QFL0341 - Estrutura e Propriedades de Compostos Orgânicos - (2019)

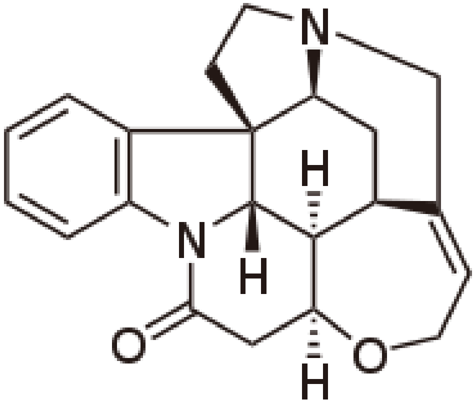
13 de agosto de 2019

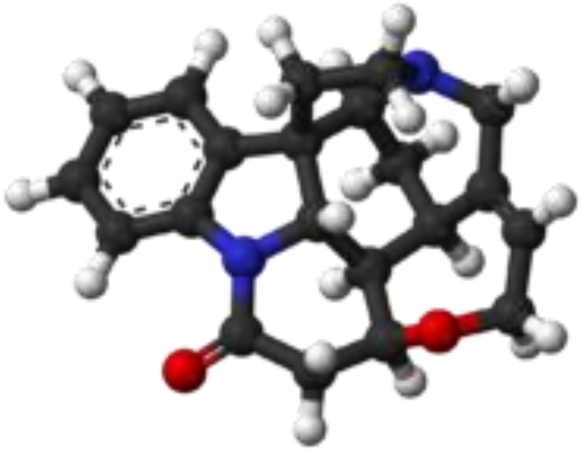
Métodos espectrométricos para caracterização de hidrocarbonetos (alcanos, alcenos e acetilenos)

História

Elucidação estrutural de produtos naturais costumava ser muito árdua e demorava.

Estriquinina *alcalóide tóxico isolado por Pelletier & Caventou (1818)*
Passado: H. Leuchs trabalhou em sua estrutura por 40 anos até que R. Woodward (1954) o venceu.
Hoje: requer <1 mg amostra;
Um fim-de-semana seria suficiente.





Etapas para elucidação estrutural

- Determinação da Fórmula Molecular
- Caracterizar Grupos funcionais
- Degradação da Molécula e Síntese de Derivados

Atualmente

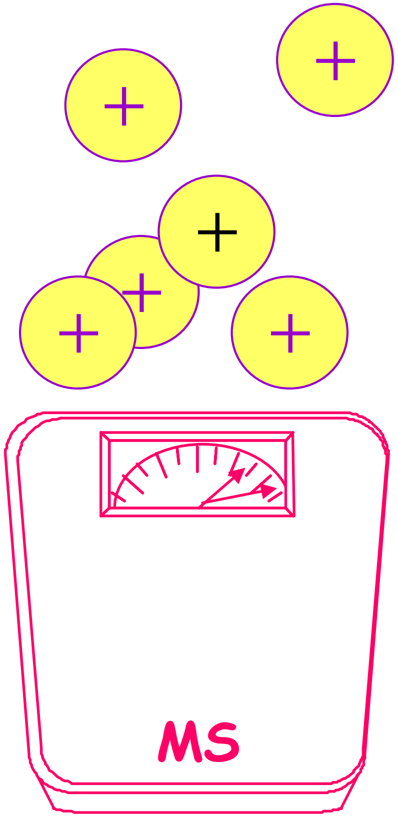
- Métodos Espectroscópicos

- **Determinação da fórmula molecular**
  - Análise elementar
  - Espectrometria de massas
- **Determinação de Grupos funcionais**
  - Análise funcional orgânica
  - Espectroscopia nas regiões do Ultravioleta e do Infravermelho
- **Conectividade da Cadeia**
  - RMN de ^{1}H
  - RMN de ^{13}C

Aplicações da Espectrometria de Massas

- Análise Farmacêutica
  - Estudos de biodisponibilidade
  - Estudos do metabolismo de fármacos, farmacocinética
  - Caracterização novos fármacos
  - Análise de produtos de degradação em medicamentos
  - Triagem de candidatos a medicamentos
  - Identificação de alvos de drogas
- Caracterização de biomoléculas
  - Proteínas e peptídeos
  - oligonucleotídeos
- Análise ambiental
  - Pesticidas em alimentos
  - Contaminação do solo e águas subterrâneas
- Análise forense / clínica

- Only gaseous ions can be detected by MS.
  - MS provides molecular weight or fragmentations for structural information
  - according to their ***mass to charge ratio***
  - output signals : ***the relative abundance of each ionic species.***



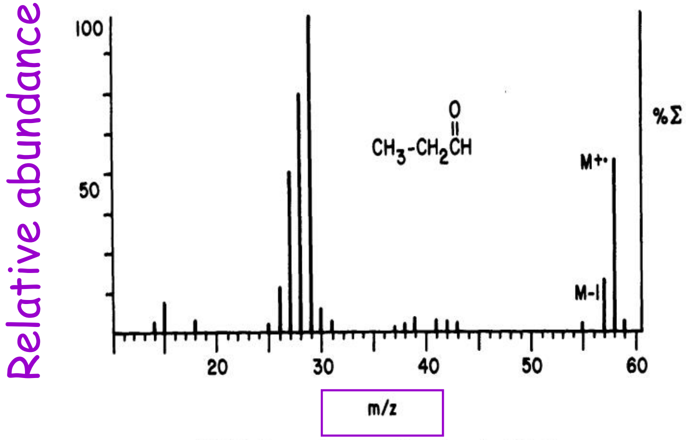


FIG. 1.7. Bar-graph mass spectrum of propionaldehyde.

Espectrometria de massas

✓técnica muito sensível – (10^{-12} g ou 10^{-15} mols);

✓ facilmente interfaceado com métodos cromatográficos de separação para misturas complexas (CG, CLAE e outros);

✓ pode ser utilizado para quantificação;

✓As massas dos fragmentos e a relativa abundância destes revelam informações importantes sobre as estruturas das substâncias.

Espectrometria de massas - um breve histórico

1897: J.J. Thompson descobre elétron e determina m/z

1912: J.J. Thompson constrói o primeiro espectrômetro de massas

1942: Demster desenvolve a fonte de impacto de elétrons

1942: Primeiro instrumento comercial para análise orgânica

1953: Quadrupolo e ion trap (W. Paul e H.S. Steinwedel, Nobel 1989)

1956: Primeiro GC-MS

Necessidade da análise do petróleo

1974: Primeiro HPLC-MS

1987: Demonstração de MALDI

1988: Demonstração de ESI

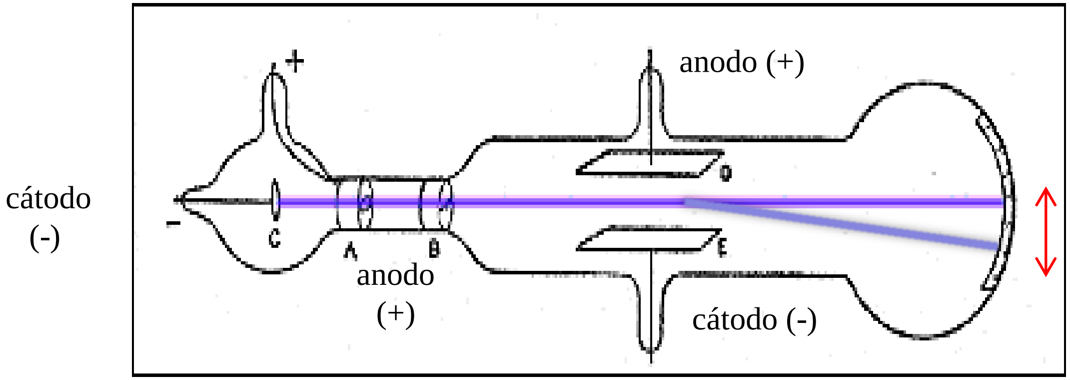
1990s: Crescimento explosivo de MS, devido a ESI e MALDI

2002: Nobel para inventores da técnica electrospray (ESI) (Fenn e Tanaka)

Development of Early Mass spectrometers

1913: J. J. Thompson descobriu os isótopos do Ne

Deflexion of the Cathode Rays by and Electrostatic Field.



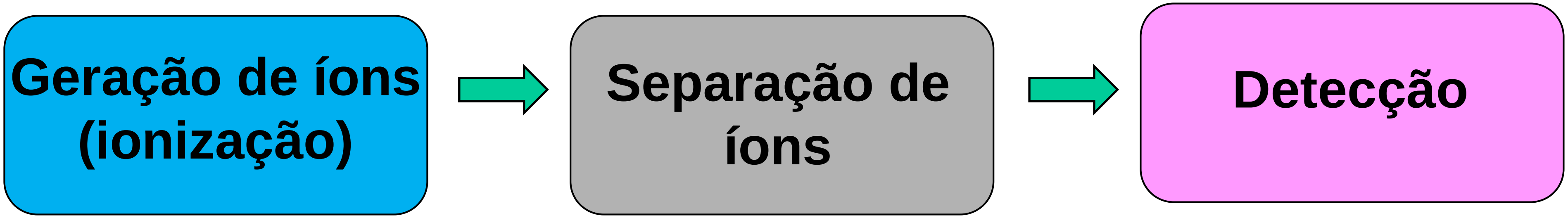
Electron:
"*corpuscles*"
in a "plum pudding"

He was awarded a Nobel Prize in 1906, "in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases".

J.J. Thomson (1936). Recollections and Reflections. G. Bell and Sons: London. p. 341.

http://masspec.scripps.edu/mshistory/mshistory.php

Componentes de um espectrômetro de massas



Geração de íons (ionização)

Separação de íons

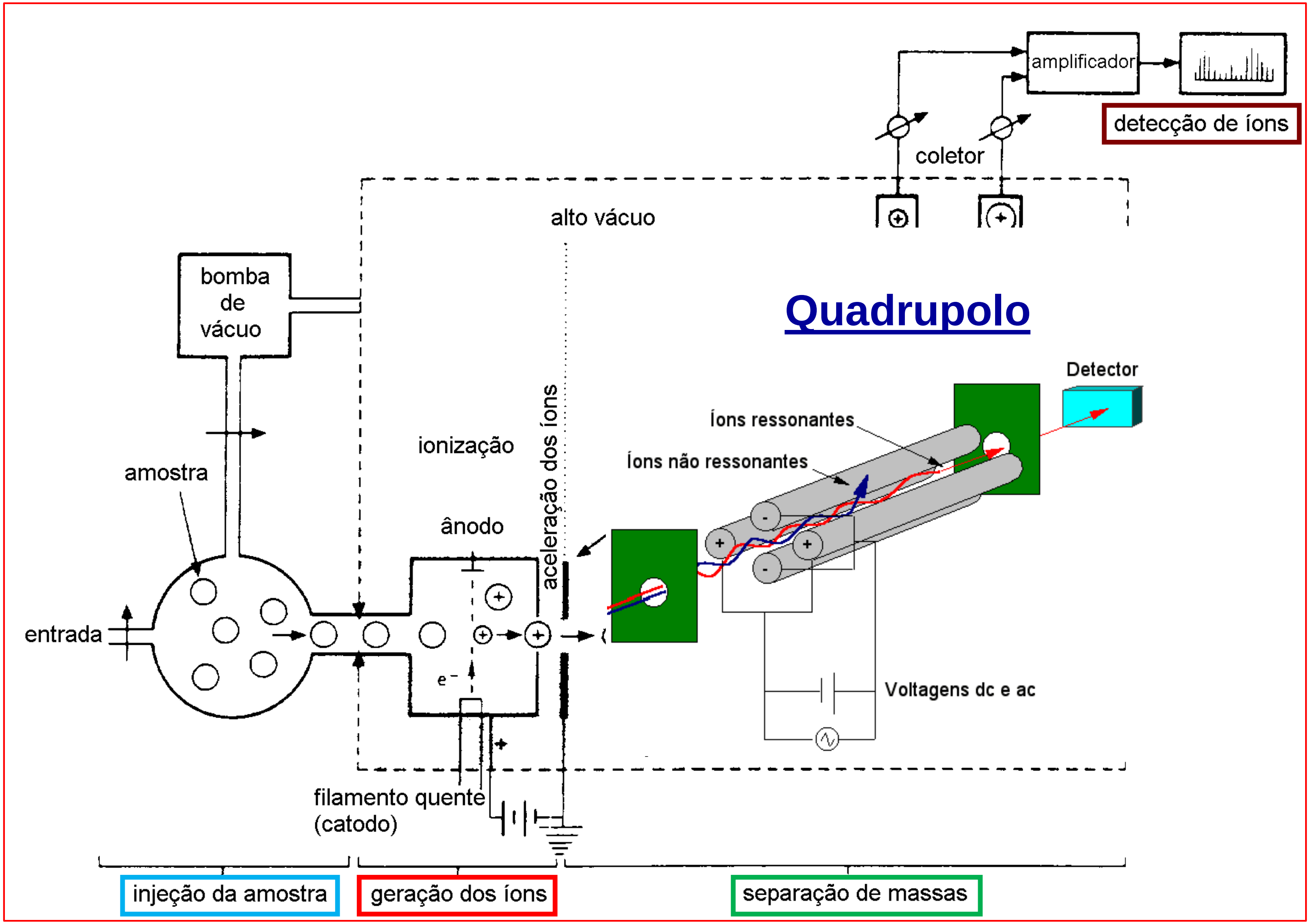
Detecção

- Impacto de elétrons
- Ionização química
- Electrospray
- MALDI

- quadrupolo
- Ion trap
- TOF

Espectrômetros de Massas

(ionização por impacto de eletrons e analisadores por quadrupolo)



Mecanismos de Ionização

Remoção de elétrons

$$M \xrightarrow{-\,e^-} M^{+\bullet}$$

Captura de elétrons

$$M \xrightarrow{+\,e^-} M^{-\,\bullet}$$

Protonação

$$M + H^+ \longrightarrow [MH]^+$$

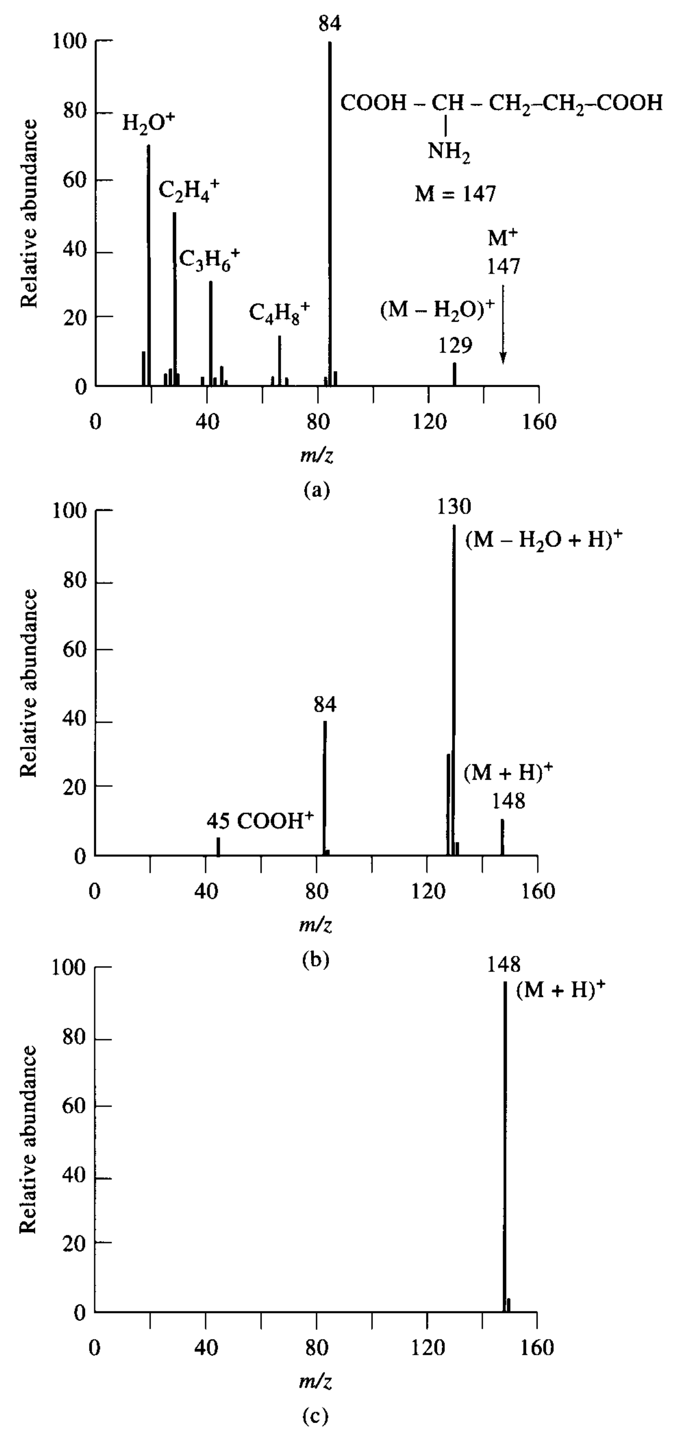
Cationização

$$M + Na^+ \longrightarrow [MNa]^+$$

Desprotonação

$$M\,H \xrightarrow{-\,H^+} M^-$$

Glutamic Acid

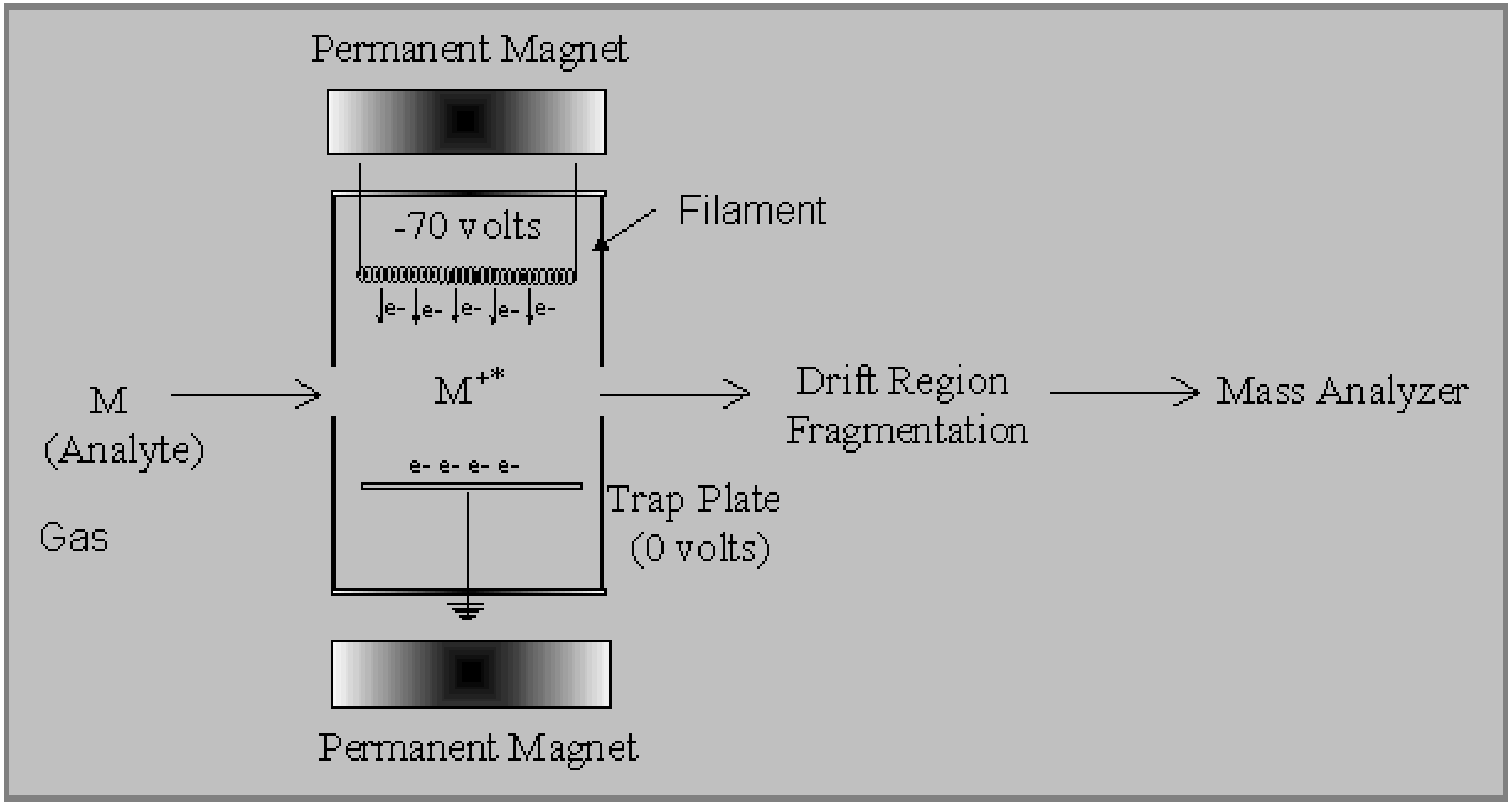


Electron Impact (EI)

Field Ionization

Field Desorption

Electron Impact



http://www.noble.org/PlantBio/MS/ion_tech_main.html

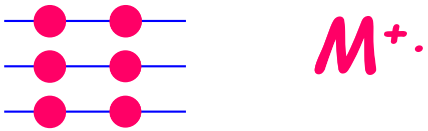
How does ionization occur?

Consider the Ionization potential

- a minimum amount of energy for ion formation to occur.

- the first ionization potential

the energy input required to remove an electron from the highest occupied atomic or molecular orbital of the neutral particle



- **First ionization potential**
  - **in the *5-15 eV* range for most elements**
  - **in the *8-12 eV* range for most organic molecules and radicals**

1 eV=1.6021 $\times 10^{-19}$ Joules=3.8291 $\times 10^{-20}$ calories

- **To remove a second, third, *etc* electron, additional energy is needed.**

- **When excess energy is available, fragmentation of the molecule may also occur during the process of ionization**

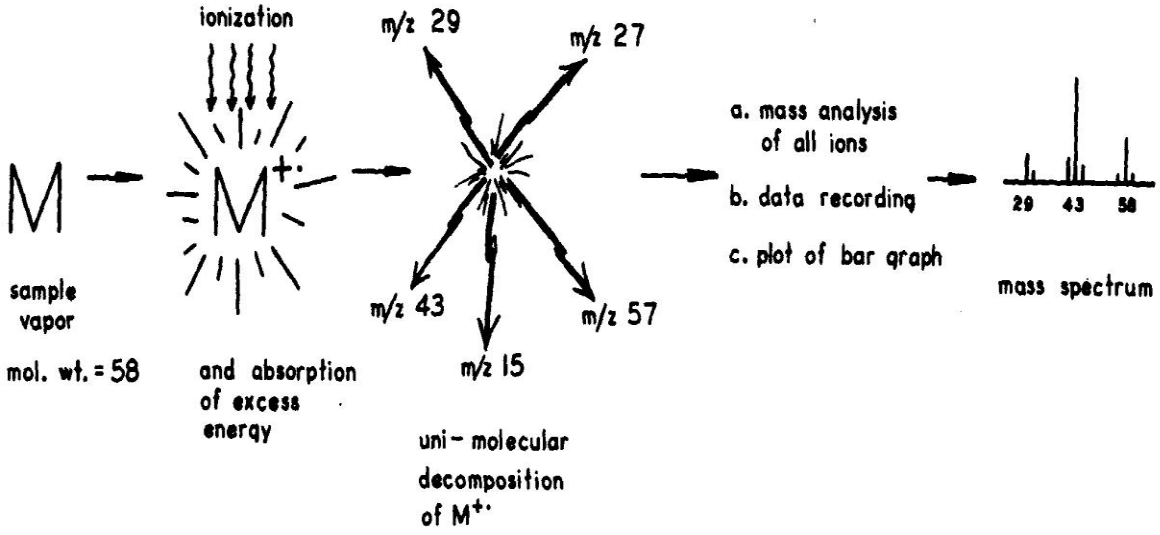
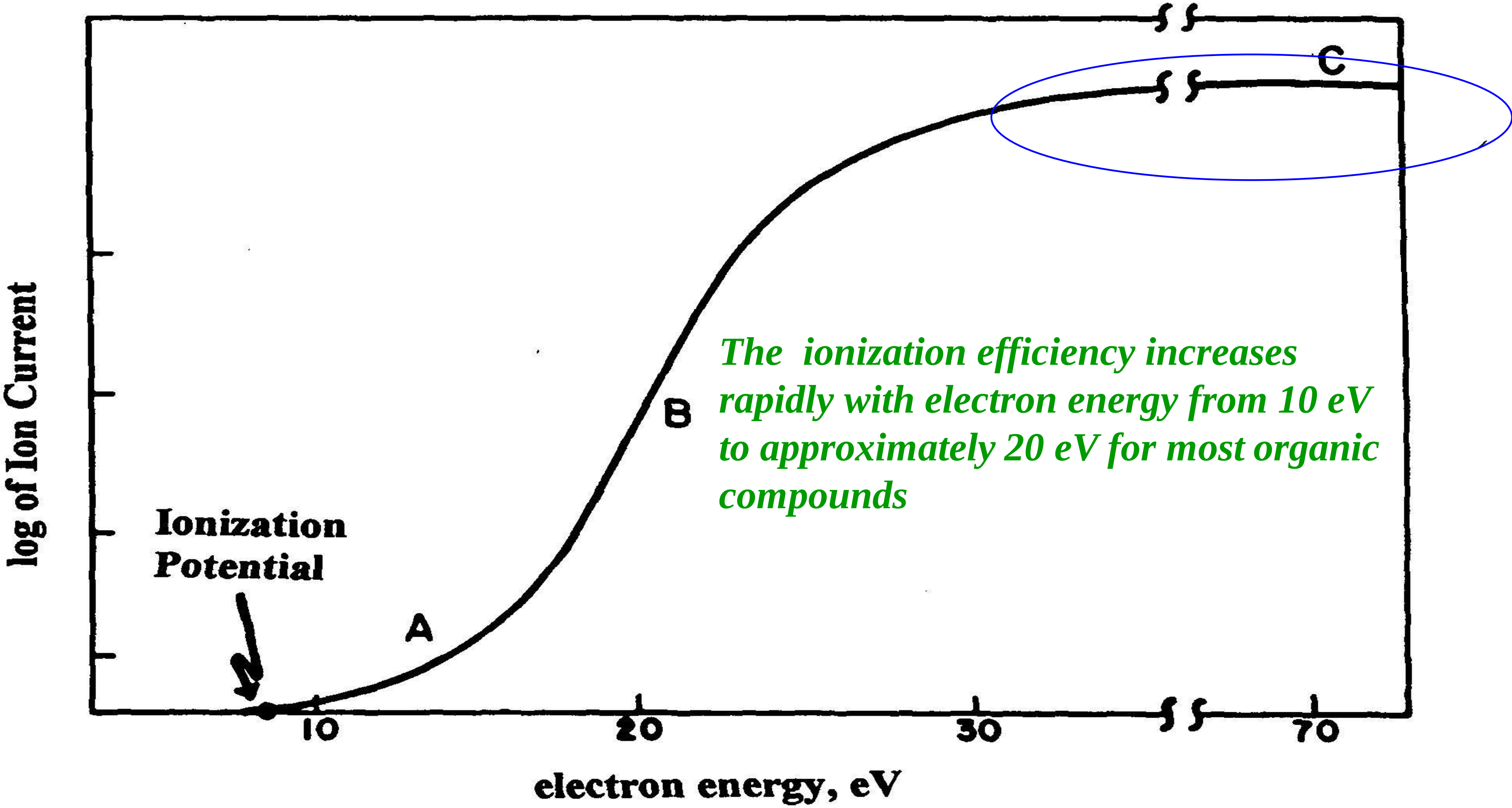


FIG. 1.1. General conceptual scheme for vapor-phase analysis by mass spectrometry.

1 eV is the energy gained (23 kcal/mole) by an electron in traversing an electric field maintained by a potential difference of 1V.



The ionization efficiency increases rapidly with electron energy from 10 eV to approximately 20 eV for most organic compounds

FIG. 7.3. Relationship between ion production and energy (electron volts) of ionizing electrons: *region A,* threshold region, principally molecular ions produced; *region B,* production of fragment ions becomes important; *region C,* routine operation, mostly fragment ions.

Electron Impact

(low picomole)

<u>Advantages</u>

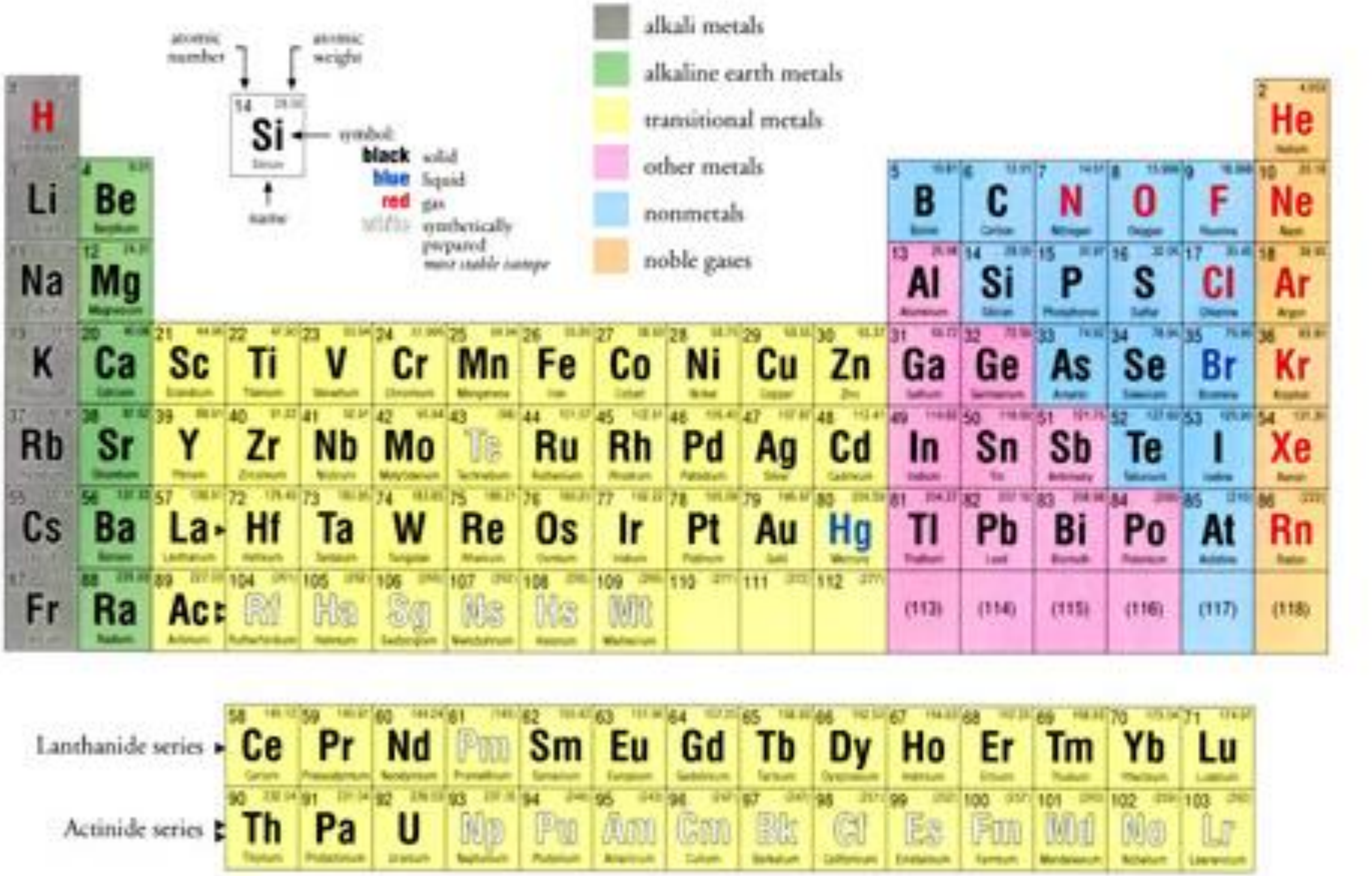
- Well-Established
- Fragmentation Libraries
- No Supression
- Insoluble Samples
- Interface to GC
- Non-Polar Samples

<u>Disadvantages</u>

- Parent Identification
- Need Volatile Sample
- Need Thermal Stability
- No Interface to LC
- Low Mass Compounds (<1000 amu)
- Solids Probe Requires Skilled Operator

Princípios da espectrometria de massas

- Elementos diferentes podem ser identificados unicamente pela suas massas



H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac	Rf	Ha	Sg	Ns	Hs	Mt	110	111	112	(113)	(114)	(115)	(116)	(117)	(118)

Lanthanide series	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Actinide series	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Isotopes

- **Most elements have more than one stable isotope.**

For example, most carbon atoms have a mass of 12 Da, but in nature, 1.1% of C atoms have an extra neutron, making their mass 13 Da.

- **Why do we care?**

Mass spectrometers can “see” isotope peaks if their resolution is high enough.

If an MS instrument has resolution high enough to resolve these isotopes, better mass accuracy is achieved.

The Mass Spectrum

Origin of Relative Ion Abundances

M contributors		M+1 contributors		M+2 contributors	
Isotope	**Natural Abundance**	**Isotope**	**Natural Abundance**	**Isotope**	**Natural Abundance**
^{1}H	99.9855%	^{2}H	0.015%	^{3}H	ppm
^{12}C	98.893	^{13}C	1.107	^{14}C	ppm
^{14}N	99.634	^{15}N	0.366		
^{16}O	99.759	^{17}O	0.037	^{18}O	0.204
^{19}F	100.0				
^{32}S	95.0	^{33}S	0.76	^{34}S	4.22
^{35}Cl	75.77			^{37}Cl	24.23
^{79}Br	50.69			^{81}Br	49.31
^{127}I	100.0				

The Mass Spectrum

Relative Intensity of Molecular Ion Peaks

Imagine a sample containing 10,000 methane molecules...

Molecule	# in sample	m/z	Relative abundance
$^{12}C^{1}H_4$	9889	12 + (4 x 1) = 16	100%
$^{13}C^{1}H_4$	110	13 + (4 x 1) = 17	(110/9889) x 100% = 1.1%*
$^{14}C^{1}H_4$	~1	14 + (4 x 1) = 18	(1/9889) x 100% = < 0.1%*

*Contributions from ions with ^{2}H are ignored because of its very small natural abundance

CH_4 mass spectrum

m/z = 16 (M; 100%), m/z = 17 (M+1; 1.1%), m/z = 18 (M+2; < 0.1%)

Formula from Mass Spectrum

M+1 Contributors

Comparing many mass spectra reveals M+1 intensity ↑ ~1.1% per C in formula

- Examples: C_2H_6 M = 100%; M+1 = ~2.2%
  C_6H_6 M = 100%; M+1 = ~6.6%

Working backwards gives a useful observation...

When relative contribution of M = 100% then relative abundance of M+1/1.1% gives the approximate number of carbon atoms in the molecular formula

Other M+1 contributors

- ^{15}N (0.37%) and ^{33}S (0.76%) should be considered
- ^{2}H (0.015%) and ^{17}O (0.037%) can be ignored

Formula from Mass Spectrum

M+2 Contributors

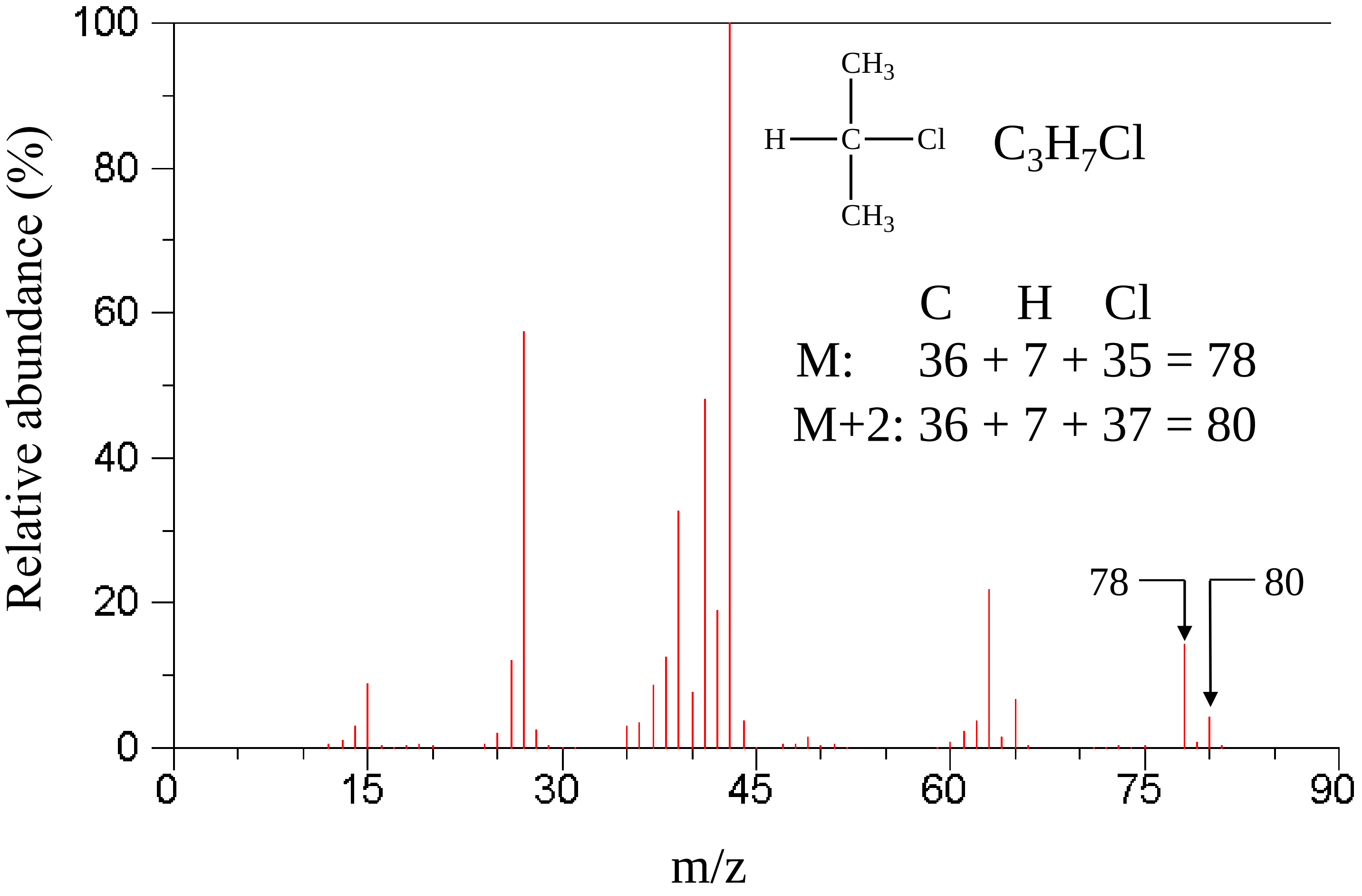
Anything useful from intensity of M+2?

Isotopes	Natural abundances	Intensity M : M+2
$^{32}S : ^{34}S$	95.0 : 4.2	100 : 4.4
$^{35}Cl : ^{37}Cl$	75.8 : 24.2	100 : 31.9
$^{79}Br : ^{81}Br$	50.7 : 49.3	100 : 97.2

Conclusion: *Mass spectra of molecules with S, Cl, or Br have significant M+2 peaks*

Formula from Mass Spectrum

M+2 Contributors



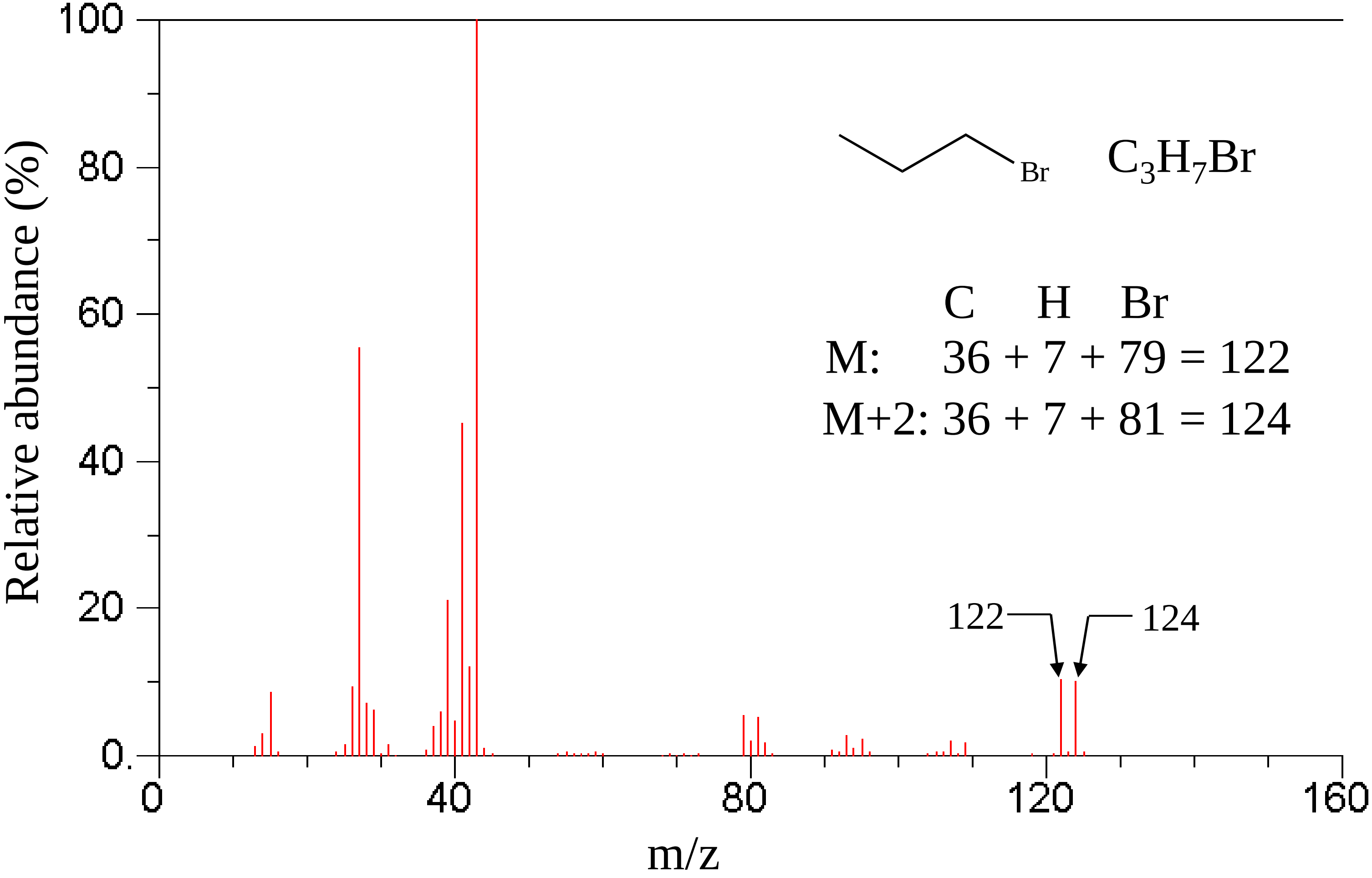
C_3H_7Cl

	C		H		Cl		
M:	36	+	7	+	35	=	78
M+2:	36	+	7	+	37	=	80

M:M+2
abundance
~3:1

Formula from Mass Spectrum

M+2 Contributors



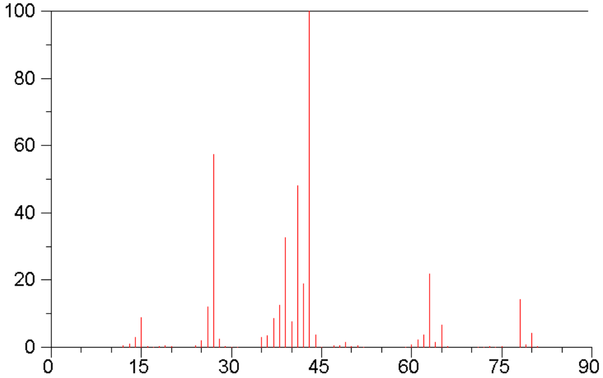
C_3H_7Br

	C	H	Br	
M:	36 +	7 +	79 =	122
M+2:	36 +	7 +	81 =	124

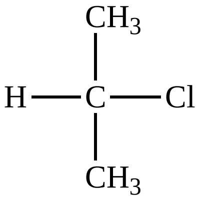
M:M+2
abundance
~1:1

Mass Spectrum → Formula → Structure

How do we derive structure from the mass spectrum?

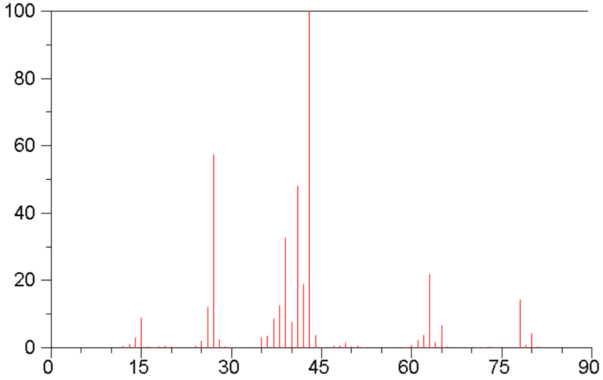


$\overset{?}{\rightarrow}$

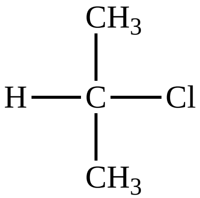


$H-\overset{CH_3}{\underset{CH_3}{C}}-Cl$

- Not trivial to do this directly
- Structure comes from formula; formula comes from mass spectrum



→ C_3H_7Cl →

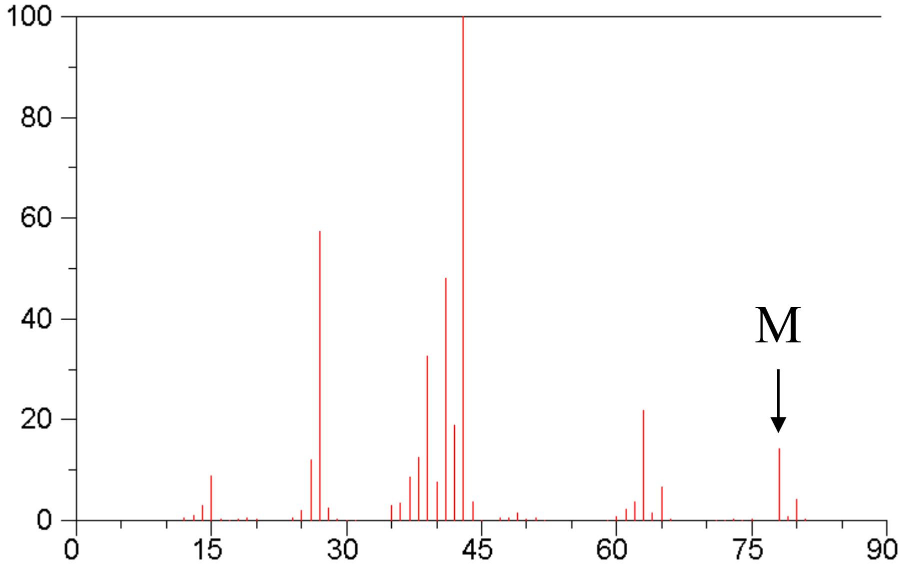


$H-\overset{CH_3}{\underset{CH_3}{C}}-Cl$

Mass Spectrum → Formula → Structure

How do we derive formula from the mass spectrum?

- m/z and relative intensities of M, M+1, and M+2



M: m/z = 78

$C_2H_6O_3$

C_3H_7Cl

C_5H_4N

C_6H_6

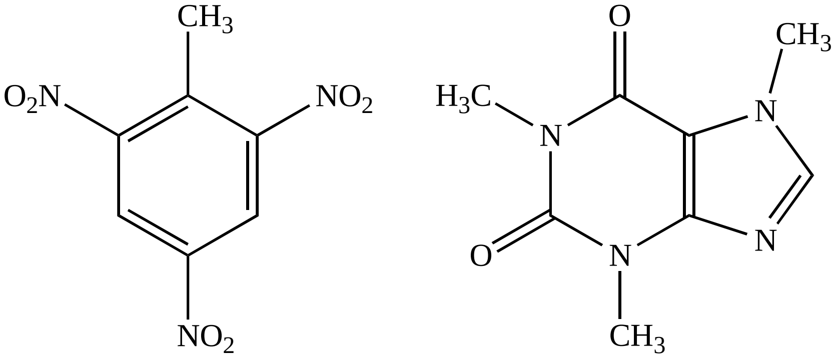
etc.

- A few useful rules to narrow the choices

How Many Nitrogen Atoms?

Consider these molecules:

NH_3 H_2NNH_2



Formula:	NH_3	N_2H_4	$C_7H_5N_3O_6$	$C_8H_{10}N_4O_2$
m/z (M):	17	32	227	194

Conclusion

- When m/z (M) = even, number of N in formula is even
- When m/z (M) = odd, number of N in formula is odd

} **The Nitrogen Rule**

How Many Nitrogen Atoms?

A Nitrogen Rule Example

Example: Formula choices from previous mass spectrum

M: m/z = 78 ← m/z even

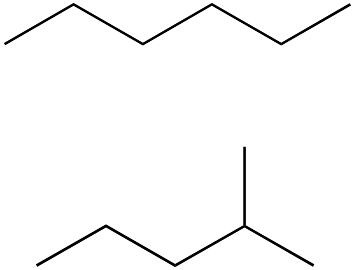
$C_2H_6O_3$ ← even nitrogen count

C_3H_7Cl ← even nitrogen count

discarded ~~C_5H_4N~~ ← odd nitrogen count

C_6H_6 ← even nitrogen count

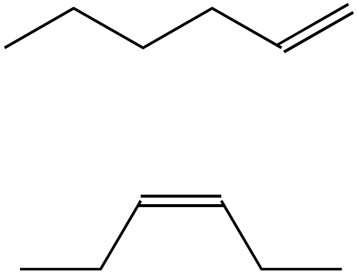
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

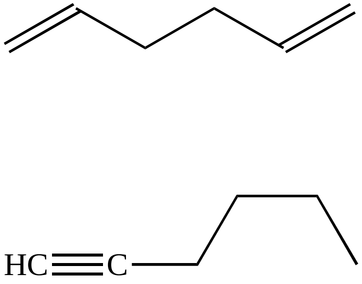
One pi bond



C_6H_{12}

H count = max - 2

Two pi bonds

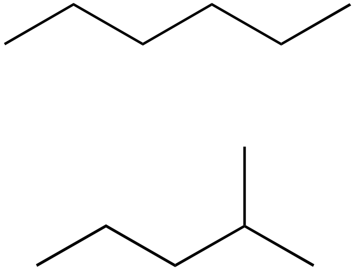


C_6H_{10}

H count = max - 4

Conclusion: Each pi bond reduces max hydrogen count by two

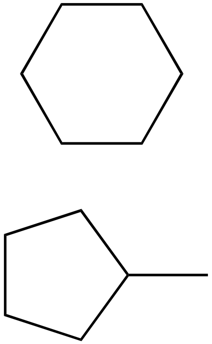
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

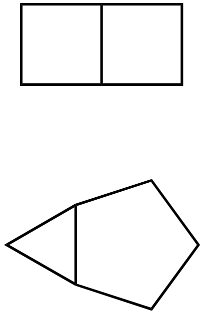
One ring



C_6H_{12}

H count = max - 2

Two rings

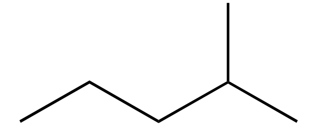


C_6H_{10}

H count = max - 4

Conclusion: Each ring reduces max hydrogen count by two

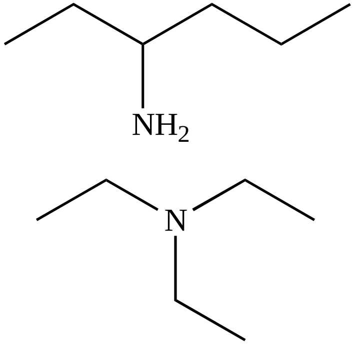
How Many Hydrogen Atoms?



C_6H_{14}

max H for 6 C

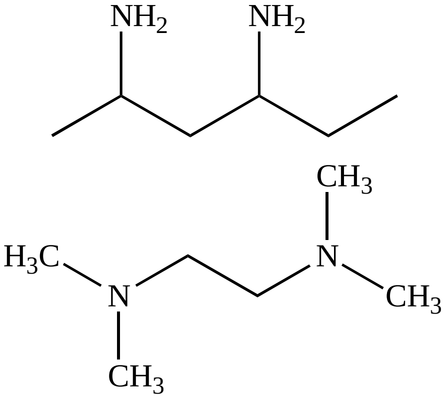
One nitrogen



$C_6H_{15}N$

H count = max + 1

Two nitrogens



$C_6H_{16}N_2$

H count = max + 2

Conclusion:

- Each nitrogen increases max H count by one
- For C carbons and N nitrogens, max number of H = 2C + N + 2

The Hydrogen Rule

Mass Spectrum → Formula

Example #1

Given information

m/z	Molecular ion	Relative abundance	Conclusions
102	M	100%	*Mass (lowest isotopes) = 102* *Even number of nitrogens*
103	M+1	6.9%	6.9 / 1.1 = 6.3 *Six carbons**
104	M+2	0.38%	*< 4% so no S, Cl, or Br* ~~*Oxygen?*~~

*Rounding: 6.00 to 6.33 = 6; 6.34 to 6.66 = 6 or 7; 6.67 to 7.00 = 7

Mass Spectrum → Formula

Example #1

Mass (M) - mass (C, S, Cl, Br, F, and I) = mass (N, O, and H)
102 - C_6 = 102 - (6 x 12) = 30 amu for N, O, and H

Oxygens	Nitrogens	30 - O - N = H	Formula	Notes
0	0	30 - 0 - 0 = 30	~~C_6H_{30}~~	Violates hydrogen rule
1	0	30 - 16 - 0 = 14	$C_6H_{14}O$	Reasonable
2	0	30 - 32 - 0 = -2	~~$C_6H_{-2}O_2$~~	Not possible
0	2*	30 - 0 - 28 = 2	$C_6H_2N_2$	Reasonable

*Nitrogen rule!

- Other data (functional groups from IR, NMR integration, etc.) further trims the list

Mass Spectrum → Formula

Example #2

m/z	Molecular ion	Relative abundance	Conclusions
157	M	100%	*Mass (lowest isotopes) = 157* *Odd number of nitrogens*
158	M+1	9.39%	9.39 / 1.1 = 8.5 *Eight or nine carbons*
159	M+2	34%	*One Cl; no S or Br*

Mass Spectrum → Formula

Example #2

Try eight carbons: M - C_8 - Cl = 157 - (8 x 12) - 35 = 26 amu for O, N, and H

Oxygens	Nitrogens	26 - O - N = H	Formula	Notes
0	1* *Nitrogen rule!	26 - 0 - 14 = 12	$C_8H_{12}ClN$	Reasonable

Not enough amu available for one oxygen/one nitrogen or no oxygen/three nitrogens

Characterization of hydrocarbons

Physical properties: mp, bp, density, solubility

Chemical properties:

Halogenation,

H^+ catalyzed polymerization for alkenes

Acidity of terminal alkynes (reaction with strong bases)

Spectroscopy:

mass spectrometry, infrared, ultraviolet and ^1H NMR

Interpretation of Mass Spectra

- Select a candidate peak for the molecular ion (M^+)
- Examine spectrum for peak clusters of characteristic isotopic patterns
- Test (M^+) peak candidate by searching for other peaks correspond to reasonable losses
- Look for characteristic low-mass fragment ions
- Compare spectrum to reference spectra

SOME COMMON AND REASONABLE LOSSES FROM THE MOLECULAR ION

M - 1	loss of hydrogen *radical*	M- $\cdot H$
M - 15	loss of methyl radical	M- $\cdot CH_3$
M - 29	loss of ethyl radical	M- $\cdot CH_2CH_3$
M - 31	loss of methoxyl radical	M- $\cdot OCH_3$
M - 43	loss of propyl radical	M- $\cdot CH_2CH_2CH_3$
M - 45	loss of ethoxyl radical	M- $\cdot OCH_2CH_3$
M - 57	loss of butyl radical	M- $\cdot CH_2CH_2CH_2CH_3$
M - 2	loss of hydrogen	M- H_2
M - 18	loss of water	M- H_2O
M - 28	loss of CO or ethylene	M- CO or M - CH_2H_4
M - 32	loss of methanol	M- CH_3OH
M - 44	loss of CO_2	M- CO_2
M - 60	loss of acetic acid	M- CH_3CO_2H
M - 90	loss of silanol: $HO\text{-}Si(CH_3)_3$	M- $HO - Si - (CH_3)_3$

Fragmentação

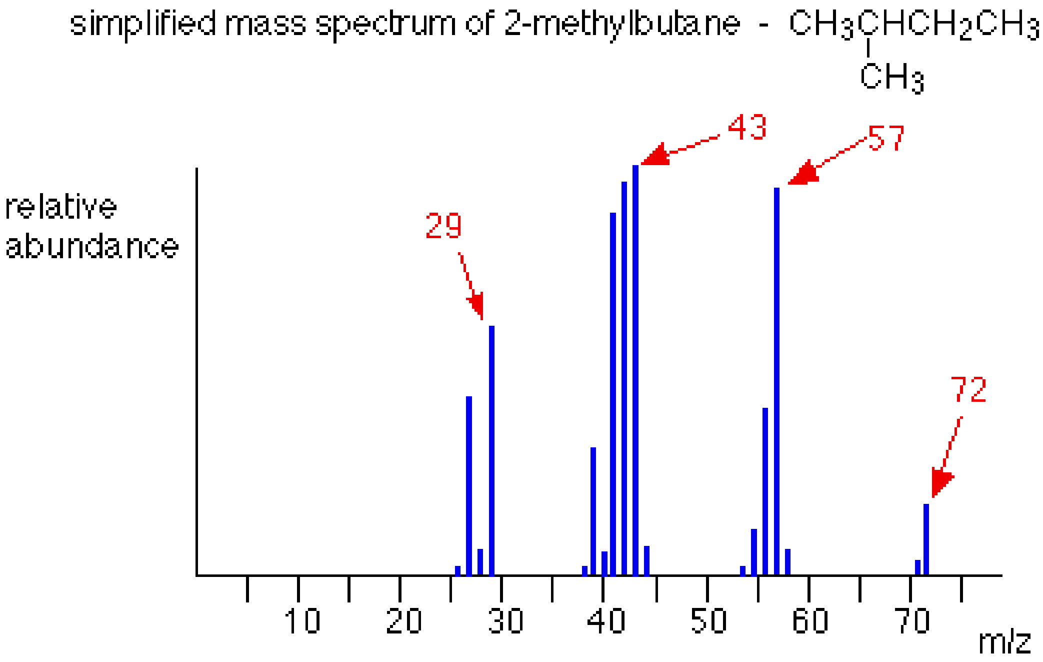
- Governada pela estabilidade do íon produzido
- consideração
    - regra do octeto
    - deslocalização por ressonância
    - polarizabilidade e hiperconjugação
    - eletronegatividade
- Regra de Stevenson:

Para quebra de uma ligações simples, o fragmento com o potencial de ionização mais baixo fica com a carga

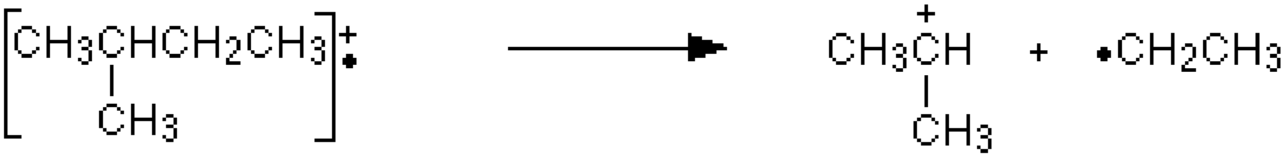
(em outras palavras, forma-se o íon mais estável)

The mass spectra of hydrocarbons are very predictable – apply the lessons of the stability of carbocations (or radicals) to predict or explain the observation of the fragments

Order of stability of carbocations
primary < secondary < tertiary


simplified mass spectrum of 2-methylbutane - $CH_3\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2CH_3$

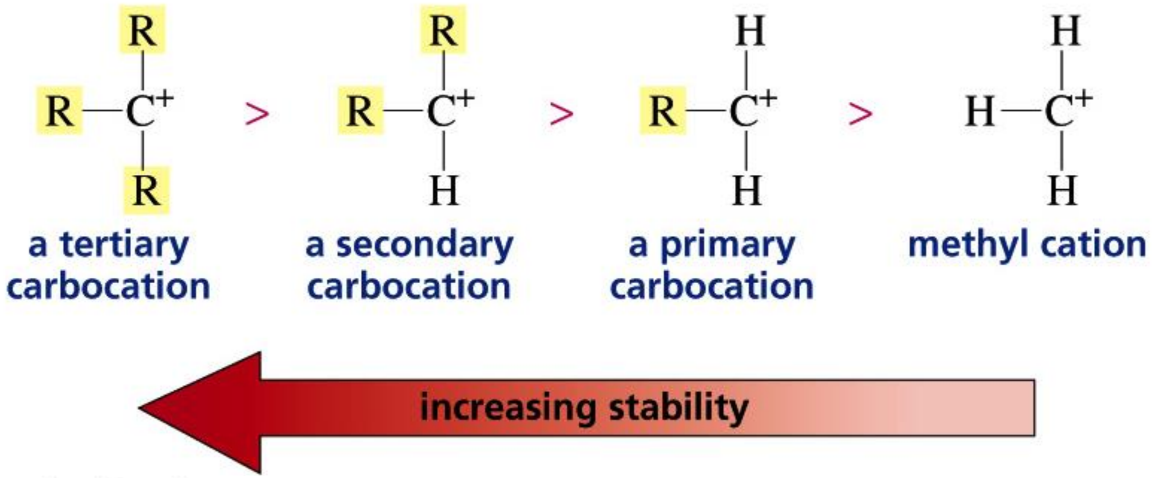
Stevenson's Rule – the fragment with the lowest ionization energy will take on the + charge; the other fragment will still have an unpaired electron

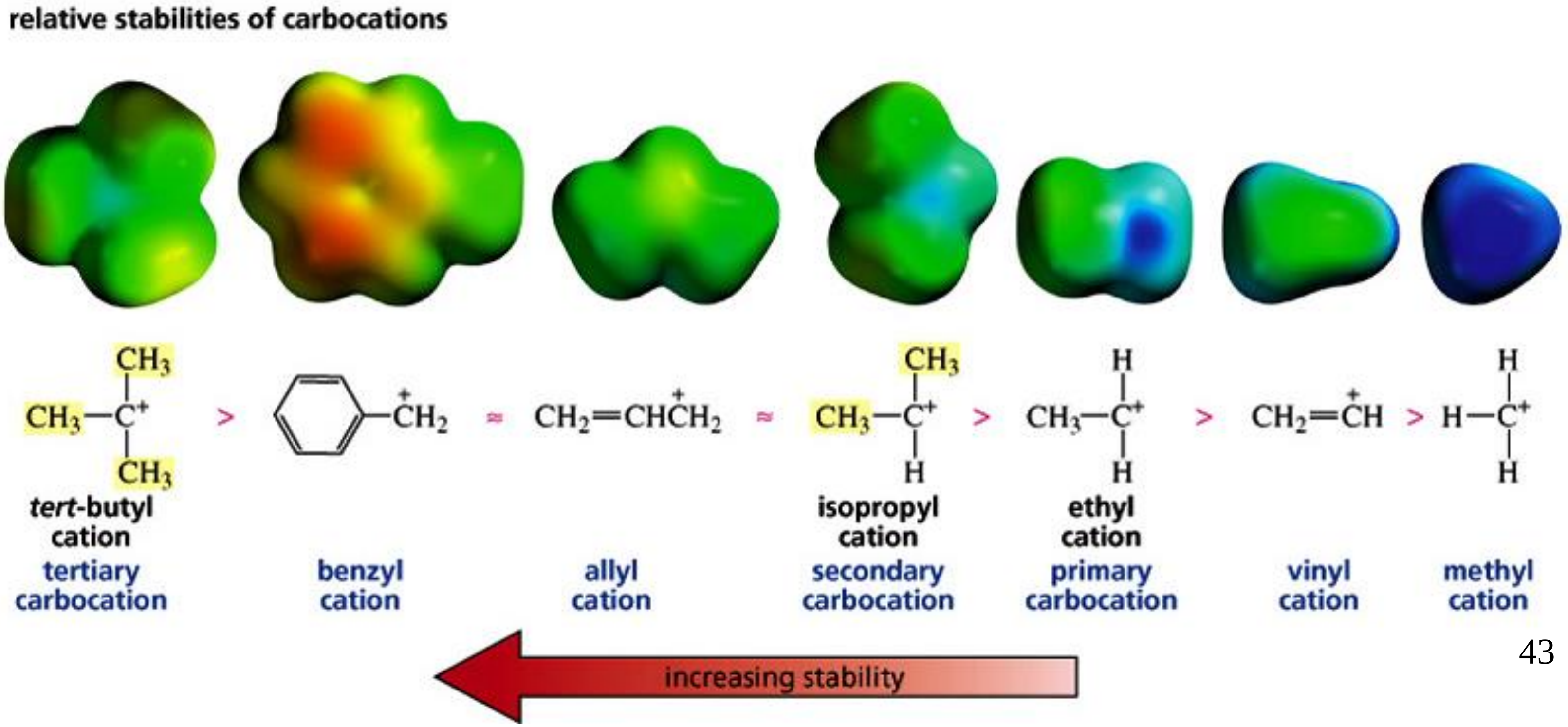

$$\left[CH_3\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2CH_3\right]^{+\bullet} \longrightarrow CH_3\underset{\displaystyle CH_3}{\underset{|}{\overset{+}{C}}}H + \bullet CH_2CH_3$$

m/z = 43

Estabilidades Relativas de Carbocátions

Somente para
lembrar

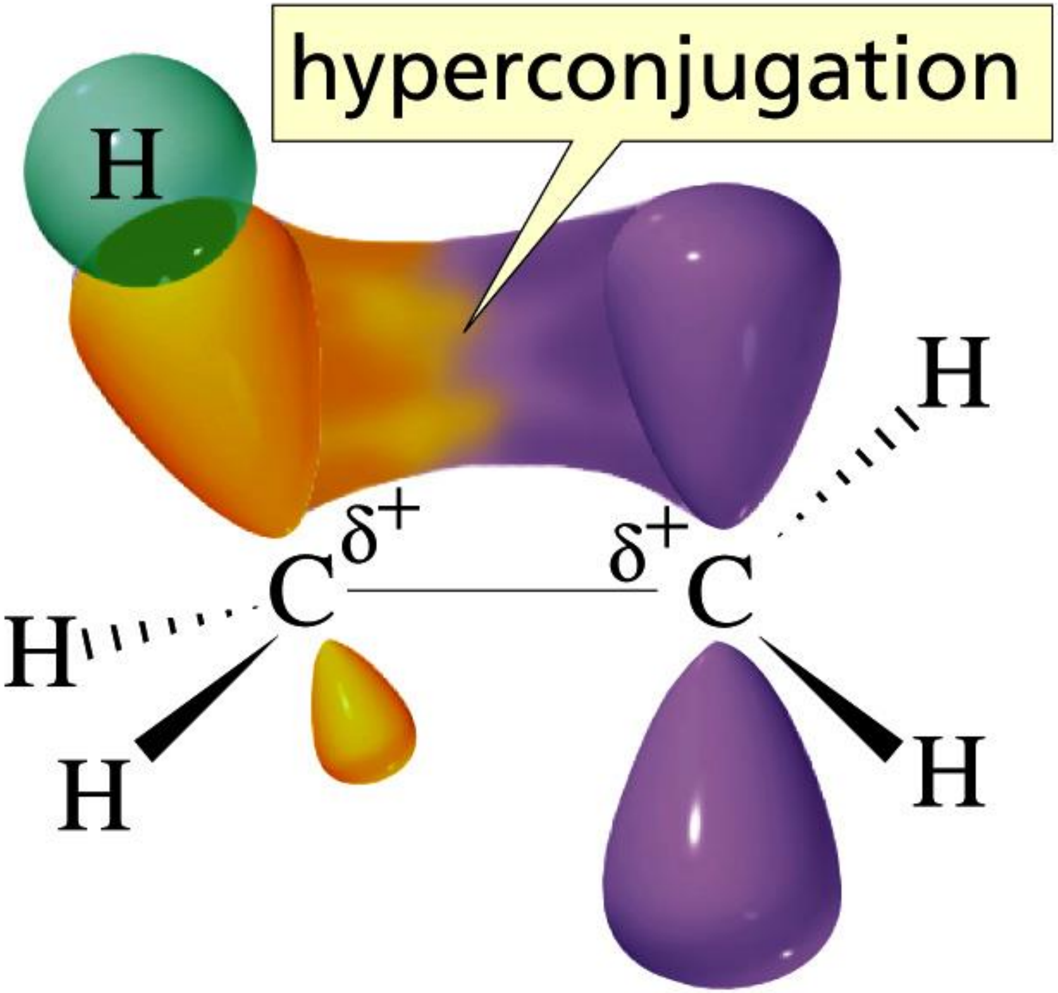




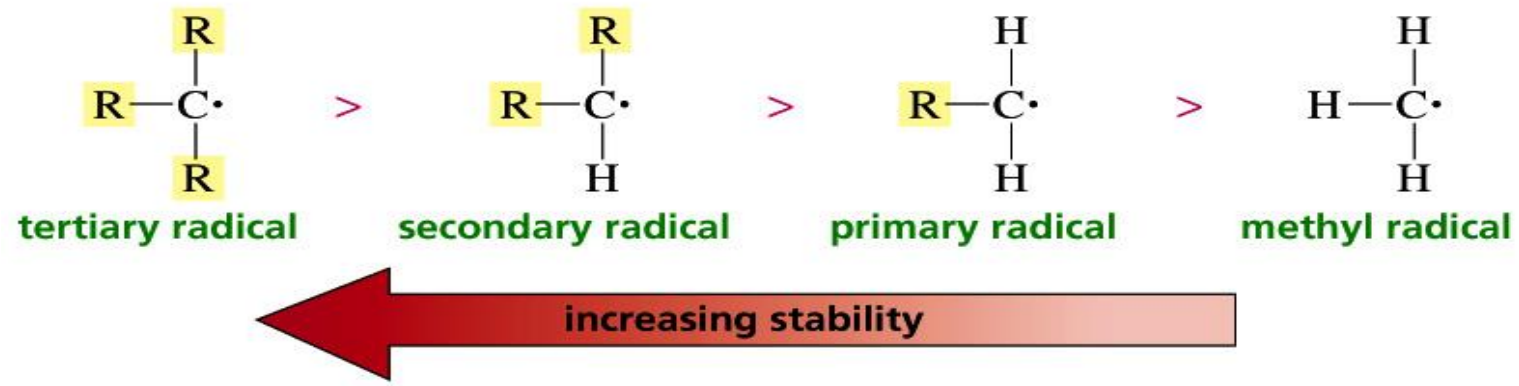
Estabilidade de Carbocátions:

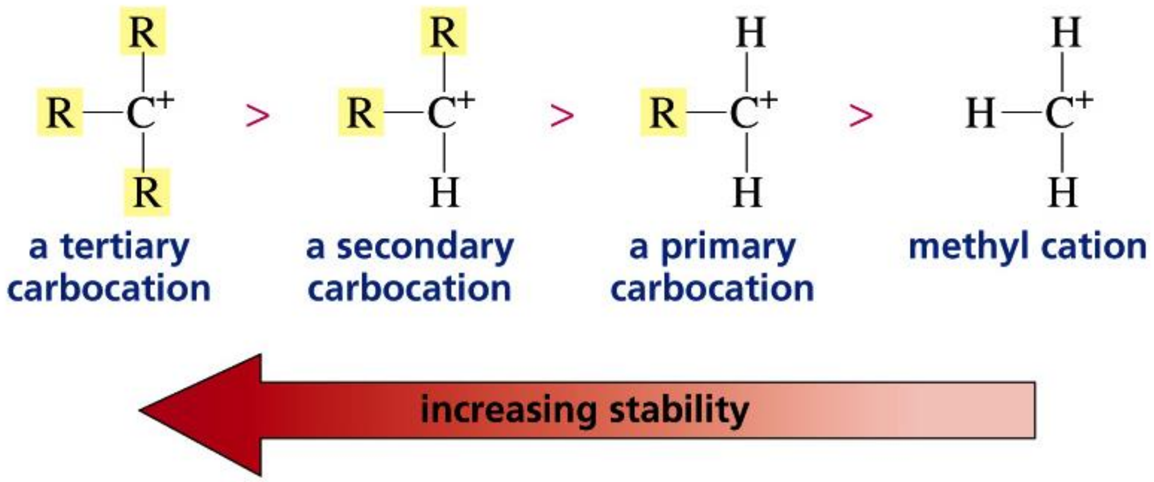
Mecanismos de Deslocalização de Elétrons: Hiperconjugação

Somente para
lembrar



Estabilidade Relativa de carbocátions e Radicais Alquílicos Seguem a mesma ordem

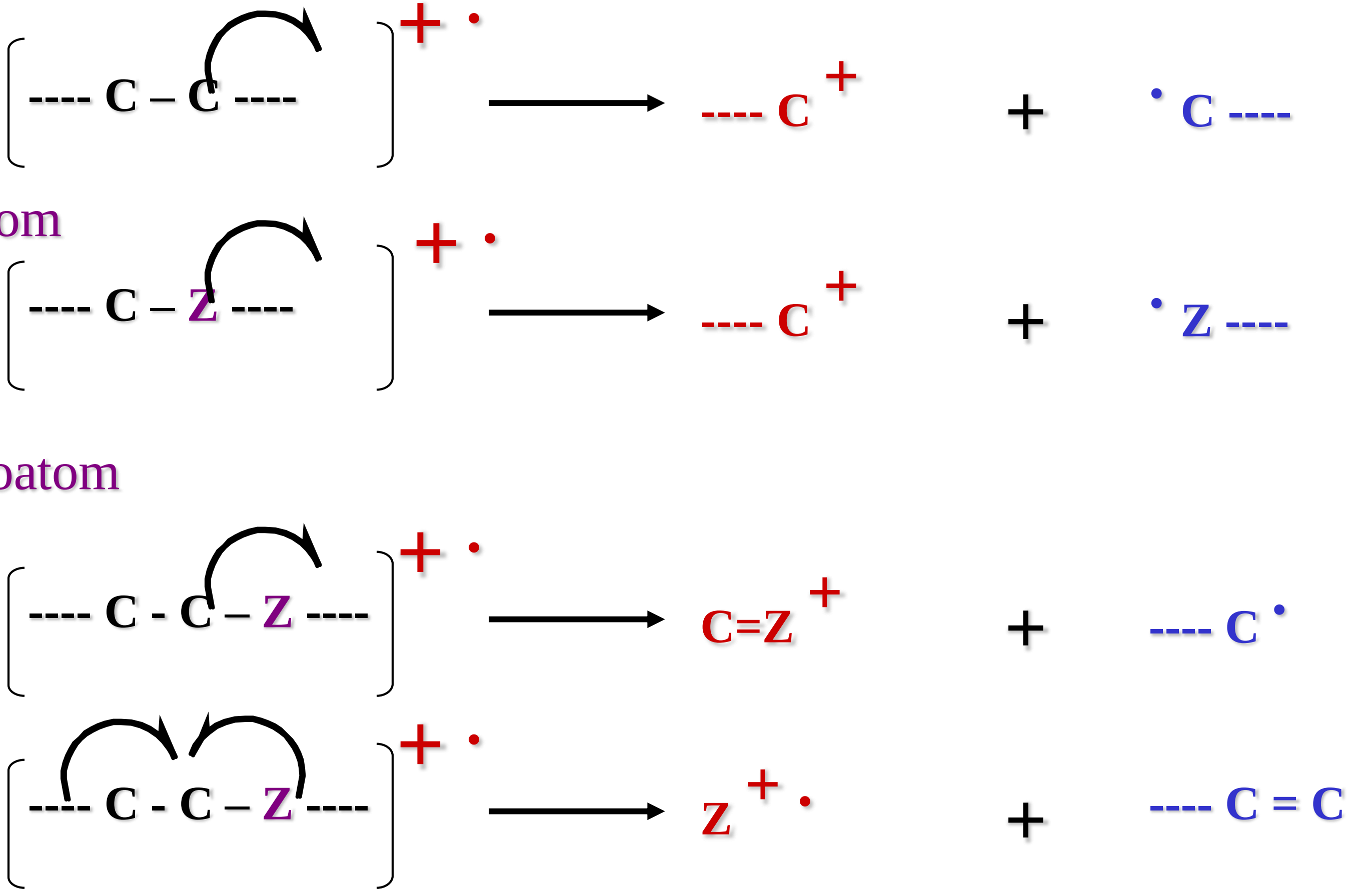




Fragmentation process

There are 3 type of fragmentations:

1) Cleavage of σ bond



$$[\text{---- C} - \text{C ----}]^{+\bullet} \longrightarrow \text{---- C}^{+} + {}^{\bullet}\text{C ----}$$

At heteroatom

$$[\text{---- C} - \text{Z ----}]^{+\bullet} \longrightarrow \text{---- C}^{+} + {}^{\bullet}\text{Z ----}$$

α to heteroatom

$$[\text{---- C - C} - \text{Z ----}]^{+\bullet} \longrightarrow \text{C=Z}^{+} + \text{---- C}^{\bullet}$$

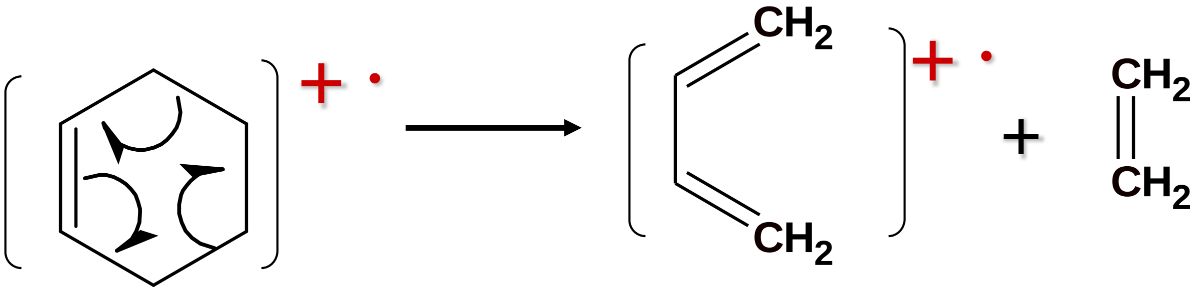
$$[\text{---- C - C} - \text{Z ----}]^{+\bullet} \longrightarrow \text{Z}^{+\bullet} + \text{---- C = C}$$

Fragmentation process

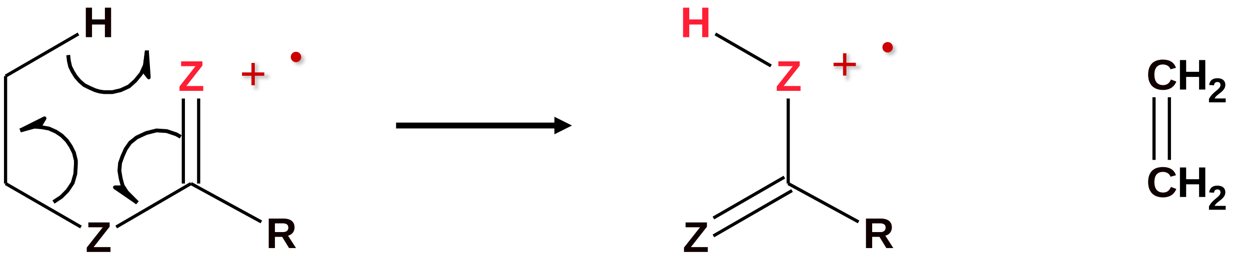
2) Cleavage of **2 σ** bond (rearrangements)

$$\left[\text{---- HC – C – Z ----}\right]^{+\cdot} \longrightarrow \text{---- C=C}^{+} \;+\; \text{HZ}$$

Retro Diels-alder



McLafferty



3) Cleavage of **Complex** rearrangements

Regras Gerais de Fragmentação

Quebra de Uma Ligação (Quebras-α)

Quebra de Duas Ligações

- Eliminação de H-X
- retro Diels-Alder
- Rearranjo de McLafferty

FRAGMENTATION PATTERNS

ALKANES

The mass spectra of simple hydrocarbons have peaks at m/z values corresponding to the ions produced by breaking C-C bonds. Peaks can occur at ...

m/z	15	29	43	57	71	85	etc.
	CH_3^+	$C_2H_5^+$	$C_3H_7^+$	$C_4H_9^+$	$C_5H_{11}^+$	$C_6H_{13}^+$	

- the stability of the carbocation formed affects its abundance
- the more stable the cation the higher the peak
- the more alkyl groups attached to the carbocation the more stable it is

most stable tertiary 3° > secondary 2° > primary 1° least stable

alkyl groups are electron releasing and stabilise the cation

Alkane Fragmentation

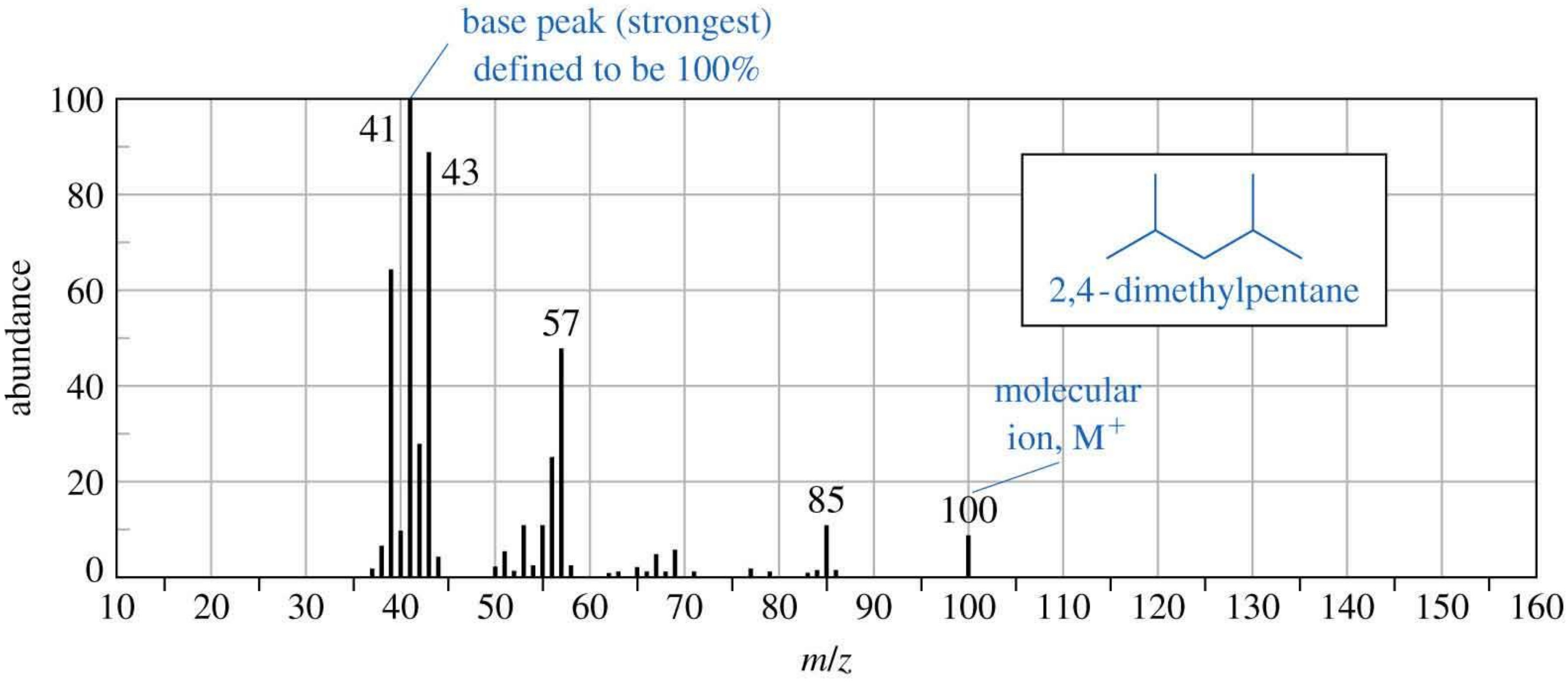
- Long chains give homologous series of m/z = 14 units
- Long chains rarely lose methyl radical
- Straight chain alkanes give primary carbocation
- branched alkanes have small or absent M^+
- enhanced fragmentation at branch points

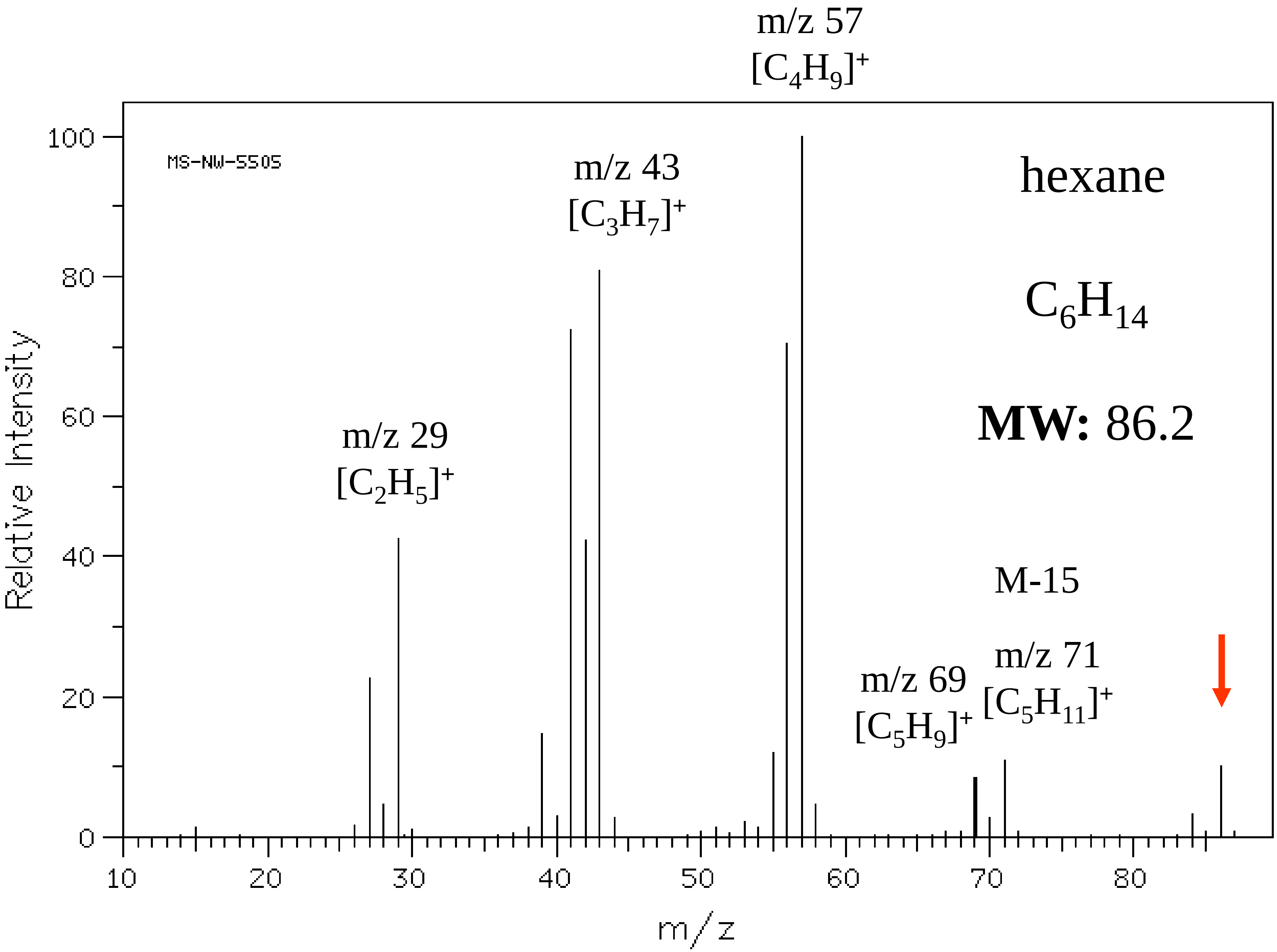
Cycloalkanes

- loss of side chain
- loss of ethylene fragments

Espectro de massas

Os íons são detectados são registrados de acordo com a sua abundância relativa



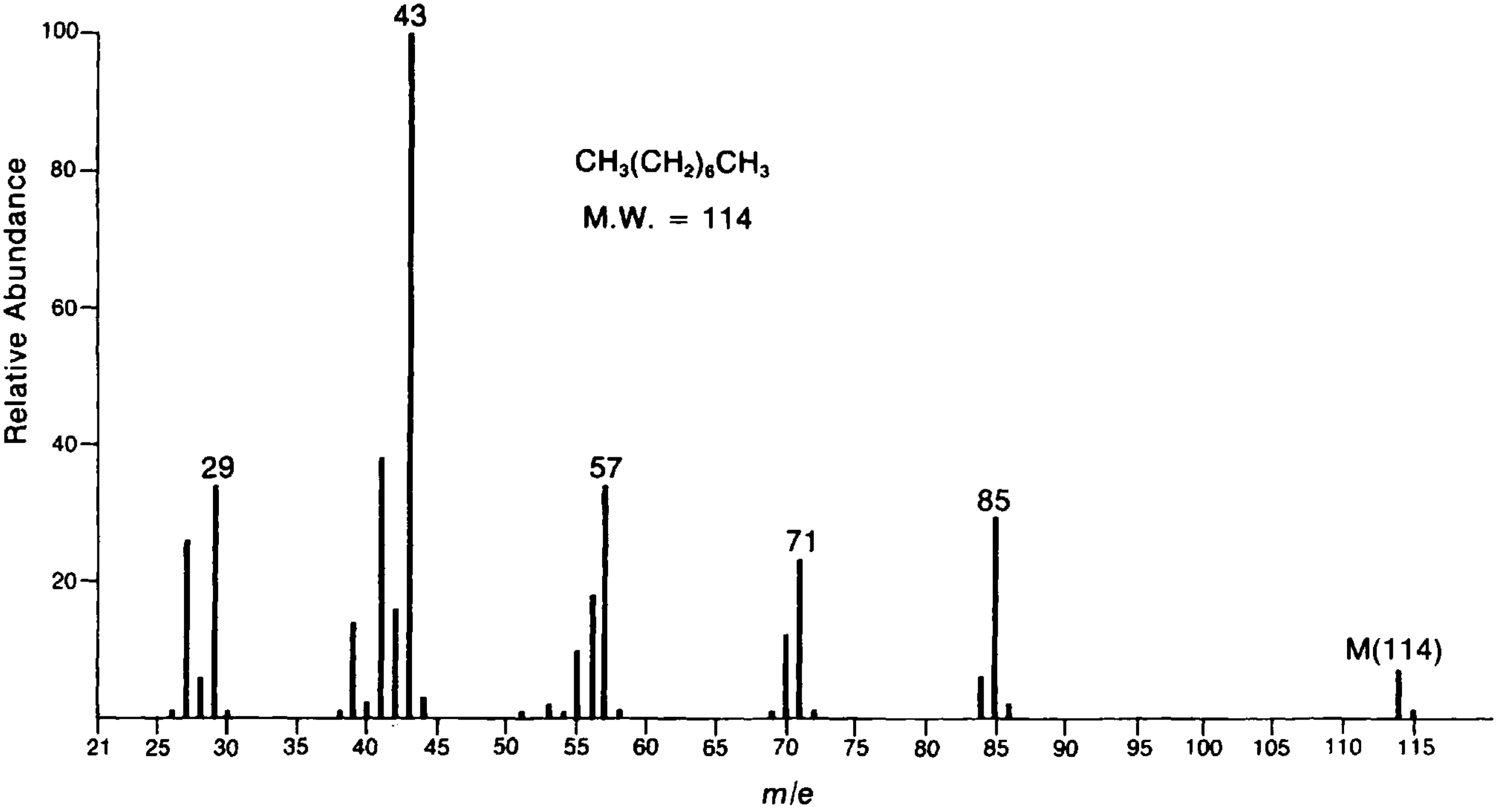


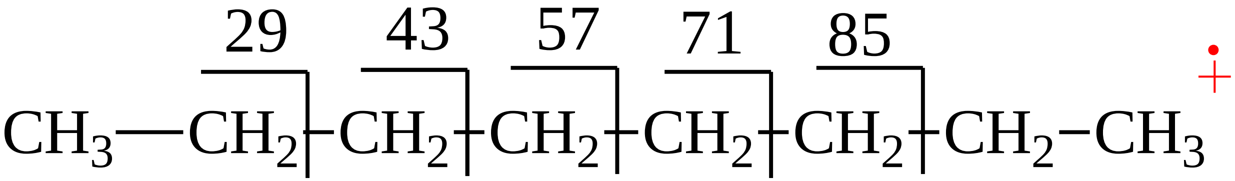
hexane

C_6H_{14}

MW: 86.2

octane


43
100
80
60
40
20
Relative Abundance
$CH_3(CH_2)_6CH_3$
M.W. = 114
29
57
71
85
M(114)
21
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
m/e



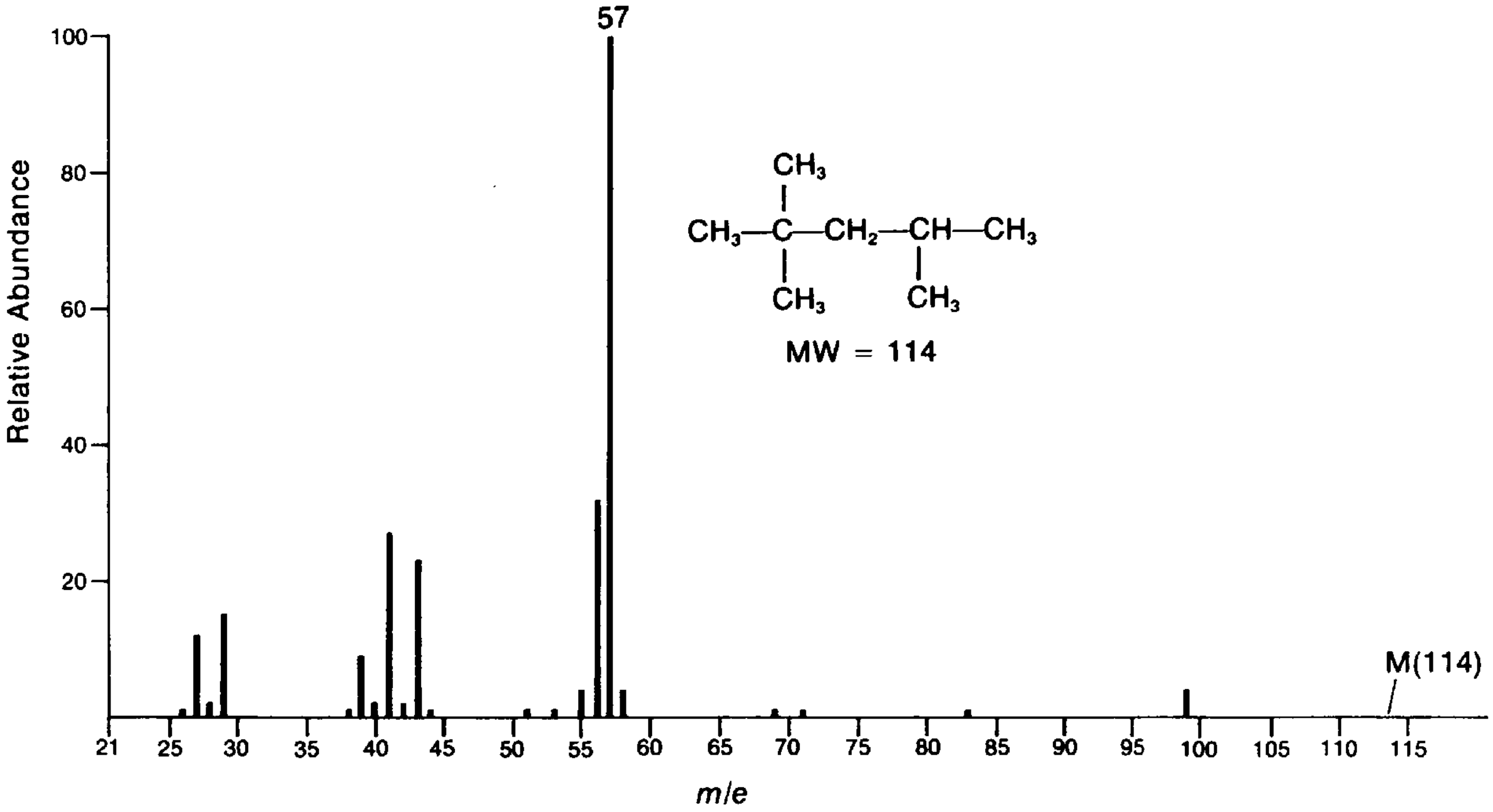
$CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3^{\cdot +}$

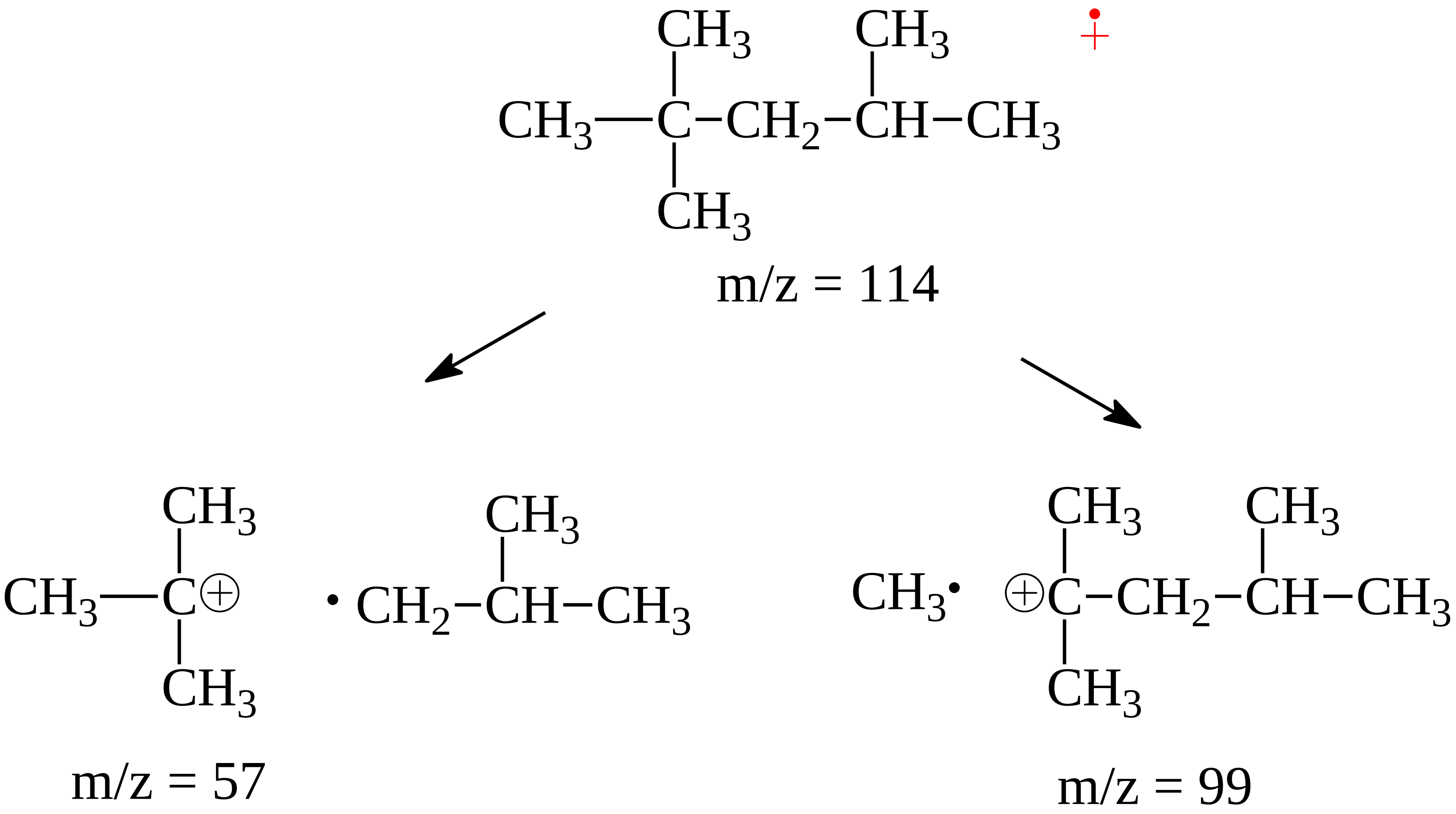
m/z = 114

linear alkane pattern

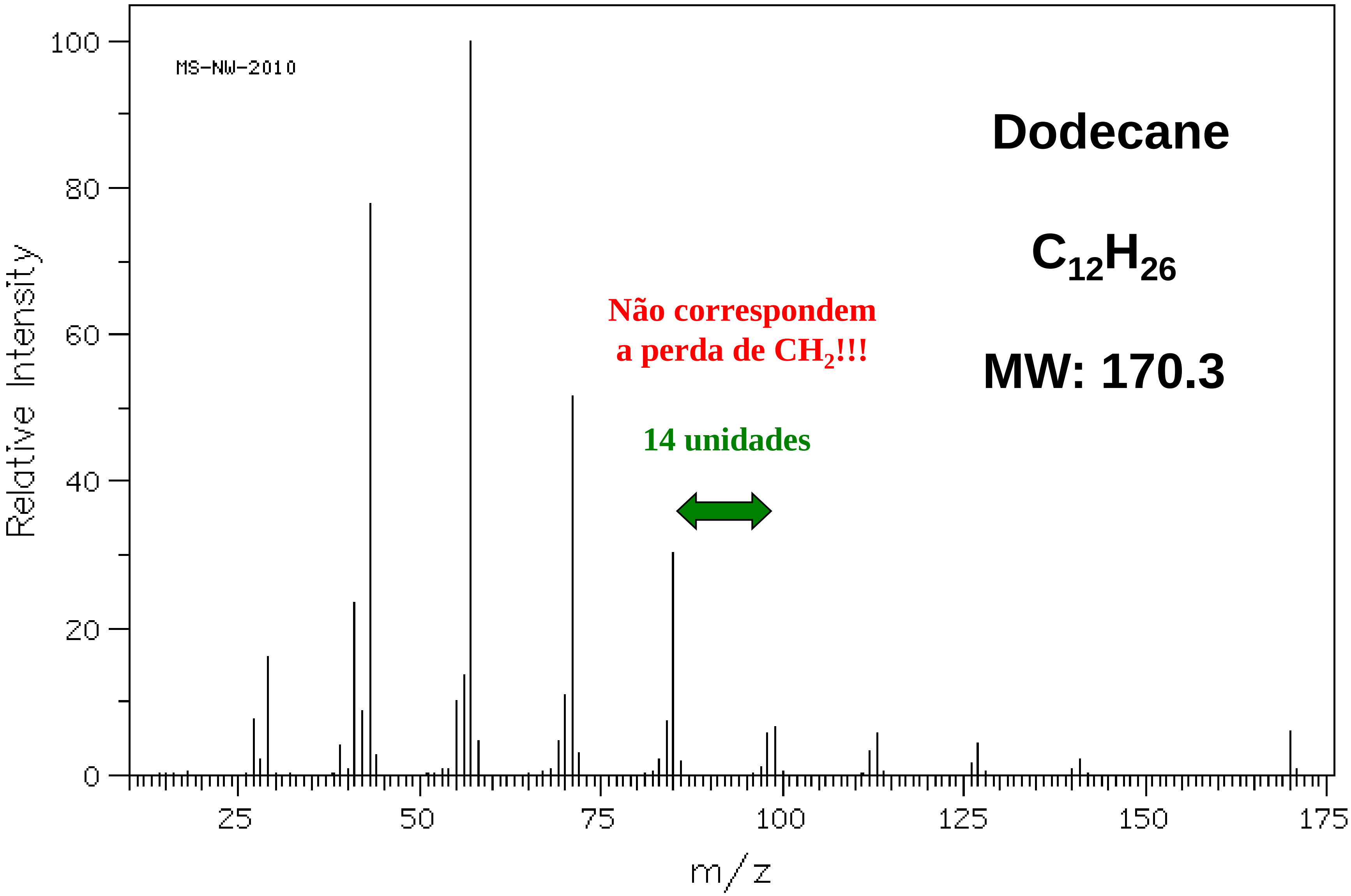
sequential peaks 14 mass units apart

isooctane


57
100
80
60
40
20
Relative Abundance
CH3
CH3—C—CH2—CH—CH3
CH3
CH3
MW = 114
M(114)
21
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
m/e



branched alkane - formation of most stable carbocation



Dodecane

$C_{12}H_{26}$

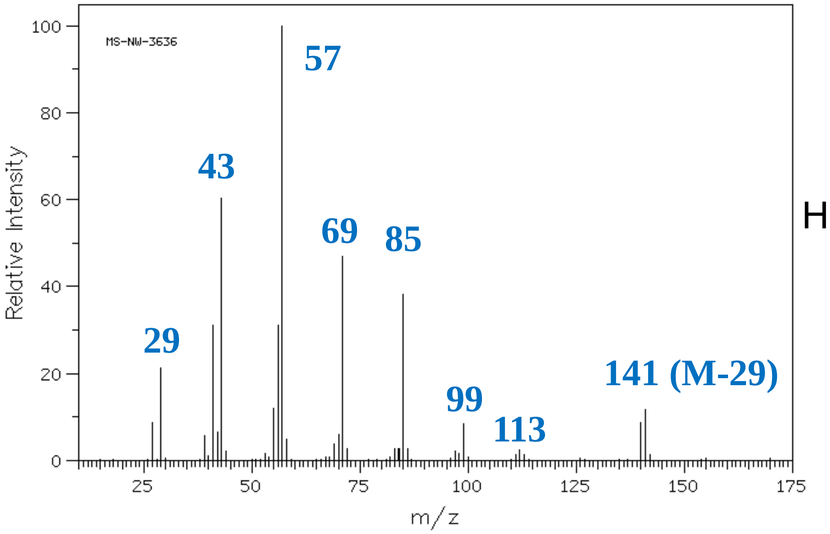
MW: 170.3

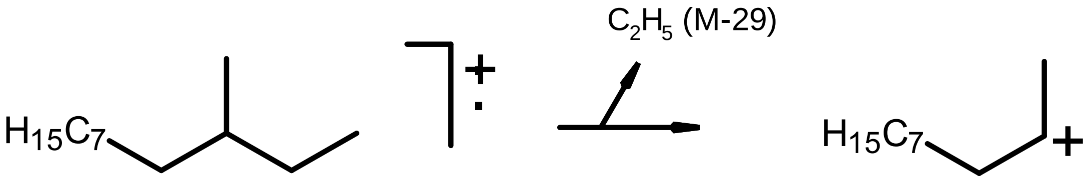
Não correspondem a perda de CH_2!!!

14 unidades

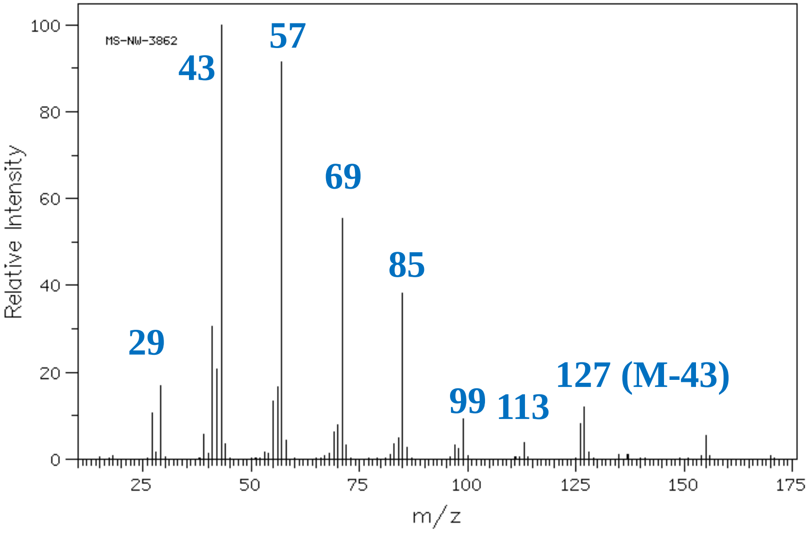
Isomeros ramificados do dodecano
MM 170.33

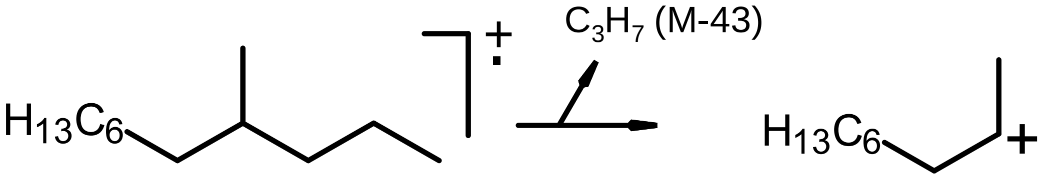
b)

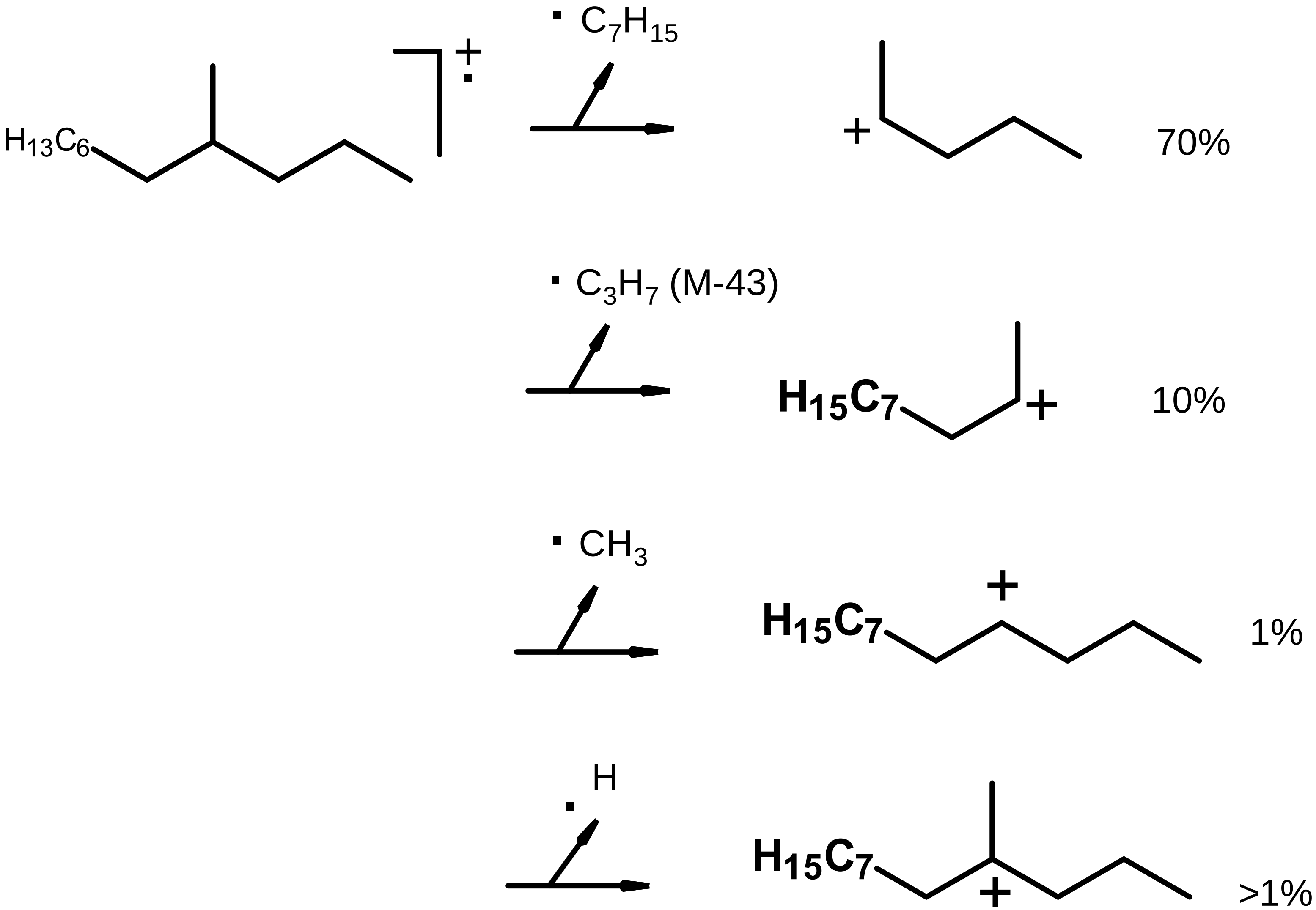


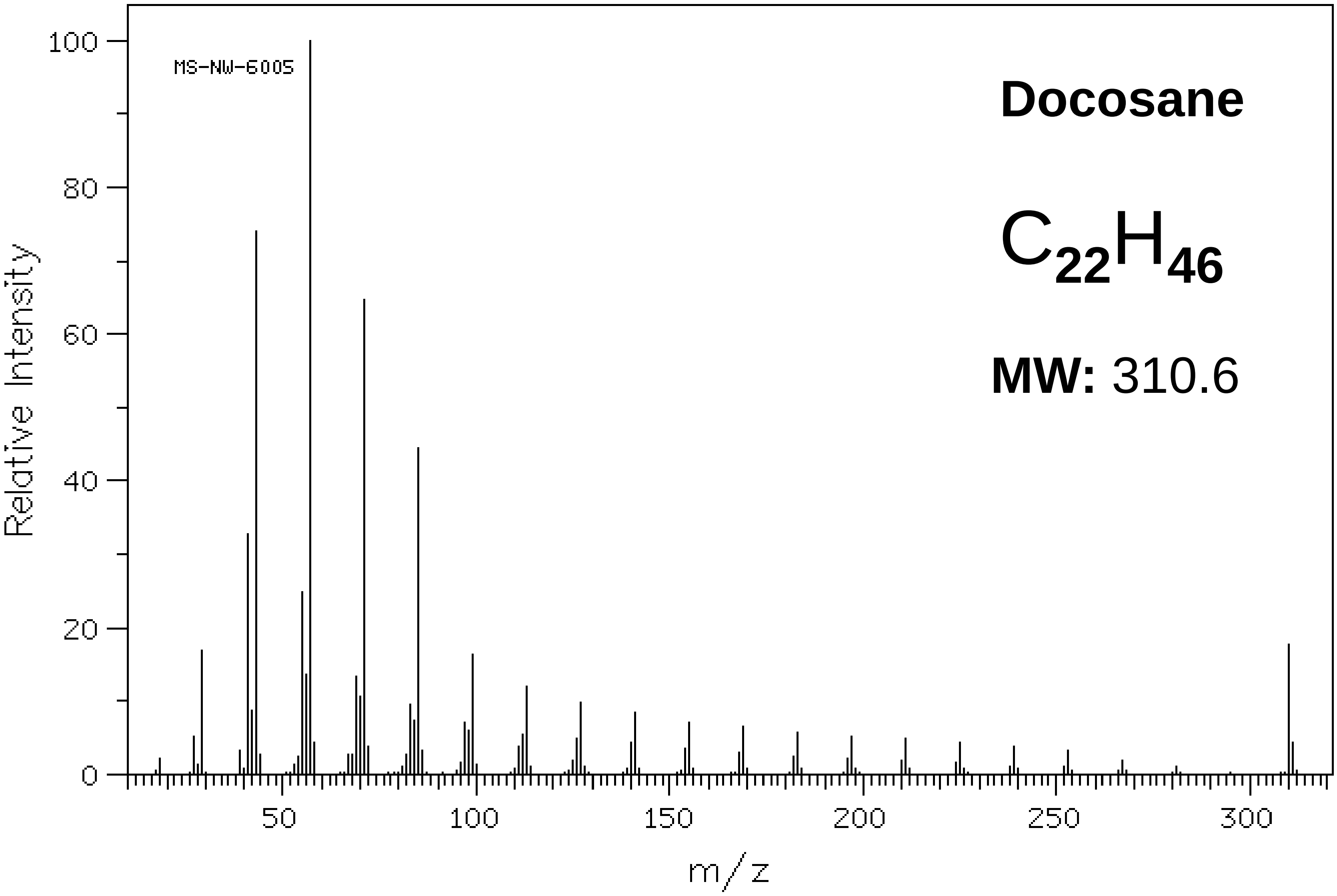


c)



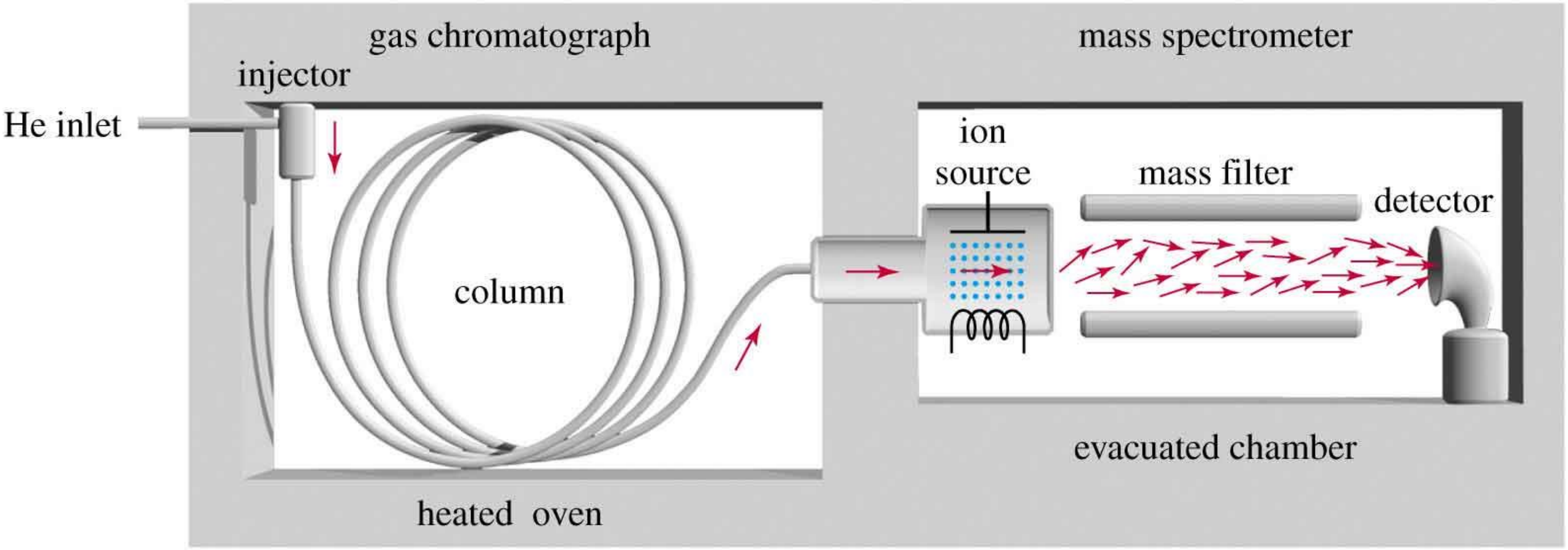



H13C6
· C7H15
70%
· C3H7 (M-43)
H15C7
10%
· CH3
H15C7
1%
· H
H15C7
>1%

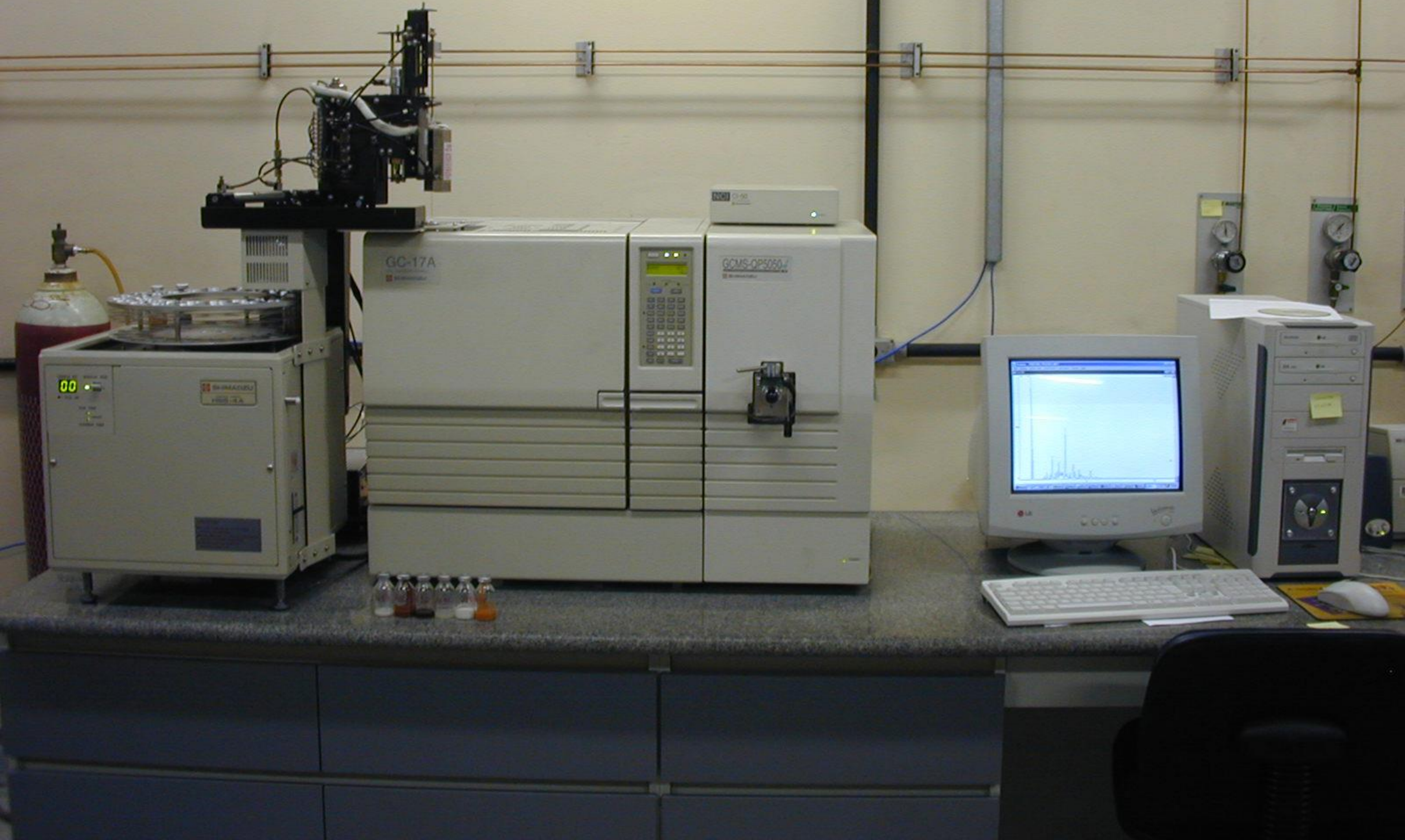

MS-NW-6005
Docosane
$C_{22}H_{46}$
MW: 310.6
Relative Intensity
0
20
40
60
80
100
50
100
150
200
250
300
m/z

GC-MS

A mixture of compounds is separated by gas chromatography, then identified by mass spectrometry.

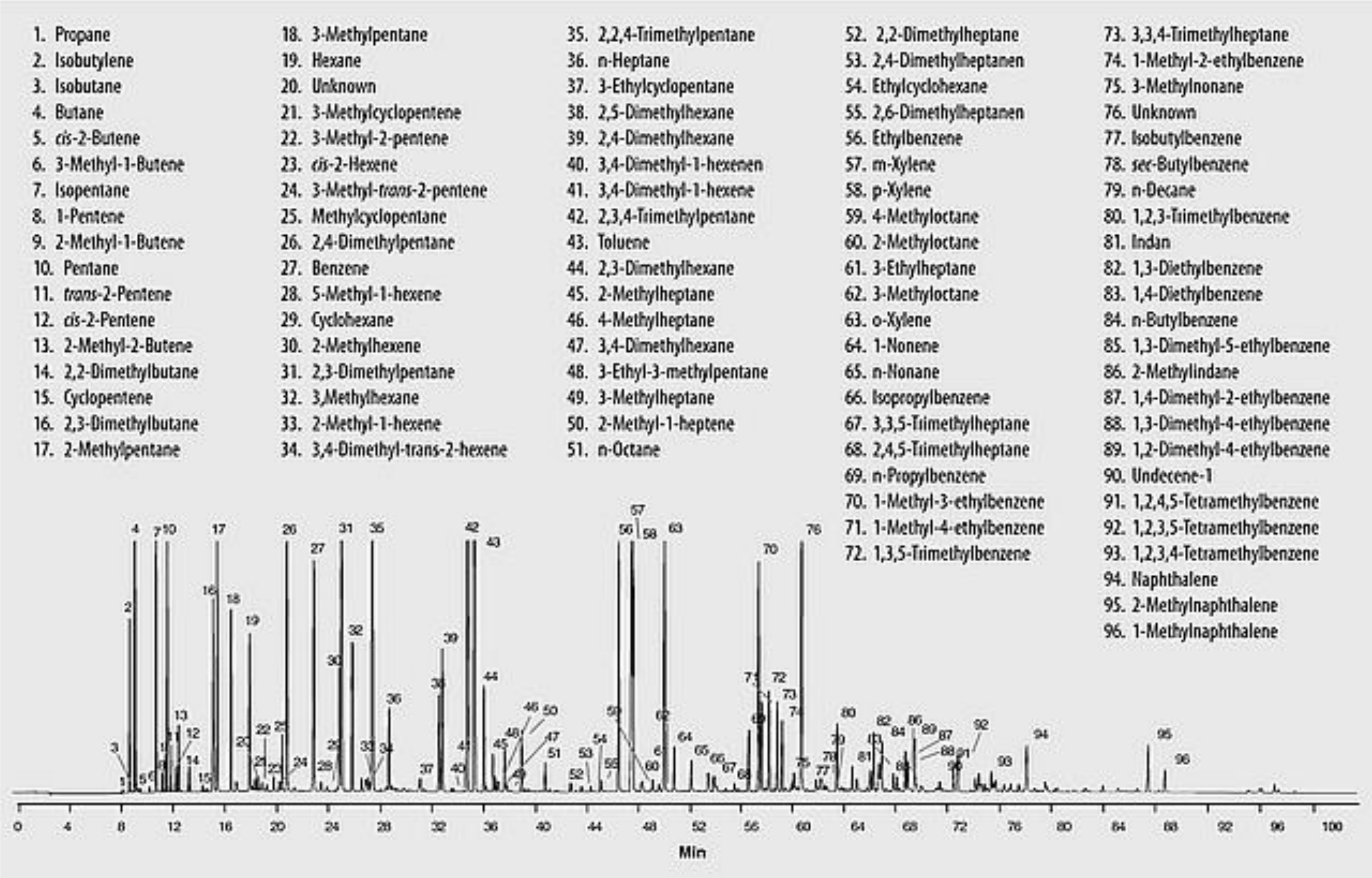


Cromatógrafo a gás acoplado a um Espectrômetro de massas



http://www.unsolvedmysteries.oregonstate.edu/

GC Chromatogram of gasoline



1. Propane
2. Isobutylene
3. Isobutane
4. Butane
5. *cis*-2-Butene
6. 3-Methyl-1-Butene
7. Isopentane
8. 1-Pentene
9. 2-Methyl-1-Butene
10. Pentane
11. *trans*-2-Pentene
12. *cis*-2-Pentene
13. 2-Methyl-2-Butene
14. 2,2-Dimethylbutane
15. Cyclopentene
16. 2,3-Dimethylbutane
17. 2-Methylpentane
18. 3-Methylpentane
19. Hexane
20. Unknown
21. 3-Methylcyclopentene
22. 3-Methyl-2-pentene
23. *cis*-2-Hexene
24. 3-Methyl-*trans*-2-pentene
25. Methylcyclopentane
26. 2,4-Dimethylpentane
27. Benzene
28. 5-Methyl-1-hexene
29. Cyclohexane
30. 2-Methylhexene
31. 2,3-Dimethylpentane
32. 3,Methylhexane
33. 2-Methyl-1-hexene
34. 3,4-Dimethyl-trans-2-hexene
35. 2,2,4-Trimethylpentane
36. n-Heptane
37. 3-Ethylcyclopentane
38. 2,5-Dimethylhexane
39. 2,4-Dimethylhexane
40. 3,4-Dimethyl-1-hexenen
41. 3,4-Dimethyl-1-hexene
42. 2,3,4-Trimethylpentane
43. Toluene
44. 2,3-Dimethylhexane
45. 2-Methylheptane
46. 4-Methylheptane
47. 3,4-Dimethylhexane
48. 3-Ethyl-3-methylpentane
49. 3-Methylheptane
50. 2-Methyl-1-heptene
51. n-Octane
52. 2,2-Dimethylheptane
53. 2,4-Dimethylheptanen
54. Ethylcyclohexane
55. 2,6-Dimethylheptanen
56. Ethylbenzene
57. m-Xylene
58. p-Xylene
59. 4-Methyloctane
60. 2-Methyloctane
61. 3-Ethylheptane
62. 3-Methyloctane
63. o-Xylene
64. 1-Nonene
65. n-Nonane
66. Isopropylbenzene
67. 3,3,5-Trimethylheptane
68. 2,4,5-Trimethylheptane
69. n-Propylbenzene
70. 1-Methyl-3-ethylbenzene
71. 1-Methyl-4-ethylbenzene
72. 1,3,5-Trimethylbenzene
73. 3,3,4-Trimethylheptane
74. 1-Methyl-2-ethylbenzene
75. 3-Methylnonane
76. Unknown
77. Isobutylbenzene
78. *sec*-Butylbenzene
79. n-Decane
80. 1,2,3-Trimethylbenzene
81. Indan
82. 1,3-Diethylbenzene
83. 1,4-Diethylbenzene
84. n-Butylbenzene
85. 1,3-Dimethyl-5-ethylbenzene
86. 2-Methylindane
87. 1,4-Dimethyl-2-ethylbenzene
88. 1,3-Dimethyl-4-ethylbenzene
89. 1,2-Dimethyl-4-ethylbenzene
90. Undecene-1
91. 1,2,4,5-Tetramethylbenzene
92. 1,2,3,5-Tetramethylbenzene
93. 1,2,3,4-Tetramethylbenzene
94. Naphthalene
95. 2-Methylnaphthalene
96. 1-Methylnaphthalene

column: Petrocol DH, **100 m** x 0.25 mm I.D., 0.50 µm (24160-U)

Spectral Data Base (SDBS)

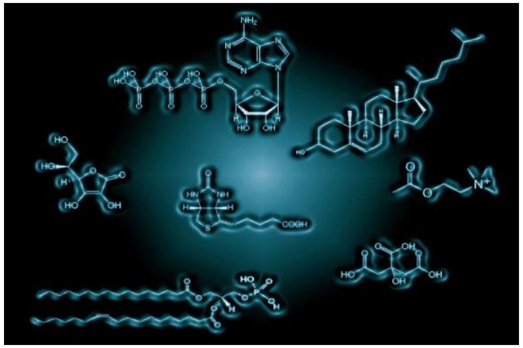
The numbers of the compounds data (May, 2015): ~ 34600

MS: ca 25000 spectra
^{1}H NMR: ca 15900 spectra
^{13}C NMR: ca 14200 spectra
FT-IR: ca 54100 spectra
Raman: ca 3500 spectra
ESR: ca 2000 spectra

http://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi

Additional tools: METABOLOMICS DATA SETS

METLIN
Metabolite &
MS/MS Database



ARM

LIPID MAPS™

NIST
National Institute of
Standards and Technology

hmp
human metabolome project

KEGG
Kyoto Encyclopedia of
Genes and Genomes

KNApSAcK

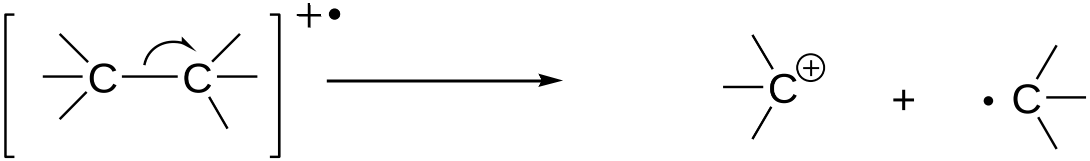
MassBank

Mass Spectrometry

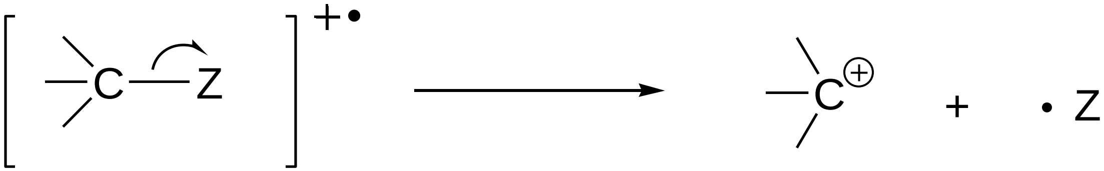
The Mass Spectrum and Structural Analysis

D. Fragmentation – Chemistry of Ions

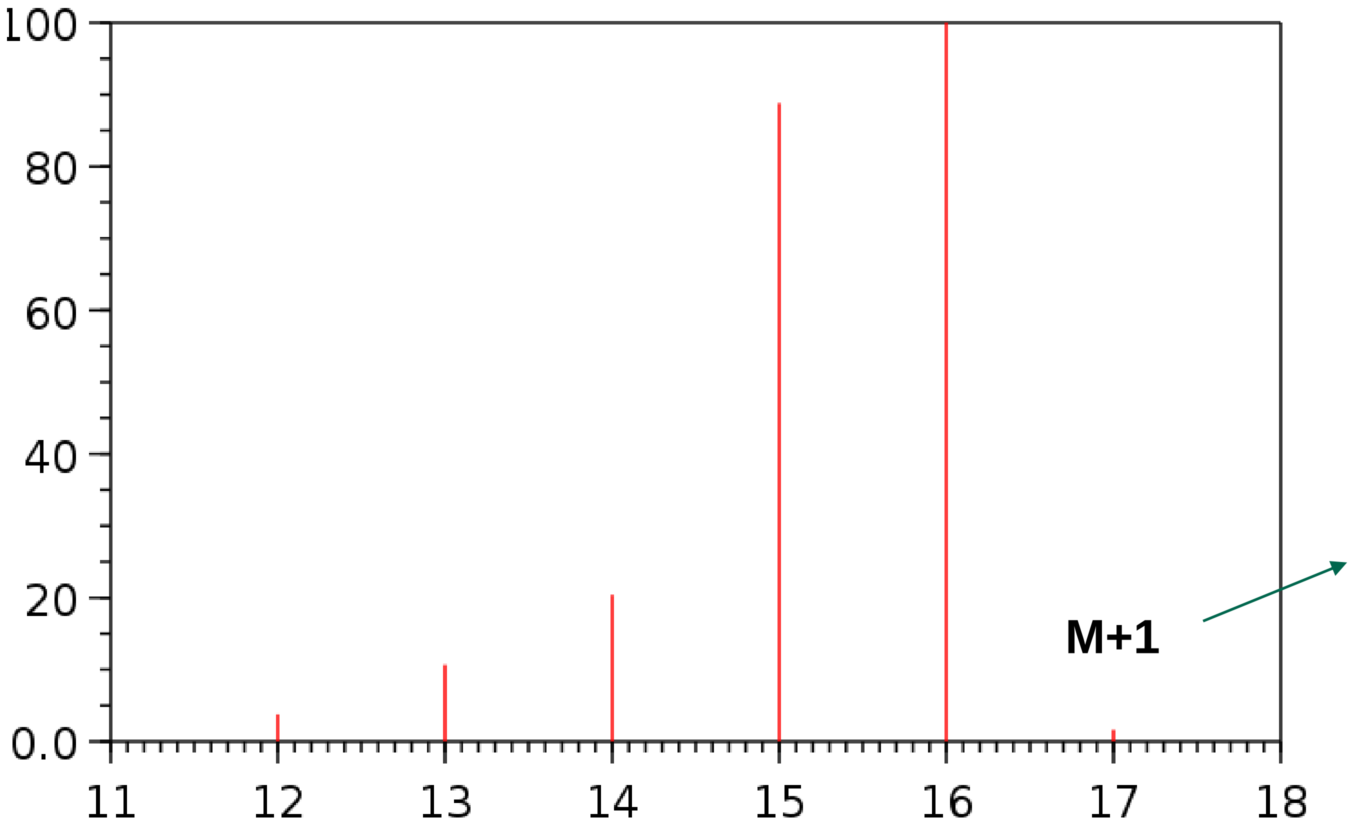
- One bond σ-cleavages:
  - a. cleavage of C-C



  - b. cleavage of C-heteroatom



Espectro de massas do metano (CH_4)



Pico base e
Íon molecular MM 16

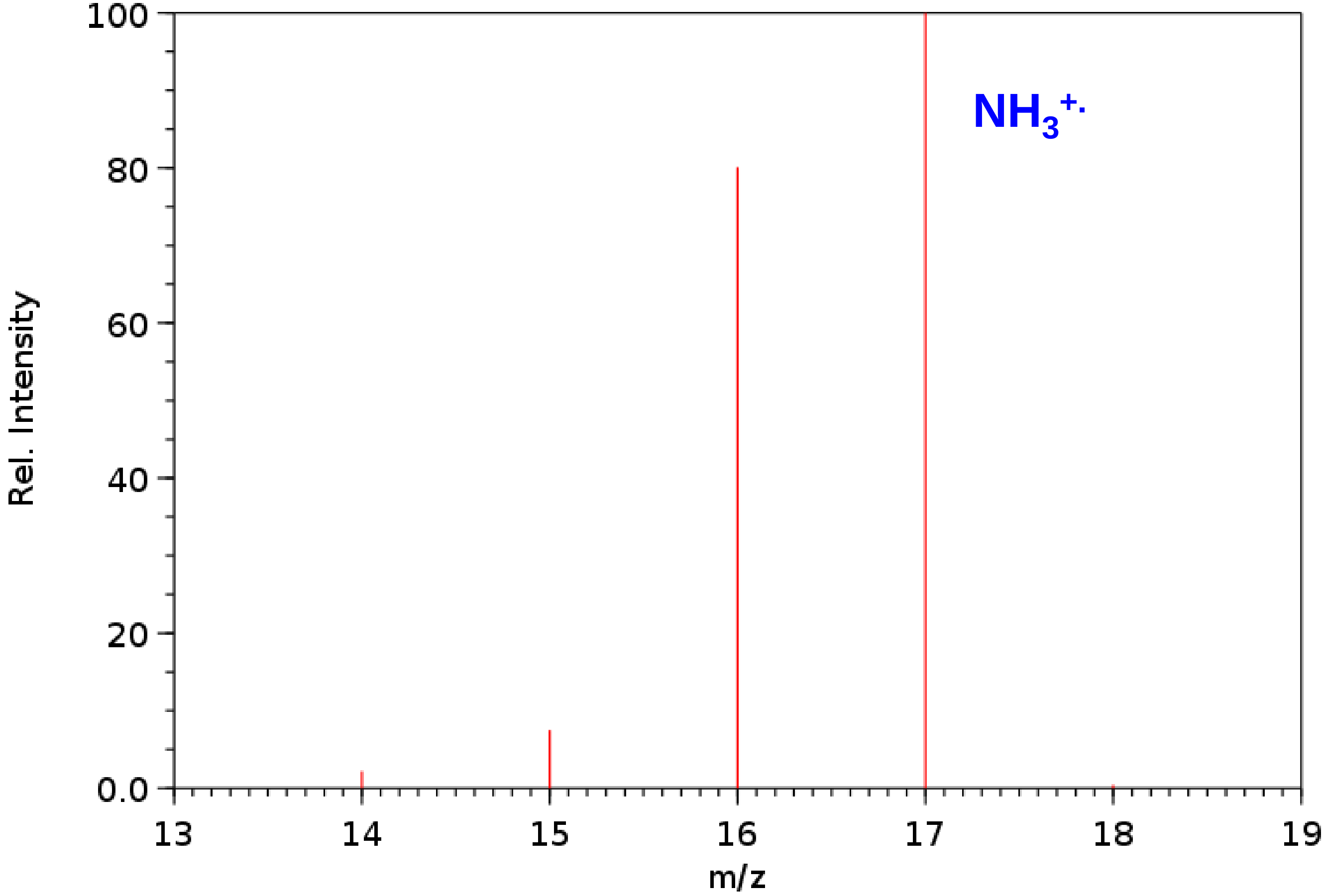
$M^{\cdot+}$

M+1

Contribuição
Isotópica do
^{13}C ($^{13}CH_4$)
(1,1%)

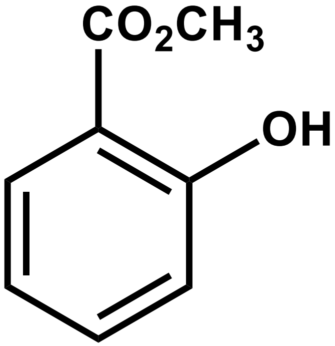
Espectro de massas da amônia (NH_3)

Pico base e
Íon molecular MM 17 (impar)



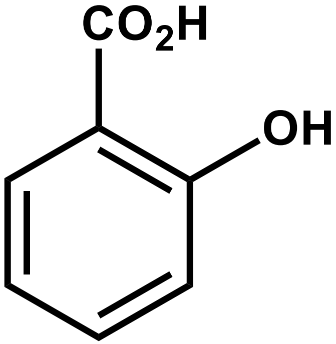
NIST Chemistry WebBook (https://webbook.nist.gov/chemistry)

Como caracterizar os derivados do ácido com base nos íons moleculares?



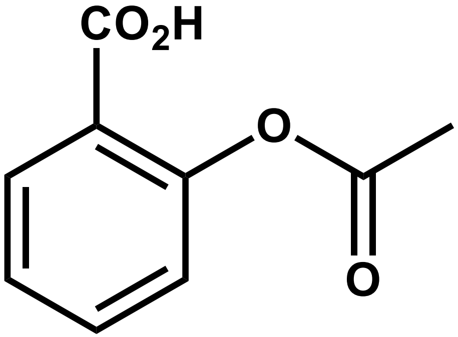
$C_8H_8O_3$

m/z 152



$C_7H_6O_3$

m/z 138

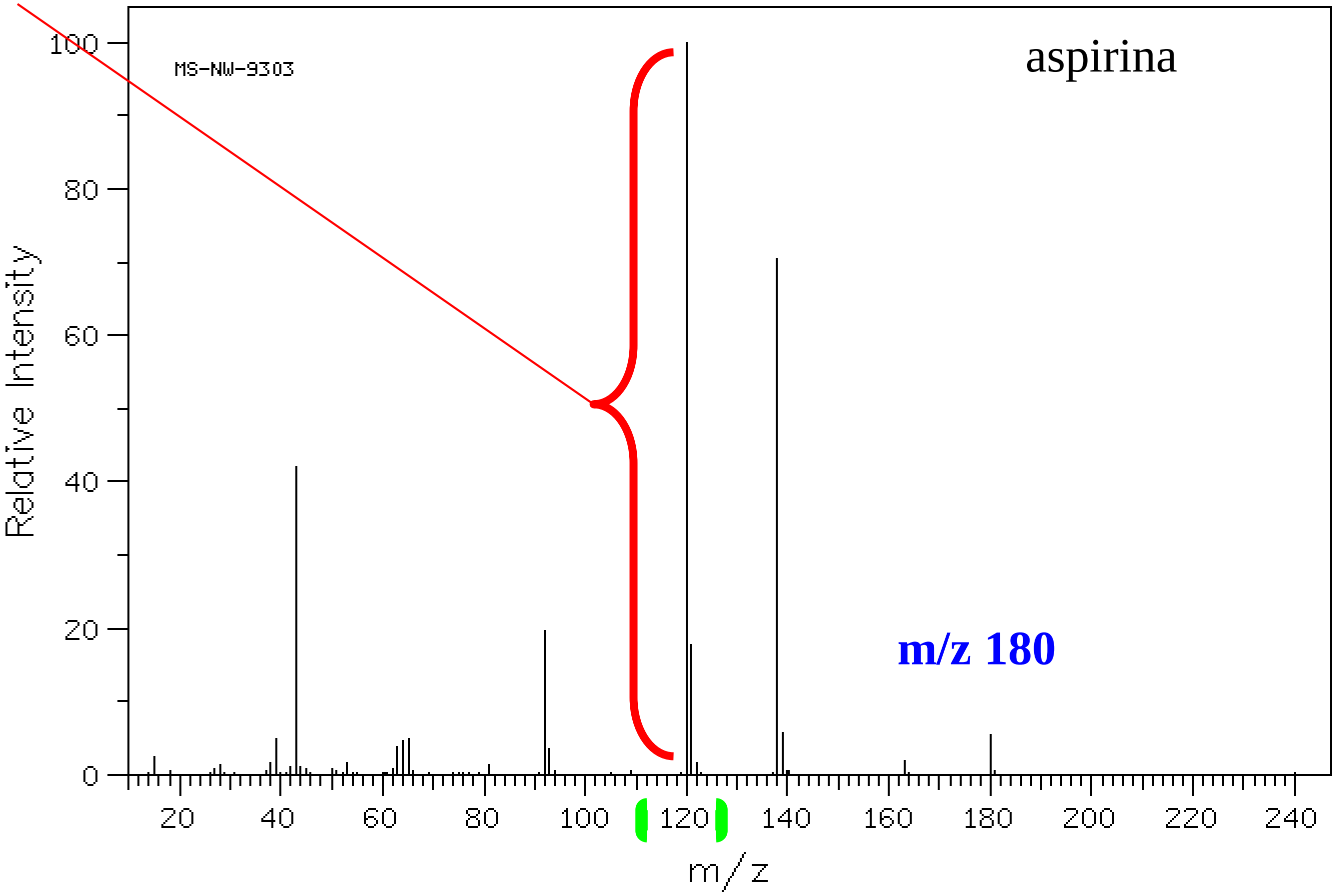


$C_9H_8O_4$

m/z 180

Espectro de massas de baixa resolução da aspirina

Abundância relativa



High Resolution MS

- Masses measured to 1 part in 20,000.
- A molecule with mass of 44 could be C_3H_8, C_2H_4O, CO_2, or CN_2H_4.
- If a more exact mass is 44.029, pick the correct structure from the table:

C_3H_8	C_2H_4O	CO_2	CN_2H_4
44.06260	44.02620	43.98983	44.03740

High Resolution Mass Spectrometers:

- Low resolution mass spectrometers report *m/z* values to the nearest whole number. Thus, the mass of a given molecular ion can correspond to many different molecular formulas.

- High resolution mass spectrometers measure *m/z* ratios to four (or more) decimal places.

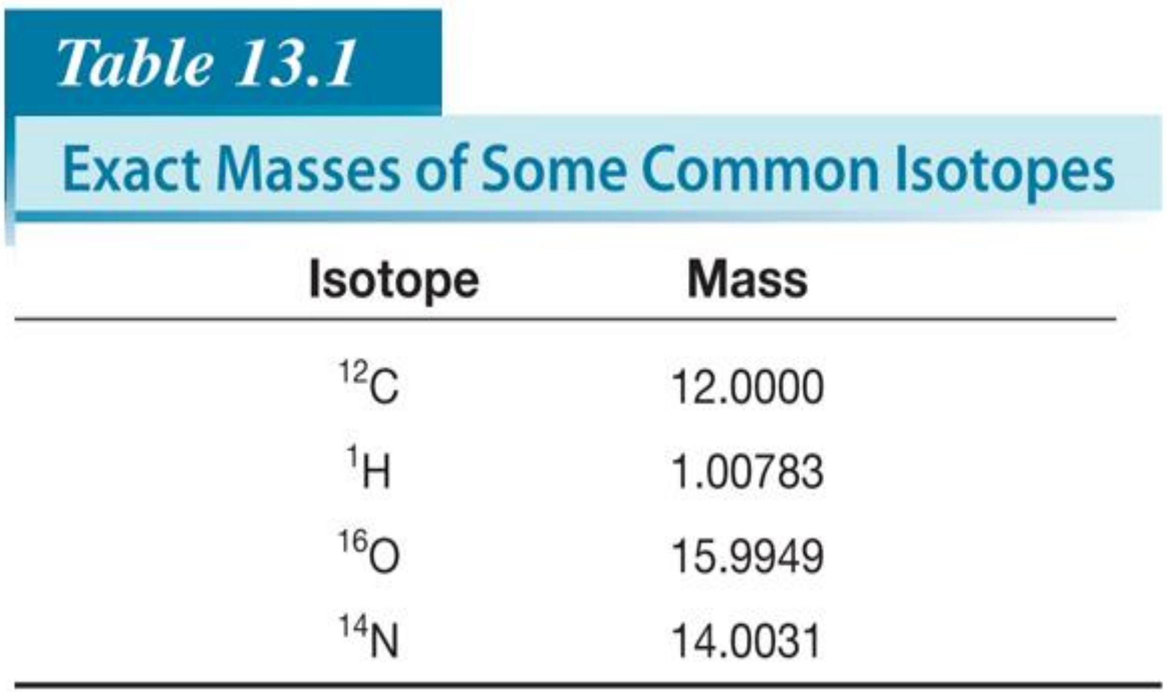


Table 13.1

Exact Masses of Some Common Isotopes

Isotope	Mass
^{12}C	12.0000
^{1}H	1.00783
^{16}O	15.9949
^{14}N	14.0031

Resolução em espectrometria de massas

- **Resolução capaz de distinguir íons com massas diferentes**

$$R = \frac{m}{\Delta m}$$

C_3H_8O	$C_2H_8N_2$	$C_2H_4O_2$	CH_4N_2O
60,05754	60,06884	60,02112	60,03242

- **Resolução necessária para distinguir estas espécies: considerar os íons com a menor diferença de massas, $\Delta m = 60{,}03242 - 60{,}02112 = 0{,}0113$**

$$R = \frac{60}{0{,}0113} = 5310$$

Measuring peak separation

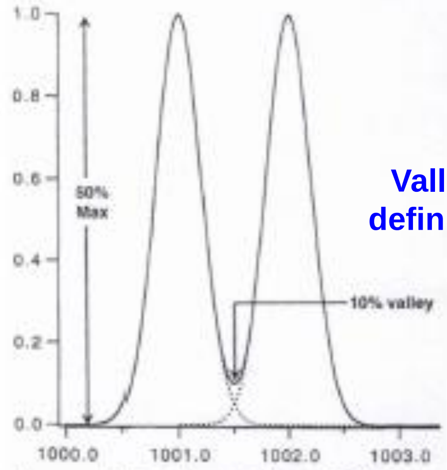


Figure 1-13. Illustration of 10% valley definition of resolving power.

Valley definition

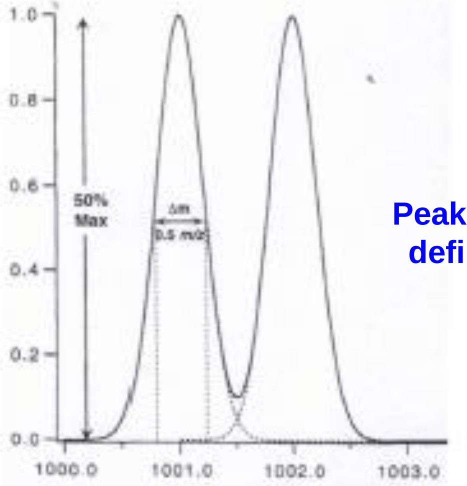
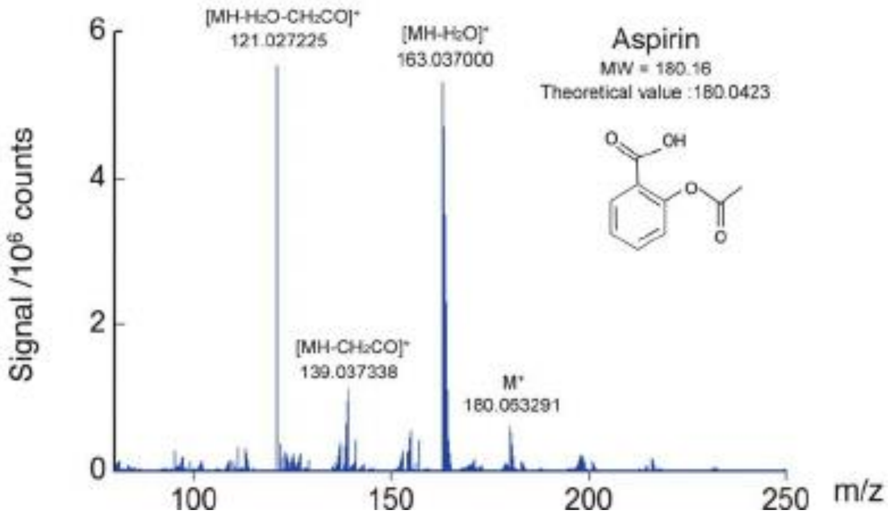


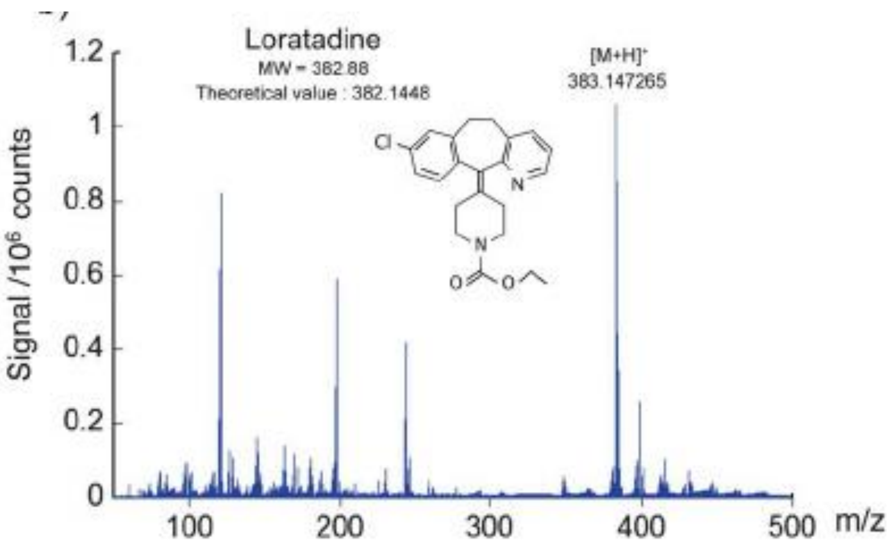
Figure 1-14. Illustration of FWHM definition of resolving power.

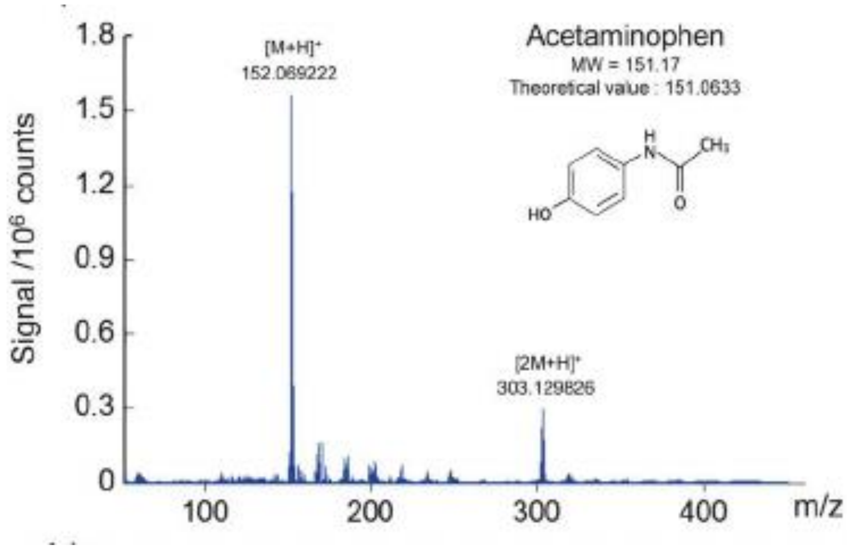
Peak width definition

FWHM: Full width at half maximum

Espectro de massas de alta resolução



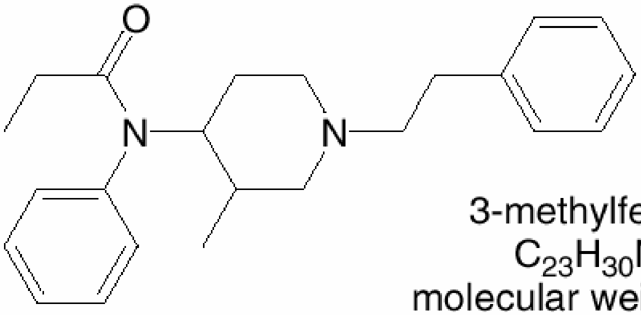




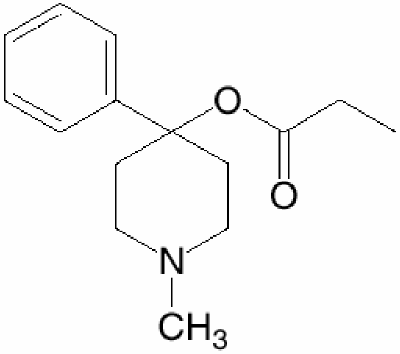
Regra do Nitrogênio para determinação da fórmula molecular

- Hidrocarbonetos como metano (CH_4) e hexano (C_6H_{14}), bem como oa compostos que contêm somente átomos de C, H e O, sempre possuem os íons moleculares com valor **pares**

- Um íon molecular **ímpar** indica a presença de **número de átomos de nitrogênio ímpares.**



3-methylfentanyl
$C_{23}H_{30}N_2O$
molecular weight = 350



MPPP
(1-methyl-4-phenyl-4-propionoxypiperidine)
$C_{15}H_{21}NO_2$
molecular weight = 247

Isotopic Abundance

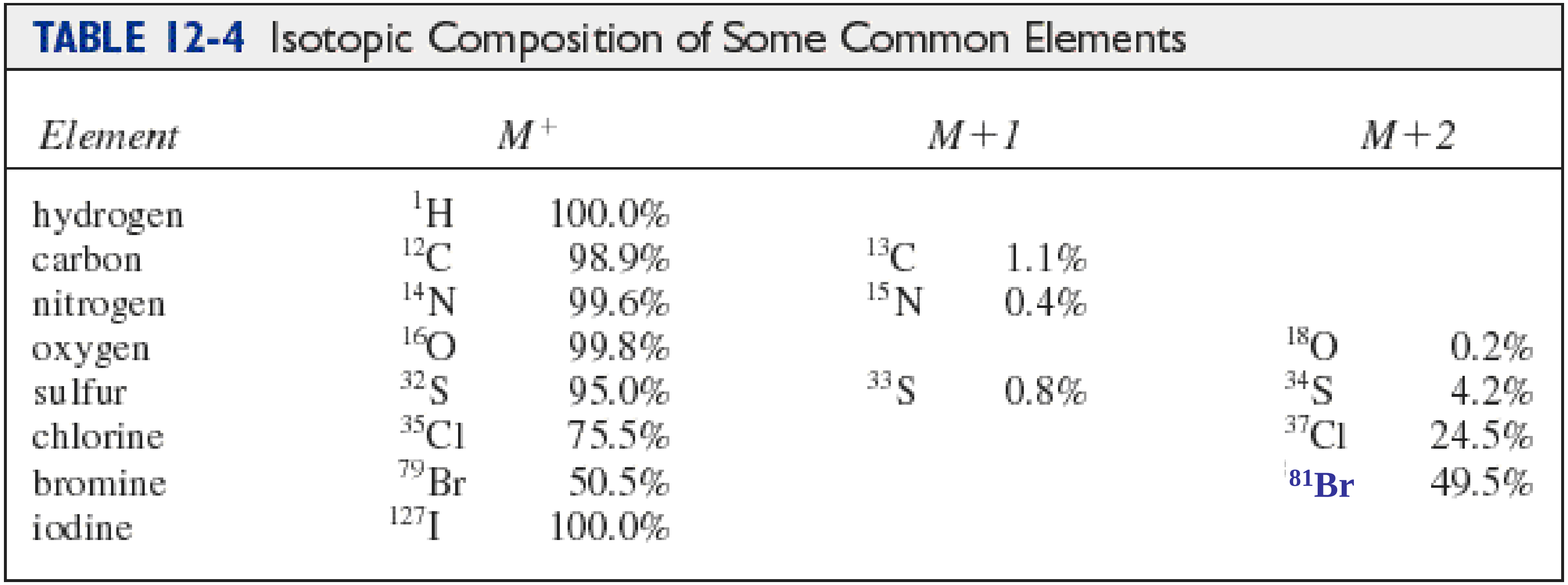
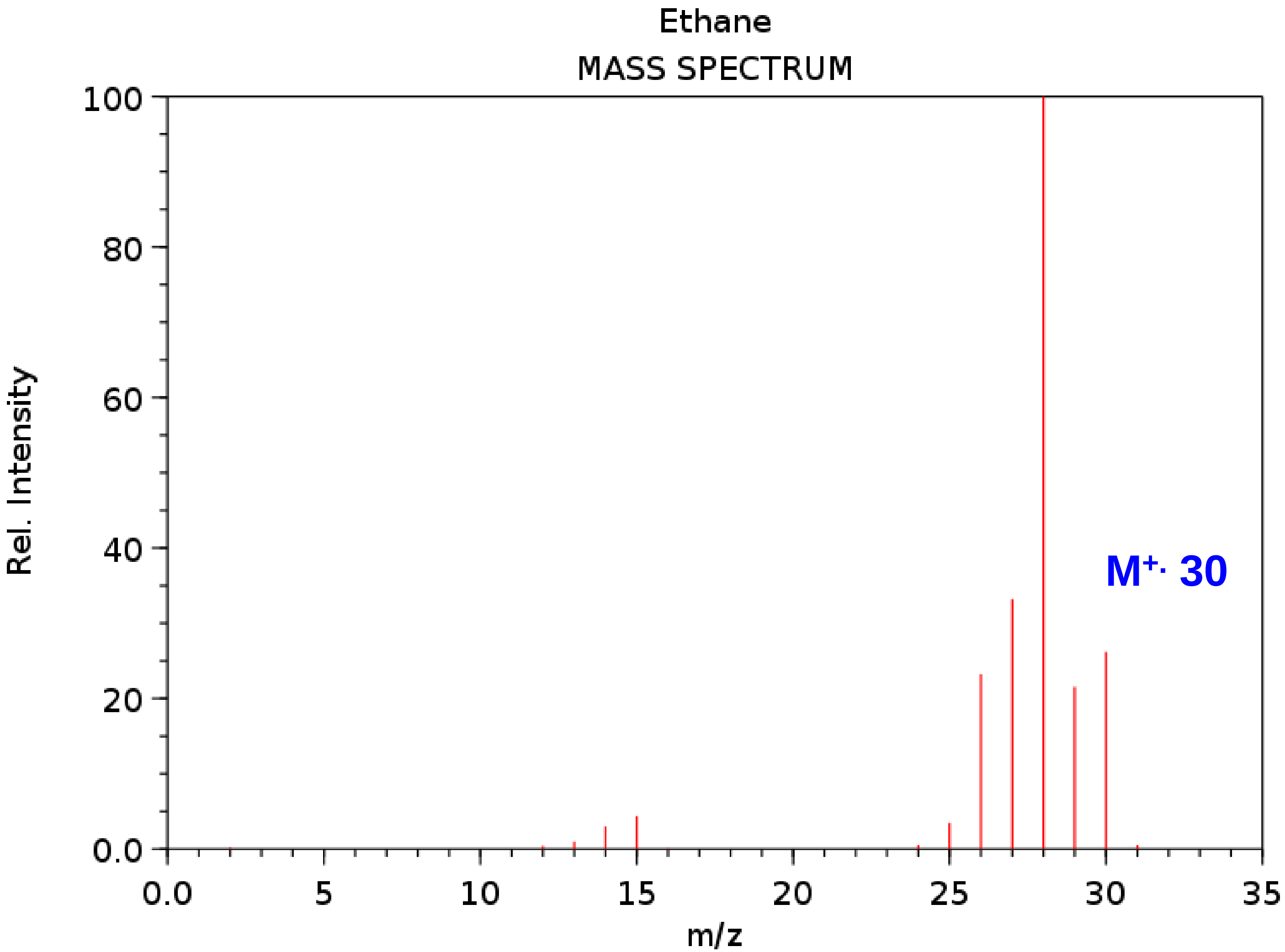


TABLE 12-4 Isotopic Composition of Some Common Elements

Element	M^+		$M+1$		$M+2$	
hydrogen	^{1}H	100.0%				
carbon	^{12}C	98.9%	^{13}C	1.1%		
nitrogen	^{14}N	99.6%	^{15}N	0.4%		
oxygen	^{16}O	99.8%			^{18}O	0.2%
sulfur	^{32}S	95.0%	^{33}S	0.8%	^{34}S	4.2%
chlorine	^{35}Cl	75.5%			^{37}Cl	24.5%
bromine	^{79}Br	50.5%			^{81}Br	49.5%
iodine	^{127}I	100.0%				

=>

Espectro de massas do etano (C_2H_6)

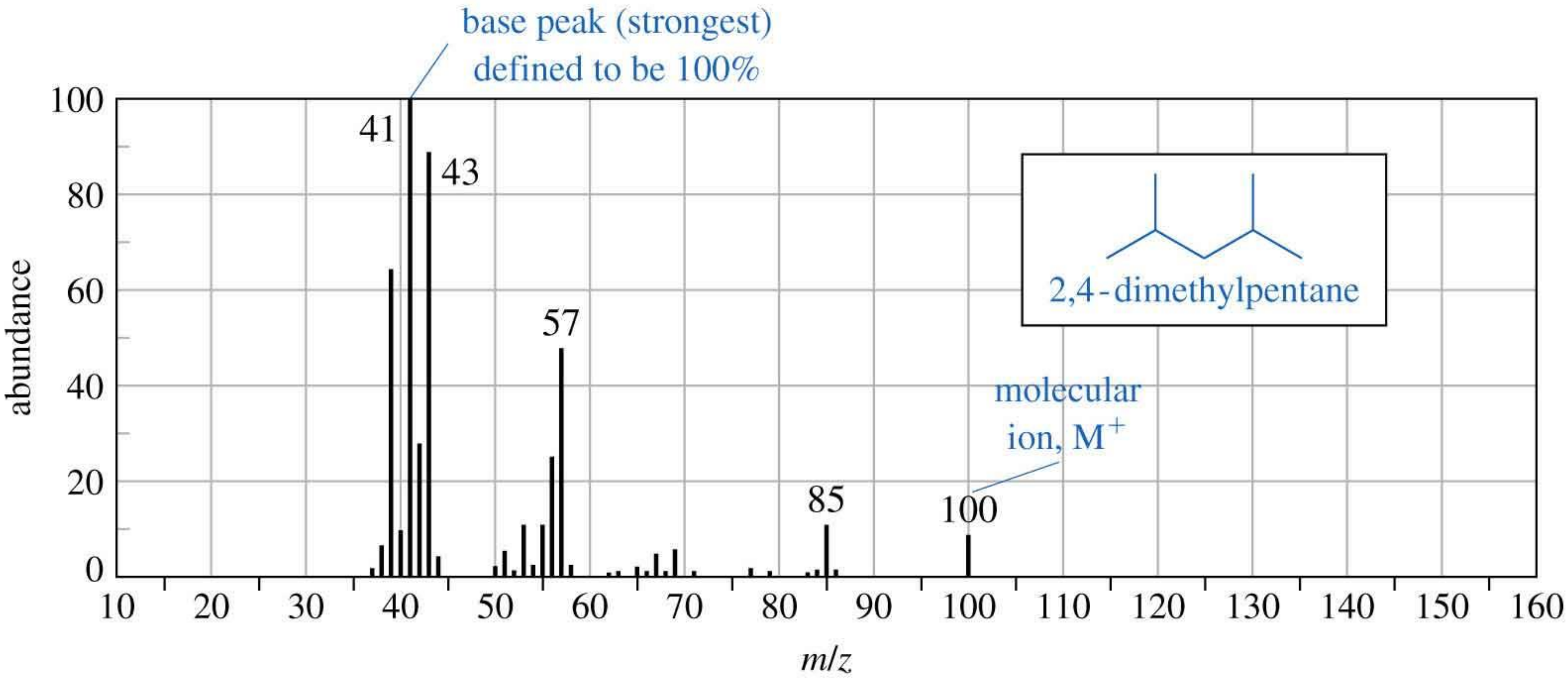


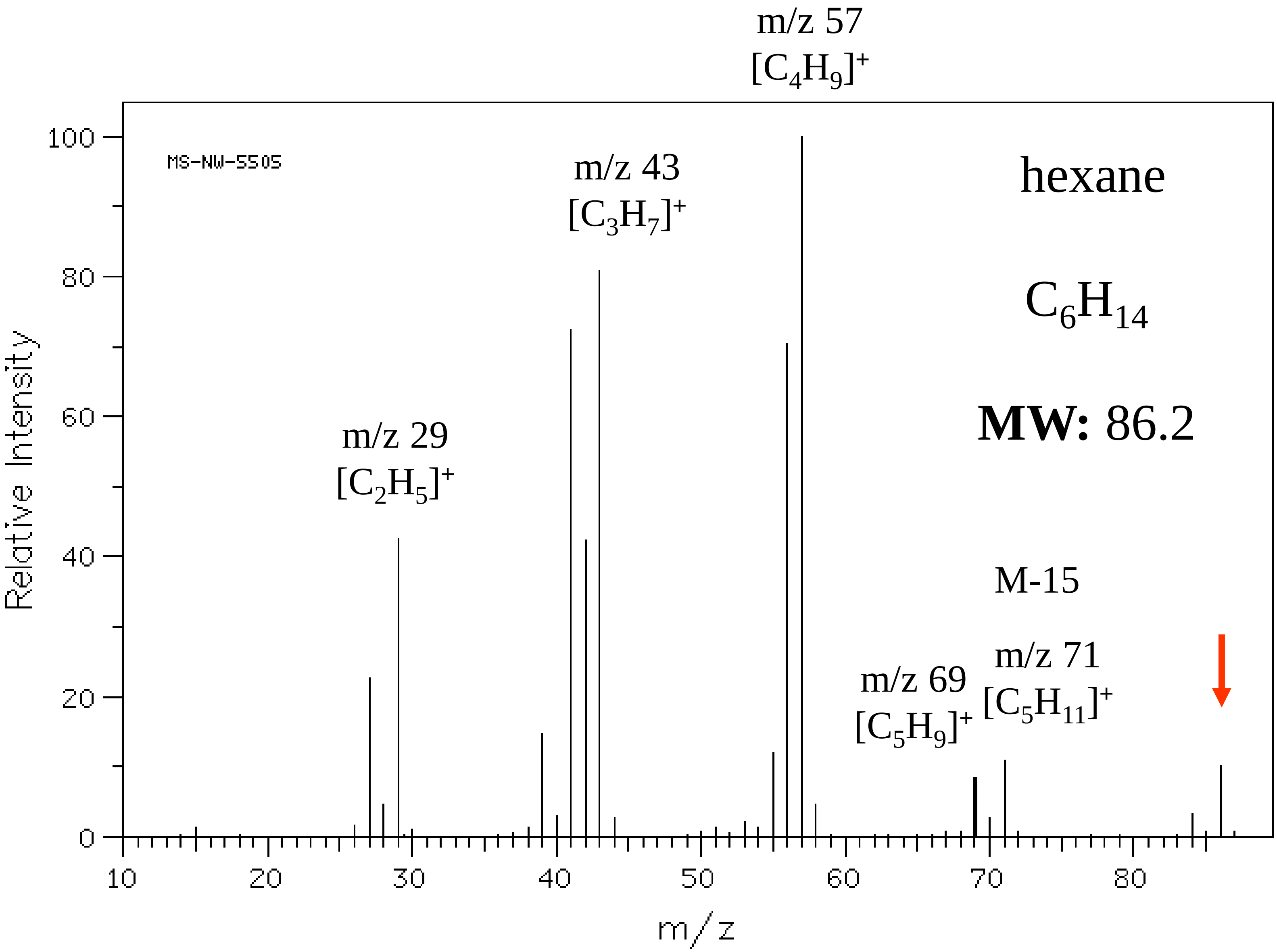
$M^{+\cdot}$ 30

NIST Chemistry WebBook (https://webbook.nist.gov/chemistry)

Espectro de massas

Os íons são detectados são registrados de acordo com a sua abundância relativa





m/z 57
$[C_4H_9]^+$

m/z 43
$[C_3H_7]^+$

m/z 29
$[C_2H_5]^+$

hexane

C_6H_{14}

MW: 86.2

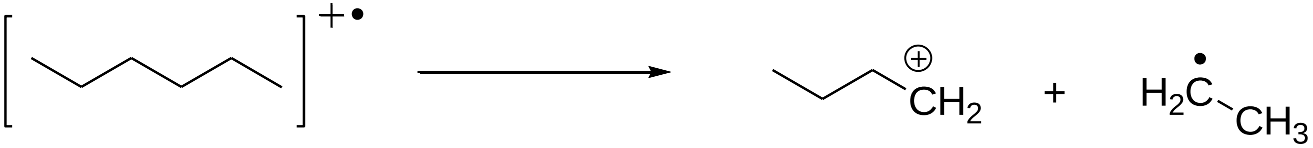
M-15

m/z 71
$[C_5H_{11}]^+$

m/z 69
$[C_5H_9]^+$

The Mass Spectrum and Structural Analysis
Fragmentation Patterns of Groups

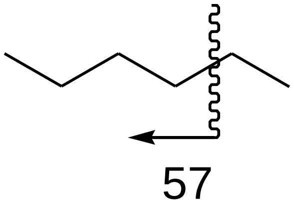
Aside: Some nomenclature – rather than explicitly writing out single bond cleavages each time:



Fragment obs. by MS

Neutral fragment inferred by its loss – not observed

Is written as:



The Mass Spectrum and Structural Analysis

Fragmentation Patterns of Groups

- Alkanes

Fragment Ions : *n*-alkanes

» For straight chain alkanes, a $M^{\dot{+}}$ is often observed

» Ions observed: clusters of peaks C_nH_{2n+1} apart from the loss of $–CH_3$, $-C_2H_5$, $-C_3H_7$, etc.

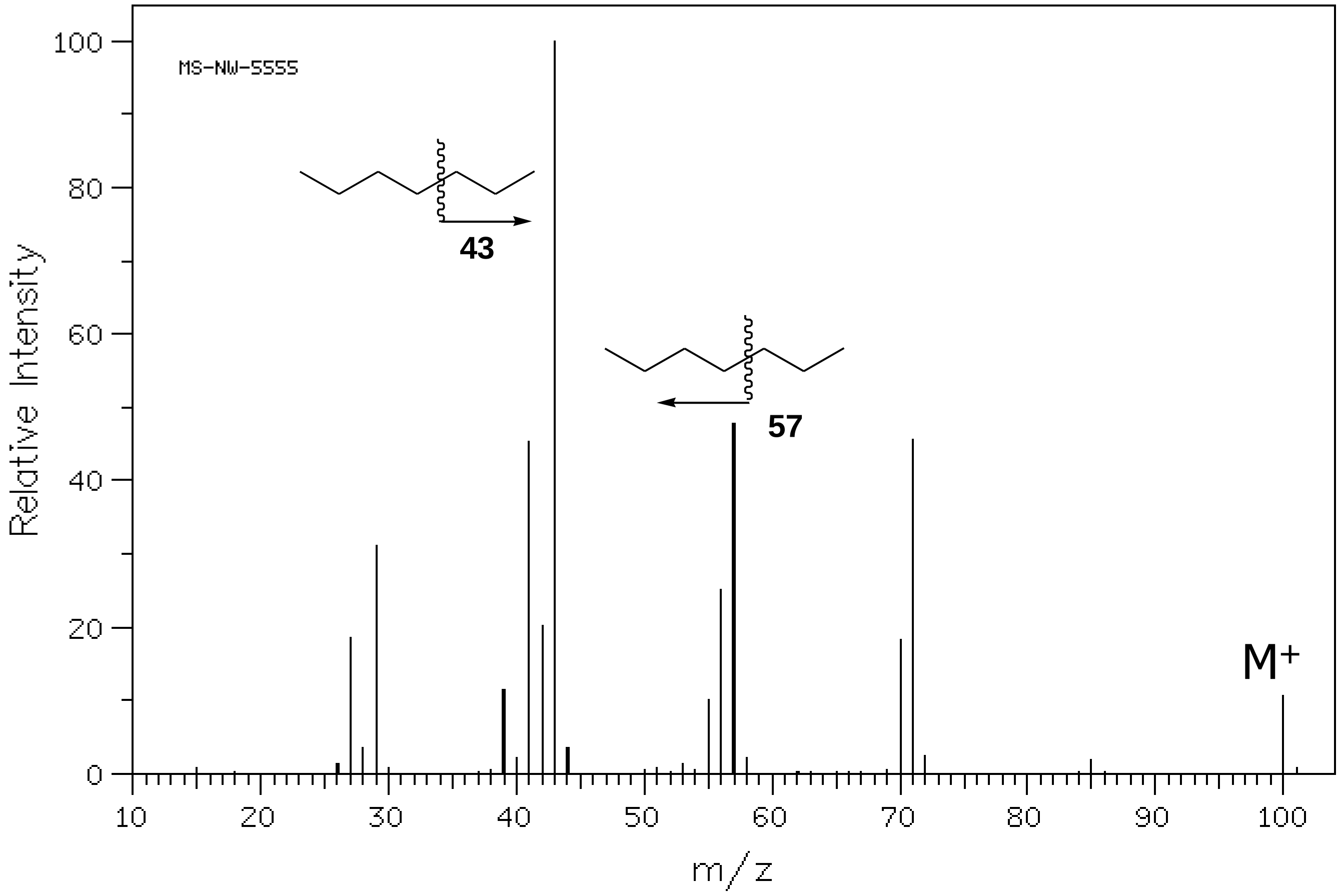
» Fragments lost: $\cdot CH_3$, $\cdot C_2H_5$, $\cdot C_3H_7$, etc.

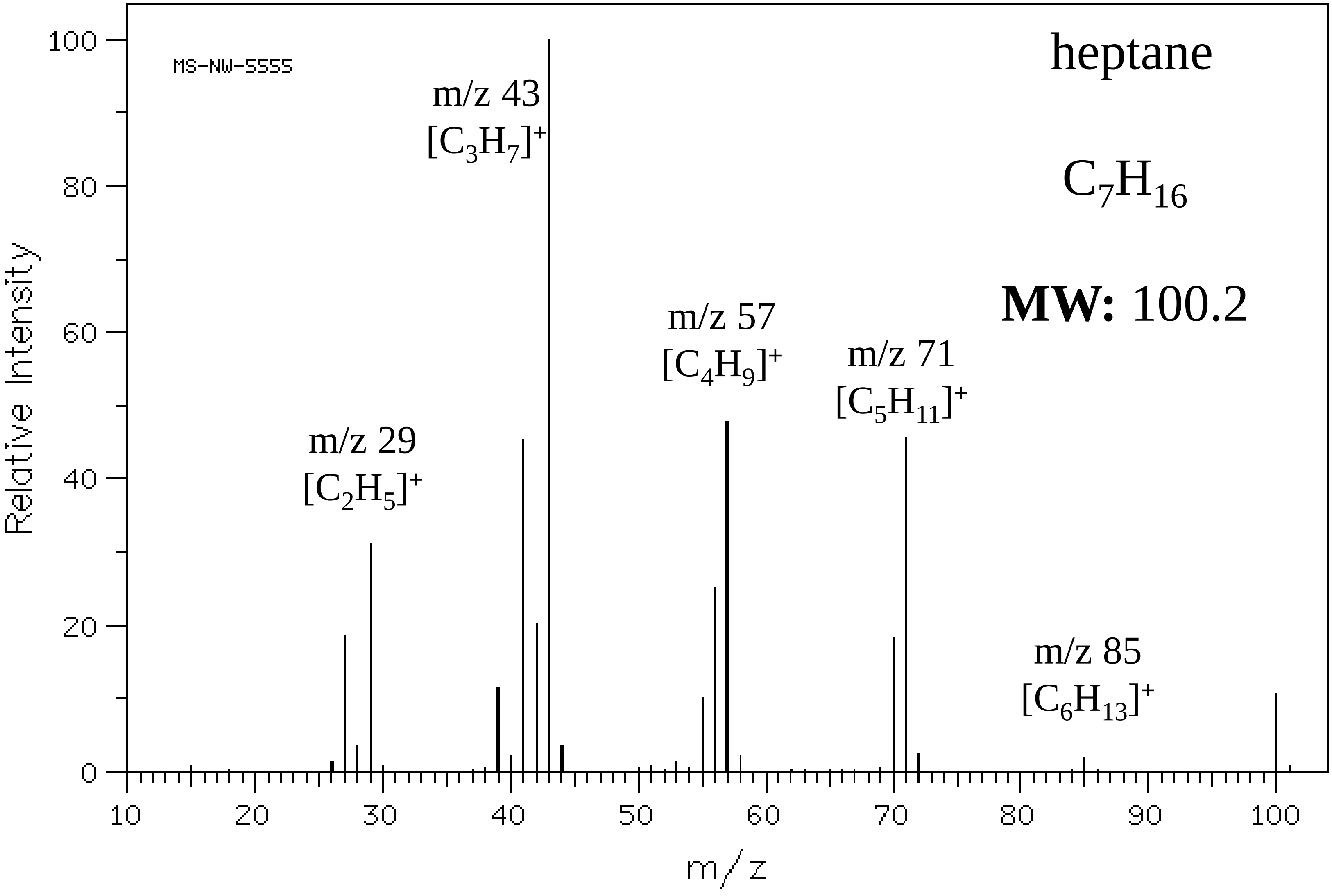
» In longer chains – peaks at 43 and 57 are the most common

The Mass Spectrum and Structural Analysis

Fragmentation Patterns of Groups

- ***n*-alkanes** – *n*-heptane

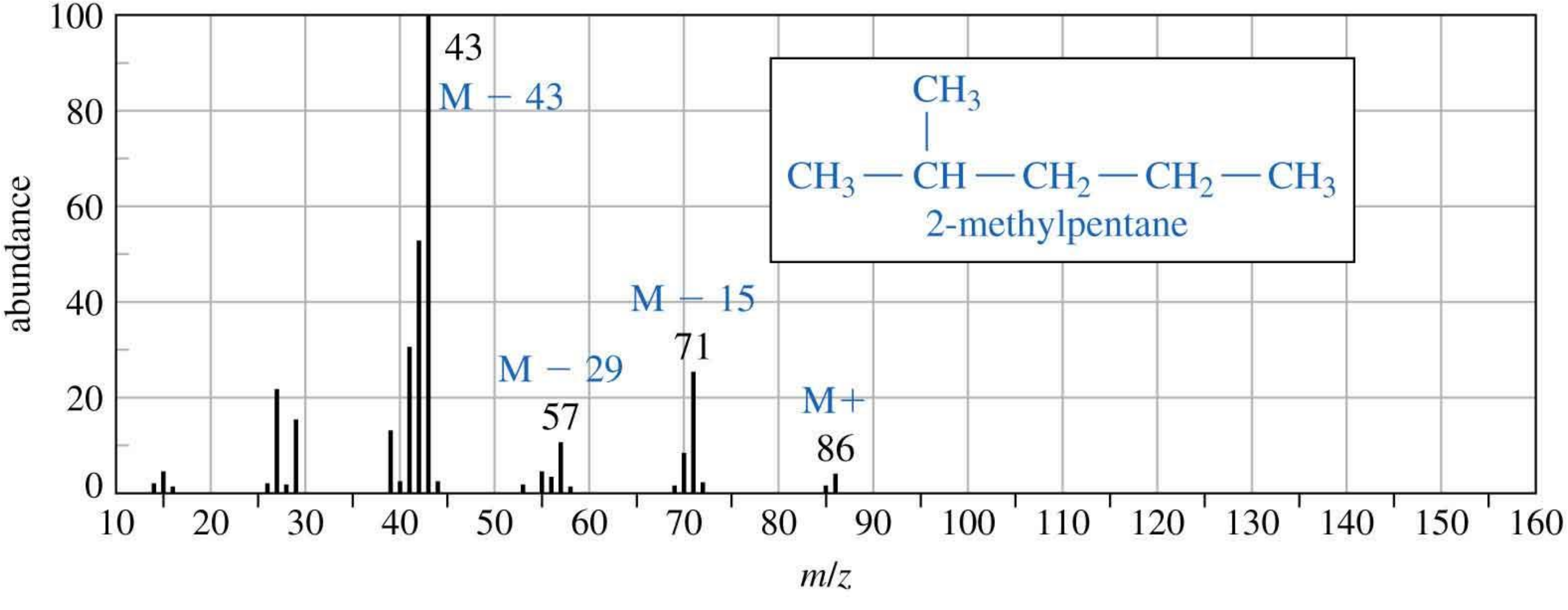




MS-NW-5555
heptane
C_7H_{16}
MW: 100.2
m/z 43
$[C_3H_7]^+$
m/z 29
$[C_2H_5]^+$
m/z 57
$[C_4H_9]^+$
m/z 71
$[C_5H_{11}]^+$
m/z 85
$[C_6H_{13}]^+$
Relative Intensity
0
20
40
60
80
100
10
20
30
40
50
60
70
80
90
100
m/z

Mass Spectra of Alkanes

More stable carbocations will be more abundant!

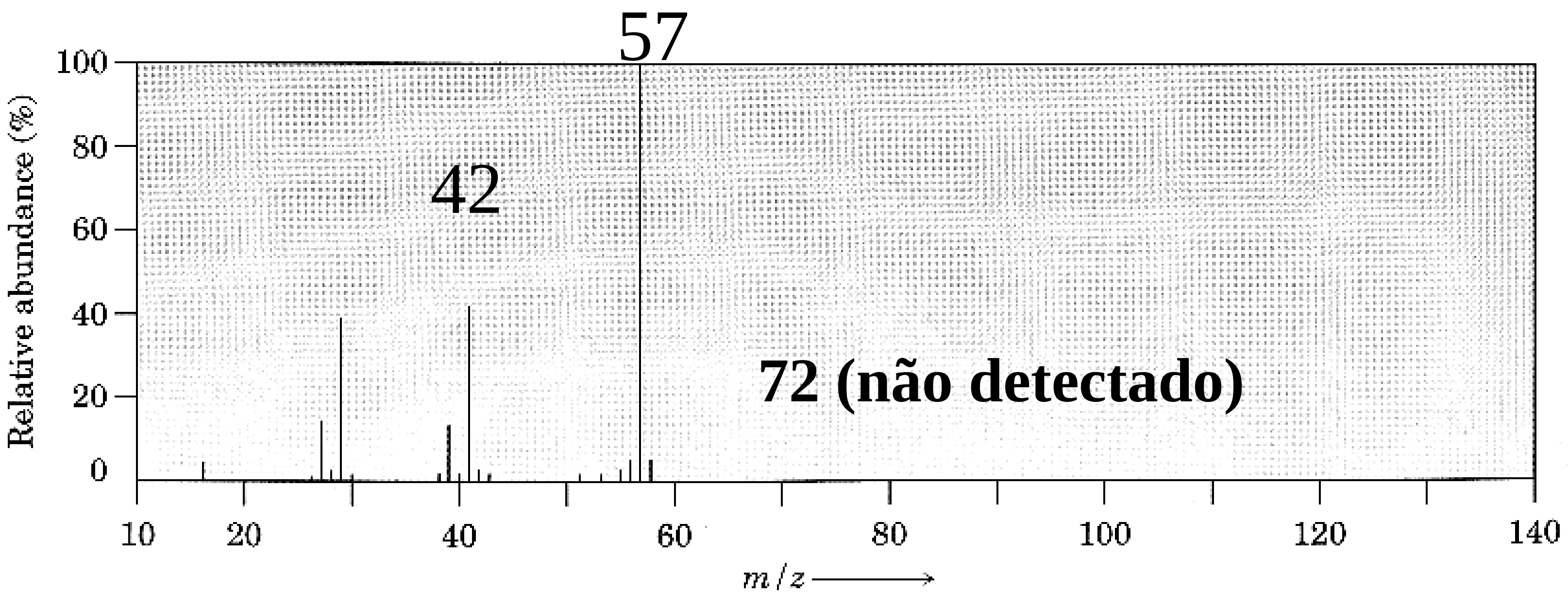


=>

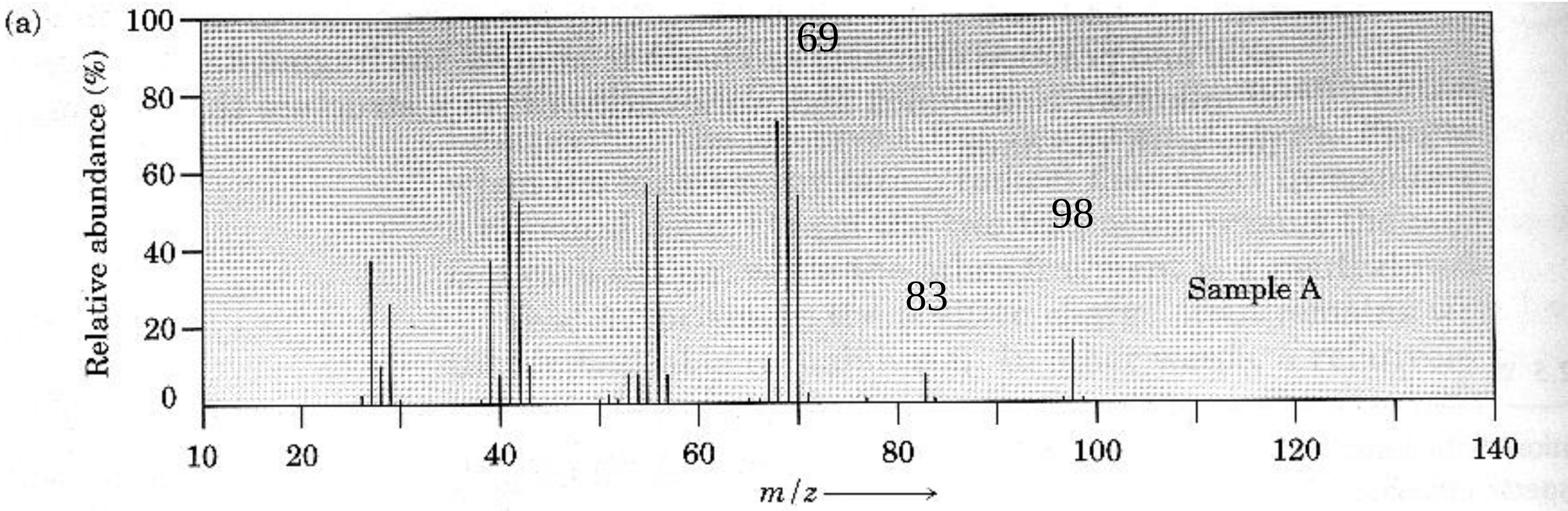
Espectro de massas do 2,2-dimetilpropano (C_5H_{12}; MM 72)

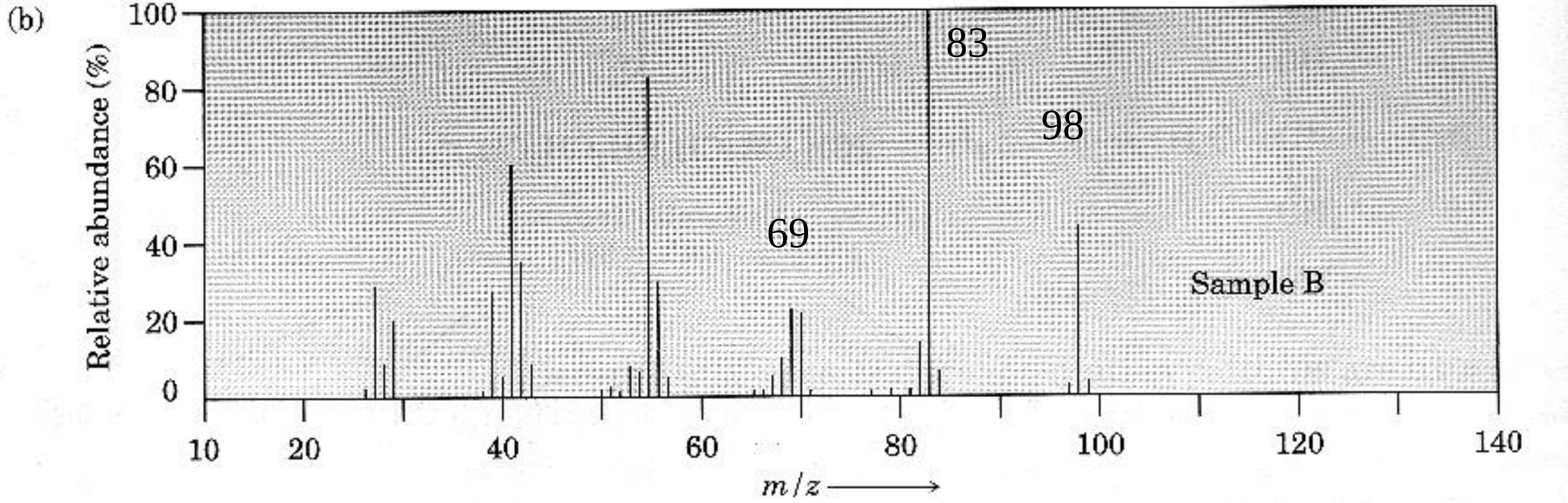
Por que não se detecta o íon molecular?

Qual a estrutura do íon 57 Da?



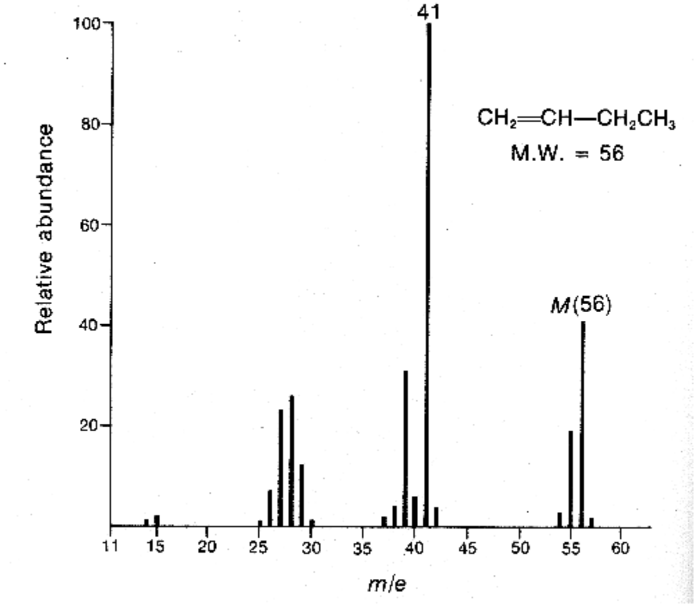
Atribua os espectros de massas (A e B) ao etilciclopentano e ao metilcicloexano

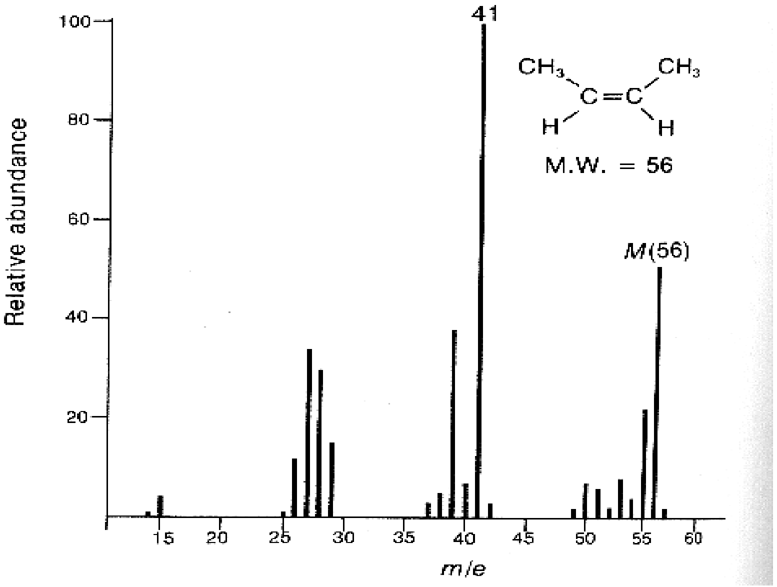




Expectro de massas de olefinas
(a possibilidade de isomerização via radicalar dificulta uma caracterização segura)

1-buteno



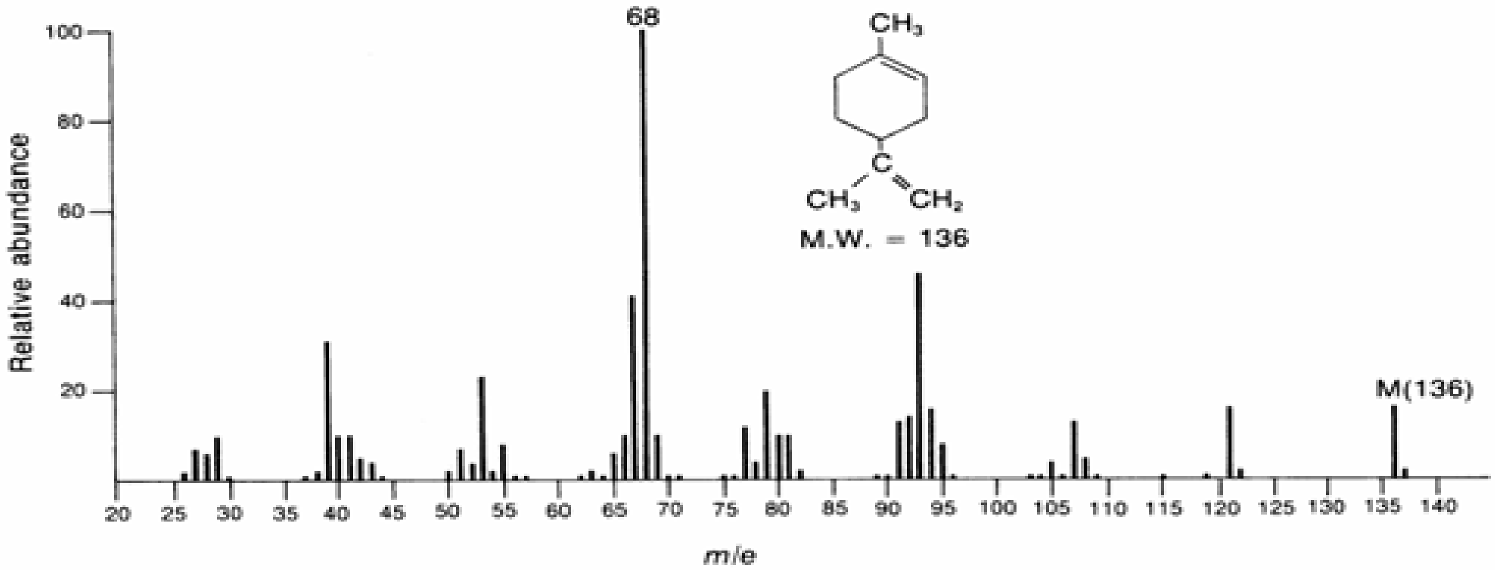


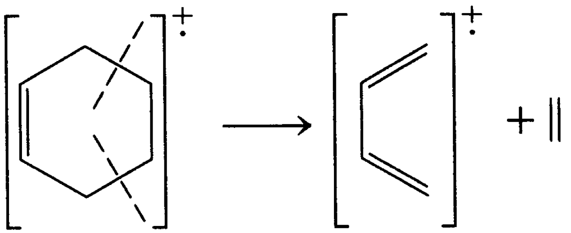
Pavia 7.12

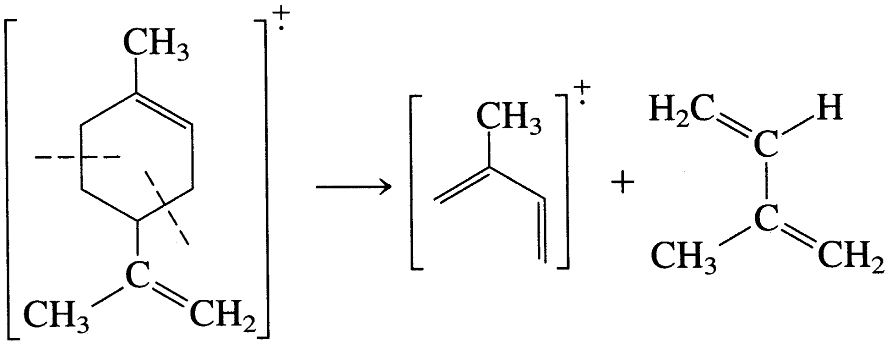
Pavia 7.13

Espectro de massas do limoneno

Qual a estrutura do cátion radicalar em 68 ?

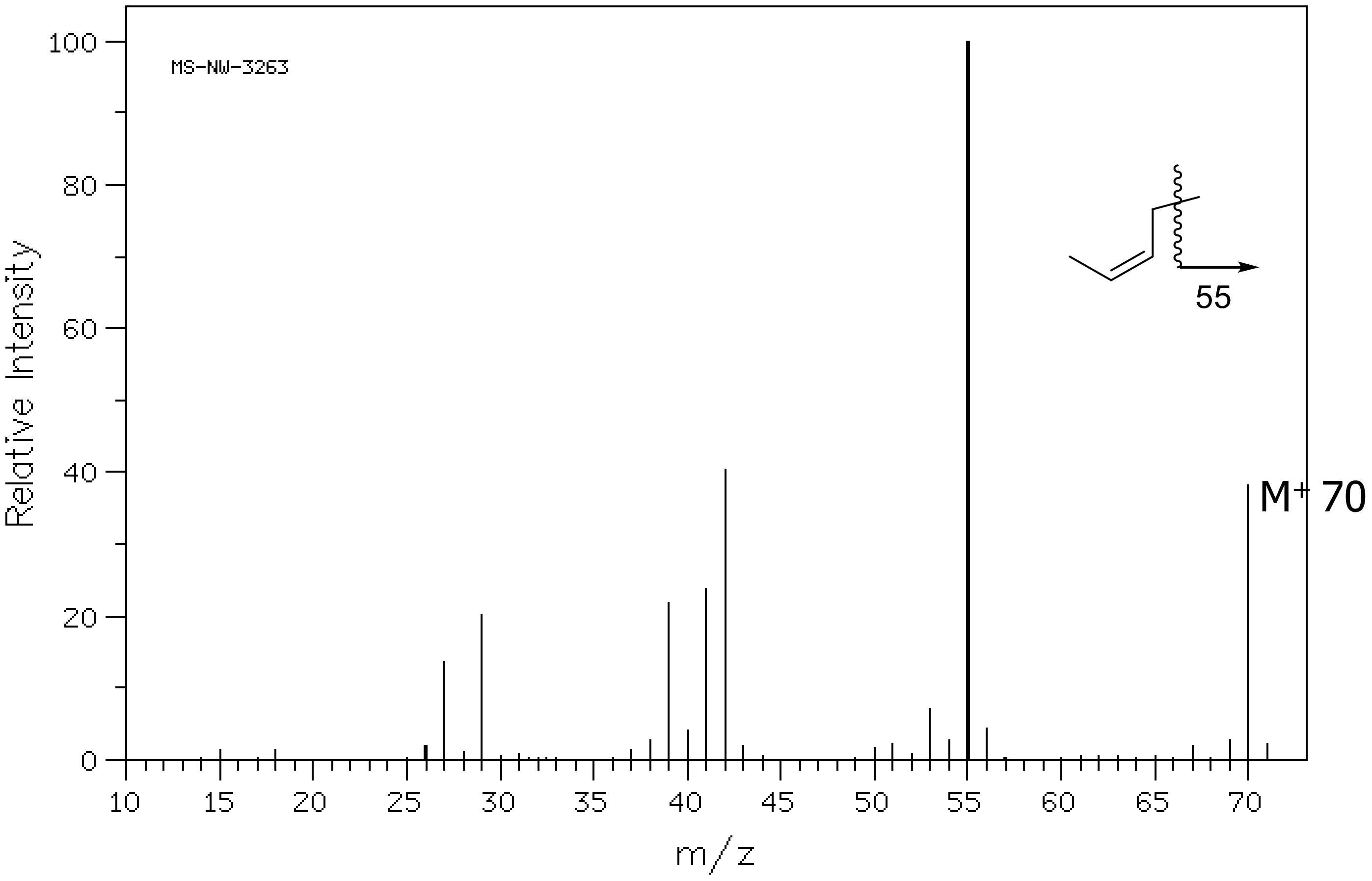




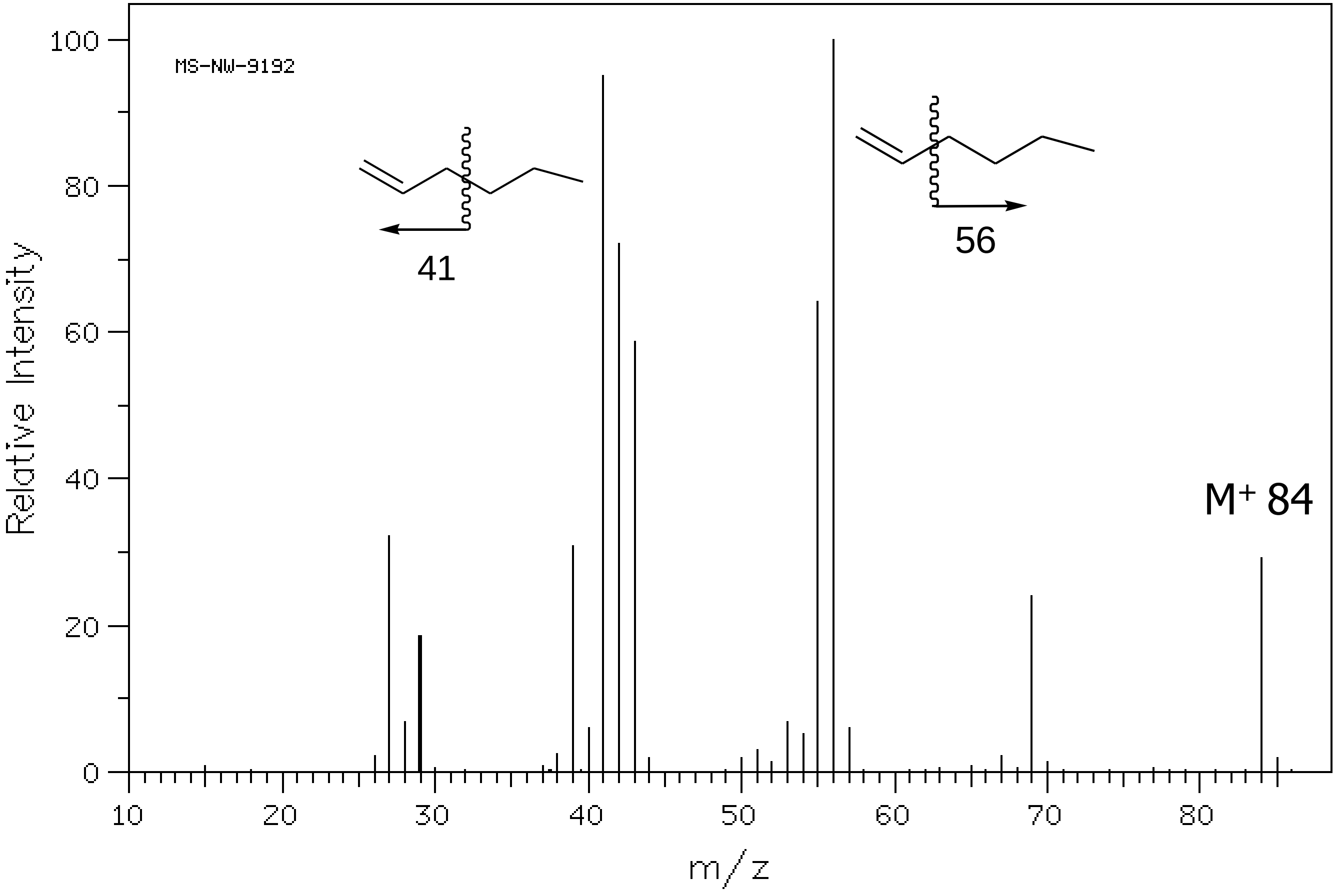


Reação retro Diels-Alder
(uma reação pericíclica)

The Mass Spectrum of Alkenes (*cis*- 2-pentene)



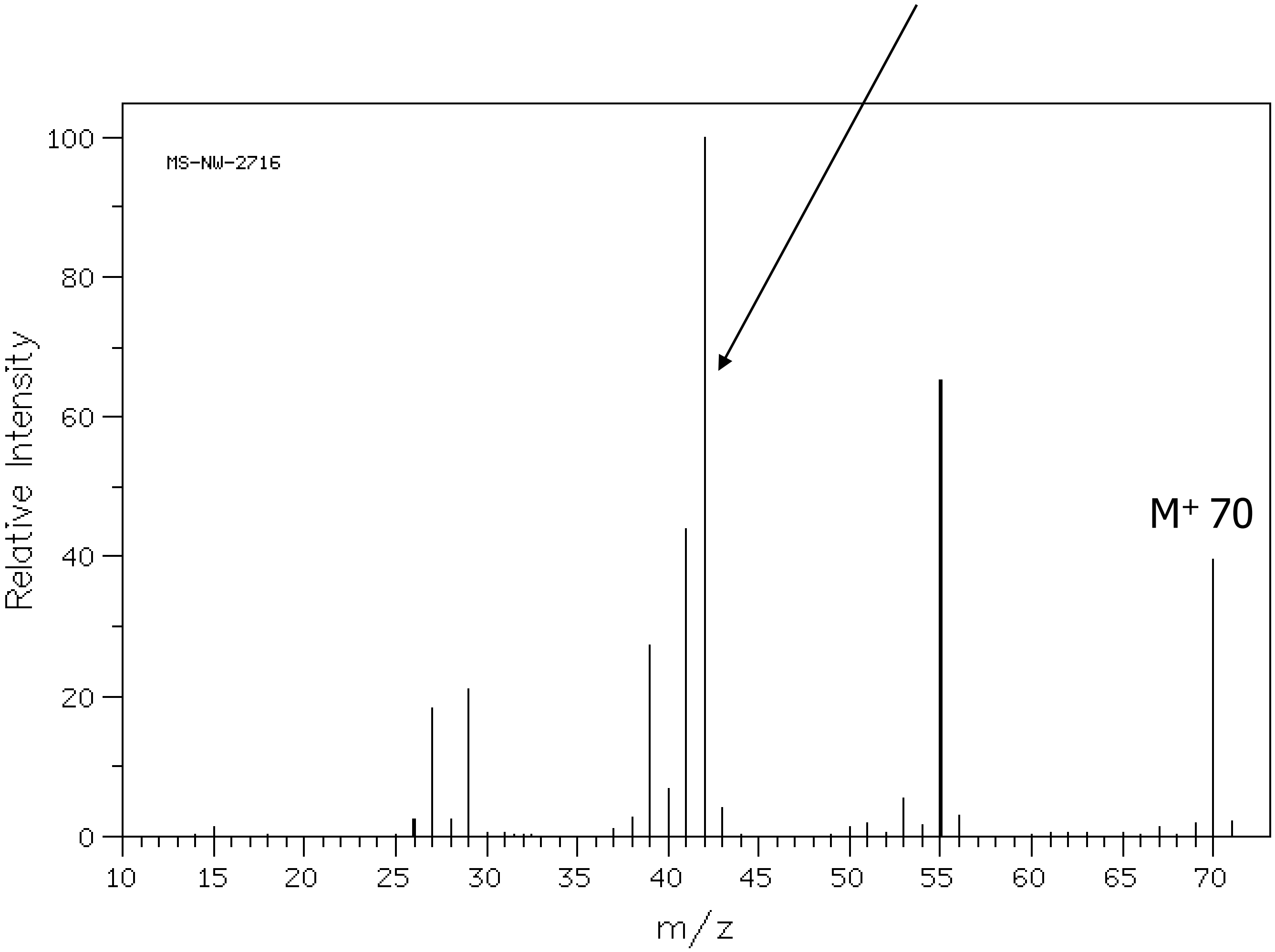
The Mass Spectrum of **alkenes (**1-hexene)



Mass Spectrometry

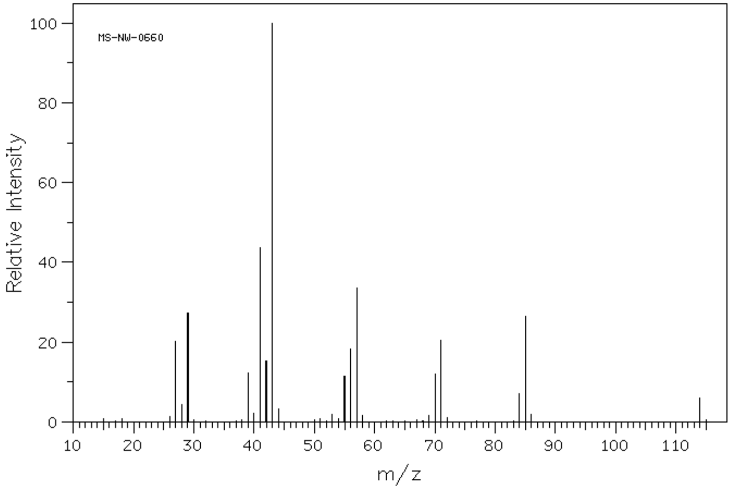
The Mass Spectrum of **alkenes (**1-pentene)

Take home assignment 2:
What is m/z 42?

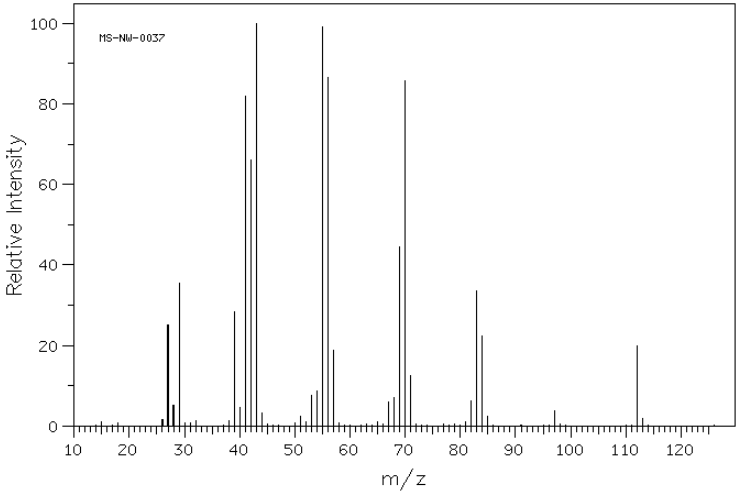


The Mass Spectrum and Structural Analysis

Fragmentation Patterns of Groups - Alkanes vs. alkenes



Octane (75 eV)
M^+ 114
m/z 85, 71, 57, 43 (base), 29



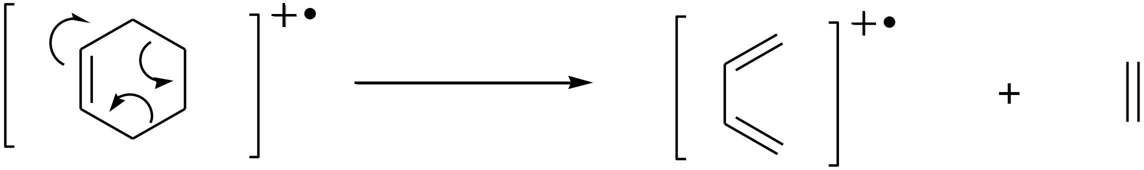
Octene (75 eV)
M^+ 112 (stronger @ 75eV than octane)
m/z 83, 69, 55, 41, 29

Fragmentation Patterns of Alkenes

Fragment Ions : **cycloalkenes**

- Molecular ions strong and commonly observed – cleavage of the ring still gives same mass value

- Retro-Diels-Alder is significant



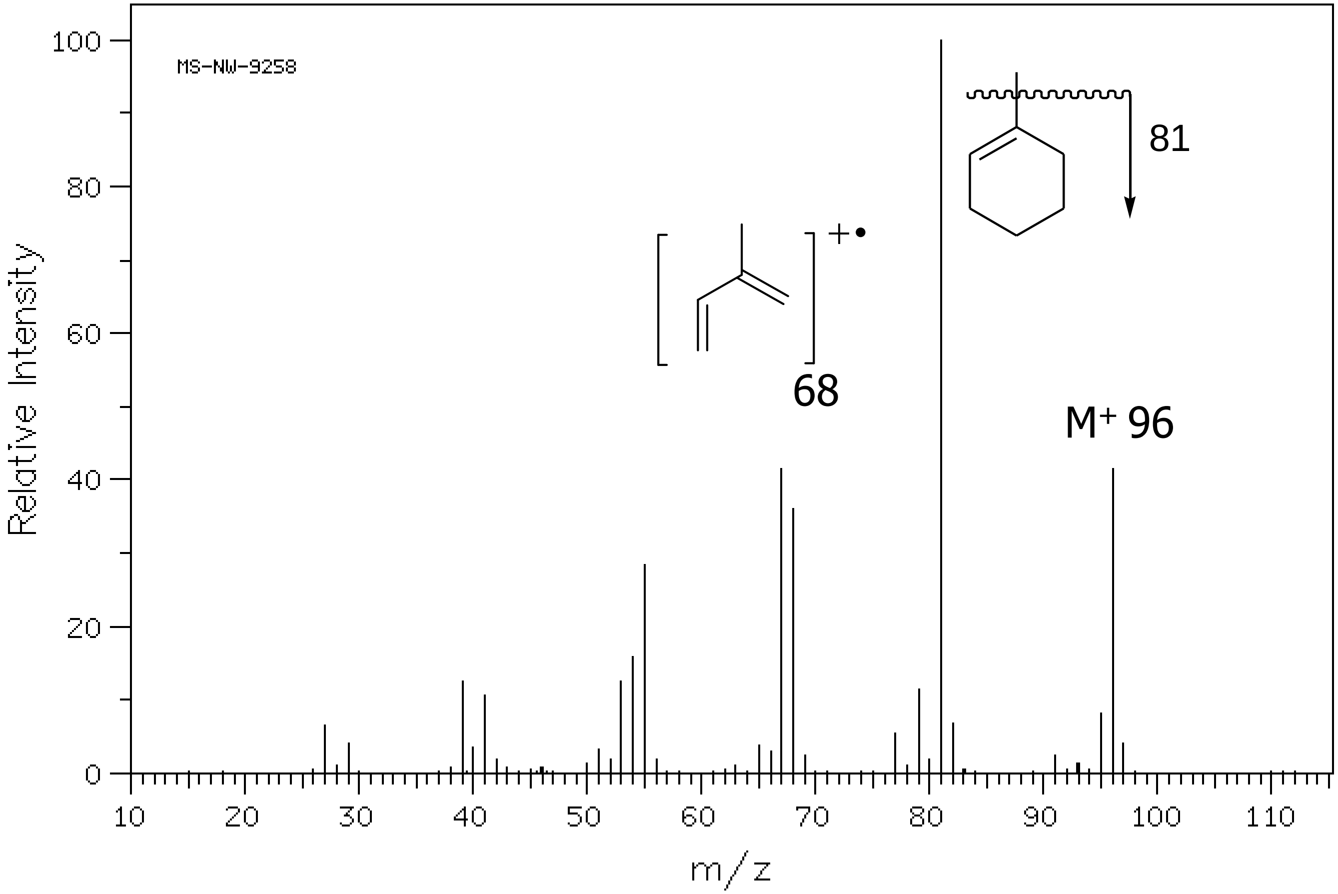
observed

loss of 28

- Side chains are easily fragmented

The Mass Spectrum and Structural Analysis

Fragmentation Patterns of **cycloalkenes (**1-methyl-1-cyclohexene)

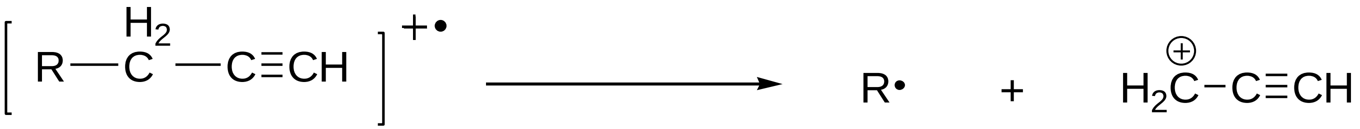


Fragmentation Patterns of **Alkynes** – **Fragment Ions**

a) The π-bond of an alkyne can also absorb substantial energy – molecular ions are commonly observed

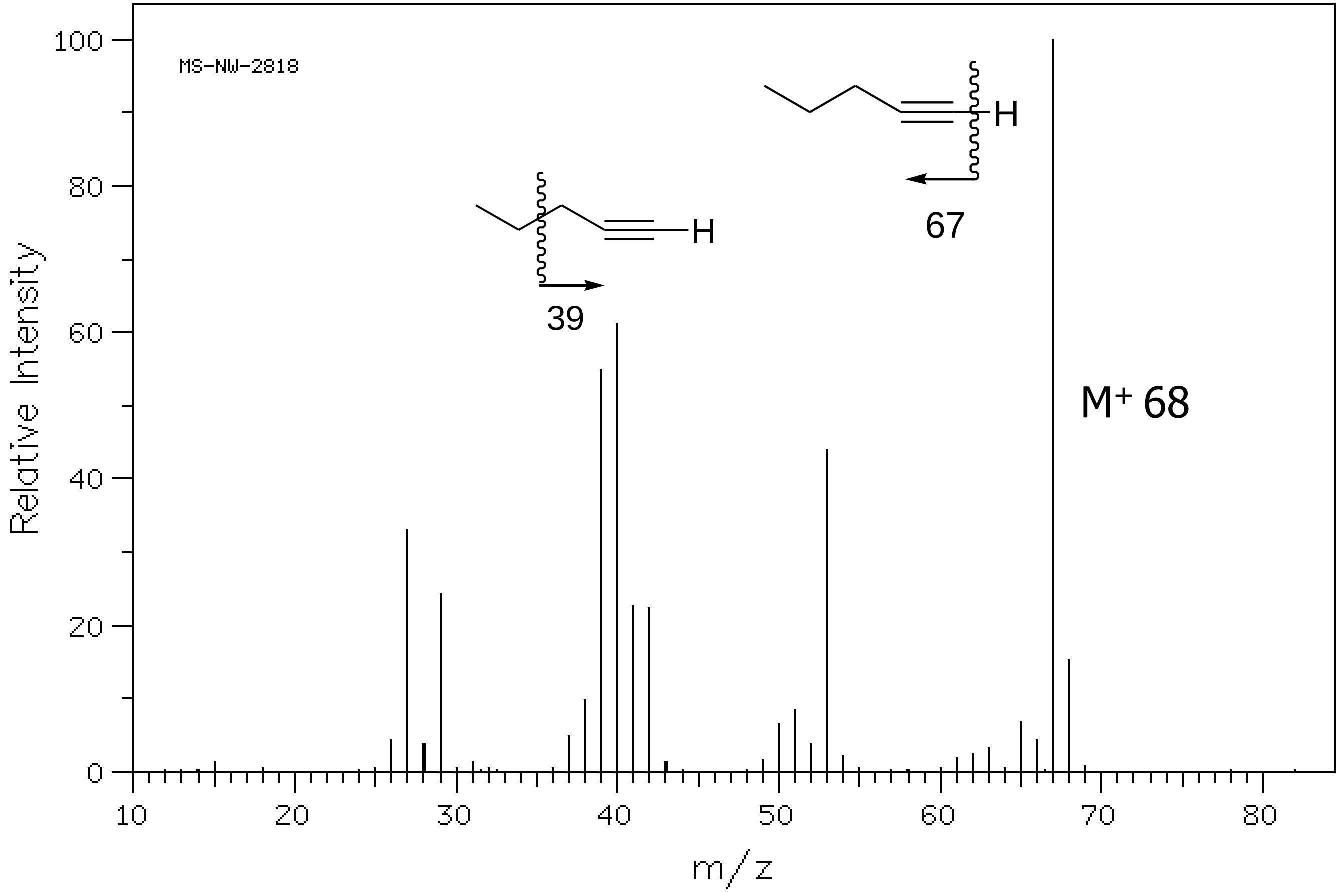
b) For terminal alkynes, the loss of terminal hydrogen is observed (M-1) – this may occur at such intensity to be the base peak or eliminate the presence of M^+

c) Terminal alkynes form the propargyl cation, m/z 39 (lower intensity than the allyl cation)

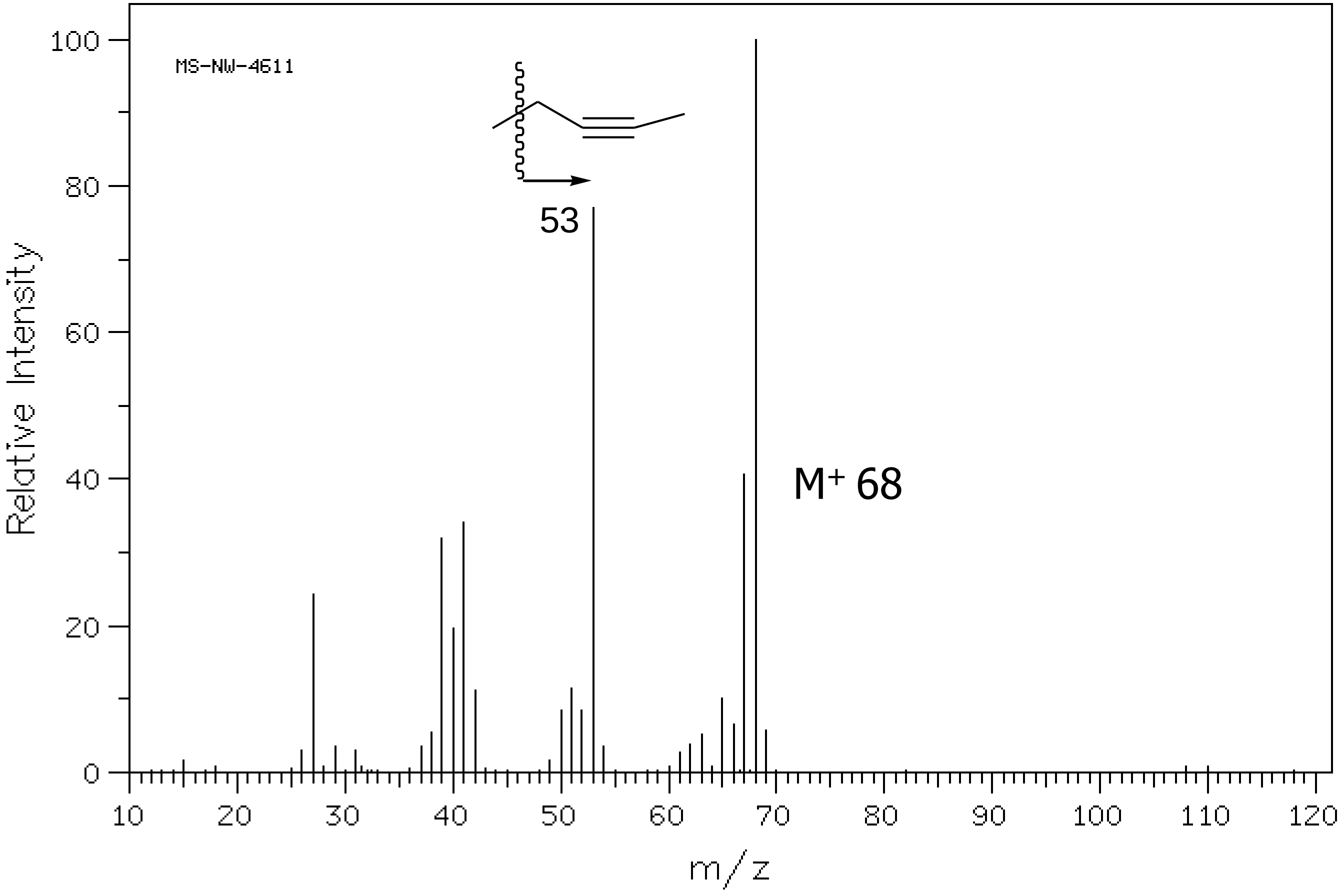


$$\left[R-\overset{H_2}{C}-C\equiv CH \right]^{+\bullet} \longrightarrow R\bullet + H_2\overset{\oplus}{C}-C\equiv CH$$

The Mass Spectrum of Alkynes (1-pentyne)



The Mass Spectrum of alkynes (2-pentyne)



Isotopic Abundance

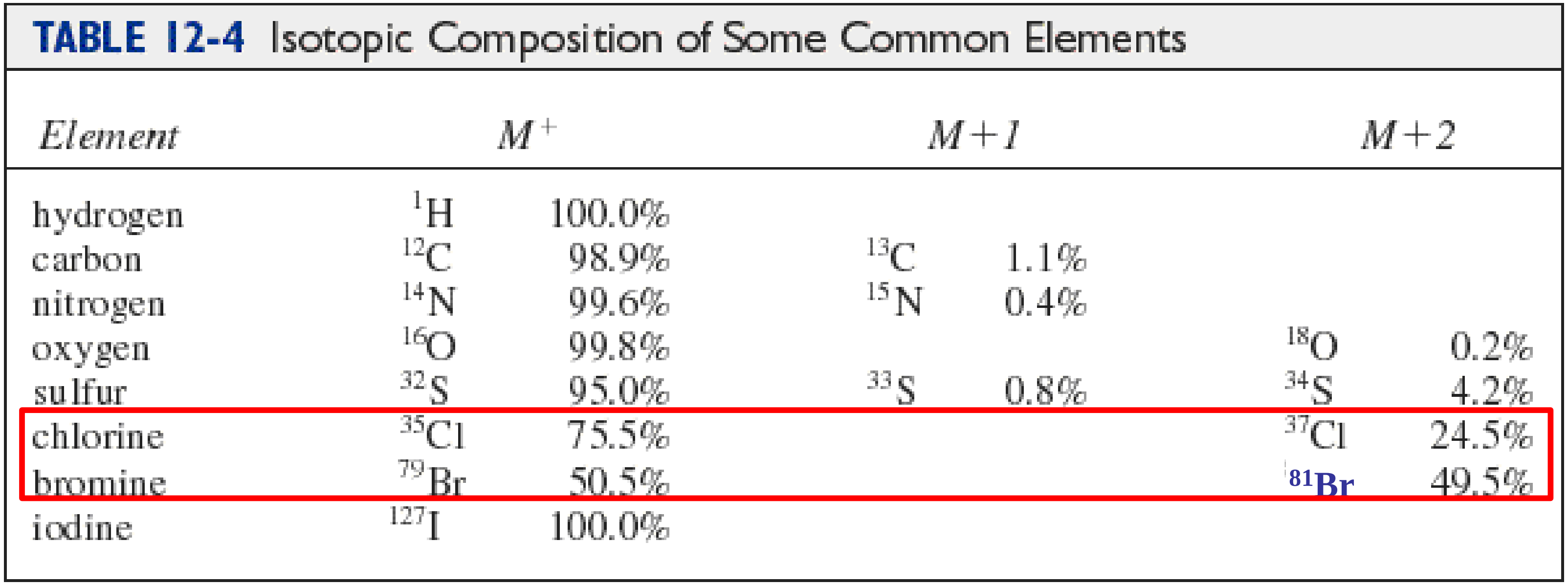
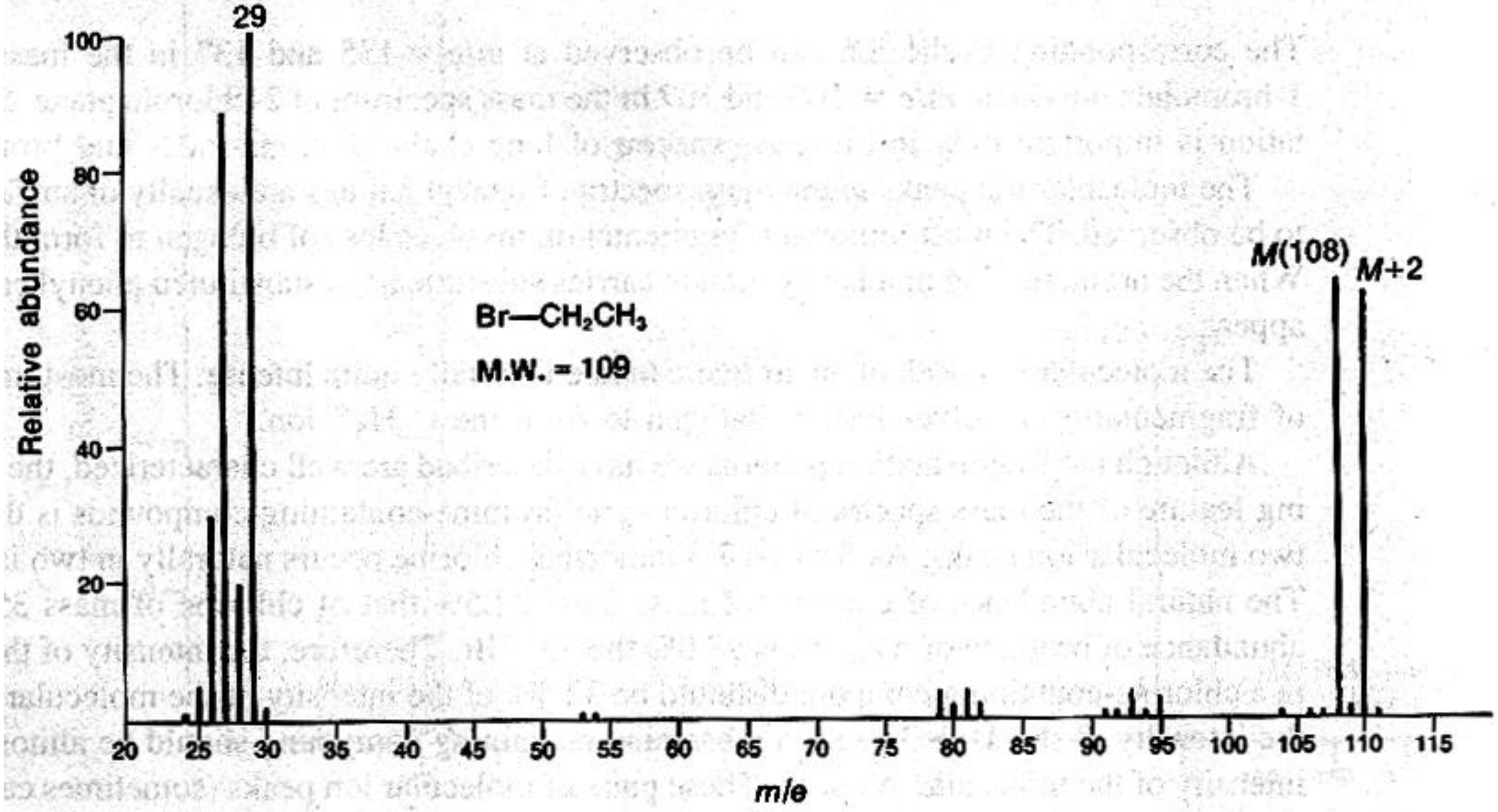


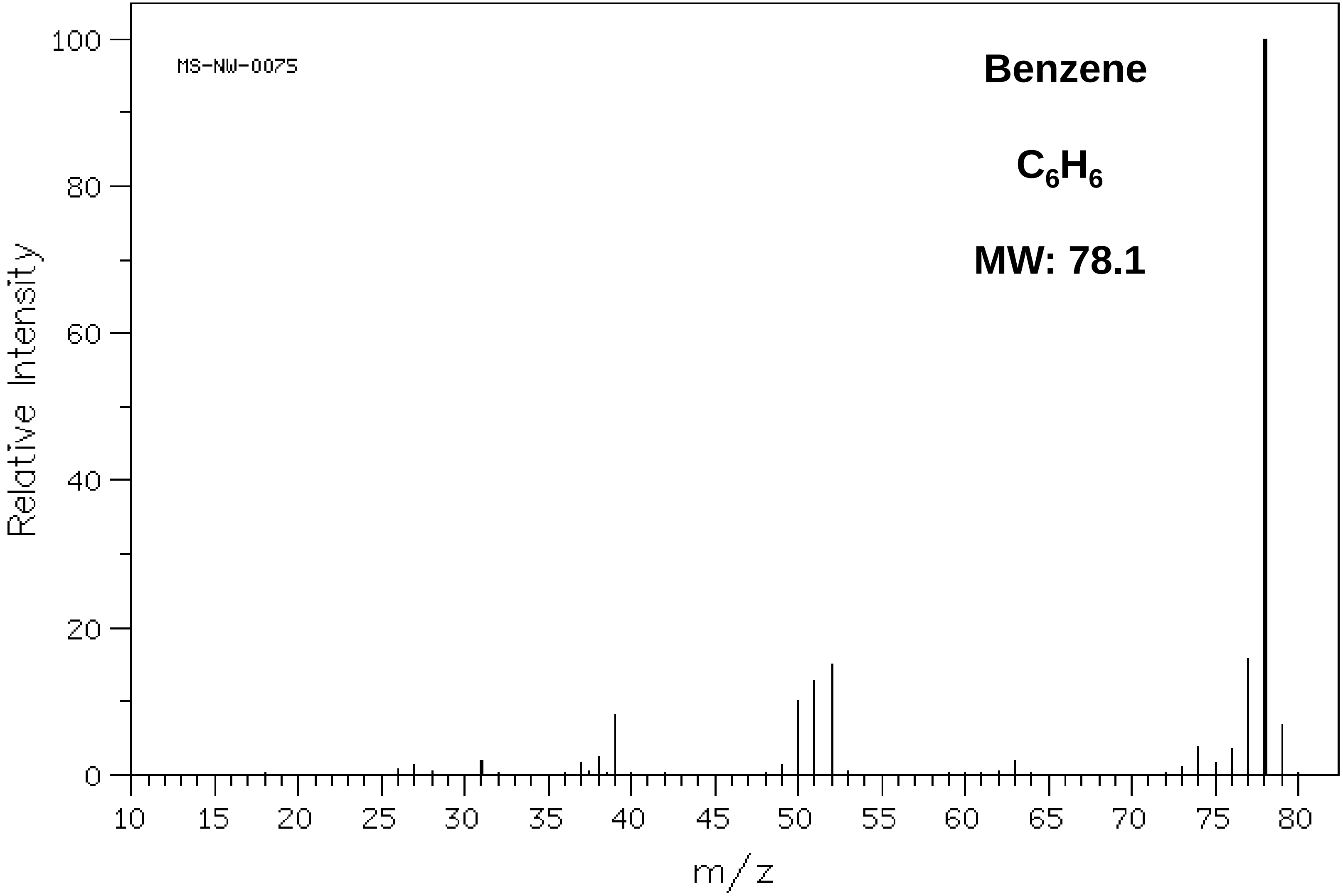
TABLE 12-4 Isotopic Composition of Some Common Elements

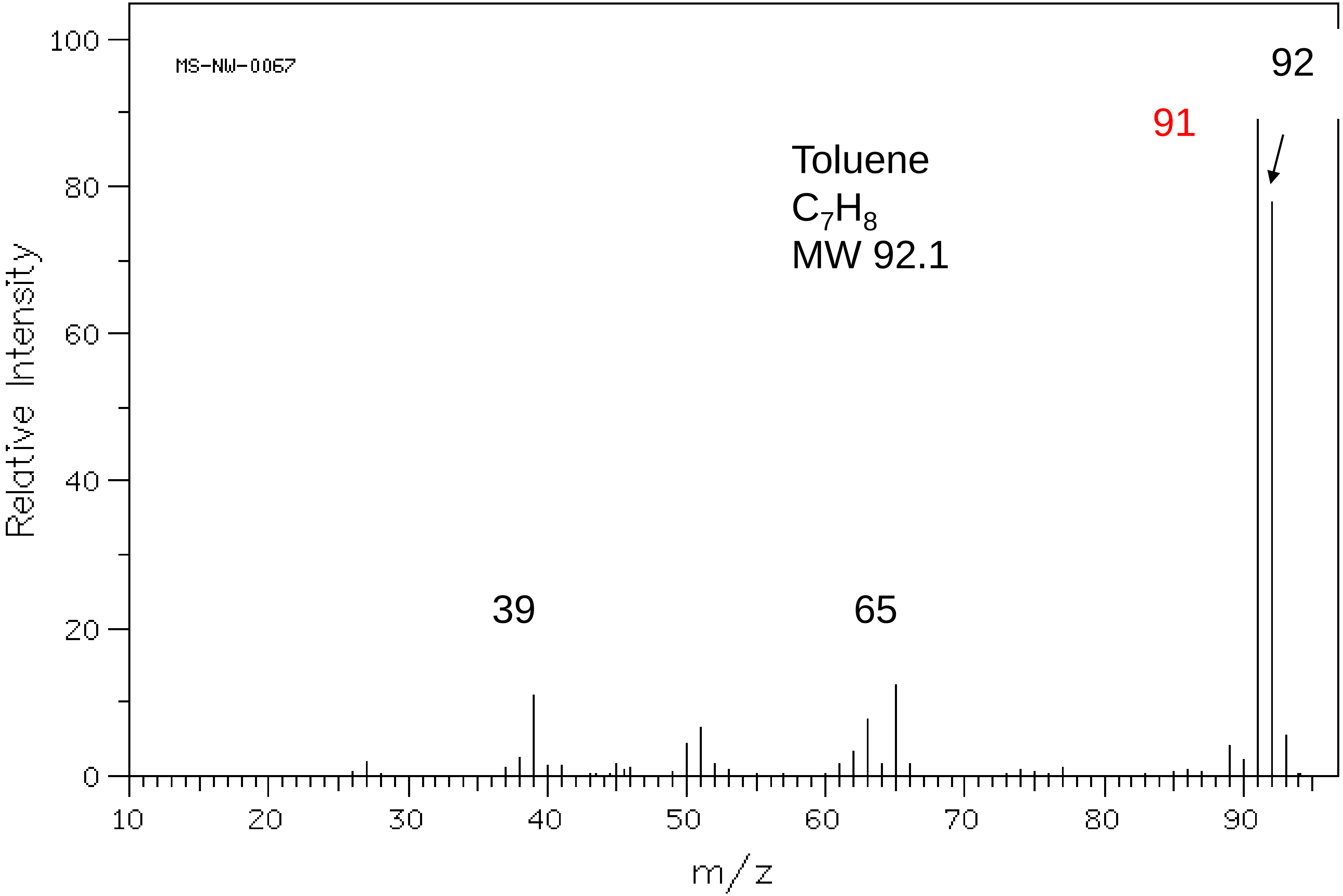
Element	M^+		$M+1$		$M+2$	
hydrogen	^{1}H	100.0%				
carbon	^{12}C	98.9%	^{13}C	1.1%		
nitrogen	^{14}N	99.6%	^{15}N	0.4%		
oxygen	^{16}O	99.8%			^{18}O	0.2%
sulfur	^{32}S	95.0%	^{33}S	0.8%	^{34}S	4.2%
chlorine	^{35}Cl	75.5%			^{37}Cl	24.5%
bromine	^{79}Br	50.5%			^{81}Br	49.5%
iodine	^{127}I	100.0%				

=>



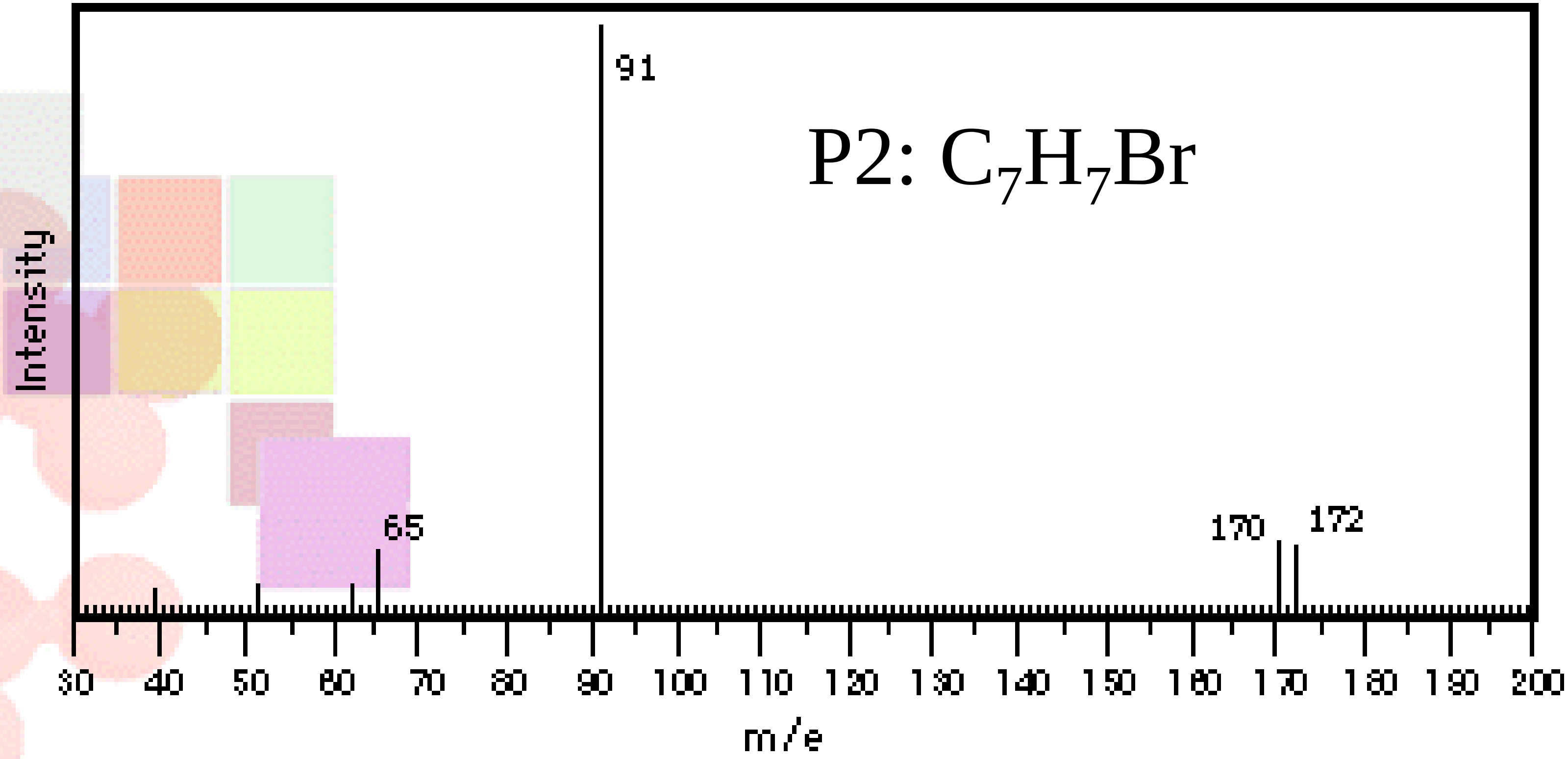
▶ **FIGURE 7.50** Mass spectrum of ethyl bromide.

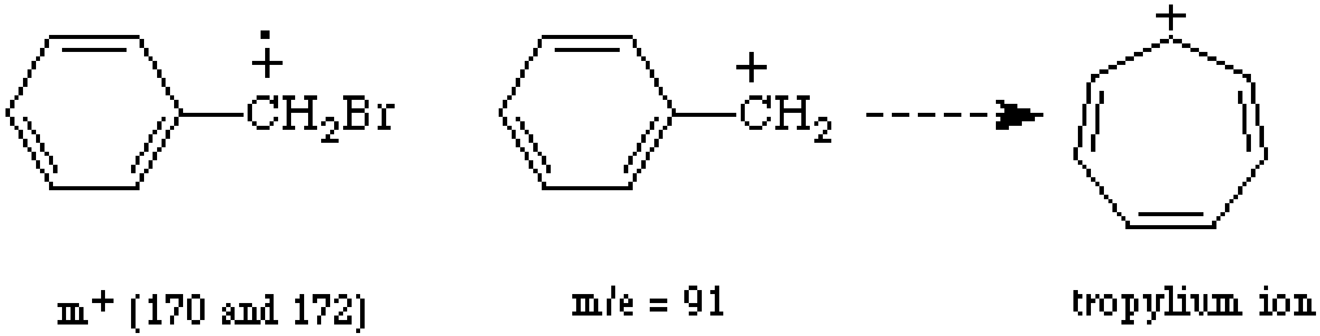
MS-NW-0075
Benzene
C_6H_6
MW: 78.1
Relative Intensity
0
20
40
60
80
100
m/z
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80



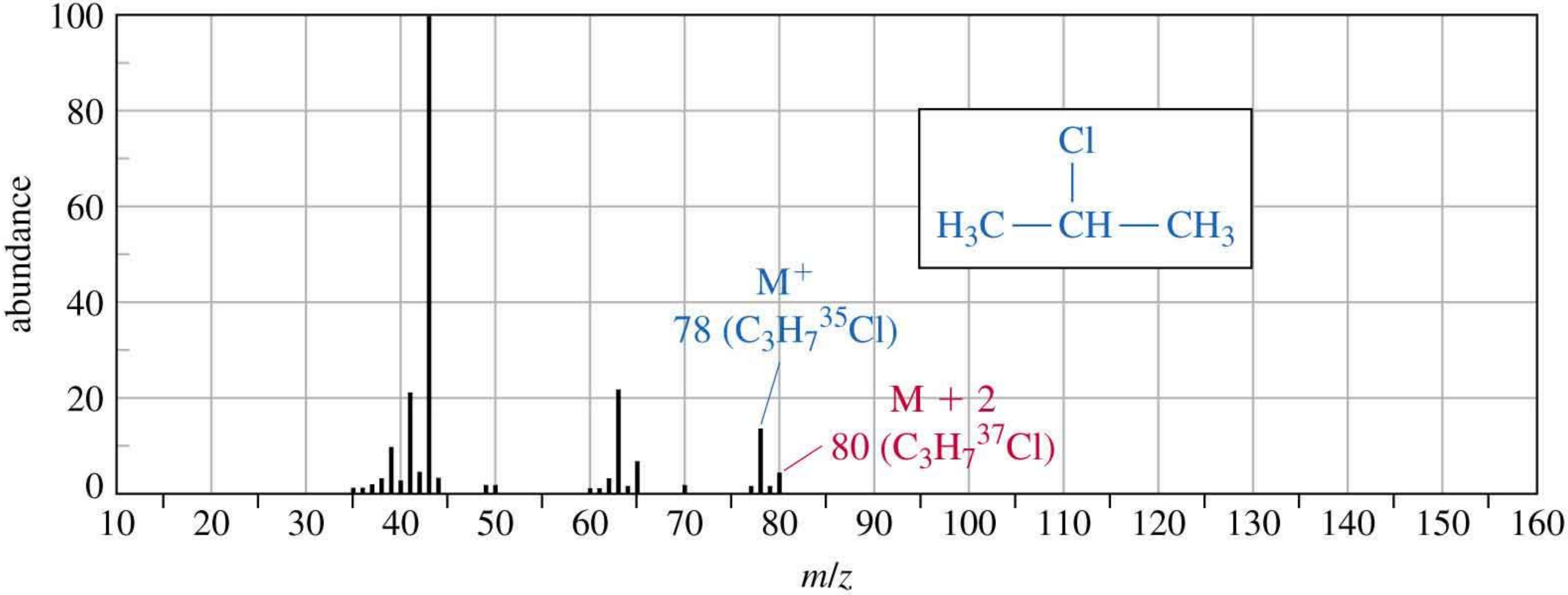
Toluene
C_7H_8
MW 92.1

P2: C_7H_7Br

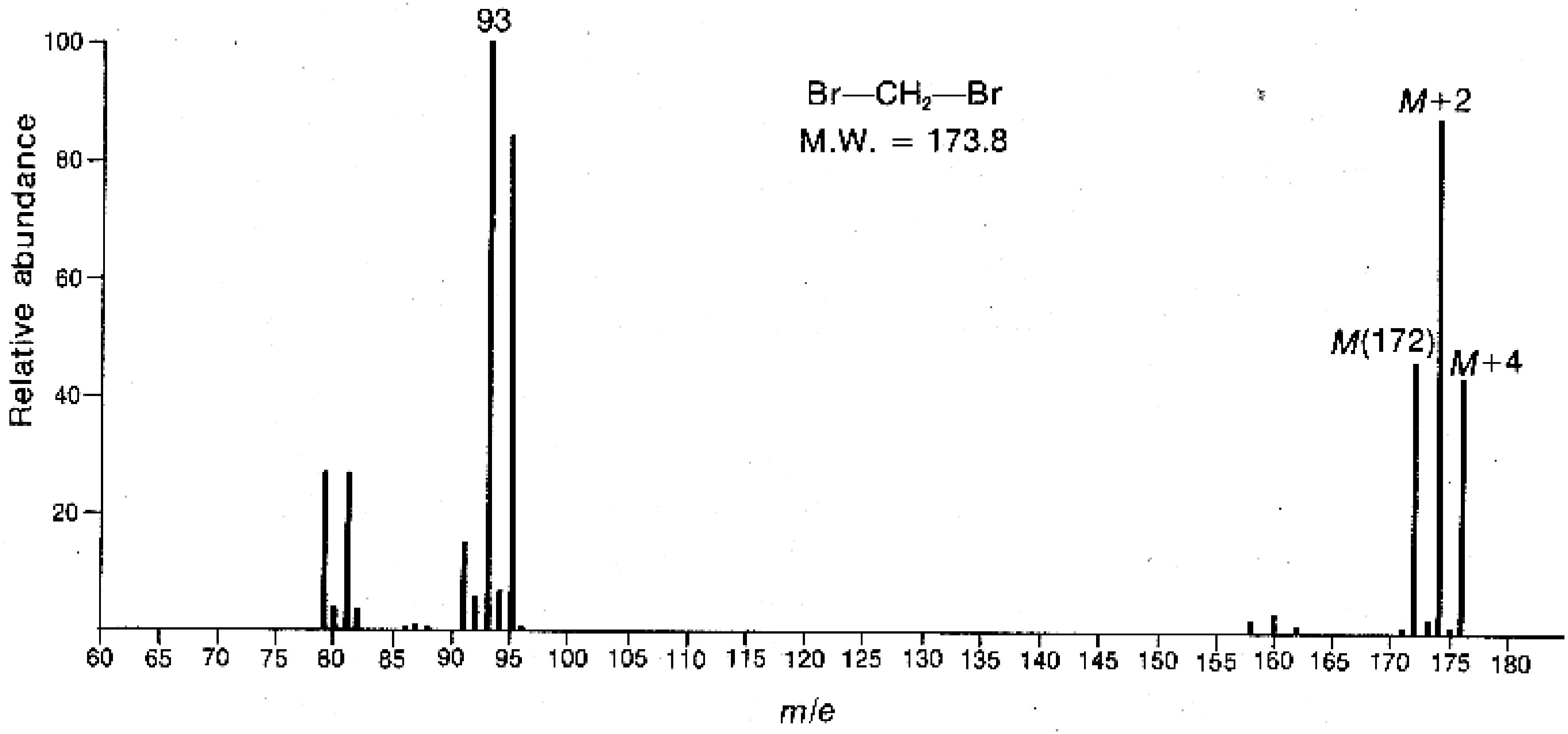

Intensity
91
65
170
172
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
m/e


CH2Br
CH2
m+ (170 and 172)
m/e = 91
tropylium ion

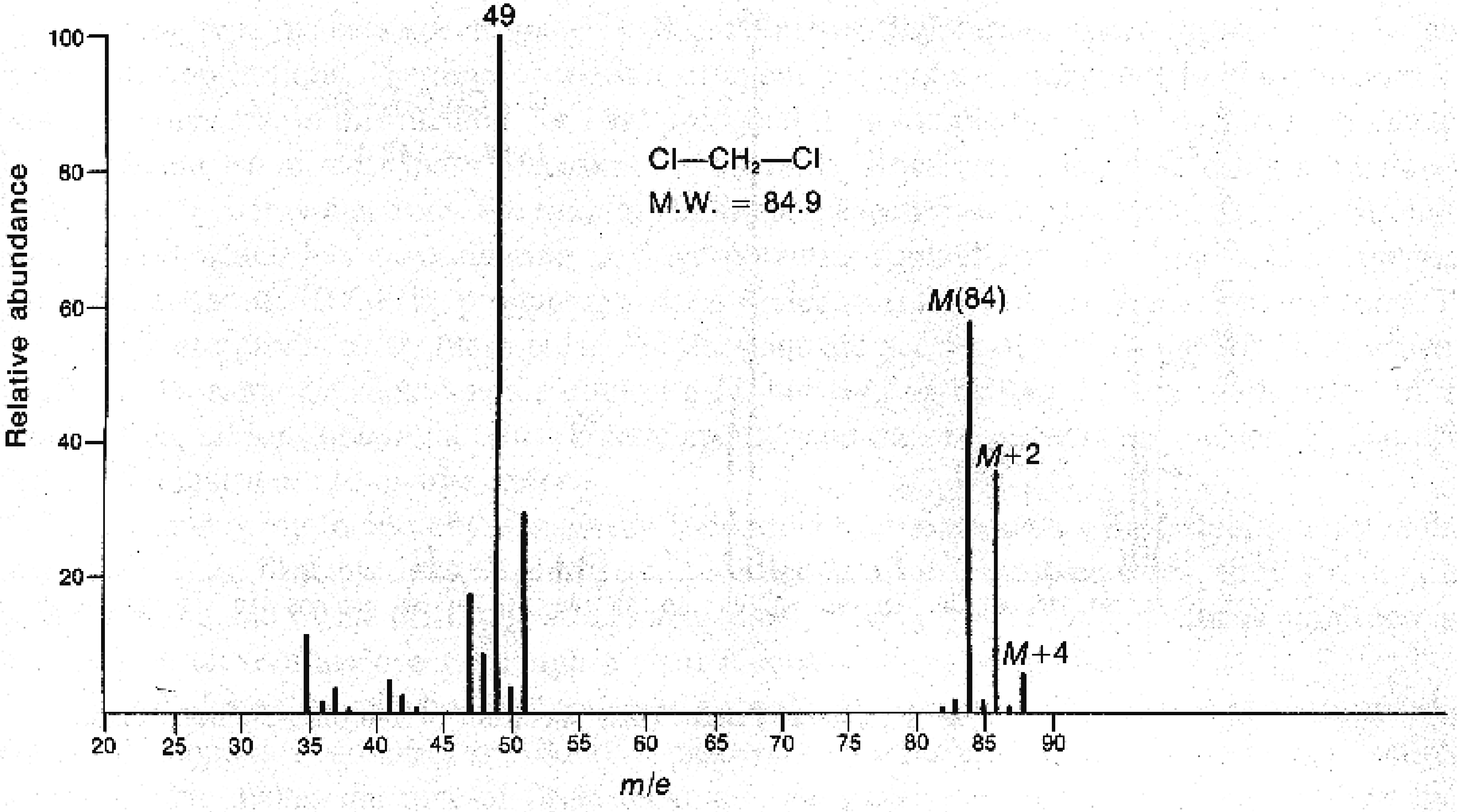
Mass Spectrum with Chlorine



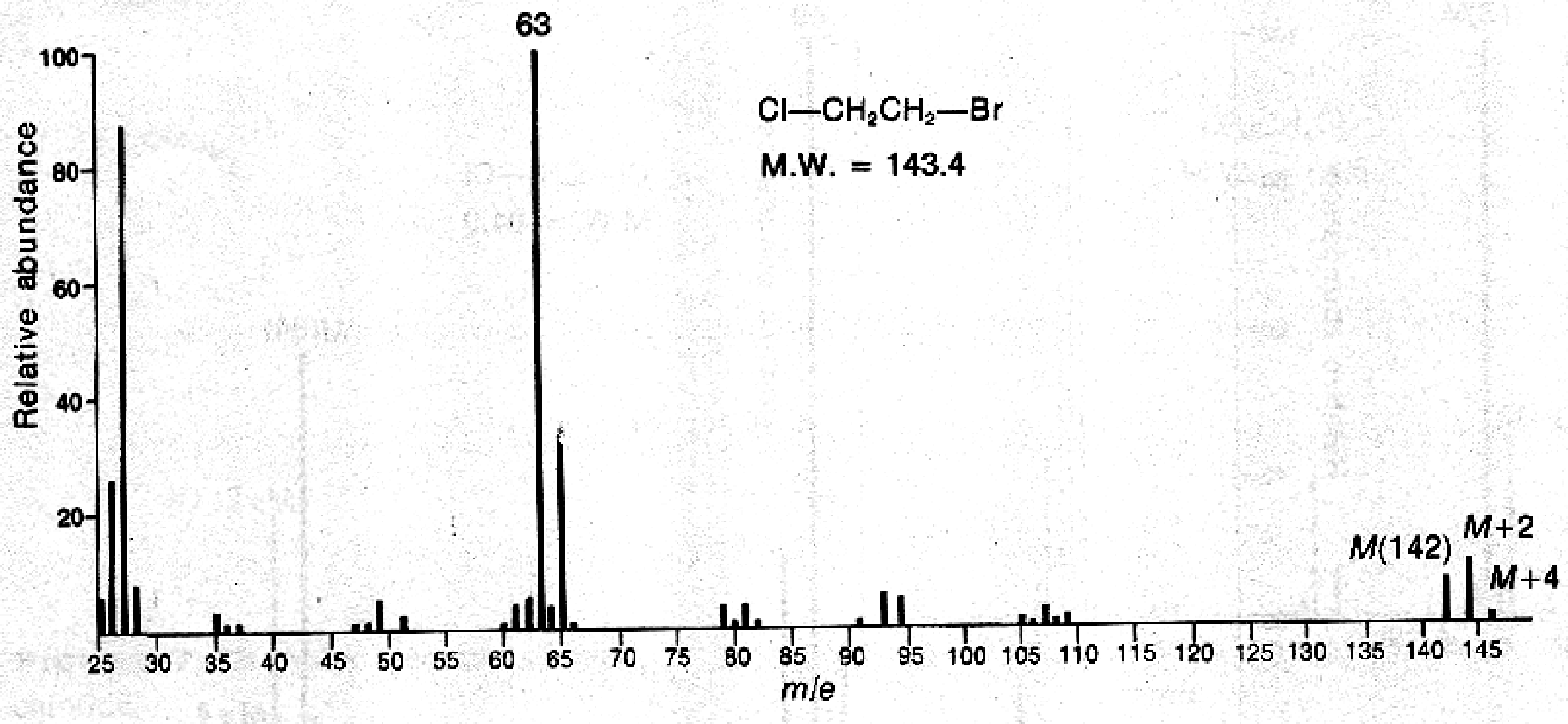
=>



▶ **FIGURE 7.52** Mass spectrum of dibromomethane.



▶ **FIGURE 7.51** Mass spectrum of dichloromethane.



▶ **FIGURE 7.53** Mass spectrum of 1-bromo-2-chloroethane.

Índice de deficiência de hidrogênio

MM 82 Compatível com C_6H_{10}

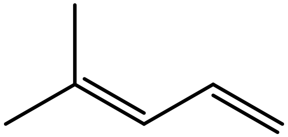
Se fosse saturado seria C_6H_{14} (CnH_2n+2)

4H de diferença = 2 H_2 (duas deficiências)

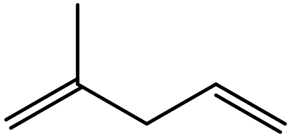
Índice de deficiência de hidrogênio

MM 82 Compatível com C_6H_{10}

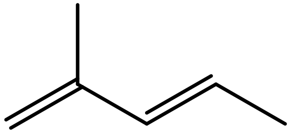
Quais estruturas seriam possíveis?



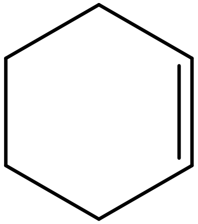
4-methylpenta-1,3-diene



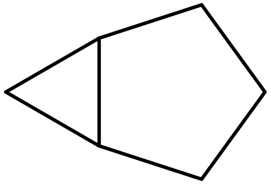
2-methylpenta-1,4-diene



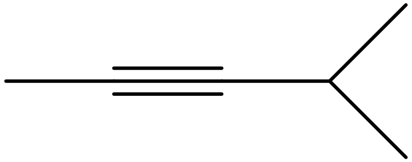
(*E*)-2-methylpenta-1,3-diene



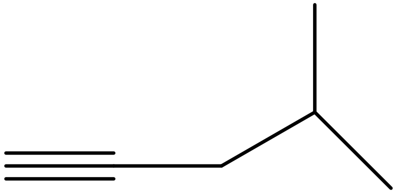
cyclohexene



bicyclo[3.1.0]hexane



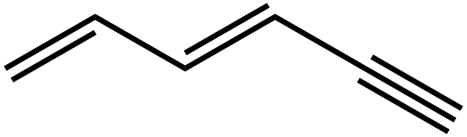
4-methylpent-2-yne



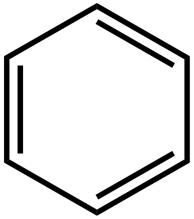
4-methylpent-1-yne

Possíveis estruturas para C_6H_6 ?

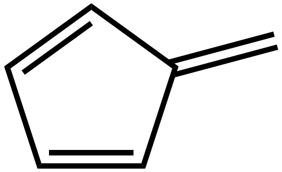
IDH = 4



(*E*)-hexa-1,3-dien-5-yne



benzene



fulvene

IDH para C_6H_5Br ? (= C_6H_6)

X (F, Cl, Br e I) são monovalentes

IDH para C_6H_6O ? (= C_6H_6)

Oxigênio é divalente