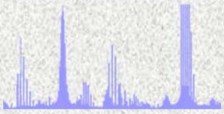


Chimica delle biomolecole

Gianluca Giorgi

Università degli Studi di Siena
Dipartimento di Biotecnologie, Chimica e Farmacia
via Aldo Moro
53100 Siena

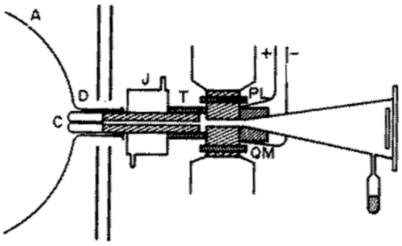
e-mail: gianluca.giorgi@unisi.it
Tel. 0577-234241

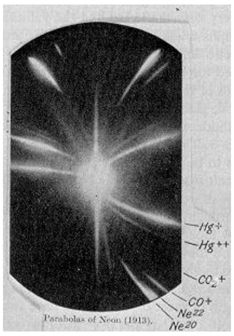
Corso di Laurea Magistrale in Biologia Molecolare e Cellulare

A.A. 2015-2016

Chimica delle Biomolecole: 6 crediti

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





2013:
100 years of mass spectrometry

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

SPETTROMETRIA
DI MASSA

Specificità

Selettività

Sensibilità

Speed

Stechiometria

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
Radiofi

Quali ioni è possibile studiare

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

Come vengono prodotti gli ioni ?

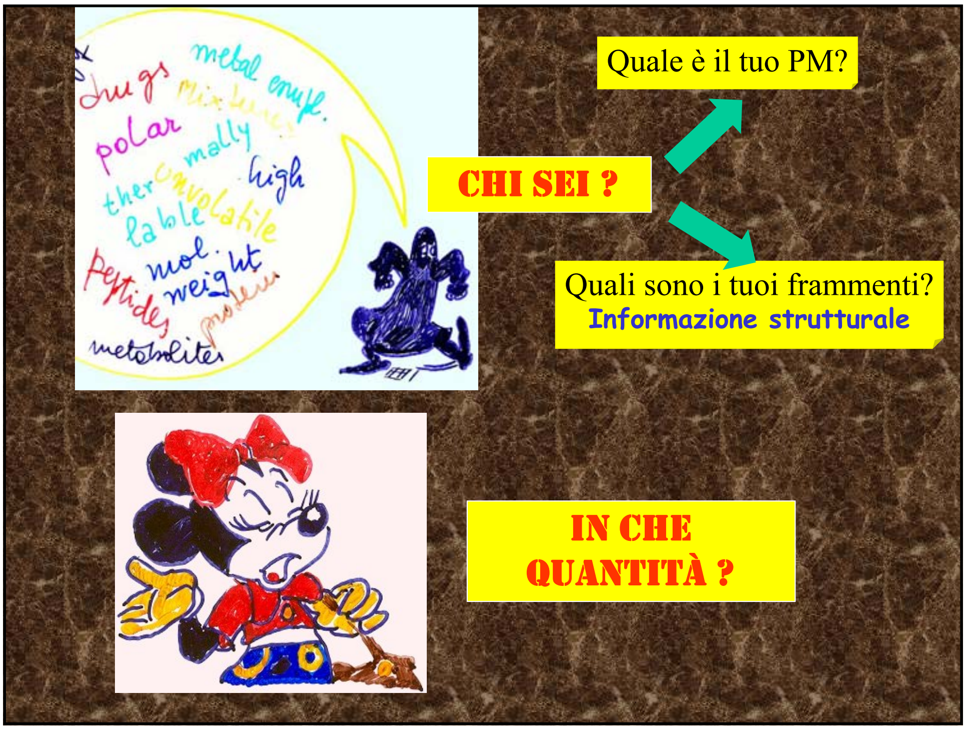
Ionizzazione

Come è possibile studiarli ?

Spettrometro di massa

Quale tipo di informazione è possibile ottenere ?

Qualitativa Quantitativa



CHI SEI ?

Quale è il tuo PM?

Quali sono i tuoi frammenti?
Informazione strutturale

IN CHE QUANTITÀ ?

Spettrometria di massa

Spettro di massa ⟶ Peso molecolare

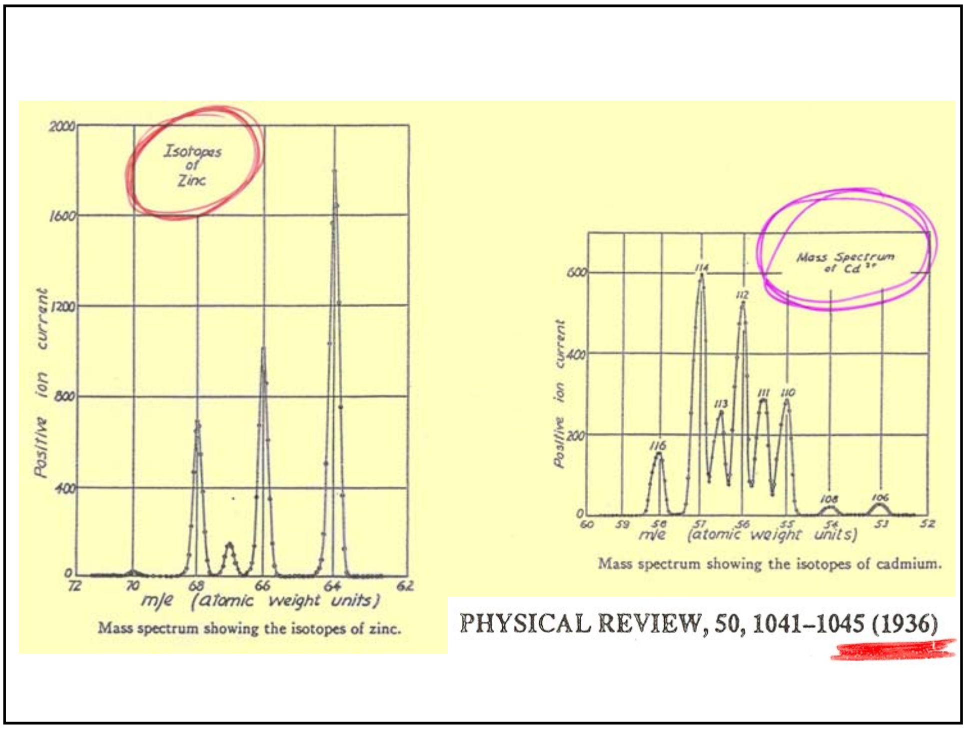
HR + massa accurata ⟶ Stechiometria

MS^n ⟶ Informazioni strutturali, an. quantitativa

Ion spectroscopy ⟶ Informazioni strutturali

Ion Mobility ⟶ Conformazione

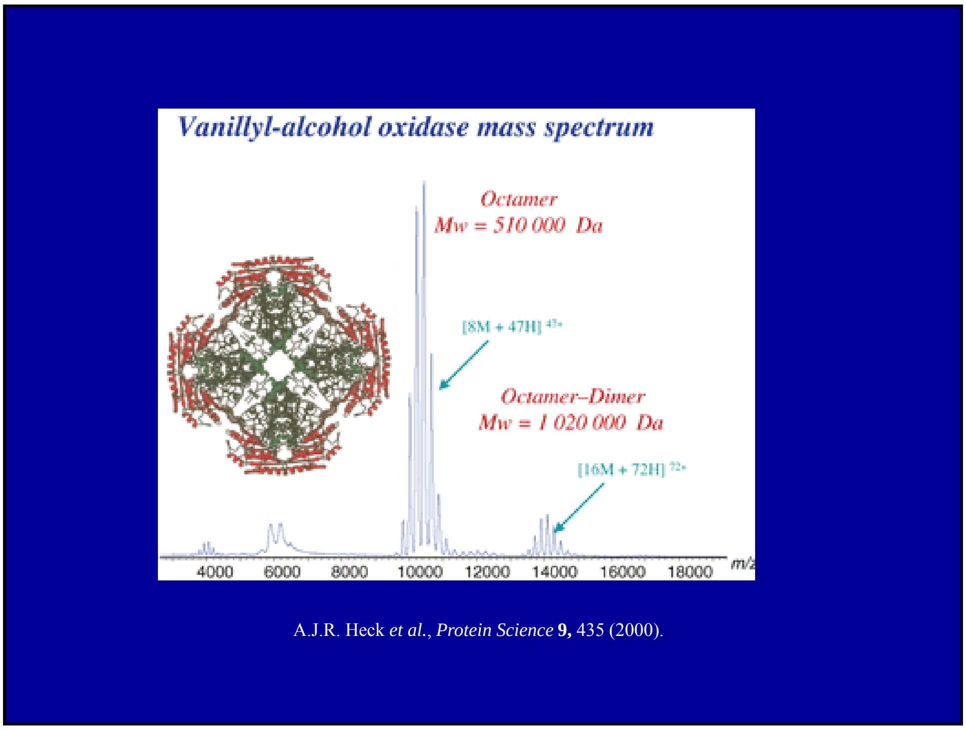
Imaging ⟶ Mappa degli analiti



Mass spectrum showing the isotopes of zinc.

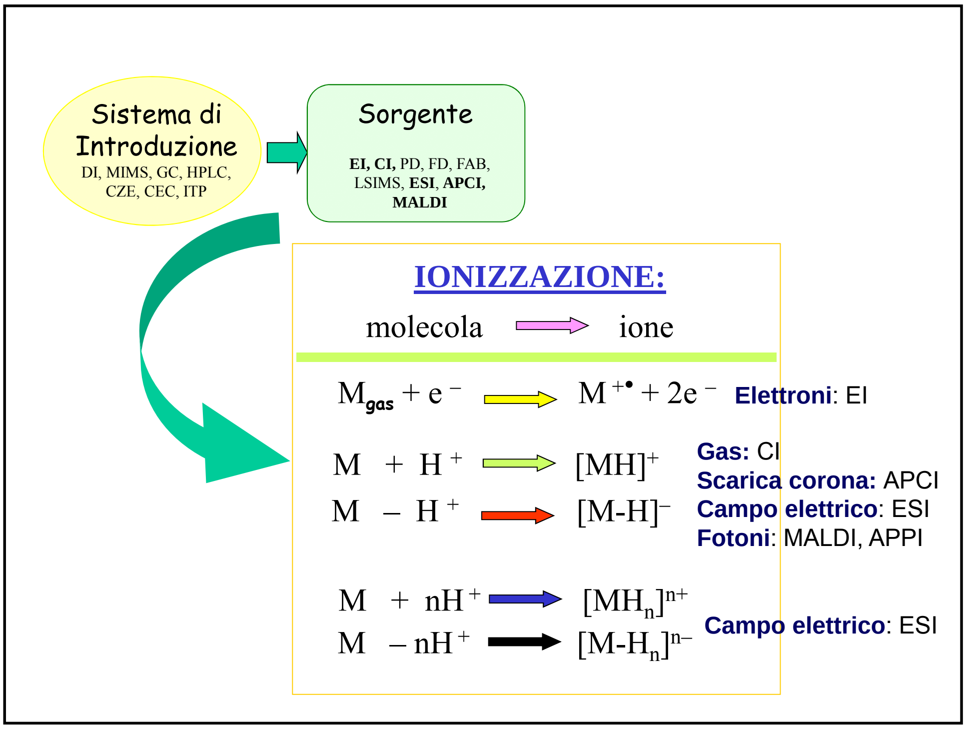
Mass spectrum showing the isotopes of cadmium.

PHYSICAL REVIEW, 50, 1041–1045 (1936)



Vanillyl-alcohol oxidase mass spectrum

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



Sistema di Introduzione
DI, MIMS, GC, HPLC, CZE, CEC, ITP

Sorgente
EI, CI, PD, FD, FAB, LSIMS, **ESI**, **APCI,** **MALDI**

IONIZZAZIONE:

molecola → ione

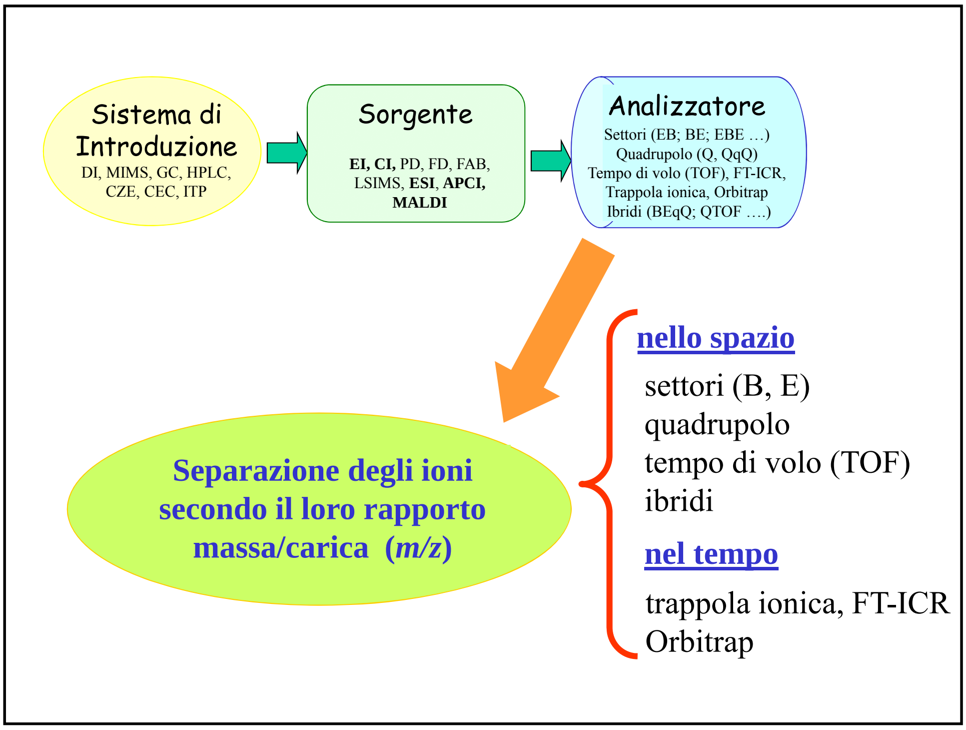
$M_{gas} + e^- \rightarrow M^{+\bullet} + 2e^-$ **Elettroni**: EI

$M + H^+ \rightarrow [MH]^+$
$M - H^+ \rightarrow [M-H]^-$

Gas: CI
Scarica corona: APCI
Campo elettrico: ESI
Fotoni: MALDI, APPI

$M + nH^+ \rightarrow [MH_n]^{n+}$
$M - nH^+ \rightarrow [M-H_n]^{n-}$

Campo elettrico: ESI



Sistema di Introduzione
DI, MIMS, GC, HPLC, CZE, CEC, ITP

Sorgente
EI, CI, PD, FD, FAB, LSIMS, **ESI**, **APCI,** **MALDI**

Analizzatore
Settori (EB; BE; EBE …)
Quadrupolo (Q, QqQ)
Tempo di volo (TOF), FT-ICR,
Trappola ionica, Orbitrap
Ibridi (BEqQ; QTOF ….)

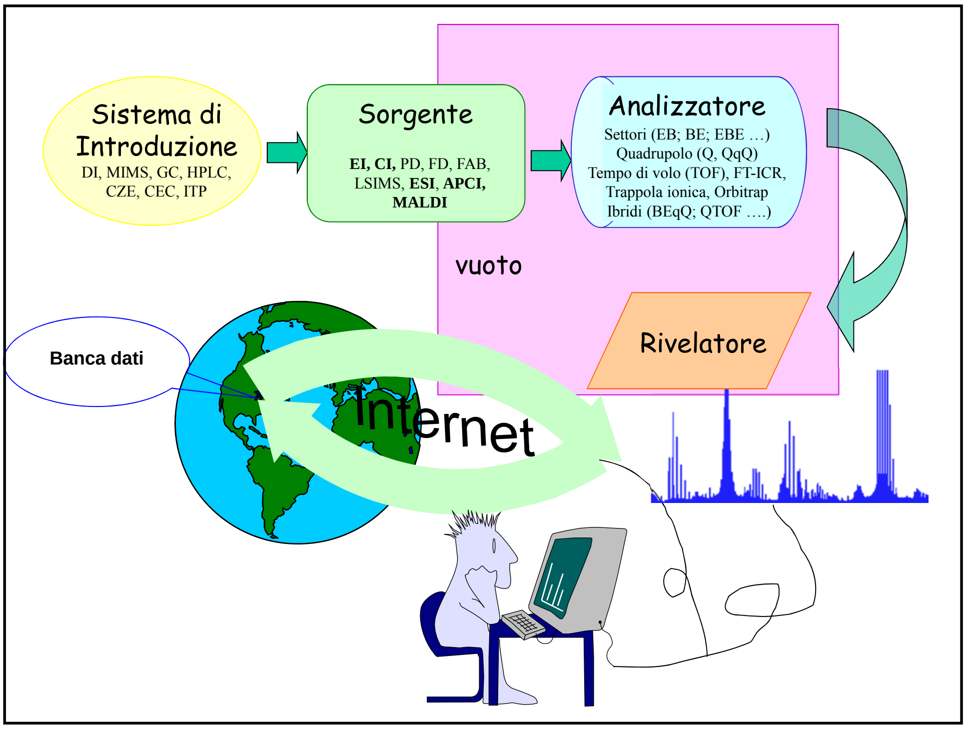
Separazione degli ioni secondo il loro rapporto massa/carica (*m/z*)

nello spazio

settori (B, E)
quadrupolo
tempo di volo (TOF)
ibridi

nel tempo

trappola ionica, FT-ICR
Orbitrap



Sistema di Introduzione
DI, MIMS, GC, HPLC, CZE, CEC, ITP

Sorgente
EI, CI, PD, FD, FAB, LSIMS, **ESI**, **APCI, MALDI**

Analizzatore
Settori (EB; BE; EBE …)
Quadrupolo (Q, QqQ)
Tempo di volo (TOF), FT-ICR,
Trappola ionica, Orbitrap
Ibridi (BEqQ; QTOF ….)

vuoto

Rivelatore

Banca dati

Internet

Nella sorgente avviene la

ionizzazione

molecola → ione

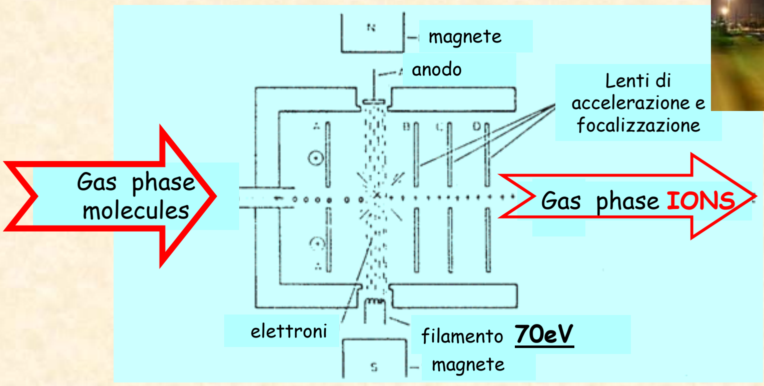
A seconda dell’energia depositata nello ione:

ionizzazione

soft hard

Ionizzazione elettronica (EI)

$$M + e^- \longrightarrow M^{+\bullet} + 2e^-$$



Analizz.

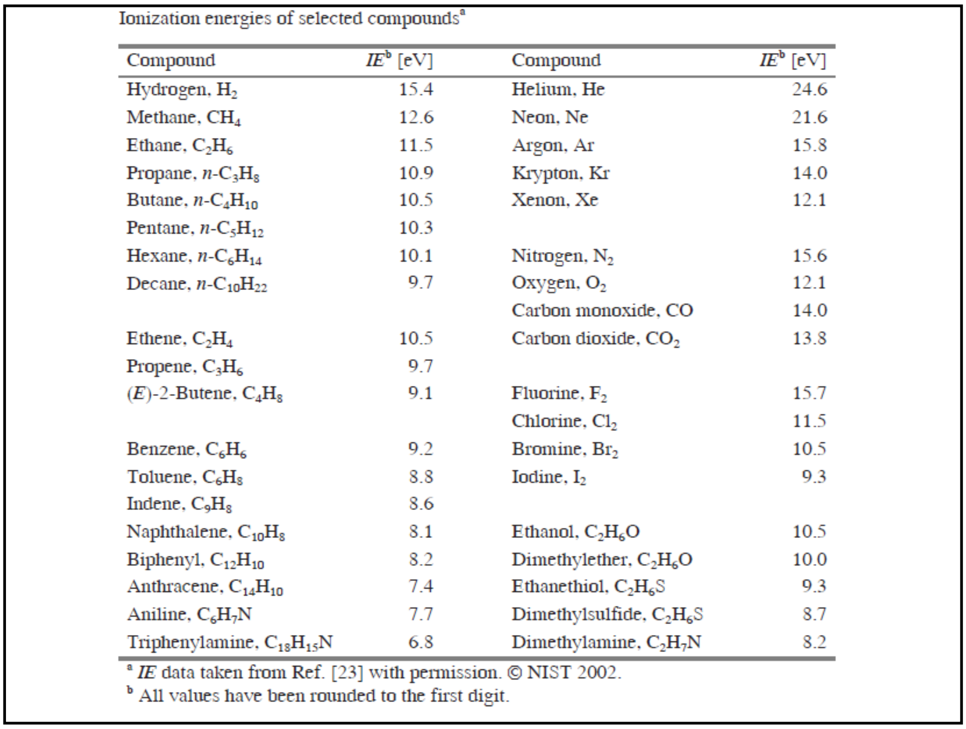
MOLECOLE **VOLATILI**↔ **APOLARI**, **TERMICAM. STABILI, BASSO PM**

---

70 eV ≡ 1610 kcal $mole^{-1}$

Potenziale 1° ionizzazione comp. organici:

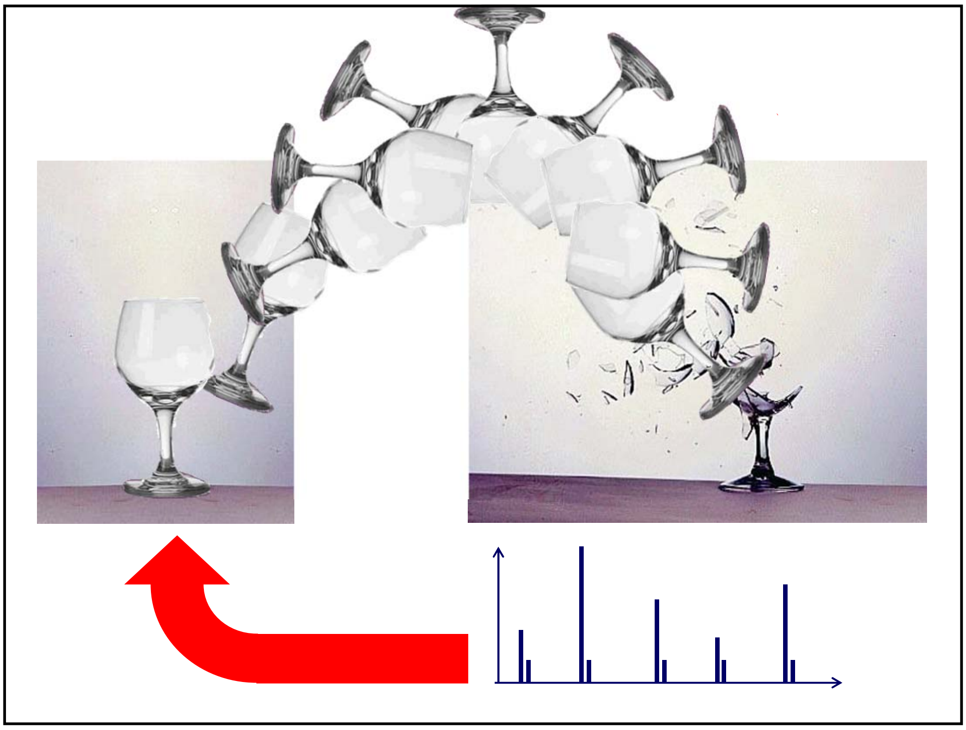
8-15 eV (180-346 kcal $mole^{-1}$)



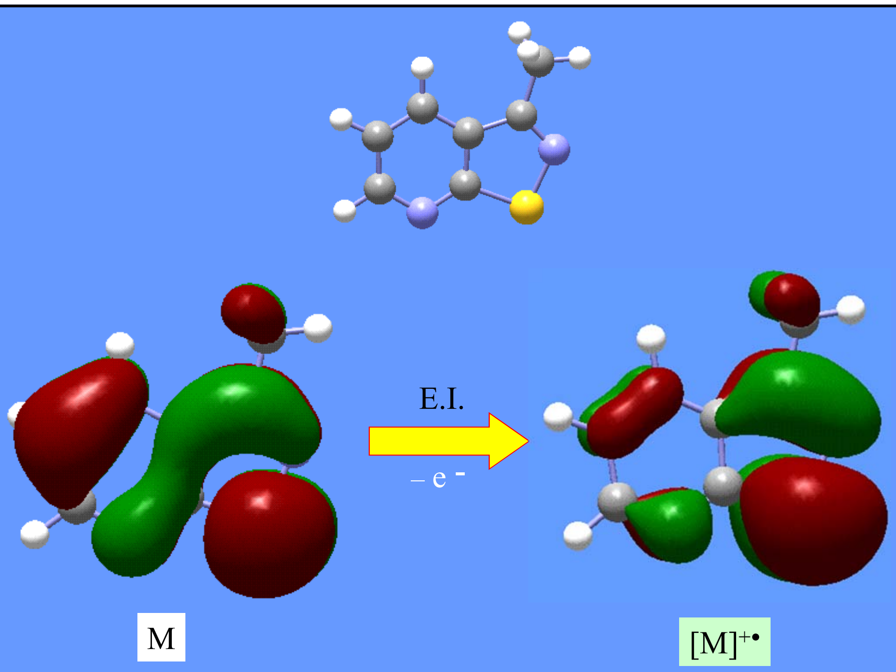
Ionization energies of selected compounds[a]

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_2H_6	11.5	Argon, Ar	15.8
Propane, n-C_3H_8	10.9	Krypton, Kr	14.0
Butane, n-C_4H_{10}	10.5	Xenon, Xe	12.1
Pentane, n-C_5H_{12}	10.3		
Hexane, n-C_6H_{14}	10.1	Nitrogen, N_2	15.6
Decane, n-$C_{10}H_{22}$	9.7	Oxygen, O_2	12.1
		Carbon monoxide, CO	14.0
Ethene, C_2H_4	10.5	Carbon dioxide, CO_2	13.8
Propene, C_3H_6	9.7		
(E)-2-Butene, C_4H_8	9.1	Fluorine, F_2	15.7
		Chlorine, Cl_2	11.5
Benzene, C_6H_6	9.2	Bromine, Br_2	10.5
Toluene, C_6H_8	8.8	Iodine, I_2	9.3
Indene, C_9H_8	8.6		
Naphthalene, $C_{10}H_8$	8.1	Ethanol, C_2H_6O	10.5
Biphenyl, $C_{12}H_{10}$	8.2	Dimethylether, C_2H_6O	10.0
Anthracene, $C_{14}H_{10}$	7.4	Ethanethiol, C_2H_6S	9.3
Aniline, C_6H_7N	7.7	Dimethylsulfide, C_2H_6S	8.7
Triphenylamine, $C_{18}H_{15}N$	6.8	Dimethylamine, C_2H_7N	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 ridistribuzione di tutti gli elettroni

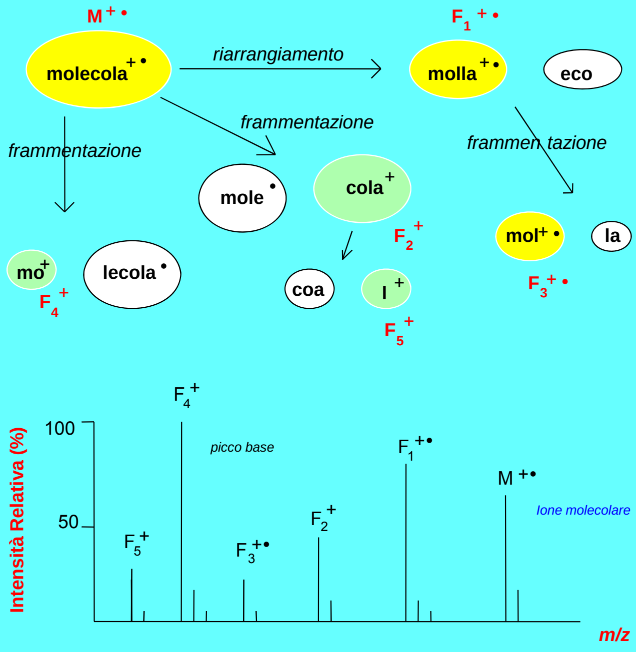


Tecniche di ionizzazione **hard** : ***ionizzazione elettronica***

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**

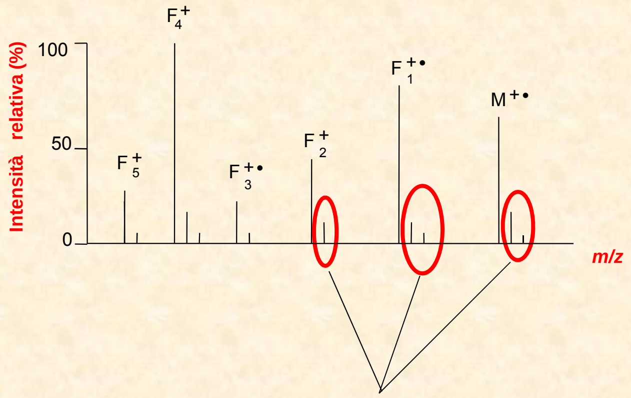
Una molecola organica contenente C, H, O, S, P, o alogeni ha una massa nominale **dispari** solo se contiene un **numero dispari di atomi di azoto**

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

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

Lo spettro di massa:

- ✓ **Informazioni sul peso molecolare e sugli elementi presenti**



Picchi isotopici

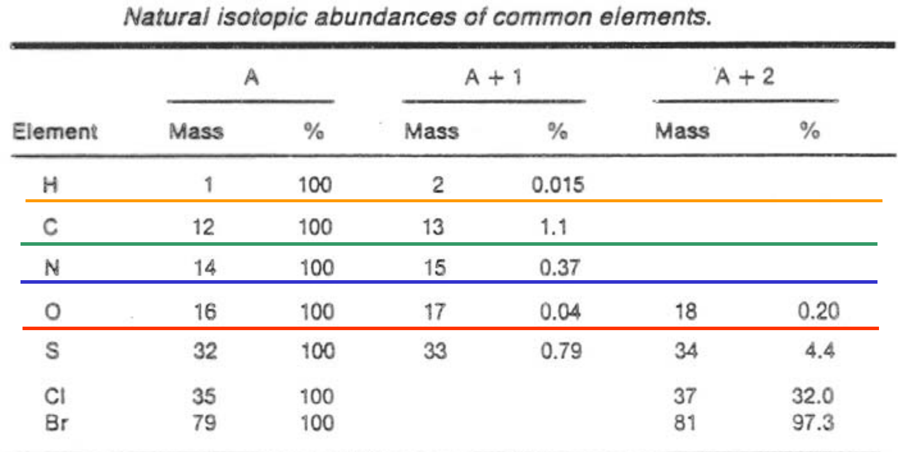
ISOTOPI

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%

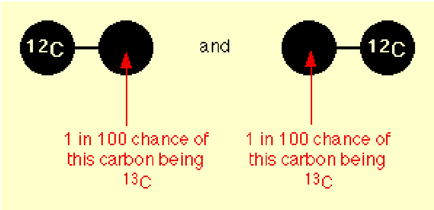


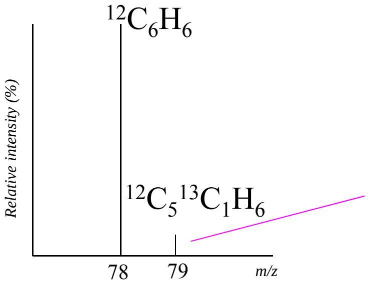
Natural isotopic abundances of common elements.

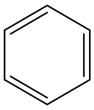
	A		A + 1		A + 2	
Element	Mass	%	Mass	%	Mass	%
H	1	100	2	0.015		
C	12	100	13	1.1		
N	14	100	15	0.37		
O	16	100	17	0.04	18	0.20
S	32	100	33	0.79	34	4.4
Cl	35	100			37	32.0
Br	79	100			81	97.3

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

C_2



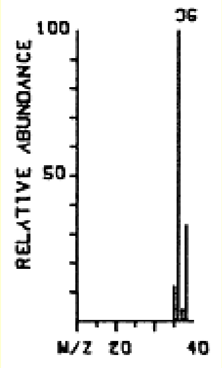


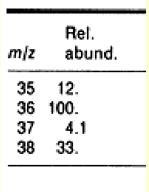


C_6H_6

Rel.int=6*1.1=6.6%

Ionizzazione elettronica

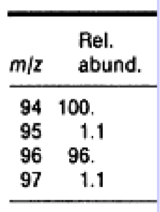




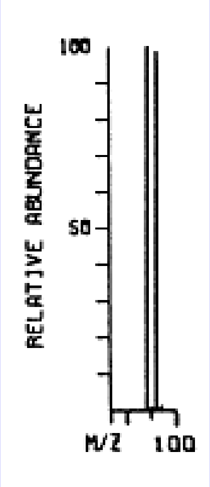
m/z	Rel. abund.
35	12.
36	100.
37	4.1
38	33.

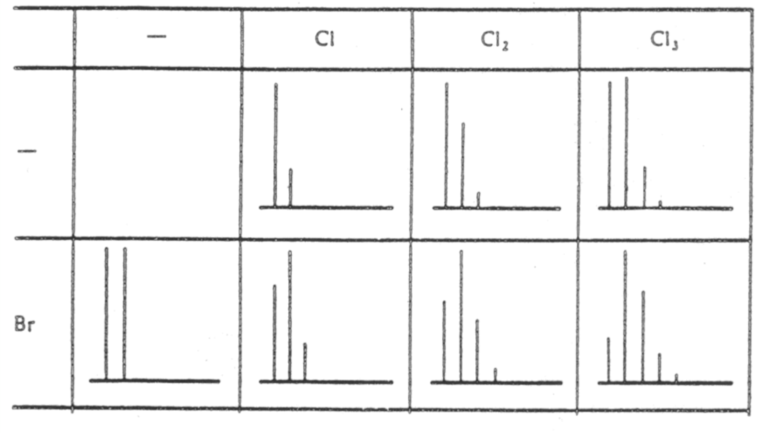
HCl

CH_3Br

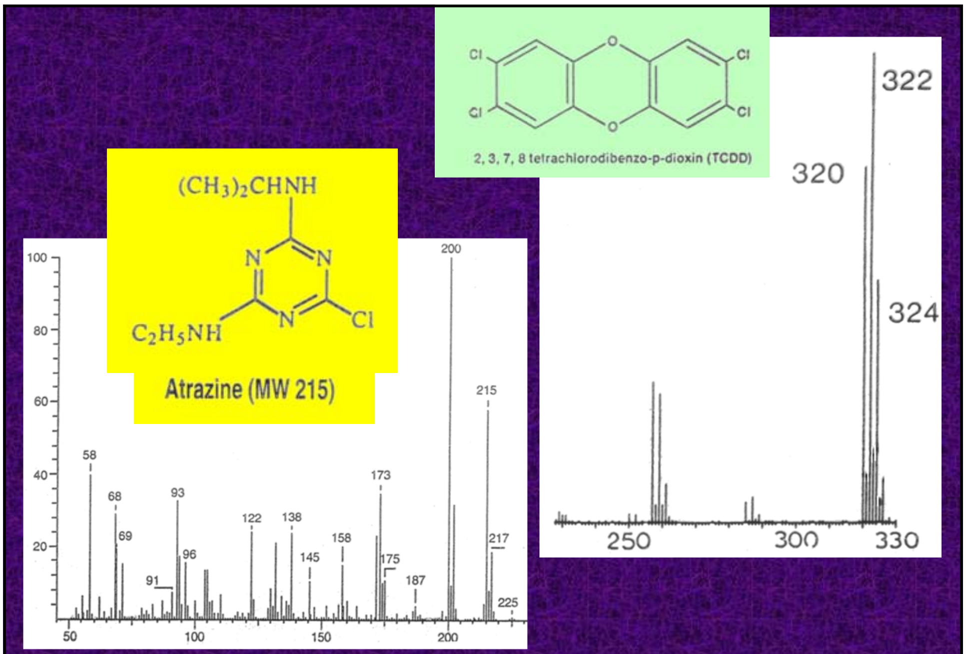


m/z	Rel. abund.
94	100.
95	1.1
96	96.
97	1.1





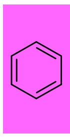
Tutti gli ioni differiscono di 2u !!



Atrazine (MW 215)

2, 3, 7, 8 tetrachlorodibenzo-p-dioxin (TCDD)

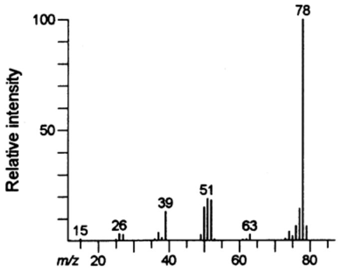
Ionizzazione elettronica

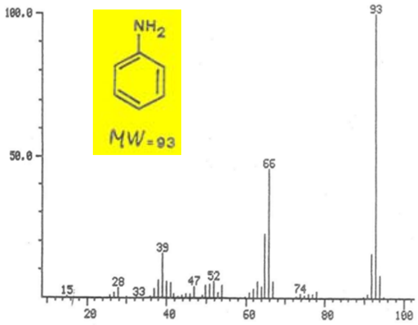


C_6H_6

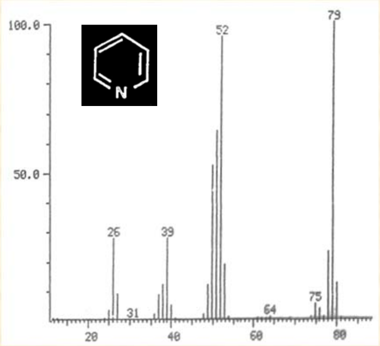
MW = 78

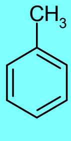
m/z	Int.	*m/z*	Int.
12	0.2	53	0.8
13	0.4	60	0.2
14	0.4	61	0.4
15	1.0	62	0.8
24	0.4	63	2.9
25	0.8	64	0.2
26	3.2	72	0.4
27	2.6	73	1.0
36	0.9	74	3.9
37	3.8	75	2.2
39	13.	76	7.0
40	0.4	77	15.
50	16.	78	100.
51	19.	79	6.8
52	20.	80	0.2





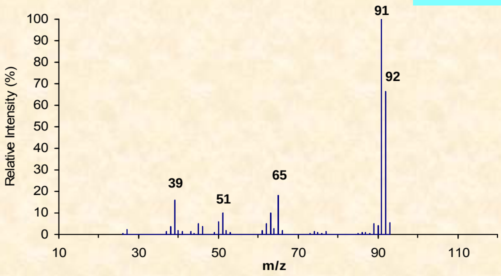
Ionizzazione elettronica





C_7H_8

MW = 92



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

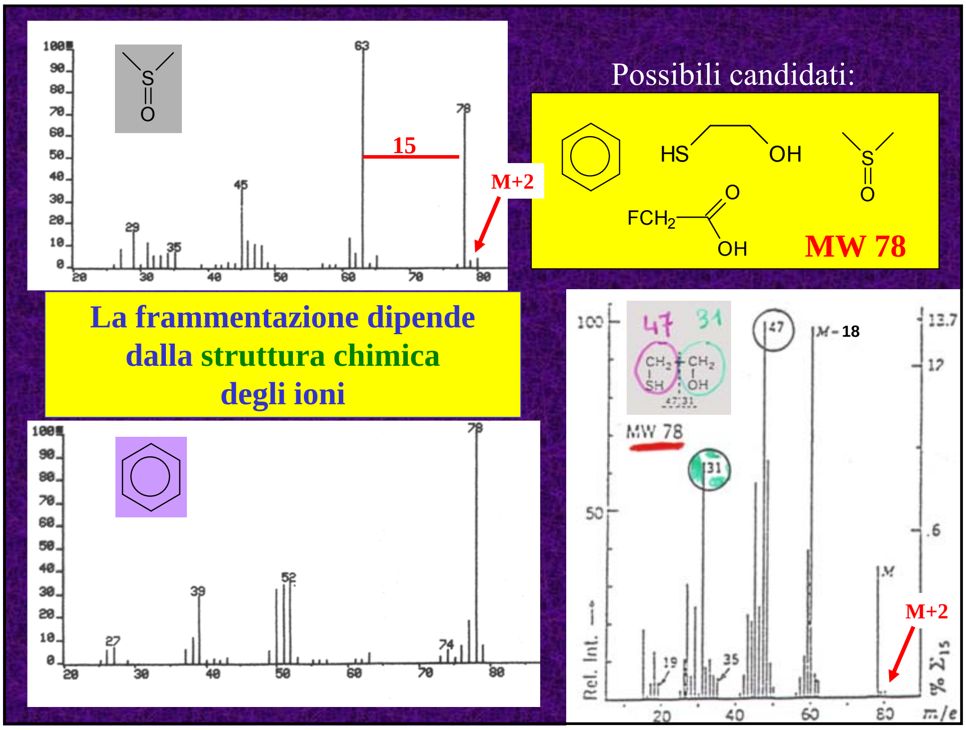
La frammentazione consiste in:

- 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:

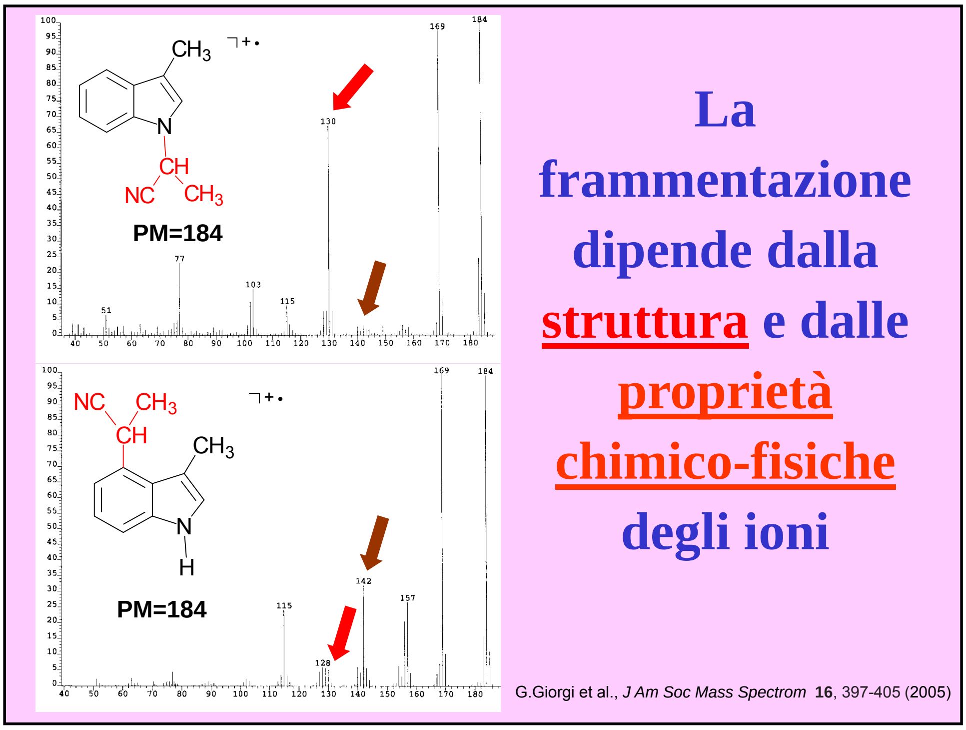
- dalla struttura dello ione
- dalla sua energia interna (vibrazionale e rotazionale)



Possibili candidati:

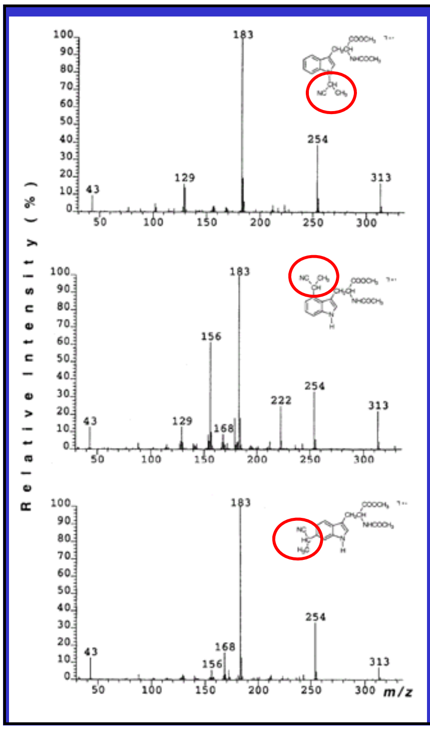
MW 78

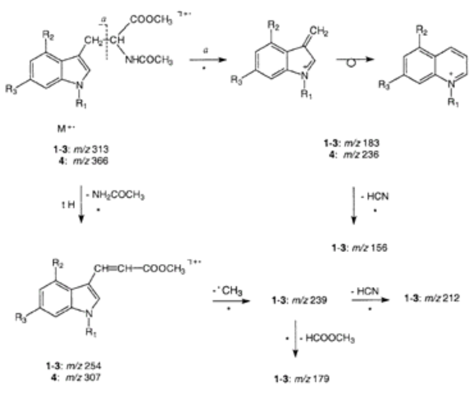
La frammentazione dipende dalla struttura chimica degli ioni



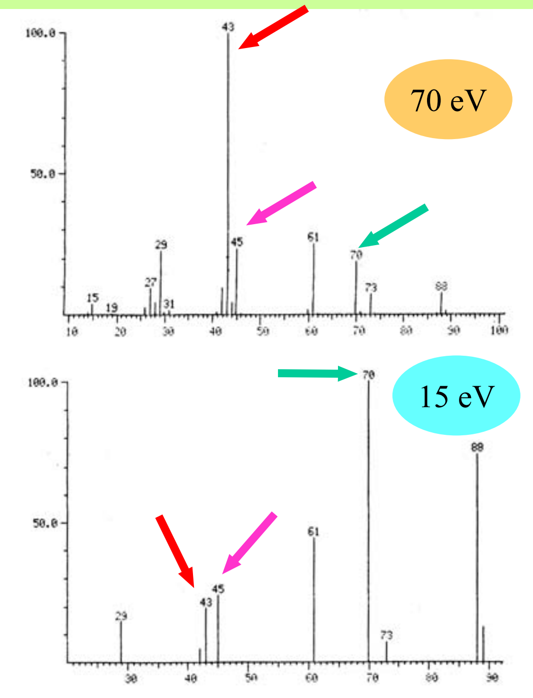
La frammentazione dipende dalla struttura e dalle proprietà chimico-fisiche degli ioni

G.Giorgi et al., *J Am Soc Mass Spectrom* **16**, 397-405 (2005)

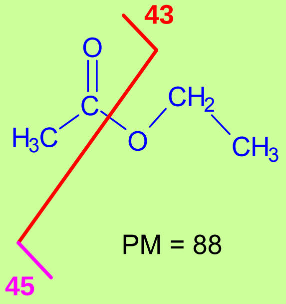




G.Giorgi et al., *J Am Soc Mass Spectrom* **2002**, *13*, 1298–1303



La frammentazione dipende dalla energia interna degli ioni



PM = 88

La frammentazione

NON È

un processo casuale

ma, operando nelle stesse condizioni sperimentali,

È

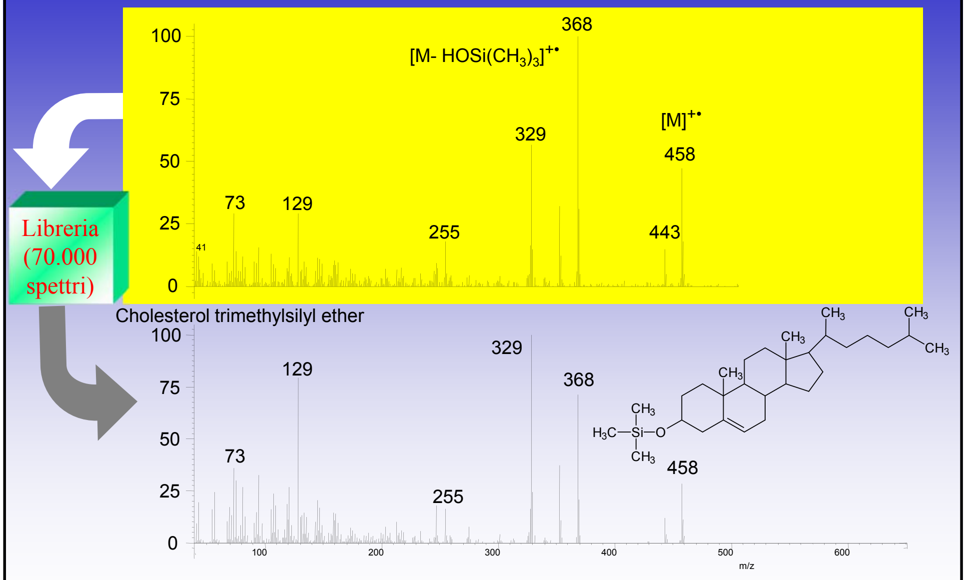
ripetibile a livello quali e quantitativo

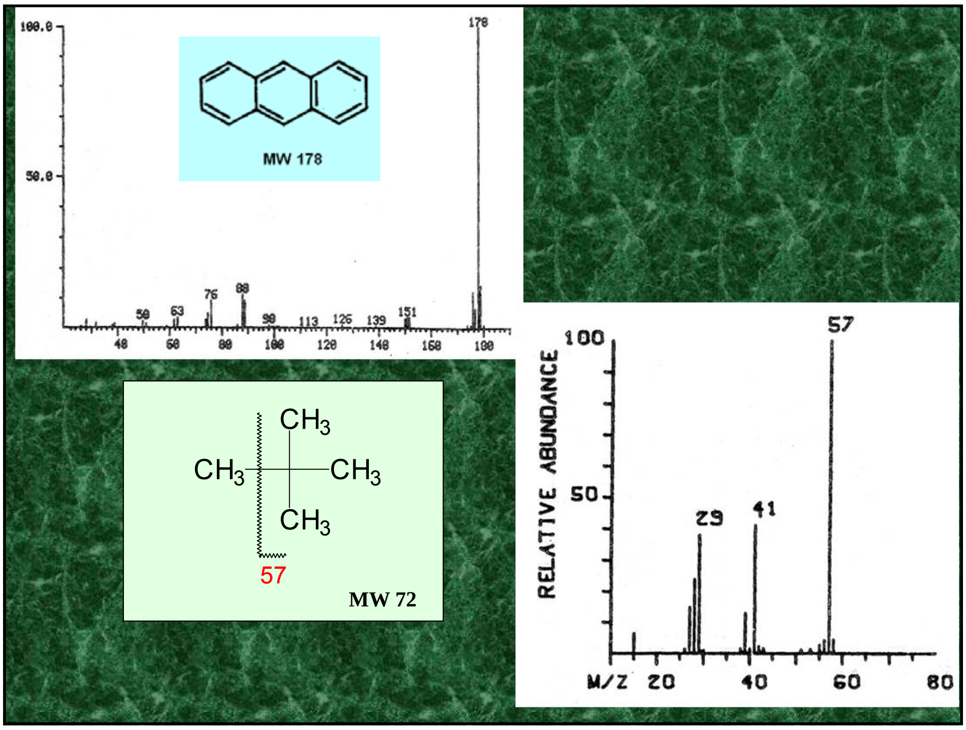
Stessi frammenti della stessa intensità

↕

Stesso spettro di massa

Ricerca in libreria …………….





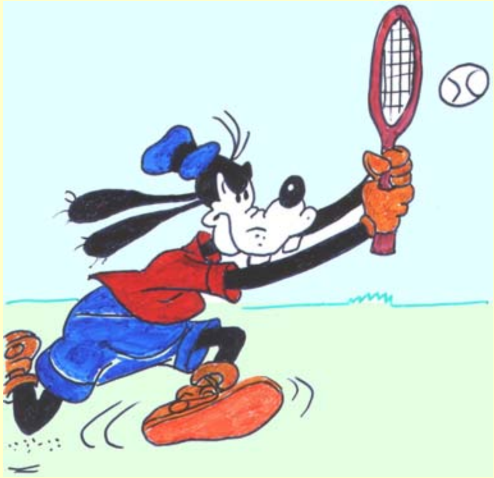
Tecniche di ionizzazione *soft*

ionizzazione chimica

Molecole protonate $[M+H]^+$ / deprotonate $[M–H]^–$
con minimo eccesso di energia

Frammentazione molto scarsa / assente

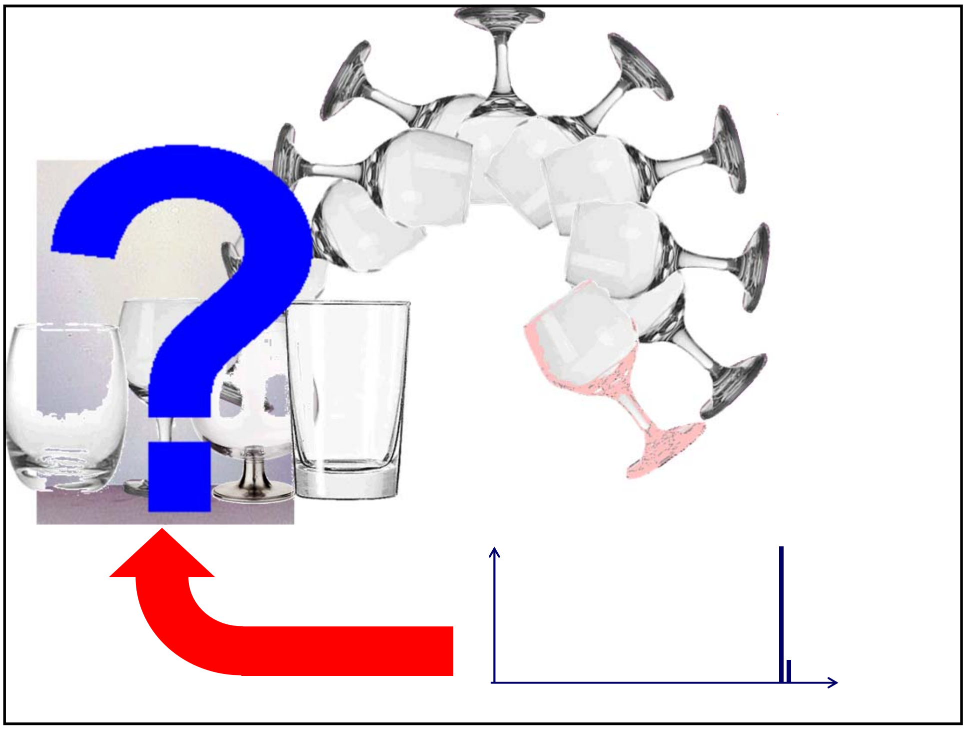
Determinazione del PM.



Ionizzazione

SOFT

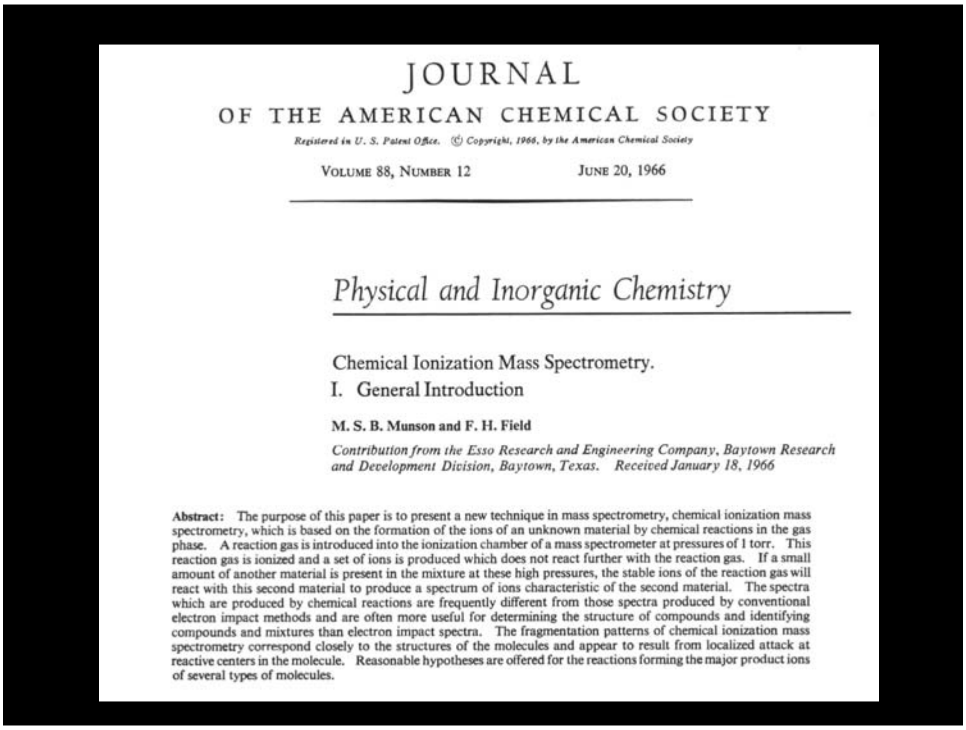
- ✓ **Frammenti assenti** → informazione strutturale?



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

Ionizzazione Chimica:

reazioni ione-molecola in fase gassosa



JOURNAL
OF THE AMERICAN CHEMICAL SOCIETY
Registered in U. S. Patent Office. © Copyright, 1966, by the American Chemical Society
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.

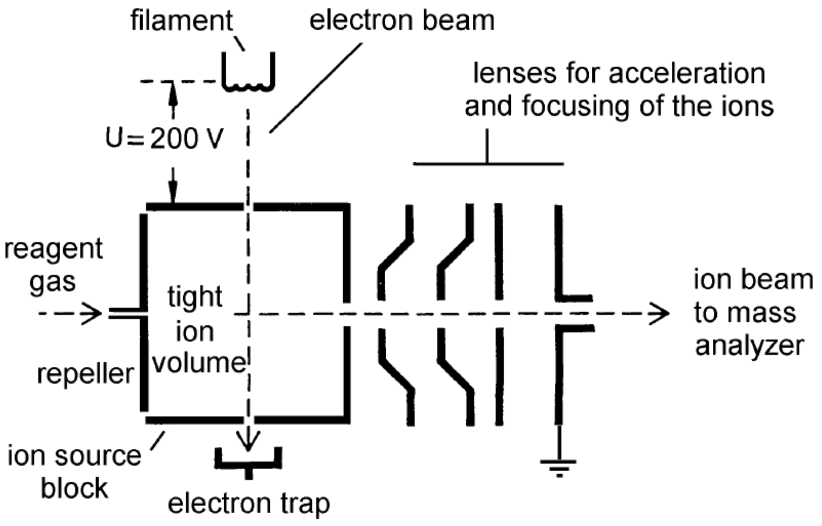
Ionizzazione chimica:

- Reazione (processo **bi**molecolare) tra
  - le molecole dell'analita
  - e gli ioni di un gas reagente in alta concentrazione

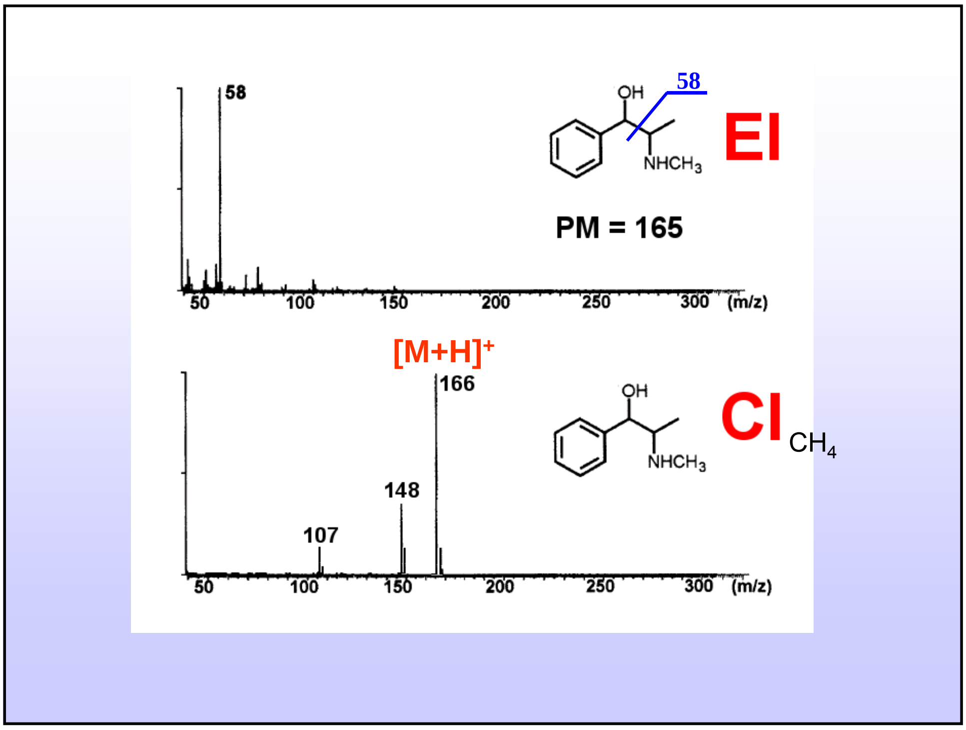
    generati da EI ad alta pressione

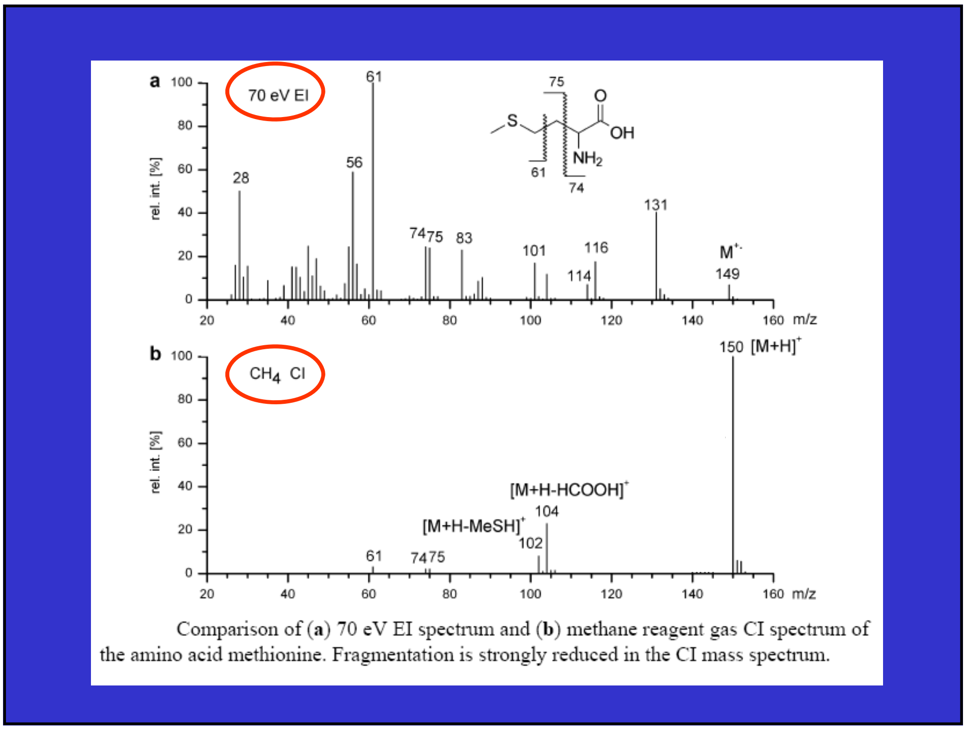
Numero di *collisioni* ione-molecola sufficientemente elevato durante il tempo di residenza dei reagenti all'interno della sorgente

Tempo resid. in sorgente ca 10^{-6}sec, P sorg.=2.5×10^{-2} Pa ➔ n. collisioni=60-70



Sorgente per ionizzazione chimica





Comparison of (**a**) 70 eV EI spectrum and (**b**) methane reagent gas CI spectrum of the amino acid methionine. Fragmentation is strongly reduced in the CI mass spectrum.

IONIZZAZIONE CHIMICA

a. *Trasferimento di protone*

$M + [BH]^+ \rightarrow [MH]^+ + B$

$PA_M > PA_B$ PA= affinità protonica in fase gassosa

b. *Rimozione di anione*

$M + X^+ \rightarrow [M\text{-}A]^+ + AX$

c. *Scambio di carica*

$M + X^{+\bullet} \rightarrow M^{+\bullet} + X$

d. *Addizione elettrofila*

$M + X^+ \rightarrow [MX]^+$

Ionizzazione chimica

per protonazione

$B + H^+ \rightarrow [BH]^+ \qquad -\Delta H^0 = PA_B$

$M + [BH]^+ \rightarrow [MH]^+ + B$

$\Delta H = PA_B - PA_M$

PA= affinità protonica in fase gassosa

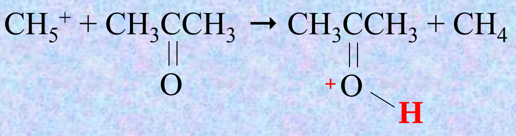
$G + e^- \rightarrow G^{+\bullet} + 2\,e^-$

$G^{+\bullet} + G \rightarrow GH^+ + [G\text{-}H]^\bullet$

$GH^+ + M \rightarrow MH^+ + G$

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

$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^\bullet$



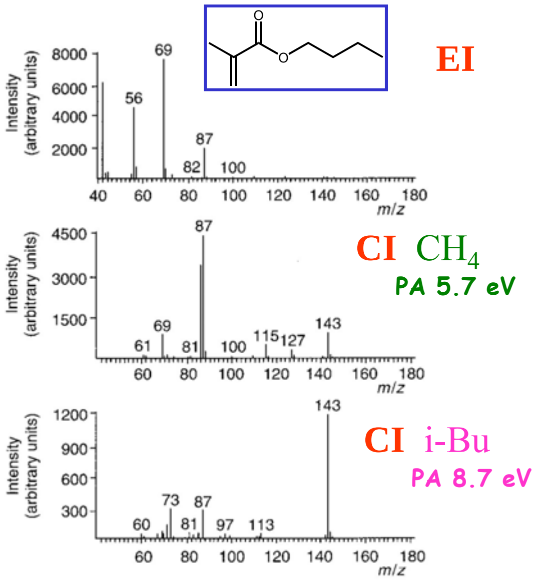
$$CH_5^+ + \underset{\substack{\| \\ O}}{CH_3CCH_3} \rightarrow \underset{\substack{\| \\ {}^+O\diagdown H}}{CH_3CCH_3} + CH_4$$

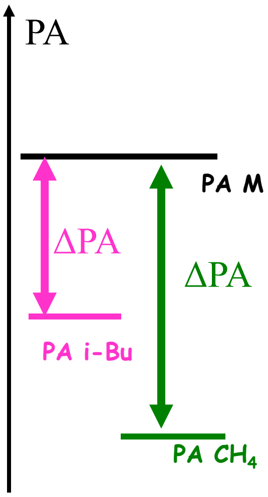
PM=58 m/z = 59

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

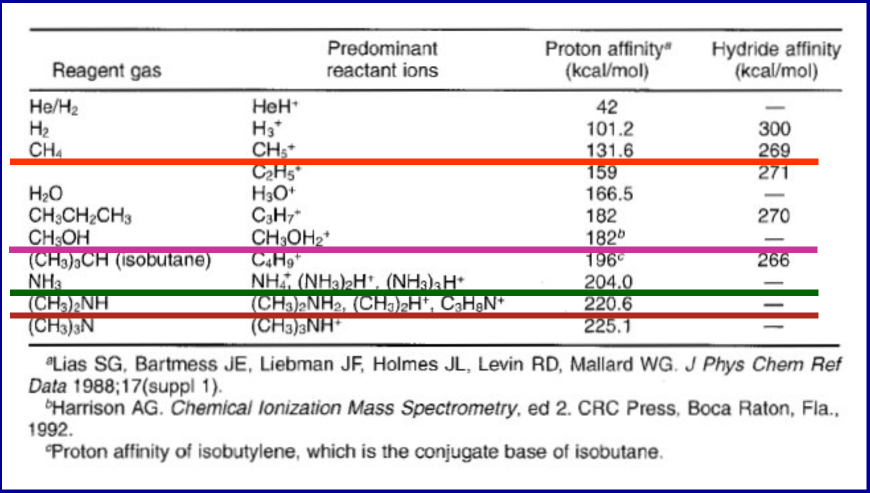
- Reagenti di uso comune:
  - CH_4 (PA = 5.7 eV)
  - NH_3 (PA = 9.7 eV)
  - Isobutano (PA = 8.7 eV)





The EI (*top*), methane CI (*middle*) and isobutane CI (*bottom*) mass spectra of butyl methacrylate. The ionization techniques (EI vs. CI) and the reagent gases (methane vs. isobutane) influence the amount of fragmentation and the prominence of the protonated molecular ions detected at 143 Th

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
	$C_2H_5^+$	159	271
H_2O	H_3O^+	166.5	—
$CH_3CH_2CH_3$	$C_3H_7^+$	182	270
CH_3OH	$CH_3OH_2^+$	182[b]	—
$(CH_3)_3CH$ (isobutane)	$C_4H_9^+$	196[c]	266
NH_3	NH_4^+, $(NH_3)_2H^+$, $(NH_3)_3H^+$	204.0	—
$(CH_3)_2NH$	$(CH_3)_2NH_2$, $(CH_3)_2H^+$, $C_3H_8N^+$	220.6	—
$(CH_3)_3N$	$(CH_3)_3NH^+$	225.1	—

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

[b] Harrison AG. *Chemical Ionization Mass Spectrometry*, ed 2. CRC Press, Boca Raton, Fla., 1992.

[c] Proton affinity of isobutylene, which is the conjugate base of isobutane.

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

$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$

$C_2H_5^+ + M \rightarrow [M(C_2H_5)]^+$ $[M+29]^+$

$C_2H_5^+ + CH_4 \rightarrow C_3H_7^+ + H_2$

$C_3H_7^+ + M \rightarrow [M(C_3H_7)]^+$ $[M+43]^+$

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

$CH_2^{+\bullet} + CH_4 \rightarrow C_2H_3^+ + H_2 + H^\bullet$

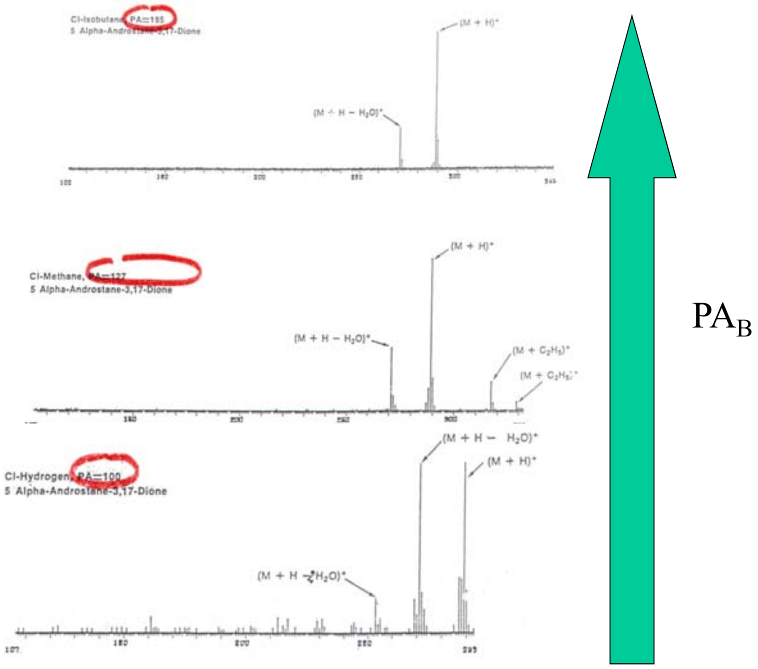
$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2$

$C_3H_5^+ + M \rightarrow [M(C_3H_5)]^+$ $[M+41]^+$

Addotti

in fase

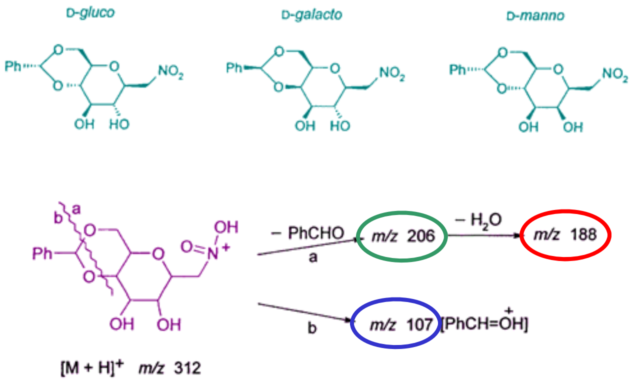
gassosa



Isobutane

chemical

ionization

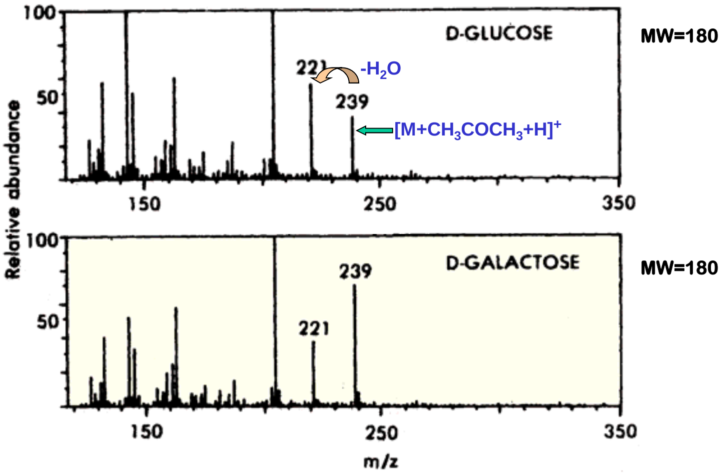


	[M + H]$^+$	m/z 206	m/z 188	m/z 107	206/107	206/188
D-gluco	312	69	58	100	0.69	1.19
D-galacto	312	31	100	66	0.47	0.31
D-manno	312	100	44	45	2.22	2.27

Fragmentation of [M + H]$^+$ ions in TOF product ion isobutane CI mass spectrometry of 4,6-*O*-benzylidene *β*-D-glycopyranosylnitromethanes 1–3.

Kováčik V. et al., *J. Mass Spectrom.*, **35**, 634 (2000)

Acetone Chemical Ionization Mass Spectrometry of Monosaccharides

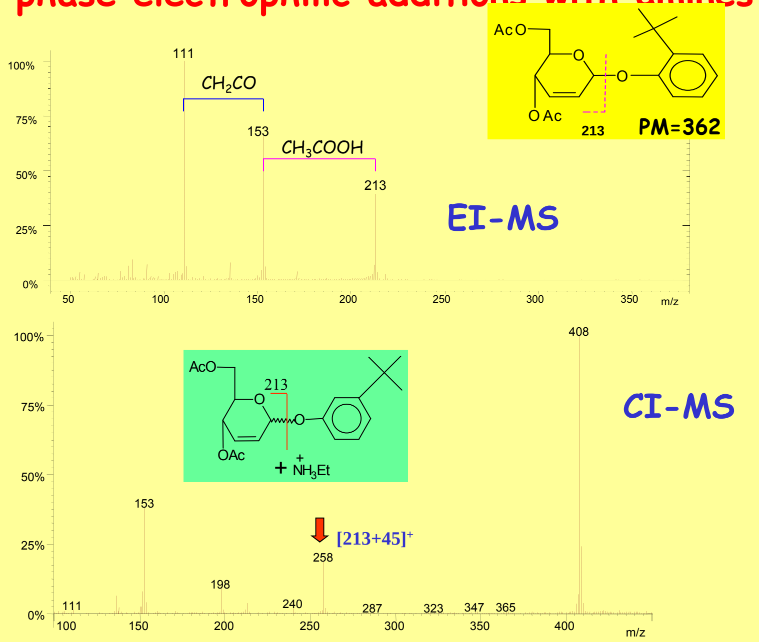


MW=180

MW=180

Wang G. et al., *Anal. Chem.*, **57**, 2283 (1985)

Gas phase electrophilic additions with amines



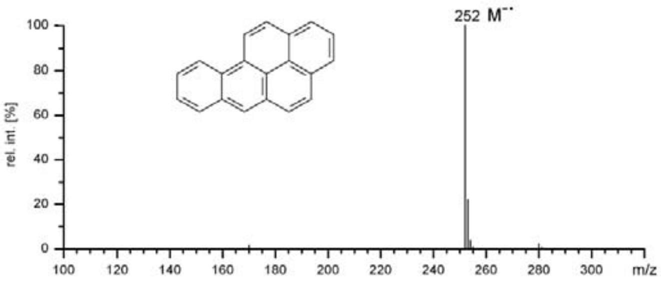
G. Giorgi et al. *Eur. J. Org. Chem.* 106-115 (2003), *J. Am. Soc. Mass Spectrom.* **14**, 851-861 (2003), **15**, 244-252 (2004)

Ionizzazione chimica

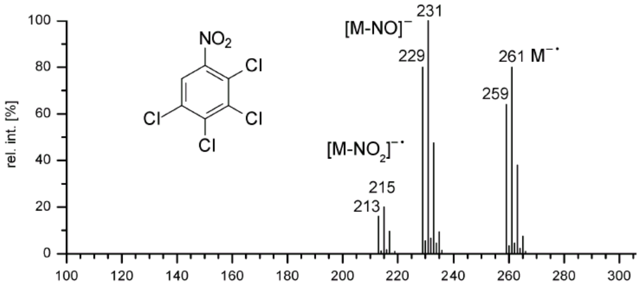
per cattura di elettroni:

studio di ioni negativi

$M + e^- \rightarrow M^{-\bullet}$	*resonance electron capture*
$M + e^- \rightarrow [M\text{–}A]^- + A^\bullet$	*dissociative electron capture*
$M + e^- \rightarrow [M\text{–}B]^- + B^+ + e^-$	*ion-pair formation*

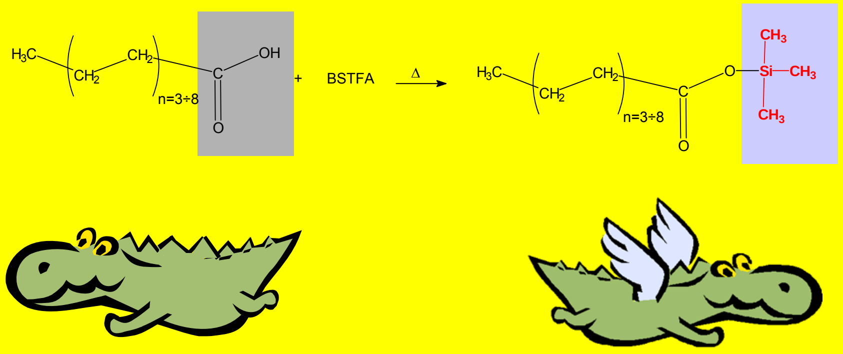


EC spectrum of benzo[a]pyrene: isobutane buffer gas, ion source 200 °C.

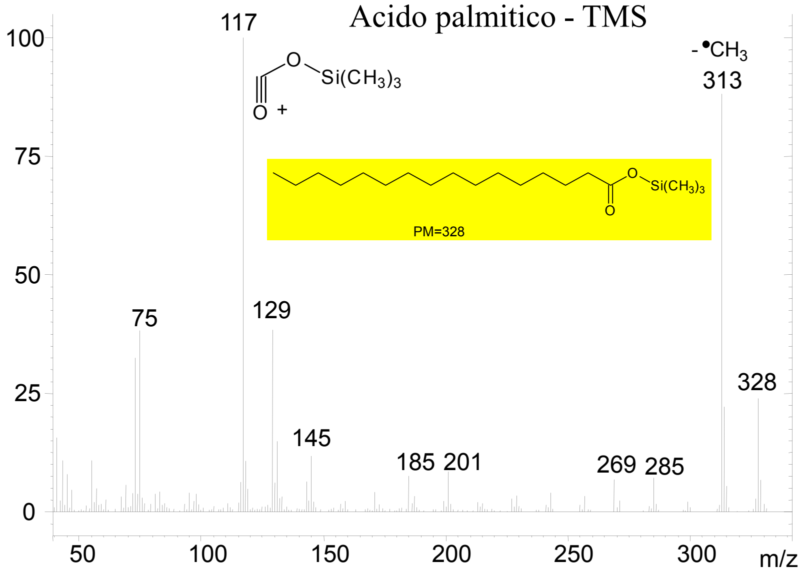


Methane EC spectrum of 2,3,4,5-tetrachloronitrobenzene.

Tutte le molecole sono …… volatili ?



Acido palmitico - TMS



MISCELE COMPLESSE

Separazione prima di MS

MISCELE COMPLESSE

Separazione prima di MS

Molecole volatili, basso PM

Gas cromatografia

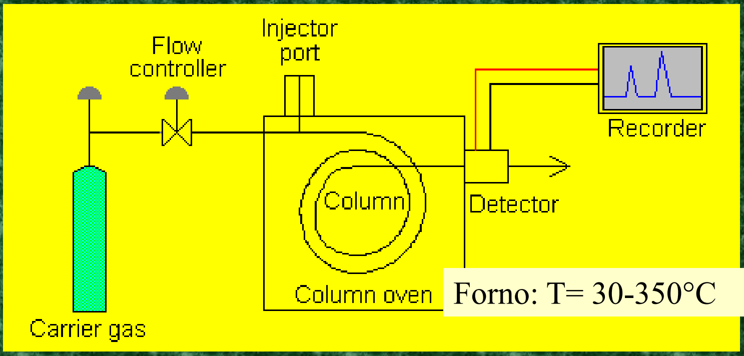
Molecole NON volatili; polari

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

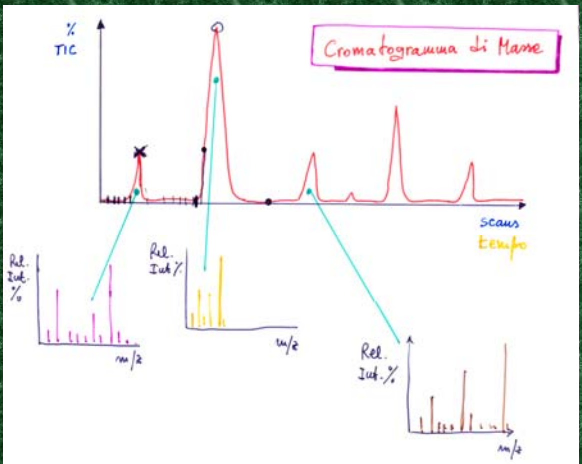


Forno: T= 30-350°C

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

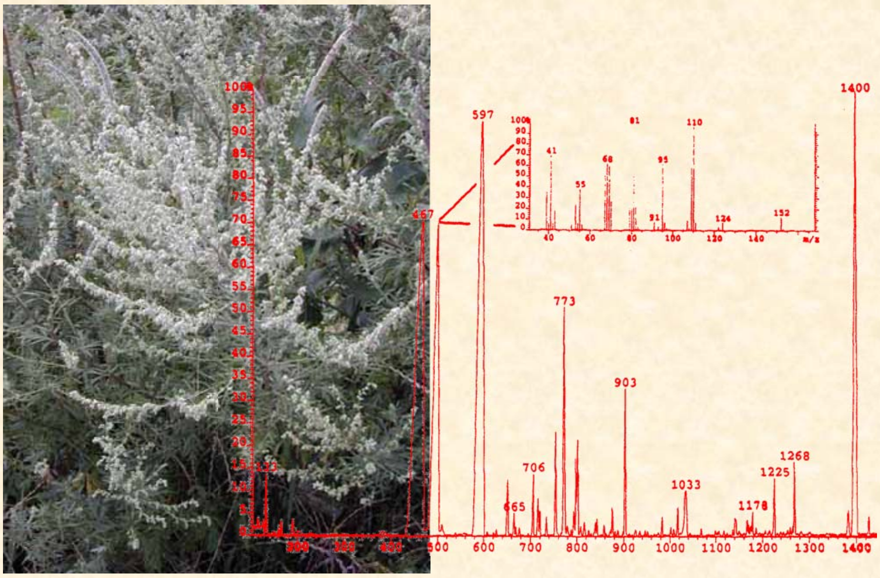


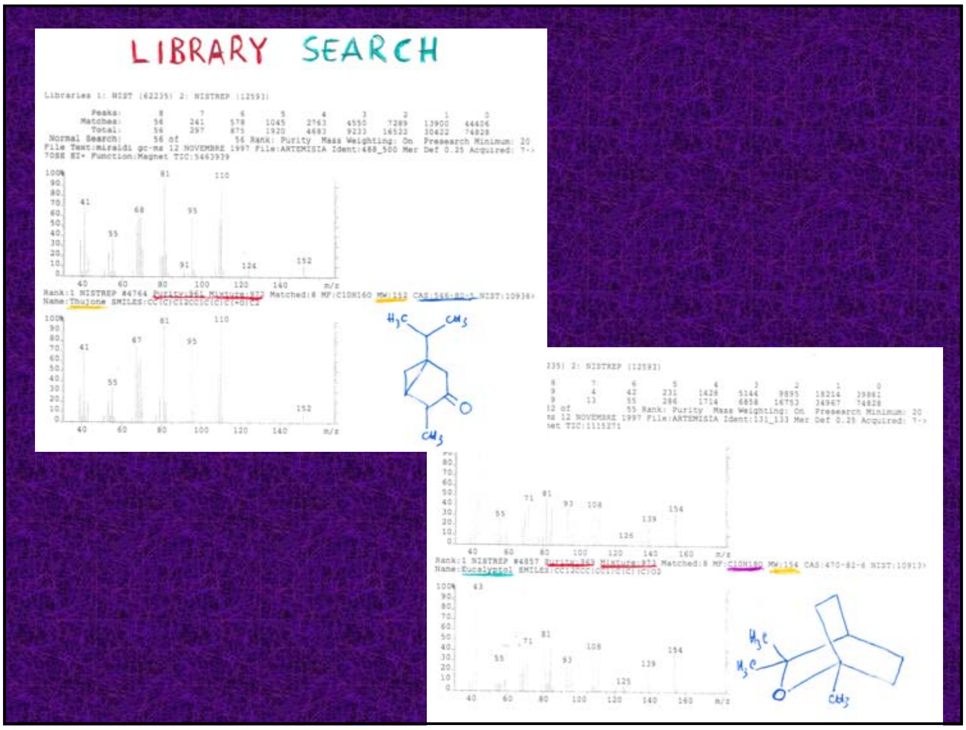
Per ogni componente la miscela:

① tempo di ritenzione GC

② spettro di massa

ALTA SELETTIVITA' e SPECIFICITA'





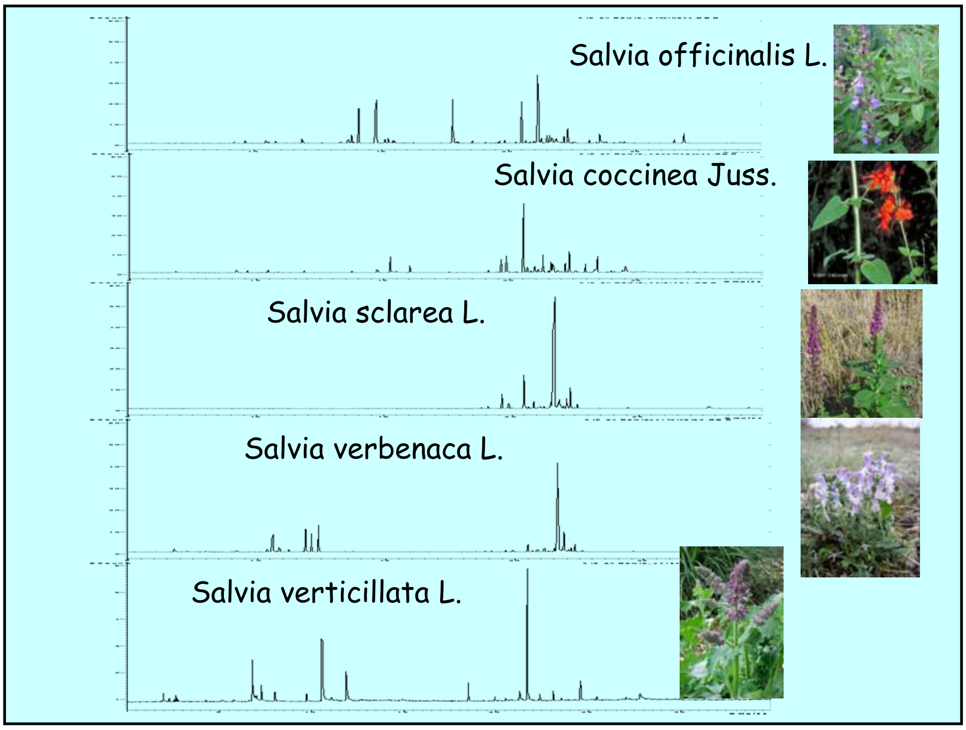
LIBRARY SEARCH

```
Libraries 1: NIST (62235) 2: NISTREP (12593)

      Peaks:       8      7      6      5      4      3      2      1      0
    Matches:      56    241    578   1045   2763   4550   7289  13900  44406
      Total:      56    297    875   1920   4683   9233  16532  30422  74828
 Normal Search:   56 of          56 Rank: Purity  Mass Weighting: On  Presearch Minimum: 20
File Text:miraldi gc-ms 12 NOVEMBRE 1997 File:ARTEMISIA Ident:488_500 Mer Def 0.25 Acquired: 7->
70SE EI+ Function:Magnet TIC:5463939
```

```
Rank:1 NISTREP #4764 Purity:961 Mixture:972 Matched:8 MF:C10H16O MW:152 CAS:546-80-5 NIST:10936>
Name:Thujone SMILES:CC(C)C12CC1C(C)C(=O)C2
```

```
235) 2: NISTREP (12593)

8      7      6      5      4      3      2      1      0
9      4     42    231   1428   5144   9895  18214  39861
9     13     55    286   1714   6858  16753  34967  74828
12 of          55 Rank: Purity  Mass Weighting: On  Presearch Minimum: 20
ms 12 NOVEMBRE 1997 File:ARTEMISIA Ident:131_133 Mer Def 0.25 Acquired: 7->
net TIC:1115271
```

```
Rank:1 NISTREP #4857 Purity:869 Mixture:971 Matched:8 MF:C10H18O MW:154 CAS:470-82-6 NIST:10913>
Name:Eucalyptol SMILES:CC12CCC(CC1)C(C)(C)O2
```



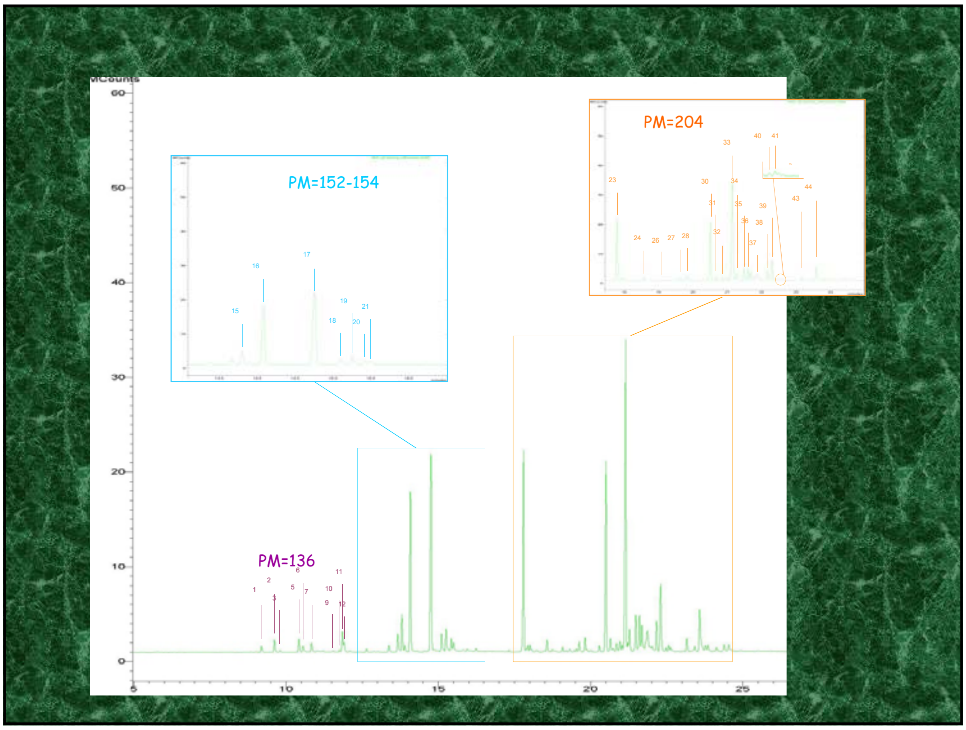
Salvia officinalis L.

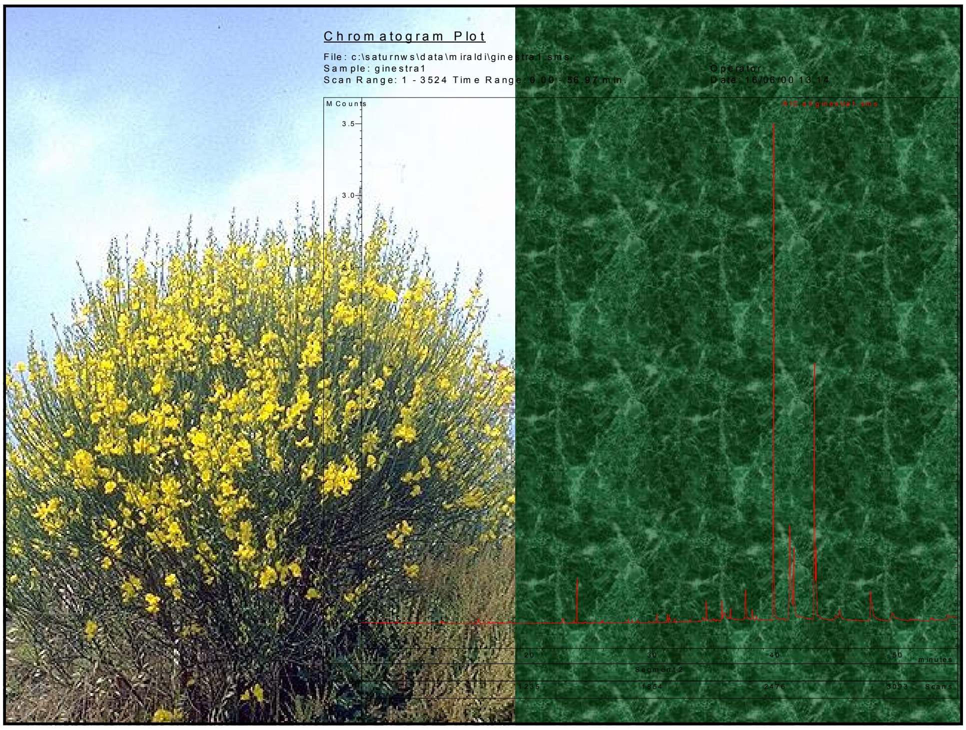
Salvia coccinea Juss.

Salvia sclarea L.

Salvia verbenaca L.

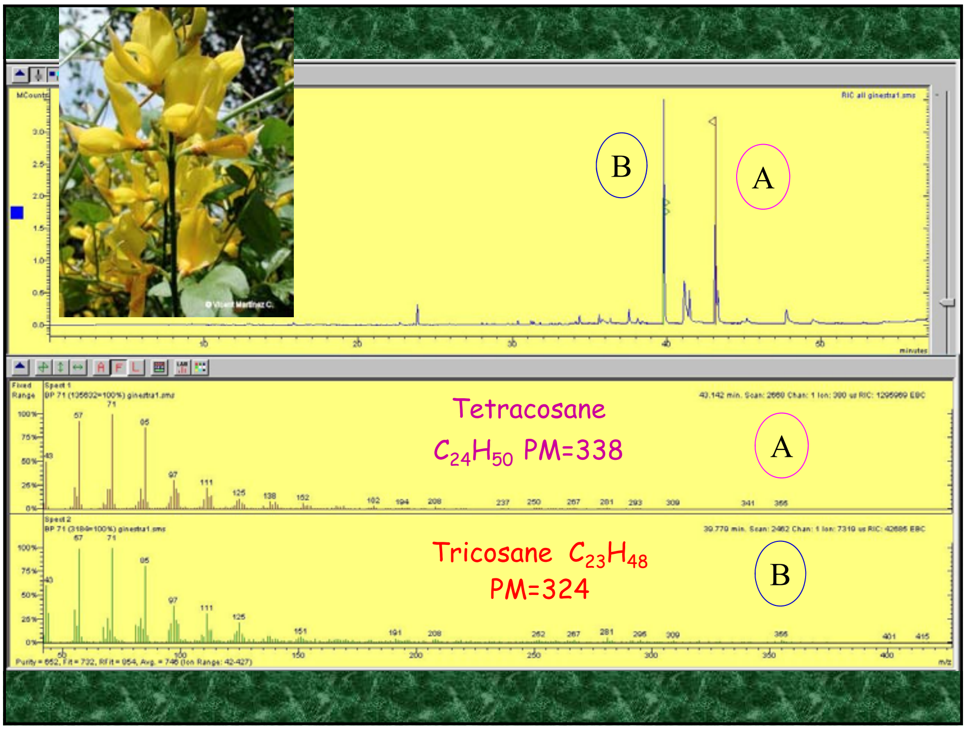
Salvia verticillata L.




Chromatogram Plot

File: c:\saturnws\data\miraldi\ginestra1.sms
Sample: ginestra1
Scan Range: 1 - 3524 Time Range: 0.00 - 56.97 min.

Operator:
Date: 16/06/00 13.14



Tetracosane

$C_{24}H_{50}$ PM=338

A

Tricosane $C_{23}H_{48}$

PM=324

B

Journal of Archaeological Science 37 (2010) 1453–1457

Contents lists available at ScienceDirect

Journal of Archaeological Science

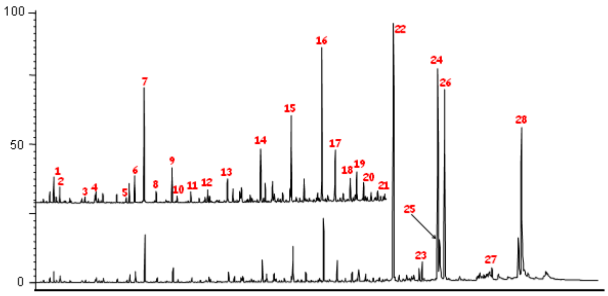
journal homepage: http://www.elsevier.com/locate/jas

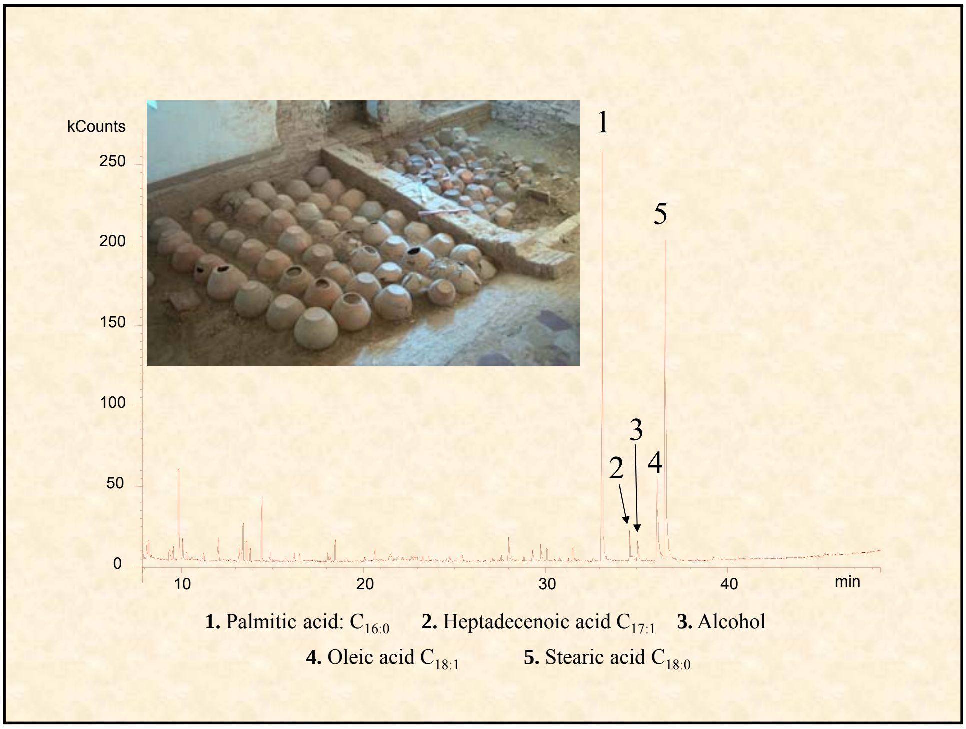
ELSEVIER

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]

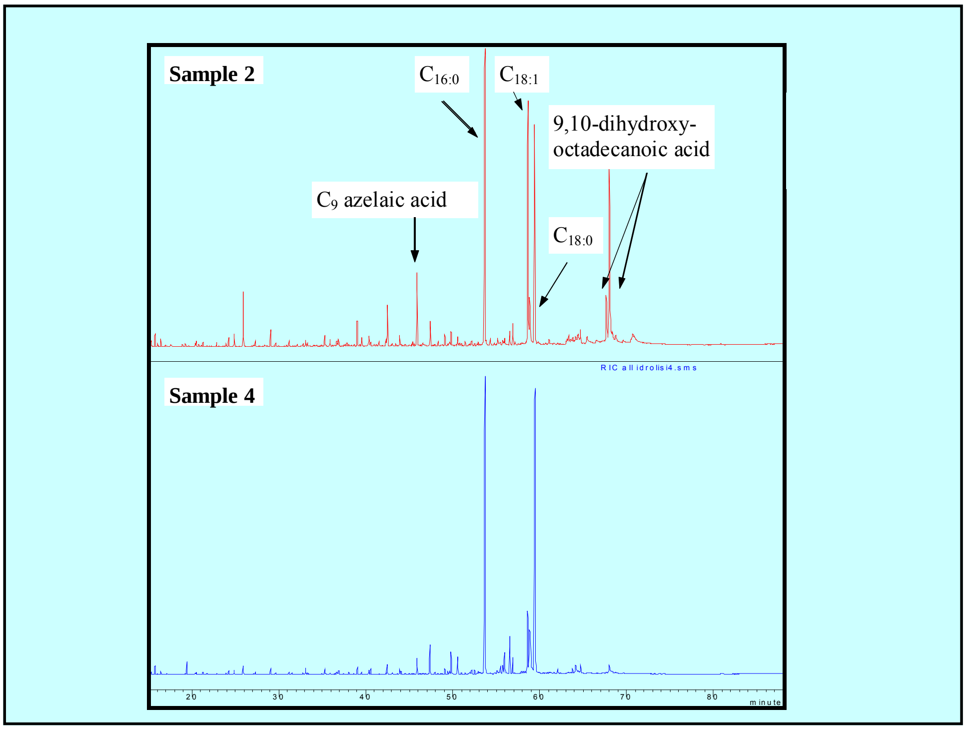
Carmine convent in Siena



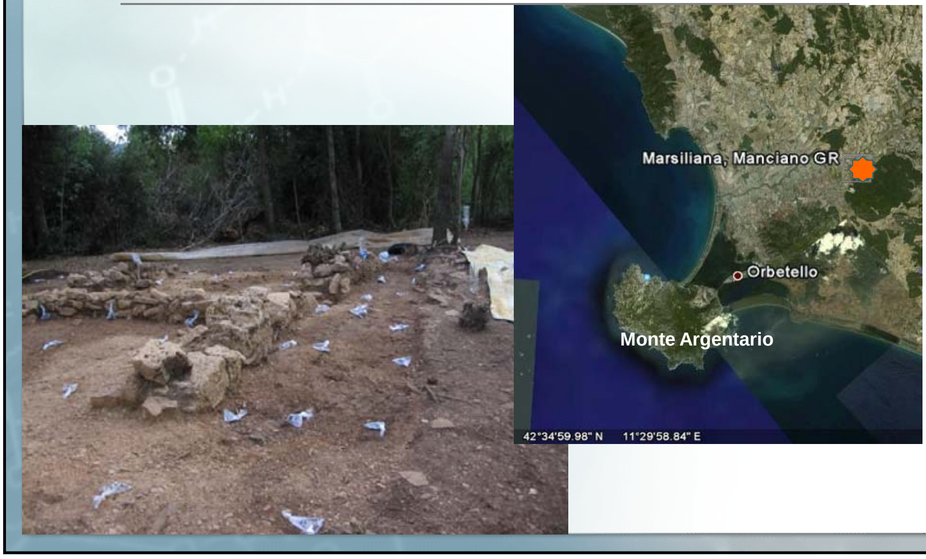


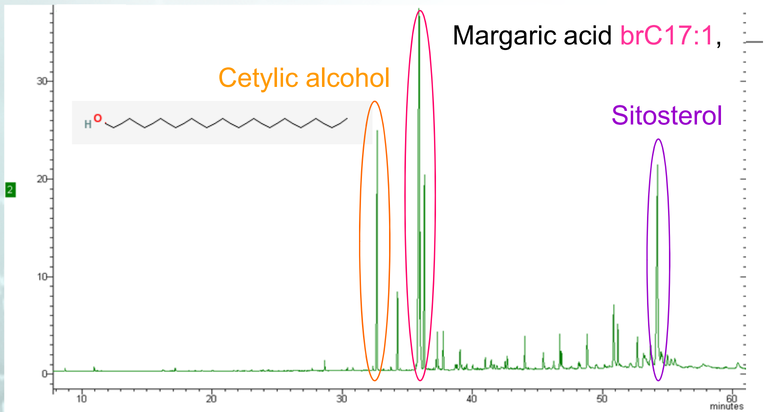
1. Palmitic acid: $C_{16:0}$ **2.** Heptadecenoic acid $C_{17:1}$ **3.** Alcohol

4. Oleic acid $C_{18:1}$ **5.** Stearic acid $C_{18:0}$

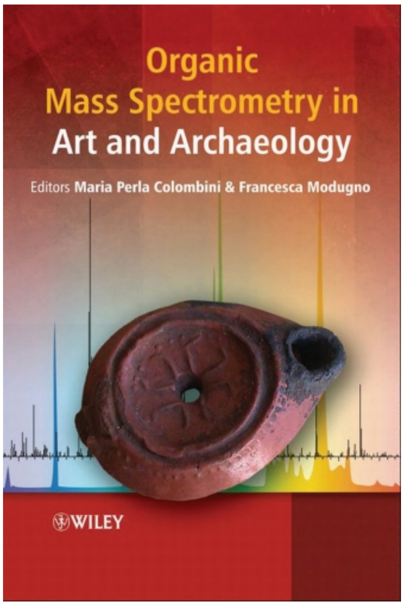


Archaeological site





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



Chapter 2

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

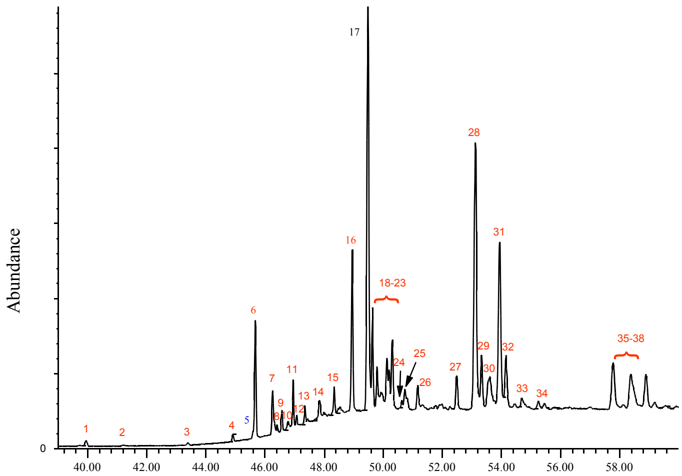
Gianluca Giorgi

2009

Sofisticate fabbriche di alcani ……

- 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



1. C_{25}
2. 13-/ 11-/ 9-, meC_{25}
3. C_{26}
4. 2-meC_{26}
5. $C_{27:1}$
6. C_{27}
7. 13-/ 11- meC_{27}
8. 7-meC_{27}
9. 5-meC_{27}
10. 9, *y* dimetil-C_{27}
11. 3-meC_{27}
12. 5, 15 dimetil-C_{27}
13. C_{28}
14. 14-/ 13-/ 12- meC_{28}
15. 2-meC_{28}
16. C_{29}
17. 15-/,13- meC_{29}
18. 7-meC_{29}
19. 5-meC_{29}
20. 11, *y* dimetil-C_{29}
21. 7, *y* dimetil-C_{29}
22. 3-meC_{29}
23. 5, *y* dimetil-C_{29}
24. C_{30}
25. *x*, y dimetil-C_{29}
26. Unknown
27. C_{31}
28. 15-/, 13-, meC_{31}
29. 7-meC31
30. 13, 17 dimetil-C_{31}
31. 7, 15 dimetil-C_{31}
32. 5, 15 dimetil-C_{31}
33. *x*, *y* dimetil-C_{33}
34. Unknown
35. 17-/, 15-/, 13-, meC_{33}
36. 13, *y* dimetil-C_{33}
37. 7, *y* dimetil-C_{33}
38. 5, *y* dimetil-C_{33}

- •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

Sofisticate fabbriche di alcani ……

$-(CH_2)_{23}-$

Pentacosane (C25)

$-(CH_2)_{24}-$

Hexacosane (C26)

$-(CH_2)_{25}-$

Heptacosane (C27)

$-(CH_2)_{26}-$

Octacosane (C28)

$-(CH_2)_{27}-$

Nonacosane (C29)

$-(CH_2)_{28}-$

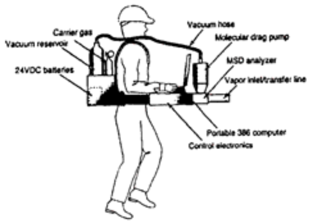
Triacontane (C30)

$-(CH_2)_{29}-$

Hentriacontane (C31)

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.

Neil S. Arnold, J. P. D., Sue Anne Sheya, William H. McClennen, and Henk L. C. Meuzelaar Design Considerations in Field-Portable GC-Based Hyphenated Instrumentation. *Field Anal. Chem. Technol.* 2000, *4*, 219-238.

Airborne GC-MS

Bruker Franzen GC-MS for German Fire Brigade

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

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

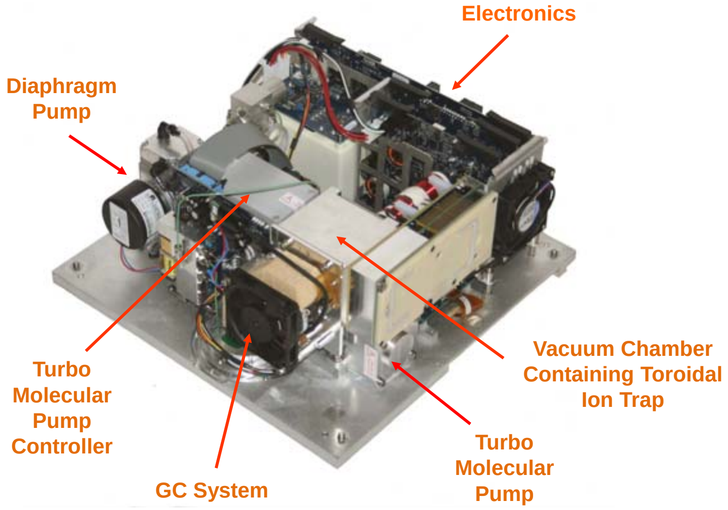
LTM gas chromatograph
5 m x 0.1 mm x 0.4 µm MTX-5
Fast temperature programming
50 - 300ºC @ 120ºC/min

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

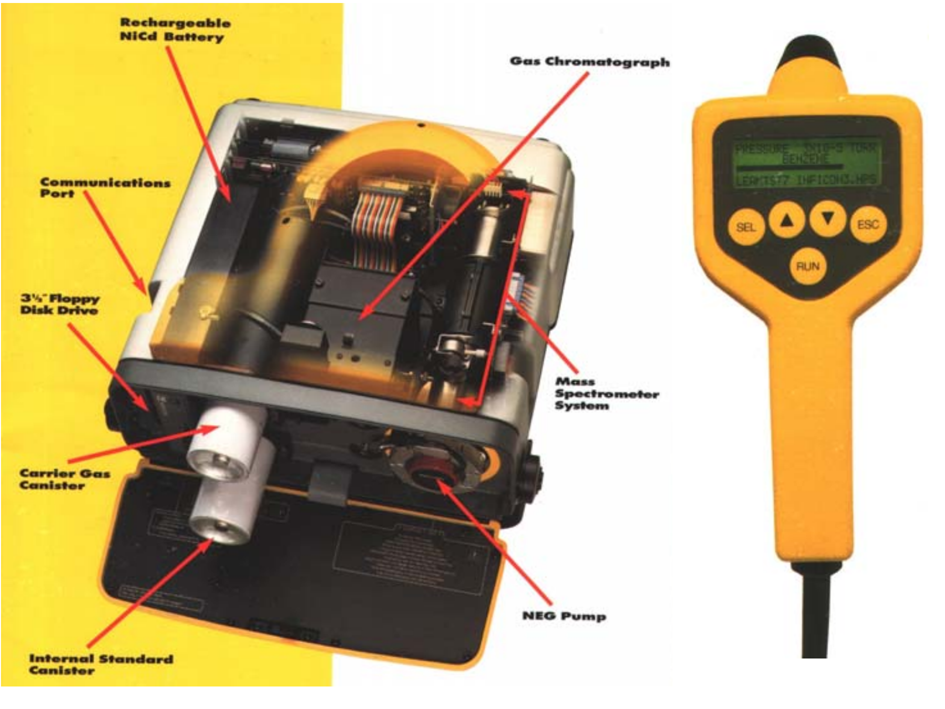
Low power
~120 W peak
~ 60 W average

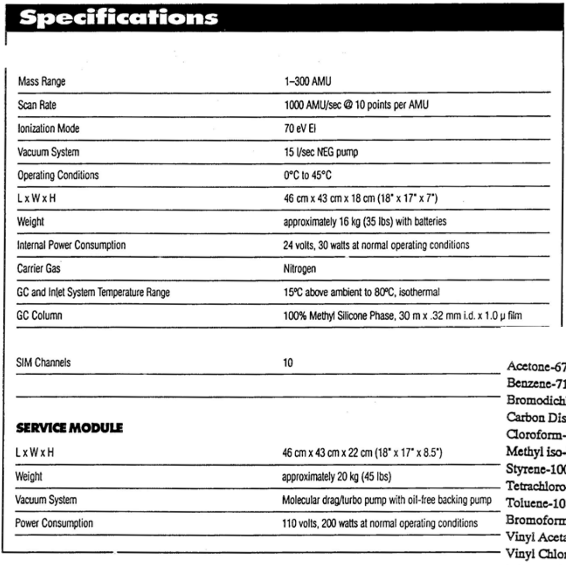
GC-TMS System Components



TORION

Designed to be operated in PPE





Specifications

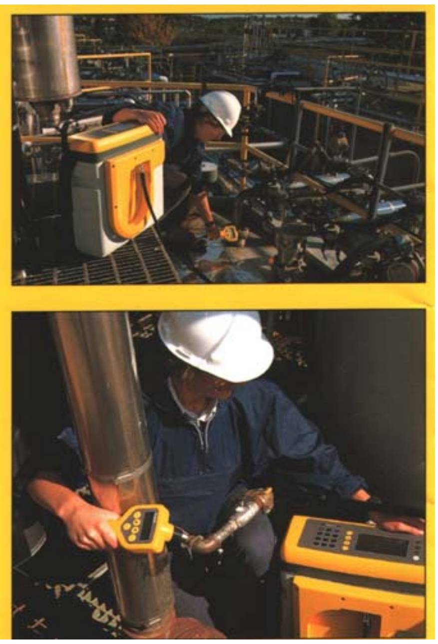
Mass Range	1–300 AMU
Scan Rate	1000 AMU/sec @ 10 points per AMU
Ionization Mode	70 eV EI
Vacuum System	15 l/sec NEG pump
Operating Conditions	0°C to 45°C
L x W x H	46 cm x 43 cm x 18 cm (18" x 17" x 7")
Weight	approximately 16 kg (35 lbs) with batteries
Internal Power Consumption	24 volts, 30 watts at 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

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

Target Analytes

Acetone-67641
Benzene-71432
Bromodichloromethane-75274
Carbon Disulfide-75150
Cloroform-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
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

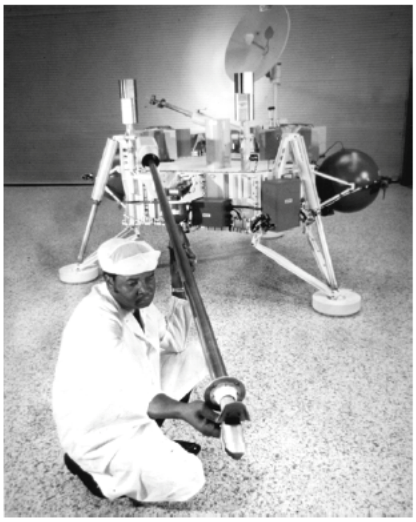


DEPENDABLE TESTING FOR VOCs

Spettrometria di massa ……

non solo sulla terra!!!

Mars Viking Lander (1975)



The Viking '76 Mass Spectrometer

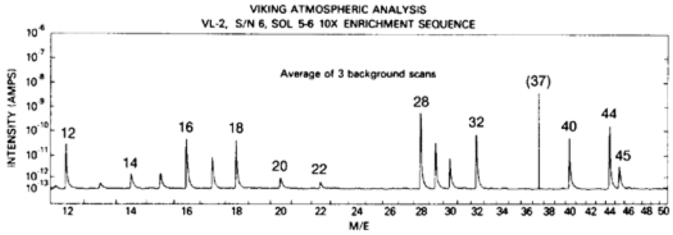


Figure 1.1. Mass spectrum (log intensity scale) of gases in the atmosphere of Mars.

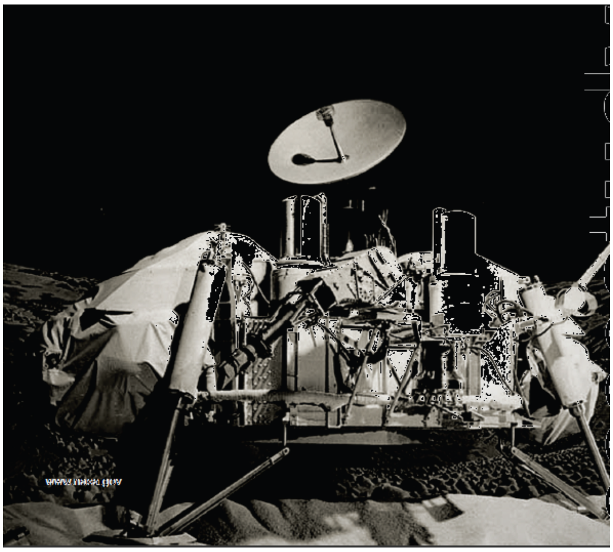
Composition of the Surface Atmosphere of Mars

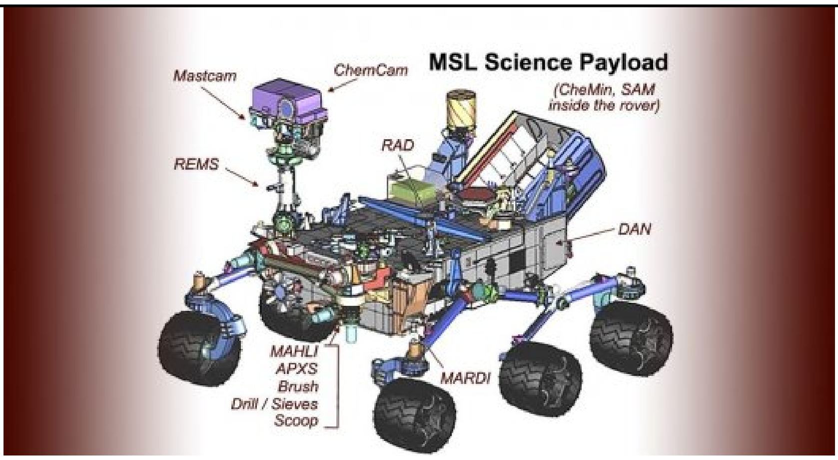
Gas		Concentration
Carbon Dioxide	CO_2	95.3%
Nitrogen	N_2	2.7%
Argon	Ar	1.6%
Carbon Monoxide	CO	(0.2)%
Oxygen	O_2	0.15%
Water Vapor	H_2O	0.03%
Neon ^{20}Ne	Ne	0.55 ppm
Krypton	Kr	0.3 ppm
Xenon	Xe	0.08 ppm
Ozone	O_3	0.03 ppm

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

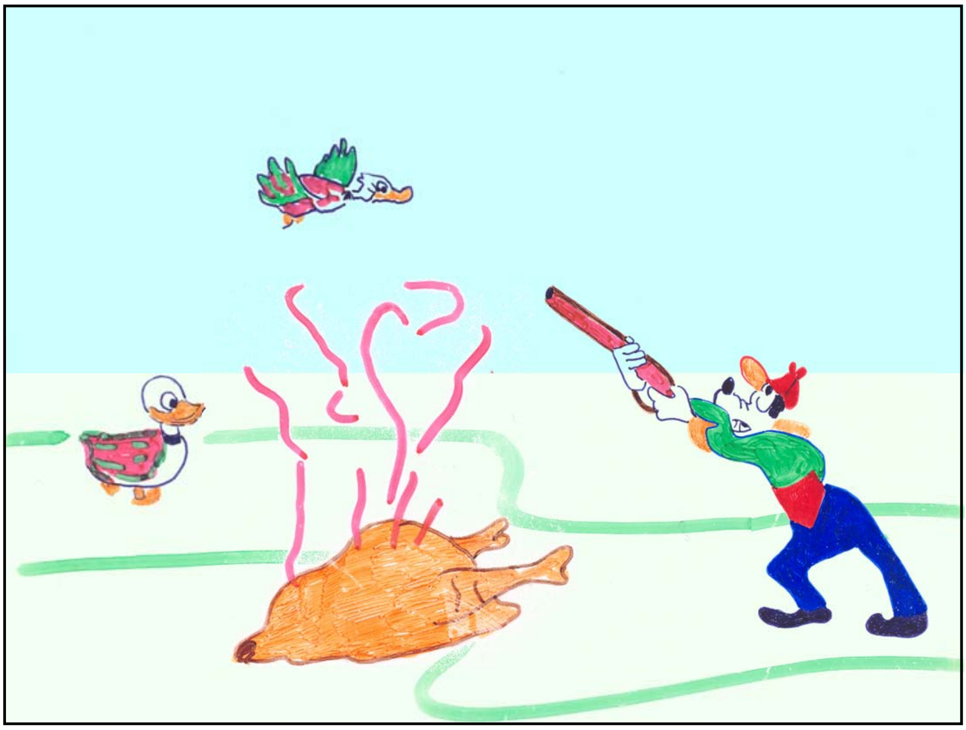
* Uncertainties in these values are presently +/-10% except for Ar and Xe.





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*



E.I.

C.I.

molecole in fase condensata

t_1 ↓ *volatilizzazione*

molecole in fase gassosa

t_2 ↓ *ionizzazione*

ioni in fase gassosa

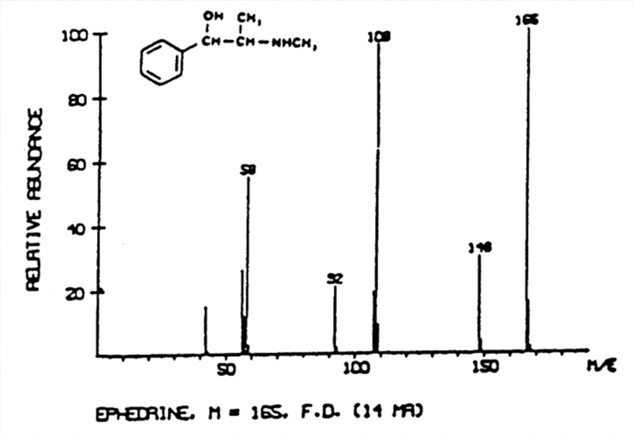
- ✓ molecole volatili
- ✓ termostabili
- ✓ basso PM

se $t_1 \approx t_2$ → **ionizzazione per desorbimento**

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**.

Tecniche di ionizzazione per desorbimento



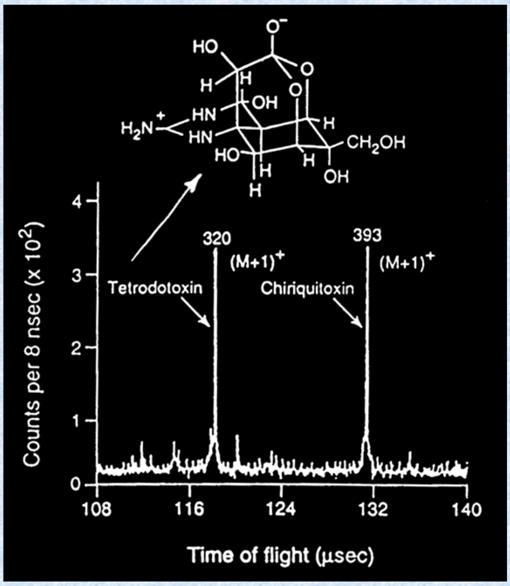
EPHEDRINE. M = 165. F.D. (14 MA)

FIELD DESORPTION (1969)

PLASMA

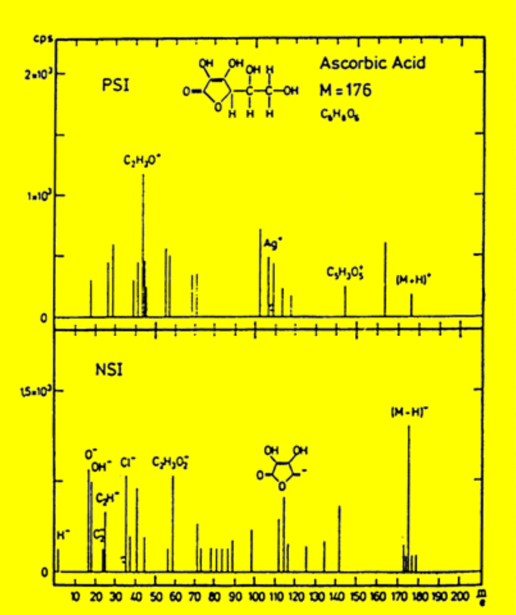
DESORPTION

(1974)



Decadimento radioattivo di un nuclide (^{252}Cf)

(MeV)



Positive and negative secondary ion spectra of ascorbic acid.

Secondary Ion Mass Spectrometry (SIMS) (1977)

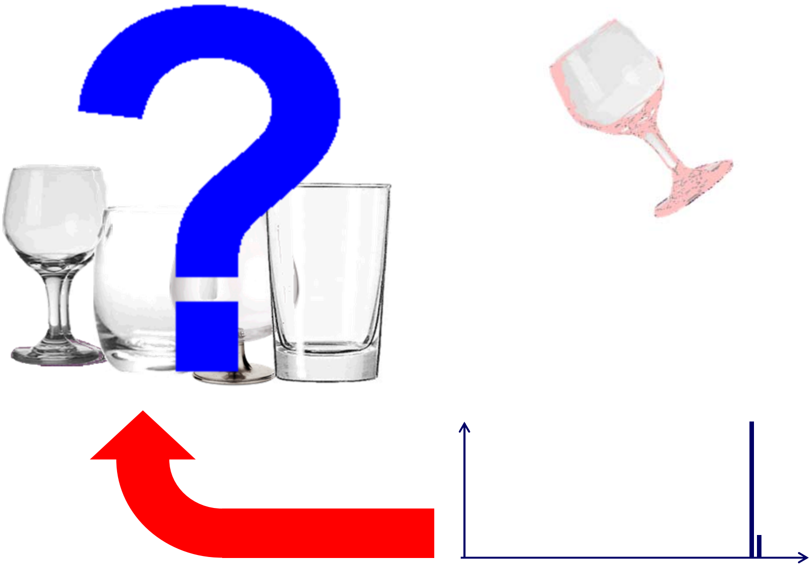
Tecniche di ionizzazione soft

ionizzazione chimica
electrospray
APCI
MALDI

Molecole protonate $[M+H]^+$ / deprotonate $[M–H]^–$ con minimo eccesso di energia

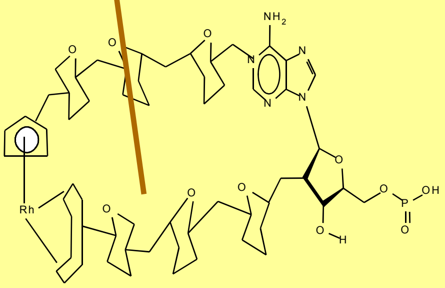
Frammentazione molto scarsa / assente

Determinazione del PM.
MS/MS necessario per ottenere informazioni strutturali



Fast Atom Bombardment

1981



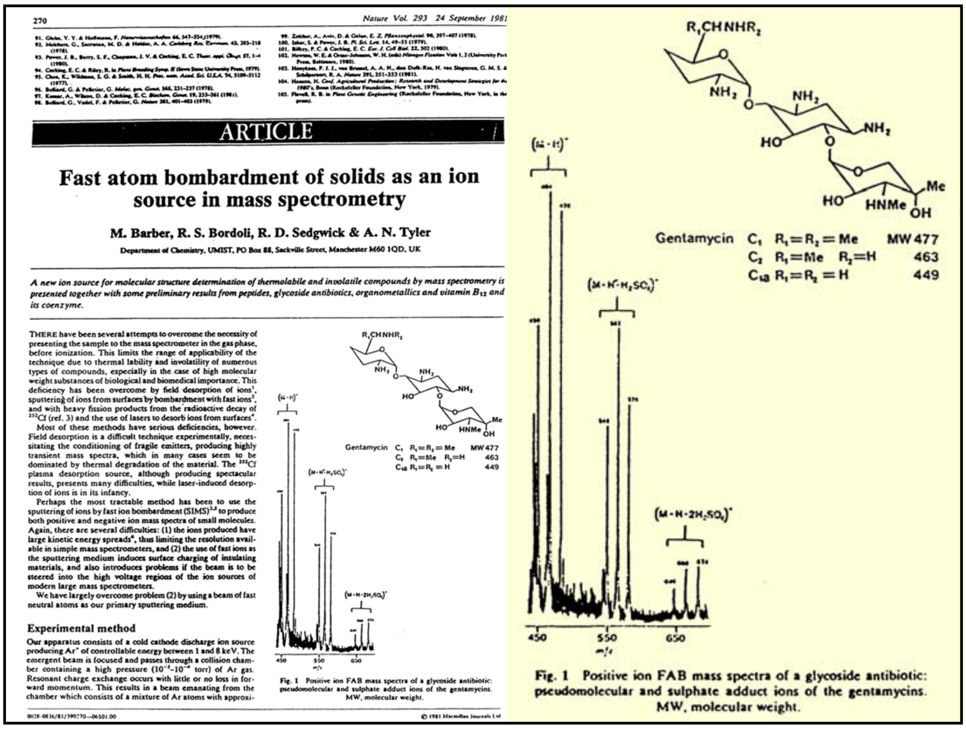
A new ion source for molecular structure determination of

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



ARTICLE

Fast atom bombardment of solids as an ion source in mass spectrometry

M. Barber, R. S. Bordoli, R. D. Sedgwick & A. N. Tyler

Department of Chemistry, UMIST, PO Box 88, Sackville Street, Manchester M60 1QD, UK

A new ion source for molecular structure determination of thermolabile and involatile compounds by mass spectrometry is presented together with some preliminary results from peptides, glycoside antibiotics, organometallics and vitamin B_{12} and its coenzyme.

THERE have been several attempts to overcome the necessity of presenting the sample to the mass spectrometer in the gas phase, before ionization. This limits the range of applicability of the technique due to thermal lability and involatility of numerous types of compounds, especially in the case of high molecular weight substances of biological and biomedical importance. This deficiency has been overcome by field desorption of ions[1], sputtering of ions from surfaces by bombardment with fast ions[2], and with heavy fission products from the radioactive decay of ^{252}Cf (ref. 3) and the use of lasers to desorb ions from surfaces[4].

Most of these methods have serious deficiencies, however. Field desorption is a difficult technique experimentally, necessitating the conditioning of fragile emitters, producing highly transient mass spectra, which in many cases seem to be dominated by thermal degradation of the material. The ^{252}Cf plasma desorption source, although producing spectacular results, presents many difficulties, while laser-induced desorption of ions is in its infancy.

Perhaps the most tractable method has been to use the sputtering of ions by fast ion bombardment (SIMS)[2,5] to produce both positive and negative ion mass spectra of small molecules. Again, there are several difficulties: (1) the ions produced have large kinetic energy spreads[6], thus limiting the resolution available in simple mass spectrometers, and (2) the use of fast ions as the sputtering medium induces surface charging of insulating materials, and also introduces problems if the beam is to be steered into the high voltage regions of the ion sources of modern large mass spectrometers.

We have largely overcome problem (2) by using a beam of fast neutral atoms as our primary sputtering medium.

Experimental method

Our apparatus consists of a cold cathode discharge ion source producing Ar^+ of controllable energy between 1 and 8 keV. The emergent beam is focused and passes through a collision chamber containing a high pressure (10^{-3}–10^{-4} torr) of Ar gas. Resonant charge exchange occurs with little or no loss in forward momentum. This results in a beam emanating from the chamber which consists of a mixture of Ar atoms with approxi-

Gentamycin C_1 $R_1 = R_2 = Me$ MW 477
C_2 $R_1 = Me$ $R_2 = H$ 463
C_{1a} $R_1 = R_2 = H$ 449

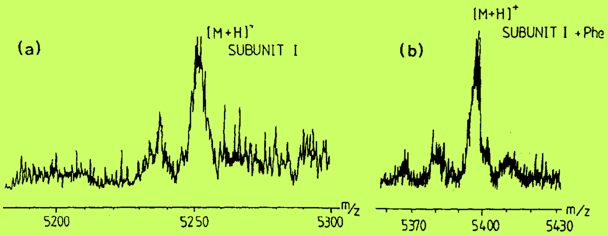
Fig. 1 Positive ion FAB mass spectra of a glycoside antibiotic: pseudomolecular and sulphate adduct ions of the gentamycins. MW, molecular weight.

0028-0836/81/390270—06$01.00

Gentamycin C_1 $R_1 = R_2 = Me$ MW 477
C_2 $R_1 = Me$ $R_2 = H$ 463
C_{1a} $R_1 = R_2 = H$ 449

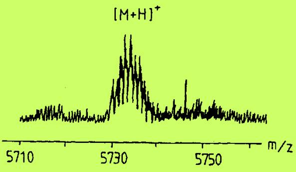
Fig. 1 Positive ion FAB mass spectra of a glycoside antibiotic: pseudomolecular and sulphate adduct ions of the gentamycins. MW, molecular weight.

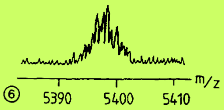
Spettrometria di massa biomolecolare



(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.

⑤ 

⑥ 

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

FAB
(1981)

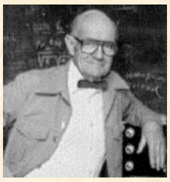
Electrospray
(1984)

MALDI
(1987)

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

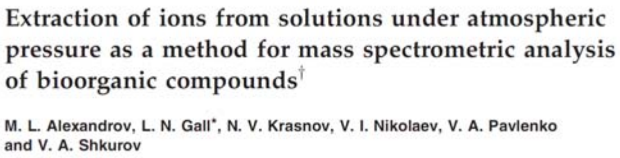
ed a

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

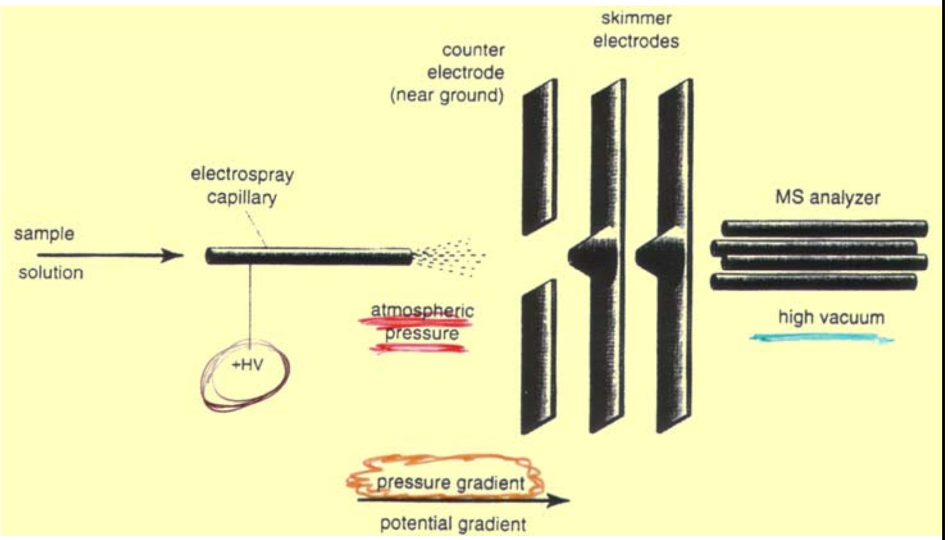
Rapid Commun. Mass Spectrom. 2008; 22: 267–270

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

Extraction of dissolved ions under atmospheric pressure (EDIAP)

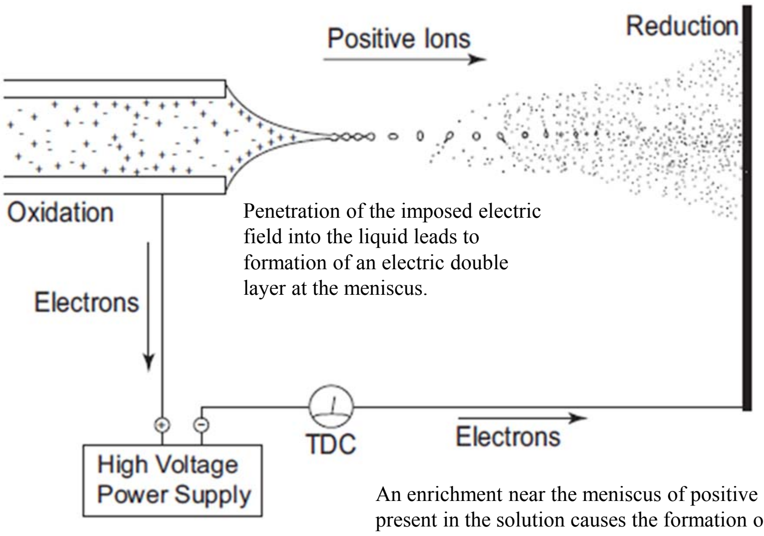
The key year in which Fenn's group and Gall's group published was 1984; Fenn et al. published papers describing an electrospray interface in *Analytical Chemistry* and *Journal of Physical Chemistry*, whilst Gall and co-workers published (in Russian) in Doklady Akademii Nauk SSSR and Bioorganicheskaya Khimiya.

ELECTROSPRAY



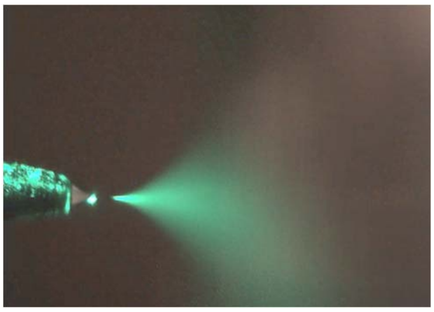
There are three major steps in the production of gas-phase ions from electrolyte ions in solution.

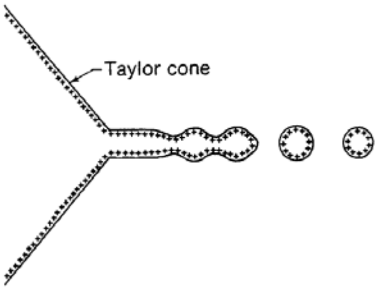
(a) **production of charged droplets** at the ES capillary tip



Penetration of the imposed electric field into the liquid leads to formation of an electric double layer at the meniscus.

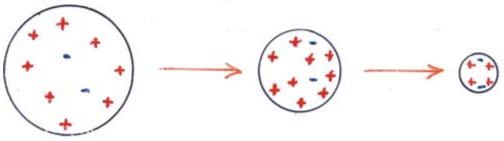
An enrichment near the meniscus of positive ions present in the solution causes the formation of a cone and a jet charged by an excess of positive ions.



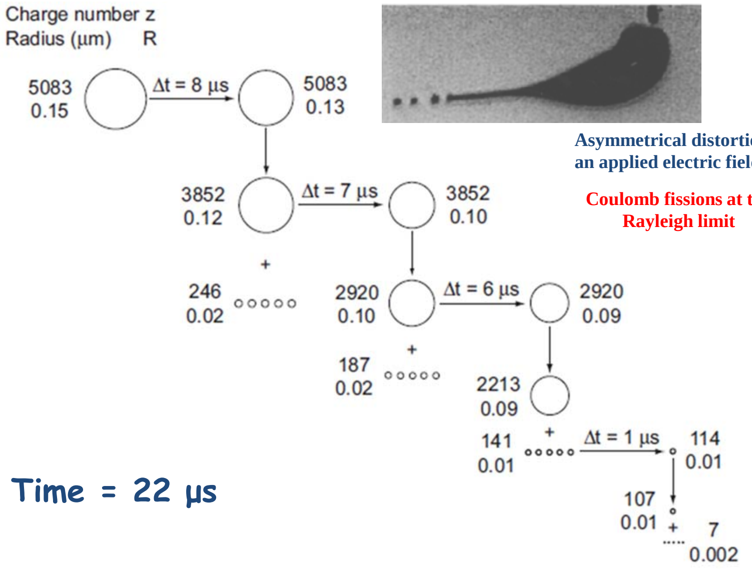


The jet splits into droplets charged with an excess of positive ions.

(b) **Shrinkage of the charged droplets by solvent evaporation** and repeated droplet disintegrations leading ultimately to very small highly charged droplets capable of producing gas-phase ions



Shrinkage = restringimento, contrazione



Asymmetrical distortions at an applied electric field

Coulomb fissions at the Rayleigh limit

Total Time = 22 µs

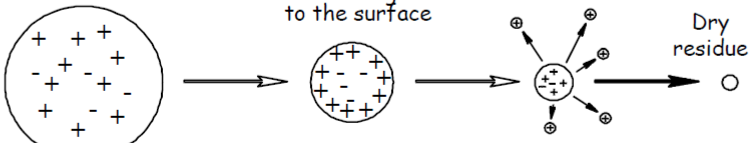
Z corresponds to the number of excess singly charged ions near the surface of the droplet.

(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



Solvent evaporation increases the electric field and the ions migrate to the surface

Dry residue

Charged droplets contain both polarity ions, with a predominant one

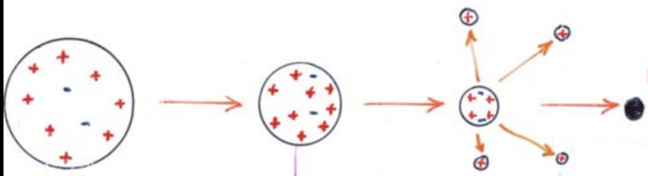
When a limit value is reached for the electric field, ions are emitted into the gas phase

direct ion emission from the droplets.

It becomes dominant over Coulomb fission for droplets with radii of R≤10 nm

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

1 nm = 10^{-9} m



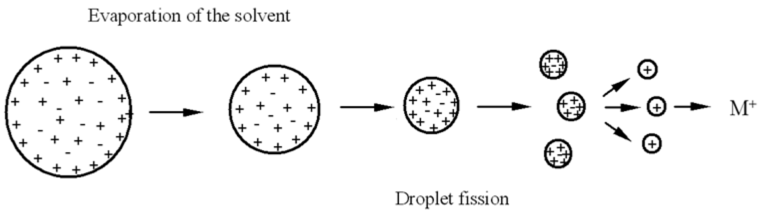
residuo involatile rimane come particella secca

- solvente + ioni ⊕ e ⊖
- polarità dominante determinata dalla polarità dello sprayer

- Al valore di campo critico gli ioni sono emessi dalla goccia

- Mentre il solvente evapora si ha un aumento del campo elettrico e gli ioni si muovono alla superficie

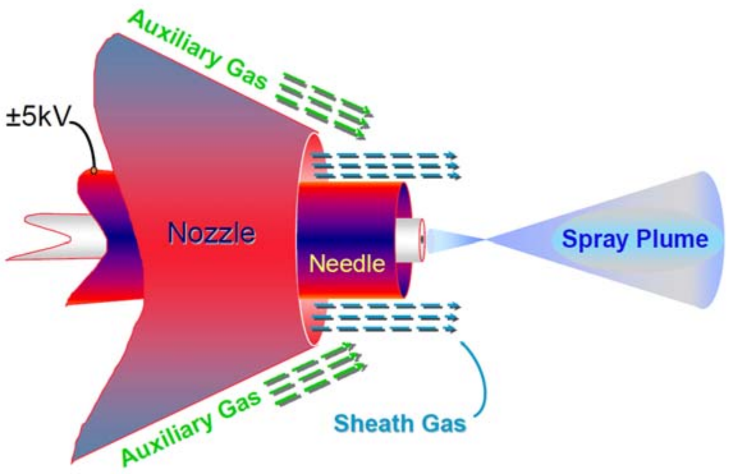
The Charged Residue Model (CRM)

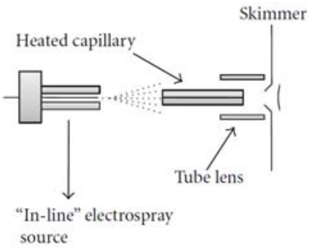


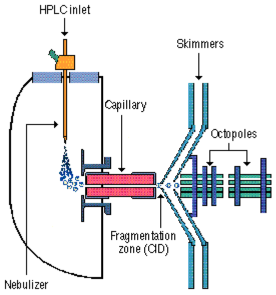
formation of extremely small droplets that could contain **one** analyte molecule and some ionic charges.

Fission = scissione

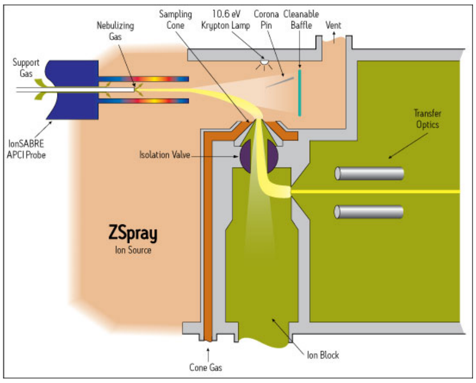
Iribarne J.V., Thomson B.A. *J. Chem. Phys.* 1976; 64: 2287







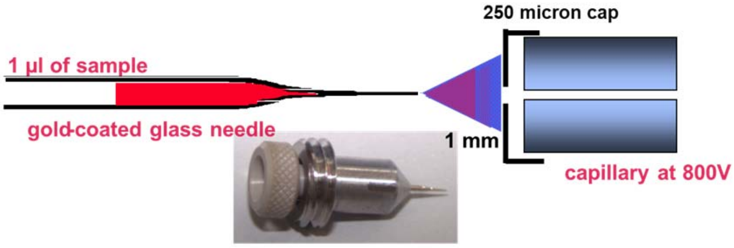
Orthogonal spray source



Nanospray Source

NanoElectrospray at flow rates down to 25 nl/min

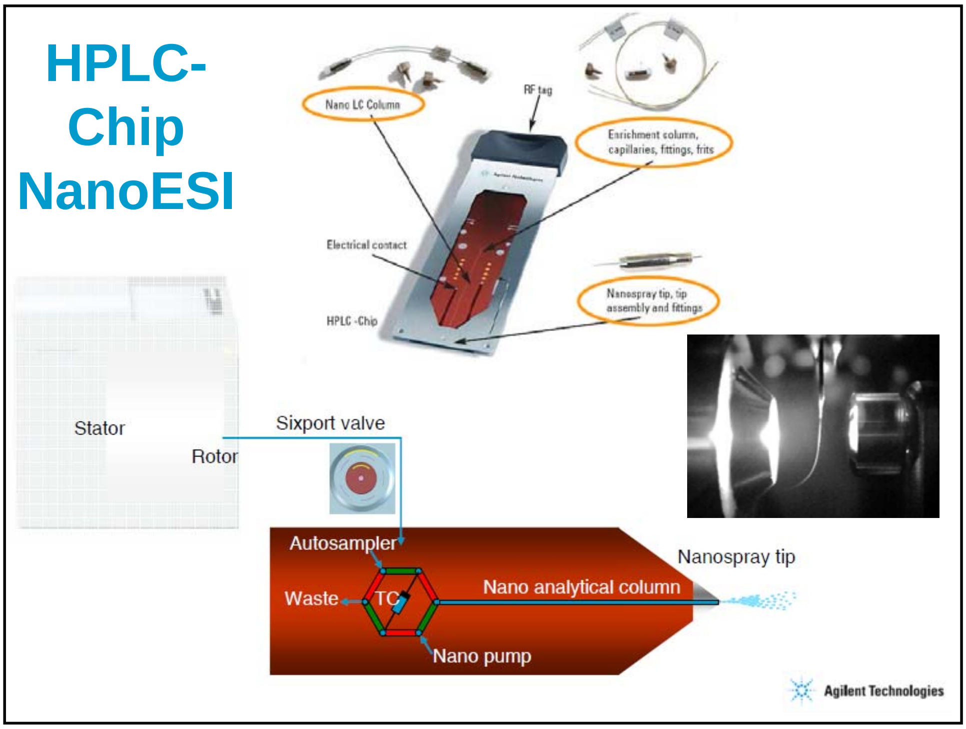
- **40 minutes of analysis time from 1 µL of sample**

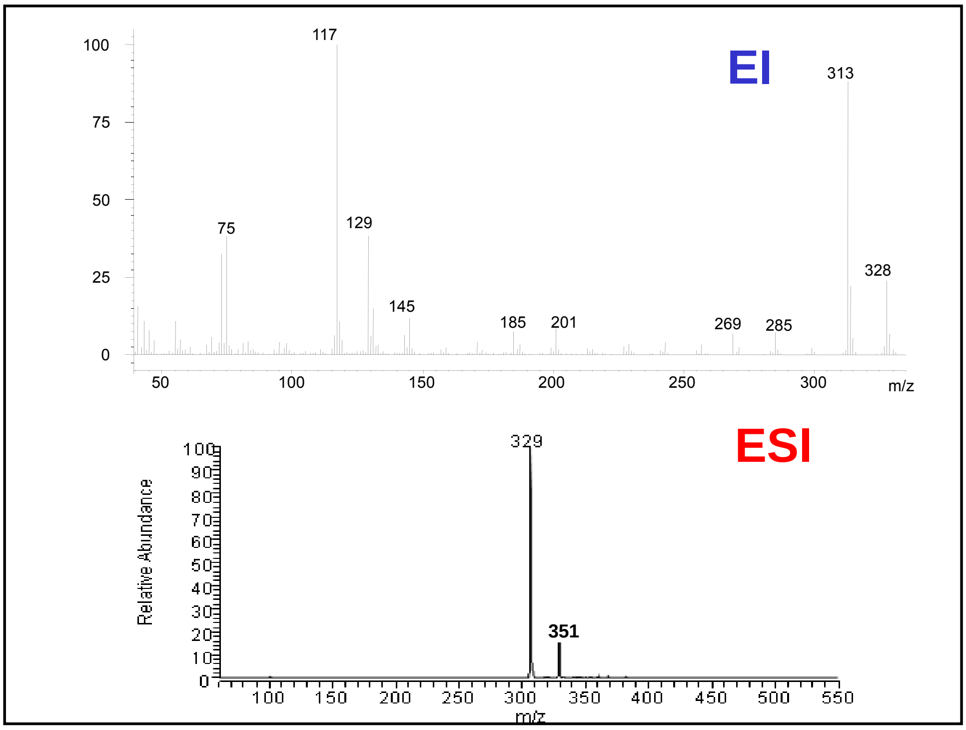


In Nanospray, a needle with an extremely small inner diameter is used. The glass needle is gold-coated for applying the correct electric potential onto the needle. For off-line nanospray, the needle is filled with about 1-5 µl of sample using a gel loader tip.
When the electric field is applied, a spray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

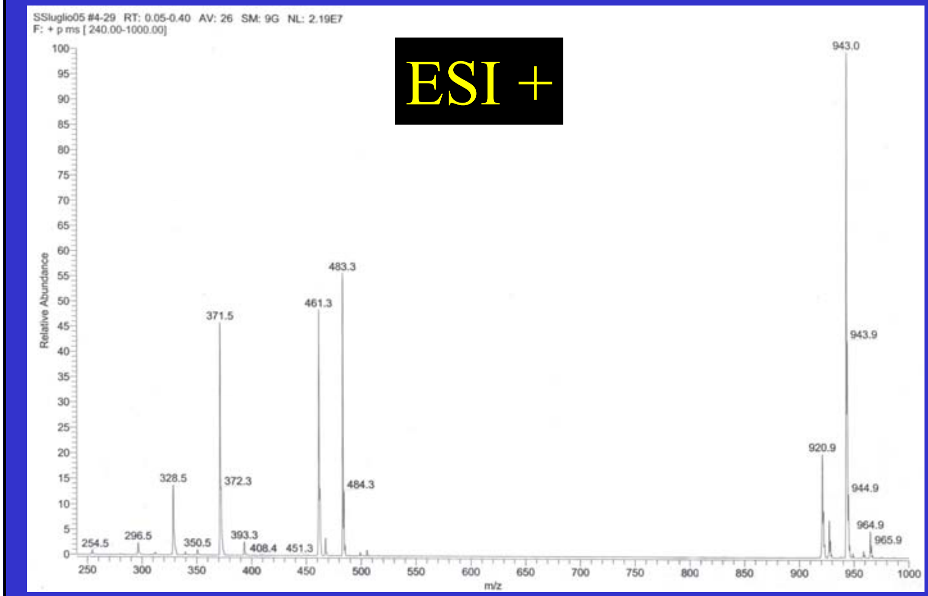
(from Bruker Daltonics)

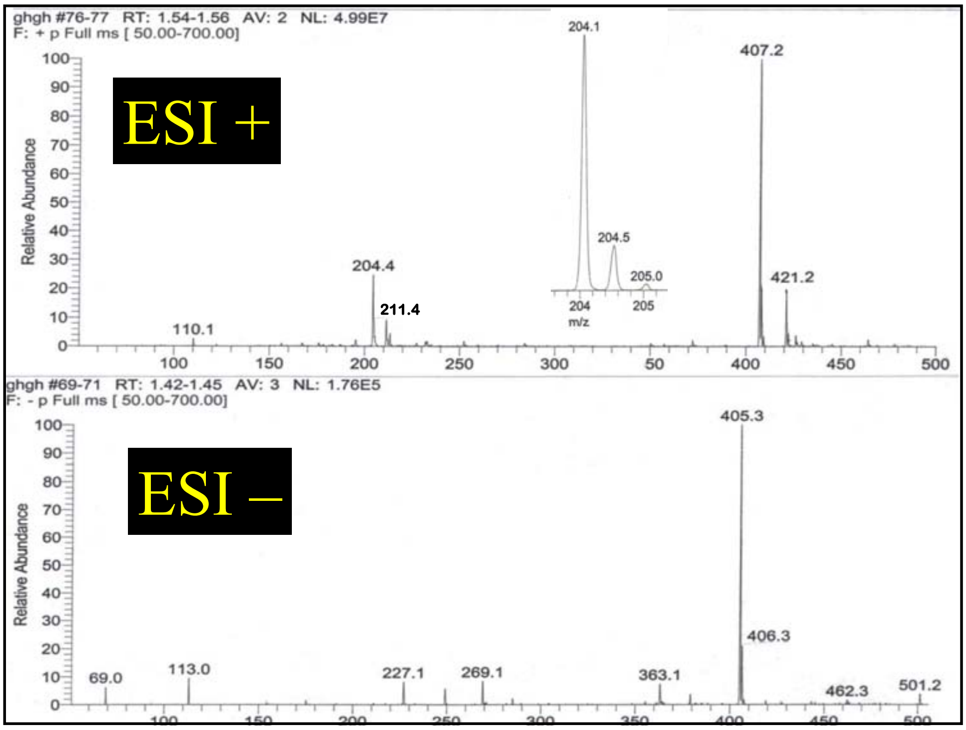
HPLC-Chip NanoESI

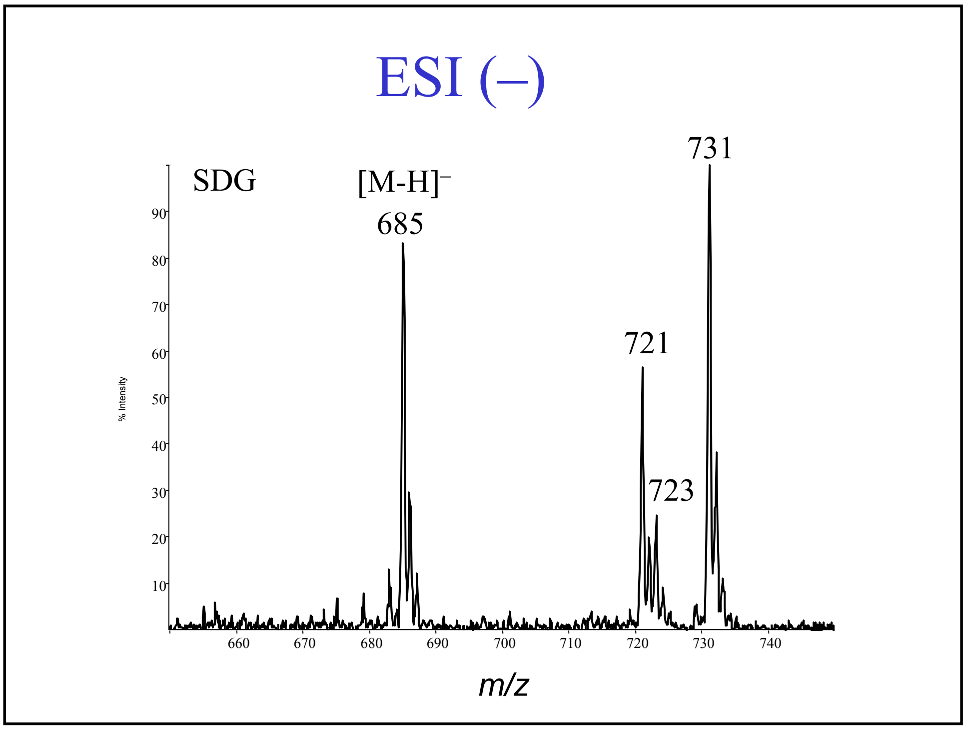




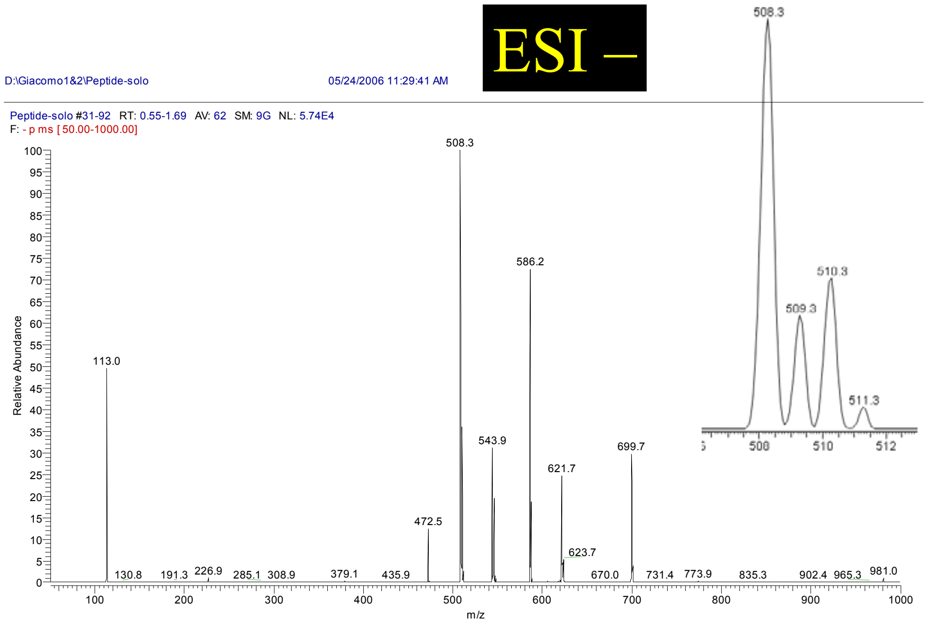
ADDUCTS			Added mass
Positive ion MS			
[M+	H]$^{+}$		1
[M+	Na]$^{+}$		23
[M+	K]$^{+}$		39
[M+	H+	NH_3]$^{+}$	18
Negative ion MS			
[M+	HCOO]$^{-}$		45
Solvent adducts			
[M+	H+	CH_3CN]$^{+}$	42
Multimer adducts			
[2M+	H]$^{+}$		2M+1



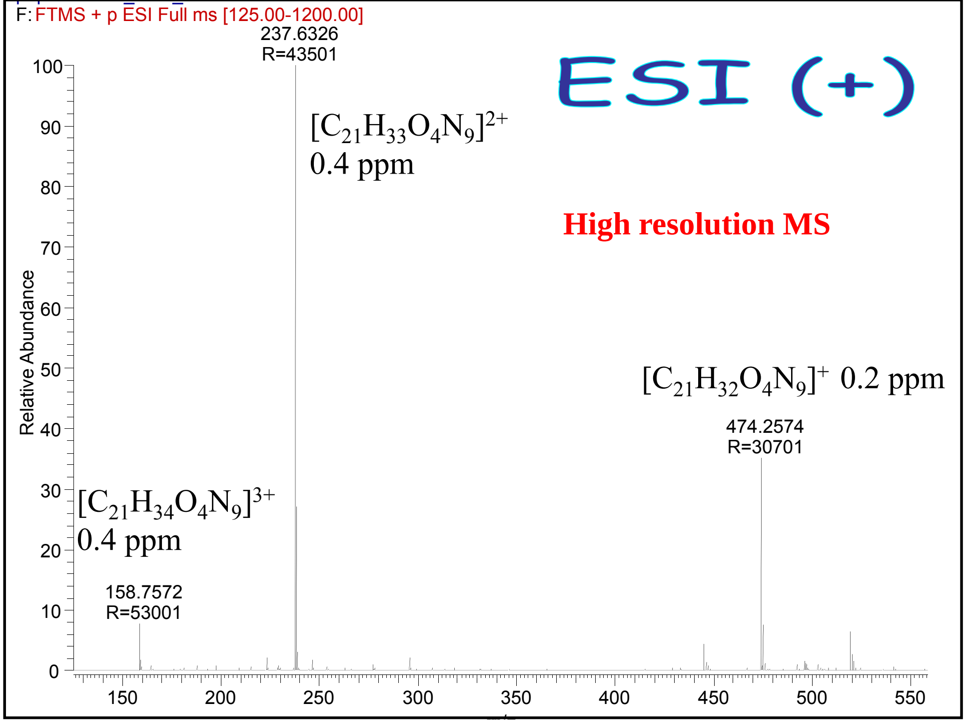

ghgh #76-77 RT: 1.54-1.56 AV: 2 NL: 4.99E7
F: + p Full ms [50.00-700.00]
ESI +
Relative Abundance
100
90
80
70
60
50
40
30
20
10
0
110.1
204.4
211.4
204.1
204.5
205.0
204
205
m/z
407.2
421.2
100
150
200
250
300
50
400
450
500
ghgh #69-71 RT: 1.42-1.45 AV: 3 NL: 1.76E5
F: - p Full ms [50.00-700.00]
ESI –
Relative Abundance
69.0
113.0
227.1
269.1
363.1
405.3
406.3
462.3
501.2
100
150
200
250
300
350
400
450
500


ESI (–)
SDG
[M-H]⁻
685
721
723
731
% Intensity
90
80
70
60
50
40
30
20
10
660
670
680
690
700
710
720
730
740
m/z

ESI –



ESI (+)



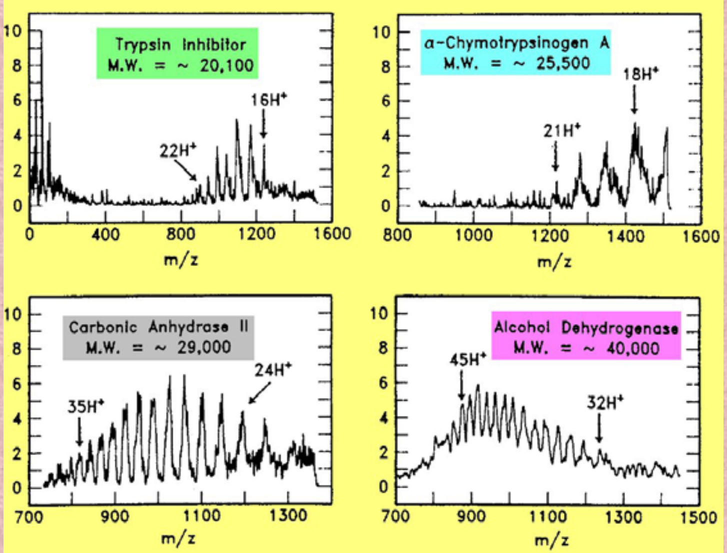
$[C_{21}H_{33}O_4N_9]^{2+}$
0.4 ppm

High resolution MS

$[C_{21}H_{32}O_4N_9]^{+}$ 0.2 ppm

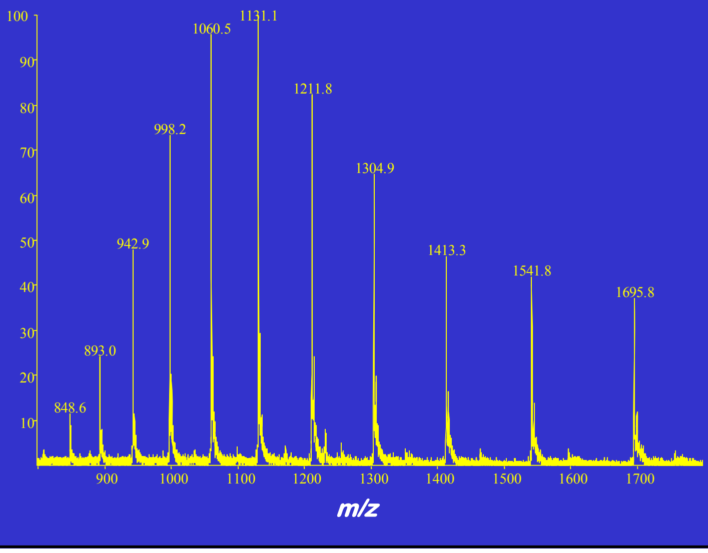
$[C_{21}H_{34}O_4N_9]^{3+}$
0.4 ppm

e
l
e
c
t
r
o
s
p
r
a
y

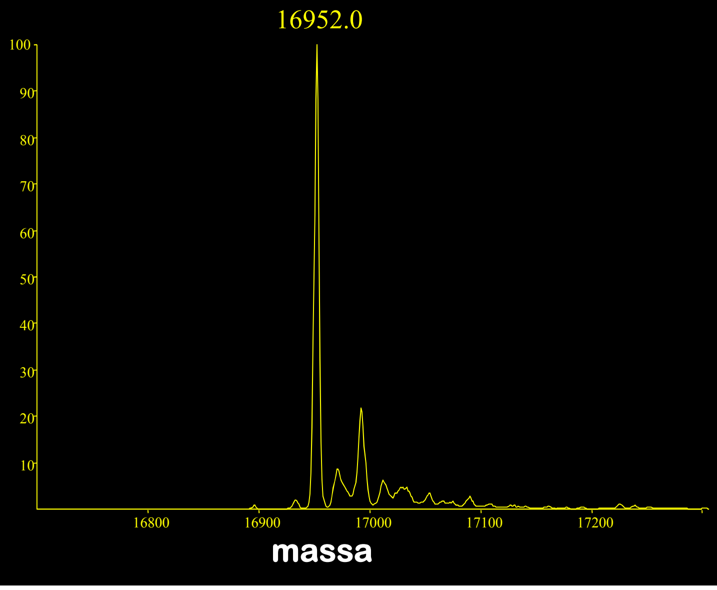


Mann, M.; Meng, C. K.; Fenn, J. B. *Proceeding of 36th Annual Conference on Mass Spectrometry and Allied Topics*; San Francisco, 1988; pp 1207-08.

Spettro ESI(+) della mioglobina di cavallo

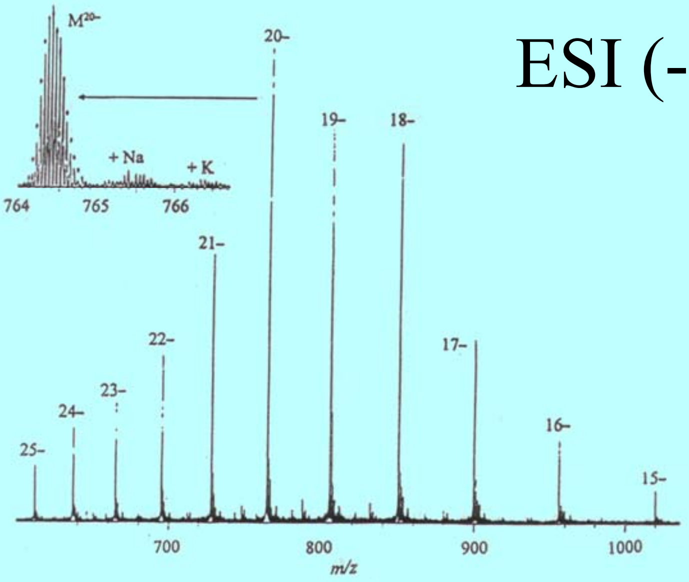


Spettro deconvoluto della mioglobina di cavallo

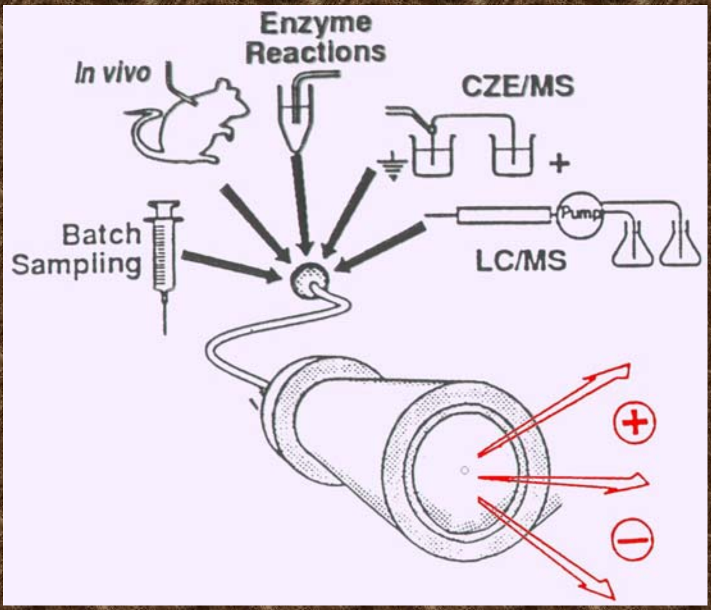


DNA sequencing by mass spectrometry

ESI (-)

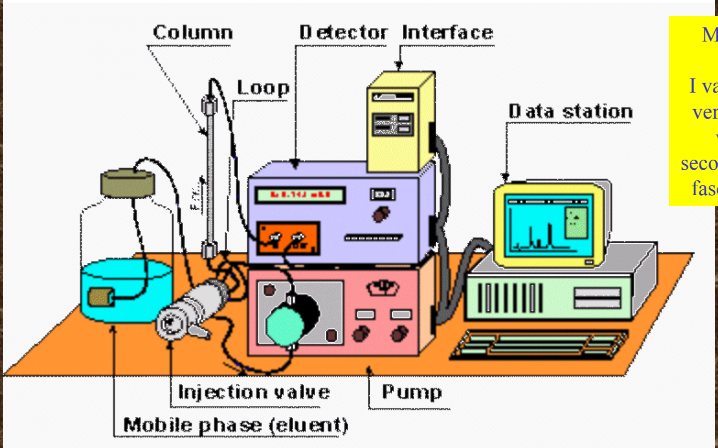


ESI FTMS mass spectrum of a single-stranded DNA 50-mer with a mass measurement accuracy of 7 ppm.



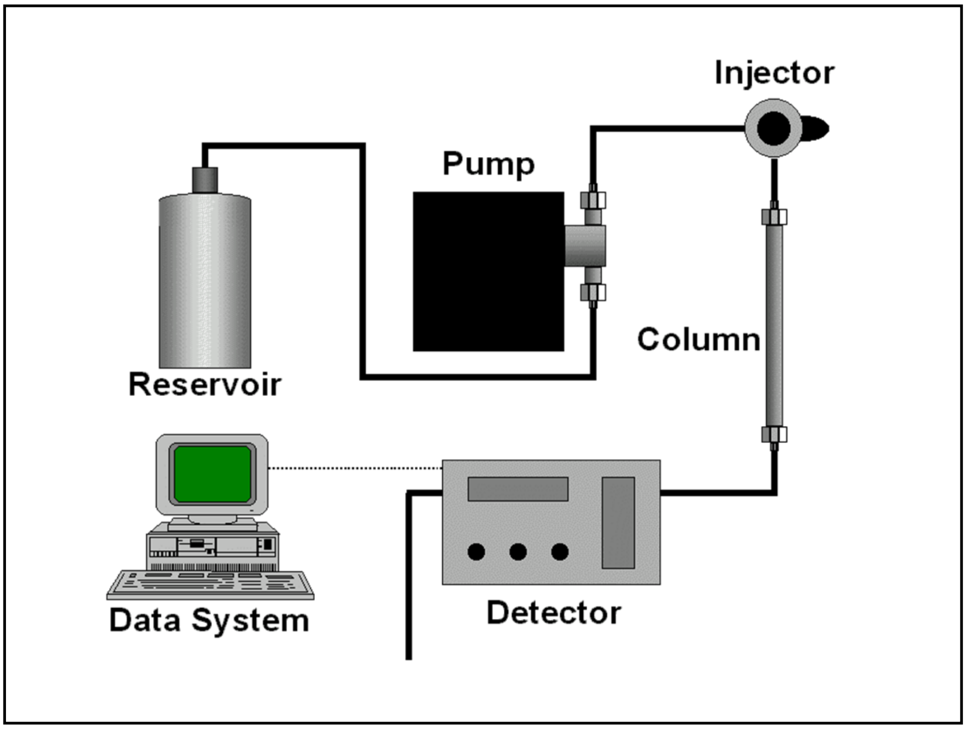
HPLC

High Performance Liquid Chromatography



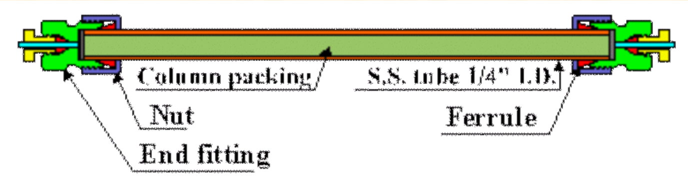
Miscela in soluzione

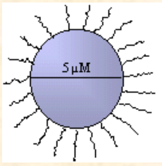
I vari componenti attraversano la colonna con velocità diverse a seconda della ripartizione fase mobile-stazionaria



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 facileda utilizzare	E' essenziale per l'identificazione di composti organici





column dimension:

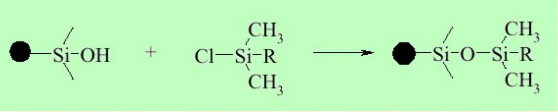
diam.: 0.05 - 4.6 mm length: 2 - 30 cm

particle size (5 – 30 µm)

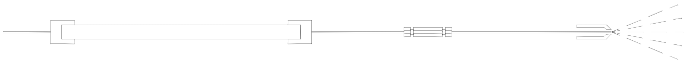
pore size (60 – 1000 Å)

stationary phase

hydrophobic alkyl chains
(C4 – C18)

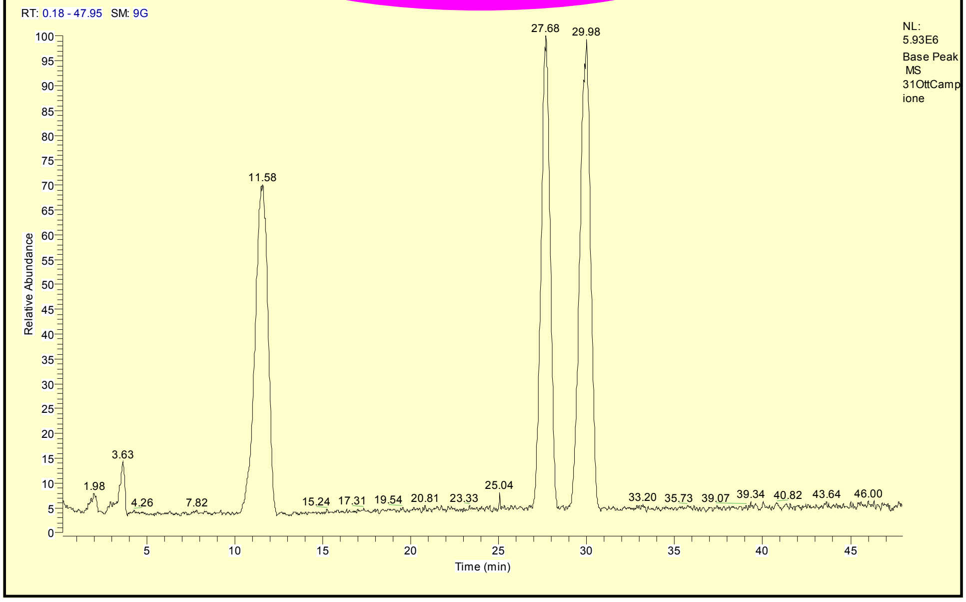


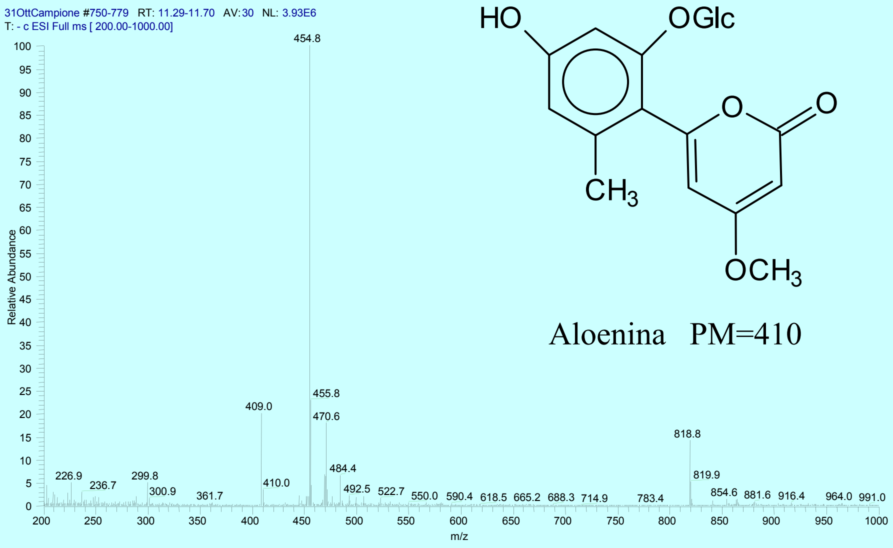
LC-ESI



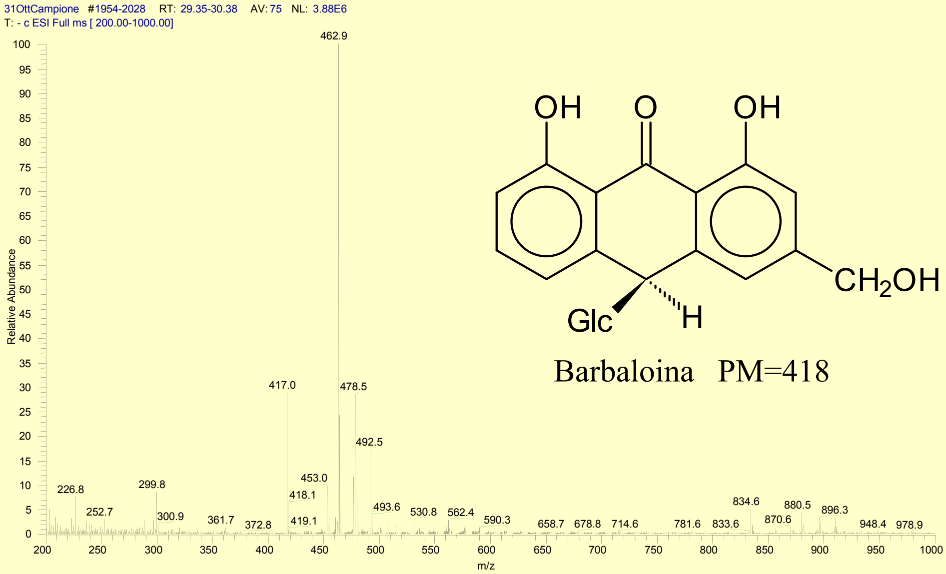
Column i.d.	Flow rate	Technique
4.6 mm	1.0 ml/min	Conventional HPLC
1.0 mm	40 µl/min	Micro LC
300 µm	4 µl/min	Capillary LC
75 µm	200 nl/min	Nano LC

HPLC-ESI neg





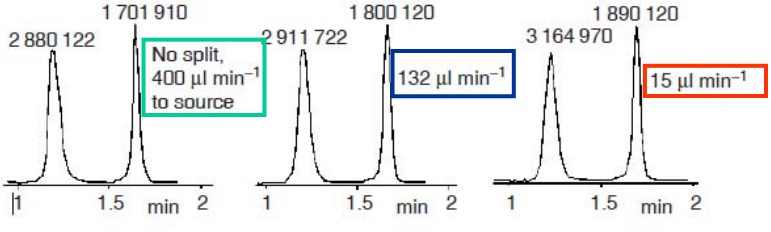
Aloenina PM=410



Barbaloina PM=418

Sensitivity to Concentration

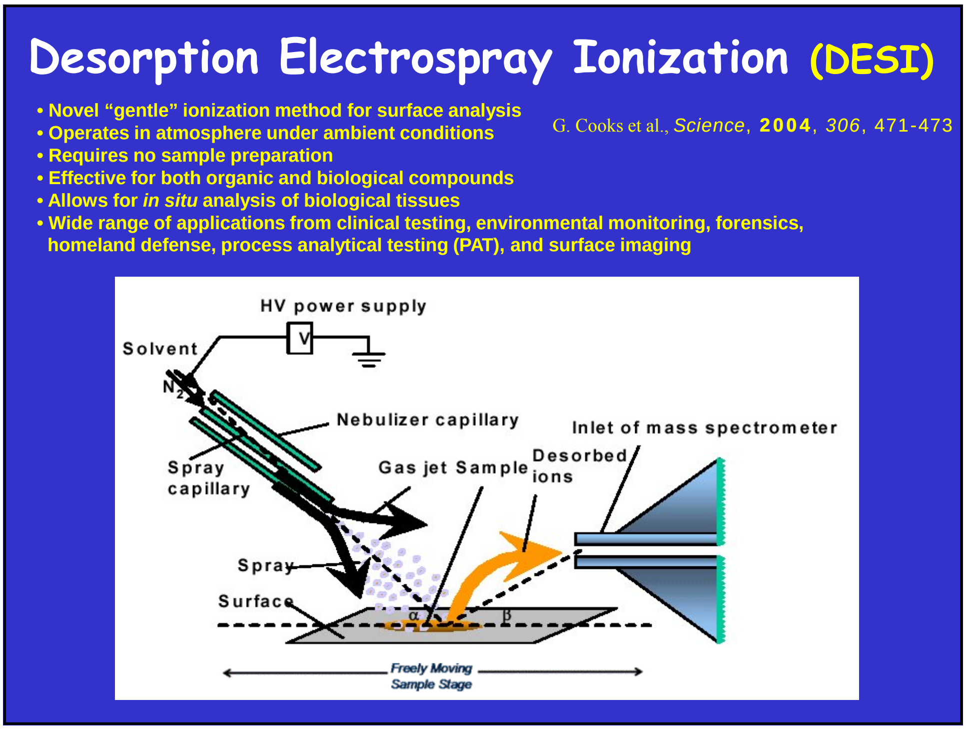
ESI is **sensitive to concentration**, and **NOT** to the total quantity of sample injected in the source



At **reduced flow rates** the **sensitivity** is slightly **increased**.

HPLC on a 2.1mm column at 400 µlmin^{-1} flow. Two drugs are monitored by selected ion monitoring.

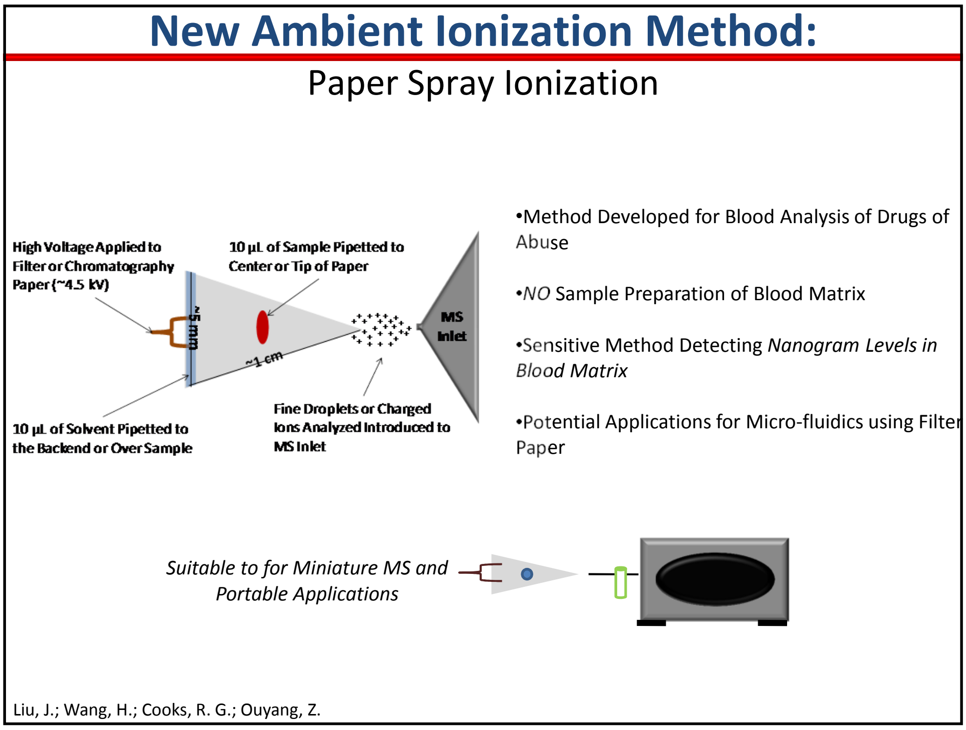
Covey T., ‘Analytical Characteristics of the Electrospray Ionization Process’ pp. 21–59 in ‘Biochemical and Biotechnological Applications of Electrospray Ionization Mass Spectrometry’, Snyder A.P., ed., ACS Symposium Series 619, American Chemical Society, 1996



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

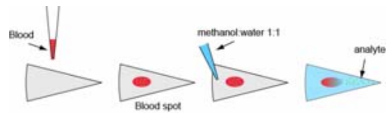
•Sensitive Method Detecting *Nanogram Levels in Blood Matrix*

•Potential Applications for Micro-fluidics using Filte Paper

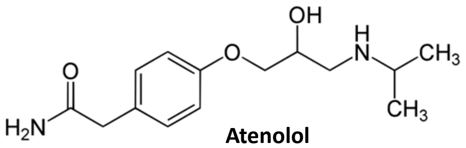
Suitable to for Miniature MS and Portable Applications

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

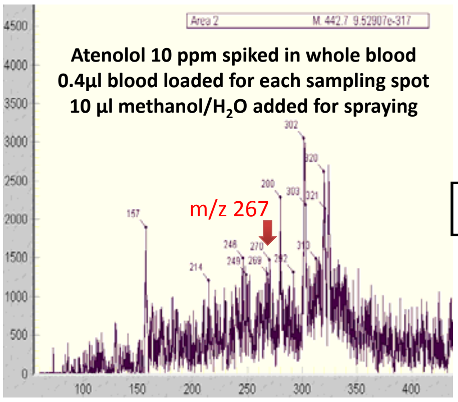
Paper spray/mini MS (direct detection Atenolol in blood)



He Wang, Jiangjiang Liu, Guangming Huang



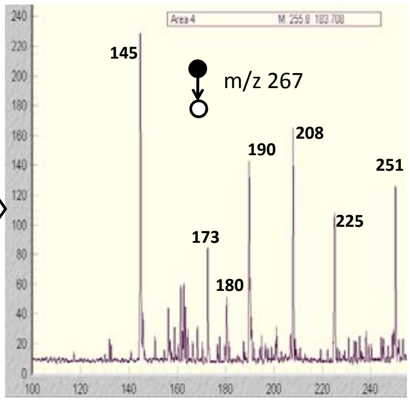
Atenolol
MW=266



Atenolol 10 ppm spiked in whole blood
0.4µl blood loaded for each sampling spot
10 µl methanol/H_2O added for spraying

MS spectrum of direct paper spray of blood spiked with atenolol (4ng/spot)

CID



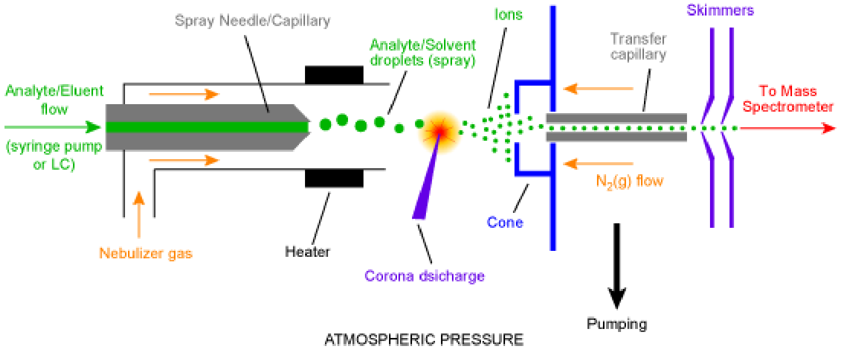
MS/MS spectrum to identify atenolol

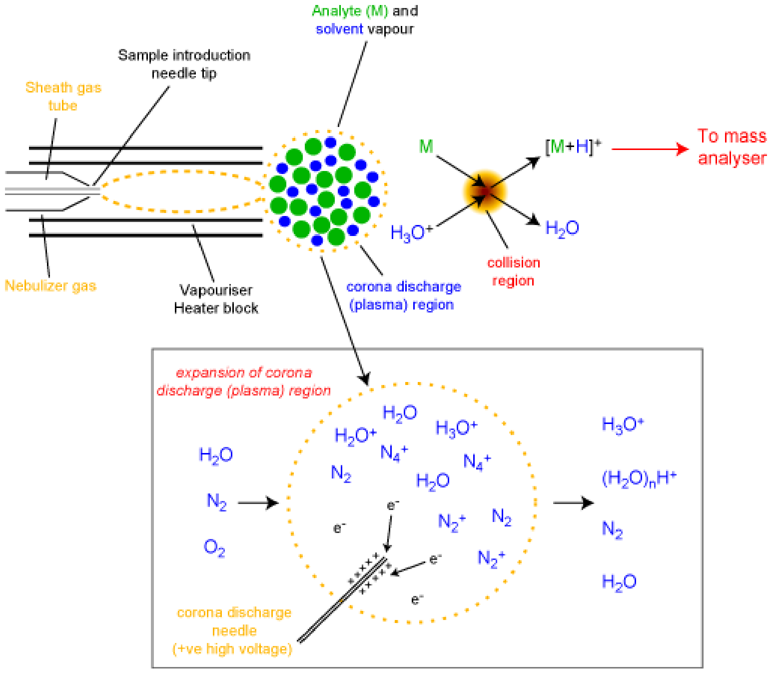
He Wang, Jiangjiang Liu, Guangming Huang, et al. unpublished

APCI Atmospheric Pressure Chemical Ionization

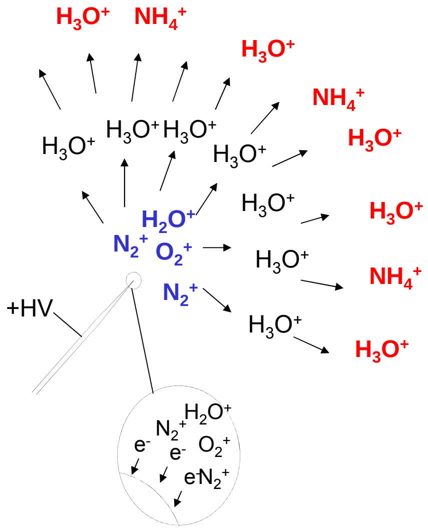
APPI Atmospheric Pressure Photo Ionization

Atmospheric Pressure Chemical Ionization



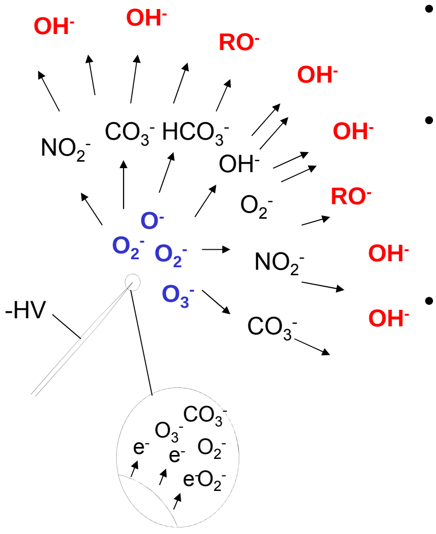


Corona Discharge
Meccanismo Ioni Positivi



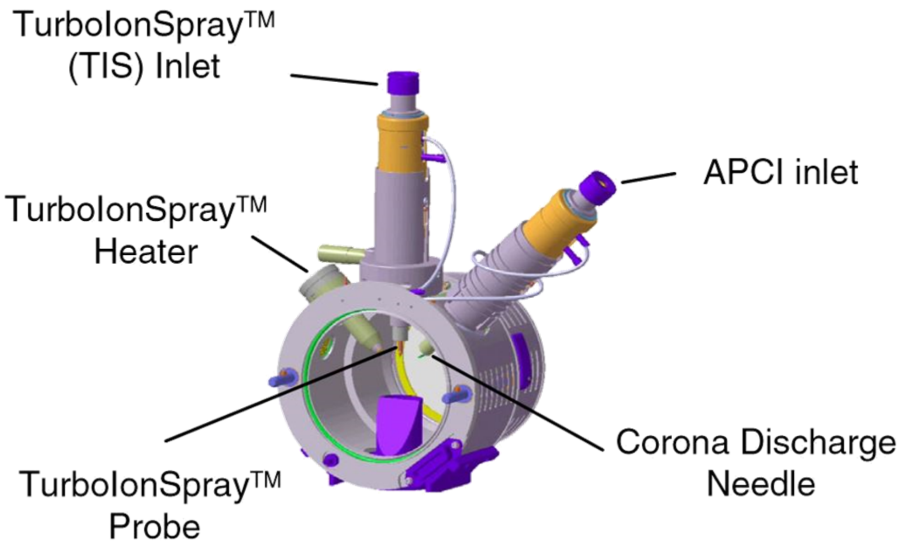
- In un primo stadio l'ago cattura elettroni formando ioni N_2^+, O_2^+, H_2O^+… (ioni primari).
- Gli ioni primari, aventi un tempo di vita molto breve, trasferiscono la carica al solvente, formando principalmente ioni H_3O^+, NH_4^+, RH_2O^+… (ioni reagenti).
- Infine, gli ioni reagenti trasferiscono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

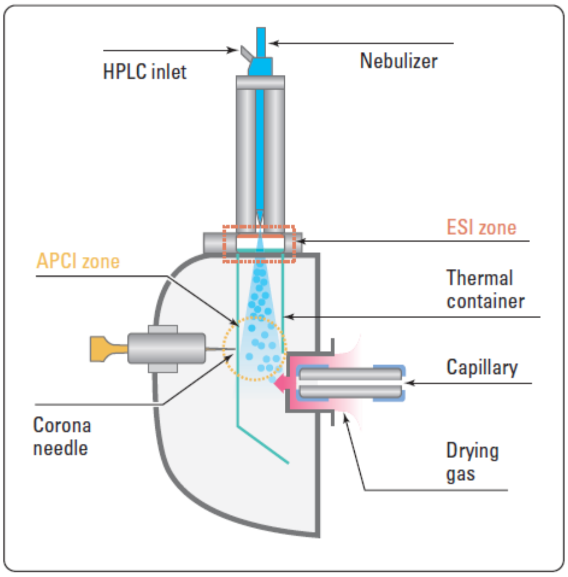
Corona Discharge
Meccanismo ioni negativi



- 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 $[M-H]^-$.

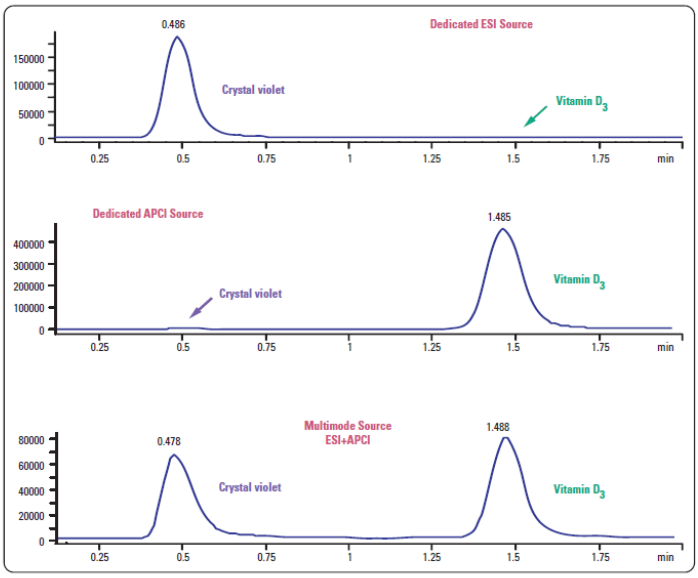
Combining ESI and APCI in the Same Source



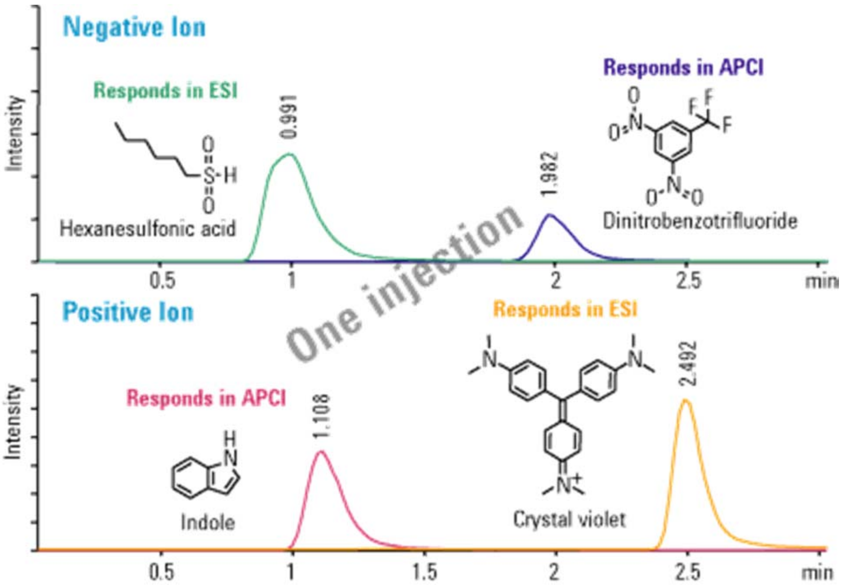


Multimode source incorporates optimized ESI and APCI zones

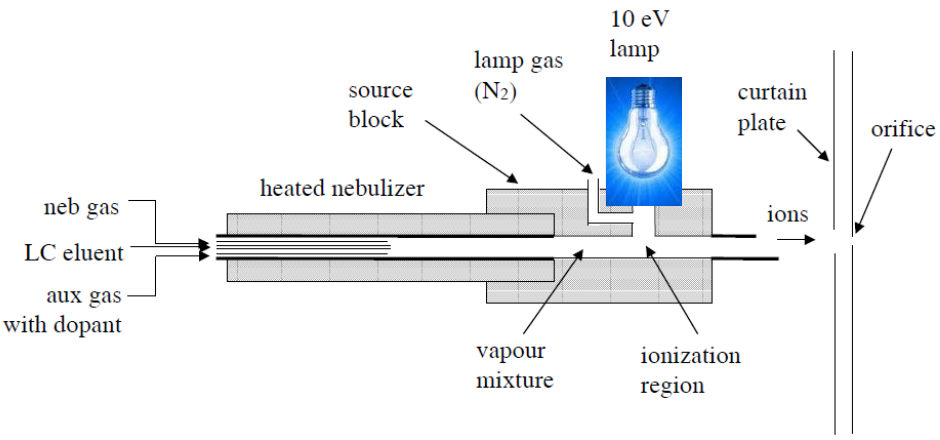
Agilent Technologies



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



Atmospheric pressure photoionization



Principi della Fotoionizzazione

Fotoionizzazione: $h\nu \geq IP$

$M + h\nu \rightarrow M^{+\bullet} + e^-$

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

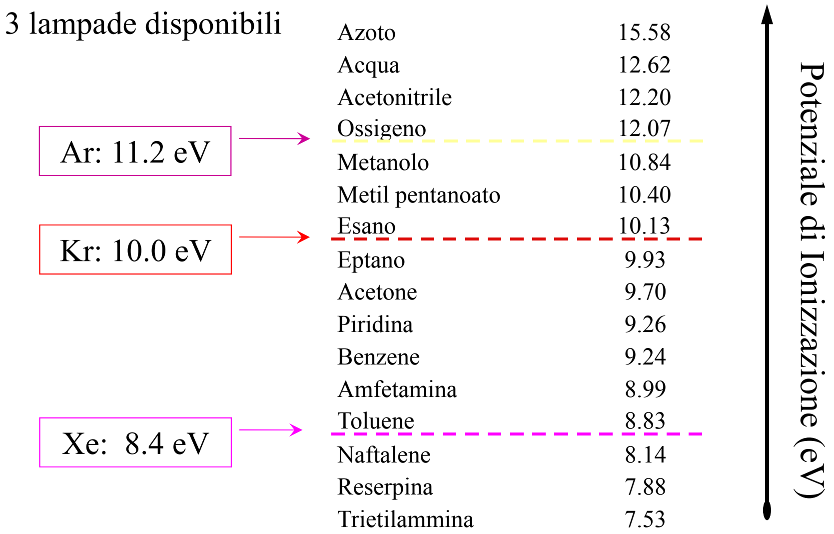
$M + h\nu \rightarrow M^*$

☞Per la maggior parte degli analiti: IP < 10 eV

☞Per i più comuni solventi HPLC: IP > 10 eV

IP = potenziale 1° ionizzazione

Ionizzazione Selettiva



3 lampade disponibili

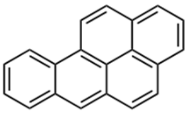
Ar: 11.2 eV

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)



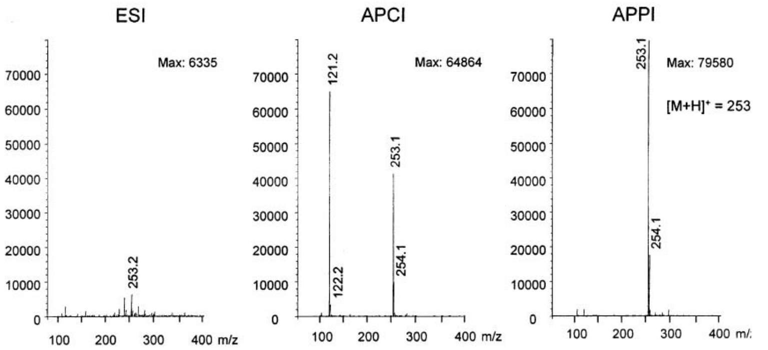
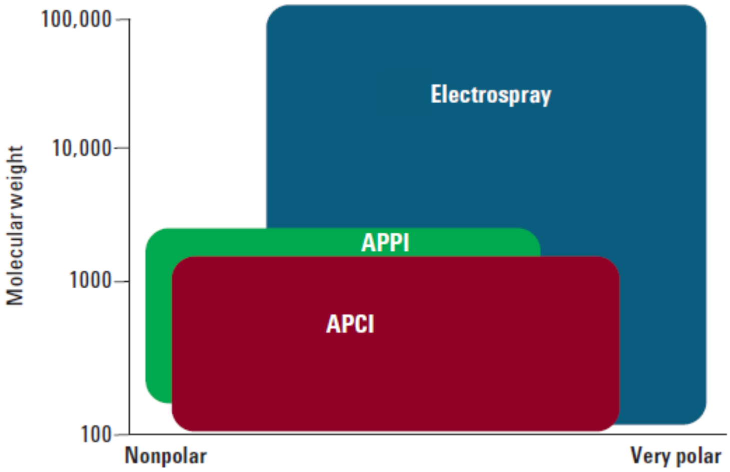


Figure 3. Comparison of ESI, APCI, and APPI for benzo[a]pyrene showing improved sensitivity for nonpolar compounds by APPI. LC conditions: flow injection analysis, flow rate 0.4 mL/min, 50% water, 50% THF. APPI source conditions: V_{cap} 1500, drying gas temperature 350 °C at 5 L/min, vaporizer temperature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


100,000
10,000
1000
100
Molecular weight
Electrospray
APPI
APCI
Nonpolar
Very polar