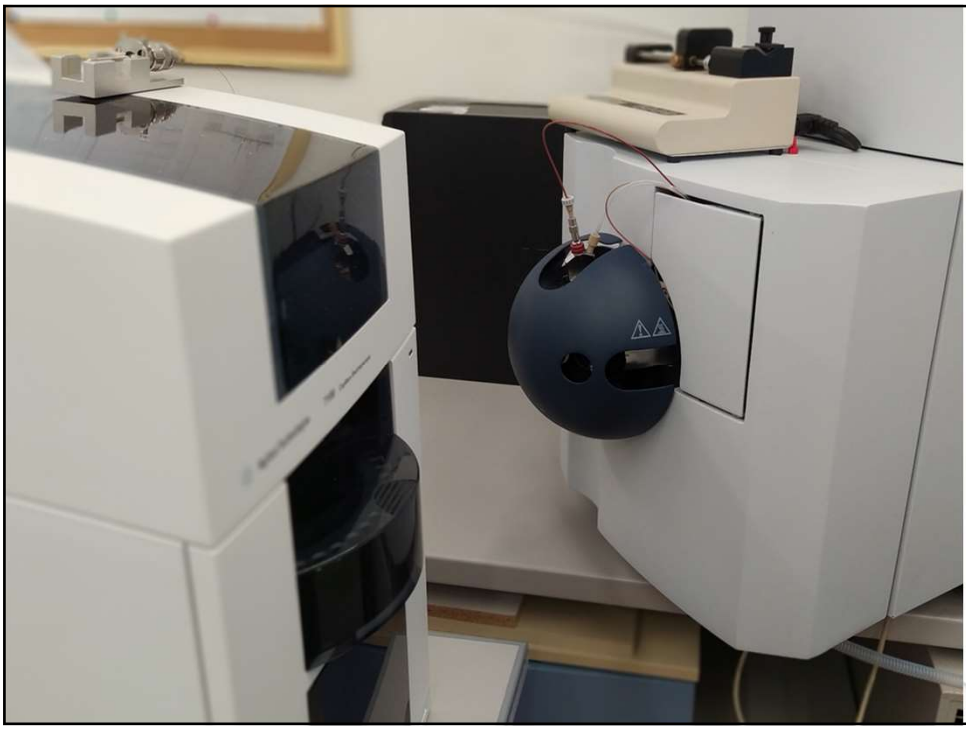

MASS SPECTROMETRY

MASS SPECTROMETRY

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- Faculty of Science
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- http://ms-servis9.webnode.cz/

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Documents

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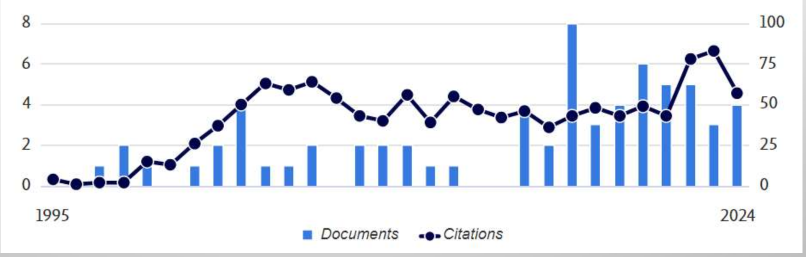
h-index



HPLC–MS/MS authentication of the eighteenth century liquorice drug remains.
Monatshefte fur Chemie, 2024, 155(8-9), pp. 813–823
HPLC-based cytotoxicity profiling and LC-ESIQTOF-MS/MS analysis of Helichrysum leucocephalum.
Heliyon, 2024, 10(5), e27230
Dereplication of natural cytotoxic products from Helichrysum oligocephalum using UHPLC-MS.
Separation Science Plus, 2024, 7(1), 2300150
Rheum turkestanicum: LCESI-QqTOF MS Characterization of phenolic compounds.
Industrial Crops and Products, 2023, 200, 116836
Authentication of two eighteenth century juniper-containing drug remains by HPLC–MS/MS.
Monatshefte fur Chemie, 2023, 154(9), pp. 977–986
Comparative LC-ESIMS-Based Metabolite Profiling of Senna italica with Senna alexandrina.
Metabolites, 2023, 13(4), 559
Chemical conversion of hardly ionizable rhenium aryl chlorocomplexes with p-substituted anilines.
Molecules, 2021, 26(11), 3427
Sensitive CE-MS method for monitoring of riociguat and desmethylriociguat levels in human serum.
Electrophoresis, 2020, 41(18-19), pp. 1564–1567

LABORATORY OF MASS SPECTROMETRY

EI GCMS-QP2010 (Shimadzu-Japan)

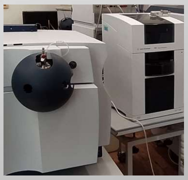
ESI, APCI, APPI **Esquire 3000** (Bruker-Germany)
AmaZon IT (Bruker-Germany)

MALDI TOF/TOF Ultraflex (Bruker-Germany)

HRMS Q-TOF Compact (Bruker-Germany)

Agilent 7100 (CE/MS) (Agilent-USA)

HPLC 1100 (HP-USA)
HPLC 1260 (Agilent-USA)
UHPLC 1290 (Agilent-USA)



LABORATORY OF MASS SPECTROMETRY

INTRODUCTION TO MASS SPECTROMETRY

Syllabus

1. Introduction, Basic Concepts, History and Ionization techniques.
2. Principles of Ion Formation. ESI; APCI; APPI; MALDI
3. Principles of Ion Separation. Sector, LQ; IT; TOF; FT-ICR; Ion detection and Vacuum Technology.
4. Interpretation of EI mass spectra of small molecules. Basic Mechanisms of Ion Fragmentation.

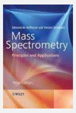
5.Elucidated interpretation procedure of Common Organic Compounds

INFORMATION SOURCES:

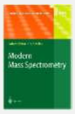
McLafferty Fred W. - Interpretation of Mass Spectra

Gross J. H. - Mass spectrometry

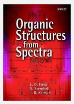
……………………………………………………………………..



E. de Hoffmann, V. Stroobant - Mass Spectrometry - Principles and Applications



Christoph.A.Schalley - Modern Mass Spectrometry



Field L.D. Sternhell, S. - Organic structures from spectra

USEFUL LINKS

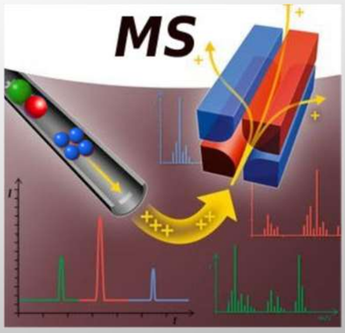
mass calculators
http://www.sisweb.com/mstools/isotope.htm http://www.chemcalc.org/ http://www.stolaf.edu/depts/chemistry/courses/toolkits/380/js/masscalc/
Databases - libraries :
NIST (National Institute for Standards and Technology) http://webbook.nist.gov/chemistry/index.html Integrated Spectral Data Base System for Organic Compounds http://sdbs.db.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng
Tutorials:
http://www.cif.iastate.edu/mass-spec/ms-tutorial.html https://www.chem.pitt.edu/facilities/mass-spectrometry/mass-spectrometry-introduction
Portals:
http://www.asms.org/ http://www.spectroscopynow.com/ms
Software:
http://www.chemistry-software.com/masspec.htm http://www.highchem.com/

INTRODUCTION TO MASS SPECTROMETRY

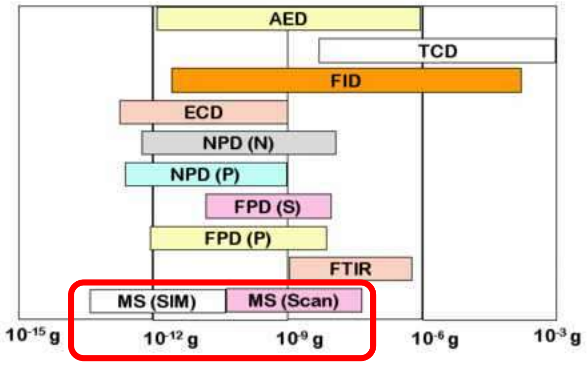
Mass spectrometry (MS) is an analytical method used to convert molecules into ions, separate these ions according to the ratio of mass-to-charge (m/z), and then record their relative intensities.

- analytical tool in chemistry, biochemistry, pharmacy, medicine…
- environmental and forensic analysis;
- drugs and food quality control;
- determination of MW and clarification of the structure of unknown substances;

- **MS** ► sensitive, destructive method, minimal sample consumption (10^{-12} g);



GC detectors



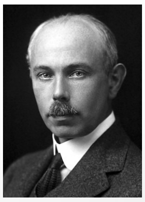
UNITS AND PHYSICAL CONSTANTS

- SCALE OF ATOMIC MASS:
- M/Z - the ratio of the ionic mass and the number of elementary charges of the respective ion;
- ATOMIC MASS UNIT – amu or Dalton – Da (BIOMED.)

  UNIFIED MASS UNIT - u
- 1 Da = 1u = 1/12 of the mass ^{12}C = 1,66054 X 10^{-27}kg
- The mass number is defined as the total number of protons and neutrons in an atom. (nominal mass);
- **NOMINAL MASS OF IONS** = the sum of the nominal masses of the constituent atoms;
- **MONOISOTOPIC MASS OF IONS** = the sum of the accurate masses of the most abundant naturally occurring stable isotope of each atom;
- **AVERAGE MASS OF IONS** = the weighted average of the masses of the naturally occurring isotopes;

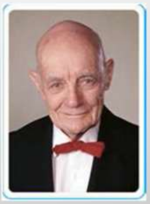
HISTORY OF MASS SPECTROMETRY

- 1898 (WIEN) - DISCOVERY OF THE CURVATURE OF THE FLIGHT PATH OF ACCELERATED IONS IN AN ELECTRIC AND MAGNETIC FIELD;
- **1906 (THOMSON) - NOBEL PRIZE IN PHYSIC – IDENTIFICATION OF ELECTRON** INVENTOR OF MS ;
- 1912 (THOMSON; ASTON) - SEPARATION OF NEON ISOTOPES ^{20}Ne AND ^{22}Ne;
- **1922 (ASTON) WAS AWARDED THE NOBEL PRIZE IN CHEMISTRY;**
- 40. YEARS OF 20. CENT. - EXPANSION OF MS IN THE PETROLEUM INDUSTRY, "FINGERPRINT" ANALYSIS WITHOUT SPECTRUM INTERPRETATION;
- 1957 (HOLMES, MORRELL) - FIRST CONNECTION ATTEMPT GC/MS;
- 1984 (FENN) - ELECTROSPRAY IONIZATION (ESI) HPLC/MS CONNECTION ;
- **1989 (PAUL) – NOBEL PRIZE IN CHEMISTRY - DEVELOPMENT OF THE ION TRAP;**
- **2002 (FENN, TANAKA) – NOBEL PRIZE IN CHEMISTRY – INVENTION OF ELECTROSPRAY AND MALDI;**



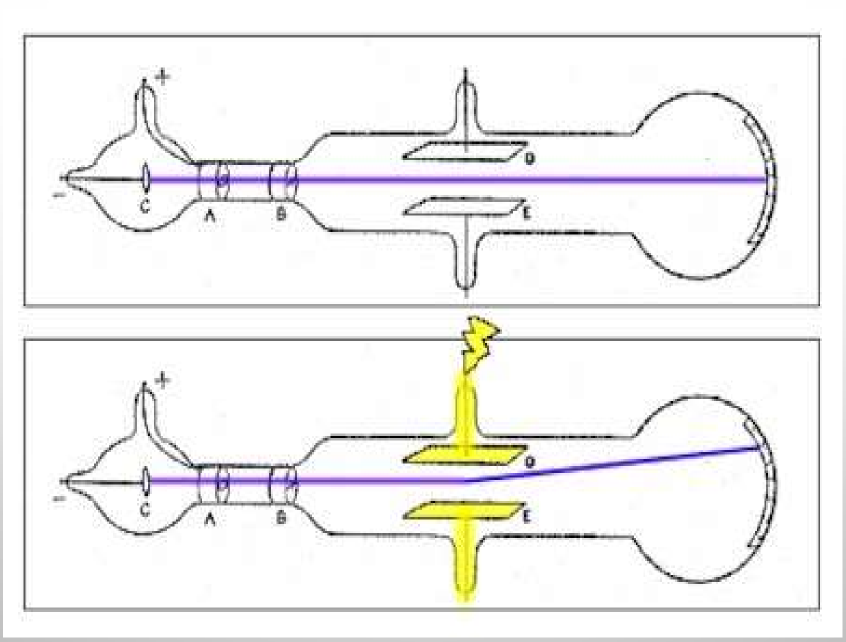
F.W.Aston

Wolfgang Paul



John B. Fenn

K. Tanaka



1912 J.J.Thomson MS

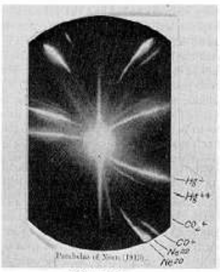
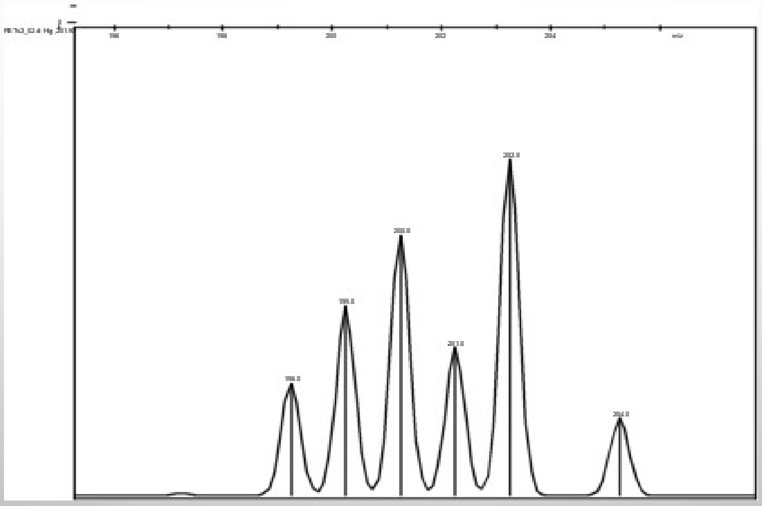
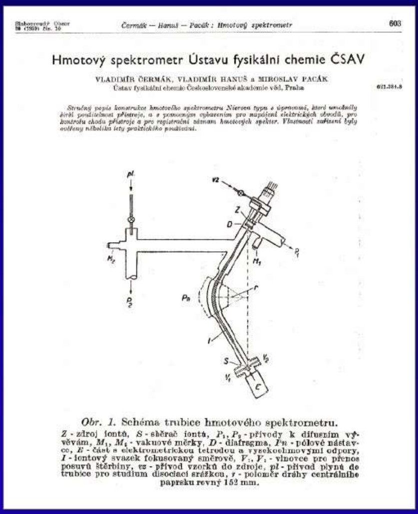


Figure 2

ISOTOPIC PATTERN OF Hg (196-204)



V. Čermák, V. Hanuš, Č. Jech, J. Cabicar, 1953



Slaboproudý Obzor 20 (1959) čís. 10 | *Čermák — Hanuš — Pacák : Hmotový spektrometr* | 603

Hmotový spektrometr Ústavu fysikální chemie ČSAV

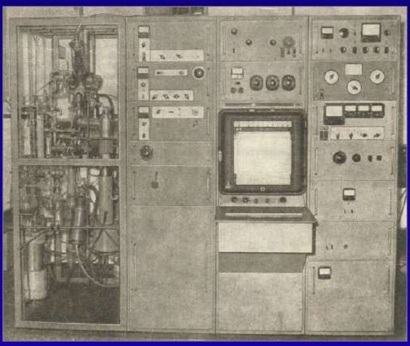
VLADIMÍR ČERMÁK, VLADIMÍR HANUŠ a MIROSLAV PACÁK
Ústav fysikální chemie Československé akademie věd, Praha

621.384.8

Stručný popis konstrukce hmotového spektrometru Nierova typu s úpravami, které umožnily širší použitelnost přístroje, a s pomocným vybavením pro napájení elektrických obvodů, pro kontrolu chodu přístroje a pro registrační záznam hmotových spekter. Vlastnosti zařízení byly ověřeny několika lety praktického používání.

Obr. 1. Schéma trubice hmotového spektrometru.
Z - zdroj iontů, *S* - sběrač iontů, P_1, P_2 - přívody k difusním vývěvám, M_1, M_2 - vakuové měrky, *D* - diafragma, *Pn* - pólové nástavce, *E* - část s elektrometrickou tetrodou a vysokoohmovými odpory, *I* - iontový svazek fokusovaný směrově, V_1, V_2 - vlnovce pro přenos posuvů štěrbiny, *vz* - přívod vzorků do zdroje, *pl* - přívod plynů do trubice pro studium disociací srážkou, *r* - poloměr dráhy centrálního paprsku rovný 152 mm.

PRESIDENT ČESKOSLOVENSKÉ REPUBLIKY
UDĚLIL NA NÁVRH VLÁDY
Dr VLADIMÍRU ČERMÁKOVI
Dr VLADIMÍRU HANUŠOVI
Dr ČESTMÍRU JECHOVI
Dr JOSEFU CABICAROVI
ZA ZKONSTRUOVÁNÍ HMOTOVÉHO SPEKTROMETRU
STÁTNÍ CENU II. STUPNĚ
S ČESTNÝM TITULEM
LAUREÁT STÁTNÍ CENY V ROCE 1954
V PRAZE DNE [illegible] KVĚTNA 1954
PŘEDSEDA VLÁDY ČESKOSLOVENSKÉ REPUBLIKY



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company	Instrument	Analyzer	Web
Agilent	GC/MS, LC/MS, CE/MS, SFC/MS, ICP-MS, IM-MS	Q, TOF, Q-TOF. IM	www.agilent.com
Bruker	GC/MS, LC/MS, HDX, MALDI-MS, IM-MS	Q, TOF, Q-TOF, IT, ICR, IM	www.bruker.com
SCIEX	LC/MS, CE/MS, IM-MS	Q, LIT, TOF, Q-TOF, IM	www.sciex.com
Shimadzu	GC/MS, LC/MS, SFC/MS, ICP-MS, MALDI-MS	Q, TOF, Q-TOF, IT	www.shimadzu.ed
Thermo Fisher Scientific	GC/MS, LC/MS, ICP-MS, IM-MS	Q, Orbitrap, LIT, IM	www.thermofisher.com
Waters	GC/MS, LC/MS, SFC/MS, IM-MS	Q, TOF, Q-TOF, IM	www.waters.com

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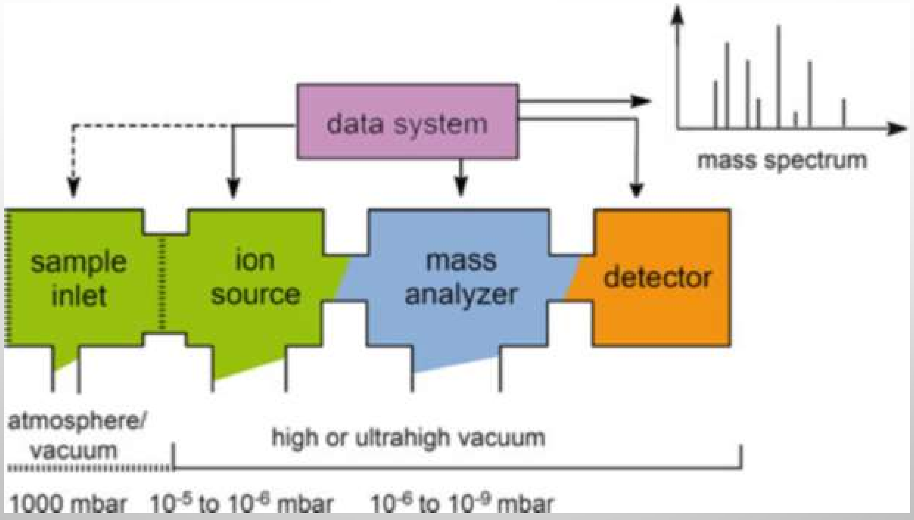
BASIC PARTS OF MASS SPECTROMETER

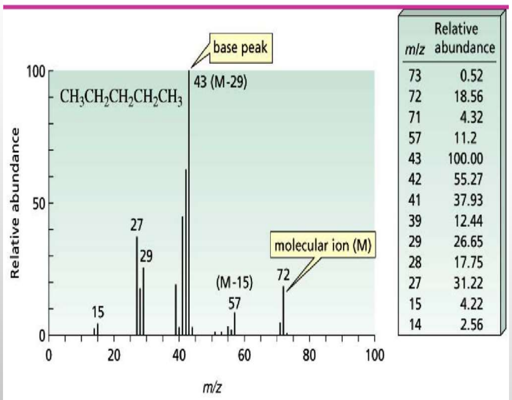
-
- 1/ **ION SOURCE**- serves to convert neutral molecules of the analyte into charged particles (so-called ionization), the construction differs according to the ionization technique used.
- 2/ **MASS ANALYZER** - used to separate ions in the gas phase under vacuum according to the ratio of mass and charge (m/z).
- 3/ **DETECTOR** - can detect ions and determine the relative intensity of individual ions.
- ► OTHER PARTS

- vacuum systém;
- input interface (probe);
- ion optics used to accelerate and focus of ions;
- computer for device control and tuning, data collection, and storage;

GENERAL SCHEME OF ANY MASS SPECTROMETER



MASS SPECTRUM – distribution of detected ions



m/z	Relative abundance
73	0.52
72	18.56
71	4.32
57	11.2
43	100.00
42	55.27
41	37.93
39	12.44
29	26.65
28	17.75
27	31.22
15	4.22
14	2.56

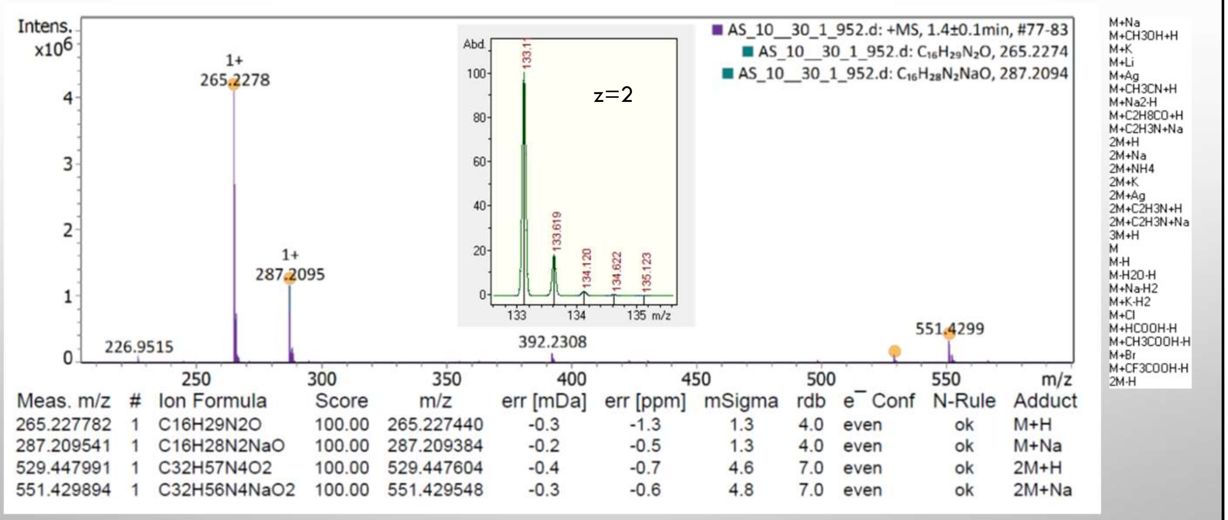
- ► MASS SPECTRUM

AXIS X = MASS TO CHARGE RATIO (M/Z) **mostly z=1, there are the masses of ions;**

AXIS Y = INTENSITY (expressed either in an absolute form or in a relative form, the most intense abundance is set to 100 %);

- CENTROID MODE (BAR GRAPH);
- PROFILE MODE;
- TABULAR LISTING;
- The mass spectrum is always affected by the used ionization technique.

ESI spectrum of memantine derivatives



Meas. m/z	#	Ion Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf	N-Rule	Adduct
265.227782	1	C16H29N2O	100.00	265.227440	-0.3	-1.3	1.3	4.0	even	ok	M+H
287.209541	1	C16H28N2NaO	100.00	287.209384	-0.2	-0.5	1.3	4.0	even	ok	M+Na
529.447991	1	C32H57N4O2	100.00	529.447604	-0.4	-0.7	4.6	7.0	even	ok	2M+H
551.429894	1	C32H56N4NaO2	100.00	551.429548	-0.3	-0.6	4.8	7.0	even	ok	2M+Na

IONIZATION TECHNIQUES

- designed to create ions from neutrals in order to be able to achieve m/z analysis of these ions.

There are a variety of ways to ionize the electroneutral molecules.

EI (Electron Ionization)	Gas phase
CI (Chemical Ionization)	
FI (Field Ionization)	
DESI (Desorption Electrospray Ionization)	Ambient ionization
DCI (Desorption Chemical Ionization)	
DAPPI (Atmospheric Pressure Desorption Photoionization)	
MALDI (Matrix-Assisted Laser Desoption/Ionization)	Solid phase
FAB (Fast Atom Bombardment)	
TSI (Thermospray Ionization)	Condensed phase
ESI (Electrospray Ionization) (API)	
APCI (Atmospheric Pressure Chemical Ionization)	
APPI (Atmospheric Pressure Photoionization)	
ICP (Inductively Coupled Plasma)	Inorganic analysis

WHY WE HAVE SO MANY TECHNIQUES?

- The current estimate is around 20 million organic compounds.
- Substances can differ in many different chemical aspects, and therefore the optimal method of ionization for a given substance must be chosen.
- The universal method does not exist.
- ▶ some ionization techniques have been replaced by others
- ▶ the most significant now:
- **ESI + APCI** – for use in HPLC/MS
- **ESI, MALDI** - for analysis of biomolecules (the softest)
- **EI** - enables comparison with databases of spectra, structural information, suitable for a limited range of substances, if a molecular ion is missing **(CI).**

HOW TO CHOOSE THE IONIZATION METHOD

- a/ **solubility and volatility (polarity)** – for volatile substances : **EI, CI, FI;**
- b/ **thermal stability** - some techniques require converting the substance into the gas phase before the ionization (EI, CI, FI). Such procedures are not suitable for thermolabile compounds. Choose e.g. ESI (more gentle);
- c/ **molecular weight** (effects volatility) – up to Mr =1000 for EI/CI, up to several thousand **TSI, APCI** and **FAB,** up to milion **ESI** a **MALDI;**
- d/ **chemical individual or mixture** - for the mixture you can use the appropriate separation technique (**GC/MS, HPLC/MS, CE/MS**);
- e/ **polarity of ionization** (soft techniques)
- - **negative ionts** - sulfonic and carboxylic acids, polyhydroxy polyhalogen compounds (easy deprotonation);
- - **positive ionts** – for most substances by the protonization or cationization (the presence of heteroatom is an advantage);
- f/ **study of non-covalent interactions and stereochemical configuration of molecules – ESI;**

ELEKTRON IONIZATION (EI)

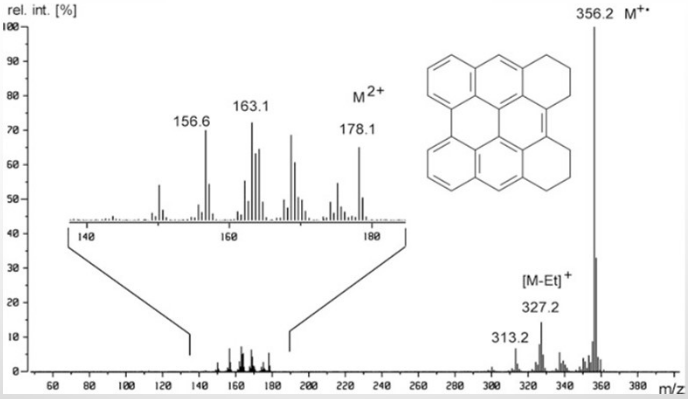
- ▶ **the most studied**, fragmentation mechanisms are described in detail, and extensive spectra libraries enable computerized comparison of the measured spectrum with a database.
- ▶ **the oldest** (since 1913), widely used, easy to control, extensive libraries of spectra;

  (NIST database contains more than 180 000 spectra)
- ▶ requirement of sufficient volatility of the substance under ionization conditions, since ionization occurs in the **gas phase.**

  (temperature 150 – 400°C, pressure - 10^{-3} - 10^{-5} Pa)
- ▶ EI cannot be used for non-volatile substances and thermolabile substances (must have sufficient thermal stability under measurement conditions)

ELECTRON IONIZATION (EI)

- The classical procedure of ionization involves shooting energetic electrons.
- ► Formerly has been termed "*Electron Impact*" It is currently not recommended because, in reality, there is no "impact" of the electron into the molecule, but only the influence of the electromagnetic fields, as a result of which the valence electron is released and the molecular ion M^+ is formed.
- **$M + e^- \rightarrow M^{+\cdot} + 2\,e^-$ RADICAL !!! (OE)**
- ► The "hardest" ionization technique because the ionized molecule acquires an excess of internal energy, which is relaxed by the fragmentation of the molecule into smaller parts (about 10% - the molecular ion is missing).
- The gas phase is highly diluted - the mean free path for the particles becomes long enough to make bimolecular interactions almost impossible within the lifetime of the particles concerned.

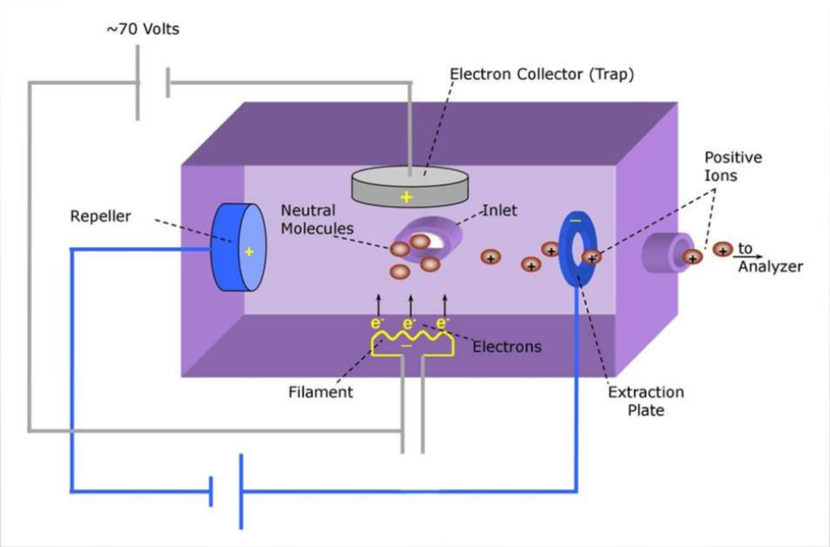


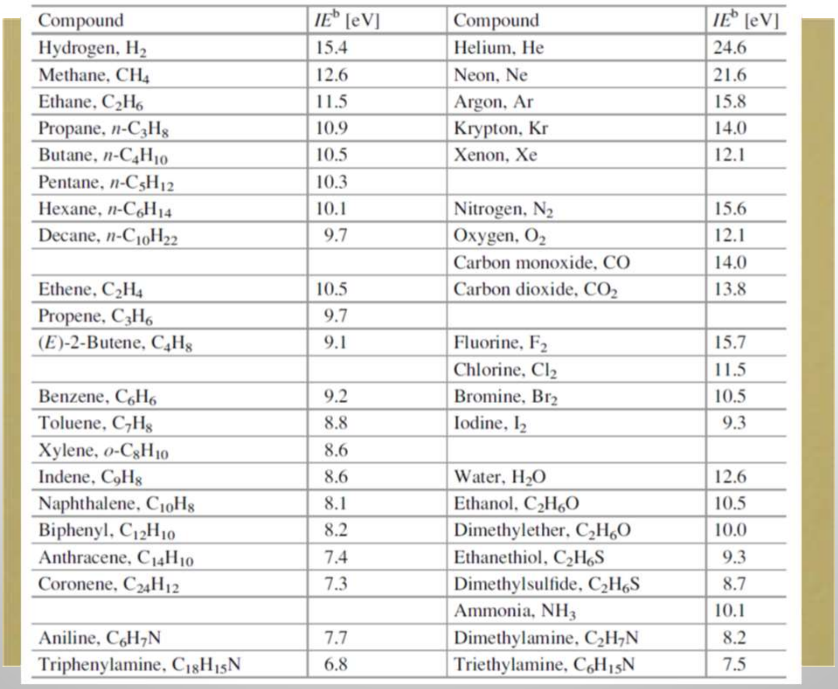
EI SPECTRUM 1,2,3,4,5,6-HEXAHYDROFENANTRO[1,10,9,8-OPQRA]PERYLEN

PRINCIPLE OF ELECTRON IONIZATION (EI)

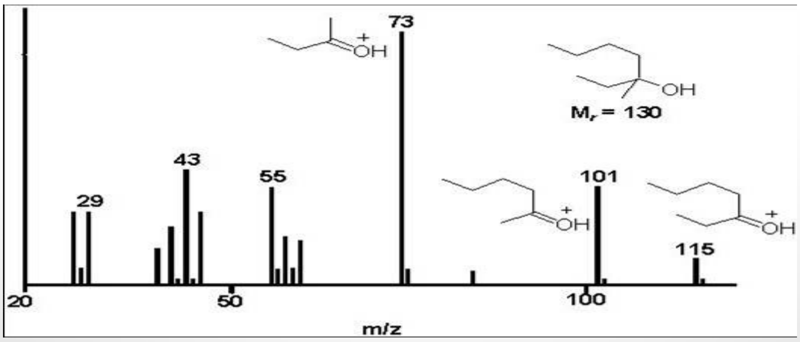
- ►filament - the heated cathode (W or Re wire) emits electrons, which are captured at the anode ("electron trap„);
- ► The accelerating potential between the cathode and the anode determines the energy of the electrons. (1 eV = $1.602*10^{-19}$ J);
- ► By approaching the emitting electron to the valence electrons of the molecule, their magnetic fields will be affected, which can lead to the release of the valence electron and, thus the formation of a radical cation **$M^{+\cdot}$**
- An electron can theoretically also be captured.
- The effectivity of EI is just $1/10^5 = 10^{-3}$ %

Scheme of EI



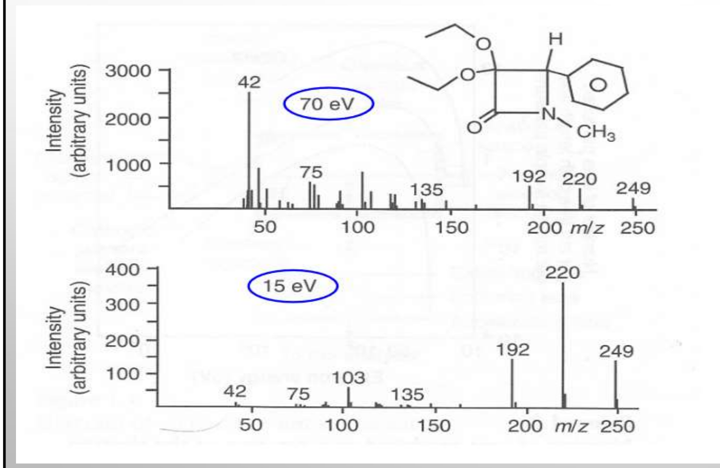


Compound	IE^b [eV]	Compound	IE^b [eV]
Hydrogen, H_2	15.4	Helium, He	24.6
Methane, CH_4	12.6	Neon, Ne	21.6
Ethane, C_2H_6	11.5	Argon, Ar	15.8
Propane, n-C_3H_8	10.9	Krypton, Kr	14.0
Butane, n-C_4H_{10}	10.5	Xenon, Xe	12.1
Pentane, n-C_5H_{12}	10.3		
Hexane, n-C_6H_{14}	10.1	Nitrogen, N_2	15.6
Decane, 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_7H_8	8.8	Iodine, I_2	9.3
Xylene, o-C_8H_{10}	8.6		
Indene, C_9H_8	8.6	Water, H_2O	12.6
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
Coronene, $C_{24}H_{12}$	7.3	Dimethylsulfide, C_2H_6S	8.7
		Ammonia, NH_3	10.1
Aniline, C_6H_7N	7.7	Dimethylamine, C_2H_7N	8.2
Triphenylamine, $C_{18}H_{15}N$	6.8	Triethylamine, $C_6H_{15}N$	7.5



- ► Excess internal energy leads to fragmentation (i.e., breaking up into smaller charged and uncharged parts); a molecular ion may even be missing.
- ► The ionization energy (IE) is the minimum amount of energy needed to form an ion.
- IE of most organic substances is 7 - 16 eV. The standard value for measuring library spectra is **70 eV.**
- **Why so high IE?** Highest sensitivity, spectrum rich in fragment ions, for most substances also molecular ion.
- In the range of 50 - 100 eV, the spectrum is less dependent on the chosen energy.

THE EFFECT OF ENERGY REDUCTION



Reasons for measuring EI spectra at 70 eV

- All atoms or molecules can be ionized
- The plateau of the ionization efficiency implies small variations in electron energy.
- better reproducibility allowing comparison of spectra from different spectrometers or mass databases.

HARDNES OF IONIZATION

- ELEKTRON IONIZATION (EI)
- CHEMICAL IONIZATION (CI)
- FAST ATOM BOMBARDMENT (FAB)
- FIELD IONIZATION (FI)
- LASER DESORPTION (LD)
- ATMOSPHERIC PRESSURE CHEMICAL IONIZATION (APCI)
- MATRIX-ASSISTED LASER DESORPTION/IONIZATION (MALDI)
- ELECTROSPRAY (ESI)

NEGATIVE IONS

- ► Rarely used under standard conditions of measuring EI spectra (70 eV);
- ►Capture e- is a low-probability process; the introduction of a "braking" gas (argon) with mutual collisions will reduce the speed of ionizing e- (formation of so-called slow e-) and thereby increase the probability of capturing.
- ► 3 basic mechanisms of the formation of negative ions strongly depend on the energy of ionizing e-;

  - a/ **AB + e-** → **$[AB]^{-\cdot}$** - **resonance capture** (0-2 eV)
  - b/ **AB + e-** → **$A^{\cdot}$ + $[B]^{-}$** - **dissociative capture** (2-10 eV)
  - c/ **AB + e-** → **$[A]^{+}$+$[B]^{-}$ + e^{-}** - **ion pair formation** (>10 eV)

- ►Suitable for substances with electronegative substituents, e.g., halogens, spectra easier to interpret (they are not rearrangement reactions); cannot be used for all substances, must have positive electron affinity (EA).

CHEMICAL IONIZATION (CI)

- ► **so-called soft ionization method;**
- Unlike EI, protonated molecules $[M+H]^{+}$ or deprotonated molecules $[M-H]^{-}$ are formed (quasi-molecular ions).
- the ionized molecule has a much smaller amount of energy compared to EI, which is why we mainly observe (de)protonated molecules and a minimum of fragment ions in the spectra.
- ► Principle is analogous to EI, but the molecules of the **reagent gas** are ionized by the ionizing e- first, then ionize the molecules of the analyte by **ion-molecular** reactions.
- all ions have an even number of electrons EE even-electron × unlike $M^{+\cdot}$ at EI);
- The pressure of reagent gas - 100 Pa;
- an excess of reagent gas compared to the sample – 10 000 :1

- THE SENSITIVITY IS USUALY LOWER THAN EI (aproximately one order less)
- strongly depends on the conditions of the ionization proces;
- ▶ the most common reactant gas: **methane** (forming ions $[CH_5]^+$, $[C_2H_5]^+$, $[C_3H_5]^+$, **isobutane** (ion $[t\text{-}C_4H_9]^+$) a **ammonia** (ions $[NH_4]^+$, $[(NH_3)_2H]^+$, $[(NH_3)_3H]^+$)
- ▶ less common: propane, methanol, water, nitrogen , Noble gases…

- ▶ The most common is **protonation** (transfer of a proton from a gaseous Brönsted acid to a neutral molecule).
- ▶ **Hydride abstraction** is seen with aliphatic hydrocarbons
- $RH + CH_5^+ \longrightarrow R^+ + CH_4 + H_2$
- ▶ The **condensation** is the reaction of ions from a methane plasma $[C_2H_5]^+$ a $[C_3H_5]^+$ leading to $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$
- ▶ **charge exchange** GC/MS: $He^{+\cdot} + M \rightarrow He + M^{+\cdot}$

ION-MOLECULAR REACTION OF METHANE:

$CH_4 + e^- \rightarrow CH_4^{+\cdot} + 2\,e^-$ (usual EI formation of radical cations, non ion-mol. r.)

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

$CH_4^{+\cdot} \rightarrow CH_3^+ + H^{\cdot}$ (monomolecular dissociation, non ion-mol. r.)

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

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

$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2$, etc.

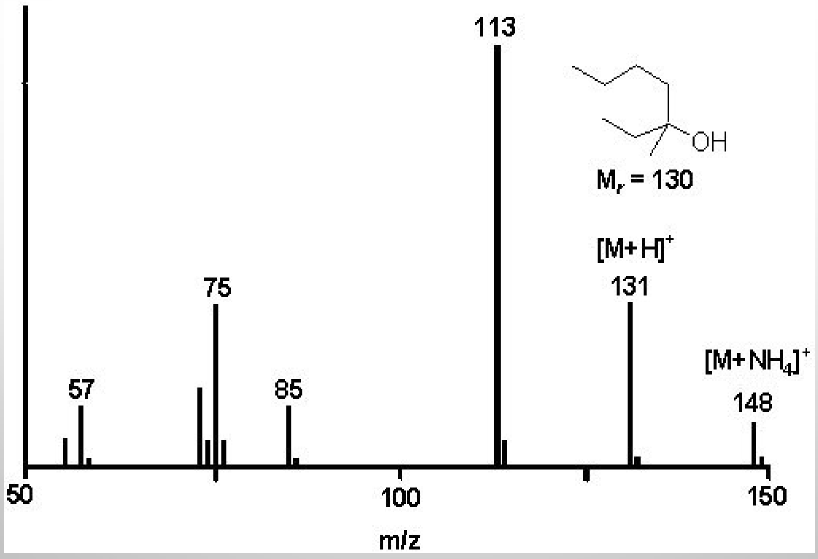
- ▶ methane reagent gas plasma **$[CH_5]^+$, $[C_2H_5]^+$ a $[C_3H_5]^+$**
- the most present ions $[M+H]^+$; $[M+C_2H_5]^+$; $[M+C_3H_5]^+$

CI FORMATION OF NEGATIVE IONS

- ► for **negative CI** several reactive gases are suitble:

  methane; isobutane; amomonia; halogenated compounds…
- very sensitive to molecules containing halogens or other electronegative elements.
- ► The most common reaction leading to the formation of negative ions:

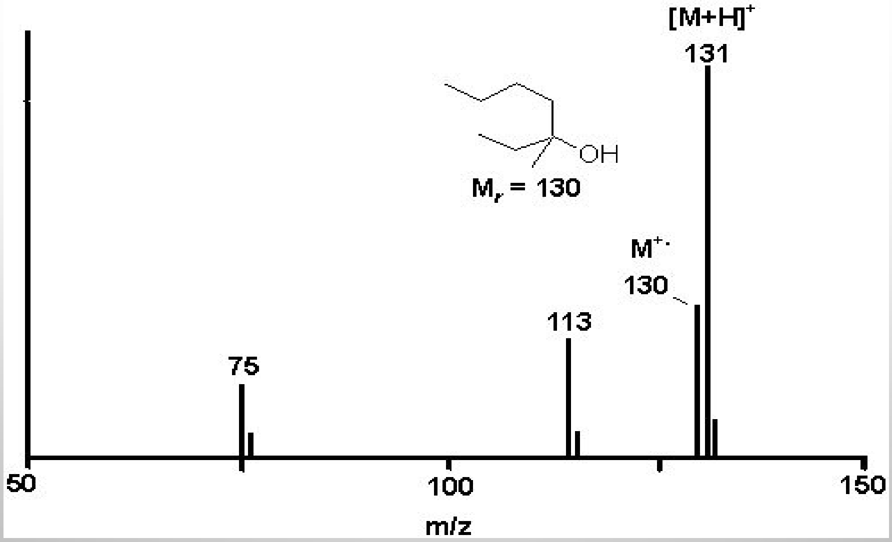
- **a/ $M + [R\text{-}H]^-$ → $[M\text{-}H]^- + R$ (deprotonation)**
- **b/ $M + X^-$ → $[M+X]^-$ (halogenide addition X^-)**
- **c/ $M + e-$ → $M^{-\cdot}$ (electron capture)**
- **d/ $M + R^{-\cdot}$ → $M^- + R^\cdot$ (charge transfer)**

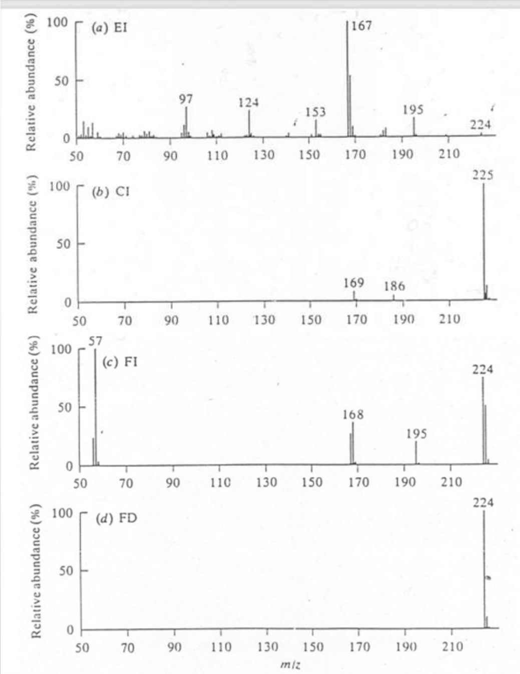


FIELD IONIZATION, FI

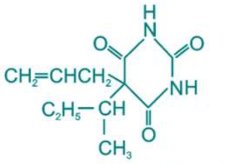
FIELD IONIZATION, FI

- ► Together with **EI** and **CI**, it belongs to 3 ionization techniques, where the analyzed molecules must be in the gas phase before ionization.
- ► anode (so-called emitter) - thin wire or sharp edge (W, diameter 10 μm), cathode - output slot; a high voltage applied (5-20 kV) creates a **high potential gradient** (10^7-10^8 V/cm) on the tip or edge, which enables tunneling of the least bound e- away from the molecule and its capture by the anode.
- **$M \rightarrow M^{+\cdot} + e^-$**
- ► The molecular ion M^+ is formed with little residual vibrational energy; therefore, there is minimum fragmentation except for $M^{+\cdot}$ $[M+H]^+$, $[M+Na]^+$ and multiply charged ions can also be formed.





Allobarbital



$M_r = 224$