

Chimica delle biomolecole



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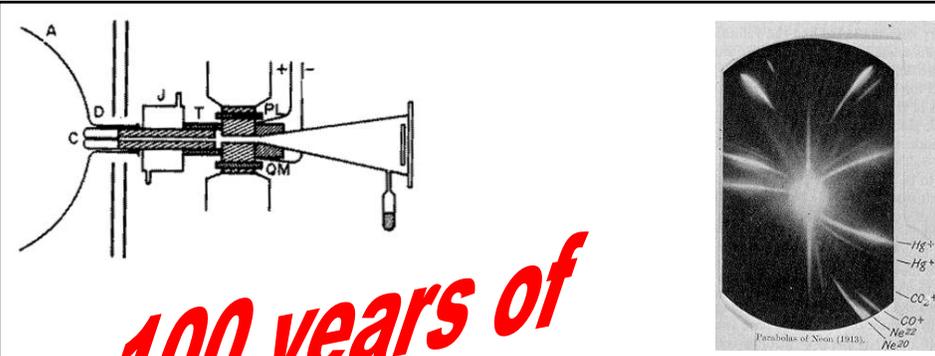
Tel. 0577-234241

Corso di Laurea Magistrale in Biologia Molecolare e Cellulare

A.A. 2014-2015

Chimica delle Biomolecole: 6 crediti

**Identificazione, caratterizzazione
strutturale, proprietà delle
biomolecole: la spettrometria di
massa**



100 years of mass spectrometry

Joseph John Thomson
 Rays of positive electricity
Proceedings of the Royal Society
 A 89, 1-20 (1913)



Parabolas of Neon (1913).
 Hg^+
 Hg^{++}
 CC_2^+
 CO^+
 Ne^{20}
 Ne^{22}

S P E T T R O M E T R I A

S P E C I F I C I T A

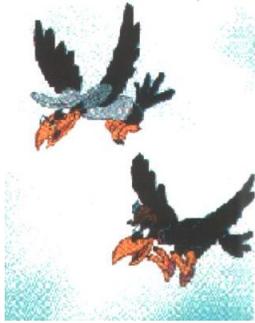
S E L E T T I V I T A

S E N S I B I L I T A

S P E E D

S T E C H I O M E T R I A





La spettrometria di massa studia

ioni in fase gassosa



La spettrometria di massa studia gli ioni in fase gassosa

Cos'è uno ione ?

Particella atomica o molecolare
avente una carica (IUPAC)

Perché gli ioni ?

Campo elettrico
Campo magnetico
Radiofrequenza

Quali ioni è possibile studiare

Inorganici, organici
a basso PM fino a 10^8
Dalton

Come vengono prodotti gli ioni ?

Ionizzazione

Come è possibile studiarli ?

Spettrometro di massa

Quale tipo di informazione è possibile ottenere ?

Qualitativa

Quantitativa

Quale è il tuo PM?

CHI SEI ?

Quali sono i tuoi frammenti?
Informazione strutturale

IN CHE QUANTITÀ ?

Spettrometria di massa

Spettro di massa → Peso molecolare

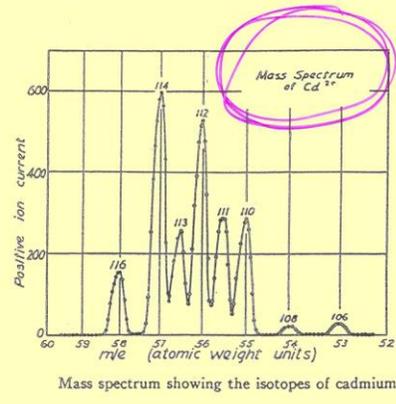
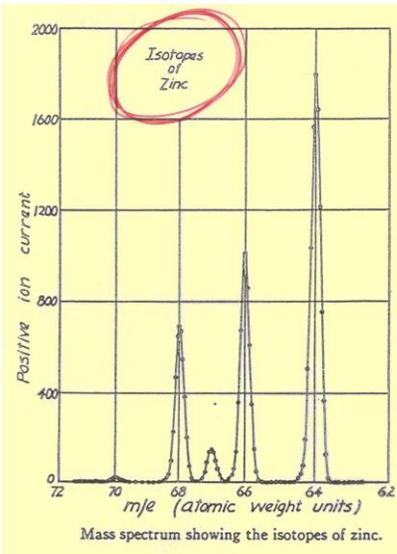
HR + massa accurata → Stechiometria

MSⁿ → Informazioni strutturali, an. quantitativa

Ion spectroscopy → Informazioni strutturali

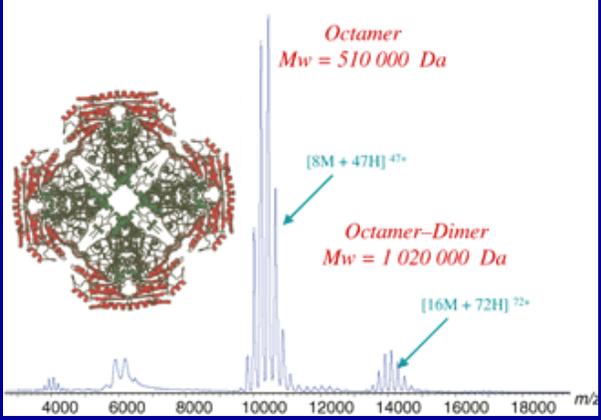
Ion Mobility → Conformazione

Imaging → Mappa degli analiti

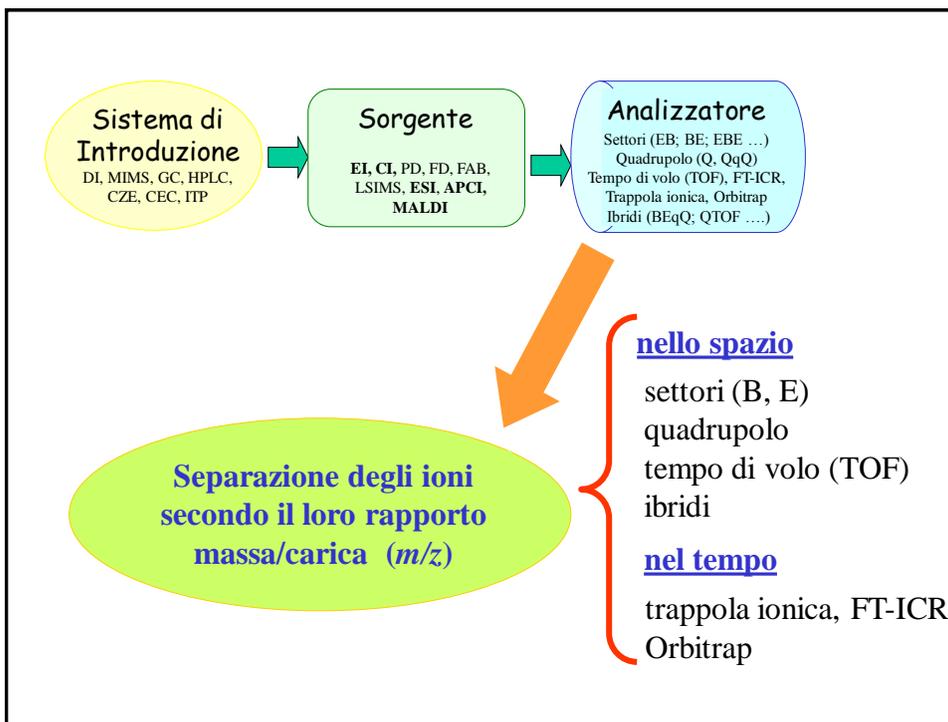
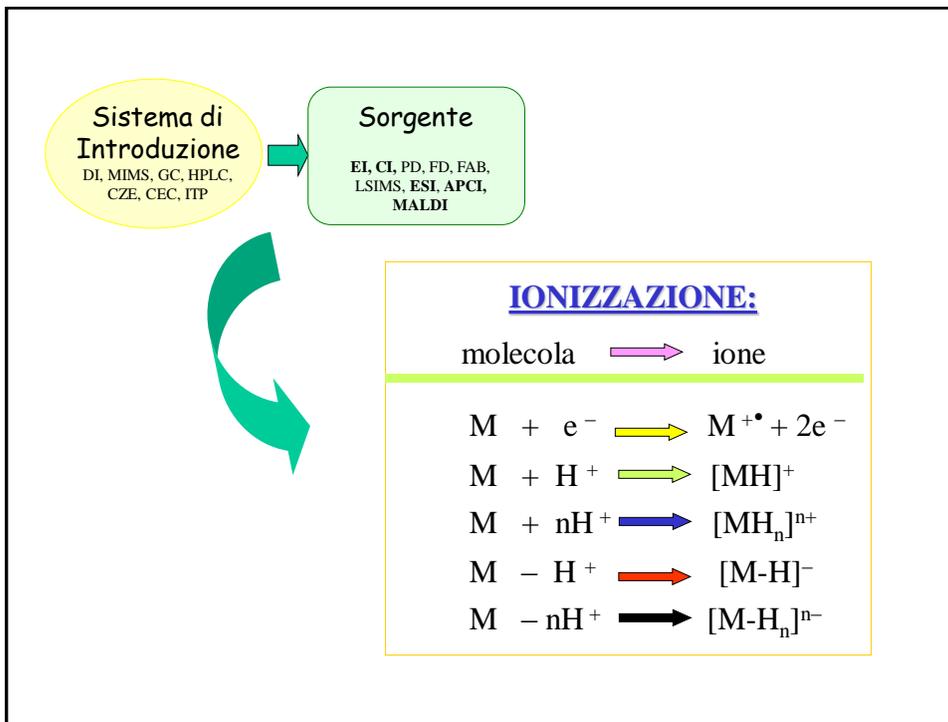


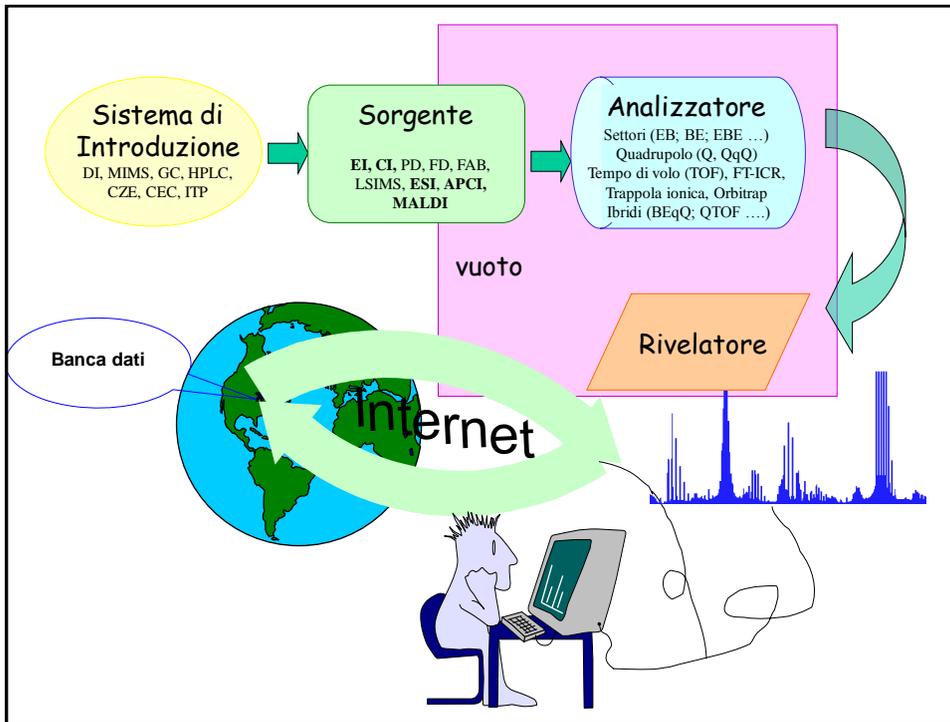
PHYSICAL REVIEW, 50, 1041-1045 (1936)

Vanillyl-alcohol oxidase mass spectrum



A.J.R. Heck et al., *Protein Science* 9, 435 (2000).





Nella **sorgente** avviene la
ionizzazione

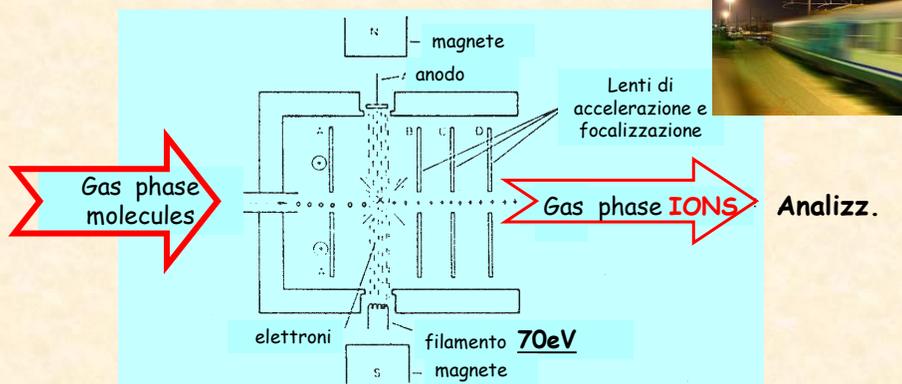
molecola → **ione**

A seconda dell'energia depositata nello ione:
ionizzazione

soft

hard

Ionizzazione elettronica (EI)



MOLECOLE **VOLATILI** ↔ **APOLARI**, **TERMICAM. STABILI**,
BASSO PM

$$70 \text{ eV} \equiv 1610 \text{ kcal mole}^{-1}$$

Potenziale 1° ionizzazione comp. organici:

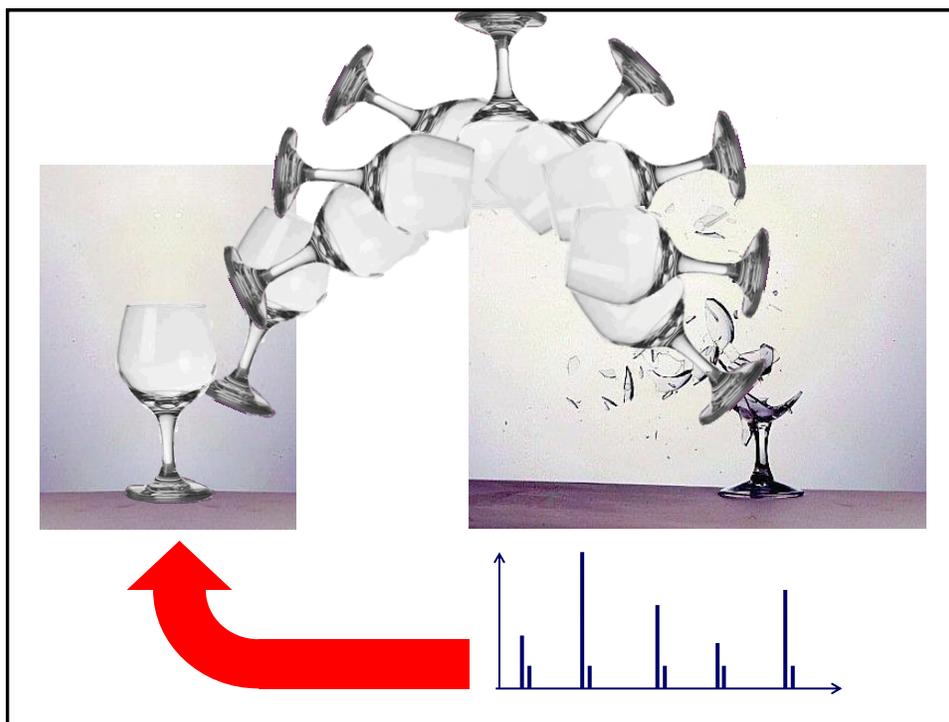
$$8\text{-}15 \text{ eV} (180\text{-}346 \text{ kcal mole}^{-1})$$

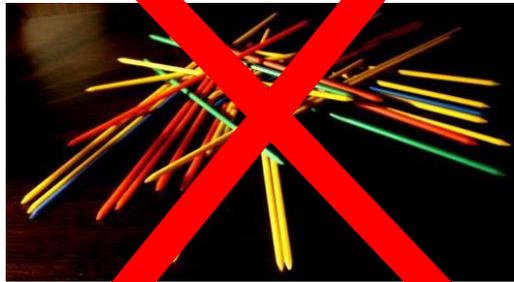
Ionization energies of selected compounds^a

Compound	IE^b [eV]	Compound	IE^b [eV]
Hydrogen, H ₂	15.4	Helium, He	24.6
Methane, CH ₄	12.6	Neon, Ne	21.6
Ethane, C ₂ H ₆	11.5	Argon, Ar	15.8
Propane, <i>n</i> -C ₃ H ₈	10.9	Krypton, Kr	14.0
Butane, <i>n</i> -C ₄ H ₁₀	10.5	Xenon, Xe	12.1
Pentane, <i>n</i> -C ₅ H ₁₂	10.3		
Hexane, <i>n</i> -C ₆ H ₁₄	10.1	Nitrogen, N ₂	15.6
Decane, <i>n</i> -C ₁₀ H ₂₂	9.7	Oxygen, O ₂	12.1
		Carbon monoxide, CO	14.0
Ethene, C ₂ H ₄	10.5	Carbon dioxide, CO ₂	13.8
Propene, C ₃ H ₆	9.7		
(<i>E</i>)-2-Butene, C ₄ H ₈	9.1	Fluorine, F ₂	15.7
		Chlorine, Cl ₂	11.5
Benzene, C ₆ H ₆	9.2	Bromine, Br ₂	10.5
Toluene, C ₆ H ₈	8.8	Iodine, I ₂	9.3
Indene, C ₉ H ₈	8.6		
Naphthalene, C ₁₀ H ₈	8.1	Ethanol, C ₂ H ₆ O	10.5
Biphenyl, C ₁₂ H ₁₀	8.2	Dimethylether, C ₂ H ₆ O	10.0
Anthracene, C ₁₄ H ₁₀	7.4	Ethanethiol, C ₂ H ₆ S	9.3
Aniline, C ₆ H ₇ N	7.7	Dimethylsulfide, C ₂ H ₆ S	8.7
Triphenylamine, C ₁₈ H ₁₅ N	6.8	Dimethylamine, C ₂ H ₇ N	8.2

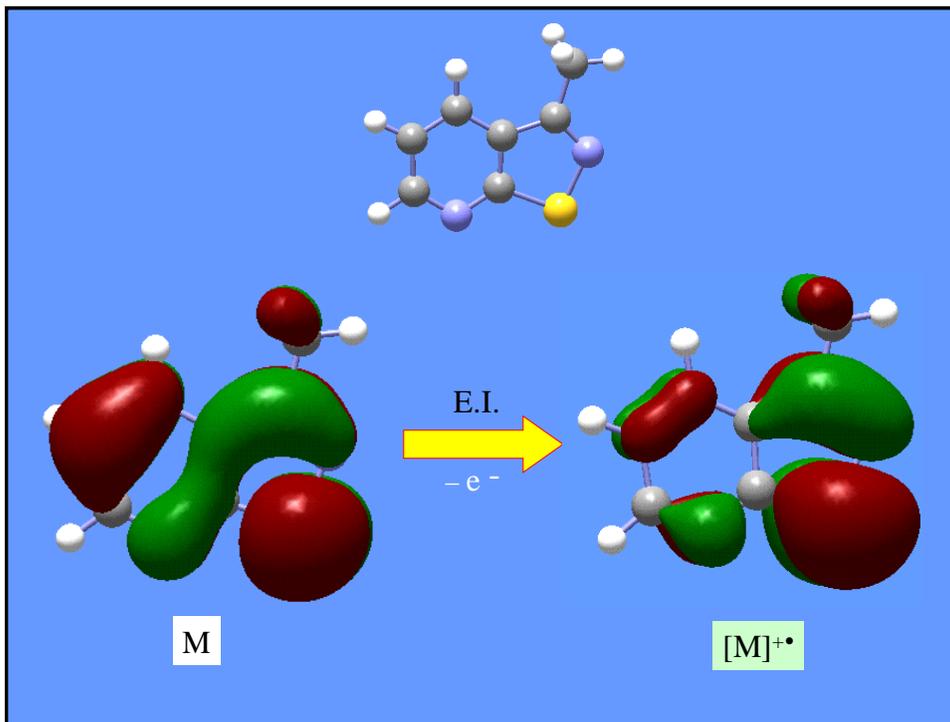
^a IE data taken from Ref. [23] with permission. © NIST 2002.

^b All values have been rounded to the first digit.





La rimozione di un elettrone dalla
molecola
causa una redistribuzione di tutti
gli elettroni



Tecniche di ionizzazione **hard** : *ionizzazione elettronica*



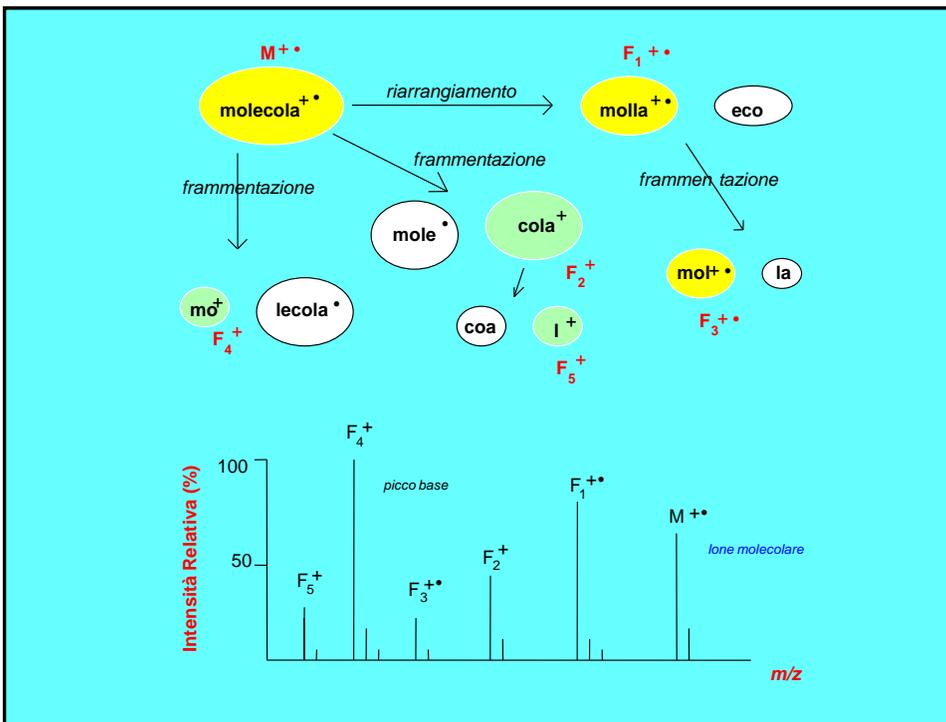
Ampio eccesso di energia sullo ione molecolare $[M]^{\bullet+}$



Estesa frammentazione



Informazioni strutturali



Lo spettro di massa:

- ✓ Informazioni sul peso molecolare e sugli elementi presenti

Una molecola organica contenente C, H, O, S, P, o alogeni ha una massa nominale **dispari**

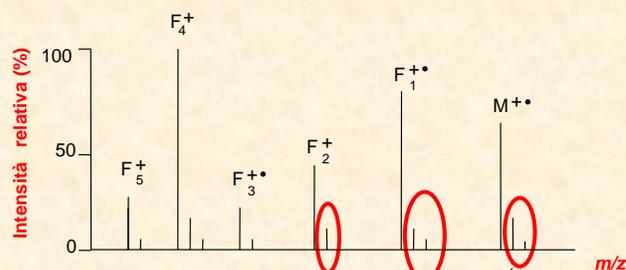
solo se contiene un **numero dispari di atomi di azoto**

N _{1,3,5,7,...} PM **dispari** ↔ $[M]^{+\bullet}$ *m/z* **dispari**

N _{0,2,4,6,...} PM **pari** ↔ $[M]^{+\bullet}$ *m/z* **pari**

Lo spettro di massa:

- ✓ Informazioni sul peso molecolare e sugli elementi presenti



Picchi isotopici

ISOTOPI

Atomi della stessa specie, con **lo stesso numero atomico** (stesso numero di protoni) ma **numero di massa diverso** (protoni + neutroni).

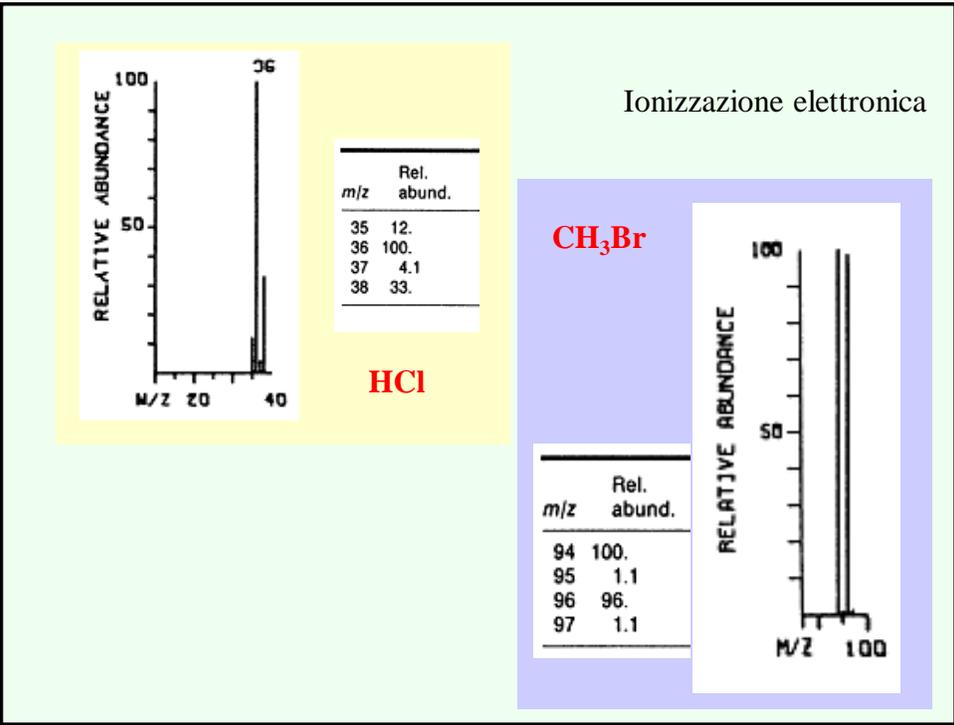
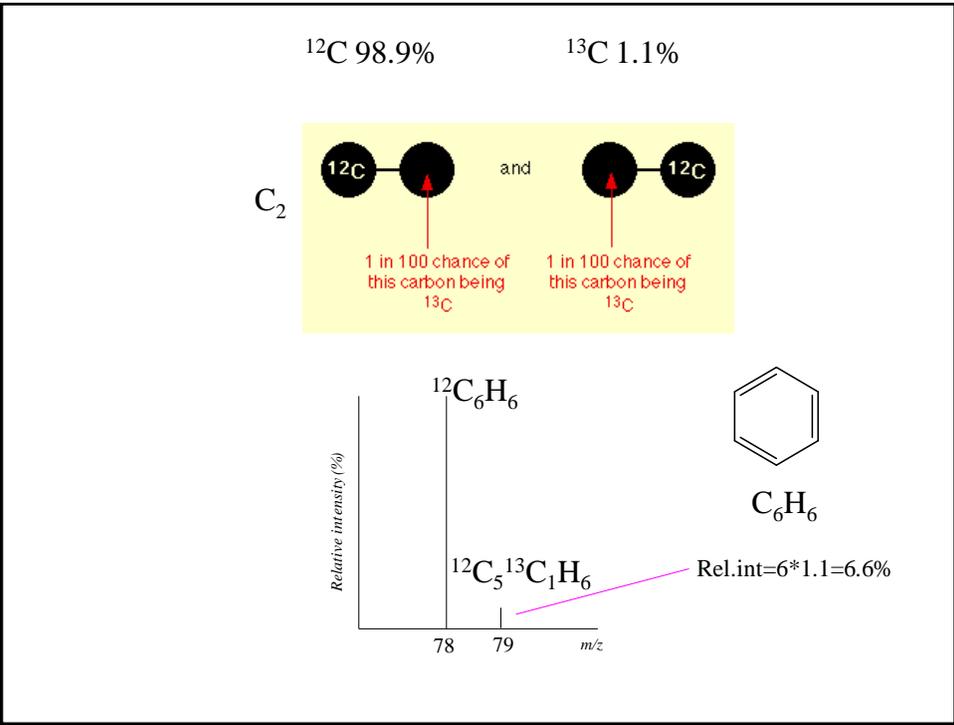
Essi hanno **proprietà chimiche identiche**, ma **peso atomico diverso**

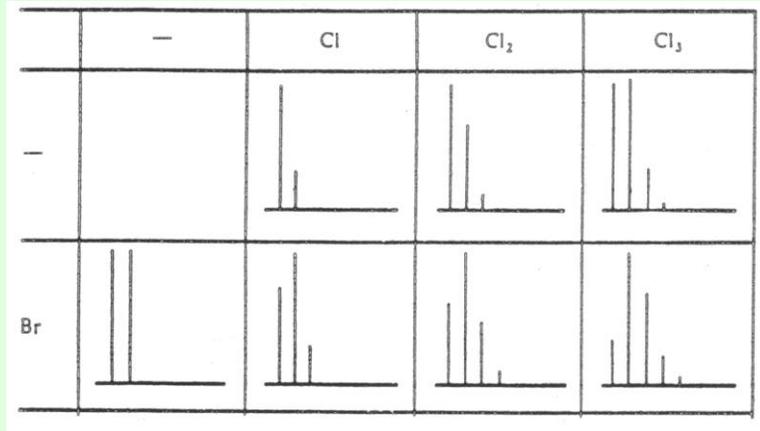
ISOTOPI STABILI: atomi senza degradazione spontanea

^{12}C 98.9% ^{13}C 1.1%

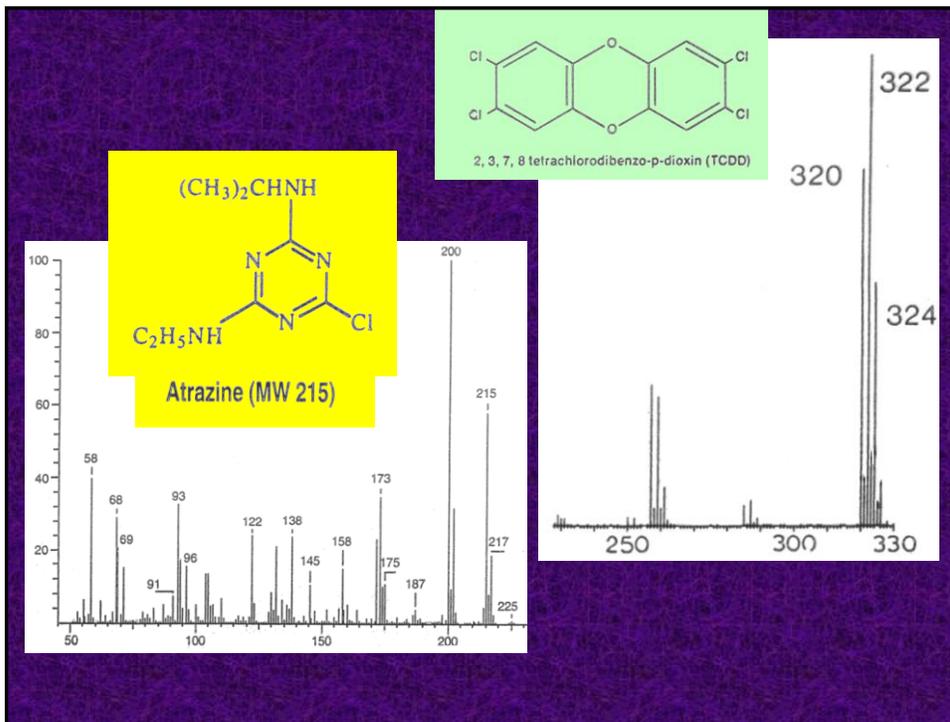
Natural isotopic abundances of common elements.

Element	A		A + 1		A + 2	
	Mass	%	Mass	%	Mass	%
H	1	100	2	0.015		
C	12	100	13	1.1		
N	14	100	15	0.37		
O	16	100	17	0.04	18	0.20
S	32	100	33	0.79	34	4.4
Cl	35	100			37	32.0
Br	79	100			81	97.3



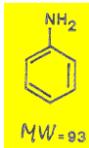
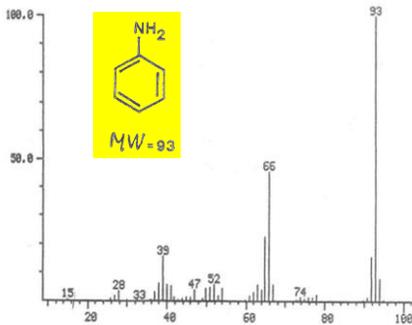
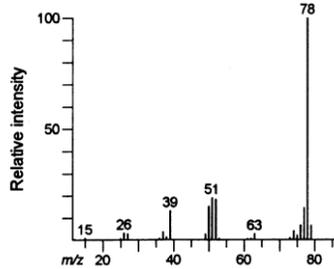
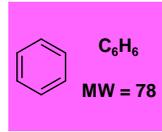


Tutti gli ioni differiscono di 2u !!

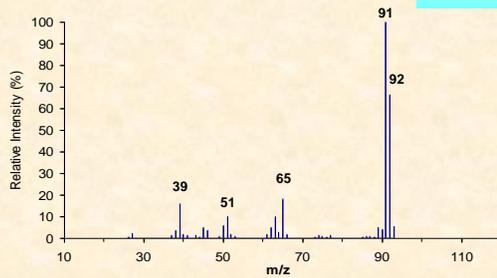
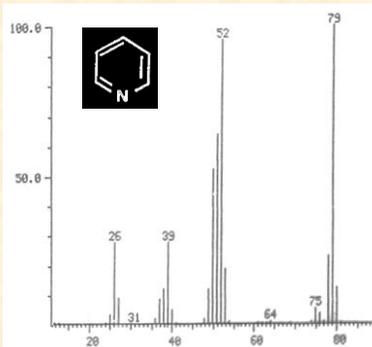


Ionizzazione elettronica

m/z	Int.	m/z	Int.
12	0.2	53	0.8
13	0.4	60	0.2
14	0.4	61	0.4
15	1.0	62	0.8
24	0.4	63	2.9
25	0.8	64	0.2
26	3.2	72	0.4
27	2.6	73	1.0
36	0.9	74	3.9
37	3.8	75	2.2
39	13.	76	7.0
40	0.4	77	15.
50	16.	78	100.
51	19.	79	6.8
52	20.	80	0.2



Ionizzazione elettronica



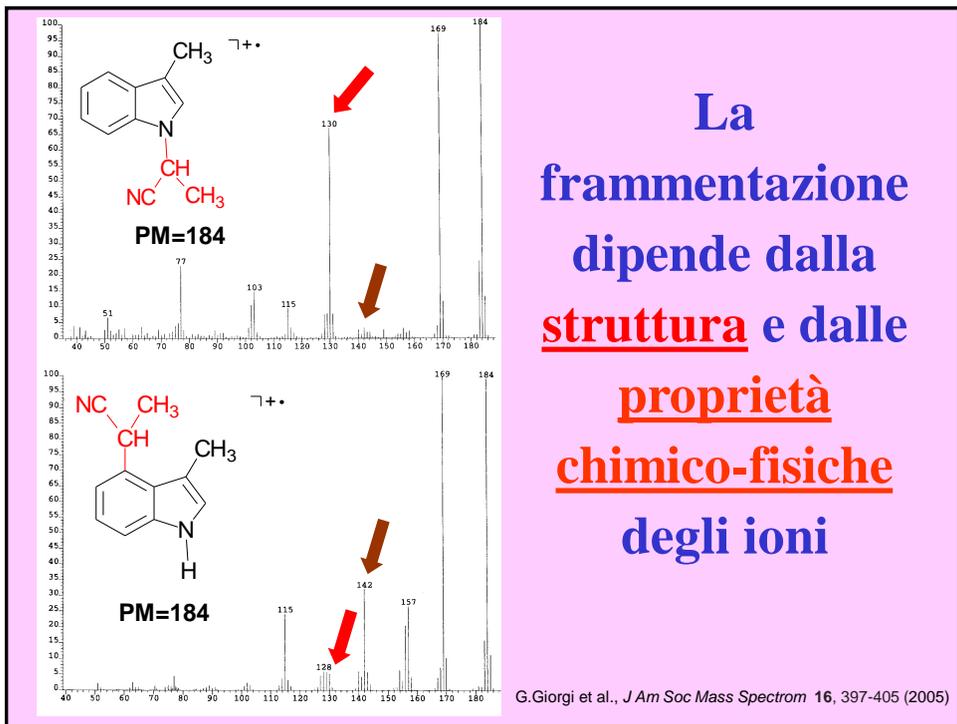
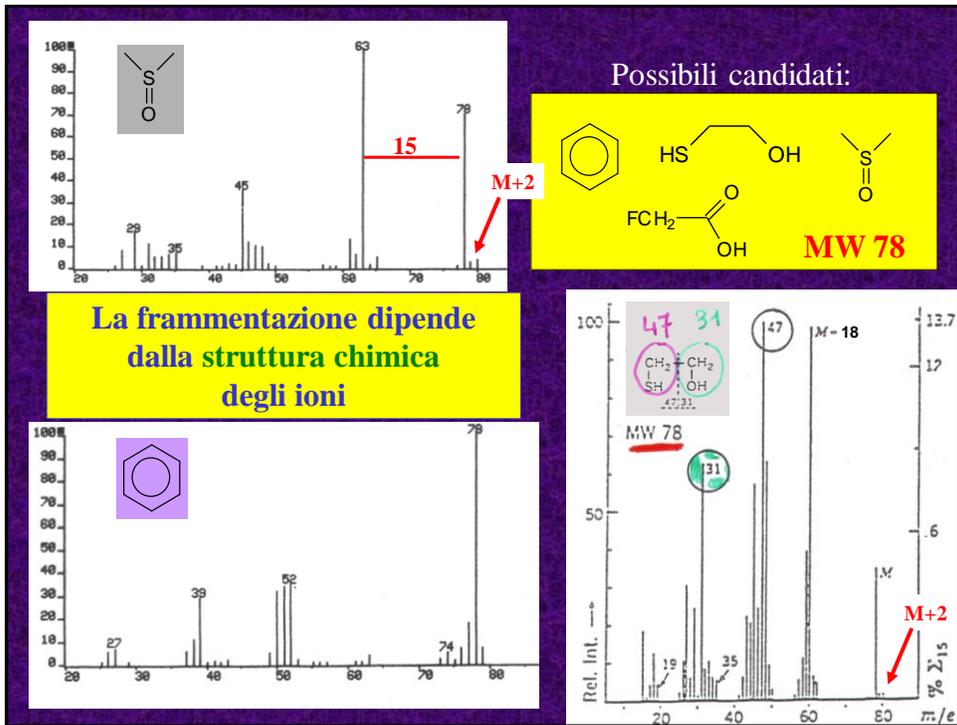
m/z	%
89	5.0
90	4.0
91	100
92	66.5
93	5.5

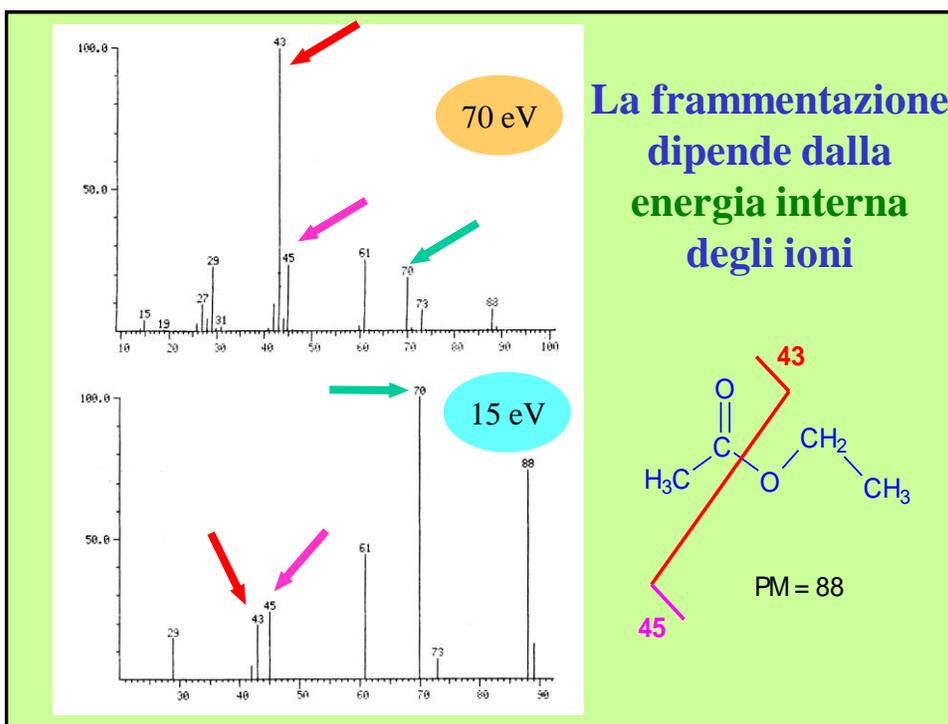
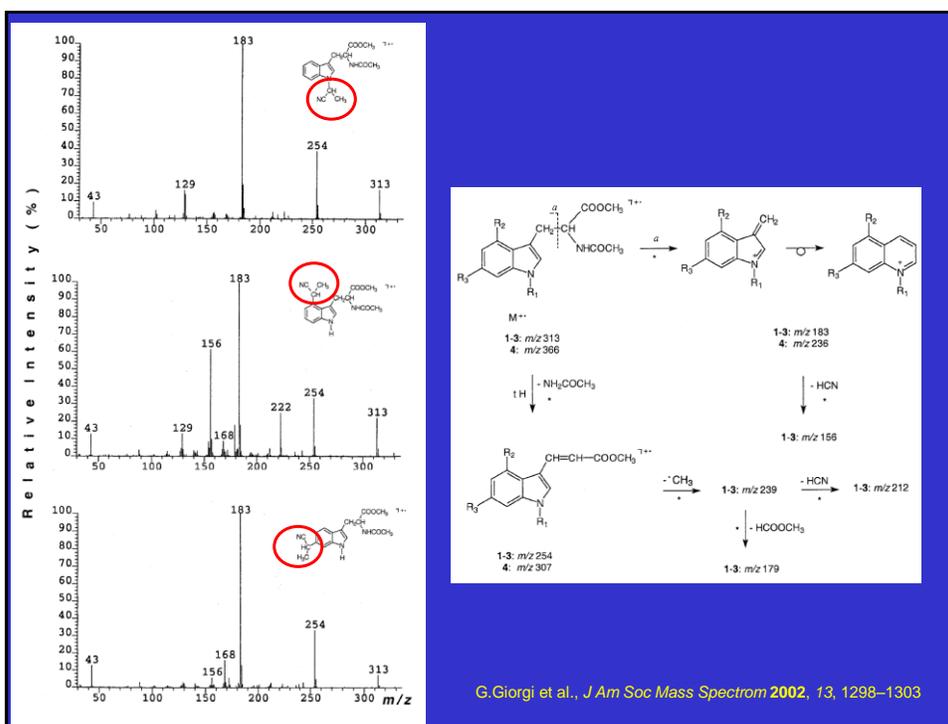
La frammentazione consiste in:

- un insieme di reazioni di decomposizione **unimolecolare** che avvengono
 - all'interno della sorgente ionica
 - in un intervallo compreso tra 10^{-10} e 10^{-6} s dalla ionizzazione

La frammentazione dipende:

- dalla **struttura** dello ione
- dalla sua **energia interna** (vibrazionale e rotazionale)





La frammentazione

NON È

un processo casuale

ma, operando nelle stesse condizioni sperimentali,

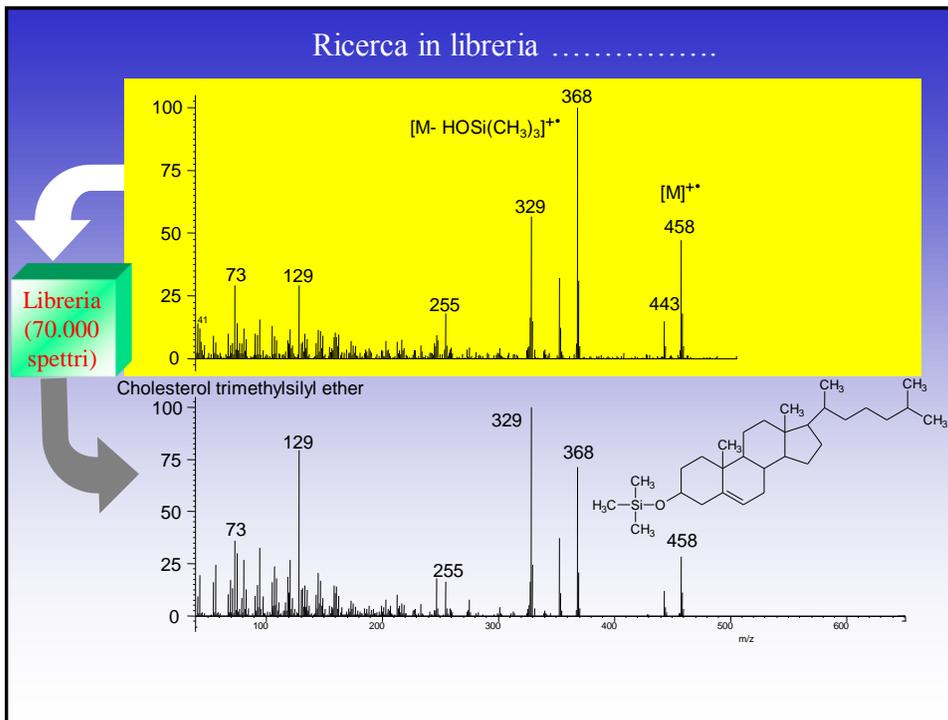
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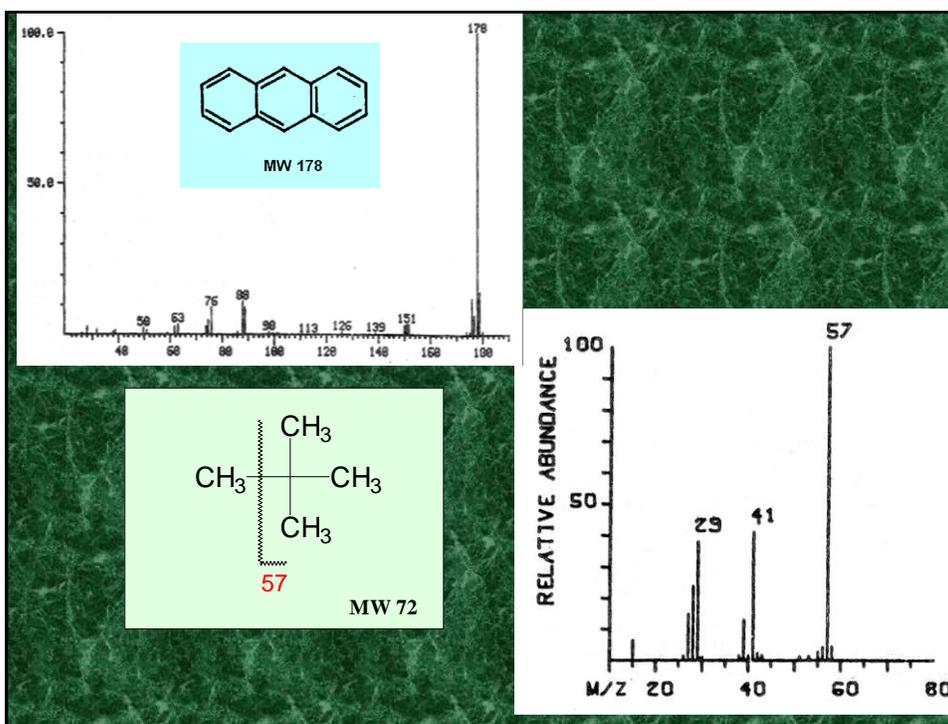
ripetibile a livello quali e quantitativo

Stessi frammenti
della stessa intensità



Stesso spettro di
massa



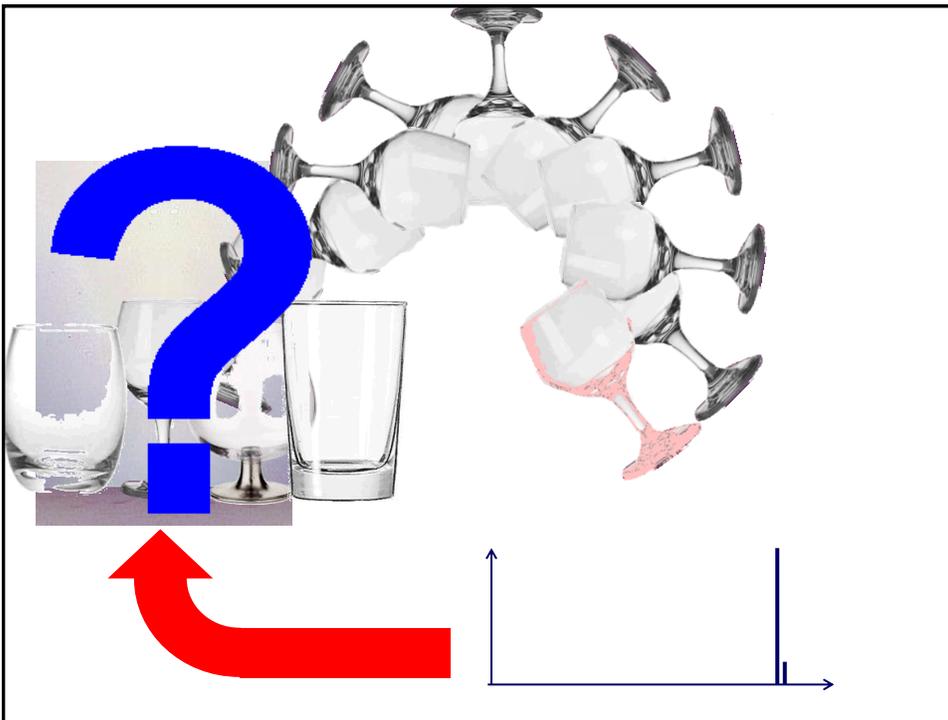




Ionizzazione

SOFT

✓ Frammenti assenti → informazione strutturale?



Lo spettrometro di massa:
un versatile laboratorio in fase gassosa.

Ionizzazione Chimica: reazioni ione-molecola in fase gassosa

JOURNAL
OF THE AMERICAN CHEMICAL SOCIETY

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VOLUME 88, NUMBER 12

JUNE 20, 1966

Physical and Inorganic Chemistry

Chemical Ionization Mass Spectrometry.

I. General Introduction

M. S. B. Munson and F. H. Field

Contribution from the Esso Research and Engineering Company, Baytown Research and Development Division, Baytown, Texas. Received January 18, 1966

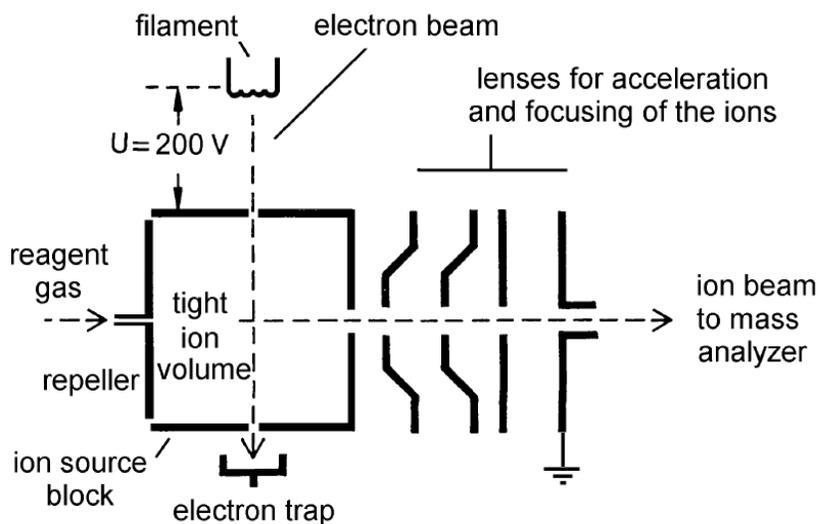
Abstract: The purpose of this paper is to present a new technique in mass spectrometry, chemical ionization mass spectrometry, which is based on the formation of the ions of an unknown material by chemical reactions in the gas phase. A reaction gas is introduced into the ionization chamber of a mass spectrometer at pressures of 1 torr. This reaction gas is ionized and a set of ions is produced which does not react further with the reaction gas. If a small amount of another material is present in the mixture at these high pressures, the stable ions of the reaction gas will react with this second material to produce a spectrum of ions characteristic of the second material. The spectra which are produced by chemical reactions are frequently different from those spectra produced by conventional electron impact methods and are often more useful for determining the structure of compounds and identifying compounds and mixtures than electron impact spectra. The fragmentation patterns of chemical ionization mass spectrometry correspond closely to the structures of the molecules and appear to result from localized attack at reactive centers in the molecule. Reasonable hypotheses are offered for the reactions forming the major product ions of several types of molecules.

Ionizzazione chimica:

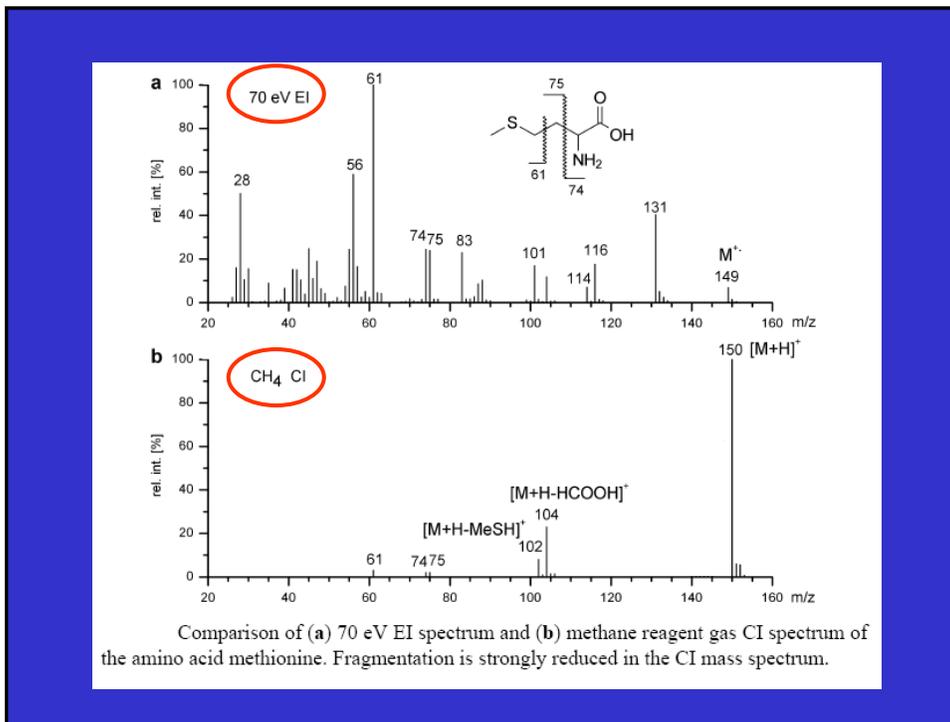
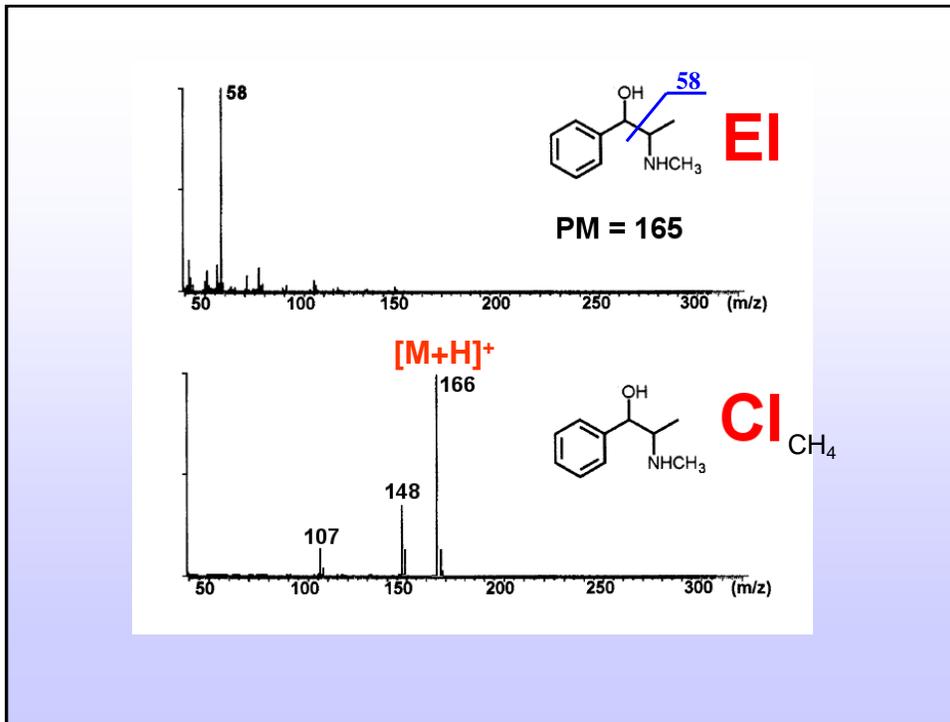
- Reazione (**processo bimolecolare**) tra
 - le molecole dell'analita
 - e gli ioni di un gas reagente in alta concentrazione generati da EI ad alta pressione

Numero di *collisioni* ione-molecola sufficientemente elevato durante il tempo di residenza dei reagenti all'interno della sorgente

Tempo resid. in sorgente ca 10^{-6} sec, $P_{\text{sorg.}}=2,5 \times 10^{-2}$ Pa \rightarrow n. collisioni=60-70

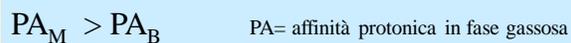
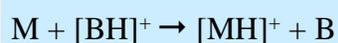


Sorgente per ionizzazione chimica



IONIZZAZIONE CHIMICA

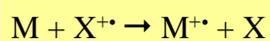
a. *Trasferimento di protone*



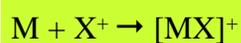
b. *Rimozione di anione*



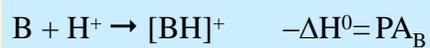
c. *Scambio di carica*



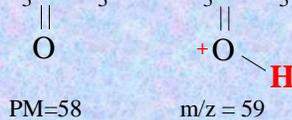
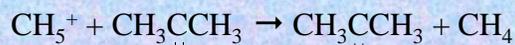
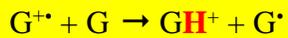
d. *Addizione elettrofila*



Ionizzazione chimica per protonazione

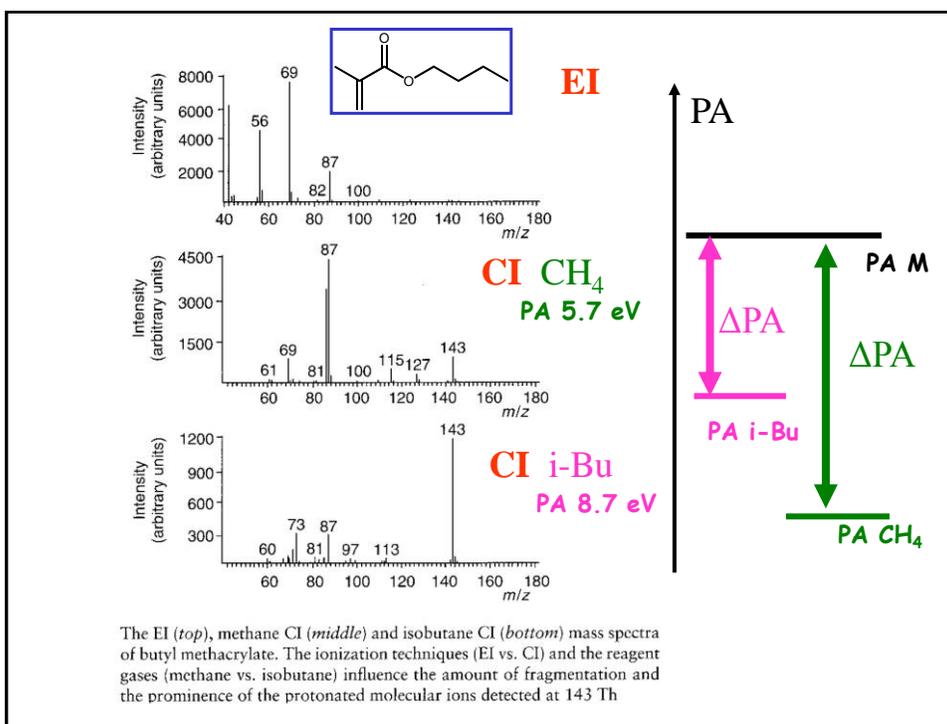


PA= affinit\`a protonica in fase gassosa



Trasferimento di protone in ionizzazione chimica

- Maggiore è la differenza di affinità protonica (PA) tra il reagente e l'analita →
 - Maggiore è l'energia dello ione MH⁺
 - Maggiore è la sua frammentazione
- Reagenti di uso comune:
 - CH₄ (PA = 5.7 eV)
 - NH₃ (PA = 9.7 eV)
 - Isobutano (PA = 8.7 eV)



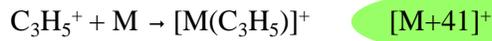
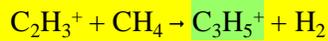
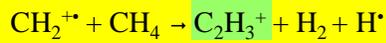
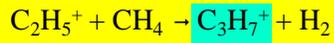
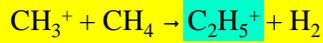
Gas reagenti usati in CI e loro affinità protoniche

Reagent gas	Predominant reactant ions	Proton affinity ^a (kcal/mol)	Hydride affinity (kcal/mol)
He/H ₂	HeH ⁺	42	—
H ₂	H ₃ ⁺	101.2	300
CH ₄	CH ₅ ⁺	131.6	269
	C ₂ H ₅ ⁺	159	271
H ₂ O	H ₃ O ⁺	166.5	—
CH ₃ CH ₂ CH ₃	C ₃ H ₇ ⁺	182	270
CH ₃ OH	CH ₃ OH ₂ ⁺	182 ^b	—
(CH ₃) ₃ CH (isobutane)	C ₄ H ₉ ⁺	196 ^c	266
NH ₃	NH ₄ ⁺ , (NH ₃) ₂ H ⁺ , (NH ₃) ₃ H ⁺	204.0	—
(CH ₃) ₂ NH	(CH ₃) ₂ NH ₂ ⁺ , (CH ₃) ₂ H ⁺ , C ₃ H ₈ N ⁺	220.6	—
(CH ₃) ₃ N	(CH ₃) ₃ NH ⁺	225.1	—

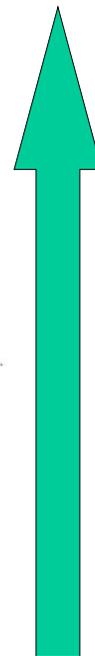
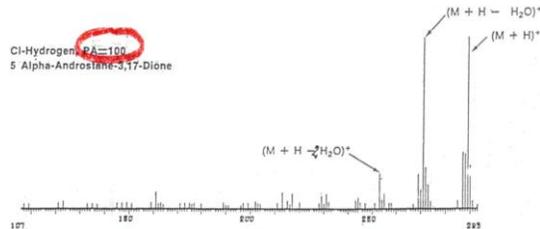
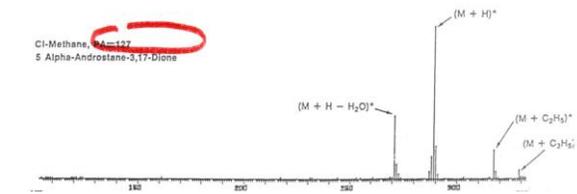
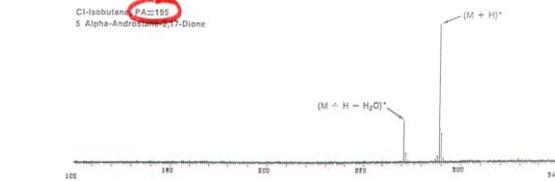
^aLias SG, Bartmess JE, Liebman JF, Holmes JL, Levin RD, Mallard WG. *J Phys Chem Ref Data* 1988;17(suppl 1).

^bHarrison AG. *Chemical Ionization Mass Spectrometry*, ed 2. CRC Press, Boca Raton, Fla., 1992.

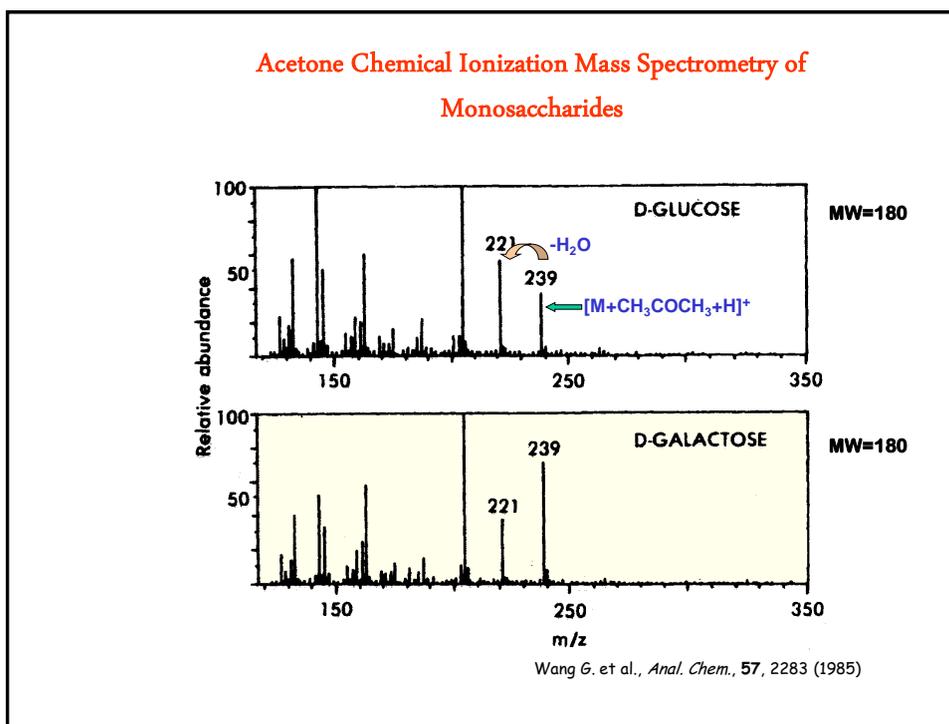
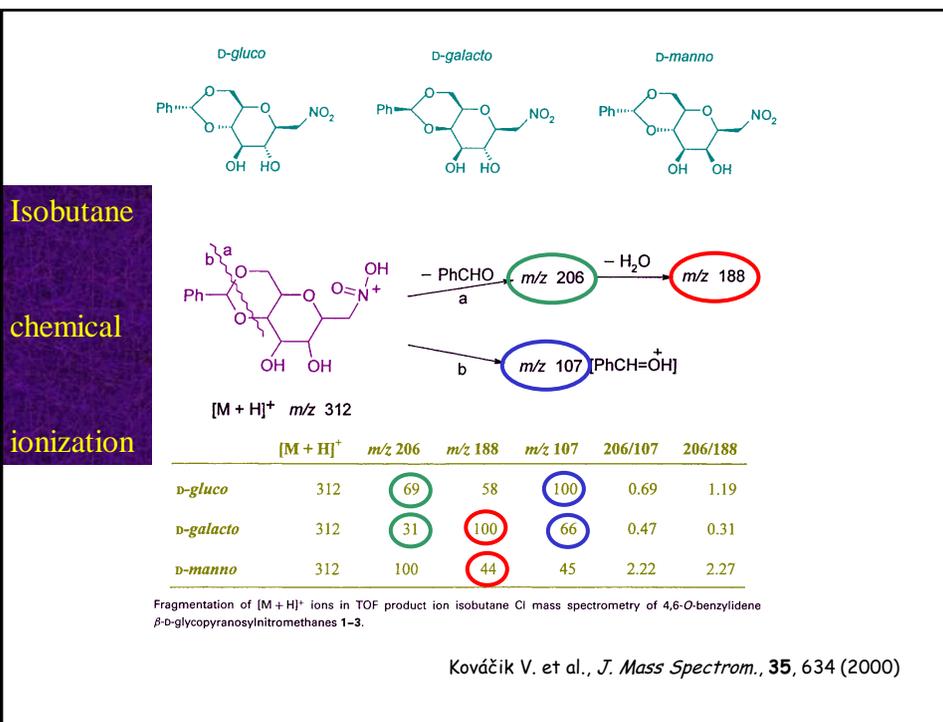
^cProton affinity of isobutylene, which is the conjugate base of isobutane.



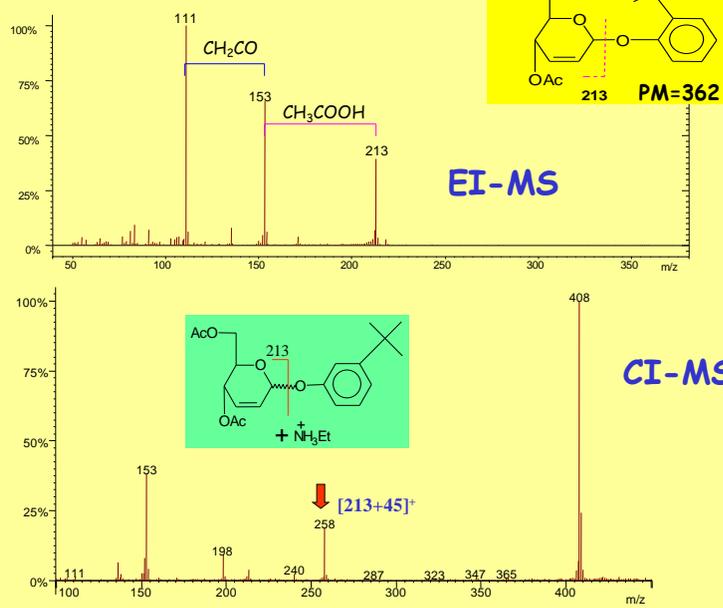
Addotti
in fase
gassosa



PA_B



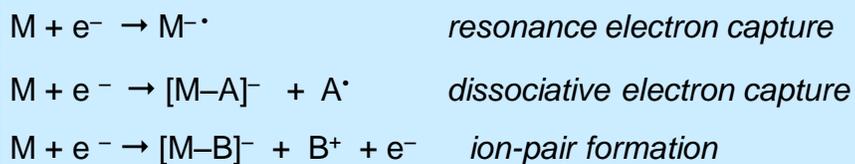
Gas phase electrophilic additions with amines

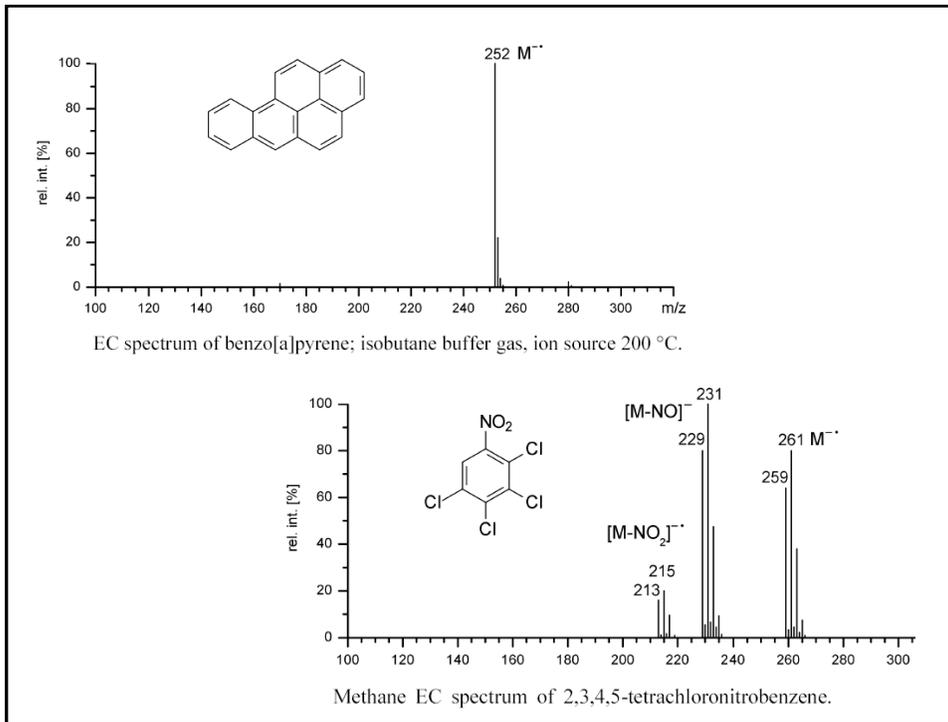


G. Giorgi et al. *Eur. J. Org. Chem.* 106-115 (2003), *J. Am. Soc. Mass Spectrom.* 14, 851-861 (2003), 15, 244-252 (2004)

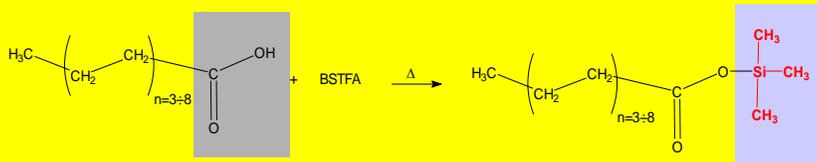
Ionizzazione chimica per cattura di elettroni:

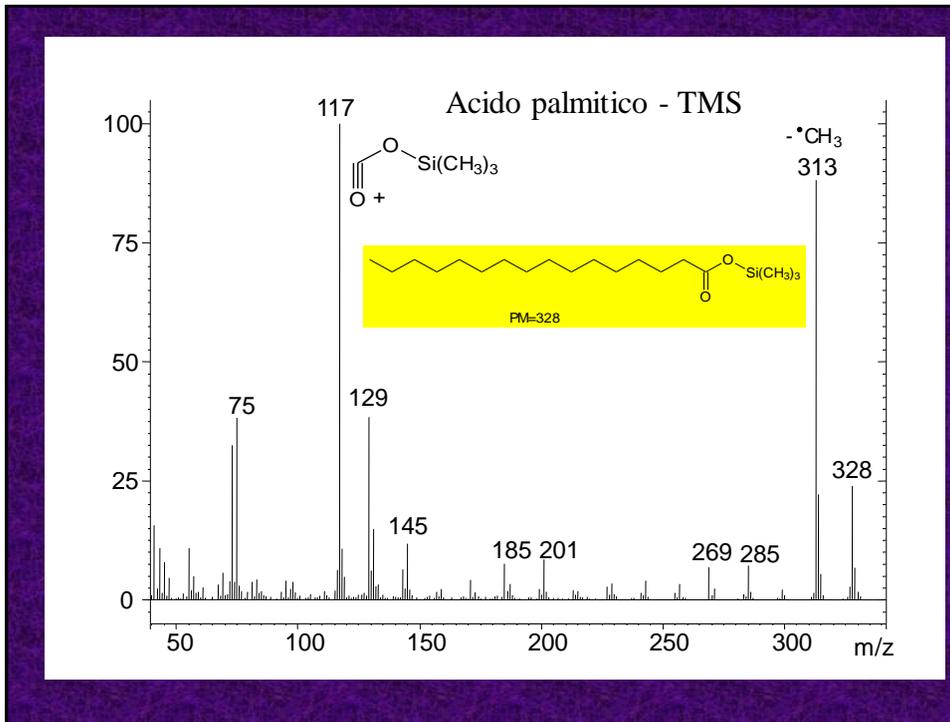
studio di ioni negativi





Tutte le molecole sono volatili ?





**MISCELE
COMPLESSE**

Separazione prima di MS

MISCELE COMPLESSE

Separazione prima di MS

Molecole volatili, basso PM



Gas cromatografia

Molecole **NON** volatili;
polari

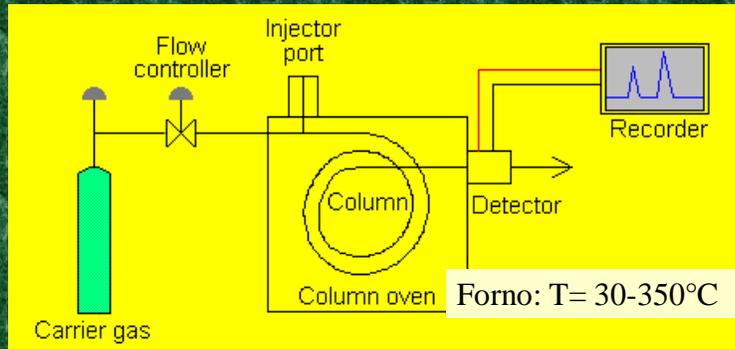


HPLC

Gas chromatography and **mass spectrometry**: two independent, but complementary techniques.

FEATURE	GAS CHROMATOGRAPHY	MASS SPECTROMETRY
Handling of mixtures	Yes	No
Ability to identify	Ambiguous	Yes
Working phase	Gas	Gas
Handling of pg amounts	Yes	Yes
Operating pressure	Atmospheric	High vacuum

Gas cromatografia



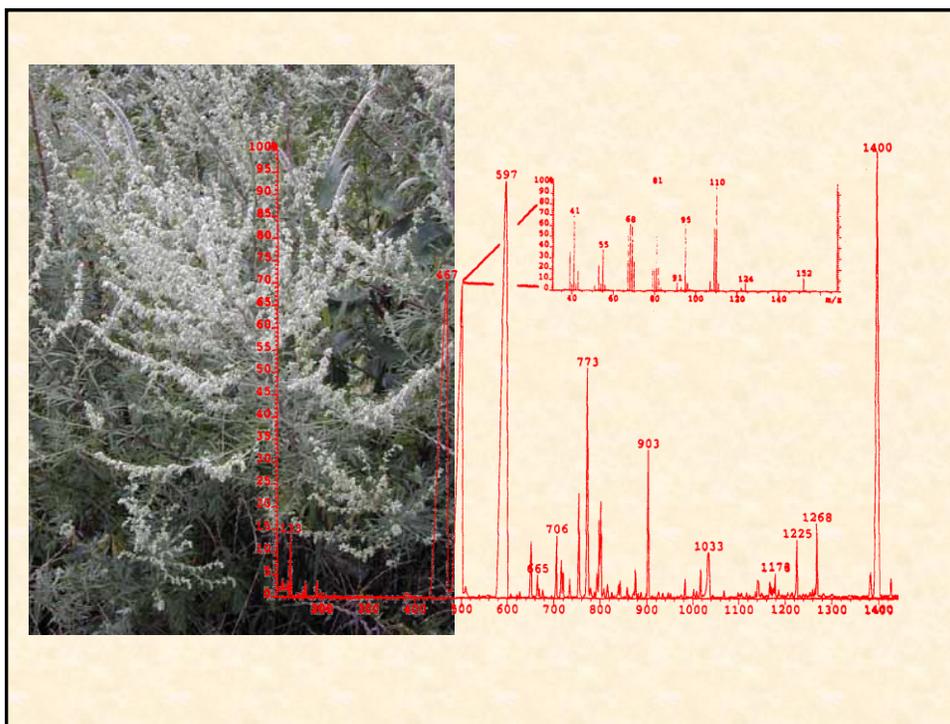
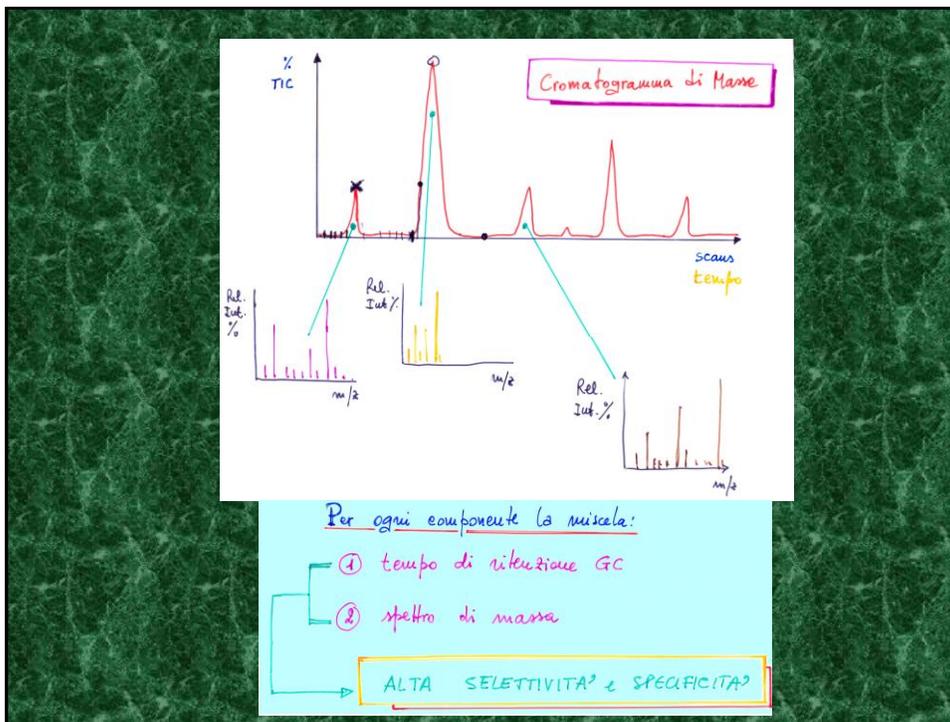
Colonne:

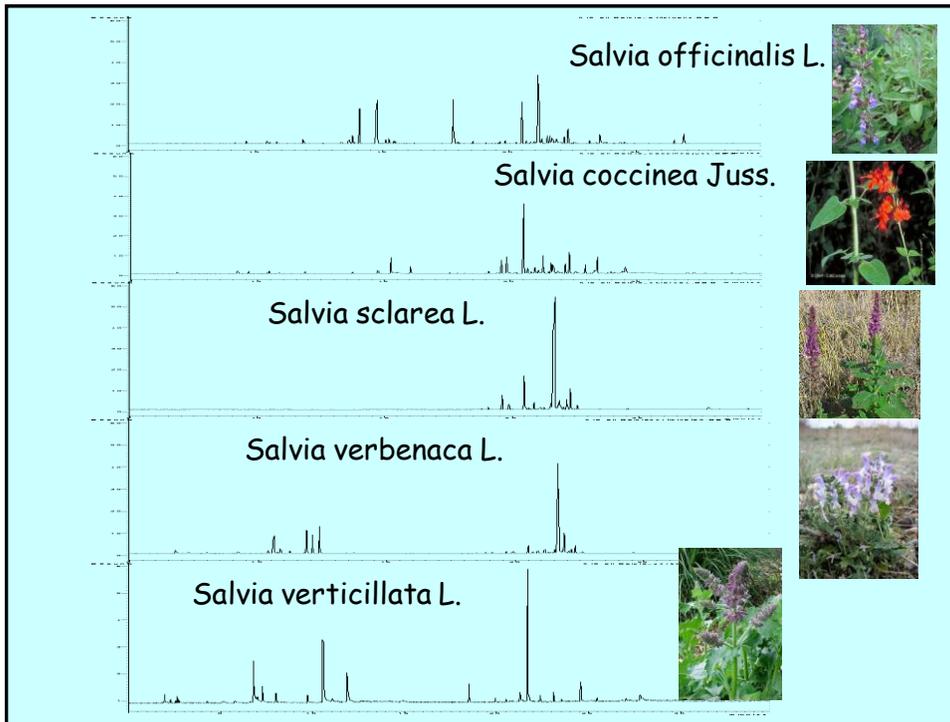
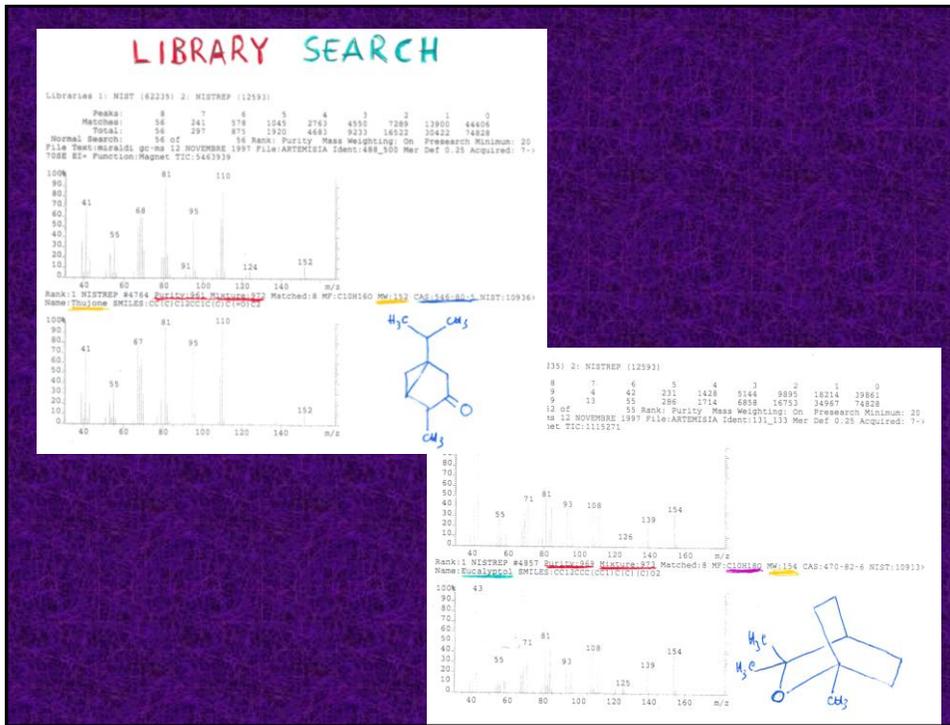


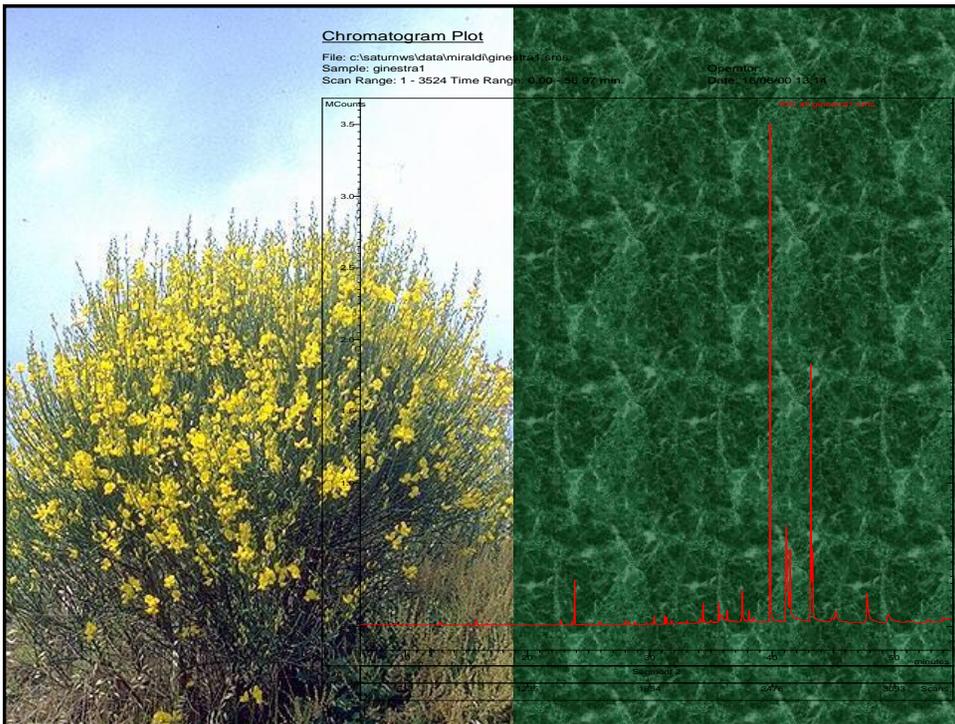
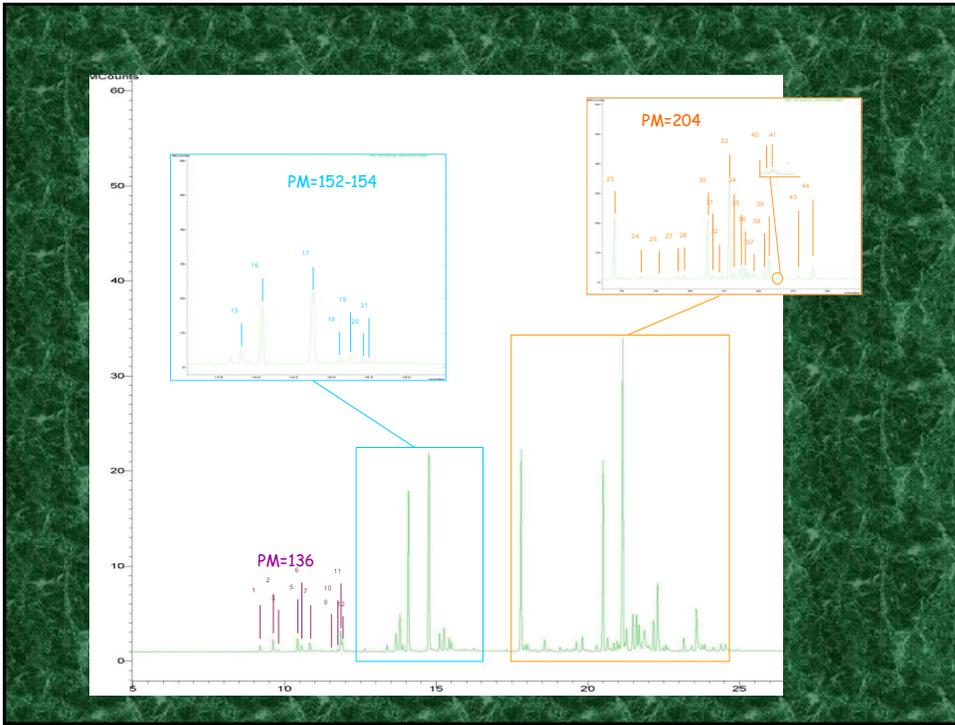
vari diametri (0.25-0.32 mm)
varie lunghezze (15-50 m)
varie fasi (polari, apolari)
vari spessori della fase (0.1-5.0 μm)

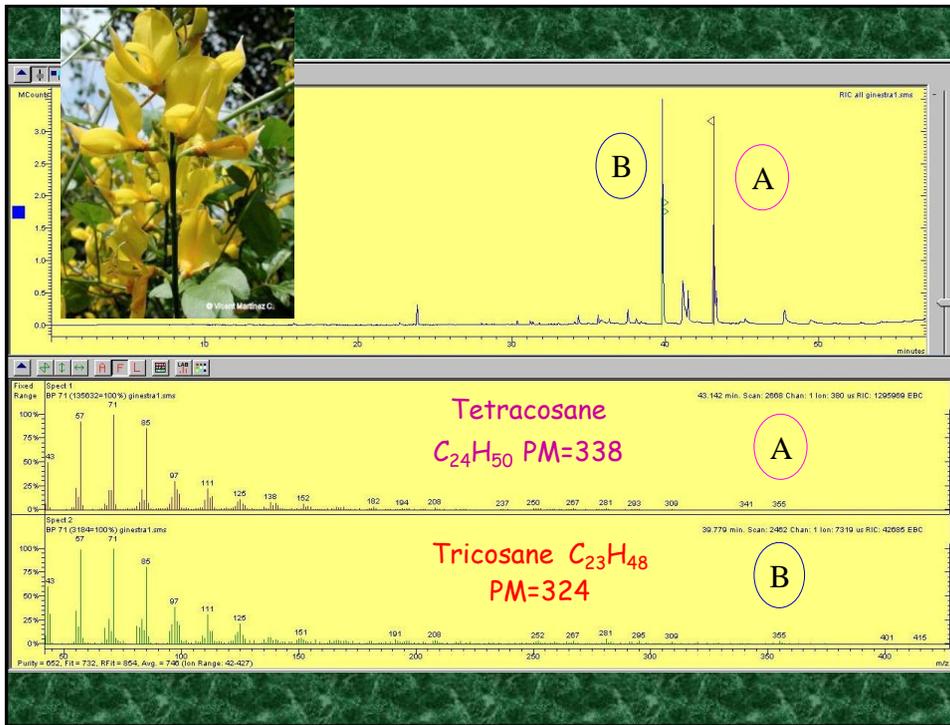
Fase mobile: elio, azoto











Journal of Archaeological Science 37 (2010) 1453–1457

Contents lists available at ScienceDirect

Journal of Archaeological Science

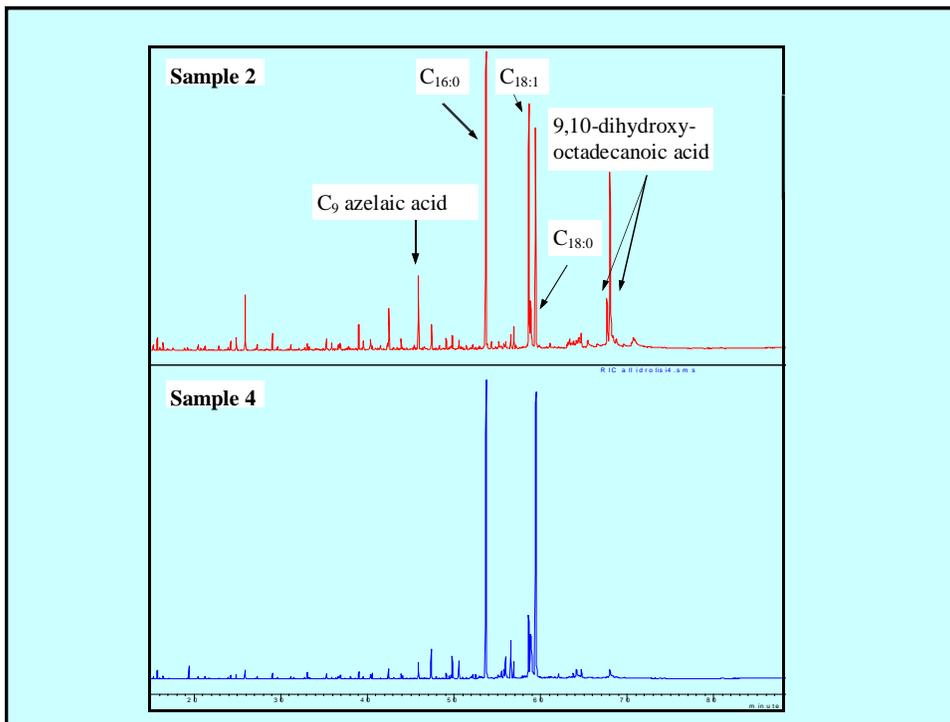
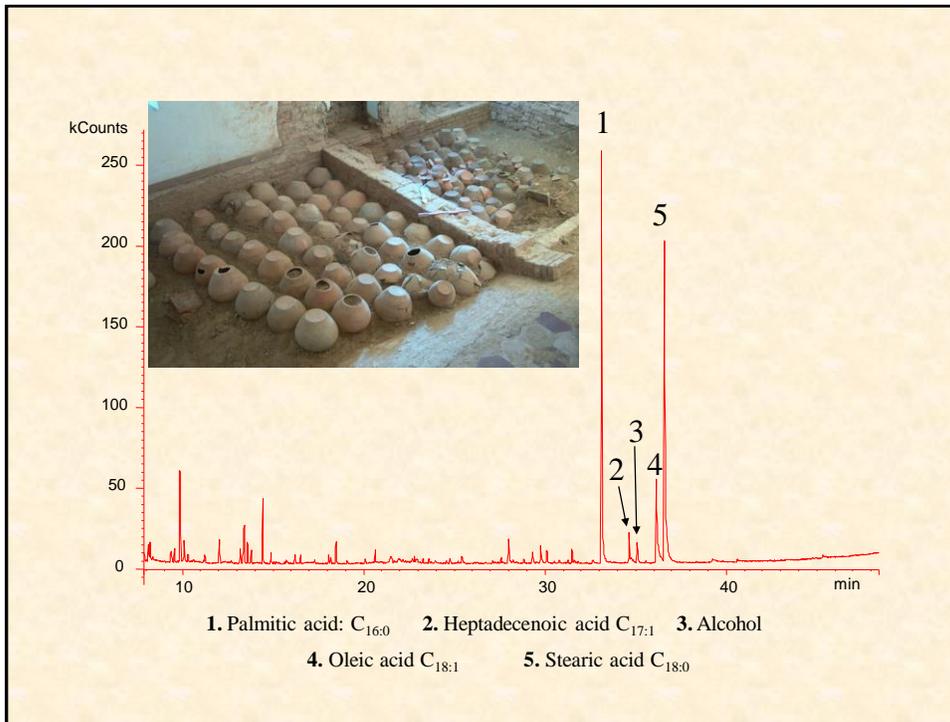
journal homepage: <http://www.elsevier.com/locate/jas>

The meals in a Tuscan building yard during the Middle Age. Characterization of organic residues in ceramic potsherds

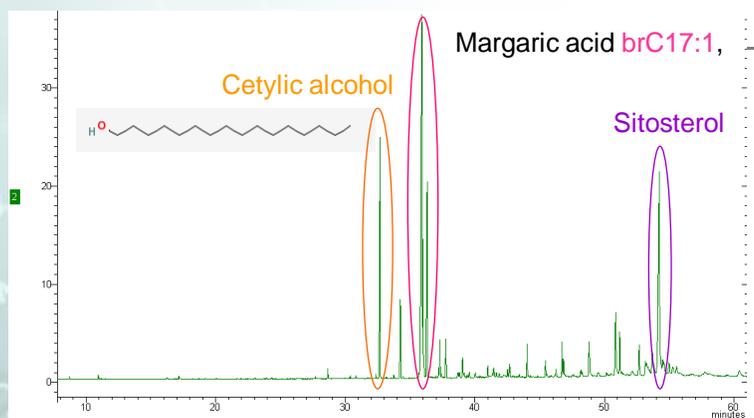
Gianluca Giorgi^{a,*}, Laura Salvini^b, Alessandra Pecci^c

Carmine convent in Siena

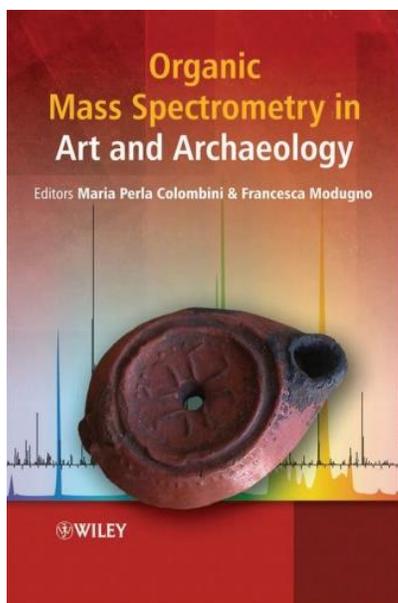
The figure shows a photograph of a ceramic potsherd on the left and its corresponding mass spectrum on the right. The mass spectrum displays a series of peaks labeled with numbers 1 through 28, indicating the presence of specific organic residues.



Archaeological site



G. Giorgi, F. Borghini, A. Pecci, manuscript in preparation



Chapter 2

Overview of Mass Spectrometric Based Techniques Applied in the Cultural Heritage Field

Gianluca Giorgi

2009

Sofisticated fabbriche di alconi

- The insect cuticle is covered by a layer of lipids. This layer protect the insect from infection and to reduce water loss.

- In social insects (wasps, bees, ants, and termites) cuticular compounds also allow individuals to recognize each other. Thus, individuals are able to discriminate colony members on the basis of the cuticular signature.

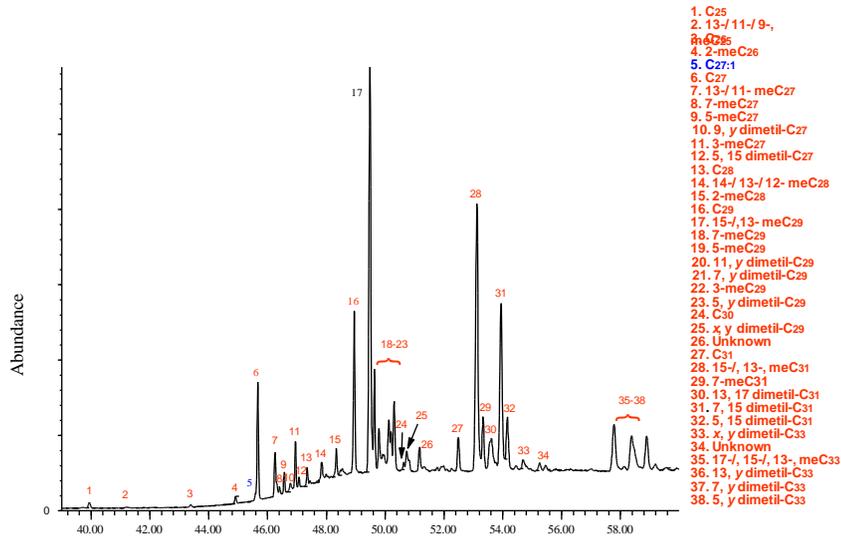
- In social insects the major compounds found on the cuticle are hydrocarbons. These are usually long-chained (C20 to C37) and may be saturated or unsaturated.

- In social wasps (*Polistes* sp.) cuticular hydrocarbons have been found to differ between colonies within species and allow colony members to recognize nestmates. Furthermore, the nest has been shown to be an important source of these hydrocarbons as well as glands present in the wasps.



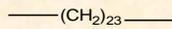


Polistes dominulus: cuticular hydrocarbons

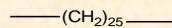


- Chromatogram after SMPE of a live individual of *Polistes dominulus*
- The cuticular signature consists mainly of saturated hydrocarbons ranging from chain length C₂₅ to C₃₃.
- Many of these are mono- or di-methylated, unsaturated hydrocarbons are present in very small

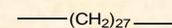
Sofisticatte fabbriche di alcani



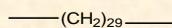
Pentacosane (C₂₅)



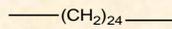
Heptacosane (C₂₇)



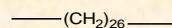
Nonacosane (C₂₉)



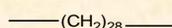
Hentriacontane (C₃₁)



Hexacosane (C₂₆)



Octacosane (C₂₈)

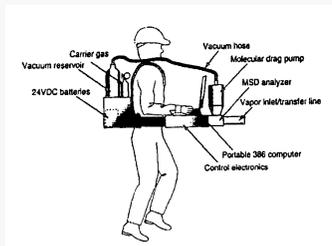


Triacontane (C₃₀)



On-site Environmental and *In Situ* Process Analysis by Mass Spectrometry

Mobile Laboratories with GC/MS and other Analytical Systems



On-site



No variation of sample composition; no evaporation of volatile components; cost reduction.
Quick response → site restoration action



In-situ

The Beginnings – Vehicle Portable GC-MS

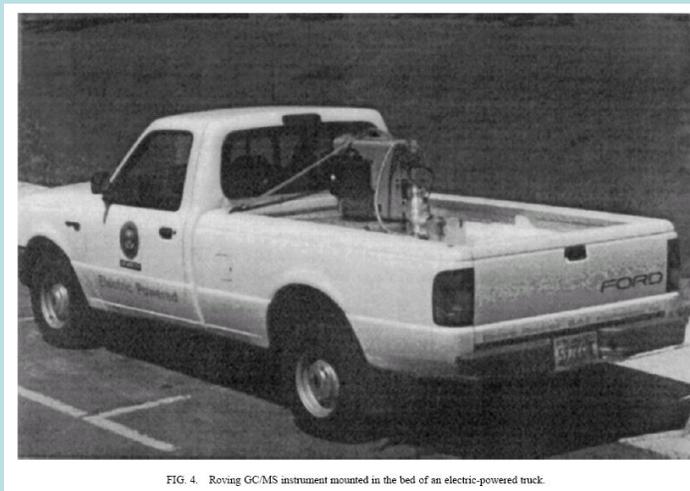


FIG. 4. Roving GC/MS instrument mounted in the bed of an electric-powered truck.

Airborne GC-MS



Bruker Franzen GC-MS for German Fire Brigade

89

Henk L.C. Meuzelaar, J. P. D., and Neil S. Arnold *Advances in Field-Portable Mobile GC/MS Instrumentation*. *Field Anal. Chem. Technol.* 2000, 4, 3-13.

Early Viking "Man"-Portable GC-MS



Early Viking Man Portable GC-MS

90

Henk L.C. Meuzelaar, J. P. D., and Neil S. Arnold *Advances in Field-Portable Mobile GC/MS Instrumentation*. *Field Anal. Chem. Technol.* 2000, 4, 3-13.

TRIDION™-9 / Guardion™ GC-TMS System



LTM gas chromatograph

5 m x 0.1 mm x 0.4 μ m MTX-5
Fast temperature programming
50 - 300°C @ 120°C/min

Toroidal ion trap

Electron ionization
Electronic pressure control
45 to 500 m/z @ 10 scans/s

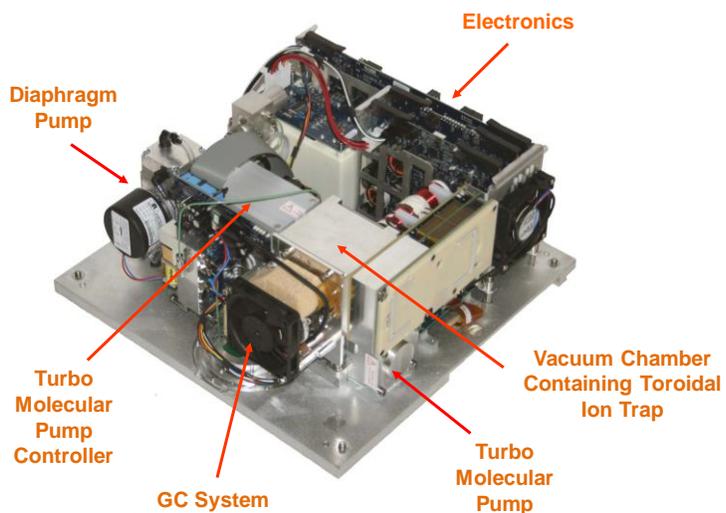
Portable

~14 kg (w/ battery and all
required utilities)

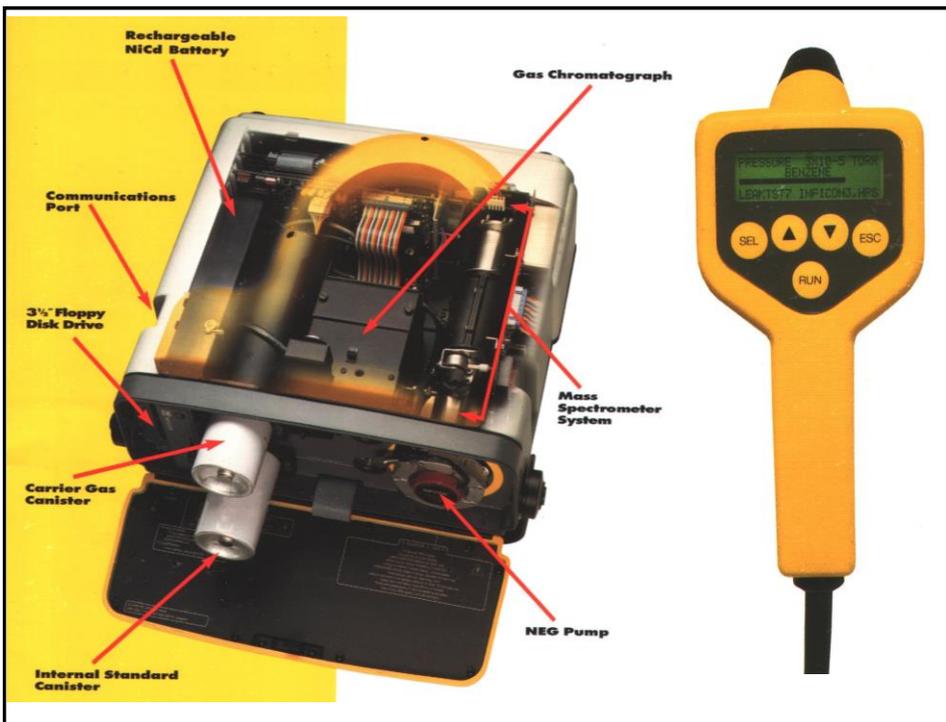
Low power

~120 W peak
~ 60 W average

GC-TMS System Components



Designed to be operated in PPE



Specifications

Mass Range	1-300 AMU
Scan Rate	1000 AMU/sec @ 10 points per AMU
Ionization Mode	70 eV EI
Vacuum System	15 l/sec NEG pump
Operating Conditions	0°C to 45°C
L x W x H	46 cm x 43 cm x 18 cm (18" x 17" x 7")
Weight	approximately 16 kg (35 lbs) with batteries
Internal Power Consumption	24 volts, 30 watts at normal operating conditions
Carrier Gas	Nitrogen
GC and Inlet System Temperature Range	15°C above ambient to 80°C, isothermal
GC Column	100% Methyl Silicone Phase, 30 m x .32 mm i.d. x 1.0 µ film

SIM Channels	10
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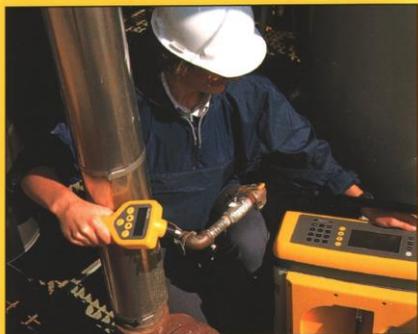
SERVICE MODULE

L x W x H	46 cm x 43 cm x 22 cm (18" x 17" x 8.5")
Weight	approximately 20 kg (45 lbs)
Vacuum System	Molecular drag/turbo pump with oil-free backing pump
Power Consumption	110 volts, 200 watts at normal operating conditions

Acetone-67641
 Benzene-71432
 Bromodichloromethane-75274
 Carbon Disulfide-75150
 Chloroform-67663
 Methyl iso-Butyl Ketone-108101
 Styrene-100425
 Tetrachloroethylene-127184
 Toluene-108883
 Bromoform-75252
 Vinyl Acetate-105084
 Vinyl Chloride-75014
 Chloromethane-74873
 cis-1,2-Dichloroethene-156592
 Dibromochloromethane-124481
 1,1-Dichloroethane-75343
 1,2-Dichloropropane-78875
 Ethyl benzene-100414
 Ethyl chloride-75003
 Methylene Chloride-75092

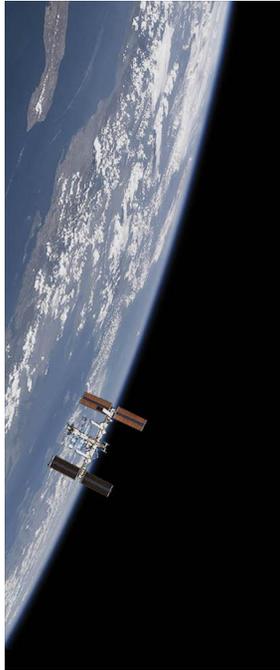
Target Analytes

1,1,2,2-Tetrachloroethane-79345
 1,1,1-Trichloroethane-71556
 1,1,2-Trichloroethane-79005
 p-Xylene-106423
 Bromomethane-74839
 Carbon Tetrachloride-56235
 Chlorobenzene-108907
 cis-1,3-Dichloropropene-10061015
 1,2-Dichloroethane-156592
 1,1-Dichloroethene-75354
 trans-1,2-Dichloroethene-156605
 Methyl Ethyl Ketone-78933
 2-Hexanone-591786
 trans-1,3-Dichloropropene-542756
 Trichloroethene-79016
 m-Xylene-108383
 o-Xylene-95476



DEPENDABLE TESTING FOR VOCs





Spettrometria di massa

non solo sulla terra!!!

Mars Viking Lander (1975)



The Viking '76 Mass Spectrometer

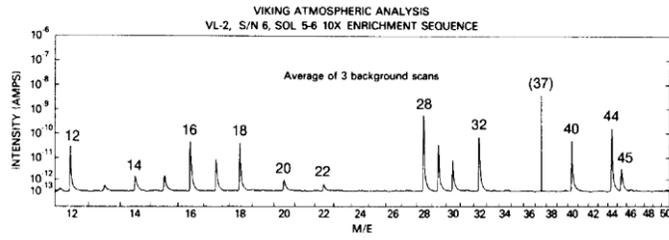


Figure 1.1. Mass spectrum (log intensity scale) of gases in the atmosphere of Mars.

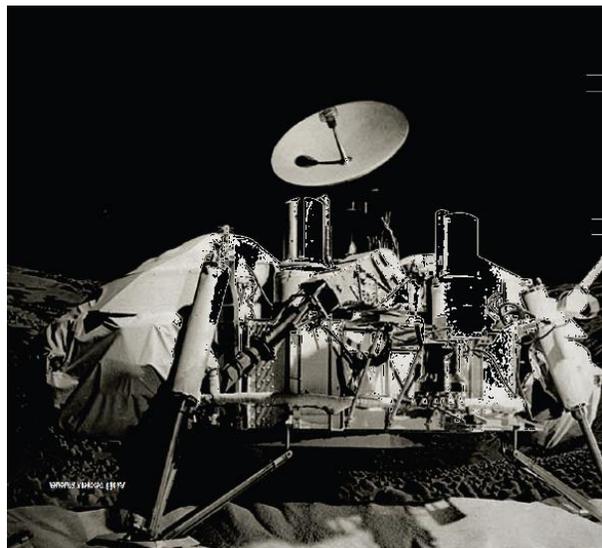
Composition of the Surface Atmosphere of Mars

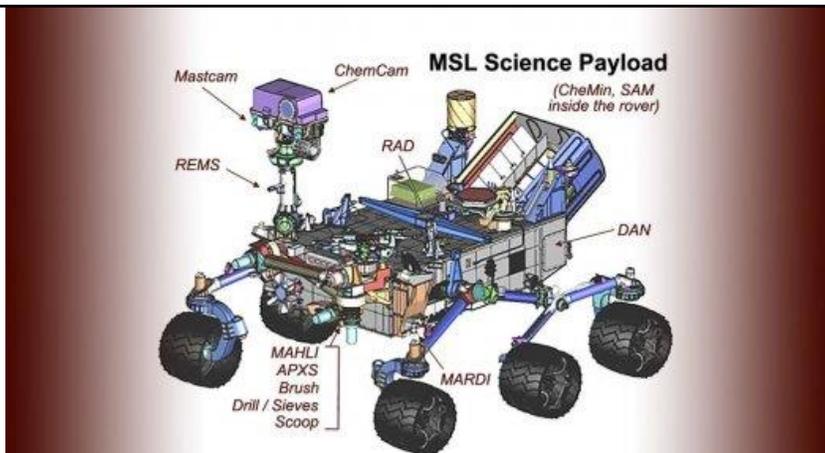
Gas		Concentration
Carbon Dioxide	CO ₂	95.3%
Nitrogen	N ₂	2.7%
Argon	Ar	1.6%
Carbon Monoxide	CO	(0.2)%
Oxygen	O ₂	0.18%
Water Vapor	H ₂ O	0.03%
Neon ²⁰ Ne	Ne	0.55 ppm
Krypton	Kr	0.3 ppm
Xenon	Xe	0.08 ppm
Ozone	O ₃	0.03 ppm

Comparison of the Isotope Ratios of Some Elements on Earth and Mars

Ratio of Element	Earth	Mars*
¹² C/ ¹³ C	89	90
¹⁶ O/ ¹⁸ O	499	500
¹⁴ N/ ¹⁵ N	277	165
⁴⁰ Ar/ ³⁶ Ar	292	3000
¹²⁹ Xe/ ¹³² Xe	0.97	2.5

* Uncertainties in these values are presently +/-10% except for Ar and Xe.

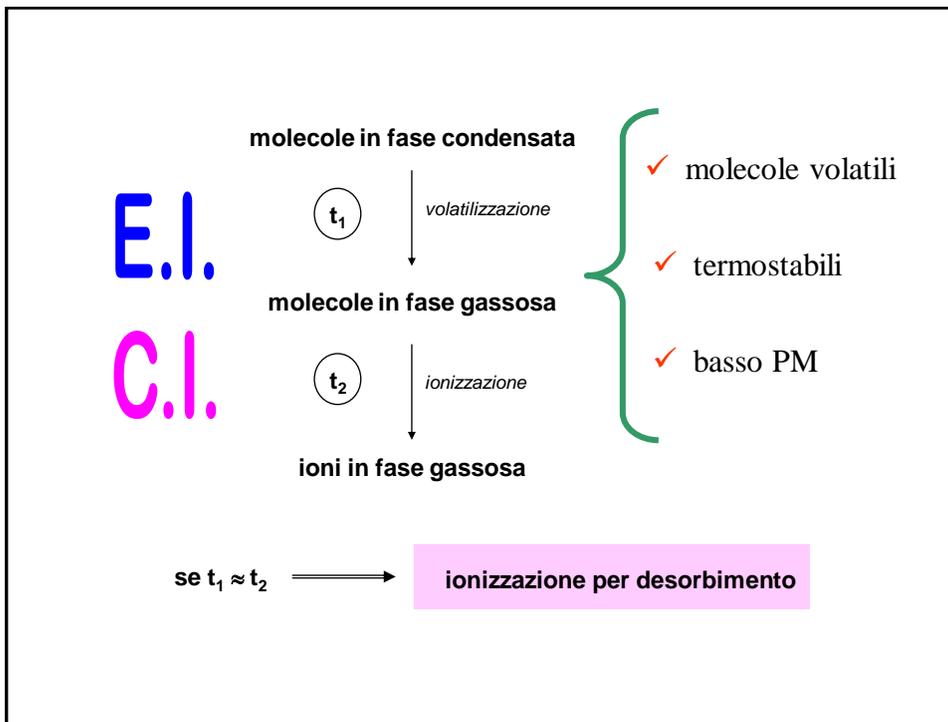




SAM = Sample Analysis at Mars **gas chromatograph/mass spectrometer**

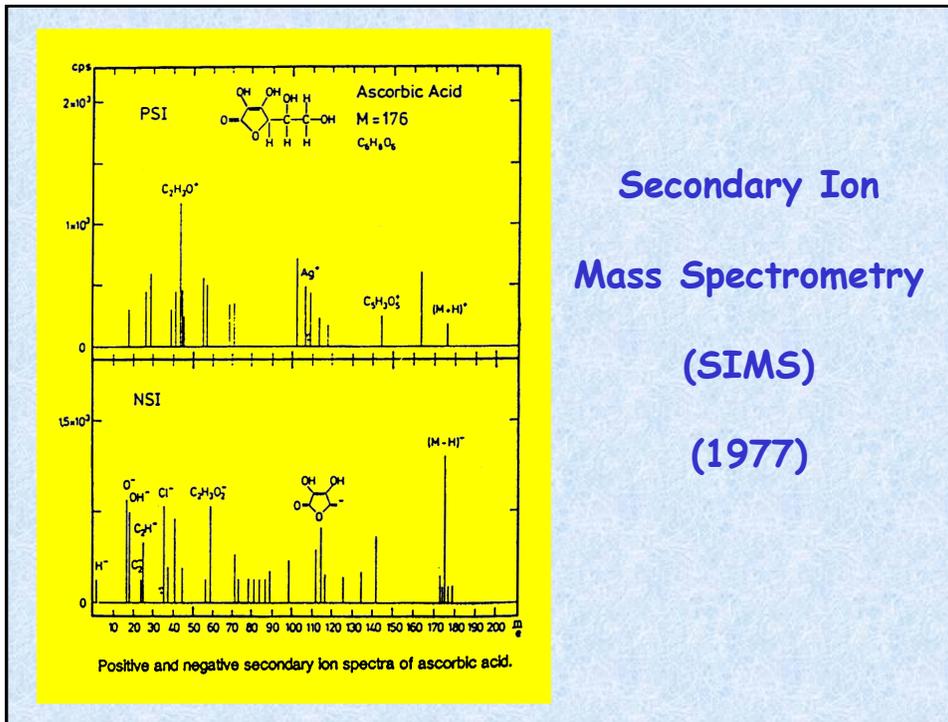
Mastcam = Mast Camera; ChemCam = Chemistry and Camera instrument; RAD = Radiation Assessment Detector; CheMin = Chemistry and Mineralogy instrument; DAN = Dynamic Albedo of Neutrons; MAHLI = Mars Hand Lens Imager; APXS = Alpha Particle X-ray Spectrometer; REMS = Rover Environmental Monitoring Station. MARDI, the Mars Descent Imager, will shoot video of the rover's descent. The brush, drill, sieves and scoop are tools on the rover's robotic arm. *NASA/JPL-Caltech*

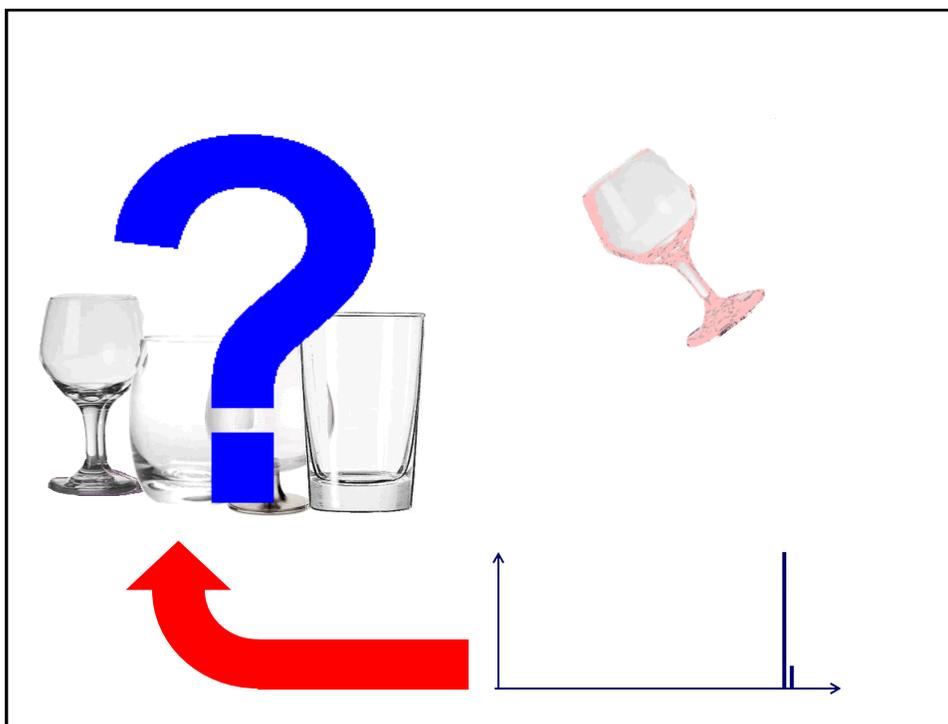




Metodi di ionizzazione per desorbimento

Rapida **addizione** di energia a **molecole in fase condensata** con la conseguente produzione di **specie ioniche stabili** in fase gassosa, generalmente **a elettroni pari**, che si frammentano solo in minima parte.

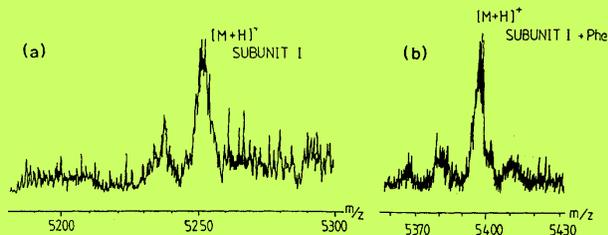




Fast Atom Bombardment
1981

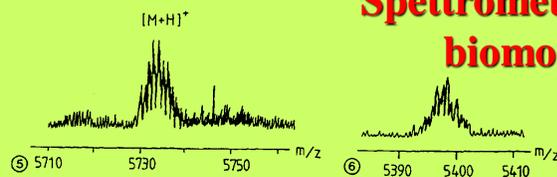
A new ion source for molecular structure determination of **thermolabile** and **involatile** compounds by mass spectrometry

M. Barber, R. S. Bordoli, G. J. Elliot, R. D. Sedgwick, A. N. Tyler, *J. Chem. Soc. Chem. Commun.*, 1981, 325



(a) FAB mass spectrum obtained by scanning from m/z 5160 to m/z 5330 over 50 sec. Protonated molecular ion cluster for subunit I is present near m/z 5250.
 (b) FAB mass spectrum obtained by scanning from m/z 5360 to m/z 5430 over 50 sec. Protonated molecular ion cluster for N-terminally extended subunit I is present near m/z 5400.

Spettrometria di massa biomolecolare



(5) FAB mass spectrum of the molecular ion region of bovine insulin obtained using a 50 sec scan over 50 mass units.
 (6) FAB mass spectrum of the molecular ion region of the minor component of subunit I. Operating conditions were the same as those used to obtain the insulin data.

1982



Premio Nobel 2002 per la Chimica

La commissione per i Nobel dell'[Accademia Reale delle Scienze Svedese](#) ha deciso di assegnare il Premio Nobel 2002 per la Chimica

Per lo sviluppo di metodi per l'identificazione e le analisi della struttura delle macromolecole biologiche



per metà congiuntamente a:

John B. Fenn, born 1917 in New York City, USA (US citizen).
Virginia Commonwealth University, Richmond, USA

ed a



Koichi Tanaka, born 1959 (43 years) in Toyama City, Japan
Shimadzu Corp., Kyoto, Japan

Per il loro lavoro sullo sviluppo di metodi di ionizzazione per desorbimento blando per le analisi di spettrometria di massa delle macromolecole biologiche

e per l'altra metà a

Kurt Wüthrich, born 1938 (64 years) in Aarberg, Switzerland
Eidgenössische Technische Hochschule (ETH), Swiss Federal Institute of Technology, Zürich, Switzerland
The Scripps Research Institute, La Jolla, USA

Extraction of ions from solutions under atmospheric pressure as a method for mass spectrometric analysis of bioorganic compounds[†]

M. L. Alexandrov, L. N. Gall*, N. V. Krasnov, V. I. Nikolaev, V. A. Pavlenko and V. A. Shkurov

Rapid Commun. Mass Spectrom. 2008; 22: 267–270

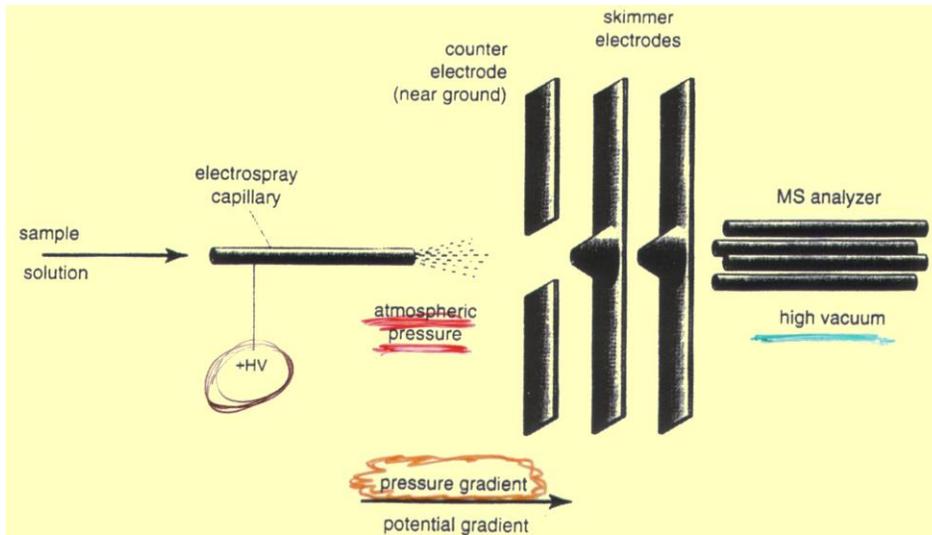


Dokl. Akad. Nauk SSSR **1984**; 277: 379–383.

Extraction of ions under atmospheric pressure (EDIAP)

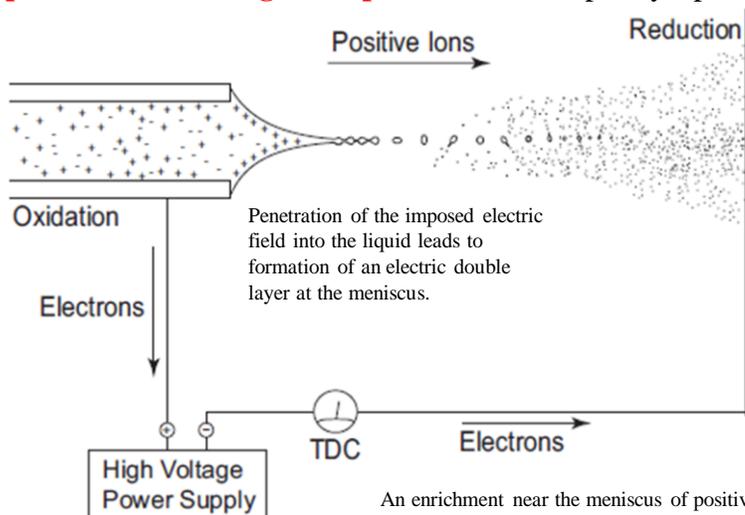
Fenn's group and Gall's group published was 1984; Fenn et al. published on an electrospray interface in *Analytical Chemistry*, whilst Gall and co-workers published (in *Dokl. Akad. Nauk SSSR* and *Bioorganicheskaya Khimiya*).

ELECTROSPRAY

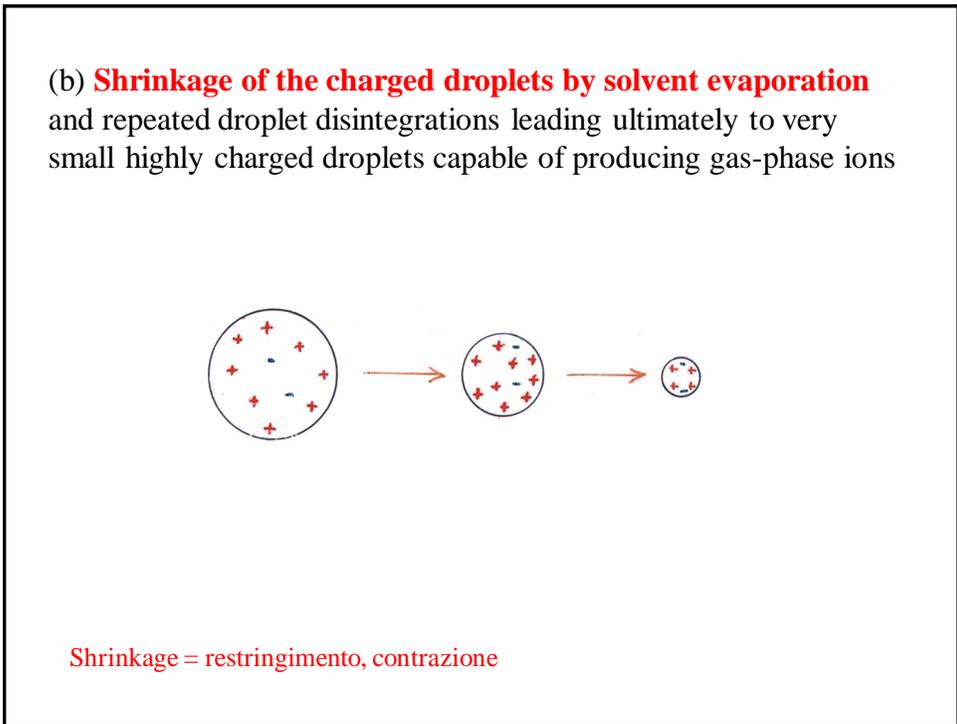
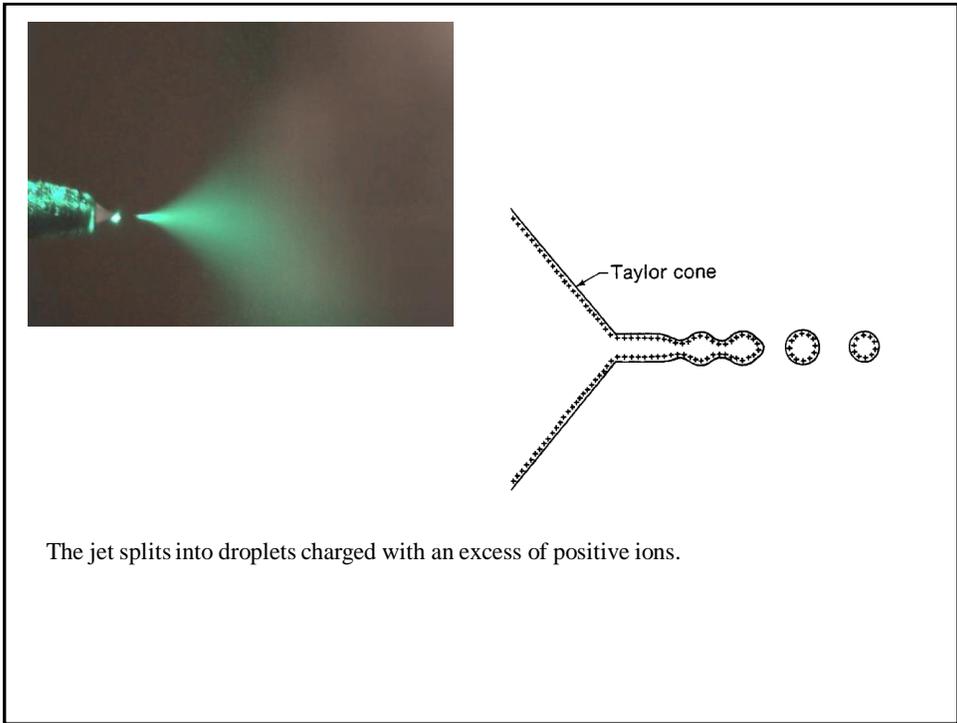


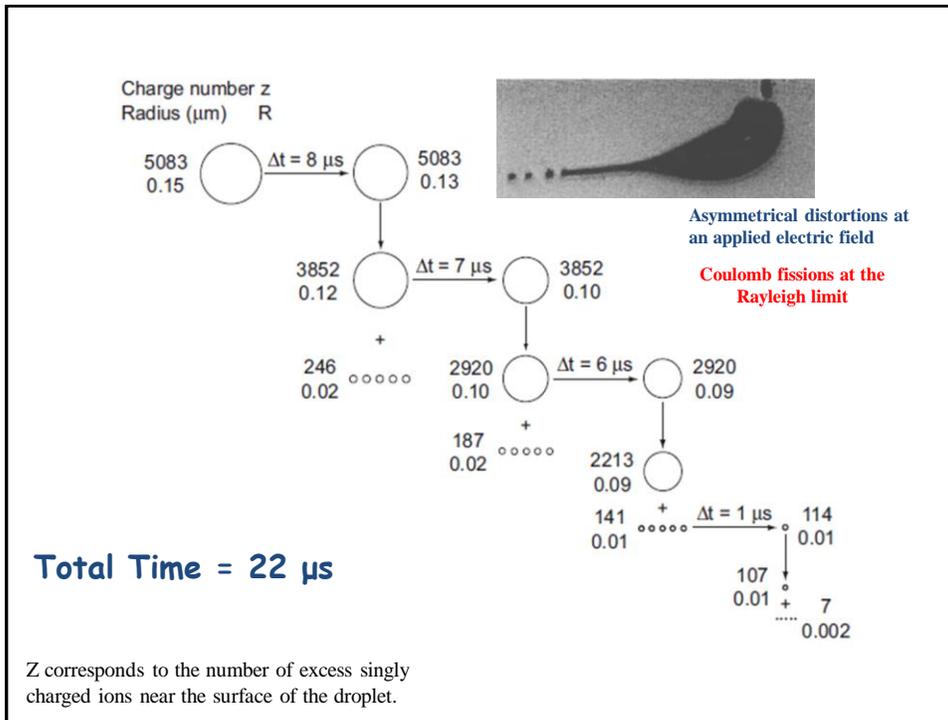
There are three major steps in the production of gas-phase ions from electrolyte ions in solution.

(a) **production of charged droplets** at the ES capillary tip



An enrichment near the meniscus of positive ions present in the solution causes the formation of a cone and a jet charged by an excess of positive ions.



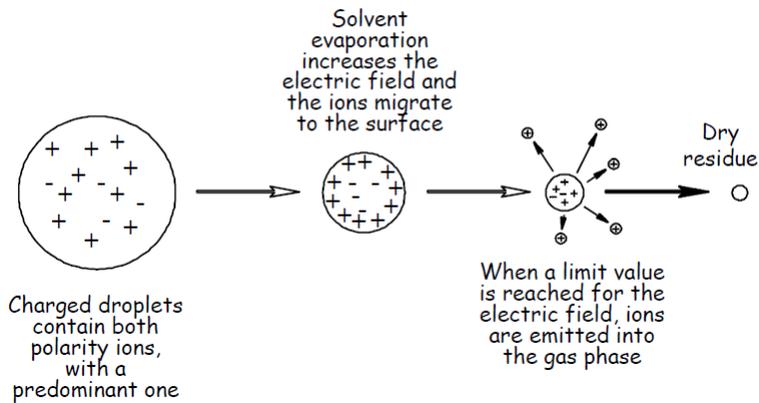


(c) the mechanism by which **gas-phase ions are produced** from the very small and highly charged droplets.

The Ion Evaporation Model (IEM)

The Charged Residue Model (CRM)

Ion Evaporation Mechanism

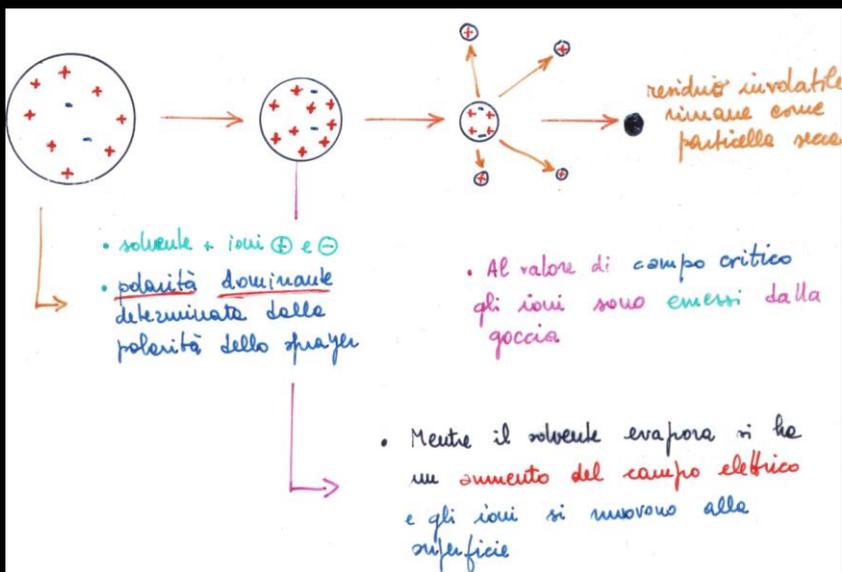


direct ion emission from the droplets.

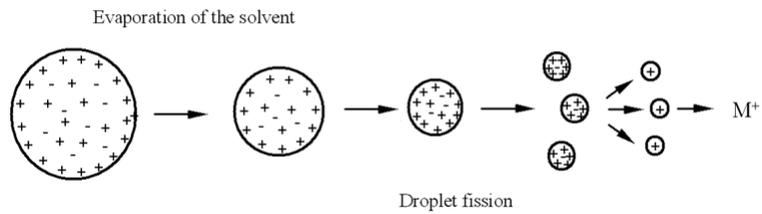
It becomes dominant over Coulomb fission for droplets with radii of $R \leq 10 \text{ nm}$

Dole M., Mack L.L. *J. Phys. Chem.* 1968; 49: 2240

$1 \text{ nm} = 10^{-9} \text{ m}$



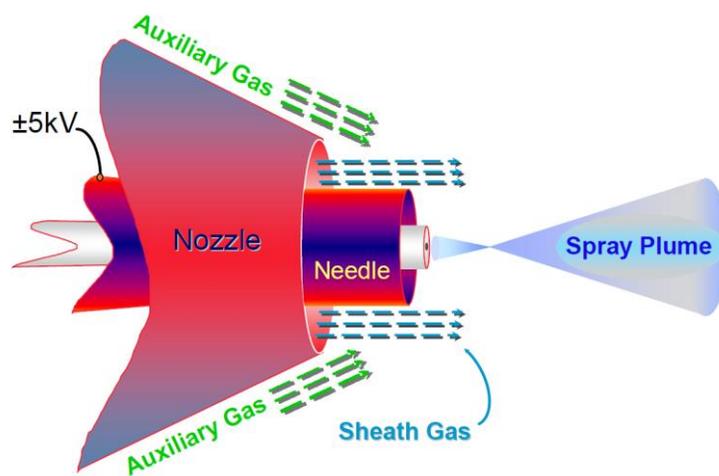
The Charged Residue Model (CRM)

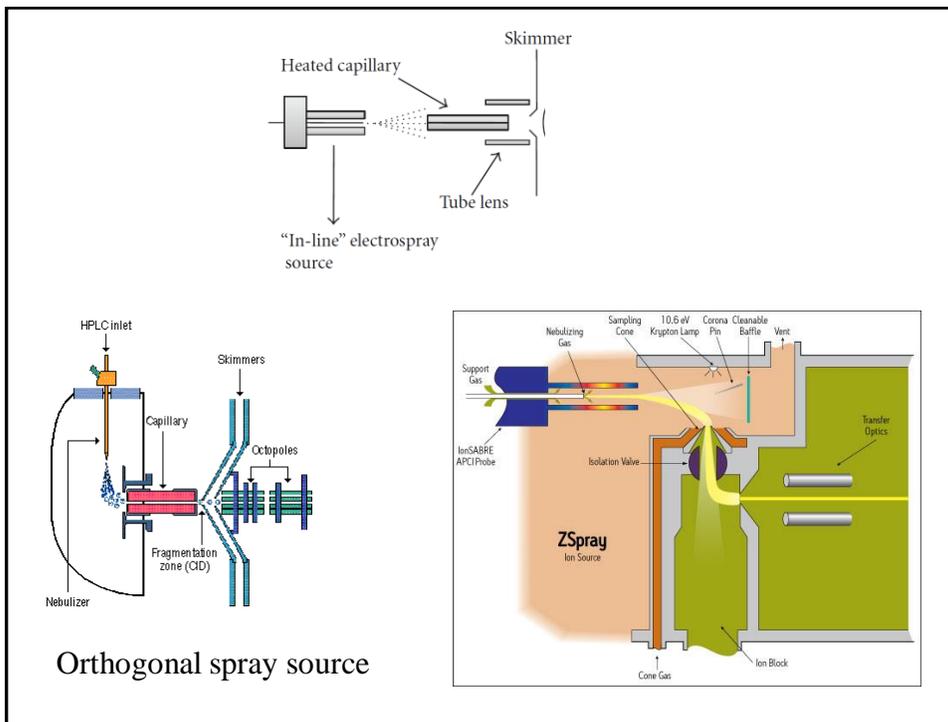


formation of extremely small droplets that could contain **one** analyte molecule and some ionic charges.

Fission = scissione

Iribarne J.V., Thomson B.A. *J. Chem. Phys.* 1976; 64: 2287



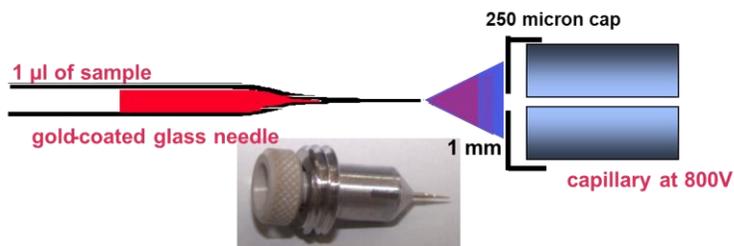


Orthogonal spray source

Nanospray Source

NanoElectrospray at flow rates down to 25 nl/min

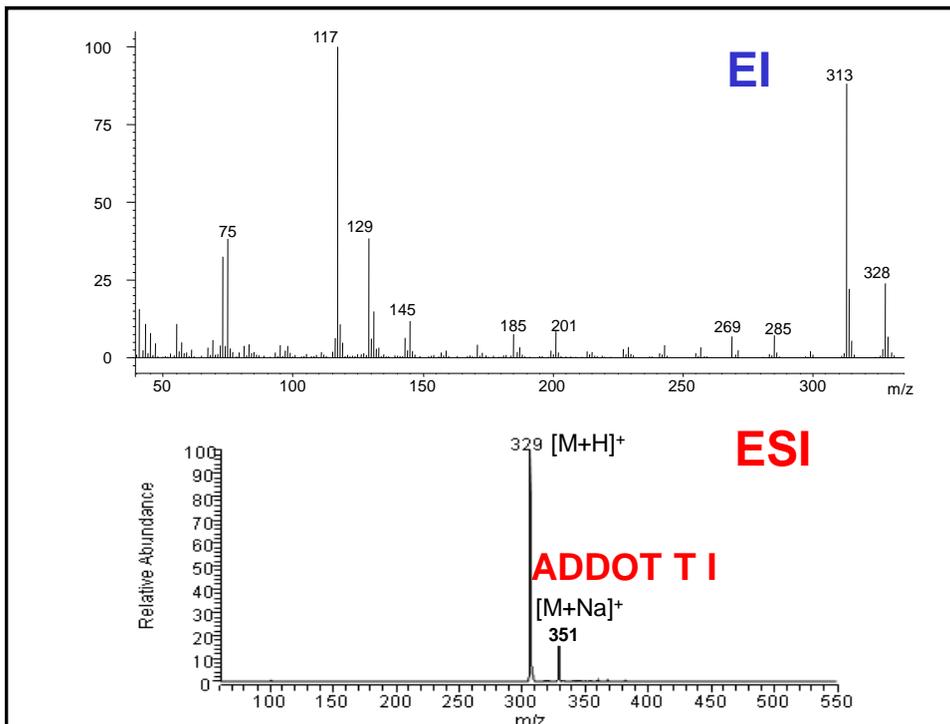
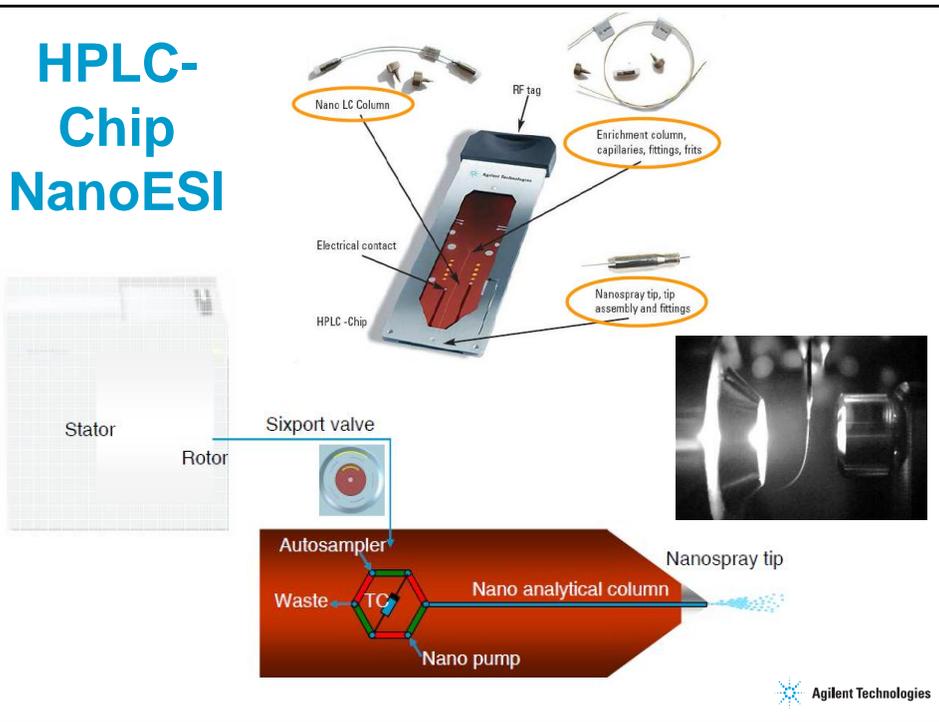
- 40 minutes of analysis time from 1 μL of sample



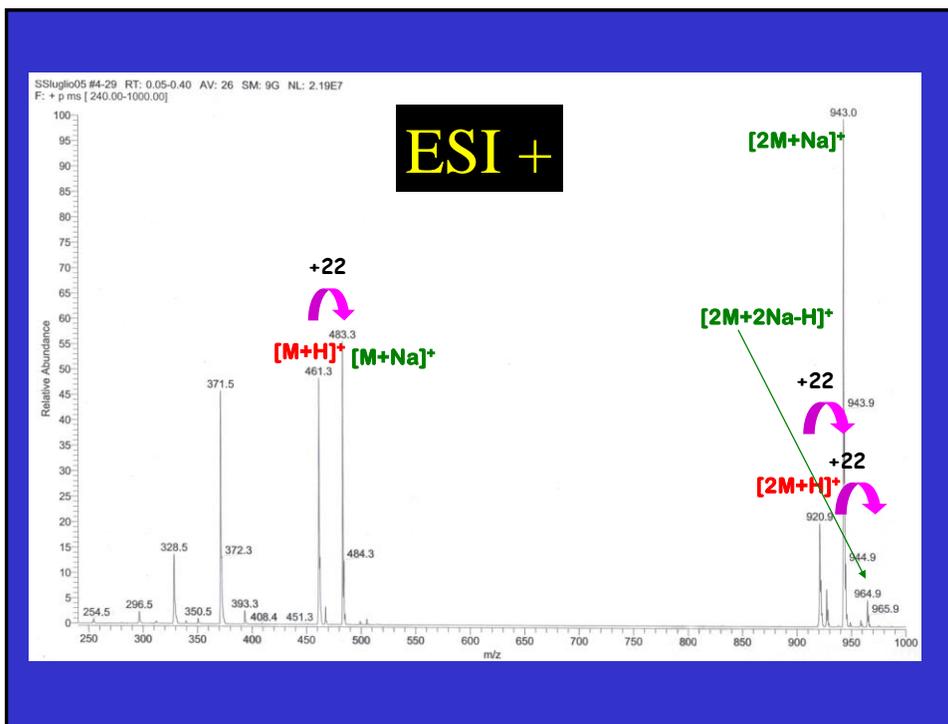
In Nanospray, a needle with an extremely small inner diameter is used. The glass needle is gold-coated for applying the correct electric potential onto the needle. For off-line nanospray, the needle is filled with about 1-5 μL of sample using a gel loader tip.

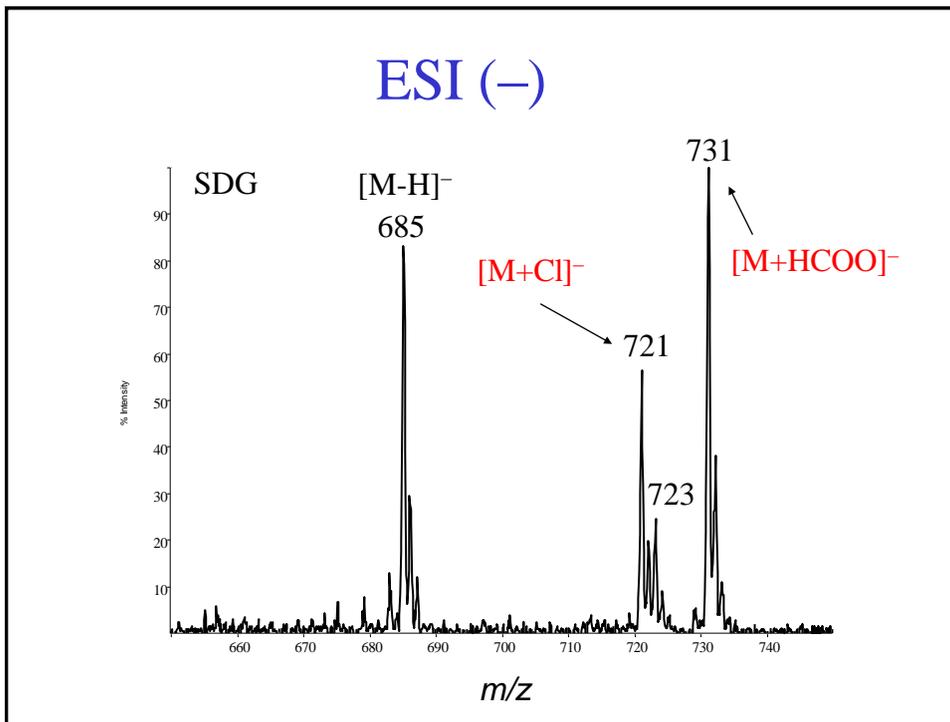
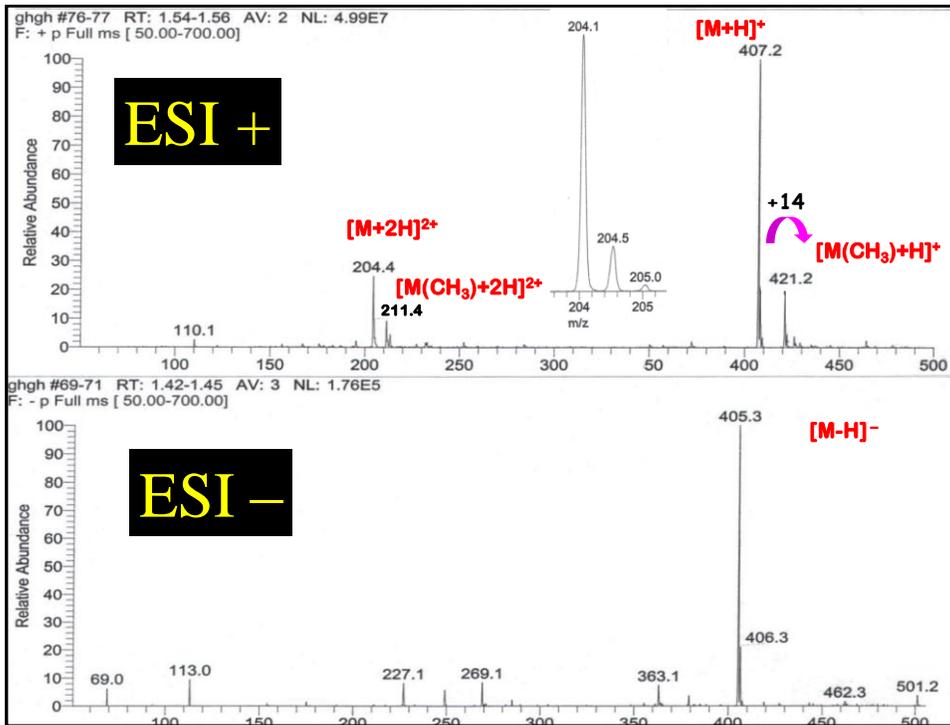
When the electric field is applied, a spray is generated with a flow rate down to 25 nl/min. That means that 1 μL of sample can be sprayed for ca. 40 min, enough time for complex MS/MS experiments with extremely high sensitivity and very low consumption of sample.

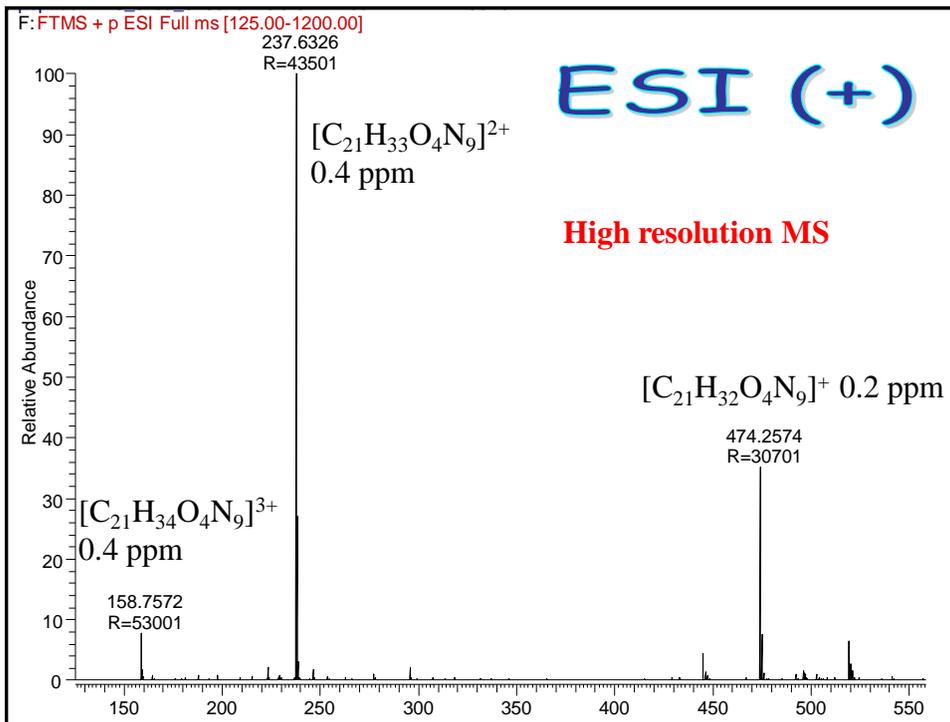
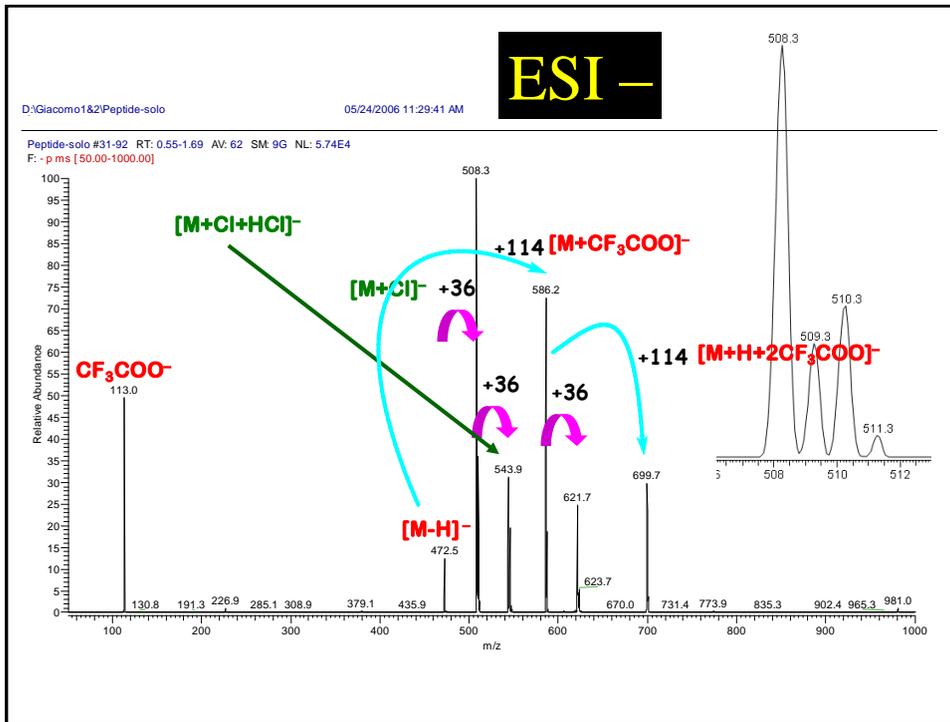
(from Bruker Daltonics)



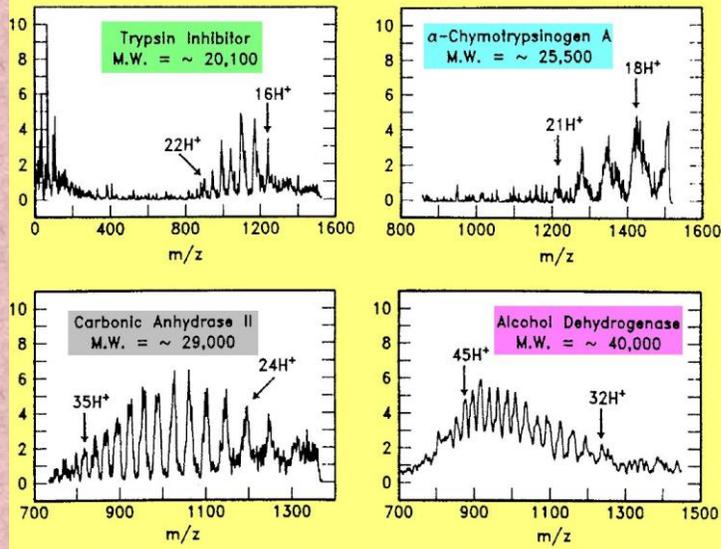
ADDUCTS			Added mass
Positive ion MS			
[M+	H] ⁺		1
[M+	Na] ⁺		23
[M+	K] ⁺		39
[M+	H+	NH ₃] ⁺	18
Negative ion MS			
[M+		HCOO] ⁻	45
Solvent adducts			
[M+	H+	CH ₃ CN] ⁺	42
Multimer adducts			
[2M+	H] ⁺		2M+1





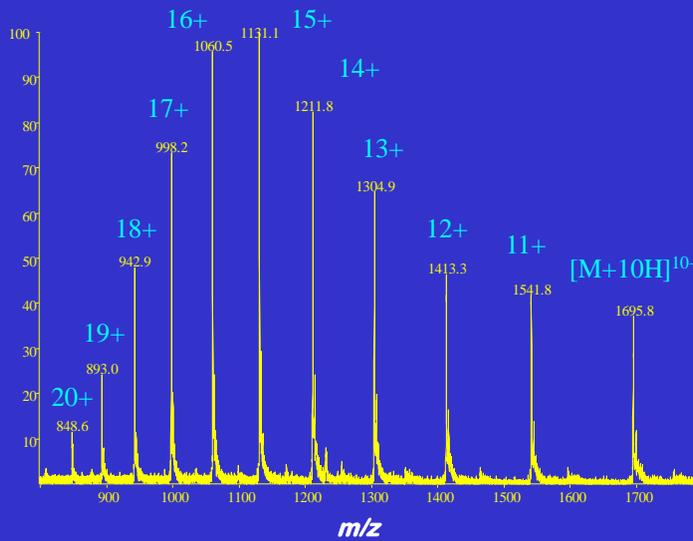


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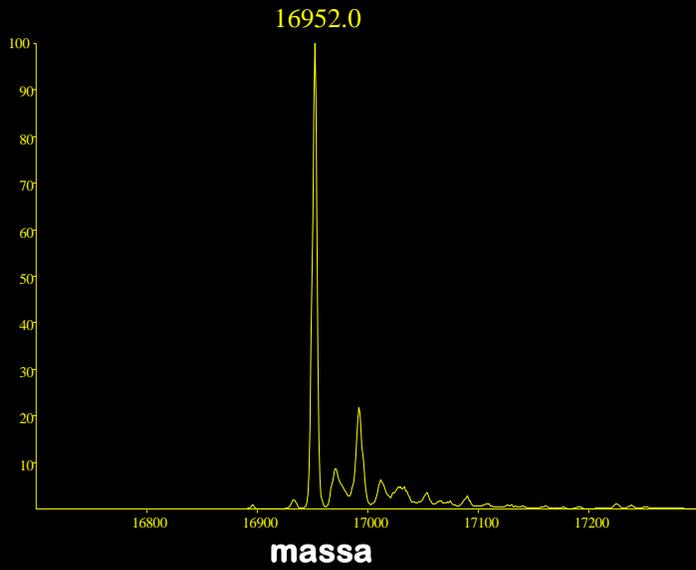


Mann, M.; Meng, C. K.; Fenn, J. B. *Proceeding of 36th Annual Conference on Mass Spectrometry and Allied Topics*; San Francisco, 1988; pp 1207-08.

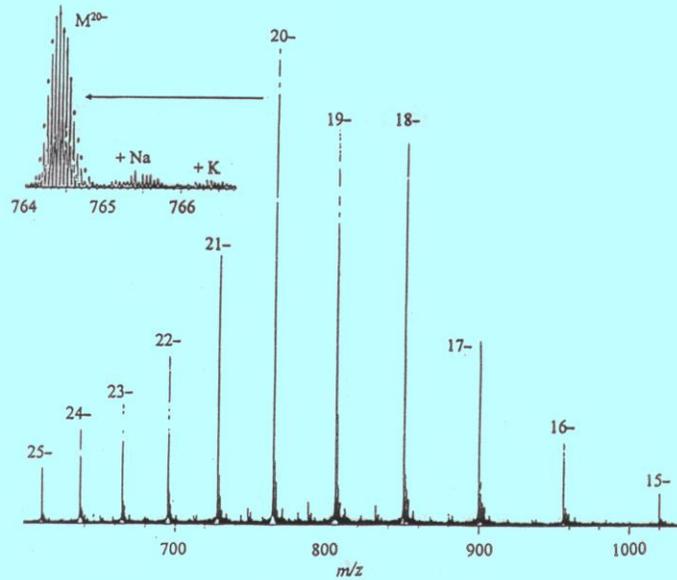
Spettro ESI della mioglobina di cavallo



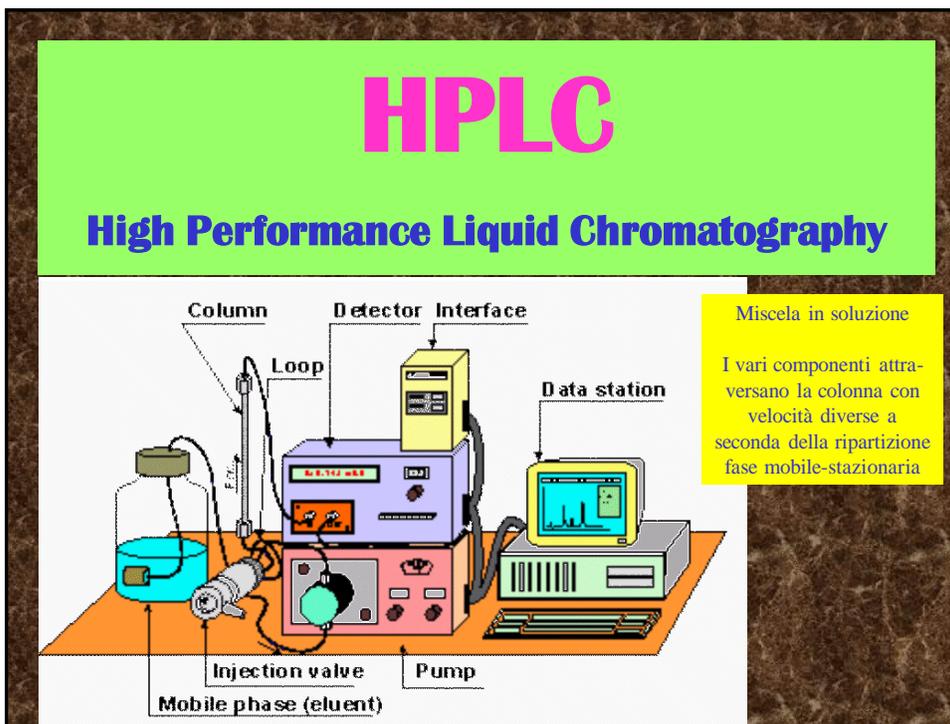
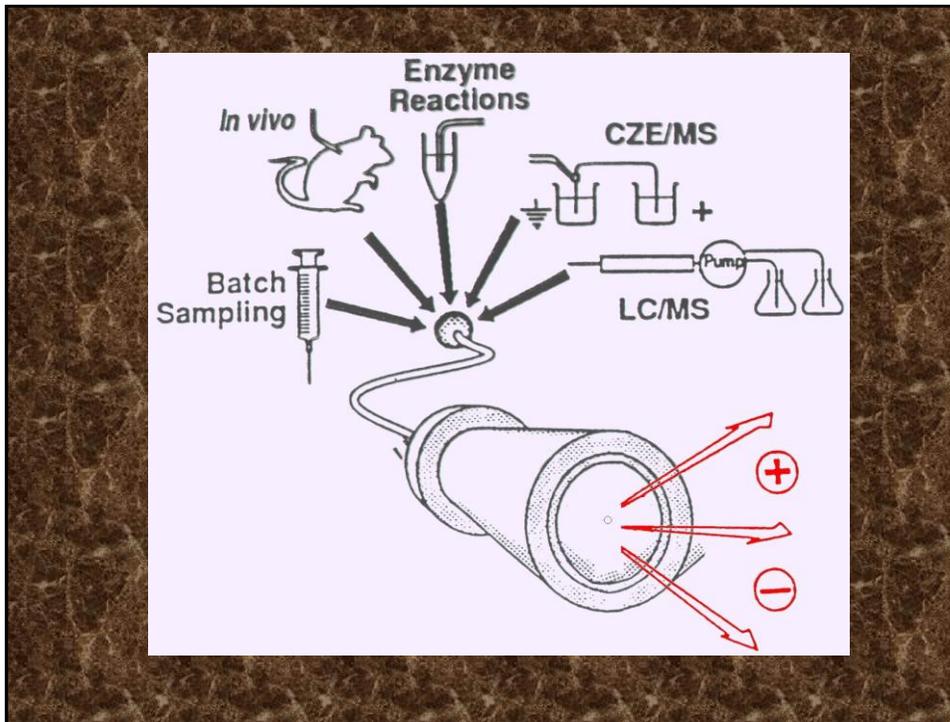
Spettro deconvoluto della mioglobina di cavallo

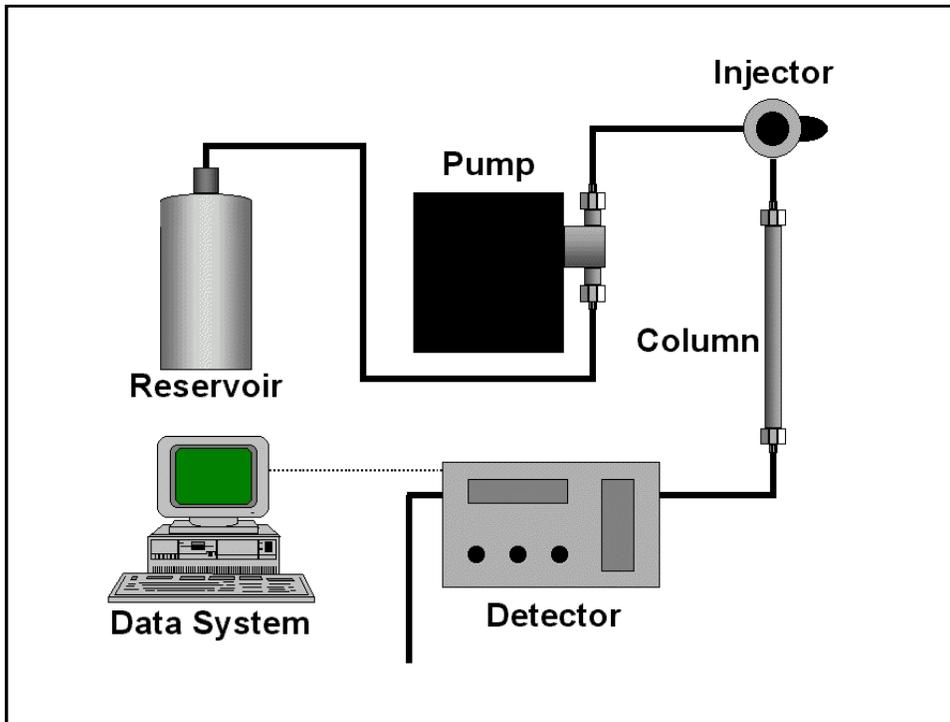


DNA sequencing by mass spectrometry



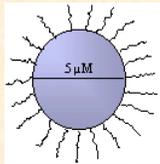
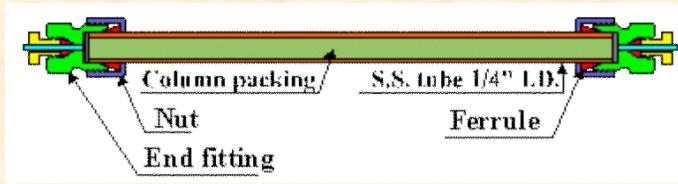
ESI FTMS mass spectrum of a single-stranded DNA 50-mer with a mass measurement accuracy of 7 ppm.





HPLC e Spettrometria di Massa

HPLC	Spettrometria di massa
E' in grado di separare miscele contenenti composti non volatili, polari , anche ad alto peso molecolare	Offre una elevata sensibilità.
Permette separazioni relativamente rapide	E' altamente specifica
Per determinazioni qualitative e quantitative	Per determinazioni qualitative e quantitative
Usa strumentazione affidabile	Usa strumentazione affidabile.
E' relativamente facile da utilizzare	E' essenziale per l'identificazione di composti organici



column dimension:

diam.: 0.05 - 4.6 mm length: 2 - 30 cm

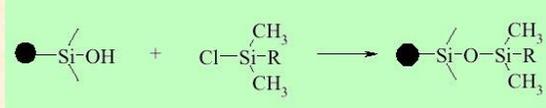
particle size (5 – 30 μm)

pore size (60 – 1000 Å)

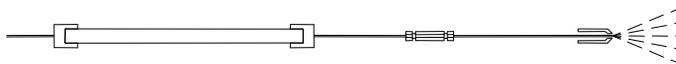
stationary phase



hydrophobic alkyl chains
(C4 – C18)

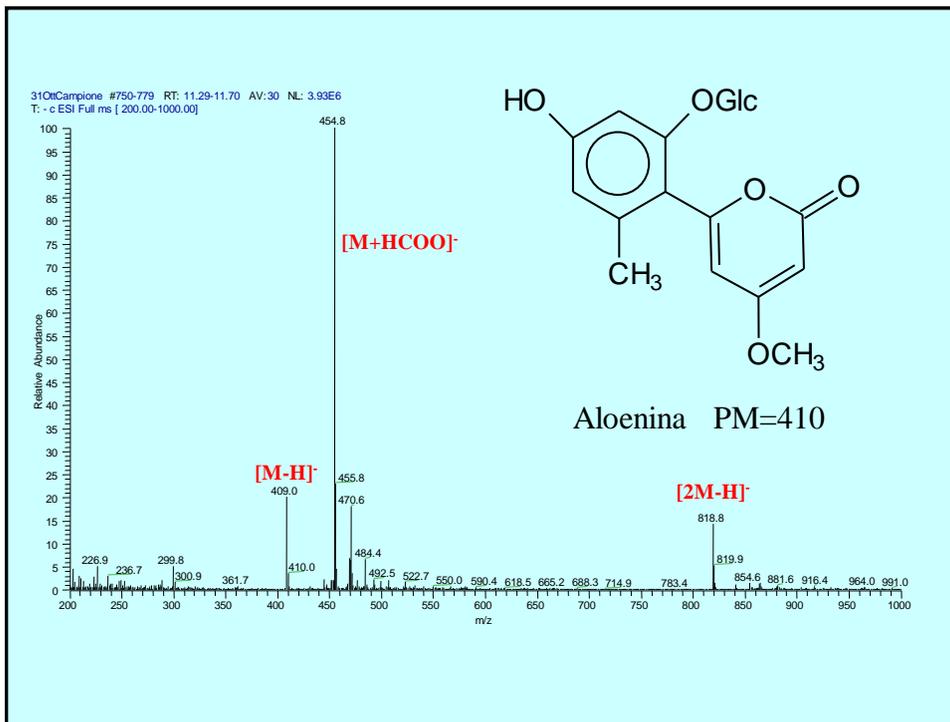
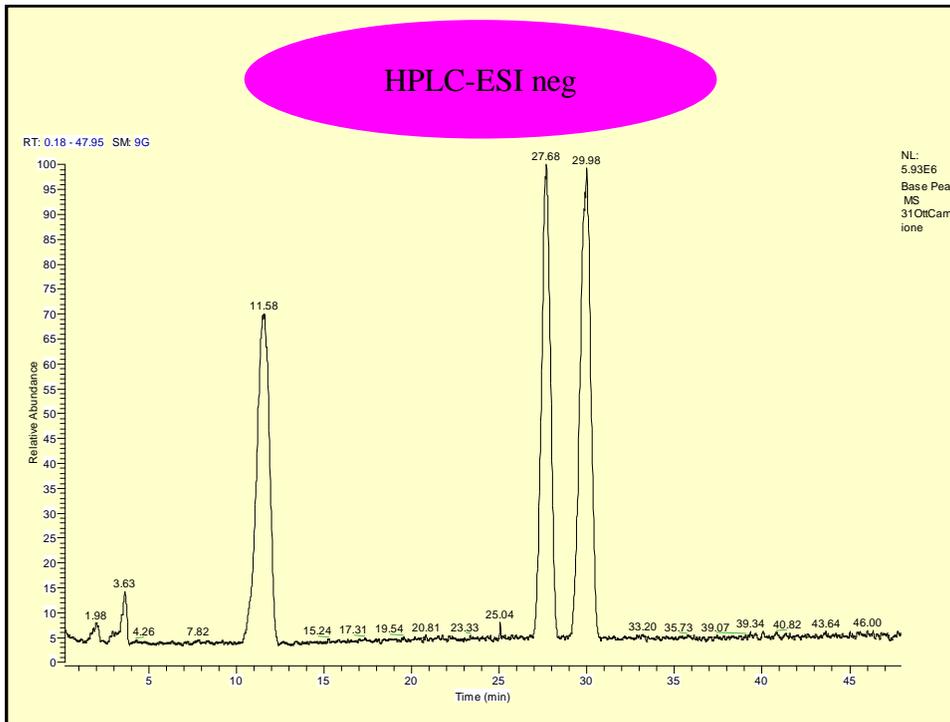


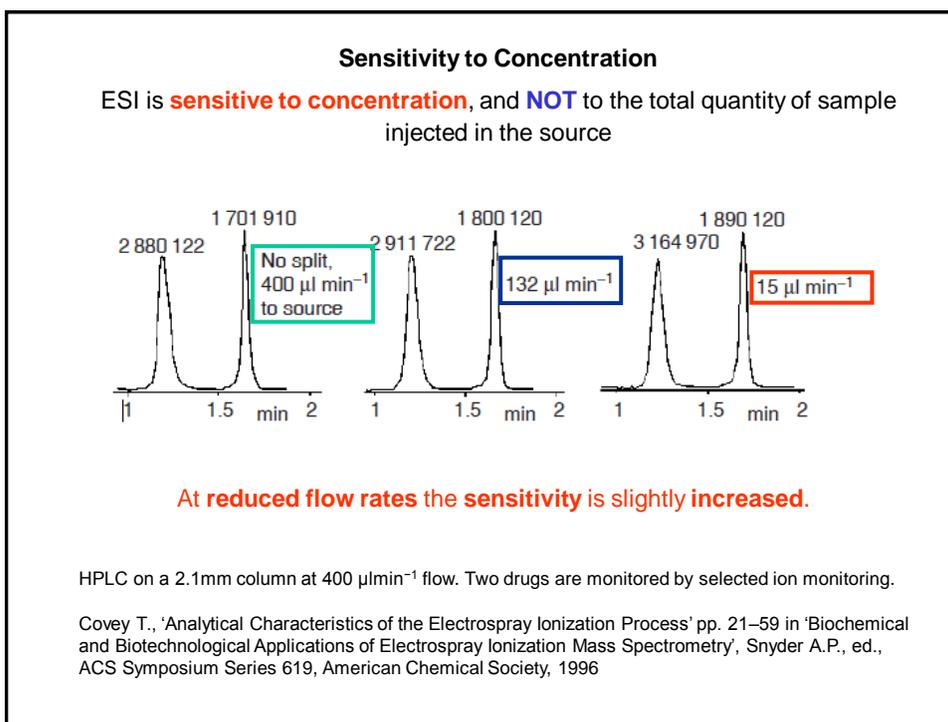
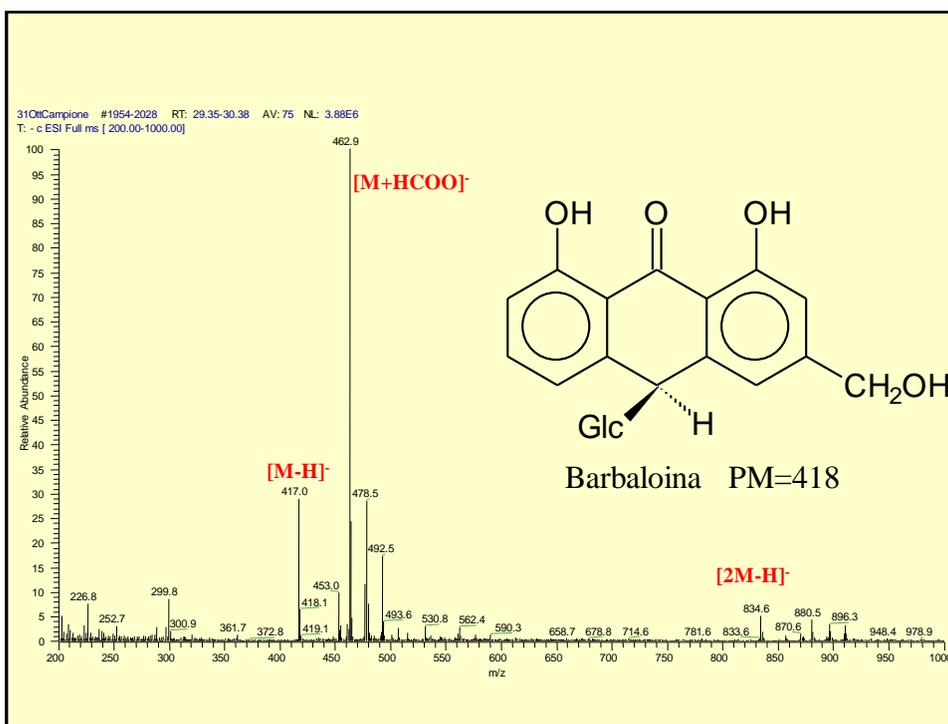
LC-ESI



Column i.d.	Flow rate	Technique
4.6 mm	1.0 ml/min	Conventional HPLC
1.0 mm	40 μl/min	Micro LC
300 μm	4 μl/min	Capillary LC
75 μm	200 nl/min	Nano LC



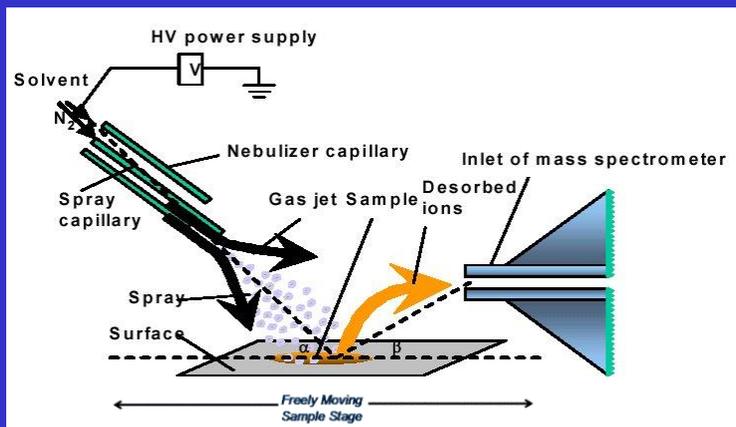




Desorption Electrospray Ionization (DESI)

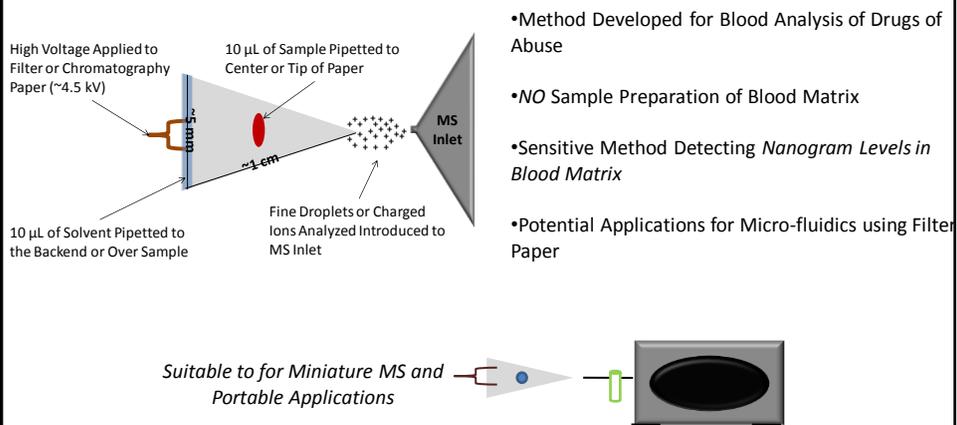
- Novel "gentle" ionization method for surface analysis
- Operates in atmosphere under ambient conditions
- Requires no sample preparation
- Effective for both organic and biological compounds
- Allows for *in situ* analysis of biological tissues
- Wide range of applications from clinical testing, environmental monitoring, forensics, homeland defense, process analytical testing (PAT), and surface imaging

G. Cooks et al., *Science*, **2004**, *306*, 471-473



New Ambient Ionization Method:

Paper Spray Ionization

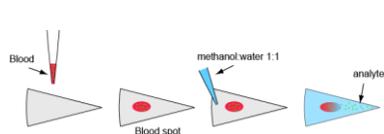


- Method Developed for Blood Analysis of Drugs of Abuse
- NO Sample Preparation of Blood Matrix
- Sensitive Method Detecting *Nanogram Levels in Blood Matrix*
- Potential Applications for Micro-fluidics using Filter Paper

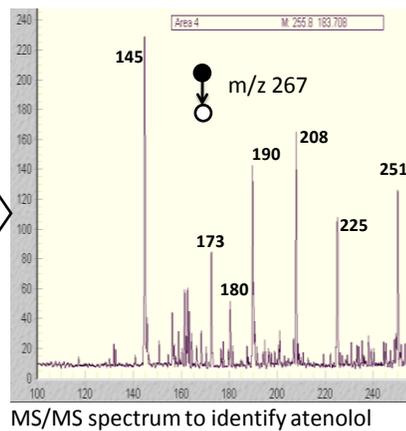
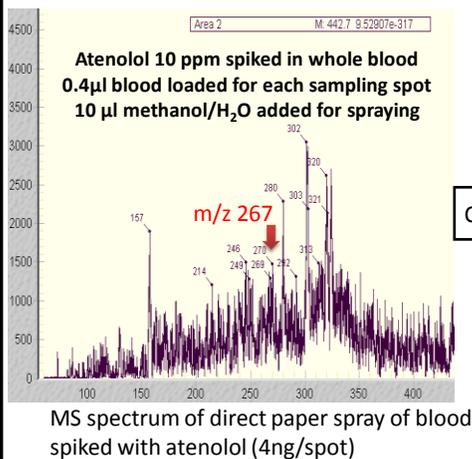
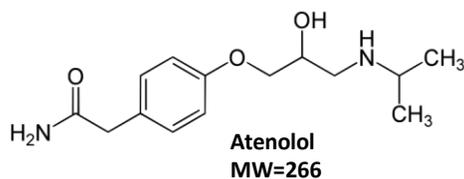
Suitable for Miniature MS and Portable Applications

Liu, J.; Wang, H.; Cooks, R. G.; Ouyang, Z.

Paper spray/mini MS (direct detection Atenolol in blood)



He Wang, Jiangjiang Liu, Guangming Huang

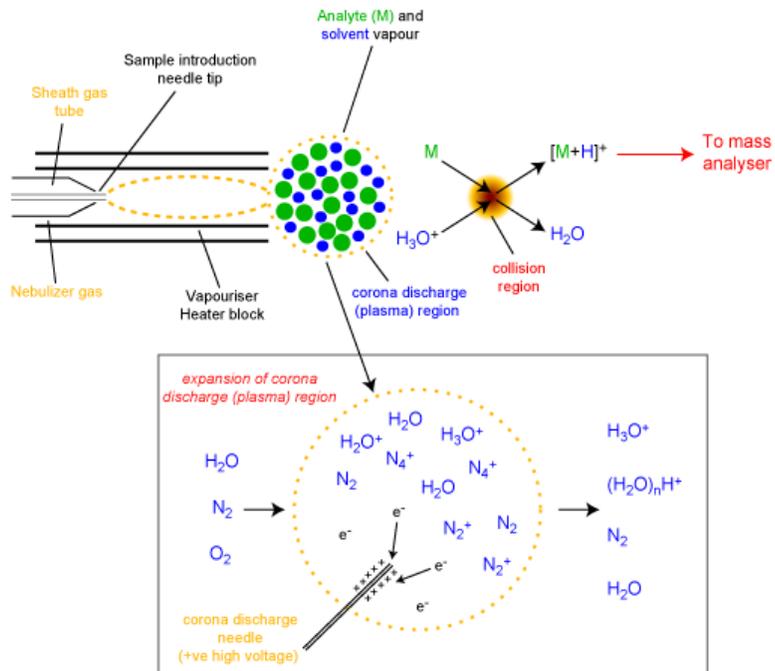
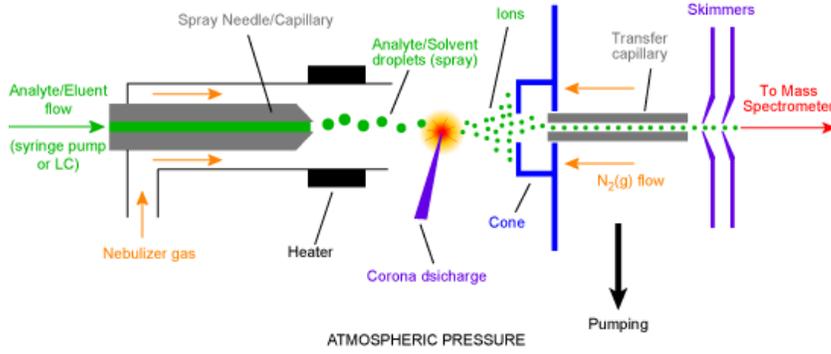


He Wang, Jiangjiang Liu, Guangming Huang, et al. unpublished

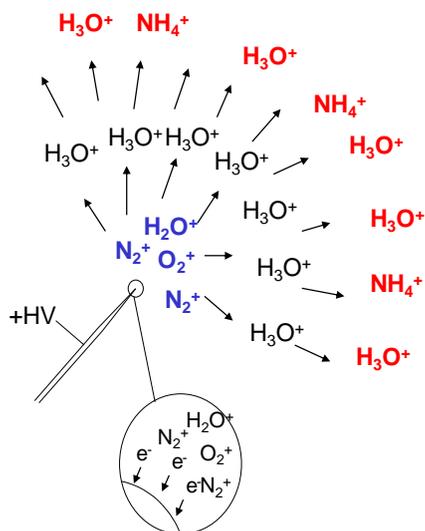
APCI Atmospheric Pressure Chemical Ionization

APPI Atmospheric Pressure Photo Ionization

Atmospheric Pressure Chemical Ionization

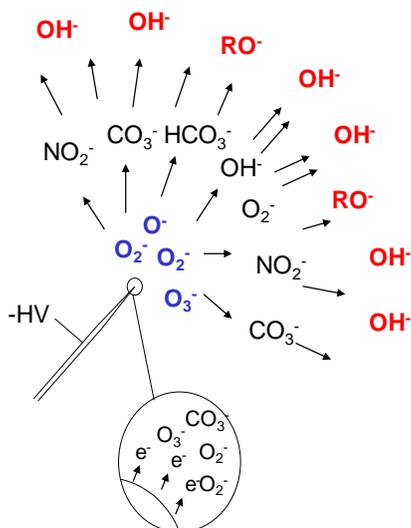


Corona Discharge Meccanismo Ioni Positivi



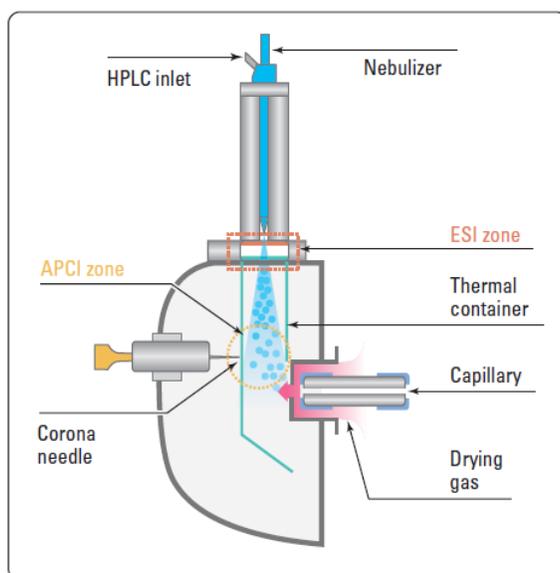
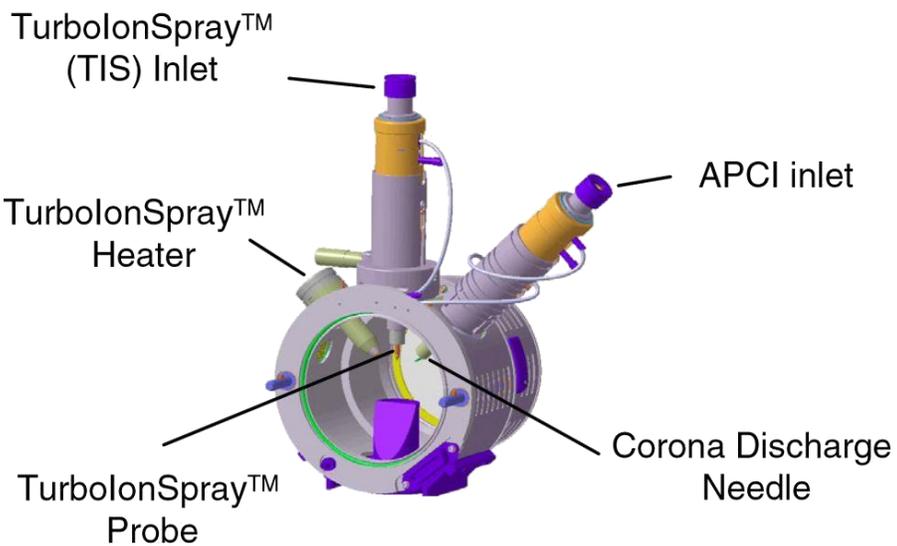
- In un primo stadio l'ago cattura elettroni formando ioni N_2^+ , O_2^+ , H_2O^+ ... (ioni primari).
- Gli ioni primari, aventi un tempo di vita molto breve, trasferiscono la carica al solvente, formando principalmente ioni H_3O^+ , NH_4^+ , RH_2O^+ ... (ioni reagenti).
- Infine, gli ioni reagenti trasferiscono la carica all'analita formando prevalentemente la specie $[M+H]^+$.

Corona Discharge Meccanismo ioni negativi

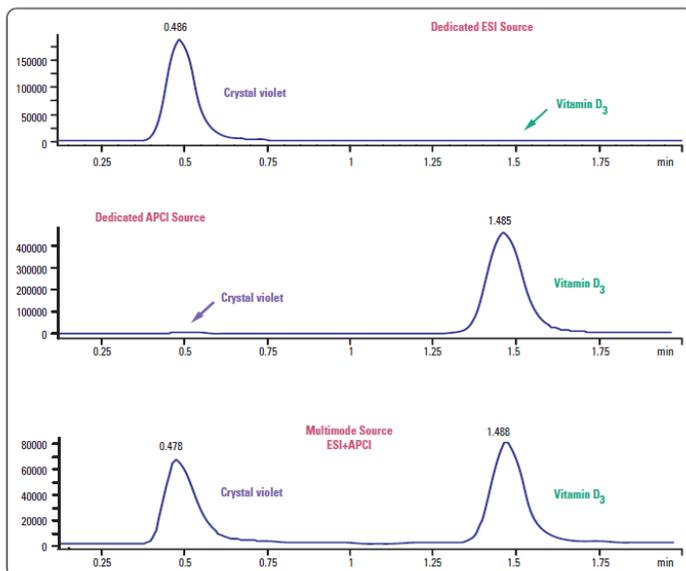


- In un primo stadio l'ago rilascia elettroni formando ioni O_3^- , O_2^- , NO_2^- , CO_3^- ... (ioni primari).
- Gli ioni primari, aventi un tempo di vita molto breve, trasferiscono la carica al solvente, formando principalmente ioni OH^- , HCO_3^- , RO^- ... (ioni reagenti).
- Infine, gli ioni reagenti trasferiscono la carica all'analita formando prevalentemente la specie $[M-H]^-$.

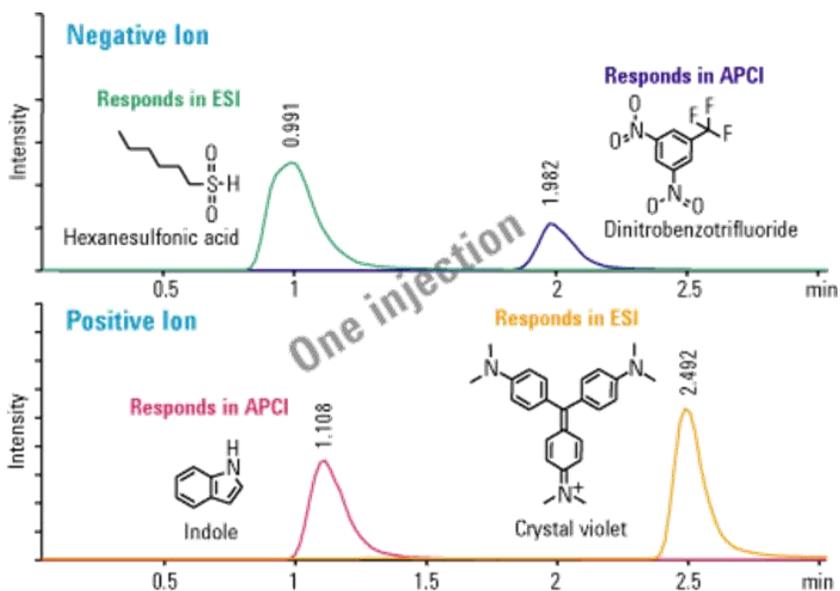
Combining ESI and APCI in the Same Source



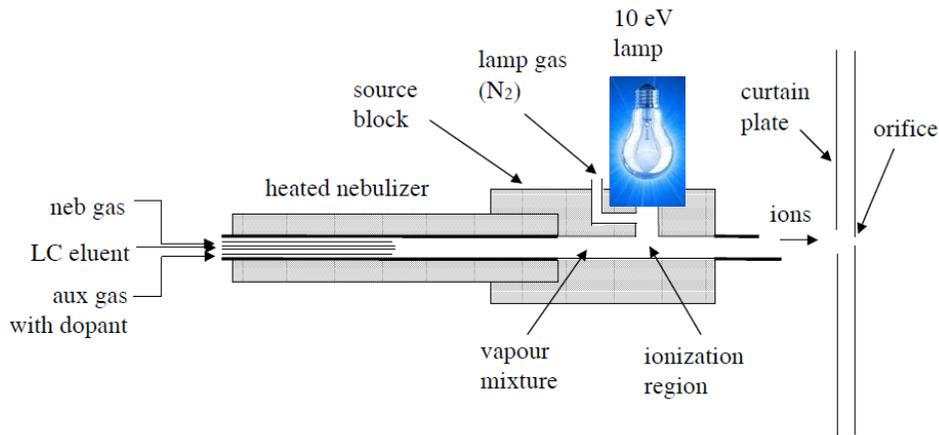
Multimode source incorporates optimized ESI and APCI zones



Simultaneous ESI and APCI maximizes throughput by eliminating the need to exchange sources and reanalyze samples

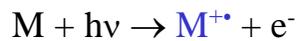


Atmospheric pressure photoionization

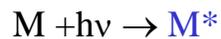


Principi della Fotoionizzazione

Fotoionizzazione: $h\nu \geq IP$



Eccitazione (ma non ionizzazione): $h\nu < IP$



☞ Per la maggior parte degli analiti: $IP < 10 \text{ eV}$

☞ Per i più comuni solventi HPLC: $IP > 10 \text{ eV}$

IP = potenziale 1° ionizzazione

Ionizzazione Selettiva

3 lampade disponibili

Ar: 11.2 eV

Kr: 10.0 eV

Xe: 8.4 eV

Azoto	15.58
Acqua	12.62
Acetonitrile	12.20
Ossigeno	12.07
Metanolo	10.84
Metil pentanoato	10.40
Esano	10.13
Eptano	9.93
Acetone	9.70
Piridina	9.26
Benzene	9.24
Amfetamina	8.99
Toluene	8.83
Naftalene	8.14
Reserpina	7.88
Trietilammina	7.53

Potenziale di Ionizzazione (eV)

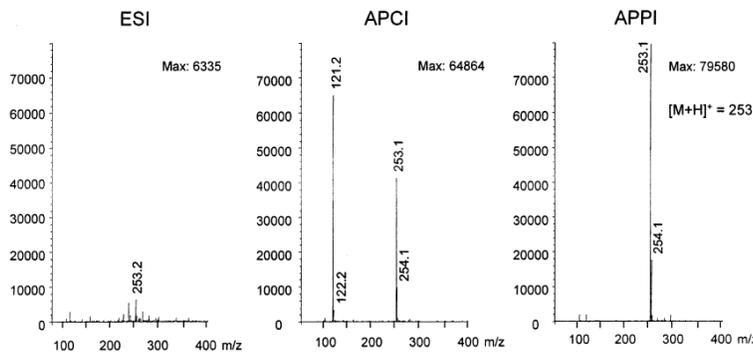
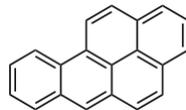


Figure 3. Comparison of ESI, APCI, and APPI for benzo[a]pyrene showing improved sensitivity for nonpolar compounds by APPI. LC conditions: flow injection analysis, flow rate 0.4 mL/min, 50% water, 50% THF. APPI source conditions: V_{cap} 1500, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 250 °C, nebulizer pressure 60 psig. APCI source conditions: V_{cap} 4000, corona current 4 μ A, drying gas temperature 350 °C at 5 L/min, vaporizer temperature 450 °C, nebulizer pressure 60 psig. ESI source conditions: V_{cap} 4000, drying gas temperature 350 °C at 13 L/min, nebulizer pressure 25 psig.

