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APPENDICES

Appendix A

Table 1A Solutions for 2D-PAGE

Solution	Chemical	Final concentration	Amount
Lysis solution	Urea	8 M	19.2 g
	CHAPS	4% (w/v)	1.6 g
	Pharmalyte 3–10	2%	800 μ l
	Double distilled H ₂ O		to 40 ml
Rehydration solution	Urea	8 M	12 g
	CHAPS	2% (w/v)	0.5 g
	Bromophenol blue	0.002% (w/v)	50 μ l
	Double distilled H ₂ O		to 25 ml
SDS equilibration buffer	Tris-HCl, pH 8.8	50 mM	10.0 ml
	Urea	6 M	72.07 g
	Glycerol	30% (v/v)	69 ml
	SDS	2% (w/v)	4.0 g
	Bromophenol blue	0.002% (w/v)	400 μ l
	Double distilled H ₂ O		to 200 ml
30% T, 2.6% C monomer stock solution	Acrylamide	30%	60.0 g
	Bisacrylamide	0.8%	1.6 g
	Double distilled H ₂ O		to 200 ml
4 \times resolving gel buffer	Tris base	1.5 M	181.7 g
	Double distilled H ₂ O		750 ml
	HCl		adjust to pH 8.8
	Double distilled H ₂ O		to 1 l
10% SDS	SDS	10% (w/v)	5.0 g
	Double distilled H ₂ O		to 50 ml
10% ammonium persulfate	Ammonium persulfate	10% (w/v)	0.1 g
	Double distilled H ₂ O		to 1 ml
SDS electrophoresis buffer	Tris-base	25 mM	30.3 g
	Glycine	192 mM	144.0 g
	SDS	0.1% (w/v)	10.0 g
	Double distilled H ₂ O		to 10 l
Agarose sealing solution	SDS Electrophoresis buffer		100 ml
	Agarose	0.5%	0.5 g
	Bromophenol blue	0.002% (w/v)	200 μ l

Table 2A Coomassie Gel stain and destain solutions

Solutions	Chemical	Amount
Coomassies Gel stain	Coomassies Blue G-250	1 g
	Methanol	450 ml
	H ₂ O	450 ml
	Glacial acetic acid	100 ml
Coomassies Gel destain	Methanol	100 ml
	H ₂ O	800 ml
	Glacial acetic acid	100 ml

Table 3A Bradford solution

Solutions	Chemical	Amount
Bradford Stock solution	95% ethanol	100 ml
	88% phosphoric acid	200 ml
	Coomassies Blue G-250	350 mg
Bradford Working solution	DI-H ₂ O	425 ml
	95% ethanol	15 ml
	88% phosphoric acid	30 ml
	Bradford Stock solution	30 ml

Table 4A Composition of Potato dextrose agar (PDA) for antifungal activity test

Composition	Amount
Potato	200 g
Glucose	20 g
Agar	12 g
DI-H ₂ O	1 l

Table 5A Composition of Nutrient Broth (NB) for antimicrobial activity test

Composition	Amount
Beef extract	3 g
Peptone	5 g
DI-H ₂ O	1 l

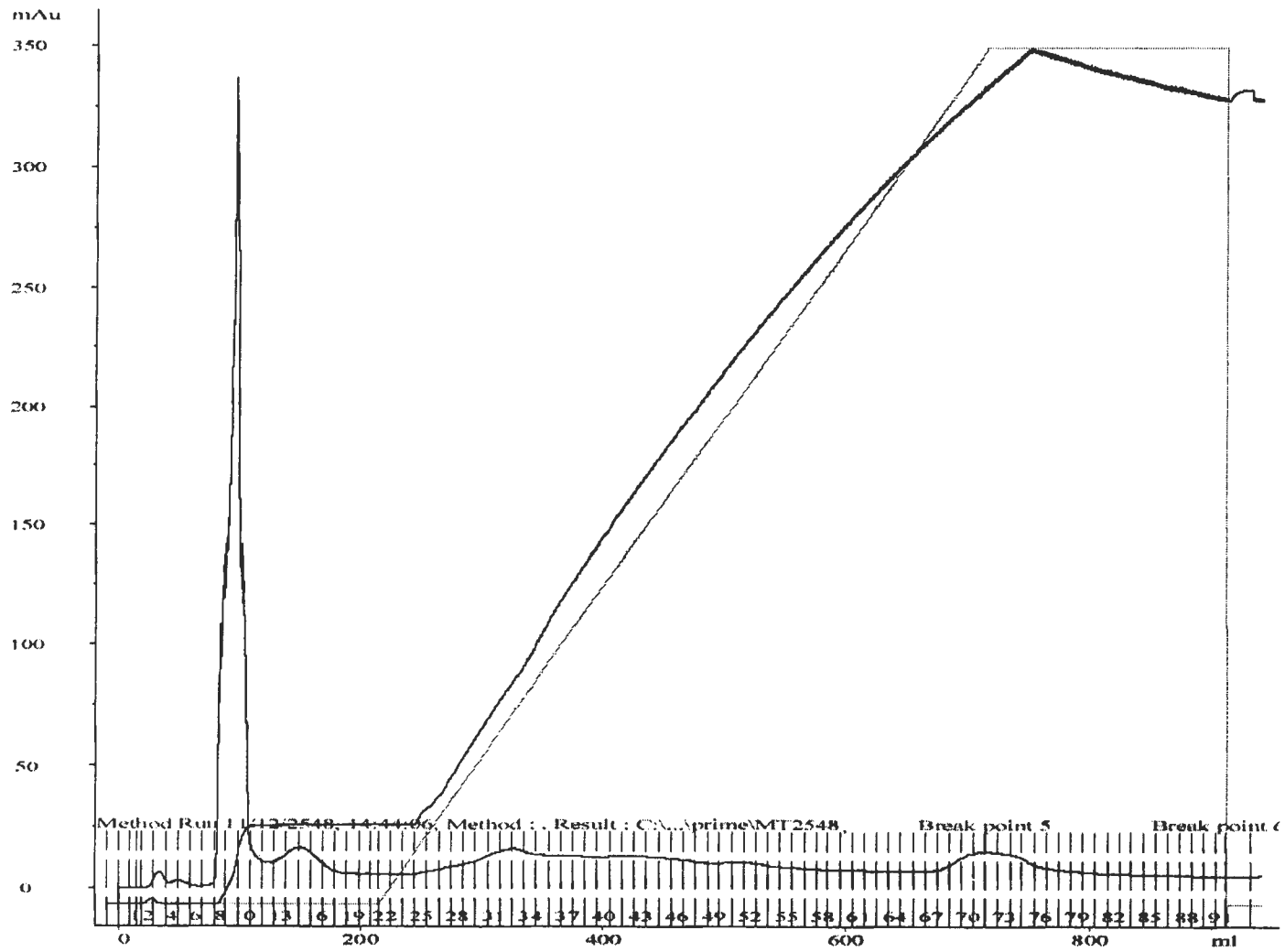


Figure 1B Q-Sepharose anion exchange chromatogram of 60% crude protein

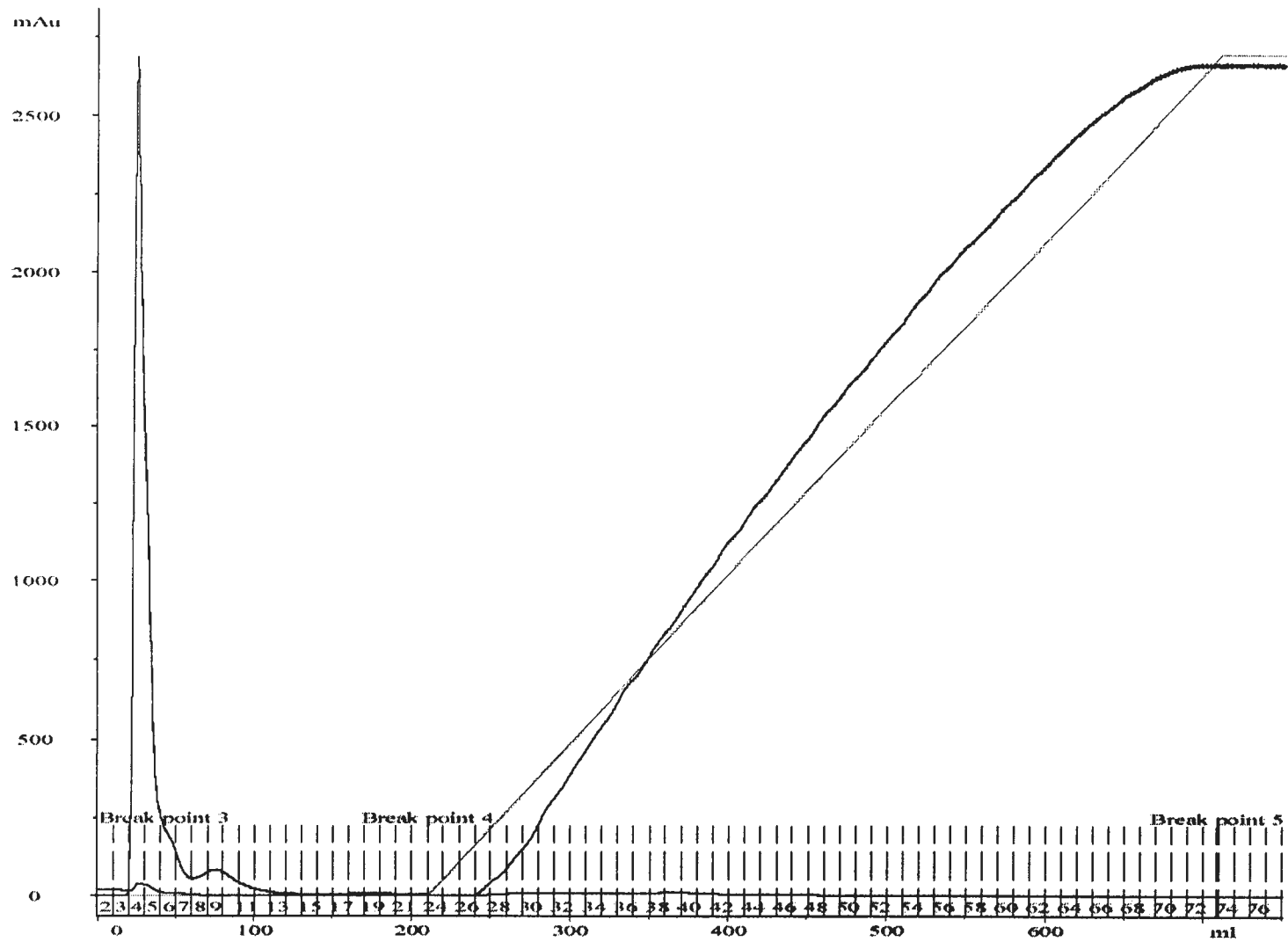


Figure 2B CM-Sepharose cation exchange chromatogram of 60% crude protein

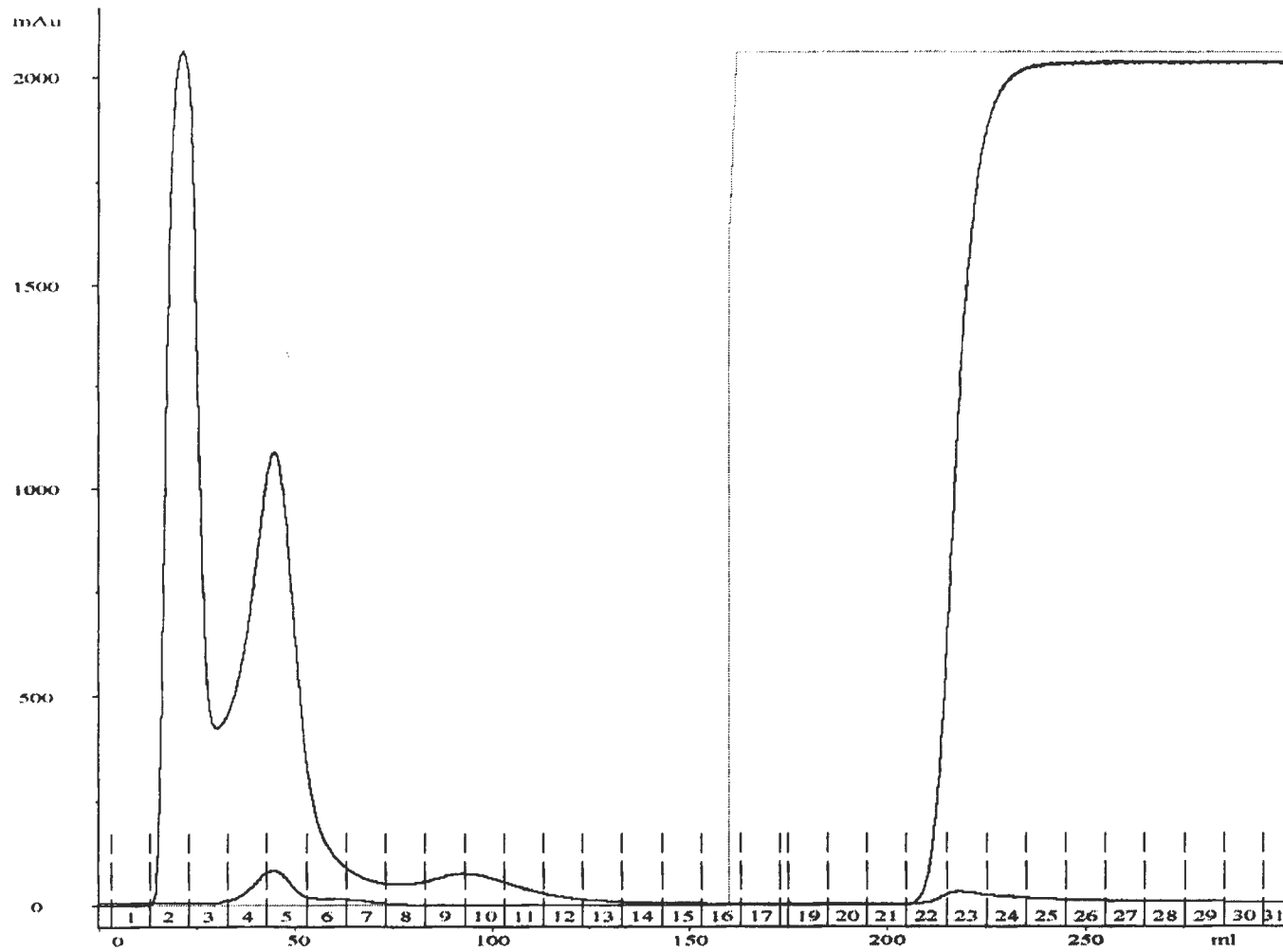


Figure 3B Affi-gel blue gel chromatogram of 60% crude protein

Appendix C

Table 1C Mass per charge of in-gel tryptic peptide of separated protein from 2D-Gel Electrophoresis of *Sesbania grandiflora* flowers crude protein

Spot No.	m/z
1	973.1072, 1043.277, 1084.192, 1235.292, 1314.364, 1497.423, 1941.328, 2084.453, 2527.536, 3219.671
2	882.2149, 926.2297, 1046.25, 1186.23, 1200.272, 1217.146, 1218.188, 1253.271, 1296.317, 1308.819, 1347.358, 1363.327, 1491.231, 1546.264, 1616.664, 1671.32, 2285.291, 2301.433, 2464.495, 2590.389, 3095.494
3	1033.855, 1049.727, 1426.278, 1560.317, 1611.228, 1739.263, 2049.212, 2157.456, 2169.387, 2179.496, 2476.4, 2559.53, 2604.496, 2741.486, 2869.529
4	1164.165, 1289.996, 1344.128, 1417.129, 1447.062, 1517.195, 1541.172, 1546.155, 1575.12, 1769.135, 1853.176, 1866.159, 1897.151, 1925.145, 1965.192, 1981.176, 2053.211, 2093.223, 2748.253, 2817.257, 2916.259
5	1046.205, 1296.276, 1308.741, 1341.269, 1347.323, 1363.3, 2150.407, 2167.422, 2464.417
6	967.3344, 1045.394, 1137.302, 1149.262, 1165.3, 1176.431, 1306.453, 1320.477, 1339.281, 1553.616, 1584.566, 1793.458, 1797.474, 1809.483, 1825.424, 2150.678, 2167.681

Table 2C Mass per charge of in-gel tryptic peptide of SGF60 separated protein from SDS-PAGE

Protein	m/z
SGF60	975.4648, 1003.471, 1005.663, 1019.452, 1115.651, 1214.699, 1219.678, 1314.663, 1516.753, 1697.991, 1735.91, 1774.77, 1826.069, 2038.921, 2043.87, 2189.017, 2198.022, 2203.895, 2357.006, 2611.102, 2661.045, 3153.165

Table 3C Mass per charge of in-gel tryptic peptide of SGF90 from SDS-PAGE

Protein	m/z
SGF90	892.5318, 898.5043, 943.5061, 948.6422, 1011.12, 1049.478, 1054.546, 1065.461, 1072.579, 1087.599, 1107.58, 1123.54, 1192.667, 1272.212, 1326.632, 1447.708, 1452.642, 1495.764, 1575.814, 1726.941, 1942.841, 1952.909, 2154.12, 2394.084, 2441.048, 2758.103, 2807.163, 2898.187, 2920.126

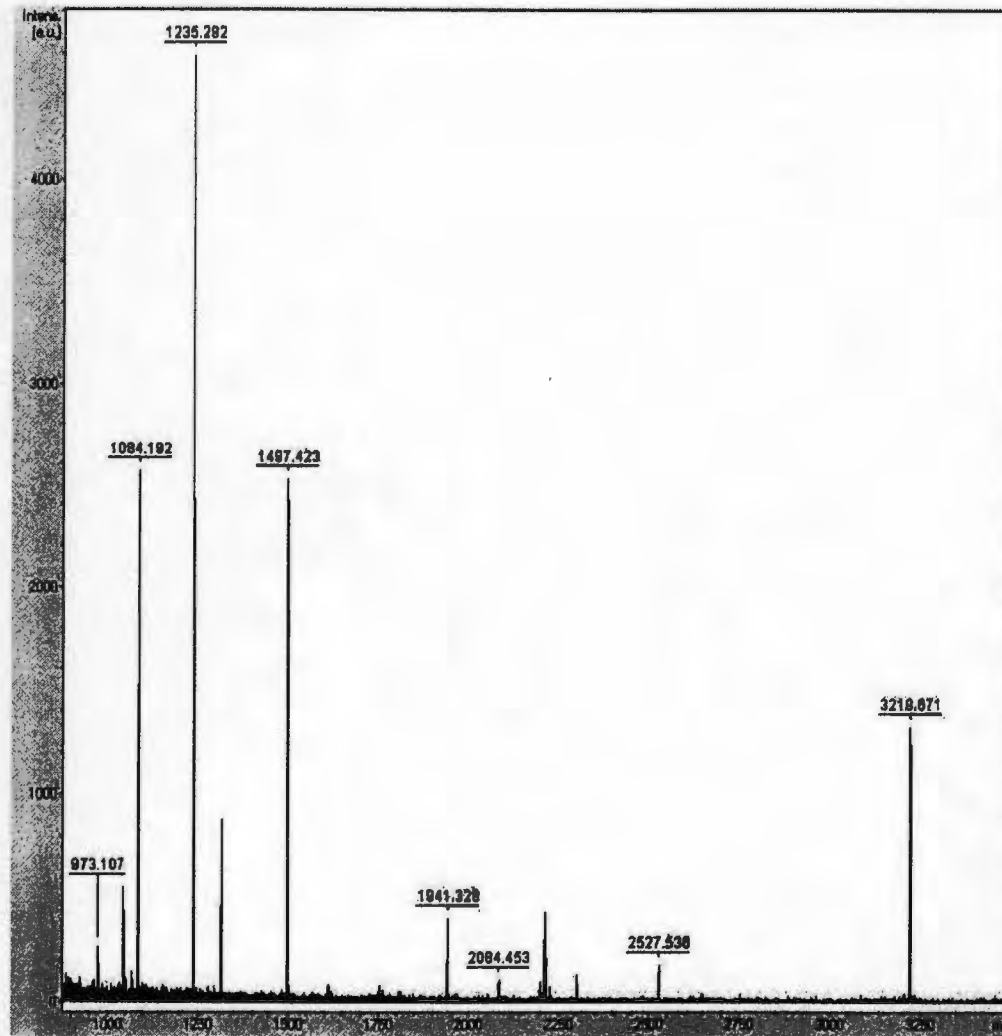


Figure 1C MALDI-MS spectrum of tryptic fragment from 2D-gel electrophoresis of spot number 1

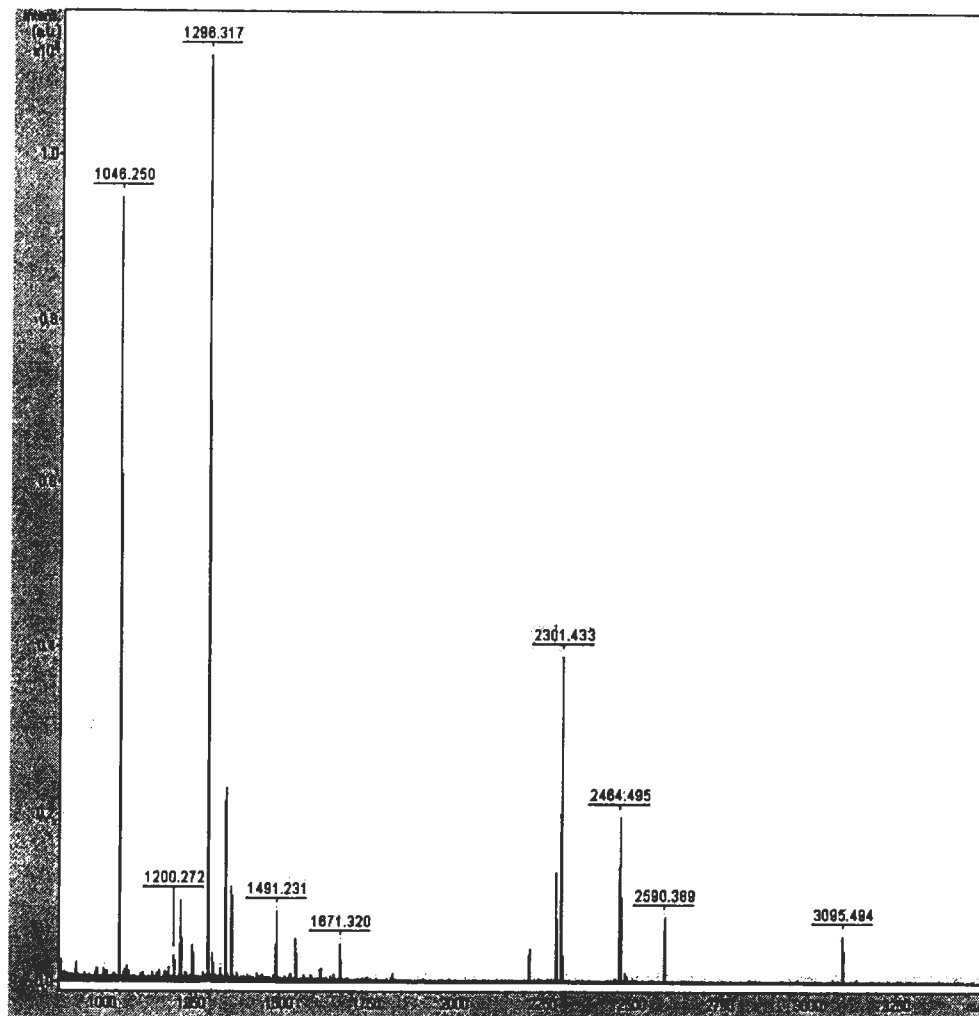


Figure 2C MALDI-MS spectrum of tryptic fragment from 2D-gel electrophoresis of spot number 2

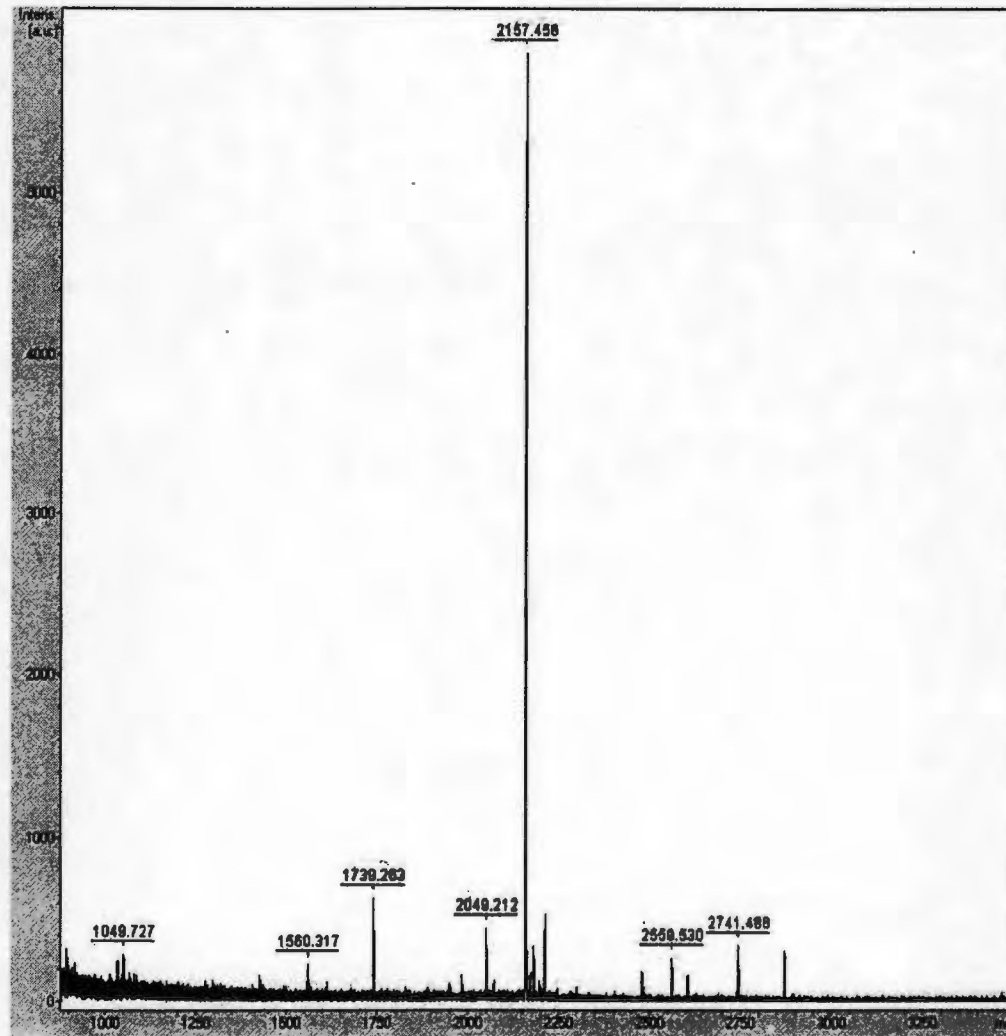


Figure 3C MALDI-MS spectrum of tryptic fragment from 2D-gel electrophoresis of spot number 3

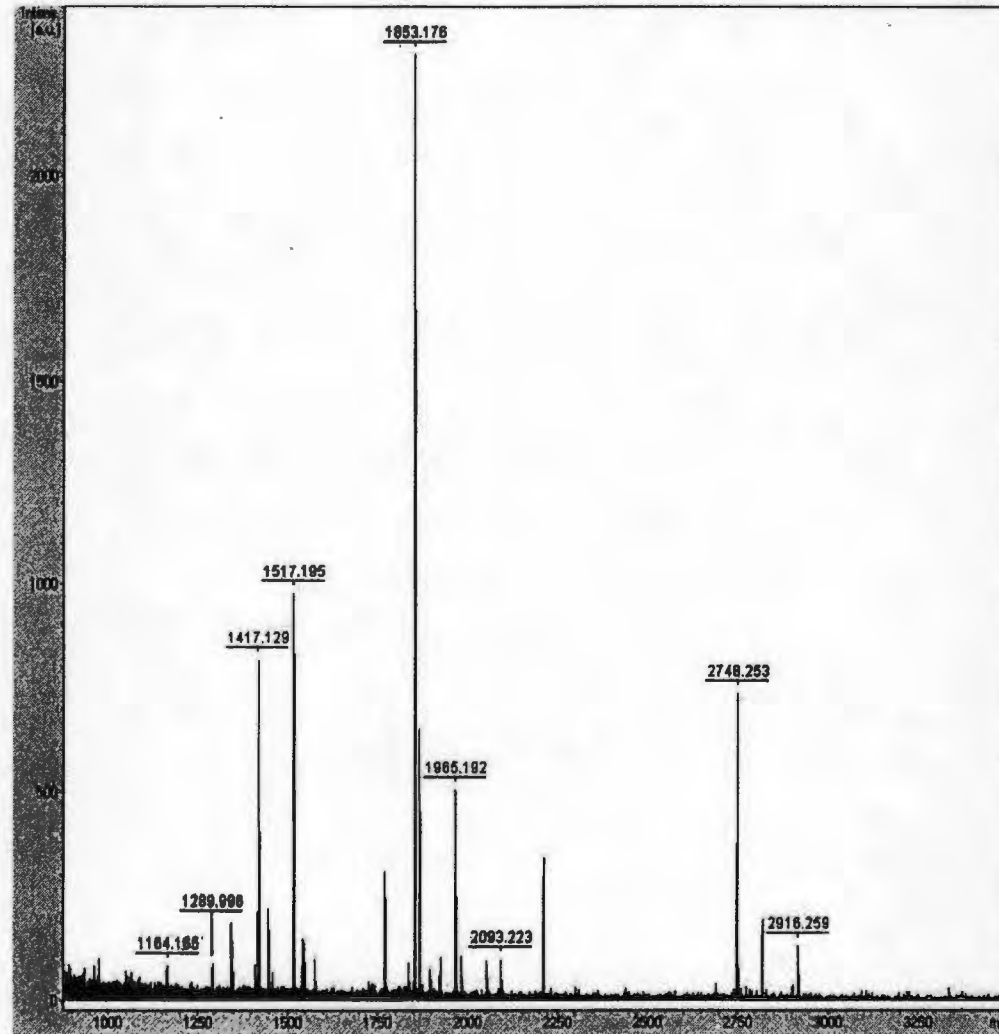


Figure 4C MALDI-MS spectrum of tryptic fragment from 2D-gel electrophoresis of spot number 4

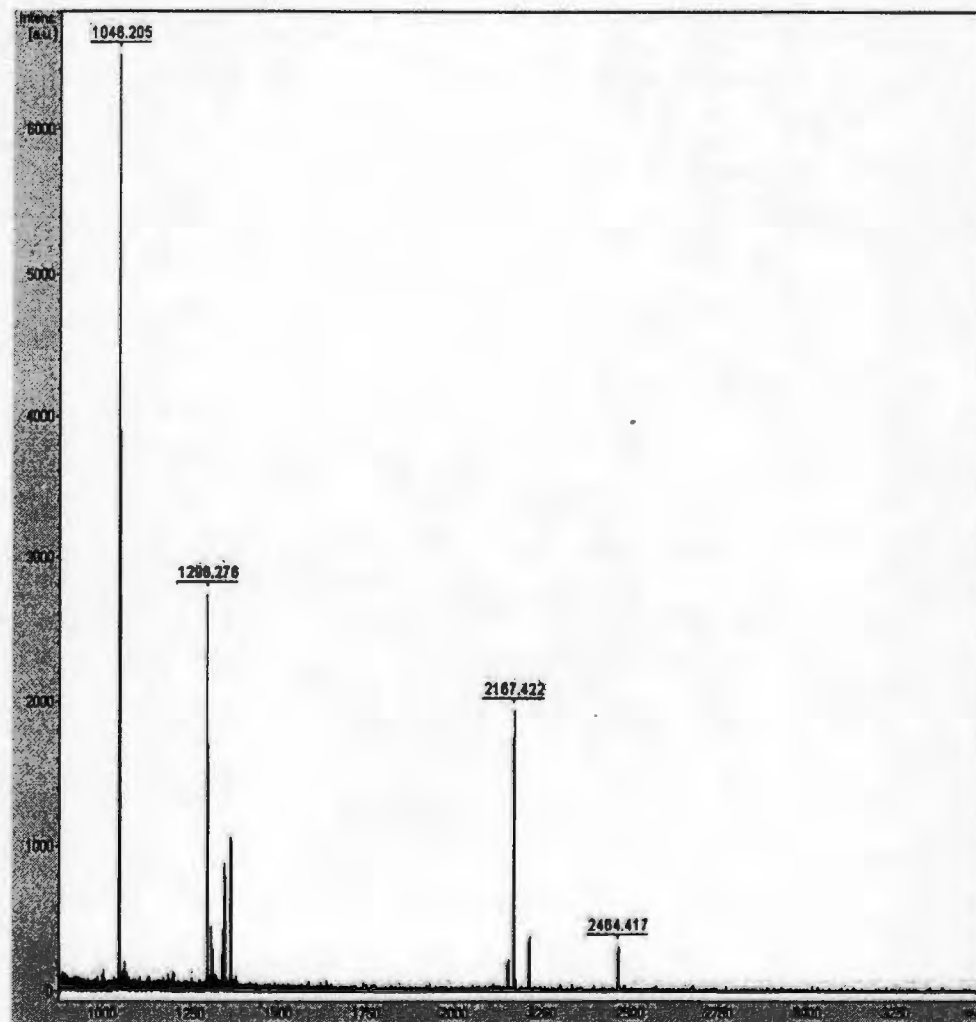


Figure 5C MALDI-MS spectrum of tryptic fragment from 2D-gel electrophoresis of spot number 5

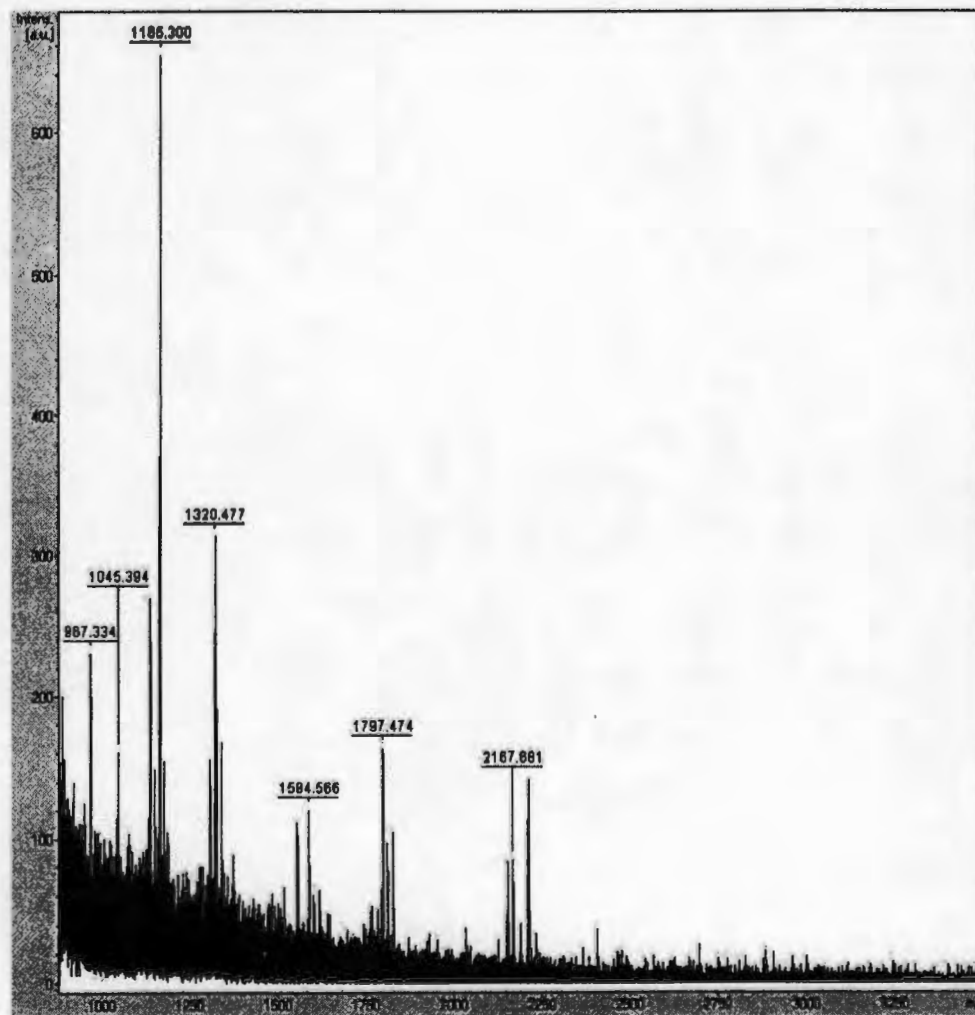


Figure 6C MALDI-MS spectrum of tryptic fragment from 2D-gel electrophoresis of spot number 6

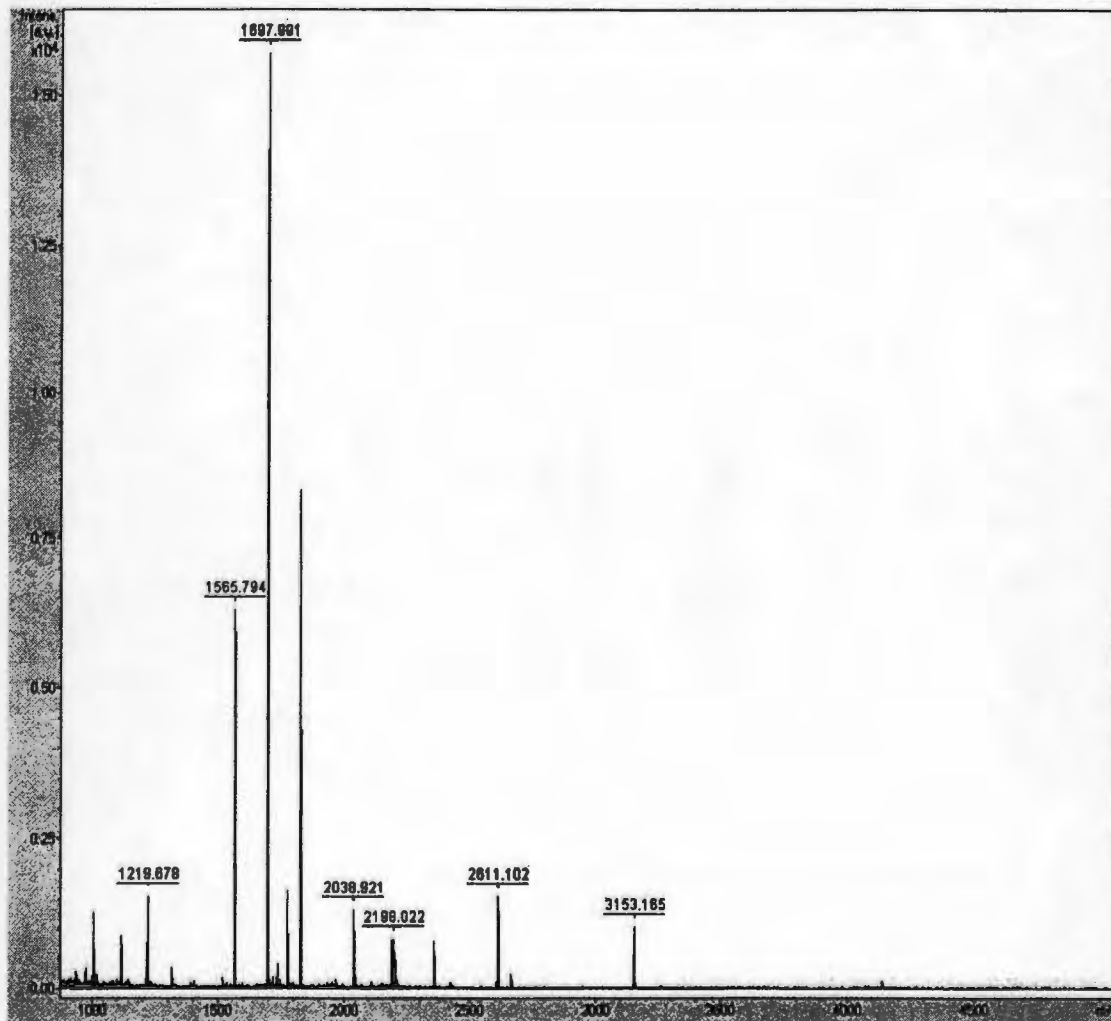


Figure 7C MALDI-MS spectrum of tryptic fragment from SGF60

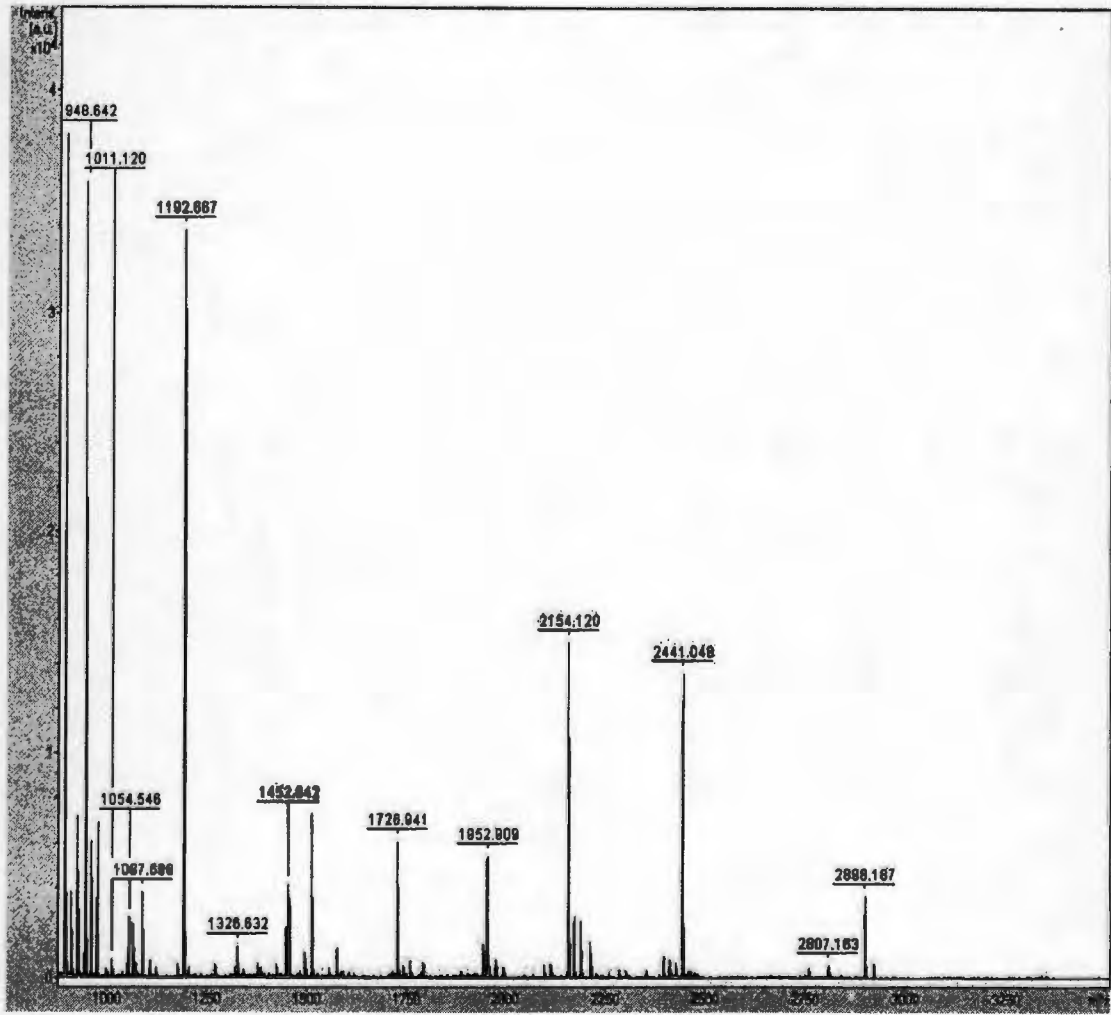


Figure 8C MALDI-MS spectrum of tryptic fragment from SGF90

Appendix D

Table 1D De Novo sequencing of peptide precursor of SGF60 protein using Peptide Sequencing program from Masslynx software

Precursor mass	Precursor ion charge	Observed MW	De Novo Sequence
412.77	2	823.5244	(-)VPGVSLPR(-)
421.77	2	841.5244	(-)VATVSLPR(-)
435.77	2	869.5244	(-)VVTVSLPR(-)
471.58	2	941.1443	(-)TAELPVANK(-), (-)ATELPVANK(-)
563.79	2	1125.5643	(-)SGGPNGATLPQK(-), (-)GSGPNGATLPQK(-), (-)SNPGGGATLPQK(-), (-)NSPGGGATLPQK(-), (-)SNPNGATLPQK(-), (-)NSPNGATLPQK(-), (-)GGSPGGGATLPQK(-)
563.81	2	1125.6044	(-)SGGPGGGATLPQK(-), (-)GSGPGGGATLPQK(-)
599.30	2	1196.5844	(-)TALTDNFVTL(-), (-)ATLTDNFVTL(-), (-)TALTDNFVTCL(-), (-)ATLTDNFVTCL(-), (-)ATLTDNFVTD(-), (-)TALTDNFVTD(-), (-)ATLTDNFVTTD(-), (-)TALTDNFVTTD(-), (-)ATLTDNFVTSE(-), (-)TALTDNFVTSE(-), (-)ATLTDNFVTES(-), (-)TALTDNFVTES(-)
609.31	2	1216.6044	(-)TNVKGKLNSSAVQ(-), (-)NTVKGKLNSSAVQ(-), (-)TNVKGKLNSSAVQ(-), (-)NTVKGKLNSSAVQ(-), (-)TNTSSNLGKPAQ(-), (-)TNTSSNLGKPAQ(-), (-)TNTSSNLGKPAQ(-)
614.72	2	1227.4243	(-)QLLVETSGRLL(-), (-)LQLVETSGRLL(-), (-)LLQVETSGRLL(-)
626.30	2	1250.5844	(-)LTDSMSGLSGAGR(-), (-)LTDSMSGLSGAGR(-), (-)LTDSMSGLSGQR(-)
676.00	2	1349.9844	(-)GSANAVDLSVSETT(-), (-)SGANAVDLSVSETT(-), (-)SGANAVDLSVSESD(-), (-)GSANAVDLSVSESD(-), (-)QSNVAVDLSVSESD(-), (-)SQNAVAVDLSVSESD(-)
718.70	2	1435.3844	(-)AHVSFLGCGVFSGR(-), (-)ESVPELGCVFSGR(-), (-)SEVPELGCVFSGR(-), (-)AHVSFLGCRFSGR(-), (-)TDVPELGCVFSGR(-), (-)PVESELGCGVFSGR(-), (-)AHSVFLGCGVFSGR(-)
735.68	2	1469.3444	(-)FYRTLGLDWT(-), (-)YFRTLGLDWT(-), (-)RFYRTLGLDWT(-), (-)RYRTLGLDWT(-), (-)FRYRTLGLDWT(-), (-)YRRTLGLDWT(-)
749.06	2	1496.1044	(-)QYRLGMTAFGAPK(-)
767.42	2	1532.8243	(-)NPLTNDLMDLFLK(-), (-)NPLTNDLMDLFLK(-), (-)NPLTNDLETYLLK(-), (-)NPLTNDLETYLLK(-), (-)NPLTNDLETYLLK(-), (-)NPLTNDLMDLFLK(-), (-)NPLTNDLMDLFLK(-)
767.73	2	1533.4443	(-)LPGTTVVTELFTR(-)
786.72	2	1571.4243	(-)YLQTSVLTAGFASGT(-), (-)LYQTSVLTAGFASGT(-), (-)YLQTSVLTAGFASSA(-), (-)LYQTSVLTAGFASSA(-), (-)YLQTSVLTAGFASTG(-), (-)YLQTSVLTAGFASAS(-), (-)LYQTSVLTAGFASAS(-), (-)YLQTSVLTAGFAASS(-), (-)LYQTSVLTAGFAASS(-), (-)YLQTSVLTAGFAGST(-), (-)LYQTSVLTAGFAGST(-), (-)YLQTSVLTAGFATSG(-), (-)LYQTSVLTAGFATSG(-), (-)YLQTSVLTAGFAGTS(-), (-)LYQTSVLTAGFATGS(-), (-)QTFLASTLTAGFANM(-), (-)TQFLASTLTAGFANM(-), (-)TQFLASTLTAGFAMN(-)
887.88	2	1773.7444	(-)HFGDTNNNDALTANR(-)
1163.02	1	1162.0122	(-)SATTFAASFYP(-), (-)ASTTFAASFYP(-), (-)ASTTFAASFME(-), (-)SATTFAASFME(-), (-)TGTTFAASFYP(-), (-)GTTTFAASFYP(-), (-)TGTTFAASFME(-), (-)GTTTFAASFME(-), (-)SATTAFASFYP(-), (-)ASTTAFASFYP(-), (-)TGTTAFASFYP(-), (-)GTTTAFASFYP(-), (-)SATTAFASFME(-), (-)SATTAFASFME(-), (-)TGTTAFASFME(-), (-)GTTTAFASFME(-)
1165.53a	1	1164.5222	(-)SLNNSAFAEGR(-), (-)LSNNSAFAEGR(-), (-)SLNNTGFAEGR(-), (-)SLNNSAFAERG(-), (-)LSNNSAFAERG(-), (-)SLNNSAFAENV(-), (-)QSVNSAFAEGR(-), (-)SLNNTGFAERG(-)
1165.53b	1	1164.5222	(-)KMTAELTEEN(-), (-)MKTAELTEEN(-), (-)GLNFELTEEN(-), (-)LGNFELTEEN(-), (-)NFVAELTEEN(-), (-)FNVAELTEEN(-), (-)NFVAELTSNGN(-), (-)FNVAELTSNGN(-), (-)NFVAELTSGGGN(-), (-)FNVAELTSGGGN(-), (-)GLYLTSVAEGR(-)
1169.76	1	1168.7522	(-)NLTYDMAEGR(-), (-)LFKYEGAEGR(-), (-)FLKYEGAEGR(-), (-)LFYKEGAEGR(-), (-)YLQFEGAEGR(-), (-)LYQFEGAEGR(-), (-)LFYQWAEGR(-), (-)YLKFEGAEGR(-), (-)LYKFEGAEGR(-), (-)LFKYWAEGR(-), (-)FLKYWAEGR(-), (-)NLTYFVAEGR(-), (-)NLTYFVAEGR(-), (-)LKYFEGAEGR(-), (-)LYVGTFGAEGR(-), (-)QSLYFVAEGR(-), (-)GFRLGENYLT(-), (-)LVYGTFGAEGR(-)
1180.08	1	1179.0721	(-)YLTAATALGNGK(-), (-)YLATATALGNGK(-), (-)YLPGGSTKGDGK(-), (-)YLVSGTAGLNGK(-)

Polkit 071206 B1

Polkit_071306_01 491 (18.873) Cm (429.560)

1: TOF MS ES+
1.25e4

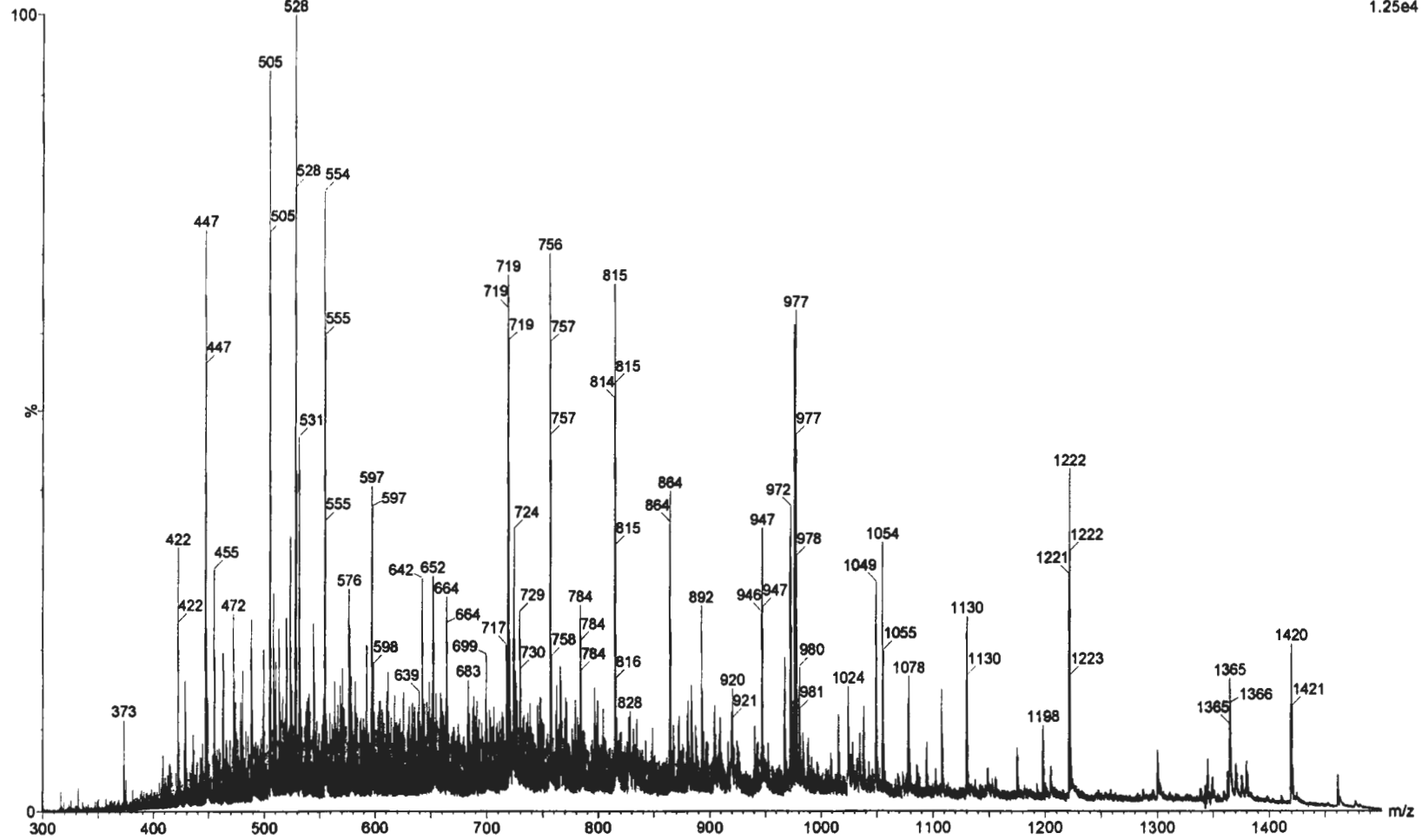


Figure 1D ESI-Q-TOF mass spectrum of tryptic fragments of protein SGF90

Polkit 071206 B6

Polkit_071306_06 491 (17.264) Cm (444:701)

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1.05e4

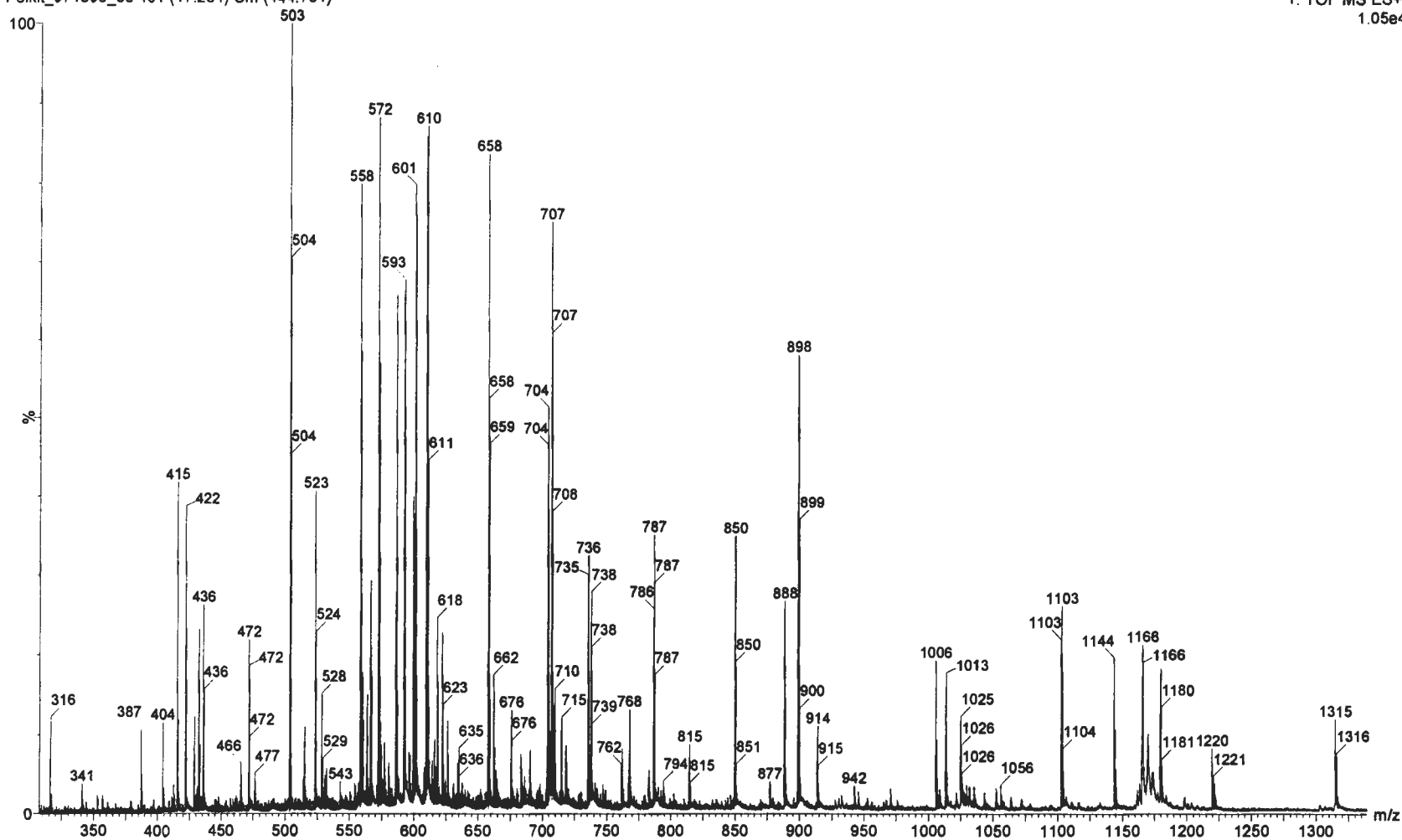


Figure 2D ESI-Q-TOF mass spectrum of tryptic fragments of protein SGF60

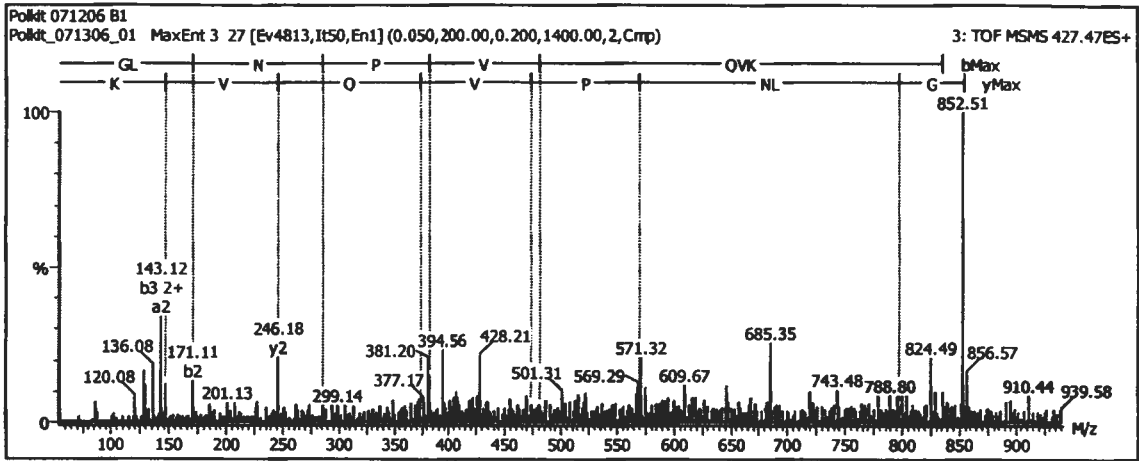


Figure 3D The product ion spectrum of peptide precursor m/z 427.47 from SGF90

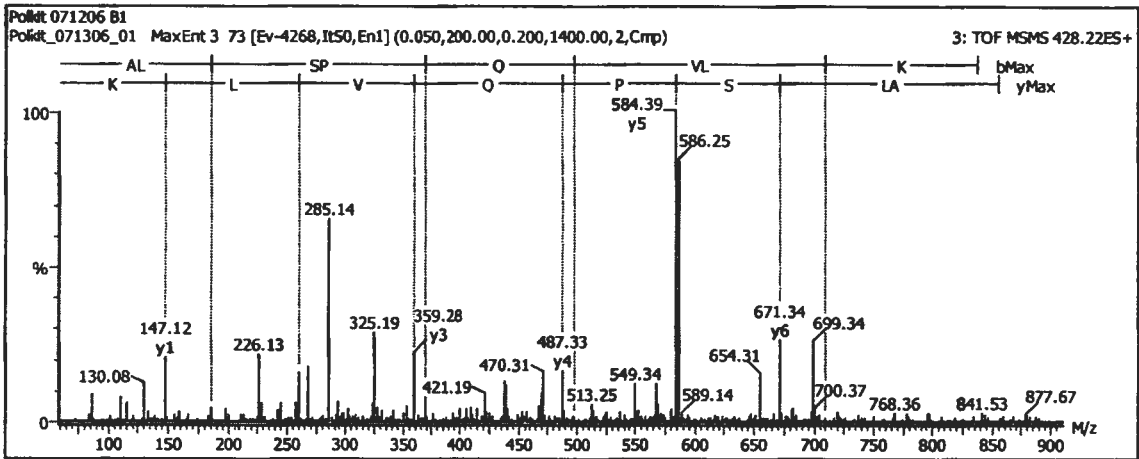


Figure 4D The product ion spectrum of peptide precursor m/z 428.22 from SGF90

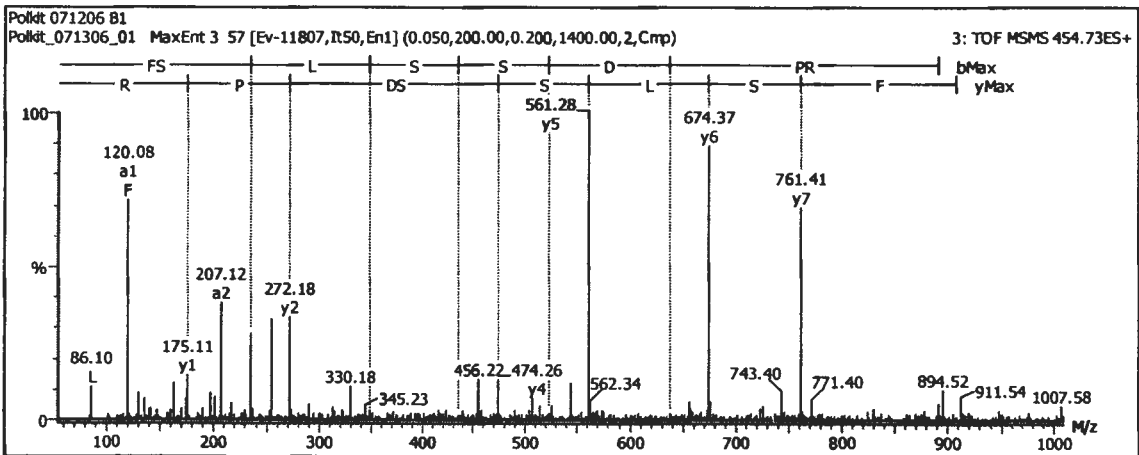


Figure 5D The product ion spectrum of peptide precursor m/z 454.73 from SGF90

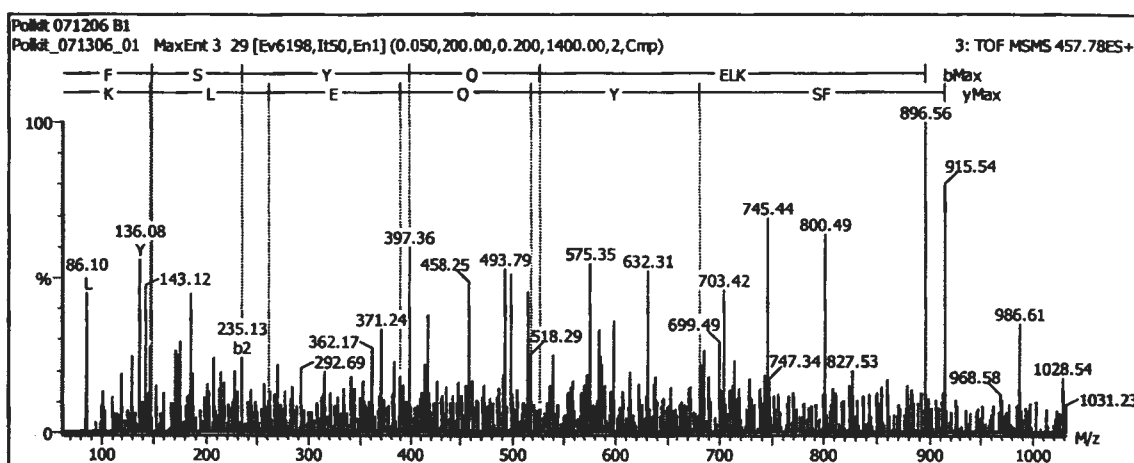


Figure 6D The product ion spectrum of peptide precursor m/z 457.78 from SGF90

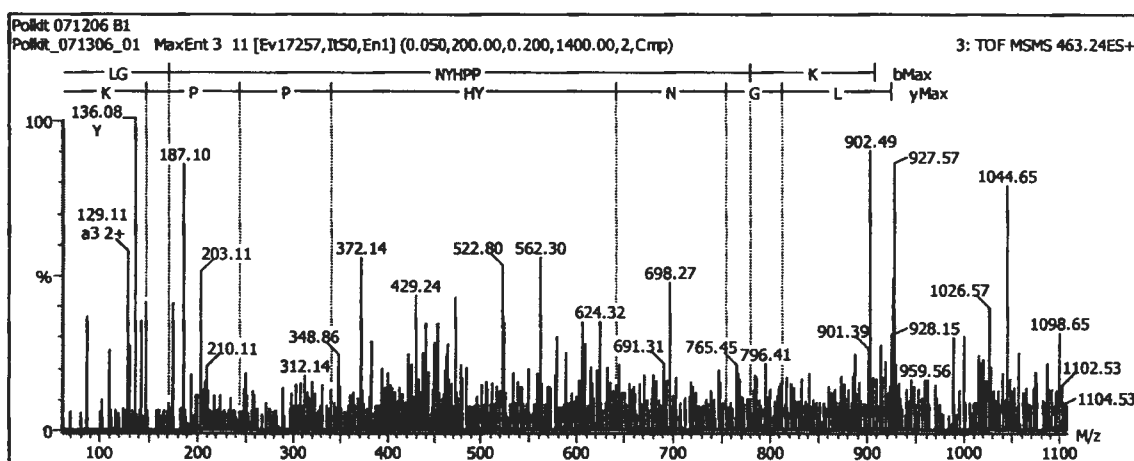


Figure 7D The product ion spectrum of peptide precursor m/z 463.24 from SGF90

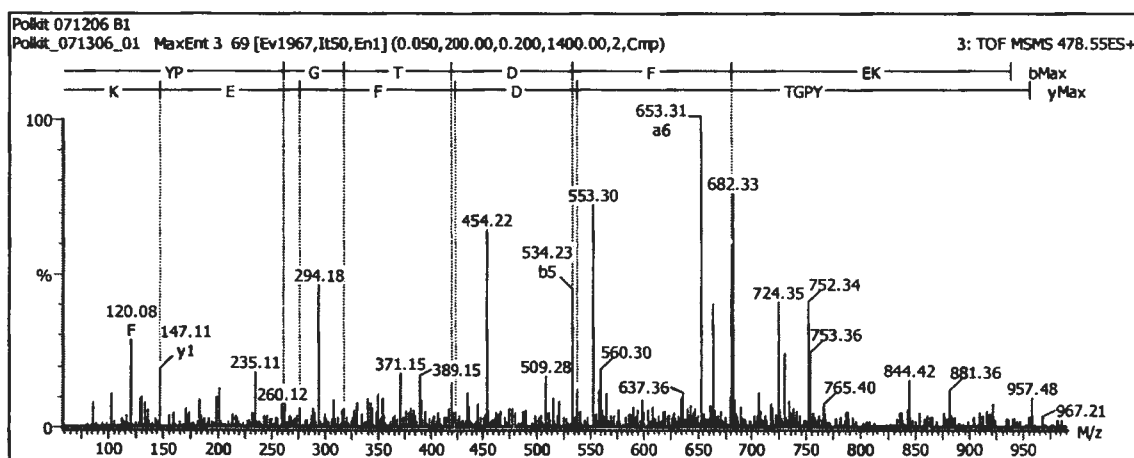


Figure 8D The product ion spectrum of peptide precursor m/z 478.55 from SGF90

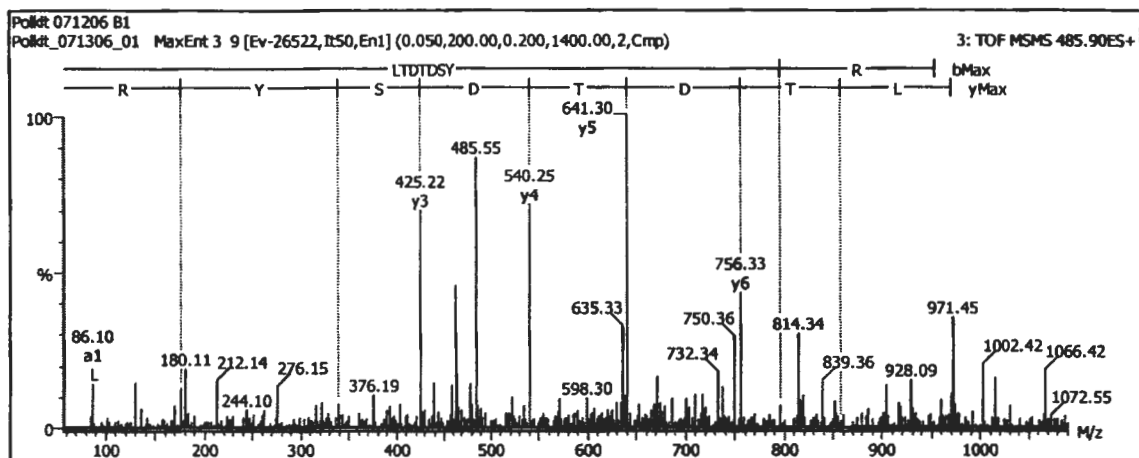


Figure 9D The product ion spectrum of peptide precursor m/z 485.90 from SGF90

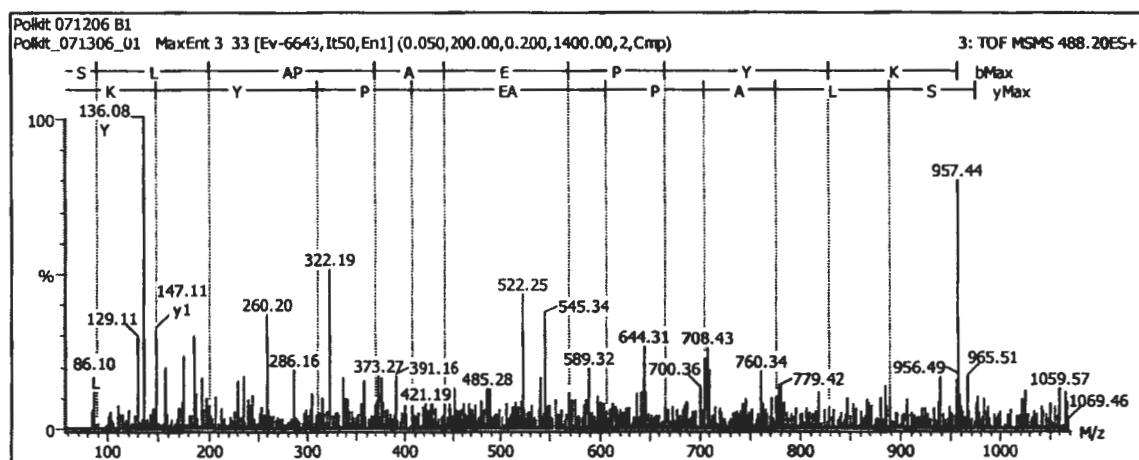


Figure 10D The product ion spectrum of peptide precursor m/z 488.20 from SGF90

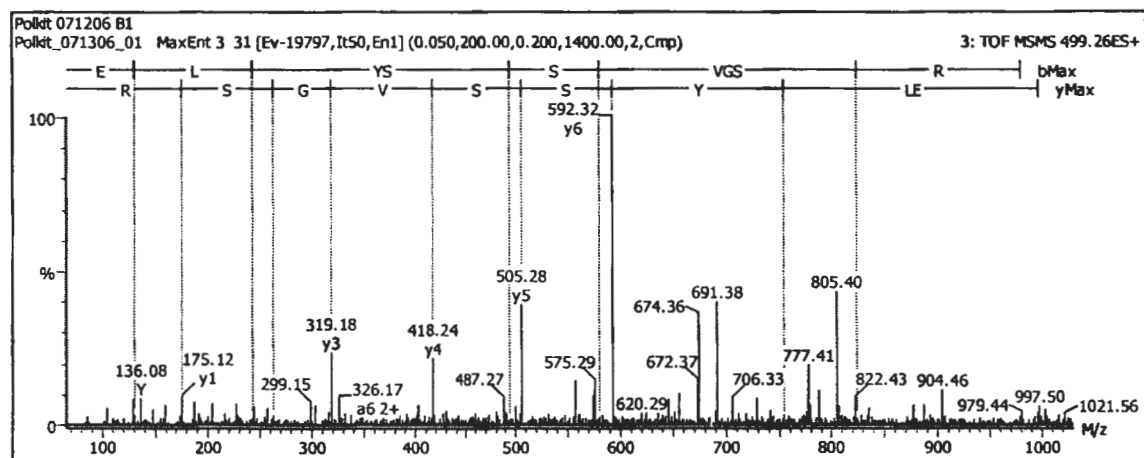


Figure 11D The product ion spectrum of peptide precursor m/z 499.26 from SGF90

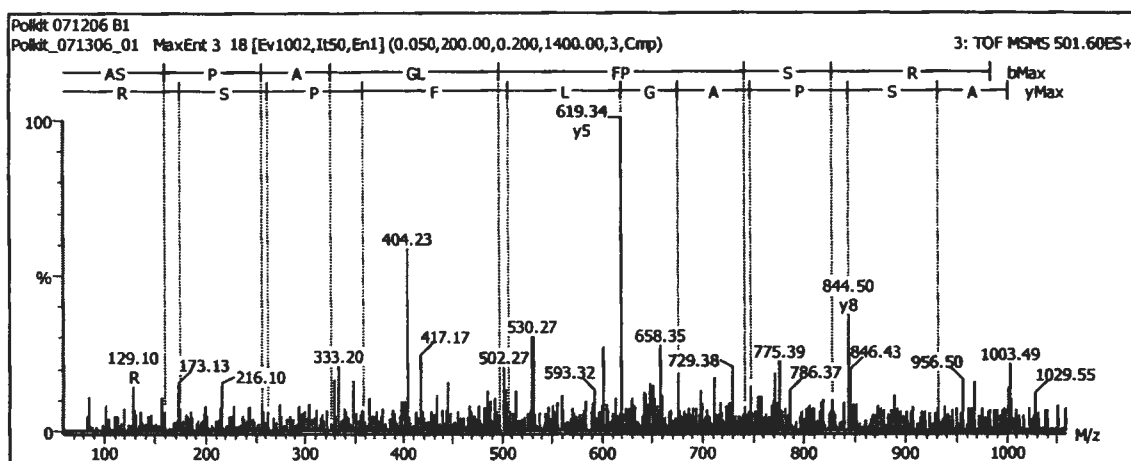


Figure 12D The product ion spectrum of peptide precursor m/z 501.60 from SGF90

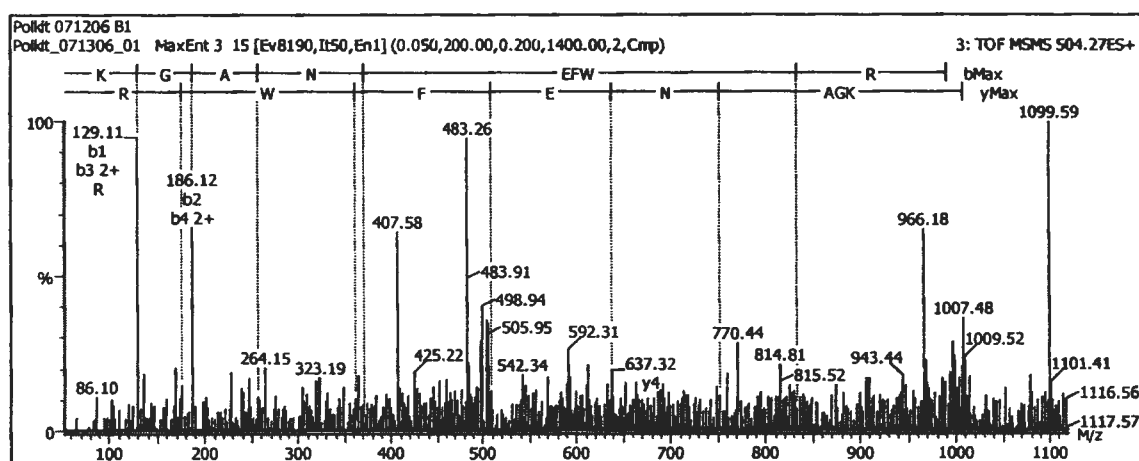


Figure 13D The product ion spectrum of peptide precursor m/z 504.27 from SGF90

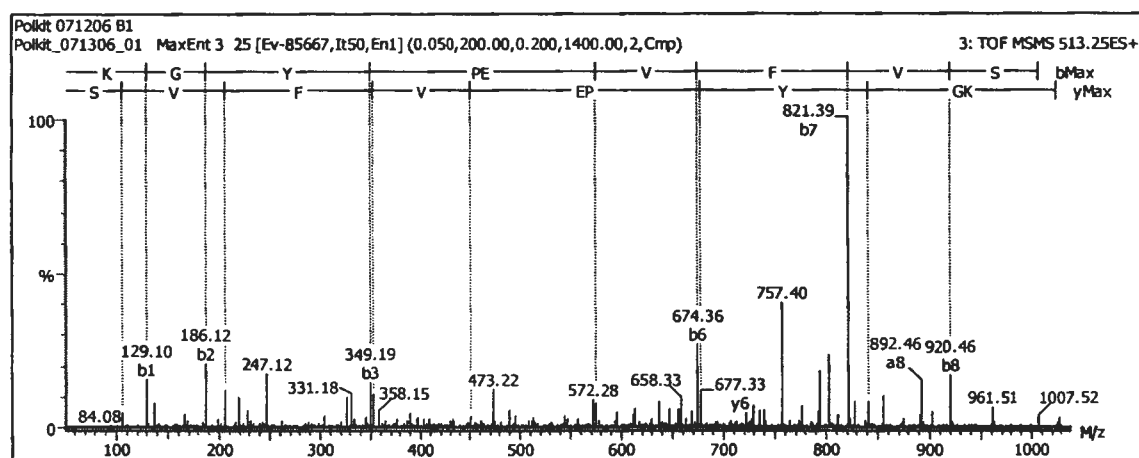


Figure 14D The product ion spectrum of peptide precursor m/z 513.25 from SGF90

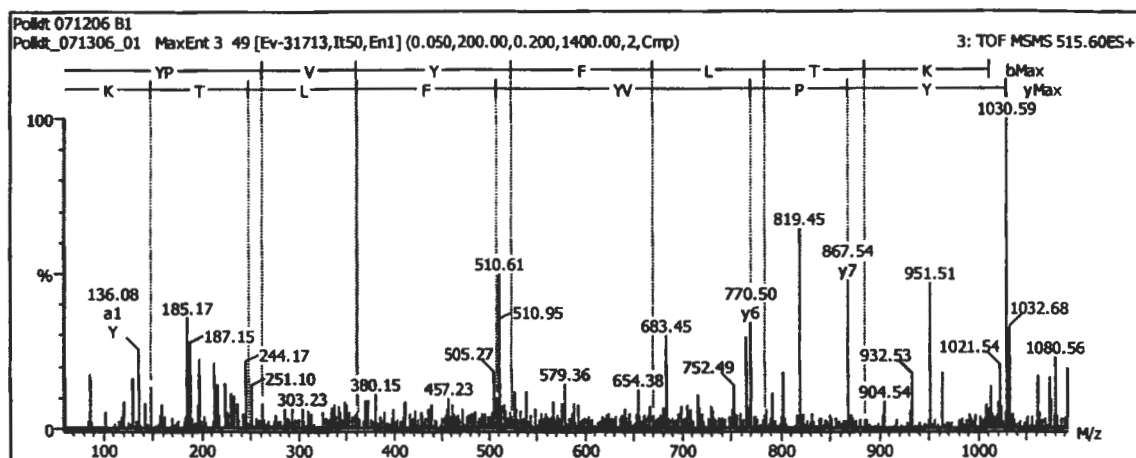


Figure 15D The product ion spectrum of peptide precursor m/z 515.60 from SGF90

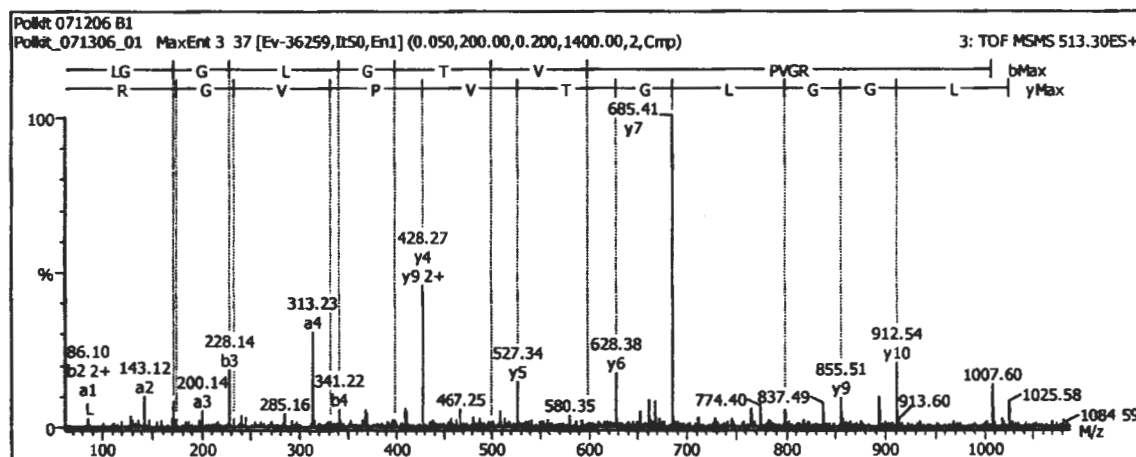


Figure 16D The product ion spectrum of peptide precursor m/z 513.30 from SGF90

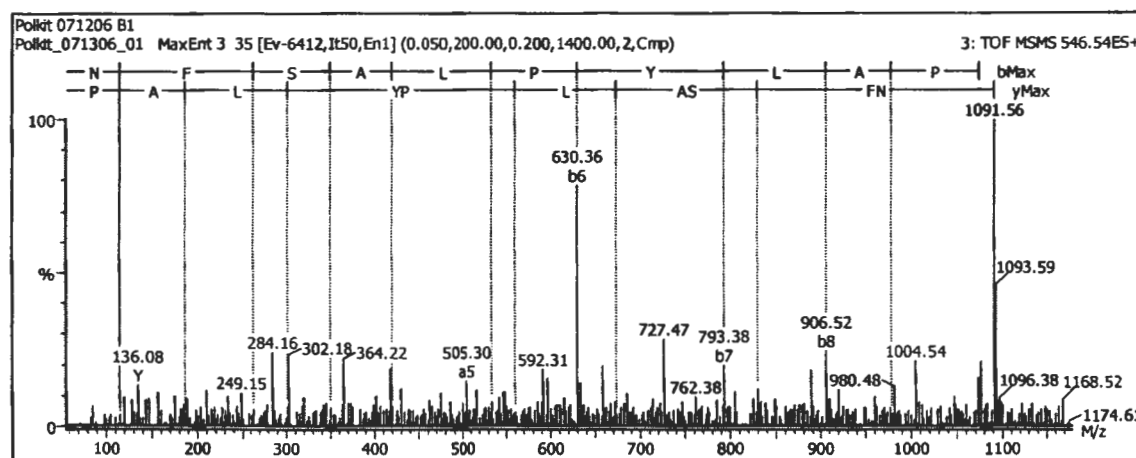


Figure 17D The product ion spectrum of peptide precursor m/z 546.54 from SGF90

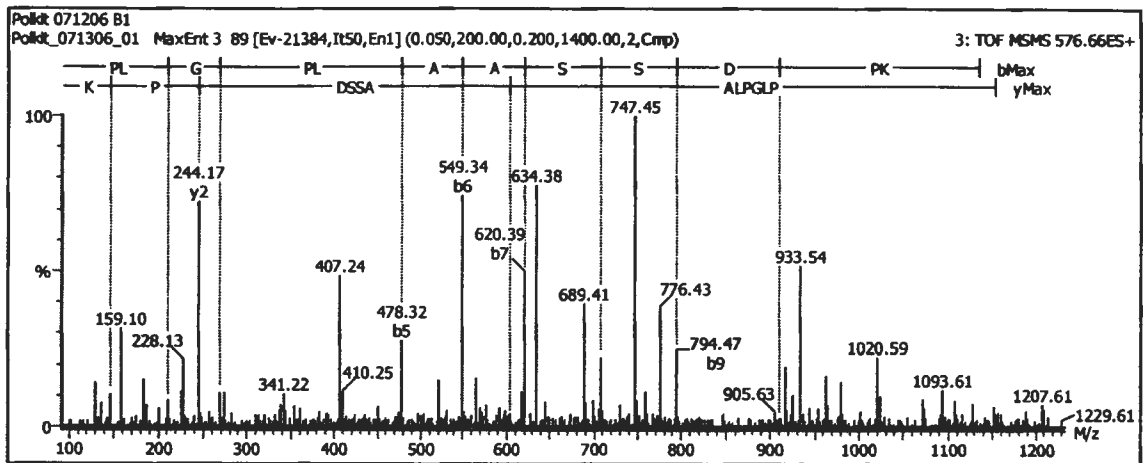


Figure 18D The product ion spectrum of peptide precursor m/z 576.66 from SGF90

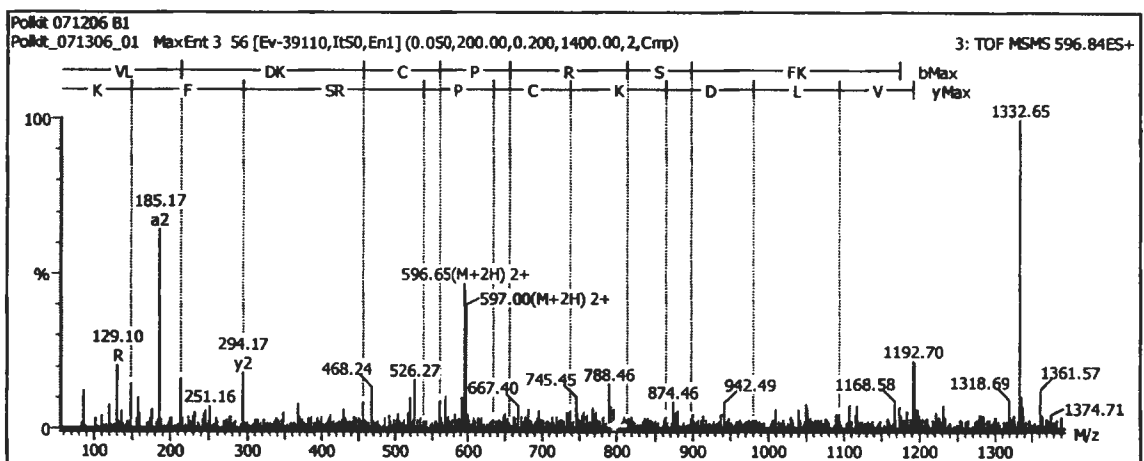


Figure 19D The product ion spectrum of peptide precursor m/z 596.84 from SGF90

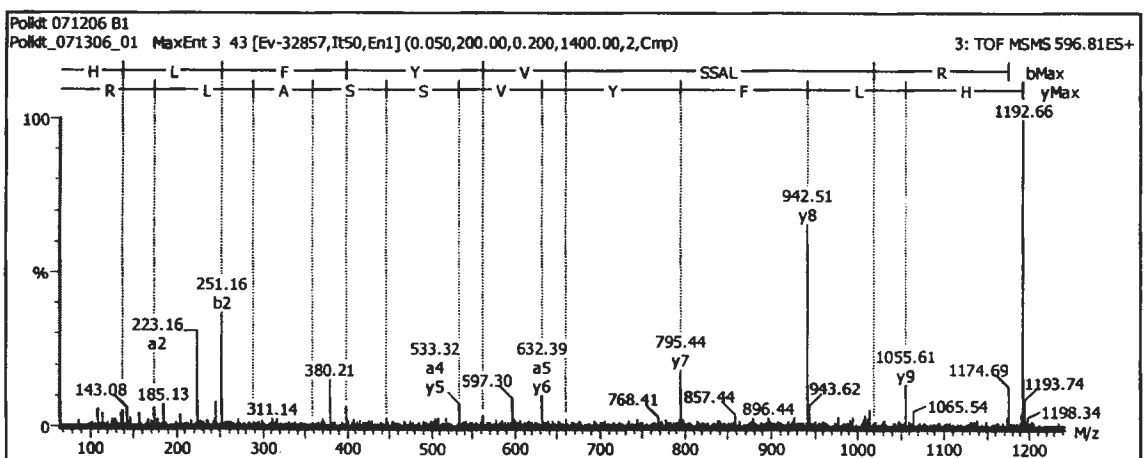


Figure 20D The product ion spectrum of peptide precursor m/z 596.81 from SGF90

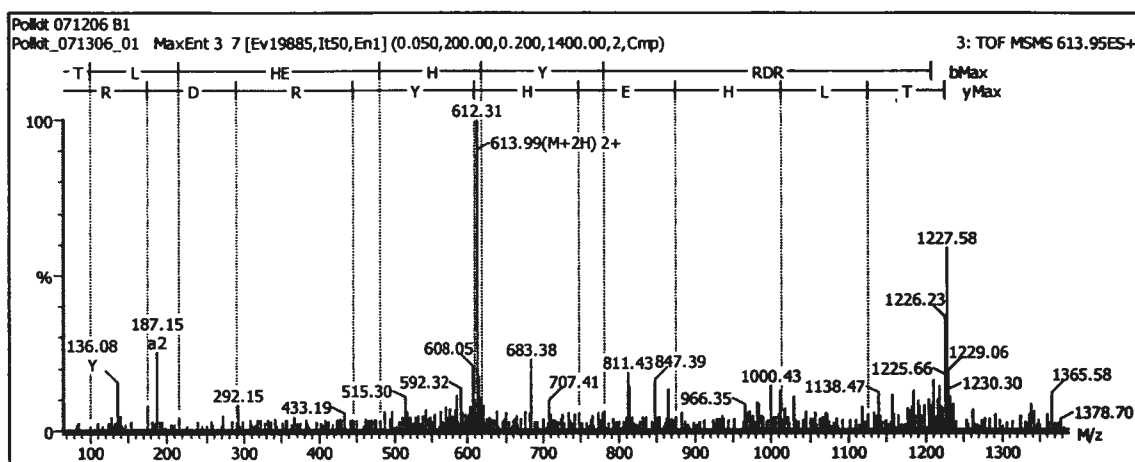


Figure 21D The product ion spectrum of peptide precursor m/z 613.95 from SGF90

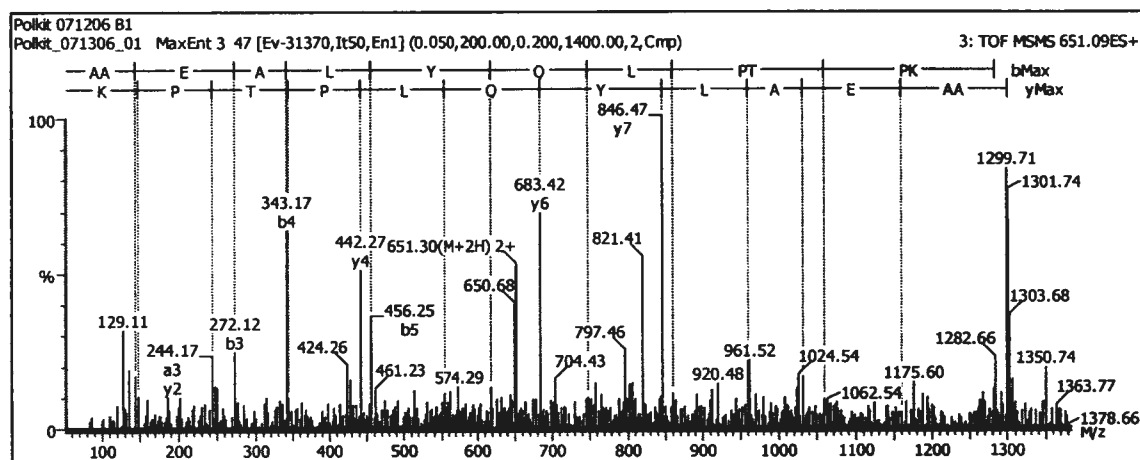


Figure 22D The product ion spectrum of peptide precursor m/z 651.09 from SGF90

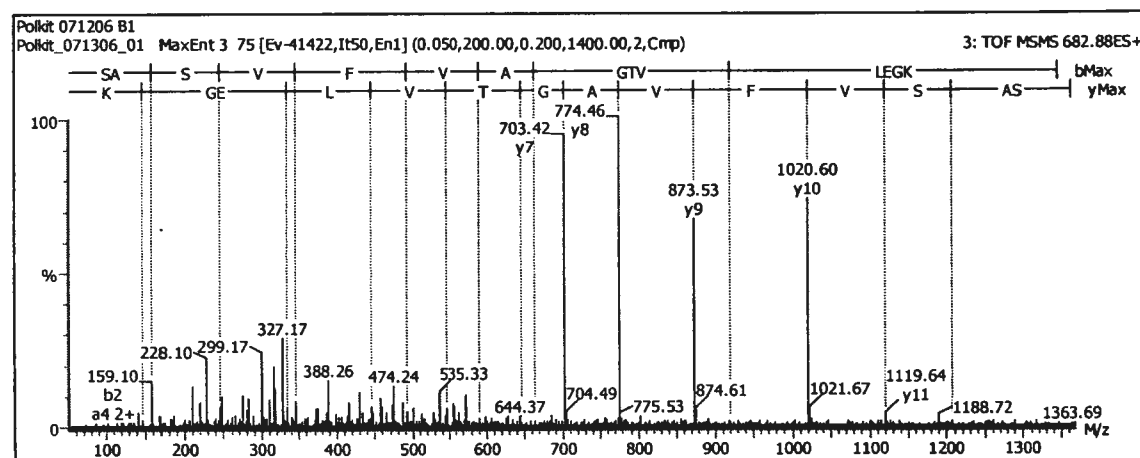


Figure 23D The product ion spectrum of peptide precursor m/z 682.88 from SGF90

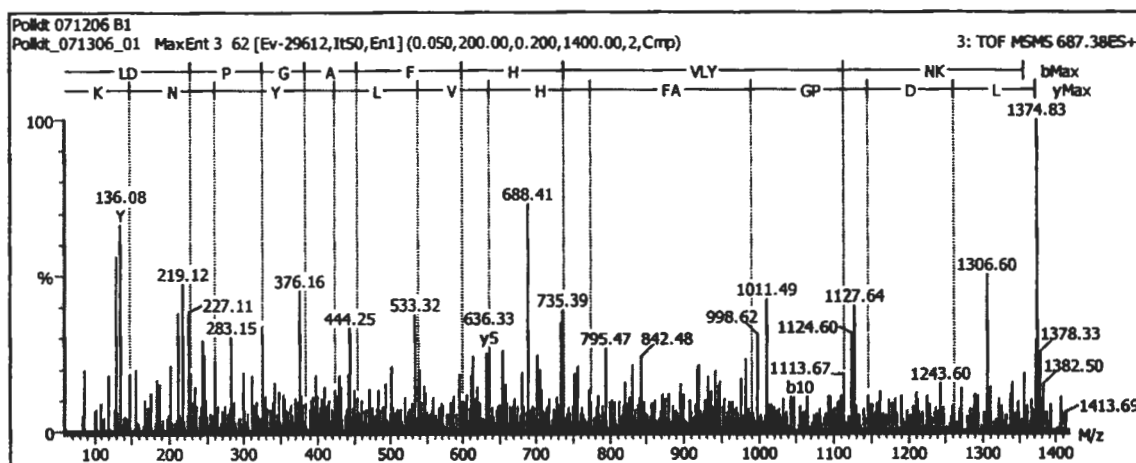


Figure 24D The product ion spectrum of peptide precursor m/z 687.38 from SGF90

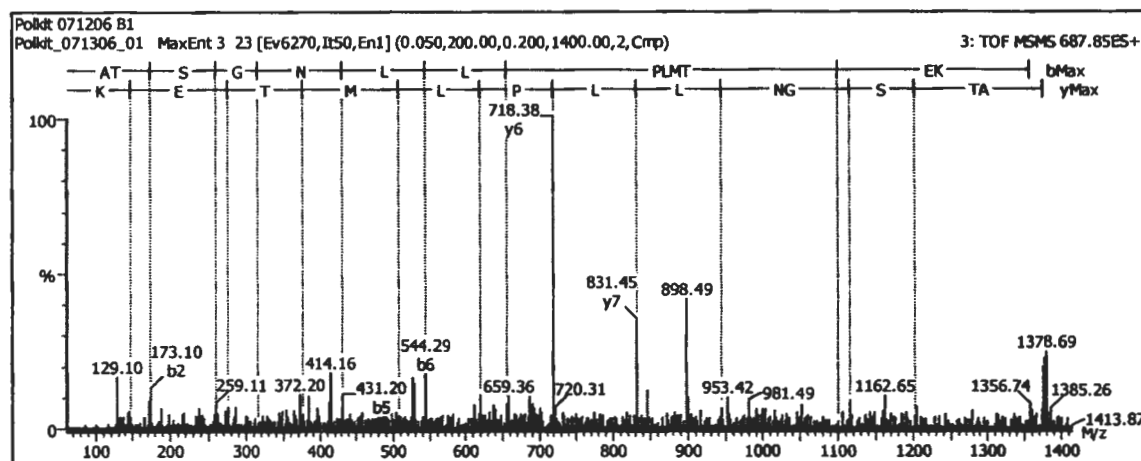


Figure 25D The product ion spectrum of peptide precursor m/z 687.85 from SGF90

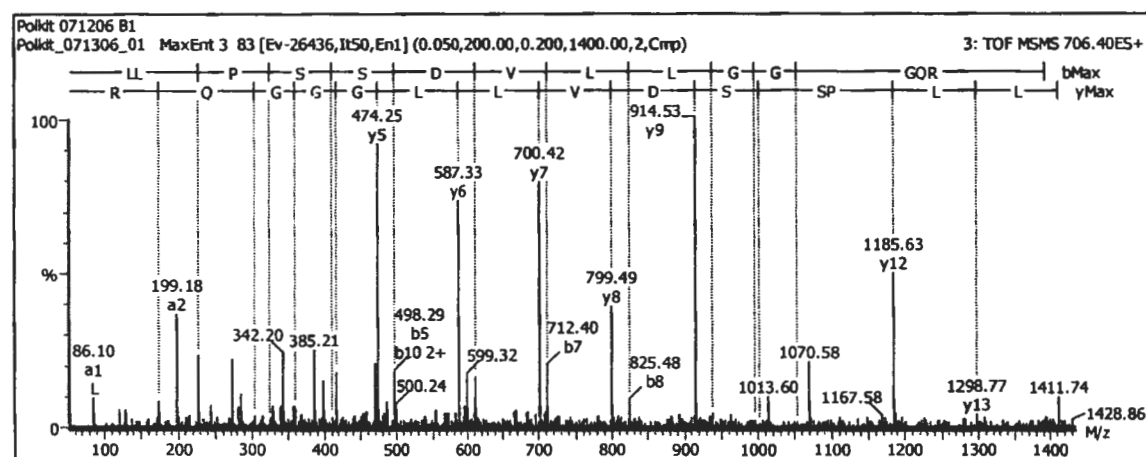


Figure 26D The product ion spectrum of peptide precursor m/z 706.40 from SGF90

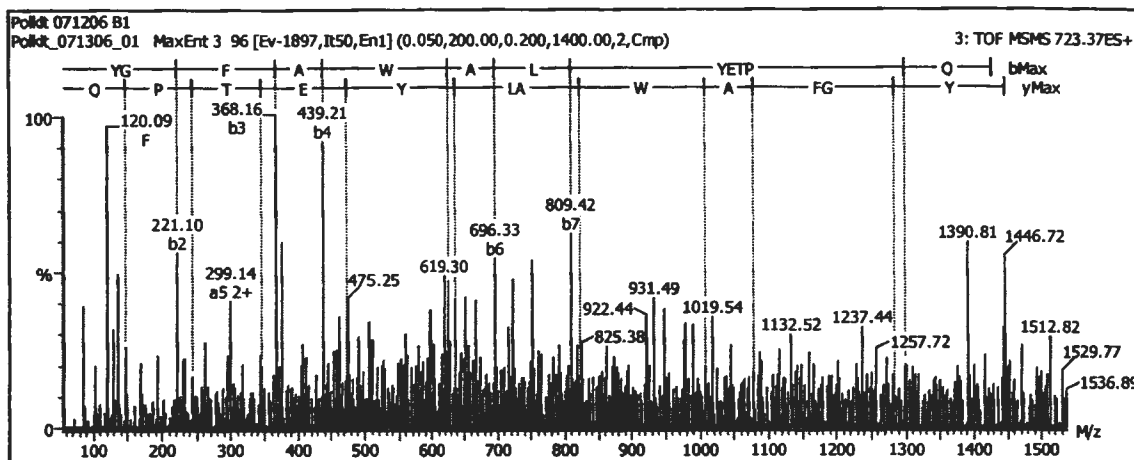


Figure 27D The product ion spectrum of peptide precursor m/z 723.37 from SGF90

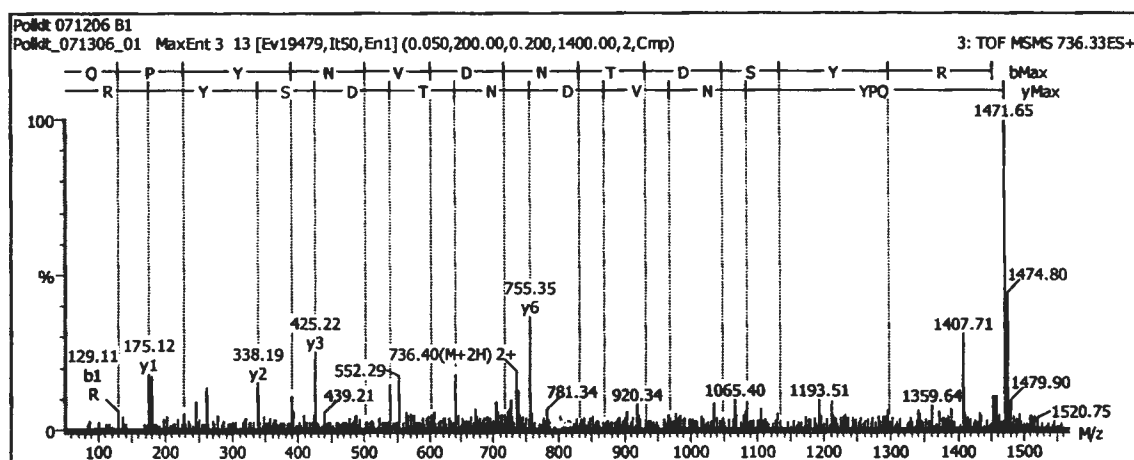


Figure 28D The product ion spectrum of peptide precursor m/z 736.33 from SGF90

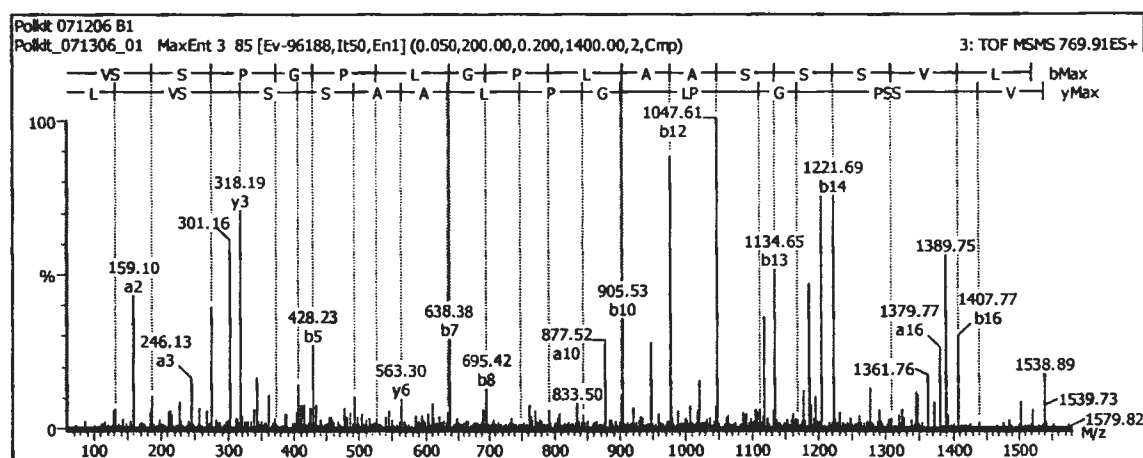


Figure 29D The product ion spectrum of peptide precursor m/z 769.91 from SGF90

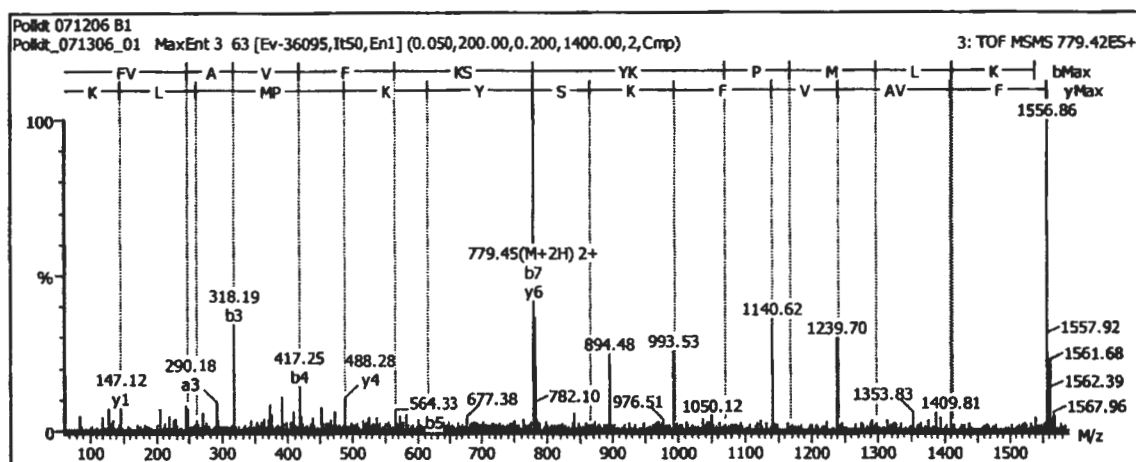


Figure 30D The product ion spectrum of peptide precursor m/z 779.42 from SGF90

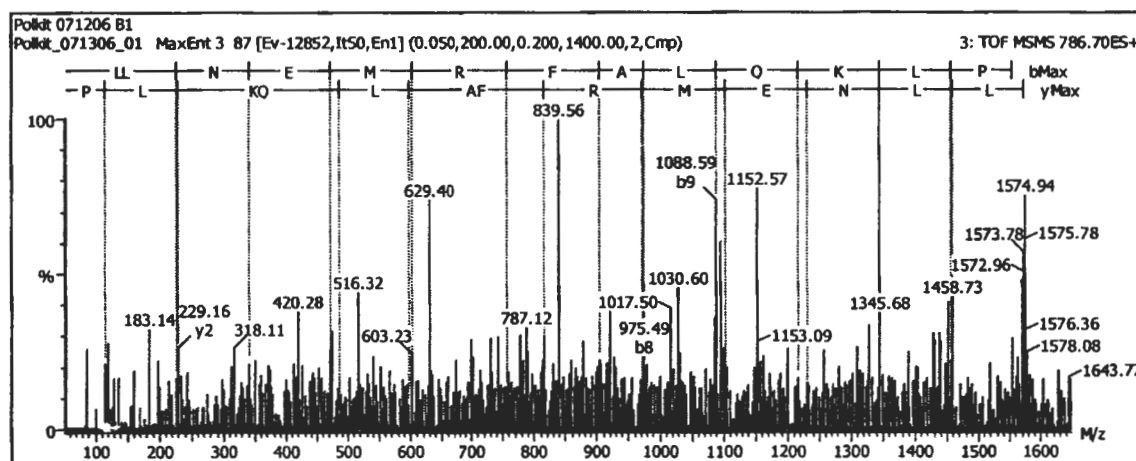


Figure 31D The product ion spectrum of peptide precursor m/z 786.70 from SGF90

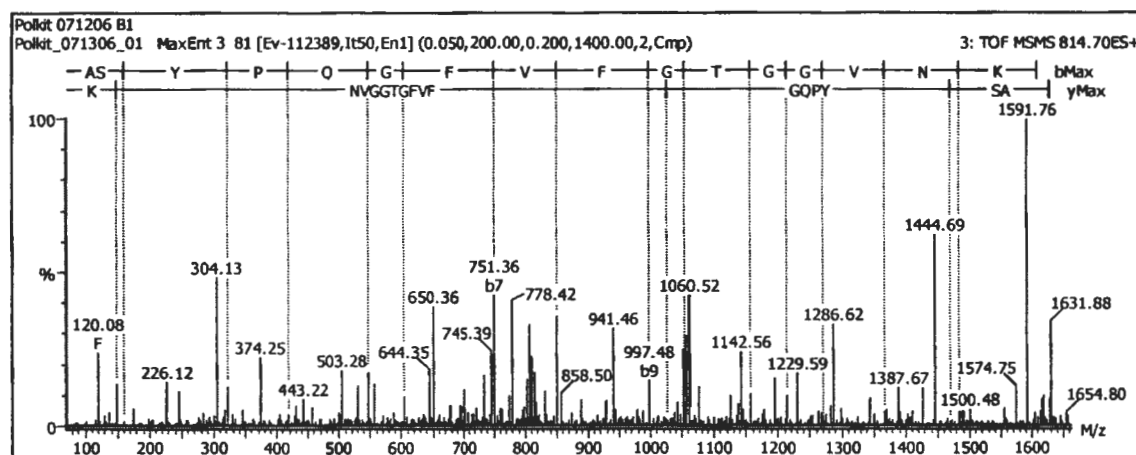


Figure 32D The product ion spectrum of peptide precursor m/z 814.73 from SGF90

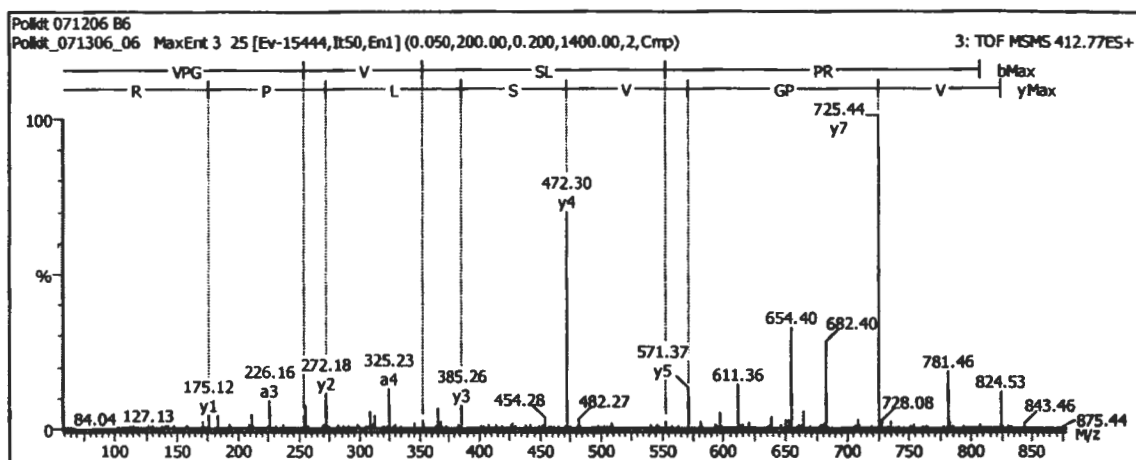


Figure 33D The product ion spectrum of peptide precursor m/z 412.77 from SGF60

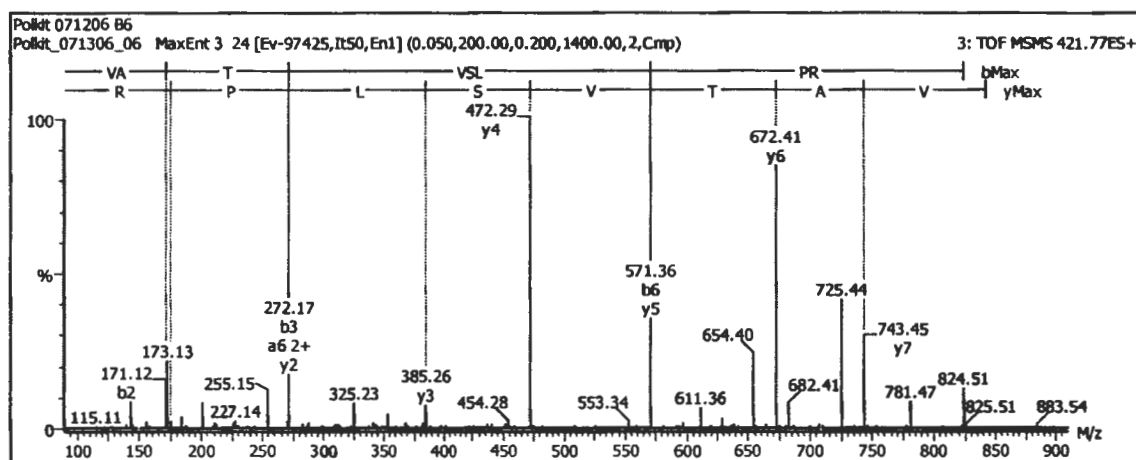


Figure 34D The product ion spectrum of peptide precursor m/z 421.77 from SGF60

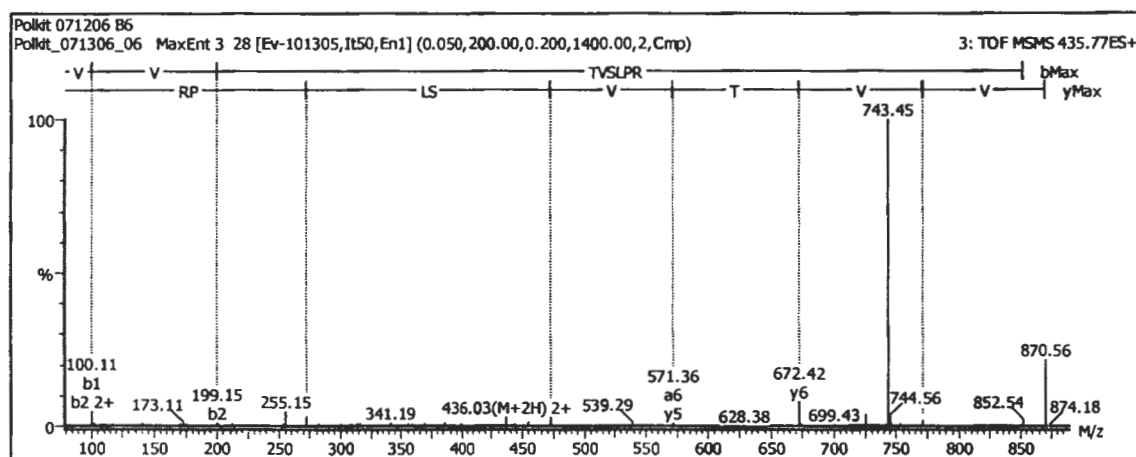


Figure 35D The product ion spectrum of peptide precursor m/z 435.77 from SGF60

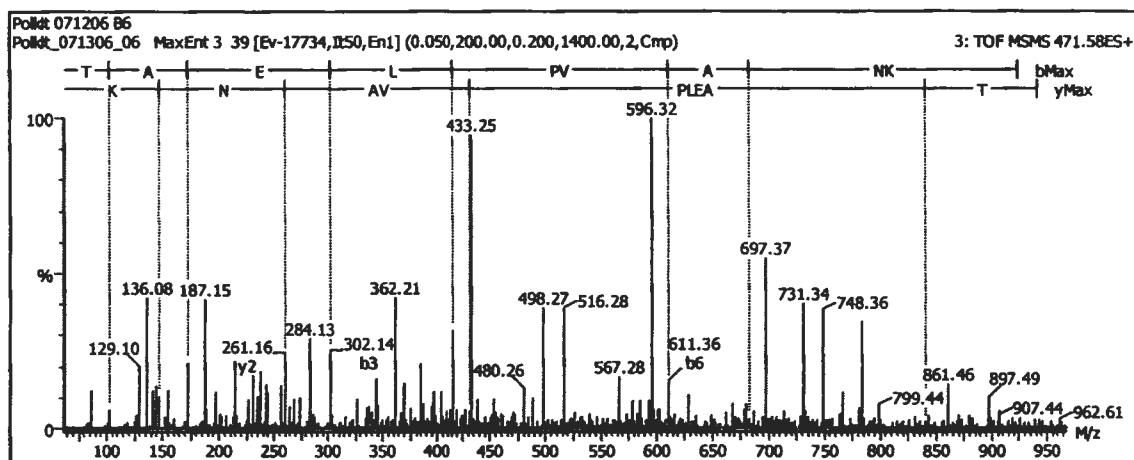


Figure 36D The product ion spectrum of peptide precursor m/z 471.58 from SGF60

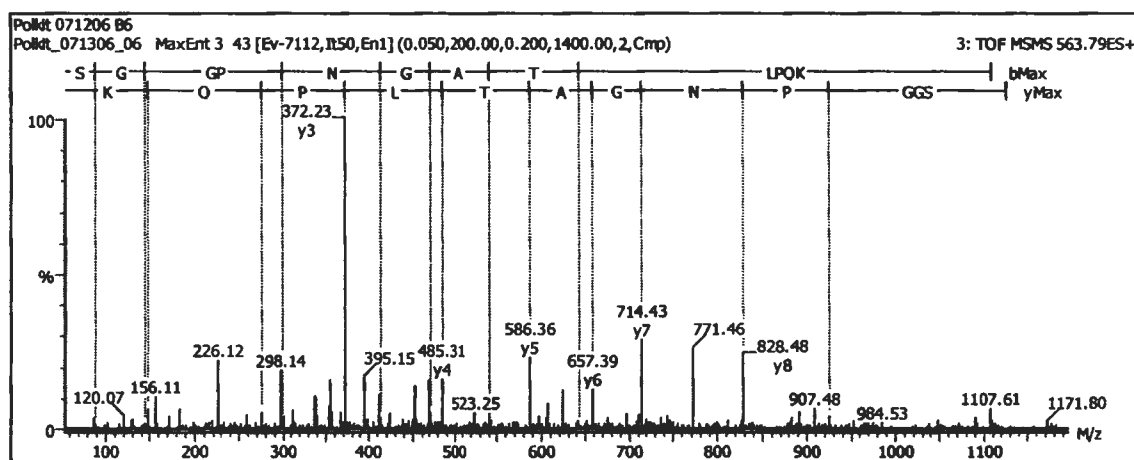


Figure 37D The product ion spectrum of peptide precursor m/z 563.79 from SGF60

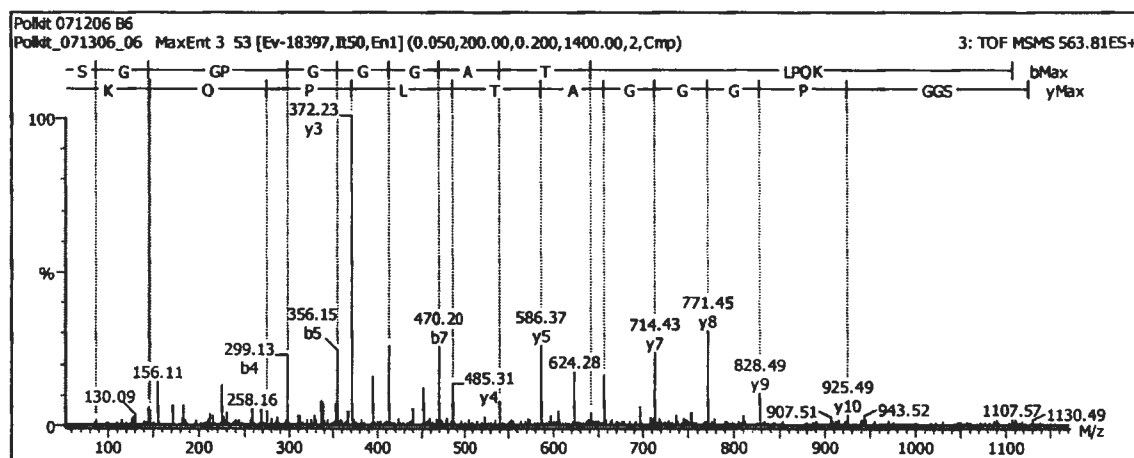


Figure 38D The product ion spectrum of peptide precursor m/z 563.81 from SGF60

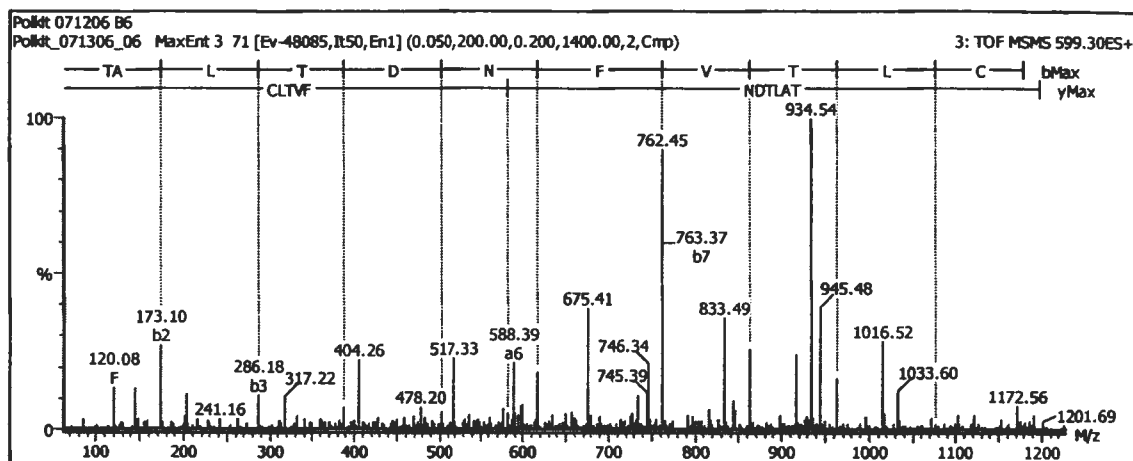


Figure 39D The product ion spectrum of peptide precursor m/z 599.30 from SGF60

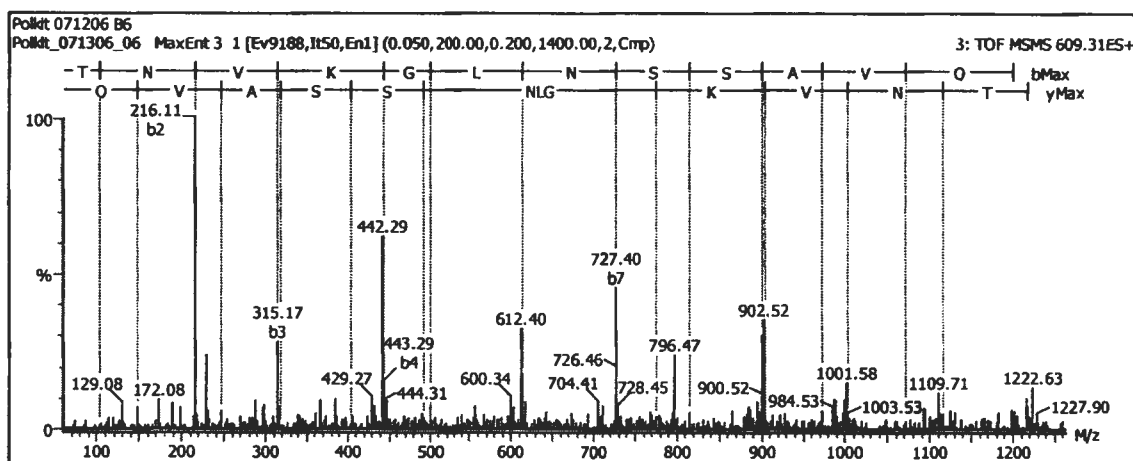


Figure 40D The product ion spectrum of peptide precursor m/z 609.31 from SGF60

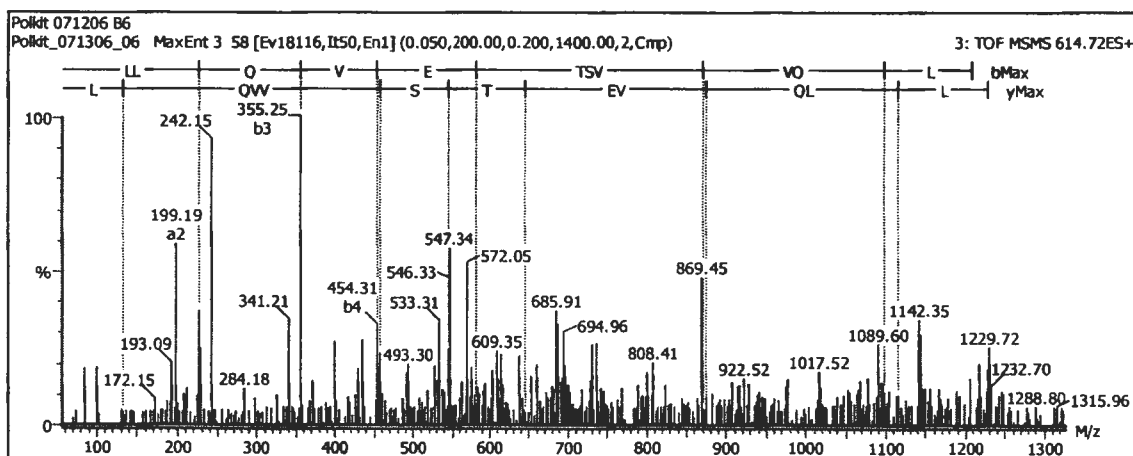


Figure 41D The product ion spectrum of peptide precursor m/z 614.72 from SGF60

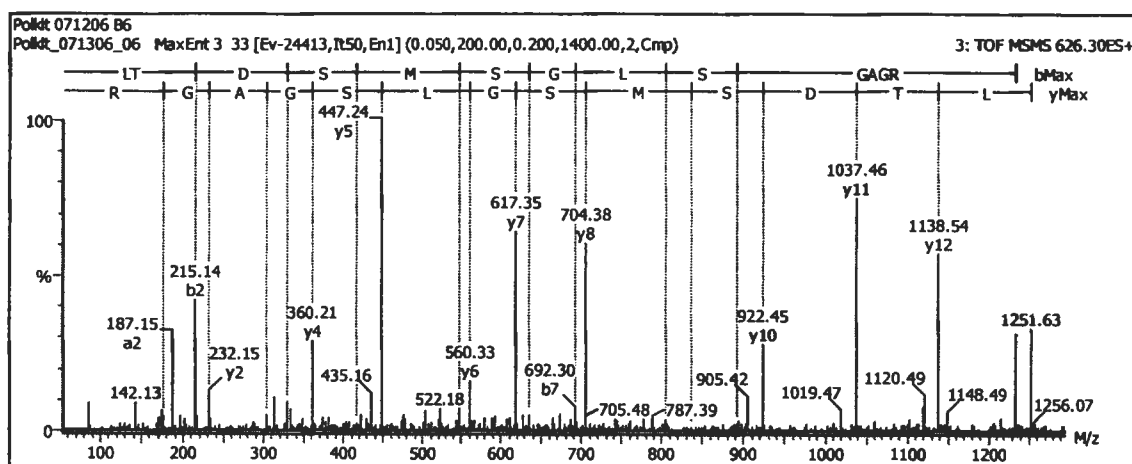


Figure 42D The product ion spectrum of peptide precursor m/z 626.30 from SGF60

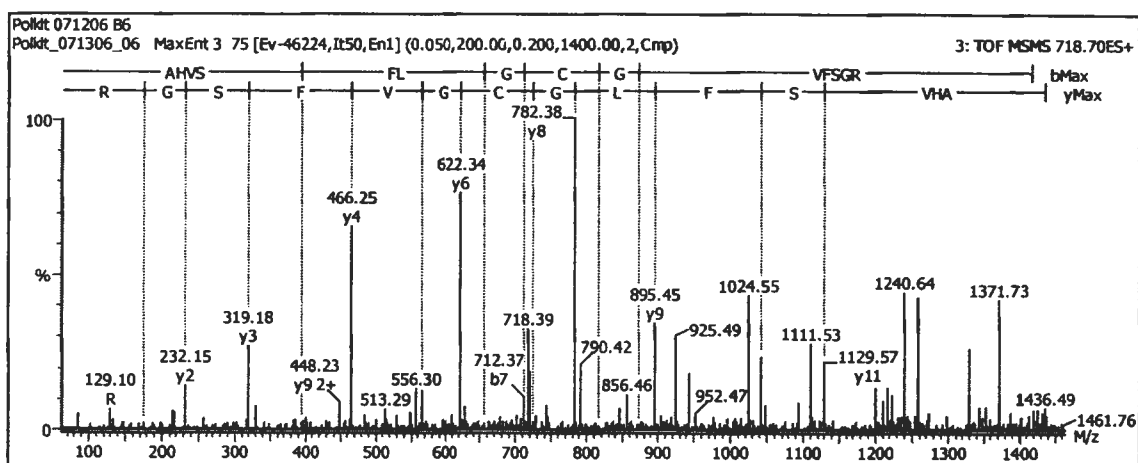


Figure 43D The product ion spectrum of peptide precursor m/z 718.70 from SGF60

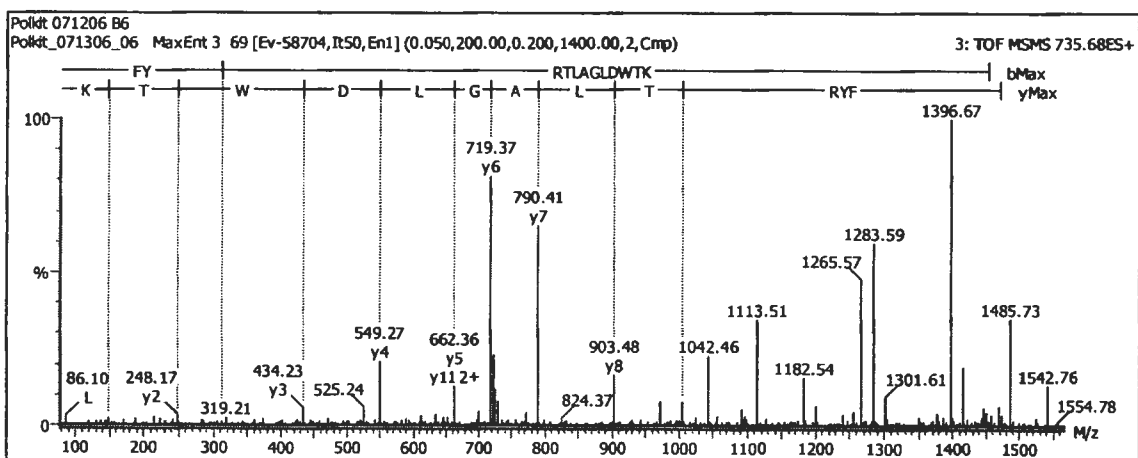


Figure 44D The product ion spectrum of peptide precursor m/z 735.68 from SGF60

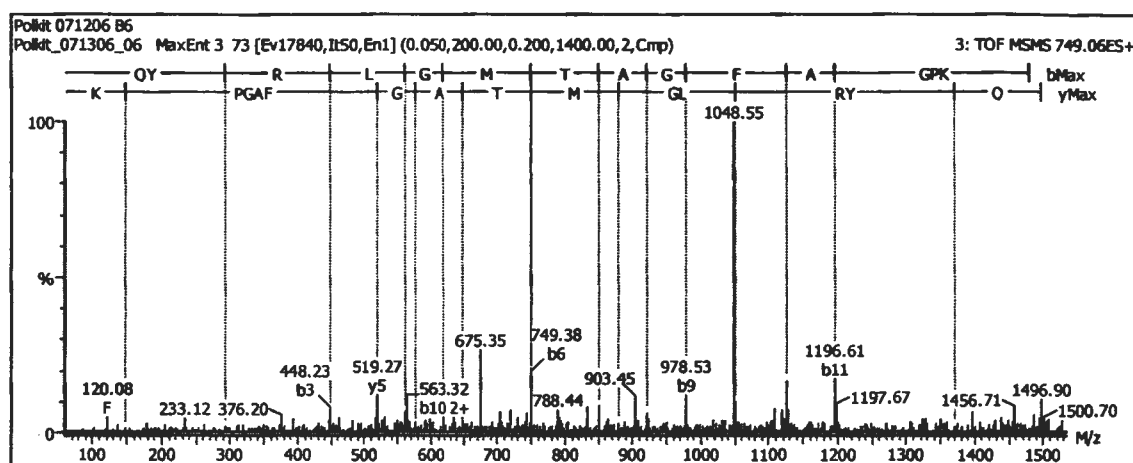


Figure 45D The product ion spectrum of peptide precursor m/z 749.06 from SGF60

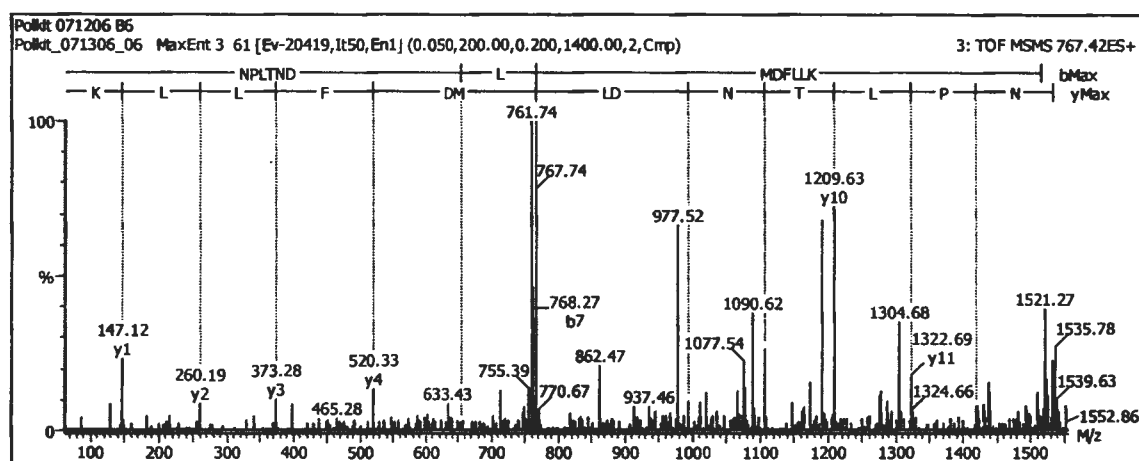


Figure 46D The product ion spectrum of peptide precursor m/z 767.42 from SGF60

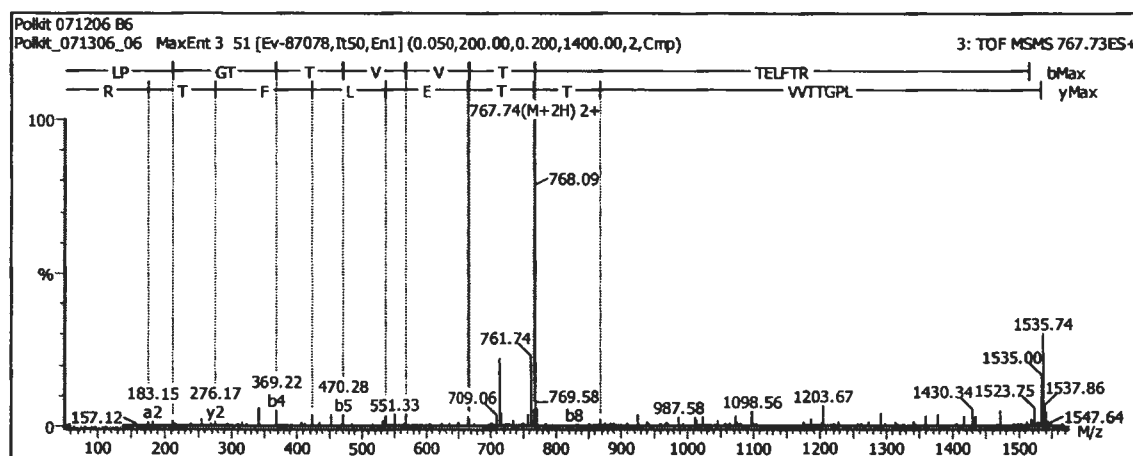


Figure 47D The product ion spectrum of peptide precursor m/z 767.73 from SGF60

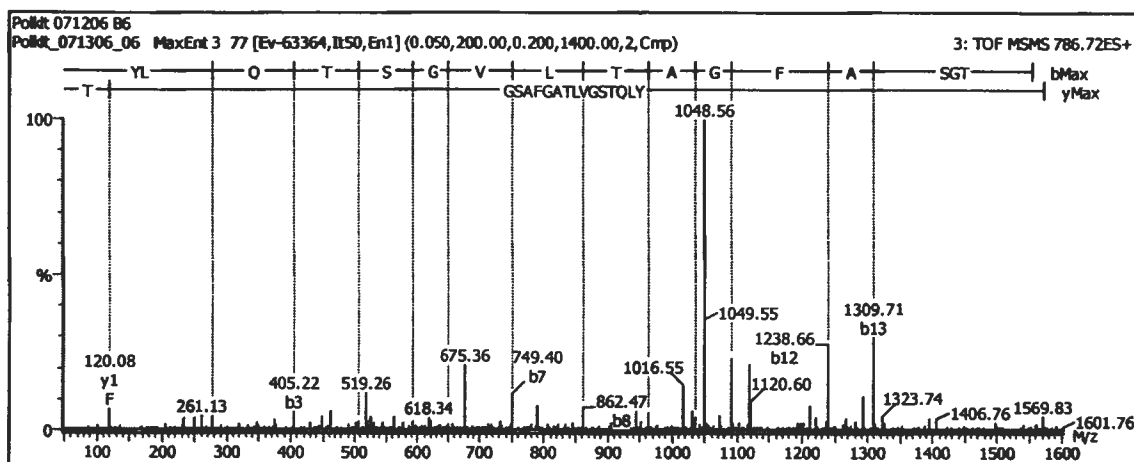


Figure 48D The product ion spectrum of peptide precursor m/z 786.72 from SGF60

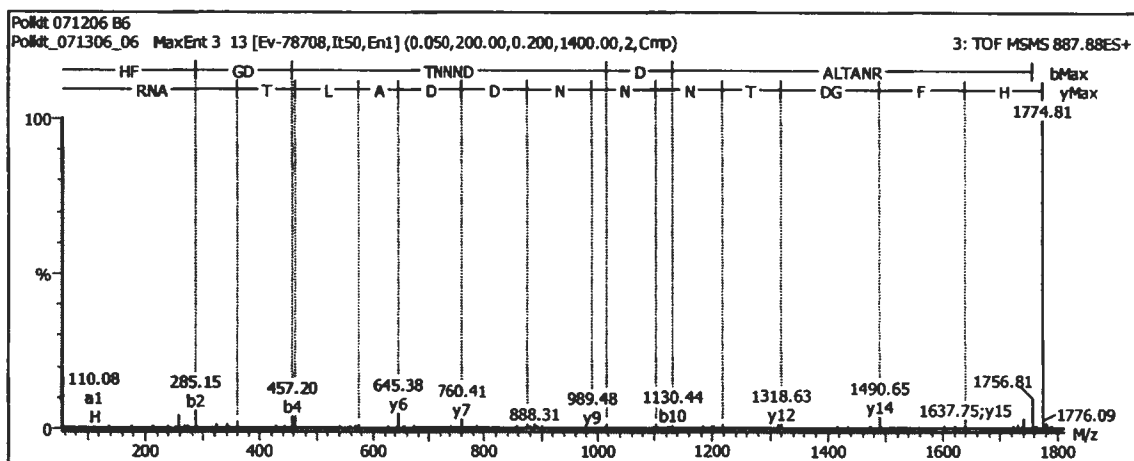


Figure 49D The product ion spectrum of peptide precursor m/z 887.88 from SGF60

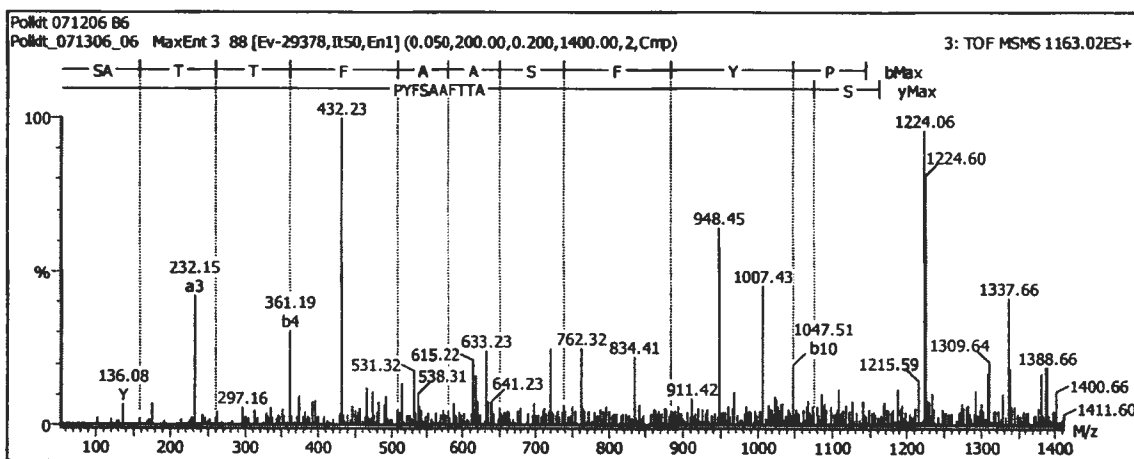


Figure 50D The product ion spectrum of peptide precursor m/z 1163.02 from SGF60

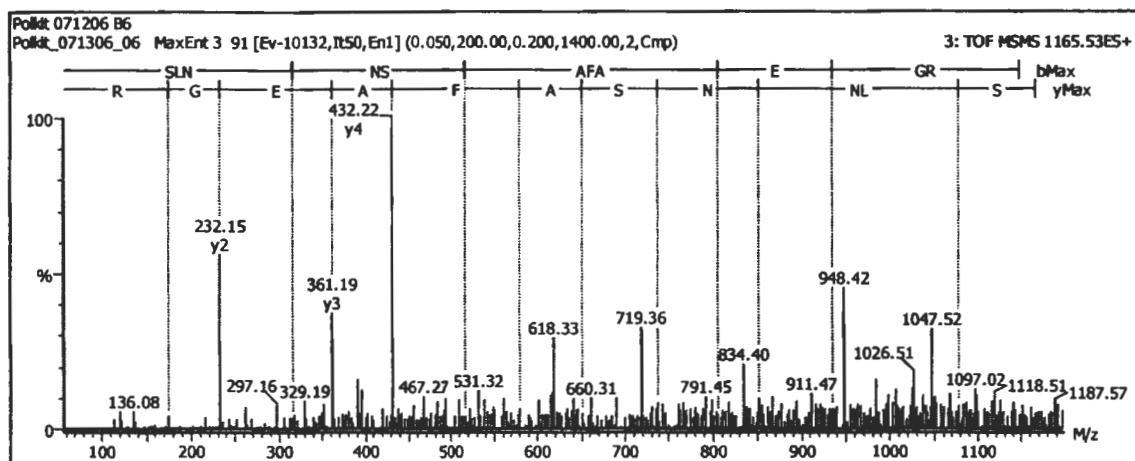


Figure 51D The product ion spectrum of peptide precursor m/z 1165.53a from SGF60

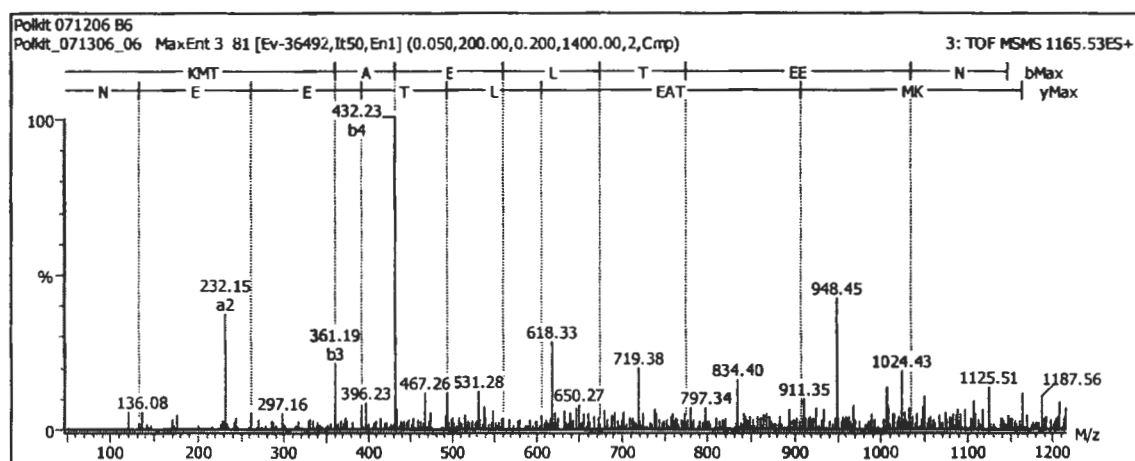


Figure 52D The product ion spectrum of peptide precursor m/z 1165.53b from SGF60

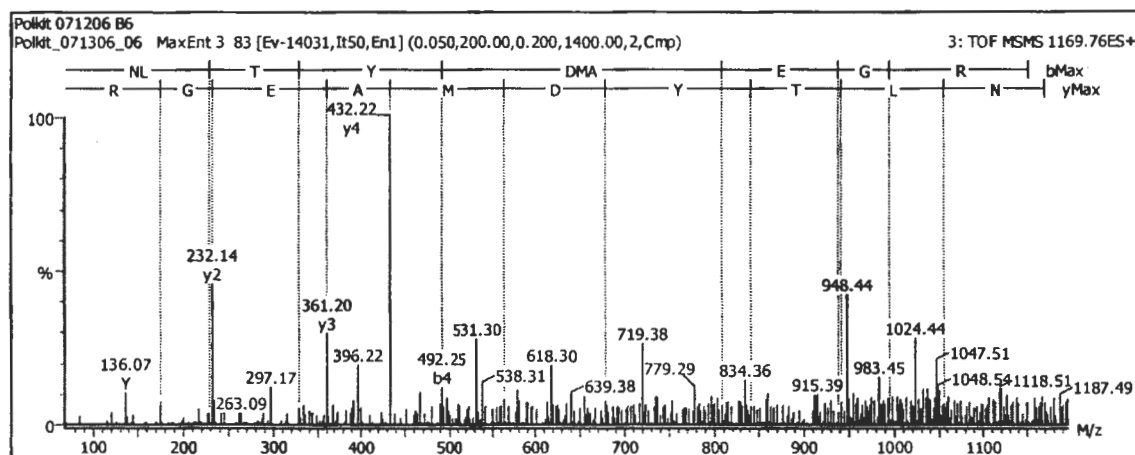


Figure 53D The product ion spectrum of peptide precursor m/z 1169.76 from SGF60

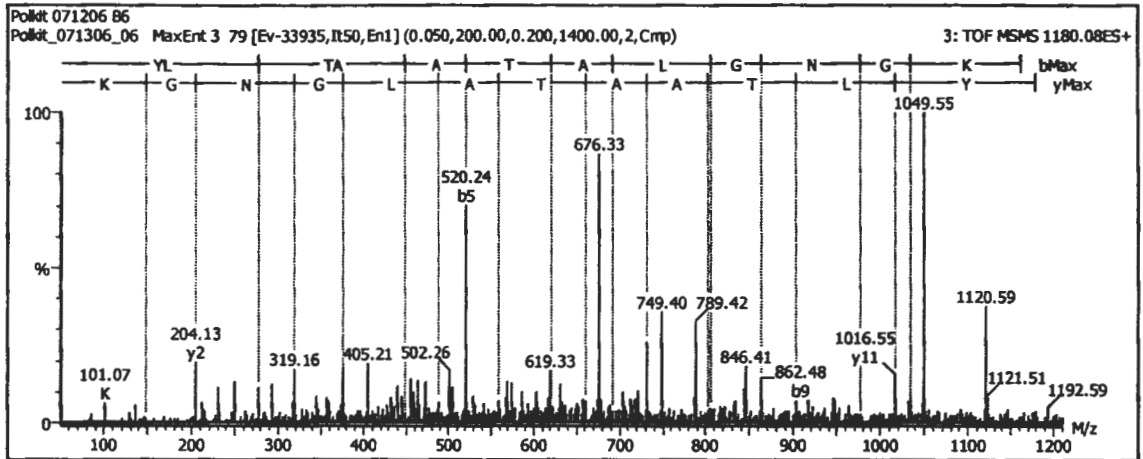


Figure 54D The product ion spectrum of peptide precursor m/z 1180.08 from SGF60

Appendix E**Table 1E** The letter code of twenty amino acids

amino acid	three letter code	single letter code
glycine	Gly	G
alanine	Ala	A
valine	Val	V
leucine	Leu	L
isoleucine	Ile	I
methionine	Met	M
phenylalanine	Phe	F
tryptophan	Trp	W
proline	Pro	P
serine	Ser	S
threonine	Thr	T
cysteine	Cys	C
tyrosine	Tyr	Y
asparagine	Asn	N
glutamine	Gln	Q
aspartic acid	Asp	D
glutamic acid	Glu	E
lysine	Lys	K
arginine	Arg	R
histidine	His	H

VITA

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