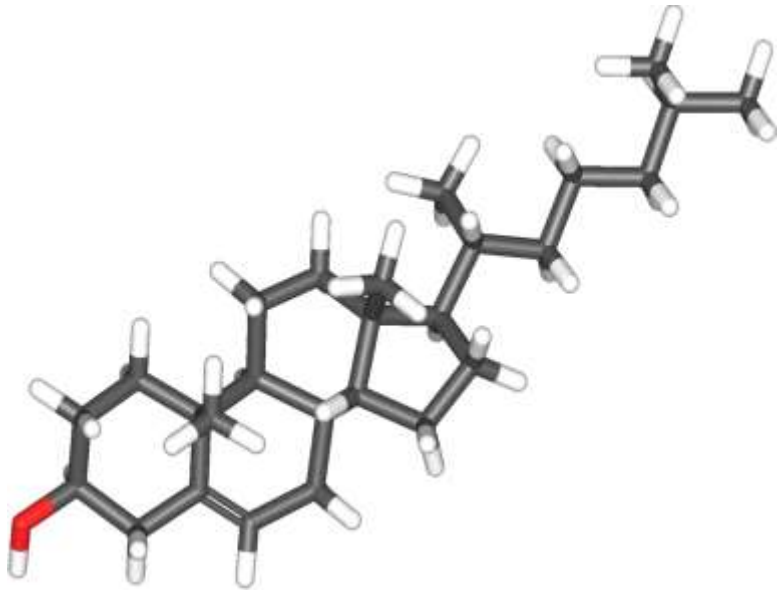
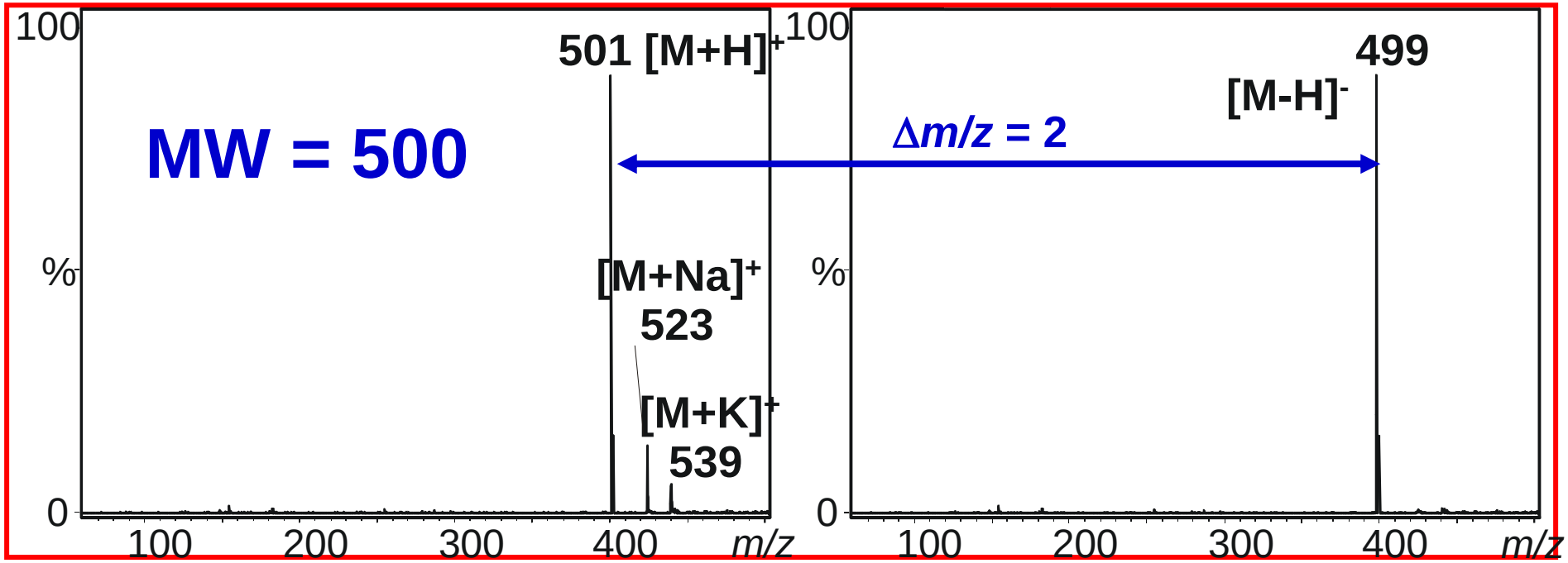
Vybrané příklady interpretace „měkkých“ MS a MS/MS spekter



Určení molekulové hmotnosti (API-MS)



$[M+H]^+$ $\Delta m/z = 22$ $[M+Na]^+$ $\Delta m/z = 16$ $[M+K]^+$

$\Delta m/z = 38$

$\Delta m/z = 17$ $[M+NH_4]^+$

Nejběžnější typy molekulárních aduktů

Singly charged molecular adducts in positive-ion API mass spectra.

Molecular adduct	Nominal mass shift[a] [ΔDa]	Exact mass shift[a] [mDa]
$[M+Li]^+$	6	+8.2
$[M+NH_4]^+$	17	+26.5
$[M+H+H_2O]^+$	18	+10.6
$[M+Na]^+$	22	−18.1
$[M+H+CH_3OH]^+$	32	+26.2
$[M+K]^+$	38	−44.1
$[M+H+CH_3CN]^+$	41	+26.5
$[M+H+H_2O+CH_3OH]^+$	50	+36.8
$[M+Na+CH_3CN]^+$	63	+8.5
$[M+Ag]^+$	106	−102.7

posun od iontu $[M+H]^+$

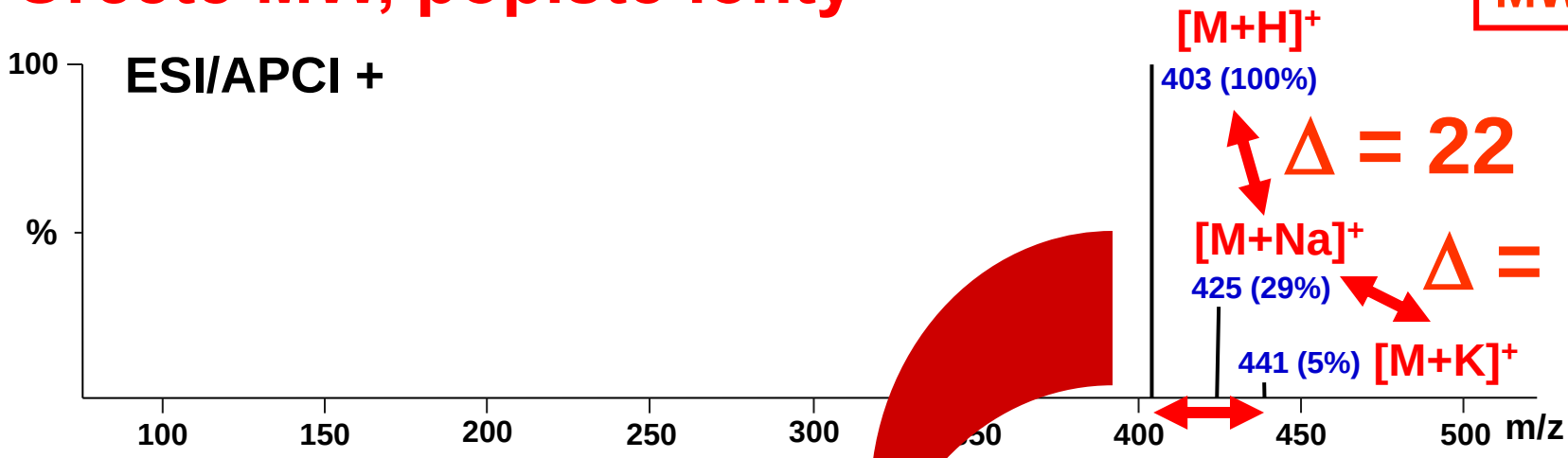
Singly charged molecular adducts in negative-ion API mass spectra.

Molecular adduct	Nominal mass shift[a] [ΔDa]	Exact mass shift[a] [mDa]
$[M-H+H_2O]^-$	18	+10.6
$[M+F]^-$	20	+6.2
$[M-H+CH_3OH]^-$	32	+26.2
$[M+Cl]^-$	36	−23.3
$[M+HCOO]^-$	46	+5.5
$[M+NO_2]^-$	47	+0.7
$[M+CH_3COO]^-$	60	+21.1
$[M+NO_3]^-$	63	−4.4
$[M+Br]^-$	80	−73.8
$[M+HSO_4]^-$	98	−32.6
$[M+H_2PO_4]^-$	98	−23.1
$[M+CF_3COO]^-$	114	−7.1
$[M+I]^-$	128	−87.7
$[2M-H]^-$	–	–

posun od iontu $[M-H]^-$

#1/ Určete MW, popište ionty

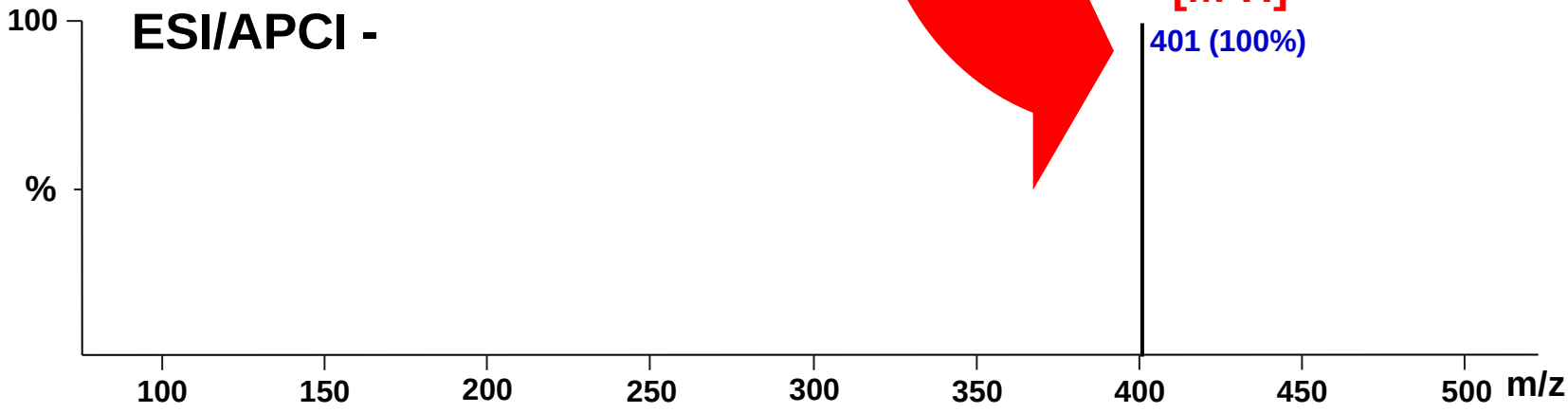
MW=402



m/z 420 $[M+NH_4]^+$
m/z 461 $[M+CH_3COO]^-$

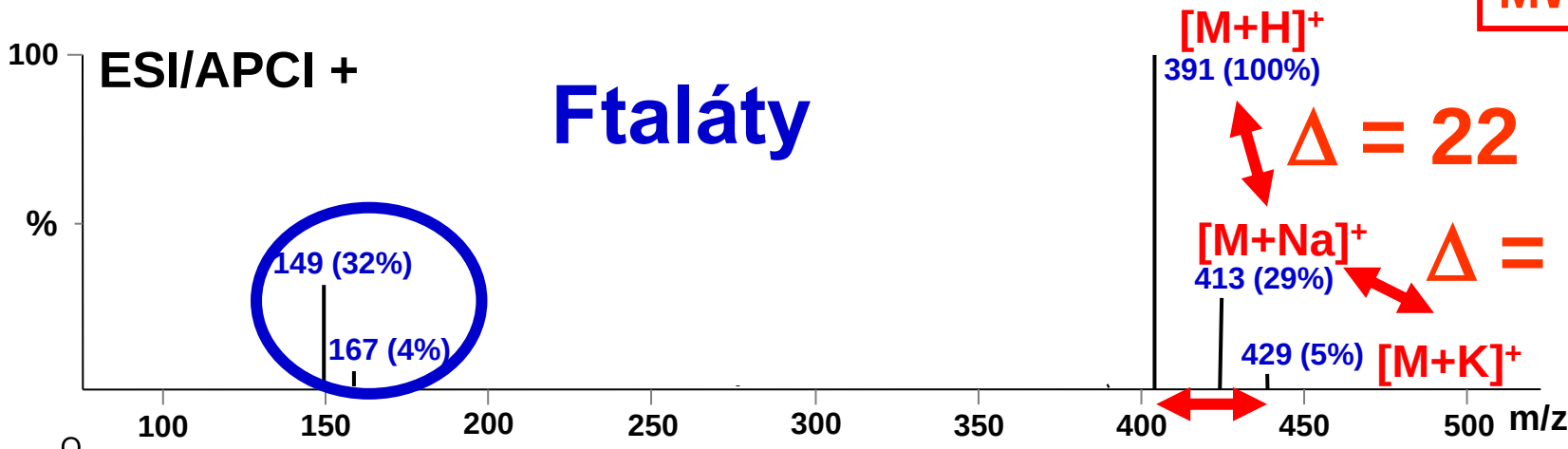
$\Delta = 2$

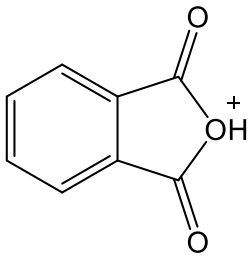
Úkol: jaké změny očekáváte po přídavku 5 mM octanu amonného do mobilní fáze



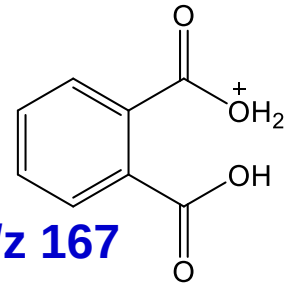
#2/ Určete MW, popište ionty

MW=390





m/z 149

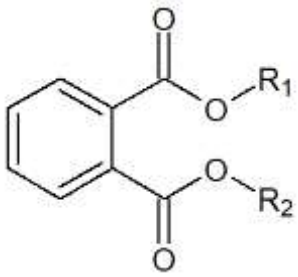


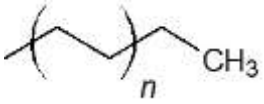
m/z 167

$[C_8H_7O_4]^+$

Výpočet elementárního složení ftalátů:

$[M+H]^+ \rightarrow [C_{16+8}H_{32+7}O_4]^+ = [C_{24}H_{39}O_4]^+$





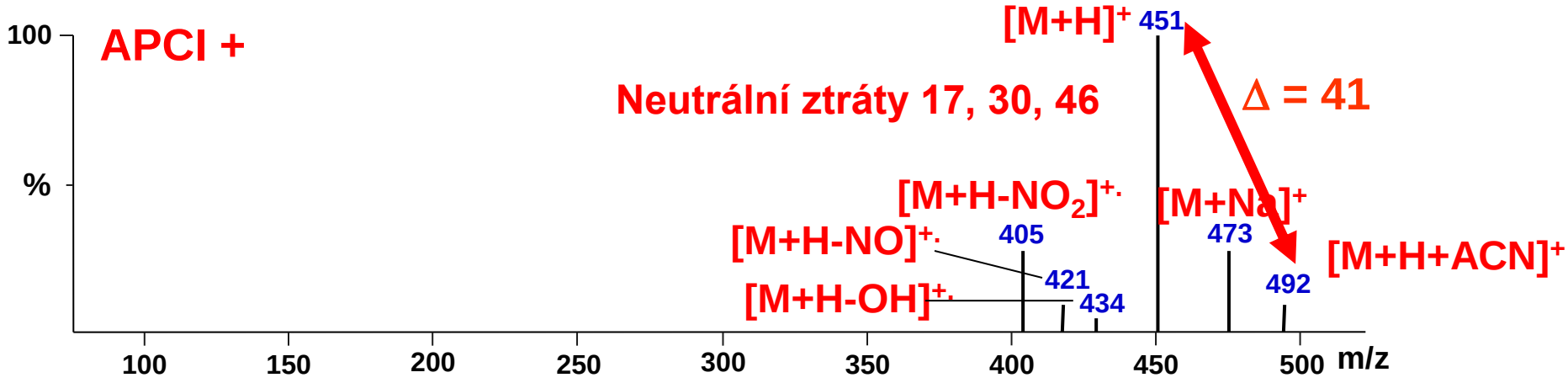
391 – 167 = 224

224/14 = 16 = 16xCH_2 = $C_{16}H_{32}$

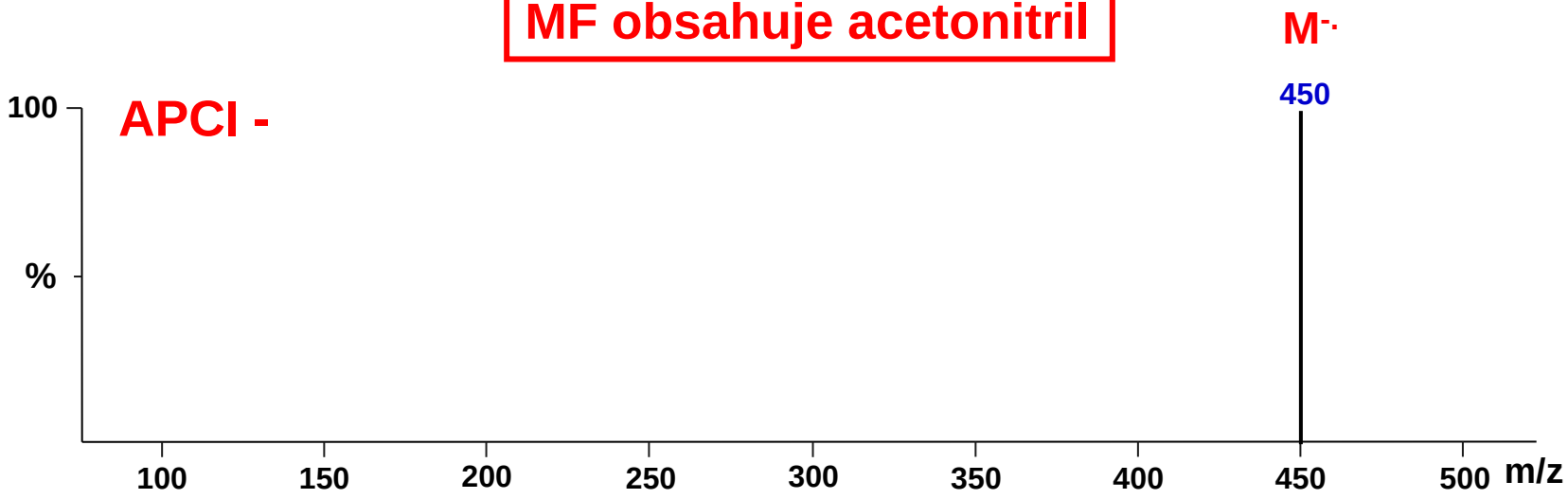
$M \rightarrow C_{24}H_{38}O_4$

Di(n-octyl) ftalát

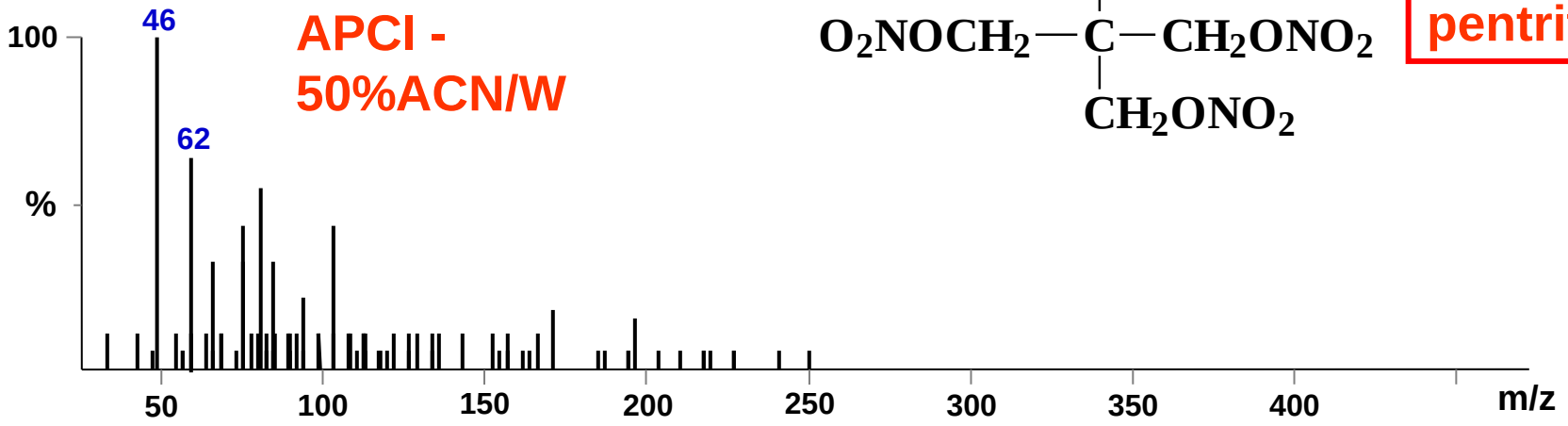
#3/ Určete MW a další strukturní informace



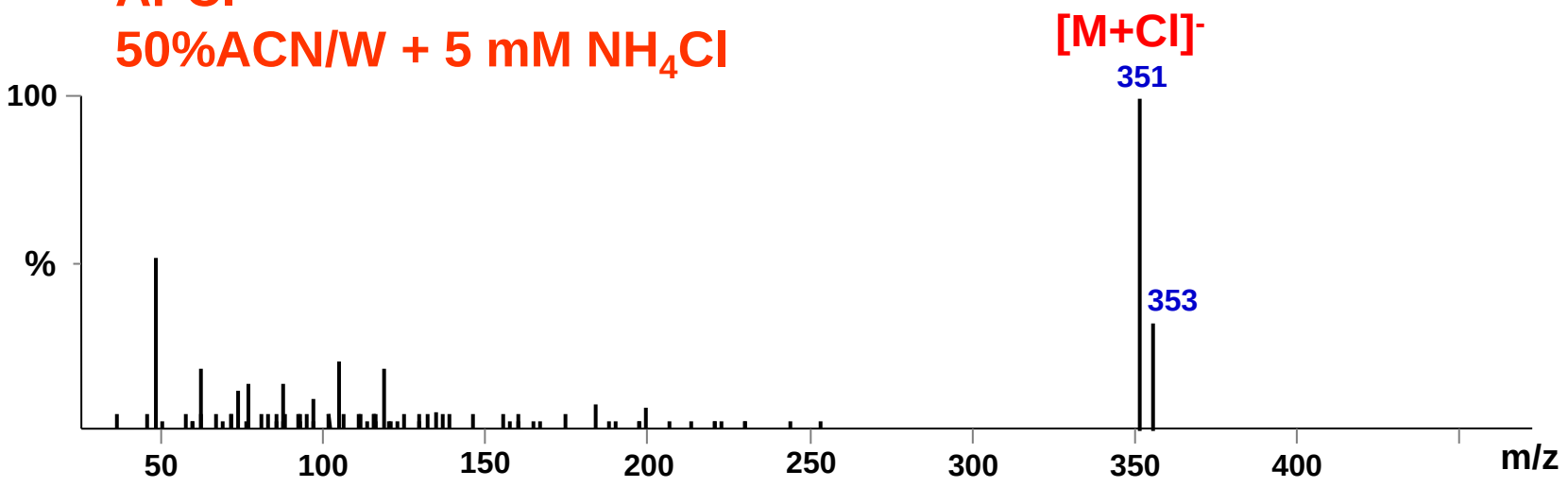
MW=450
nitroskupina
sudý počet dusíků
MF obsahuje acetonitril



#4/ O jaký typ sloučeniny se jedná? Tvorba aduktů může vést ke stabilizaci iontu.



MW=316
pentrit

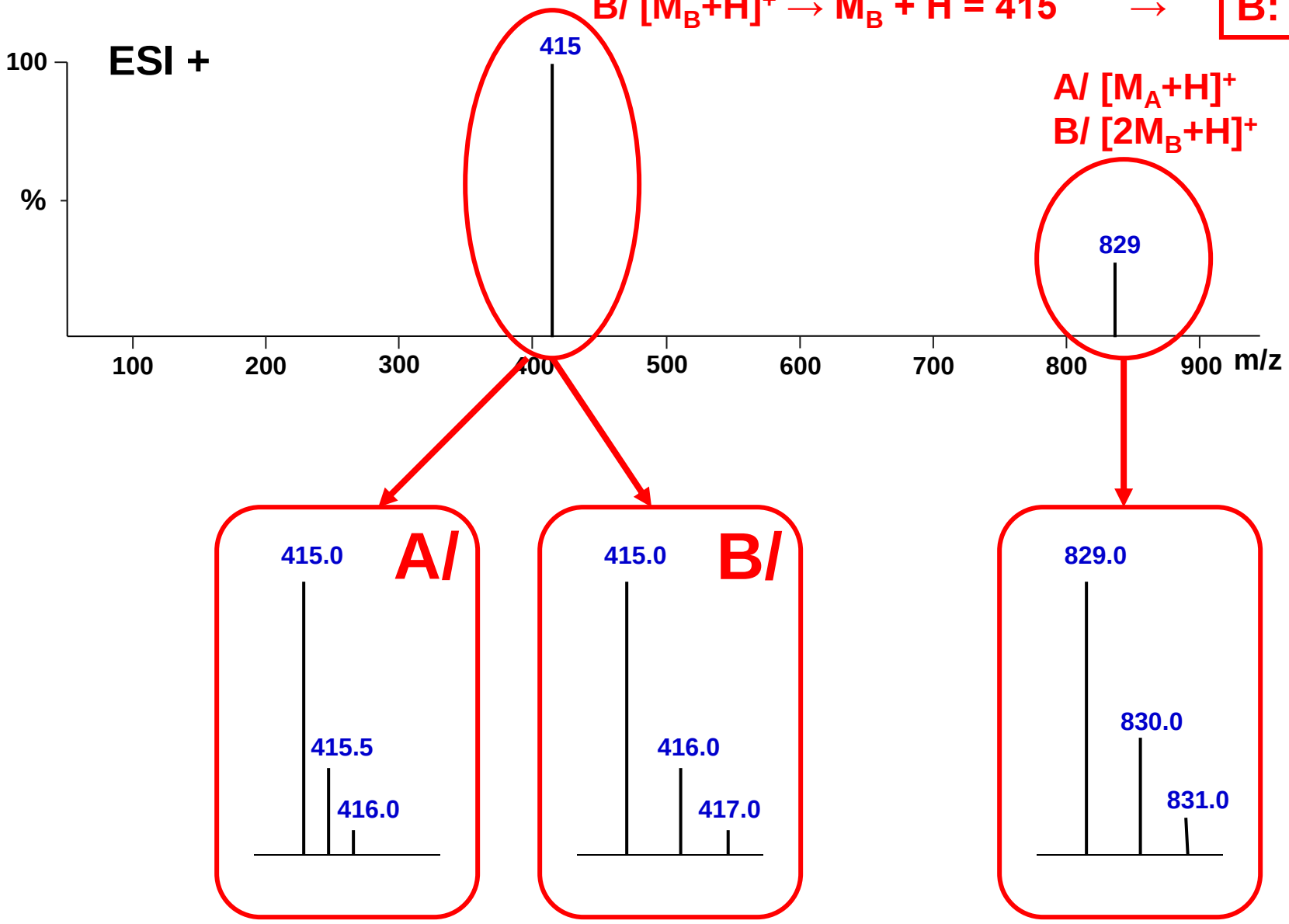


#5/ Určete MW

A/ $[M_A+2H]^{2+} \rightarrow (M_A + 2) / 2 = 415 \rightarrow$

B/ $[M_B+H]^{+} \rightarrow M_B + H = 415 \rightarrow$

A: M_A=828
B: M_B=414

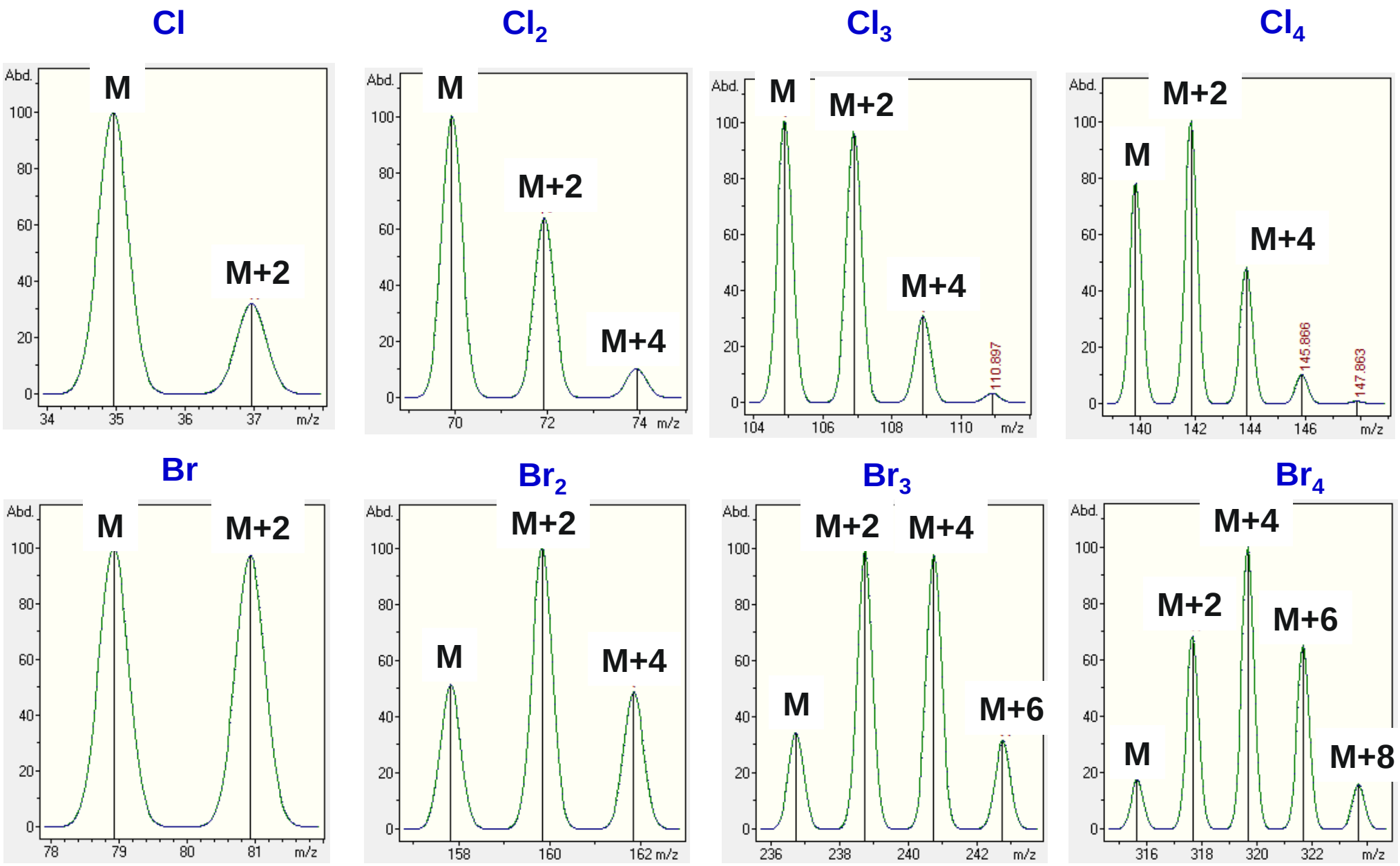


Určení elementárního složení

Defekty atomových hmotností Izotopické zastoupení

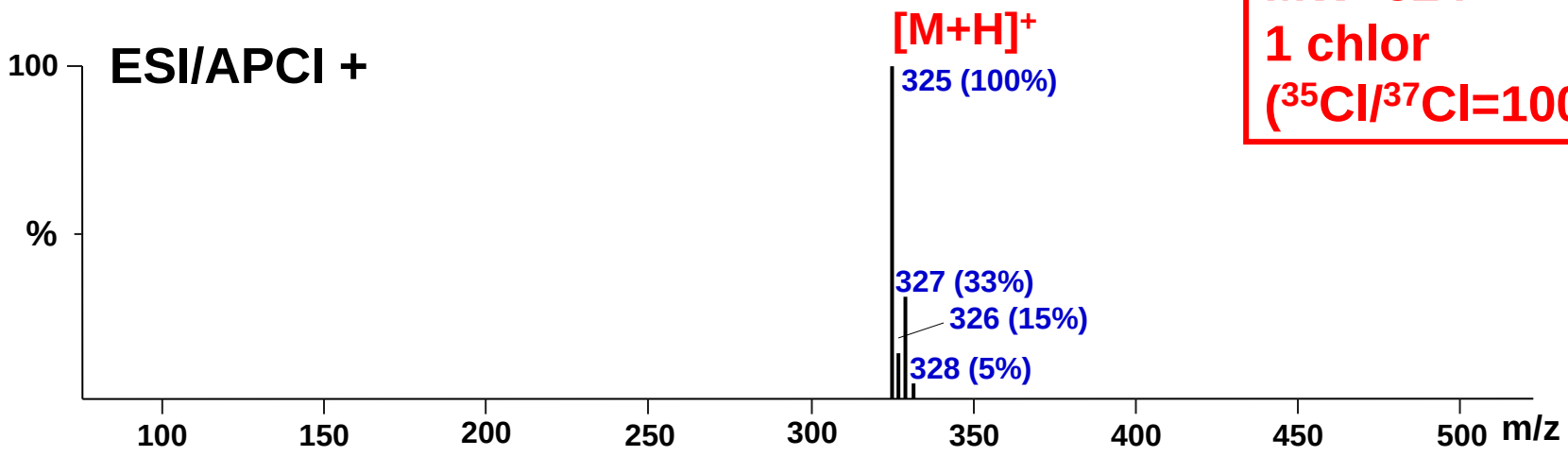
Prvek	Nominální atomová hmotnost [Da]	Hmotnostní schodek [mDa]	Přírodní zastoupení izotopů			Typ prvku
			M [%]	M+1 [%]	M+2 [%]	
H	1	7.8	100	0.015		"M"
C	12	0	100	1.1		"M+1"
N	14	3.1	100	0.37		"M+1"
O	16	-5.1	100	0.04	0.2	"M+2"
F	19	-1.6	100			"M"
Si	28	-23.1	100	5.1	3.4	"M+2"
P	31	-26.2	100			"M"
S	32	-27.9	100	0.79	4.4	"M+2"
Cl	35	-31.1	100		32	"M+2"
Br	79	-81.7	100		97.3	"M+2"
I	127	-95.5	100			"M"
Na	23	-10.2	100			"M"

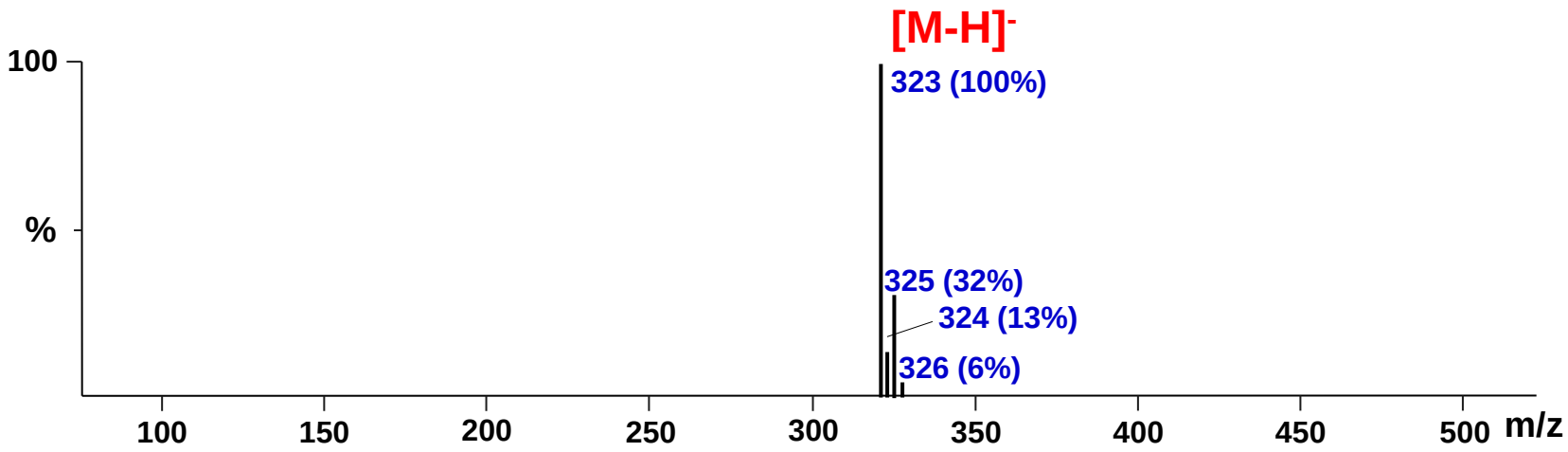
Izotopická obálka pro atomy chloru a bromu



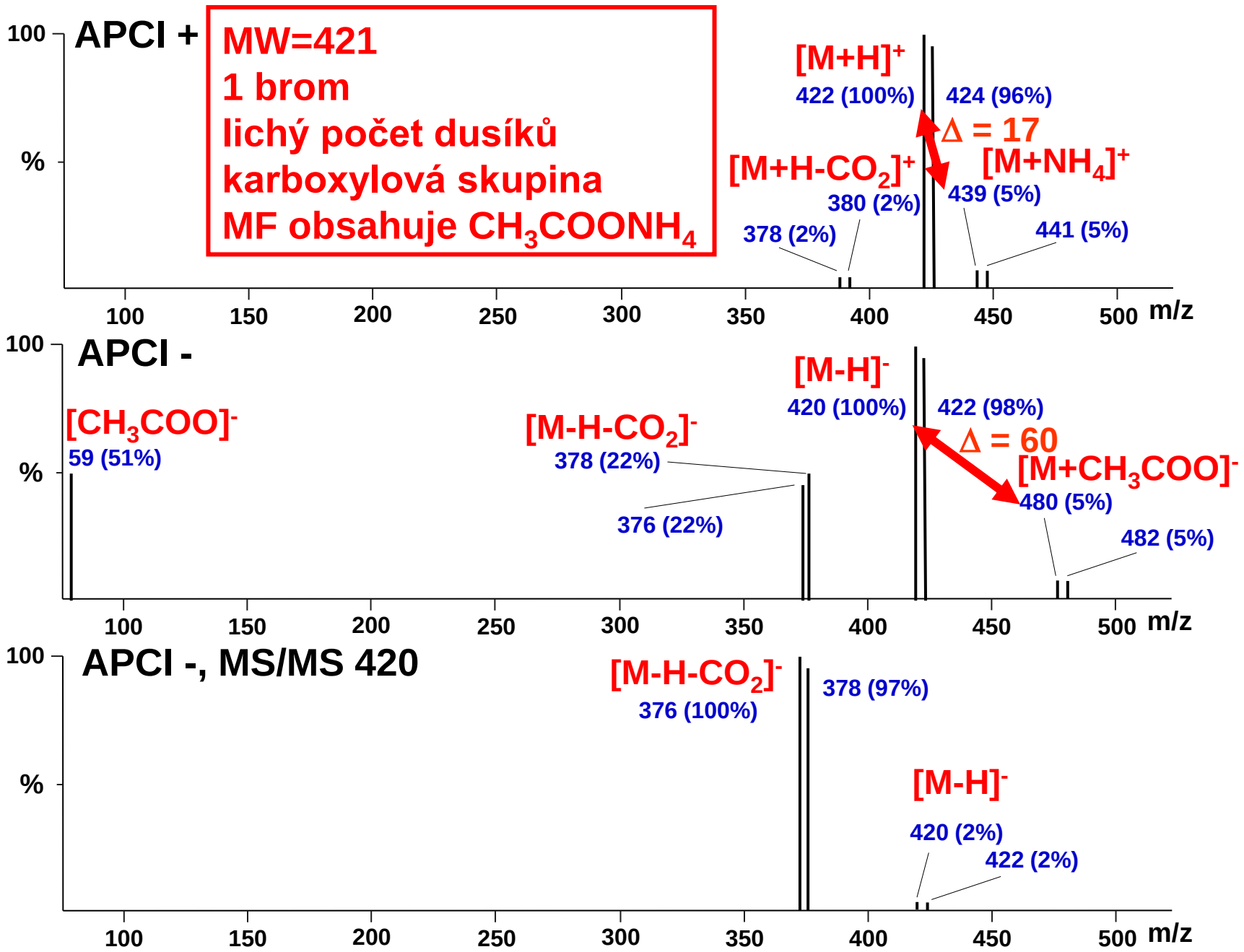
#6/ Určete MW a vysvětlete izotopy

MW=324
1 chlor
(^{35}Cl/^{37}Cl=100:32)

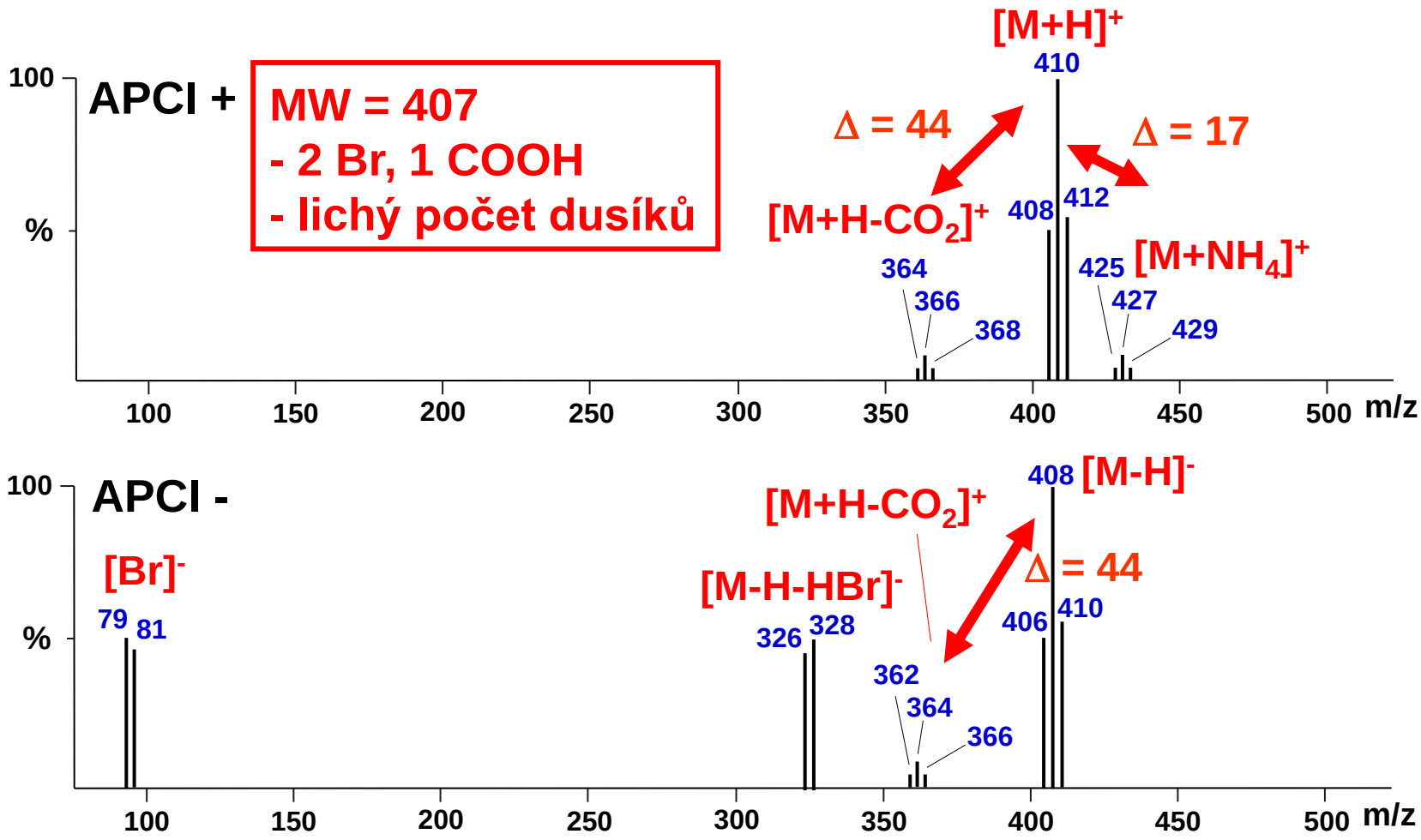




#7/ Určete MW, popište ionty



#8/ Určete MW a maximum informací



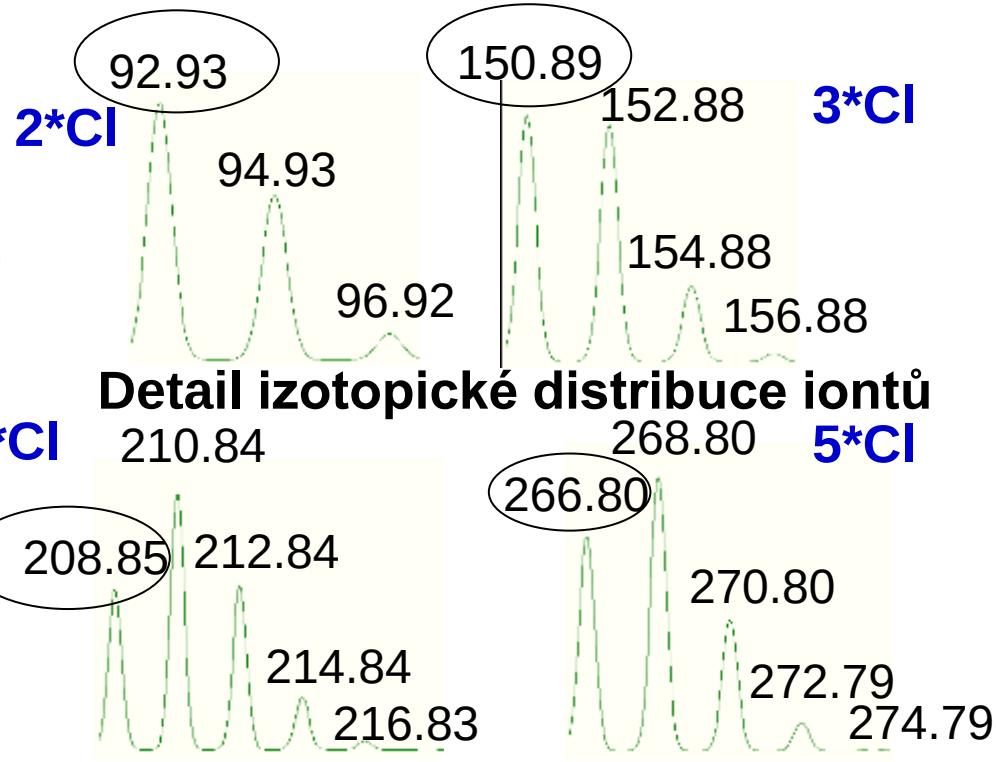
#9/ Určete o jakou sloučeninu se jedná?

Výchozí informace: bílá krystalická látka

Hodnoty m/z ve spektru odpovídají nejintenzivnějším píkům izotopické distribuce jednotlivých iontů

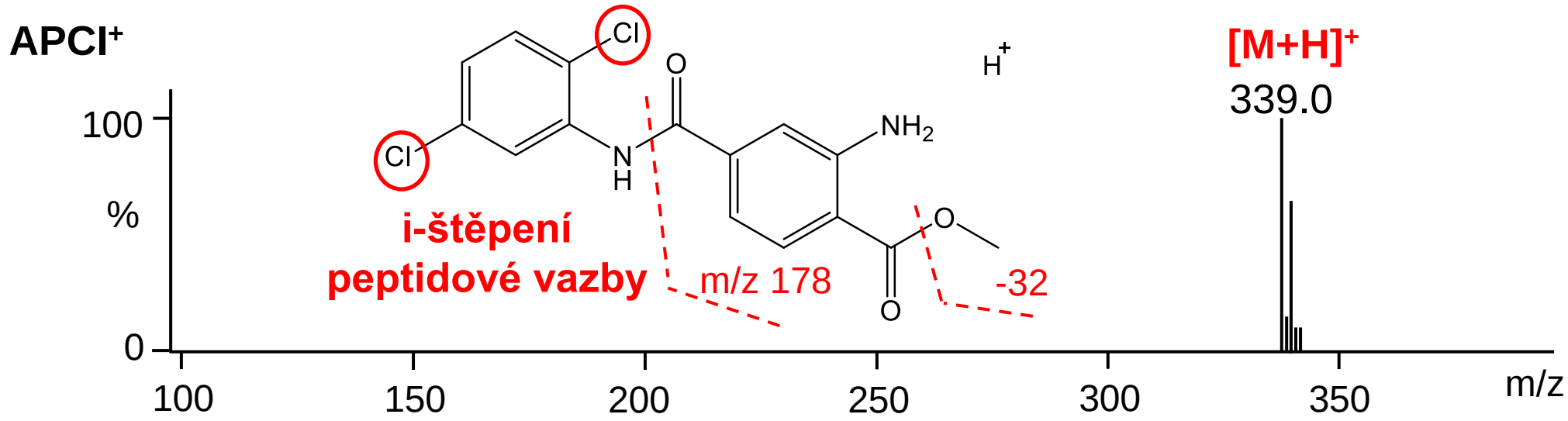
58 – 35 = 23

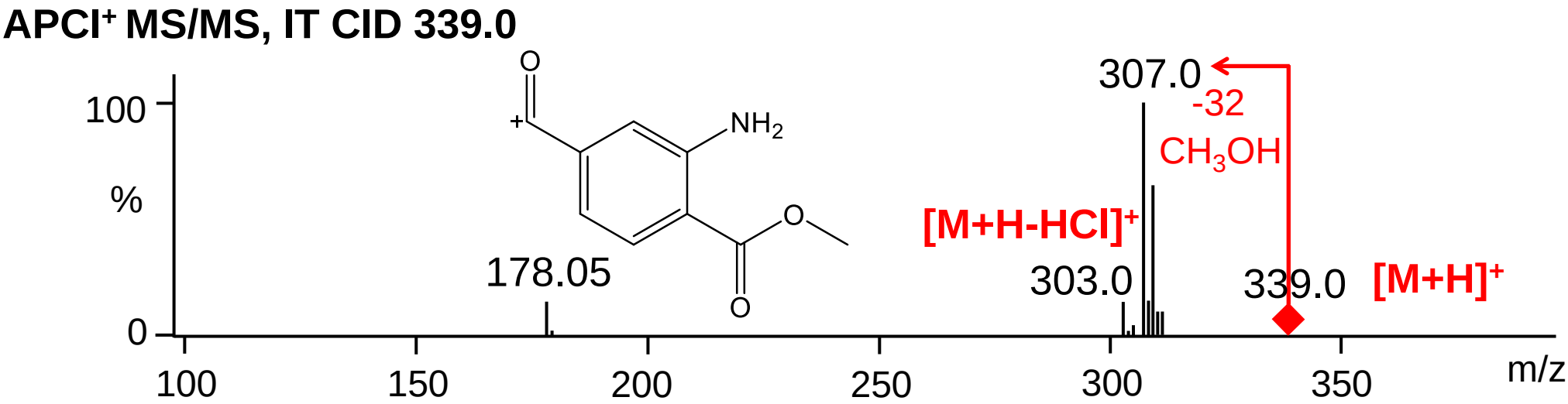
$[(NaCl)_n+Cl]^-$



U molekul složených z atomů, které nemají M+1 izotop se nevyskytují píky M+1, M+3, atd.

#10/ Vysvětlete produktové ionty





Pokud možné, tak kontrola izotopické distribuce
(musí být ale širší izolační šířka při izolaci prekurzoru

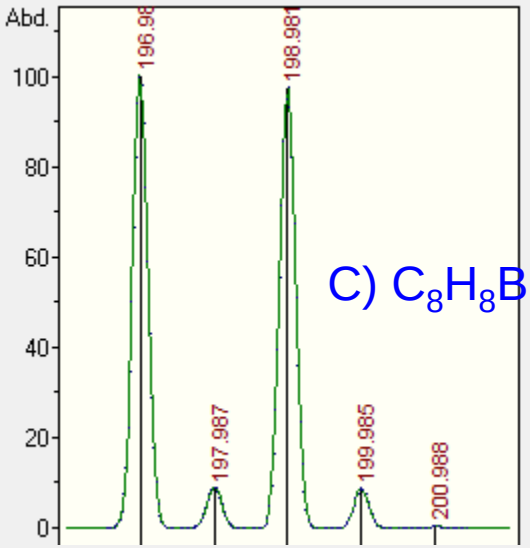
#11/ Přiřaďte jednotlivé elementární složení k izotopickým obálkám iontu m/z 197:

A) $(NaCl)_3Na$ klástrový ion

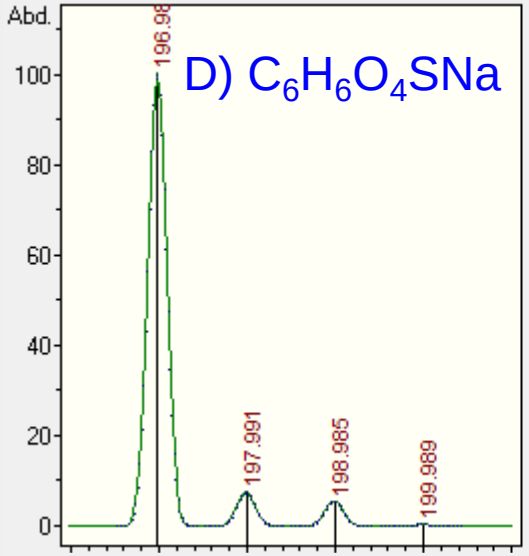
B) sodný adukt argininu $C_6H_{14}N_4O_2Na$

C) 5-Bromoindolin ($M^{+\cdot}$) C_8H_8BrN;

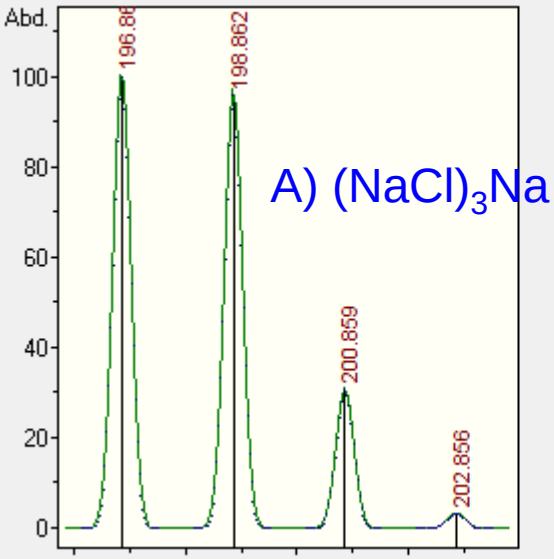
D) sodný adukt 4-hydroxybenzensulfonové kyseliny $C_6H_6O_4SNa$



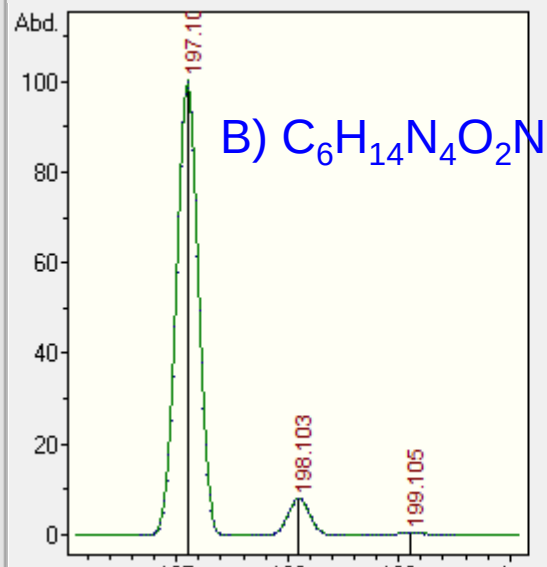
#	m/z	Abundance
1	196.983463	100.000
2	197.986591	9.114
3	198.981448	97.645
4	199.984553	8.874
5	200.987641	0.358



#	m/z	Abundance
1	196.987900	100.000
2	197.990877	7.511
3	198.985383	5.585
4	199.988552	0.369



#	m/z	Abundance
1	196.865088	100.000
2	198.862138	95.883
3	200.859188	30.645
4	202.856238	3.265

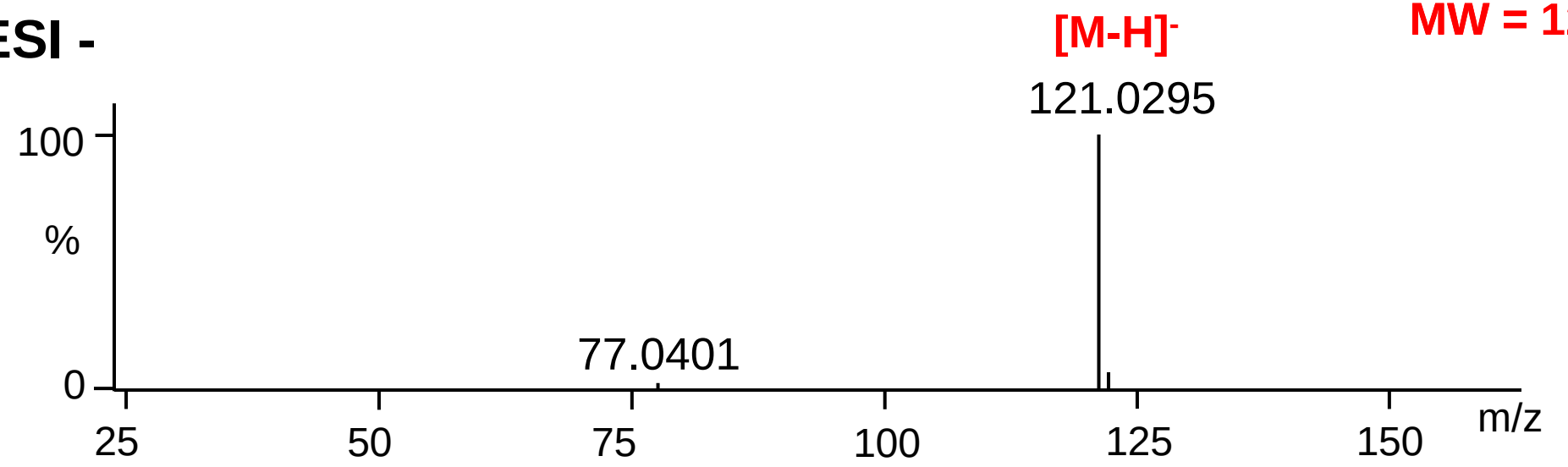


#	m/z	Abundance
1	197.100897	100.000
2	198.103179	8.204
3	199.105211	0.710

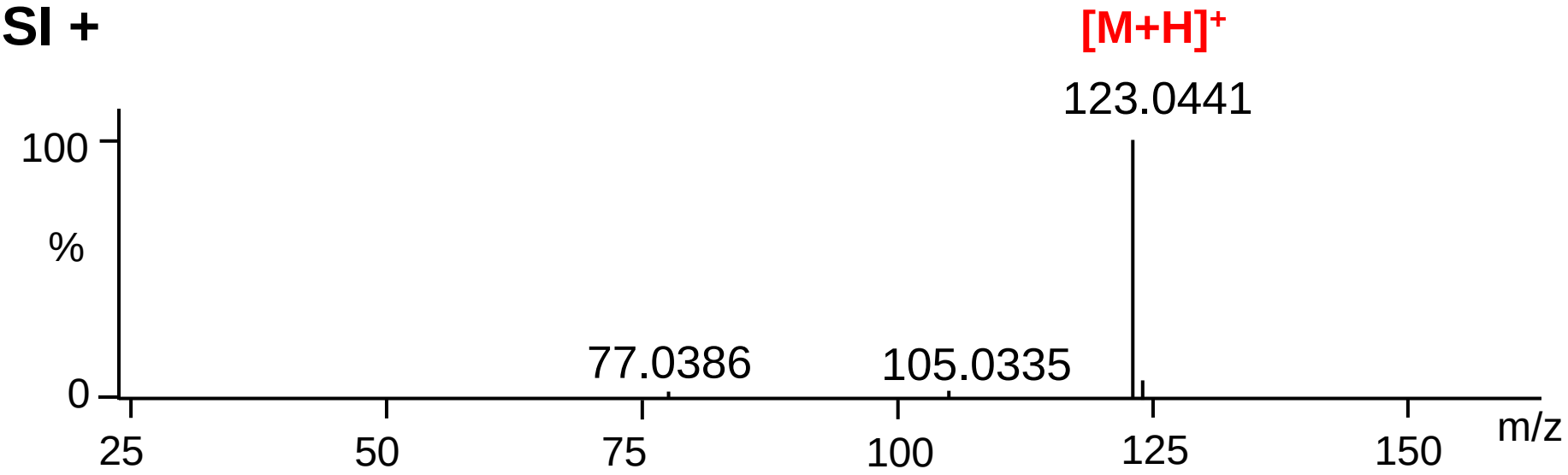
#12/ Určete o jakou sloučeninu se jedná

MW = 122

ESI -



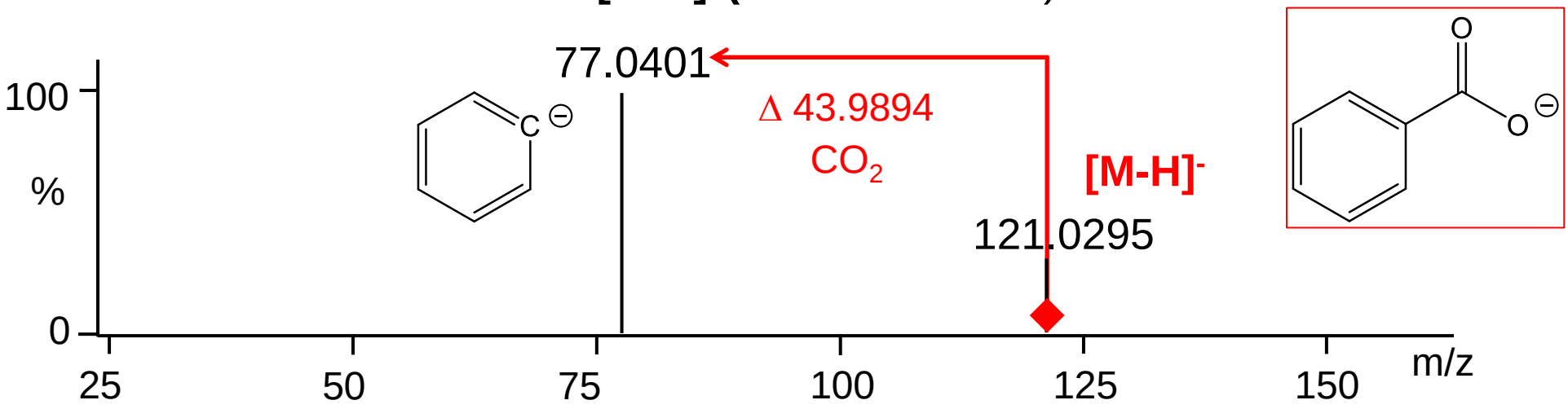
ESI +



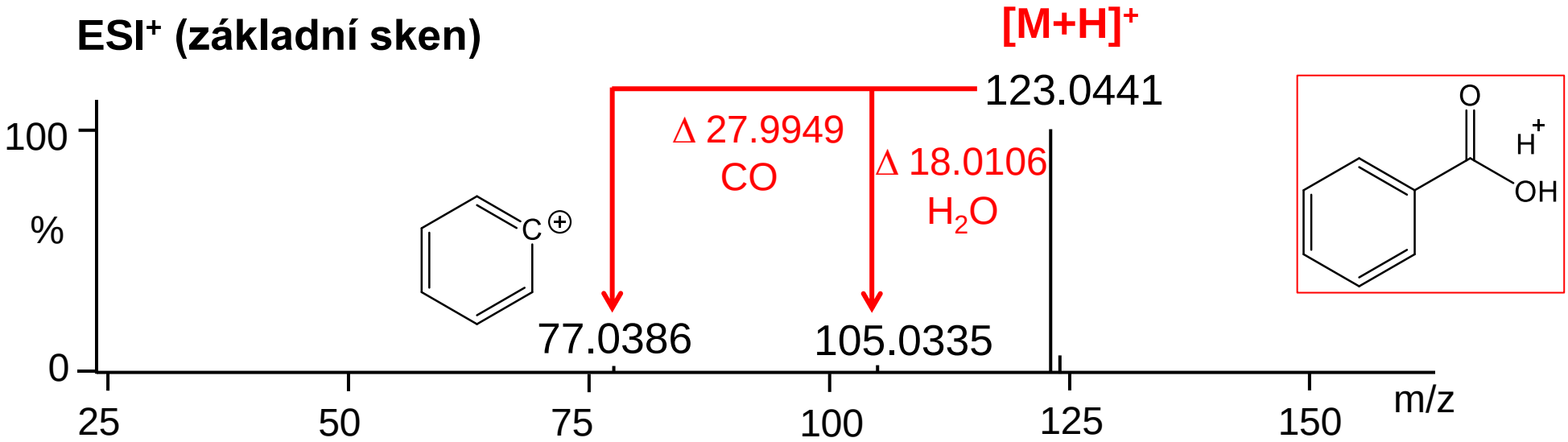
Pokračování 12.

Liché hmoty → 0 nebo sudý počet N

ESI⁻ MS/MS iontu m/z 121 [M-H]⁻ (FT CID 25 NCE)

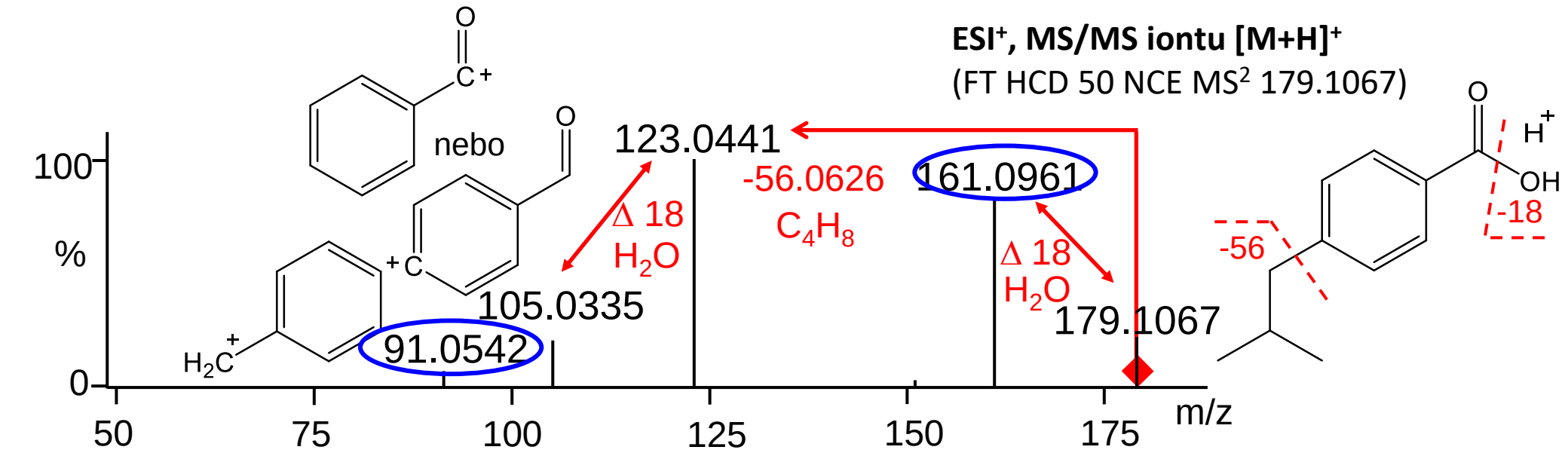


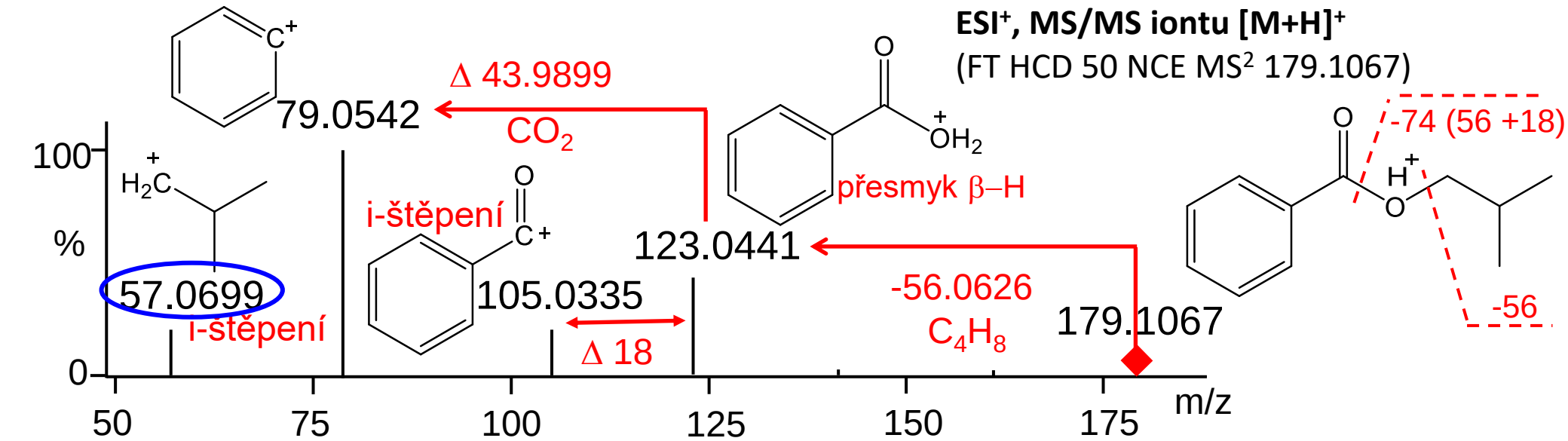
ESI⁺ (základní sken)



Zdroj: mzCloud (https://www.mzcloud.org/)

#13/ Popište ionty (neutrální ztráty). Které ionty slouží pro jednoznačné rozlišení studovaných sloučenin?

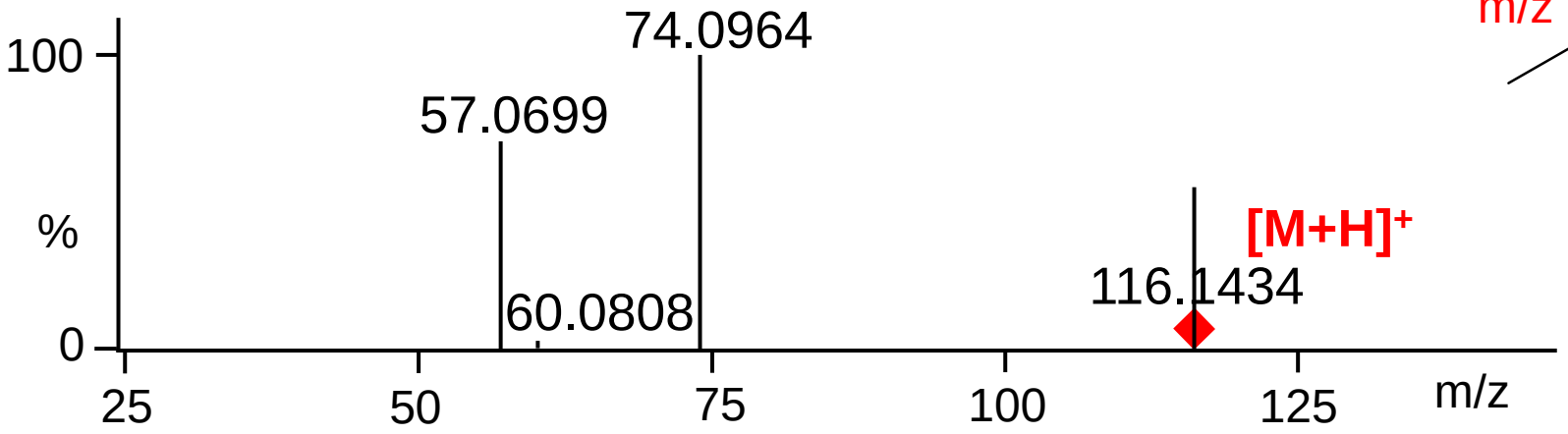


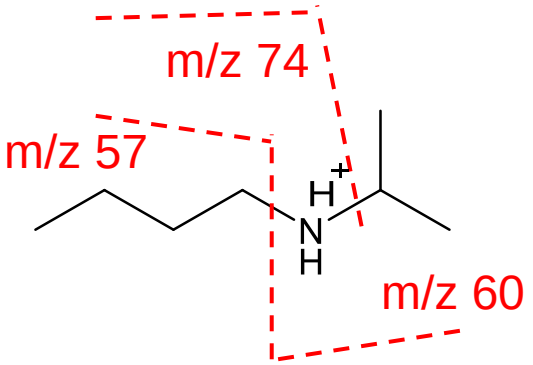


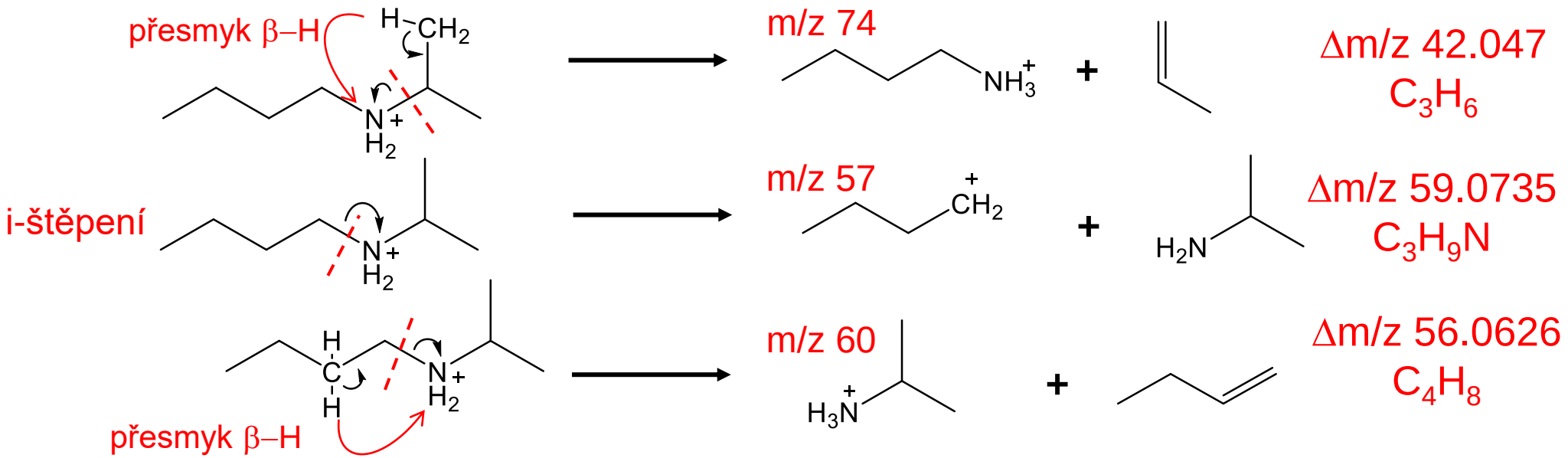
Zdroj: mzCloud (https://www.mzcloud.org/)

#14/ Navrhněte mechanismus tvorby iontů v MS/MS $[M+H]^+$ butylisopropylaminu

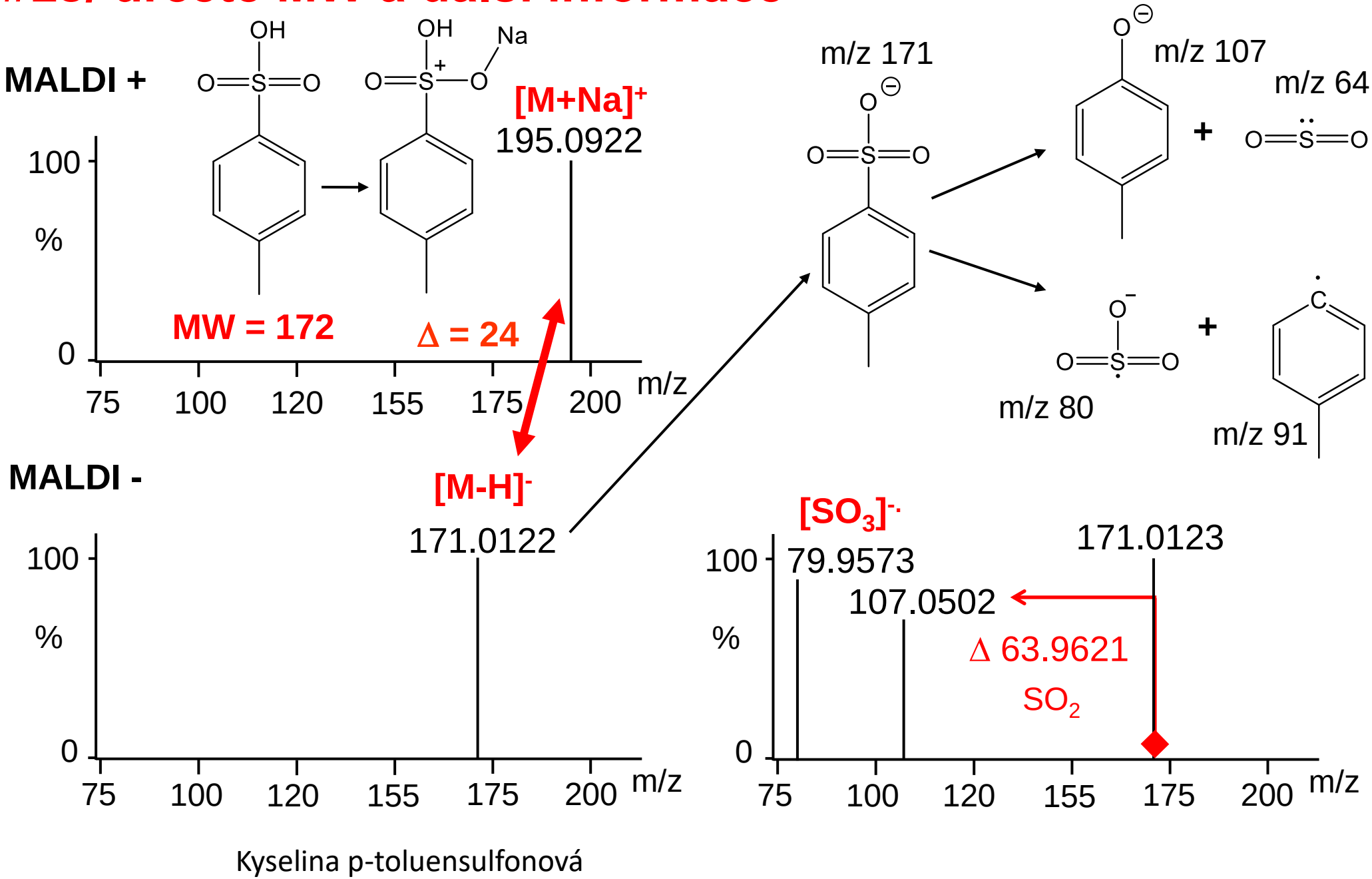
ESI^+ MS/MS iontu $[M+H]^+$ (FT HCD 50 NCE MS^2 179.1067)





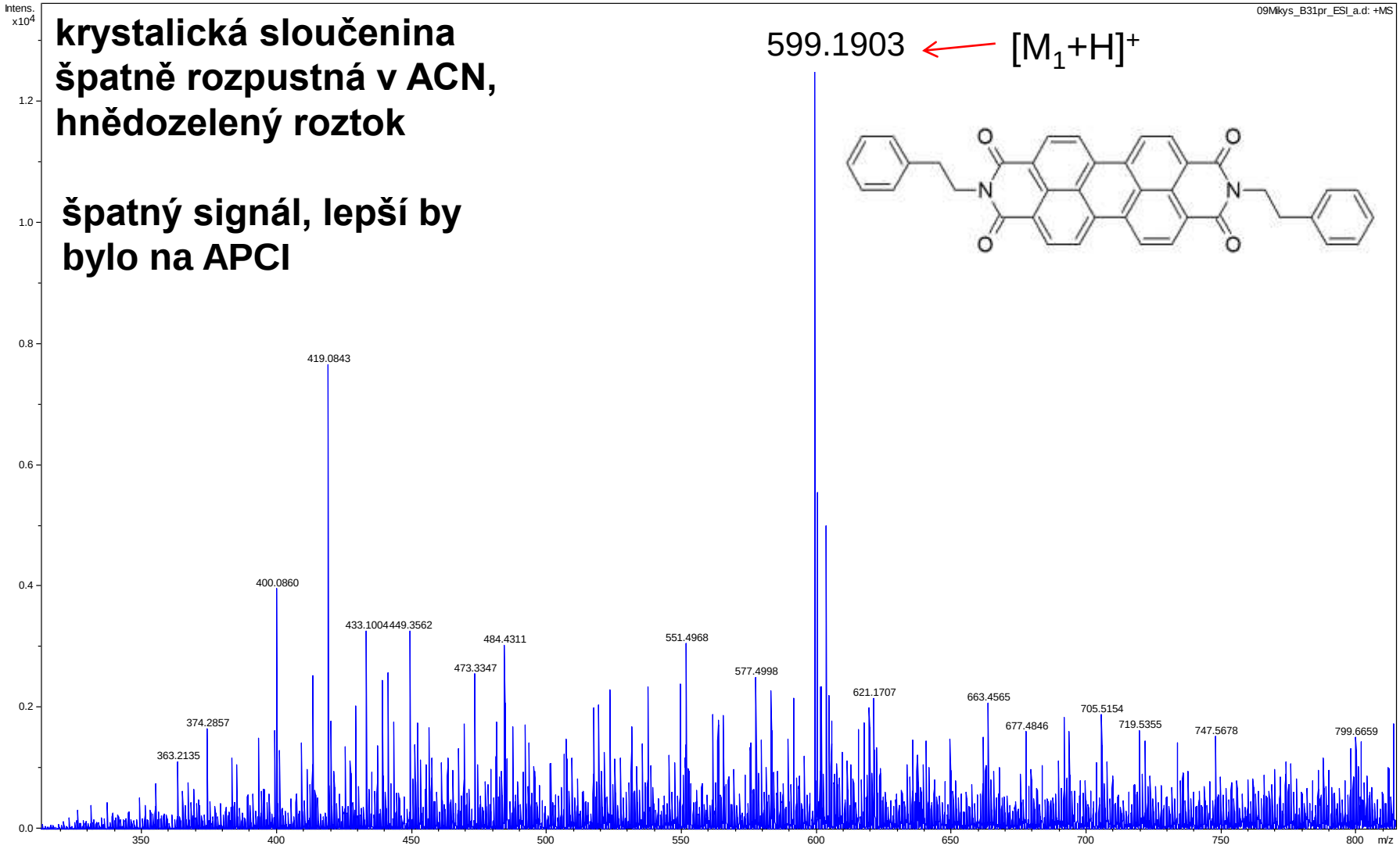


#15/ určete MW a další informace



Kyselina p-toluensulfonová

#16/ Nepřečištěný produkt syntézy (ESI-QqTOF)

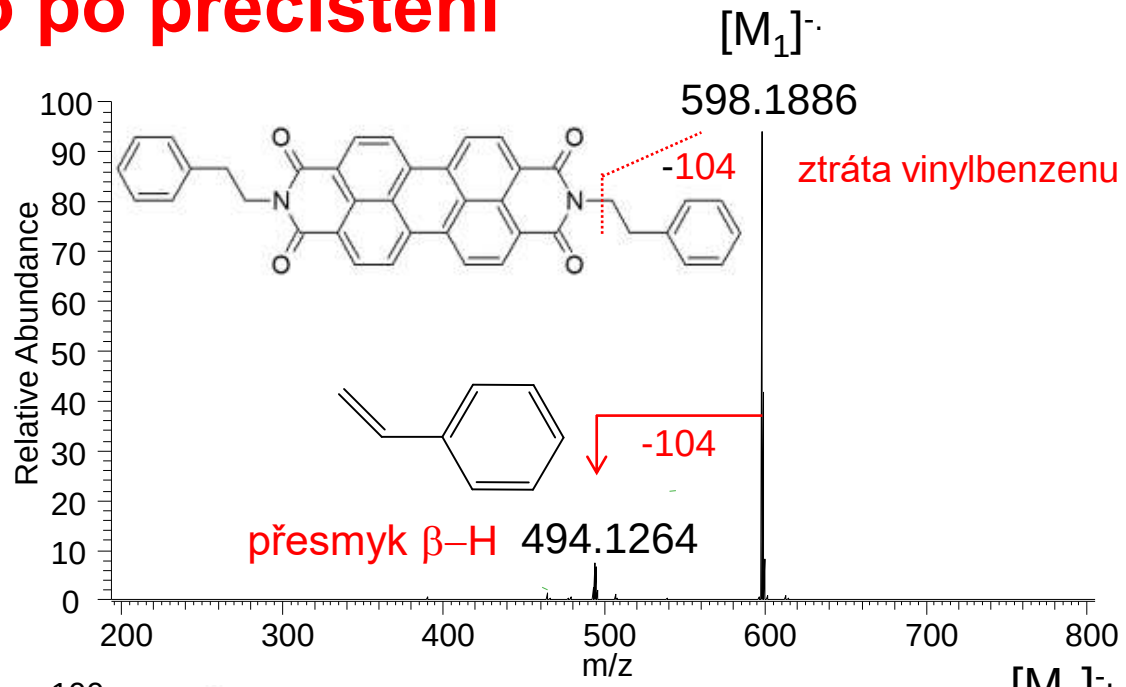


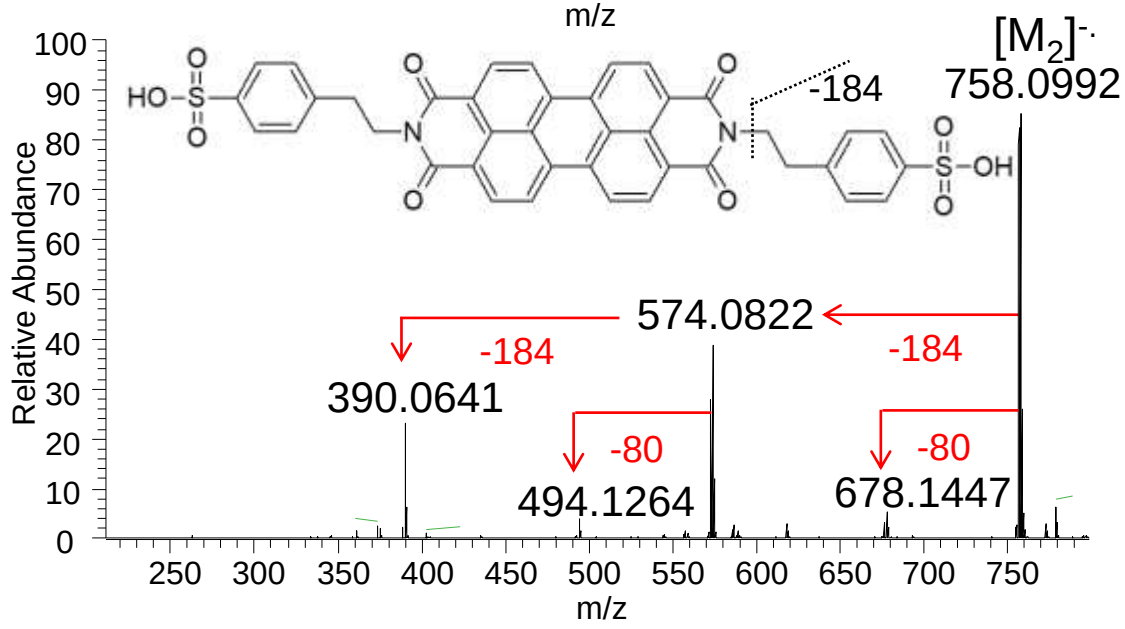
Pokračování 16 - na základě informací popište ionty a vysvětlete, co se stalo po přečištění

- krystalická sloučenina špatně rozpustná ACN, hnědozelený roztok

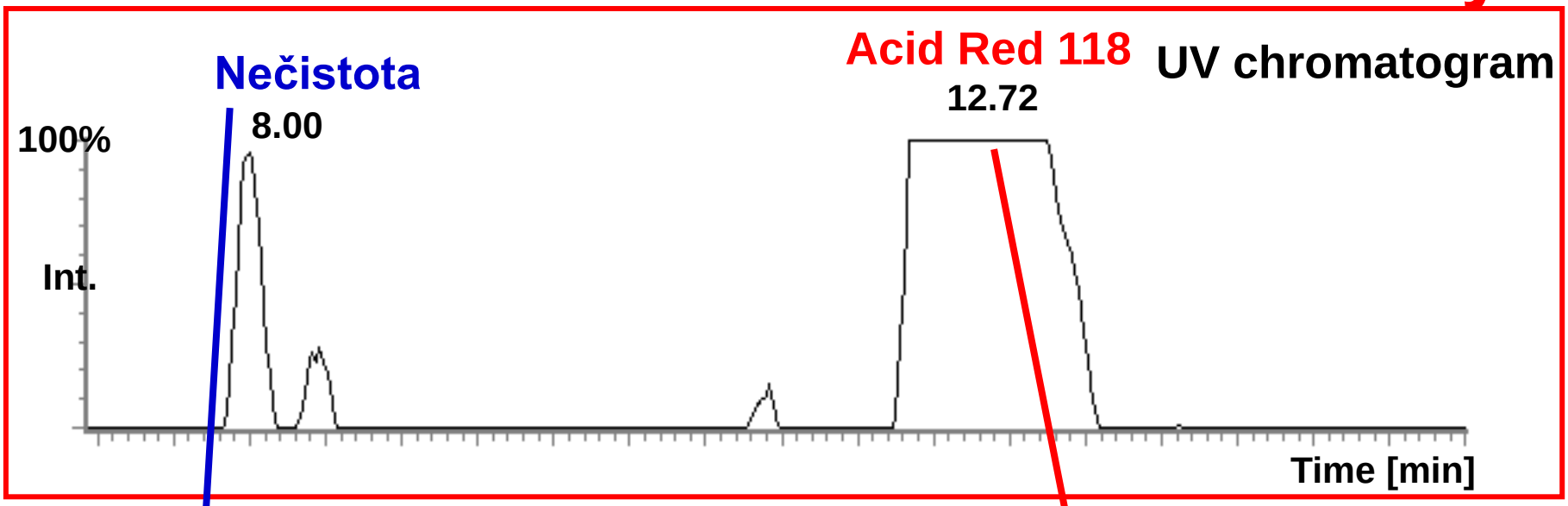
- po přečištění v H_2SO_4 dobře rozpustná v ACN/H_2O, červený roztok

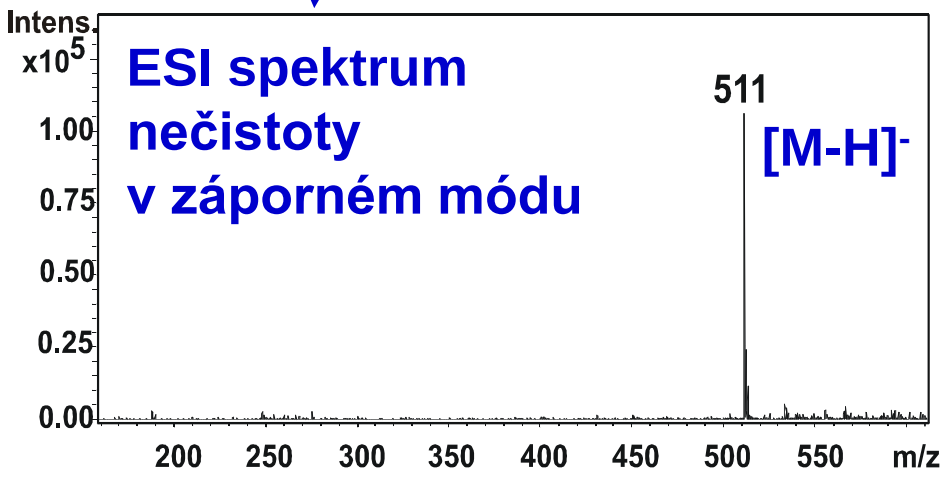
Číslo za desetinou čárkou je u fragmentového iontu vyšší Δm/z 79,9545 (hmotnostní defekt typický pro síru) –SO_3

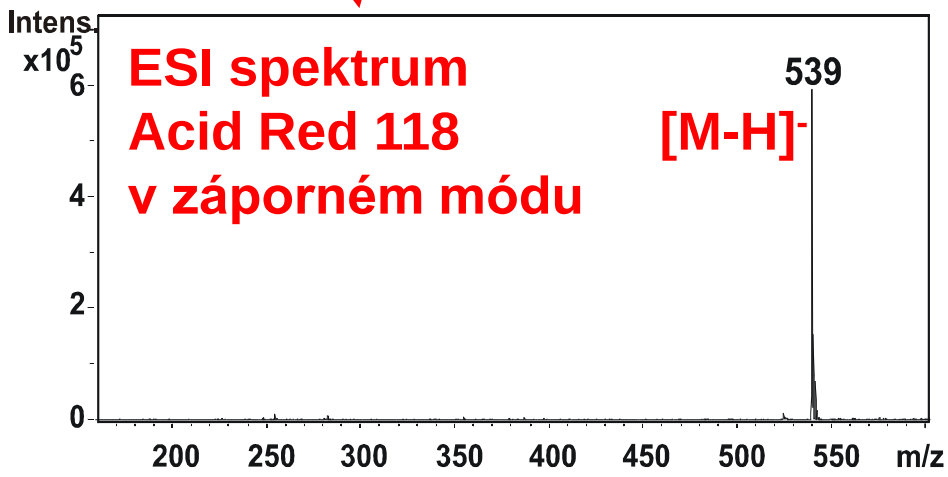




#17/ HPLC/MS/MS identifikace nečistoty





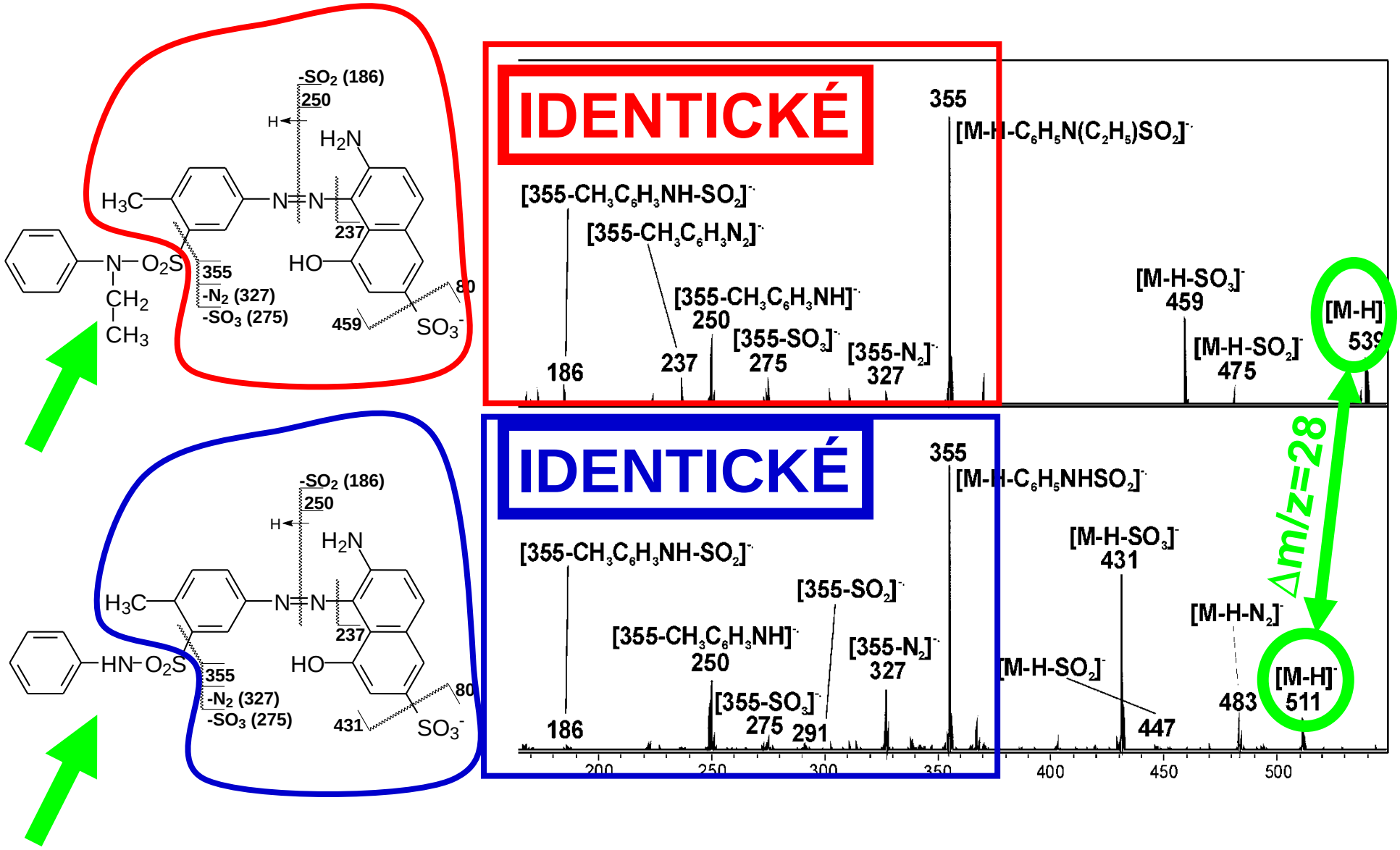


M_R=512

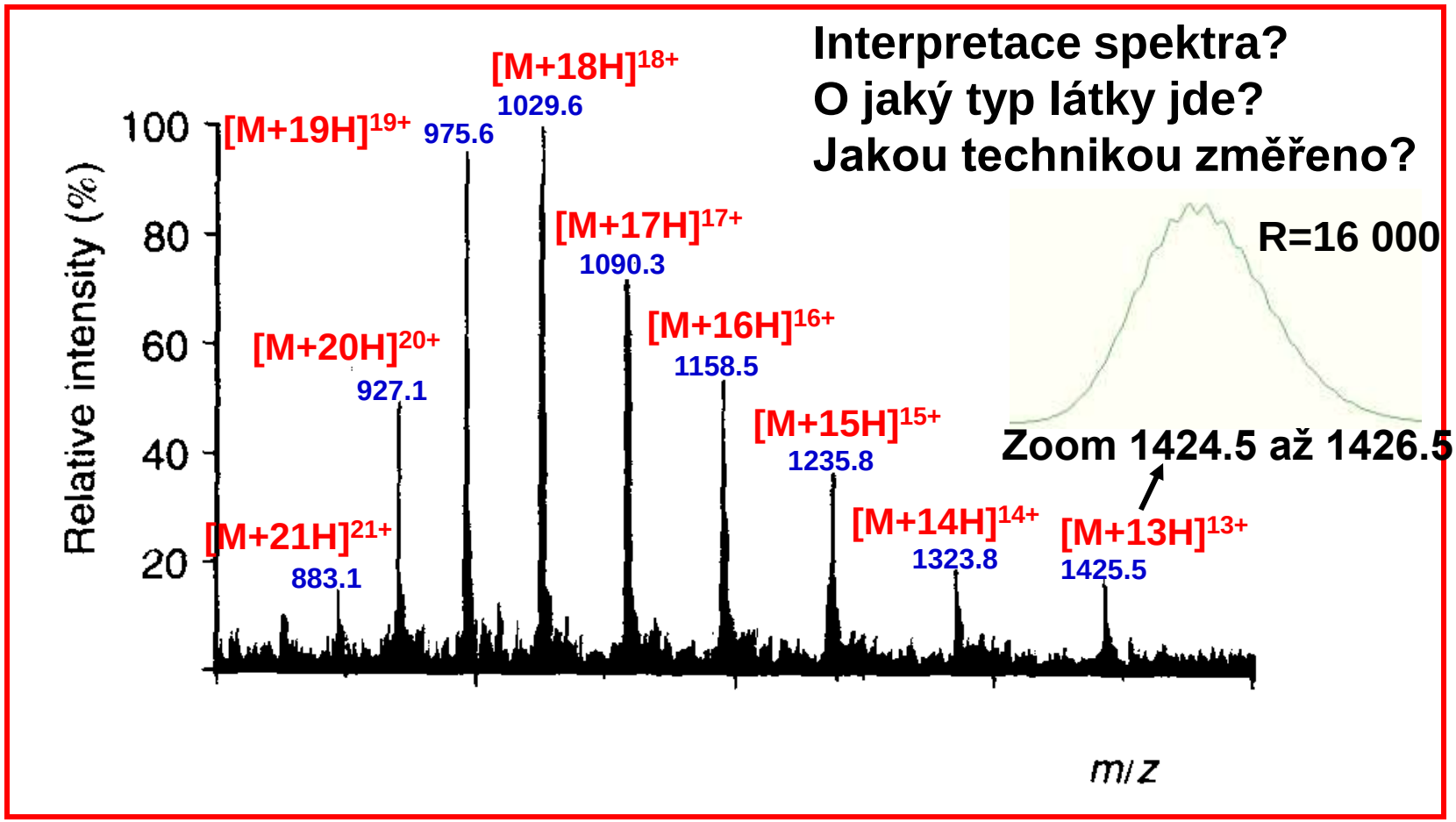
ΔM_R=28

M_R=540

HPLC/MS/MS barviva a nečistoty



#18/



Příklad výpočtu MW:

A = 1029.6 = (MW + z) / z

B = 975.6 = (MW + z + 1) / (z + 1)

- řešením vyjde z = 18.05 = 18

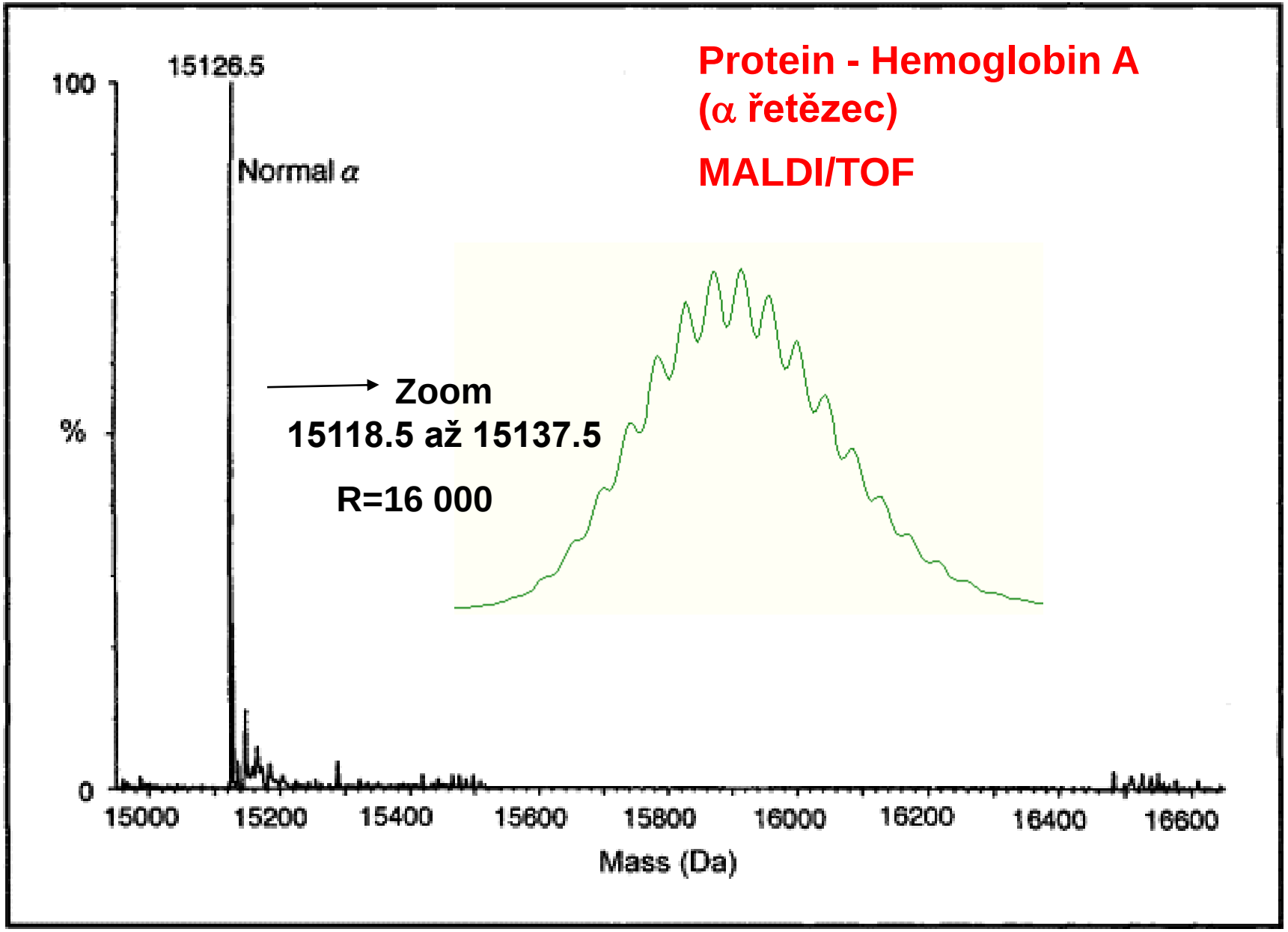
A: MW = 1029.6 * 18 – 18 = 18514.8

B: MW = 975.6 * 19 – 19 = 18517.4, atd.

Změřeno pomocí ESI+

Protein
MW=18521 Da

Zadání: Určete o jaký typ sloučeniny se jedná a určete její molekulovou hmotnost. Jakou instrumentací bylo změřeno níže uvedené MS spektrum?

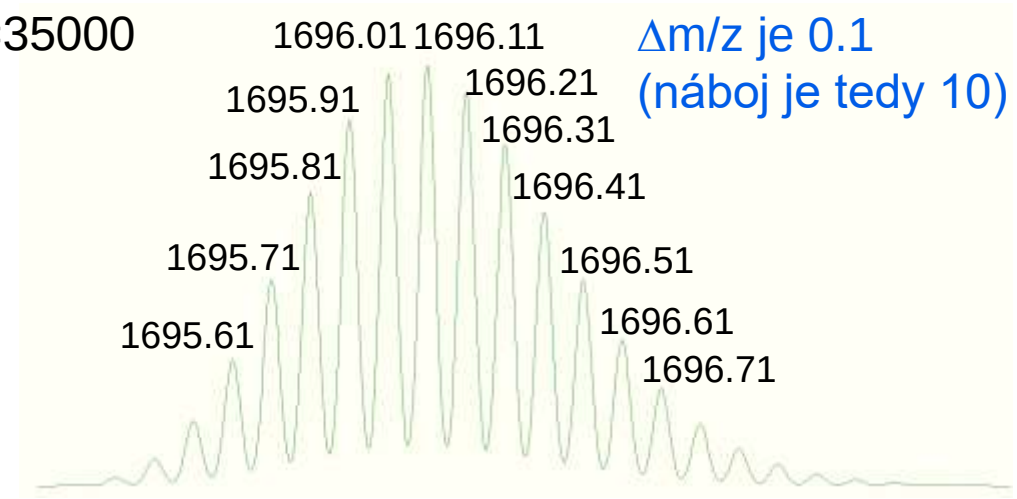


Interpretace izotopické obálky

Protein v ESI+

$[C_{769}H_{1202}N_{210}O_{218}S_2+10H]^{10+}$

R=35000

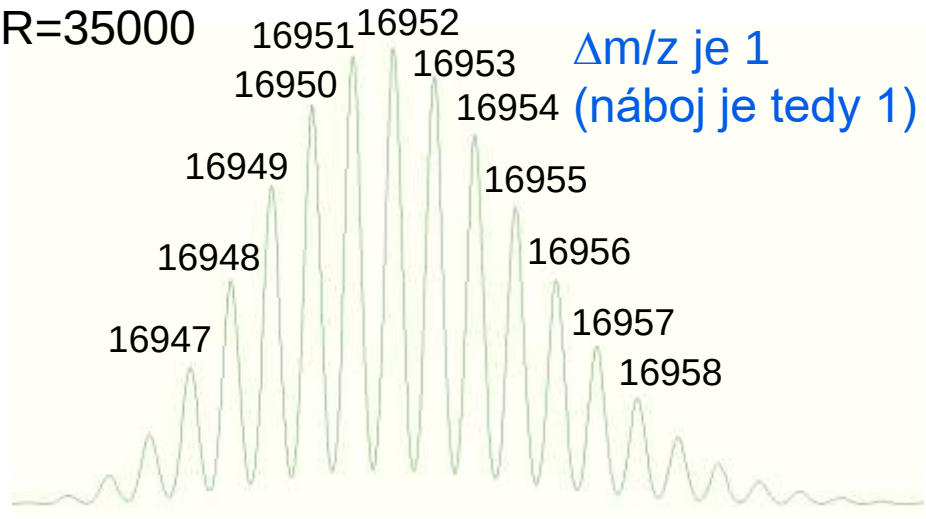


Δm/z je 0.1
(náboj je tedy 10)

Protein jednou nabitý

$[C_{769}H_{1212}N_{210}O_{218}S_2+H]^{+}$

R=35000

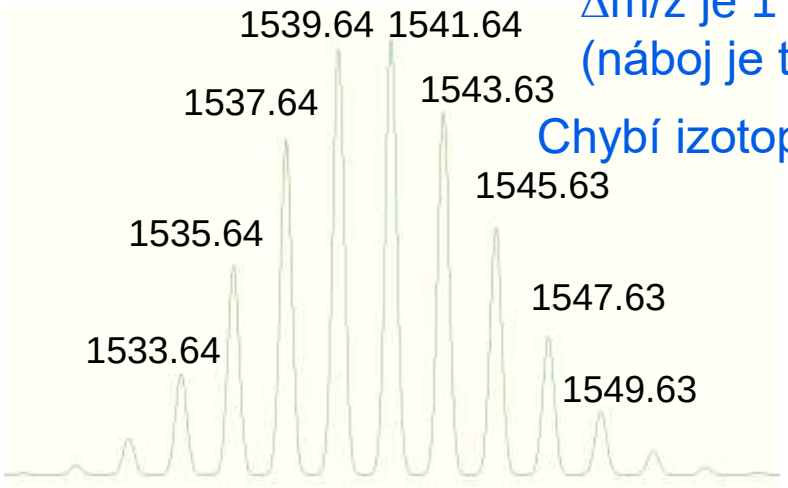


Δm/z je 1
(náboj je tedy 1)

Klástr AgCl

R=3000

$[(AgCl)_{10}Ag]^{+}$



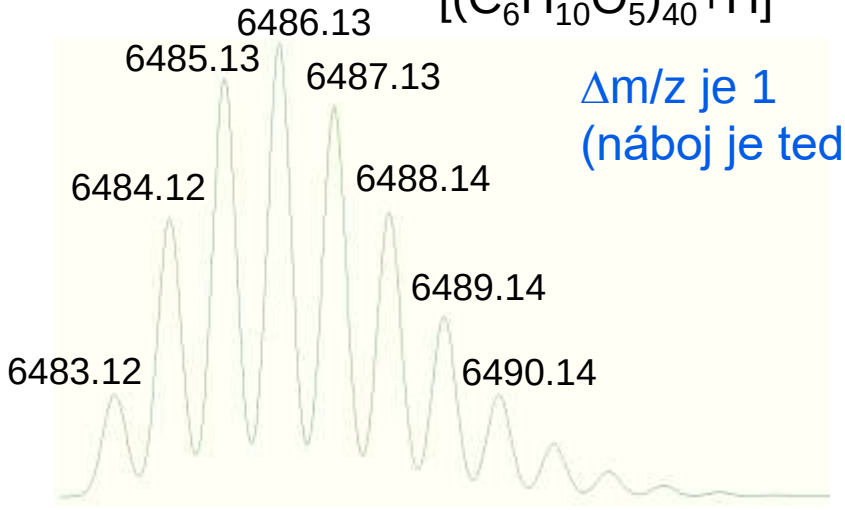
Δm/z je 1
(náboj je tedy 1)

Chybí izotopy M+1 atd.

polysacharid

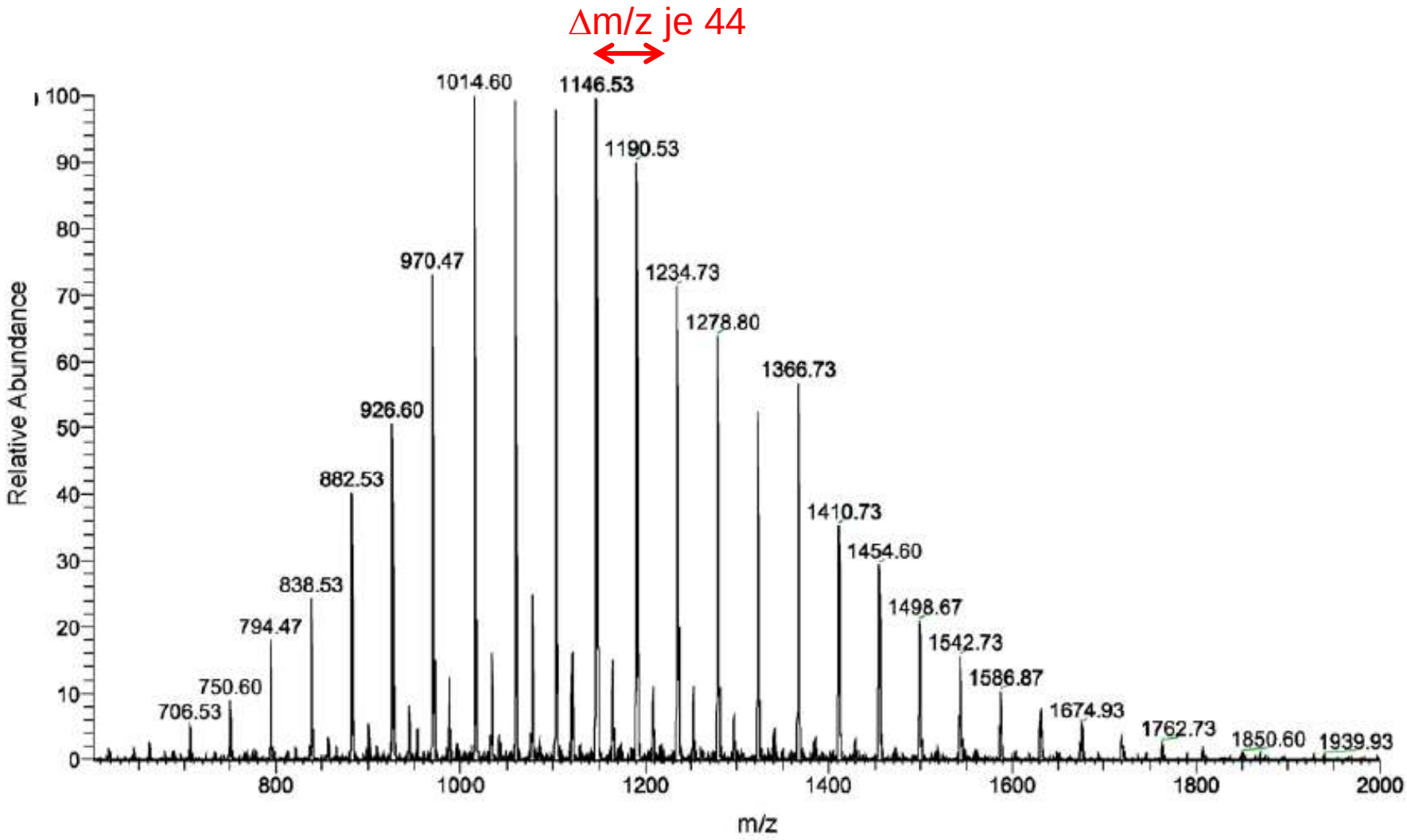
R=13000

$[(C_6H_{10}O_5)_{40}+H]^{+}$



Δm/z je 1
(náboj je tedy 1)

Určete o jaký typ sloučenin se jedná (všechny ionty jsou jednou nabité)



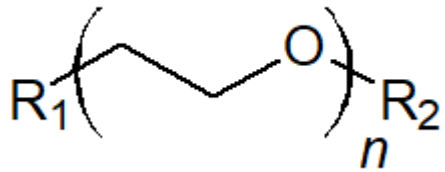
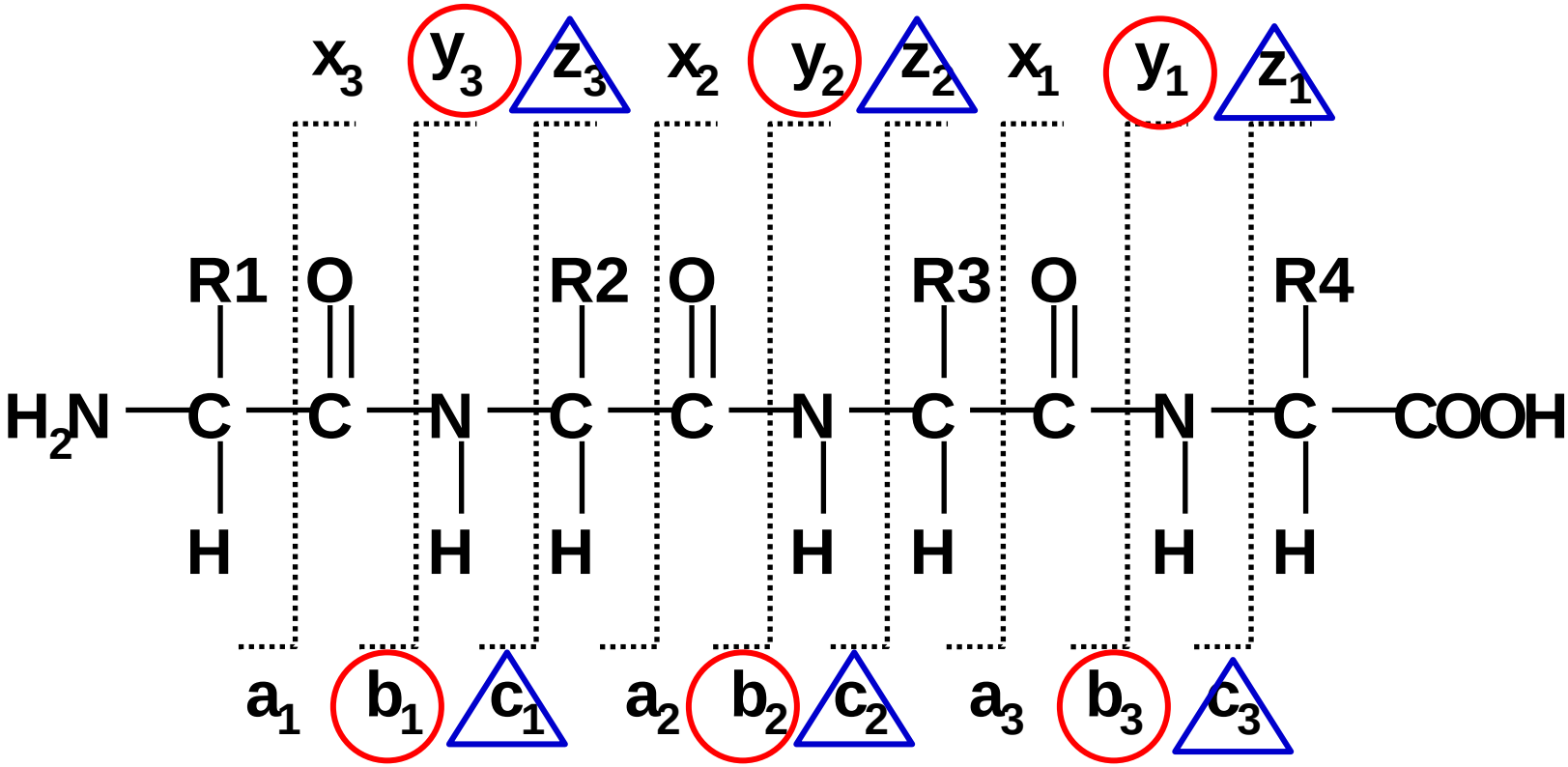


Schéma značení fragmentových iontů peptidů

Typické fragmenty pro CID MS/MS
Typické fragmenty pro ETD, UVPD

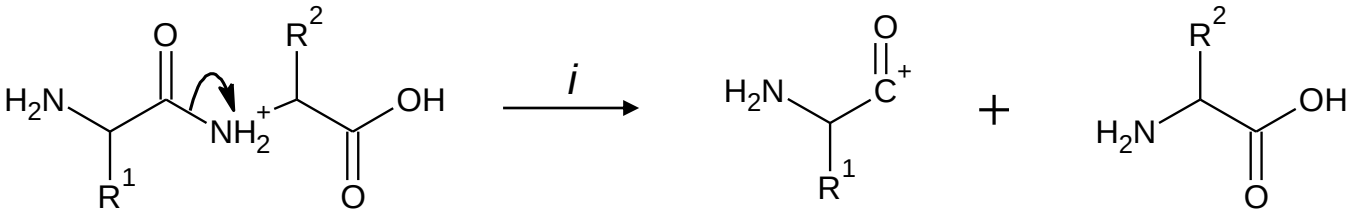


C-C (a, x); peptidová vazba (b, y); N-Cα (c, z)

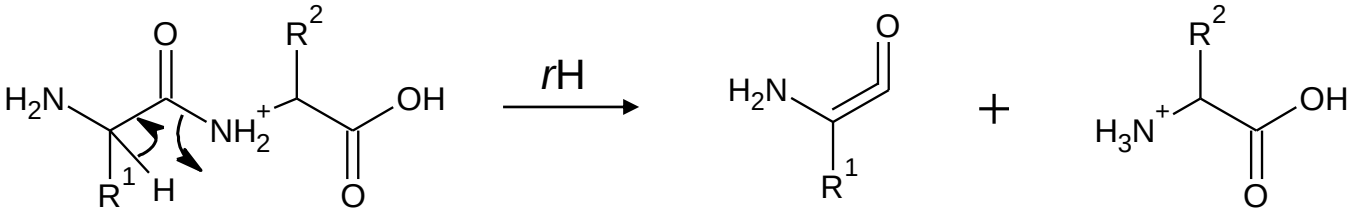
Štěpení peptidové vazby

Zjednodušený mechanismus fragmentace $[M + H]^+$ peptidu

– indukční štěpení, vznik acyliového iontu (iont typu ***b***)

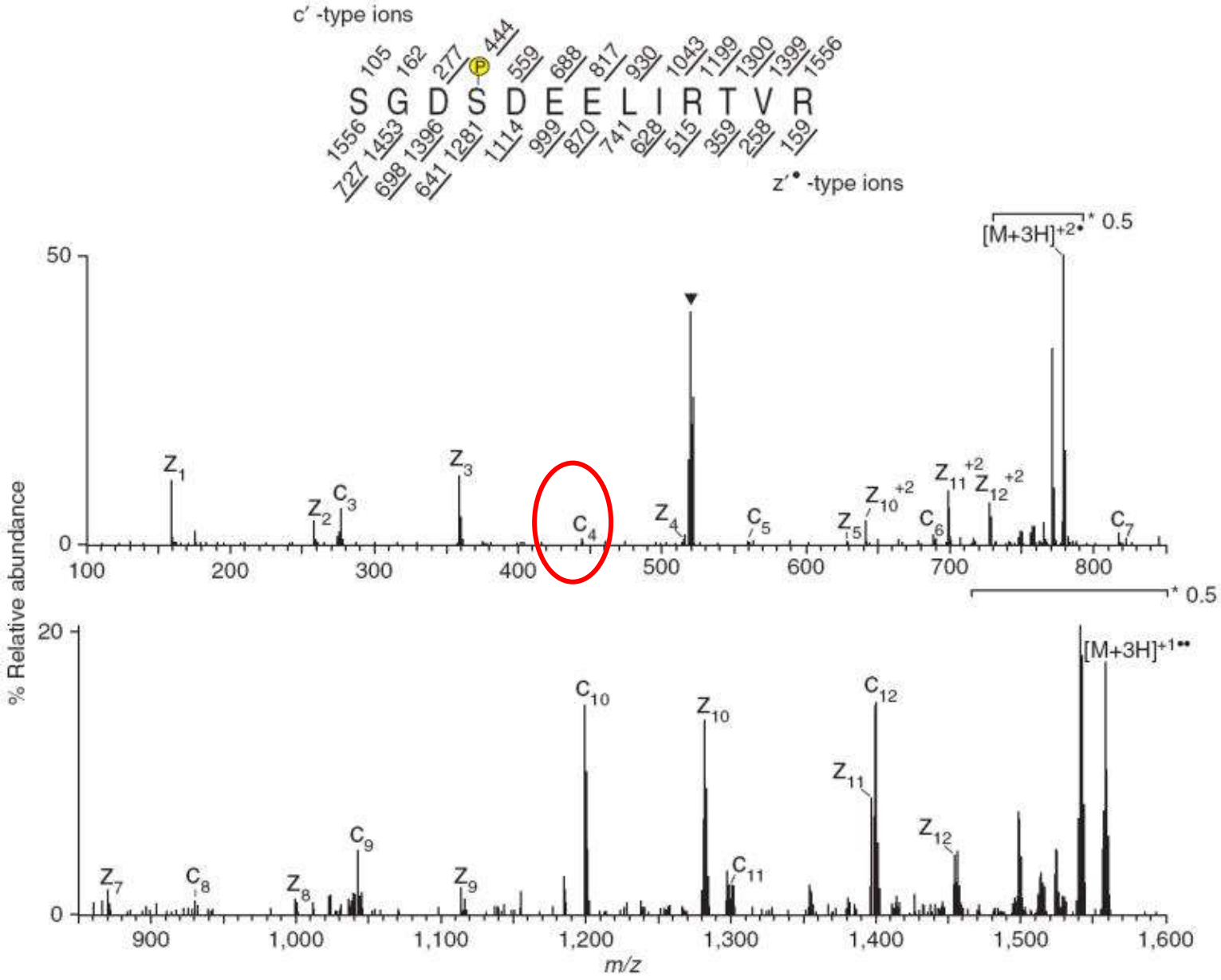


– štěpení po přesmyku ß-vodíku, vznik kratšího proton. peptidu (iont typu ***y***)



snímek převzat z přednášky doc. J. Cvačky (ÚOCHB, Praha)

Zjištění sekvence aminokyselin v peptidu - ETD



Zjištění sekvence aminokyselin v peptidu - ETD

Table of Amino Acids and Their Abbreviations

Full Name	Abbreviation (3 Letter)	Abbreviation (1 Letter)
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Aspartate or Asparagine	Asx	B
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glutamate or Glutamine	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V