

Fig. 4 SDS-PAGEゲルを組み合わせた解析法

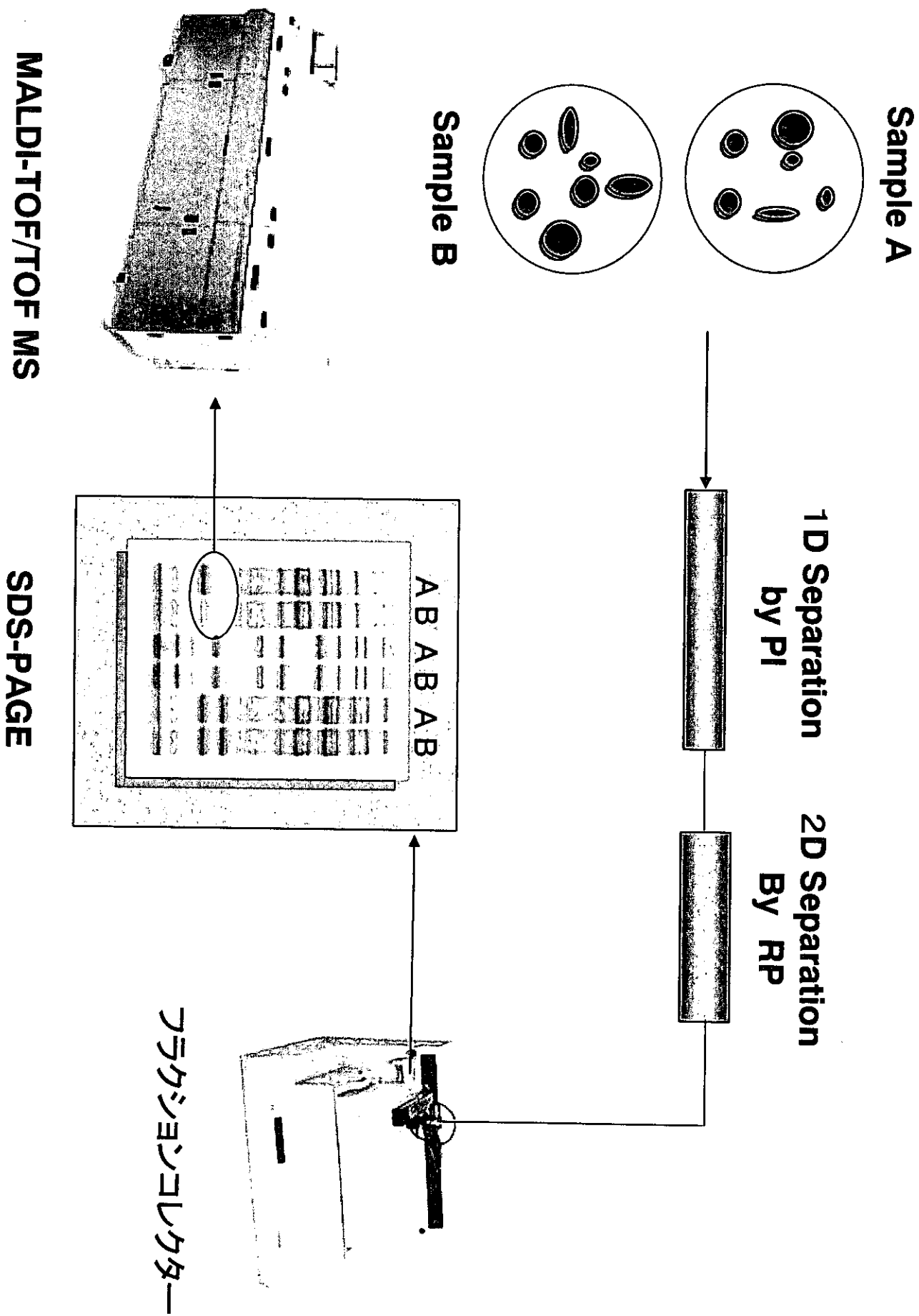


Fig. 5 ICAT法を用いた網羅的蛋白発現解析

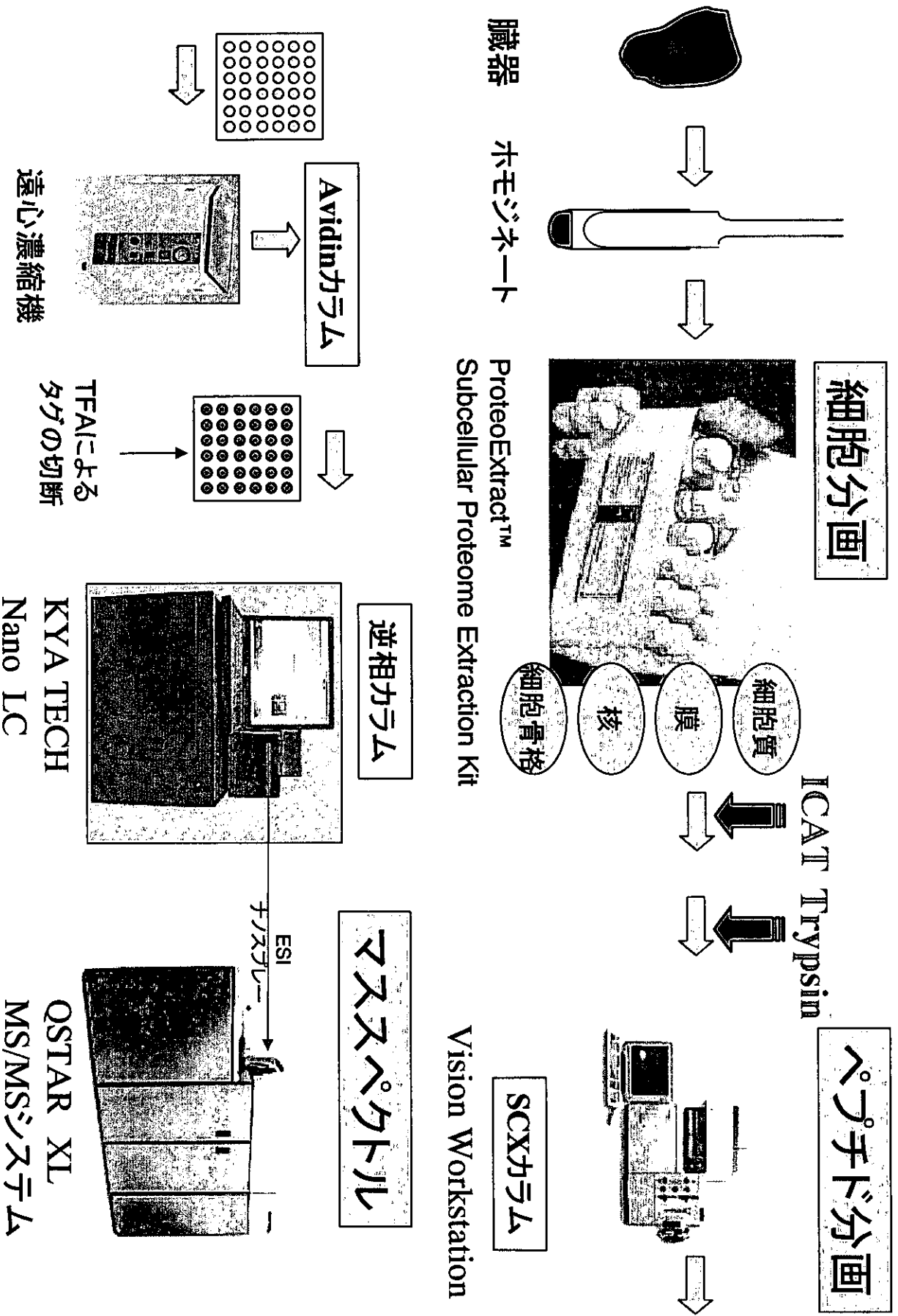
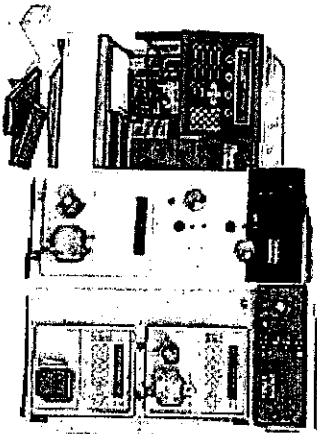
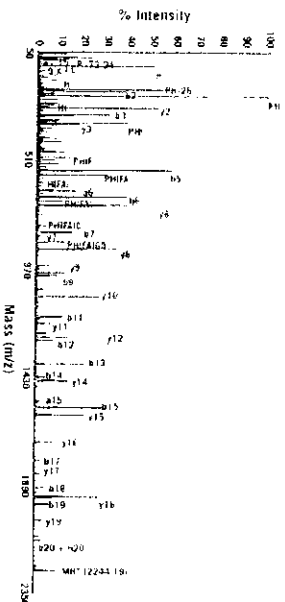


Fig. 6 LCからMALDI-TOF/TOF型マスへの展開

二次元ナノLC  
Ultimate (LC Packings)

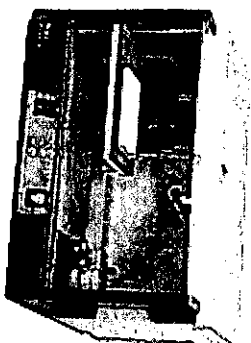


TNPPHFAQGDVVGQPMIAHK identities  
dihydroliipoamide dehydrogenase ec 1.8.1  
(sp | HQ3573 | Induc2)

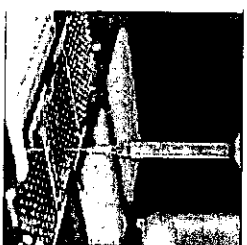


マイクロラジヨシコクタ  
Probot

オンライン



MALDIプレートへの  
スポットティング



AB 4700 MALDI-TOF/TOF Mass

シーケンスタグによるタンパクの同定

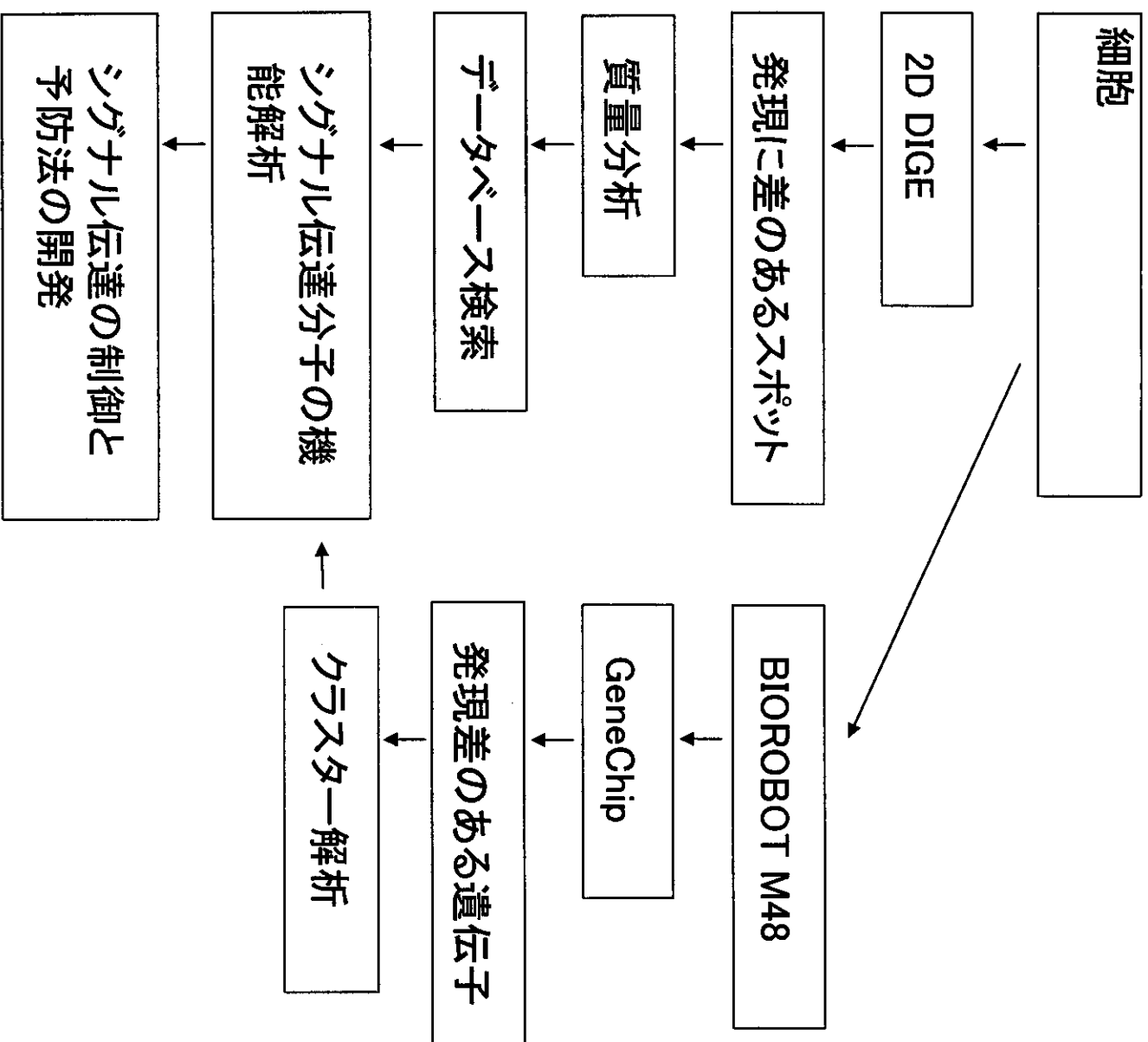
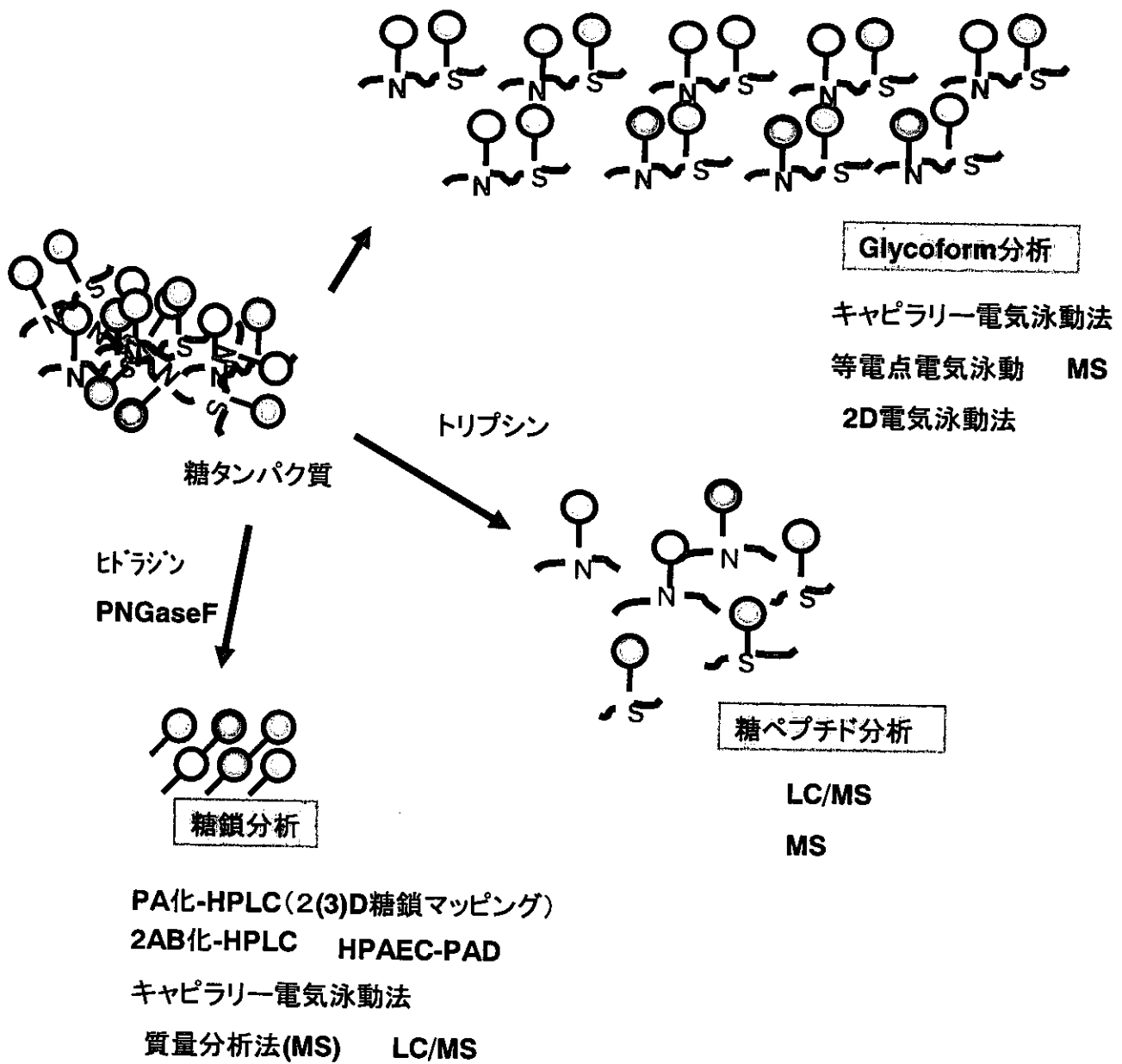


Fig. 7. シグナル伝達のプロファイリング方法

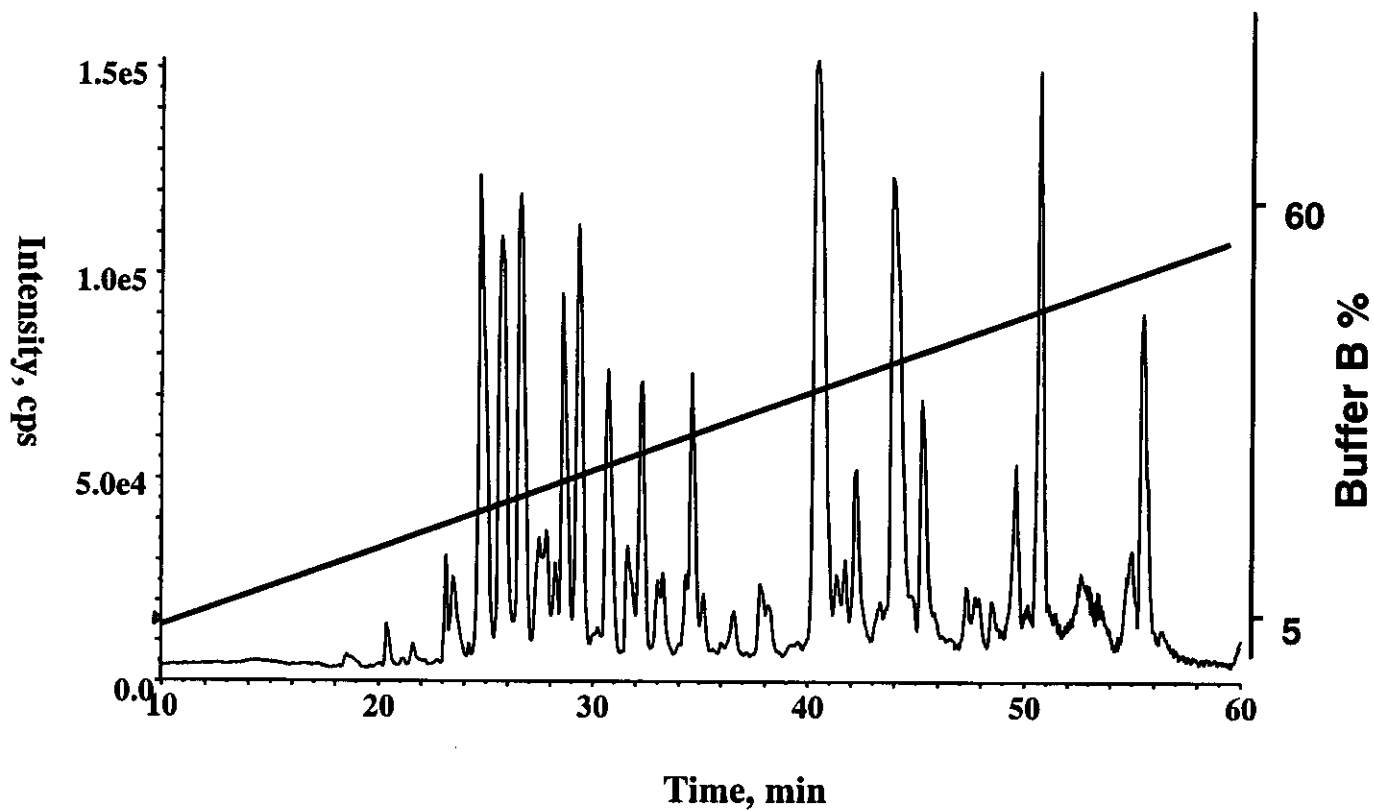


**Fig. 8 Methods for the structural analysis of glycoproteins**

<b>mkwvesifli</b>	<b>fllnftesrt</b>	<b>lhrneygias</b>	<b>ildsyqctae</b>	<b>isladlatif</b>
<b>faqfvqeaty</b>	<b>kevskmvkda</b>	<b>ltaiekptgd</b>	<b>eqssgclenq</b>	<b>lpafleelch</b>
<b>ekeilekygh</b>	<b>sdccsqseeg</b>	<b>rhncflahkk</b>	<b>ptpasiplfq</b>	<b>vpepvtscea</b>
<b>yedretfmn</b>	<b>kfiyeiarrh</b>	<b>pflyaptill</b>	<b>waarydkiiip</b>	<b>scckaenave</b>
<b>cfqtkaatvt</b>	<b>kelresslln</b>	<b>qhacavmknf</b>	<b>gtrtfqaitv</b>	<b>tklsqkftkv</b>
<b><u>n</u>fteiqklvl</b>	<b>dvahvhehcc</b>	<b>rgdvldclqd</b>	<b>gekimsyics</b>	<b>qqdtlsnkit</b>
<b>eccklttler</b>	<b>gqcihaend</b>	<b>ekpeglspl</b>	<b>nrflgdrdfn</b>	<b>qfssgekknif</b>
<b>lasfvheysr</b>	<b>rhpqlavsvi</b>	<b>lrvakgyqel</b>	<b>lekcfqtenp</b>	<b>lecqdkgeee</b>
<b>lqkyiqesqa</b>	<b>lakrscglfq</b>	<b>klgeyylna</b>	<b>flvaytkkap</b>	<b>qлтsselmai</b>
<b>trkmaataat</b>	<b>ccqlsedkll</b>	<b>acgegaadii</b>	<b>ighlcirhem</b>	<b>tpvnpgvgqc</b>
<b>ctssyanrrp</b>	<b>cfsslvvdet</b>	<b>yppafsddk</b>	<b>fifhkdlcqa</b>	<b>qgvalqtmkq</b>
<b>eflinlvkqk</b>	<b>pqiteeqlea</b>	<b>viadfsglle</b>	<b>kccqgqeqev</b>	<b>cfaeegqkli</b>
<b>sktraalgv</b>				

Fig. 9 Amino acid sequence and N-glycosylation sites of alpha-Fetoprotein  
n: N-glycosylation site

**(A) TIC for the full scan  $m/z$  700-2000**



**Fig. 10 LC-MS/MS of tryptic digest of  $\alpha$ -fetoprotein**

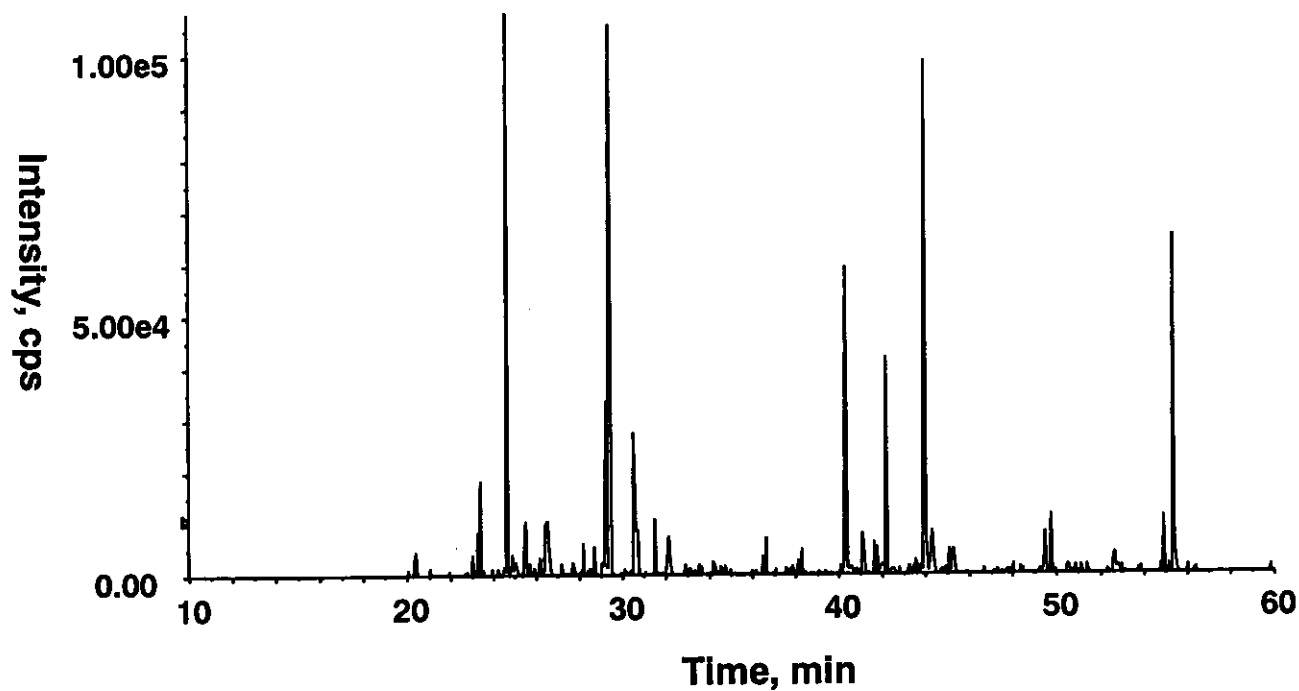
**Column Magic C18**

**3  $\mu$ , 0.2\*50 mm, 2  $\mu$ l/min**

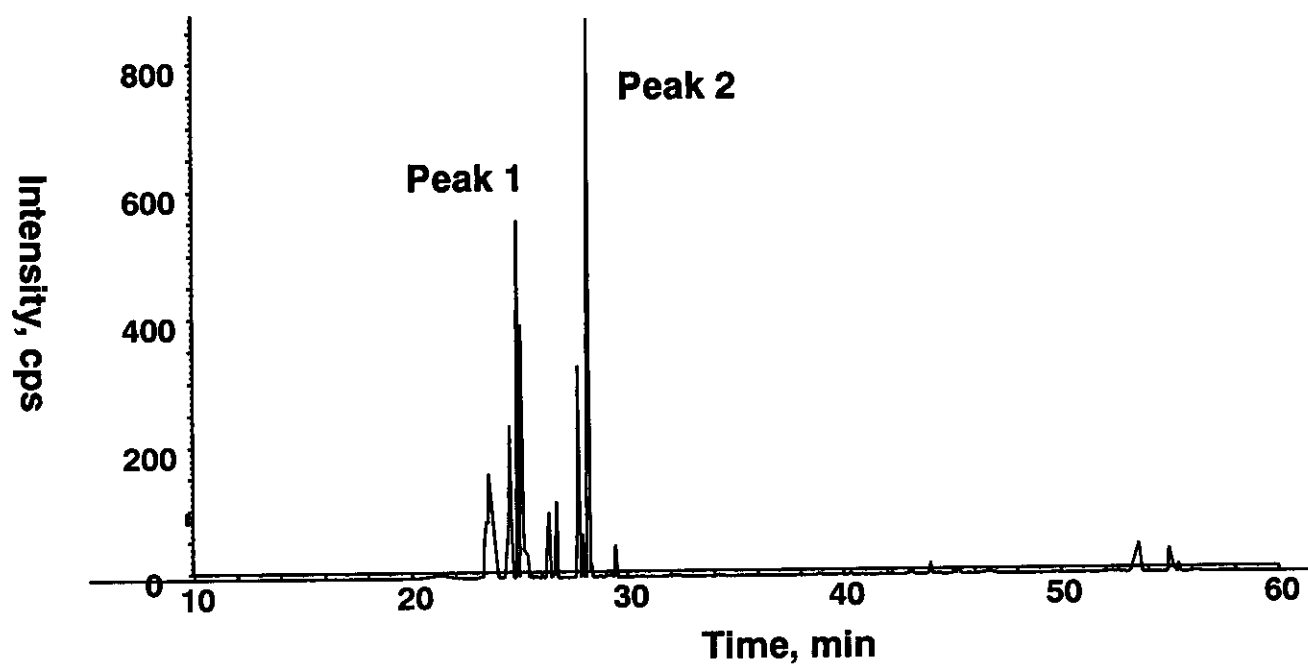
**A: 2 %  $\text{CH}_3\text{CN}$  + 0.1 % formic acid**

**B: 90 %  $\text{CH}_3\text{CN}$  + 0.1 % formic acid**

**(B) TIC for the product ion scan at  $m/z$  100 - 2000**



**(C) Product ion scan at  $m/z$  204**





Residue	Mass	b	b-NH3	y	y-NH3
V	99.07	100.08	83.05	978.53	961.50
N	114.04	214.12	197.09	879.46	862.43
F	147.07	361.19	344.16	765.41	748.39
T	101.05	462.23	445.21	618.35	601.32
E	129.04	591.28	574.25	517.30	500.27
I	113.08	704.36	687.33	388.26	371.23
Q	128.06	832.42	815.39	275.17	258.14
K	128.10	960.51	943.49	147.11	130.09

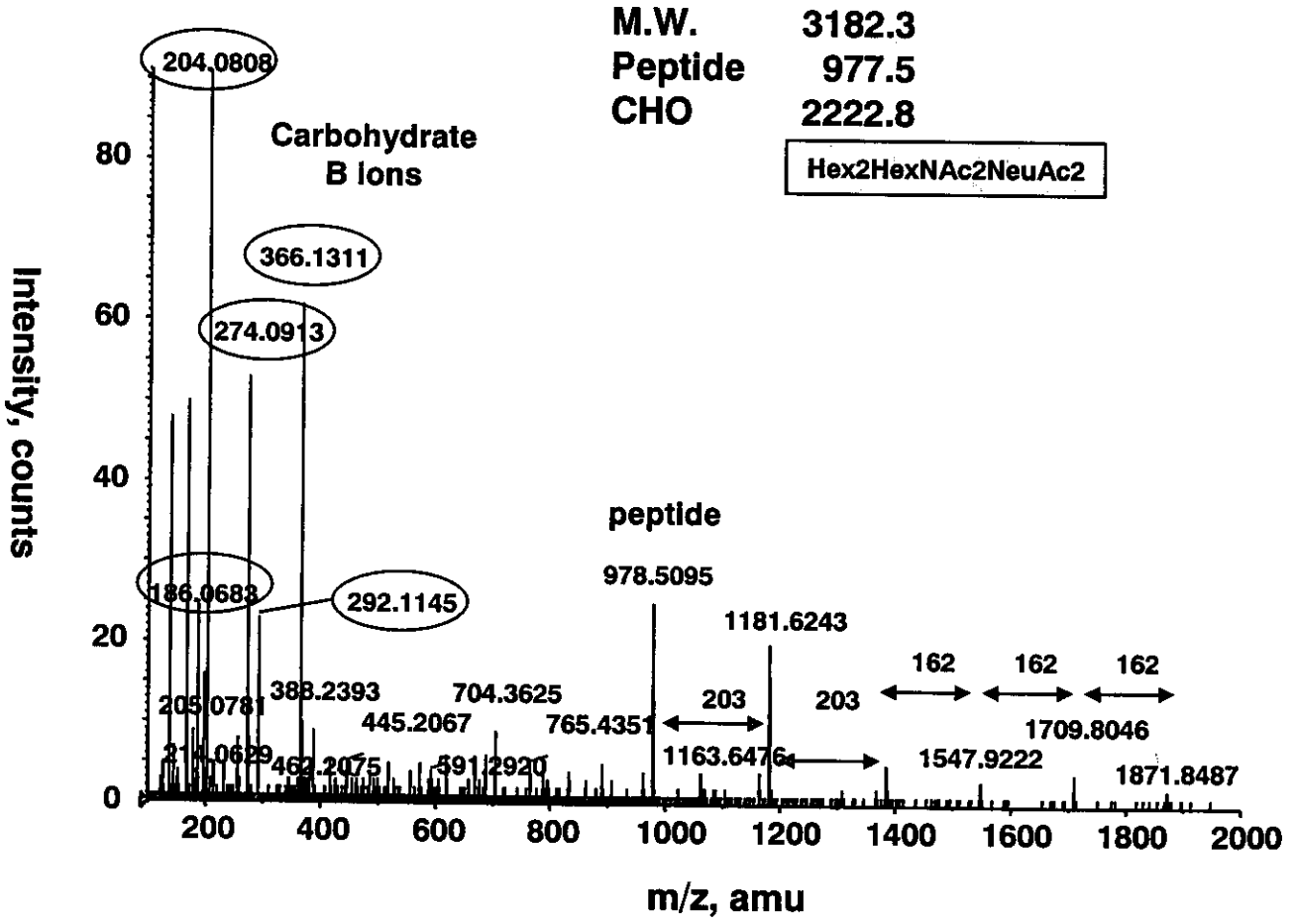



Fig. 11 Product ion spectrum of  $M^+(m/z1061.8^{3+})$  at 25 min


Table 4 Glycosylation analysis of alpha-fetoprotein

Retention time (min)	m/z	Charge	M.W.	Peptide Sequences	M.W.	Oligosaccharide Structures	M.W.
23	1013.43	+3	3037.3	K/vntfqlqk/I	977.5	[HexNAc]4[Hex]5[Neu5Ac]1[Fuc]1	2077.8
23	1519.67	+2	3037.3	K/vntfqlqk/I	977.5	[HexNAc]5[Hex]5[Neu5Ac]1[Fuc]1	2281.4
23	1081.29	+3	3240.9	K/vntfqlqk/I	977.5	[HexNAc]4[Hex]5[Neu5Ac]1	1931.8
23	1621.44	+2	3240.9	K/vntfqlqk/I	977.5	[HexNAc]3[Hex]4	1275.9
23	1446.62	+2	2891.2	K/vntfqlqk/I	977.5	[HexNAc]3[Hex]4	1275.9
23	1118.67	+2	2235.3	K/vntfqlqk/I	977.5	[HexNAc]3[Hex]4	1275.9
23	1264.05	2	2526.1	K/vntfqlqk/I	977.5	[HexNAc]3[Hex]4[Neu5Ac]1	1566.6
	1110.48	3	2526.1	K/vntfqlqk/I	977.5	[HexNAc]3[Hex]4[Neu5Ac]1	1566.6
24	1665.44	+2	3328.9	K/vntfqlqk/I	977.5	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2369.4
24	1178.33	+3	3532.0	K/vntfqlqk/I	977.5	[HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1	2572.5
24	1061.78	3	3182.3	K/vntfqlqk/I	977.5	[HexNAc]4[Hex]5[Neu5Ac]2	2222.9
25	1592.17	2	3182.3	K/vntfqlqk/I	977.5	[HexNAc]4[Hex]5[Neu5Ac]2	2222.9
27	1236.04	+3	3705.1	K/fttkvntfqlqk/I	1353.7	[HexNAc]4[Hex]5[Neu5Ac]2[Fuc]1	2369.4
27	1853.29	+2	3704.6	K/fttkvntfqlqk/I	1353.7	[HexNAc]4[Hex]5[Neu5Ac]2	2222.8
27	1187.17	+3	3558.5	K/fttkvntfqlqk/I	1353.7	[HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1	2572.0
28	1780.23	+2	3558.5	K/fttkvntfqlqk/I	1353.7	[HexNAc]5[Hex]5[Neu5Ac]2[Fuc]1	2572.0
28	1303.56	+3	3907.7	K/fttkvntfqlqk/I	1353.7	[HexNAc]4[Hex]5[Neu5Ac]1	2077.8
28	1138.84	+3	3413.5	K/fttkvntfqlqk/I	1353.7	[HexNAc]5[Hex]5[Neu5Ac]1[Fuc]1	2280.9
28	1206.52	+3	3616.6	K/fttkvntfqlqk/I	1353.7	[HexNAc]4[Hex]5[Neu5Ac]1	1931.7
28	1090.14	+3	3267.4	K/fttkvntfqlqk/I	1353.7	[HexNAc]4[Hex]5[Neu5Ac]1	1931.7
28	1322.75	+3	3965.3	K/fttkvntfqlqk/I	1353.7	[HexNAc]6[Hex]5[Neu5Ac]2	2629.6

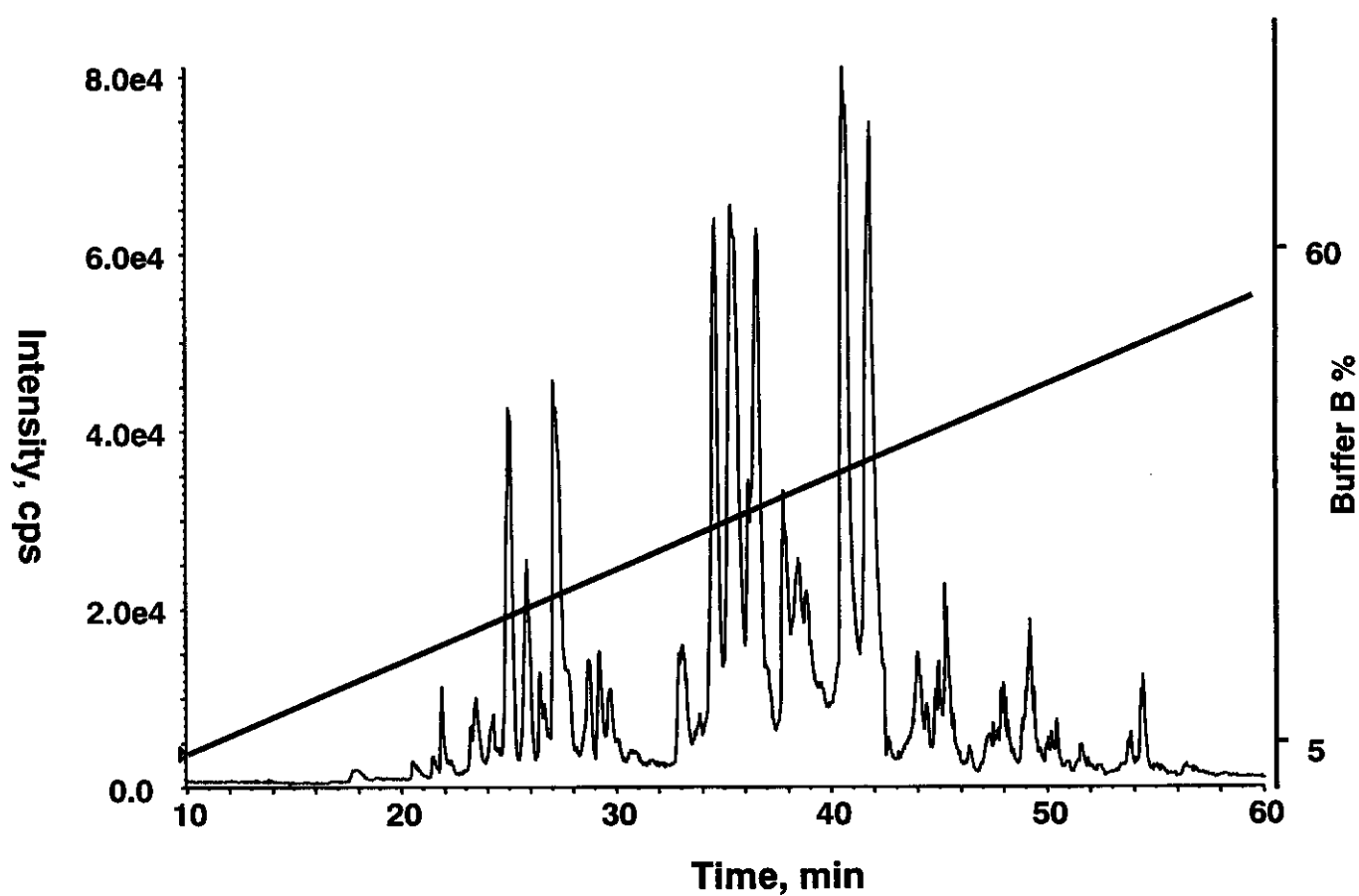
mkililgifi	flcstpawak	ekhyyigiie	ttwdyasdhg	ekklisvdte
hsniylqngp	drigrlykka	lylqytdetf	rttiekpowl	gflgpiikae
tgdkvyvhlk	nlasrpytfh	shgityykeh	egaiypdntt	dfqraddkvy
pgeqytmll	ateeqspgeg	dgnvcvtriyh	shidapkdia	sgligpliic
kkdsldkeke	khidrefvvm	fsvvdeffsw	yledniktyc	sepekvdkdn
edfquesrmy	svngytfgsi	pglsmcaedr	vkwyifgmgn	evdvhaaffh
gqaltknknyr	idtinlfpat	lfdaymvaqn	pgewmlscqn	lnhikaglqa
ffqvqecfks	sskdnirgkh	vrhyyiaaee	iiwnyapsgi	diftkefta
pgsdsavffe	qgttriggisy	kklyvreytd	asftnrkerg	peeelgilg
pviwaevgdt	irvtfhnkga	yplsiepigv	rfnknnegty	yspnynpqsr
svppsashva	ptetftyewt	vpkevgptna	dpvclakmyy	savdptkdif
tgligpmkic	kkgsllhangr	qkdvdkefyl	fptvfdehes	lllednirmf
ttapdqvdke	dedfquesnm	hsmngfmygn	qpgltmckgd	svvwyfhsag
neadvhgiyf	sgntylwrge	rrdtanlfpq	tslthmwtpd	tegtfnvecl
ttdhytggmk	qkytvnqerr	qsedstfylg	ertyyiaave	vewdyspqre
wekelhlhqe	qfvsnafldk	gefyigskyk	kvvyrqytds	tfrvpverka
eeehlgilgp	qlhadvgdkv	kiifknmatr	pysihahgvq	tesstvtptl
pgetltyvwk	ipersgagte	dsacipwayy	stvdqvkdy	sgligplivc
rrpylkvfnp	rrklefallf	lvfdeheswy	lddniktysd	hpekvnkdde
efiesnkmha	ingrmfgnlq	gltmhvgdev	nwyimgmgne	idlhtvhfhg
hsfqykhrgv	yssdvfdifp	gtyqtlemfp	rtpgiwllhc	hvtddihagm
ettytvlqne	dtksg			

Fig. 12 Amino acid sequence and N-glycosylation sites of ceruloplasmin (CP)

: Potential N-glycosylation sites

: N-glycosylation sites

**(A) TIC for the full scan  $m/z$  1000-2000**



**Fig. 13 LC-MS/MS of tryptic digest of ceruloplasmin**

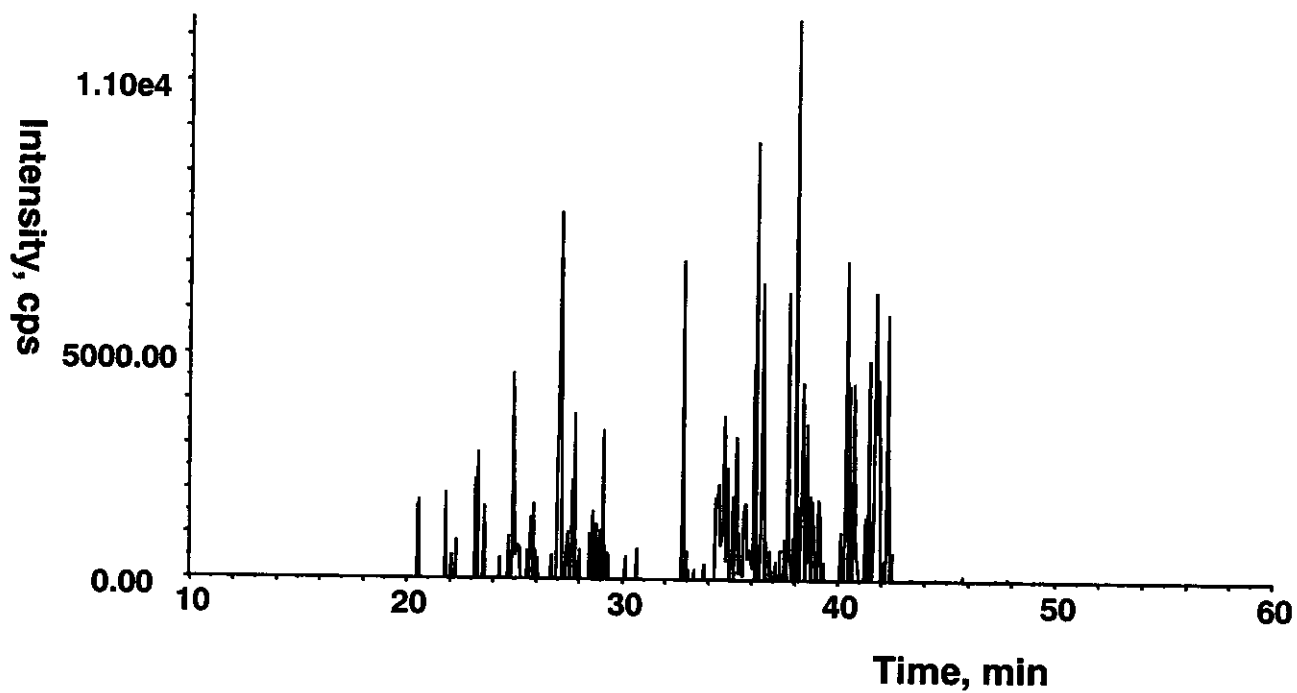
**Column Magic C18**

**3  $\mu$ , 0.2\*50 mm, 2  $\mu$ l/min**

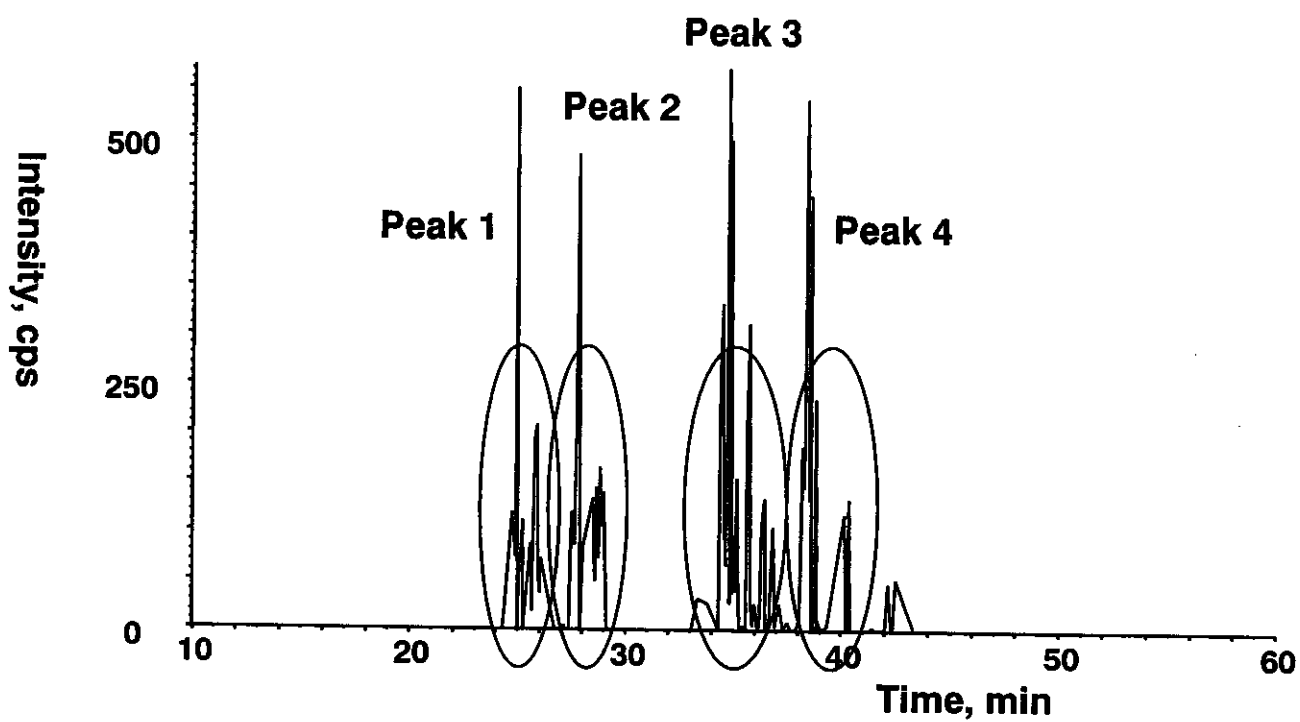
**A: 2 %  $\text{CH}_3\text{CN}$  + 0.1 % formic acid**

**B: 90 %  $\text{CH}_3\text{CN}$  + 0.1 % formic acid**

**(B) TIC for the product ion scan at  $m/z$  100 - 2000**



**(C) Product ion scan at  $m/z$  204**



M.W. 4096.6  
 Peptide 1891.8  
 CHO 2222.8

Hex<sub>2</sub>HexNAc<sub>2</sub>NeuAc<sub>2</sub>

Residue	Mass	b	b-NH3	y	y-NH3
E	129.04	130.05	113.02	1892.84	1875.81
H	137.06	267.11	250.08	1763.80	1746.77
E	129.04	396.15	379.12	1626.74	1609.71
G	57.02	453.17	436.15	1497.70	1480.67
A	71.04	524.21	507.18	1440.68	1423.65
I	113.08	637.29	620.27	1369.64	1352.61
Y	163.06	800.36	783.33	1256.55	1239.53
P	97.05	897.41	880.38	1093.49	1076.46
D	115.03	1012.44	995.41	996.44	979.41
N	114.04	1126.48	1109.45	881.41	864.38
T	101.05	1227.53	1210.50	767.37	750.34
T	101.05	1328.58	1311.55	666.32	649.29
D	115.03	1443.60	1426.58	565.27	548.25
F	147.07	1590.67	1573.64	450.25	433.22
Q	128.06	1718.73	1701.70	303.18	286.15
R	156.10	1874.83	1857.80	175.12	158.09

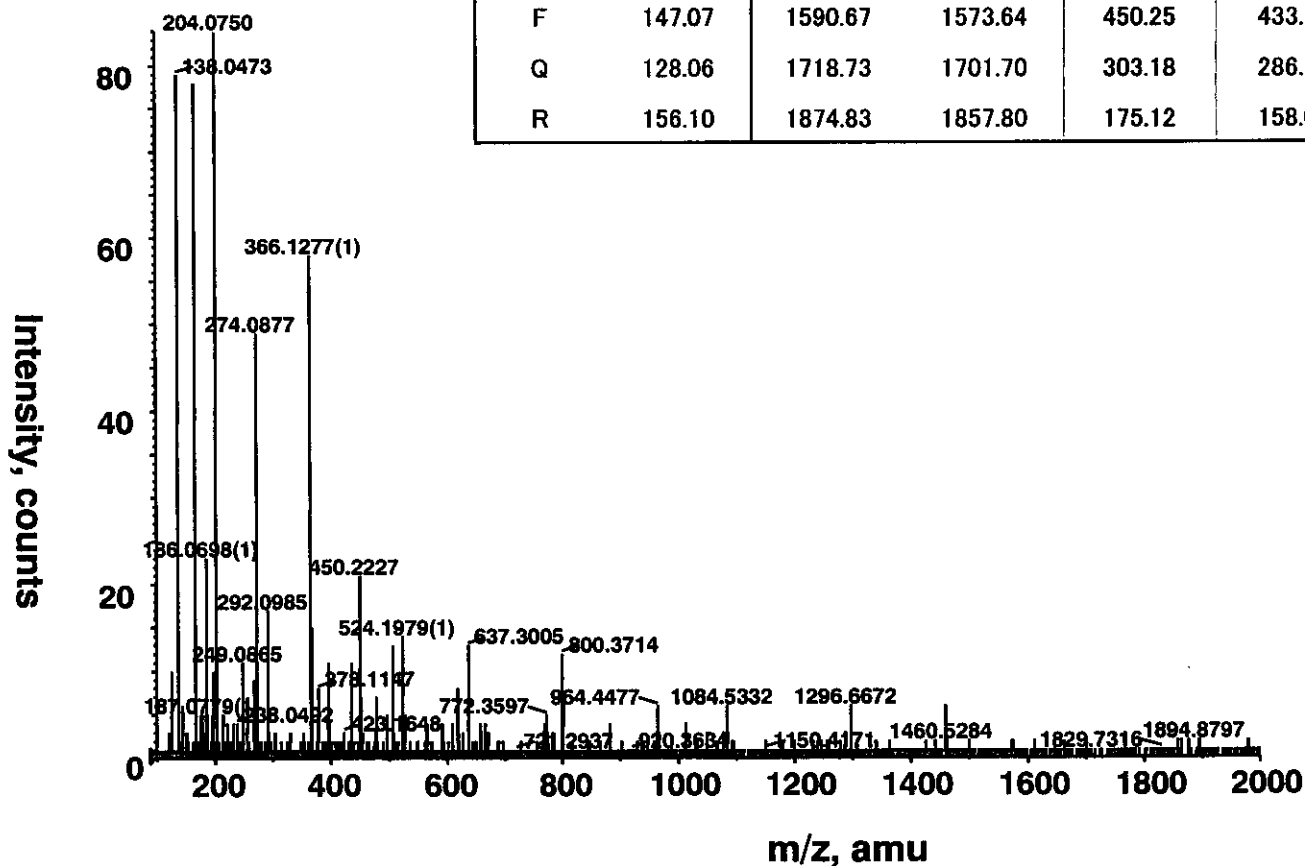
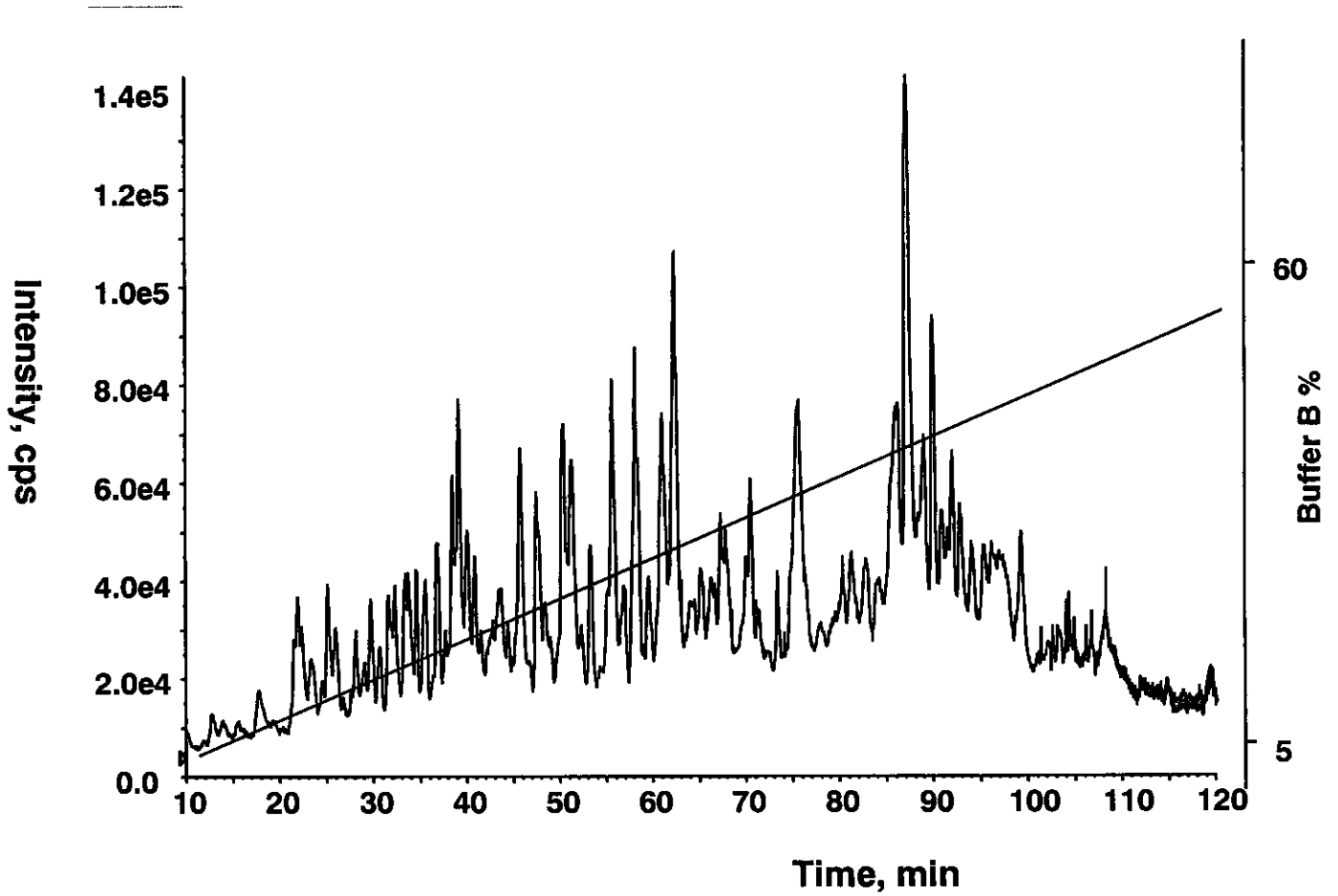


Fig. 14 Product ion spectrum of M<sup>+</sup>(m/z1366.6<sup>3+</sup>) at 25 min

**Table 5 Glycosylation analysis of ceruloplasmin**

Retention time (min)	m/z	Charge	M.W.	Peptide Sequences	M.W.	Oligosaccharide Structures	M.W.
24	1025.17	+4	4096.7	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2	2222.9
24	1366.54	+3	4096.6	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2+Na	2244.9
24	1373.88	+3	4118.6	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.9
24	1061.67	+4	4242.7	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.9
25	1415.23	+3	4242.7	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.9
25	1262.24	+4	5045.0	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]5[Hex]5[Neu5Ac]3[Luc]2	3171.2
25	1682.65	+3	5045.0	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]5[Hex]5[Neu5Ac]3[Luc]2	3171.2
25	1189.21	+4	4752.8	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]5[Hex]6[Neu5Ac]3	2879.1
25	1585.27	+3	4752.8	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]5[Hex]6[Neu5Ac]3	2879.1
25	1225.72	+4	4898.9	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]5[Hex]6[Neu5Ac]3[Luc]1	3025.1
25	1633.96	+3	4898.9	EHEGAIYPDNITTDFOQR	1891.8	[HexNAc]5[Hex]6[Neu5Ac]3[Luc]1	3025.1
27	1093.95	+4	4371.8	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.8
27	1458.27	+3	4371.8	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.8
27	1057.43	+4	4225.7	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
27	1409.57	+3	4225.7	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
28	1257.99	+4	5028.0	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]5[Hex]6[Neu5Ac]3[Luc]1	3025.0
28	1677.01	+3	5028.0	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]5[Hex]6[Neu5Ac]3[Luc]1	3025.0
28	1221.48	+4	4881.9	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]5[Hex]6[Neu5Ac]3	2878.9
28	1628.30	+3	4881.9	ELHHLQEQNVSNAFLDK	2021.0	[HexNAc]5[Hex]6[Neu5Ac]3	2878.9
33	1347.54	+3	4039.6	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]1	1931.6
34	1450.24	+3	4347.7	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]1	1931.6
34	1083.68	+4	4330.7	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2+NH3	2239.7
34	1444.56	+3	4330.7	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
34	1493.28	+3	4476.8	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
34	1089.17	+4	4352.7	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.8
35	1668.97	+3	5003.9	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]4[Hex]5[Neu5Ac]2+Na	2244.7
35	1663.29	+3	4986.9	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]5[Hex]6[Neu5Ac]3+NH3	2895.9
35	1247.72	+4	4986.9	ENLTAPGSDSAVFEEQGTTR	2126.0	[HexNAc]5[Hex]6[Neu5Ac]3	2878.9
37	1282.48	+3	3844.4	AGLQAFQVQEQCNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
37	1923.24	+2	3844.5	AGLQAFQVQEQCNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2	2222.7
38	1331.17	+3	3990.5	AGLQAFQVQEQCNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2[Luc]1	2368.8
38	1289.81	+3	3866.4	AGLQAFQVQEQCNK	1639.7	[HexNAc]4[Hex]5[Neu5Ac]2+Na	2244.7

**(A) TIC for the full scan  $m/z$  400-2000**



**Fig. 15 LC-MS/MS of tryptic digest of human serum**

**Column Magic C18**

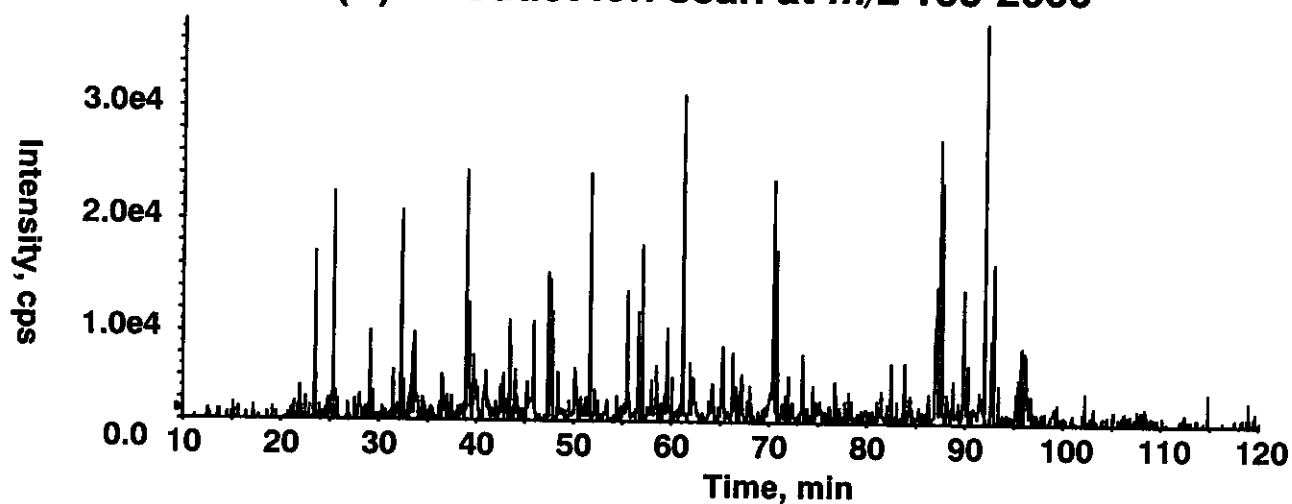
**3  $\mu$ , 0.2\*50 mm, 2  $\mu$ l/min**

**A: 2 %  $\text{CH}_3\text{CN}$  + 0.1 % formic acid**

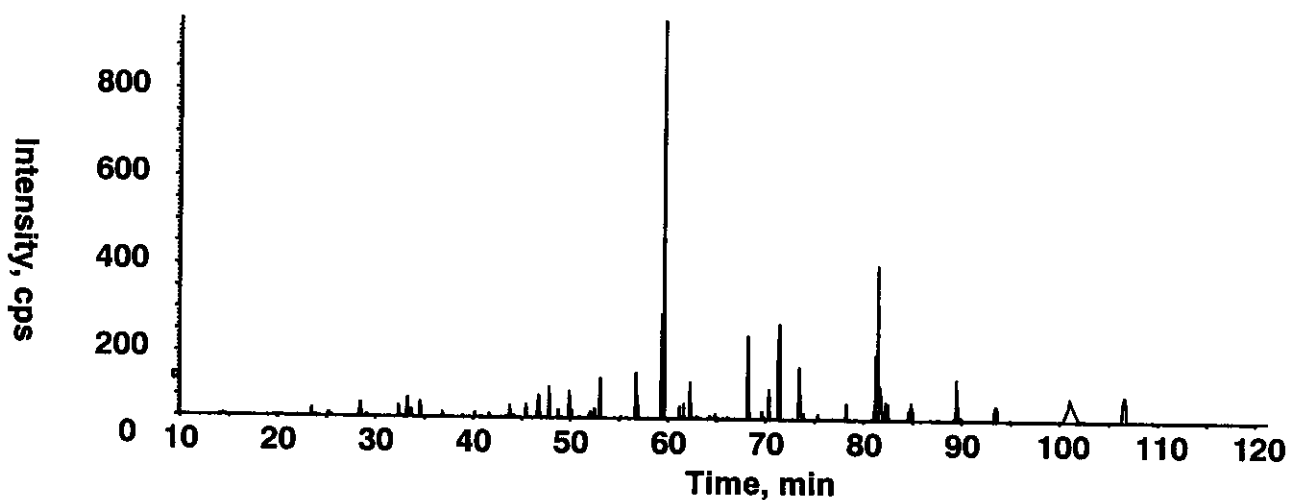
**B: 90 %  $\text{CH}_3\text{CN}$  + 0.1 % formic acid**



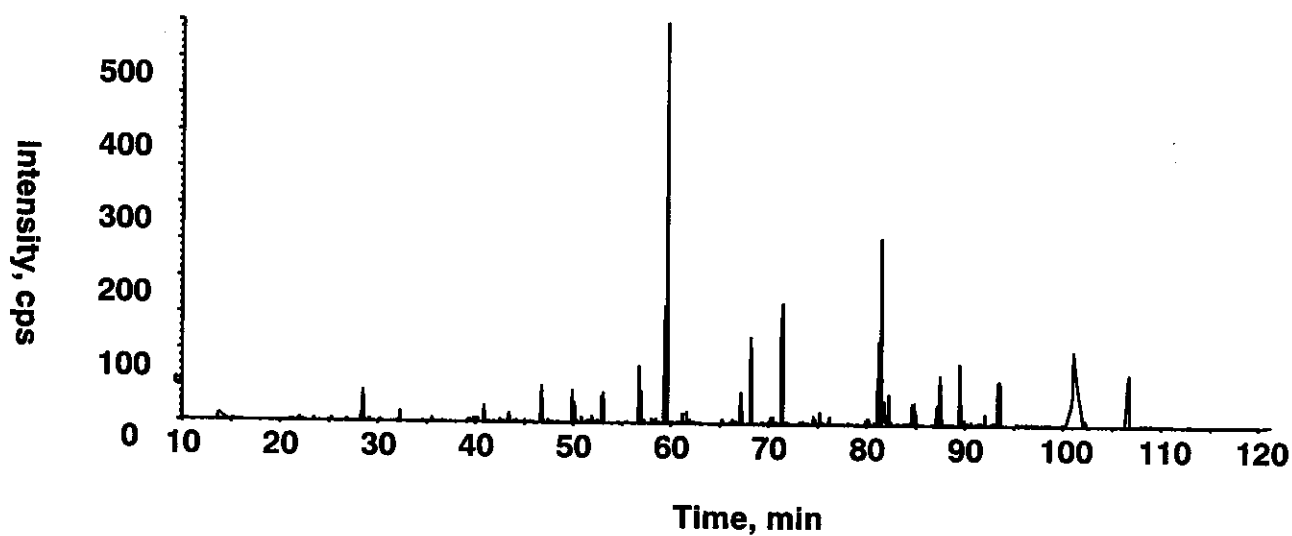
**(B) Product ion scan at  $m/z$  100-2000**



**(C) Product ion scan at  $m/z$  204**

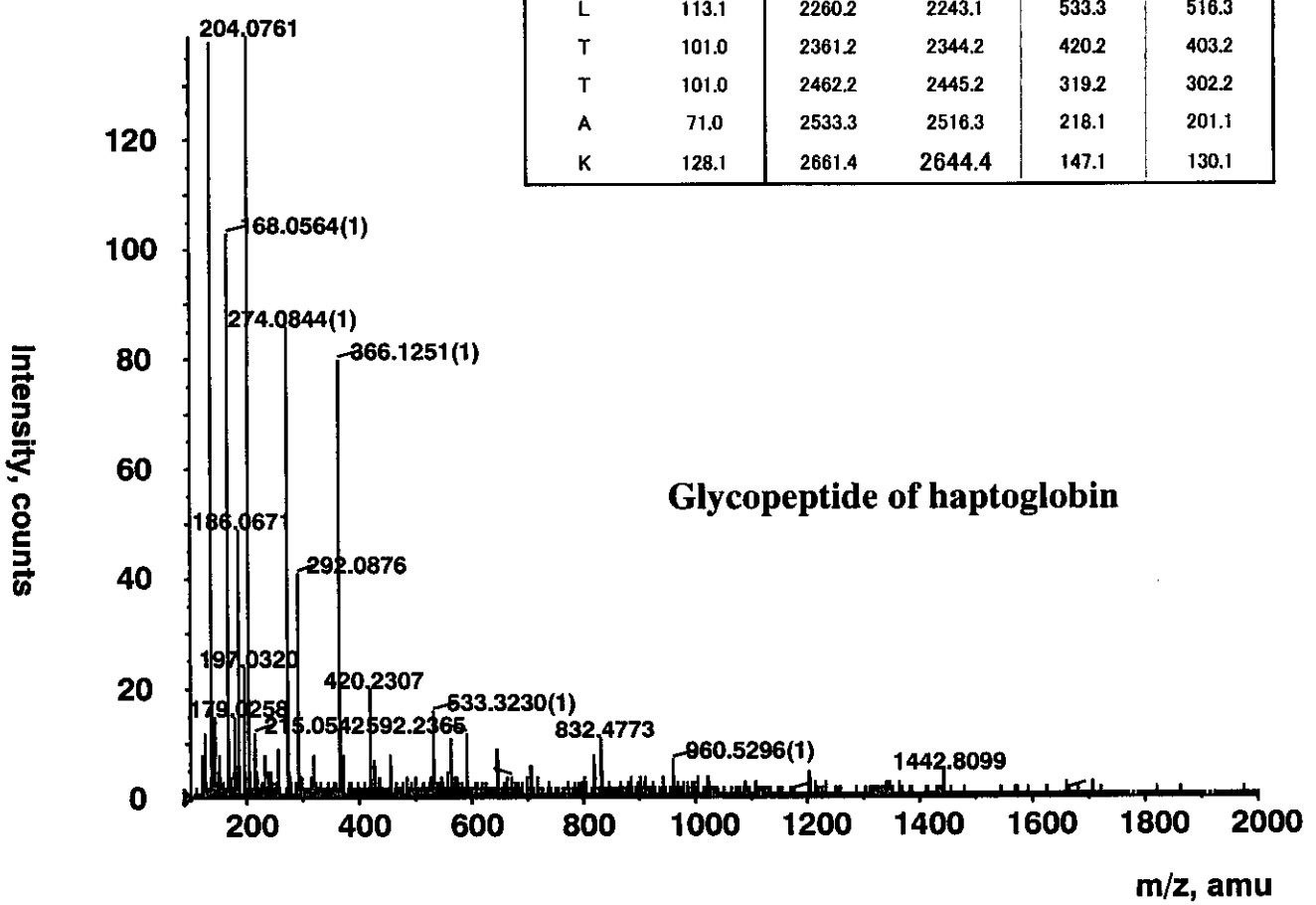


**(D) Product ion scan at  $m/z$  366**



**M.W. 4882.8**  
**Peptide 2678.4**  
**CHO 2222.8**

Residue	Mass	b	b-NH3	y	y-NH3
M	131.0	132.0	115.0	2879.4	2662.4
V	99.1	231.1	214.1	2548.4	2531.3
S	87.0	318.1	301.1	2449.3	2432.3
H	137.1	455.2	438.2	2362.3	2345.2
H	137.1	592.3	575.2	2225.2	2208.2
N	114.0	706.3	689.3	2088.1	2071.1
L	113.1	819.4	802.4	1974.1	1957.1
T	101.0	920.4	903.4	1861.0	1844.0
T	101.0	1021.5	1004.5	1760.0	1742.9
G	57.0	1078.5	1061.5	1658.9	1641.9
A	71.0	1149.5	1132.5	1601.9	1584.9
T	101.0	1250.6	1233.6	1530.9	1513.8
L	113.1	1363.7	1346.7	1429.8	1412.8
I	113.1	1476.8	1459.7	1316.7	1299.7
N	114.0	1590.8	1573.8	1203.6	1186.6
E	129.0	1719.8	1702.8	1089.6	1072.6
Q	128.1	1847.9	1830.9	960.6	943.5
W	186.1	2034.0	2017.0	832.5	815.5
L	113.1	2147.1	2130.0	646.4	629.4
L	113.1	2260.2	2243.1	533.3	516.3
T	101.0	2361.2	2344.2	420.2	403.2
T	101.0	2462.2	2445.2	319.2	302.2
A	71.0	2533.3	2516.3	218.1	201.1
K	128.1	2661.4	2644.4	147.1	130.1



**Fig. 16 Product ion spectrum of M<sup>+</sup>(m/z1221.7<sup>3+</sup>) at 26 min**

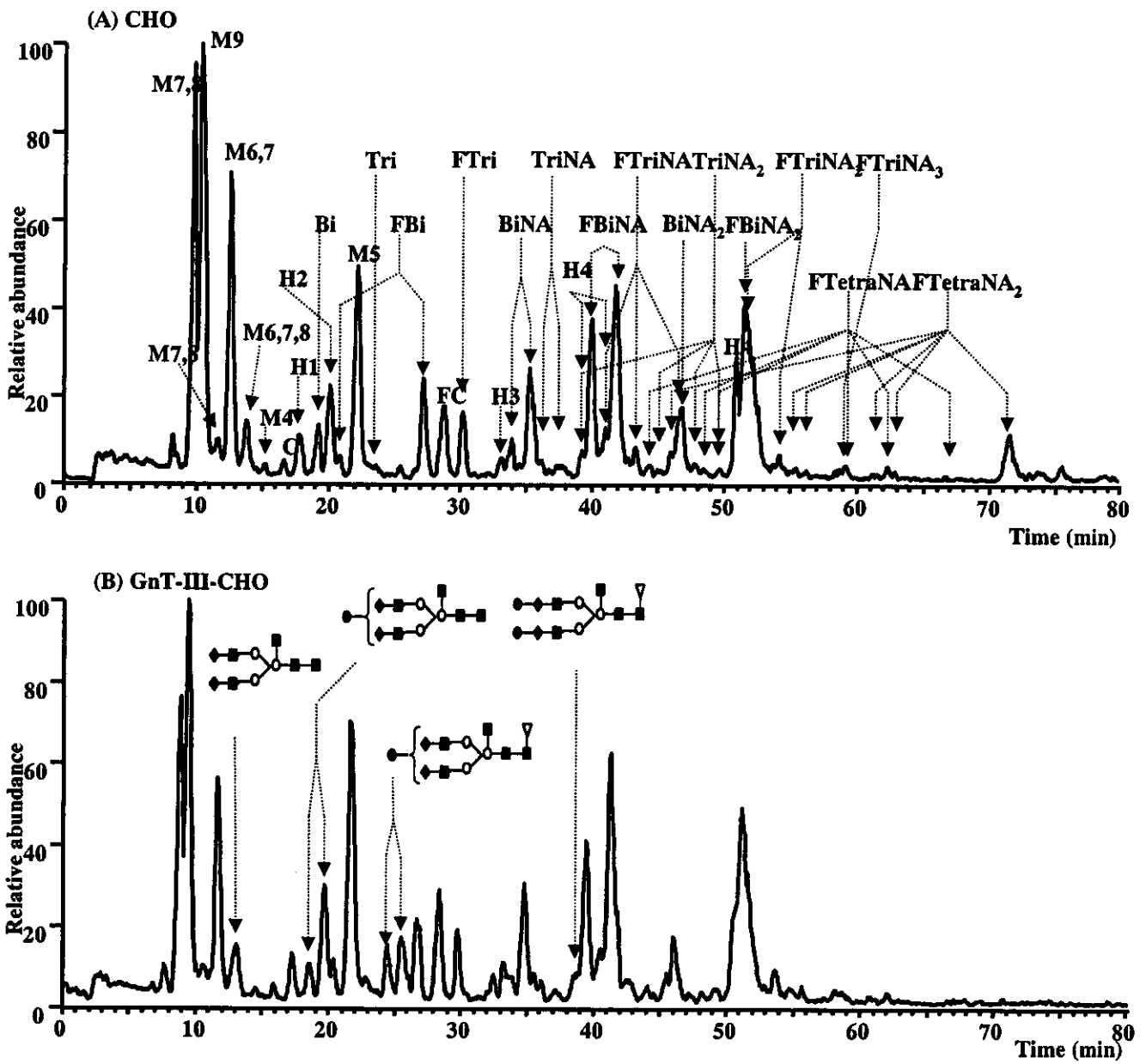
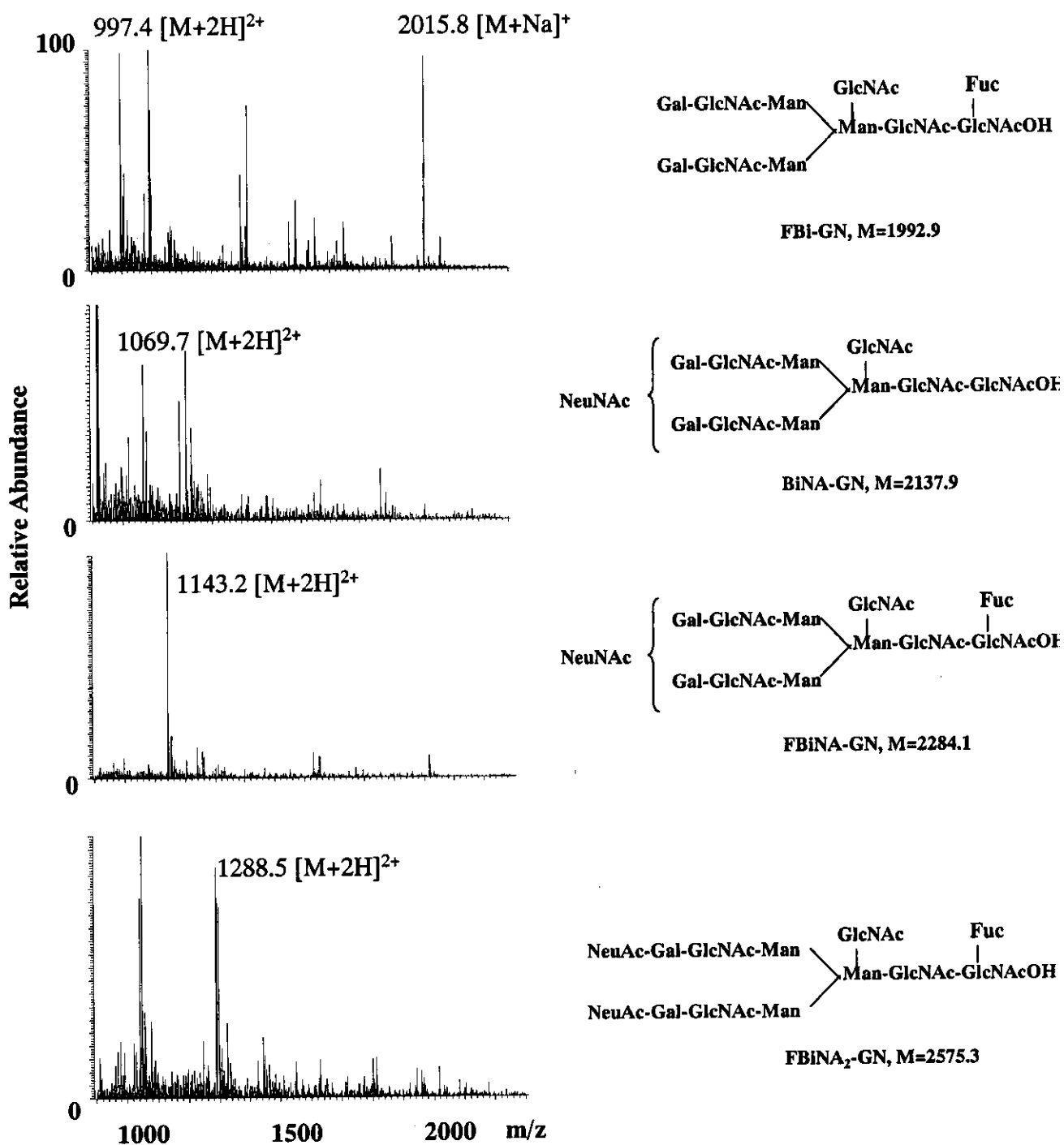


Fig. 17 Oligosaccharide profiling of CHO (A) and CHO-III (B) cells



**Fig. 18** Mass spectra of bisecting biantennaries from CHOIII membrane