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 $[M-H]^-$

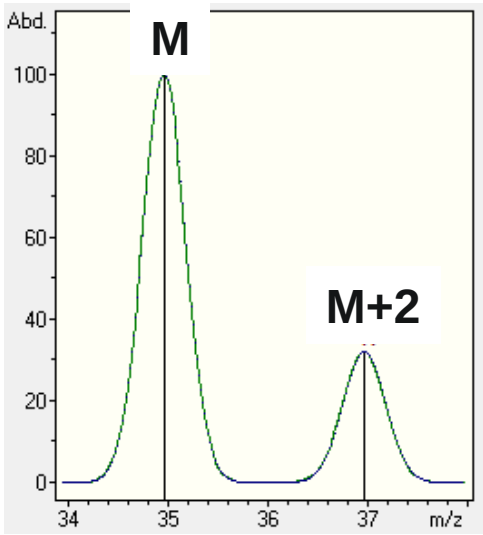
Určování elementárního složení, izotopy

Defekty atomových hmotností Izotopické zastoupení

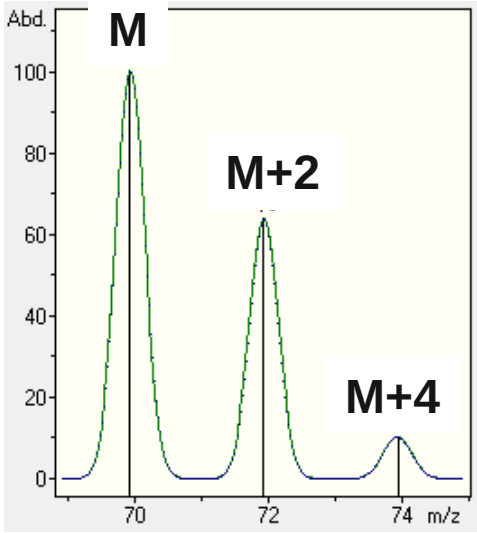
Prvek	Nominální atomová hmotnost [Da]	Hmotnostní schodek [mDa]	Přírodní zastoupení izotopů			Typ prvku
			M [%]	M+1 [%]	M+2 [%]	
H	1	7.8	100	0.015		"M"
C	12	0	100	1.1		"M+1"
N	14	3.1	100	0.37		"M+1"
O	16	-5.1	100	0.04	0.2	"M+2"
F	19	-1.6	100			"M"
Si	28	-23.1	100	5.1	3.4	"M+2"
P	31	-26.2	100			"M"
S	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"

3. Určení elementárního složení - izotopy

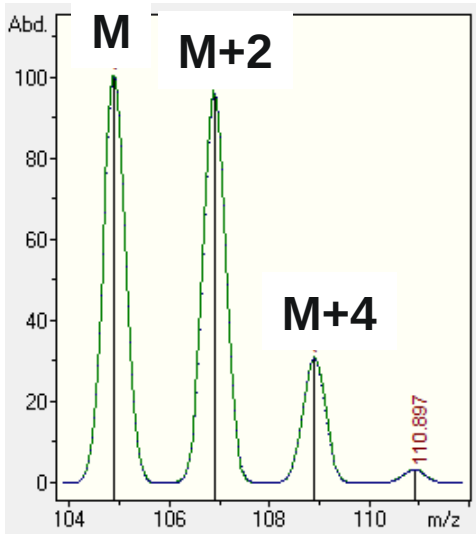
Cl



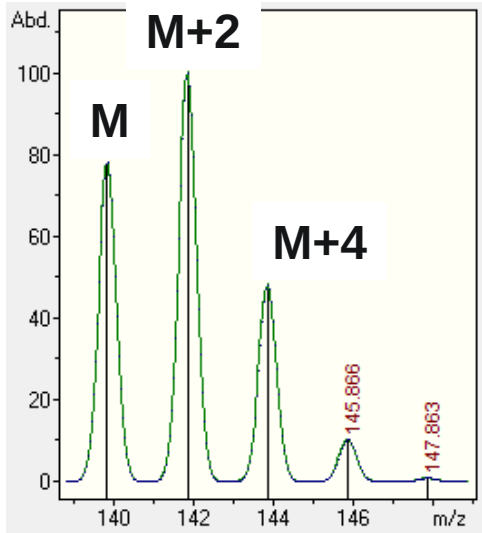
Cl_2



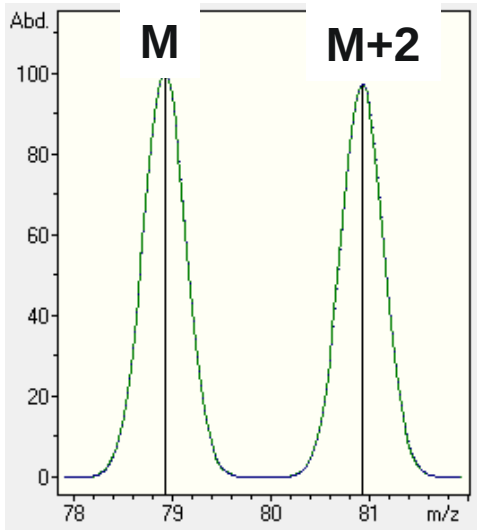
Cl_3



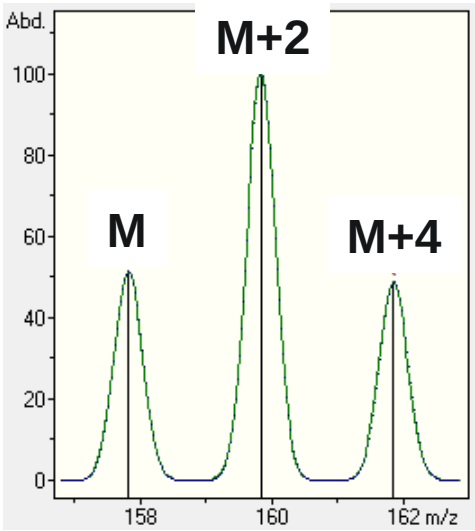
Cl_4



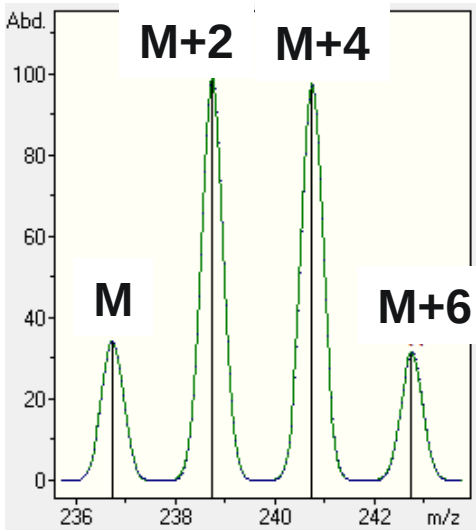
Br



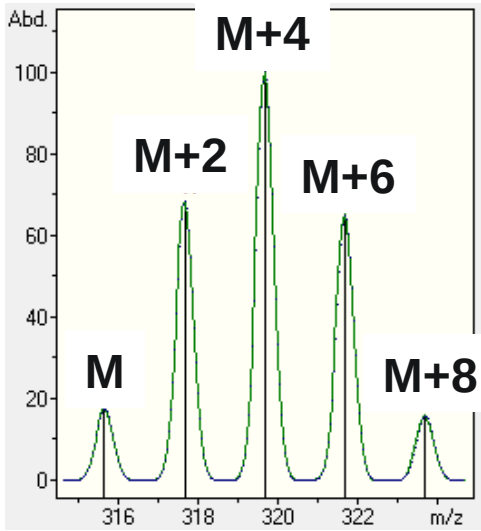
Br_2



Br_3



Br_4



4. MS/MS - vliv funkčních skupin na fragmentaci

Funkční skupina / Substituent	**Neutrální ztráta / Produktový ion (*m/z*)**
Alkyl a aryl	alken, alkan (zejména u druhé ztráty), C_6H_4 (76) nebo C_6H_6 (78)
Fosfáty (RPO_4H_2)	HPO_3 (80) a H_3PO_4 (98), *m/z* 97 $[H_2PO_4]^-$, *m/z* 79 $[PO_3]^-$
Sulfát (RSO_4H)	SO_3 (80) a H_2SO_4 (98), *m/z* 97 $[HSO_4]^-$
Amid (R^1CONH_2, R^1CONHR^2, $R^1CONR^2R^3$)	$[M+H-\overset{17}{NH_3}]^+$, $[M+H-R^2NH_2]^+$, $[M+H-R^2R^3NH]^+$
Amin (R^1NH_2, R^1NHR^2, $R^1NR^2R^3$)	$[M+H-\overset{17}{NH_3}]^+$, $[M+H-R^2NH_2]^+$, $[M+H-R^2R^3NH]^+$, $[M+H-\overset{27}{HCN}]^+$
Azo ($R^1N{=}NR^2$)	$[M+H-\overset{28}{N_2}]^+$, $[M+H-R^i]^+$, $[M+H-R^iNH]^+$, $[M+H-R^iN_2]^+$
Nitril (RCN)	$[M+H-\overset{27}{HCN}]^+$
Nitrát (RNO_3)	$[M-H-\overset{30}{NO}]^{-\cdot}$, $[M-\overset{46}{NO_2}]^-$, $[M-H-\overset{46}{NO_2}]^{-\cdot}$, $[M-H-\overset{62}{ONO_2}]^{-\cdot}$
Nitro (RNO_2)	$[M+H-\overset{17}{OH}]^{+\cdot}$, $[M+H-\overset{18}{H_2O}]^+$, $[M+H-\overset{30}{NO}]^{+\cdot}$, $[M+H-\overset{46}{NO_2}]^{+\cdot}$, $[M-\overset{17}{OH}]^-$, $[M-\overset{30}{NO}]^-$, $[M-H-\overset{30}{NO}]^{-\cdot}$, $[M-H-\overset{31}{HNO}]^-$, $[M-H-\overset{17}{OH}]^{-\cdot}$, $[M-\overset{46}{NO_2}]^-$, $[M-H-\overset{46}{NO_2}]^{-\cdot}$, $[\overset{46}{NO_2}]^-$

Funkční skupina / Substituent	**Neutrální ztráta / Produktový ion (*m/z*)**
Nitroso (RNO)	$[M+H-\overset{30}{NO}]^+$
N-oxid (RN^+O^-)	$[M+H-\overset{16}{O}]^+$, $[M+H-\overset{17}{OH}]^{+\cdot}$, $[M+H-\overset{18}{H_2O}]^+$
Halogenid (RX)	$[M+H-X]^{+\cdot}$, $[M+H-HX]^+$, $[M-H-X]^{-\cdot}$, $[M-H-HX]^-$, $[\overset{19}{F}]^-$, $[\overset{35}{Cl}]^-$, $[\overset{79}{Br}]^-$, $[\overset{127}{I}]^-$
Ester (R^1COOR^2)	$[M+H-R^2OH]^+$, $[M+H-R^2OH-\overset{28}{CO}]^+$, (např. $[M-\overset{46}{C_2H_5OH}-\overset{28}{CO}]^+$)
Keton (R^1COR^2)	$[M+H-\overset{18}{H_2O}]^+$, $[M+H-\overset{28}{CO}]^+$, $[R^iCO]^+$
Aldehyd (RCHO)	$[M+H-\overset{28}{CO}]^+$, $[M-H-\overset{28}{CO}]^-$
Karboxyl (RCOOH)	$[M+H-\overset{18}{H_2O}]^+$, $[M+H-\overset{44}{CO_2}]^+$, $[M+H-\overset{28}{H_2O-CO}]^+$, $[M-H-\overset{44}{CO_2}]^-$
Hydroxyperoxid (ROOH)	$[M+H-\overset{18}{H_2O}]^+$, $[M+H-\overset{34}{H_2O_2}]^+$ (pozn. Δm/z 34 je i u SH skupiny) *HR-MS rozliší*
Epoxid/Alkohol (RO/ROH)	$[M+H-\overset{18}{H_2O}]^+$
Fenol (ROH)	$[M+H-\overset{18}{H_2O}]^+$, $[M+H-\overset{28}{CO}]^+$, $[M-H-\overset{18}{H_2O}]^-$, $[M-H-\overset{28}{CO}]^-$
Methoxy ($ROCH_3$)	$[M+H-\overset{15}{CH_3}]^{+\cdot}$, $[M+H-\overset{31}{CH_3O}]^{+\cdot}$, $[M+H-\overset{32}{CH_3OH}]^+$, $[M+H-\overset{30}{HCOH}]^+$

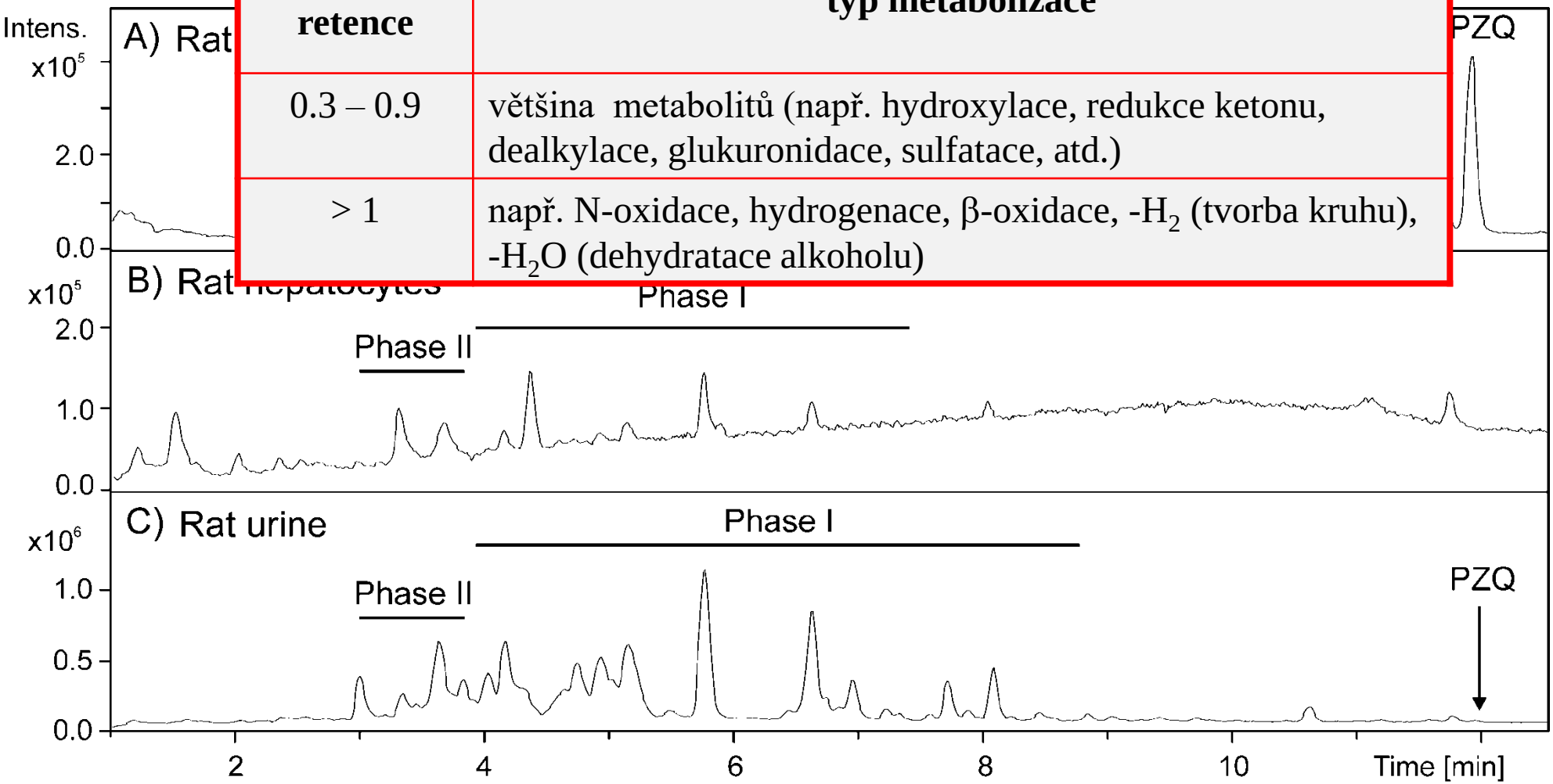
Typické neutrální ztráty < Δm/z 100

Δ m/z (nominální)	Δ m/z (přesná)	Ztráta
15	15,0235	$CH_3^{\cdot}$
17	17,0027	$OH^{\cdot}$
17	17,0265	NH_3
26	26,0157	C_2H_2
18	18,0106	H_2O
20	20,0062	HF
27	27,0109	HCN
28	27,9949	CO
28	28,0061	N_2
28	28,0313	C_2H_4
30	29,9980	NO
30	30,0106	HCOH
30	30,0470	C_2H_6
31	31,0184	$CH_3O^{\cdot}$
31	31,0422	CH_3NH_2
32	32,0262	CH_3OH
34	33,9877	H_2S
35	34,9689	$Cl^{\cdot}$
36	35,9767	HCl
36	36,0211	$2*H_2O$
42	42,0470	C_3H_6

Δ m/z (nominální)	Δ m/z (přesná)	Ztráta
44	43,9898	CO_2
46	45,9929	NO_2
46	46,0055	$H_2O + CO$
46	46,0419	C_2H_5OH
56	56,0626	C_4H_8
58	58,0783	C_4H_{10}
60	60,0211	CH_3COOH
60	60,0575	C_3H_7OH
62	61,9878	$ONO_2^{\cdot}$
64	63,9619	SO_2
74	74,0732	C_4H_9OH
76	76,0313	C_6H_4
77	77,0391	$C_6H_5^{\cdot}$
78	78,0470	C_6H_6
79	78,9186	$Br^{\cdot}$
80	79,9262	HBr
80	79,9568	SO_3
80	79,9663	HPO_3
98	97,9668	H_2SO_4
98	97,9769	H_3PO_4

Metabolity léčiv: retence v závislosti na polaritě

relativní retence	typ metabolizace
0.3 – 0.9	většina metabolitů (např. hydroxylace, redukce ketonu, dealkylace, glukuronidace, sulfatace, atd.)
> 1	např. N-oxidace, hydrogenace, β-oxidace, $-H_2$ (tvorba kruhu), $-H_2O$ (dehydratace alkoholu)



Význam měření s vysokou správností určení *m/z*

Defekty atomových hmotností

Element	Nominal atomic mass [Da]	Mass defect [mDa]
H	1	+7.8
C	12	0
N	14	+3.1
O	16	-5.1
F	19	-1.6
Si	28	-23.1
P	31	-26.2
S	32	-27.9
Cl	35	-31.1
Br	79	-81.7
I	127	-95.5

Nejběžnější metabolické reakce I. fáze

Nominal mass shift [ΔDa]	Metabolic reaction (elemental composition change)	Exact mass shift [mDa]
-44	Decarboxylation ($-CO_2$)	+10.2
-18	Alcohol dehydration ($-H_2O$)	+10.6
-14	Demethylation ($-CH_2$)	-15.7
-2	Ring formation ($-H_2$)	-15.7
+2	Ring opening ($+H_2$)	+15.7
+14	Hydroxylation and cyclization ($+O-H_2$)	-20.7
+16	Hydroxylation (+O)	-5.1
	Epoxidation (+O)	-5.1
	Oxidation (+O)	-5.1
+34	Epoxidation and hydration ($+H_2O_2$)	+5.5

Nejběžnější metabolické reakce II. fáze

Nominal mass shift [ΔDa]	Conjugation reaction (elemental composition change)	Drug functional group	Exact mass shift [mDa]
+14	Methylation (+CH_2)	NH_2, OH, SH	+15.7
+42	Acetyl conjugation (+C_2H_2O)	NH_2, $NHNH_2$, SO_2NH_2, OH	+10.6
+57	Glycine conjugation (+C_2H_3ON)	COOH	+21.5
+79	Phosphorylation (+PO_3)	OH	-41.5
+80	Sulfation (+SO_3)	NH_2, SO_2NH_2, OH	-43.2
+162	Glucosylation (+$C_6H_{10}O_5$)	OH, COOH	+52.8
+176	Glucuronidation (+$C_6H_8O_6$)	OH	+32.1
+220	Indirect carbamate glucuronidation of amines (+$C_7H_8O_8$)	NH_2 + CO_2	+21.9
+306-X	Glutathione conjugation – halide substitution (-X+$C_{10}H_{16}O_6N_3S$)	Halide (X)	+76.0
+305	Glutathione conjugation *via* epoxidation (+$C_{10}H_{15}O_6N_3S$)	Aromatic	+68.2

M. Holčapek, L. Kolářová, M. Nobilis, Anal. Bioanal. Chem, 216 (2008) 1962