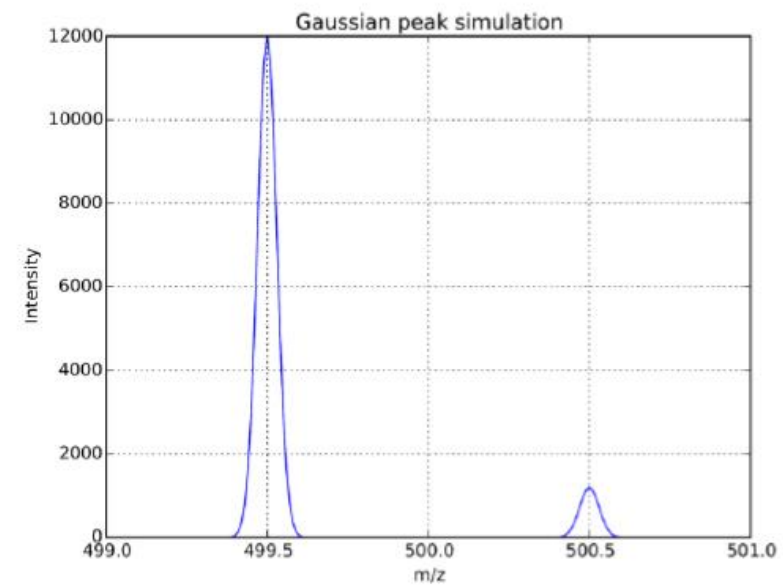
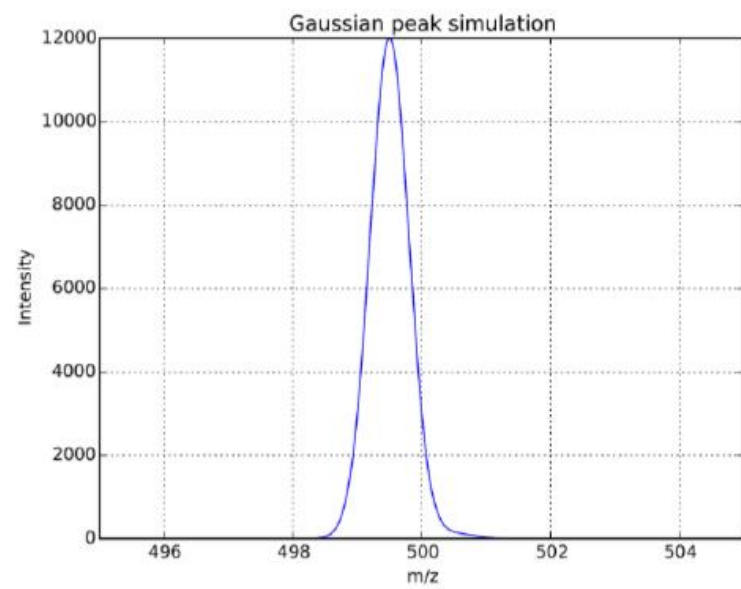
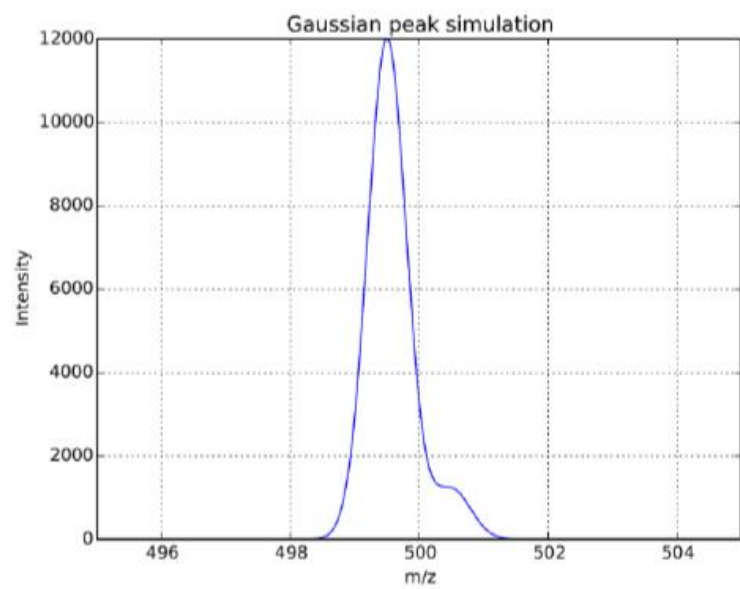
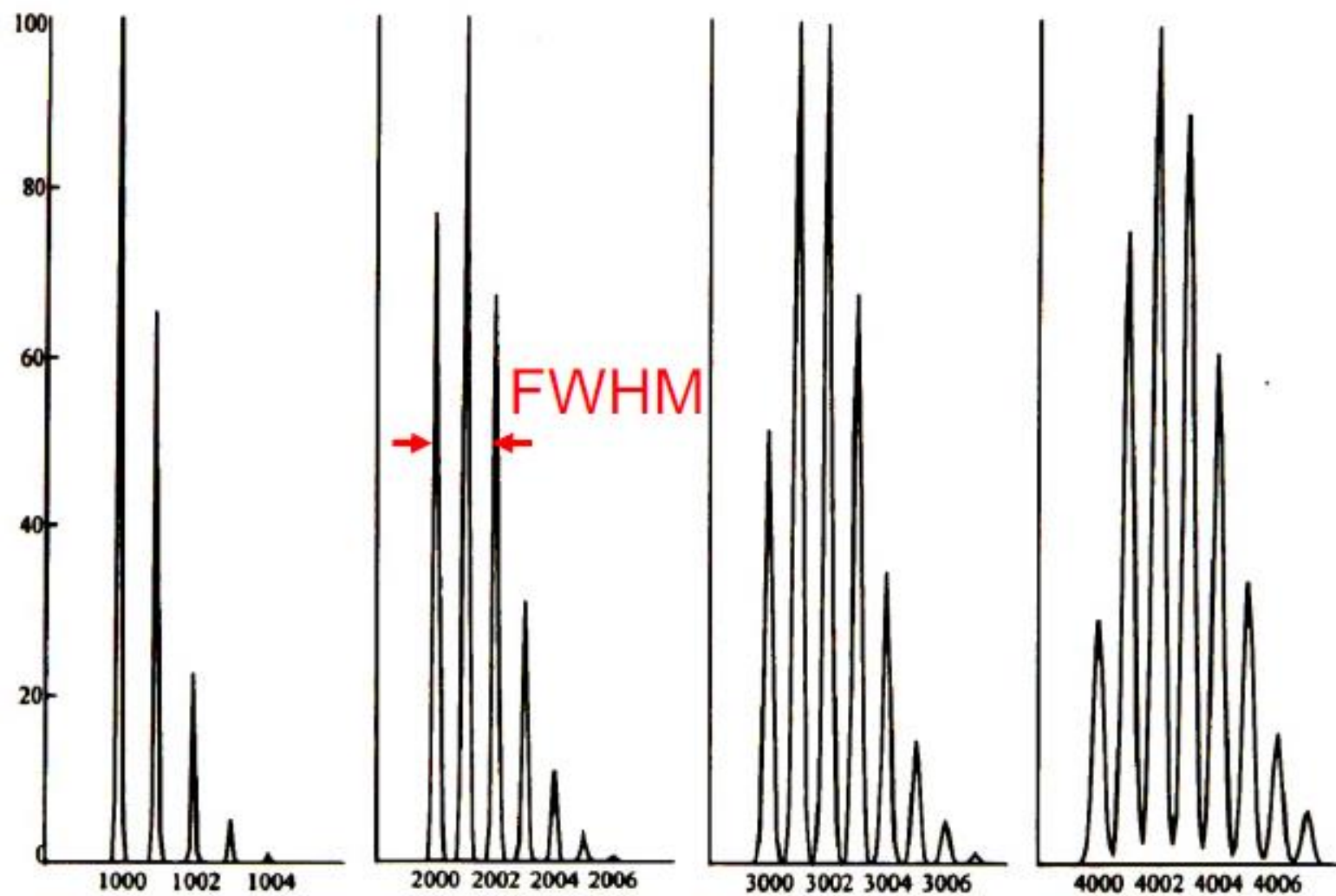


SPETTROMETRIA DI MASSA
Potere Risolutivo, Risoluzione e
accuratezza di massa





Mass Analyzer	Mass Range (u)	Mass Resolution	Mass Accuracy (ppm)
Fourier Transform Ion Cyclotron Resonance (FT ICR)	30.000	1.000.000+	< 1 (@ 400 u)
Orbitrap	50.000	500.000	< 2 (@ 100-2000)
Magnetic Sector (BE)	20.000	100.000	< 10
Time-of-Flight (TOF, RTOF)	> 1.000.000	5.000-20.000	200; 5-10
Quadrupole (Q)	4.000	2.000	100
Iontrap (IT)	6.000	4.000	100



Theoretical isotope distributions of peptides of 1000, 2000, 3000 and 4000 Da.

<i>Isotope</i>	<i>Mass</i>	<i>Abundance</i>	<i>Chemical mass</i>	<i>Deviation from the whole number</i>
¹ H	1.00782510	99.9852%	1.00794	+0.0079
² H (D)	2.01410222	0.0148%		
¹² C	12.0(0)	98.892%	12.011	+0.011
¹³ C	13.0033544	1.108%		
¹⁴ N	14.00307439	99.635%	14.00674	+0.007
¹⁵ N	15.0001077	0.365%		
¹⁶ O	15.99491502	99.759%	15.9994	-0.0006
¹⁷ O	16.9991329	0.037%		
¹⁸ O	17.99916002	0.204%		
³¹ P	30.9737647	100%	30.9737647	-0.0262
³² S	31.9720737	95.0%	32.066	+0.066
³³ S	32.9714619	0.76%		
³⁴ S	33.9678646	4.22%		
³⁶ S	35.967090	0.014%		

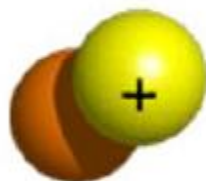
The Nuclei of the Three Isotopes of Hydrogen

Protium



1 proton

Deuterium

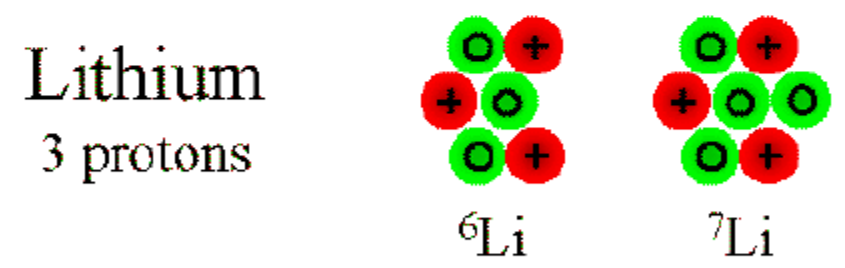
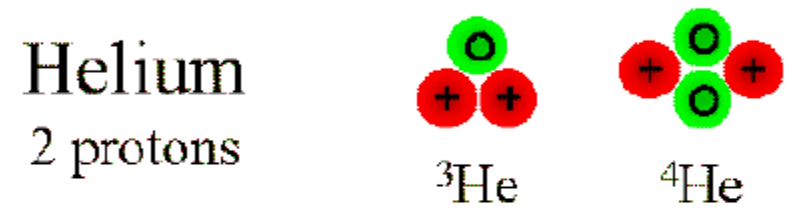



1 proton
1 neutron


Tritium

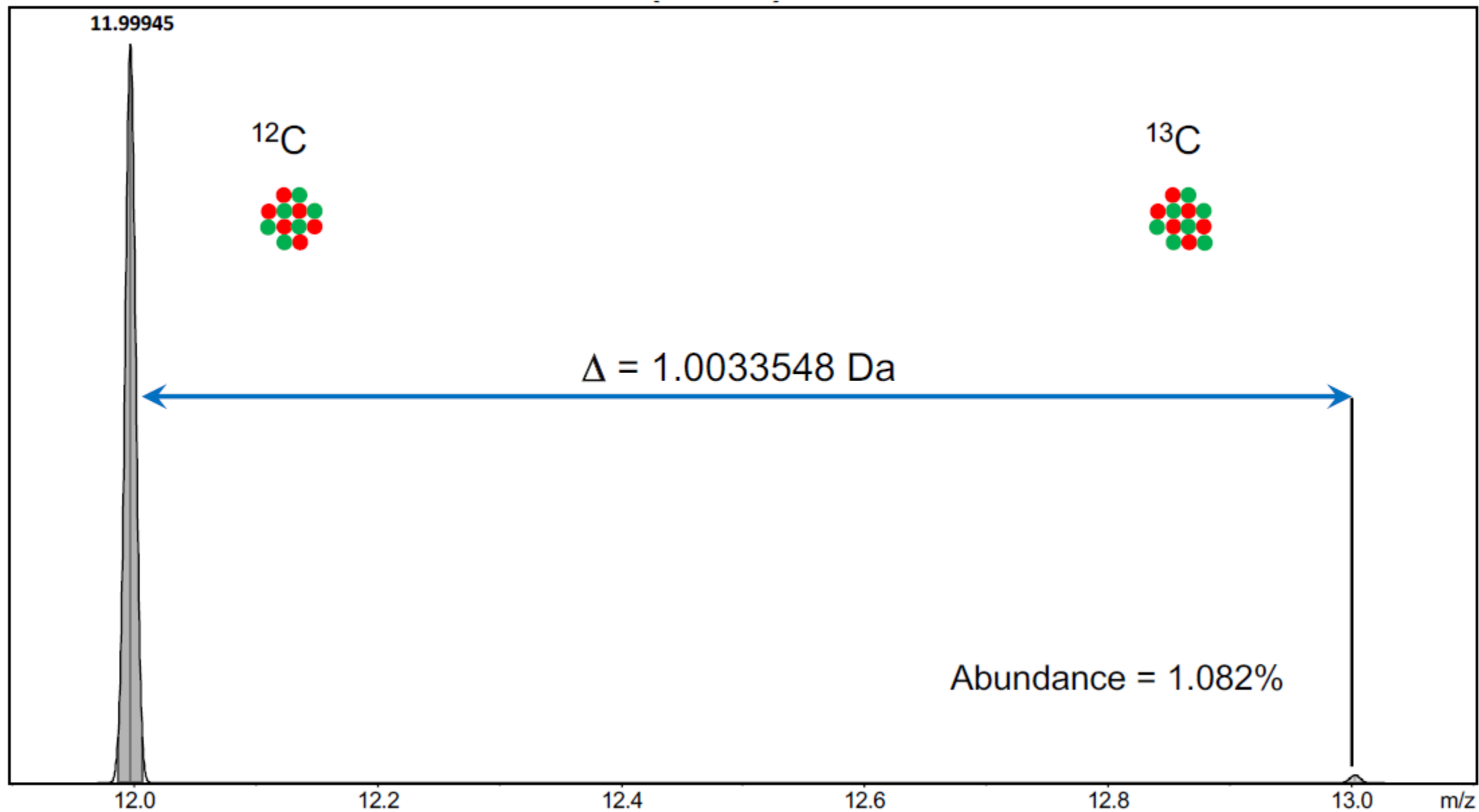


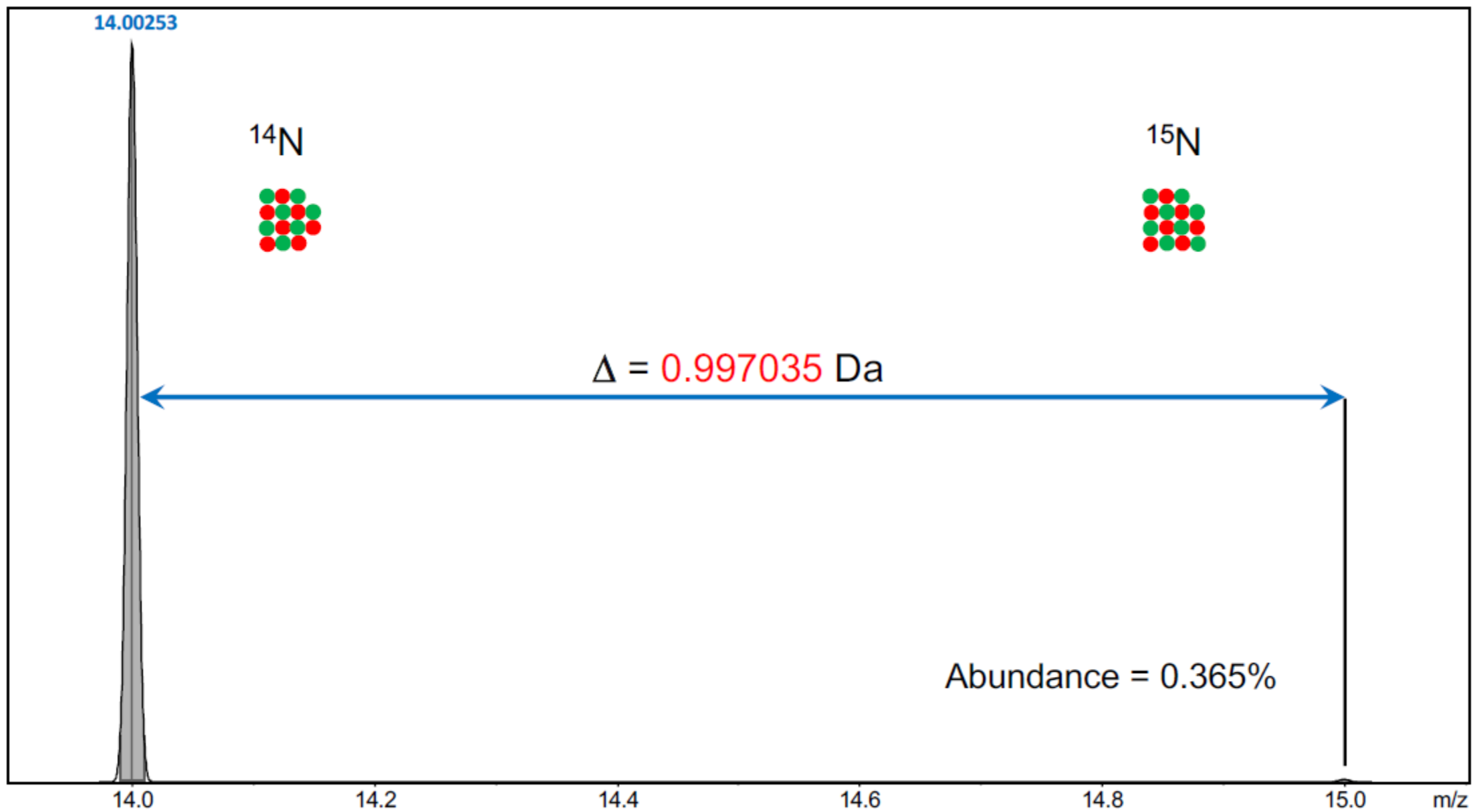
1 proton
2 neutrons

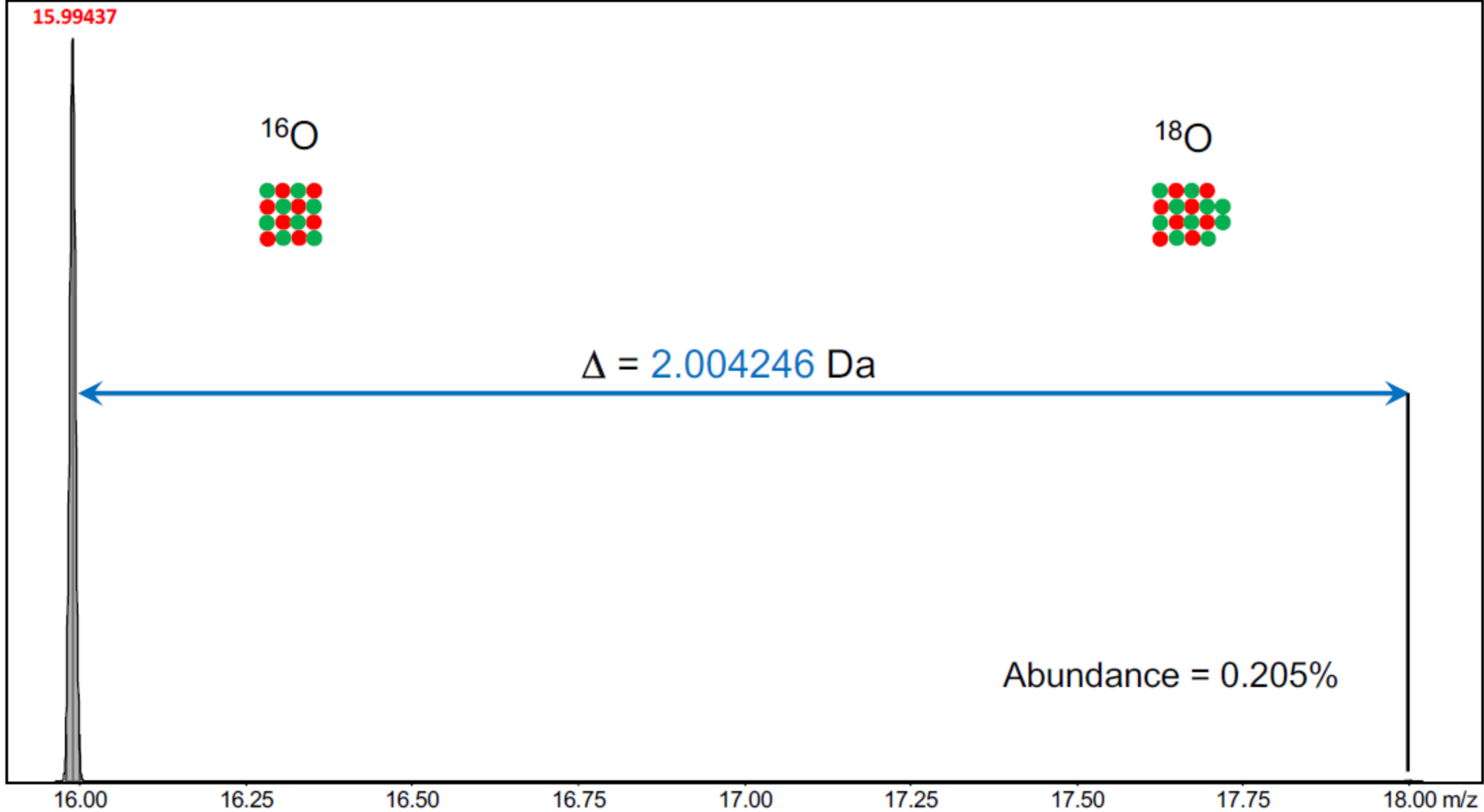


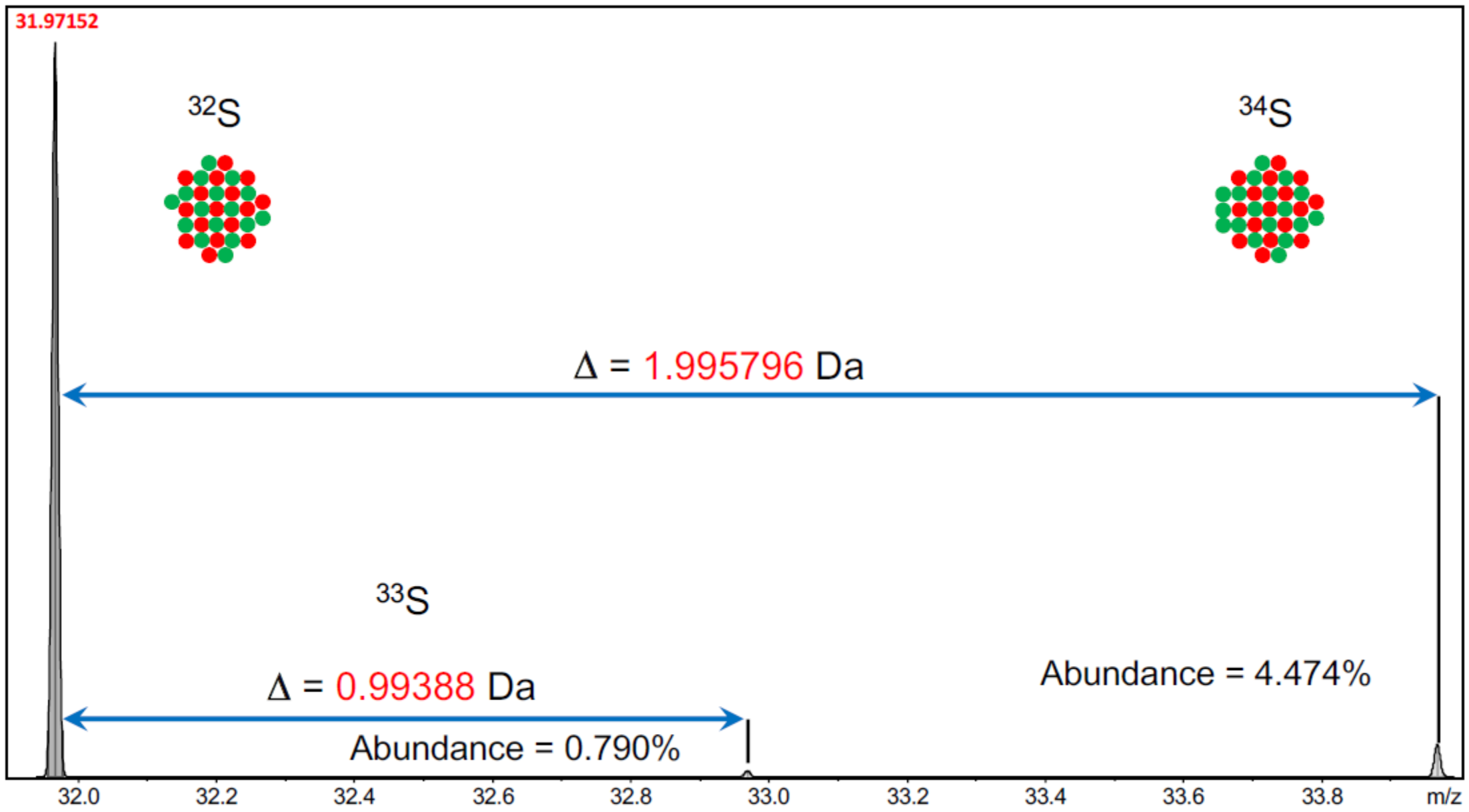
Proton: 
1.00727

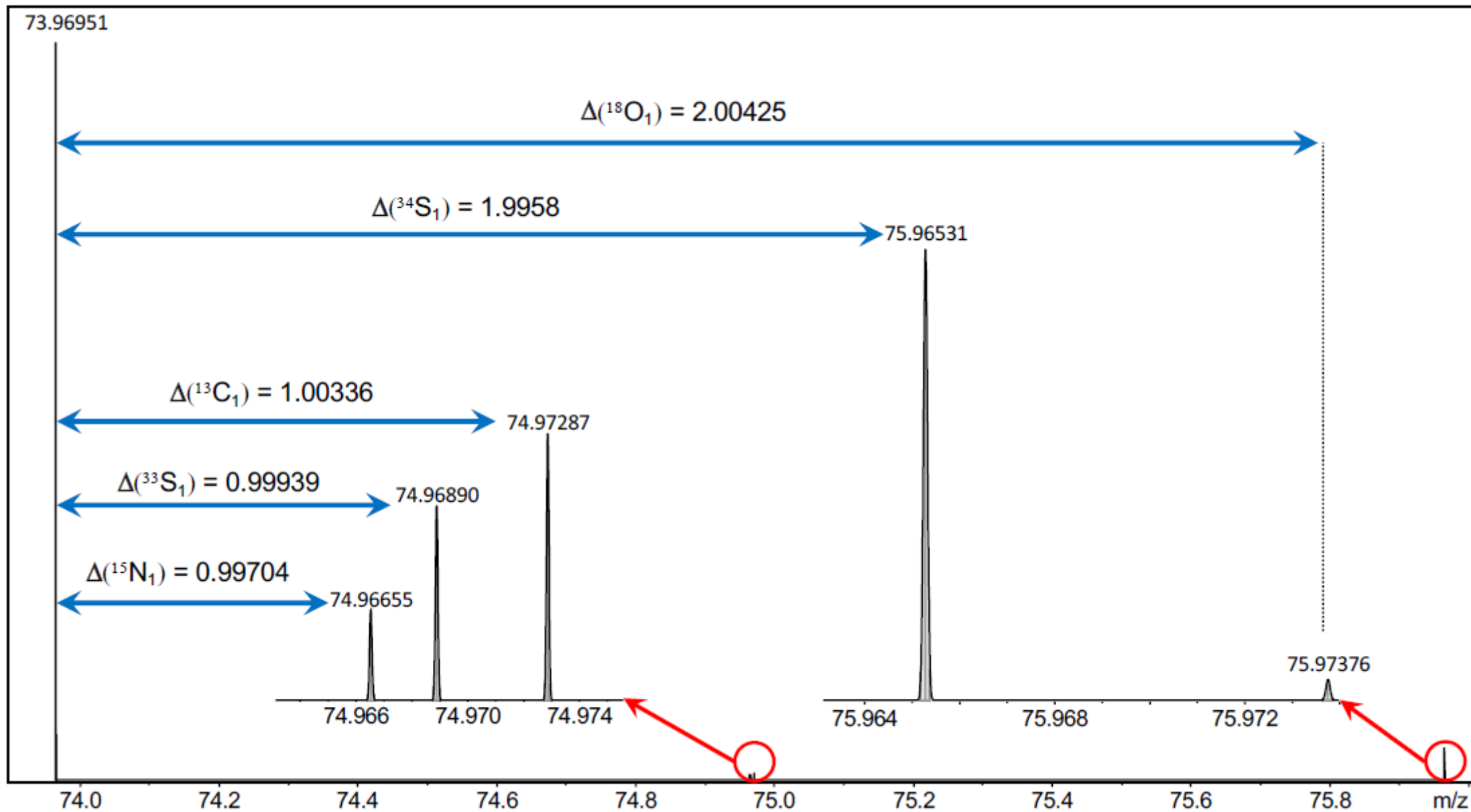
Neutron: 
1.00866





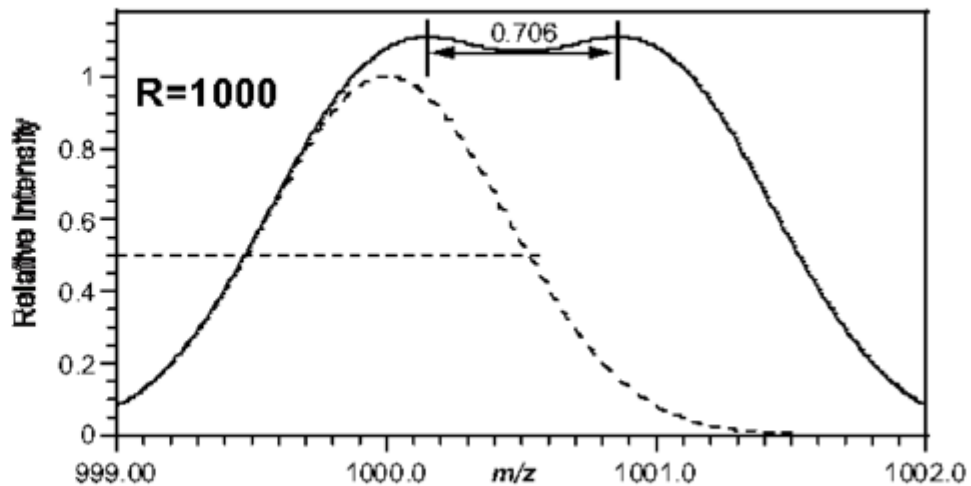
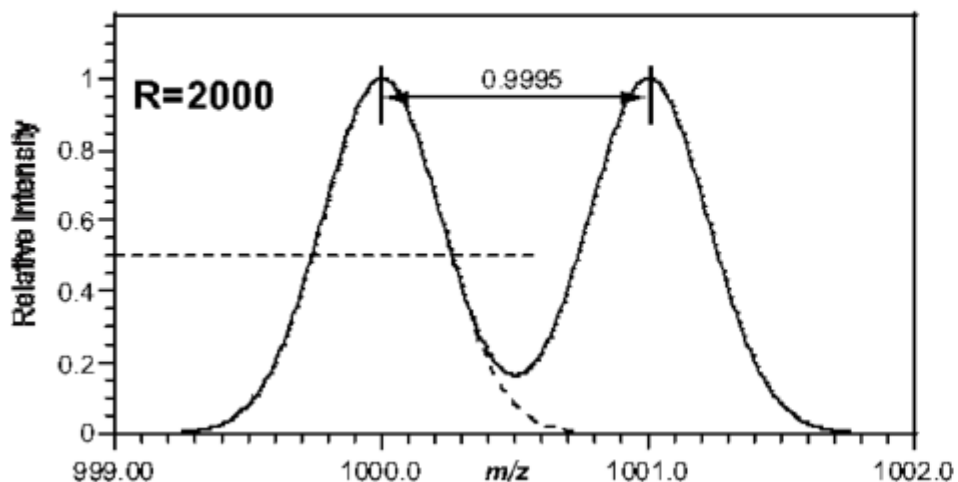
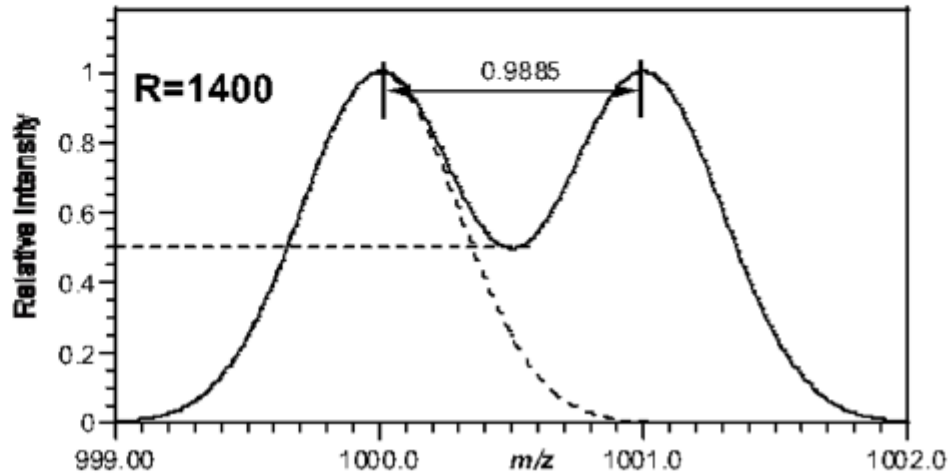
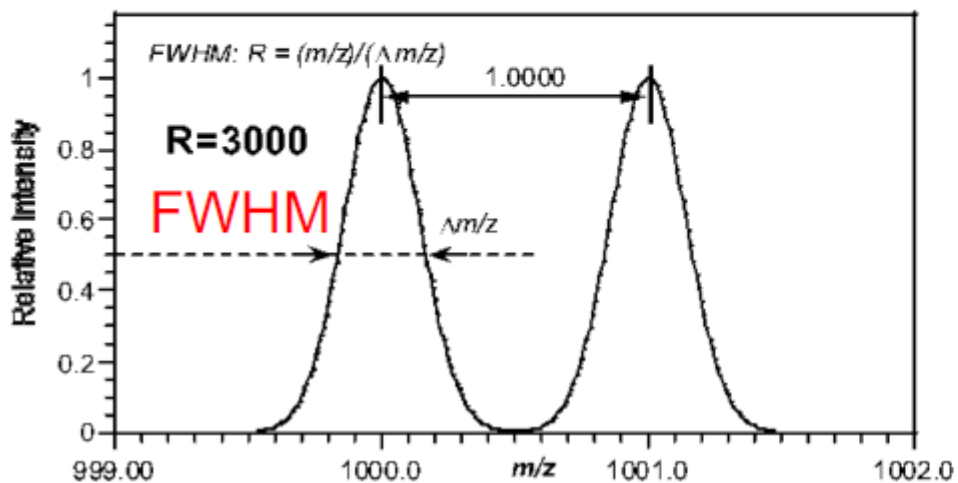




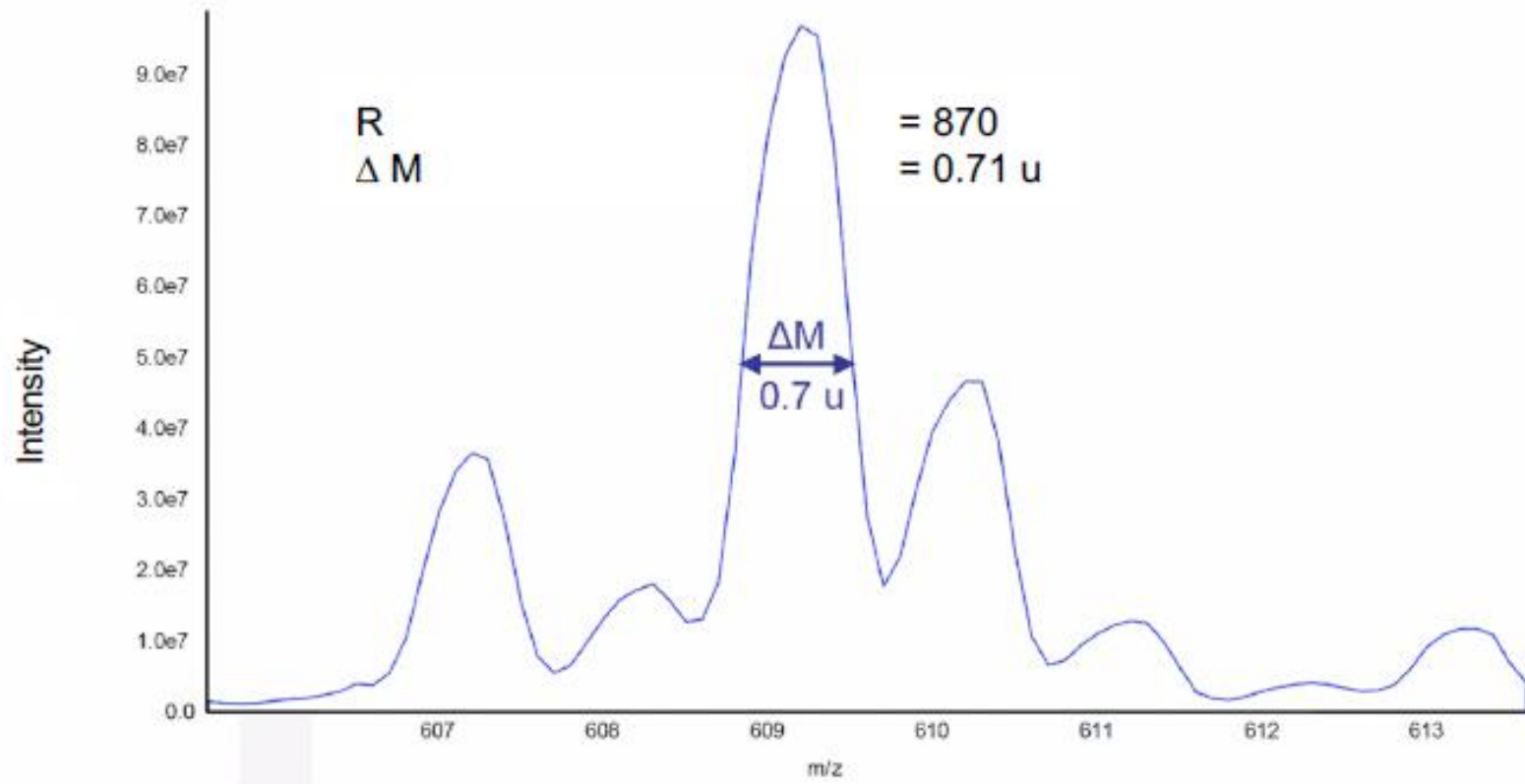


	mass	probability	¹ H	² H	¹² C	¹³ C	¹⁴ N	¹⁵ N	¹⁶ O	¹⁷ O	¹⁸ O	³² S	³³ S	³⁴ S	³⁶ S
1	5731.6075806688	0.1123023514	377	0	252	2	65	0	75	0	0	6	0	0	0
2	5732.6109355040	0.1028778936	377	0	251	3	65	0	75	0	0	6	0	0	0
3	5730.6042258336	0.0814037470	377	0	253	1	65	0	75	0	0	6	0	0	0
4	5733.6142903392	0.0704027660	377	0	250	4	65	0	75	0	0	6	0	0	0
5	5734.6176451744	0.0383896060	377	0	249	5	65	0	75	0	0	6	0	0	0
7	5733.6033765247	0.0301636876	377	0	252	2	65	0	75	0	0	5	0	1	0
6	5729.6008709984	0.0293871014	377	0	254	0	65	0	75	0	0	6	0	0	0
8	5734.6067313599	0.0276323390	377	0	251	3	65	0	75	0	0	5	0	1	0
9	5732.6046155640	0.0266824062	377	0	252	2	64	1	75	0	0	6	0	0	0

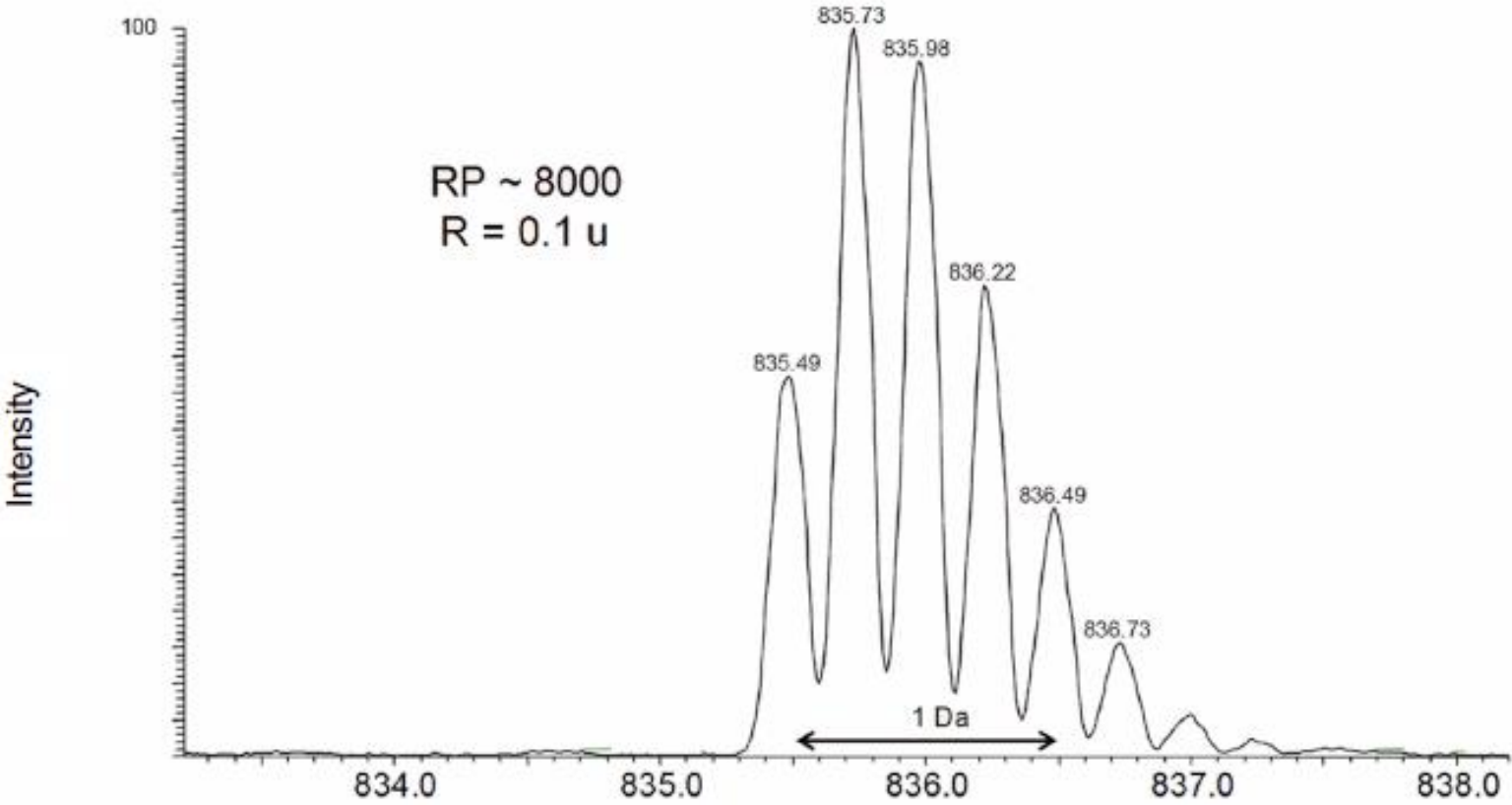
$R = (m/z)_0 / \text{FWHM}(m/z)$
 FWHM: full width at half maximum



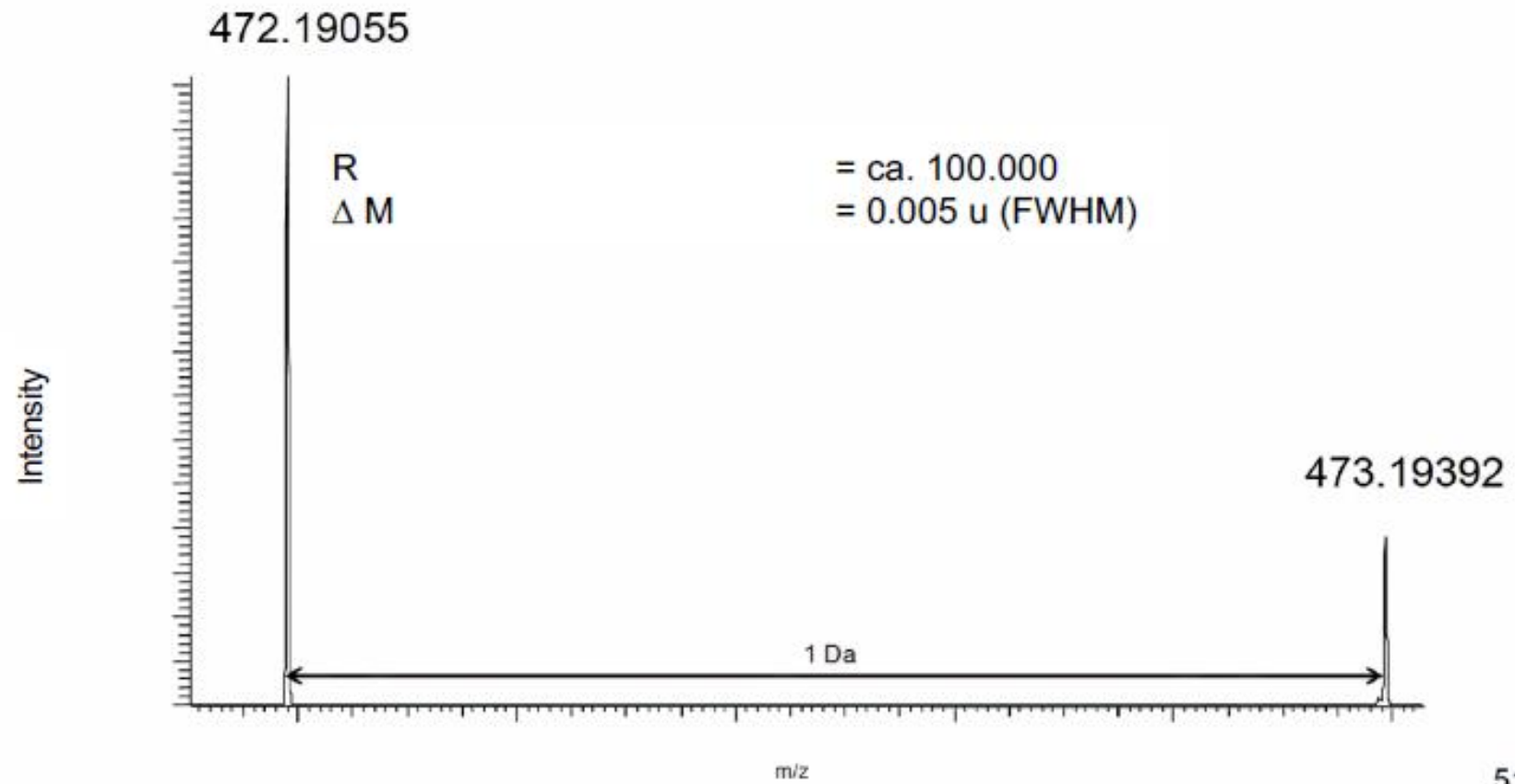
low resolution



moderate resolution



high resolution



Average Mass e.g. C=12.01115

Mass of an ion or molecule weighted for its *isotopic composition*.

Monoisotopic Mass (Exact Mass) e.g. C=12.000000

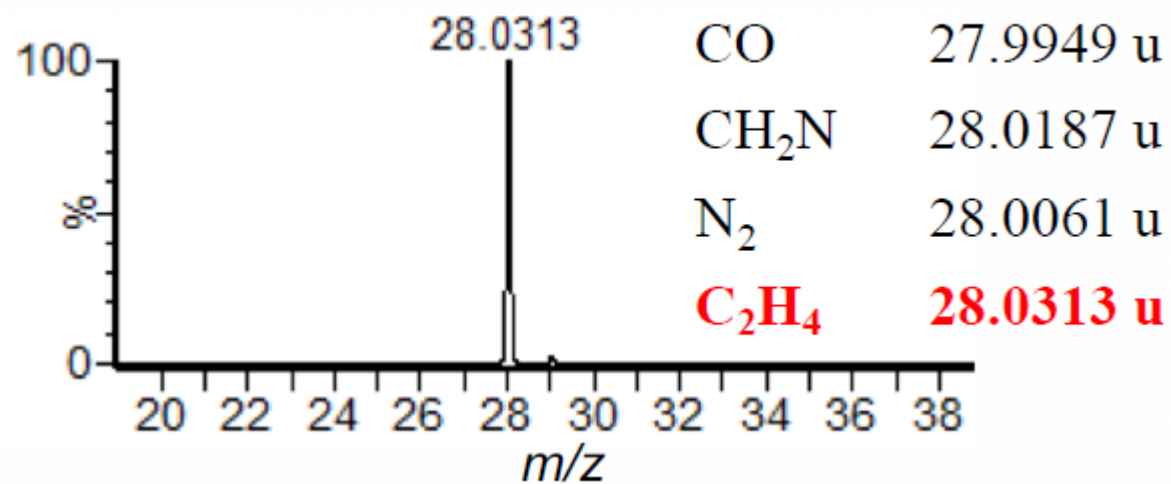
Exact mass of an ion or molecule calculated using the *mass of the most abundant isotope* of each element.

Nominal Mass e.g. C=12

Mass of a molecular ion or molecule calculated using the *isotope mass of the most abundant constituent* (ignoring mass defect) element isotope of each element rounded to the *nearest integer* value and multiplied by the number of atoms of each element (C=12, H=1, N=14, O=16,)

e.g. m/z 28 can have 4 different elemental compositions with different exact mass values.

CO	27.9949 u	CH ₂ N	28.0187 u
N ₂	28.0061 u	C ₂ H ₄	28.0313 u

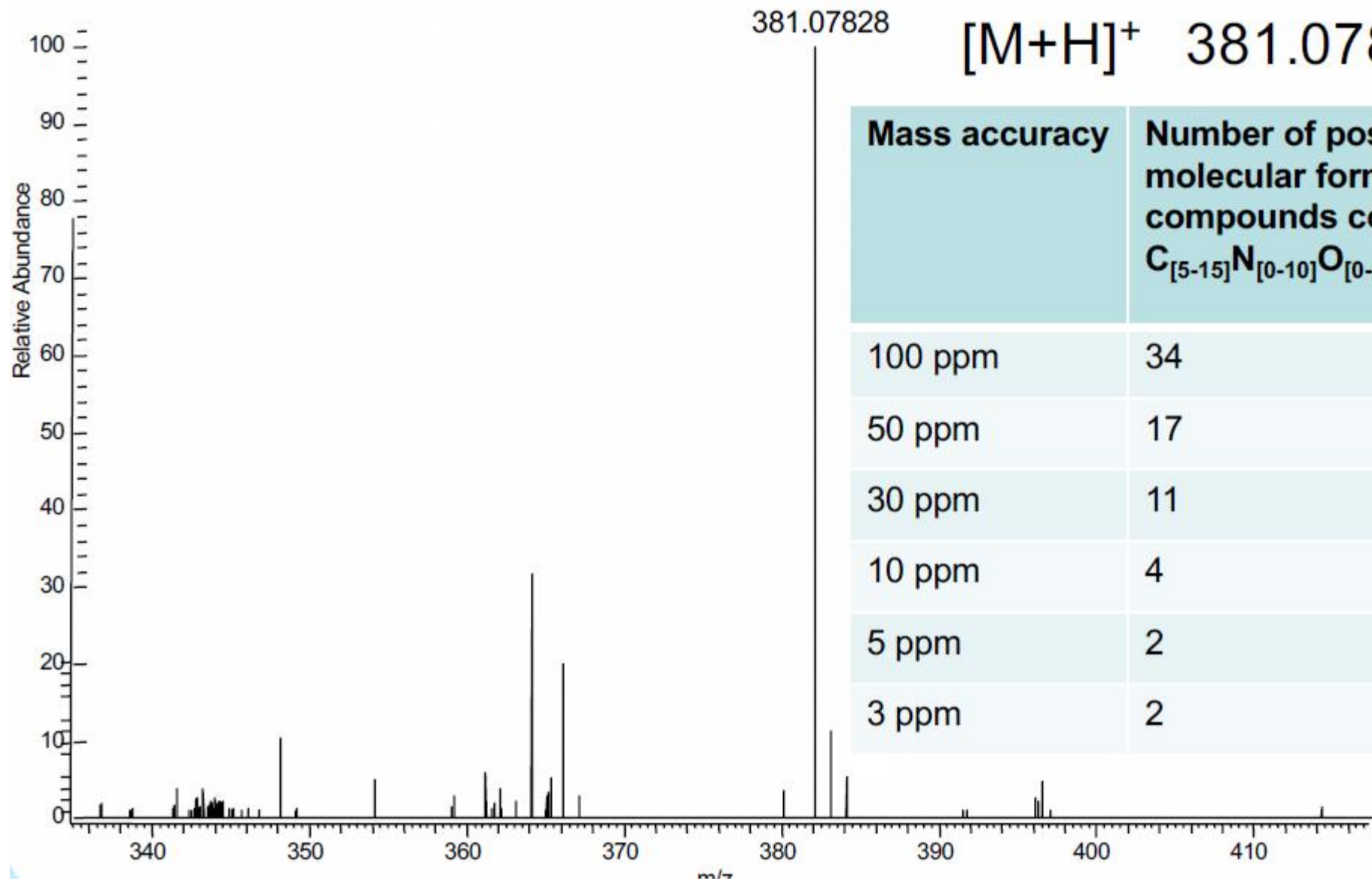


- Mass error in millimass units (mmu)

$$[\text{measured mass (u)} - \text{theoretical mass (u)}] \times 1000$$
- Mass error in parts per million (ppm)

$$\frac{[\text{measured mass (u)} - \text{theoretical mass (u)}] \times 1000000}{\text{theoretical mass (u)}}$$

Measured Mass	Theoretical Mass	Mass Difference (u)	ppm error
200.0020	200.0000	0.002	$(0.002/200)1e^6 = 10 \text{ ppm}$
400.0020	400.0000	0.002	$(0.002/400)1e^6 = 5 \text{ ppm}$
800.0020	800.0000	0.002	$(0.002/800)1e^6 = 2.5 \text{ ppm}$
1000.0020	1000.0000	0.002	$(0.002/1000)1e^6 = 2 \text{ ppm}$
		Constant with mass	Varies with mass

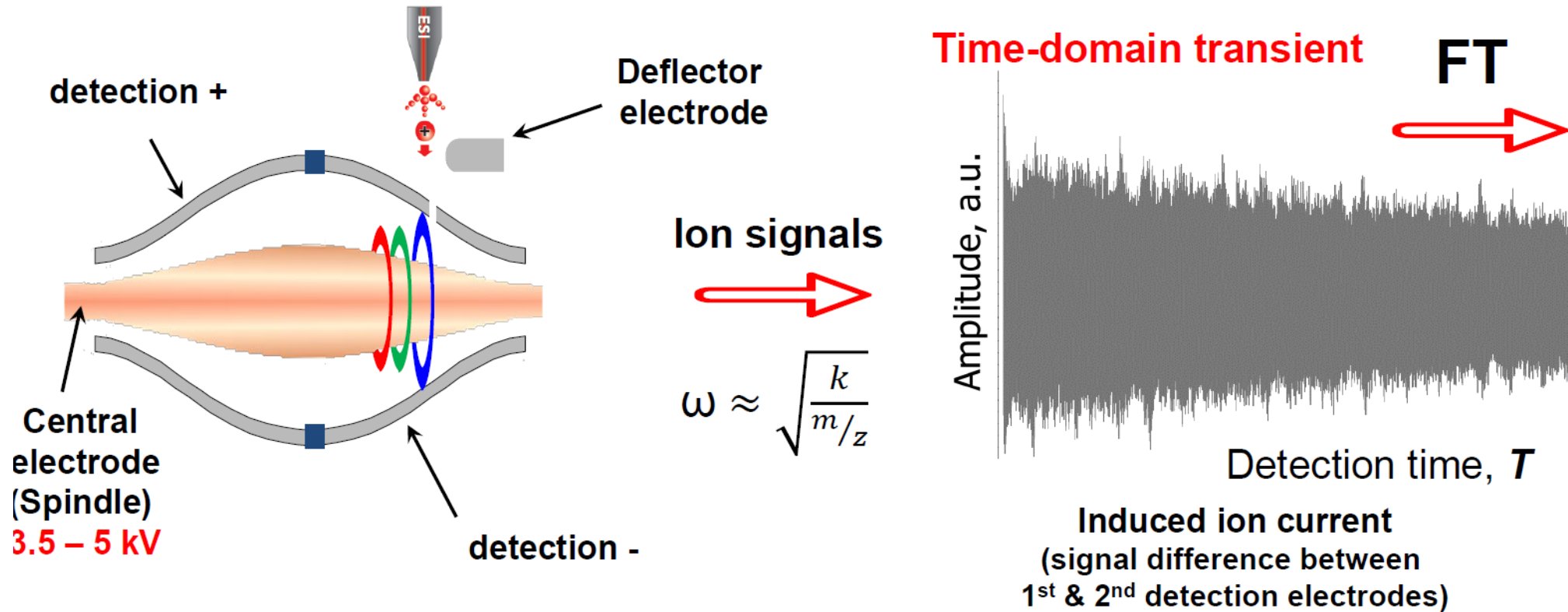


$[M+H]^+$ 381.07828

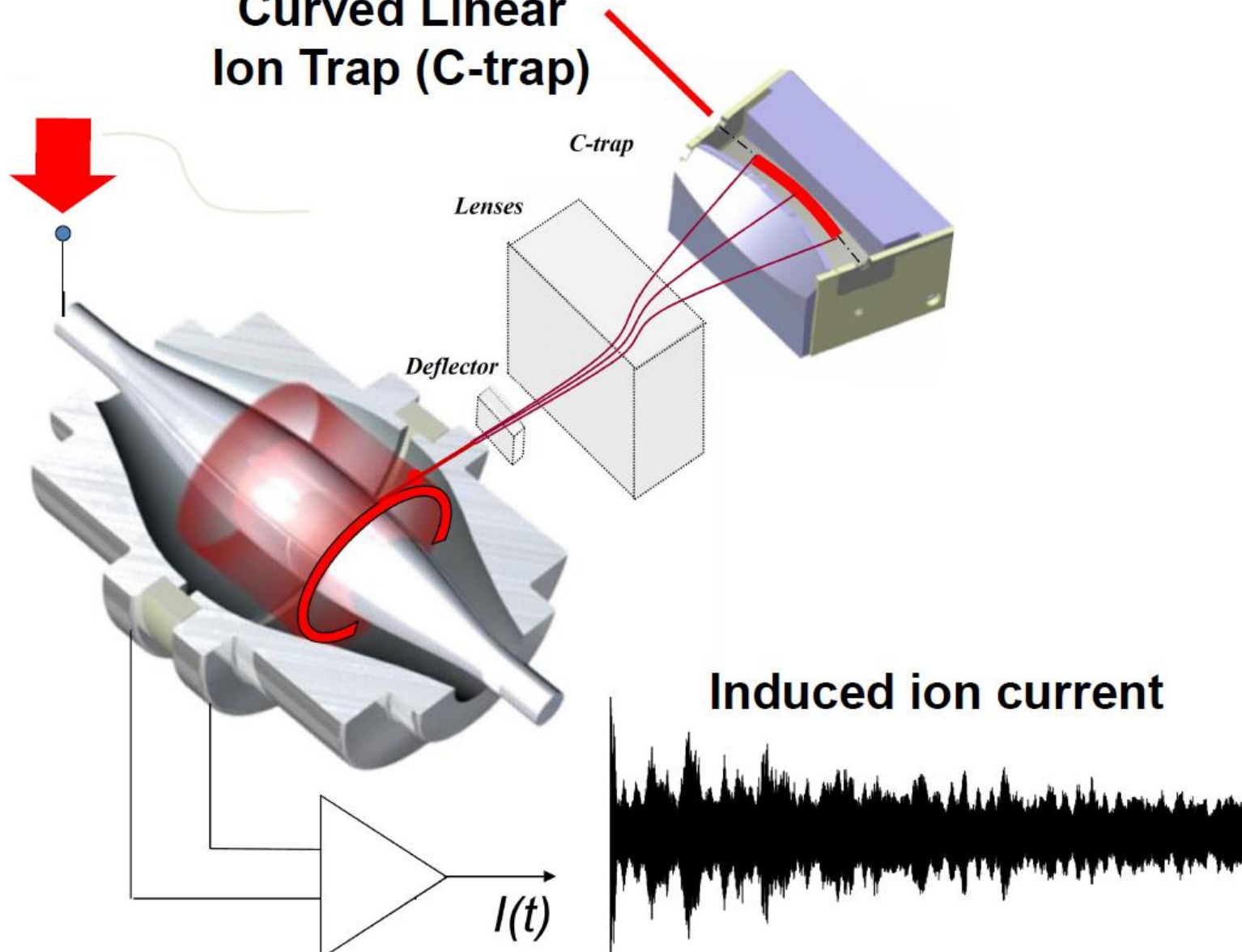
Mass accuracy	Number of possible molecular formulae for compounds containing $C_{[5-15]}N_{[0-10]}O_{[0-15]}H_{[0-40]}$
100 ppm	34
50 ppm	17
30 ppm	11
10 ppm	4
5 ppm	2
3 ppm	2

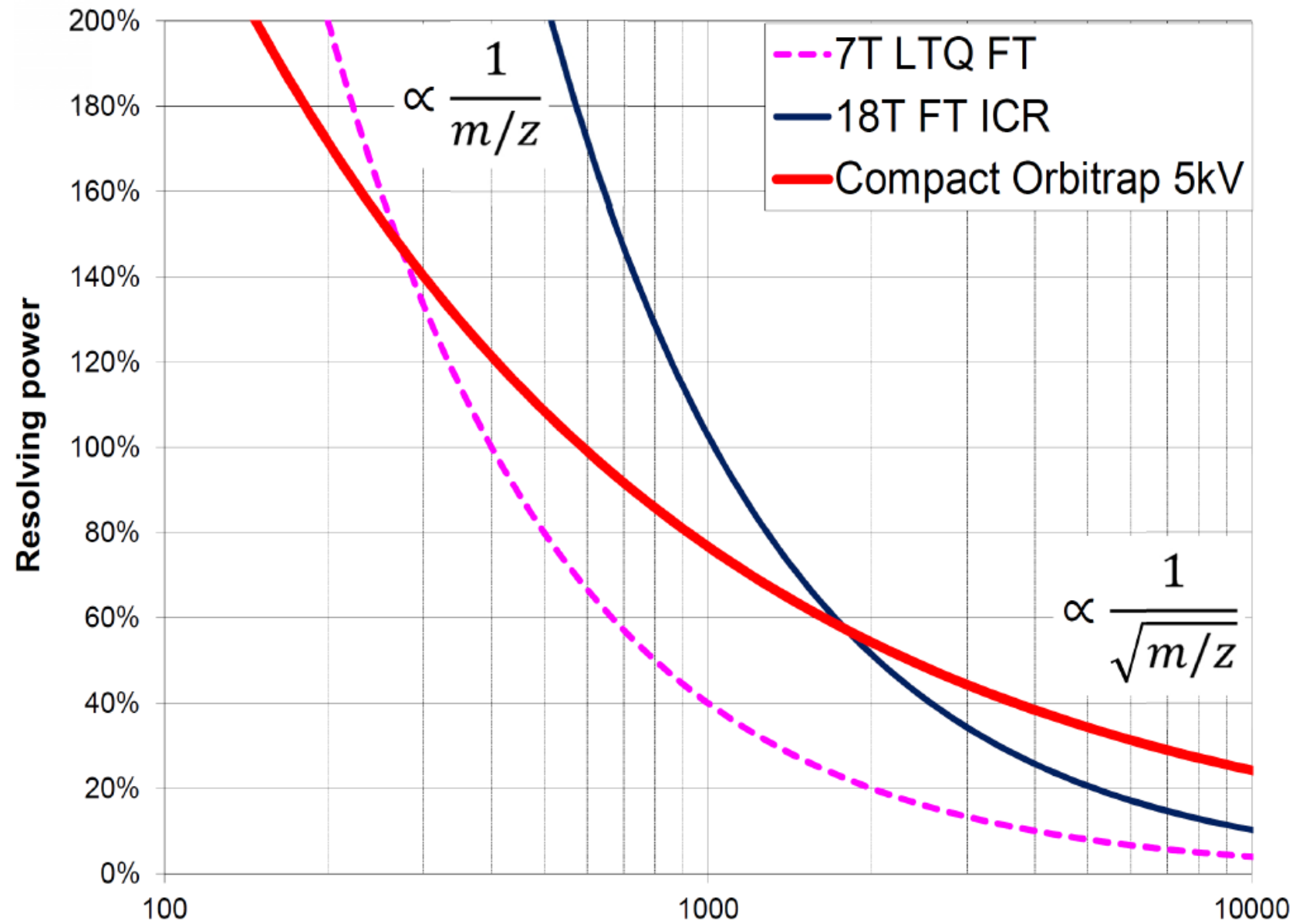
ANALIZZATORI DI MASSA AD ALTA RISOLUZIONE

ORBITRAP

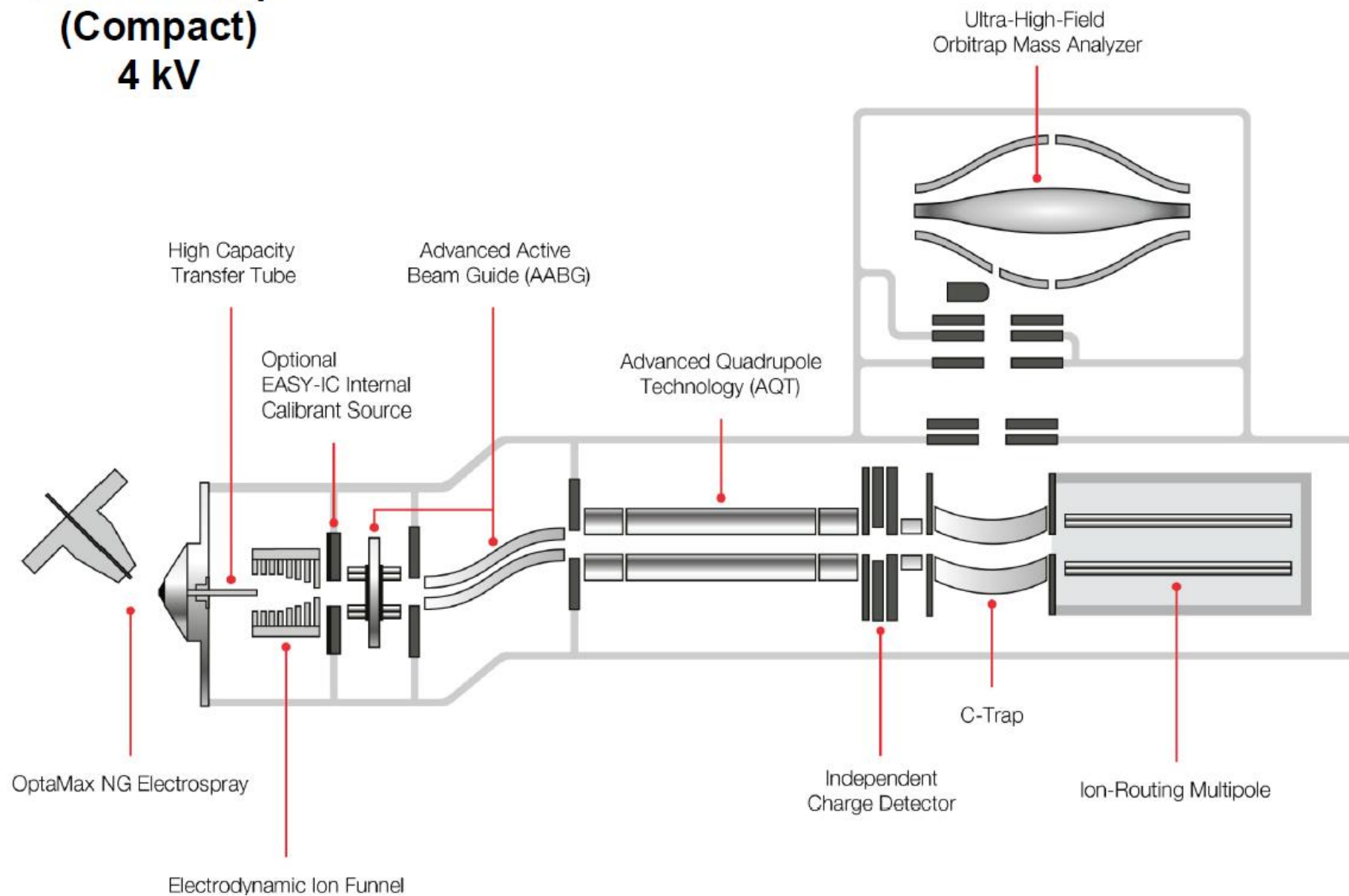


Curved Linear Ion Trap (C-trap)





D20 Orbitrap (Compact) 4 kV



TIME OF FLIGHT

$$E_p = qU$$

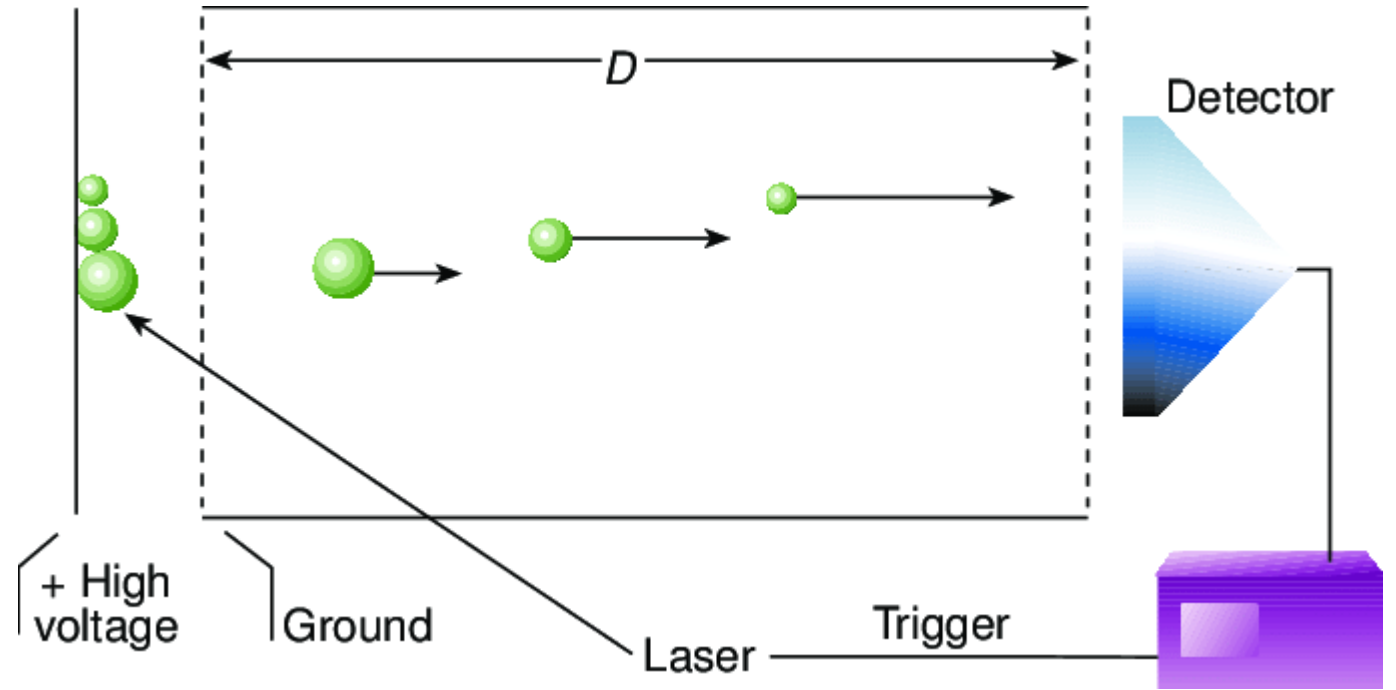
$$E_k = \frac{1}{2}mv^2$$

$$E_p = E_k$$

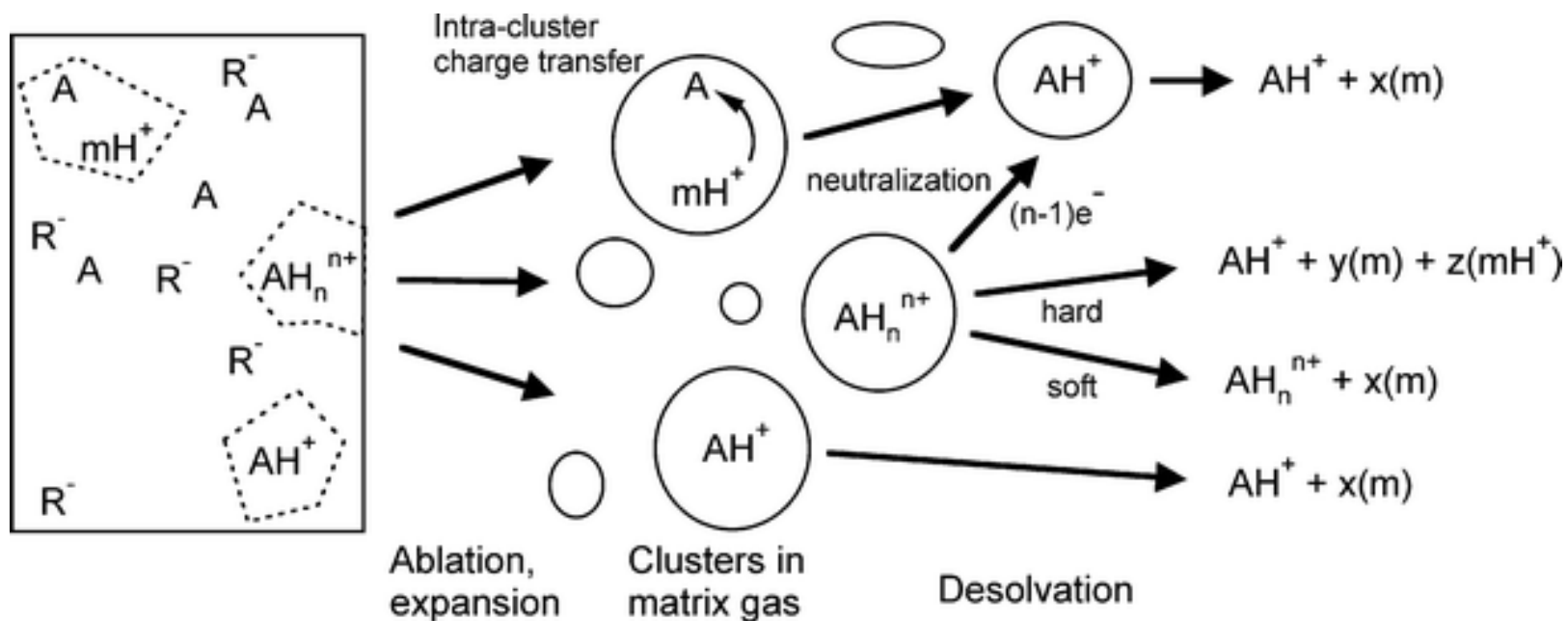
$$qU = \frac{1}{2}mv^2$$

$$v = \frac{d}{t}$$

$$t = k \sqrt{\frac{m}{q}}$$



FENOMENO LUCKY SURVIVOR



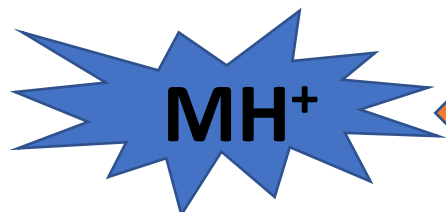
Durante il desorbimento gli ioni tendono a neutralizzarsi



Più cariche avrà la molecola più velocemente questa tenderà a neutralizzarsi



Le molecole monocariche tenderanno a neutralizzarsi più lentamente

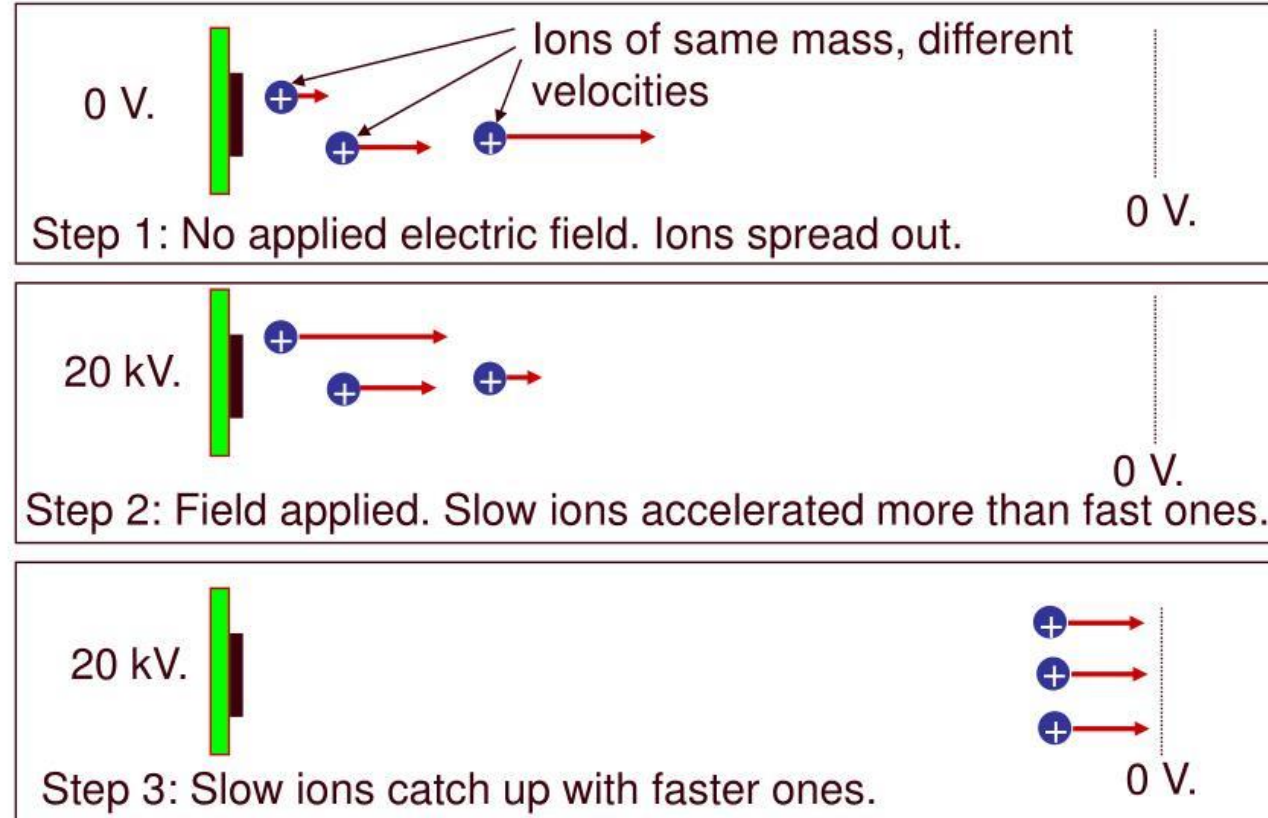


ABBONDANZA DI IONI MONOCARICATI



DELAYED EXTRACTION

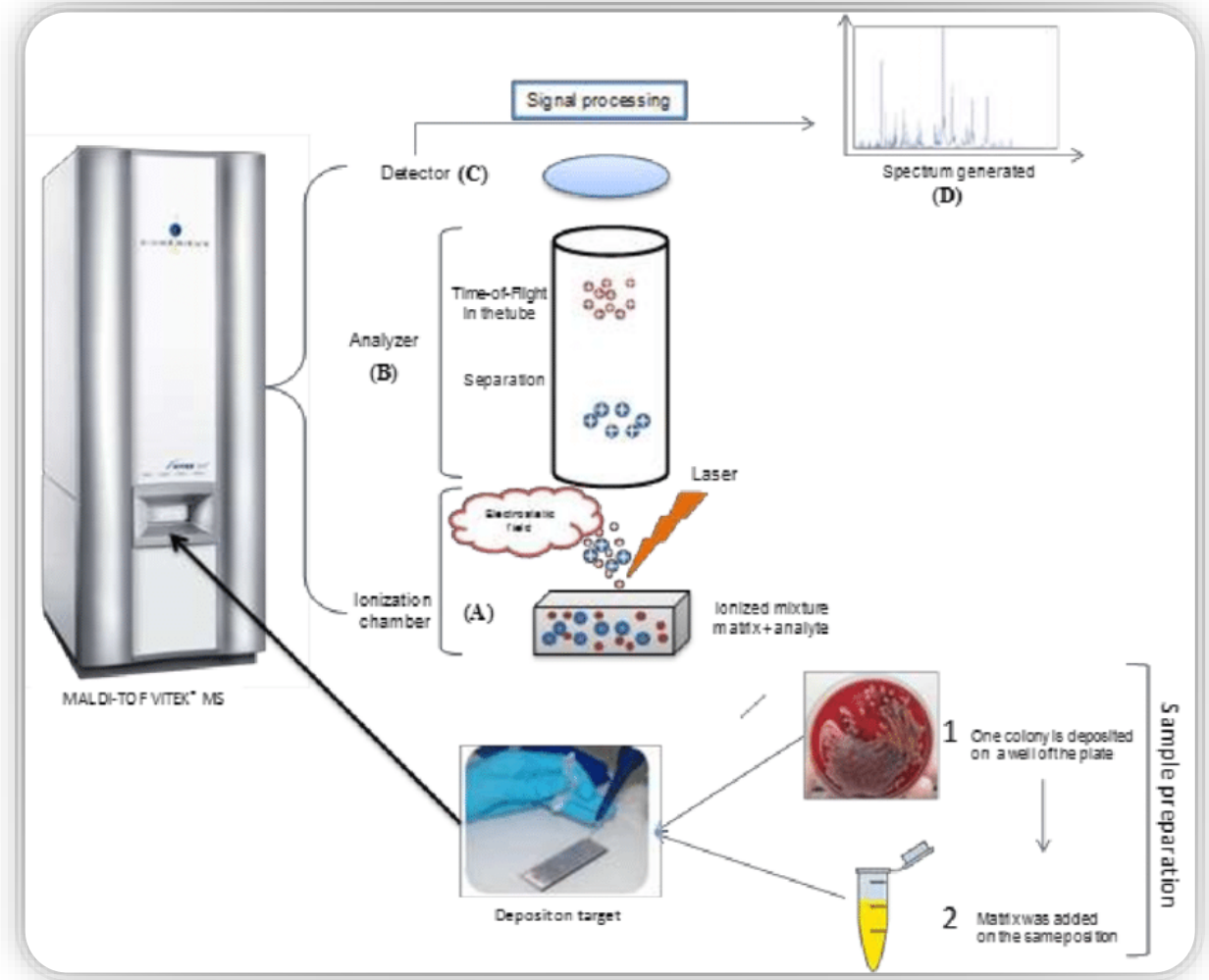
Delayed Extraction (DE) improves performance



MALDI-TOF

PRINCIPI ED APPLICAZIONI

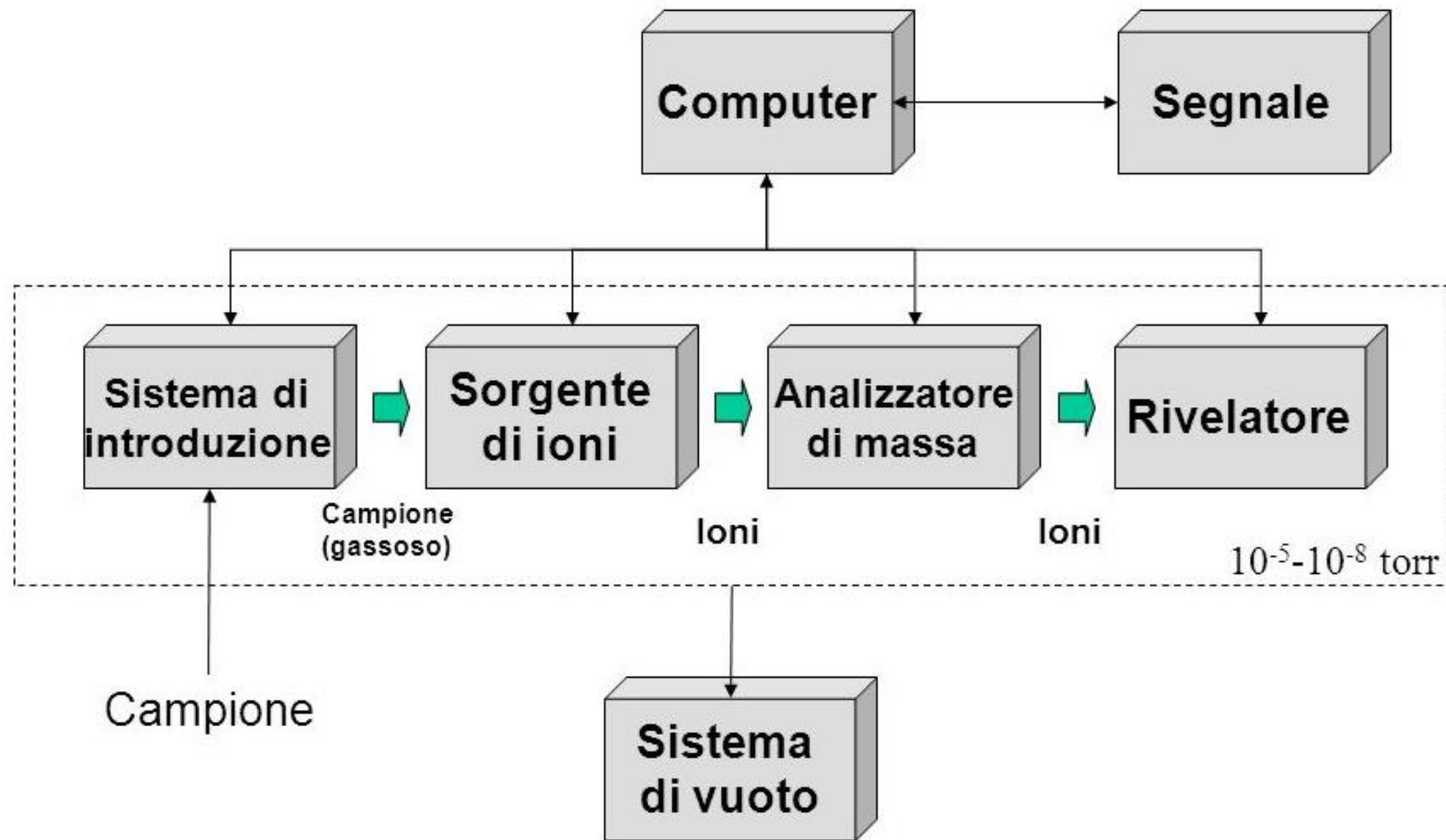
In spettrometria di massa il desorbimento/ionizzazione laser assistito da matrice, comunemente indicato con l'acronimo MALDI (dall'inglese Matrix-Assisted Laser Desorption/Ionization), è una tecnica di ionizzazione soft usata in spettrometria di massa a partire dagli anni 1990. La tecnica MALDI è normalmente condotta sotto vuoto (10 mTorr o meno di pressione), ma è possibile anche lavorare a pressione ambiente (AP-MALDI) perdendo però sensibilità e restringendo l'intervallo di rivelabilità.

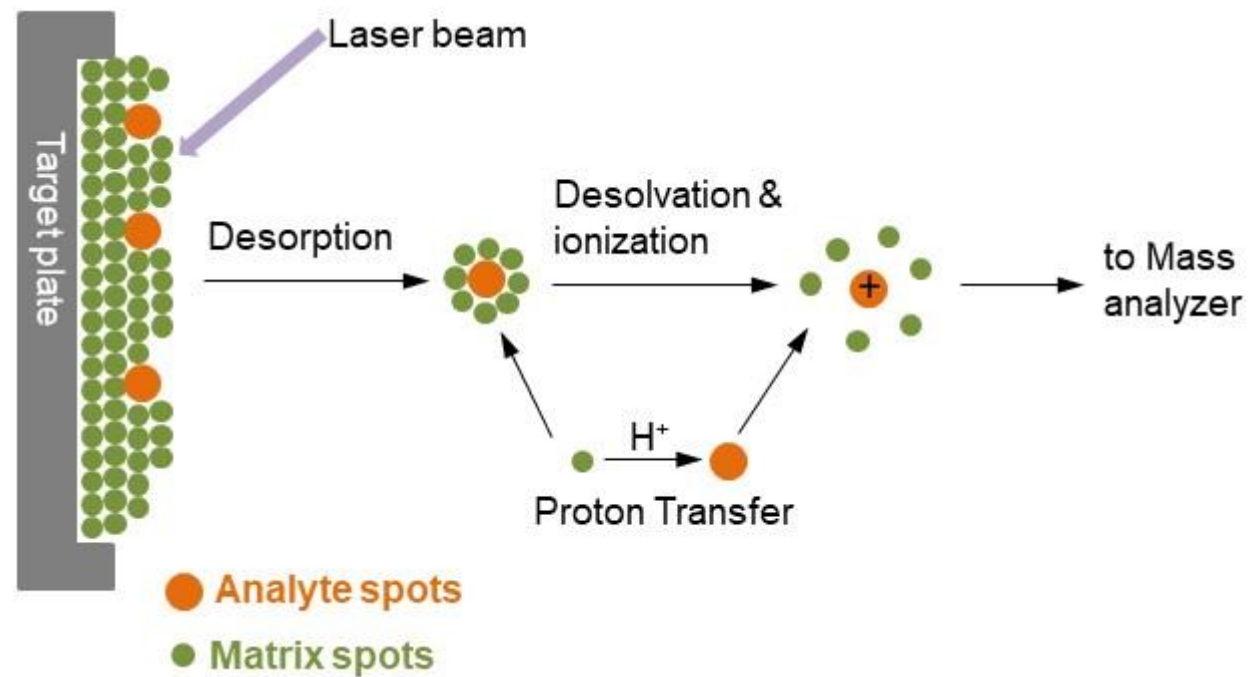
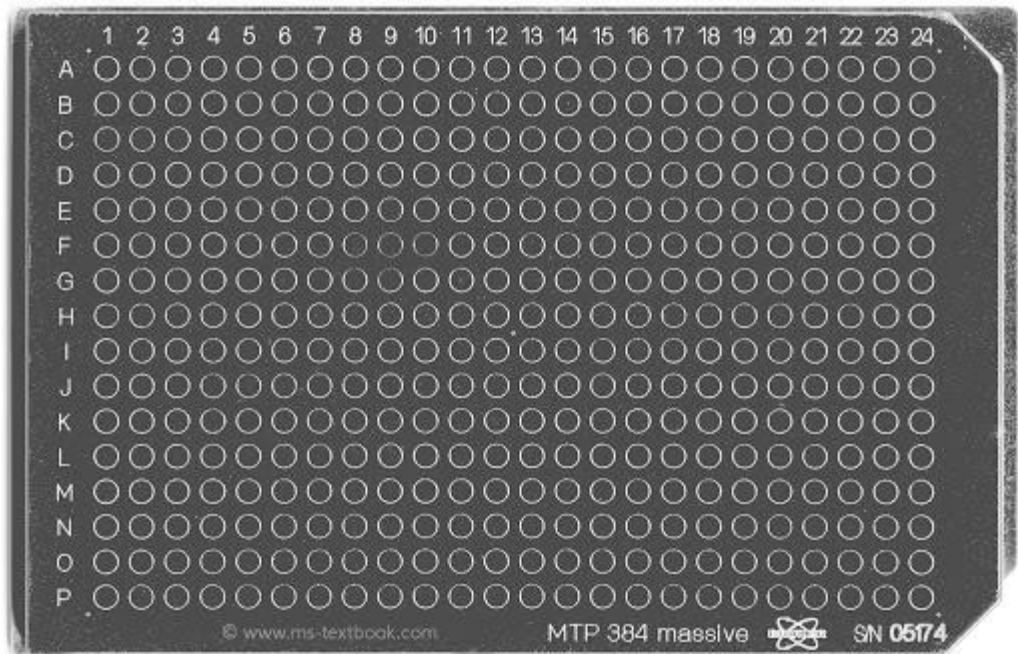


La tecnica consiste nell'assorbire il campione su una matrice, che può essere realizzata in vari materiali, specialmente organici (glicerolo, acido picolinico, acido succinico, acido caffeico, acido sinapico ecc.), e una volta portata in soluzione viene successivamente bombardata con un fascio laser (spesso un laser ad azoto). La matrice deve possedere determinate caratteristiche chimico-fisiche, tra le quali: deve essere facilmente evaporabile ma tale evaporazione non deve essere significativa durante la preparazione del campione o prima dell'effettuazione delle misurazioni, deve avere un certo carattere acido in modo da fungere da fonte di protoni incoraggiando la ionizzazione dell'analita, possedere un forte assorbimento ottico nella regione UV tale che le permetta di assorbire la radiazione laser in modo efficiente, deve infine possedere gruppi polari ed essere idrosolubile.

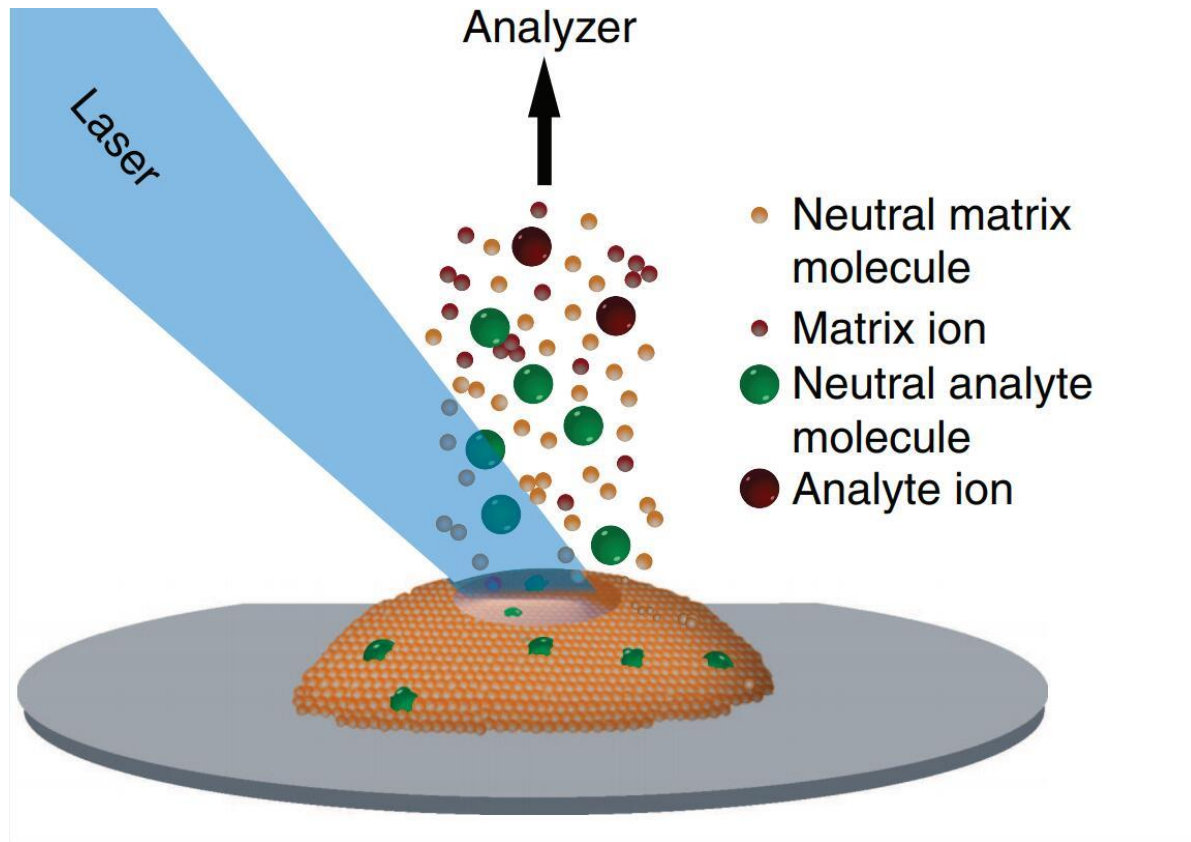


Grazie al fenomeno del desorbimento, il campione viene rilasciato in forma "clusterizzata", ovvero complessato con la matrice. La matrice smorza gli effetti del fascio laser assicurando un'adeguata protezione all'analita che viene ionizzato e vaporizzato tramite l'energia in eccesso ceduta secondariamente dalla matrice stessa. Vengono così ottenuti ioni molecolari generalmente a singola carica, come quelli creati dall'acquisizione o dalla perdita di un protone. Molto spesso la tecnica MALDI viene abbinata a spettrometri dotati di analizzatore a tempo di volo.





DESORBIMENTO LASER



Il trasferimento di energia è dovuto all'assorbimento della luce del laser su un campione solido, andando a colpire su una porzione molto piccola del campione: 0.05-0.2 mm

L'energia viene assorbita dalla matrice e trasferita agli analiti

TIPOLOGIE DI LASER

Intervallo spettrale	Lunghezza d'onda	Energia dei fotoni	Tipo laser
UV	193 nm	6.4 eV	Laser a eccimeri ArF
UV	248 nm	5.0 eV	Laser a eccimeri KrF
UV	266 nm	4.7 eV	Laser a Nd:YAG QIF
UV	308 nm	3.8 eV	Laser a eccimeri di XeCl
UV	337 nm	3.7 eV	Laser ad azoto
UV	355 nm	3.5 eV	Laser a Nd:YAG TIF
IR	1.06 μm	1.2 eV	Laser a Nd:YAG
IR	2.94 μm	0.4 eV	Laser a Er:YAG
IR	1.7-2.5 μm	0.7-0.5 eV	Laser OPO
IR	10.6 μm	0.1 eV	Laser a CO2

MATRICI MALDI

Devono formare solidi cristallini con un bassa pressione di vapore in modo da non sublimare all'interno della sorgente MALDI

MATRICI IR

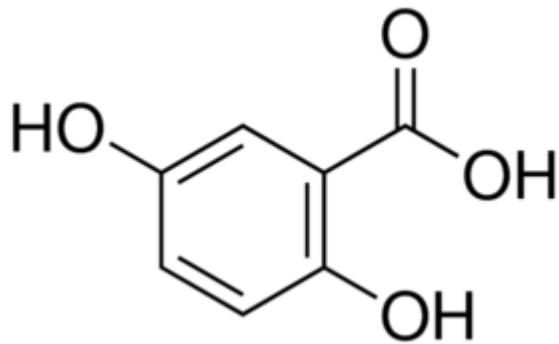
- Devono contenere gruppi OH o NH per assorbire a 3 μm tramite vibrazioni di stretching
- Oppure contenere un gruppo CO o NH che assorbono rispettivamente tramite vibrazioni di stretching e bending a 10 μm

MATRICI UV

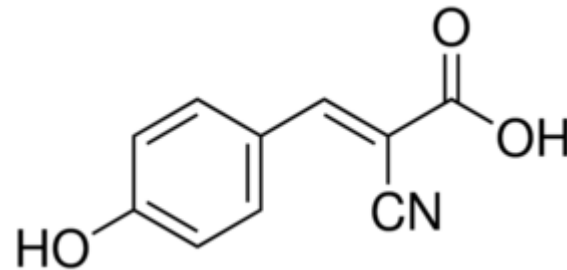
- Devono contenere gruppi cromofori che assorbono alla lunghezza d'onda del laser

TIPOLOGIE DI MATRICE

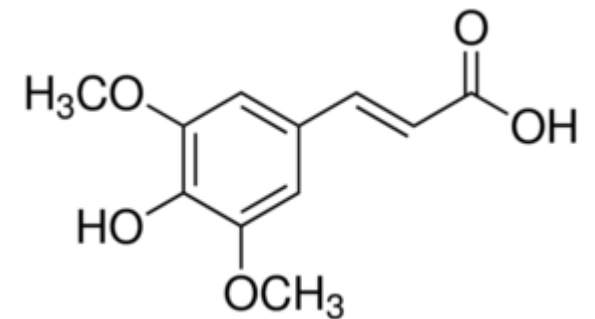
Composto	Nome alternativo	Solvente	Lunghezza d'onda	Applications
Acido 2,5-dihydroxybenzoico	DHB	Acetonitrile, acqua, metanolo, acetone, cloroformio	337, 355, 266	Peptidi, nucleotidi, oligonucleotidi, oligosaccaridi
Acido Sinapinico	SA	Acetonitrile, acqua, acetone, cloroformio	337, 355, 266	Peptidi, proteine, lipidi
Acido Ferulico	FA	Acetonitrile, acqua, propanolo	337, 355, 266	Proteine
Acido α -ciano-4-idrossicinnamico	CHCA	Acetonitrile, acqua, etanolo, acetone	337, 355	Pepptide, lipidi, nucleotidi
Acido pinolinico	PA	Etanolo	266	Oligonucleotidi
Acido 3-idrossipinolinico	HPA	Etanolo	337, 355	Oligonucleotidi



DHB



CHCA



SA

RESA IONICA E FLUENZA DEL LASER

Fluenza = $F = E/A$ = energia del laser su un area



10-100 mJ/cm²

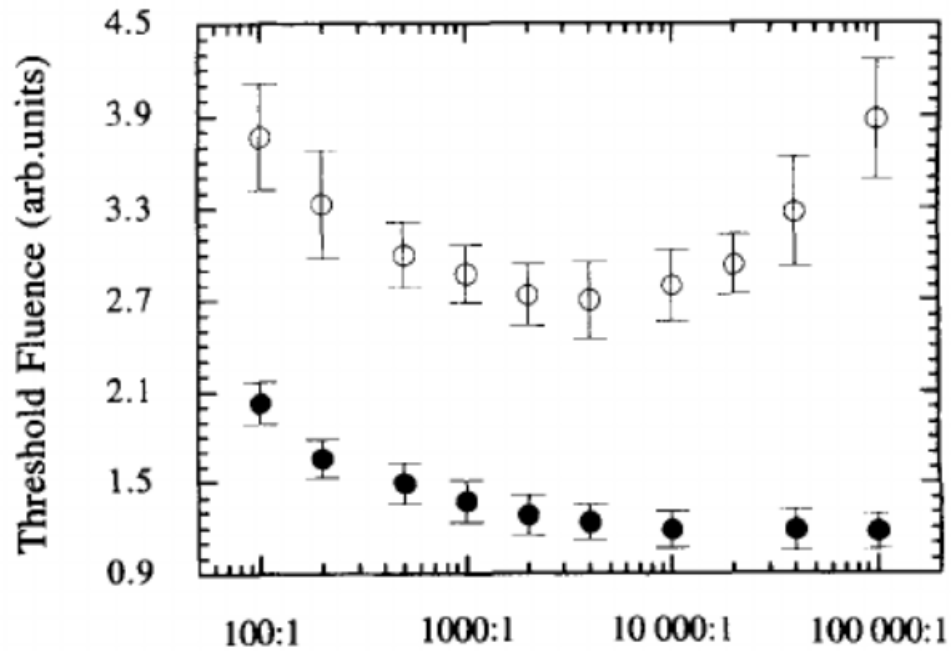
Irradianza = $I = F/A$ = fluenza su area



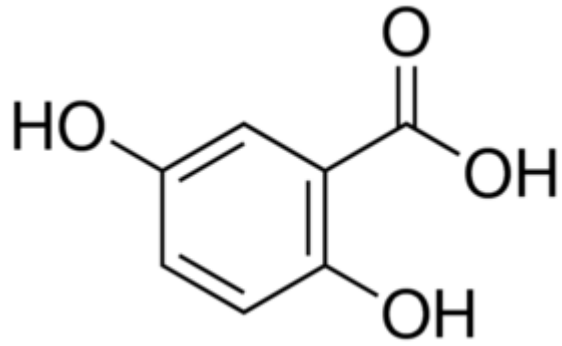
10⁶-10⁷ mJ/cm²



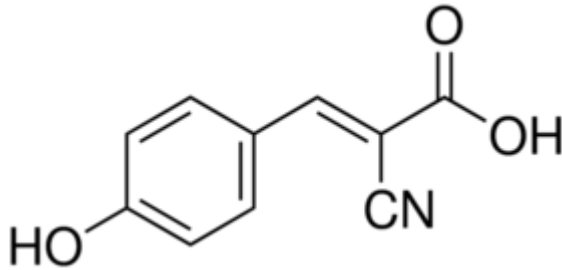
Incomincia la ionizzazione



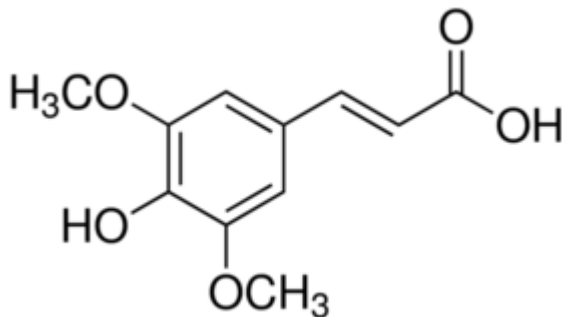
FORMAZIONE DEGLI IONI



DHB



CHCA



SA

Protonazione influenzata dal pH

La ionizzazione in MALDI necessita dell'incorporazione degli analiti nei cristalli prodotti nella matrice in forma pre-protonata, anche se la protonazione di piccole molecole può avvenire anche in fase gassosa dopo il desorbimento.

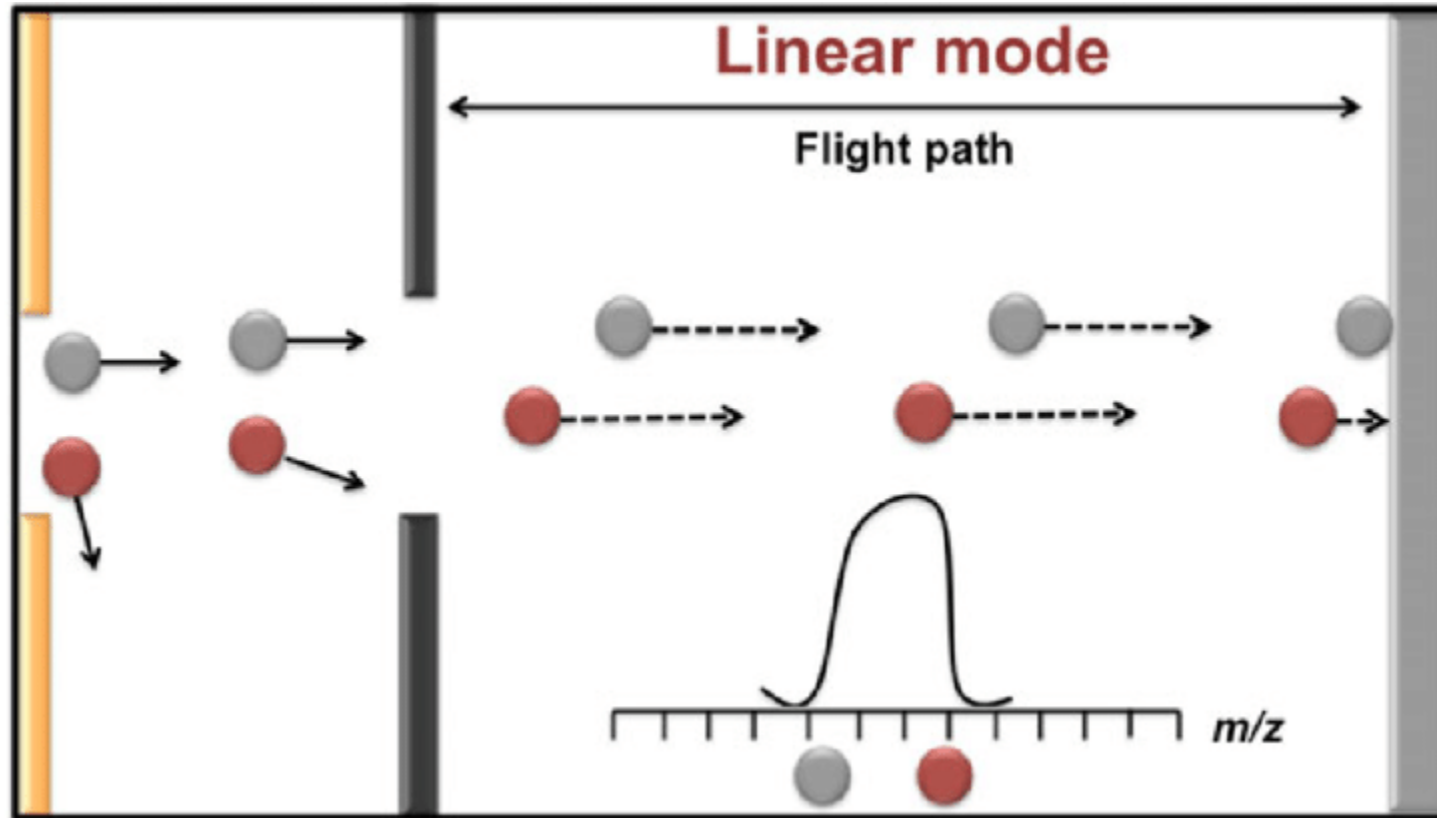
Ionizzazione per trasferimento di carica

Ci sono anche composti che non riescono a ricevere l'H⁺ dai gruppi carbossilici (analiti leggermente basici) e la cationizzazione può avvenire anche per trasferimento protonico dovuto all'eccitazione conseguente al laser

Ionizzazione per assorbimento

Si possono avere quando gli analiti stessi assorbono alla lunghezza d'onda del laser ovvero di creano ioni radicali come M^{o+} oppure M^{o-}

MODALITA' DI ACQUISIZIONE: LINEAR



METHODS MANUSCRIPT

MALDI-TOF MS protein fingerprinting of mixed samples

Michael A. Reeve* and Denise Bachmann

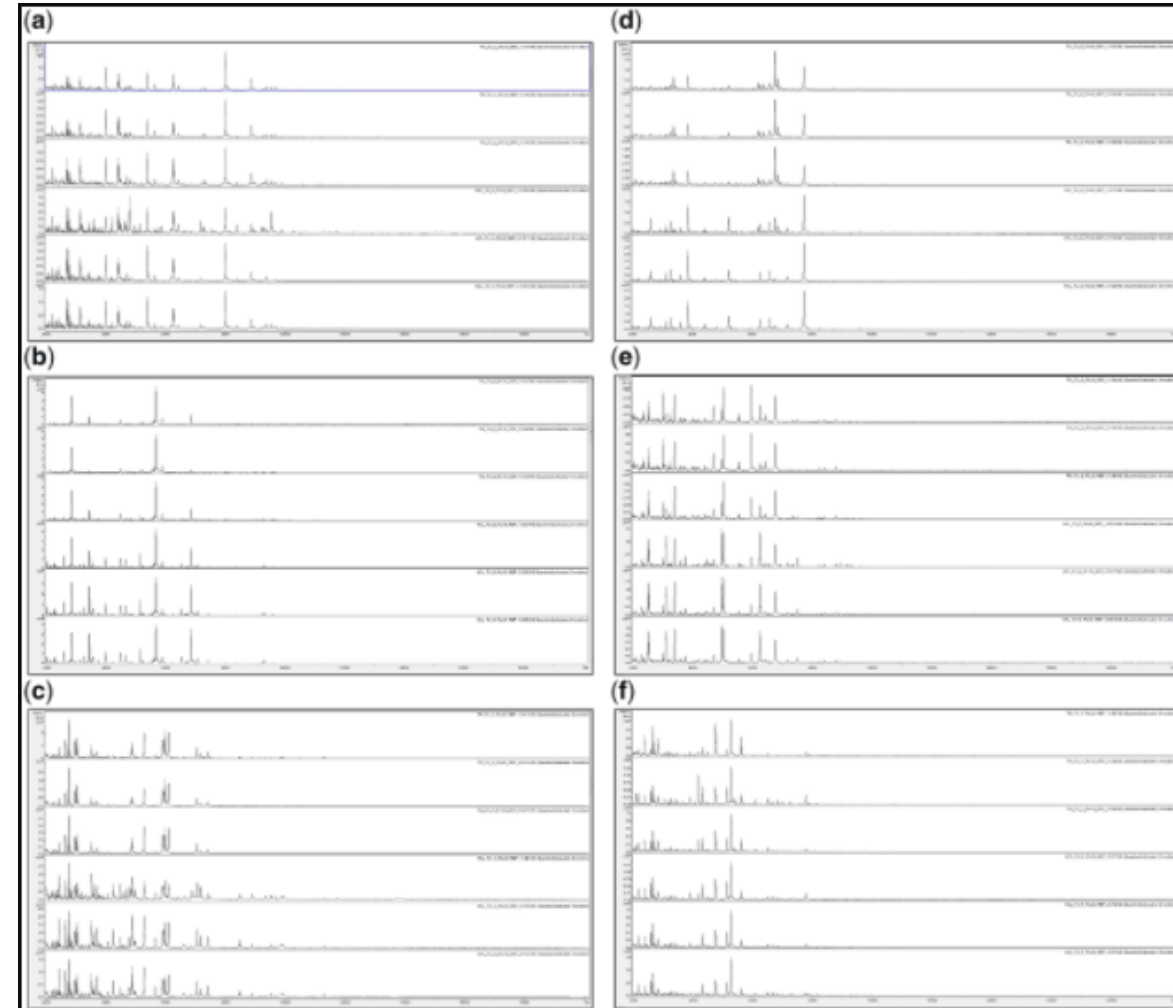
Department of Bioscience, CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK

*Correspondence address. Department of Bioscience, CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK. Tel: +44 (0)1491-829033; Fax: +44 (0)1491-829100; E-mail: M.Reeve@cabi.org

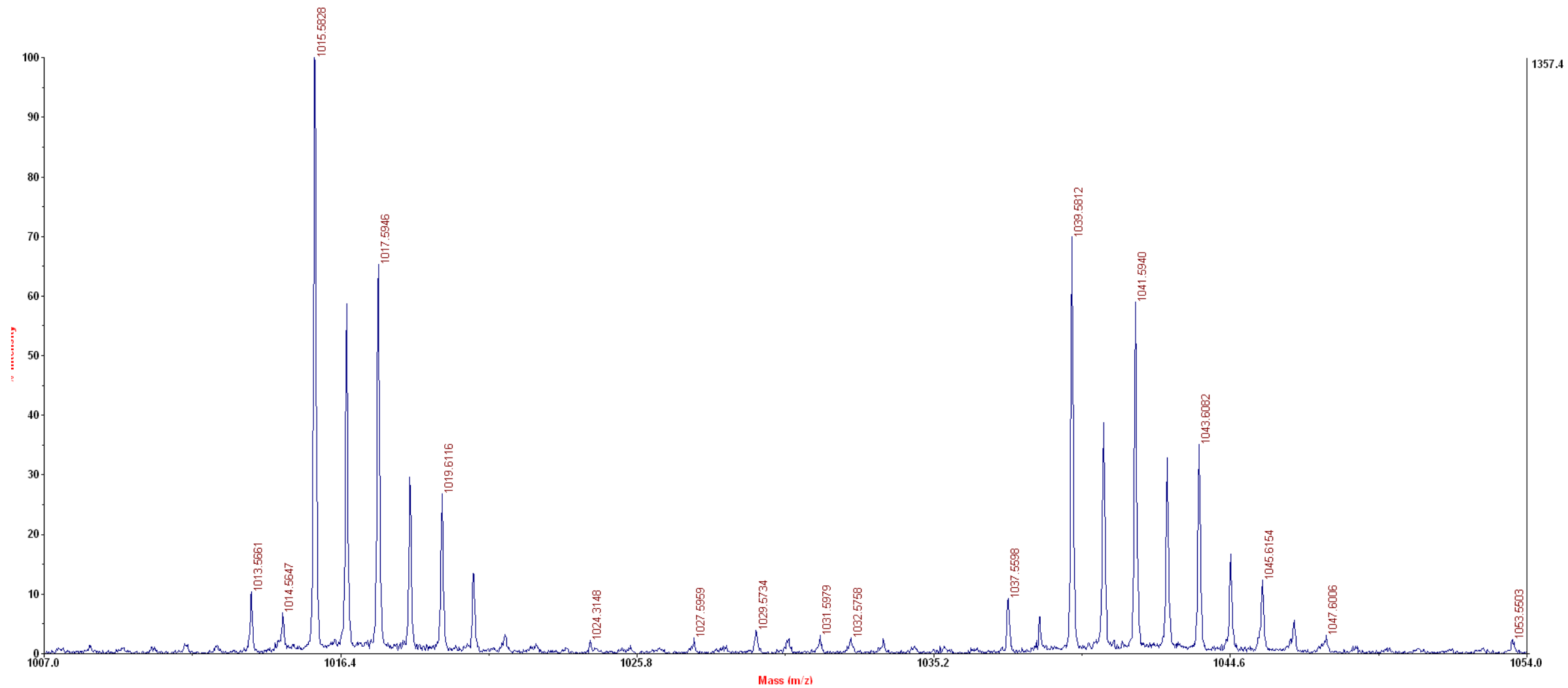
Downloaded from <https://academic.oup.com/biomethods>

Matrix: HCCA + TFA 1%

Mode: Linear Positive



MODALITA' DI ACQUISIZIONE: REFLECTRON

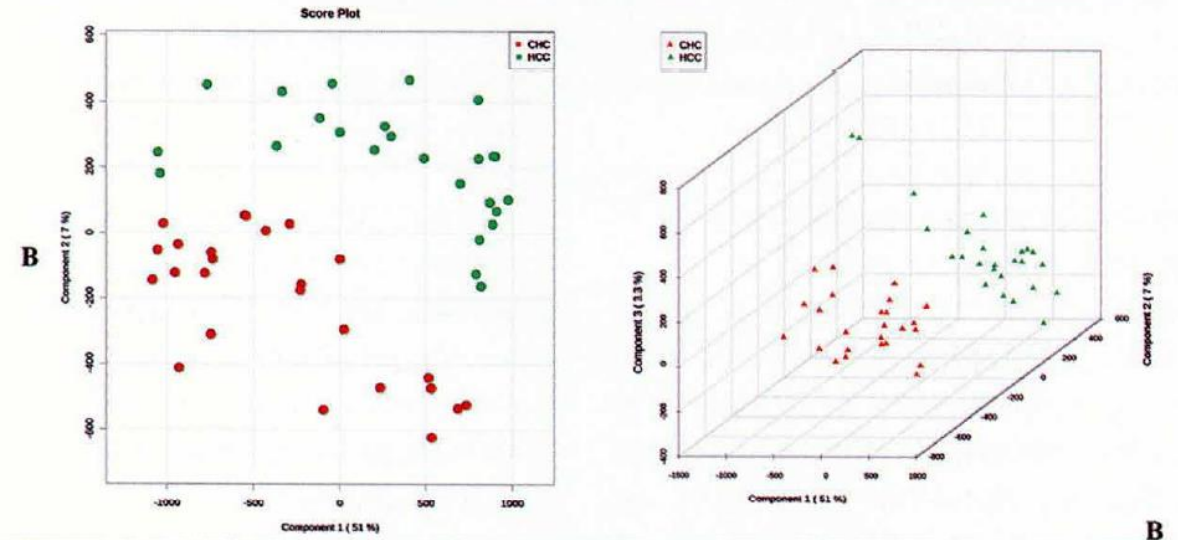
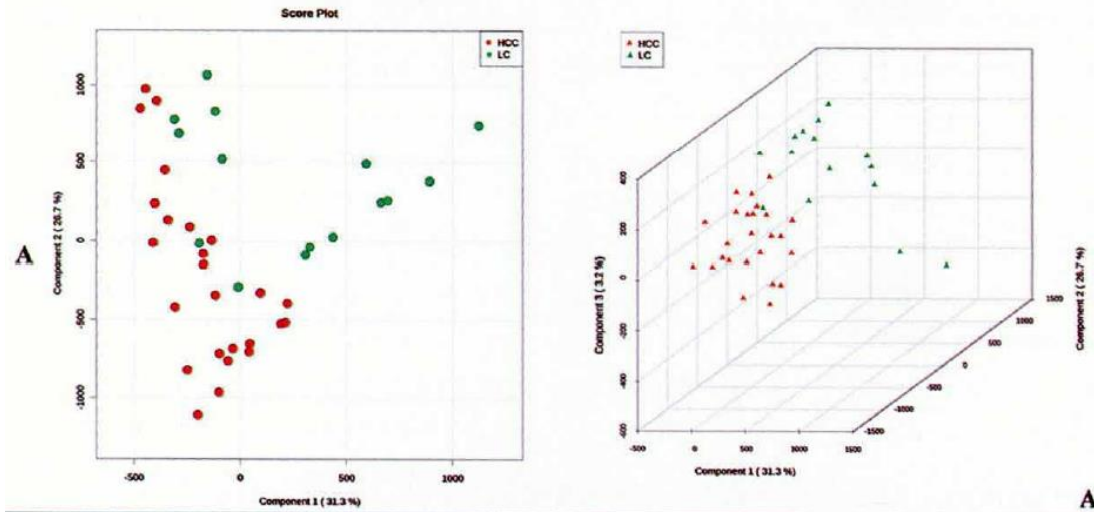
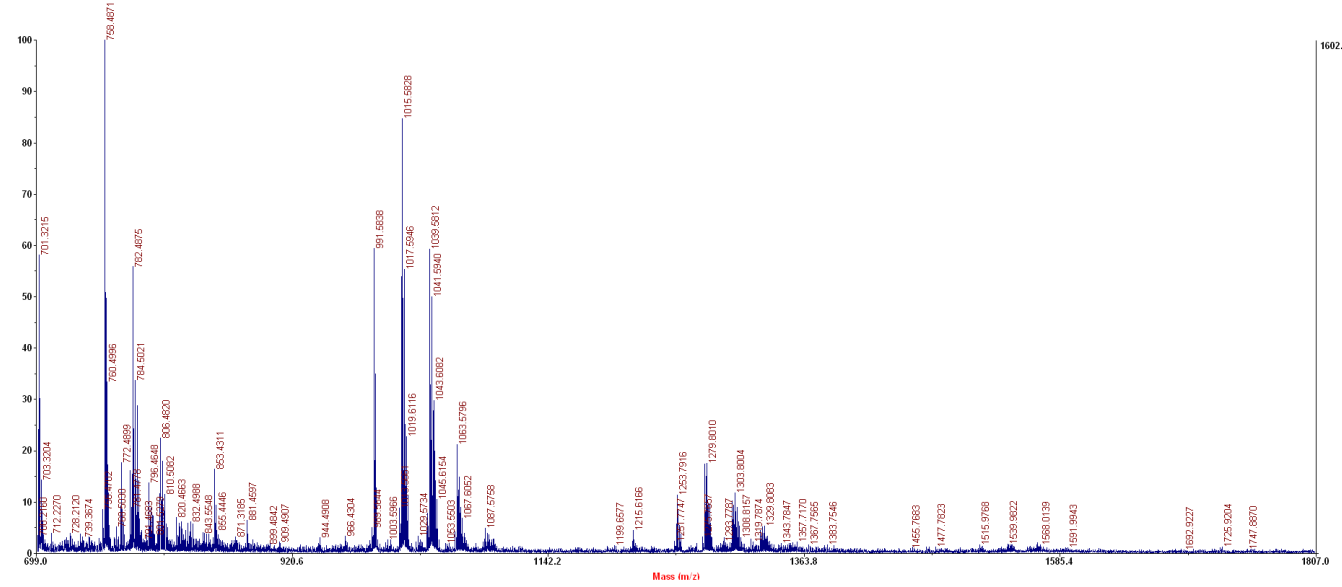


ORIGINAL PAPER

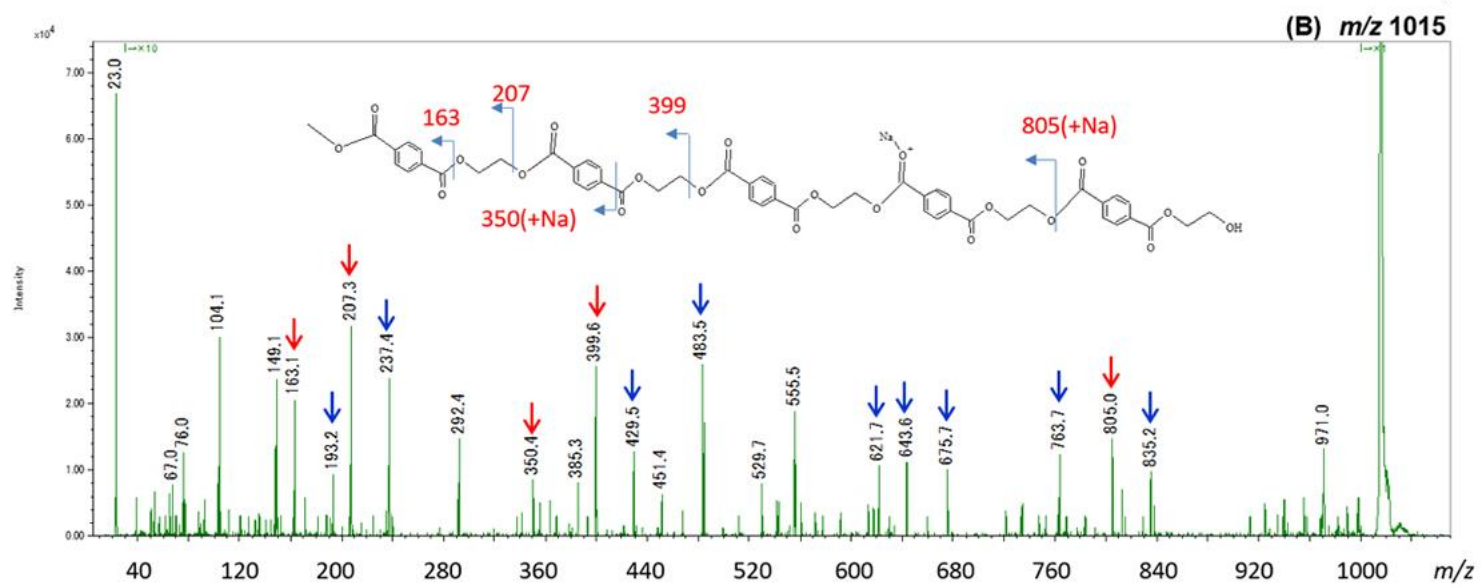
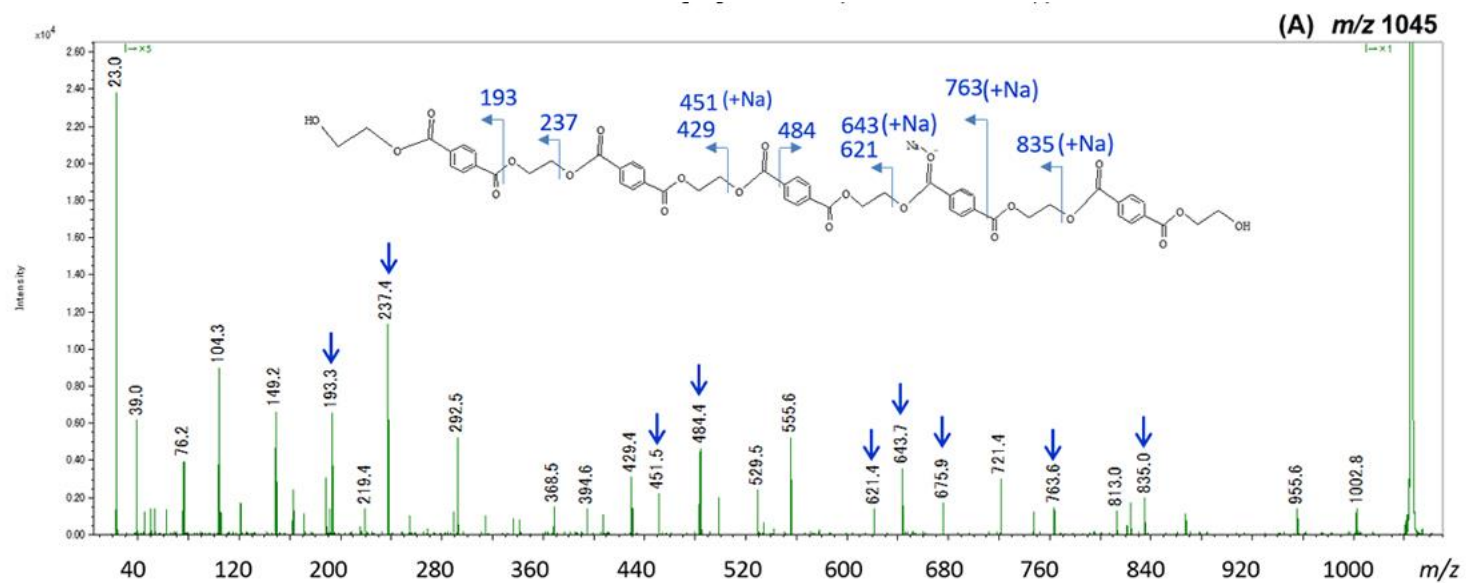
Available from: URL: <http://www.jgld.ro/2015/1/10.html>
 DOI: <http://dx.doi.org/10.15403/jgld.2014.1121.pas>

Plasma Lipidomic Fingerprinting to Distinguish among Hepatitis C-related Hepatocellular Carcinoma, Liver Cirrhosis, and Chronic Hepatitis C using MALDI-TOF Mass Spectrometry: a Pilot Study

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MODALITA' DI ACQUISIZIONE: MS/MS

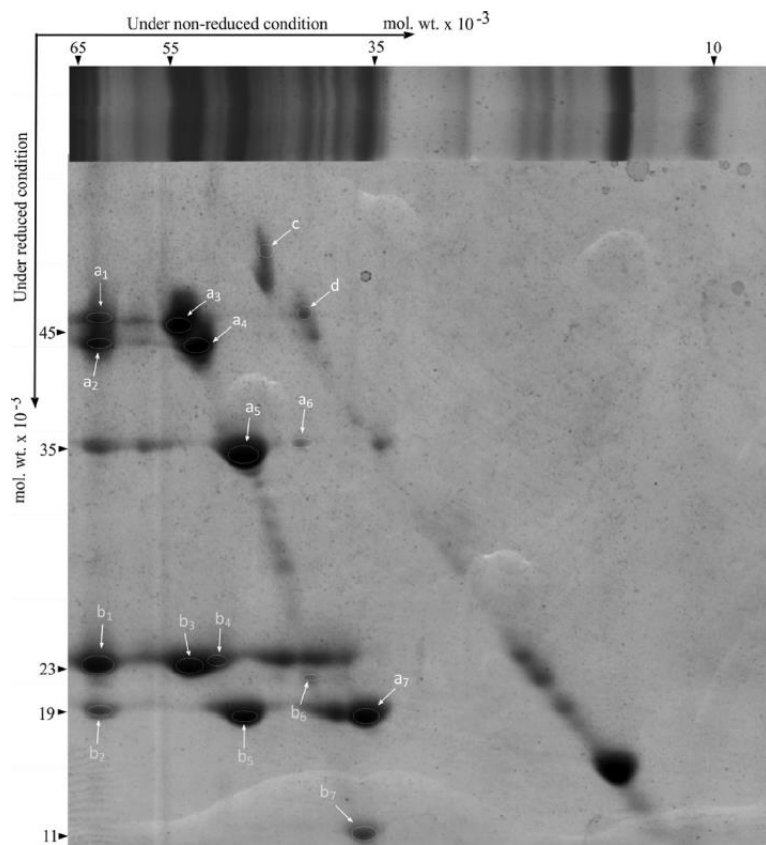


Identificazione di proteine

Proteome Profiling of Seed Storage Proteins Reveals the Nutritional Potential of *Salicornia brachiata* Roxb., an Extreme Halophyte

Bhavanath Jha,* Nater Pal Singh, and Avinash Mishra

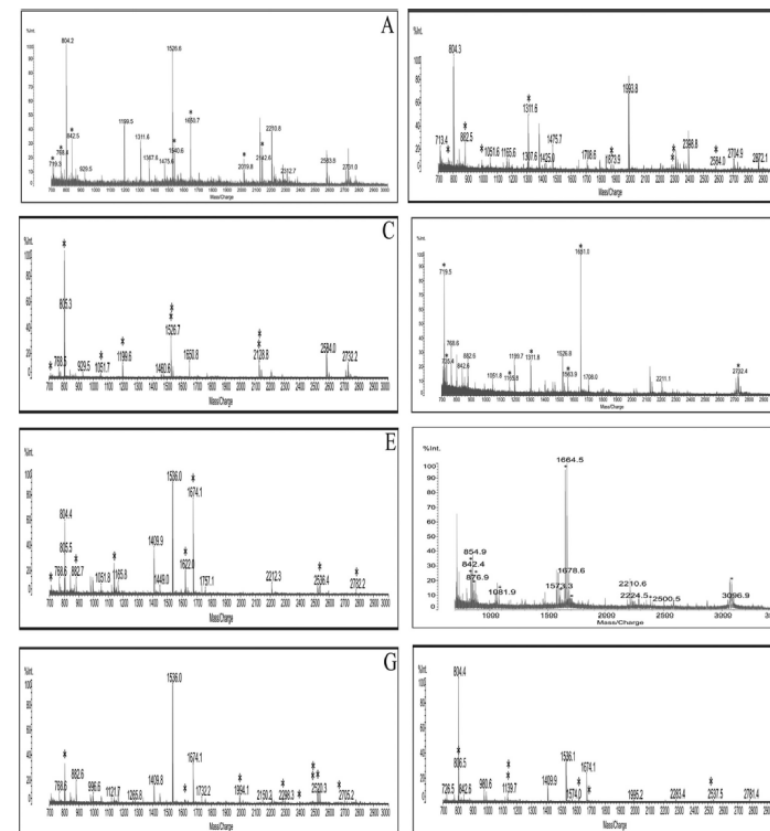
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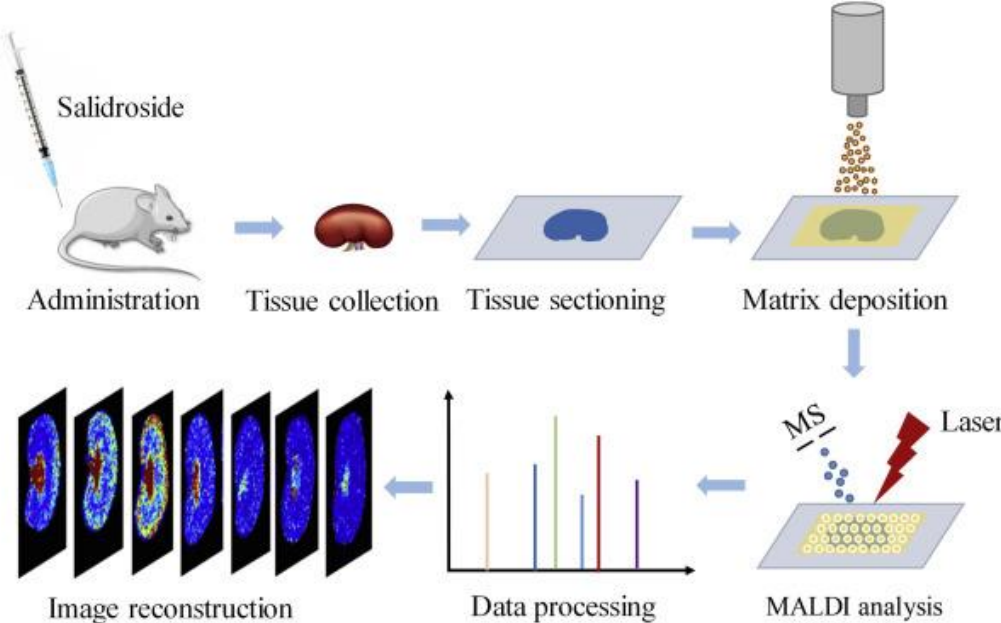
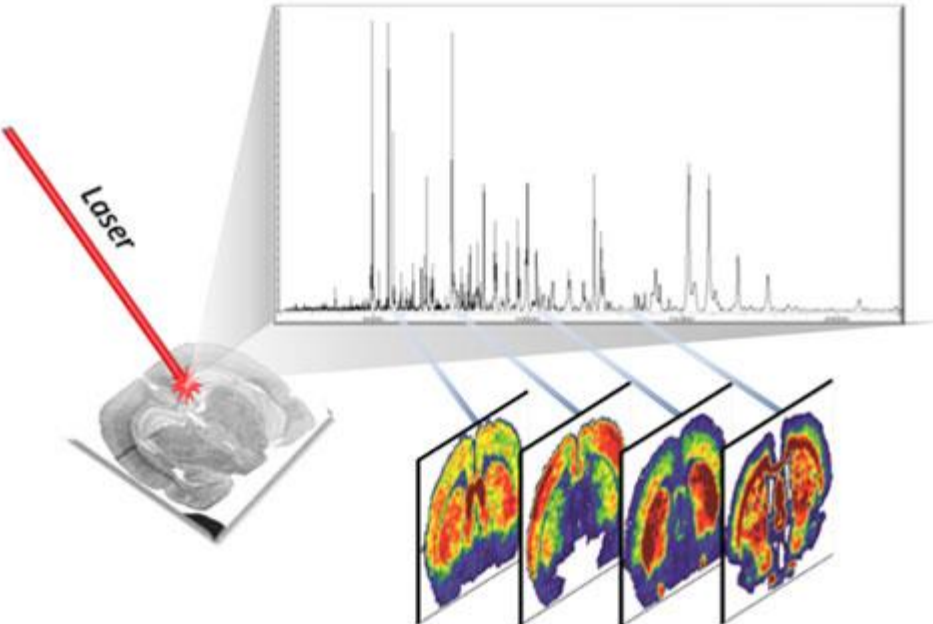
Digestione in tripsina

CHCA + 0.1%TFA

Reflectron positive mode

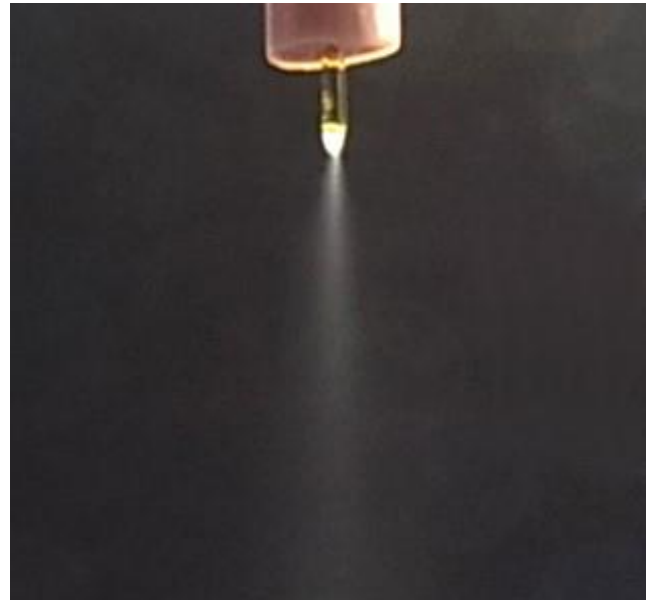
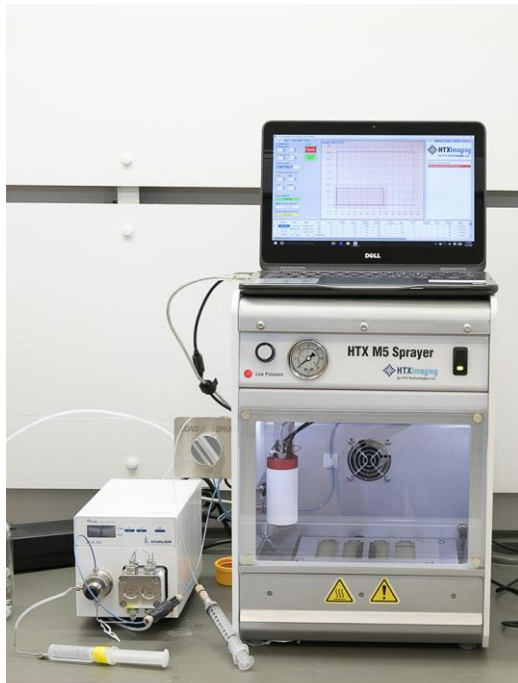


MALDI IMAGING



MALDI IMAGING

Taglio preciso
~ 10 μm



Matrice uniforme

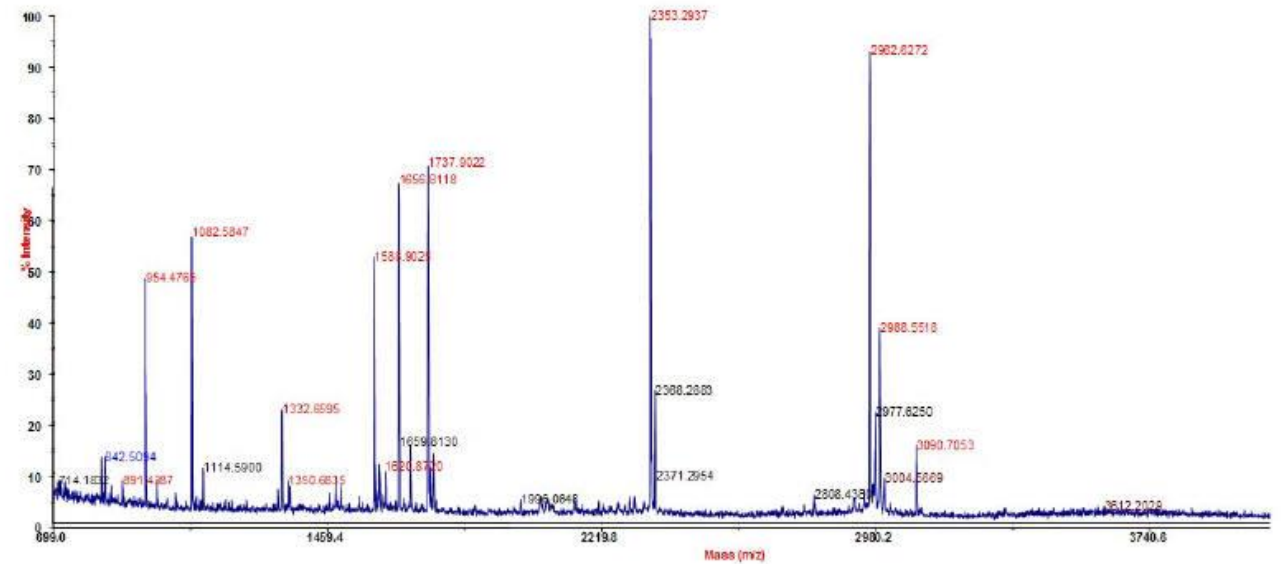
ANALISI DI PROTEINE CON LA TECNICA MALDI-MS

Fingerprinting proteico

E' la tecnica di analisi MALDI più utilizzata ma anche la più semplice

Le matrici comunemente utilizzate sono DHB e SA

Viene utilizzato il TOF in modalità linear



RESEARCH

Open Access



Distinct deposition of amyloid- β species in brains with Alzheimer's disease pathology visualized with MALDI imaging mass spectrometry

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