SPECTRA INTERPRETATION

IN MASS SPECTROMETRY

The result of measurements in mass spectrometry is a spectrum that corresponds to the applied ionization technique, the type of mass analyzer and the method of recording spectra.

The interpretation is based on knowledge of the ionization process, the natural occurrence of element isotopes and the accuracy of *m/z* measurements.

Atom composition - expression of mass

Particle	Symbol	Charge	m [kg]	amu / Dalton (Da)
Electron	e	-1	9.109×10^{-31}	0.0005486
Proton	p	+1	1.673×10^{-27}	1.007276
Neutron	n	0	1.675×10^{-27}	1.008665

amu (atomic mass unit) → uamu (unified atomic mass unit)

1 amu = 1/16 m (^{16}O) → 1 u = 1/12 m (^{12}C)

1903 - 1961 → 1961 - present

1 u = 1 m_u = (1 amu) = 1 Da = 1/12 m (^{12}C) = 1.660539×10^{-27} kg

Isotopes

Average natural isotope representation of selected elements

Element	Isotope 1 (%)	Isotope 2 (%)	Isotope 3 (%)	Group
Hydrogen	^{1}H (99.985)	^{2}H (0.015)	-	A
Carbon	^{12}C (98.9)	^{13}C (1.1)	-	A+1
Nitrogen	^{14}N (99.63)	^{15}N (0.37)	-	A+1
Oxygen	^{16}O (99.76)	^{17}O (0.04)	^{18}O (0.20)	A+2
Fluorine	^{19}F (100)	-	-	A
Silicon	^{28}Si (92.16)	^{29}Si (4.7)	^{30}Si (3.14)	A+2
Phosphorus	^{31}P (100)	-	-	A
Sulphur	^{32}S (95.05)	^{33}S (0.76)	^{34}S (4.19)	A+2
Chlorine	^{35}Cl (75.47)	-	^{37}Cl (24.53)	A+2
Bromine	^{79}Br (50.5)	-	^{81}Br (49.5)	A+2
Iodine	^{127}I (100)	-	-	A

Isotopes

Normalized isotope ratios and integer *m/z*

Element	A (*m/z*) - %	A+1 (*m/z*) - %	A+2 (*m/z*) - %	Group
Hydrogen	1 - 100	2 - 0.015	-	A
Carbon	12 - 100	13 - 1.1	-	A+1
Nitrogen	14 - 100	15 - 0.037	-	A+1
Oxygen	16 - 100	17 - 0.04	18 - 0.2	A+2
Fluorine	19 - 100	-	-	A
Silicon	28 - 100	29 - 5.1	30 - 3.4	A+2
Phosphorus	31 - 100	-	-	A
Sulphur	32 - 100	33 - 0.79	34 - 4.4	A+2
Chlorine	35 - 100	-	37 - 32	A+2
Bromine	79 - 100	-	81 - 97.3	A+2
Iodine	127 - 100	-	-	A

Ion mass (*m/z*)

Numeric formats of m/z value (definition for z=1):

1. **Integer – integer sum of monoisotopic A_r**
2. **Monoisotopic - sum of monoisotopic A_r expressed with selected number of decimals**
3. **Average – sum of average A_r including natural abundances of isotopes**

Examples (exact mass calculator from internet):

Numeric format of *m/z*	C_6H_6	C_2Cl_6
Integer	78	234
Monoisotopic	78.04695	233.81312
Average	78.112	236.738

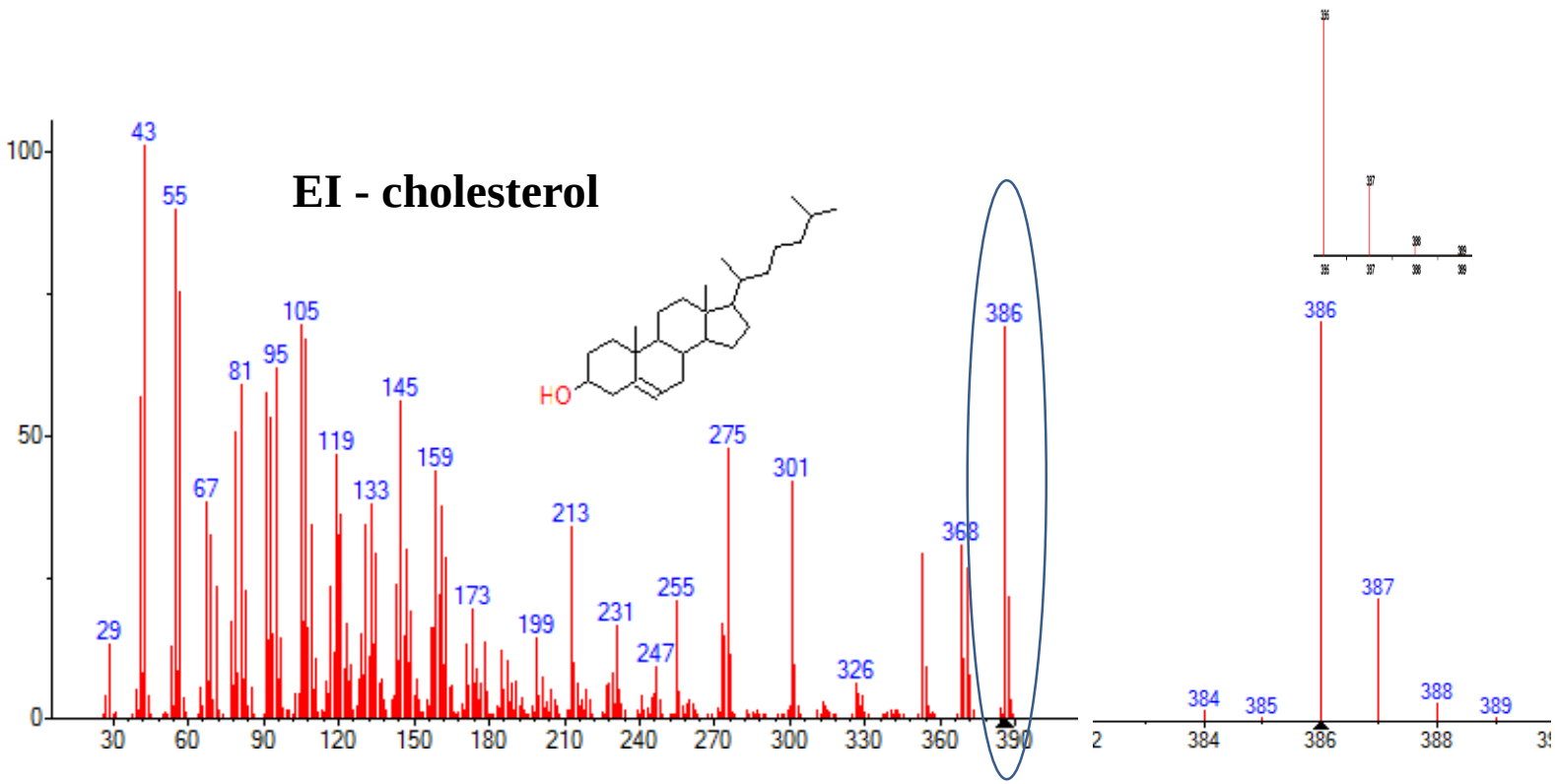
Evaluation of information contained in mass spectrum

1. Evaluation of molecular isotope cluster

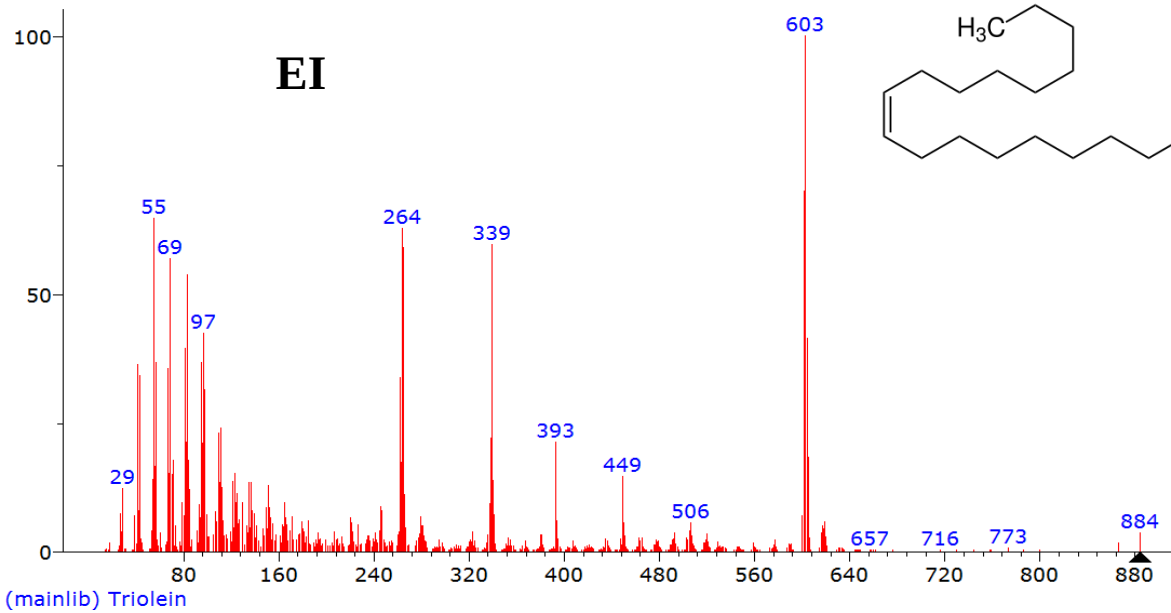
EI - fragmentation ionization - often absent or low in intensity

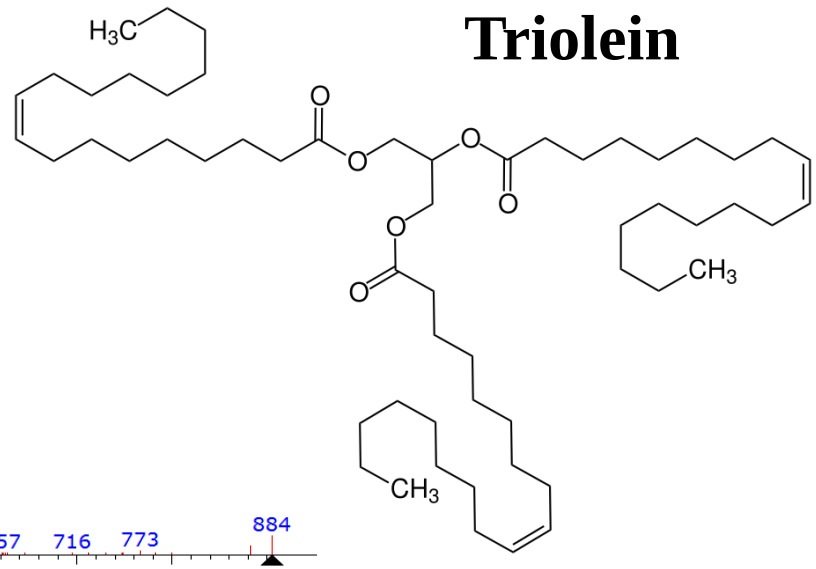
ESI/CI - often the only significant ion (pseudomolecular - adduct)

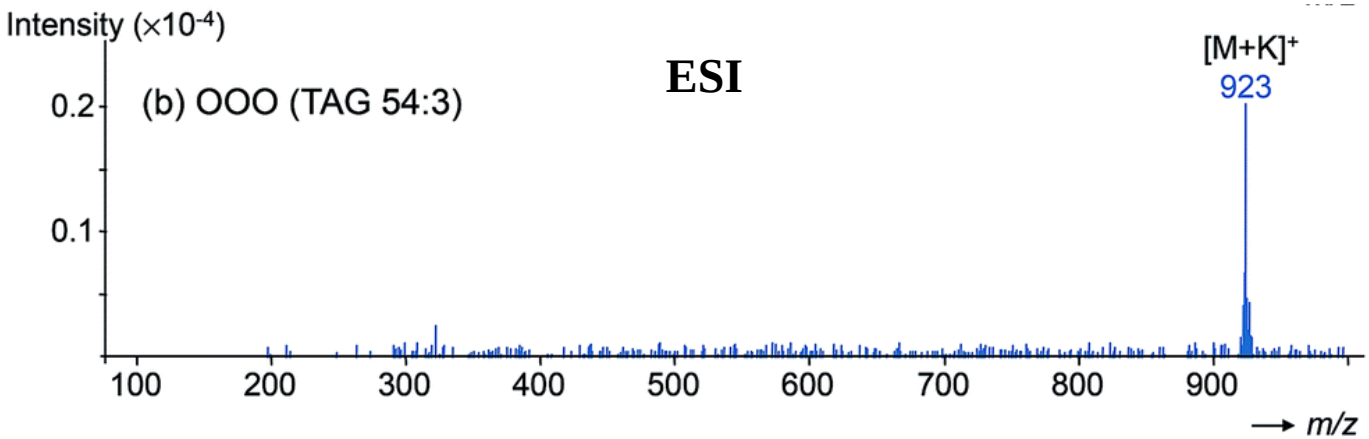
m/z → M_r → $C_xH_yO_z$ X_n **(exact mass) → confirmation - isotope cluster**



Evaluation of information contained in mass spectrum







Evaluation of information contained in mass spectrum

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: Carbon; Cl & Br; Sulphur

Monoisotopic Mass, Even Electron Ions

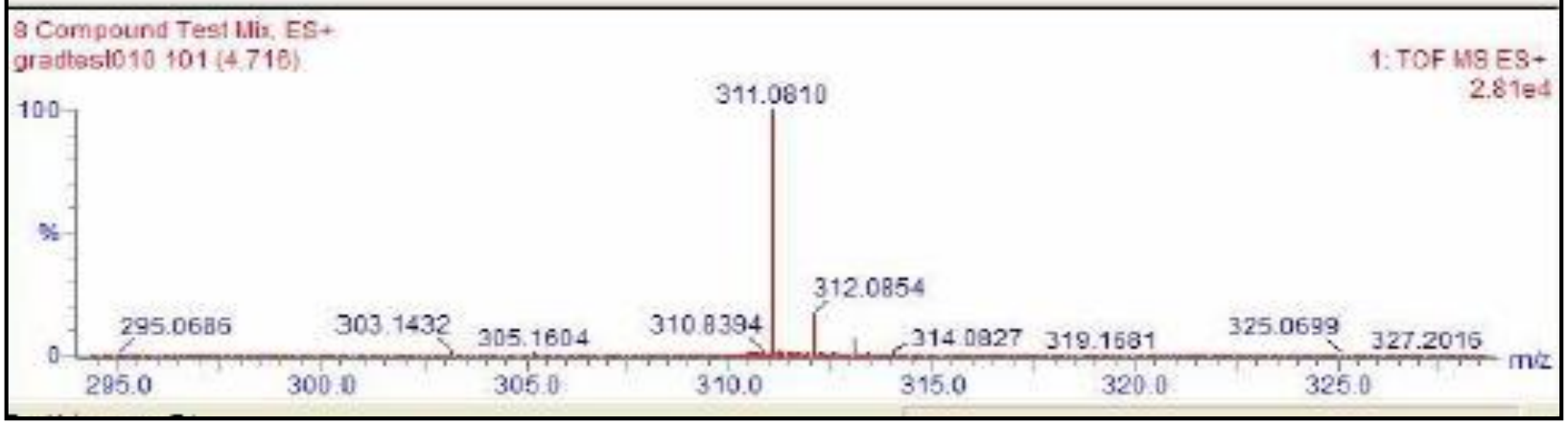
7665 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10

Br: 0-10

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	[illegible]	12	15	4	4	1		
[illegible]	[illegible]	0.9	2.9	2.5	C11 H19 O8 S	42.9	11	19		8	1		



Evaluation of information contained in mass spectrum

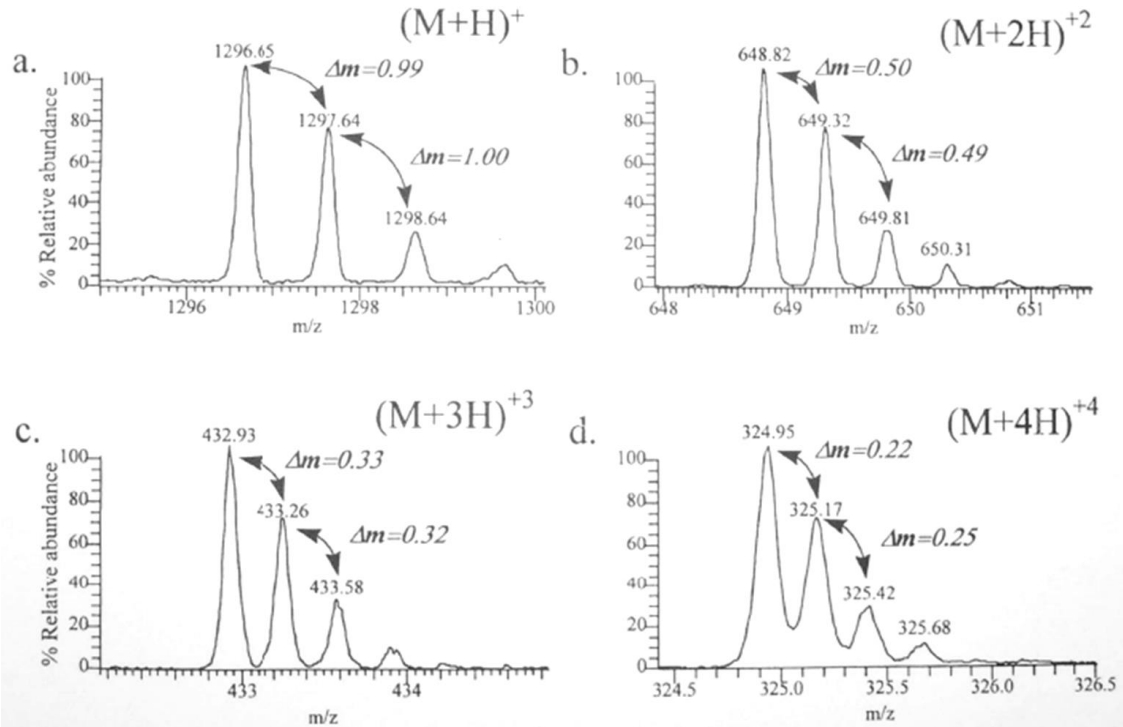
2. Fragmentation evaluation and comparison with spectra library

- suitable for EI - fragmentation ionization

The degree of agreement with library spectrum plus retention index

3. Determination of ion charge (difference between isotopic ions ≈ 1 /z)

Calculation for neighboring ions: $z = 1/(M_2 - M_1)$



A Global View of LC/MS: How to Solve Your Most Challenging Analytical Problems, R. Willoughby, E. Sheehan, S. Mitrovich, Global View Publ., 1998

Evaluation of information contained in mass spectrum

2. Estimation of M_r from a cluster of multiple charged ions

Ionization to various multiply charged ions → $[M + zH]^{z+}$

Calculation (e.g.):

18^+: $[m/z] = (M_r+z)/z \rightarrow 991.5 = (M_r+ 18)/18 \rightarrow M_r = 17829$

19^+: $[m/z] = (M_r+z)/z \rightarrow 939.2 = (M_r+ 18)/18 \rightarrow M_r = 17825.8$

20^+: $[m/z] = (M_r+z)/z \rightarrow 892.4 = (M_r + 20)/20 \rightarrow M_r = 17828$