Newborn Screening

It identifies biochemical or other inherited conditions that may produce mental retardation, other disabilities and/or death.

Babies are screened for these conditions during the newborn period.

These conditions are identified using tests on blood collected from a heel stick onto filter paper

Legge italiana n. 104/1992

...nei primi giorni di vita, ancora in ospedale, il bimbo viene sottoposto al cosiddetto "screening neonatale", una serie di esami che permettono di individuare precocemente alcune malattie congenite (cioè presenti alla nascita), ma che si manifestano in genere più tardivamente. Grazie a questo test, che deve essere eseguito dopo quarantotto ore di vita, è possibile individuare e curare precocemente queste malattie, che possono, altrimenti, avere gravi conseguenze sullo sviluppo psicomotorio e sull'accrescimento del bambino. Dal 1992 (legge-quadro n. 104 del 5-5-1992) questo esame deve essere eseguito su tutti i neonati italiani.

Lo "screening neonatale" viene effettuato per identificare alcuni disturbi molto seri, che se vengono individuati precocemente possono essere curati con ottimi risultati. Queste malattie sono congenite, presenti cioè già dalla nascita, ma nei primi giorni di vita non si manifestano e, se non viene eseguito il test, possono essere individuate solo più tardi. I disturbi individuabili con questo esame sono tre: la fenilchetonuria, una malattia ereditaria che provoca problemi nell'assimilazione di una sostanza, la fenilalanina (monitorando il dosaggio di quest'ultima); l' ipotiroidismo congenito, un problema della tiroide, le ghiandola che regola lo sviluppo e la crescita (in base al dosaggio del TSH o ormone tireotropo) e la fibrosi cistica, una malattia respiratoria molto seria (verificata tramite la concentrazione di un enzima la tripsina).

I fase 01/01/2002-31/10/2004

Progetto Pilota autorizzato sui neonati di Firenze, Prato e Pistoia
42371 neonati sottoposti a screening neonatale

Delibera Regione Toscana n° 800 del 3/8/2004:

.....tutti i neonati toscani dovranno essere sottoposti a screening allargato mediante spettrometria di massa tandem...

Centro regionale: Ospedale Meyer di Firenze

01/01/2006 ASL 1 Umbria effettua lo screening allargato presso l'ospedale Meyer di Firenze

~400,000 newborns

PREVALENCE 1:1500

265 DIAGNOSES

since 01/01/2010

Florence has performed the expanded newborn screening also for Umbria

MEYER

8500 newborns/year

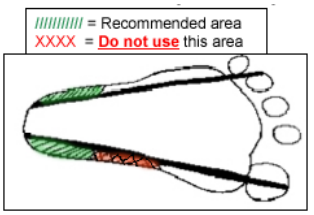
PRELIEVO DAL TALLONE

il prelievo per lo screening neonatale deve per legge essere effettuato tra la 48°e la 72° ora di vita

Riscaldare il tallone con un panno caldo (39-41°C) per 3-5 minuti

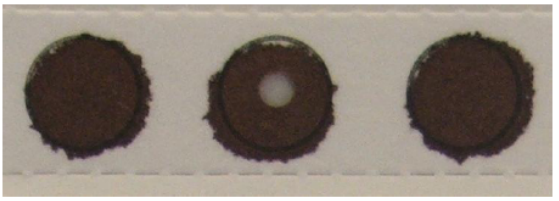
Disinfettare il punto del prelievo con alcool e rimuoverne l'eccesso con una garza sterile

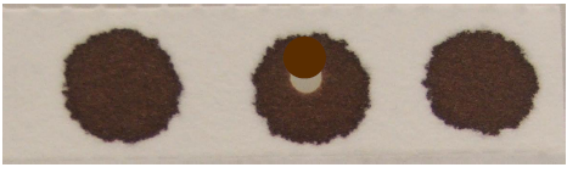
Fare una puntura con una lancetta sterile nella zona laterale del tallone



Rimuovere la prima goccia di sangue perché diluito da liquido interstiziale e aspettare che si formi una nuova goccia. Applicare leggermente la carta bibula sulla goccia lasciandola diffondere

SPOT ESEGUITI CORRETTAMENTE





3.2 mm - 3,4 µl di sangue

SCREENING IN MS/MS

AMINOACIDOPATIE
IPERFENILALANINEMIA
IPERGLICINEMIA NON CHETOTICA
MALATTIA DELLE URINE A SCIROPPO D'ACERO
TIROSINEMIA TIPO I
TIROSINEMIA TIPO II
IPERMETIONINEMIA
SINDROME HHH
ATROFIA GIRATA DELLA COROIDE E RETINA
CITRULLINEMIA TIPO II (defict di citrina)
OMOCISTINURIA

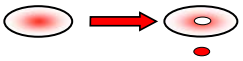
DIFETTI DEL CICLO DELL'UREA
CITRULLINEMIA
ARGININOSUCCINICOACIDURIA
ARGININEMIA
DEFICIT DI CARBAMIL FOSFATO SINTETASI
DEFICIT DI ORNITINA TRANSCARBAMILASI

DIFETTI DELLA β-OSSIDAZIONE
DEFICIT DEL TRASPORTATORE DI CARNITINA
DEFICIT DI CPT I
DEFICIT DI CPT II
DEFICIT DI CARNITINA/ACILCARNITINA TRANSLOCASI
DEFICIT DI SCAD
DEFICIT DI MCAD
DEFICIT DI VLCAD
DEFICIT DI LCHAD/PROTEINA TRIFUNZIONALE
DEFICIT DI MAD (GLUTARICO ACIDURIA II)

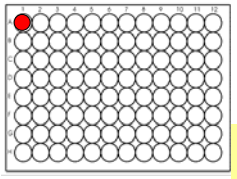
ACIDURIE ORGANICHE
ACIDEMIA PROPIONICA
METILMALONICO ACIDURIA
ISOVALERICO ACIDURIA
GLUTARICO ACIDURIA TIPO I
DEFICIT DI BIOTINIDASI
DEFICIT DI OLOCARBOSSILASI SINTETASI
DEFICIT DI 3-OH-3-METIL-GLUTARIL CoA LIASI
METILGLUTACONICO ACIDURIA
3-METILCROTONIL GLICINURIA
DEFICIT DI β-CHETOTIOLASI
DEFICIT DI 2 METILBUTIRRIL-CoA DEIDROGENASI

METODO

dried blood spot (DBS)



Diametro del cerchietto 3.2 mm



200 µL di MeOH contenente standard interni e

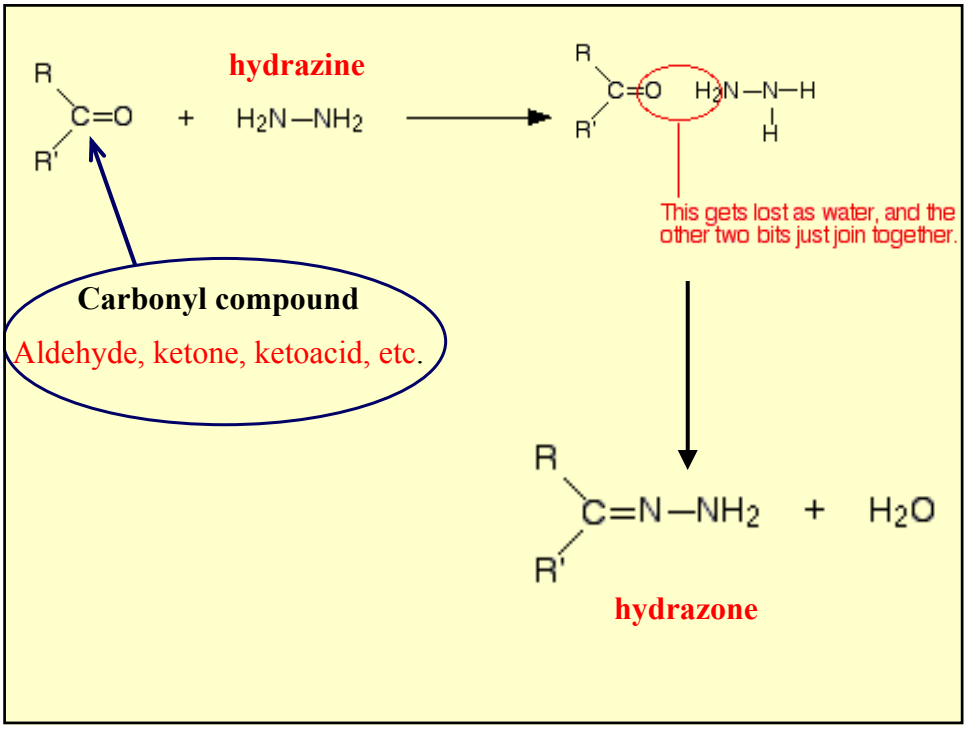
100 µL di idrazina 3mmol/L
25 min in agitazione a 37°C

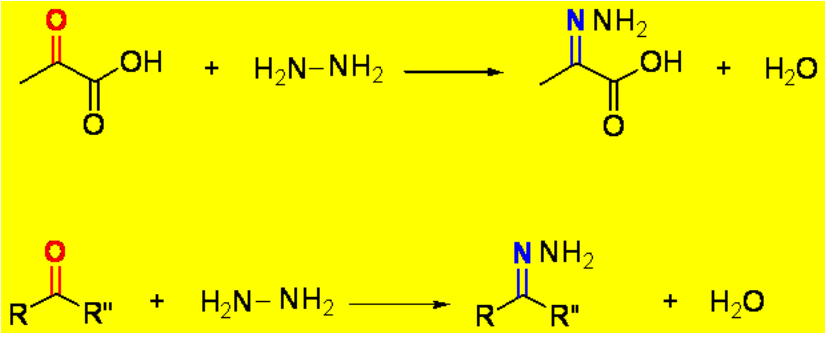
Evaporazione sotto flusso di azoto a 40°C

Derivatizzazione in Butanolo-HCl 3N 65°C per 25 minuti

Evaporazione sotto flusso di azoto a 40°C

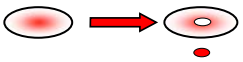
Risospensione in acqua-Acetonitrile +0.05% acido formico 30:70 e iniezione diretta in infusione diretta ESI(+)-MS/MS


hydrazine
Carbonyl compound
Aldehyde, ketone, ketoacid, etc.
This gets lost as water, and the other two bits just join together.
hydrazone

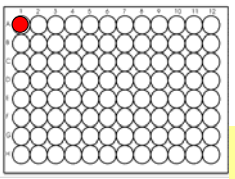


METODO

dried blood spot (DBS)



Diametro del cerchietto 3.2 mm



200 µL di MeOH contenente standard interni e

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25 min in agitazione a 37°C

Evaporazione sotto flusso di azoto a 40°C

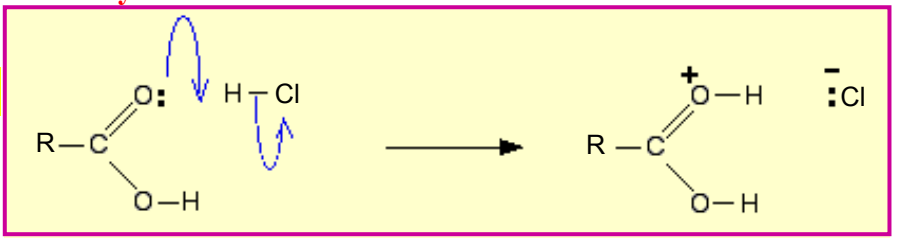
Derivatizzazione in Butanolo-HCl 3N 65°C per 25 minuti

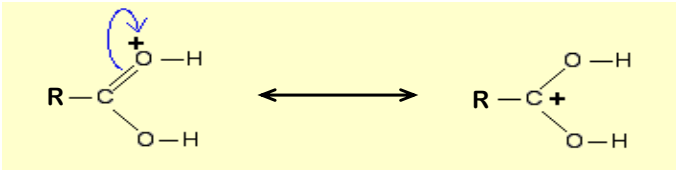
Evaporazione sotto flusso di azoto a 40°C

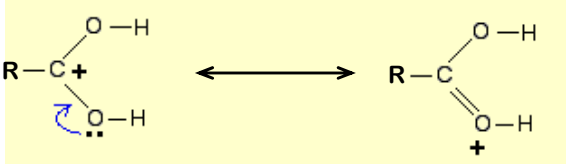
Risospensione in acqua-Acetonitrile +0.05% acido formico 30:70 e iniezione diretta in TIS-triplo quadrupolo (LC-MS/MS)

Carboxylic acid

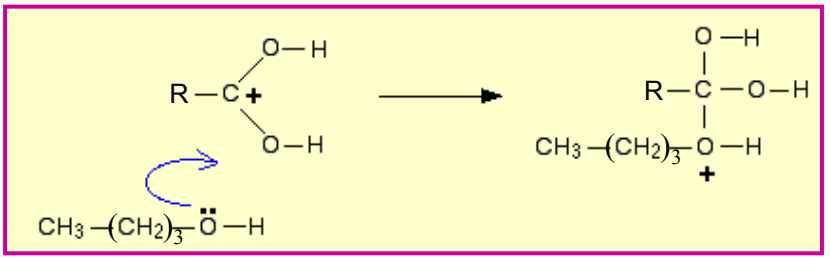
I



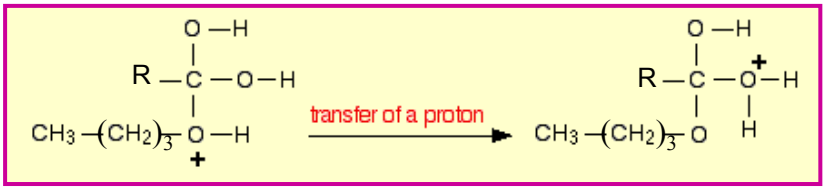




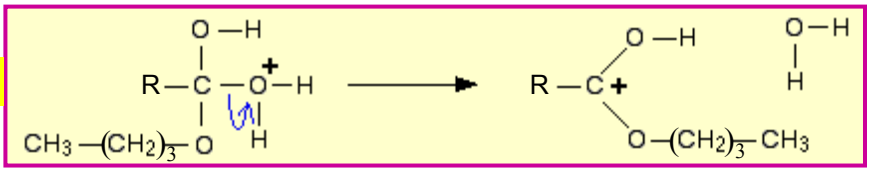
II



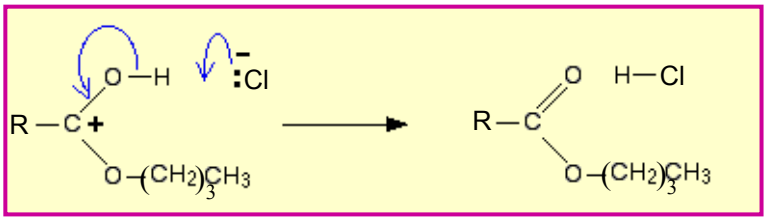
III



IV



V

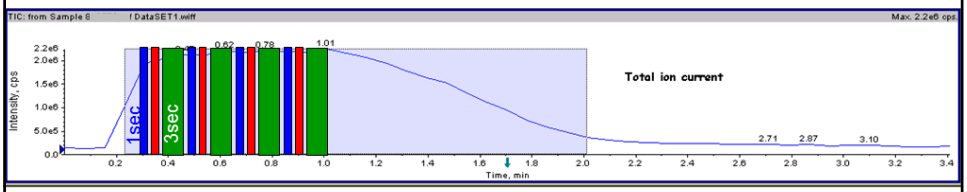


Butyl ester

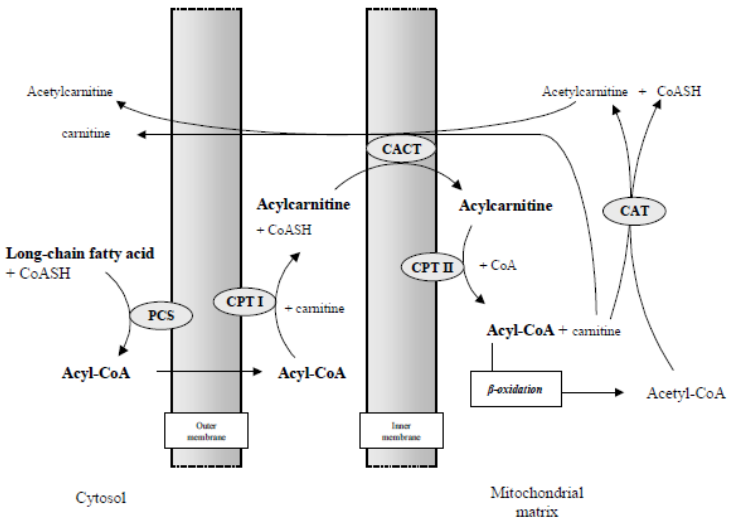
Precursor ion scans

Neutral loss scans

Multiple reaction monitoring



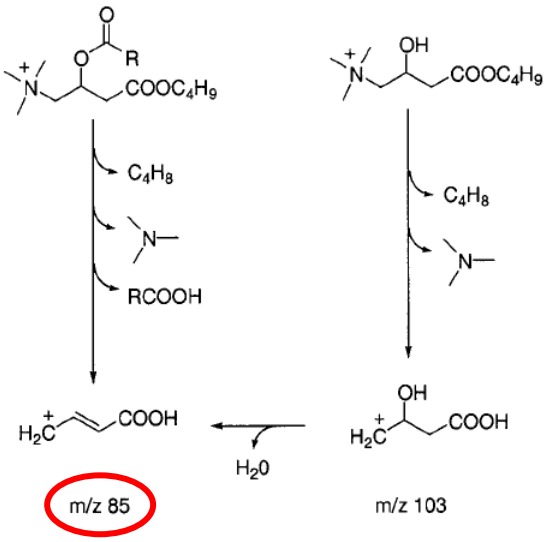
CARNITINE



Schematic representation of mitochondrial long-chain fatty acid metabolism and regulation of intramitochondrial acyl-CoA/CoA ratio

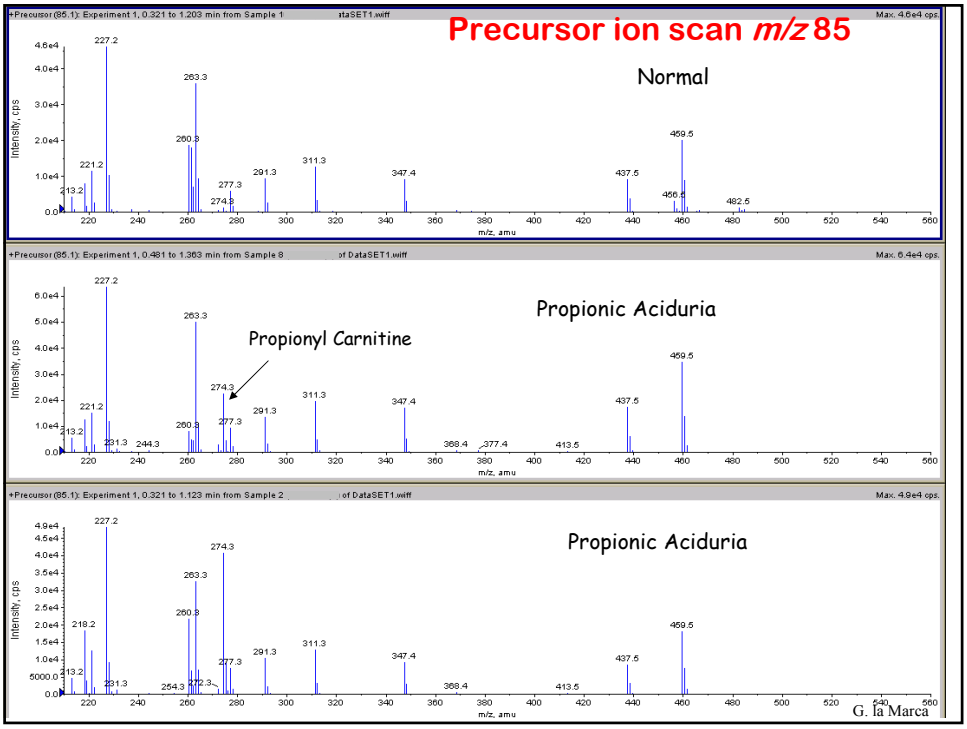
http://edoc.unibas.ch/320/1/DissB_7322.pdf

acylcarnitines **butyl esters** carnitine *m/z* 218

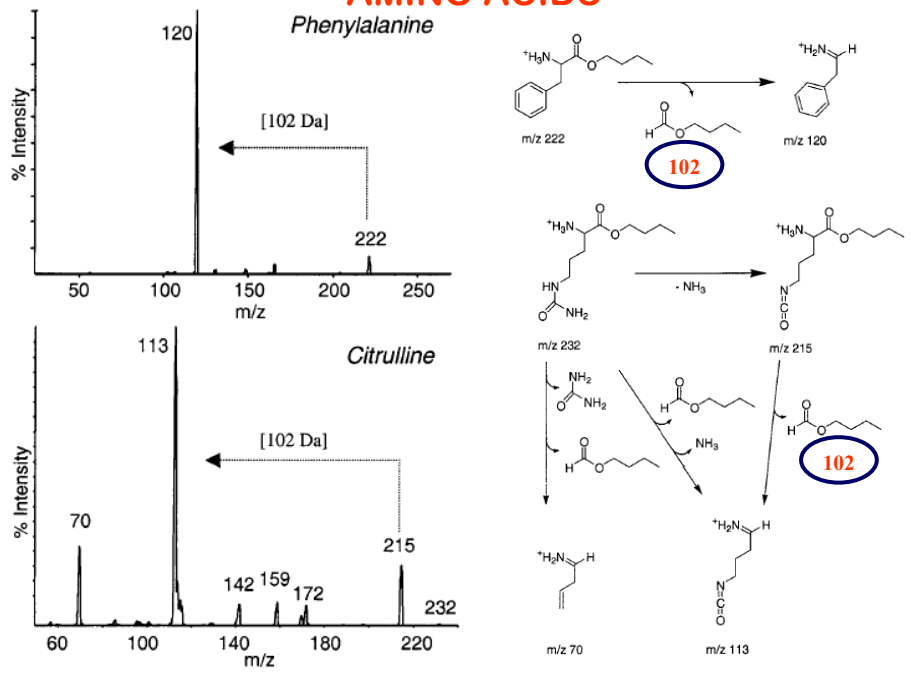


Schematic of collision-induced dissociation (CID) of the protonated butyl esters of carnitine and acyl carnitine.

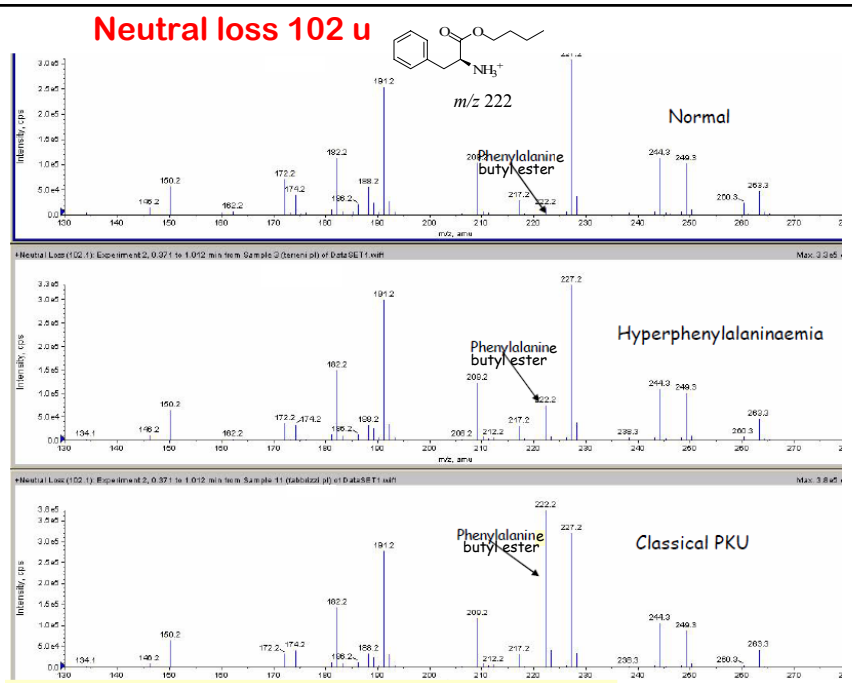
R indicates a fatty acid of 2-20 carbon atoms



AMINO ACIDS

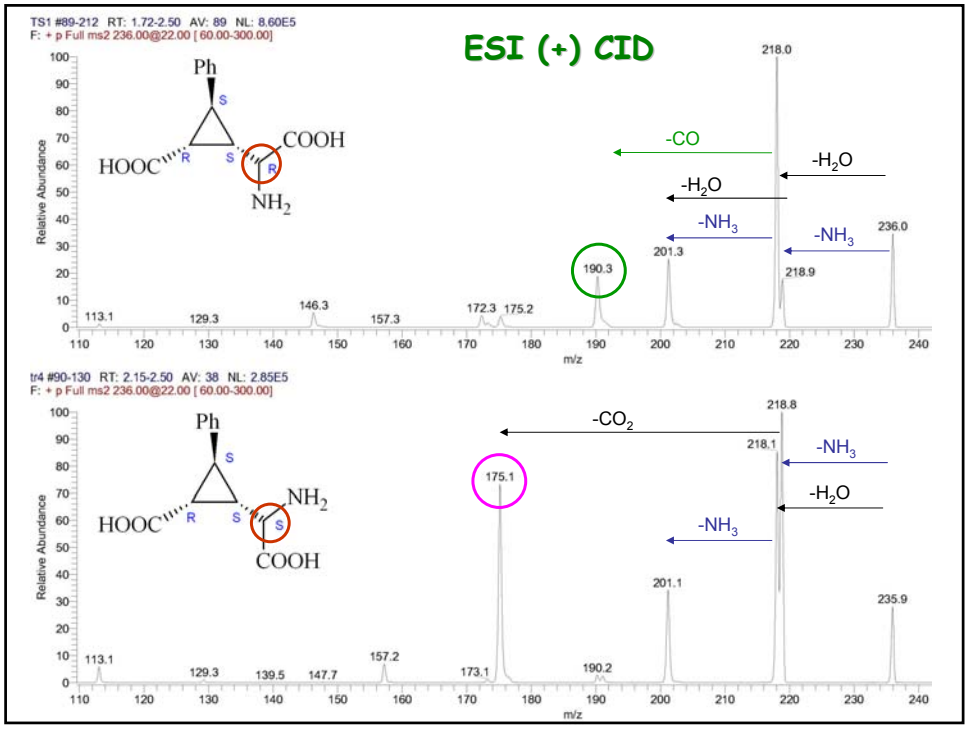


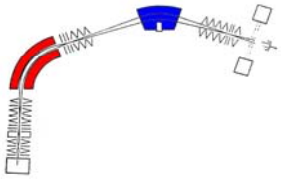
Neutral loss 102 u



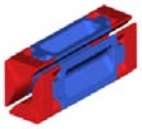
G. la Marca

Reazioni di decomposizione stereoselettive e stereospecifiche

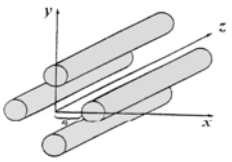




E = 10^4 eV **Path length = 1 cm**
Time scale=0.25µs **N. coll. = 1-5**



Energy : 1-10 eV
Path length: 300-3000 cm
Time scale: 10^5-10^6 µs
Number of collisions: 1-5



E= 10-100 eV **Path length=15 cm**
Time scale= 100µs **N. coll= 1-20**

What is meant by *low* and *high energy* CID?

Low energy CID:

- 10-100 ev collision energy in the laboratory frame (E_{lab})
- multiple collisions used to build up internal energy in steps
- tendency to break the weakest bond
- poor for labile or oppositely-charge PTMs
- used on quadrupole, quadrupole ion trap, linear ion trap, QTOF and FTMS

High energy CID:

- 1keV to 20 keV in the laboratory frame (E_{lab})
- single collision resulting in randomized (charge-remote) fragmentation
- used on TOF/TOF and BE mass spectrometers

One of the major challenges in tandem mass spectrometry is the difficulty to achieve efficient fragmentation of large molecules, using traditional high energy single-collision activation.
In fact the efficient fragmentation of a large molecule requires the deposition of an amount of internal energy above their dissociation threshold to fragment on the time scale of a mass spectrometer.

This is difficult because there is a combination of effects:

a) **The dramatic increase in density of states with increasing internal degrees of freedom of the ion decreases the rate of dissociation by many orders of magnitude at a given internal energy.**

b) **The center-of-mass collision energy -the absolute upper limit of energy transfer in a collision process- decreases with increasing mass of the ion for fixed ion kinetic energy and neutral mass.**

CID of large molecules

The problem is that the real collision energy (E_{rel}) decreases when the molecular ion gets larger:

$$E_{rel} = \frac{m_n}{m_n + m_M} E_{Lab}$$

where E_{lab} = kinetic energy of the molecular ion M hitting a stationary neutral gas molecule n.

Protein	*MW*	*1 keV*	*8 keV*	*20 keV*
Substance P	1,348	2.97 ev	23.7 ev	59.4 ev
Ubiquitin	8,566	0.47 ev	3.7 ev	9.3 ev
Cytochrome C	12,328	0.32 ev	2.6 ev	2.5 ev
C fragment of tetanus toxin	51,819	0.08 ev	0.6 ev	1.5 ev
Bovine serum albumin	66,430	0.06 ev	0.5 ev	1.2 ev

Decomposizioni indotte da:

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

interazioni con elettroni (electron capture dissociation ECD)
(electron transfer dissociation ETD)

interazioni con superfici (collisioni ioni/superficie)

interazioni con fotoni (IRMPD, ion spectroscopy)

THE ROLE OF ELECTRON CAPTURE DISSOCIATION IN BIOMOLECULAR ANALYSIS

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[1]*School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom*
[2]*Department of Chemistry, University of Michigan, 930 North University Avenue, Ann Arbor, Michigan 48109-1055*
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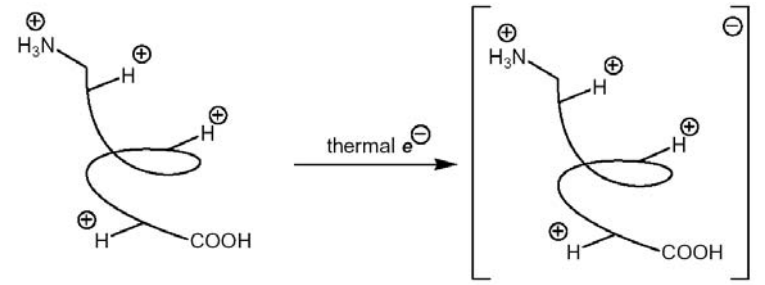
Mass Spectrometry Reviews, **2005**, *24*, 201–222

Electron Capture Dissociation

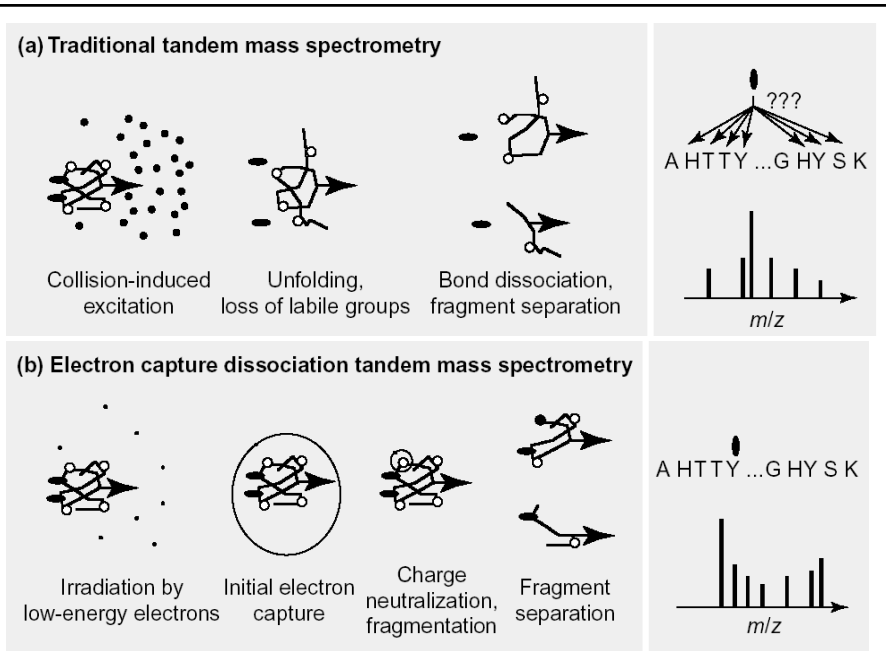


Multiply-protonated protein

Reduced-charge ion

Dissociation products

Zubarev, McLafferty, et al. *Anal. Chem.* **2000**, *72*, 563-573.



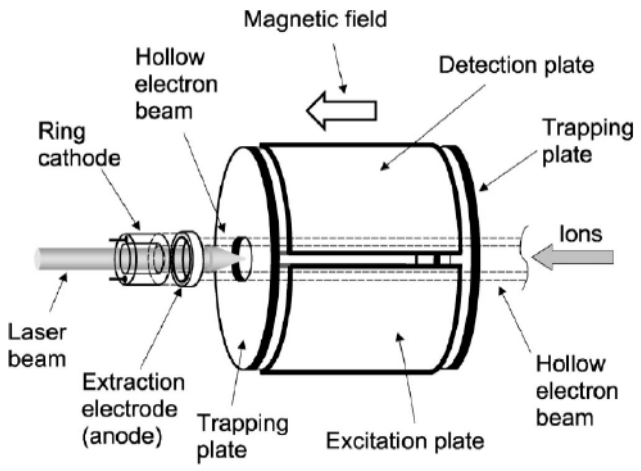
Comparison of **(a)** the traditional (collision-based) MS/MS and **(b)** ECD-based MS/MS techniques in the analysis of modified polypeptides. Whereas collisional excitation leads to polypeptide chain unfolding and losses of labile groups, ECD largely preserves the secondary structure and

Electron Capture Dissociation

- Multiply charged (ESI) ions and also their fragments can be neutralized by low energy electrons
- Electrons produced by a conventional heated filament outside the FT-MS magnet opposite to the ESI source (10^{-5} torr Ar for cooling, < 0.2 eV)
- Mostly c and z type ions are formed
- Gentle fragmentation, good for detecting post-translational modification sites (e.g., phosphorylation, O-glycosilation, sulfatation, gamma-carboxylation)
- Disadvantage: reduced charge state may require extended m/z ion analyzer range

Zubarev, R. A.; Kelleher, N. L.; McLafferty, F. W. *J. Am. Chem. Soc.* **1998**, *120*, 3265-3266.

Instrumentation for ECD



Schematic drawing of the combined IRMPD and ECD FTICRMS experimental setup. The electron injection system is based on an indirectly heated ring dispenser cathode. The IR-laser beam passes through the ring cathode into the ICR trap. Electron and photon beams can simultaneously enter the ICR trap.

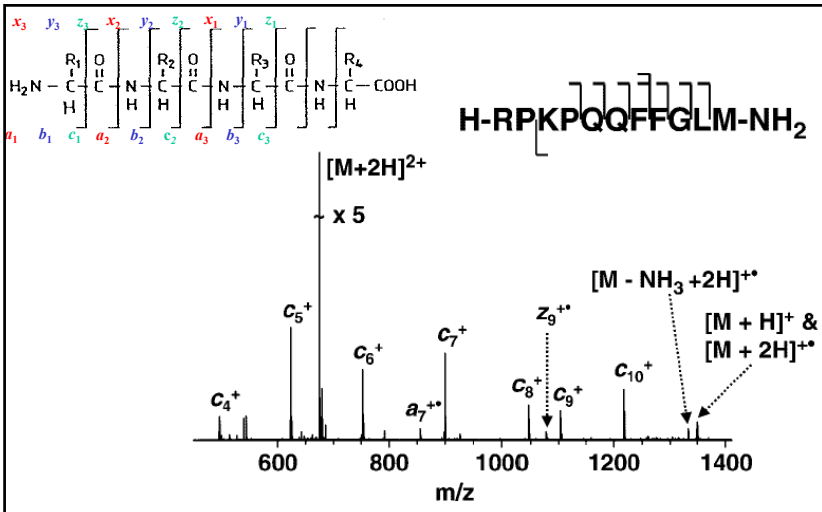
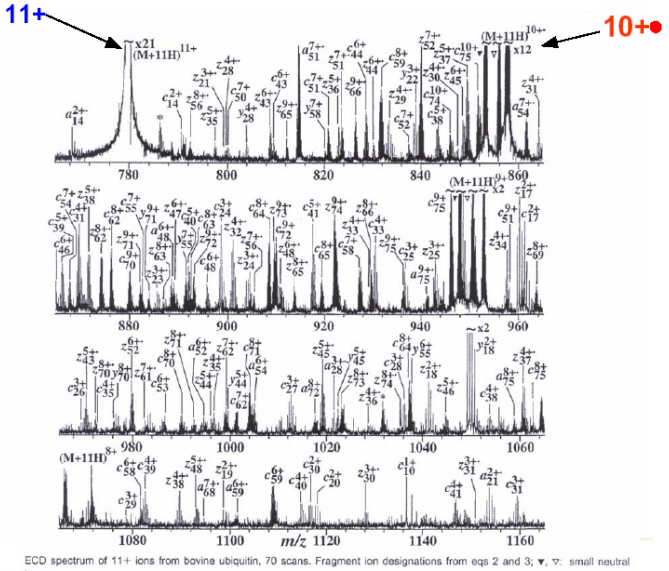


FIGURE 1. Electron capture dissociation (ECD) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrum obtained from the peptide, substance P $[M+2H]^{2+}$ ions. Spectrum obtained from the 9.4 T homebuilt instrument at the National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL. (Figure reproduced from Håkansson et al., 2003a, with permission from American Chemical Society, copyright 2003.)

ECD Spectrum of (Ubiquitin + 11H)$^{11+}$



ECD spectrum of 11+ ions from bovine ubiquitin, 70 scans. Fragment ion designations from eqs 2 and 3; ▼, ▽: small neutral losses.

Zubarev, Kelleher, McLafferty, *JACS* **1998**, *120*, **3265-3266**.

interazioni con elettroni

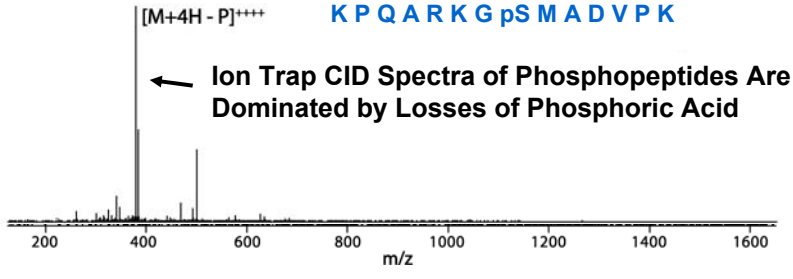
ETD

Electron Transfer Dissociation

Origins of ETD

Donald F. Hunt

"Ok. Now I want you to figure out how to do ETD on our ion traps so we can sequence more phosphopeptides."

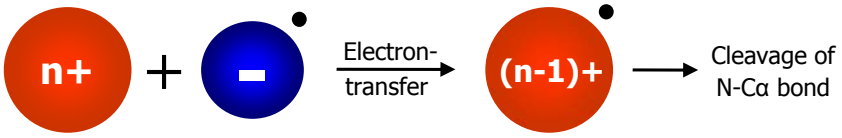


ETD Reaction Scheme

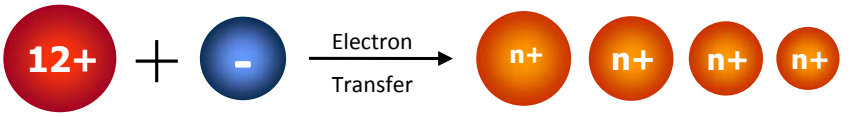
Multiply charged analyte (n≥ 2)

Reagent radical anion

odd-electron protonated peptide



Prerequisite: multiply charged precursor ions, n ≥ 2 !
ETD is not applicable to 1+ or negatively charged ions



multiply charged fragment ions **n =11, 10, 9, 8, …**

Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry

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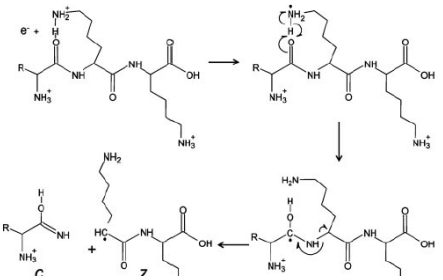
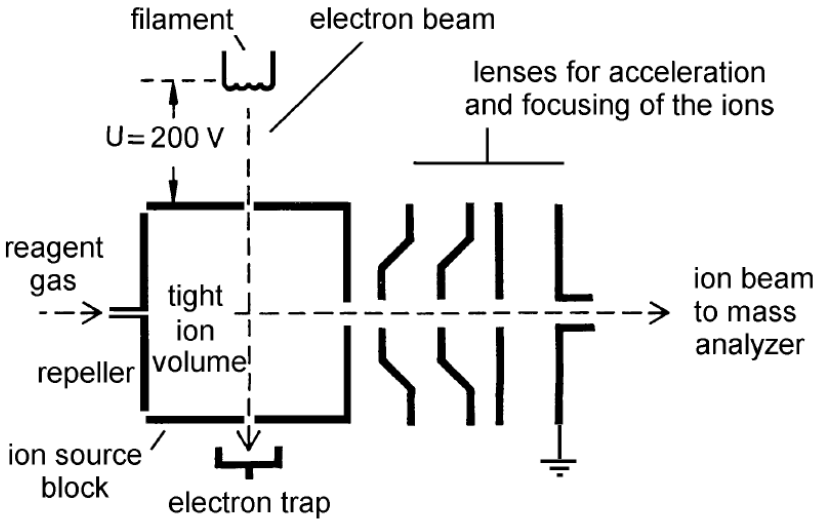
Edited by Fred W. McLafferty, Cornell University, Ithaca, NY, and approved May 17, 2004 (received for review April 15, 2004)

Peptide sequence analysis using a combination of gas-phase ion/ion chemistry and tandem mass spectrometry (MS/MS) is demonstrated. Singly charged anthracene anions transfer an electron to multiply protonated peptides in a radio frequency quadrupole linear ion trap (QLT) and induce fragmentation of the peptide backbone along pathways that are analogous to those observed in electron capture dissociation. Modifications to the QLT that enable this ion/ion chemistry are presented, and automated acquisition of high-quality, single-scan electron transfer dissociation MS/MS spectra of phosphopeptides separated by nanoflow HPLC is described.

electron capture dissociation | fragmentation | ion/ion reactions | charge transfer | ion trap





Sorgente per ionizzazione chimica

Ionizzazione chimica

per cattura di elettroni:

studio di ioni negativi

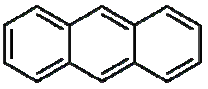
$M + e^- \rightarrow M^{-\bullet}$ *resonance electron capture*

$M + e^- \rightarrow [M–A]^- + A^\bullet$ *dissociative electron capture*

$M + e^- \rightarrow [M–B]^- + B^+ + e^-$ *ion-pair formation*

$$CH_4 + e^- \rightarrow CH_4^{+\bullet} + CH_3^+ + CH_2^{+\bullet} + CH^+ + C_2H_5^+$$

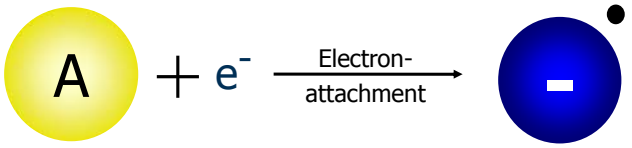
$$+ \quad e^-_{\text{termici}}$$



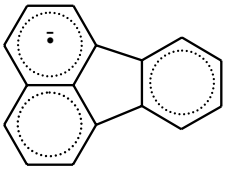
$$C_{14}H_{10} + e \rightarrow C_{14}H_{10}^{-\bullet}$$ Electron capture

Fluoranthene Reagent Anion

Electron Carrier → **Reagent Anion**

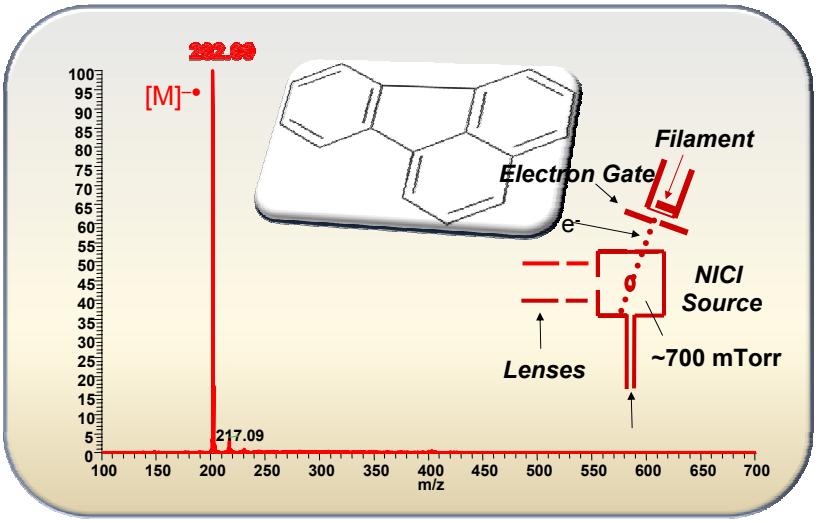


m/z = 202



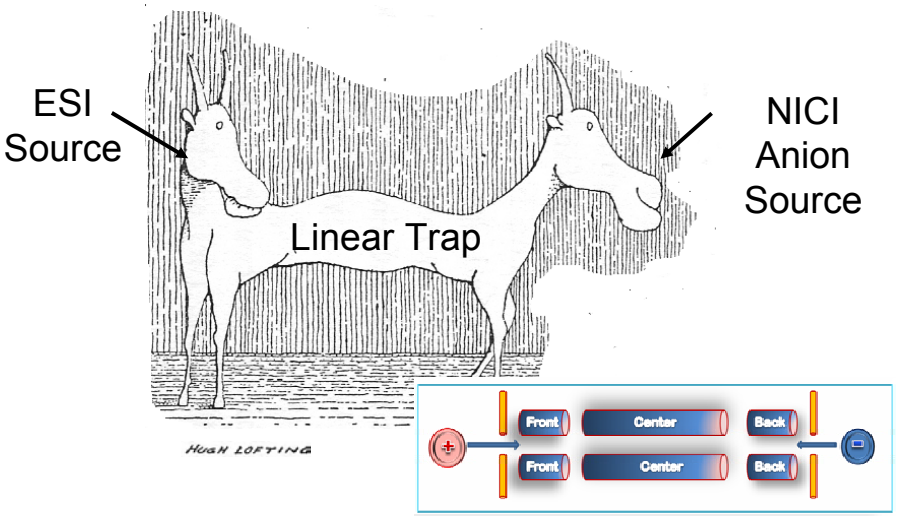
Flouranthene
Radical Anion
$C_{16}H_{10}^{\bullet -}$

NCI spectrum of Fluoranthene



Courtesy by G. Vago Thermo

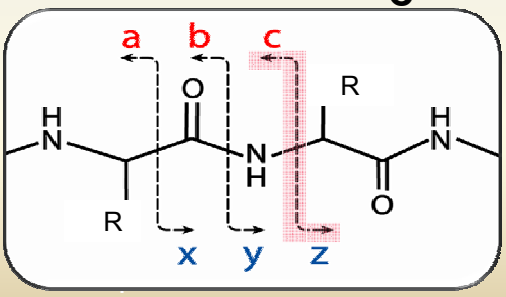
Pushmi-Pullyou Geometry



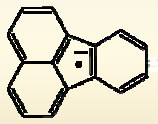
Electron Transfer Dissociation (ETD)

$$[M + 3H]^{3+} + A^{-} \rightarrow [M + 3H]^{2+\bullet} + A$$

$$[M + 3H]^{2+\bullet} \rightarrow \underbrace{[C+2H]^{1+}}_{C} + \underbrace{[Z+H]^{1+\bullet}}_{Z}$$

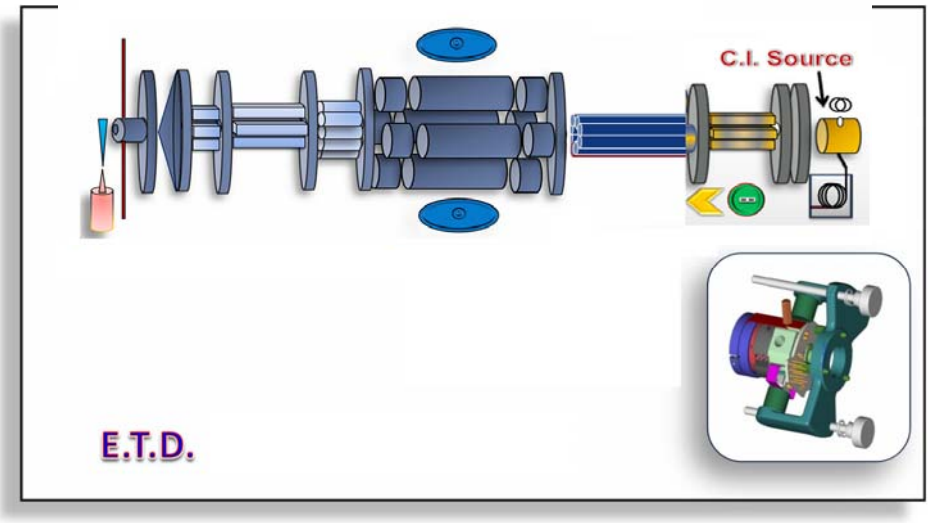


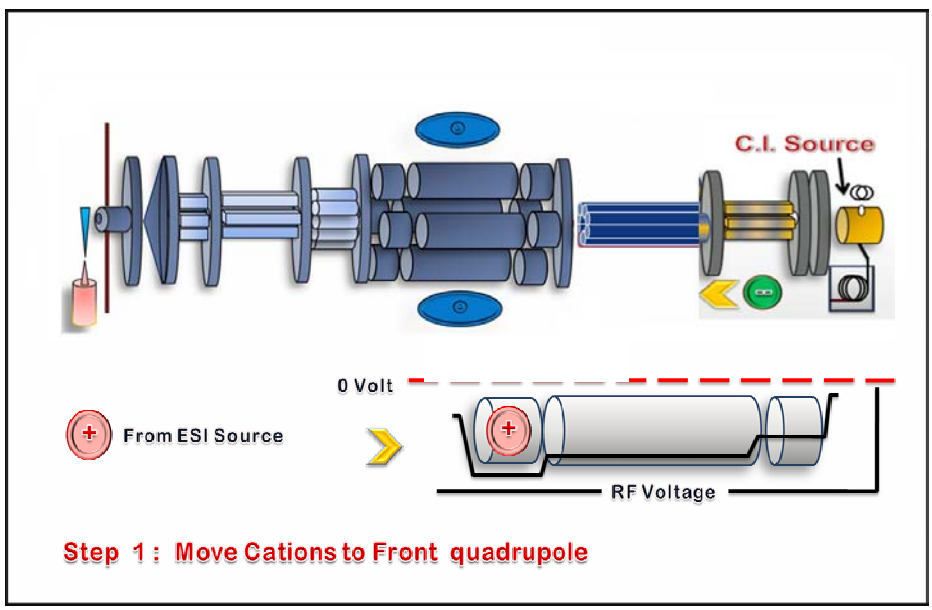
Electron Carrier/Donor



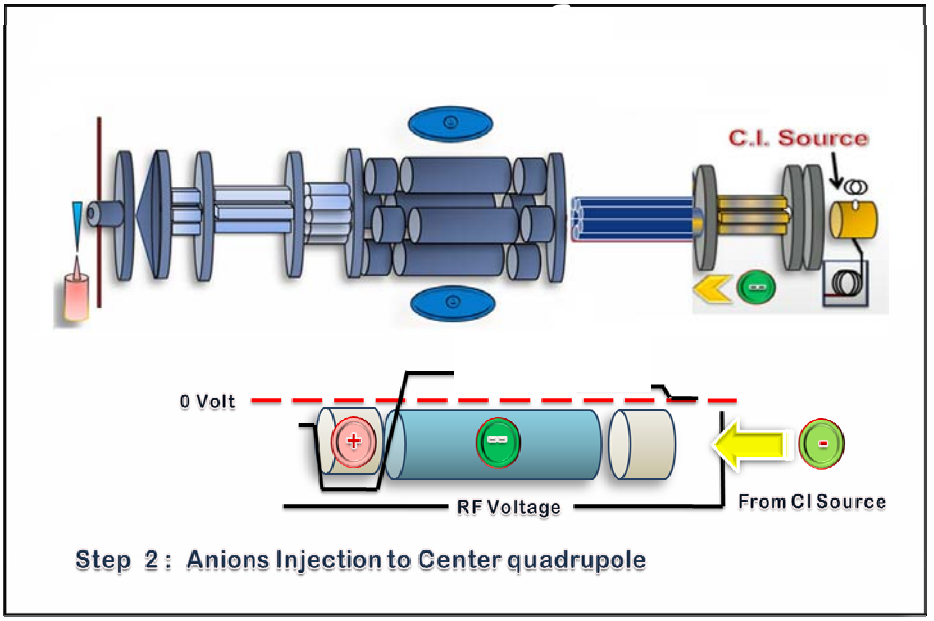
Fluoranthene
Radical Anion
m/z 202

The principle of ETD.
Ion with multiple positive charges gets an electron from an anion (A-). This reduces the charge by 1 unit (will not work with a singly charged ion – looses the charge and becomes 'invisible' in the mass spectrometer), and induces fragmentation. Peptide fragments along the backbone, cracking the bond between N and alfaC of the peptide backbone. The resulting ions are called C and z (radical – marked with a dot).



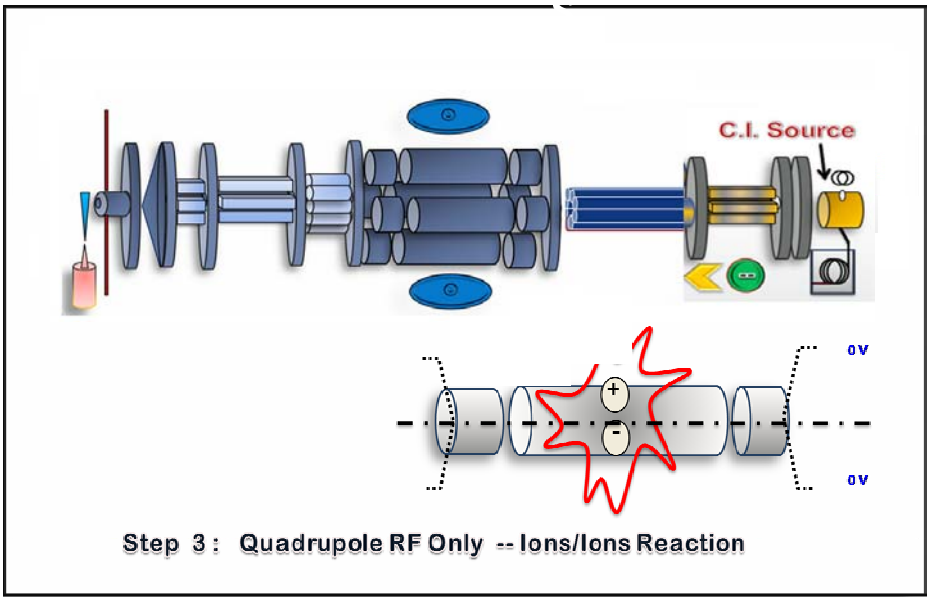


Courtesy by G. Vago Thermo



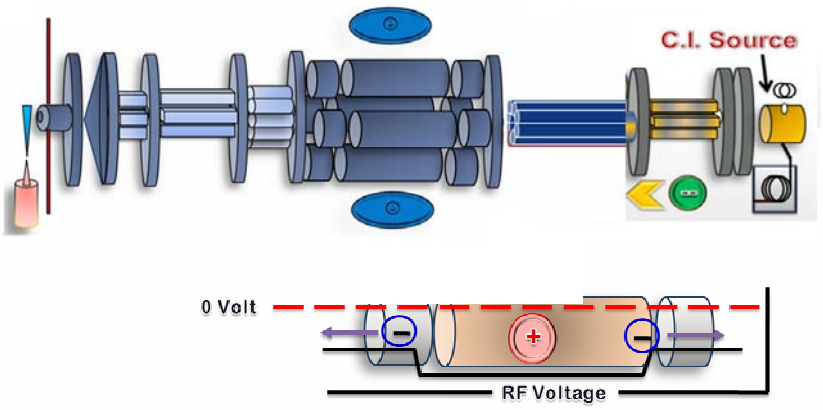
Step 2 : Anions Injection to Center quadrupole

Courtesy by G. Vago Thermo



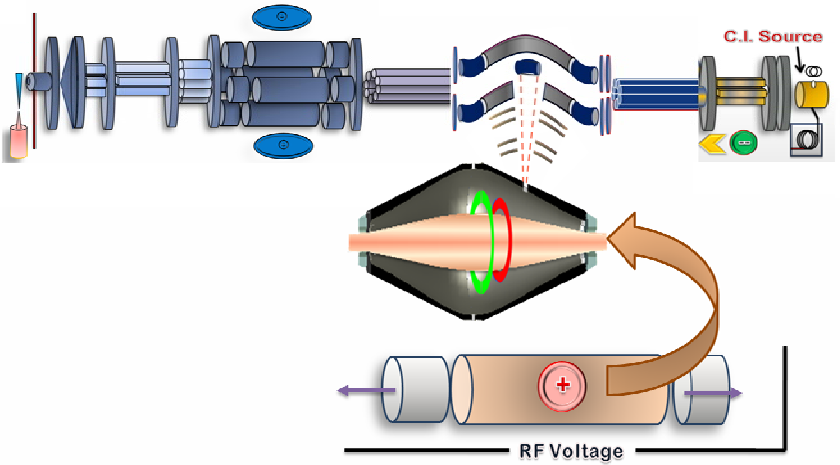
Step 3 : Quadrupole RF Only -- Ions/Ions Reaction

Courtesy by G. Vago Thermo



Step 4: RF Lens Off and Ready to Scan

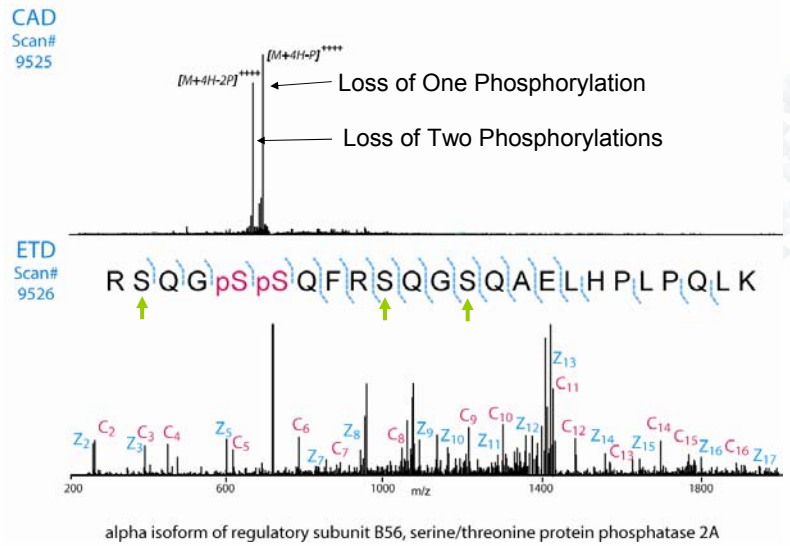
Courtesy by G. Vago Thermo



Step 5: FT or IT Scan

Courtesy by G. Vago Thermo

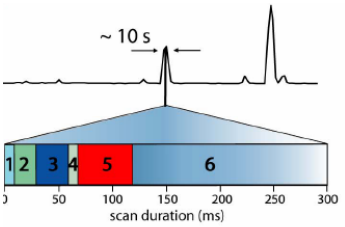
CID vs ETD for Phosphorylated Peptides



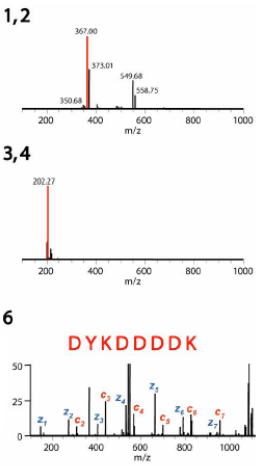
alpha isoform of regulatory subunit B56, serine/threonine protein phosphatase 2A

Courtesy by G. Vago Thermo

ETD is Fast - Less Than 300 ms Per MS/MS Scan



		time (ms)
1	Cation injection	1 - 10
2	Precursor isolation/storage	20
3	Anion injection	0.1-10
4	Anion isolation	2
5	Ion/ion reaction	10-100
6	Scan products	200

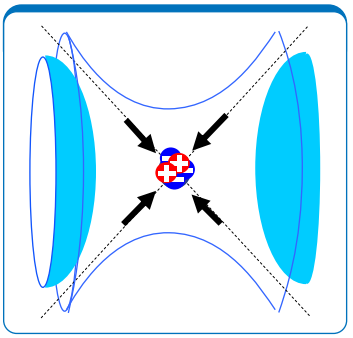


The "3D Advantage"

ETD in a tridimensional ion trap

Non-linear Paul Trap:

Dual injection and storage of ions of both polarities
peptide cations & reagent anions



Cations and anions are pushed towards the center of the trap

Direct ETD reaction as soon as anions enter the trap

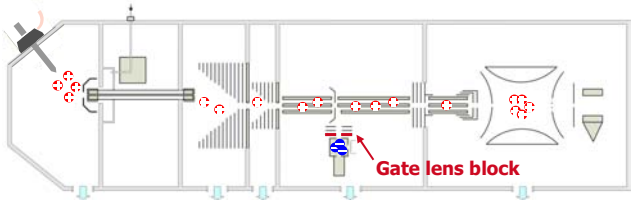
Better cross sections for ion-ion-reactions in 3D trap due to compression into the same globular volume

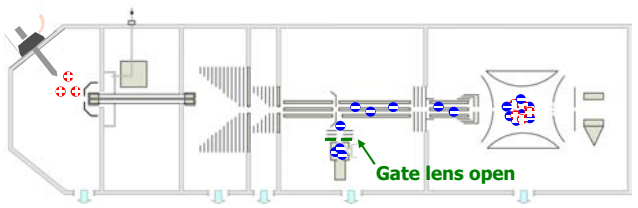
→ highly efficient ETD reaction

Spec: ≥ 18 unique peptides from 5 fmol BSA on column (Easy-nLC)

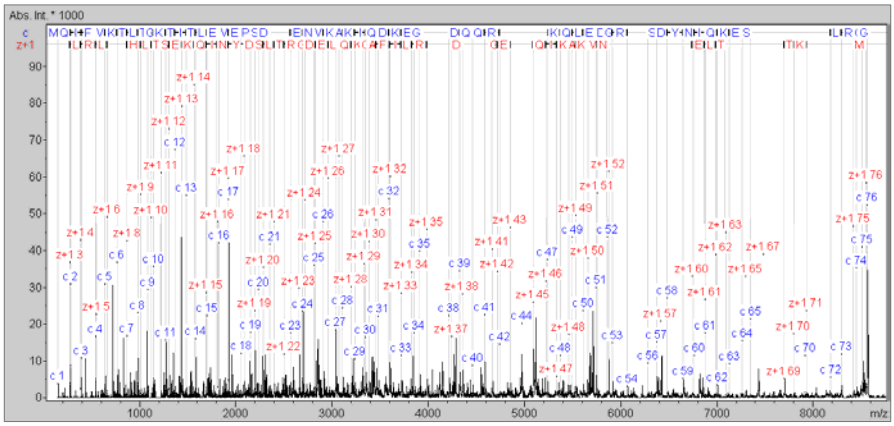
ETD Process

1. electrospray ion accumulation (positive mode)
2. precursor ion isolation
3. NCI ions accumulation
4. ETD fragmentation
5. scan





Ubiquitin, bovine (MW = 8559.6 Da)



Applications: e.g. QC of recombinant proteins, isolated proteins e.g. from cell lysates
Advantages: no 1/3 cut-off, PTMs visible, good sequence coverage, N/C-termini included!
Limitations: slow for LC separations, off-line techniques may be required (direct infusion, off-line nanospray, e.g. Nanomate™)

Decomposizioni indotte da:

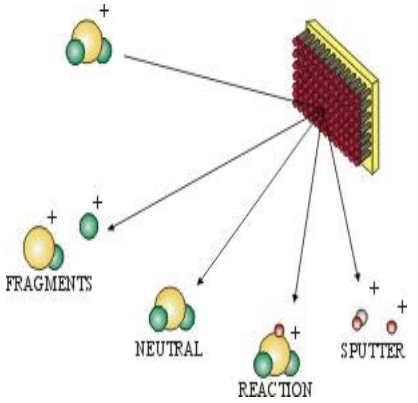
collisioni con un gas (Collision induced dissociation (CID)
collision activated dissociation (CAD))

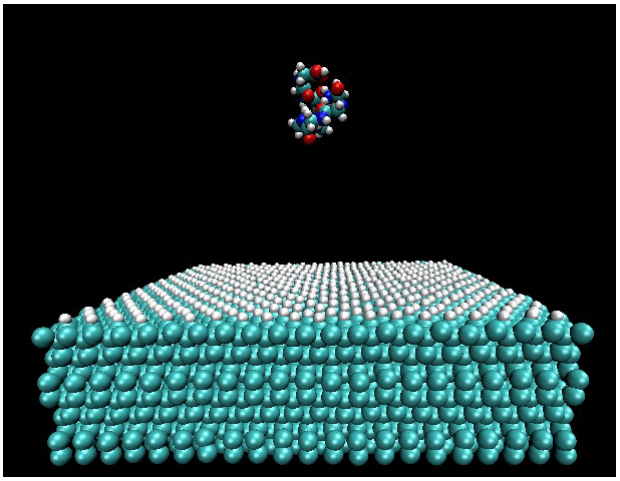
interazioni con elettroni (electron capture dissociation ECD)
(electron transfer dissociation ETD)

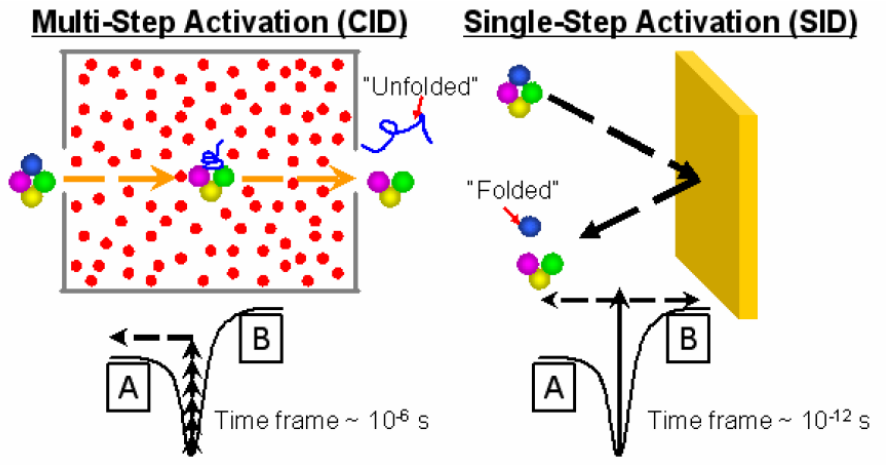
interazioni con superfici (collisioni ioni/superficie)

interazioni con fotoni (IRMPD, ion spectroscopy)

Interazioni con superfici (collisioni ioni/superficie)

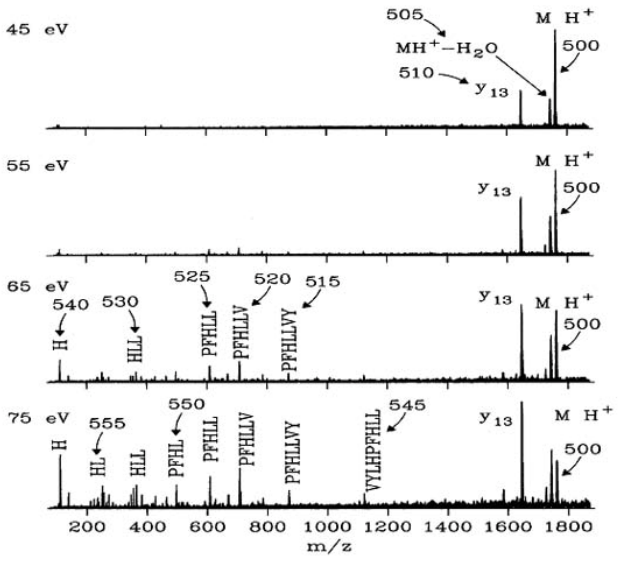






Alternative pathways that precede significant structural change seem to become accessible.

Comparison of CID and SID activation methods, illustrating a possible explanation for the different dissociation pathways observed from these techniques



Fragmentation spectra generated by Surface Induced Dissociation showing wide sequence coverage and strong evidence of mixing of low and high energy dissociation channels

Decomposizioni indotte da:

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

interazioni con elettroni (electron capture dissociation ECD)
(electron transfer dissociation ETD)

interazioni con superfici (collisioni ioni/superficie)

interazioni con fotoni (IRMPD, ion spectroscopy)

interazioni con fotoni (IRMPD, ion spectroscopy)

$$[M + H]^+ + n\,h\nu \dashrightarrow [M + H]^{+**} \longrightarrow \text{fragments}$$

Infrared Multiphoton Dissociation (IRMPD)

Wavelength = 943 cm^{-1} Power = 25 - 40 W

Ion spectroscopy

Wavelength = 800-1800 cm^{-1} Power = 0.5 - 100 MW

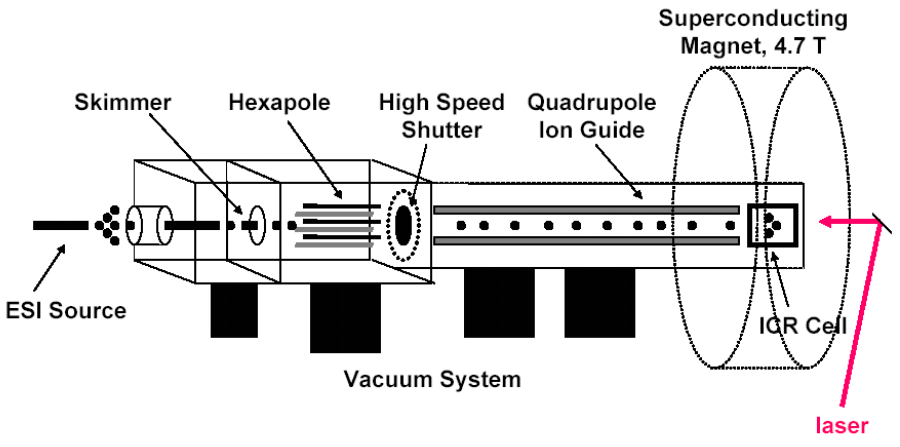
IRMPD
infrared multi photon dissociation

IRMPD can be used on any instrument that traps and confines ions in a small volume; this raises the cross-section and interaction time for reaction between the ions and a focused IR laser beam.

- quadrupole and linear ion traps, FTICR instruments are OK.
- tandem TOFs (TOF/TOF or TOF2) are not.

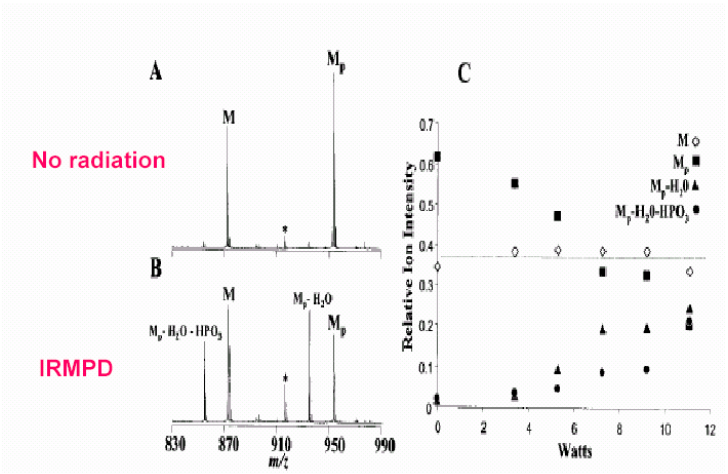
- does not depend on molecular size
- however, the absorption of multiple photons is similar to multiple collisions favoring breakage of weak bonds and loss of labile groups

Infrared multiphoton dissociation (IRMPD) in FT-ICR



IonSpec 4.7 T FT-ICR, Electrospray Ionization (ESI) Source and Ion Guide

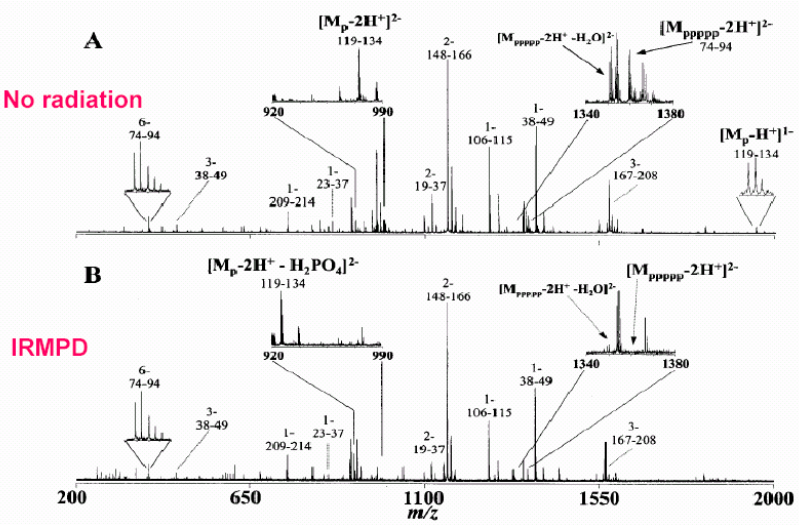
Characteristic IRMPD fragmentation of phosphorilated peptides



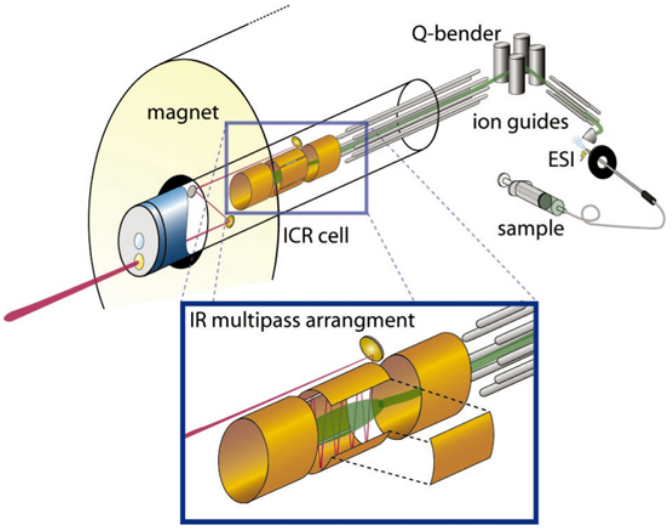
Flora, J. W.; Muddiman D. C. *Anal. Chem.* **2001**, *73*, 3305-3311

Tryptic digest of β-casein

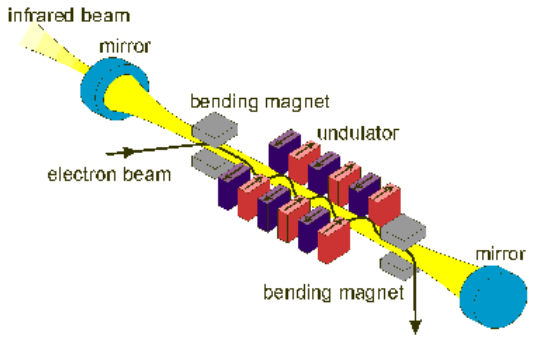
Flora, J. W.; Muddiman D. C. *Anal. Chem.* **2001**, *73*, 3305-3311



Ion spectroscopy



Free Electron Laser



'Conventional' lasers use excited atoms or molecules to amplify light.
Free-electron lasers (FEL) use a high-energy electron beam as an amplifying medium.

The electron beam emits light as it wiggles through a periodic magnetic structure called ondulator. The light is stored in in an optical cavity, and can interact back with the electrons. This interaction leads to a modulation of the electronic density, and a growth in intensity and coherence of the emitted light.

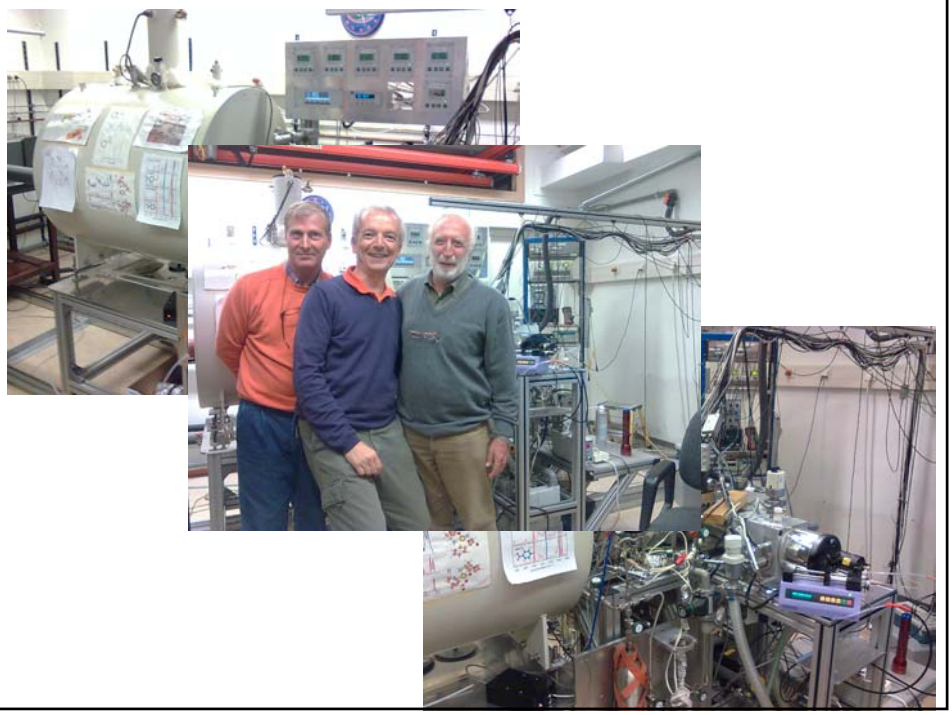
FELIX

The FELIX facility consists of the FELIX beamlines (FEL1 and FEL2) and the FELICE beam line, which is described elsewhere. The FELIX beam lines produce coherent radiation in the wavelength range of 3 - 250 µm. The specifications of FELIX can be found in the table.

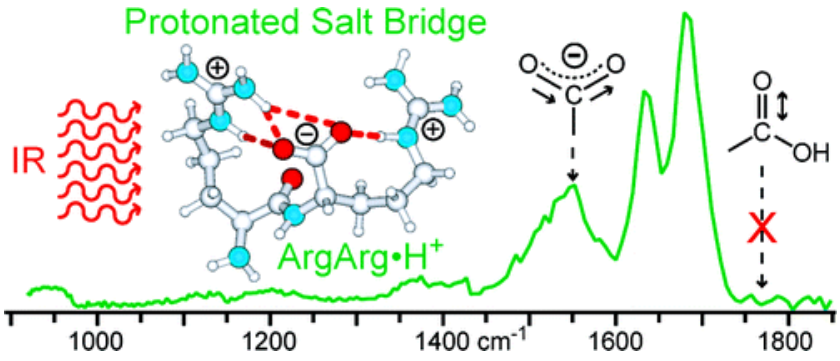
FELIX Specifications

wavelenght range	3-250 µm
continuous tuning range	factor 3
micropulse energy	1 - 50 µJ
micropulse power	0.5 - 100 MW
micropulse repetition rate	1 GHz or 25 MHz
macropulse repetition rate	5 (10) Hz
micropulse duration	6 - 100 optical cycles
macropulse duration	< 10 µs
spectral bandwidth	0.4 - 7 %
polarization (linear)	> 99 %

terahertz radiation refers to electromagnetic waves sent at frequencies in the terahertz range. It is also referred to as **submillimeter radiation**, **terahertz waves**, **terahertz light**, **T-rays**, **T-light**, **T-lux** and **THz**.
The term is normally used for the region of the electromagnetic spectrum between 300 gigahertz ($3x10^{11}$ Hz) and 3 terahertz ($3x10^{12}$ Hz), corresponding to the submillimeter wavelength range between 1 millimeter (high-frequency edge of the microwave band) and 100 micrometer (long-wavelength edge of far-infrared light).

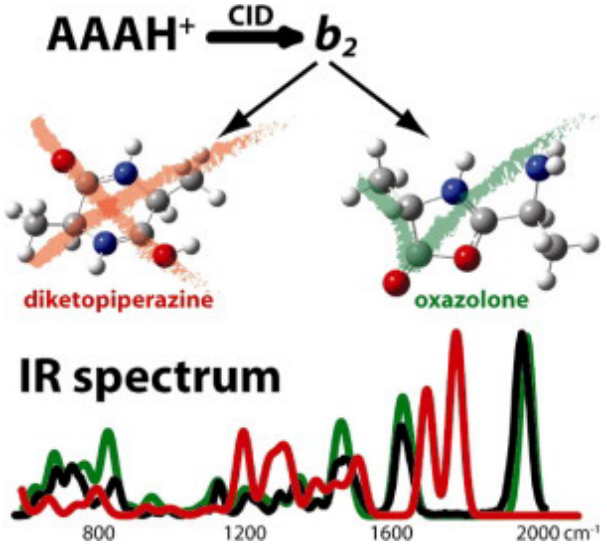


Structures of Protonated Dipeptides: The Role of Arginine in Stabilizing Salt Bridges



Prell JS et al, *J. Am. Chem. Soc.* **131**, 11442-1149 (2009)

Spectroscopic Evidence for an Oxazolone Structure of the b_2 Fragment Ion from Protonated Tri-Alanine



Oomens J et al, *J. Am. Soc. Mass. Spectrom.* **20**, 334 (2009)

Spettrometria di massa

Spettro di massa ⟶ Peso molecolare

HR + massa accurata ⟶ Stechiometria

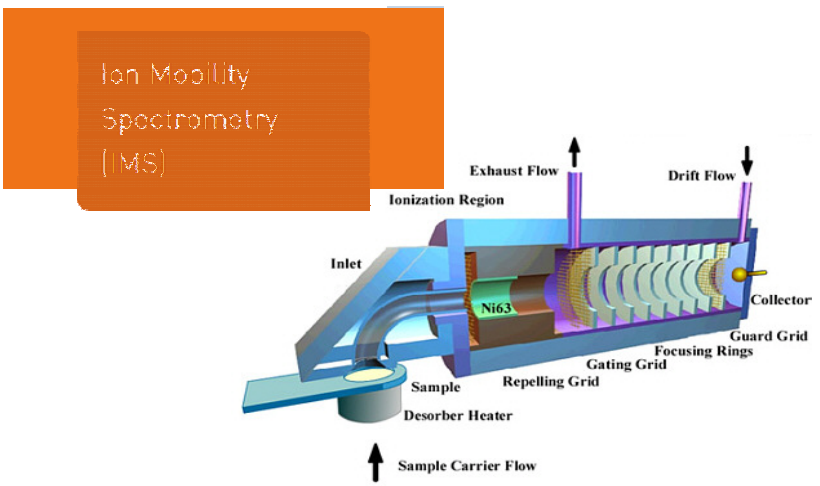
MS^n ⟶ Informazioni strutturali

? ⟵ Conformazione

Ion mobility

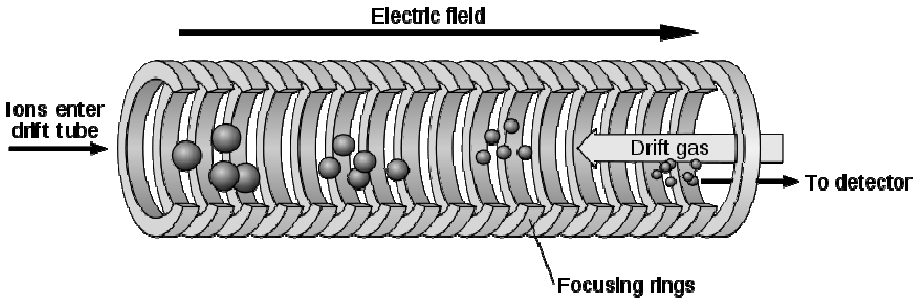
IMS was first developed primarily by Earl W. McDaniel in the 1950s and 1960s

- An ion mobility experiment consists in introducing ions into a pressure region (called “drift tube”) across which an electric field is uniformely applied.
- The uniform field is generated by connecting a series of evenly spaced rings with equal value resistors.



IMS uses soft ionization techniques, such as **^{63}Ni** or **corona discharge** (APCI).
^{63}Ni’s β emission acts as an ionization source, stripping electrons from the molecules that are given off by a material and analyzing the resulting ions in the device.
Another ionization technique is APPI.

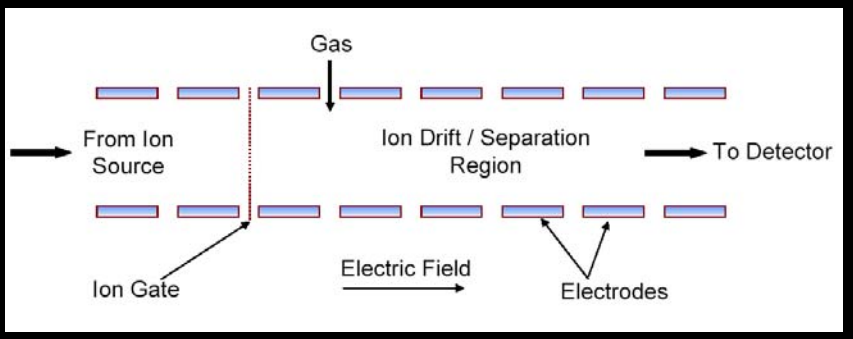
Scheme of an IMS Device

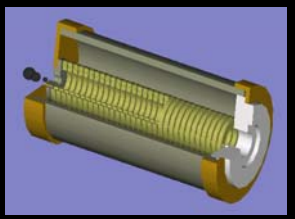


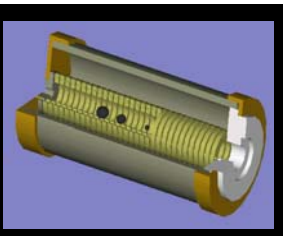
$$v_d = K\,E$$

The physical quantity ion mobility K is defined as the proportionality factor of the ion's drift velocity v_d in a gas and the strength E of the electric field.

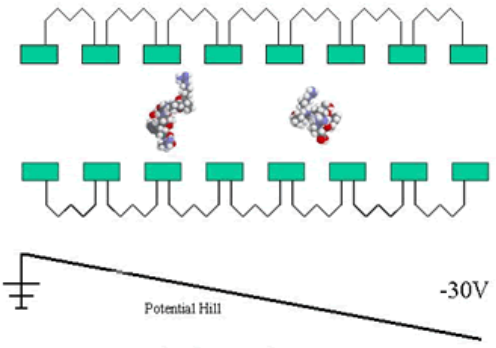
Ion Mobility





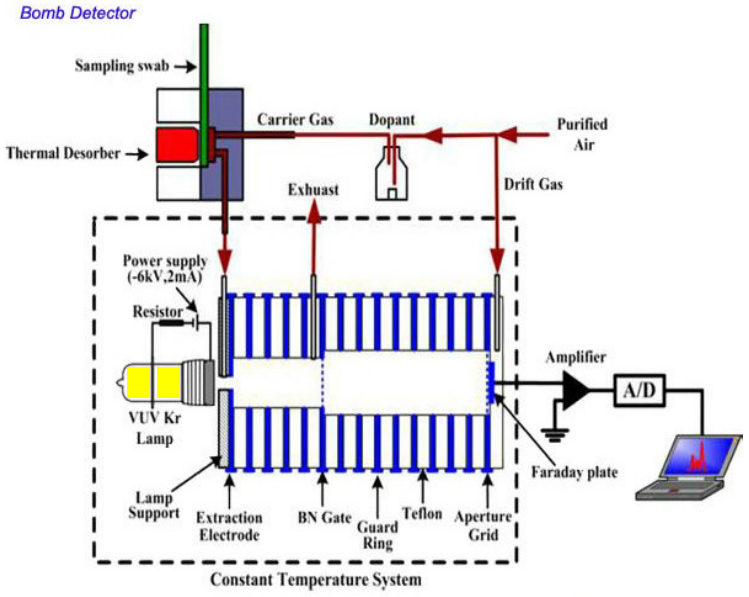


Ion Mobility

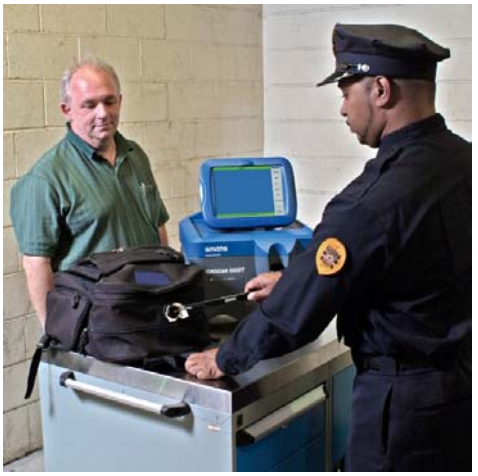


$$K = \frac{\sqrt{18 \cdot \pi}}{16} \cdot \sqrt{\frac{1}{m} + \frac{1}{m_b}} \cdot \frac{z \cdot e}{\sqrt{k_b \cdot T}} \cdot \frac{1}{\Omega} \cdot \frac{1}{N}$$

PIMS technology: Photoionization Ion Mobility Spectrometry



Ion Mobility in the Common Life



Over **50000** stand-alone ion mobility spectrometers are currently employed throughout the world for the detection of **explosives**, **drugs**, and **chemical-warfare** agents.

Explosives Detectors for Airport Security
EDS – Explosives Detection Systems

www.xraysecuscan.com

Agents Detected Nerve, blood, blister, choking and a selected library of Toxic Industrial Chemicals

http://www.smithsdetection.com/index.php/products-solutions/chemical-agents-detection/59-chemical-agents-detection/lcd-3-3.html#.VIxdGk10zDc

Explosives, Narcotics, CWA/TICs

Analysis time Detection in 10 seconds, complete analysis in 20 seconds

Explosives detected	RDX, PETN, TNT, Semtex, TATP, NG, Ammonium Nitrate, H_2O_2 and others
Drugs detected	Cocaine, Heroin, THC, Methamphetamine and others
Toxic industrial chemicals detected	Hydrogen Cyanide (HCN), Phosgene, SO, NH and others
Chemical warfare agents detected	Nerve and blister agents such as Tabun, Sarin, Soman, Cyclosarin, Agent VX and Vx

Dual ion mobility spectrometry

Feature Highlights

- **Simultaneous detection of explosives and narcotics**
- **Over 40 substances detected and identified in 5-8 seconds**
- **Large touch-screen color display**
- **Built-in thermal printer**
- **Internal data storage**

General Specifications

Technology	Dual Ion Mobility Spectrometry (IMS)
Operating modes	Explosives/Narcotics simultaneous, Explosives only, Narcotics only
Explosives detected	RDX, PETN, NG, TNT, HMX, TATP and others
Narcotics detected	Cocaine, Heroin, Amphetamine, Methamphetamine, MDA, THC and others
Sensitivity	Explosives: picogram range Narcotics: sub-nanogram range
Analysis time	5-8 seconds

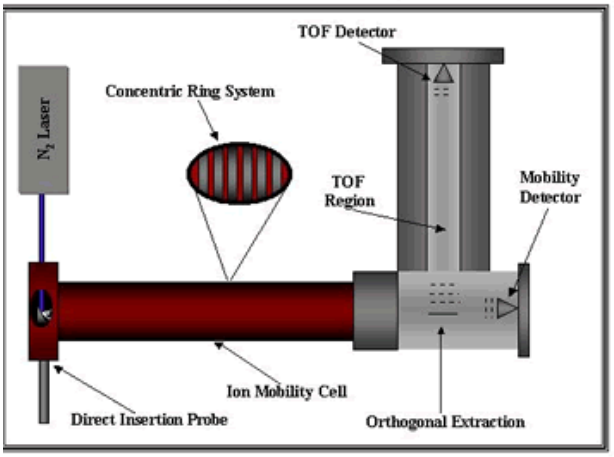
Detectors based on ion mobility spectrometry using ^{63}Ni can now satisfy enhanced Homeland Security requirements at airports and other sensitive locations.

Ion Mobility Mass Spectrometry (IMMS)

Inserire un dispositivo ion mobility all'interno di uno spettrometro di massa

David E. Clemmer , 1995

Ion Mobility-Time-of-Flight

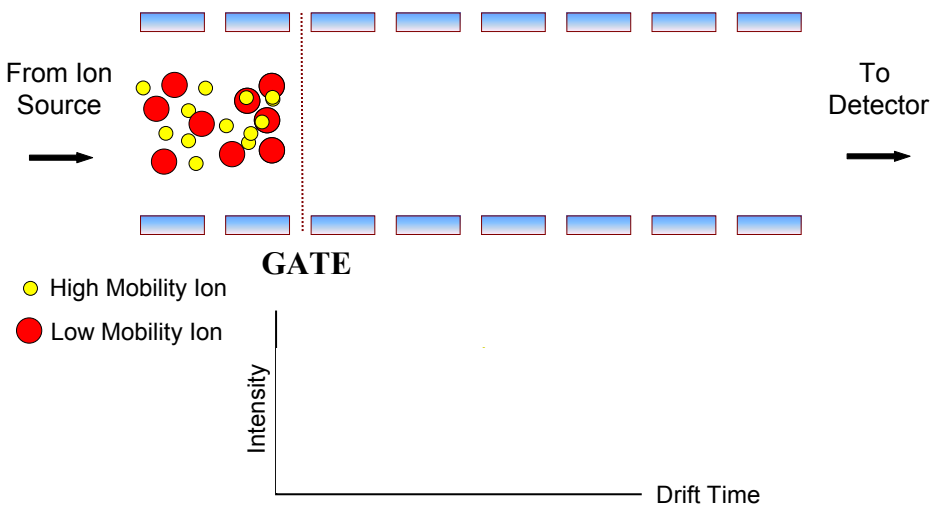


Areas of Interest

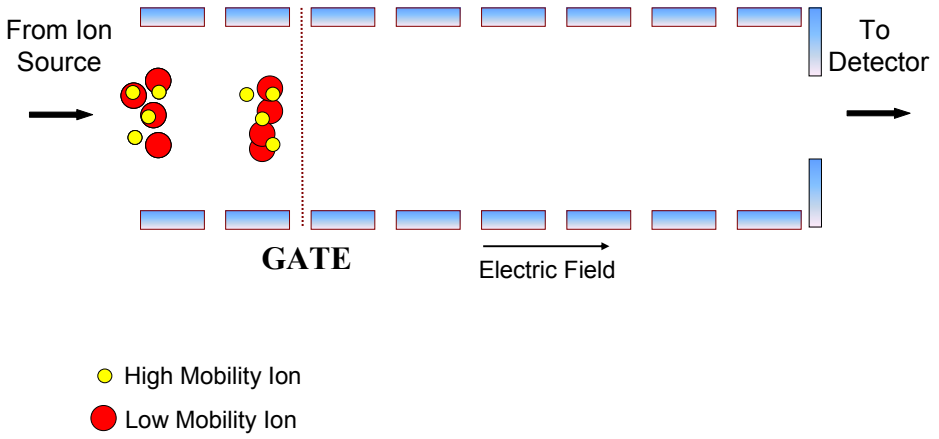
- Proteomics
- Gas Phase Clusters
- Peptide/Protein Conformation
- MALDI Fundamentals
- DNA/RNA
- Further Instrument Development
- Non-covalent complexes

The MALDI-IM-o-TOF instrument was built in-house, and consists of a 12 inch drift-cell (which separates ions based on their collision cross-section) and a 20cm time-of-flight (for post-separation mass analysis). MALDI is performed at the operating pressure of the drift cell (5-10 torr He), and separation occurs in a linear electric field, supplied by a system of concentric ring electrodes.

Conventional ion mobility spectrometers



Pre-Trapping Ion Mobility



The time required for the ions to reach the detector depends upon the ion's

- **Collision cross section** (averaged over all possible orientation of the ion);
- **Charge state** ions with higher charge states experience a higher electric force, and hence travel at a higher velocity, compared to ions with lower charge states
- **Mass**
- **Drift tube operating parameters** (electric field strength, drift tube length, buffer gas pressure, temperature)

Some definitions:

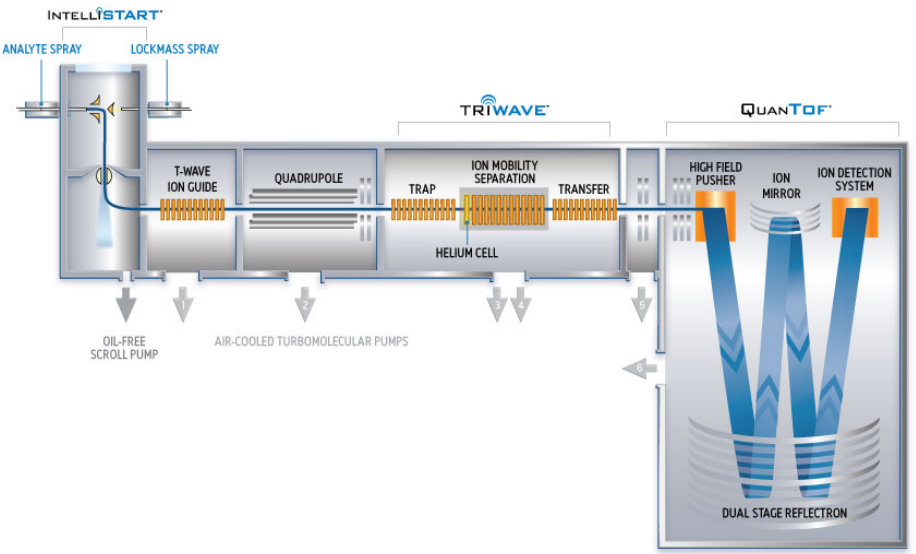
Reduced ion mobility

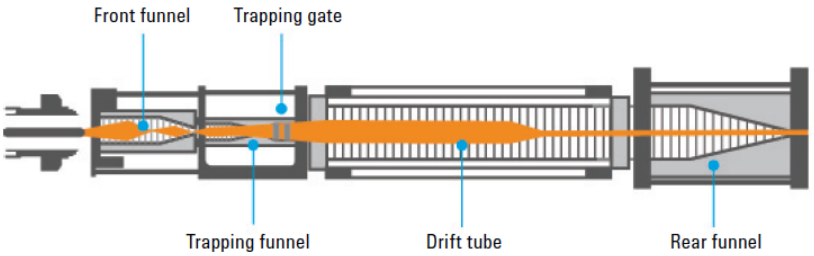
$$K_0 = L*P*273 / t_D*E*760*T$$

Where t_D is the drift time, L the length of the drift tube, P the pressure, E the electric field strength and T the temperature

Collision cross section (Ω)

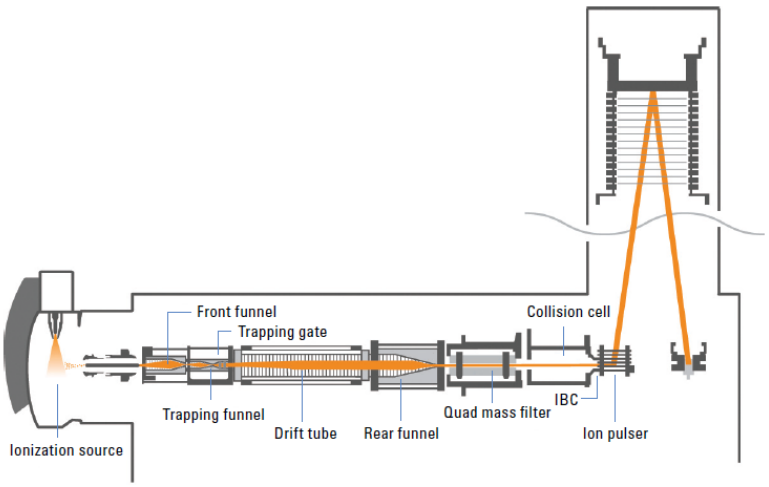
$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_B} \right]^{1/2} \frac{t_D E}{L} \frac{760}{P} \frac{T}{273} \frac{1}{N}$$



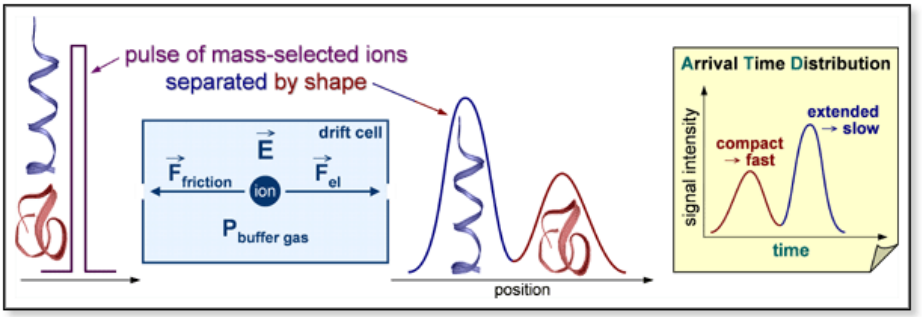


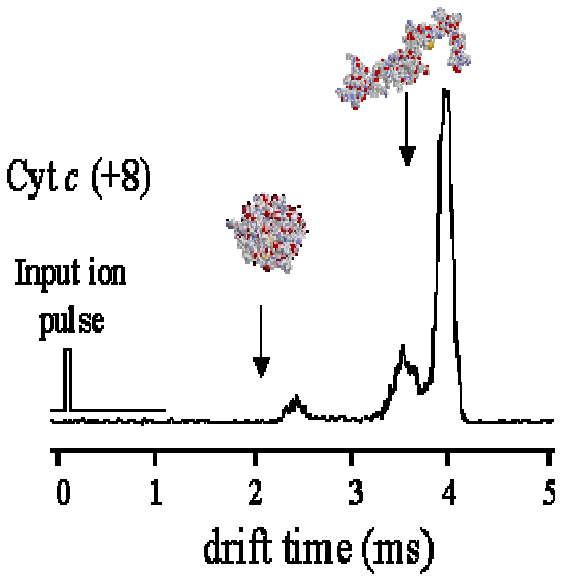
Schematic diagram of the ion mobility instrument. Ions generated in the source region are carried into the front ion funnel through a single bore capillary. The front ion funnel improves the sensitivity by efficiently transferring gas phase ions into the trapping funnel while pumping away excess gas and neutral molecules. The trapping funnel accumulates and releases ions into the drift tube. The drift cell is ~80 cm long and generally operated at 20 V/cm drift field. Ions exiting the drift tube enter the rear ion funnel that efficiently refocuses and transfers ions to the mass analyzer.

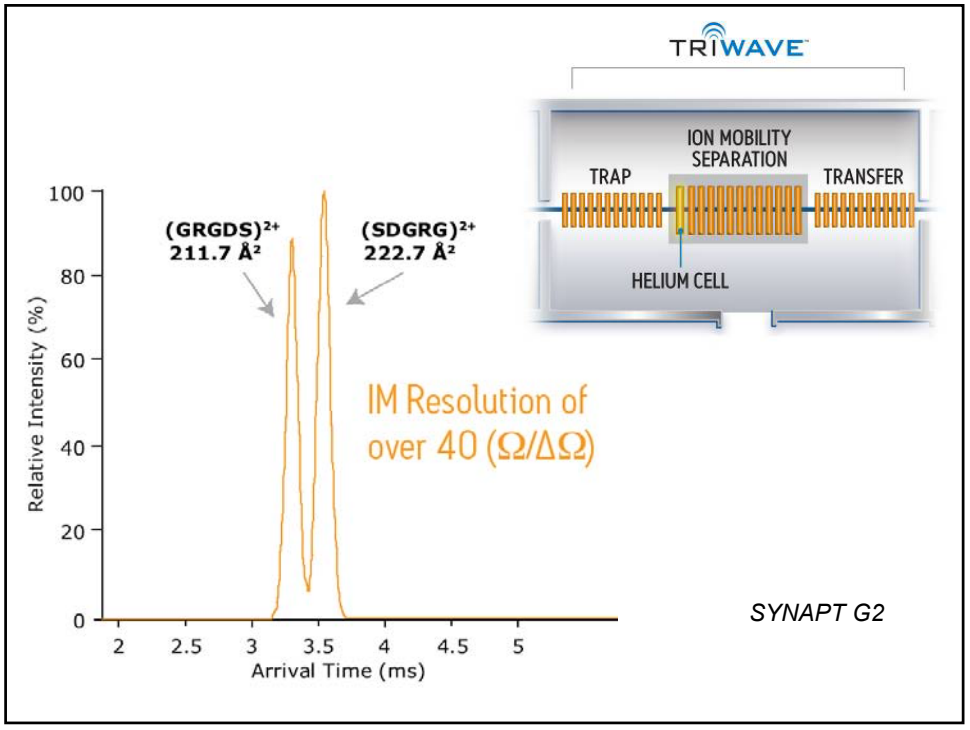
The key function of the front ion funnel is to enrich the sample ions and remove excess gas. The continuous ion beam from the electrospray process has to be converted into a pulsed ion beam prior to ion mobility separation. The trapping funnel operates by first storing and then releasing discrete packets of ions into the drift cell.

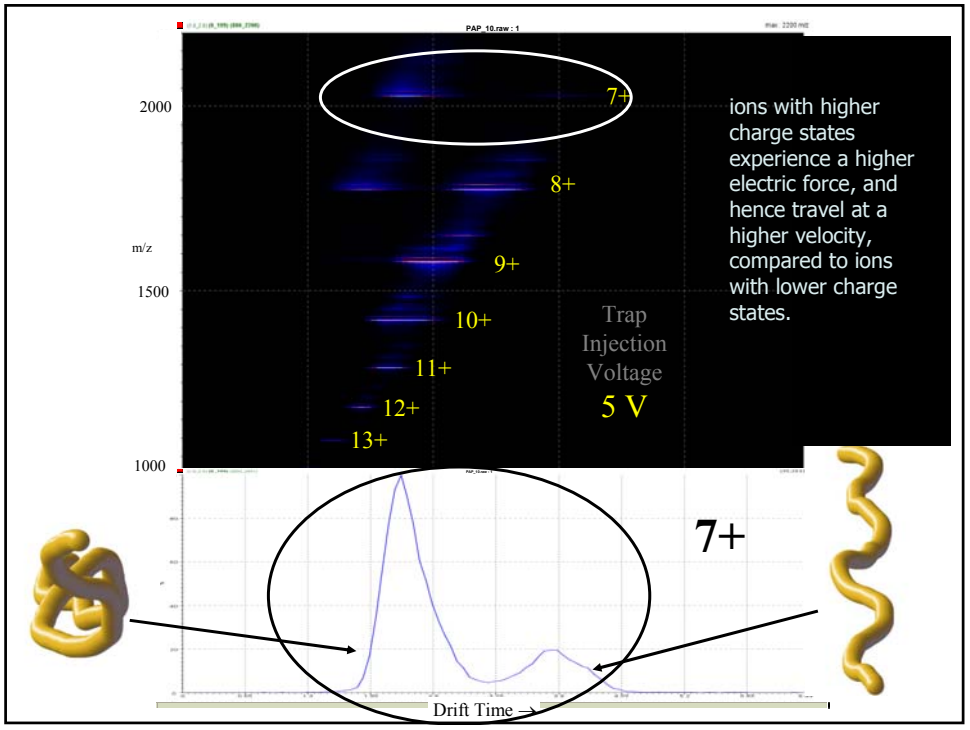


Schematic of the Agilent IM-QTOF instrument. The ion mobility spectrometer is coupled to a quadrupole time-of-flight mass spectrometer using a hexapole ion guide.


pulse of mass-selected ions
separated by shape
drift cell
E
F friction
ion
F el
P buffer gas
position
Arrival Time Distribution
signal intensity
compact
→ fast
extended
→ slow
time

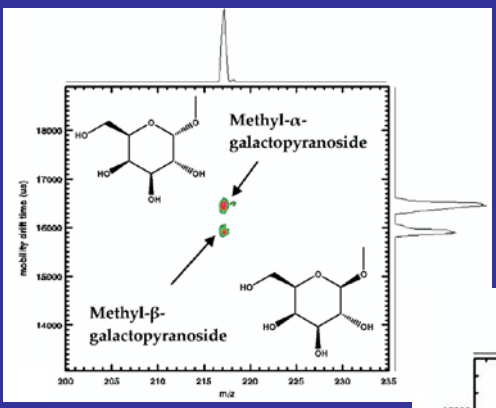

Cyt c (+8)
Input ion
pulse
0
1
2
3
4
5
drift time (ms)

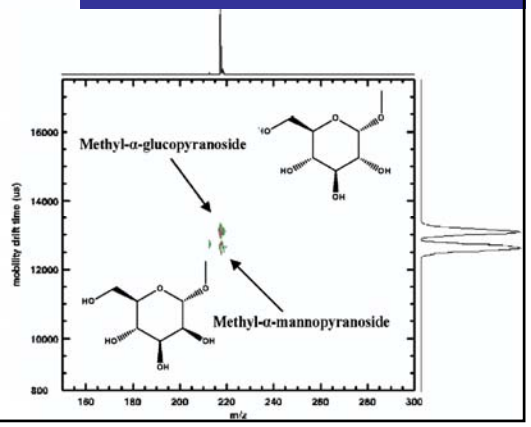

TRIWAVE
ION MOBILITY SEPARATION
TRAP
TRANSFER
HELIUM CELL
(GRGDS)2+ 211.7 Å2
(SDGRG)2+ 222.7 Å2
IM Resolution of over 40 (Ω/ΔΩ)
Relative Intensity (%)
Arrival Time (ms)
SYNAPT G2


m/z
2000
1500
1000
7+
8+
9+
10+
11+
12+
13+
Trap Injection Voltage 5 V
ions with higher charge states experience a higher electric force, and hence travel at a higher velocity, compared to ions with lower charge states.
7+
Drift Time

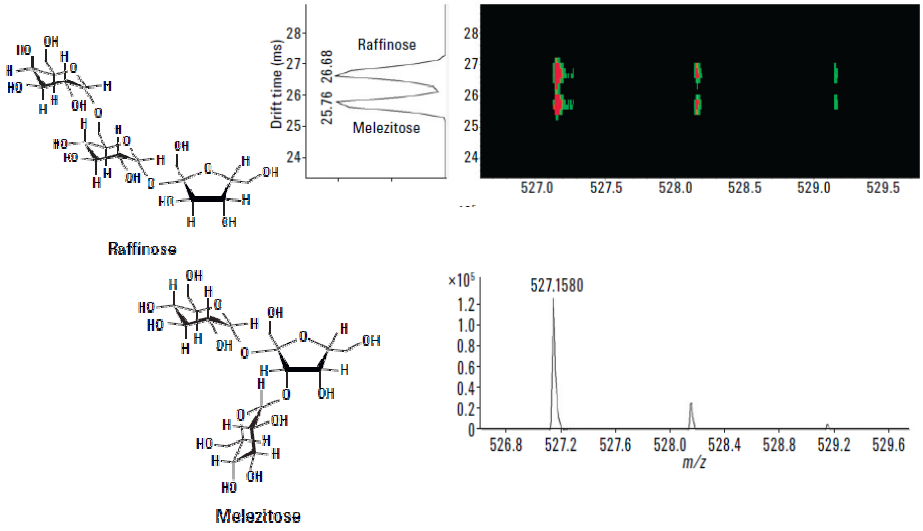
ESI-APIMS-TOFMS

[Sugar-Na]$^+$ adducts

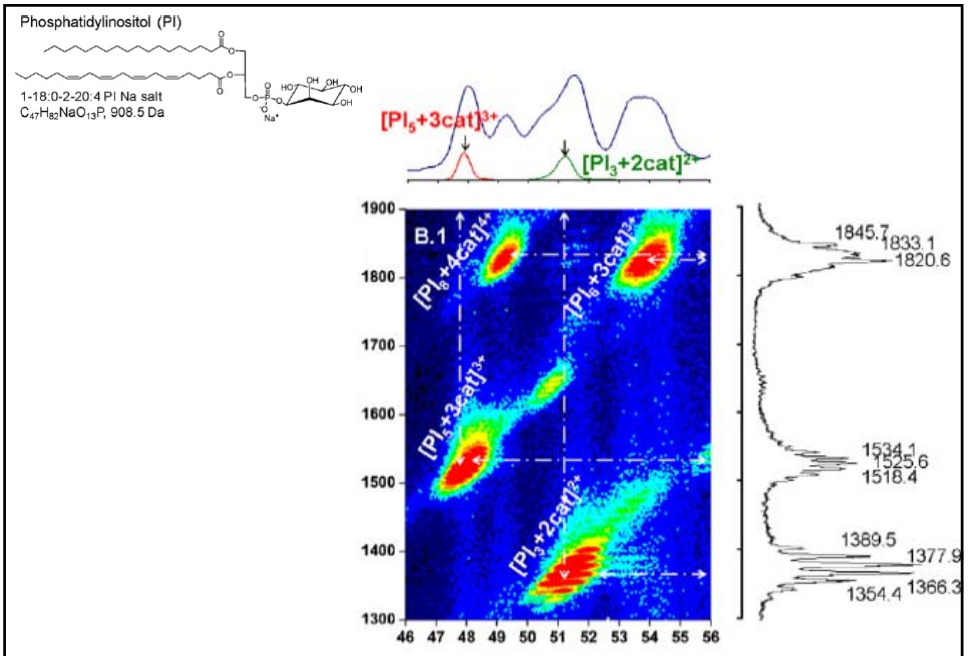




P Dwivedi, B Bendiak, BH Clowers, HH. Hill Jr, *J. Am. Soc. Mass Spectrom.* **18**, 1163 (2007)



Separation of isobaric tri-saccharides using the IM-QTOF. A 1:1 mixture of melezitose and raffinose was infused using a syringe pump. These two carbohydrates can be baseline separated using the ion mobility drift cell and detected using the Q-TOF mass analyzer as sodium adducts. The ion mobility resolving power for this separation is 60.



D.E. Clemmer et al. *Int. J. Mass Spectrom.* **287**, 58–69 (2009)

Spettrometria di massa

Spettro di massa ⟶ Peso molecolare

HR + massa accurata ⟶ Stechiometria

MS^n ⟶ Informazioni strutturali

Ion Mobility ⟶ Conformazione

? ⟵ **Mappa degli analiti**

Spettrometria di massa

Spettro di massa ⟶ Peso molecolare

HR + massa accurata ⟶ Stechiometria

MS^n ⟶ Informazioni strutturali

Ion Mobility ⟶ Conformazione

MS Imaging ⟶ **Mappa degli analiti**

? ⟵ Analisi quantitativa

Analisi quantitativa

Campione biologico
matrice organica complessa

↓

Aliquota di campione ← **Aggiunta Standard Interno**

↓

Estrazione

↓

Purificazione/Separazione

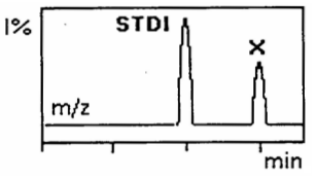
↓

ANALISI **GC-, HPLC-MS, MS^n**

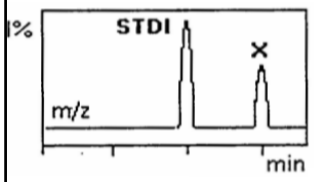
Si monitora uno ione (MS, SIM) o una reazione (MS^n, SRM) o più reazioni (MS^n, MRM)

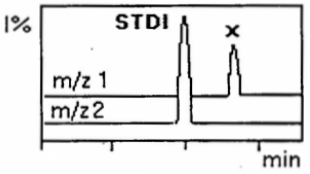
SCELTA DELLO STANDARD INTERNO

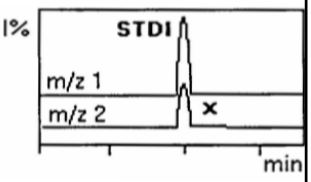
1 - Omologo dell'analita in esame; si può focalizzare sullo stesso valore di m/z ma deve avere un tempo di ritenzione (rt) diverso:



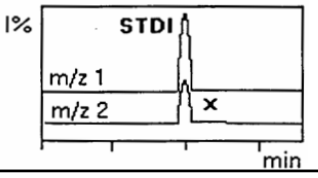
2 – Composto della stessa classe che può avere lo stesso valore di m/z (nel qual caso deve avere diverso rt) o un valore diverso di m/z per cui può avere o meno lo stesso rt:





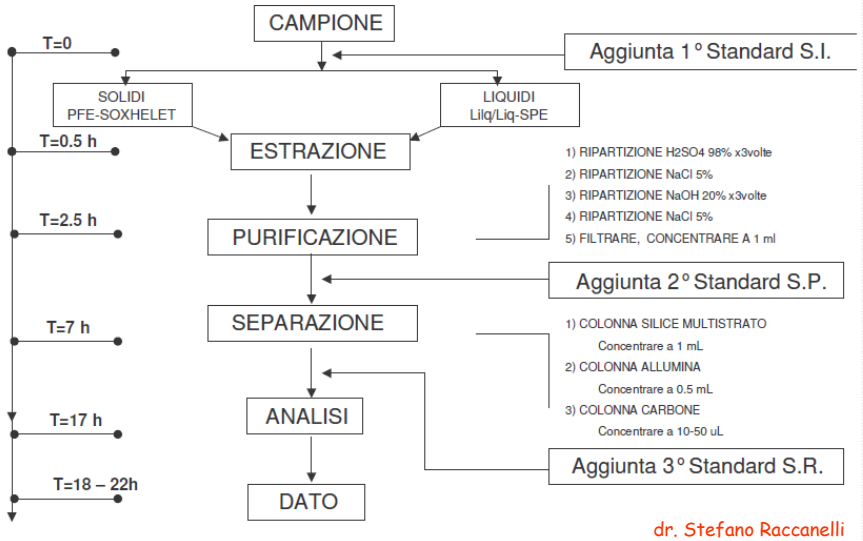


3 – Un isotopomero dell'analita (^2H, ^{13}C, ^{18}O, ^{15}N), che avrà quindi lo stesso rt ma un valore di m/z diverso:



Consorzio Interuniversitario Nazionale la Chimica per l'Ambiente

ANALISI PCDD/F: PREPARAZIONE DEI CAMPIONI



dr. Stefano Raccanelli

ANALISI PCDD/F: PERCHE' LA DILUIZIONE ISOTOPICA?

RESE % ???

(1)	Standard Interno S.I.	15 congeneri 2,3,7,8 $^{13}C_{12}$ da TCDD/F a OCDD (purezza ≥ 99%)
(2)	Standard Purificazione S.P.	2,3,7,8 $T^{37}C_{14}DD$ (purezza ≥ 96%)
(3)	Standard Recupero S.R.	1,2,3,4 $T^{13}C_{12}DD$ 1,2,3,7,8,9 $Hx^{13}C_{12}DD$ (purezza ≥ 99%)

$$RESA = \frac{pg\ S.I.\ x}{pg\ S.R.\ y} \times 100$$

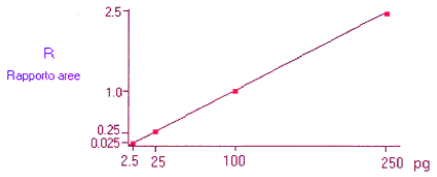
dr. Stefano Raccanelli

Analisi quantitativa

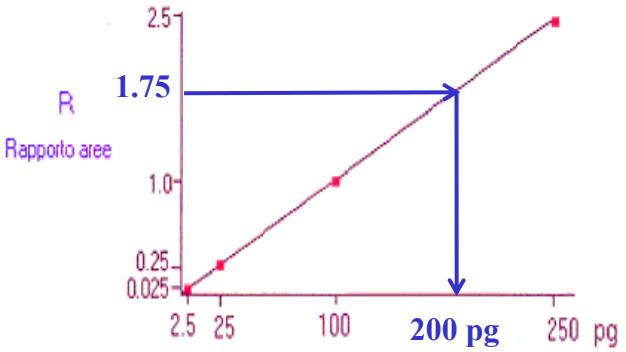
Metodo della diluizione isotopica (ID)

	⓪	①	②	③	④	
Analita	0	2.5	25	100	250	pg/ul
Analita-d_3	100	100	100	100	100	pg/ul
Rapporto	0	0.025	0.25	1.0	2.5	

Si monitora uno ione (MS, SIM) o una reazione (MS^n, SRM) o più reazioni (MS^n, MRM)

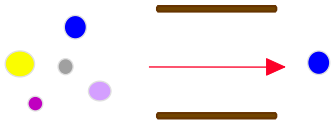


Campione reale: rapporto aree= 1.75



Selected Ion Monitoring (SIM)

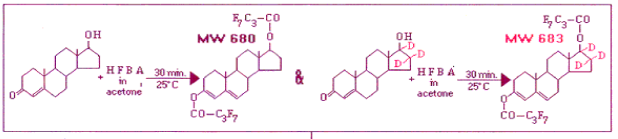
Selected Ion Recording (SIR)



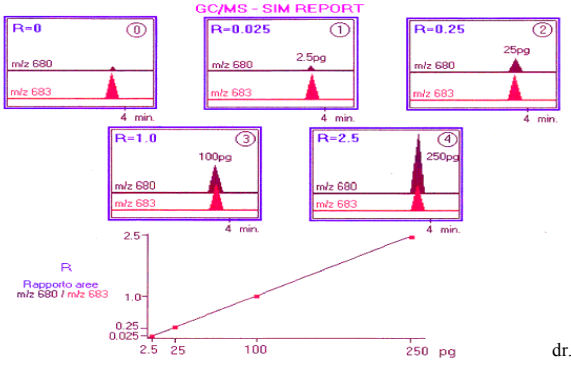
CURVA DI TARATURA TESTOSTERONE

	0	1	2	3	4	
testosterone	0	2.5	25	100	250	pg/ul
testosterone-d_3	100	100	100	100	100	pg/ul
Rapporto	0	0.025	0.25	1.0	2.5	

DERIVATIZZAZIONE



GC/MS - SIM REPORT



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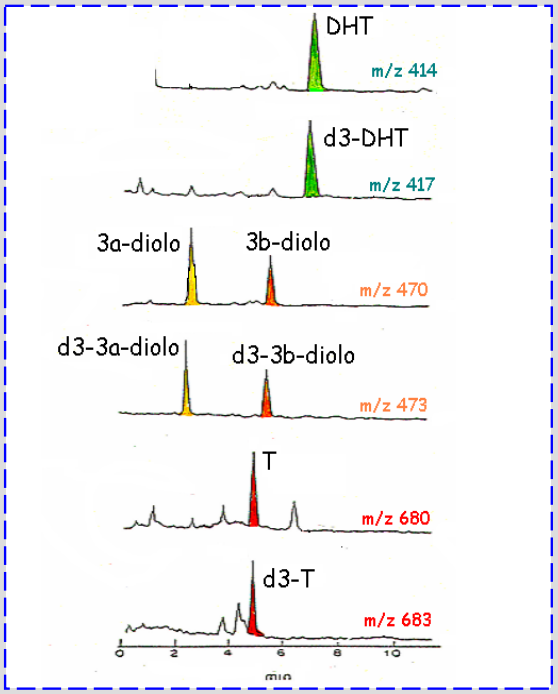

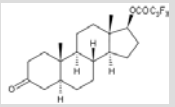

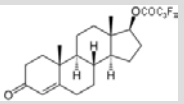

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DOSAGGIO DI STEROIDI IN SIERO UMANO IN *GC-MS ID*

CORTISOLO

(0.5 - 1 ml)
↓ +STDI ^2H_2C
una notte a 5°C
↓ estrazione su Extrelut
eluito con 6 ml di CH_2Cl_2
↓ portati a secco
+ metossiamina cloridrato in piridina anidra
↓ 2 ore a 60°C
a secco sotto azoto a 60°C
↓
+ 20µl di BSTFA con il 2% di bromosilano
↓ 1 ora a 90°C
a secco e ripreso con eptano
↓
GC/MS
SIM
m/z 636 m/z 638
area ratio 636/638

PROGESTERONE

(1 - 3 ml)
↓ +STDI ^2H_3P
una notte a 5°C
↓ estrazione su Extrelut
eluito con 10 ml di esano
↓ portati a secco
+ 75µl di acetone e25µl di HFBA
↓ 1 ora a T.A.
a secco e ripreso con eptano
↓
GC/MS
SIM
m/z 510 m/z 513
area ratio 510/513

TESTOSTERONE

(2 - 6 ml)
↓ +STDI ^2H_3T
una notte a 5°C
↓ estrazione su Extrelut
eluito con 15 ml di CH_2Cl_2
↓ portati a secco
ripreso con 150µl di eluente per Sephadex
↓
depositato su colonne di Sephadex LH20 (0.8g)
↓
eluito con esano:$CHCl_3$:MeOH (80:10:10)
↓ portati a secco
+ 75µl di acetone e25µl di PFPA
↓ 1 ora a T.A.
a secco e ripreso con eptano
↓
GC/MS
SIM
m/z 580 m/z 583
area ratio 580/583

ESTRADIOLO

(3 - 6 ml)
↓ +STDI $^2H_3E_2$
una notte a 5°C
↓ estrazione su Extrelut
eluito con 15 ml di CH_2Cl_2 con l'1% di CH_3COOH
↓ portati a secco
ripreso con 200µl di eluente per Sephadex
↓
depositato su colonne di Sephadex LH20 (1.0g)
↓
eluito con CH_2Cl_2:MeOH:CH_3COOH (94:5:1)
↓ portati a secco
+ 75µl di acetone e25µl di PFPA
↓ 1 ora a T.A.
a secco e ripreso con eptano
↓
GC/MS
SIM
m/z 564 m/z 567
area ratio 564/567

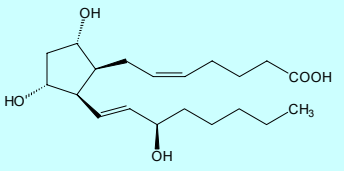
dr. Giuseppe Pieraccini

GC-NICI-ITMS/MS

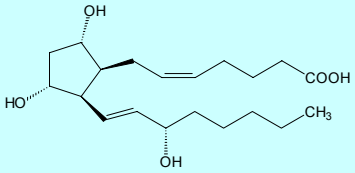
Ion Trap Tandem Mass Spectrometric Determination of F_2-Isoprostanes

Cinzia Signorini, Mario Comporti, Gianluca Giorgi *, *J. Mass Spectrom.* **38**, 1067-1074 (2003)

- F_2-isoprostanes, are formed *in vivo* by non-enzymatic free radical-induced oxidation of arachidonic acid.
- 8-epi-$PGF_{2\alpha}$, the most abundant isomer formed, can exist as two main distereoisomers differing in the stereochemistry at C(15), namely 15(R) and 15(S).
- F_2-Isoprostanes have many characteristics to be considered as a reliable index of oxidative stress *in vivo*.

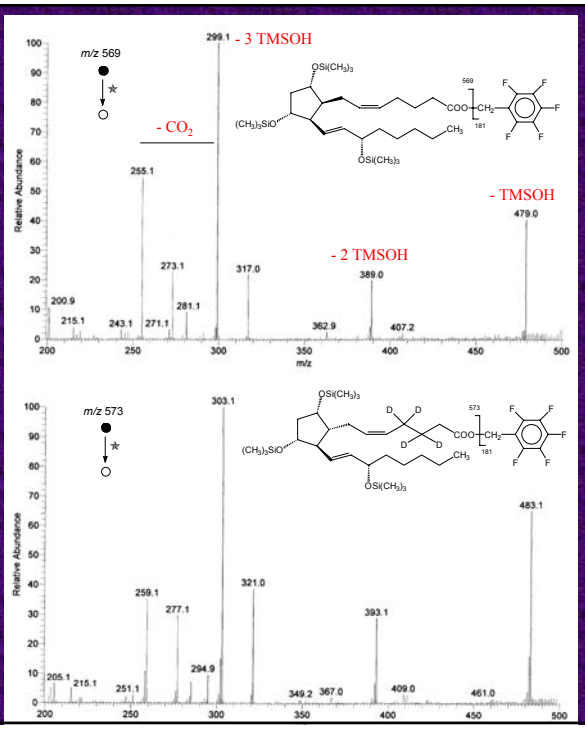


8-epi-15(R)-$PGF_{2\alpha}$

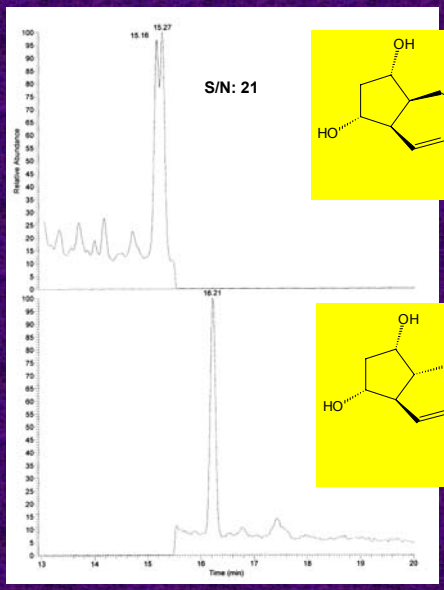


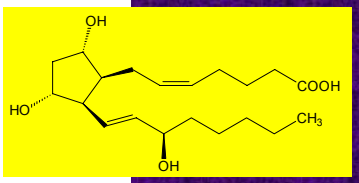
8-epi-15(S)-$PGF_{2\alpha}$

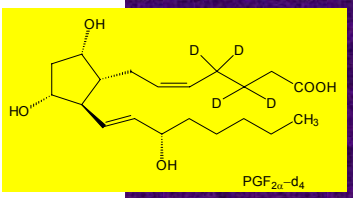
GC-NICI-ITMS/MS of isoprostanes



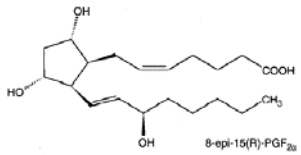
GC-NICI-ITMS of isoprostanes



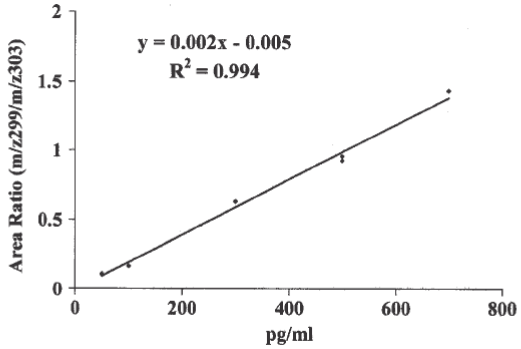




Reconstructed ion chromatogram obtained under GC-NICI conditions by μscans with an ion trap mass analyser. Top: 8-epi-$PGF_{2\alpha}$ (*m/z* range: 568.5÷569.5). Bottom: $PGF_{2\alpha}$-d_4 (*m/z* range: 572.5÷573.5).

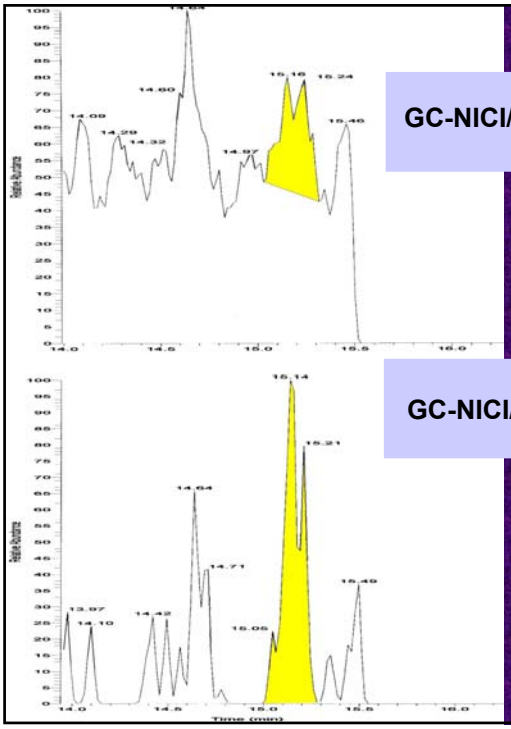


Metodo della diluizione isotopica (ID)



Calibration curve obtained by varying the concentration of 8-epi-$PGF_{2\alpha}$ in a solution containing 500 pg of $PGF_{2\alpha}$-d_4.

G. Giorgi et al. *J. Mass Spectrom.* **38**, 1067-1074 (2003)



GC-NICI/SIM

GC-NICI/MS/MS

healthy adult human plasma

GC-NICI-ITMS/MS of isoprostanes

SUMATRIPTAN

CAS Registry number: [103628-46-2]

CA name(s): 3-[2-(Dimethylamino)ethyl]-N-methyl-1H-indole-5-methanesulfonamide;

Drug code(s): GR-43175.

Derivative: Succinate

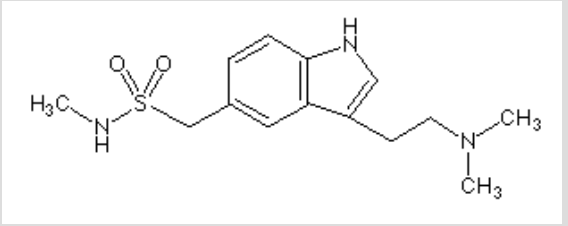
CAS Registry number: [103628-48-4]

Drug code(s): GR-43175C

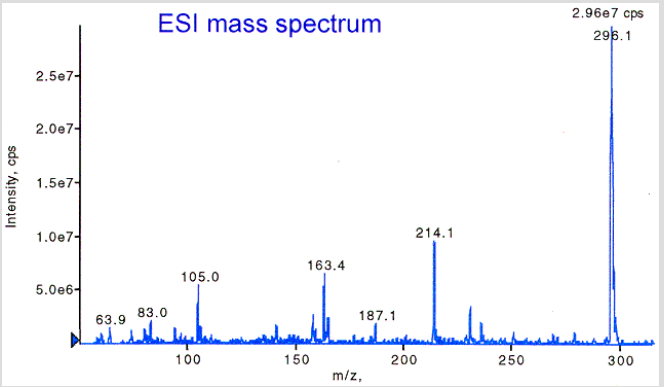
Trade name(s): Imigran (Glaxo), Imitrex (Glaxo).

THERAP. CAT.: Antimigraine.

Molecular formula: $C_{14}H_{21}N_3O_2S \cdot C_4H_6O_4$

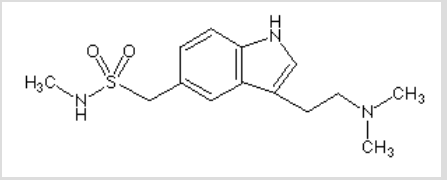


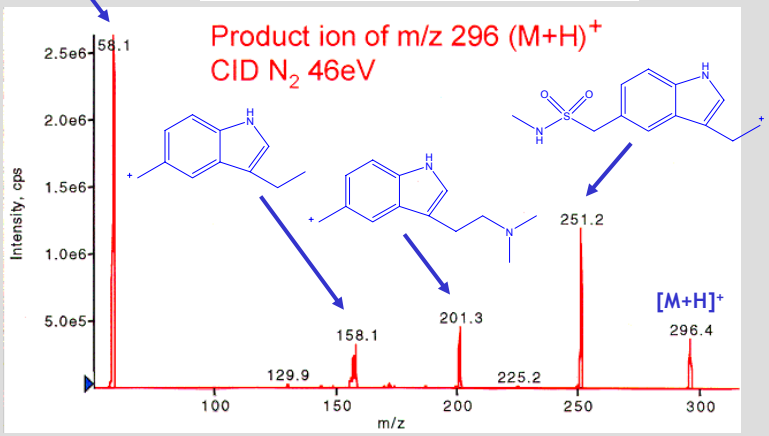
SUMATRIPTAN

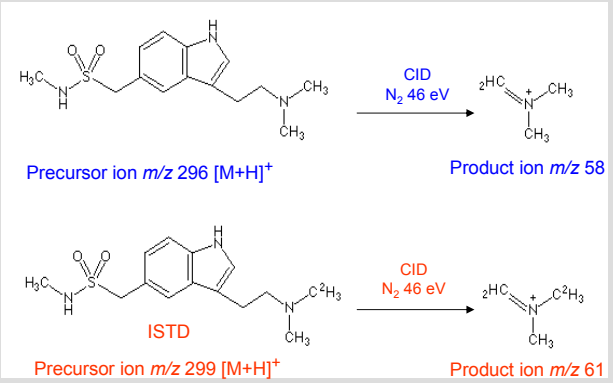


Prof. Gloriano Moneti

SUMATRIPTAN







PREPARAZIONE DEL CAMPIONE DI CSF PER L'ANALISI µHPLC-MS/MS DEL SUMATRIPTAN

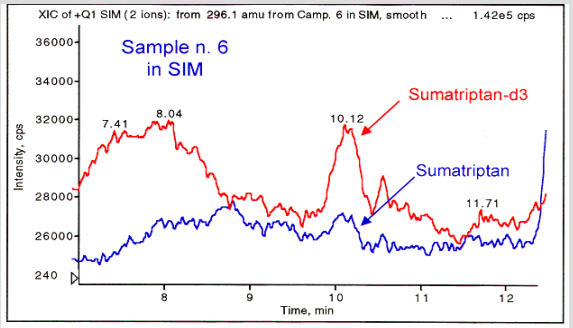
20 µL di CSF

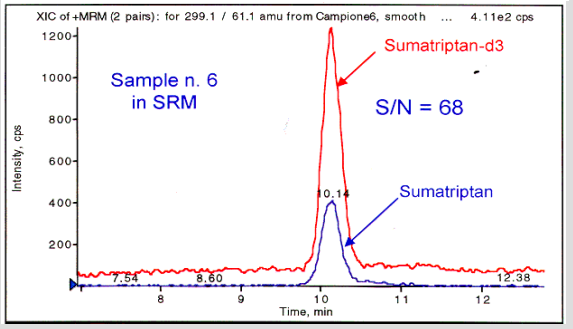
↓ • add. 40 µL IS in H_2O + 0.1%FoAc

iniettare 5 µL in µHPLC

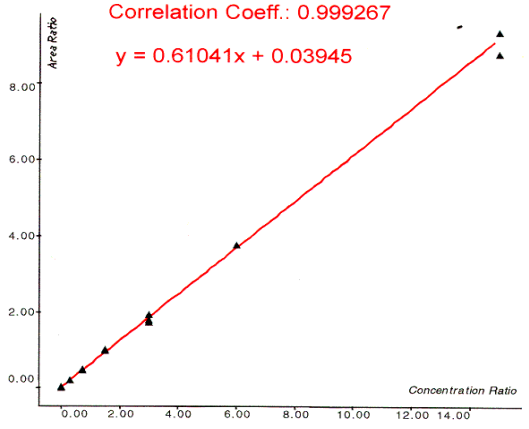
µHPLC: *ULTIMATE* - LC Packing
- Colonna: PrepMap™C18
- I.D. 300 µm, 150 mm, 3 µm
- Flusso: **5** µL/min
- A: H_2O + 0.1% FoAc
- B: CH_3CN + 0.1% FoAc
- Gradiente: time 0 min A = 95%
  time 8 min A = 40%
  time 11 min A = 40%

COMPARISON OF SIM AND SRM ANALYSES





SUMATRIPTAN CALIBRATION CURVE



Calibration range: 0 - 15 ng/mL

LOD: 300 pg/mL (0.5 pg Inj) S/N = 5.8