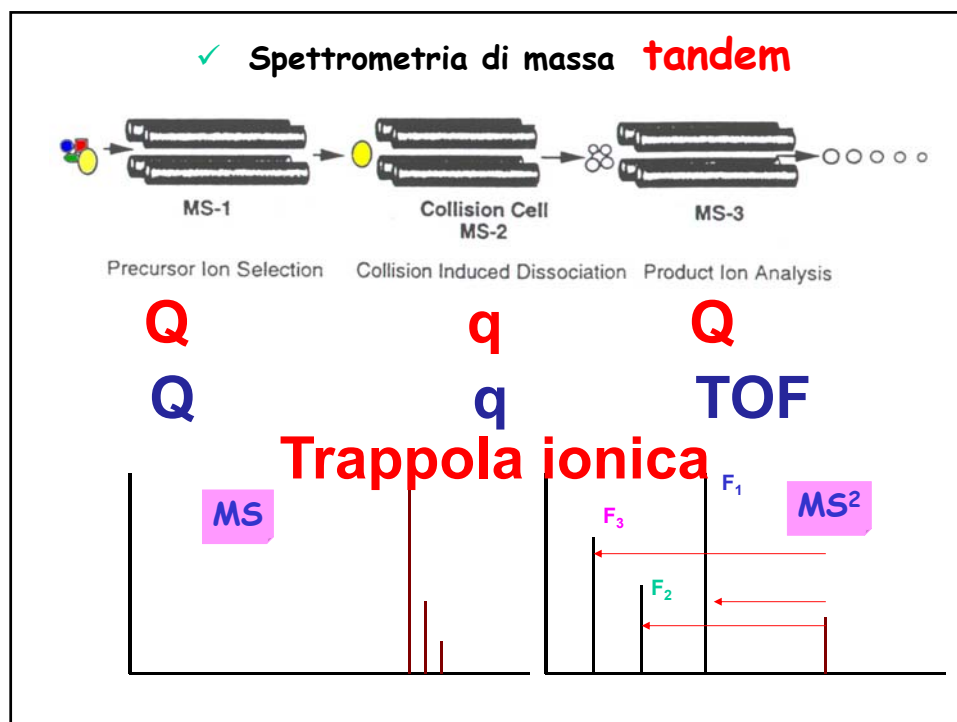
La spettrometria di massa tandem impiega
due o più stadi di analisi di massa
con lo scopo di esaminare
selettivamente le frammentazioni di una
famiglia di ioni

MS/MS

MS²

MSⁿ





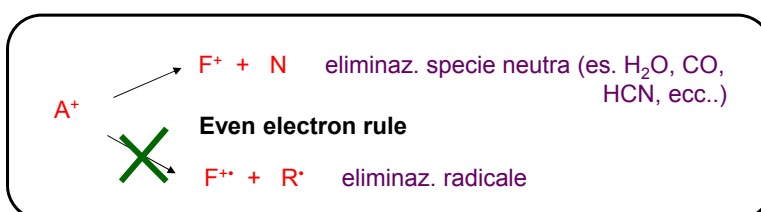
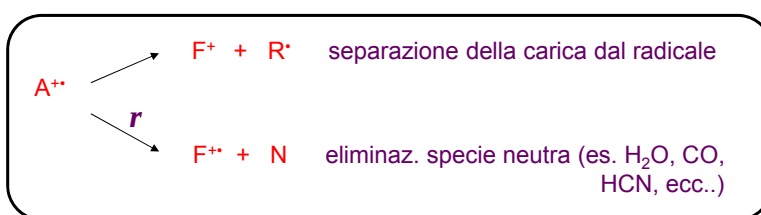


Il triplo quadrupolo: trent'anni di successi !
Attualità, strumentazione, applicazioni, prospettive

Milano, 18 dicembre 2008



Reazioni di decomposizione



Decomposizioni spontanee:

decomposizioni metastabili

Decomposizioni indotte da:

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

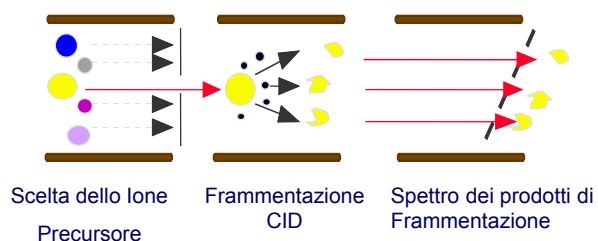
interazioni con elettroni (ECD, ETD)

interazioni con superfici (SID)

interazioni con fotoni (IRMPD, ion spectroscopy)

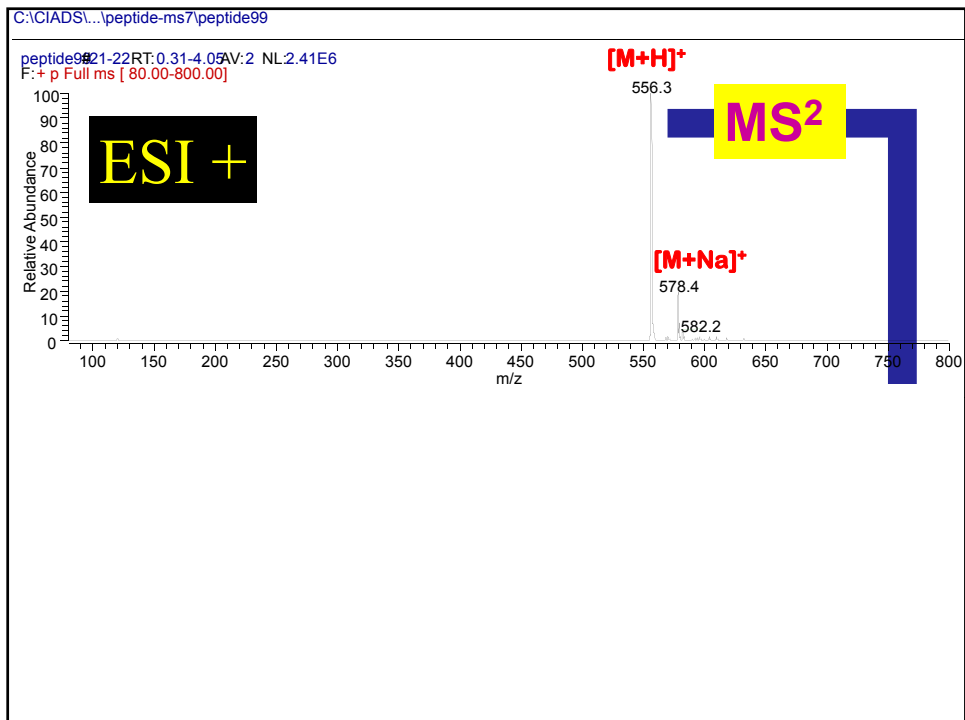
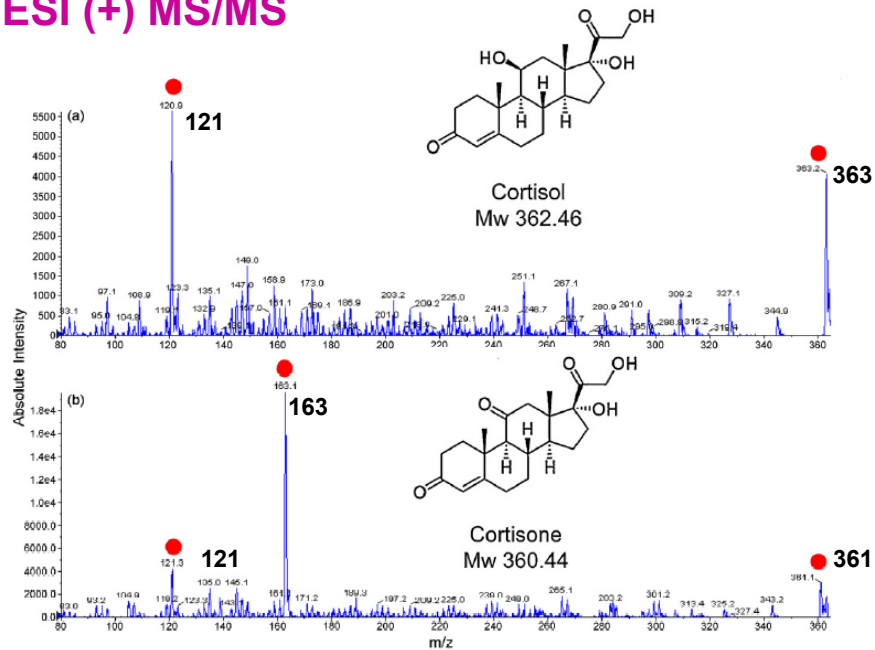
MS/MS

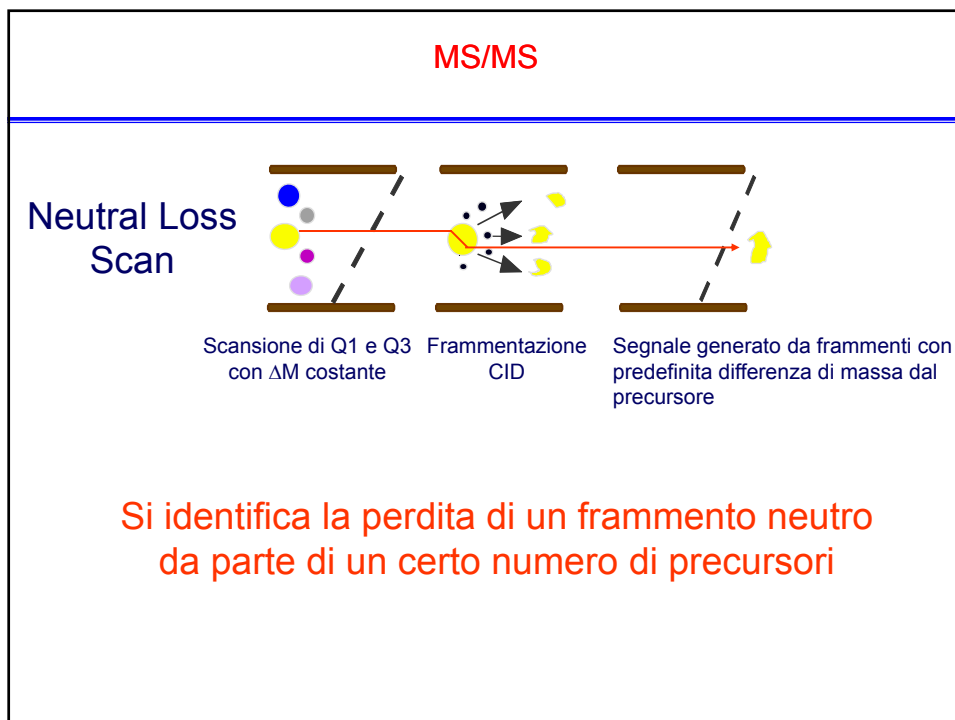
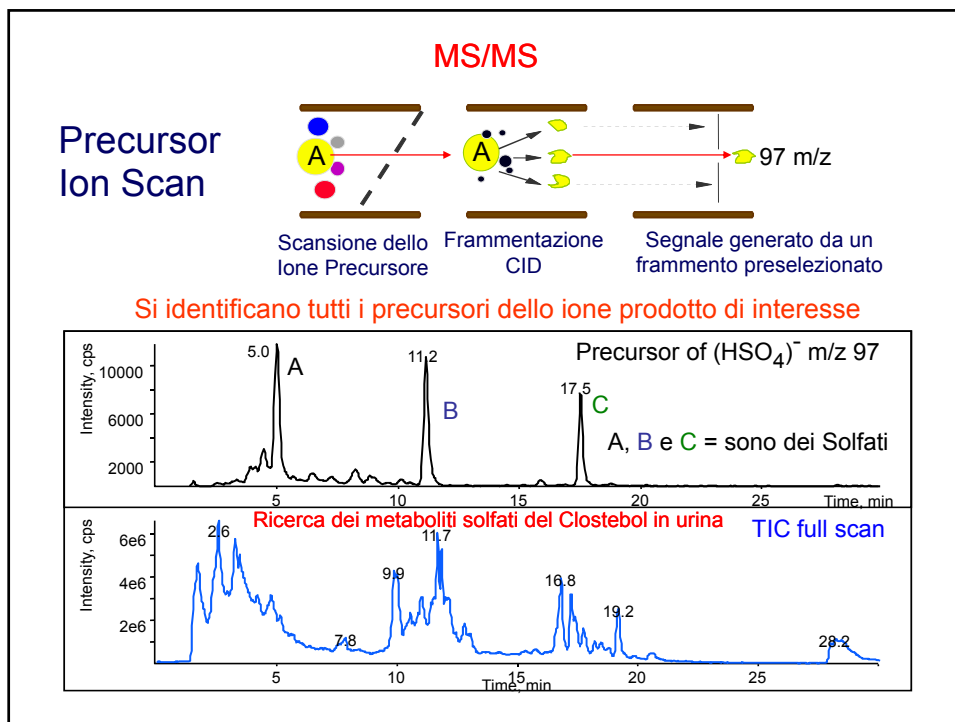
Product Ion
Scan



Si identificano tutti i prodotti dello ione precursore selezionato

ESI (+) MS/MS

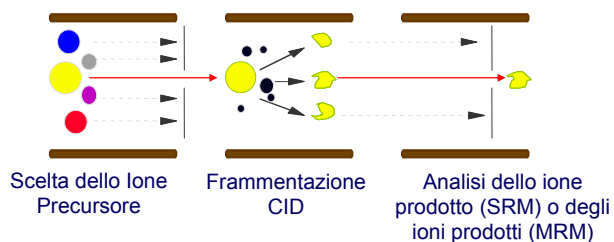




MS/MS

SRM

MRM

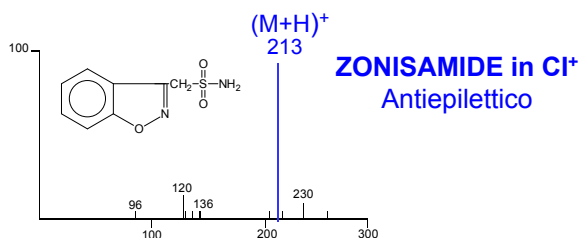


Si monitora una transizione specifica (SRM)
o più transizioni (MRM)
sia con scansioni di ioni prodotto, di ioni precursori
o neutral loss

Identificazione rapida di metaboliti di farmaci

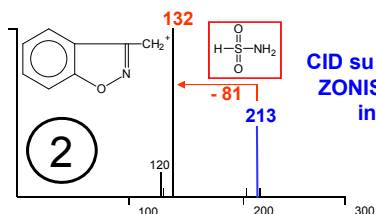
1

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



TIPI DI SCANSIONI IN MS/MS

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

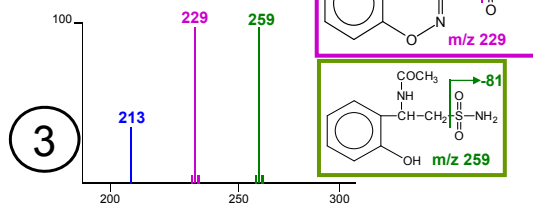


**CID su m/z 213
ZONISAMIDE
in Cl^-**

**SUL CAMPIONE BIOLOGICO ESTRATTO DA
PLASMA O DA URINE IDROLIZZATE**

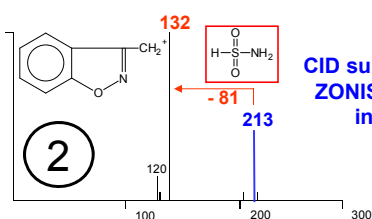
-Spettro **PERDITA NEUTRA** selezionando la perdita del frammento **neutro (-81)** gruppo sulfonamidico. Gli ioni presenti sono possibili **METABOLITI**

**SPETTRO
NEUTRAL LOSS
(-81)**



TIPI DI SCANSIONI IN MS/MS

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

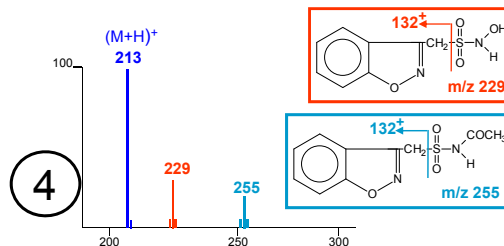


**CID su m/z 213
ZONISAMIDE
in Cl^-**

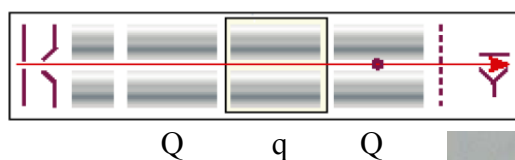
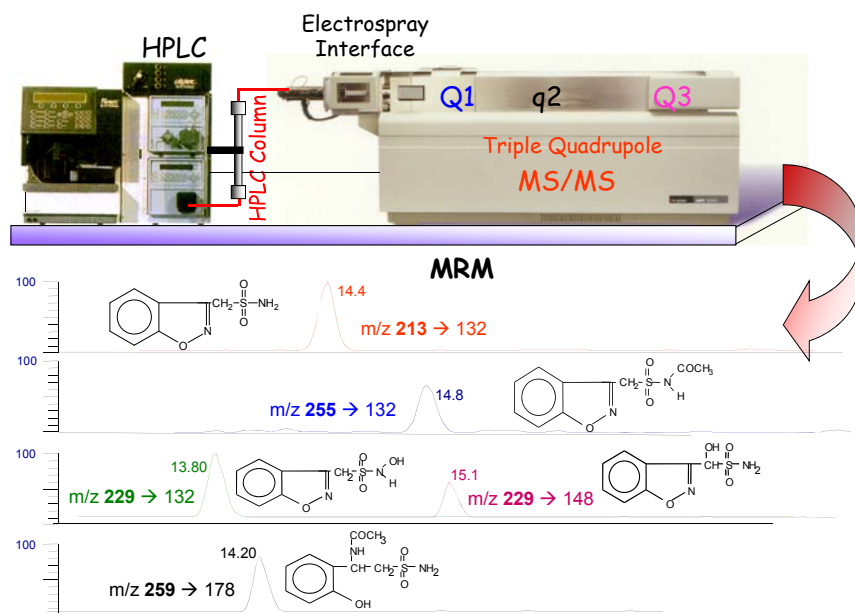
**SUL CAMPIONE BIOLOGICO ESTRATTO DA
PLASMA O DA URINE IDROLIZZATE**

-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)**



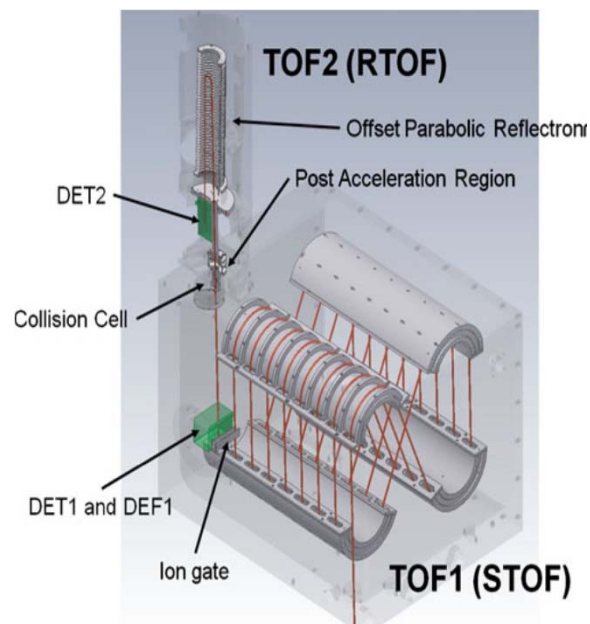
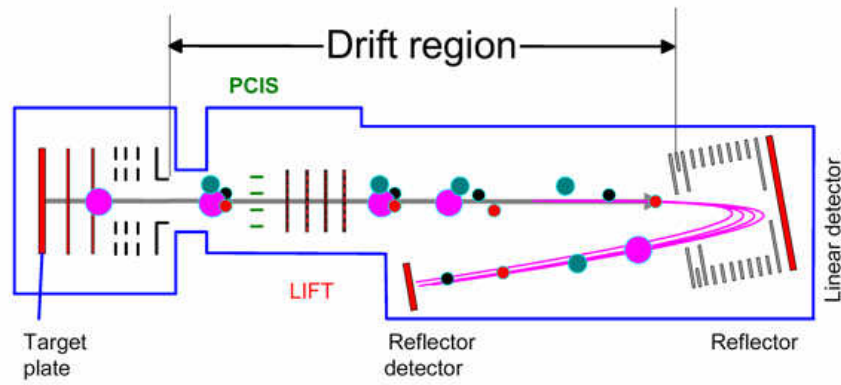
LC-ESI-MS/MS



QqQ con cella di collisione a 180°

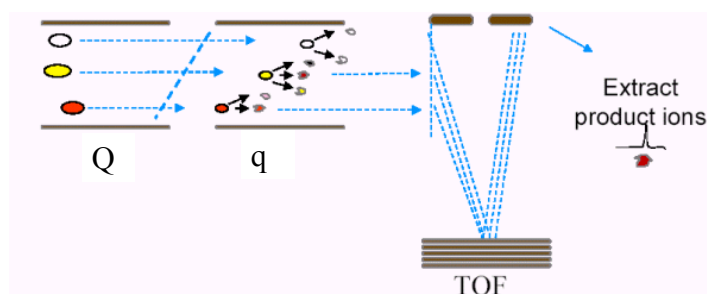


TOF-TOF

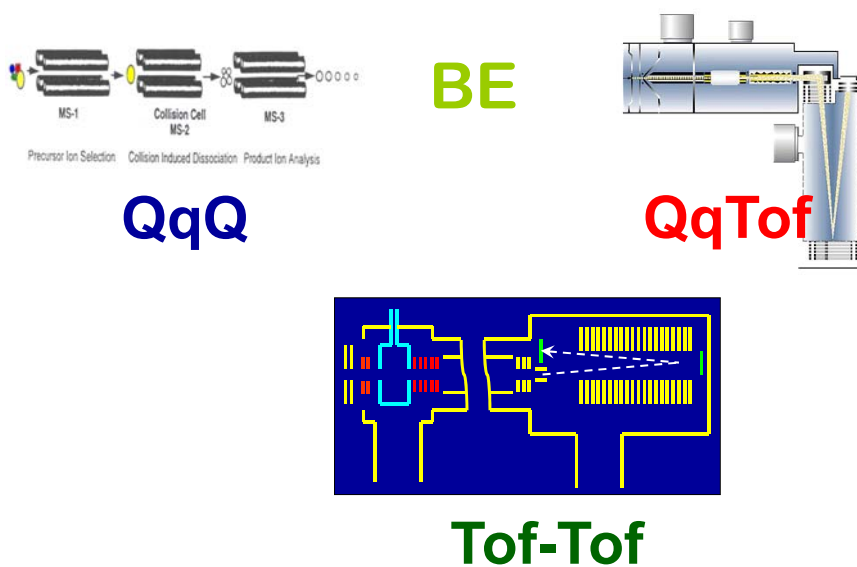


MS/MS Analizzatori ibridi

QqTOF

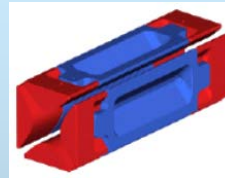


MS/MS nello spazio

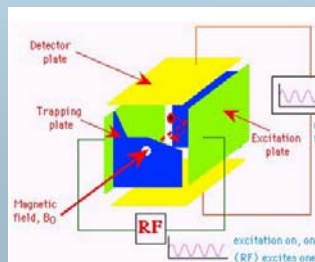


MS/MS nel tempo

3D

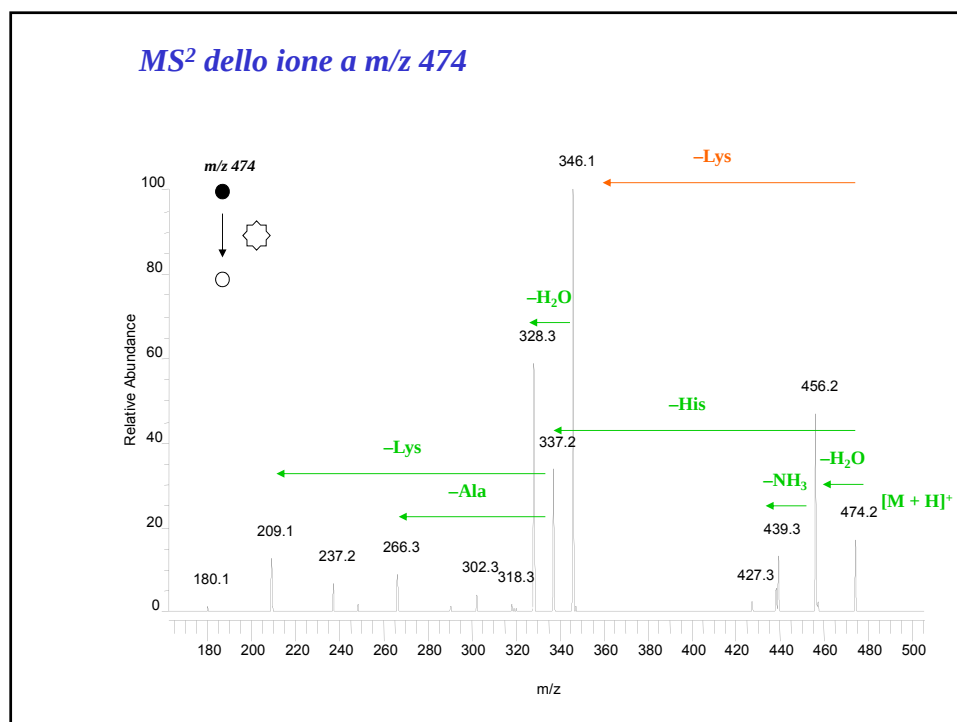
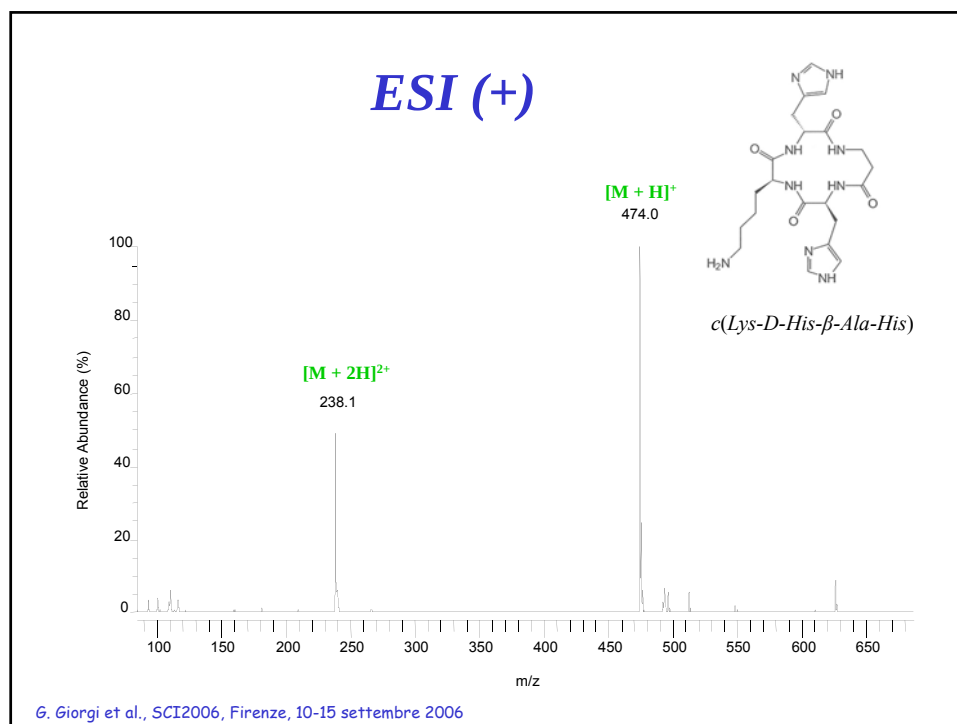


2D

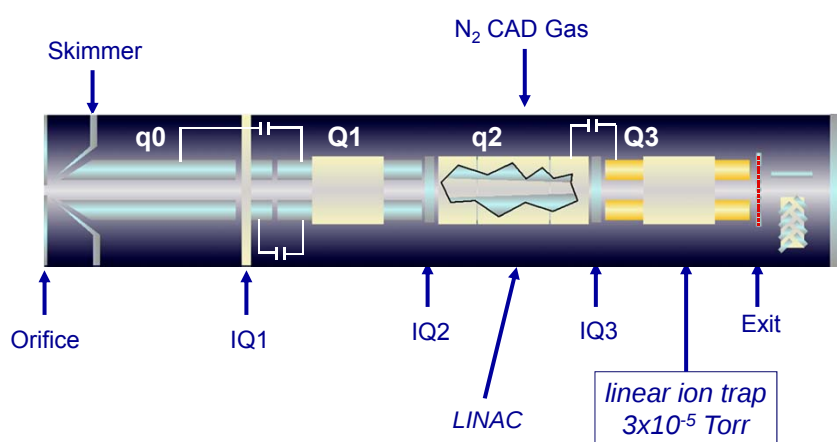


Cella a risonanza ciclotronica

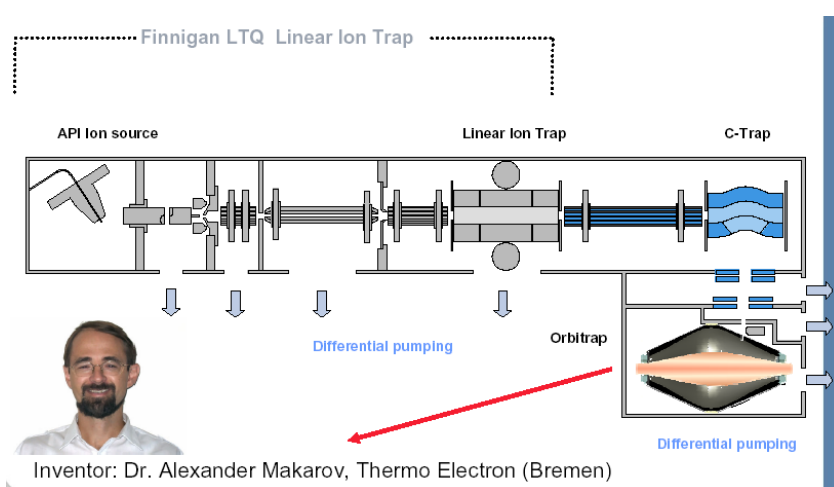
Animazione IT 3D e 2D

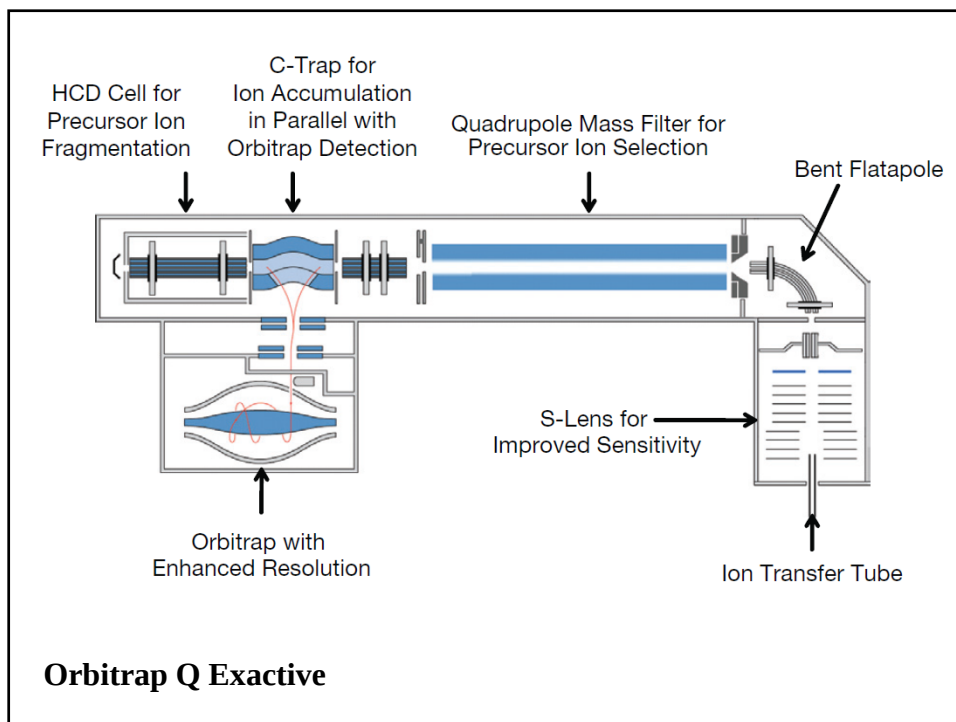
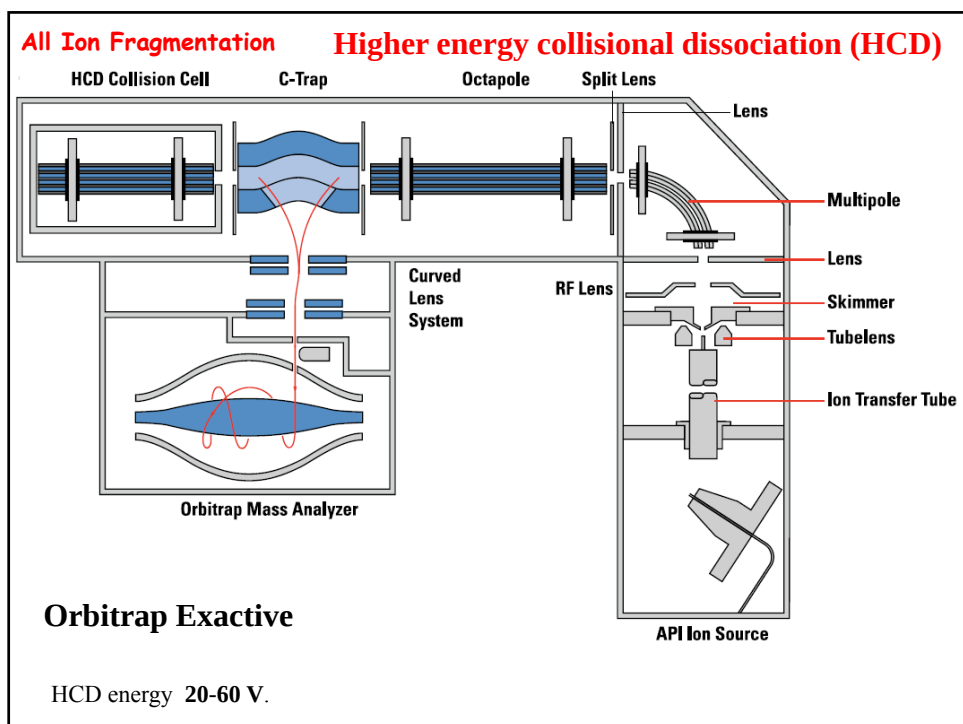


Hybrid Instrument with Q3 Linear Ion Trap MS

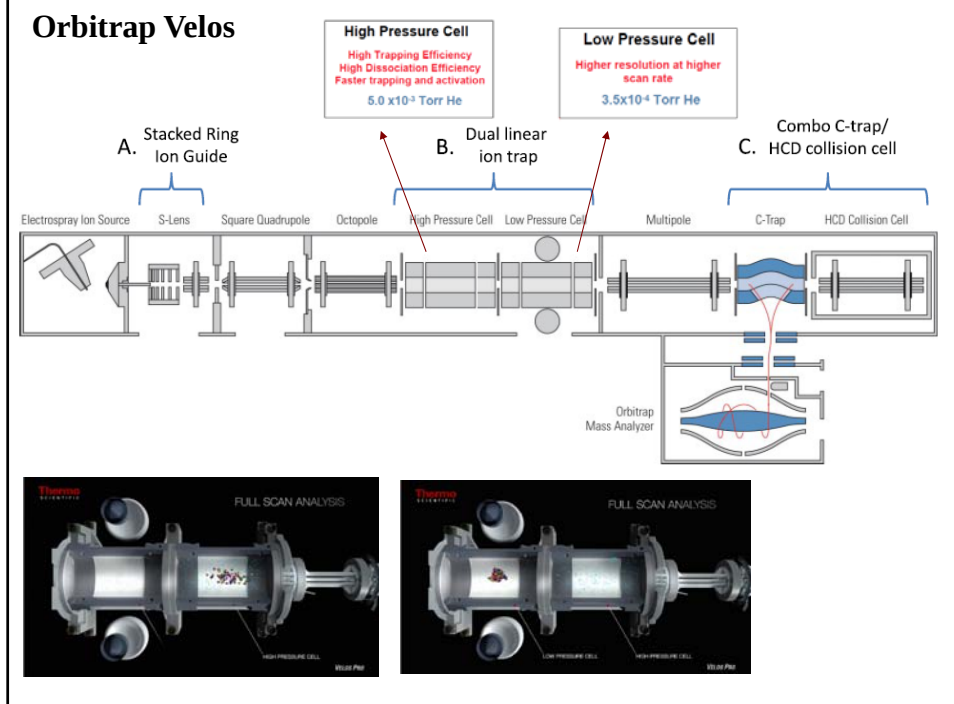


Linear Ion Trap - Orbitrap Hybrid

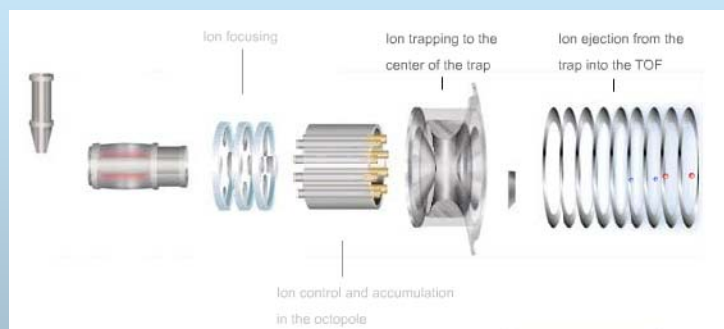




Orbitrap Velos



MS/MS IT-TOF ibrido



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

