

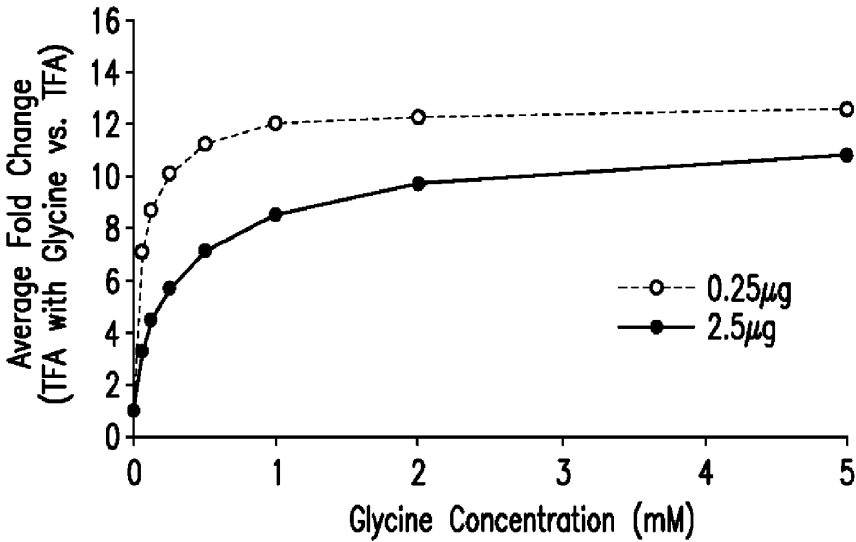


FIG.1A

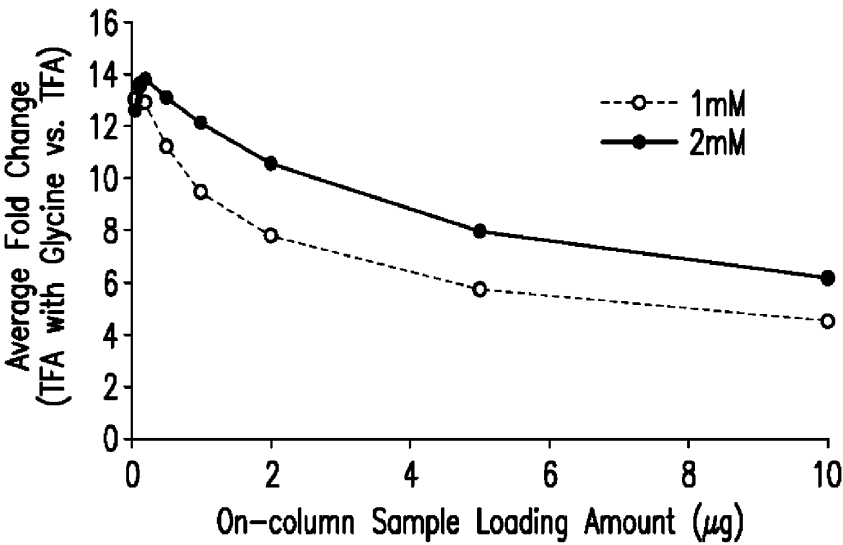


FIG.1B

SEQ ID NO: 1

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWDDKKHYNP SLKDRLTISKDT SKNQVVLKVTNMDP
ADTATYYCARDMIENFYFDWGGGTTTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVMNSGALTSGVHTFPAVLQSS
GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKOTLMISRTPEVTCVVDVDSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVWSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNIHYTEQKSLSLSPGK

SEQ ID NO: 2

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPKGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTITSSLPDDFATYYCFQSG
YPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVWCLLNNFYPREAKVQWIKVDNALQSGNSQESVTEQDSKDSITYSLSSITLTKAD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC

FIG.1C

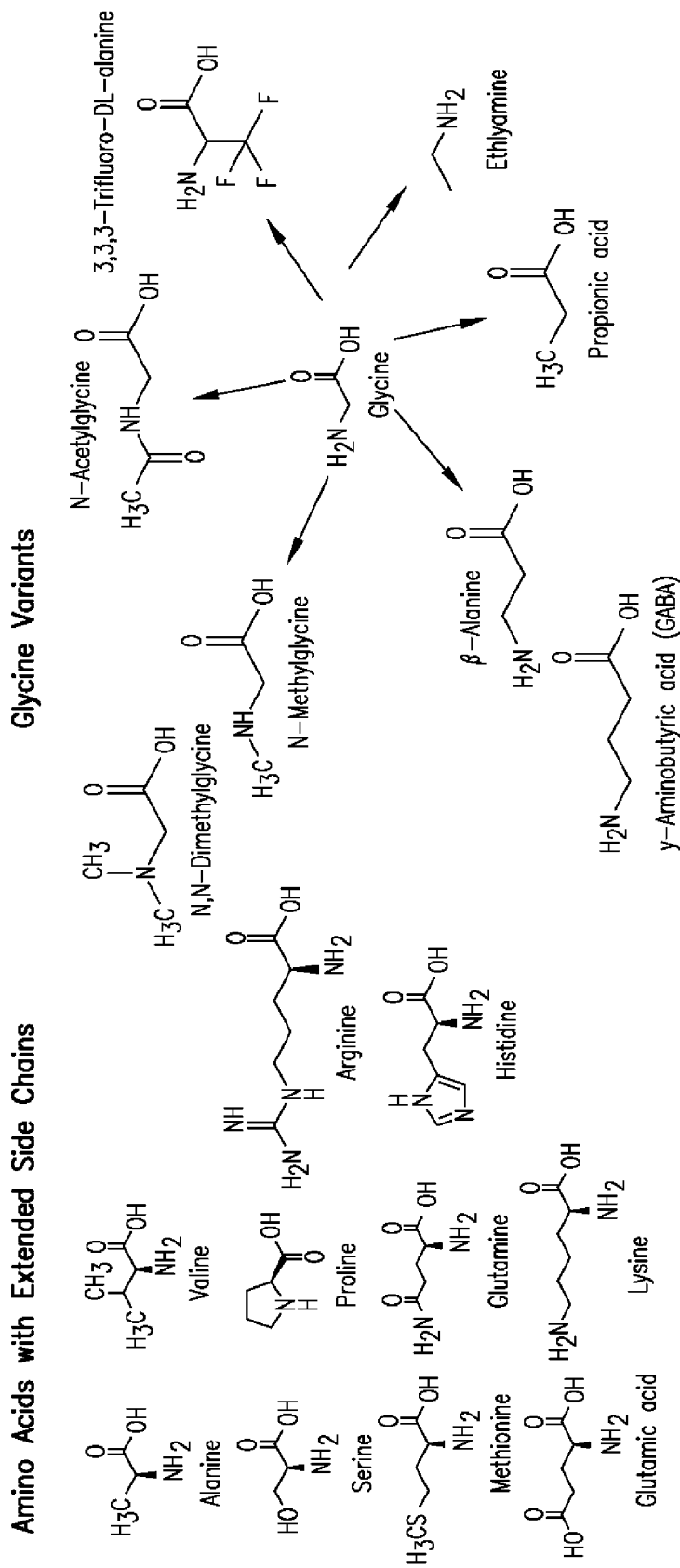


FIG.2

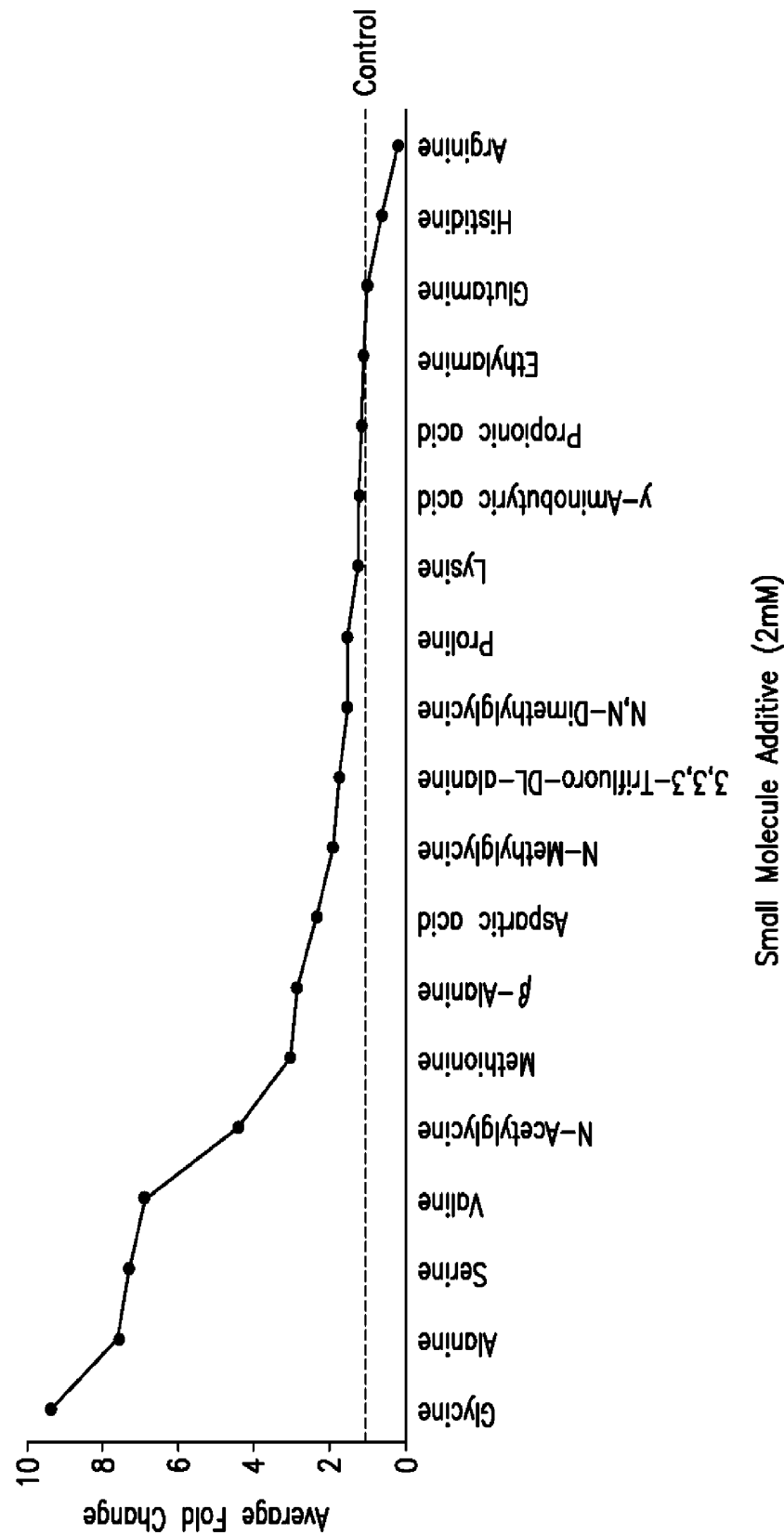


FIG.3

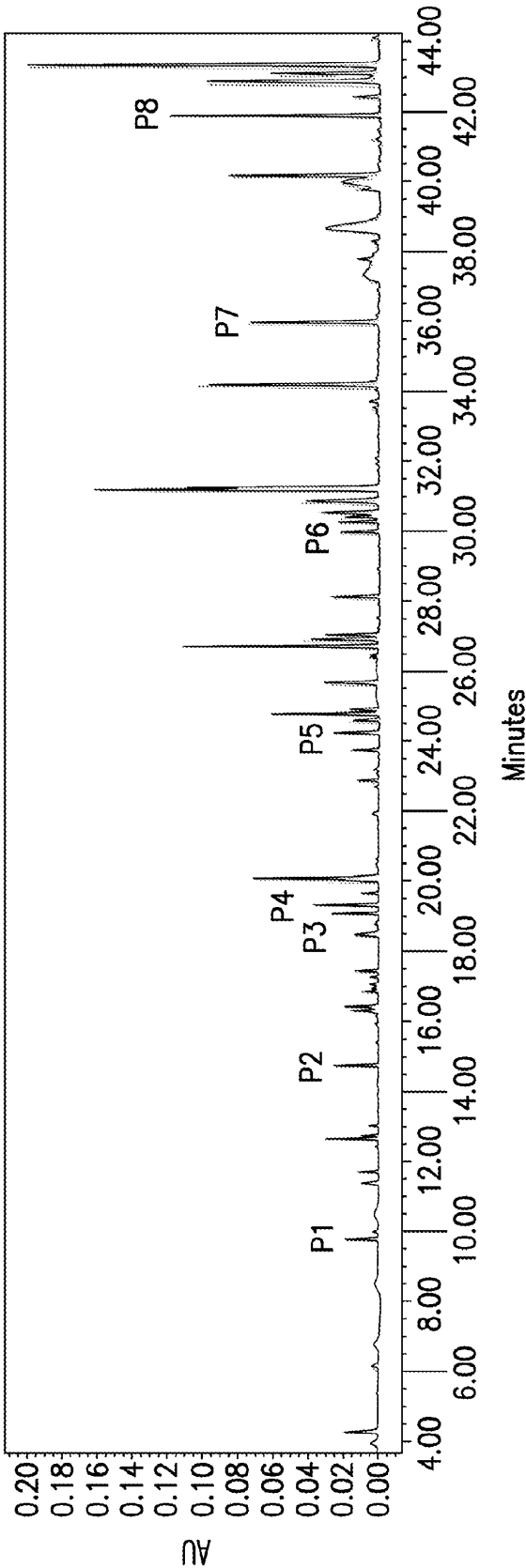


FIG.4

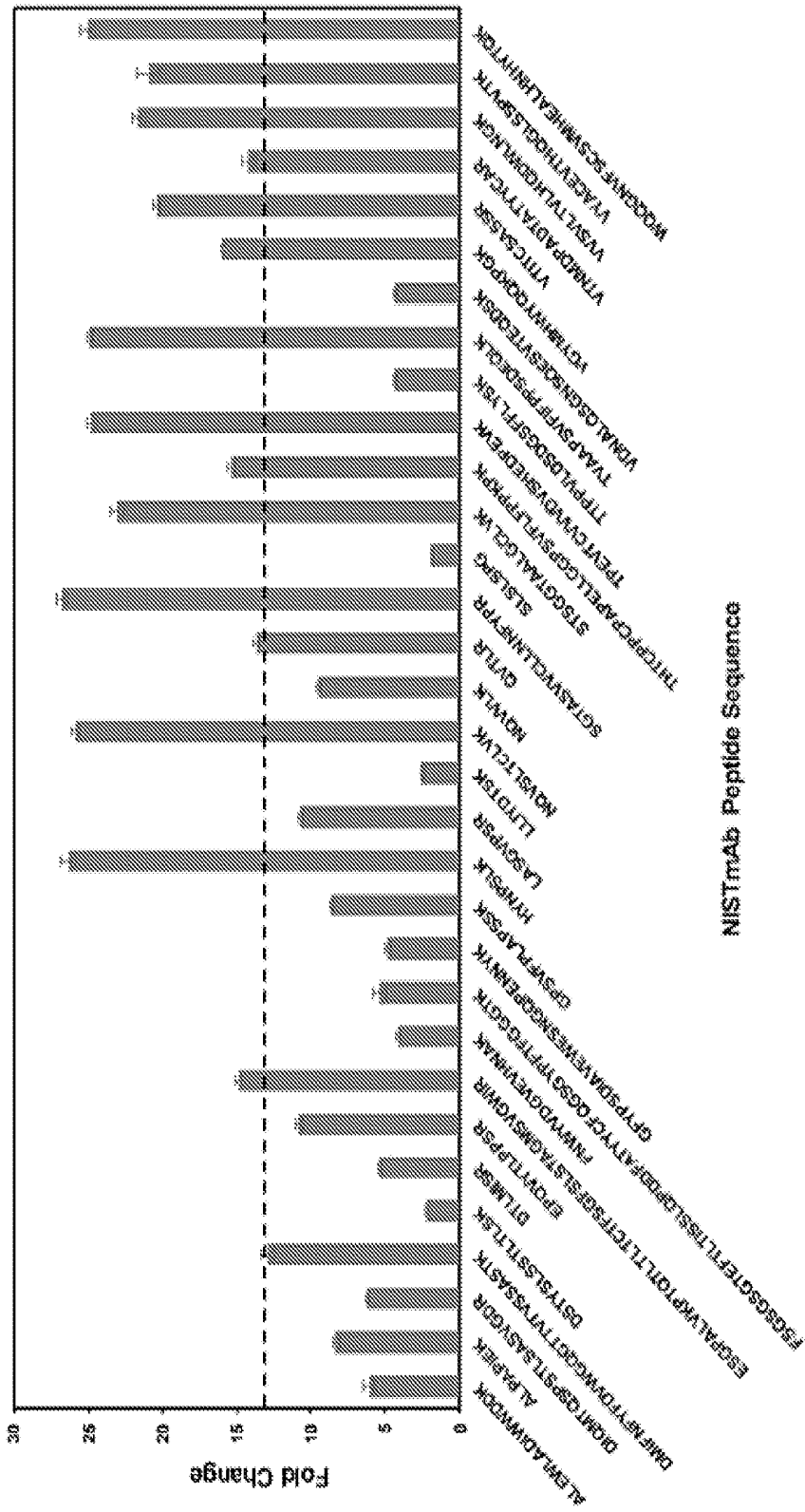


FIG. 5

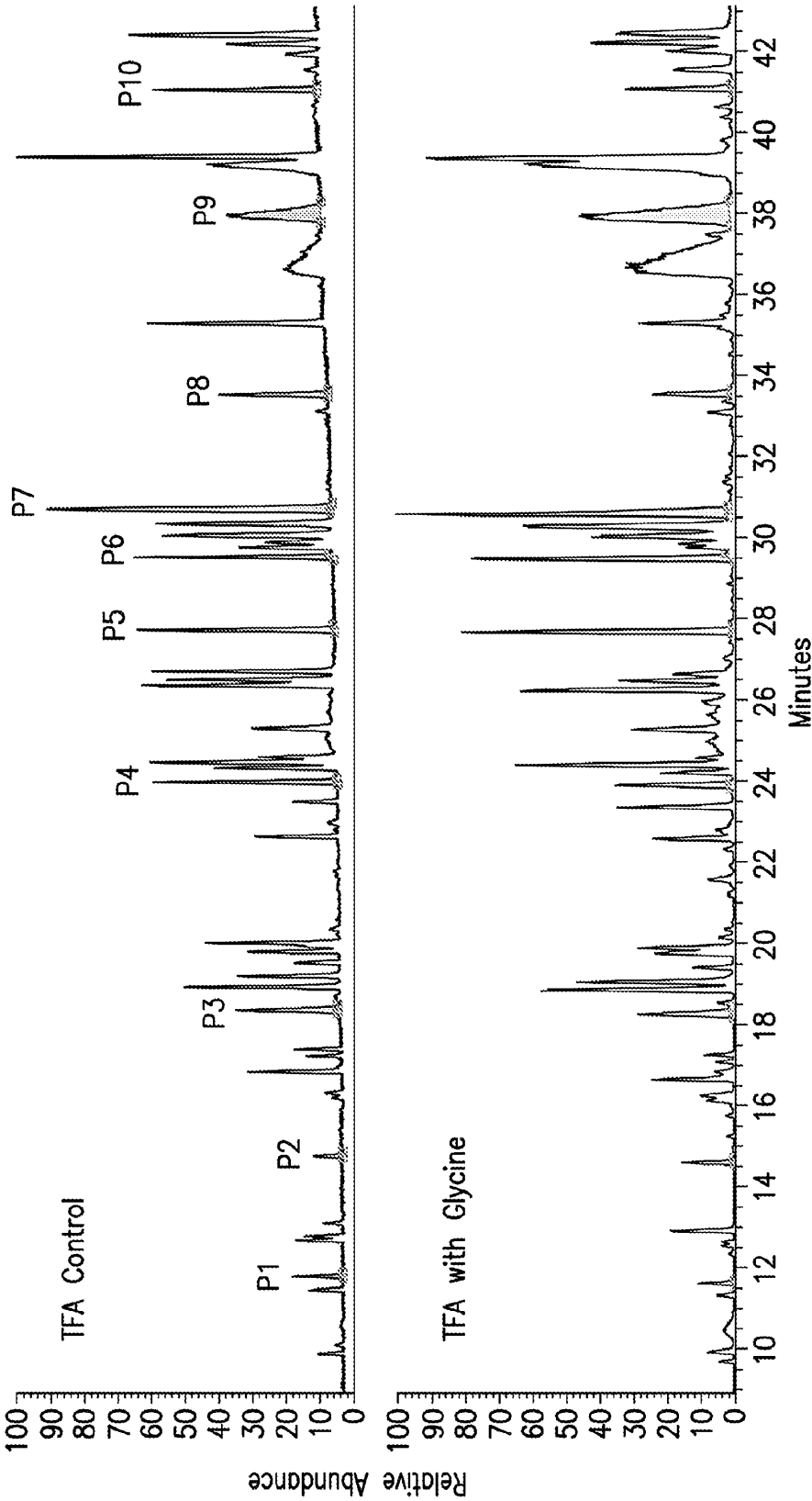
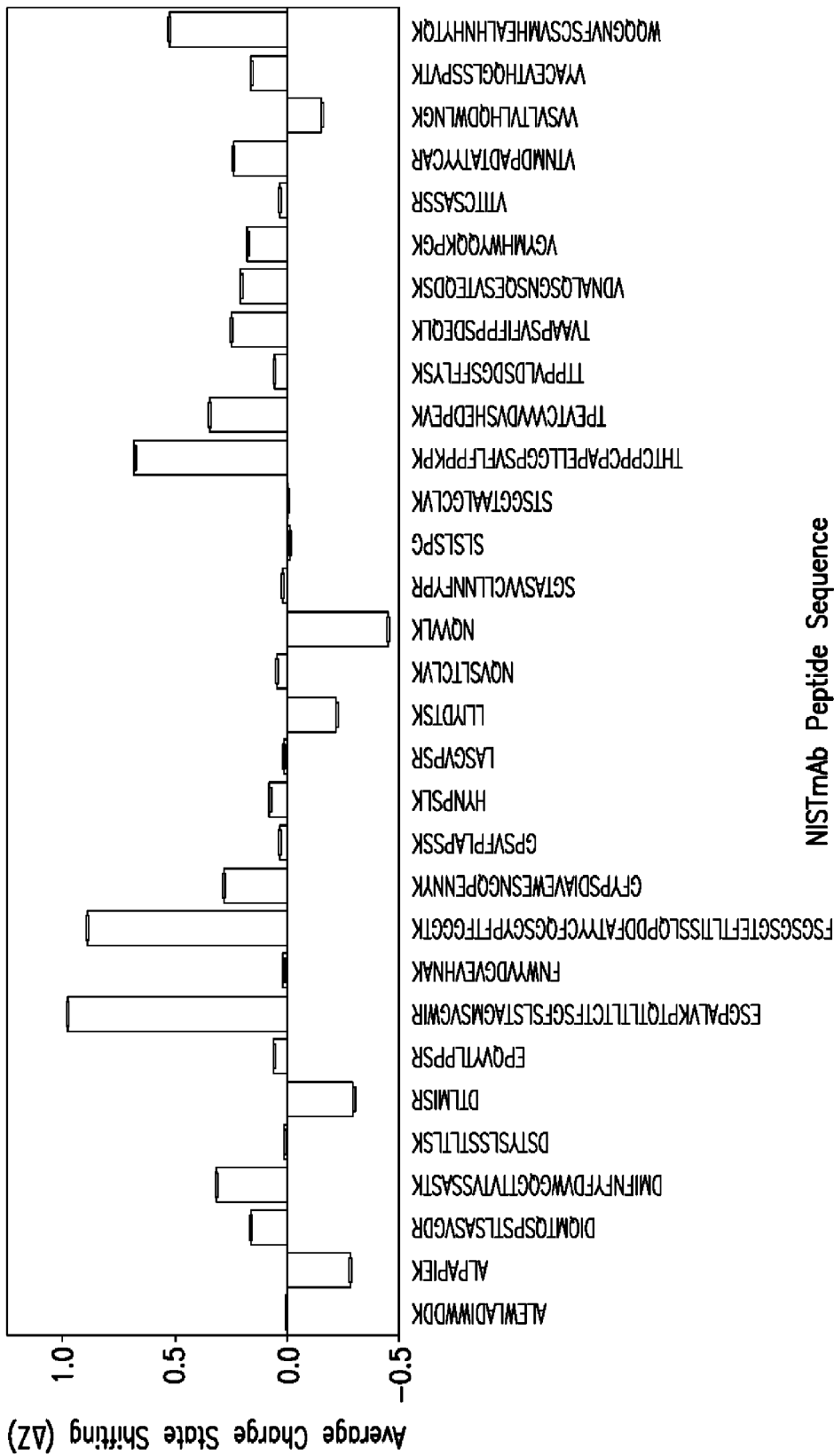


FIG.6



NISTmAb Peptide Sequence

FIG. 7

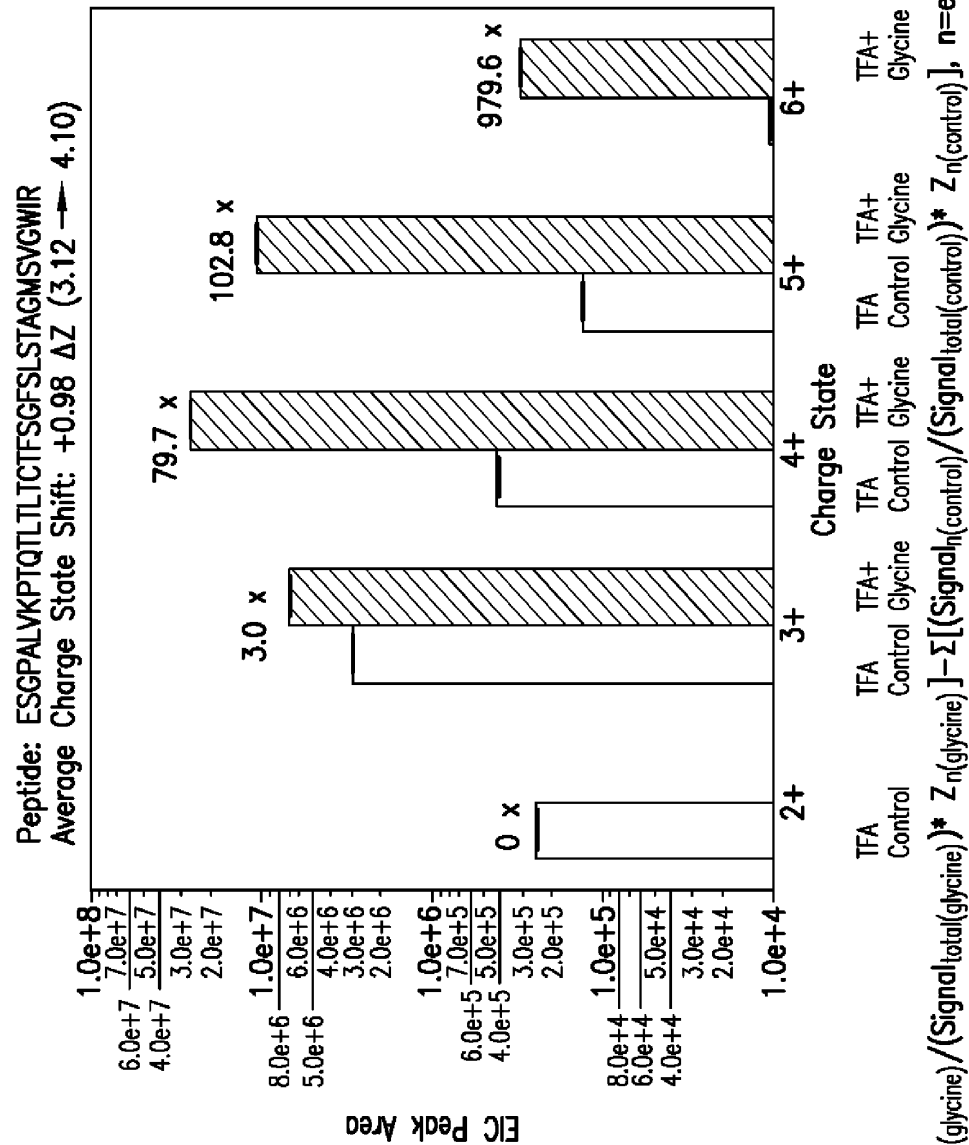


FIG.8A

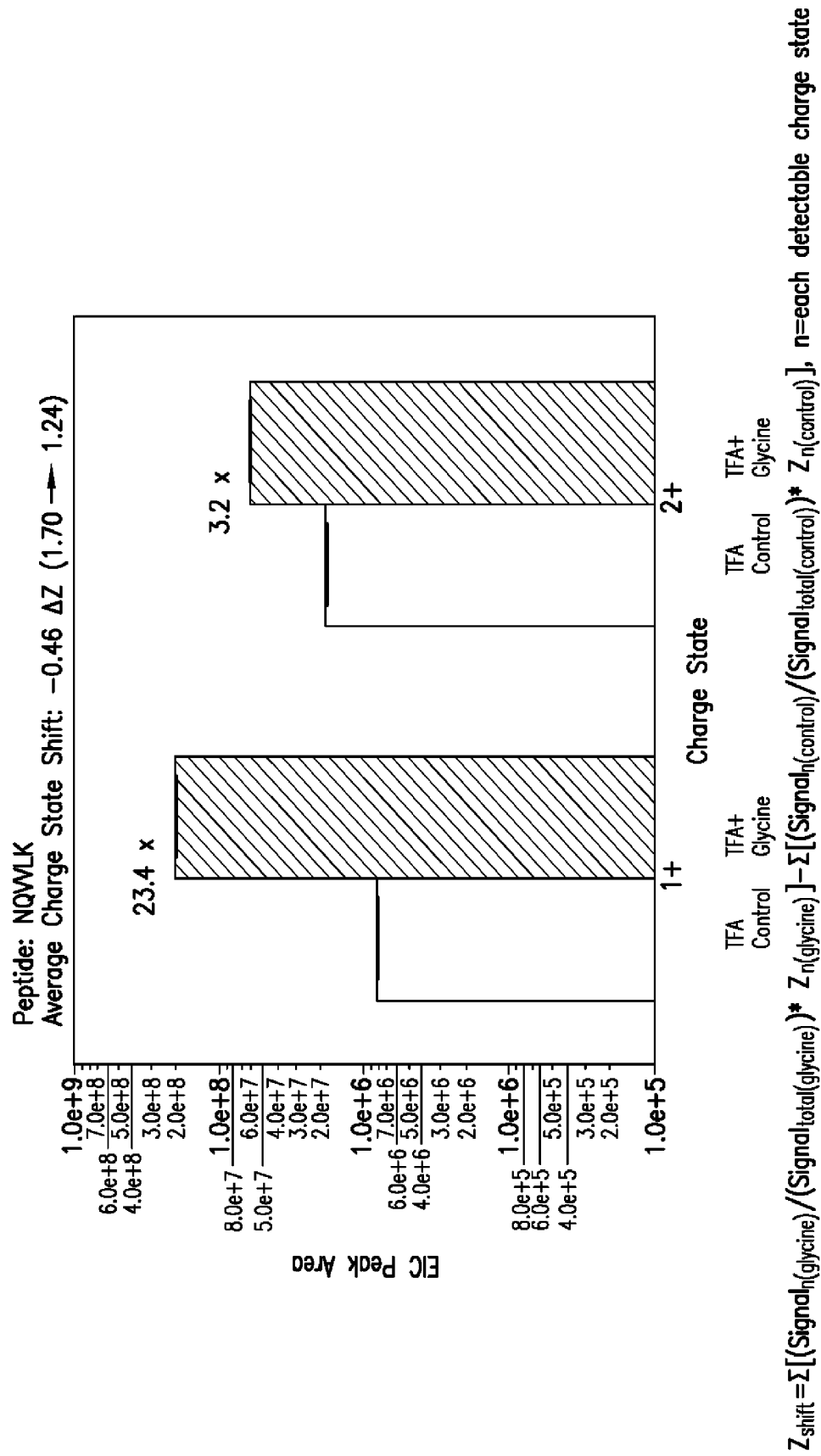


FIG.8B

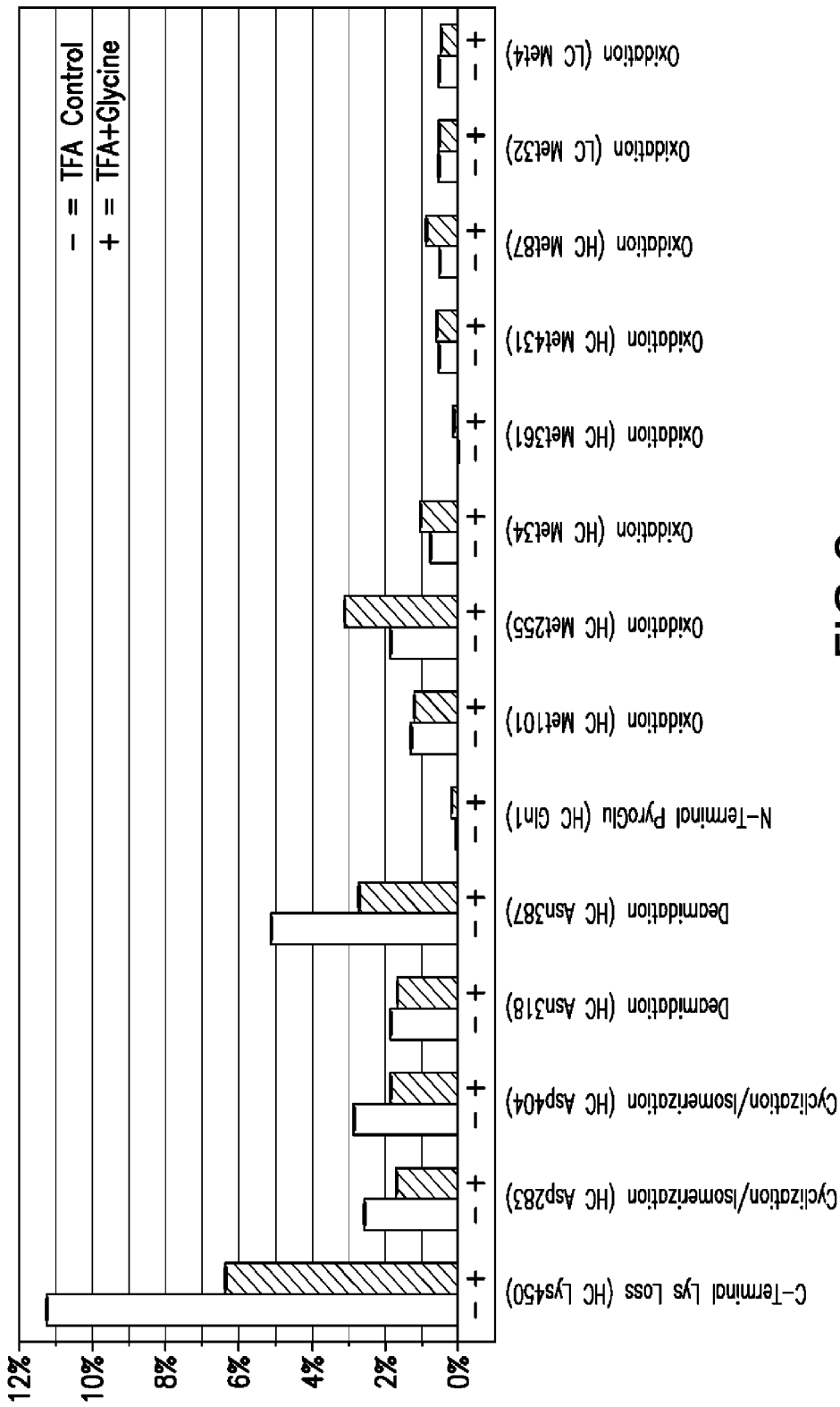
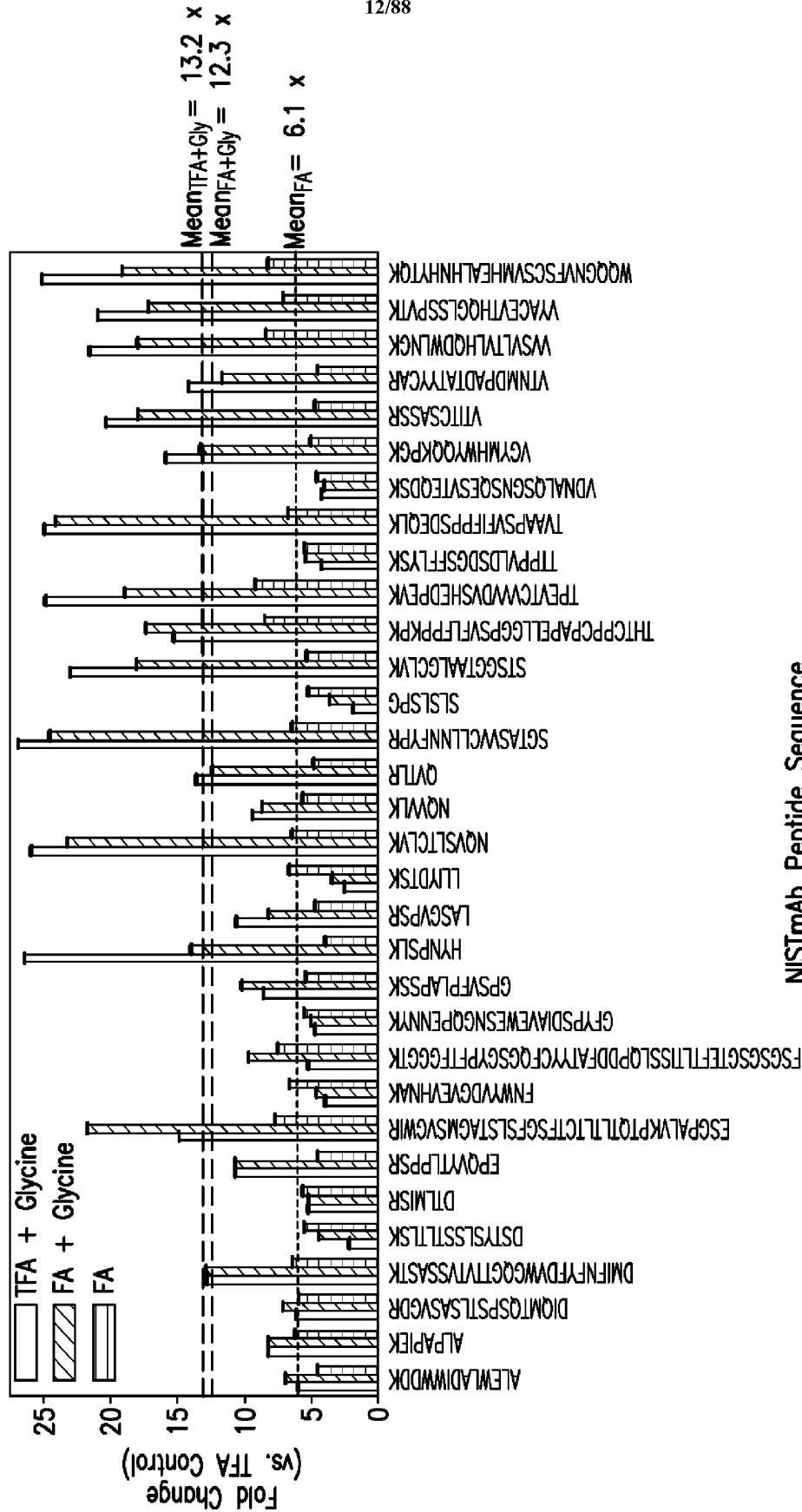


FIG.9



NISTmAb Peptide Sequence

FIG.10

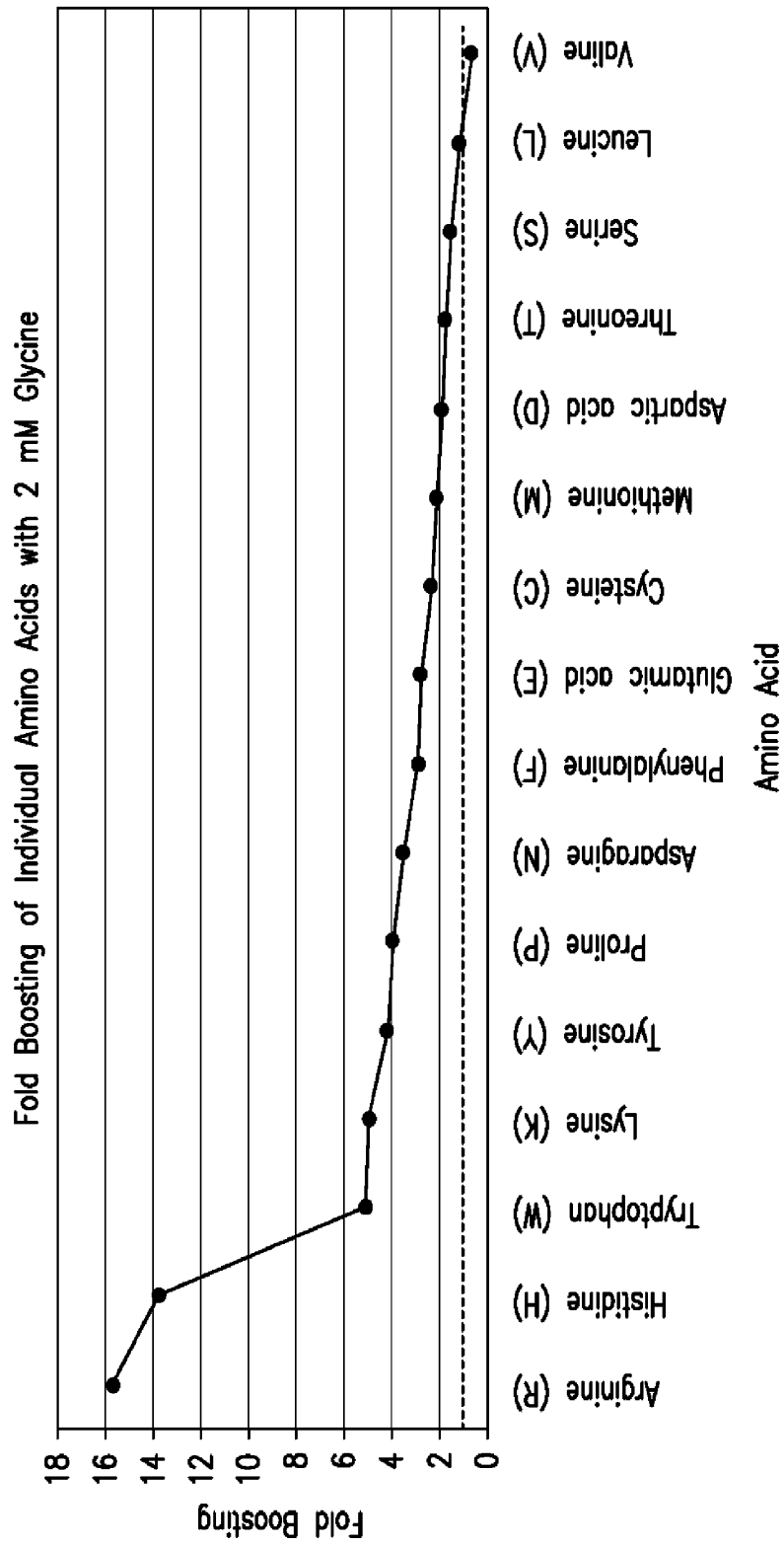


FIG.11

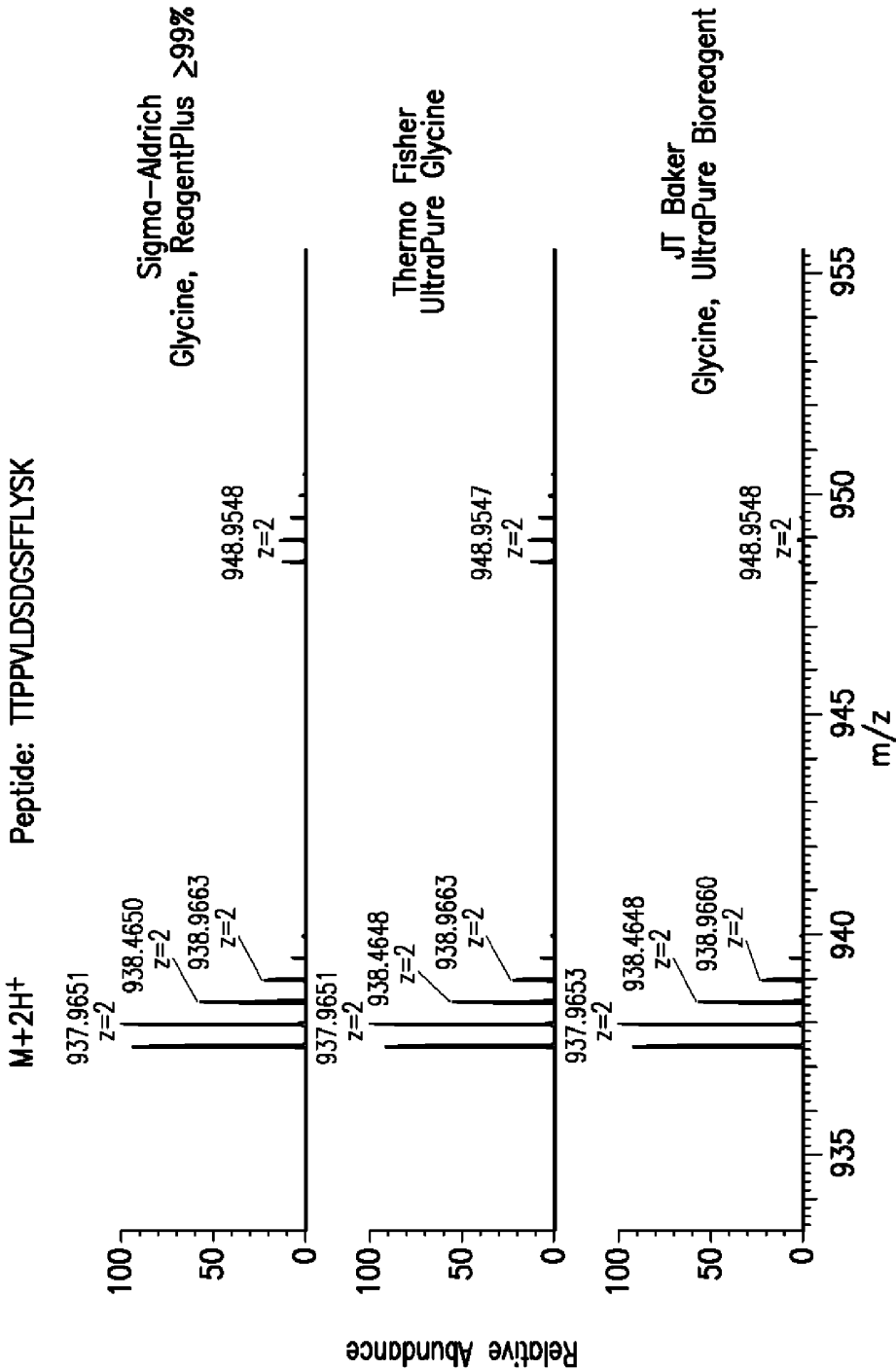


FIG.12

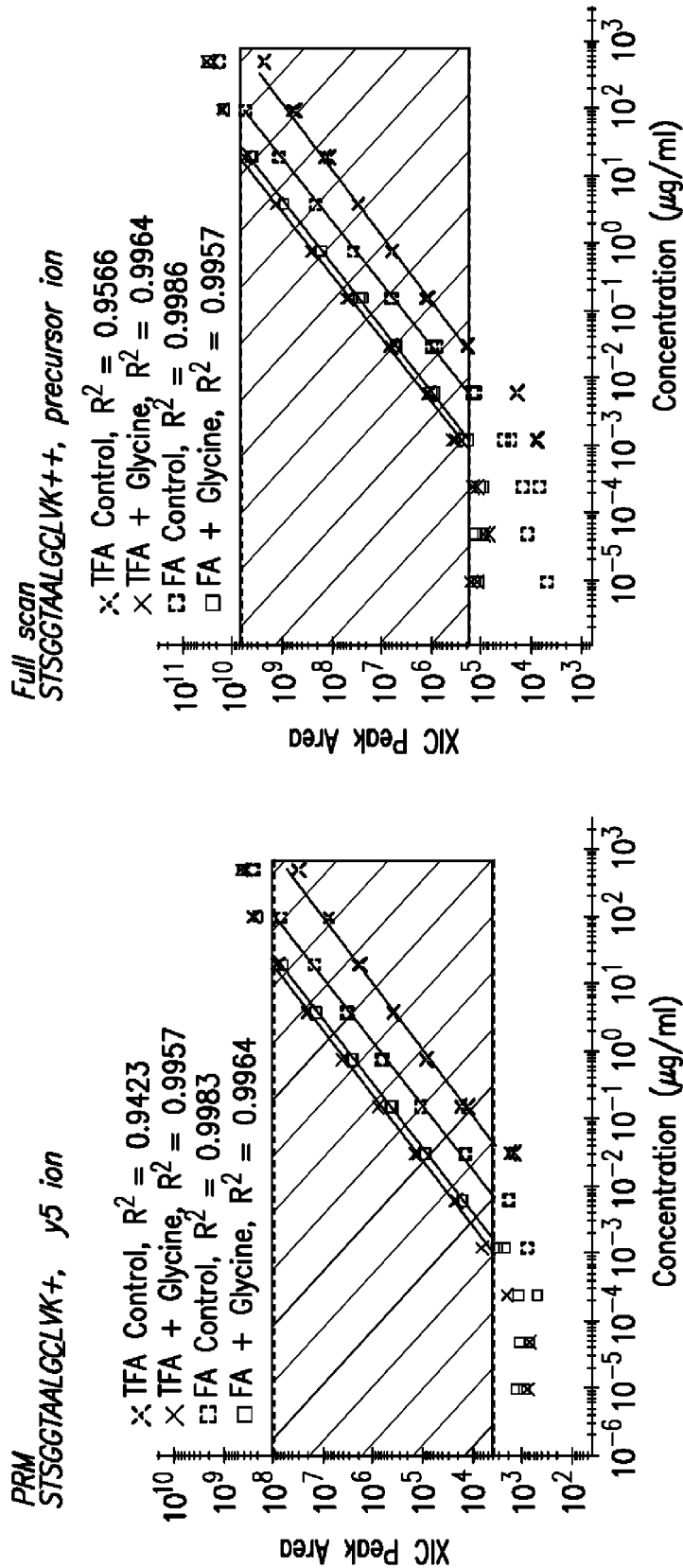


FIG.13

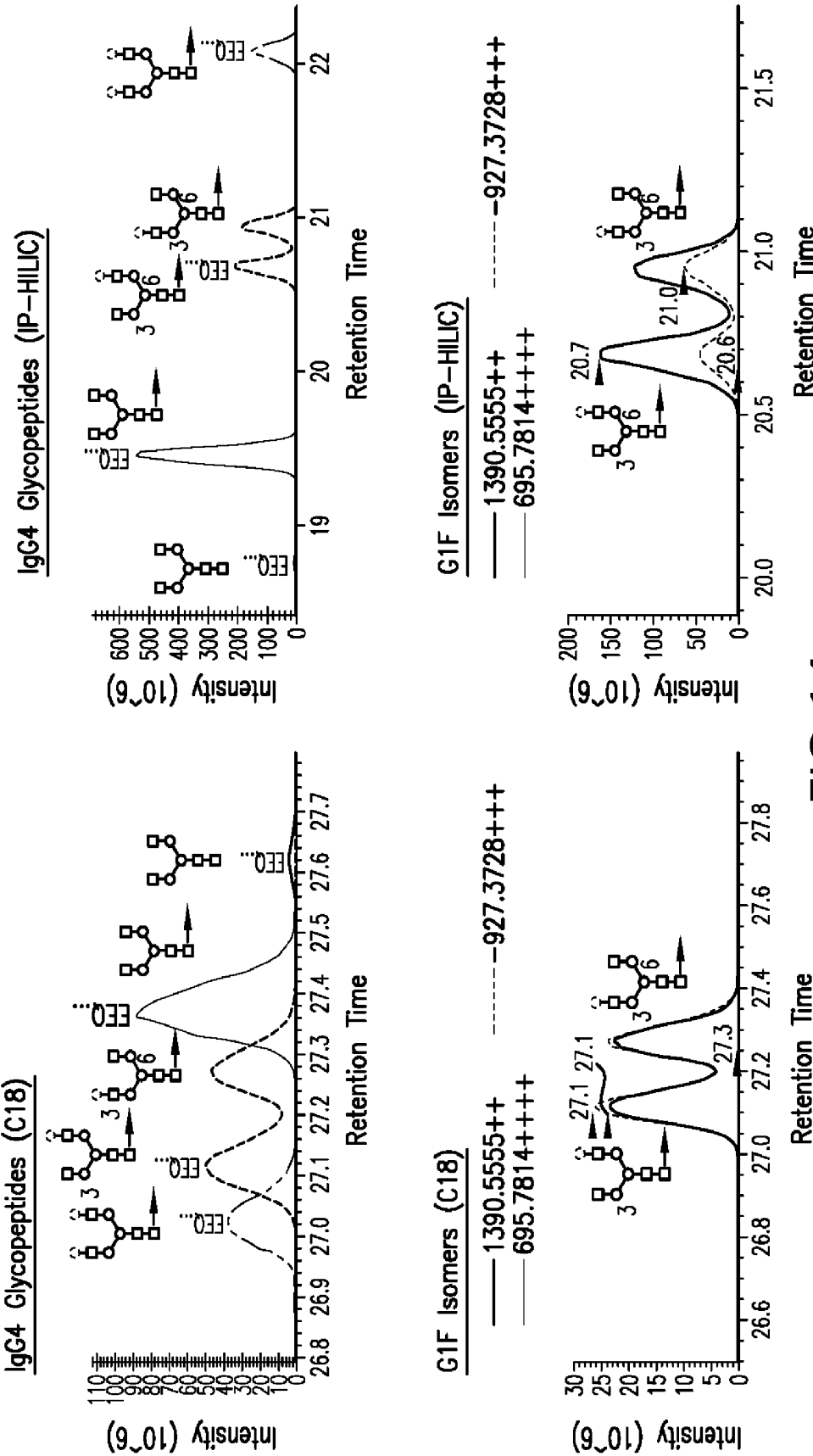


FIG.14

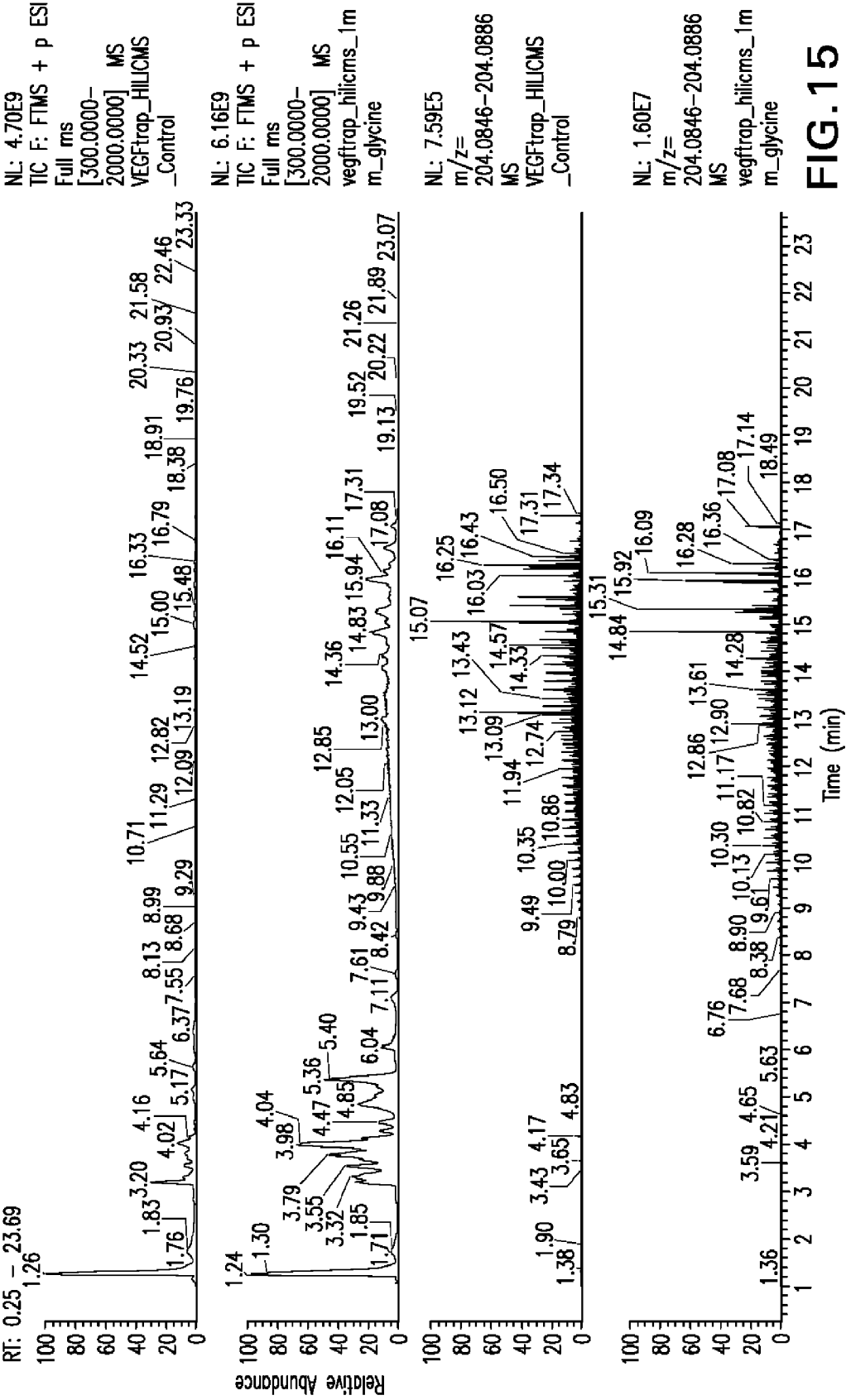
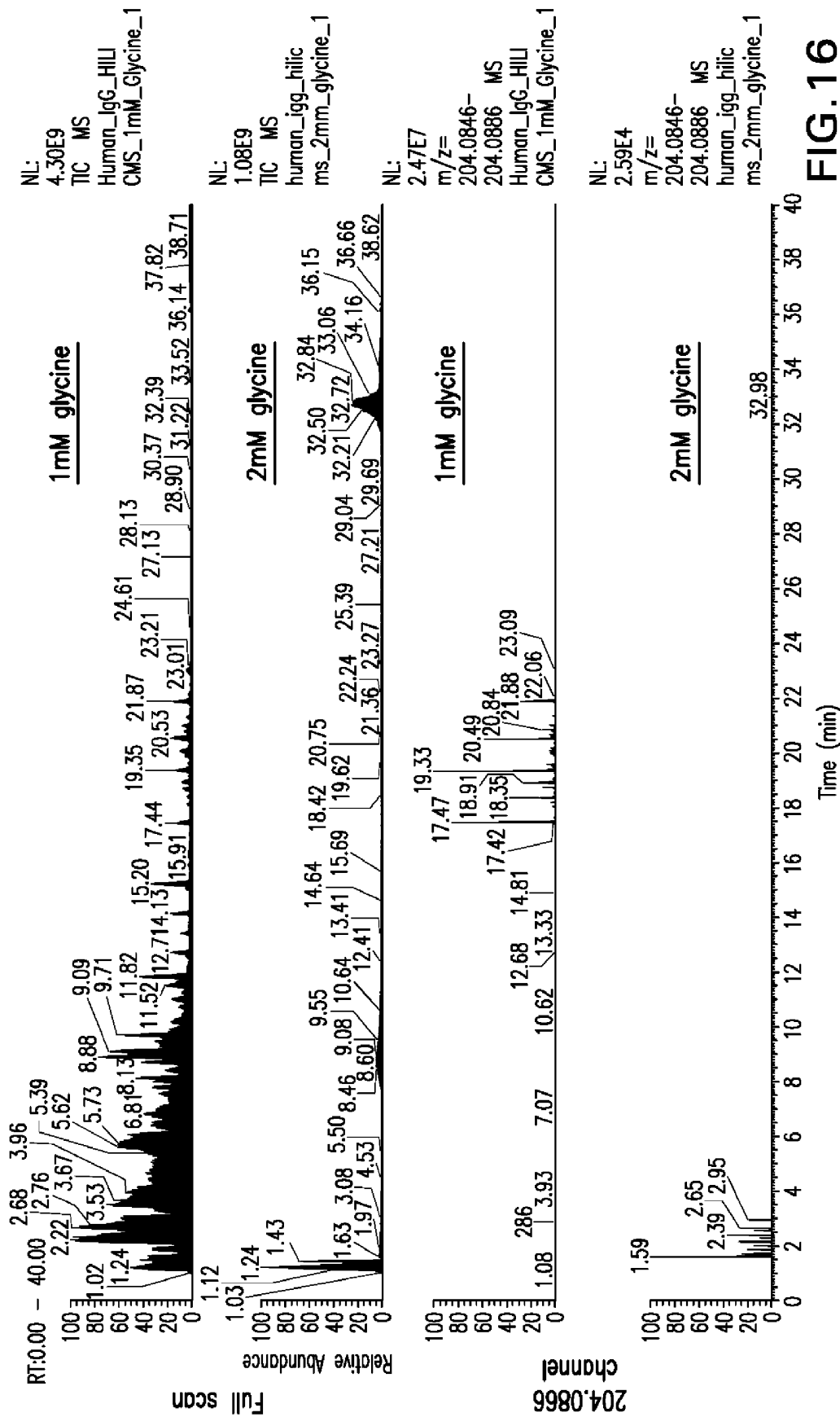


FIG.15



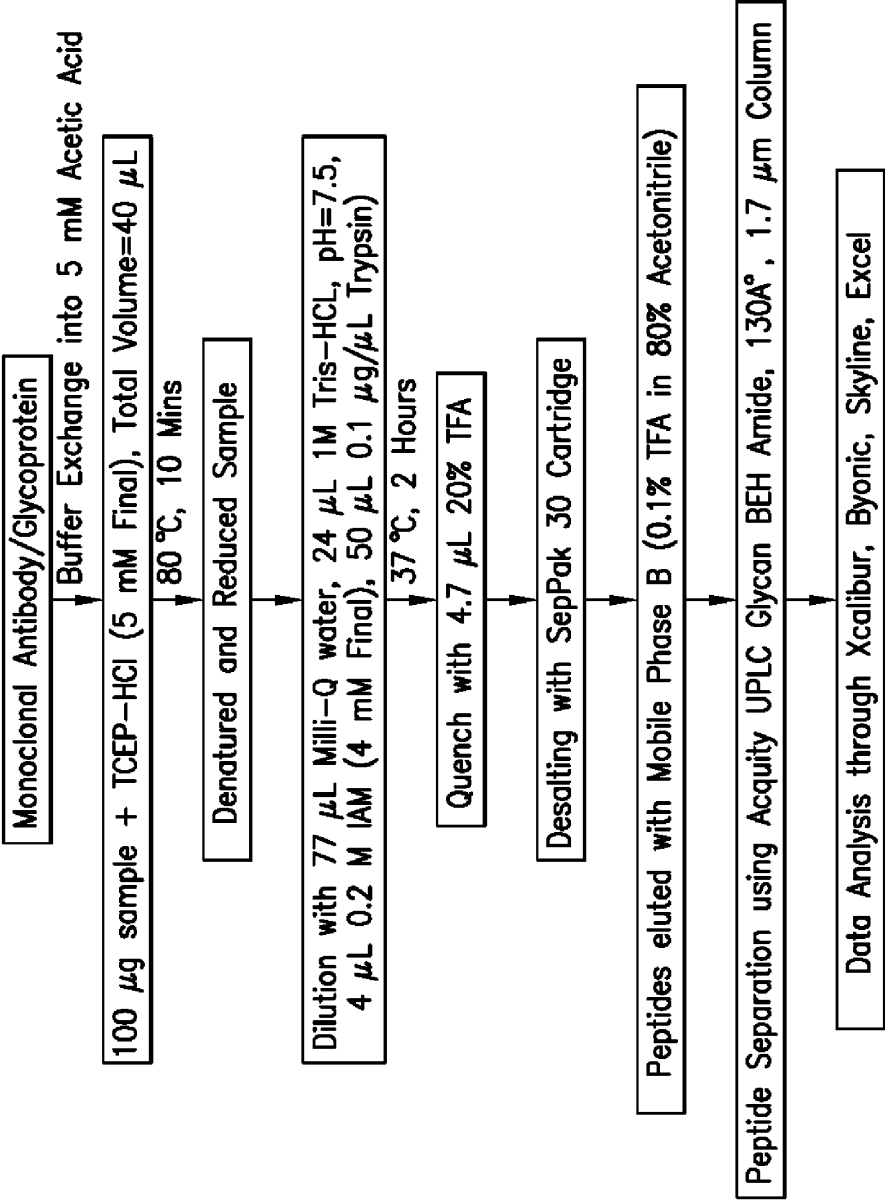


FIG.17

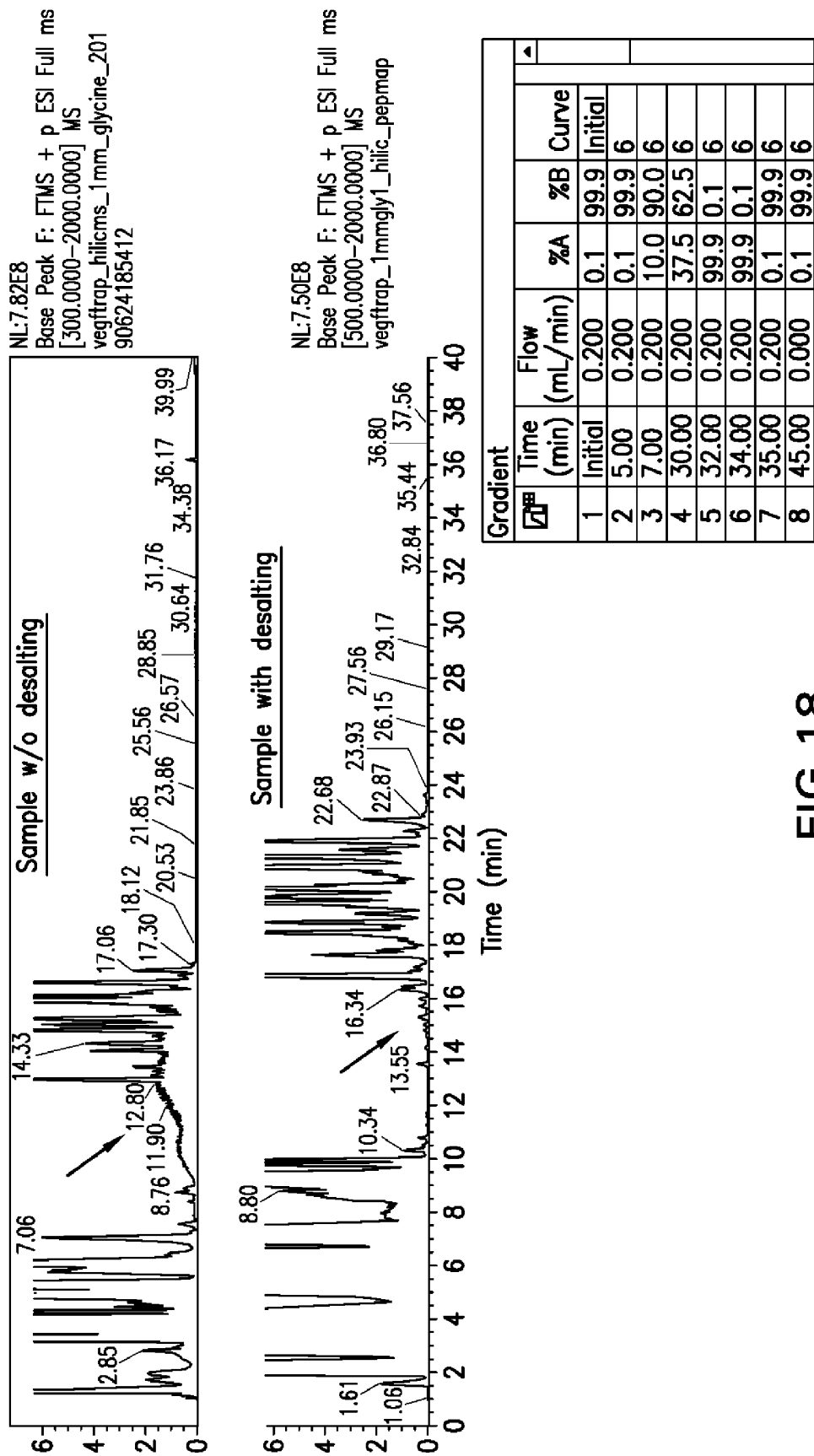


FIG.18

Glycan Annotation*	Average Peak Area**		Average Fold Change	Relative level	
	Peptide 1_Glycine	Peptide 1_Control		Peptide 1_Glycine	Peptide 1_Control
G0F	1.2e10 ± 8.3e7	7.5e8 ± 7.5e7	16.6	43.2% ± 0.1%	46.5% ± 0.7%
G1F	9.1e9 ± 6.0e7	5.1e8 ± 6.8e7	17.7	31.5% ± 0.1%	31.7% ± 0.5%
G0	2.7e9 ± 6.3e7	1.4e8 ± 1.6e7	19.5	9.2% ± 0.1%	8.4% ± 0.0%
G2F	1.7e9 ± 1.7e7	7.7e7 ± 1.1e7	22.7	6.0% ± 0.0%	4.7% ± 0.1%
G1	9.4e8 ± 2.1e7	4.3e7 ± 6.0e6	21.5	3.2% ± 0.0%	2.7% ± 0.1%
Man5	5.3e8 ± 2.8e6	2.3e7 ± 2.8e6	22.8	1.8% ± 0.0%	1.4% ± 0.0%
G0F-GlcNAc	4.2e8 ± 2.7e6	2.3e7 ± 2.4e6	18.5	1.5% ± 0.0%	1.4% ± 0.0%
G1F-GlcNAc	2.2e8 ± 4.7e6	1.0e7 ± 1.3e6	21.8	0.77% ± 0.01%	0.63% ± 0.01%
G0-GlcNAc	1.8e8 ± 3.8e5	1.0e7 ± 1.3e6	17.6	0.63% ± 0.00%	0.64% ± 0.00%
Man3	1.1e8 ± 3.1e5	6.0e6 ± 6.7e5	18.8	0.39% ± 0.00%	0.37% ± 0.00%
G1-GlcNAc	7.6e7 ± 1.9e6	3.8e6 ± 5.4e5	20.3	0.27% ± 0.00%	0.23% ± 0.01%
G2	7.7e7 ± 1.0e6	4.2e6 ± 5.2e5	18.6	0.27% ± 0.00%	0.26% ± 0.00%
G2FS	5.9e7 ± 1.9e6	3.0e6 ± 4.7e5	19.5	0.20% ± 0.00%	0.19% ± 0.01%
Man6	3.8e7 ± 1.4e6	2.0e6 ± 2.4e5	19.3	0.13% ± 0.00%	0.12% ± 0.00%
Man8	3.4e7 ± 1.2e6	2.0e6 ± 2.6e5	16.9	0.12% ± 0.00%	0.13% ± 0.00%
G0FB	3.3e7 ± 5.8e5	1.9e6 ± 2.3e5	17.9	0.12% ± 0.00%	0.11% ± 0.00%
Man4_G1	2.9e7 ± 1.7e6	1.5e6 ± 1.4e5	20.0	0.10% ± 0.01%	0.09% ± 0.00%
Man7	2.7e7 ± 7.4e5	1.3e6 ± 1.6e5	20.9	0.09% ± 0.00%	0.08% ± 0.00%
G1F+GlcNAc	2.5e7 ± 1.8e5	1.2e6 ± 1.9e5	21.4	0.09% ± 0.00%	0.08% ± 0.00%
G0F+GlcNAc	2.7e7 ± 2.7e5	1.3e6 ± 1.6e5	20.6	0.09% ± 0.00%	0.07% ± 0.00%
Man5_G1	2.1e7 ± 5.1e5	1.1e6 ± 1.8e5	18.8	0.07% ± 0.00%	0.07% ± 0.00%
G1FS-GlcNAc	1.8e7 ± 2.4e4	1.1e6 ± 1.6e5	16.8	0.06% ± 0.00%	0.07% ± 0.00%
Man4	1.1e7 ± 8.7e4	4.1e5 ± 1.3e5	27.0	0.04% ± 0.00%	0.02% ± 0.01%
Man3F	8.2e6 ± 2.4e5	4.4e5 ± 6.6e4	18.9	0.03% ± 0.00%	0.03% ± 0.00%
G0B	5.9e6 ± 3.7e5	2.5e5 ± 5.7e4	23.1	0.02% ± 0.00%	0.02% ± 0.00%
Man5F	2.3e6 ± 7.7e3	ND	---	0.01% ± 0.00%	0.00% ± 0.00%
Man4F	1.1e7 ± 8.8e6	ND	---	0.04% ± 0.03%	0.00% ± 0.00%

FIG.19

Glycan Annotation*	Average Peak Area**		Fold Change***	Relative level	
	Peptide2	Peptide2		Peptide2	Peptide2
	+Glycine	Control		+Glycine	Control
G0F	2.4e10 ± 4.5e7	1.3e9 ± 9.2e6	18.4	70.4% ± 0.1%	72.6% ± 0.7%
G1F	5.3e9 ± 5.9e6	2.8e8 ± 1.6e7	19.2	15.4% ± 0.0%	15.2% ± 0.6%
G0	1.7e9 ± 2.2e7	7.2e7 ± 3.9e5	23.7	5.0% ± 0.0%	4.0% ± 0.0%
G0F-GlcNAc	1.1e9 ± 1.2e7	5.3e7 ± 1.8e6	21.4	3.3% ± 0.0%	2.9% ± 0.1%
G2F	6.6e8 ± 1.6e7	3.3e7 ± 1.8e6	20.1	1.9% ± 0.0%	1.8% ± 0.1%
Man5	3.4e8 ± 2.7e6	1.5e7 ± 4.3e5	22.9	1.0% ± 0.0%	0.80% ± 0.0%
G0-GlcNAc	3.1e8 ± 7.3e6	1.3e7 ± 4.2e5	23.8	0.89% ± 0.02%	0.71% ± 0.01%
Man8	1.9e8 ± 3.3e6	9.3e6 ± 3.1e5	20.1	0.54% ± 0.01%	0.51% ± 0.01%
G1	1.8e8 ± 9.0e5	9.2e6 ± 2.1e5	19.4	0.50% ± 0.00%	0.50% ± 0.00%
G0F+GlcNAc	1.3e8 ± 6.7e5	7.2e6 ± 2.2e5	18.1	0.38% ± 0.00%	0.40% ± 0.01%
Man6	4.7e7 ± 1.2e5	2.1e6 ± 9.7e4	22.1	0.14% ± 0.00%	0.12% ± 0.00%
G1F+GlcNAc	4.5e7 ± 4.5e5	2.6e6 ± 7.9e4	17.6	0.13% ± 0.00%	0.14% ± 0.00%
G2FS	3.2e7 ± 3.2e4	1.7e6 ± 2.5e4	19.4	0.09% ± 0.00%	0.09% ± 0.00%
Man7	3.2e7 ± 1.2e5	1.5e6 ± 6.4e4	22.1	0.09% ± 0.00%	0.08% ± 0.00%
G1FS	2.8e7 ± 1.3e6	1.6e6 ± 5.2e4	17.7	0.08% ± 0.00%	0.09% ± 0.00%
G2	2.0e7 ± 5.7e5	1.1e6 ± 1.9e4	18.8	0.06% ± 0.00%	0.06% ± 0.00%
G2F+GlcNAc	1.3e7 ± 7.5e5	6.1e5 ± 2.2e4	20.7	0.04% ± 0.00%	0.03% ± 0.00%
G2FS2	4.4e6 ± 4.6e4	7.9e4 ± 1.1e4	(55.9)	0.01% ± 0.00%	0.00% ± 0.00%
G3F	2.5e6 ± 1.9e5	5.4e4 ± 7.5e3	(47.0)	0.01% ± 0.00%	0.00% ± 0.00%

FIG.20

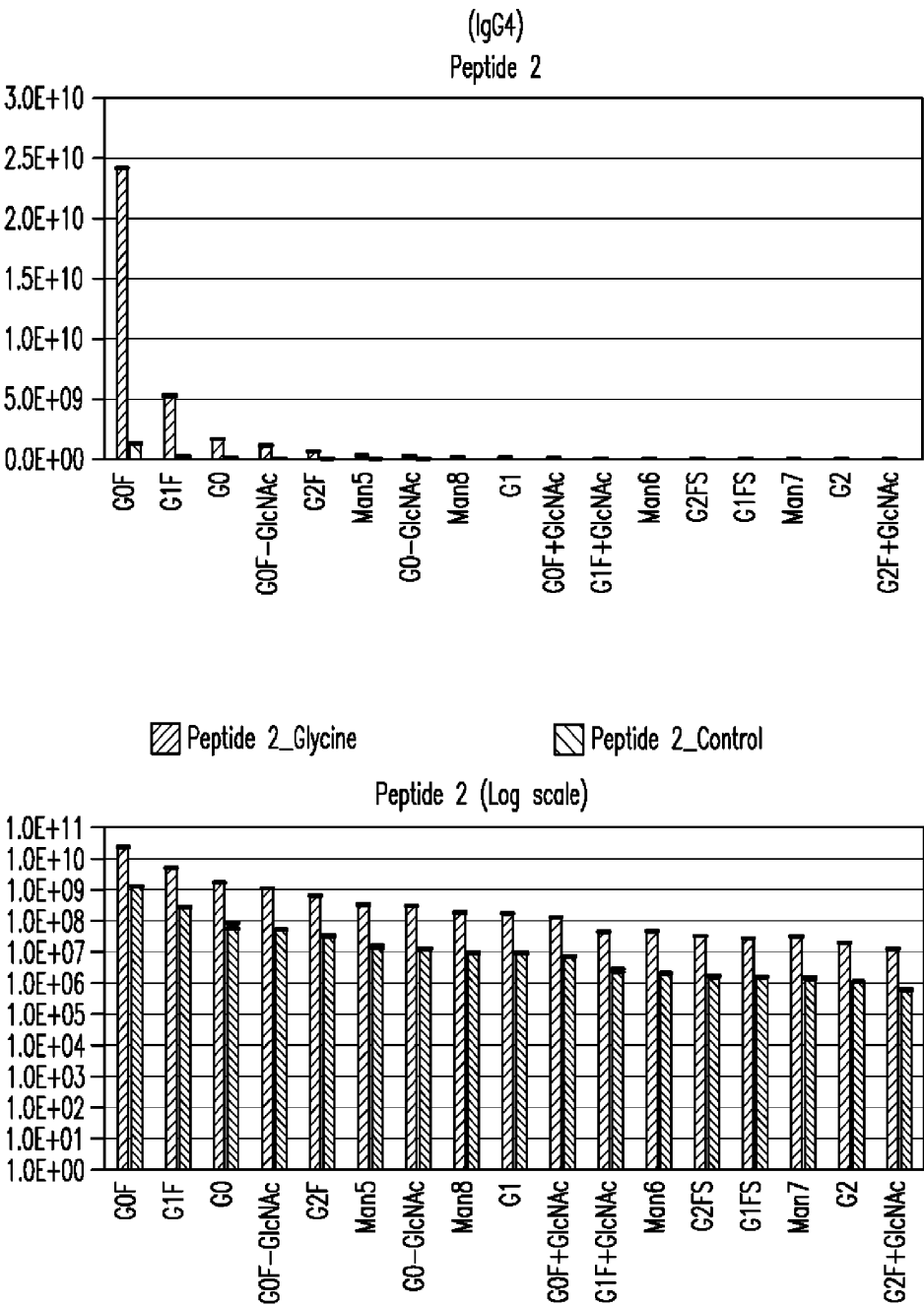


FIG.21A

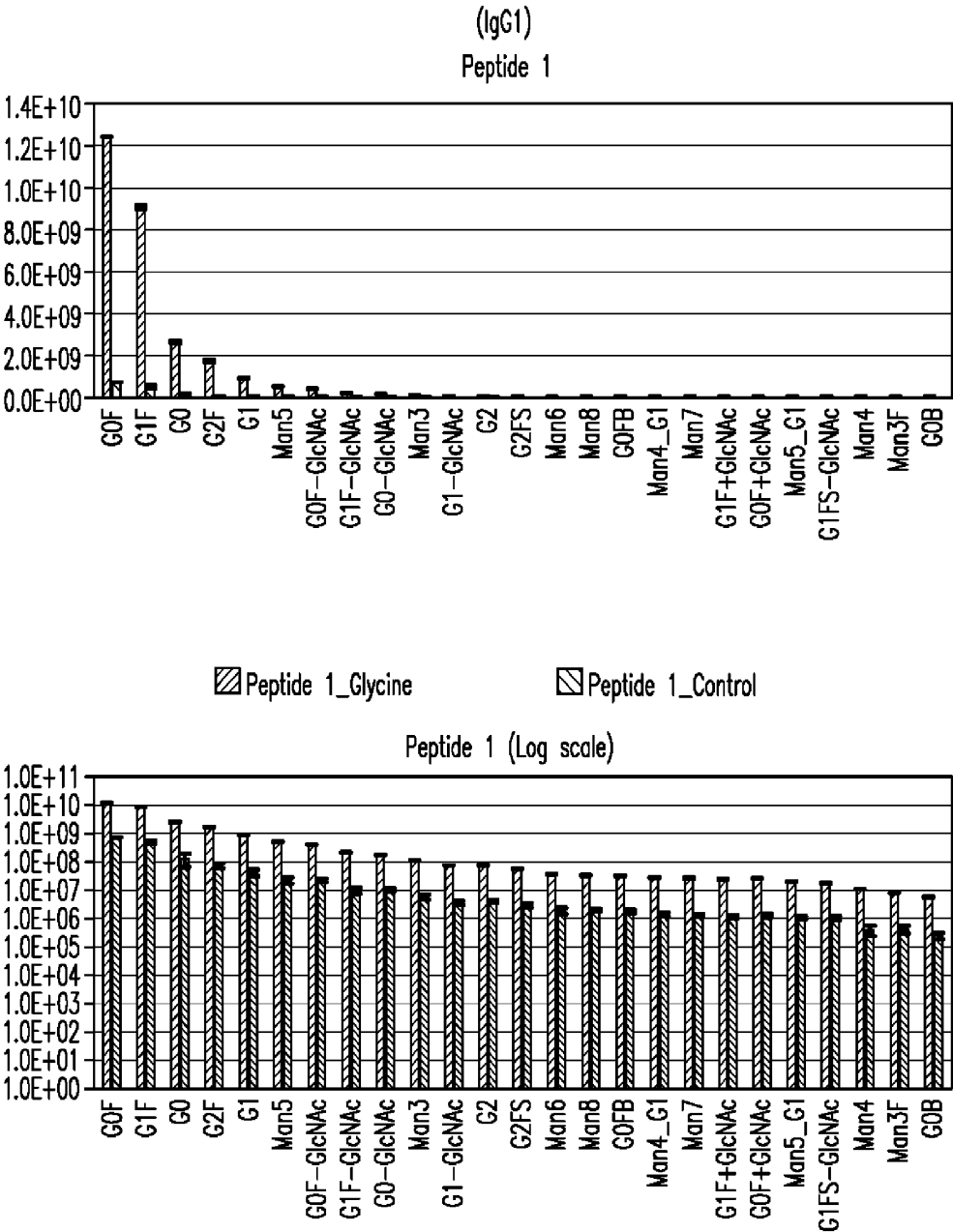


FIG.21B

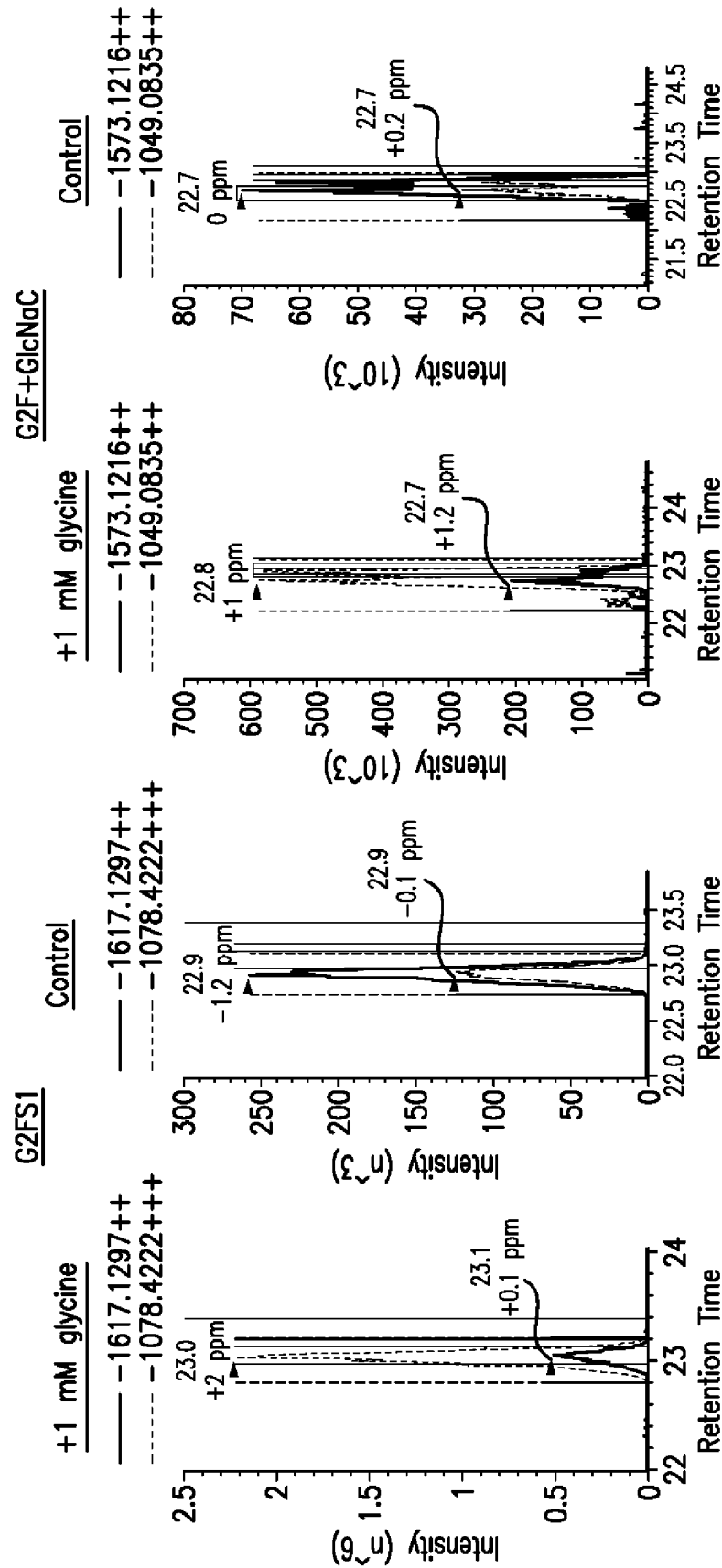


FIG.22

Mobile Phase	N36		N68		N123		N196		N282		Total	
	# PSM	# Glycoform	# PSM	# Glycoform	# PSM	# Glycoform	# PSM	# Glycoform	# PSM	# Glycoform	# PSM	# Glycoform
HILIC 0.1%TFA	1	1	0	0	0	0	0	0	1	1	2	2
C18 (RPLC) 0.05% TFA	16	6	5	3	8	3	2	1	3	2	34	15
HILIC 0.1%TFA +1mM Glycine	15	9	45	12	112	23	139	31	29	12	340	87

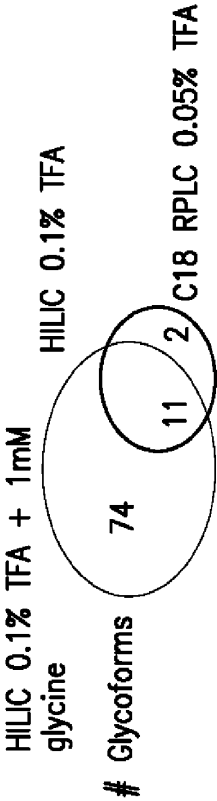


FIG.23

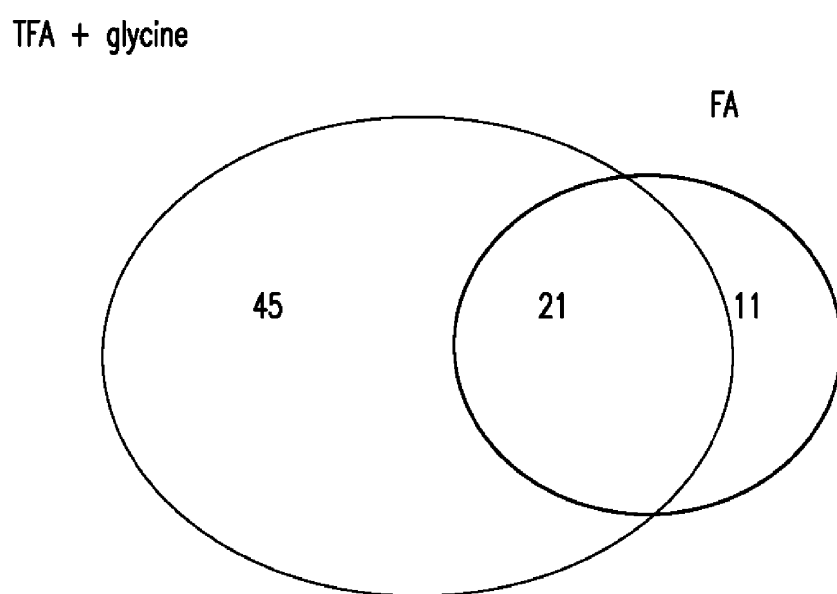
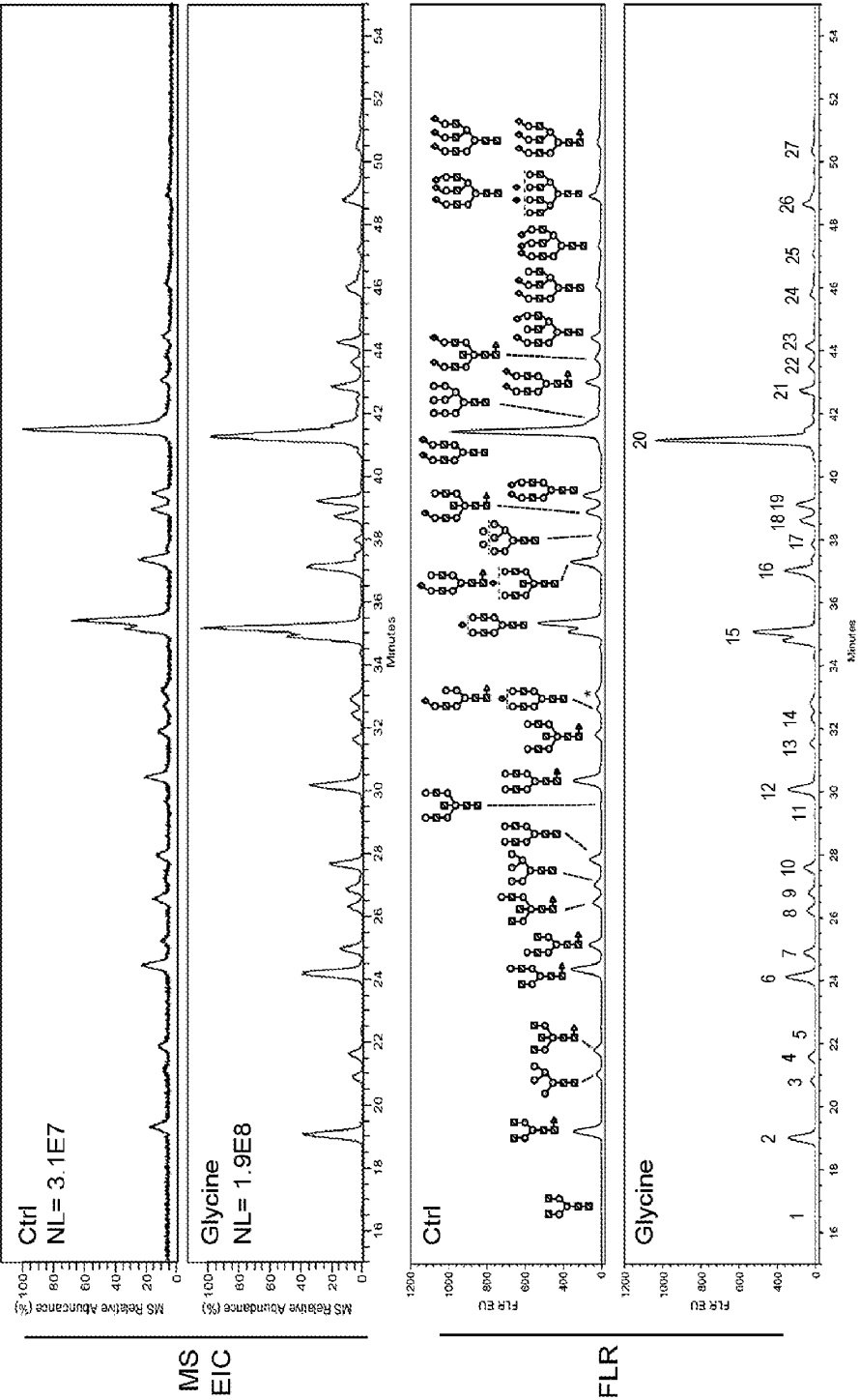


FIG.24



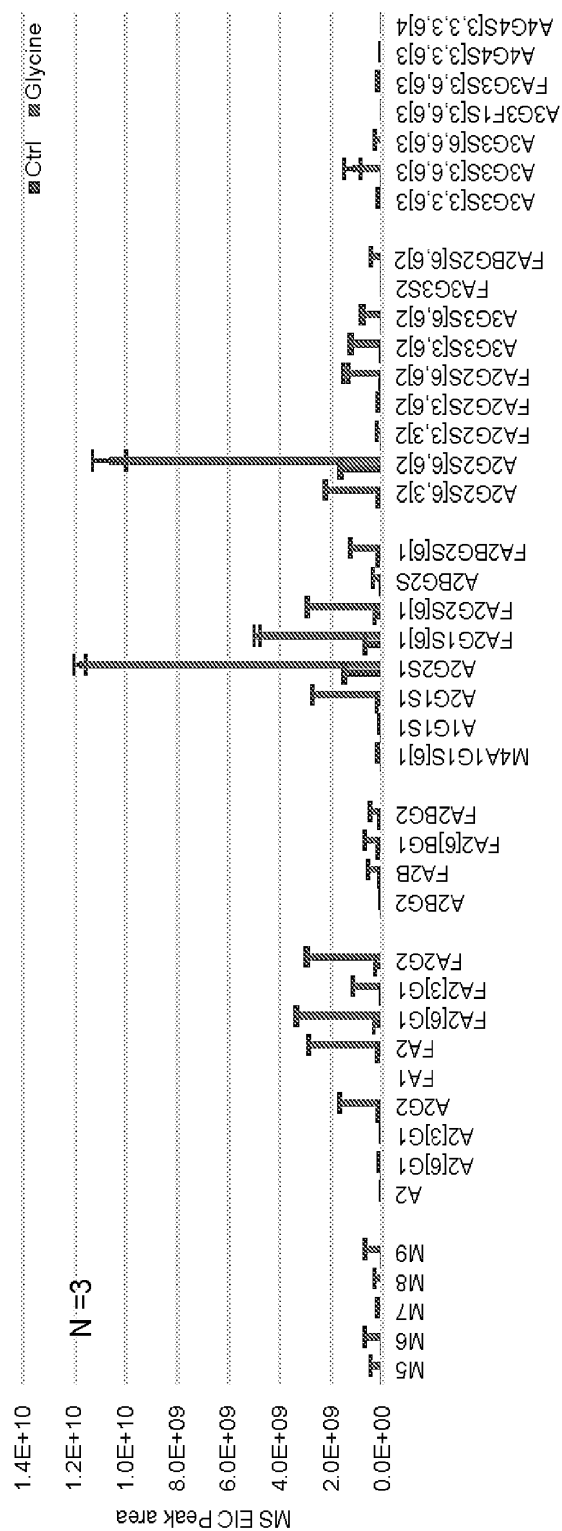
EIC: m/z 600-2000
* The peaks is composed of 5 different glycans

FIG. 25

Composition	Average Charge state		Total MS signal boost by glycine
	Ctrl	Glycine	
M5	1.6	1.9	13.1
M6	1.7	1.9	21.9
M7	1.9	2.0	23.3
M8	1.8	2.0	35.6
M9	2.0	2.0	36.9
A2	1.5	1.9	9.8
A2[6]G1	1.8	2.0	10.6
A2[3]G1	1.6	2.0	11.4
A2G2	1.9	2.0	9.6
FA1	1.5	1.9	25.1
FA2	1.6	2.0	13.8
FA2[6]G1	1.9	2.0	9.9
FA2[3]G1	1.7	2.0	15.4
FA2G2	2.0	2.0	10.1
A2BG2	2.0	2.3	3.1
FA2B	1.8	2.0	5.1
FA2[6]BG1	2.0	2.1	3.6
FA2BG2	2.0	2.3	3.6
M4A1G1S[6]1	2.0	2.0	12.2
A1G1S1	1.9	2.0	11.4
A2G1S1	2.0	2.0	11.8
A2G2S1	2.0	2.2	7.9
FA2G1S[6]1	2.0	2.0	7.3
FA2G2S[6]1	2.0	2.3	9.0
A2BG2S	2.0	2.6	5.1
FA2BG2S[6]1	2.0	2.7	6.6
A2G2S[6,3]2	2.0	2.6	13.1
A2G2S[6,6]2	2.0	2.6	6.5
FA2G2S[3,3]2	2.0	2.6	15.0
FA2G2S[3,6]2	2.0	2.7	16.8
FA2G2S[6,6]2	2.0	2.7	13.8
A3G3S[3,6]2	2.0	2.8	16.6
A3G3S[6,6]2	2.0	2.8	18.0
FA3G3S2	2.0	2.9	47.9
FA2BG2S[6,6]2	2.0	2.8	10.5
A3G3S[3,3,6]3	-	2.9	∞
A3G3S[3,6,6]3	2.2	2.9	26.3
A3G3S[6,6,6]3	2.1	2.9	33.3
A3G3F1S[3,6,6]3	-	3.0	∞
FA3G3S[3,6,6]3	-	3.0	∞
A4G4S[3,3,6]3	-	3.0	∞
A4G4S[3,3,3,6]4	-	3.0	∞

N=3

FIG. 26A



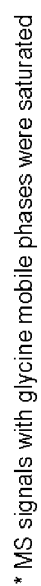


FIG. 26C

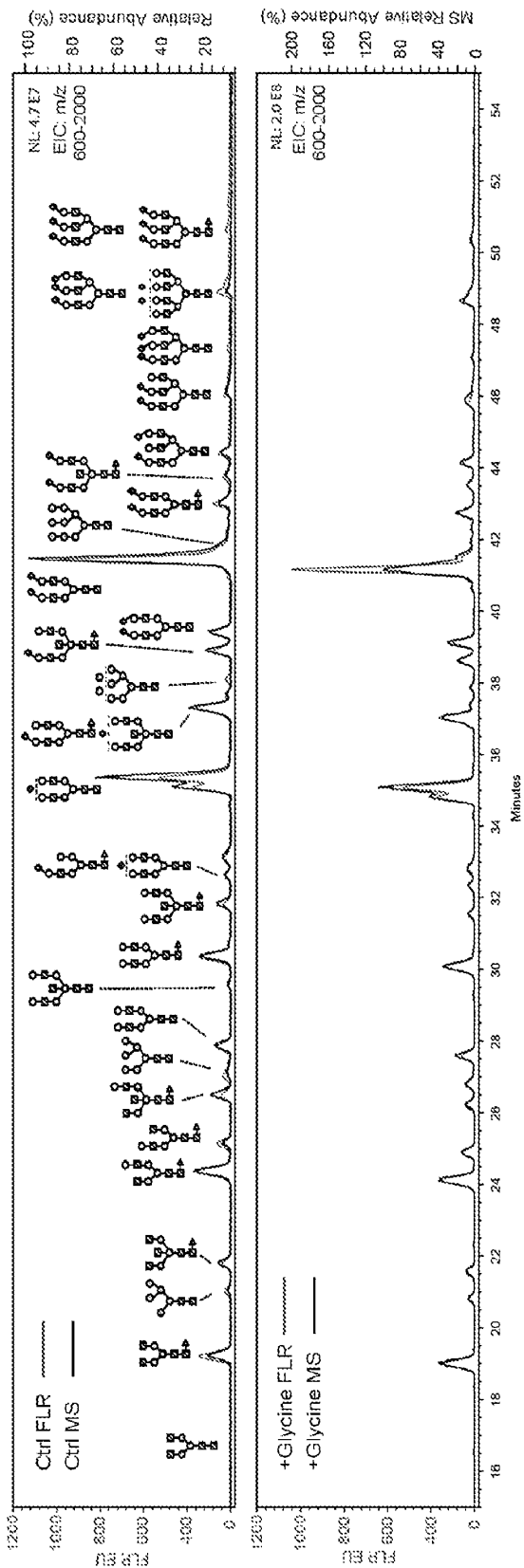


FIG. 27

Peak #	Composition	Relative levels to FA2G2					
		FLR		MS			
		Ctrl	Glycine	Ctrl		Glycine	
1	A2	2.6%	2.6%	1.9%		1.8%	
2	FA2	97.2%	97.1%	71.4%		98.2%	
3	M5	16.4%	16.4%	12.4%		16.1%	
4	FA2B	26.6%	26.6%	38.8%	44.0%	19.5%	25.0%
	A2[6]G1			5.2%		5.5%	
5	A2[3]G1	2.8%	2.9%	2.2%		2.5%	
6	FA2[6]G1	109.4%	109.3%	116.3%		114.2%	
7	FA2[3]G1	40.6%	40.5%	26.0%		39.8%	
8	FA2[6]BG1	29.0%	29.0%	66.5%		24.0%	
9	M6	28.8%	28.8%	11.0%		23.9%	
10	A2G2	41.7%	41.6%	59.6%		57.1%	
11	A2BG2	7.0%	7.2%	11.0%		3.4%	
12	FA2G2	100.0%	100.0%	100.0%		100.0%	
13	FA2BG2	19.2%	19.3%	46.7%		16.8%	
14	M4A1G1S[6]1	16.3%	16.4%	5.8%	19.7%	7.1%	18.3%
	A2G2S1			13.9%		11.2%	
15	A2G2S1	285.9%	285.3%	509.9%		399.8%	
16	A2BG2S1	105.3%	106.8%	25.4%	136.6%	12.8%	112.2%
	FA2G2S[6]1			111.3%		99.4%	
17	M8	11.7%	11.9%	2.9%		10.3%	
18	FA2BG2S[6]1	45.3%	45.0%	64.7%	69.6%	42.3%	49.7%
	FA2G2S[3,3]2			5.0%		7.4%	
19	A2G2S[6,3]2	54.9%	56.3%	57.9%		75.4%	
20	A2G2S[6,6]2	469.7%	498.6%	559.3%	565.7%	361.2%	384.5%
	M9			6.4%		23.3%	
21	FA2G2S[6,6]2	46.7%	48.3%	35.5%		48.6%	
22	FA2BG2S[6,6]2	19.1%	21.3%	15.1%		15.7%	
23	A3G3S[3,6]2	29.8%	30.1%	25.8%		42.6%	
24	A3G3S[6,6]2	18.5%	21.7%	15.0%		26.9%	
25	A3G3S[3,3,6]3	5.2%	5.3%	0.9%	1.2%	5.6%	6.9%
	FA3G3S2			0.3%		1.4%	
26	A3G3S[3,6,6]3	38.5%	40.8%	15.3%		39.9%	
27	A3G3S[6,6,6]3	17.5%	19.7%	3.1%	3.1%	10.2%	16.4%
	FA3G31S[6,6,6]3			0.0%		6.2%	

FIG. 28A

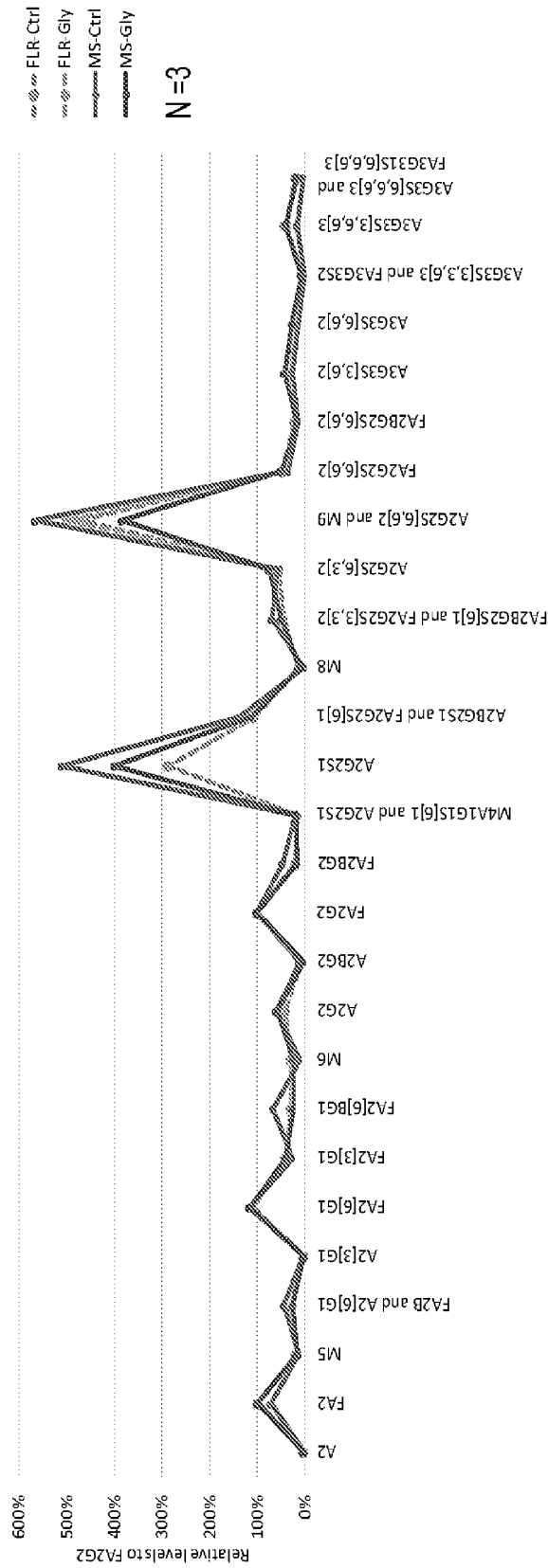


FIG. 28B

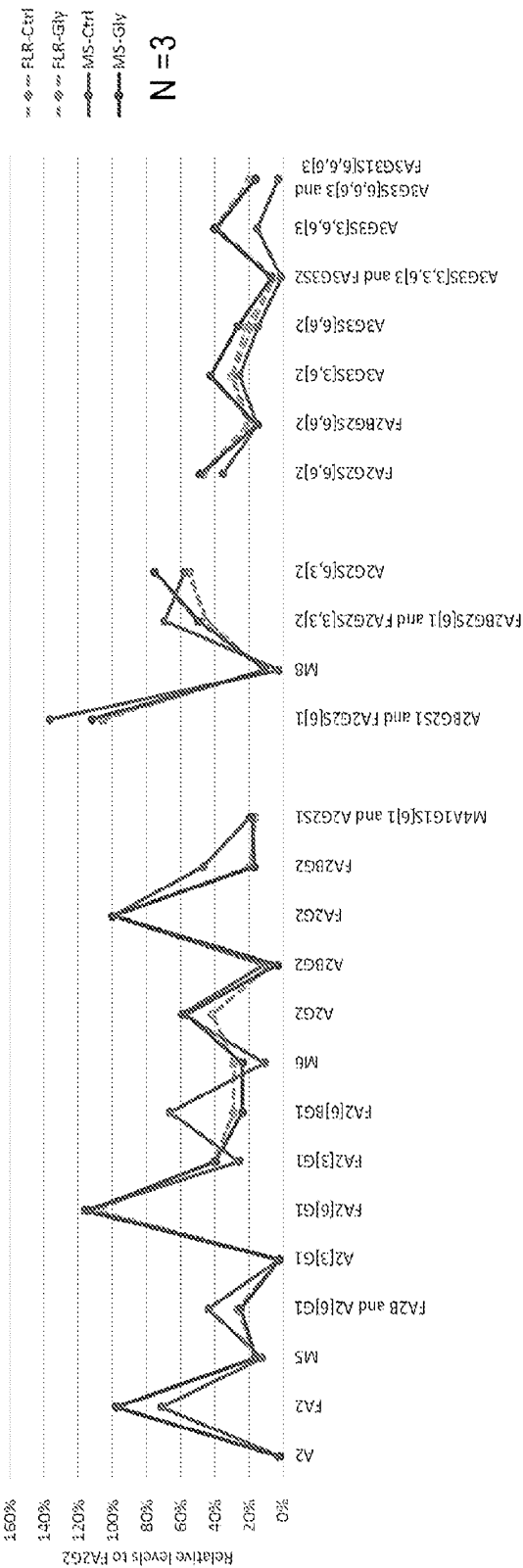


FIG. 28C

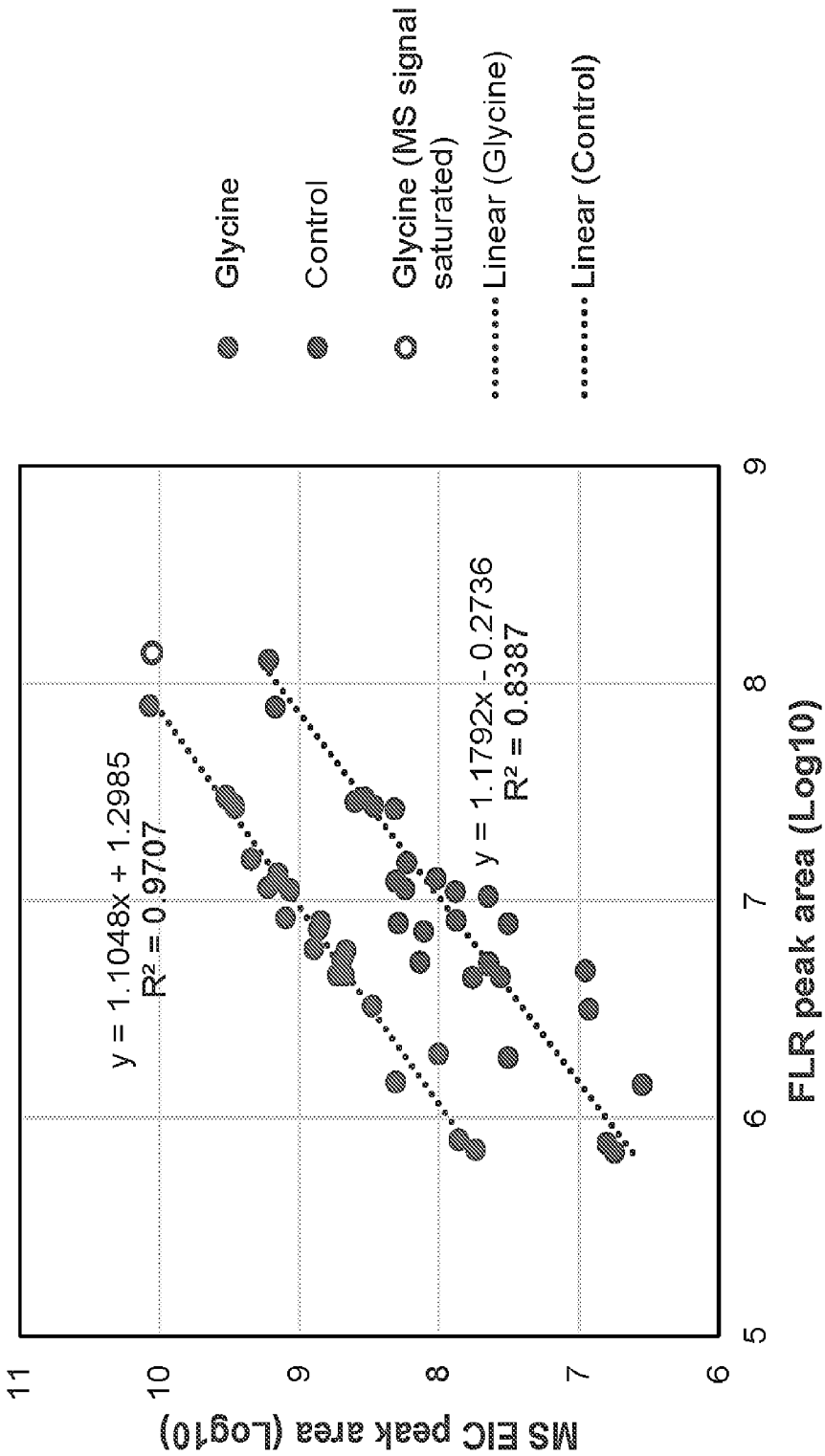


FIG. 29

Composition	Average Charge state		Total signal boost by glycine
	Ctrl	Glycine	
M5	2.0	2.0	6.1
M6	2.0	2.0	5.7
M7	2.0	2.0	6.5
M8	2.0	2.0	11.2
M9	2.0	2.0	16.4
A2	2.0	2.0	2.8
A2[6]G1	2.0	2.0	4.7
A2[3]G1	2.0	2.0	3.7
A2G2	2.1	2.1	7.8
FA2	2.1	2.0	9.1
FA2[6]G1	2.1	2.0	10.6
FA2[3]G1	2.1	2.1	10.8
FA2G2	2.2	2.1	11.8
A2BG2	2.3	2.5	10.5
FA2B	2.0	2.0	11.6
FA2[6]BG1	2.2	2.2	10.3
FA2BG2	2.3	2.5	14.9
M4A1G1S[6]1	2.1	2.1	10.8
A2G1S1	2.1	2.0	13.4
A2G2S[6]1	2.4	2.4	11.0
FA2G1S[6]1	2.2	2.1	11.1
FA2G2S[6]1	2.4	2.5	12.6
A2BG2S	2.5	2.8	14.5
FA2BG2S[6]1	2.6	2.8	14.0
A2G2S[6,3]2	2.5	2.7	30.2
A2G2S[6,6]2	2.6	2.8	19.0
FA2G2S[3,3]2	2.4	2.7	38.7
FA2G2S[3,6]2	2.5	2.8	36.8
FA2G2S[6,6]2	2.6	2.9	21.3

N =3

FIG. 30A

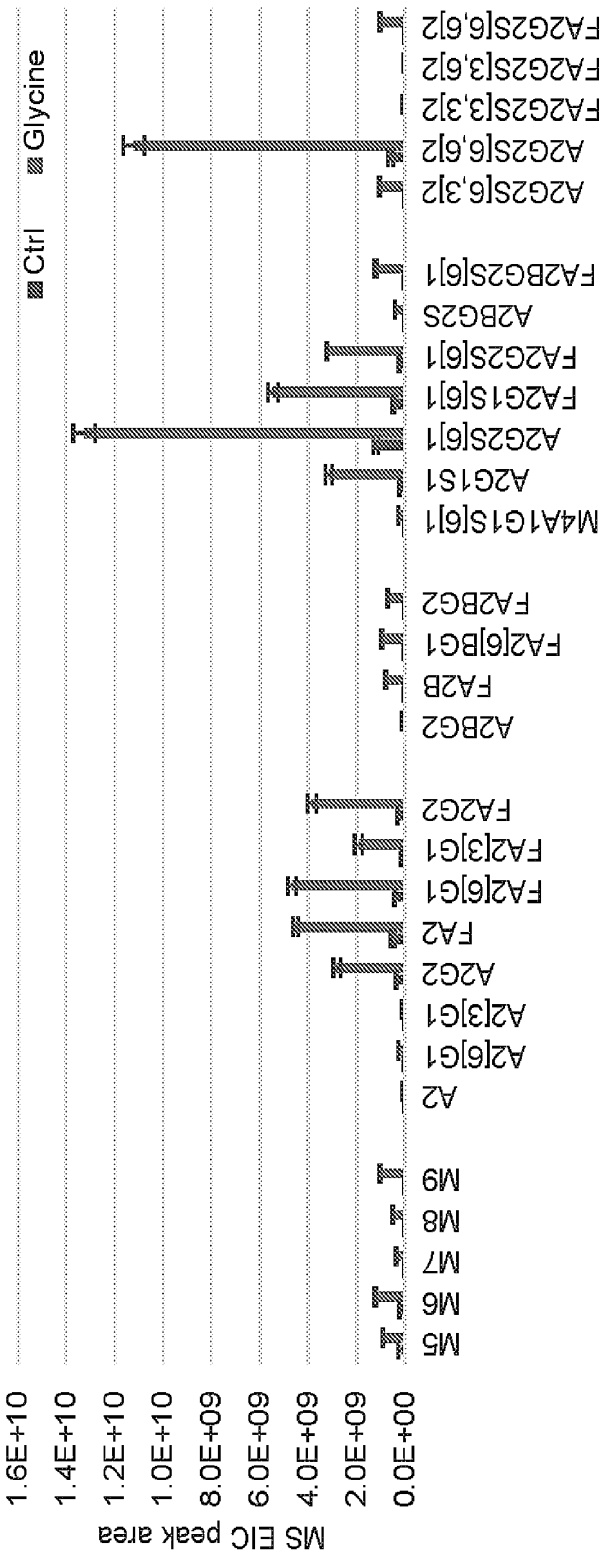


FIG. 30B

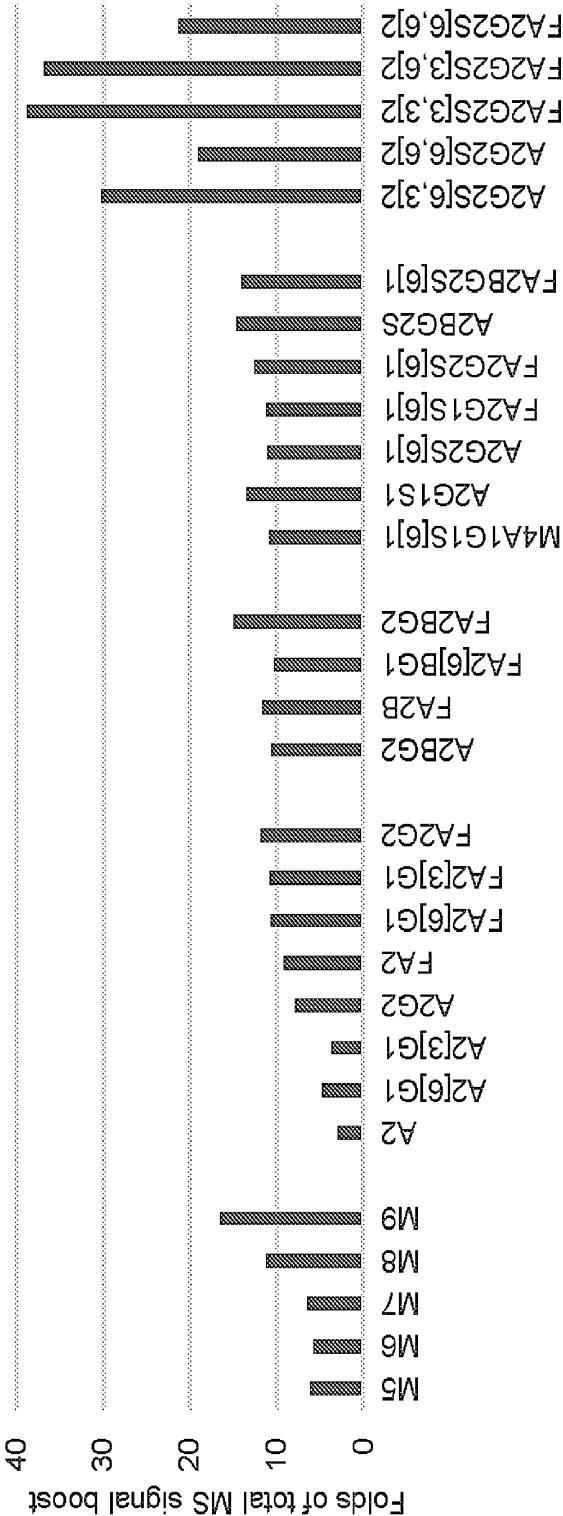


FIG. 30C

Composition	Relative levels to FA2					Average Charge state		Total signal boost by glycine
	FLR	MS				Ctrl	Glycine	
	Ctrl	Ctrl		Glycine				
FA1	0.7%	-		0.4%		2.0	2.0	-
A2	1.8%	1.6%		2.2%		2.0	2.0	1.7
FA2	102.6%	124.1%		110.2%		2.0	2.0	1.1
M5	48.5%	14.8%	49.7%	13.9%	41.5%	2.0	2.0	1.2
FA2B		34.8%		27.6%		2.0	2.0	1.0
A2[6]G1	2.5%	1.4%		2.3%		2.0	2.0	2.1
A2[3]G1	1.7%	1.1%		1.6%		2.0	2.0	1.7
FA2[6]G1	136.3%	140.8%		124.0%		2.0	2.0	1.1
FA2[3]G1	56.7%	60.5%		54.6%		2.0	2.1	1.1
FA2[6]BG1	49.2%	48.6%		46.2%		2.0	2.3	1.2
M6	18.2%	14.1%		14.2%		1.9	2.0	1.2
A1G1S1	4.1%	4.7%		5.1%		2.0	2.0	1.3
A2G2	8.0%	7.5%		8.7%		2.0	2.1	1.4
FA2G2	100.0%	100.0%		100.0%		2.0	2.1	1.2
A2G1S1	27.0%	11.6%	24.6%	14.4%	31.0%	2.0	2.3	1.5
FA2BG2		13.0%		16.7%		2.0	2.6	1.6
M4A1G1S[6]1	47.1%	5.5%	30.1%	6.7%	38.9%	2.0	2.1	1.5
A2G2S1		5.9%		6.6%		2.0	2.2	1.4
M7		1.5%		3.5%		2.0	2.0	2.8
FA2G1S[6]1		17.2%		22.2%		2.0	2.3	1.6
A2G2S[6]1	266.6%	288.0%		390.5%		2.0	2.4	1.7
FA2G2S[6]1	131.1%	129.7%		154.1%		2.0	2.6	2.2
M8	27.0%	2.8%	7.9%	4.7%	12.0%	2.0	2.0	2.1
A2G2S[3,3]2		2.3%		3.7%		2.0	2.7	2.0
M5A1G1S1		2.8%		3.6%		2.0	2.1	1.6
FA2BG2S[6]1	48.0%	37.7%		63.1%		2.0	2.8	2.1
FA2G2S[3,3]2	18.6%	10.5%		15.8%		2.0	2.7	1.9
A2G2S[3,6]2	175.8%	150.6%		254.5%		2.0	2.7	2.1
FA2G2S[3,6]2	6.8%	9.3%		19.3%		2.0	2.8	2.6
A2G2S[6,6]2	1221.0%	1010.0%	1024.3%	1684.0%	1701.8%	2.0	2.7	2.1
M9		14.3%		%		17.8%	%	2.0
FA2G2S[6,6]2	100.4%	68.6%		125.9%		2.0	2.8	2.3
FA2BG2S[6,6]2	33.7%	19.4%		41.2%		2.2	2.9	2.6
A3G3S[3,6]2	24.8%	9.6%		24.8%		2.2	3.0	3.2
A3G3S[6,6]2	7.6%	2.3%		6.4%		2.0	3.0	3.4
A3G3S[3,3,6]3	34.7%	21.0%		41.9%		2.5	3.0	2.5
A3G3S[3,6,6]3	236.3%	161.5%		294.7%		2.5	3.0	2.3
A3G3F1S[3,6,6]3	94.3%	39.7%	67.4%	74.4%	142.4%	2.6	3.0	2.3
A3G3S[6,6,6]3	30.8%	27.6%		68.0%		2.4	3.0	3.0
FA3G31S[6,6,6]3		-	8.5%	2.4%	17.3%	2.7	3.0	-
A4G4S[3,3,6]3		8.5%		15.0%		3.0	3.0	2.2
A4G4S[3,3,3,6]4	19.2%	15.2%		24.5%		2.9	3.1	2.0
A4G4S[3,6,6,6]4	15.7%	9.9%	9.9%	17.6%	20.2%	3.0	3.0	2.2
A4G4FS[3,3,6,6]4		-		2.6%		3.0	3.0	-

N =3

FIG. 31A

Composition	Average Charge state		Total signal boost by glycine
	Ctrl	Glycine	
A2	1.0	1.3	0.9
FA2	1.1	1.5	2.3
FA2B	1.5	1.9	1.8
M5	1.0	1.0	0.8
FA2[6]G1	1.1	1.7	2.4
FA2[3]G1	1.1	1.8	2.2
FA2[6]BG1	1.7	2.0	1.7
M6	1.0	1.0	0.6
A2G2	1.2	1.9	2.9
FA2G2	1.4	1.9	2.2
FA2BG2	1.9	2.0	1.3
FA2G1S[6]1	1.8	2.0	1.6
A2G2S[6]1	1.6	2.0	2.1
FA2G2S[6]1	1.8	2.0	1.1
FA2BG2S[6]1	2.0	2.0	0.7
A2G2S[3,6]2	1.9	2.0	0.8
A2G2S[6,6]2	1.8	2.0	1.2
M9	1.3	1.9	1.5
FA2G2S[6,6]2	2.0	2.0	0.7
FA2BG2S[6,6]2	2.0	2.0	0.6
A3G3S[3,6,6]3	2.0	2.2	0.6
A3G3S[6,6,6]3	2.0	2.1	0.5
A3G3F1S[3,6,6]3	2.0	2.2	0.3
FA3G31S[6,6,6]3	2.0	2.3	0.4

FIG. 32

UPLC System	Waters Acquity UPLC I-Class				
Mobile Phase	A: 50 mM ammonium formate, pH 4.4, with or without 1 mM glycine				
	B: 15% 50 mM ammonium formate, pH 4.4/85% acetonitrile, with or without 1 mM total concentration of glycine				
Column	ACQUITY UPLC Glycan BEH Amide Column, 130Å, 1.7 µm, 2.1 × 150 mm from Waters (Catalog No. 186004742)				
Column Temperature	60°C ± 1°C				
Autosampler Temperature	10°C ± 4°C				
Gradient	Time (min)	% A	% B	Flow (mL/min)	Curve
	Initial	11.8	88.2	0.45	Initial
	29	20.2	79.8	0.45	Linear
	65	25.3	64.7	0.45	Linear
	66.5	100.0	0.0	0.2	Isocratic
	69.5	100.0	0.0	0.2	Linear
	74	11.8	88.2	0.2	Isocratic
	77	11.8	88.2	0.45	Isocratic
Detector	85				
	Excitation wavelength at 310 nm and emission wavelength at 370 nm PMT gain set at 1.00				

- MS Tune Settings for N-Glycan Analysis
- Polarity: Positive
 - Spray Voltage: 3.8 kV
 - Capillary Temperature: 350°C
 - Sheath Gas: 40
 - Aux Gas: 10
 - Probe Heater Temperature: 250°C
 - S-Lens RF Level: 50
 - Ion Source: HESI
 - Range: 400 to 2000 m/z

FIG. 33

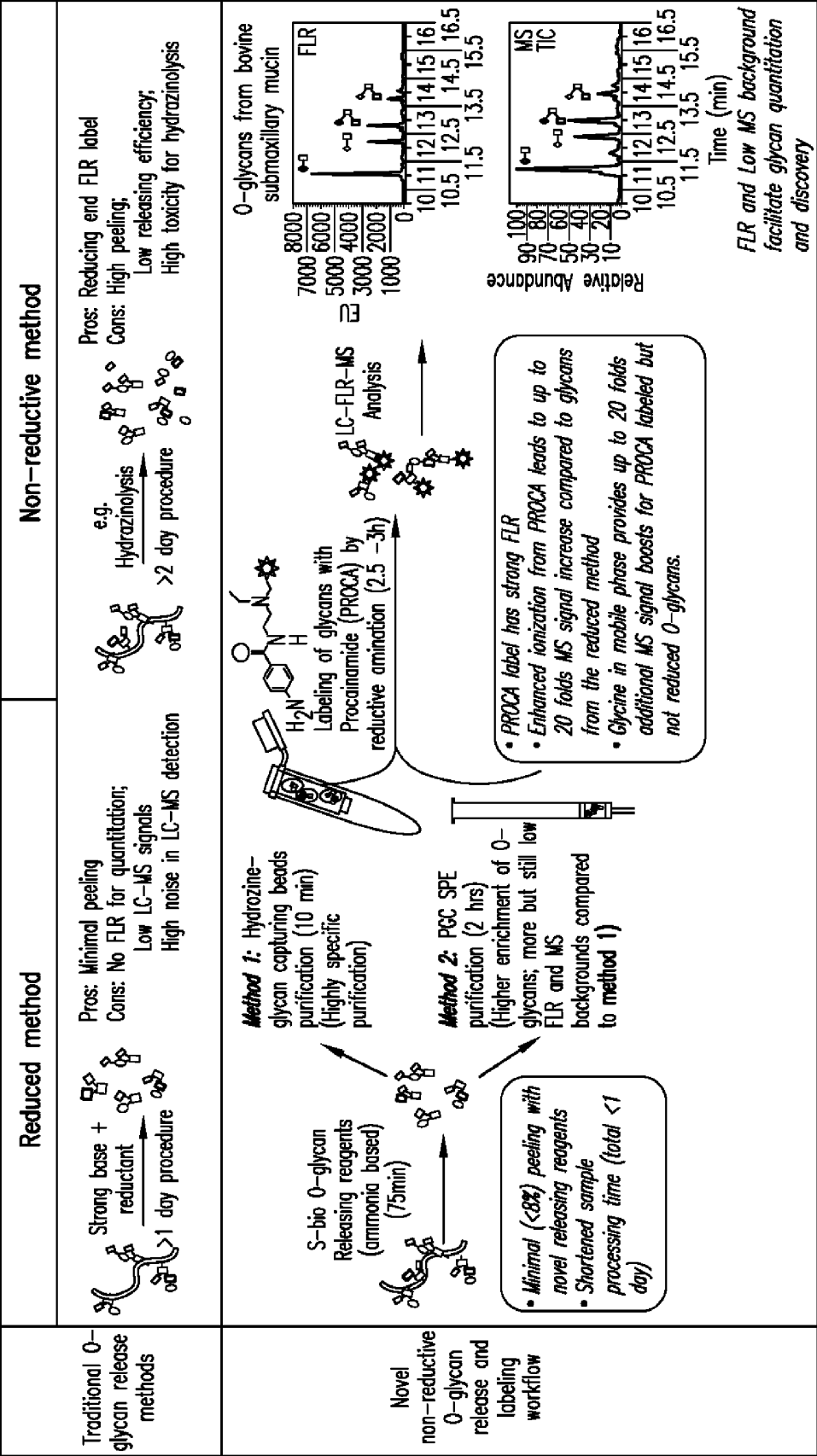


FIG. 34

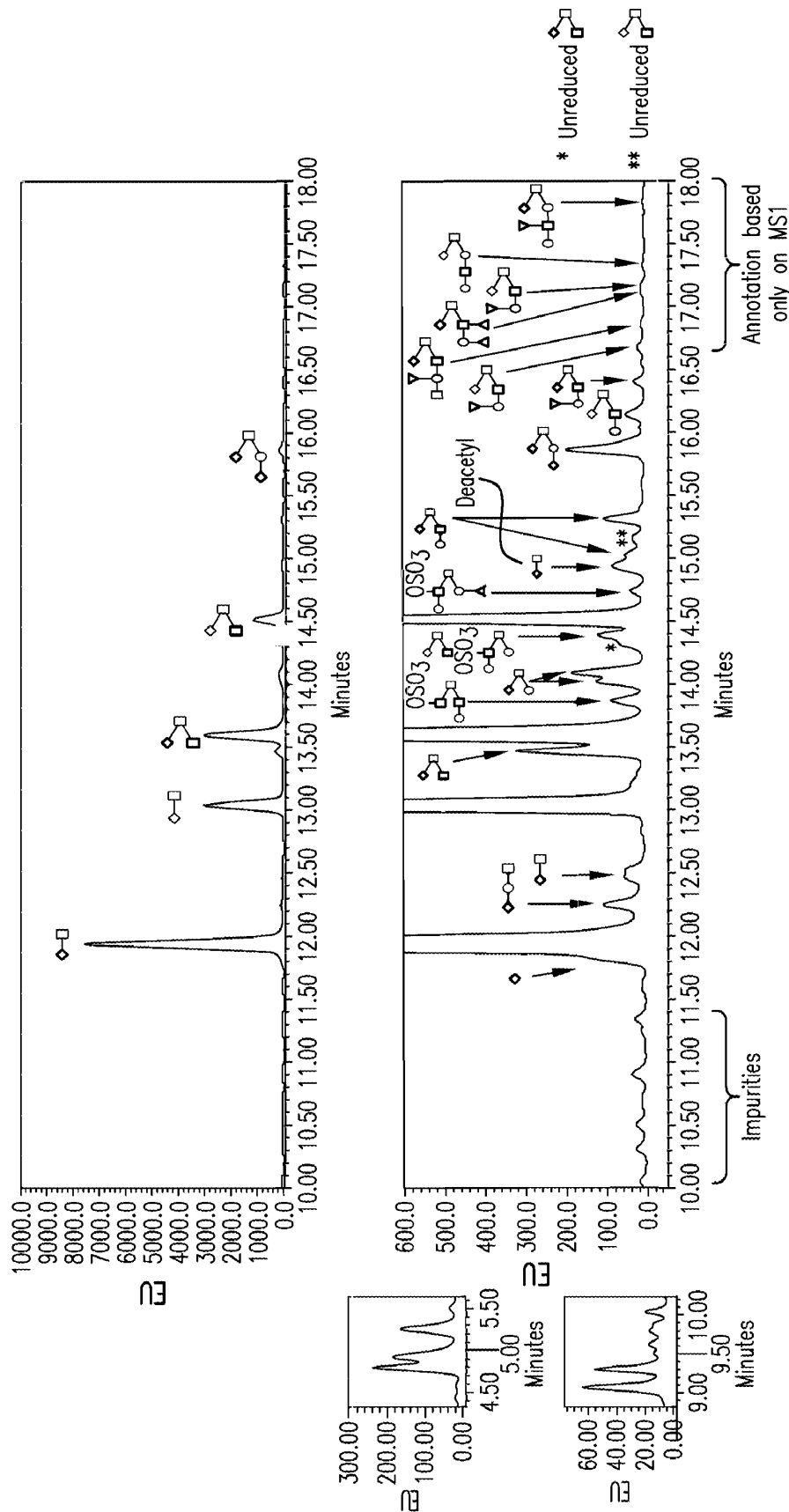


FIG. 35

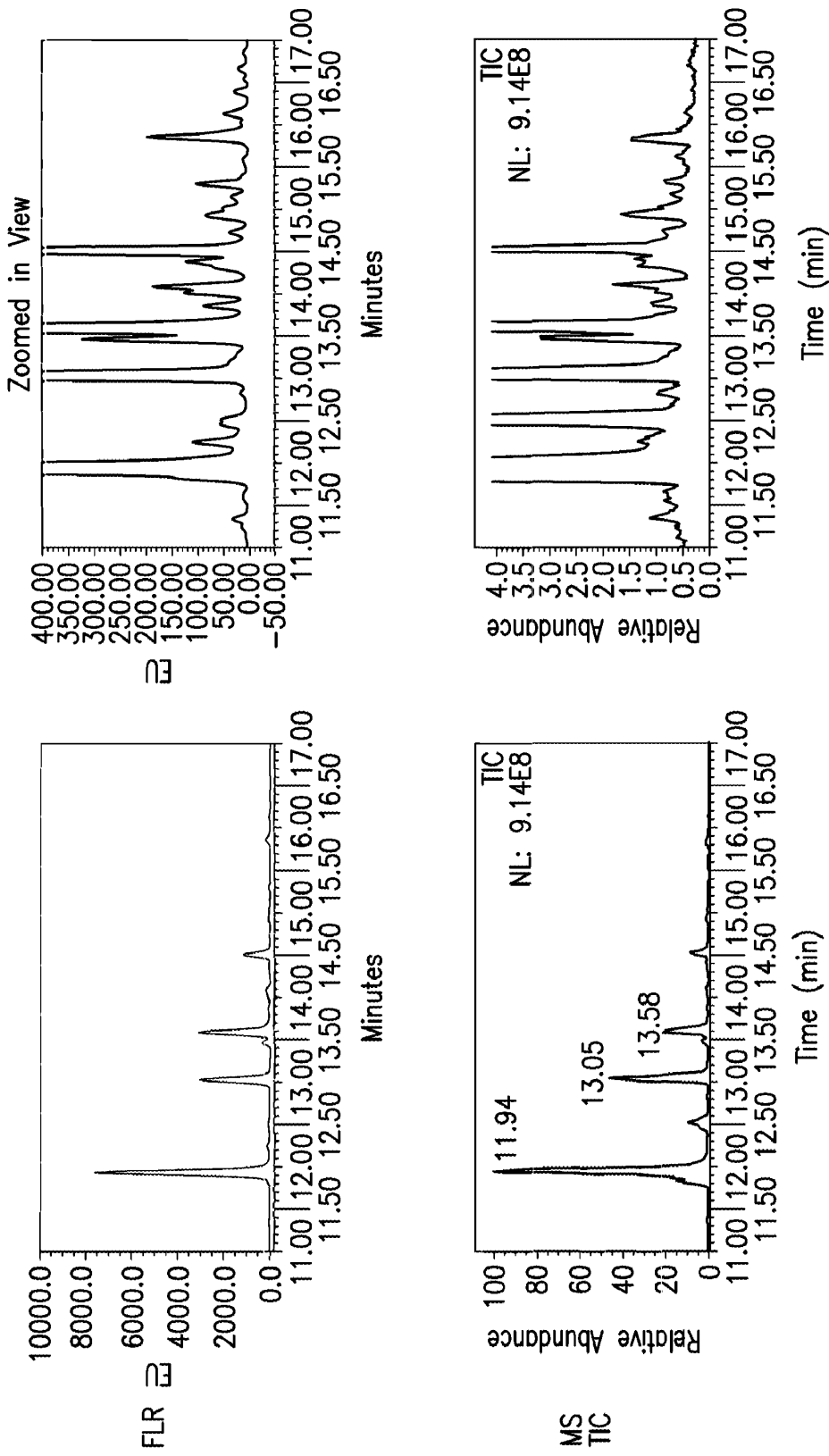
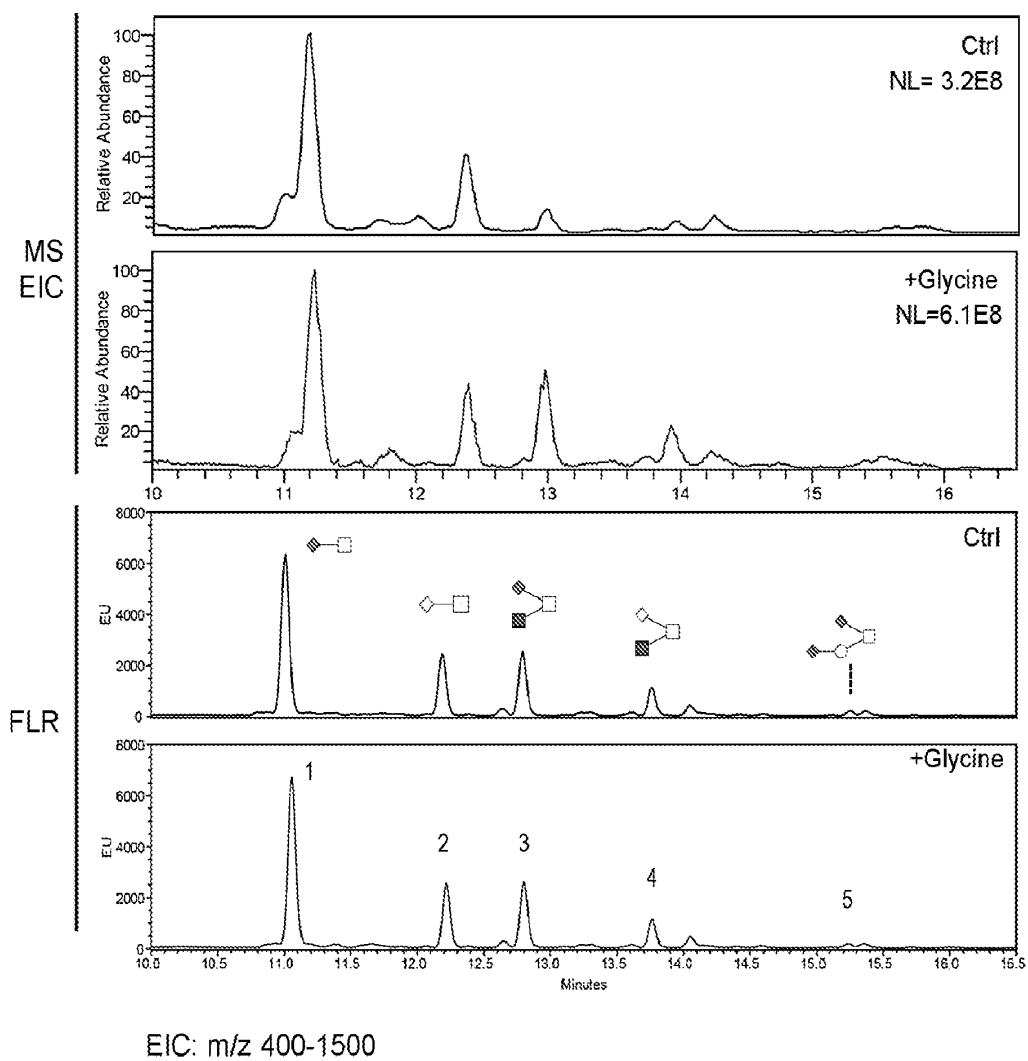


FIG. 36

**FIG. 37A**

Composition	Strcuture	Relative levels to HexNac1NeuAc1			
		FLR		MS	
		Ctrl	Glycine	Ctrl	Glycine
HexNac1NeuAc1		100.0%	100.0%	100.0%	100.0%
HexNac1NeuGc1		34.9%	34.9%	36.4%	37.8%
HexNac2NeuAc1		38.1%	38.1%	9.7%	36.0%
HexNac2NeuGc1		17.0%	17.0%	3.5%	12.7%
Hex1HexNac1NeuAc2		3.1%	3.1%	0.2%	2.1%

FIG. 37B

ProCA labeled BSM O-glycans

Composition	Average charge state		Total signal boost by glycine
	Ctrl	Glycine	
HexNAc	1.0	1.0	1.8
HexNAc2	1.0	1.0	3.2
HexNAc3	1.0	1.2	2.1
Hex1HexNAc1	1.0	1.0	3.7
HexNAc1NeuAc1	1.0	1.0	2.6
HexNAc1NeuGc1	1.0	1.0	2.7
HexNAc2NeuAc1	1.1	1.5	8.1
HexNAc2NeuGc1	1.1	1.5	9.0
Hex1HexNAc1NeuAc1	1.0	1.2	8.7
Hex1HexNAc1NeuAc1dHex1	1.1	1.6	8.8
Hex1HexNAc2NeuAc1	1.6	1.9	18.6
Hex1HexNAc2NeuGc1	1.6	1.9	21.7
Hex1HexNAc2NeuAc1dHex1	1.7	2.0	21.3
Hex1HexNAc2NeuGc1dHex1	1.7	2.0	23.7
Hex1HexNAc4dHex1	2.0	2.0	8.3
Hex3HexNAc4dHex1	2.0	2.0	14.0
Hex1HexNAc1NeuAc2	1.5	2.0	34.3
Hex1HexNAc1NeuAc1NeuGc1	1.2	2.0	156.5
Hex1HexNAc3NeuAc1	2.0	2.0	22.8
Hex1HexNAc3NeuAc1dHex	2.0	2.0	16.1
Hex1HexNAc2NeuAc1dHex2	1.7	2.0	30.5
Hex2HexNAc2NeuAc1dHex1	-	2.0	∞
Hex2HexNAc2NeuGc1	-	2.0	∞
Hex1HexNAc3Sulf1	1.0	1.4	6.3
Hex2HexNAc2Sulf1	1.0	1.1	4.0
Hex2HexNAc2dHex1Sulf1	1.0	1.2	4.6

N=3

FIG. 38A

2AB labeled BSM O-glycans

Composition	Average charge state		Total signal boost by glycine
	Ctrl	Glycine	
HexNAc2	1.0	1.0	2.7
HexNAc3	1.0	1.0	2.8
HexNAc1NeuAc1	1.0	1.0	2.2
HexNAc1NeuGc1	1.0	1.0	1.4
HexNAc2NeuAc1	1.0	1.0	1.3
HexNAc2NeuGc1	1.0	1.0	1.0

FIG. 38B

Reduced BSM O-glycans

Composition	Average charge state		Total signal boost by glycine
	Ctrl	Glycine	
HexNAc	1.0	1.0	5.5
HexNAc2	1.0	1.0	5.5
HexNAc3	1.0	1.0	3.9
Hex1HexNAc1	1.0	1.0	12.2
HexNAc1NeuAc1	1.0	1.0	2.2
HexNAc1NeuGc1	1.0	1.0	2.6
HexNAc2NeuAc1	1.0	1.0	1.8
HexNAc2NeuGc1	1.0	1.0	1.7
Hex1HexNAc1NeuAc1	1.0	1.0	2.1
Hex1HexNAc1NeuAc1dHex1	1.0	1.0	1.7
Hex1HexNAc2NeuAc1	1.0	1.0	0.9
Hex1HexNAc2NeuGc1	1.0	1.0	1.4
Hex1HexNAc2NeuAc1dHex1	1.0	1.0	1.1
Hex1HexNAc2NeuGc1dHex1	1.0	1.0	0.2
Hex1HexNAc4dHex1	1.0	1.0	0.8
Hex3HexNAc4dHex1	1.0	1.0	0.3
Hex1HexNAc1NeuAc2	1.0	1.0	0.2
Hex1HexNAc1NeuAc1NeuGc1	1.0	1.0	0.1

N =3

FIG. 38C

UPLC System	Waters Acquity UPLC I-Class				
Mobile Phase	A: 50 mM ammonium formate, pH 4.4, with or without 1 mM glycine				
	B: 15% 50 mM ammonium formate, pH 4.4/85% acetonitrile, with or without 1 mM total concentration of glycine				
Column	ACQUITY UPLC Glycan BEH Amide Column, 130Å, 1.7 µm, 2.1x150 mm from Waters (Catalog No. 186004742)				
Column Temperature	60°C ± 1 °C				
Autosampler Temperature	10°C ± 4 °C				
Gradient	Time (min)	% A	% B	Flow (mL/min)	Curve
	Initial	0	100.0	0.45	Initial
	5	0	100.0	0.45	Linear
	20	29.5	70.5	0.45	Linear
	23.5	100.0	0.0	0.2	Isocratic
	26.5	100.0	0.0	0.2	Linear
	30	0	100	0.2	Isocratic
	33	0	100	0.45	Isocratic
	40	0	100	0.45	Isocratic
Detector Wavelength for PROCA labeled O-glycans	Excitation wavelength at 310 nm and emission wavelength at 370 nm PMT gain set at 1.00				

MS Tune Settings for O-Glycan Analysis

- Polarity: Positive
- Spray Voltage: 3.8 kV
- Capillary Temperature: 350 °C
- Sheath Gas: 40
- Aux Gas: 10
- Probe Heater Temperature: 250 °C
- S-Lens RF Level: 50
- Ion Source: HESI
- Range: 220 to 1500 m/z

FIG. 39

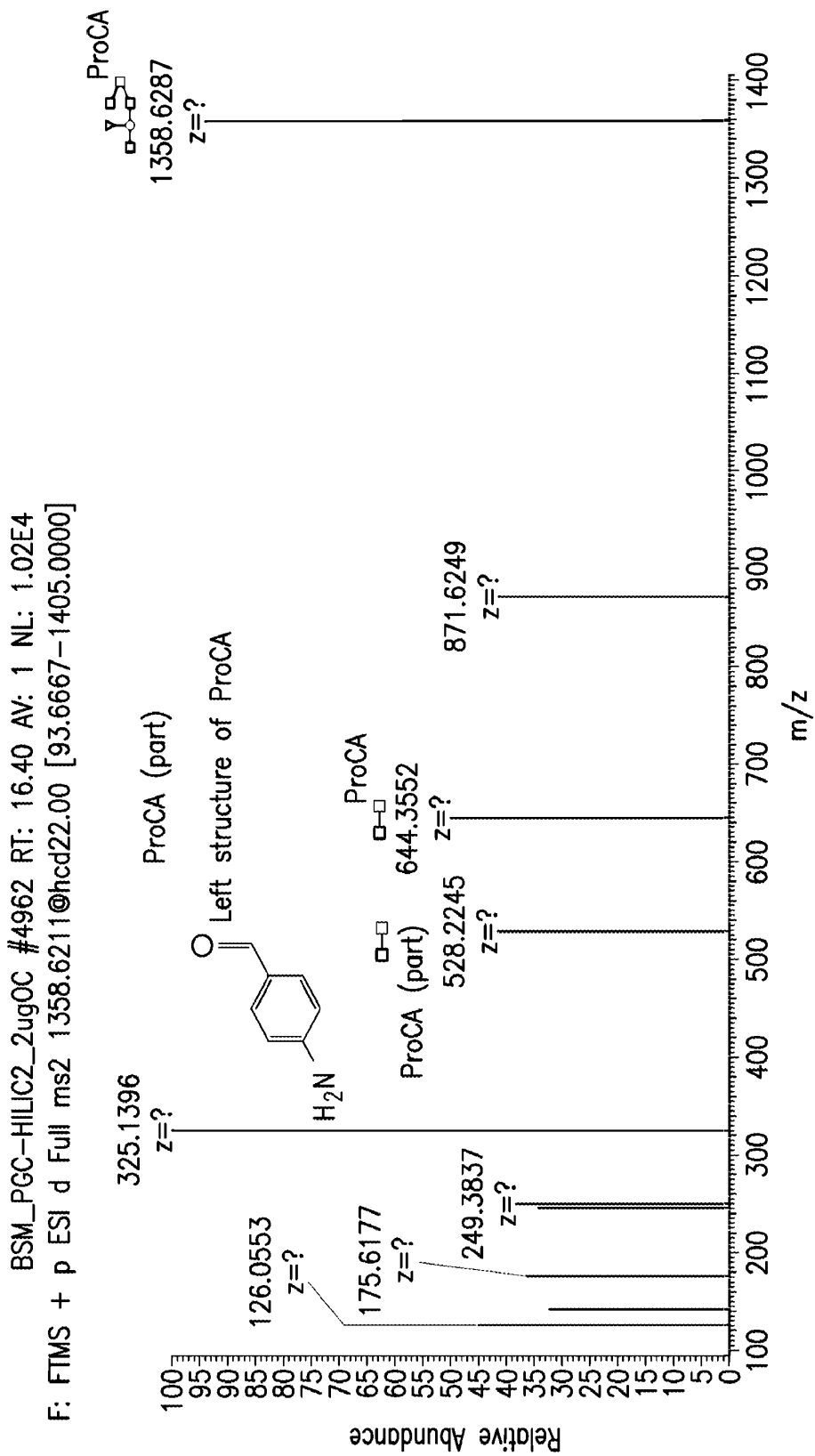


FIG. 40

BSM-Glycan-ProCA-Sbio_1ugOC_Glycine #5339 RT: 15.87 AV: 1 NL: 9.45E4
F: FTMS + p ESI d Full ms2 679.8138@hcd22.00 [93.6667-1405.0000]

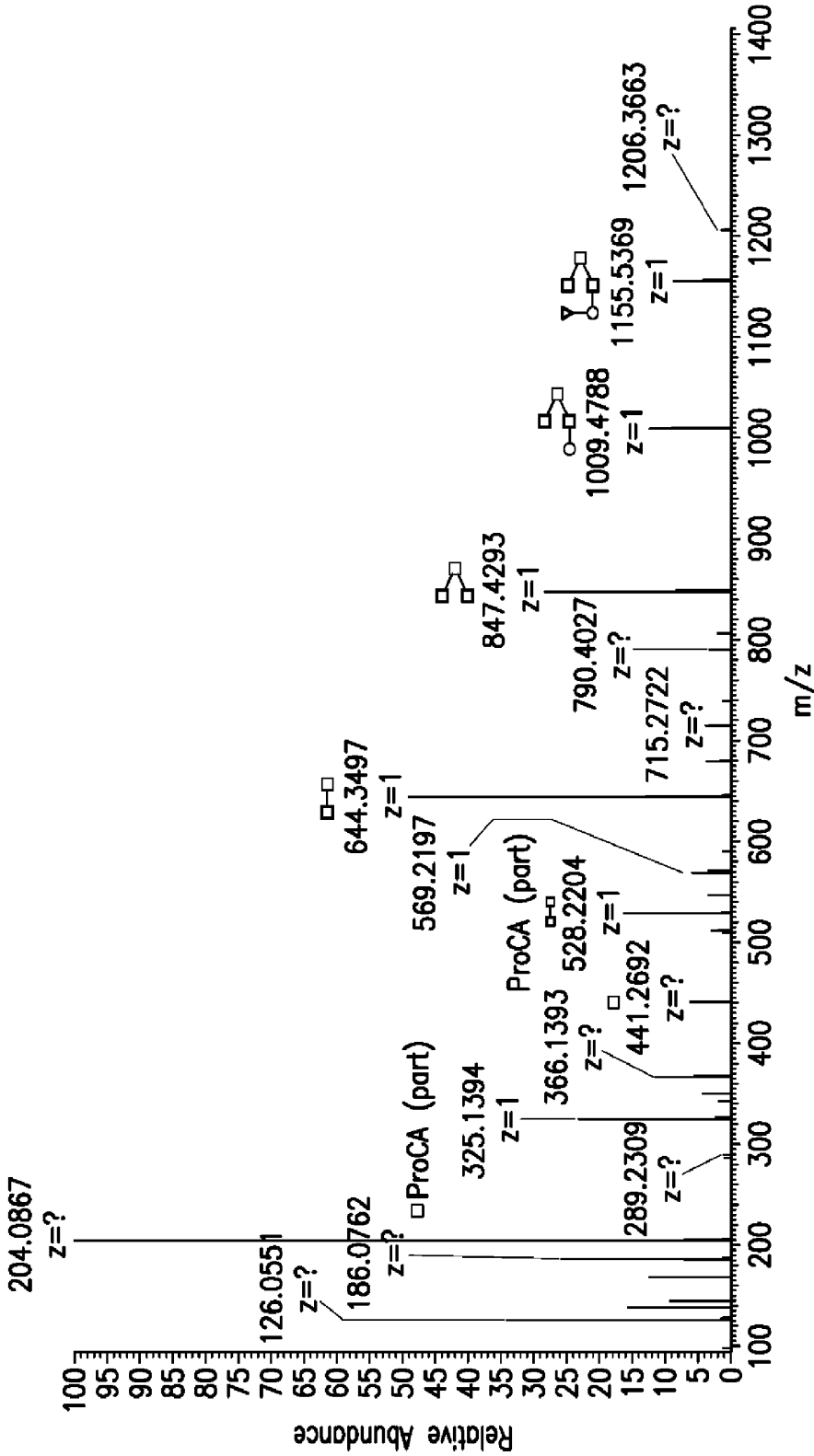


FIG. 41

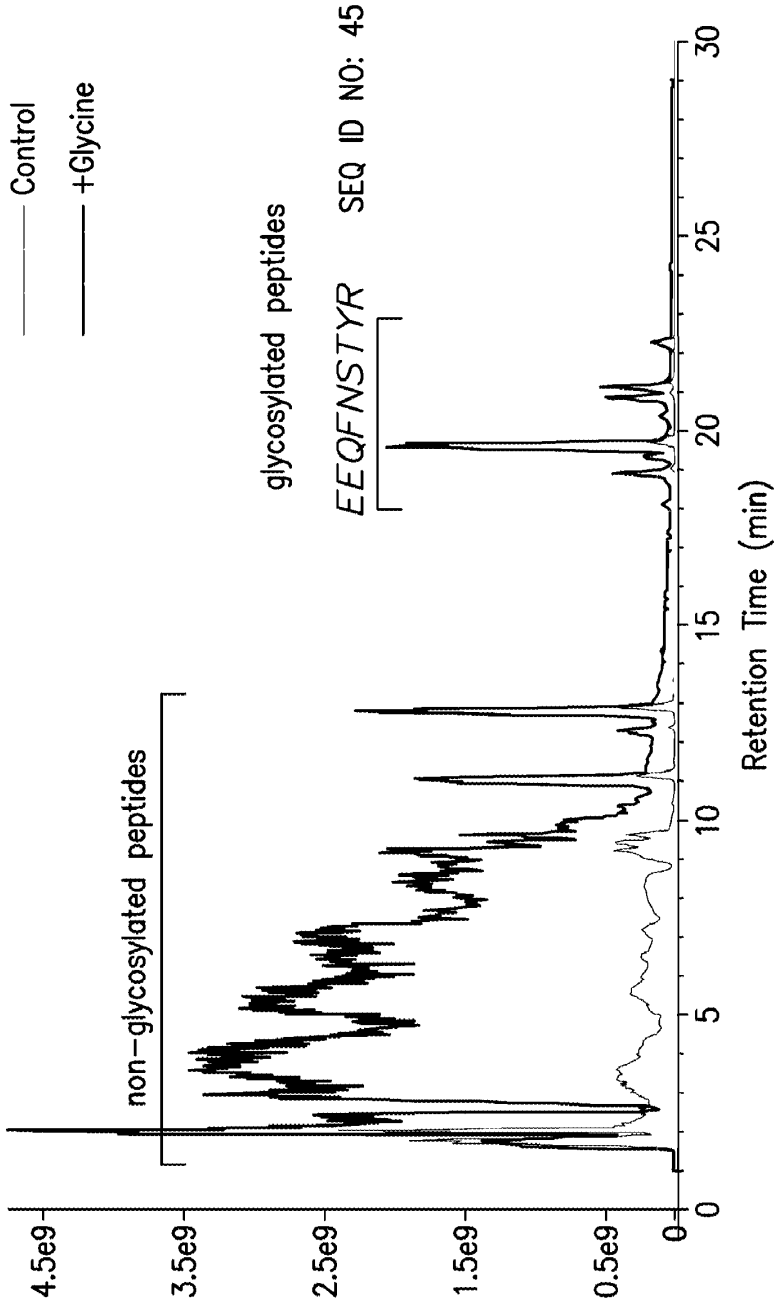


FIG. 42A

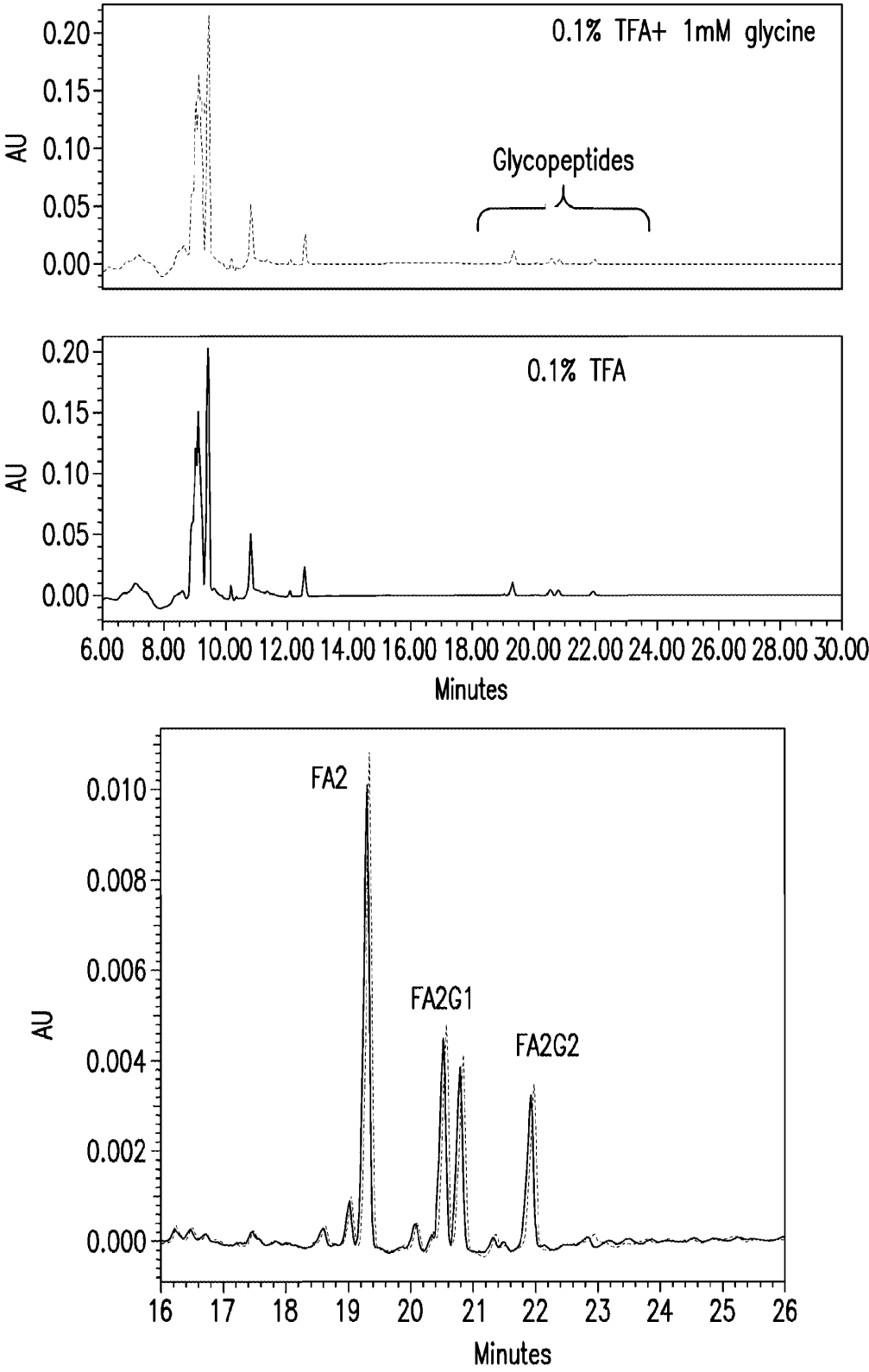


FIG.42B

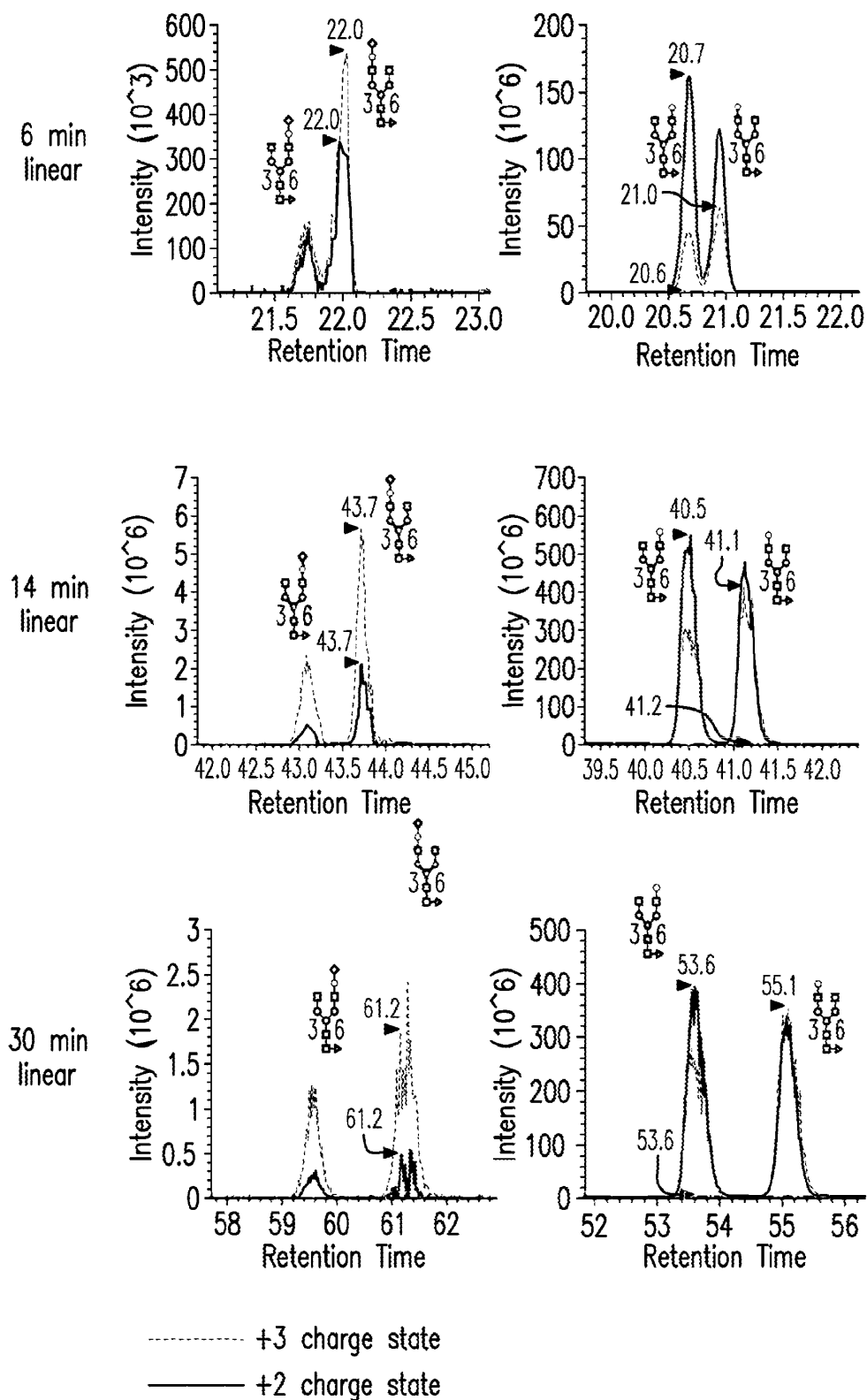


FIG. 42C-1

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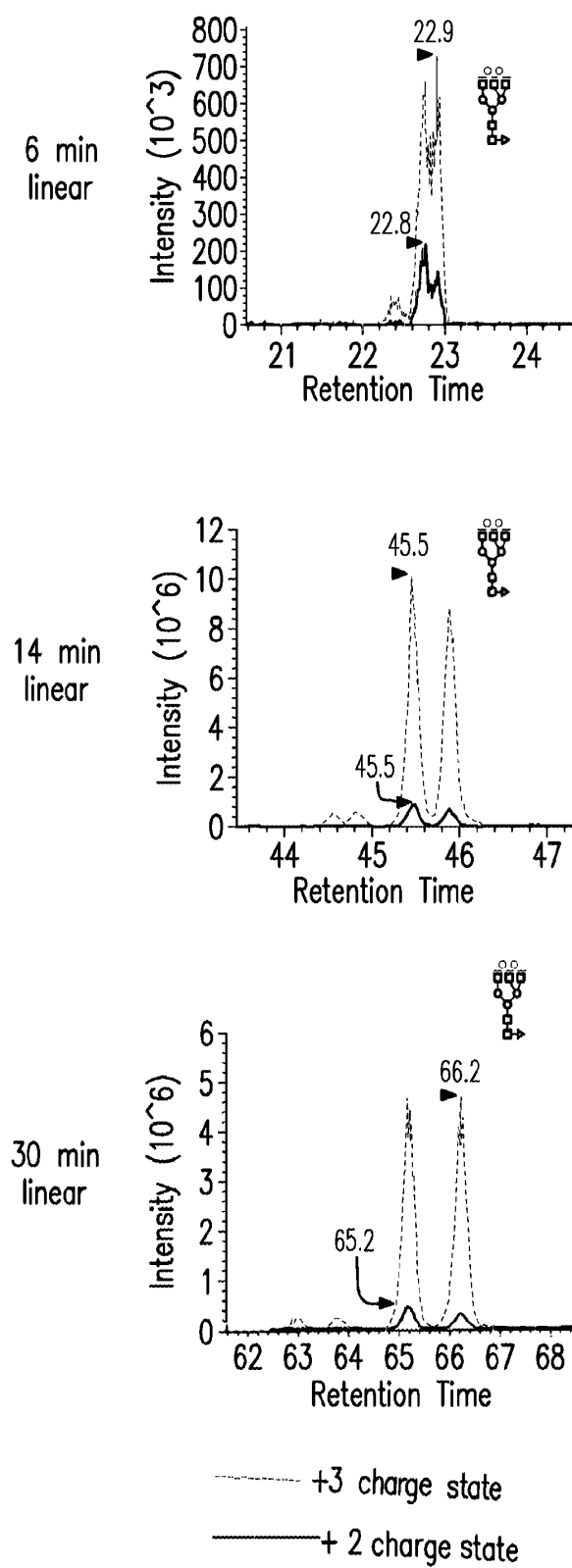


FIG. 42C-2

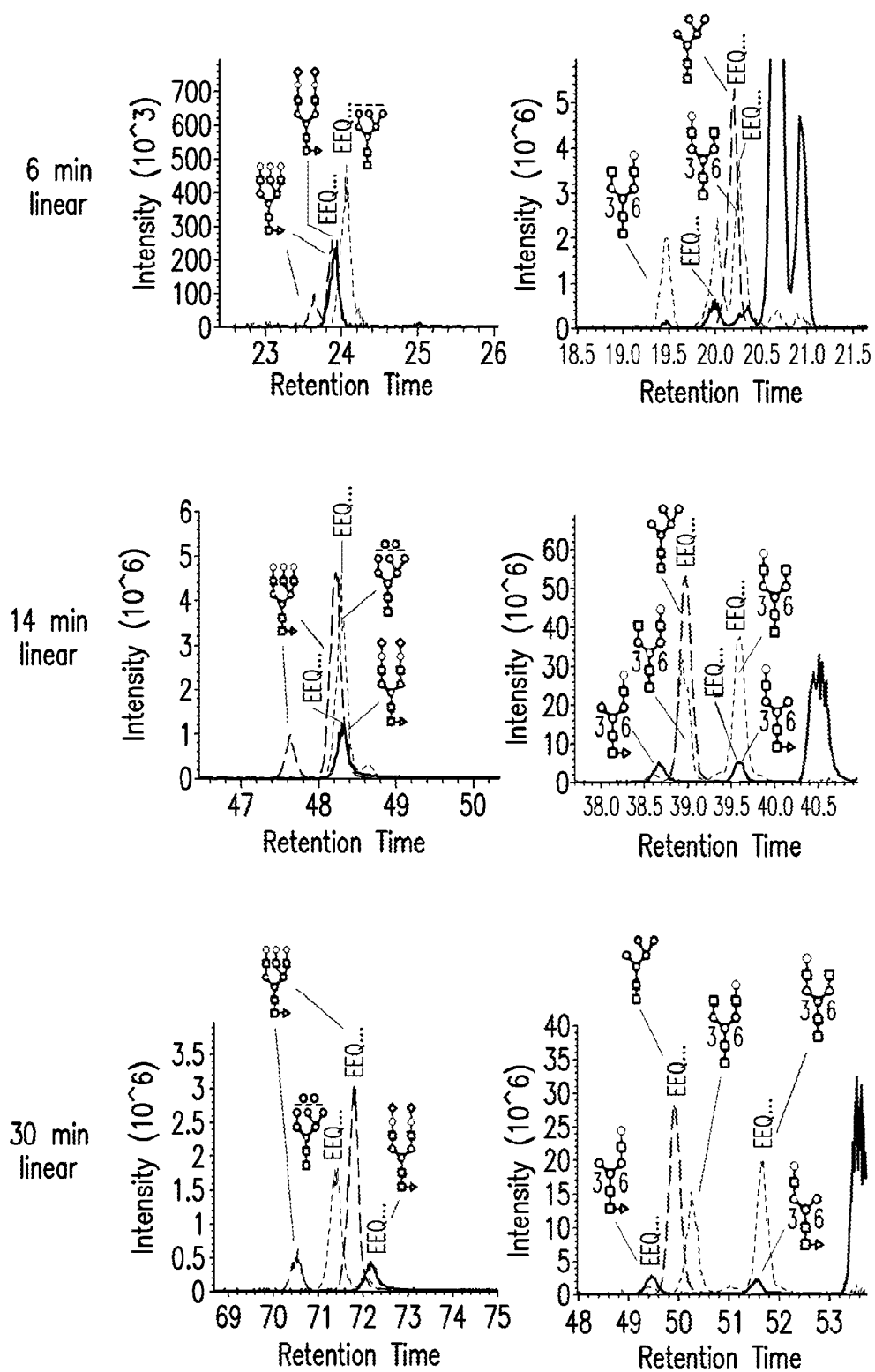


FIG. 42C-3

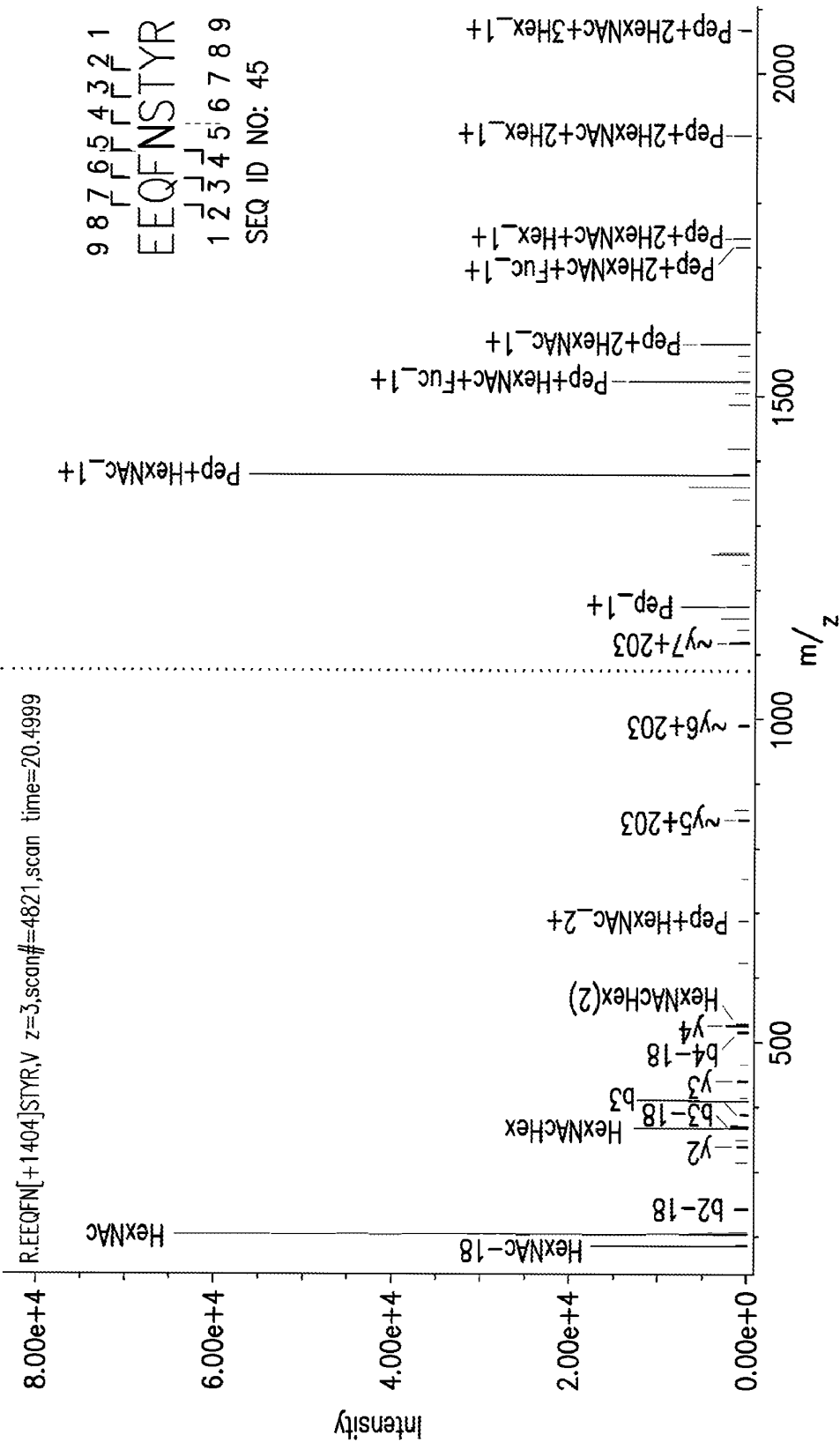


FIG. 42D

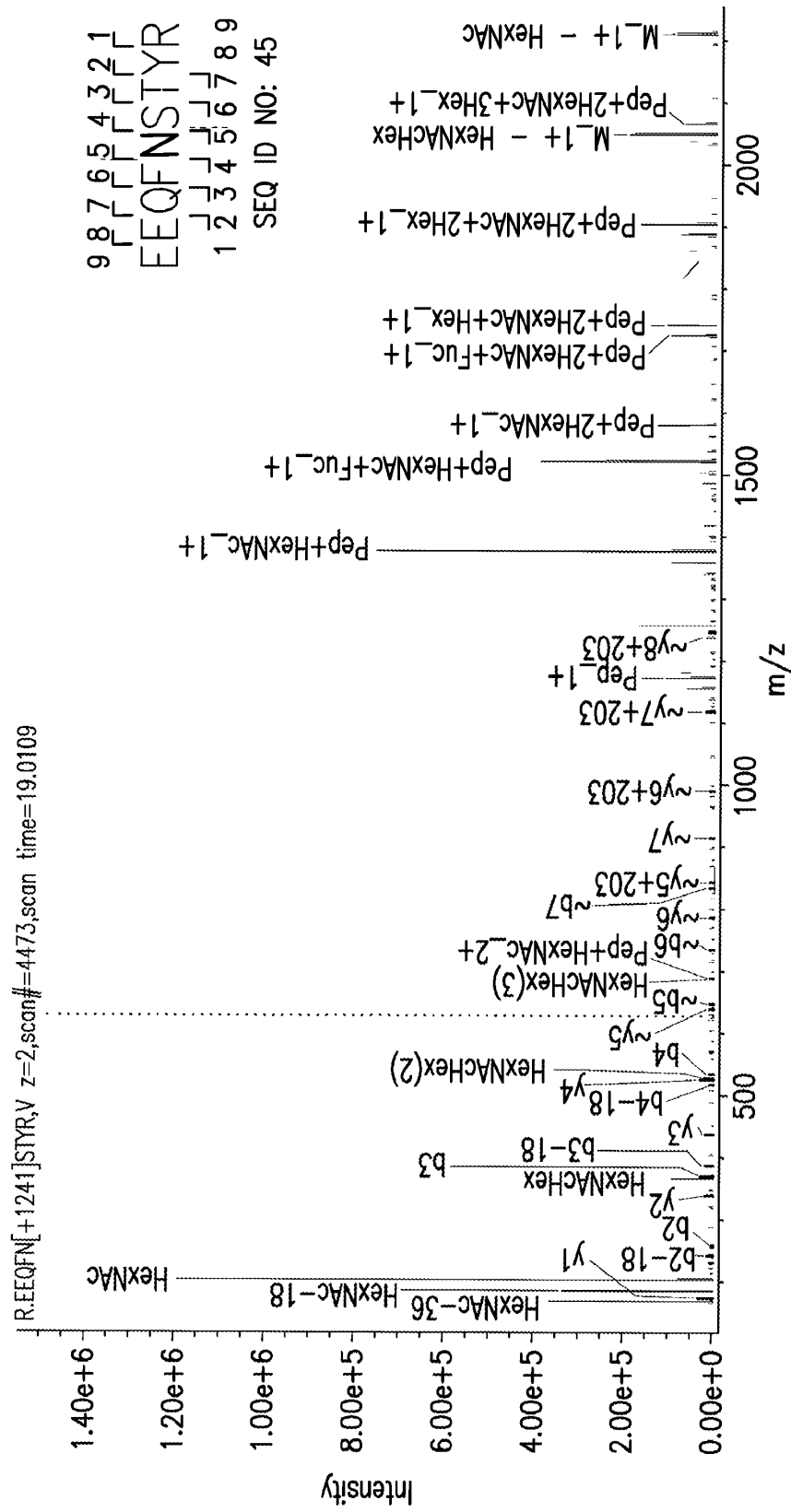


FIG. 42E

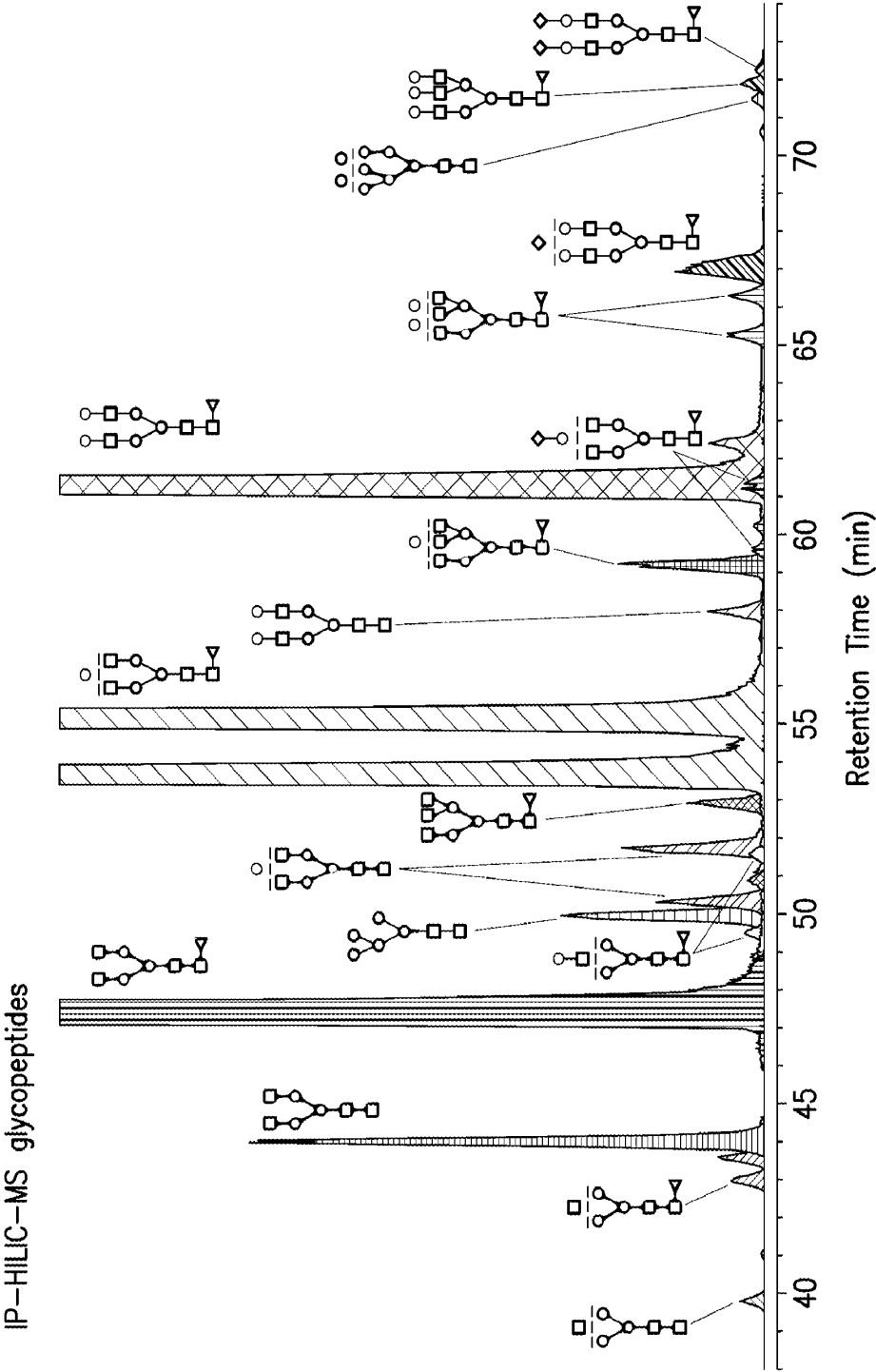
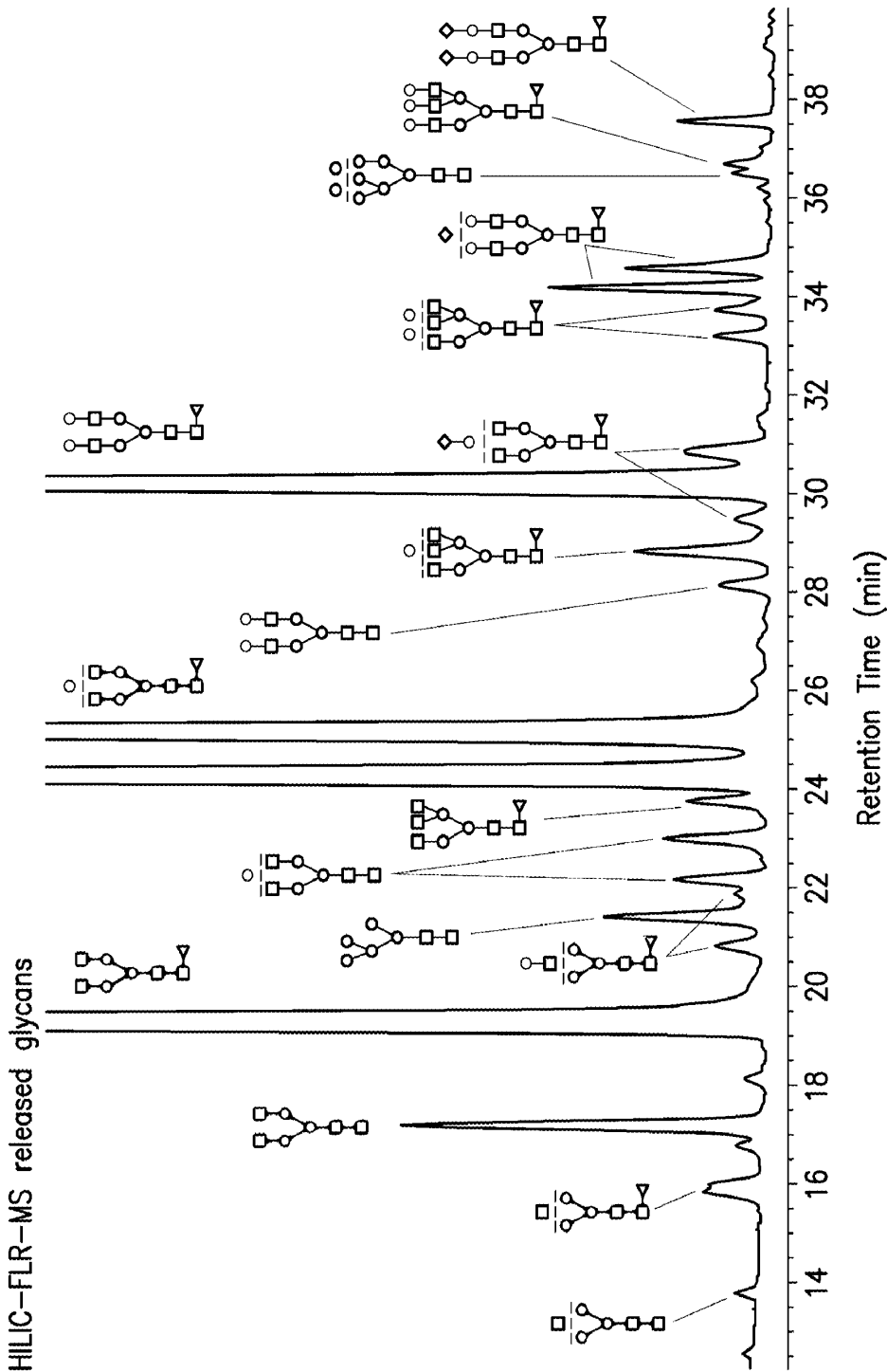


FIG. 42F



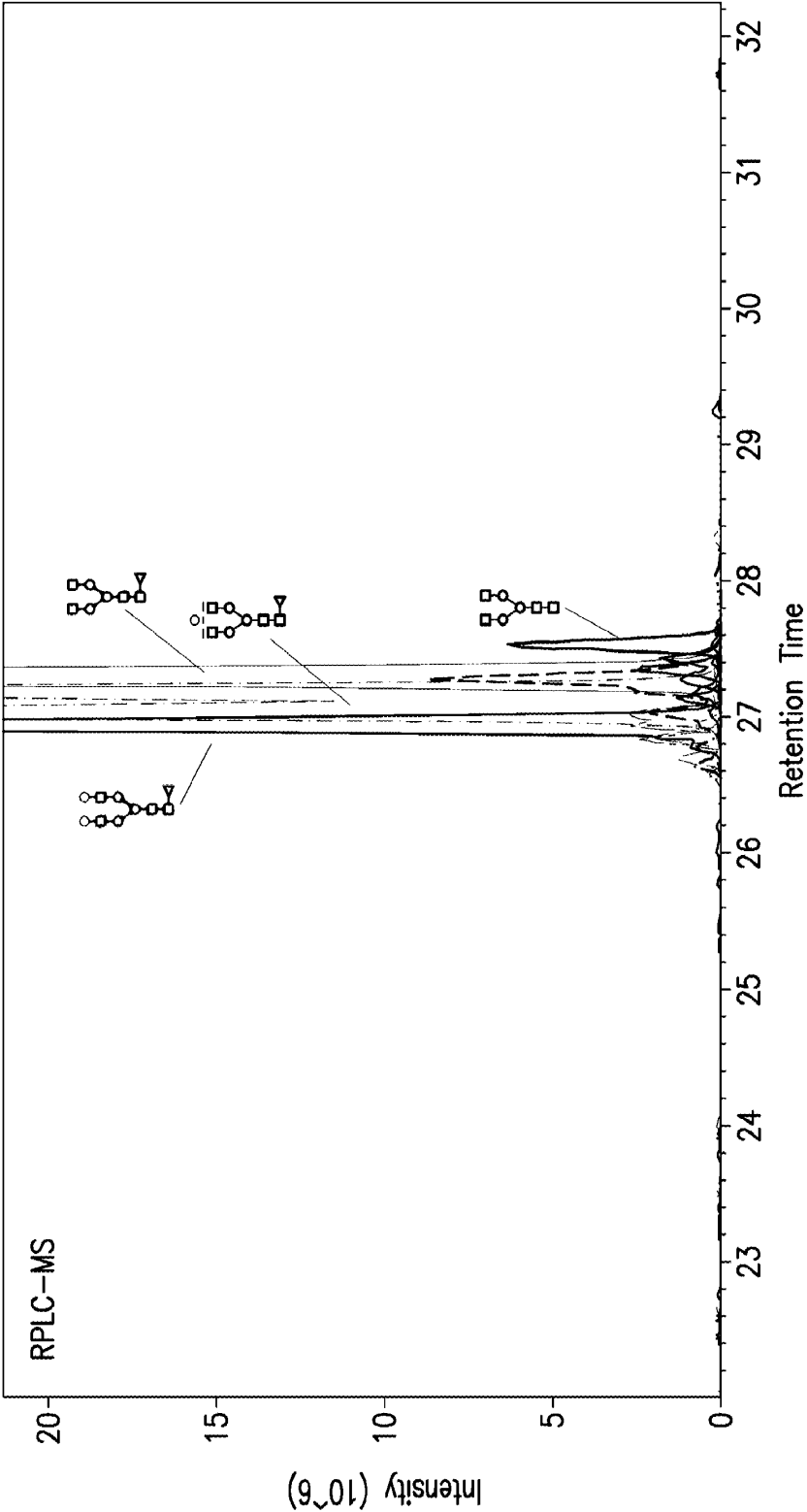


FIG. 42H-1

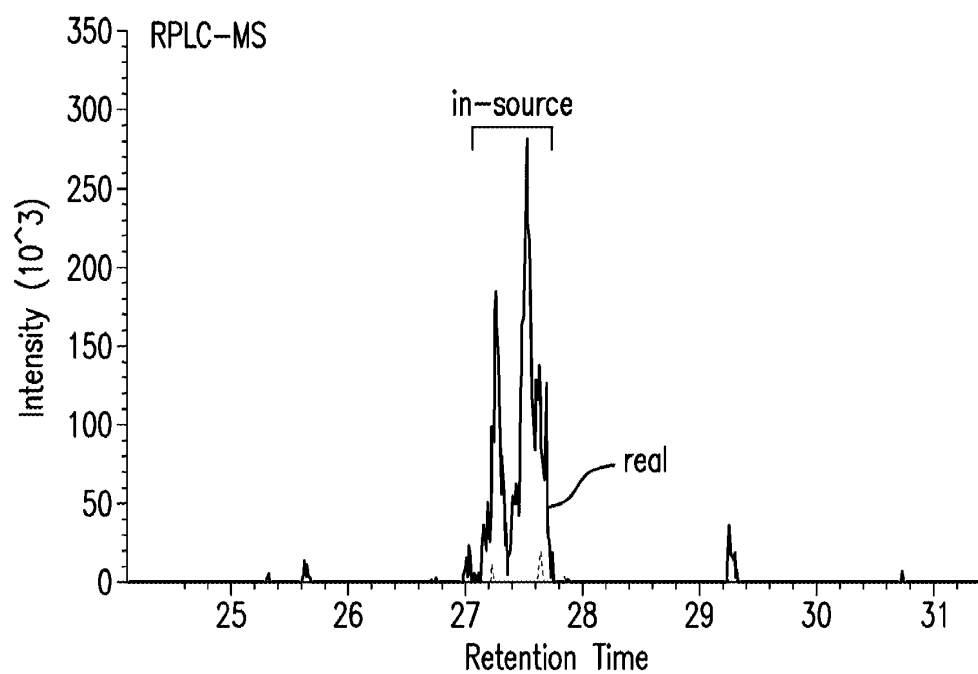
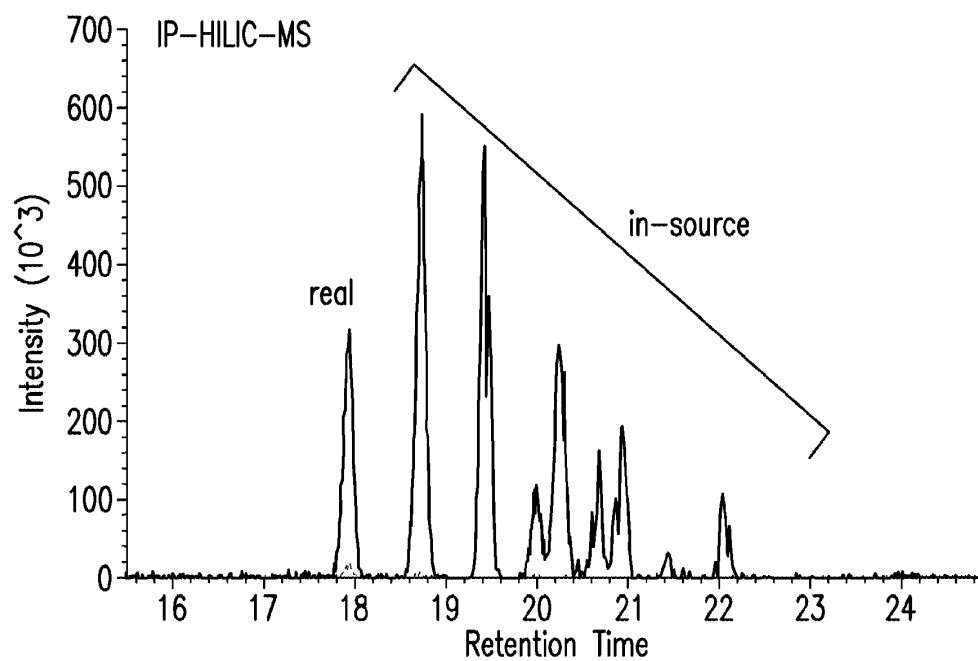


FIG. 42H-2

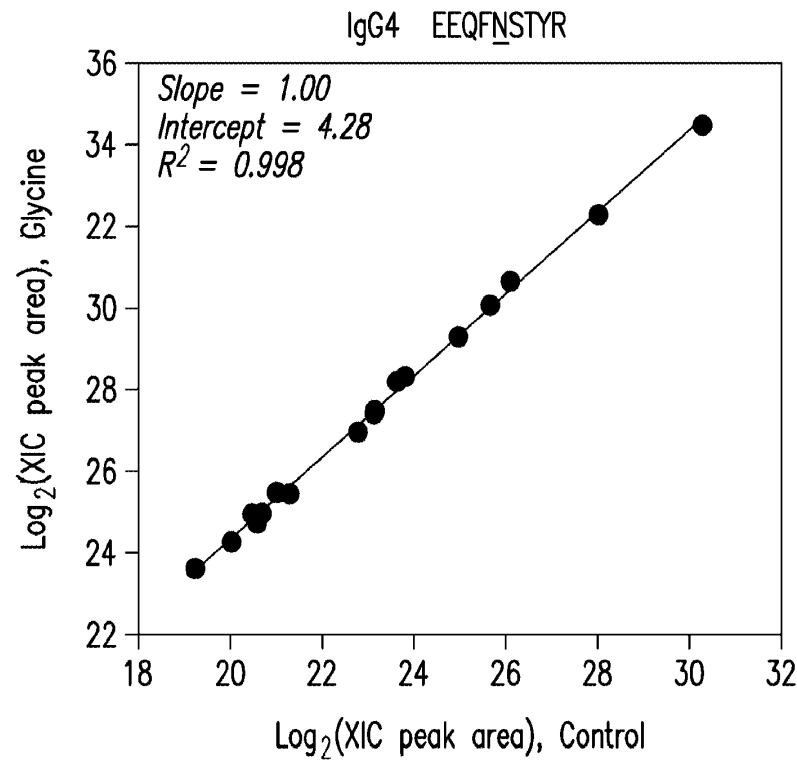


FIG. 43A

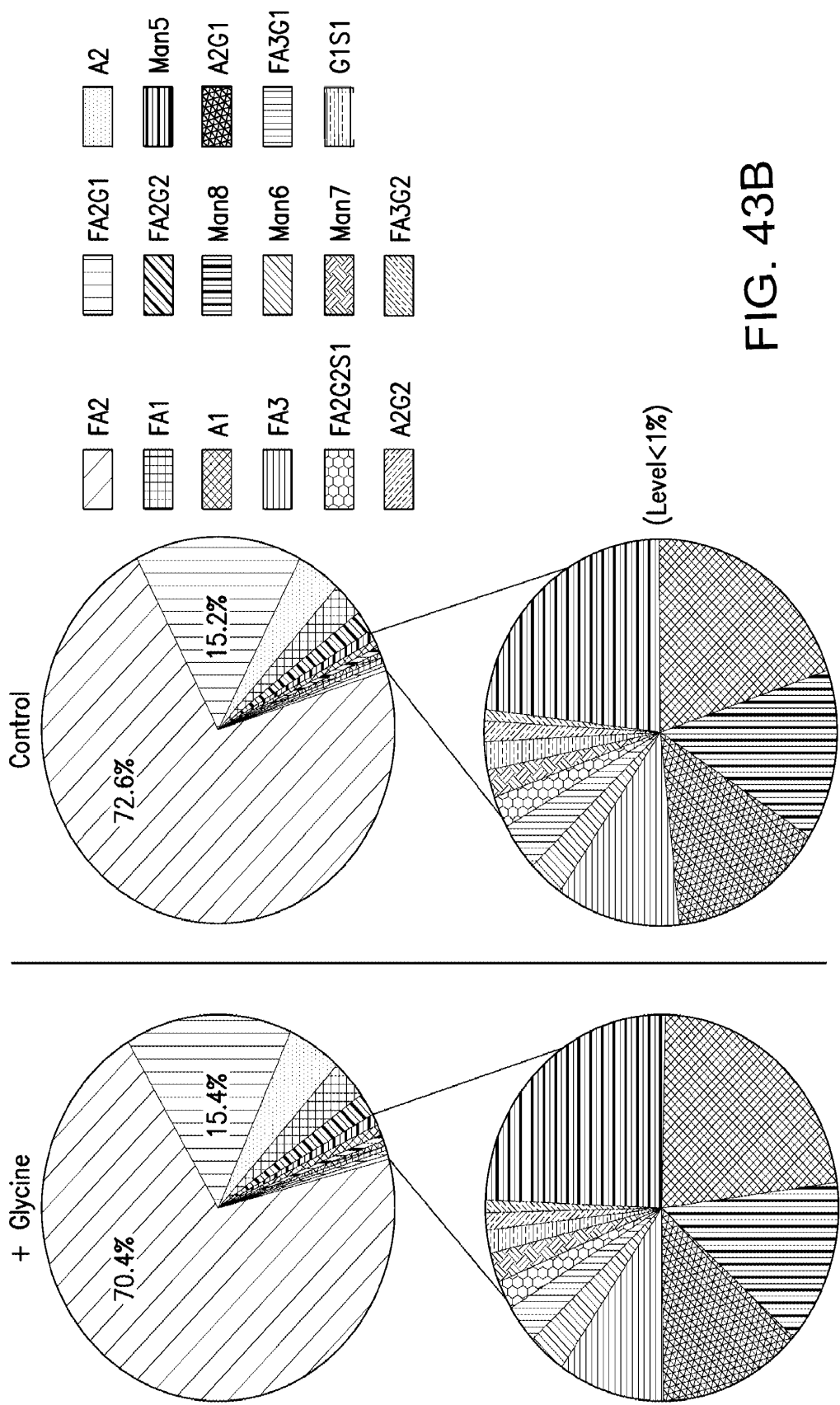


FIG. 43B

Glycan Name	0.1% TFA + 1 mM Glycine				0.1% TFA				Average fold change
	Replicate 1		Replicate 2		Replicate 1		Replicate 2		
	Peak area	Level	Peak area	Level	Peak area	Level	Peak area	Level	
A1	1.8E+08	0.63%	1.8E+08	0.64%	9.0E+06	0.63%	1.2E+07	0.64%	17.6
A1G1	7.8E+07	0.27%	7.5E+07	0.26%	3.2E+06	0.23%	4.3E+06	0.24%	20.3
FA1	4.3E+08	1.47%	4.2E+08	1.47%	2.0E+07	1.44%	2.5E+07	1.41%	18.5
FA1G1	2.3E+08	0.78%	2.2E+08	0.76%	8.9E+06	0.62%	1.1E+07	0.63%	21.8
FA1G1S1	1.8E+07	0.06%	1.8E+07	0.06%	9.1E+05	0.06%	1.2E+06	0.07%	16.8
A2	2.7E+09	9.35%	2.6E+09	9.08%	1.2E+08	8.40%	1.5E+08	8.41%	19.5
A2G1	9.6E+08	3.29%	9.2E+08	3.21%	3.7E+07	2.62%	4.9E+07	2.74%	21.5
A2G2	7.8E+07	0.27%	7.6E+07	0.27%	3.6E+06	0.26%	4.7E+06	0.26%	18.6
FA2	1.3E+10	43.06%	1.2E+10	43.29%	6.7E+08	47.20%	8.2E+08	45.73%	16.6
FA2G1	9.1E+09	31.44%	9.0E+09	31.60%	4.4E+08	31.14%	5.8E+08	32.19%	17.7
FA2G2	1.8E+09	6.05%	1.7E+09	6.05%	6.6E+07	4.61%	8.7E+07	4.84%	22.7
FA2G2S1	6.1E+07	0.21%	5.7E+07	0.20%	2.5E+06	0.18%	3.5E+06	0.19%	19.5
A3	6.2E+06	0.02%	5.5E+06	0.02%	2.0E+05	0.01%	3.1E+05	0.02%	23.1
FA3	2.7E+07	0.09%	2.7E+07	0.09%	1.1E+06	0.08%	1.5E+06	0.08%	20.6
FA3G1	2.5E+07	0.09%	2.5E+07	0.09%	9.9E+05	0.07%	1.4E+06	0.08%	21.4
Man3	1.1E+08	0.39%	1.1E+08	0.39%	5.3E+06	0.37%	6.7E+06	0.37%	18.8
Man3F	8.5E+06	0.03%	8.0E+06	0.03%	3.7E+05	0.03%	5.0E+05	0.03%	18.9
Man4	1.1E+07	0.04%	1.1E+07	0.04%	2.8E+05	0.02%	5.4E+05	0.03%	27.0
Man4_A1G1	3.1E+07	0.11%	2.8E+07	0.10%	1.3E+06	0.09%	1.6E+06	0.09%	20.0
Man4_FA1G1	3.4E+07	0.12%	3.3E+07	0.11%	1.6E+06	0.11%	2.1E+06	0.12%	17.9
Man5	5.3E+08	1.82%	5.2E+08	1.83%	2.0E+07	1.42%	2.6E+07	1.44%	22.8
Man5_A1G1	2.1E+07	0.07%	2.0E+07	0.07%	9.3E+05	0.06%	1.3E+06	0.07%	18.8
Man6	3.9E+07	0.13%	3.6E+07	0.13%	1.7E+06	0.12%	2.2E+06	0.12%	19.3
Man7	2.8E+07	0.10%	2.7E+07	0.09%	1.1E+06	0.08%	1.5E+06	0.08%	20.9
Man8	3.6E+07	0.12%	3.3E+07	0.12%	1.8E+06	0.12%	2.3E+06	0.13%	16.9

FIG. 43C

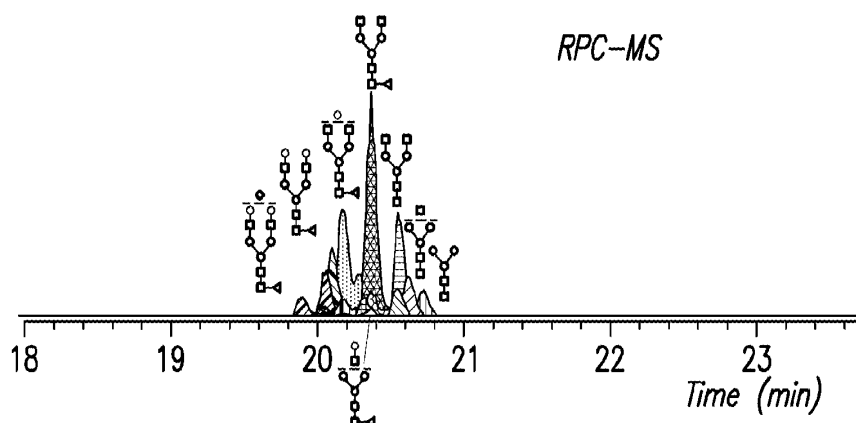
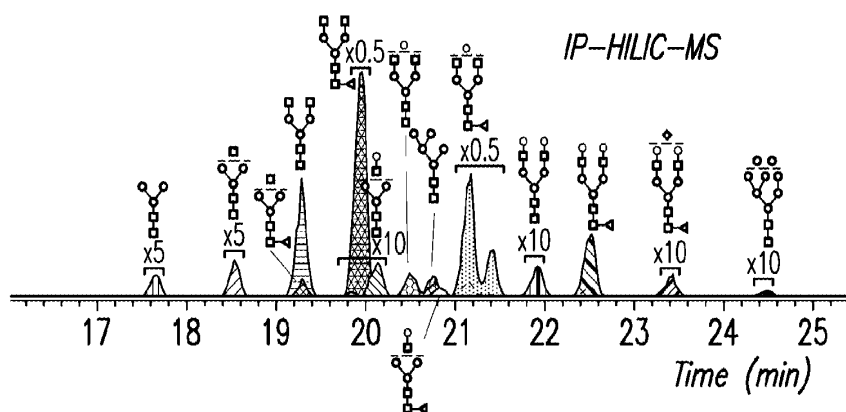
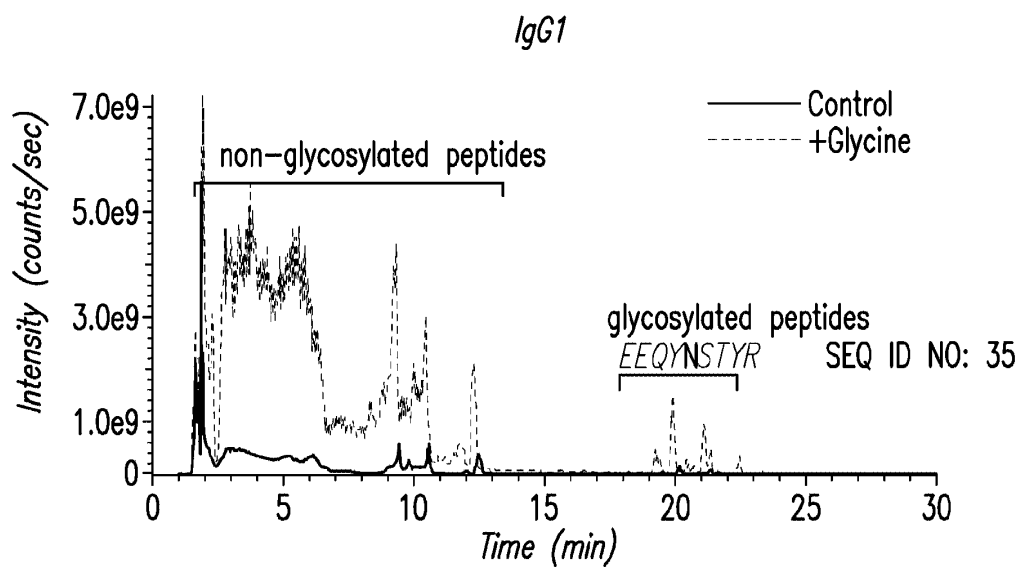


FIG. 43D-1

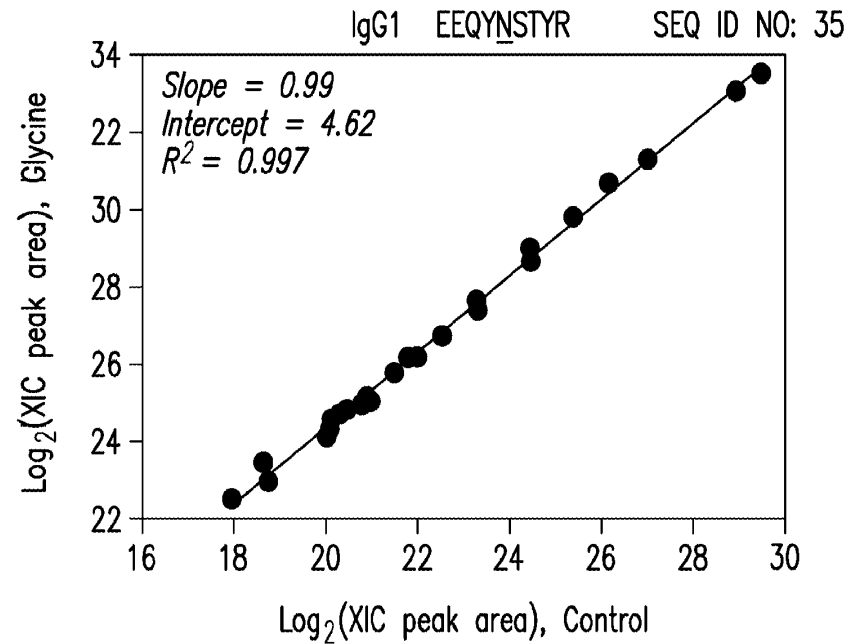


FIG. 43D-2

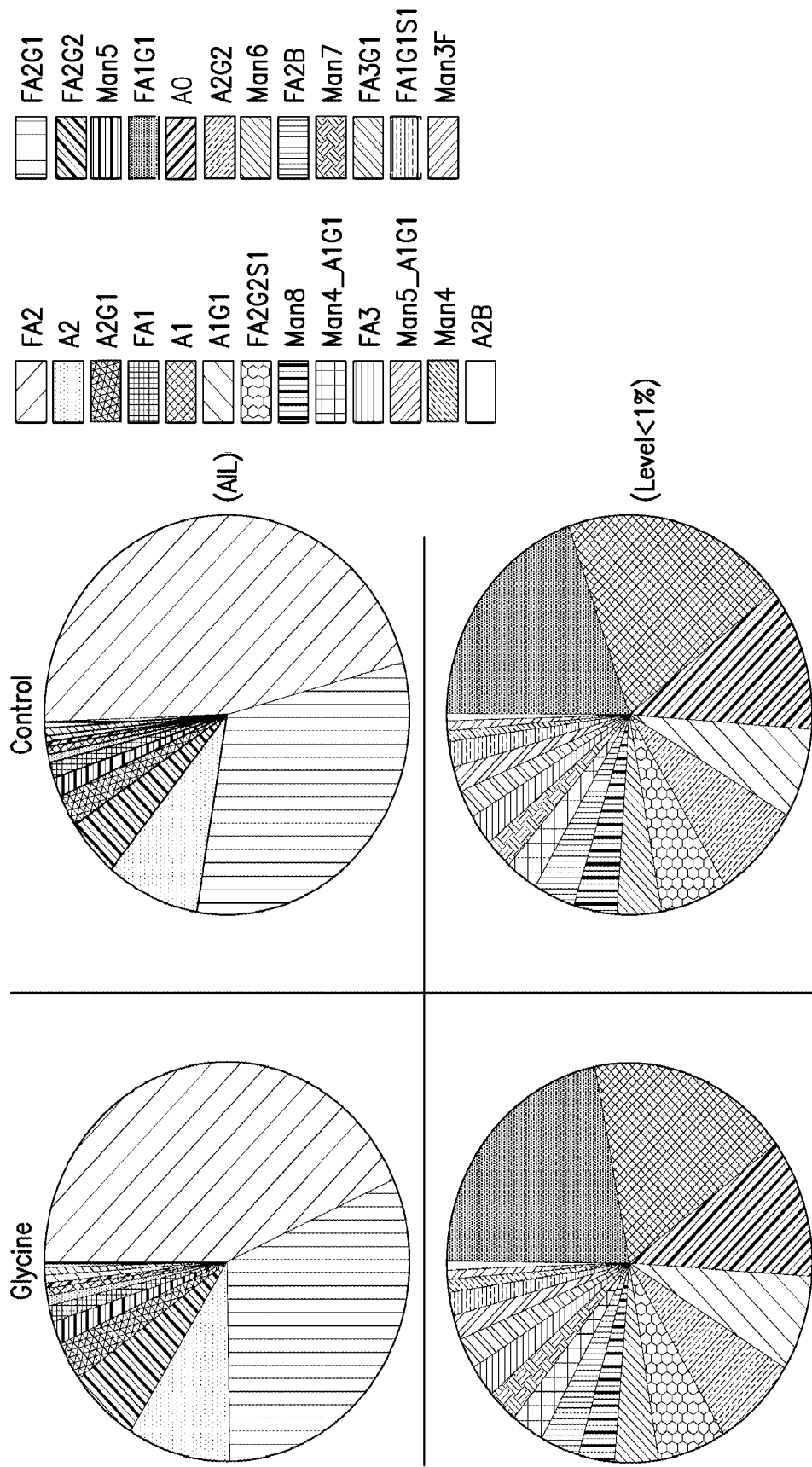


FIG. 43D-3

Glycan Name	0.1% TFA + 1 mM Glycine						0.1% TFA						Average fold change
	Replicate 1			Replicate 2			Replicate 1			Replicate 2			
	Peak area	Level		Peak area	Level		Peak area	Level		Peak area	Level		
A1	3.1E+08	0.91%		3.0E+08	0.88%		1.2E+07	0.70%		1.3E+07	0.72%		23.8
FA1	1.1E+09	3.33%		1.1E+09	3.28%		5.1E+07	2.87%		5.5E+07	2.97%		21.4
A2	1.7E+09	5.00%		1.7E+09	4.91%		7.2E+07	4.01%		7.2E+07	3.91%		23.7
A2G1	1.8E+08	0.51%		1.8E+08	0.52%		9.0E+06	0.50%		9.4E+06	0.51%		19.4
A2G2	2.0E+07	0.06%		1.9E+07	0.06%		1.0E+06	0.06%		1.1E+06	0.06%		18.8
FA2	2.4E+10	70.31%		2.4E+10	70.53%		1.3E+09	73.32%		1.3E+09	71.87%		18.4
FA2G1	5.3E+09	15.39%		5.3E+09	15.46%		2.6E+08	14.56%		2.9E+08	15.76%		19.2
FA2G1S1	2.9E+07	0.08%		2.6E+07	0.08%		1.5E+06	0.08%		1.6E+06	0.09%		17.7
FA2G2	6.8E+08	1.96%		6.5E+08	1.89%		3.1E+07	1.74%		3.5E+07	1.88%		20.1
FA2G2S1	3.3E+07	0.09%		3.2E+07	0.09%		1.7E+06	0.09%		1.7E+06	0.09%		19.4
FA2G2S2	4.4E+06	0.01%		4.4E+06	0.01%		6.8E+04	0.00%		8.9E+04	0.00%		55.9*
FA3	1.3E+08	0.38%		1.3E+08	0.38%		7.0E+06	0.39%		7.4E+06	0.40%		18.1
FA3G1	4.5E+07	0.13%		4.6E+07	0.13%		2.5E+06	0.14%		2.7E+06	0.14%		17.6
FA3G2	1.3E+07	0.04%		1.2E+07	0.03%		5.9E+05	0.03%		6.4E+05	0.03%		20.7
FA3G3	2.7E+06	0.01%		2.3E+06	0.01%		4.6E+04	0.00%		6.1E+04	0.00%		47.0*
Man5	3.4E+08	0.99%		3.3E+08	0.98%		1.4E+07	0.80%		1.5E+07	0.82%		22.9
Man6	4.6E+07	0.13%		4.7E+07	0.14%		2.0E+06	0.11%		2.2E+06	0.12%		22.1
Man7	3.2E+07	0.09%		3.2E+07	0.09%		1.4E+06	0.08%		1.5E+06	0.08%		22.1
Man8	1.9E+08	0.55%		1.8E+08	0.53%		8.9E+06	0.50%		9.6E+06	0.52%		20.1

FIG. 43E

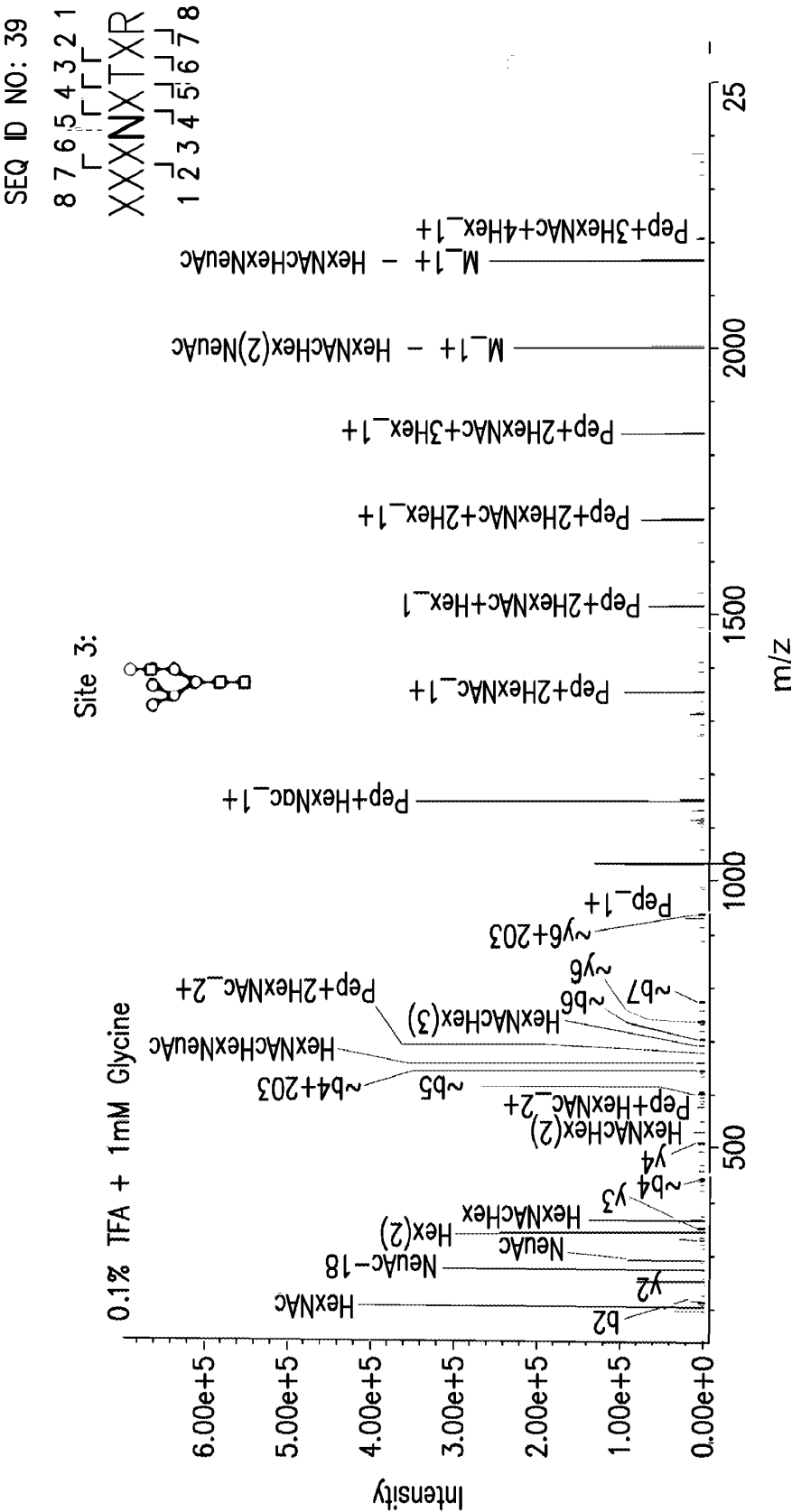


FIG. 43F

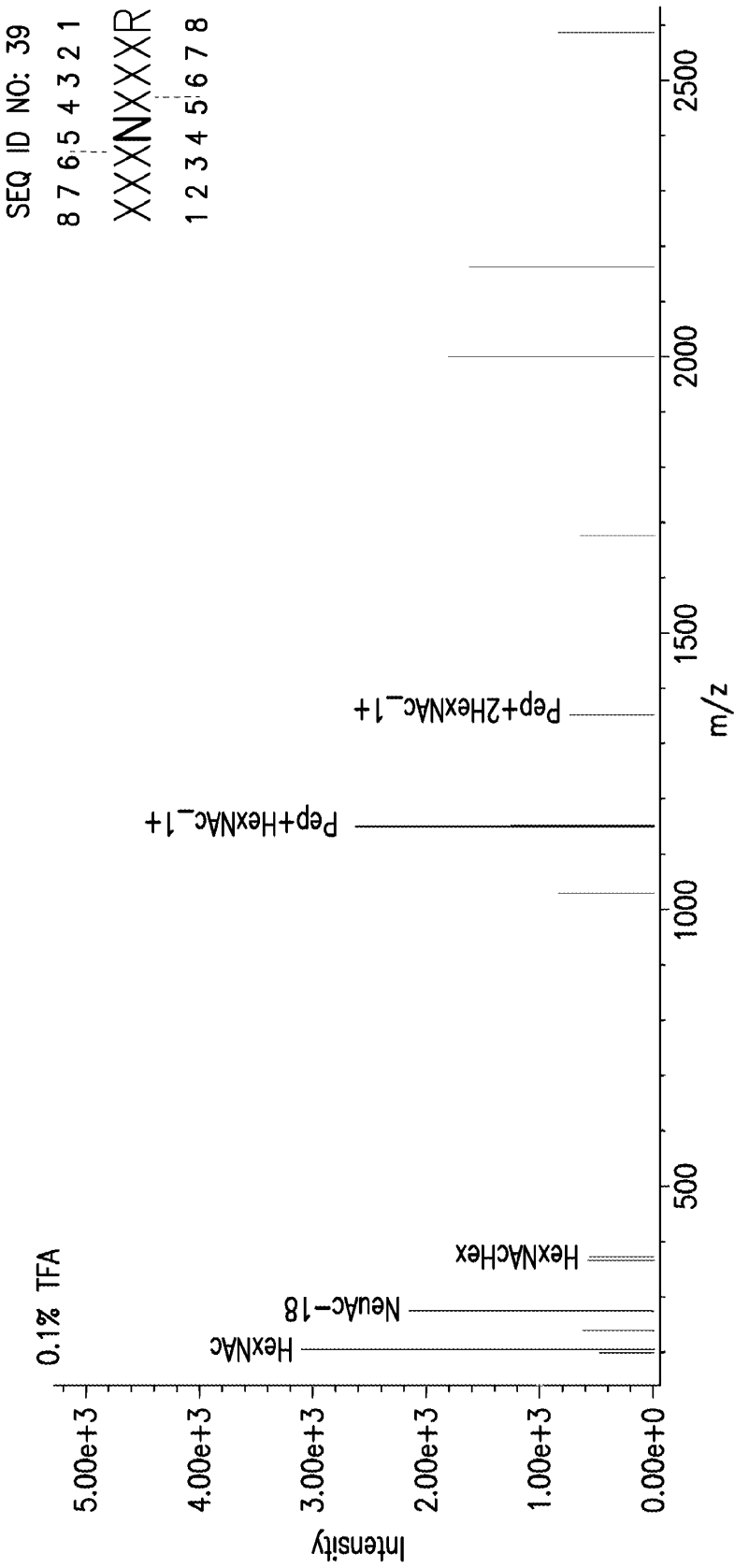


FIG. 43G

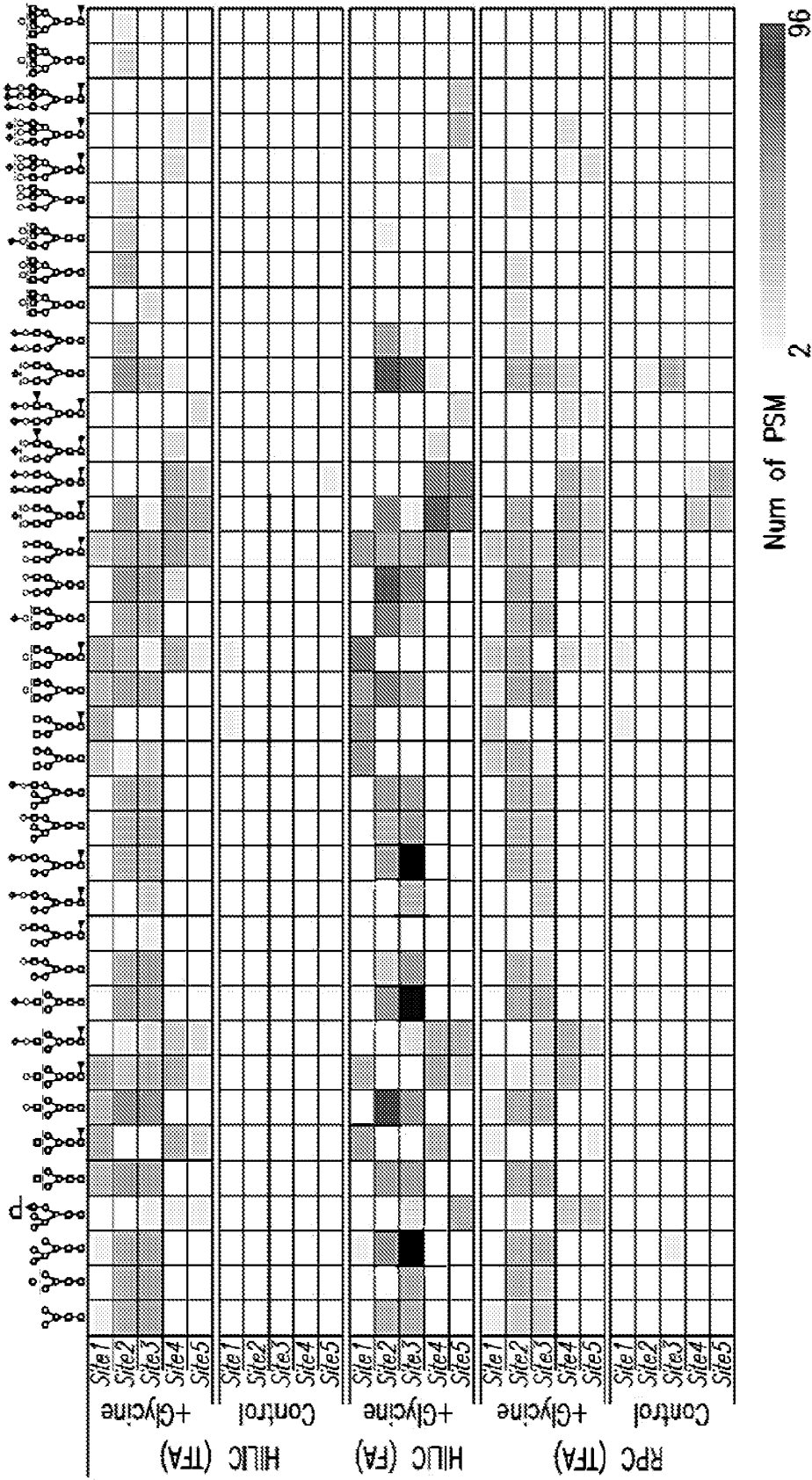


FIG. 44A

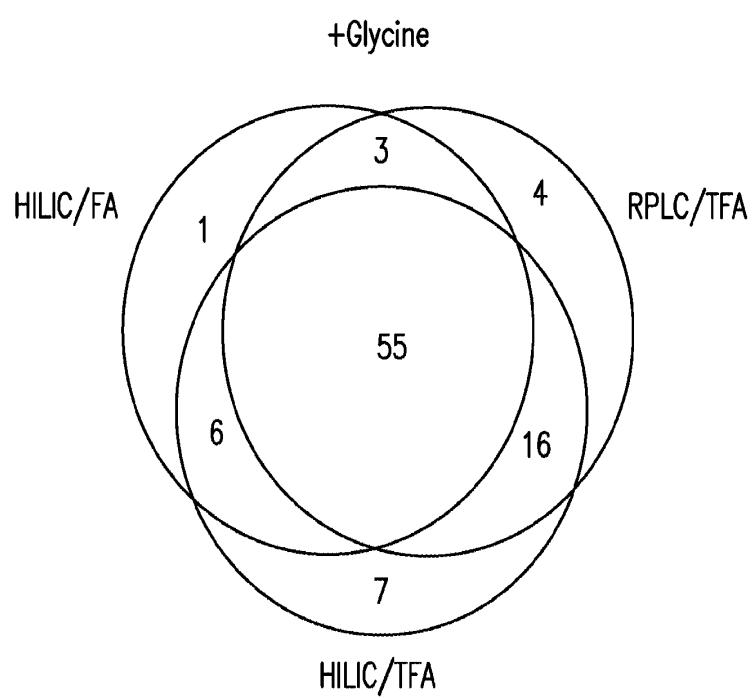


FIG. 44B

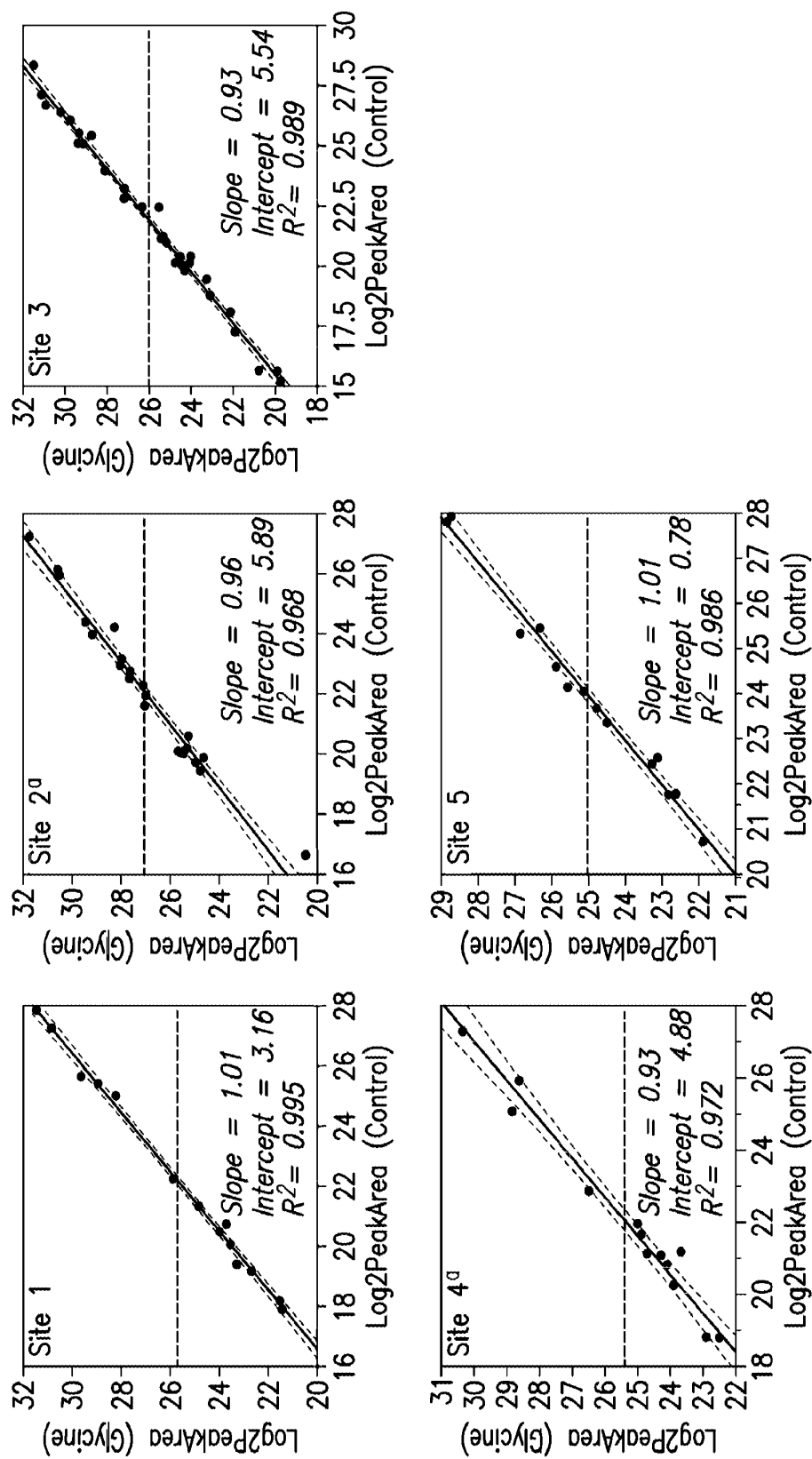


FIG. 44C

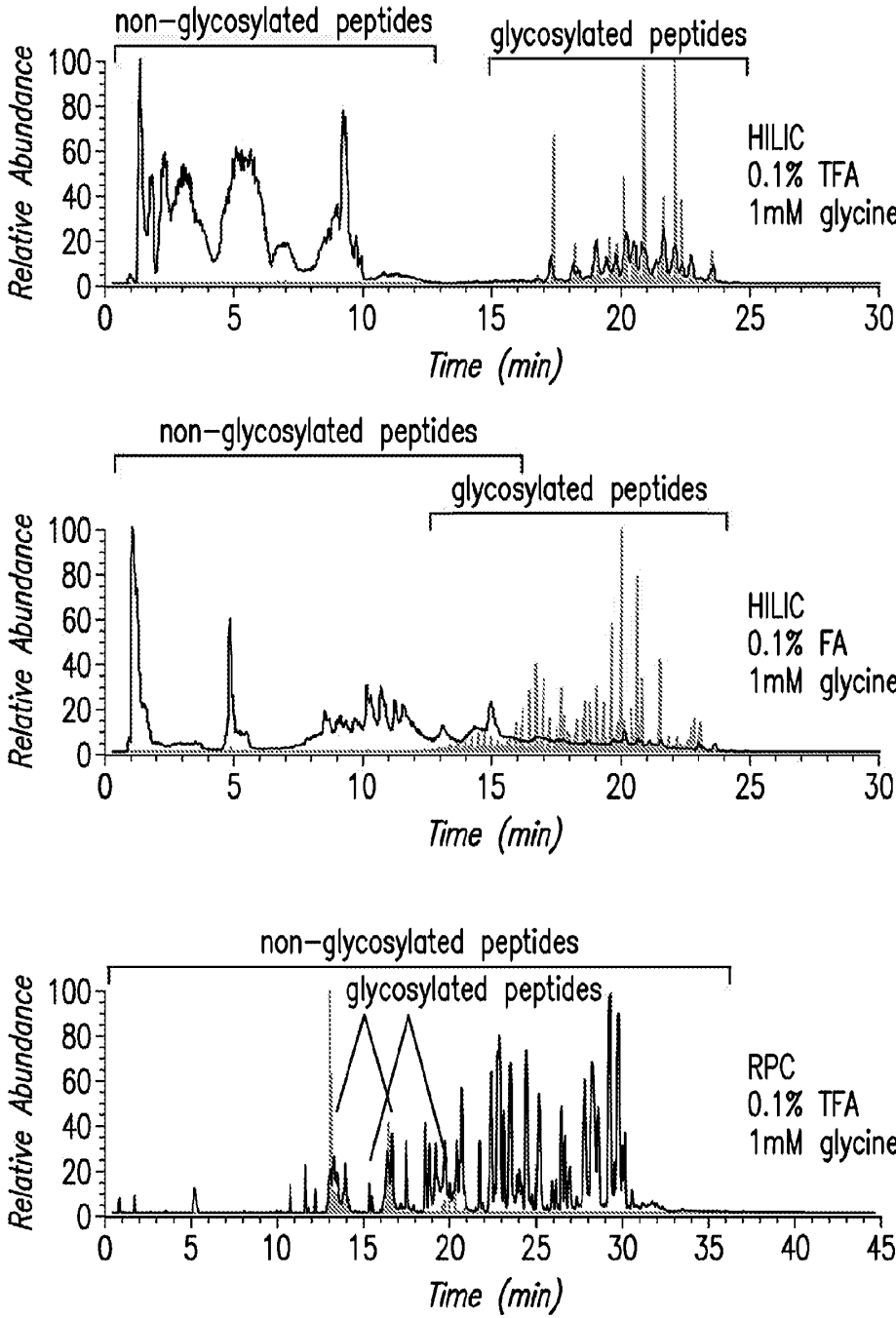


FIG. 44D

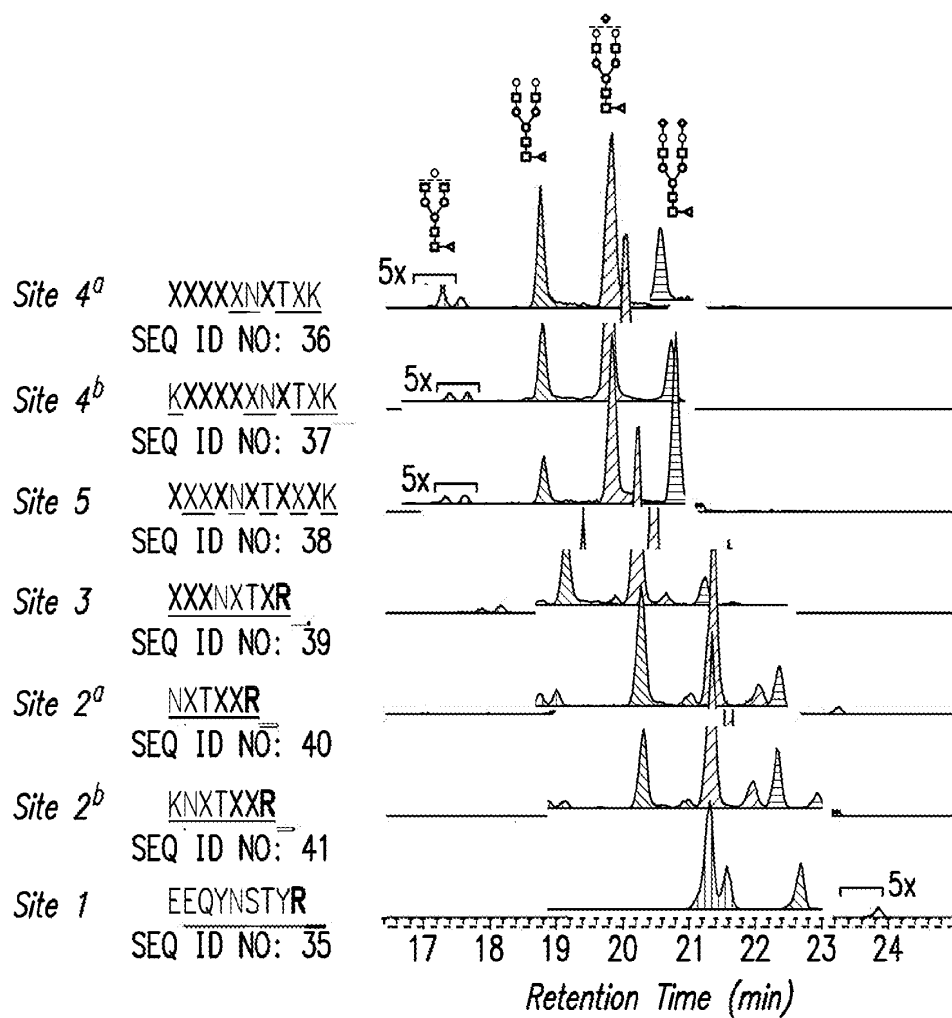
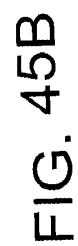


FIG. 45A



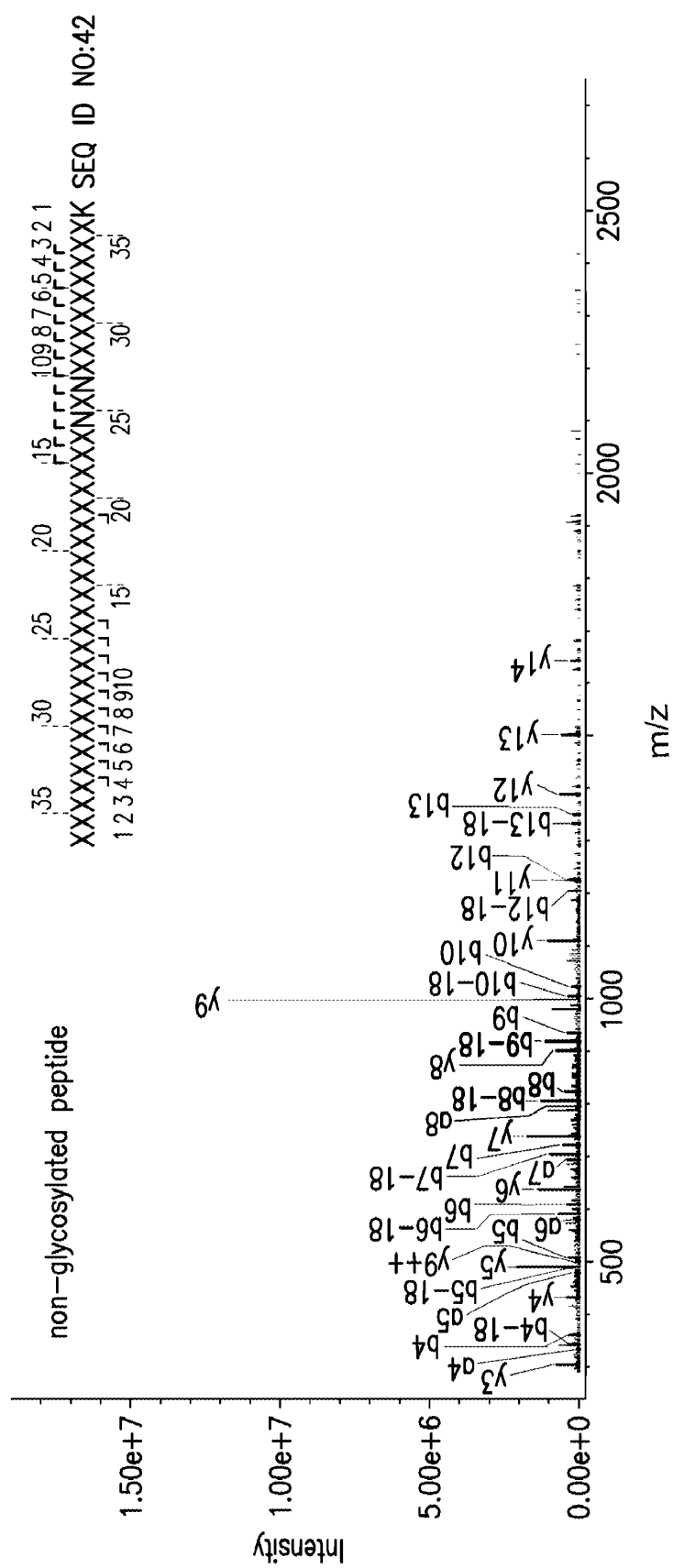


FIG. 45C

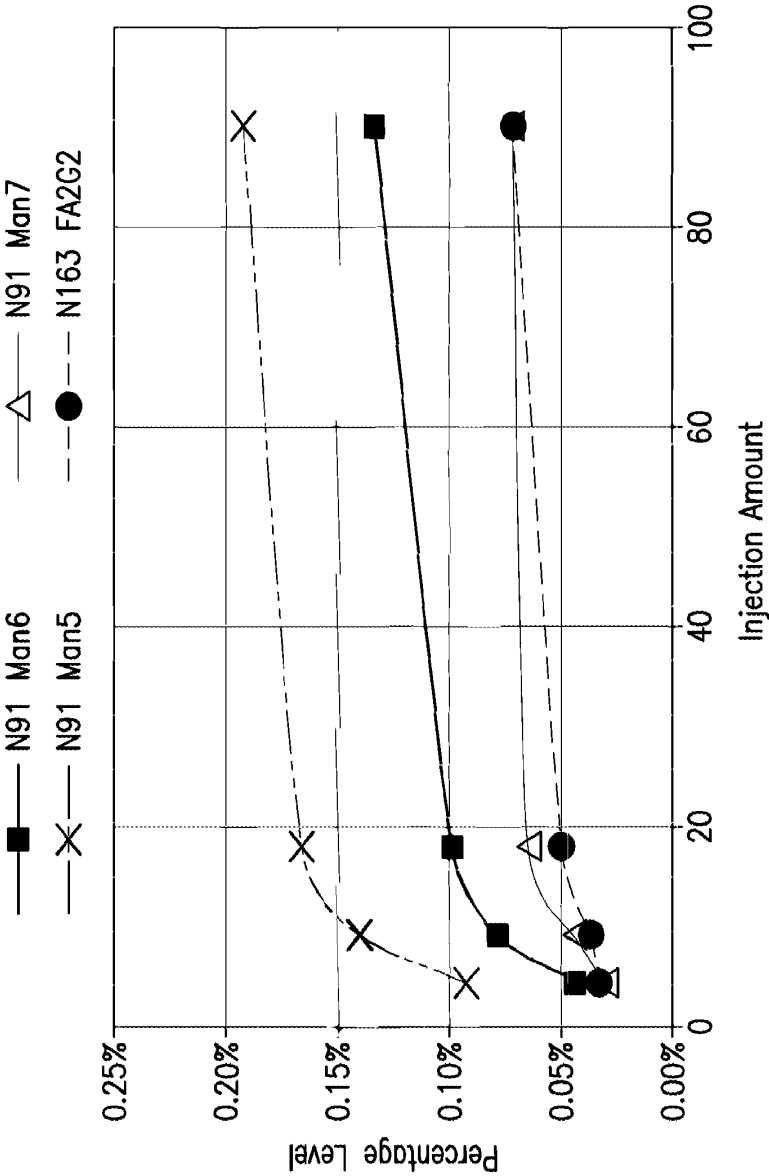
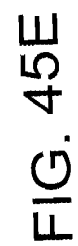


FIG. 45D



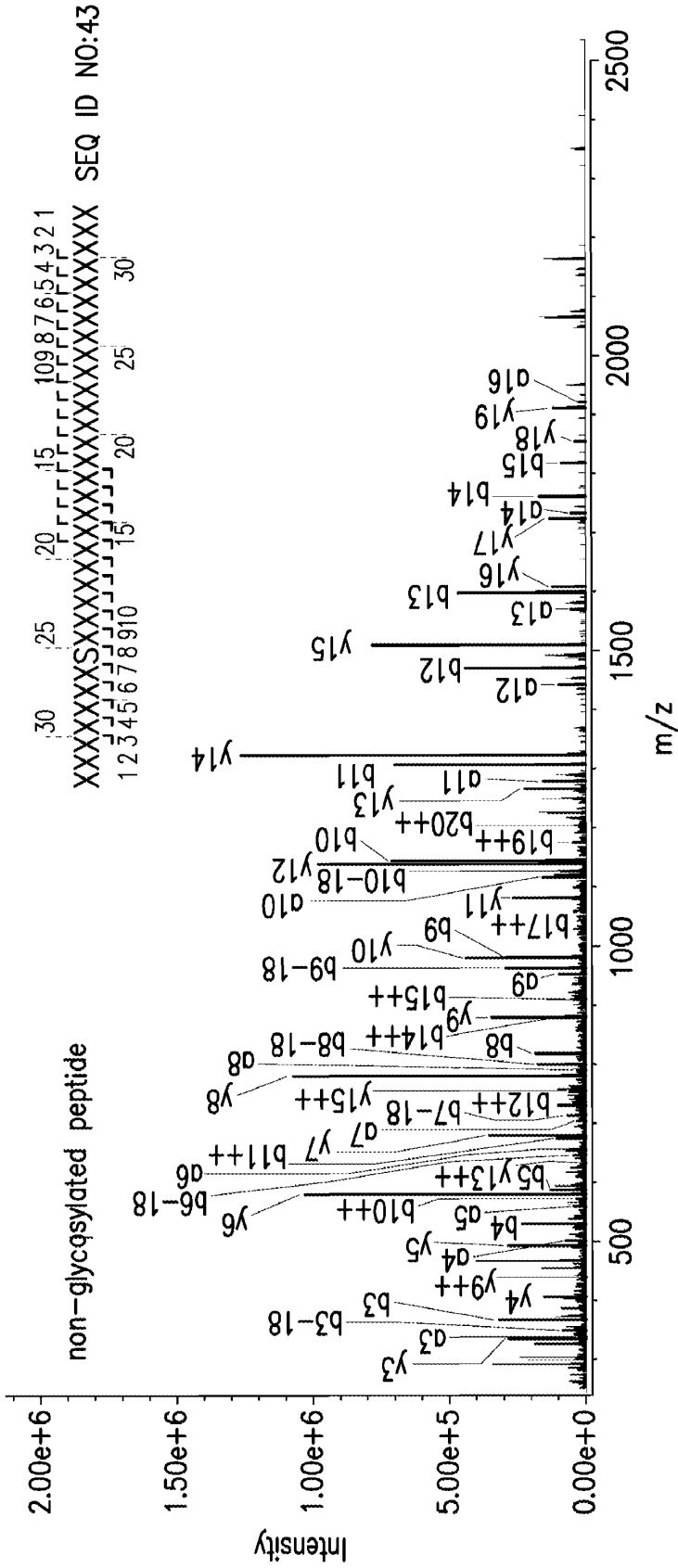


FIG. 45F

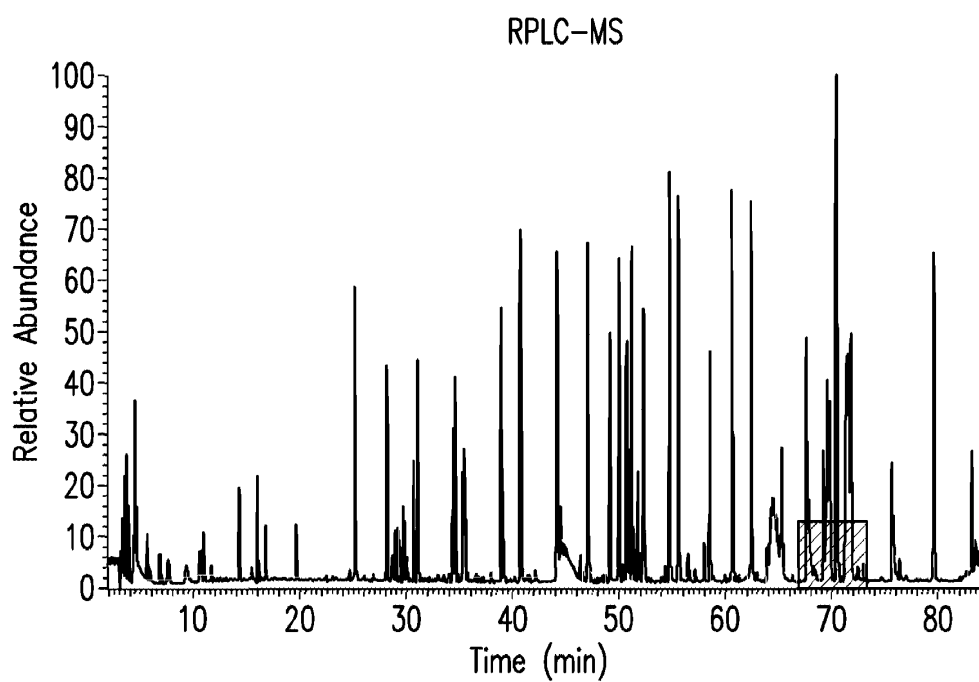


FIG.46A

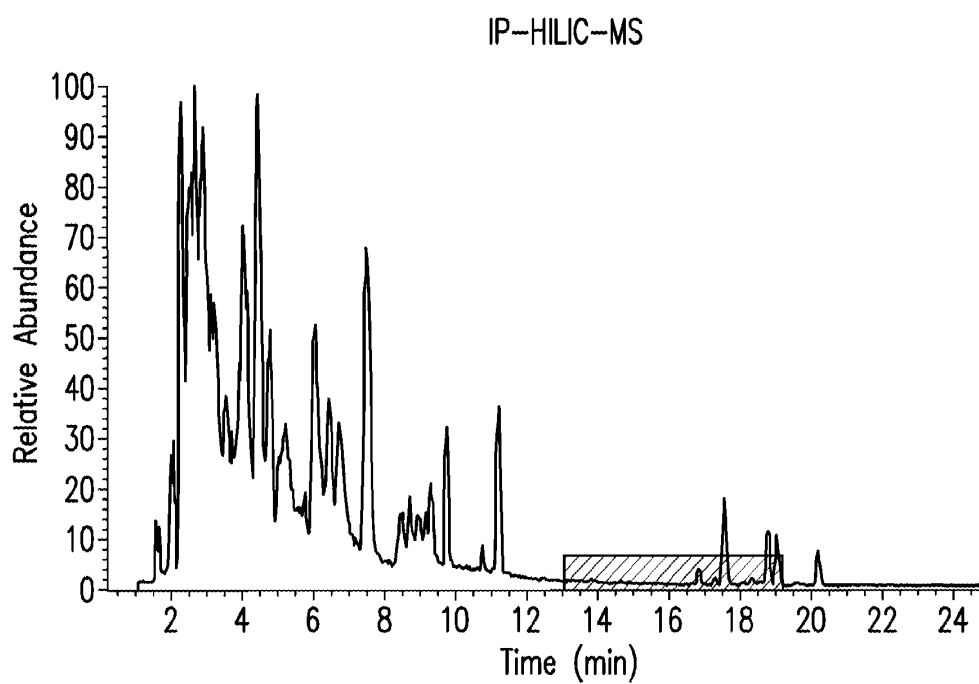


FIG.46B

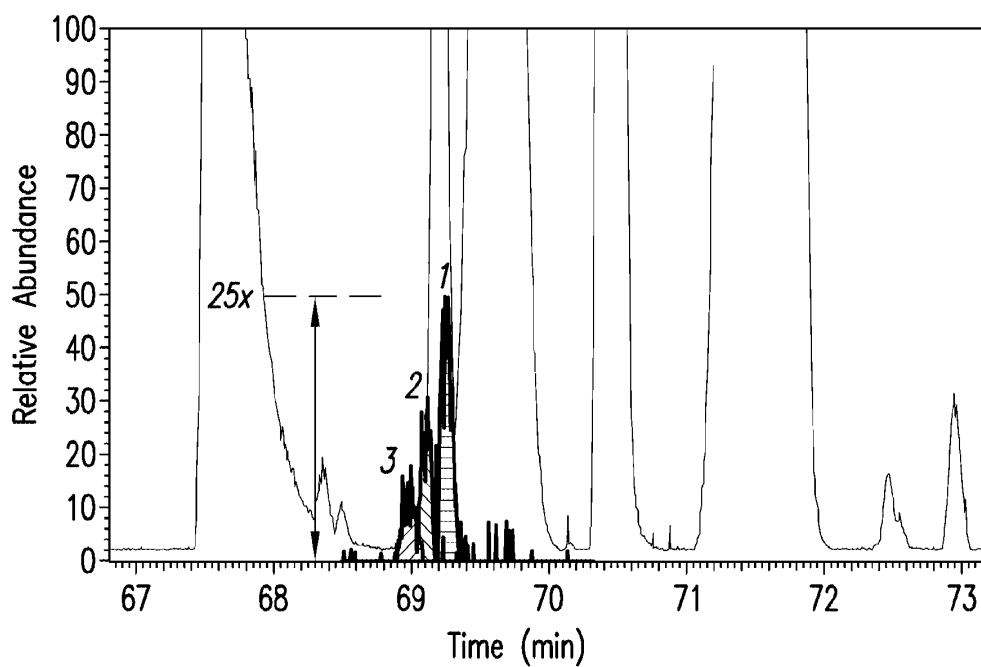


FIG. 46C

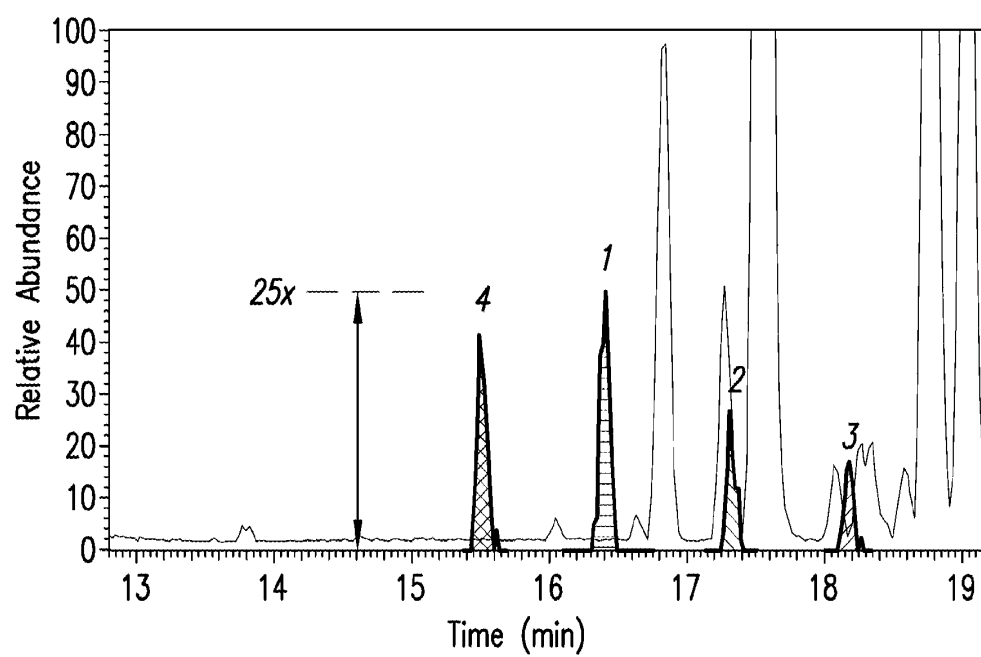


FIG. 46D

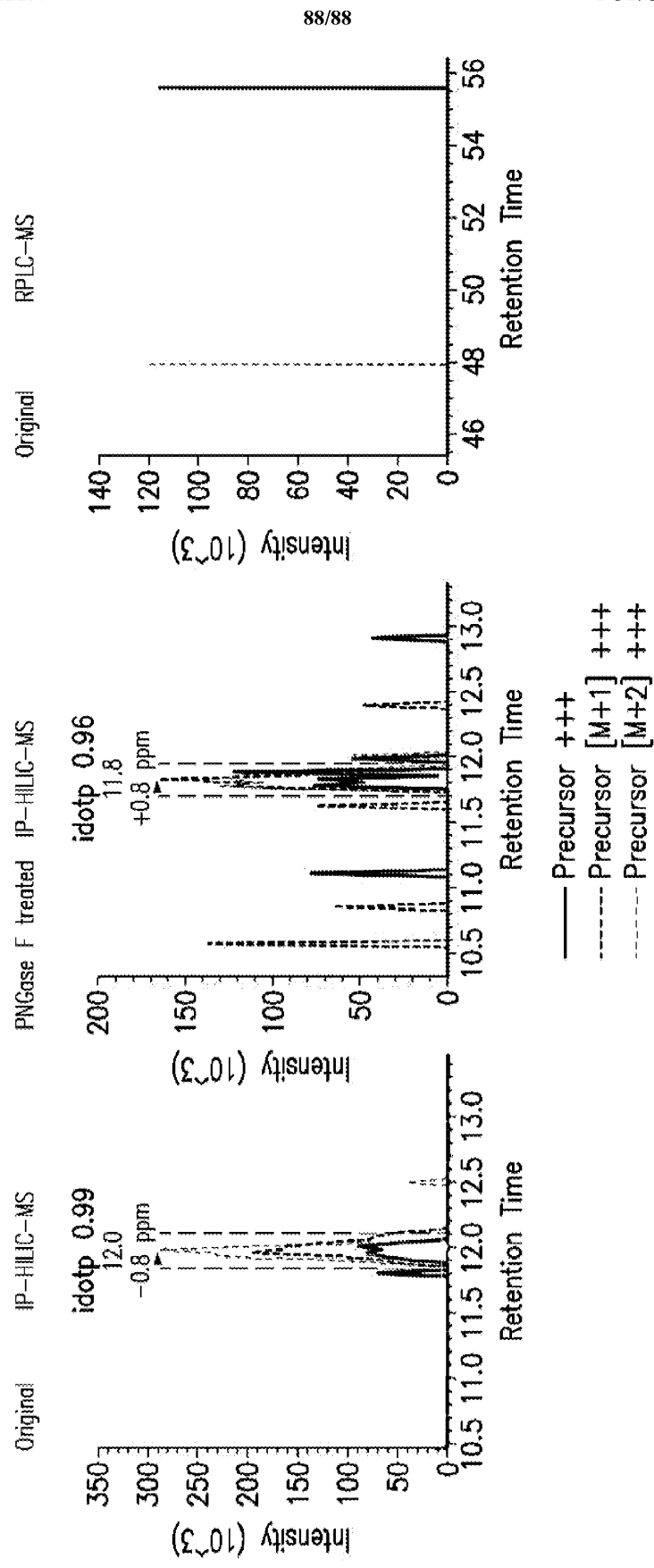


FIG. 46E