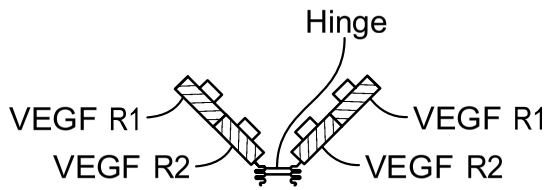
Fabricator cleaved mini Trap: Fabricator enzyme cleaved product from aflibercept



Amino Acid Sequence

SDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKKFPLDTLIPDGKRIIWDSRK
GFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTIID

VVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGS
EMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK

DKTHTCPPCPAPELLG

GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Hinge Domain Fragment

Cleaved off Fc Fragment

VEGFR1

VEGFR2

FIG.1

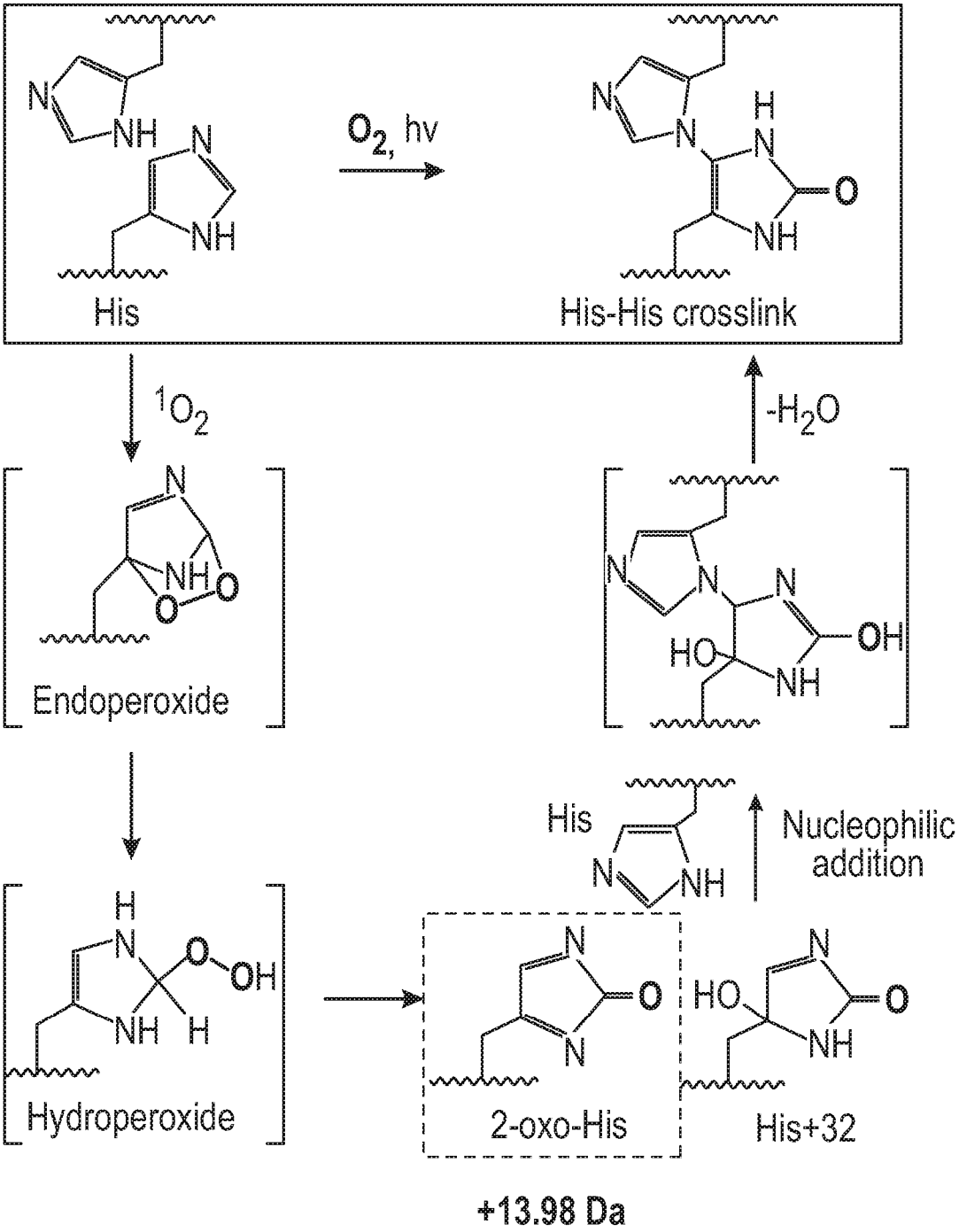
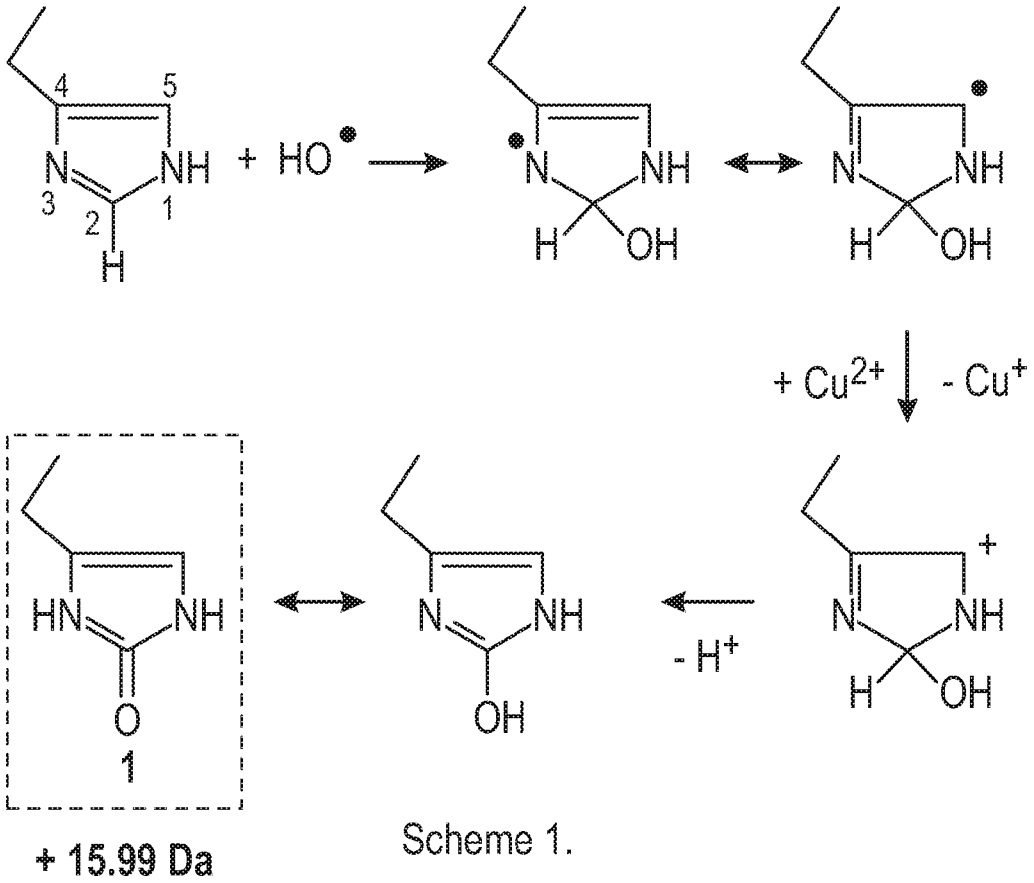

His
O_2, hv
His-His crosslink
$^1O_2$
$-H_2O$
Endoperoxide
Nucleophilic addition
His
Hydroperoxide
2-oxo-His
His+32
+13.98 Da

FIG. 2



Scheme 1.

FIG. 3

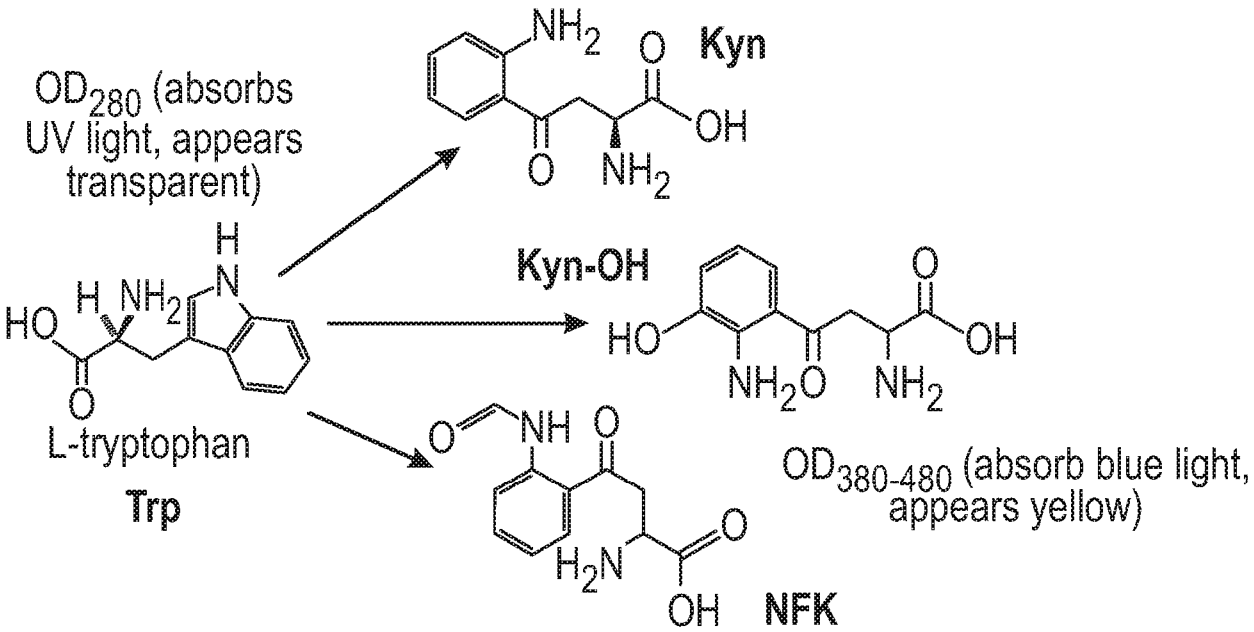


FIG. 4

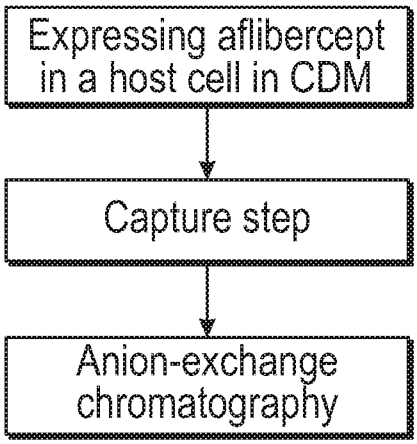


FIG. 5

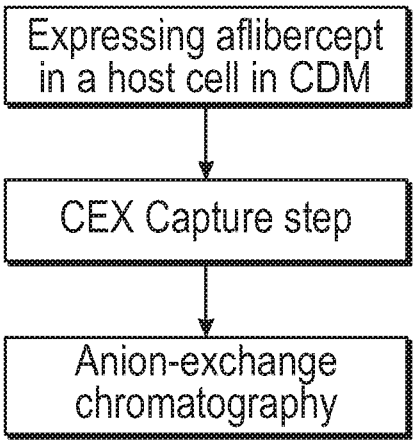


FIG. 7

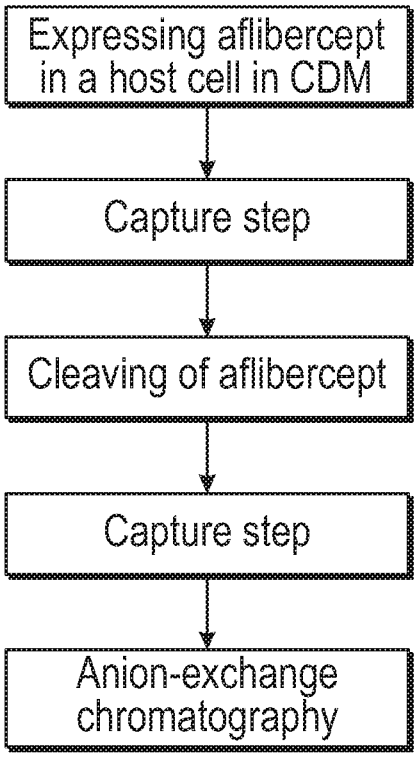


FIG. 6

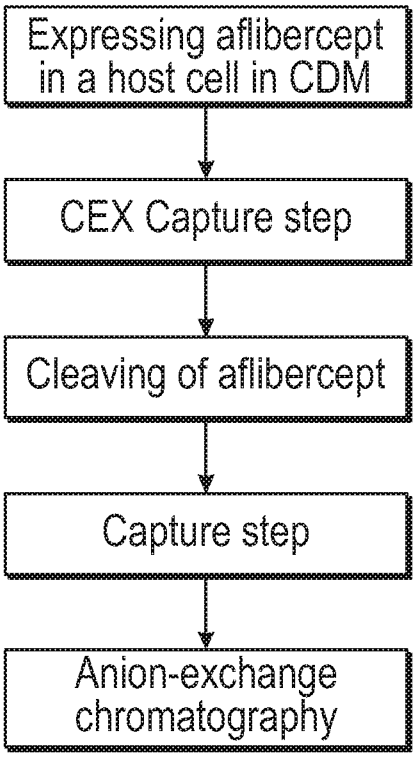


FIG. 8

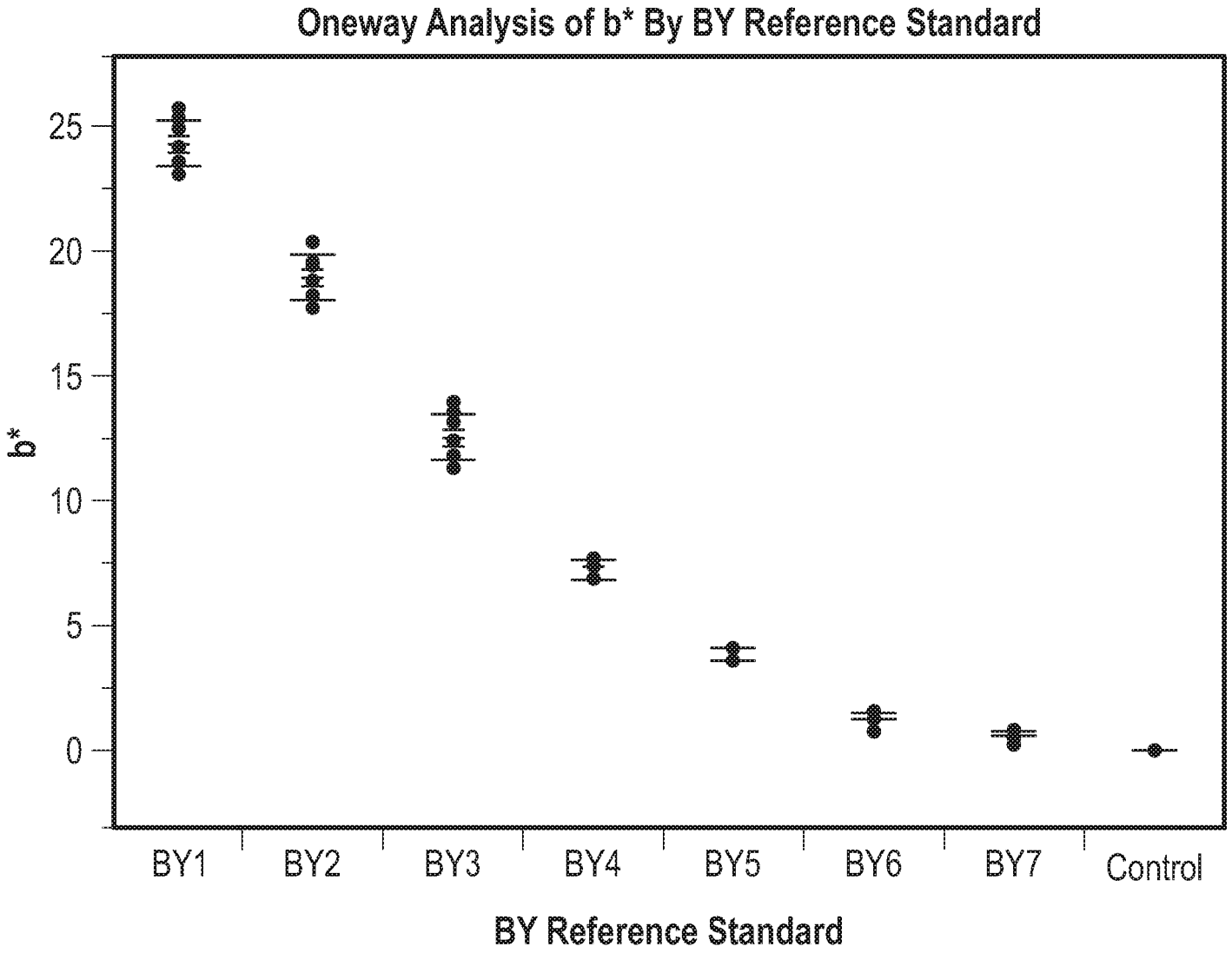

Oneway Analysis of b* By BY Reference Standard
b*
25
20
15
10
5
0
BY1
BY2
BY3
BY4
BY5
BY6
BY7
Control
BY Reference Standard

FIG. 9

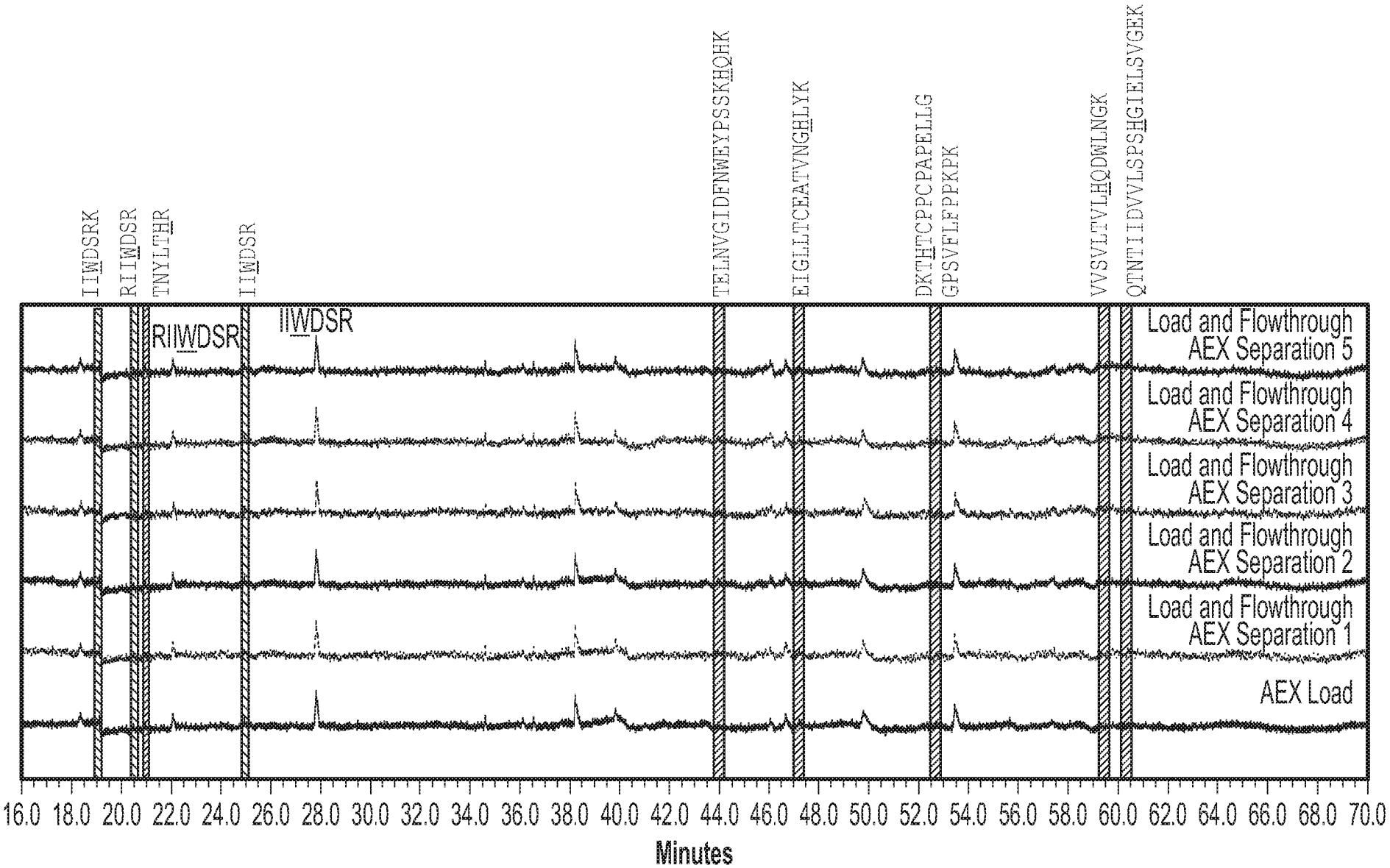

IIWDSRK
RIIWDSR
TNYLTHR
IIWDSR
TELNVGIDFNWEYPSSKHQHK
EIGLLTCEATVNGHLYK
DKTHTCPPCPAPELLG
GPSVFLFPPKPK
VVSVLTVLHQDWLNGK
QTNTIIDVVLSPSHGIELSVGEK
RIIWDSR
IIWDSR
Load and Flowthrough AEX Separation 5
Load and Flowthrough AEX Separation 4
Load and Flowthrough AEX Separation 3
Load and Flowthrough AEX Separation 2
Load and Flowthrough AEX Separation 1
AEX Load
16.0 18.0 20.0 22.0 24.0 26.0 28.0 30.0 32.0 34.0 36.0 38.0 40.0 42.0 44.0 46.0 48.0 50.0 52.0 54.0 56.0 58.0 60.0 62.0 64.0 66.0 68.0 70.0
Minutes

FIG. 10

PTM	Site	Peptide Sequence	Modification	Aflibercept AEX Load	Aflibercept AEX Pool				
					POROS 50 HQ pH 8.4 2.0 mS/cm	QsFF pH 8.0 2.5 mS/cm	POROS 50 HQ pH 8.0 2.5 mS/cm	QsFF pH 7.8 4.0 mS/cm	POROS 50 HQ pH 7.8 4.0 mS/cm
					AEX Separation 1	AEX Separation 2	AEX Separation 3	AEX Separation 4	AEX Separation 5
				3.06	1.05	0.96	1.33	1.35	1.55
His Oxidation	H19	SDTGRPFVEMYSEIPEIIHMTEGR	+14	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%
	H86	EIGLLTC*EATVNGHLYK	+14	0.013%	0.006%	0.007%	0.008%	0.009%	0.010%
	H95	TNYLTHR	+14	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%
	H110	QTNTIIDVVLSPSHGIELSVGEK	+14	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%
	H145	TELNVGIDFNWEYPSSKHQHK	+14	0.067%	0.039%	0.033%	0.035%	0.038%	0.042%
	H209	THTC*PPC*PAPELLGGPSVFLFPPKPK	+14	0.056%	0.027%	0.037%	0.040%	0.045%	0.040%
	H295	VVSVLTVLHQDWLNGK	+14	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%
Trp Oxidation	W58	IIWDSR	+4	0.012%	0.008%	0.007%	0.009%	0.009%	0.011%
			+16	0.027%	0.017	0.022%	0.021%	0.021%	0.023%
			+32	0.114%	0.102%	0.111%	0.121%	0.114%	0.135%
			+48	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%
	W138	TELNVGIDFNWEYPSSK	+4	0.069%	0.055%	0.040%	0.036	0.039%	0.038%
			+16	0.131%	0.078%	0.070%	0.089	0.077%	0.081%
			+32	0.086%	0.057%	0.040%	0.059%	0.046%	0.068%

*: ALKYLATED CYSTEINE

FIG. 11

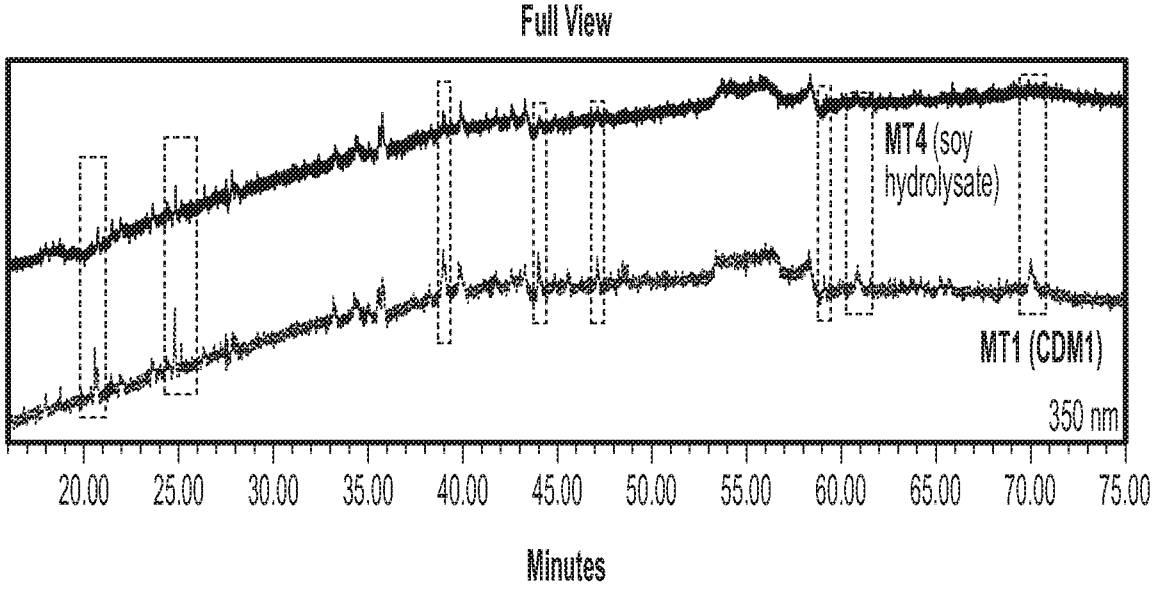


FIG. 12A

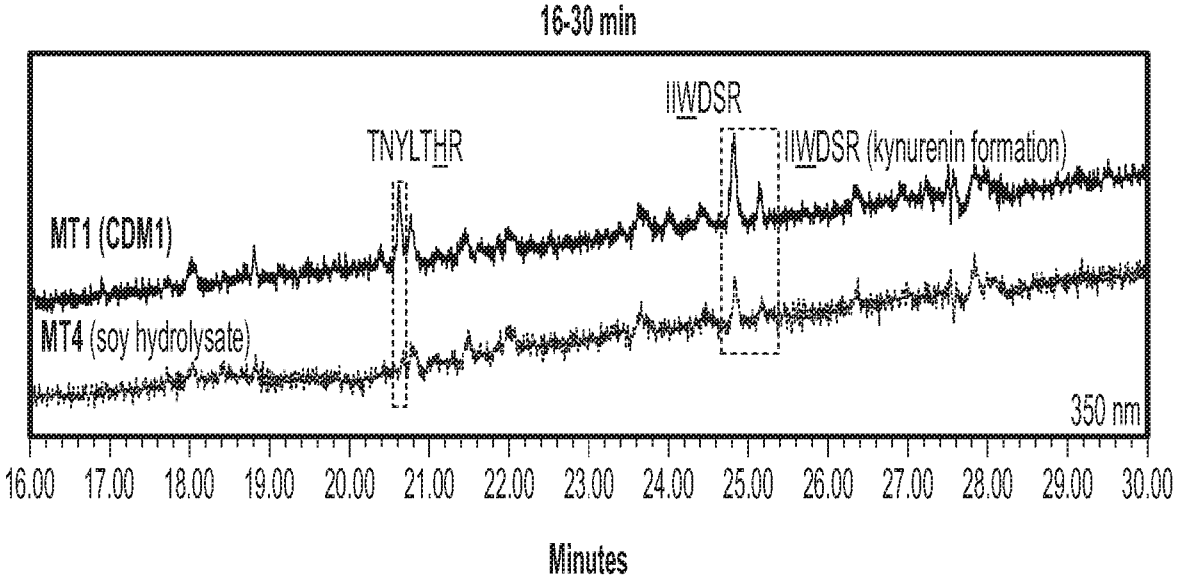


FIG. 12B

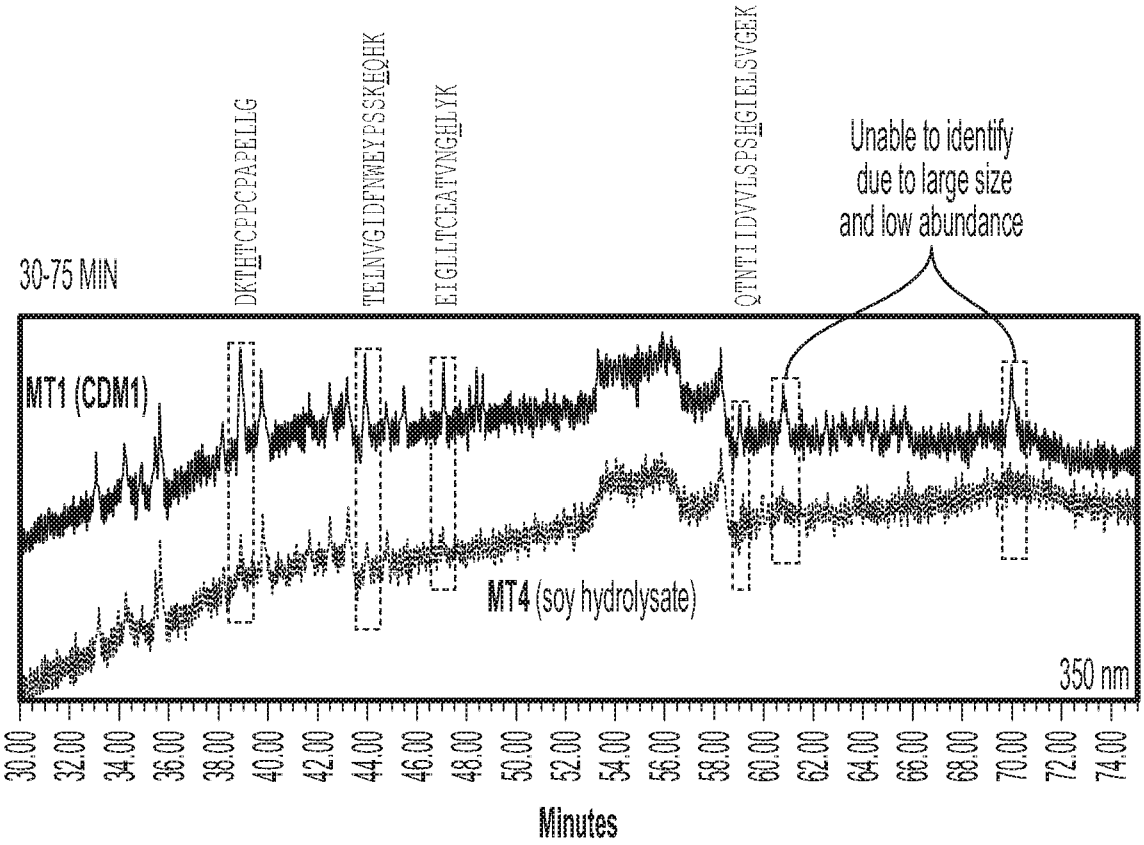


FIG. 12C

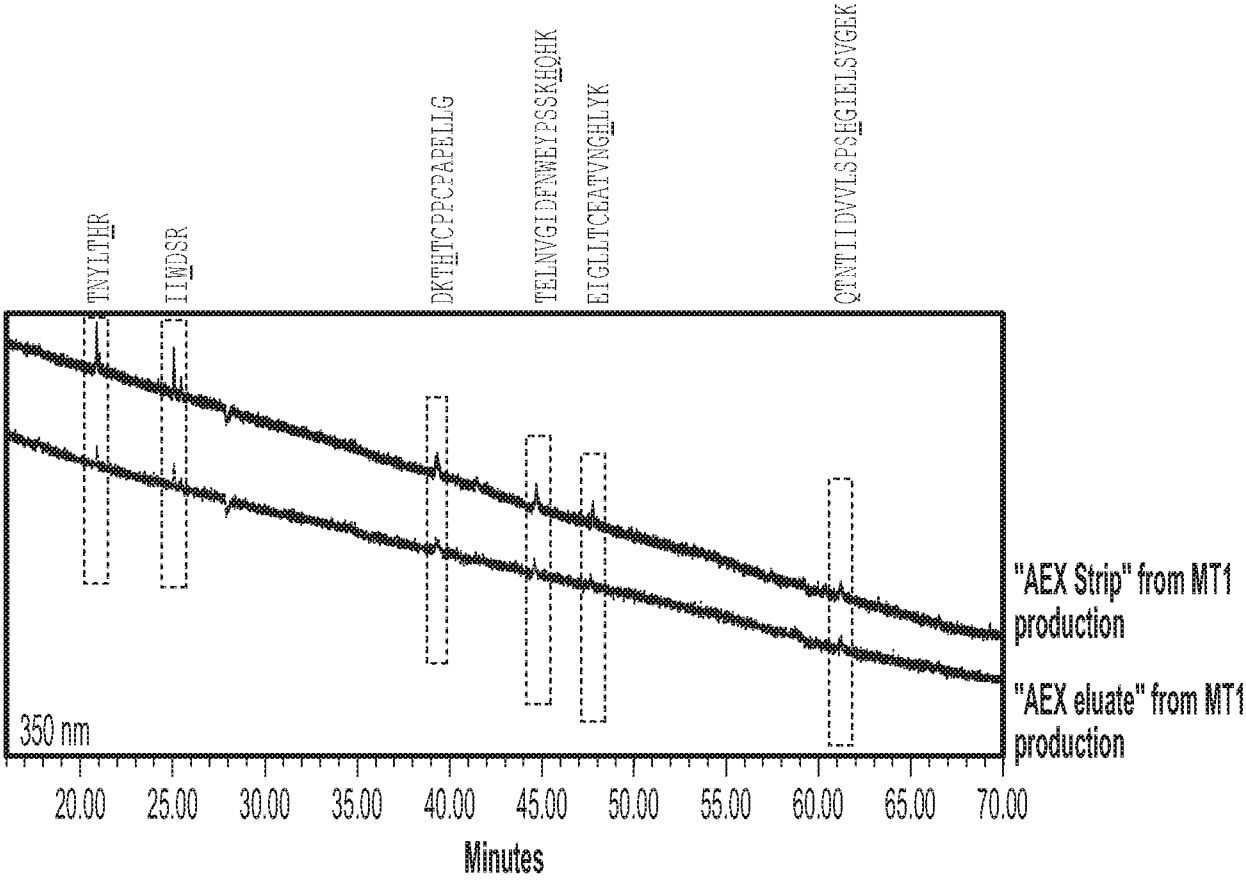


FIG. 13

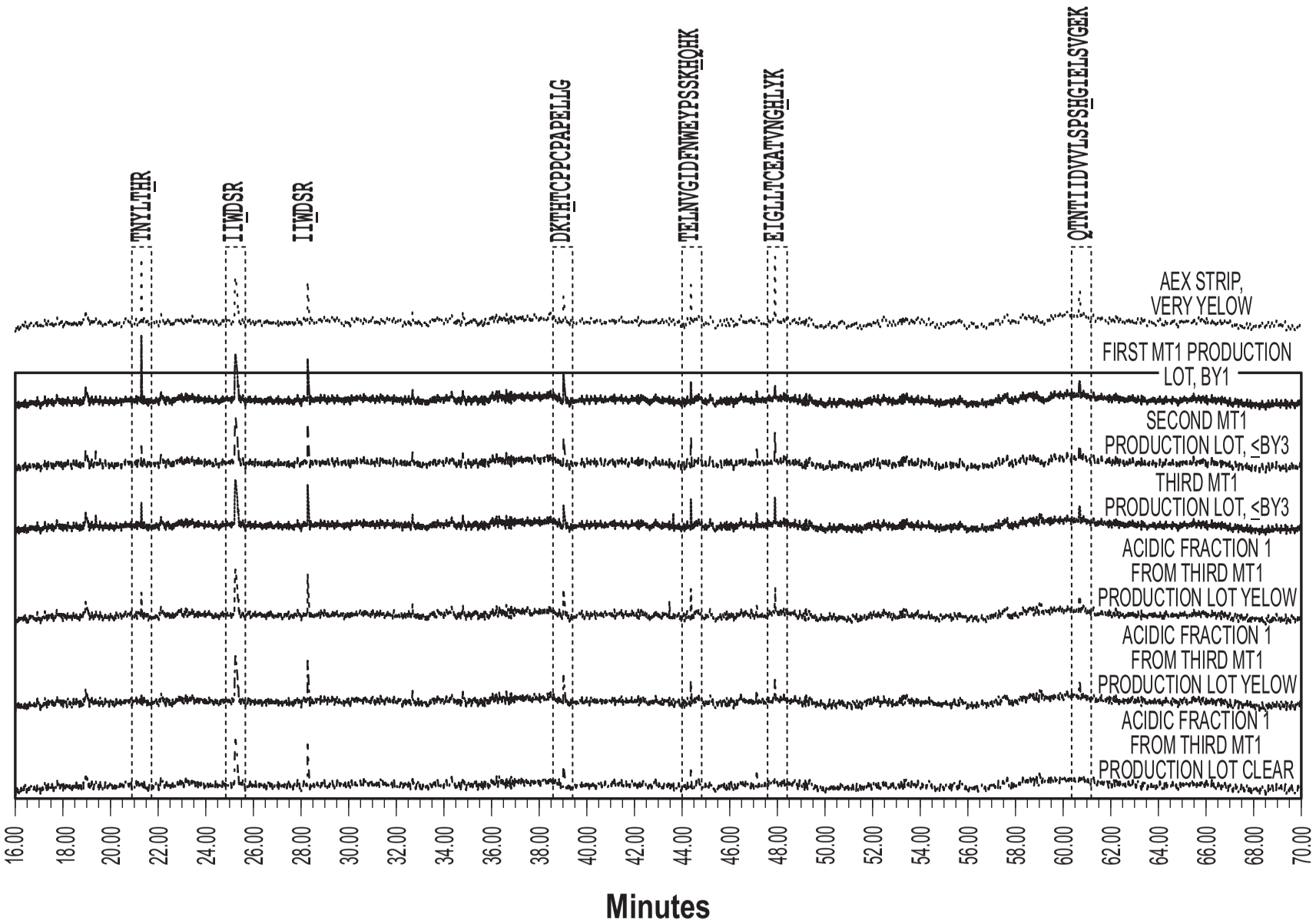

TNYLTHR
IIWDSR
IIWDSR
DKTHTCPPCPAPELLG
TELNVGIDFNWEYPSSKHQHK
EIGLITCEATVNGHLYK
QTNYIIDVVLSPSHGIELSVGEK
AEX STRIP, VERY YELOW
FIRST MT1 PRODUCTION LOT, BY1
SECOND MT1 PRODUCTION LOT, ≤BY3
THIRD MT1 PRODUCTION LOT, ≤BY3
ACIDIC FRACTION 1 FROM THIRD MT1 PRODUCTION LOT YELOW
ACIDIC FRACTION 1 FROM THIRD MT1 PRODUCTION LOT YELOW
ACIDIC FRACTION 1 FROM THIRD MT1 PRODUCTION LOT CLEAR
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
38.00
40.00
42.00
44.00
46.00
48.00
50.00
52.00
54.00
56.00
58.00
60.00
62.00
64.00
66.00
68.00
70.00
Minutes

FIG. 14

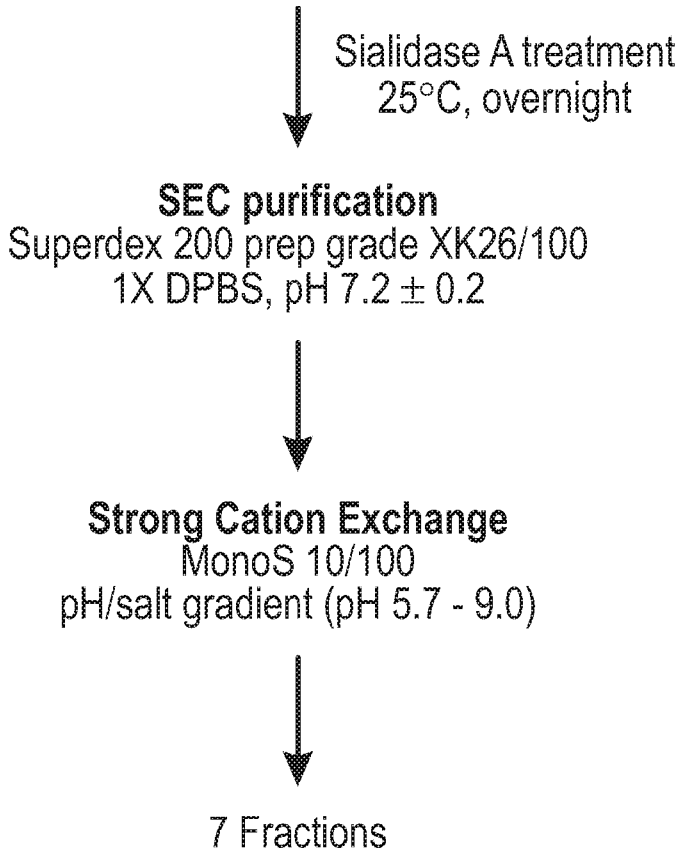

Sialidase A treatment
25°C, overnight
SEC purification
Superdex 200 prep grade XK26/100
1X DPBS, pH 7.2 ± 0.2
Strong Cation Exchange
MonoS 10/100
pH/salt gradient (pH 5.7 - 9.0)
7 Fractions

FIG. 15

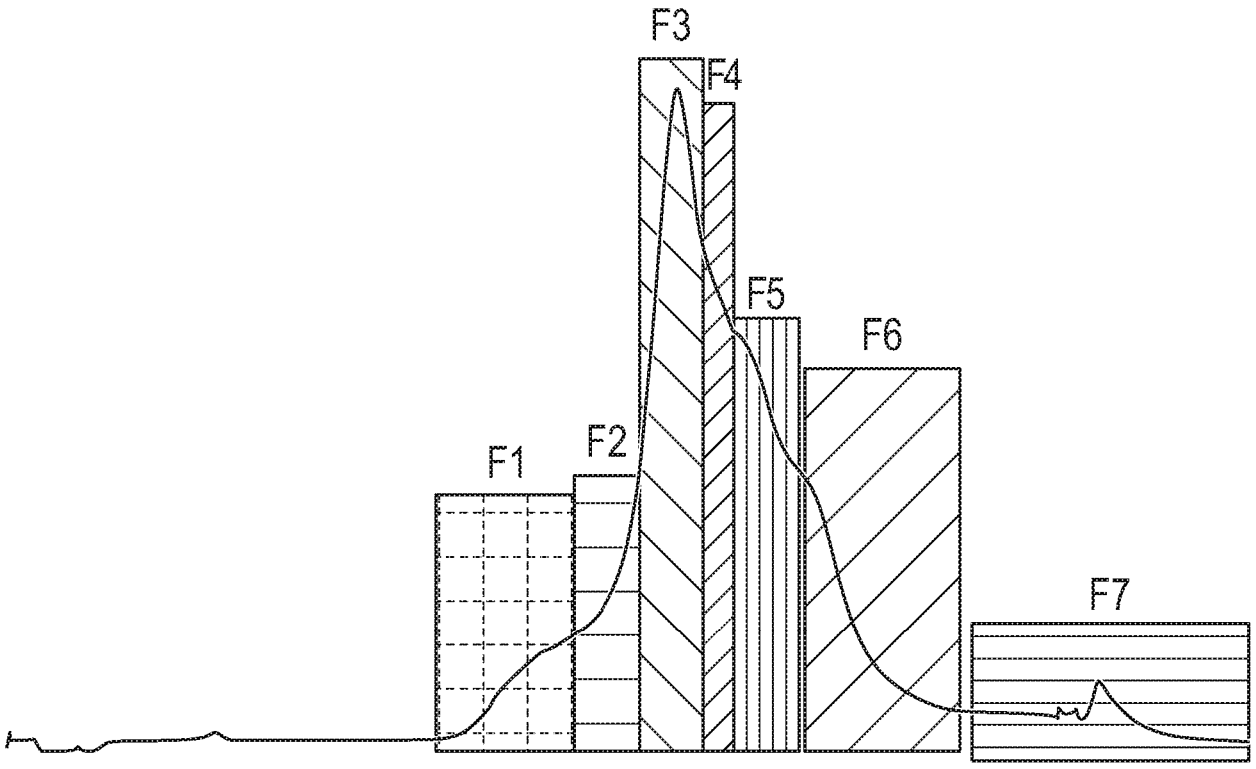

F1
F2
F3
F4
F5
F6
F7

FIG. 16

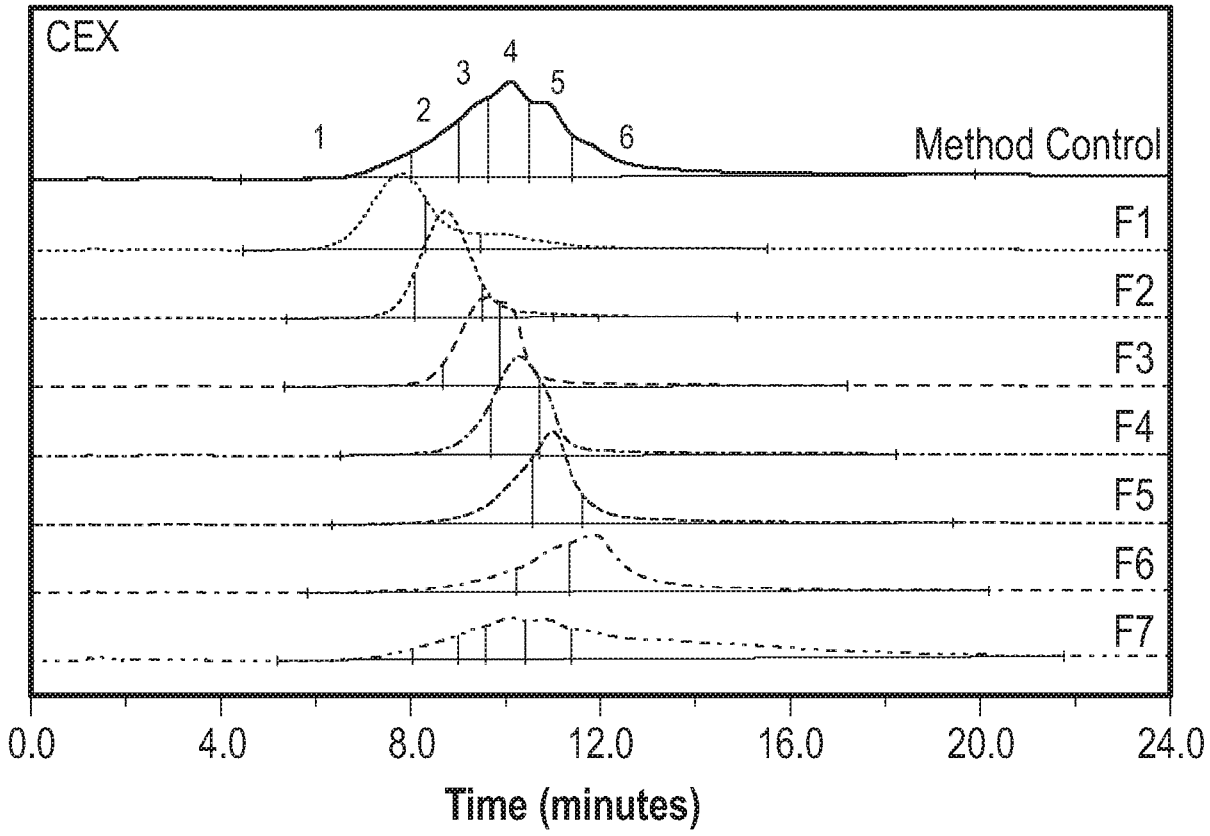

CEX
1
2
3
4
5
6
Method Control
F1
F2
F3
F4
F5
F6
F7
0.0
4.0
8.0
12.0
16.0
20.0
24.0
Time (minutes)

FIG. 17

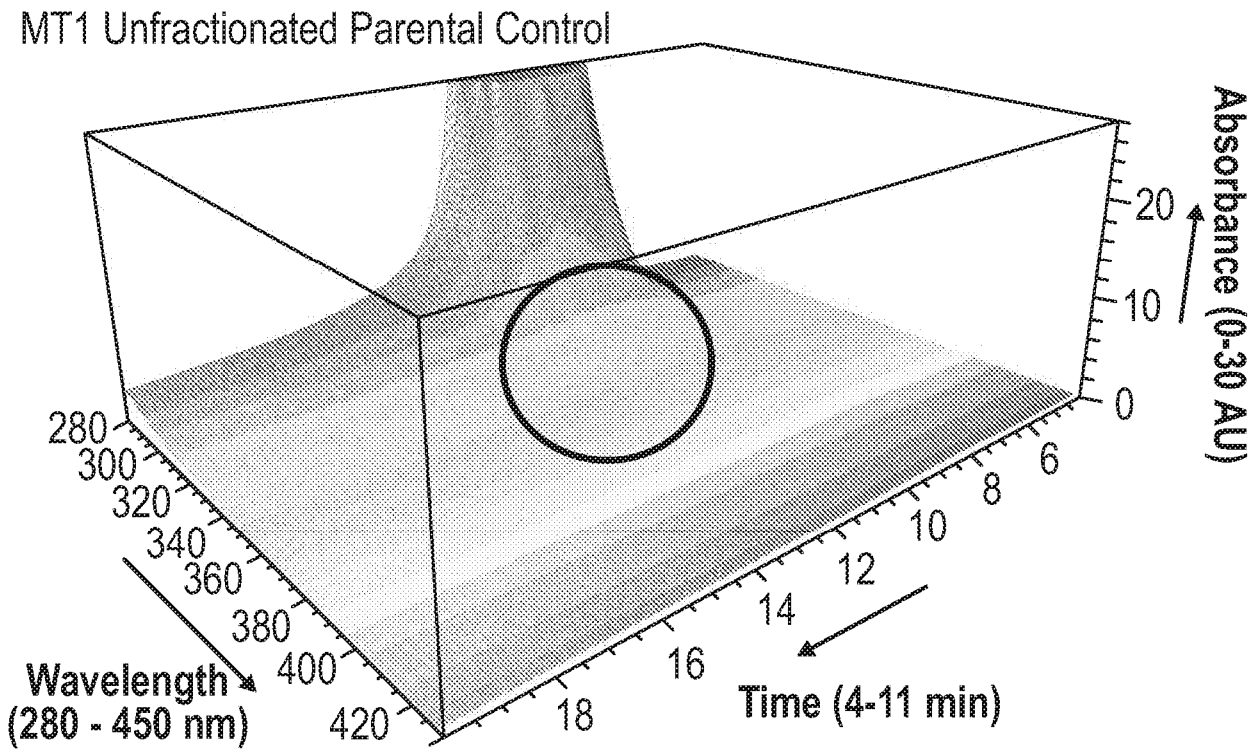


FIG. 18A

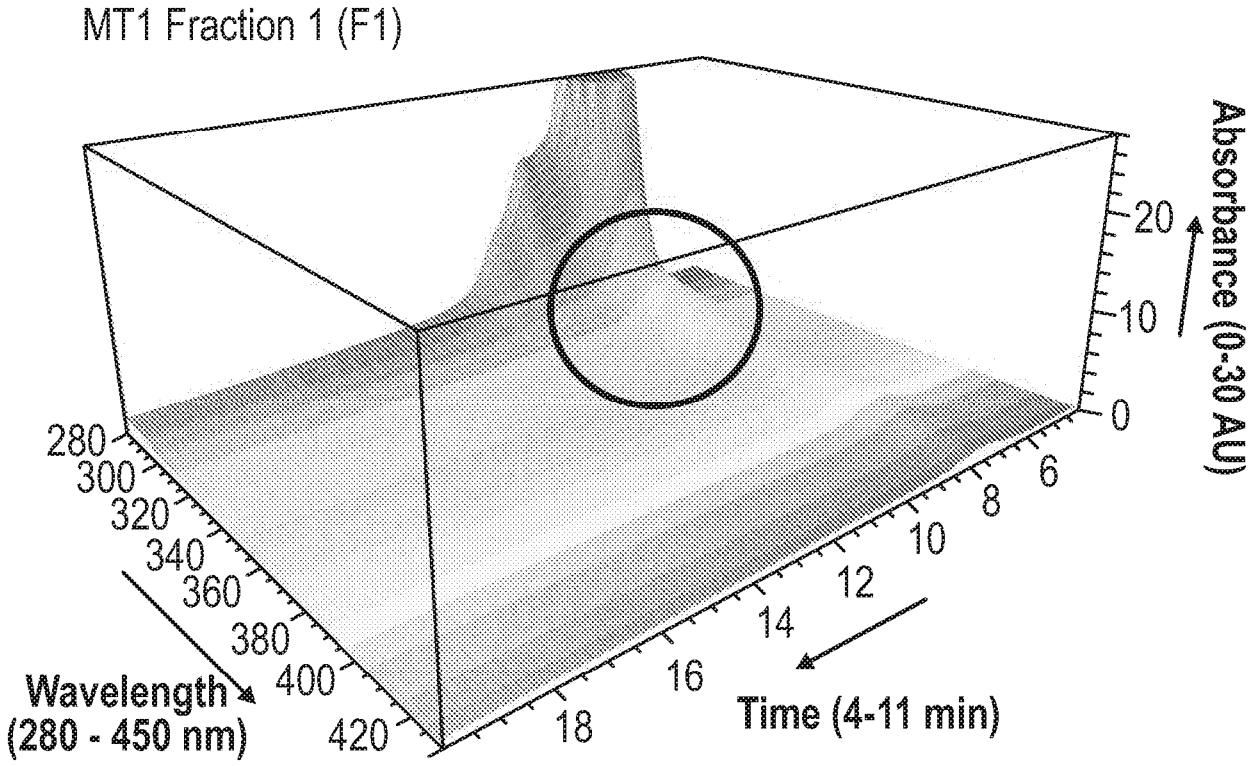


FIG. 18B

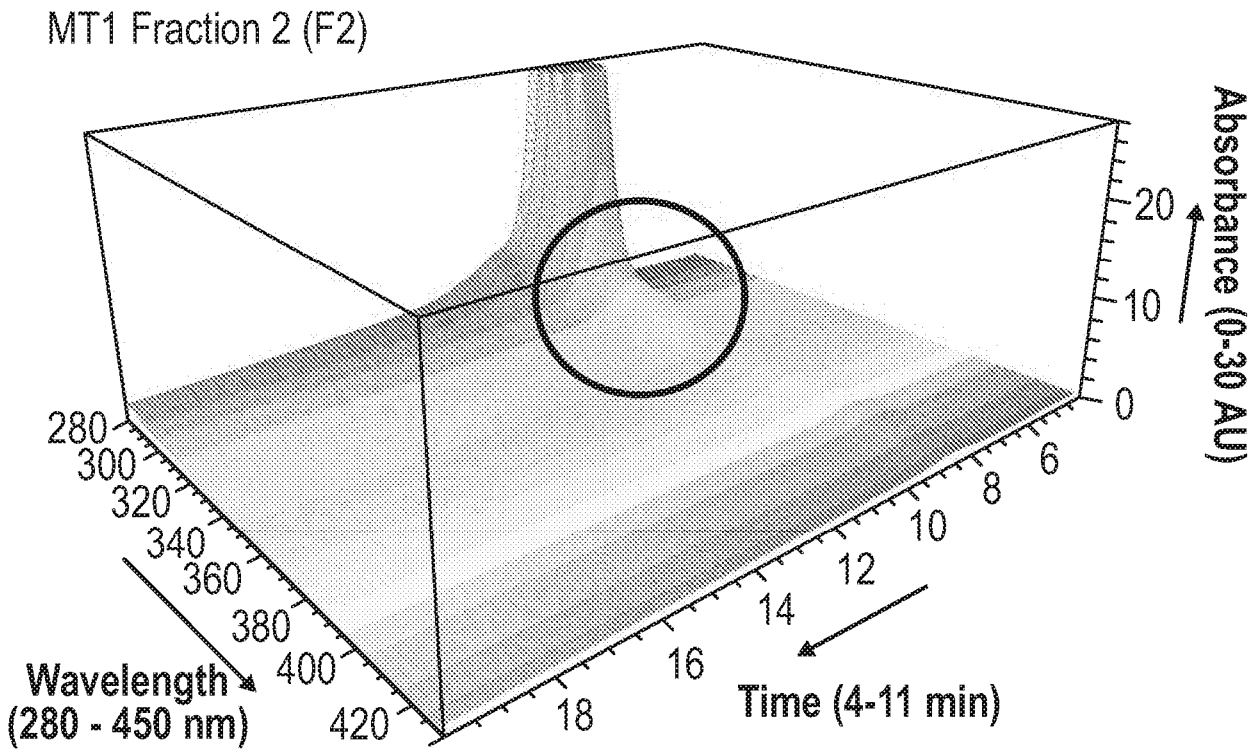


FIG. 18C

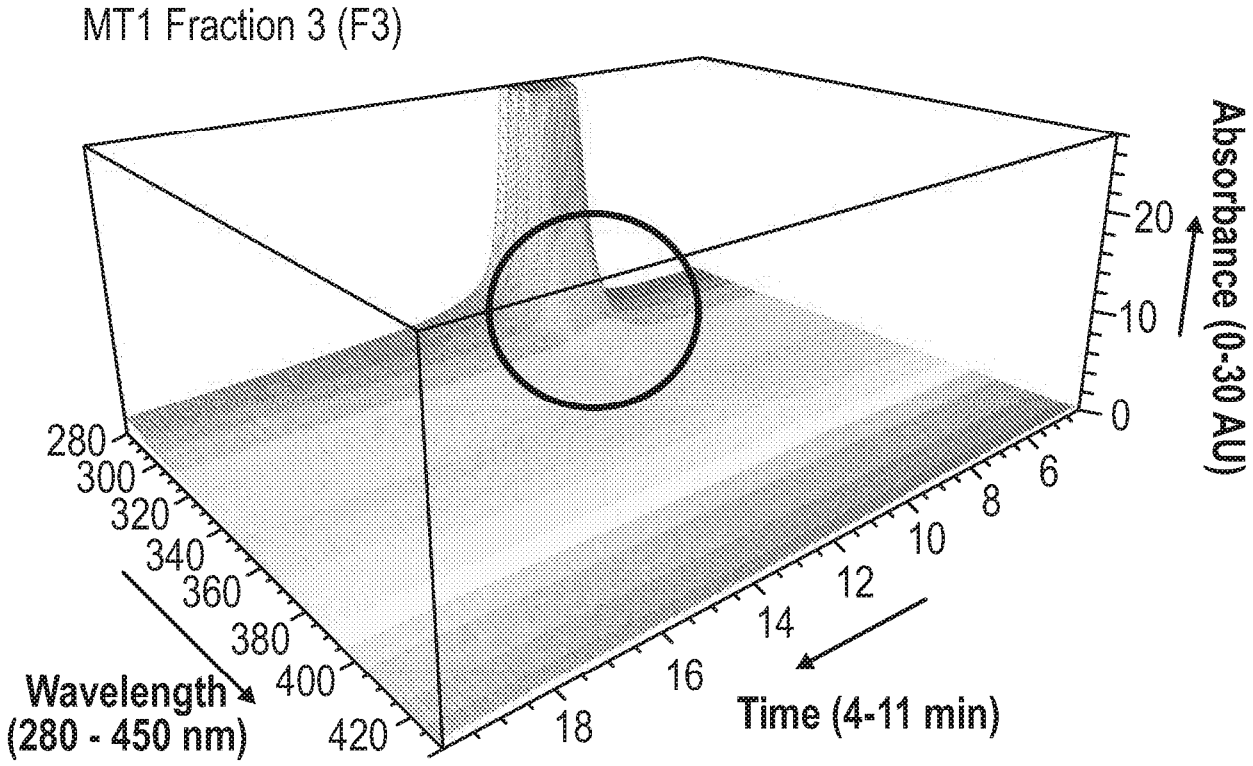


FIG. 18D

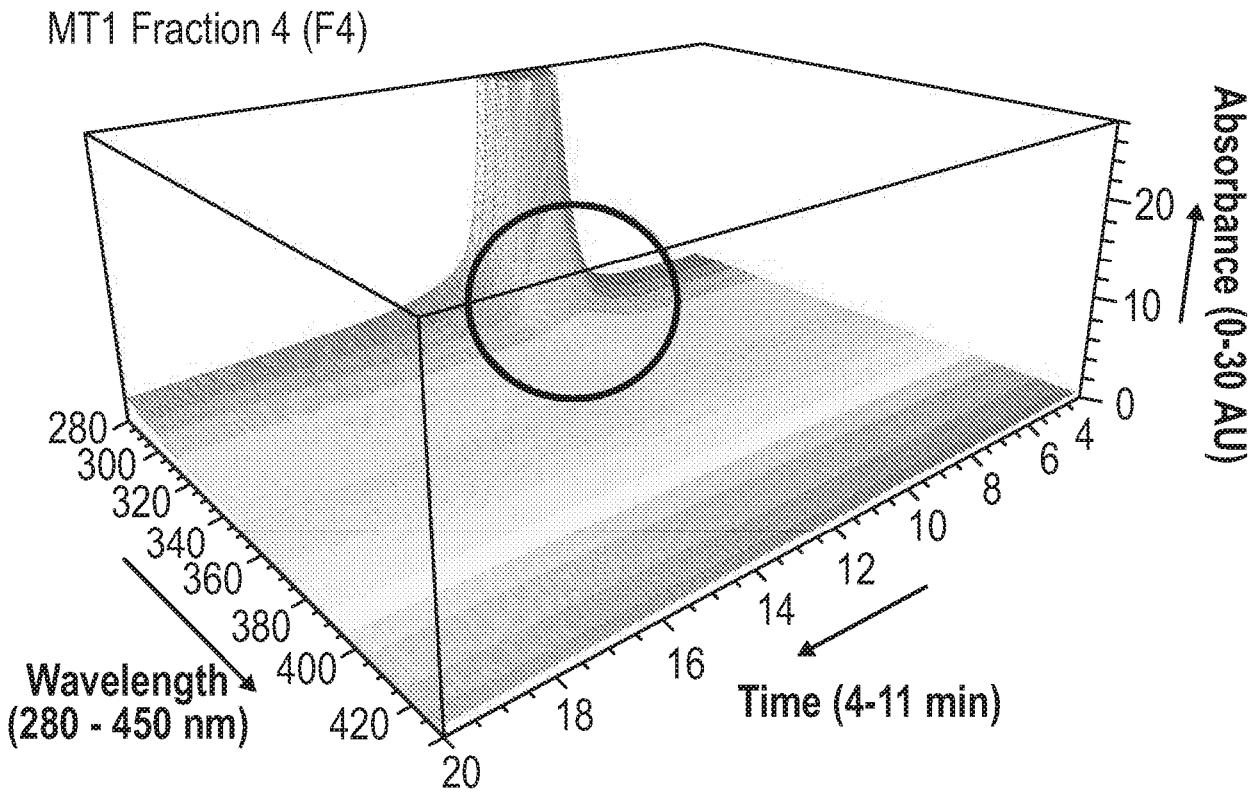


FIG. 18E

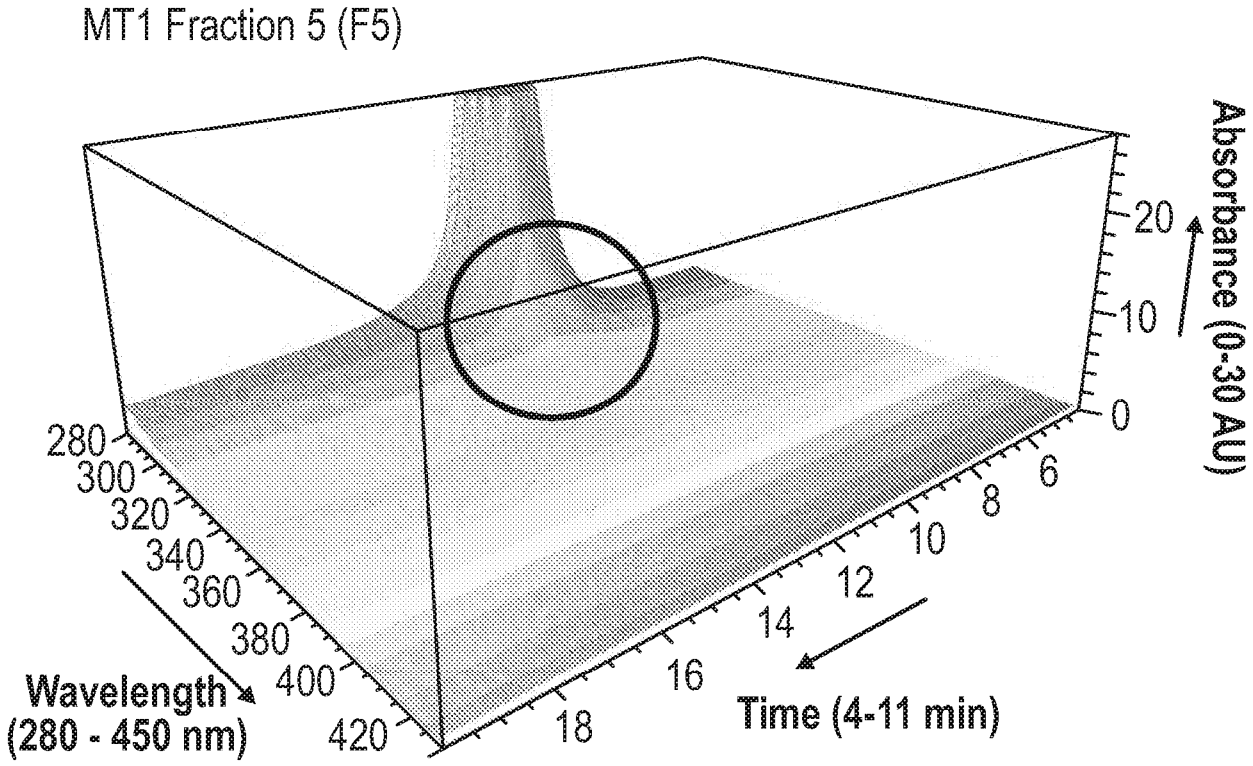


FIG. 18F

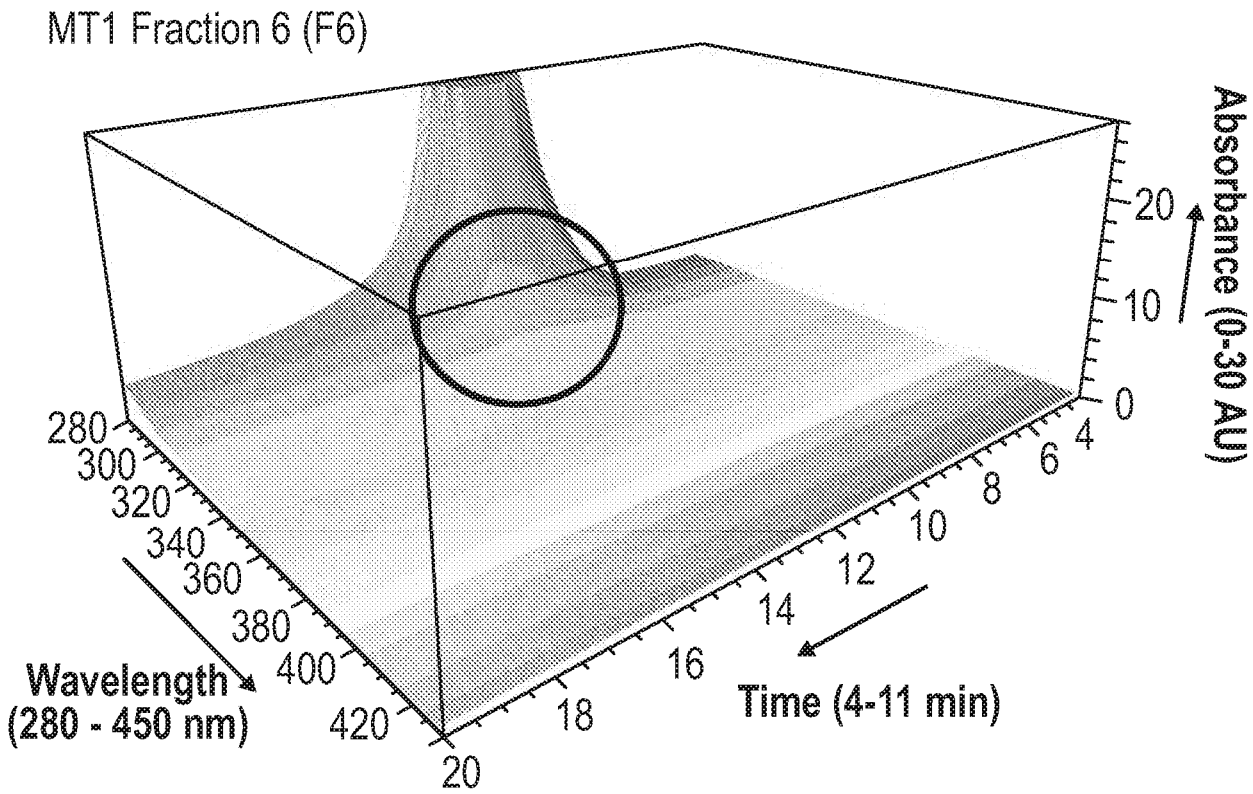


FIG. 18G

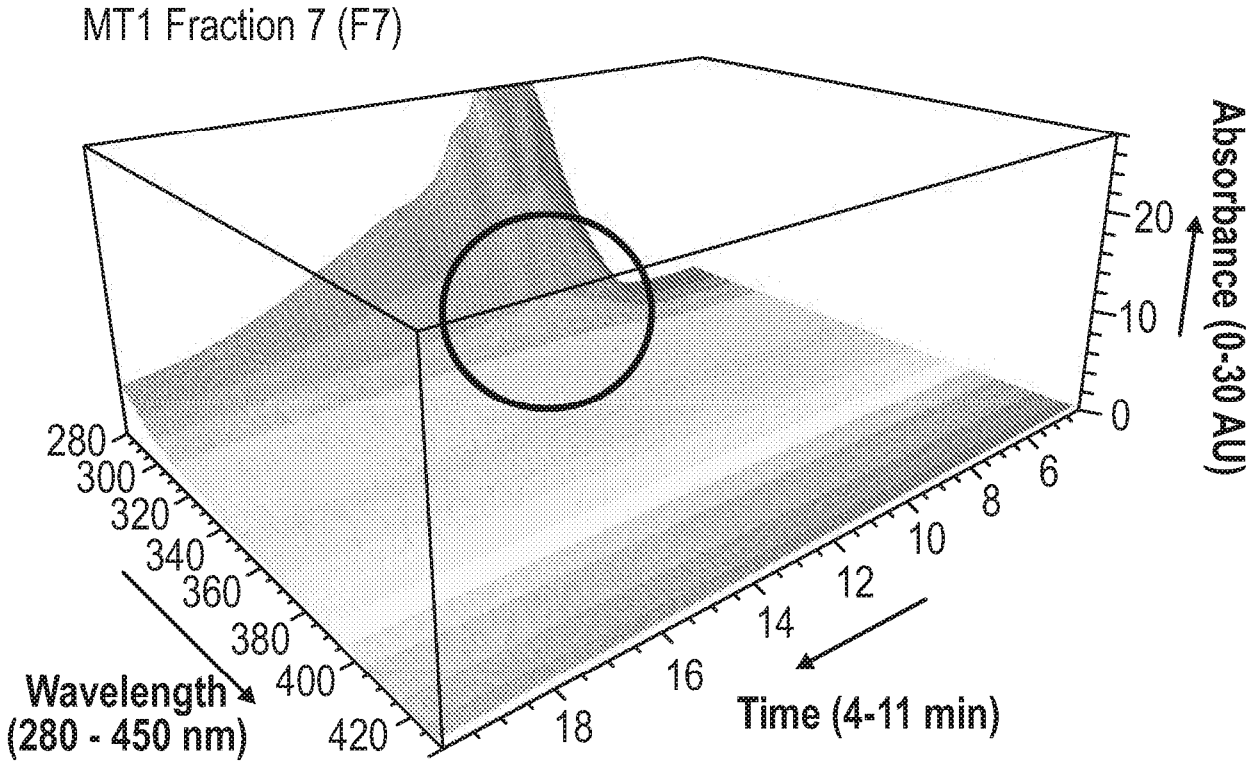


FIG. 18H

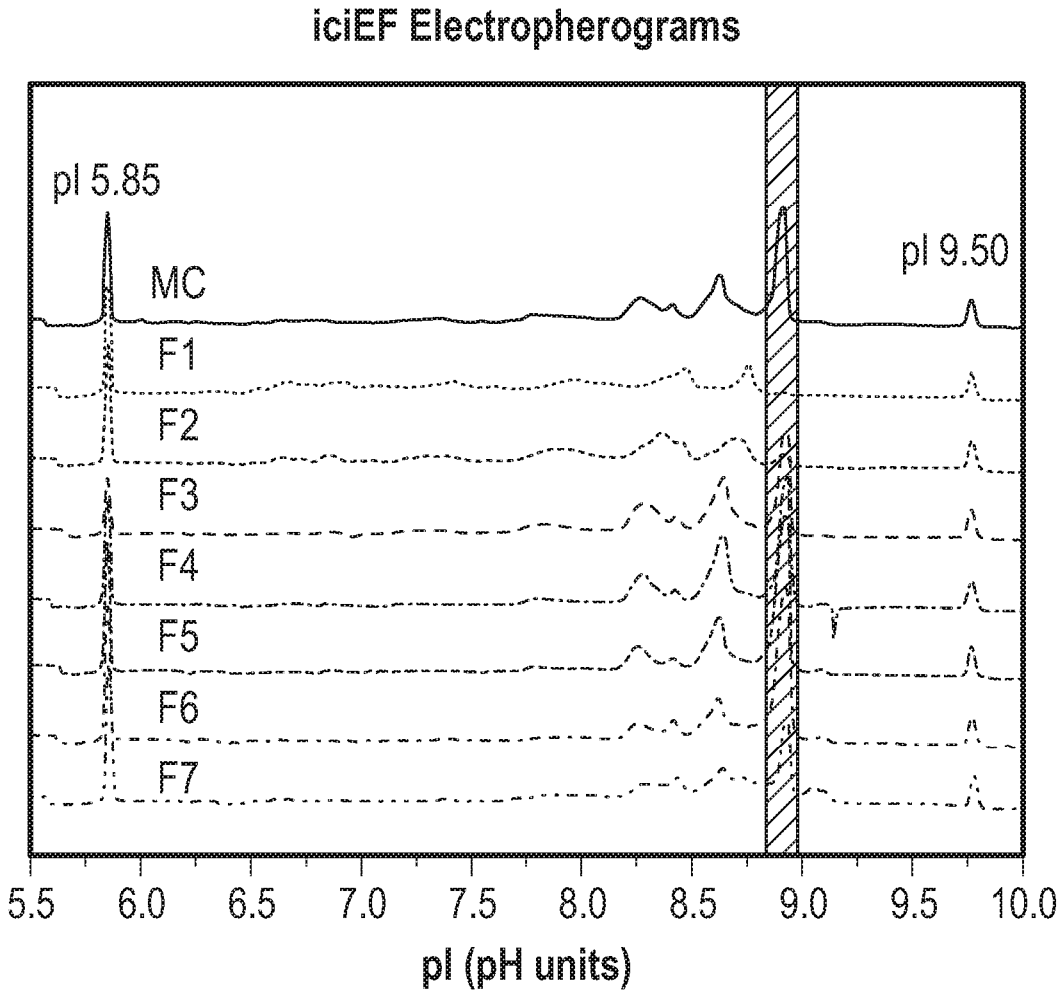

iciEF Electropherograms
pI 5.85
pI 9.50
MC
F1
F2
F3
F4
F5
F6
F7
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
pI (pH units)

FIG. 19

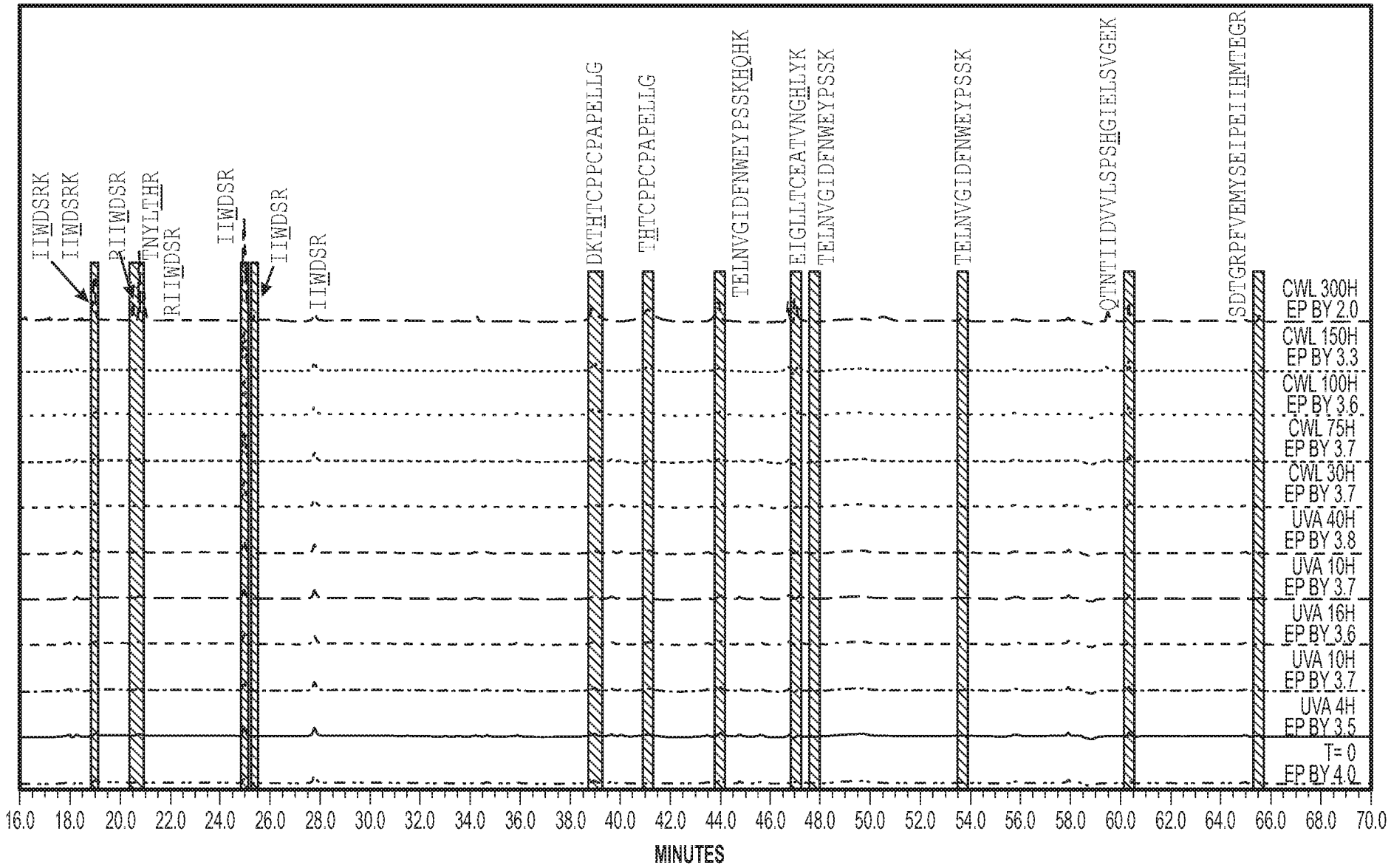
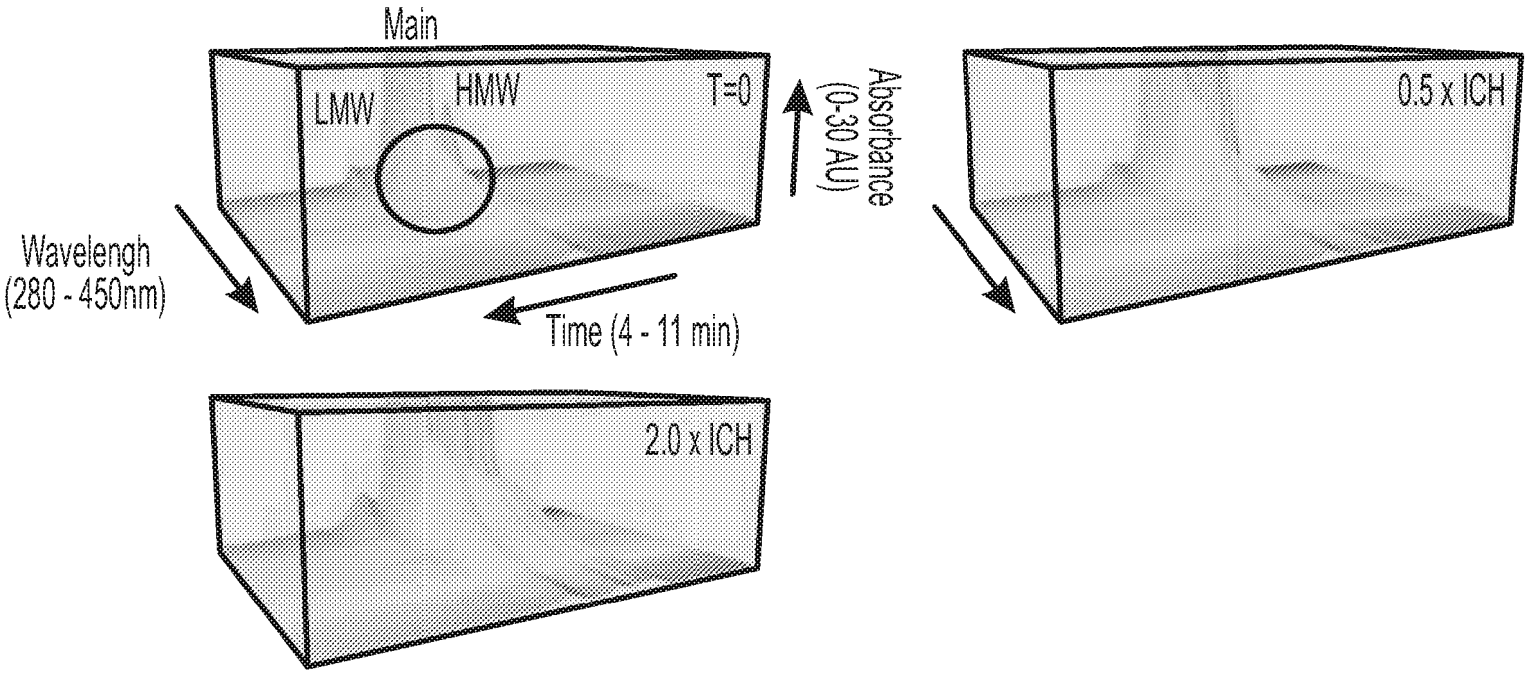

IIWDSRK
IIWDSRK
RIIWDSR
TNYLTHR
RIIWDSR
IIWDSR
IIWDSR
IIWDSR
DKTHTCPPCPAPELLG
THTCPPCPAPELLG
TELNVGIDFNWEYPSSKHQHK
EIGLLTCEATVNGHLYK
TELNVGIDFNWEYPSSK
TELNVGIDFNWEYPSSK
QTNTIIDVVLSPSHGIELSVGEK
SDTGRPFVEMYSEIPEIIHMTEGR
CWL 300H EP BY 2.0
CWL 150H EP BY 3.3
CWL 100H EP BY 3.6
CWL 75H EP BY 3.7
CWL 30H EP BY 3.7
UVA 40H EP BY 3.8
UVA 10H EP BY 3.7
UVA 16H EP BY 3.6
UVA 10H EP BY 3.7
UVA 4H EP BY 3.5
T= 0 EP BY 4.0
16.0 18.0 20.0 22.0 24.0 26.0 28.0 30.0 32.0 34.0 36.0 38.0 40.0 42.0 44.0 46.0 48.0 50.0 52.0 54.0 56.0 58.0 60.0 62.0 64.0 66.0 68.0 70.0
MINUTES

FIG. 20


Main
LMW
HMW
T=0
Absorbance
(0-30 AU)
0.5 x ICH
Wavelengh
(280 - 450nm)
Time (4 - 11 min)
2.0 x ICH

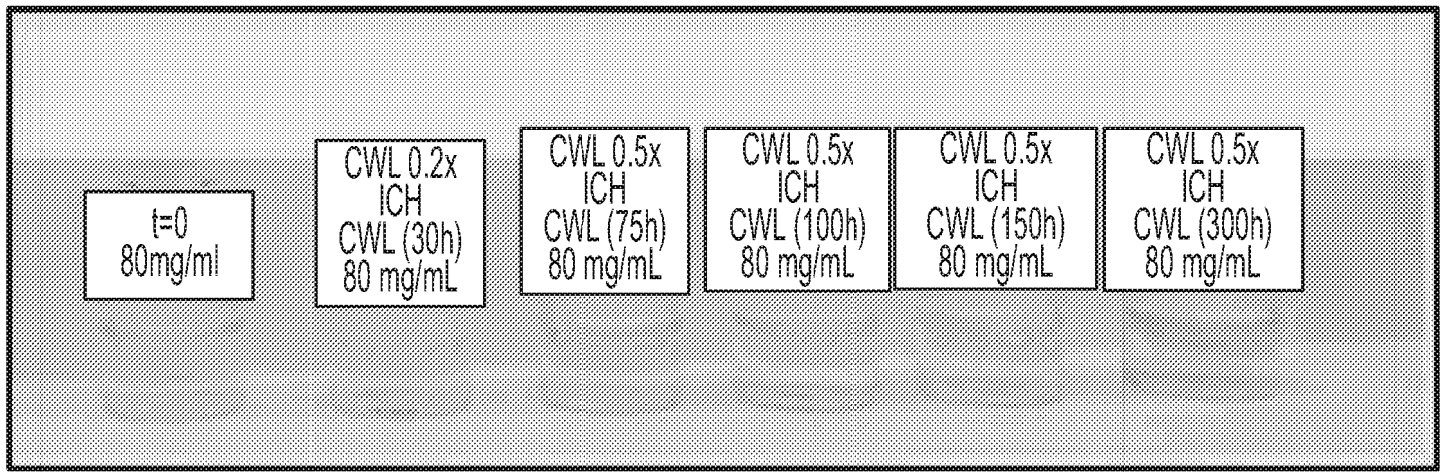

t=0
80mg/ml
CWL 0.2x
ICH
CWL (30h)
80 mg/mL
CWL 0.5x
ICH
CWL (75h)
80 mg/mL
CWL 0.5x
ICH
CWL (100h)
80 mg/mL
CWL 0.5x
ICH
CWL (150h)
80 mg/mL
CWL 0.5x
ICH
CWL (300h)
80 mg/mL

FIG. 21

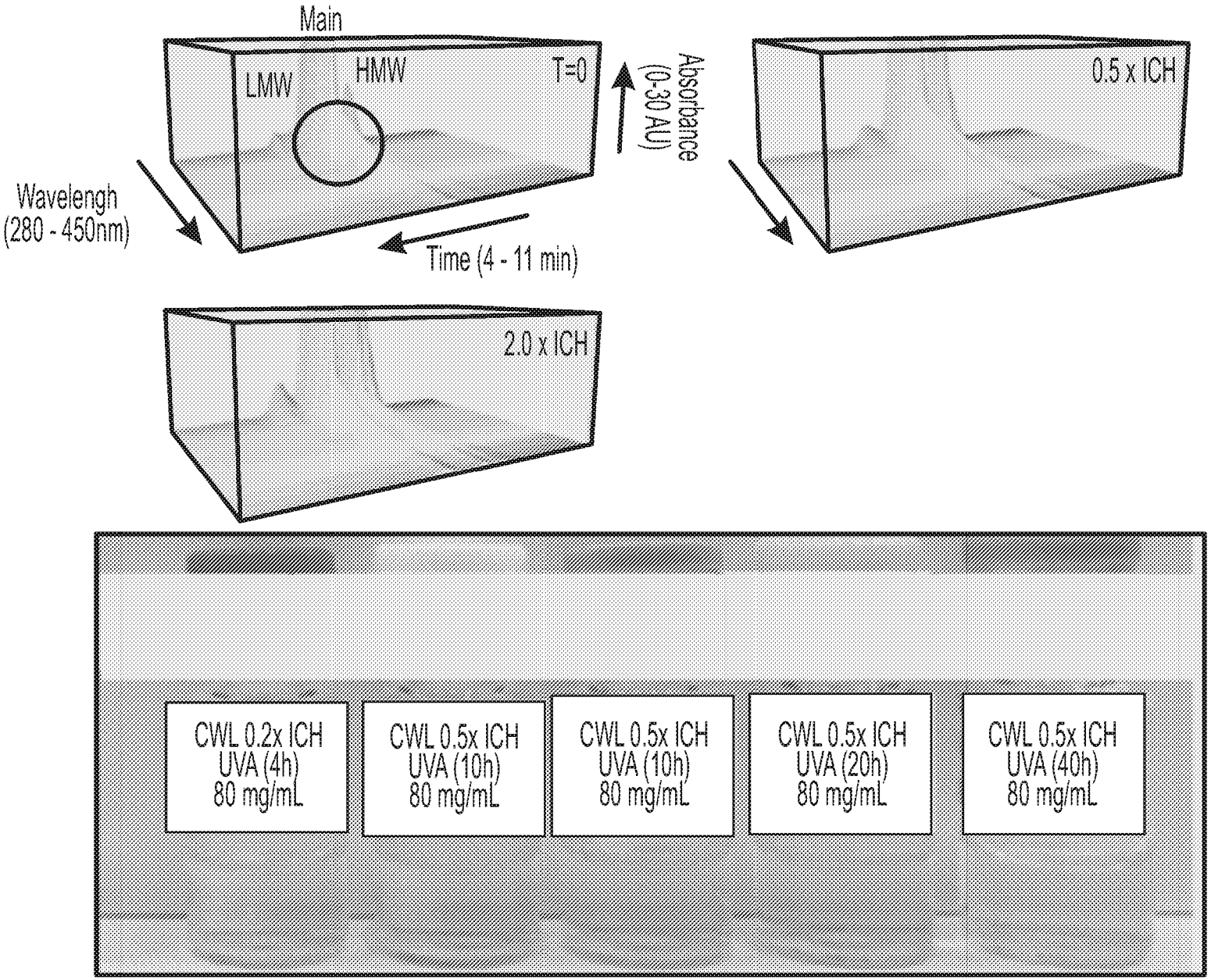

Main
LMW
HMW
T=0
Absorbance
(0-30 AU)
0.5 x ICH
Wavelengh
(280 - 450nm)
Time (4 - 11 min)
2.0 x ICH
CWL 0.2x ICH
UVA (4h)
80 mg/mL
CWL 0.5x ICH
UVA (10h)
80 mg/mL
CWL 0.5x ICH
UVA (10h)
80 mg/mL
CWL 0.5x ICH
UVA (20h)
80 mg/mL
CWL 0.5x ICH
UVA (40h)
80 mg/mL

FIG. 22

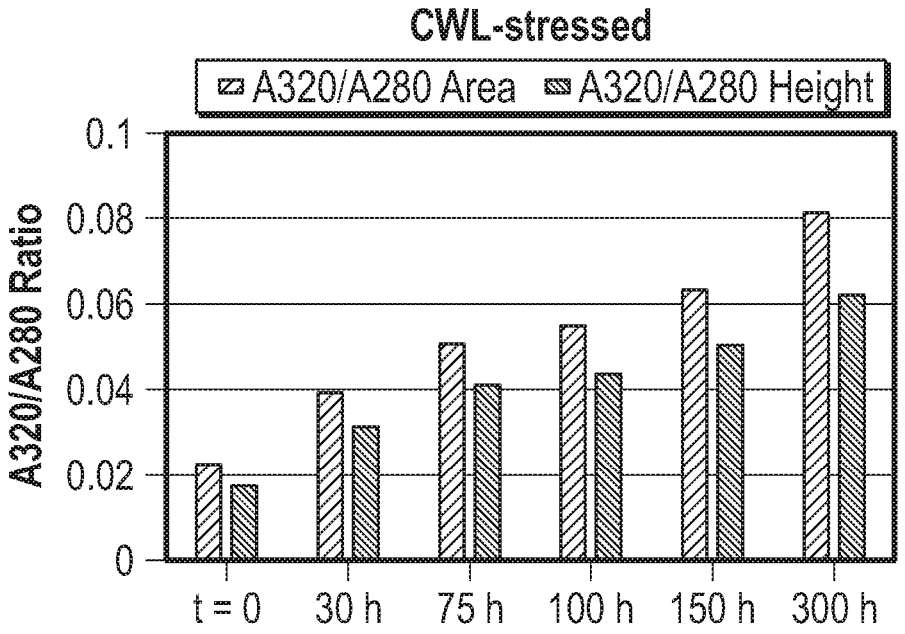


FIG. 23A

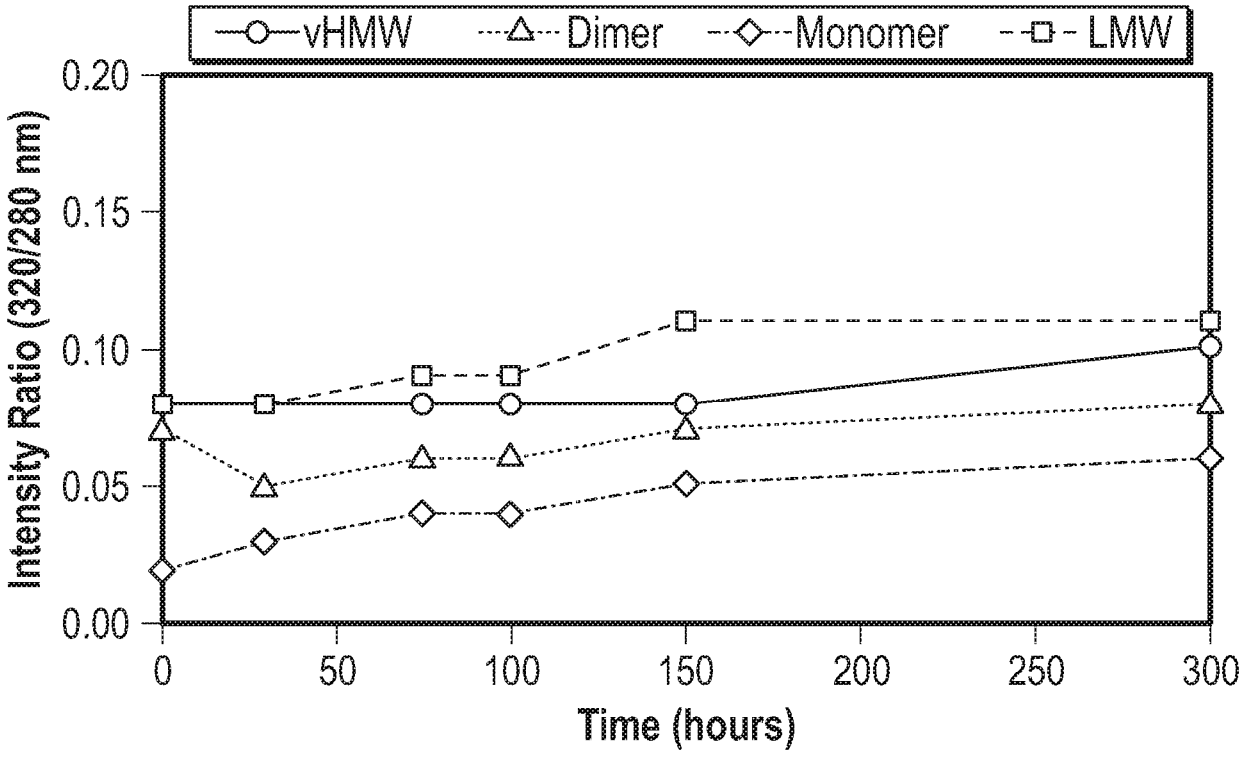


FIG. 23B

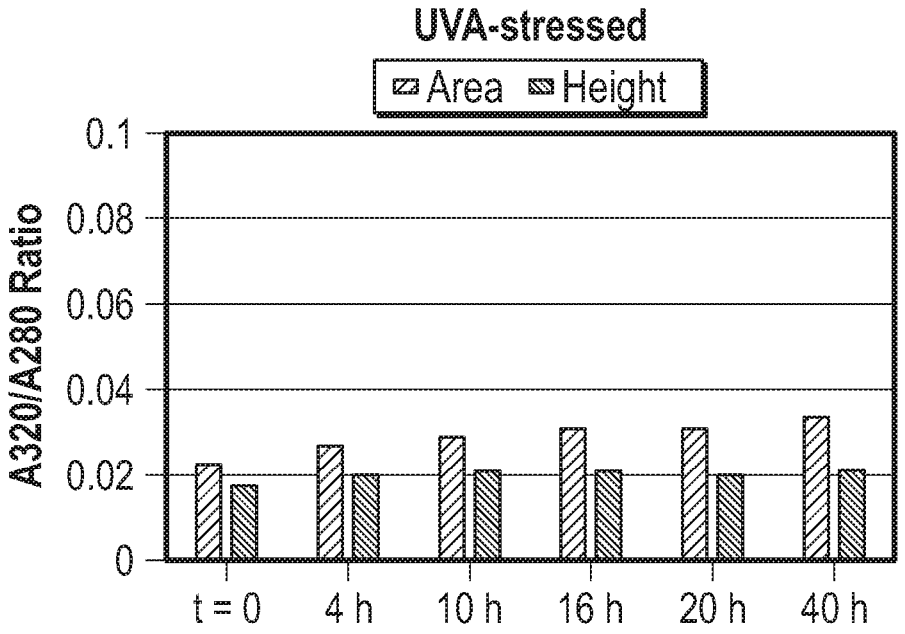


FIG. 24A

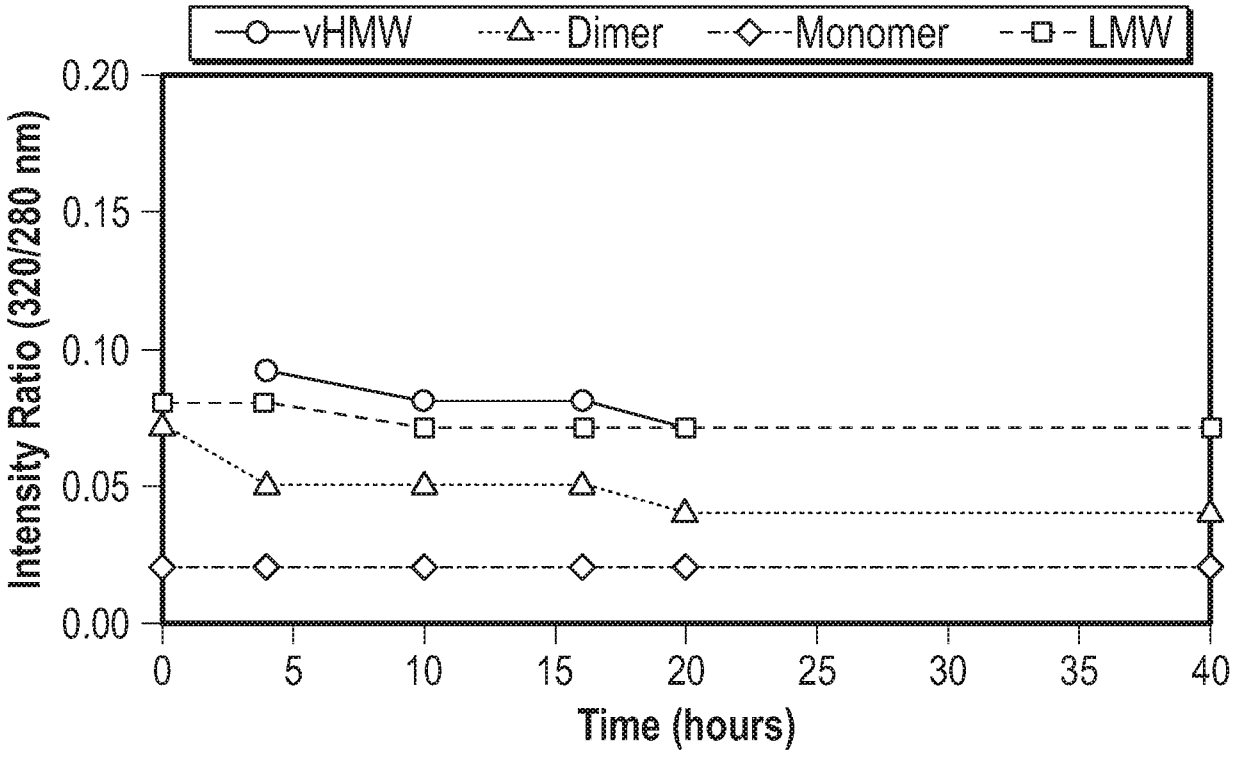


FIG. 24B

Scaled Estimates					
Term	Scaled Estimate		Std Error	t Ratio	Prob> |t|
Intercept	0.4682071		0.009776	47.89	<.0001*
Cysteine(0,1)	0.3470799		0.009806	35.40	<.0001*
B-Vits Group(0,1)	0.0373611		0.009897	3.78	0.0008*
Iron(0,1)	0.0698208		0.010074	6.93	<.0001*
Zinc(0,1)	0.0538585		0.010255	5.25	<.0001*
Folic Acid(0,1)	0.037899		0.010172	3.73	0.0009*
Cysteine*B-Vits Group	0.0253685		0.009837	2.58	**0.0155***
Cysteine*Iron	0.07244		0.01011	7.17	<.0001*
Cysteine*Zinc	0.0339284		0.009973	3.40	0.0020*
Cysteine*Folic Acid	0.0298402		0.010218	2.92	0.0068*
B-Vits Group*Iron	0.0229271		0.009945	2.31	**0.0288***
B-Vits Group*Folic Acid	-0.022614		0.010243	-2.21	**0.0356***

FIG. 25A

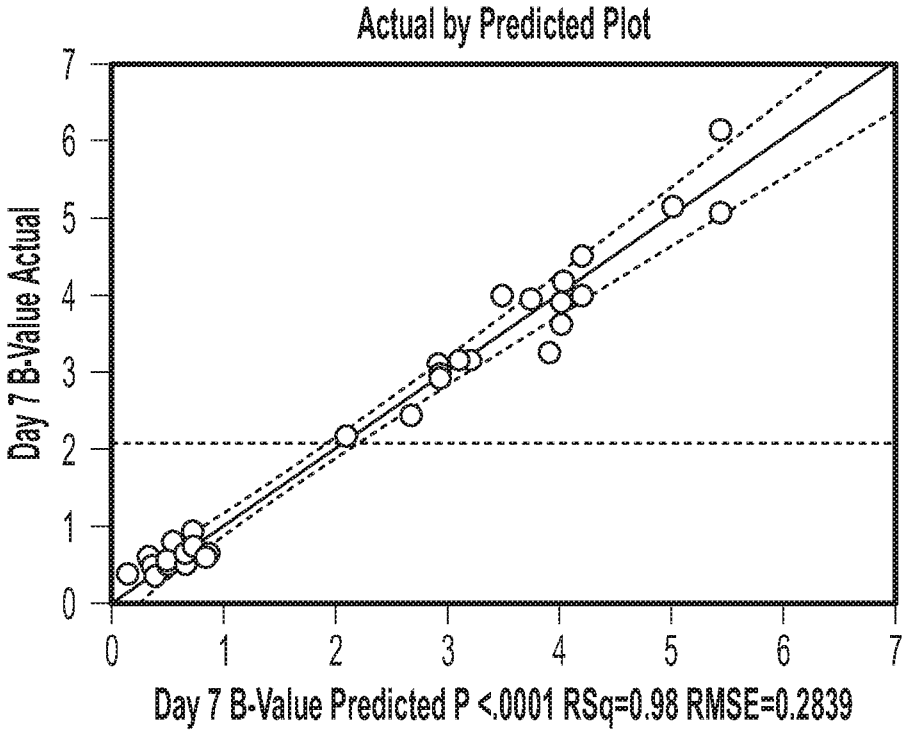


FIG. 25B

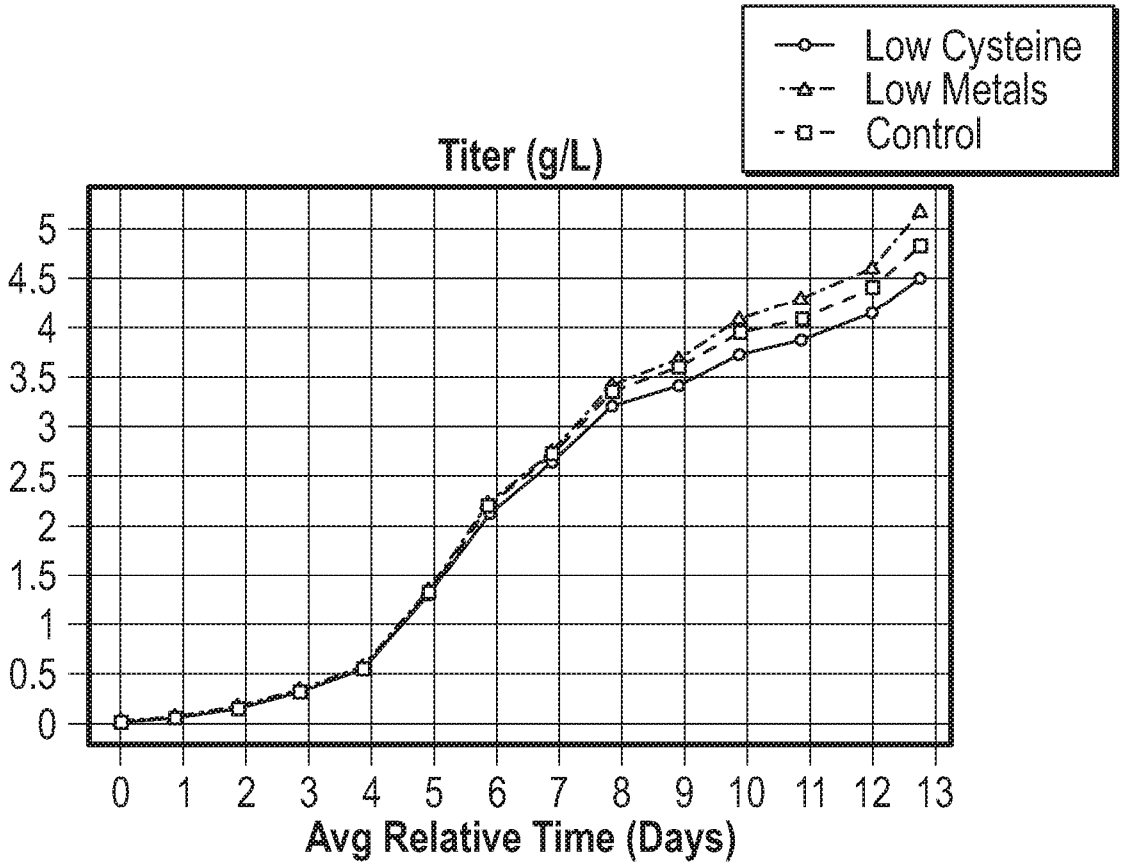


FIG. 26A

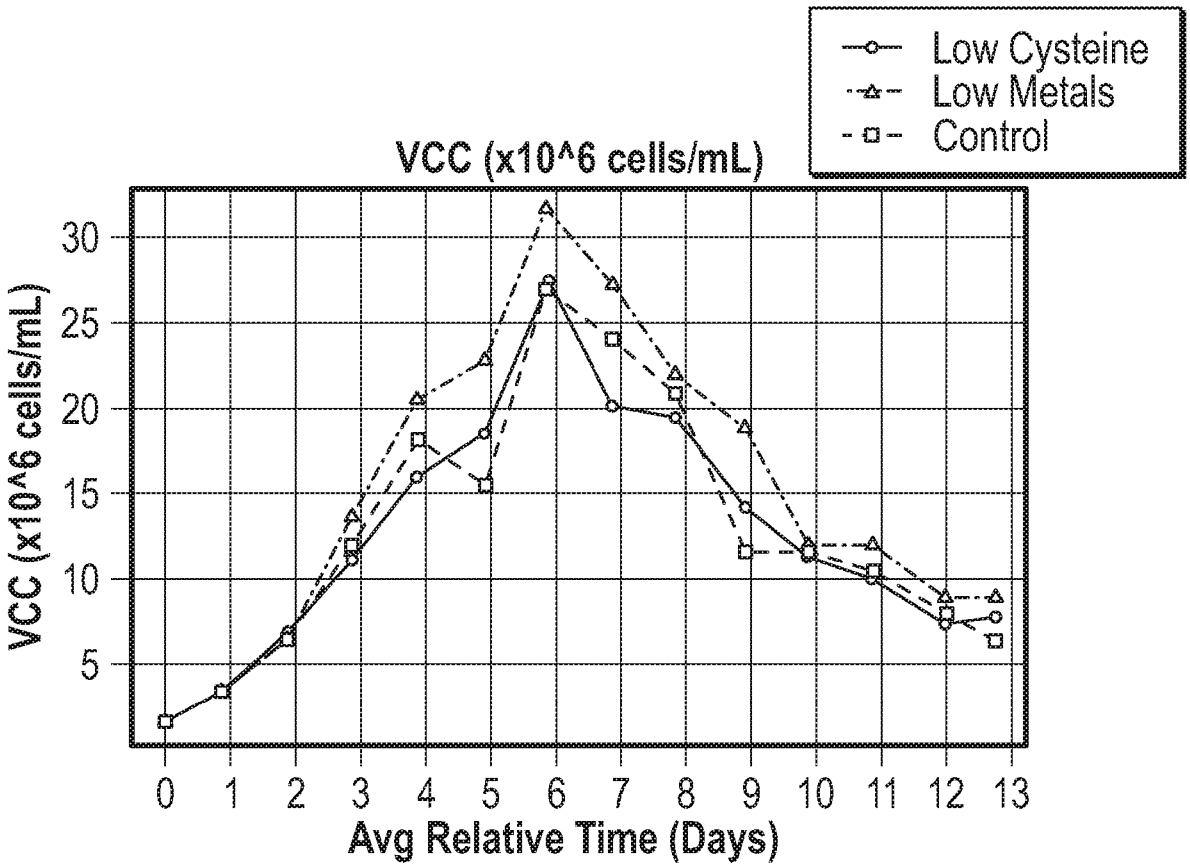


FIG. 26B

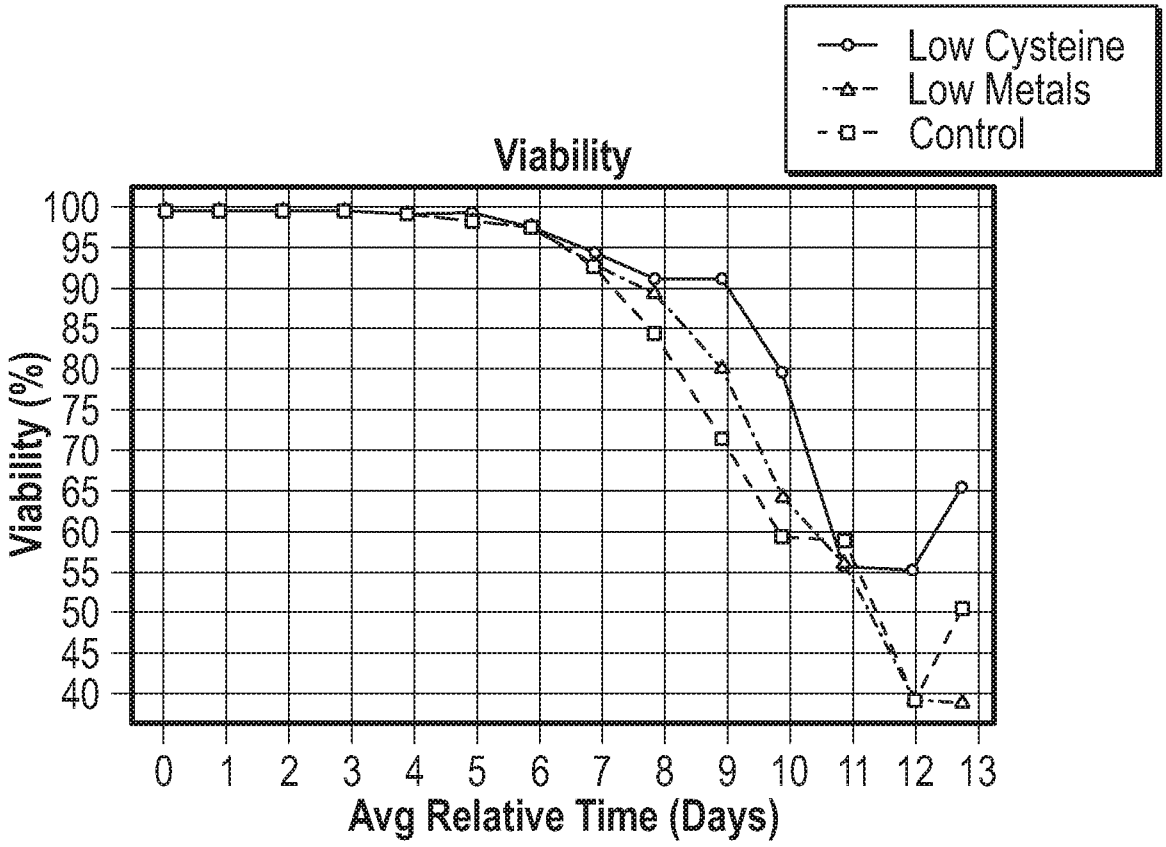


FIG. 26C

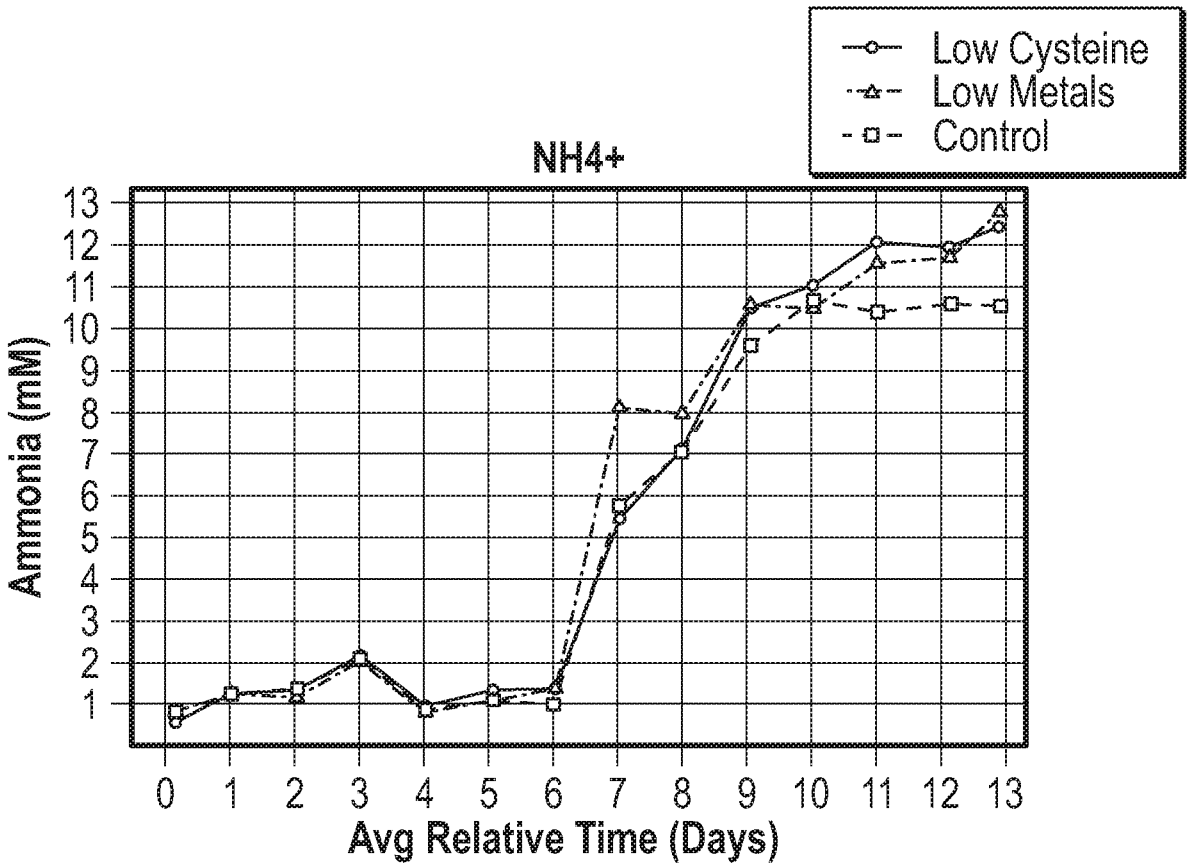


FIG. 26D

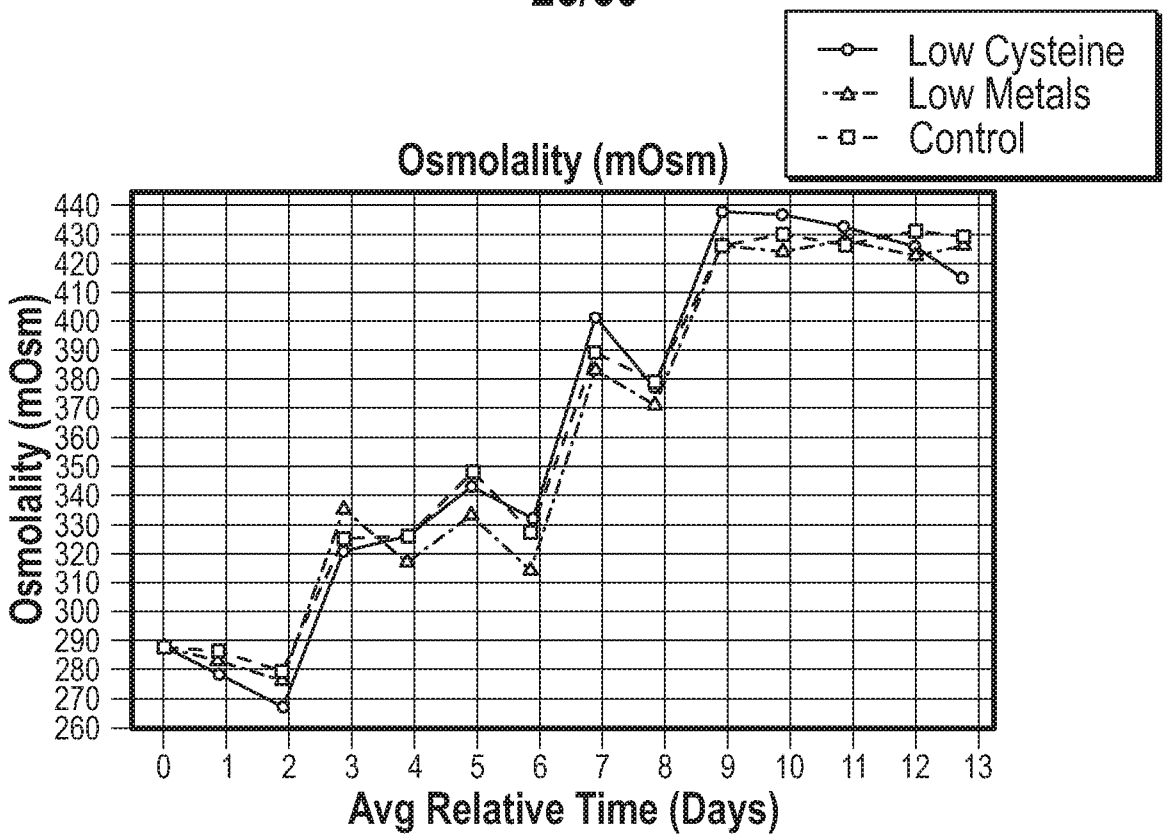
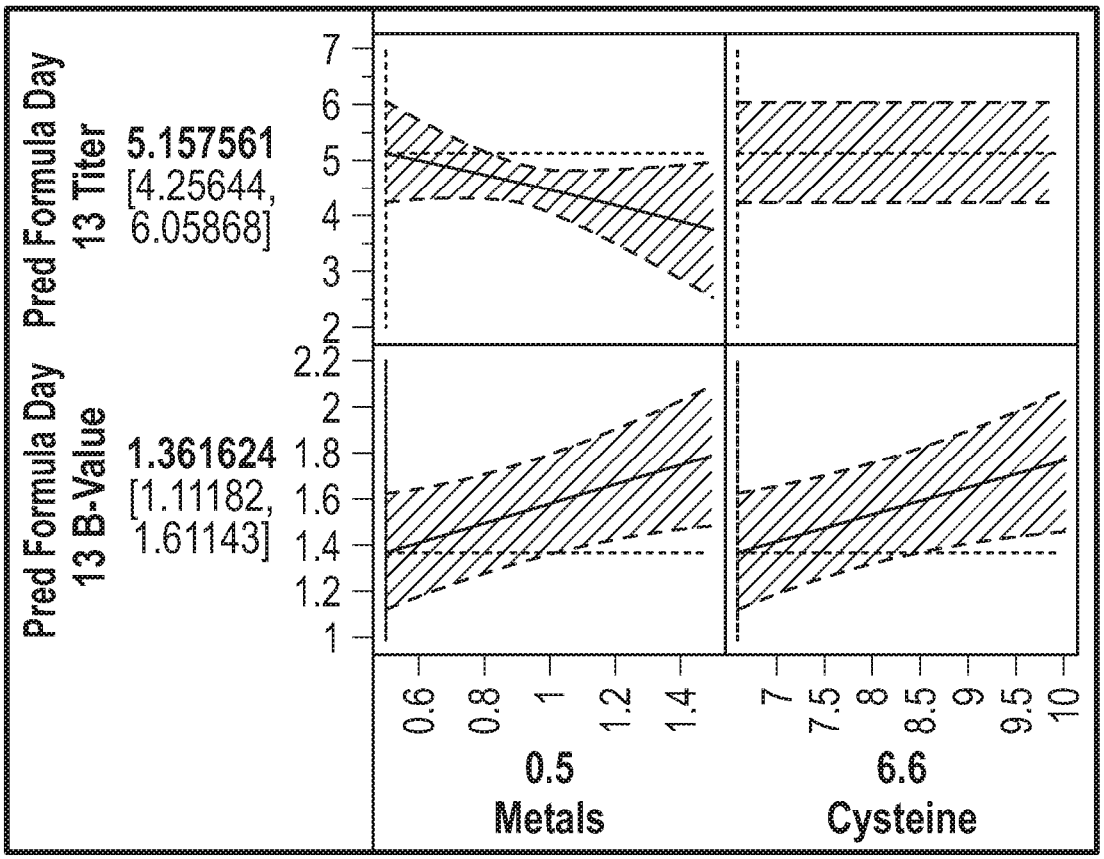


FIG. 26E



Control b-value = 1.67

FIG. 27

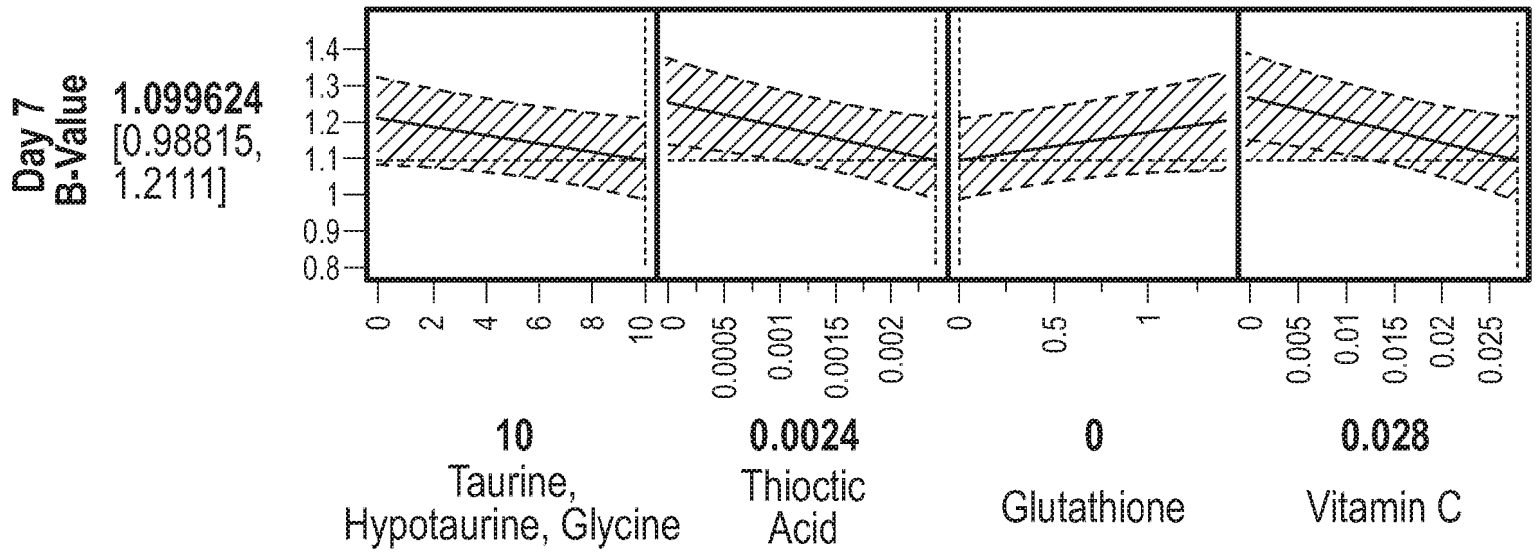


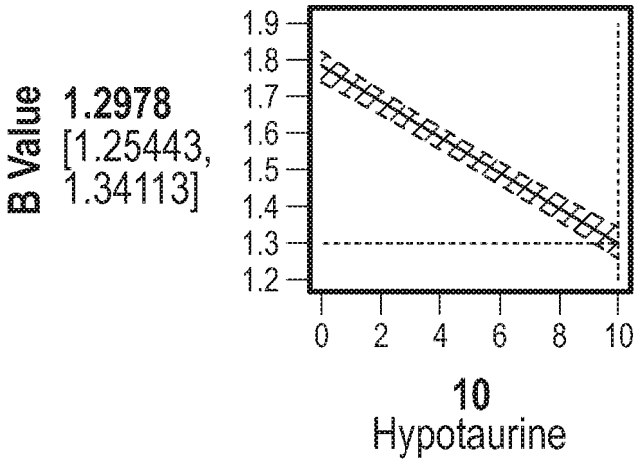
FIG. 28A

Scaled Estimates					
Term	Scaled Estimate		Std Error	t Ratio	Prob> |t|
Intercept	1.3997181		0.022019	63.57	<.0001*
Taurine, Hypotaurine, Glycine(0,1)	-0.153727		0.02201	-6.98	<.0001*
Thioctic Acid(0,1)	-0.010756		0.022019	-0.49	0.6293
Glutathione(0,1)	0.152492		0.022112	6.90	<.0001*
Vitamin C(0,1)	-0.017603		0.02201	-0.80	0.4311
Taurine, Hypotaurine, Glycine*Glutathione	-0.101323		0.022186	-4.57	0.0001*
Thioctic Acid*Vitamin C	-0.06684		0.02201	-3.04	0.0054*

FIG. 28B

Term	Scaled Estimate	Std Error	t Ratio	Prob> |t|
Intercept	1.079804	0.126434	8.54	**0.0010***
Taurine (0,1)	-0.030846	0.040585	-0.76	0.4896
Hypotaurine (0,1)	-0.085952	0.044048	-1.95	0.1228
Glycine (0,1)	-0.181863	0.047269	-3.85	**0.0183***
Thioctic Acid (0,1)	0.0575148	0.0465	1.24	0.2838
Vitamin C (0,1)	-0.093266	0.0392	-2.38	0.0761
Taurine*Hypotaurine	0.1943093	0.042535	4.57	**0.0103***
Taurine*Thioctic Acid	-0.031889	0.044591	-0.72	0.5140
Taurine*Vitamin C	0.0948234	0.046063	2.06	0.1086
Hypotaurine*Trioctic Acid	-0.009982	0.045049	-0.22	0.8355
Hypotaurine*Vitamin C	0.1509187	0.043708	3.45	**0.0260***
Thioctic Acid*Vitamin C	-0.176346	0.043781	-4.03	**0.0158***
Taurine*Taurine	0.3679087	0.117837	3.12	**0.0355***
Hypotaurine*Hypotaurine	0.192703	0.110017	1.75	0.1547
Glycine*Glycine	-0.270392	0.11851	-2.28	0.0846

FIG. 28C



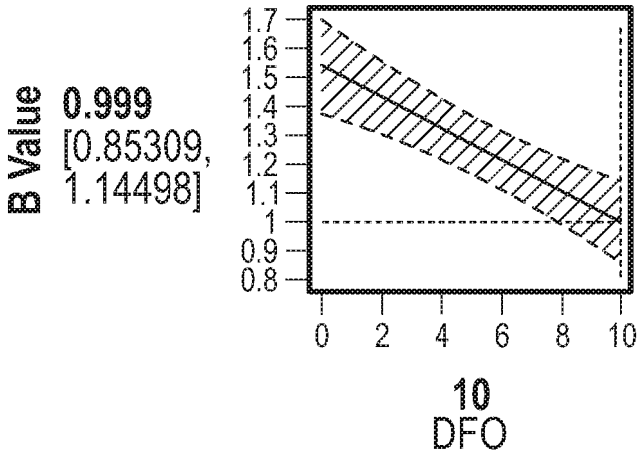


FIG. 28D

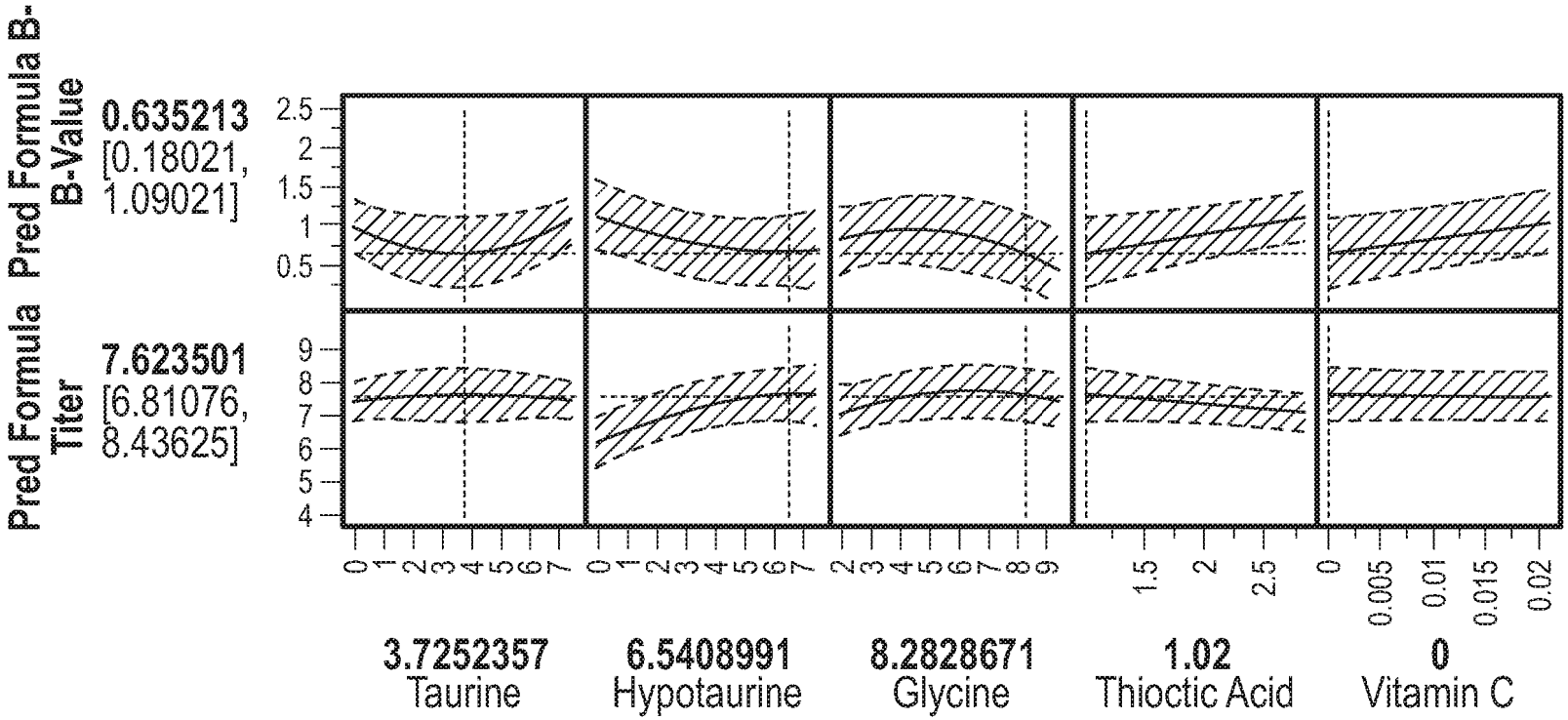


FIG. 28E

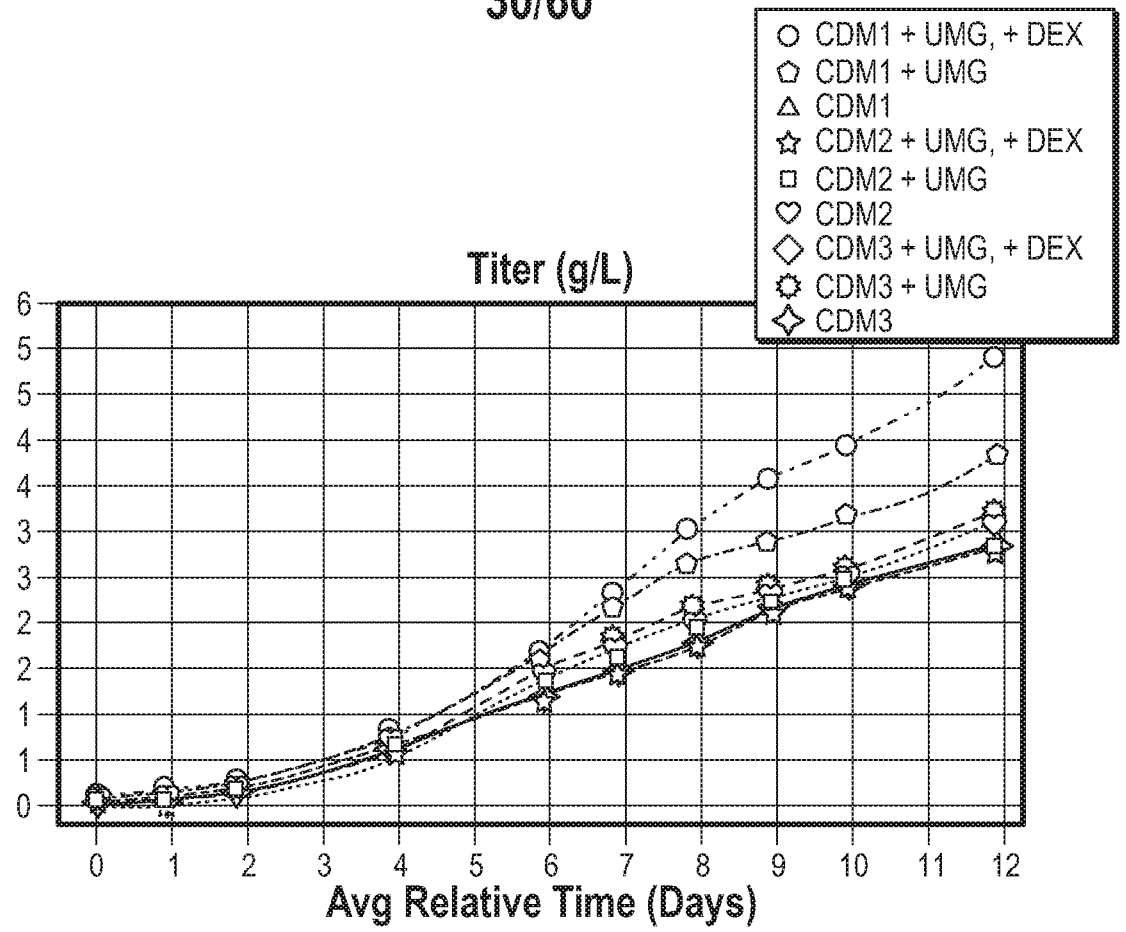

CDM1 + UMG, + DEX
CDM1 + UMG
CDM1
CDM2 + UMG, + DEX
CDM2 + UMG
CDM2
CDM3 + UMG, + DEX
CDM3 + UMG
CDM3
Titer (g/L)
6
5
5
4
4
3
3
2
2
1
1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Avg Relative Time (Days)

FIG. 29

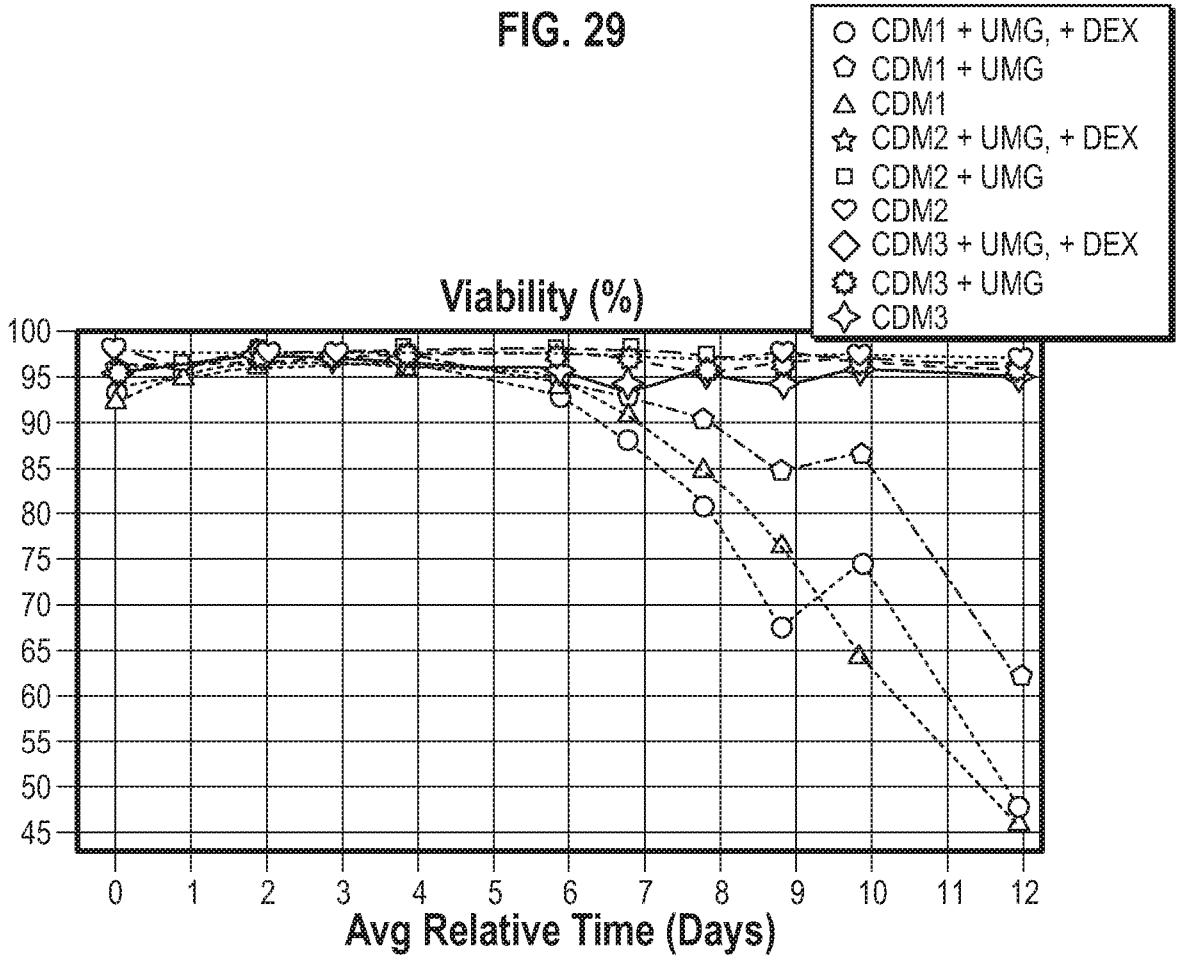

CDM1 + UMG, + DEX
CDM1 + UMG
CDM1
CDM2 + UMG, + DEX
CDM2 + UMG
CDM2
CDM3 + UMG, + DEX
CDM3 + UMG
CDM3
Viability (%)
100
95
90
85
80
75
70
65
60
55
50
45
0 1 2 3 4 5 6 7 8 9 10 11 12
Avg Relative Time (Days)

FIG. 30

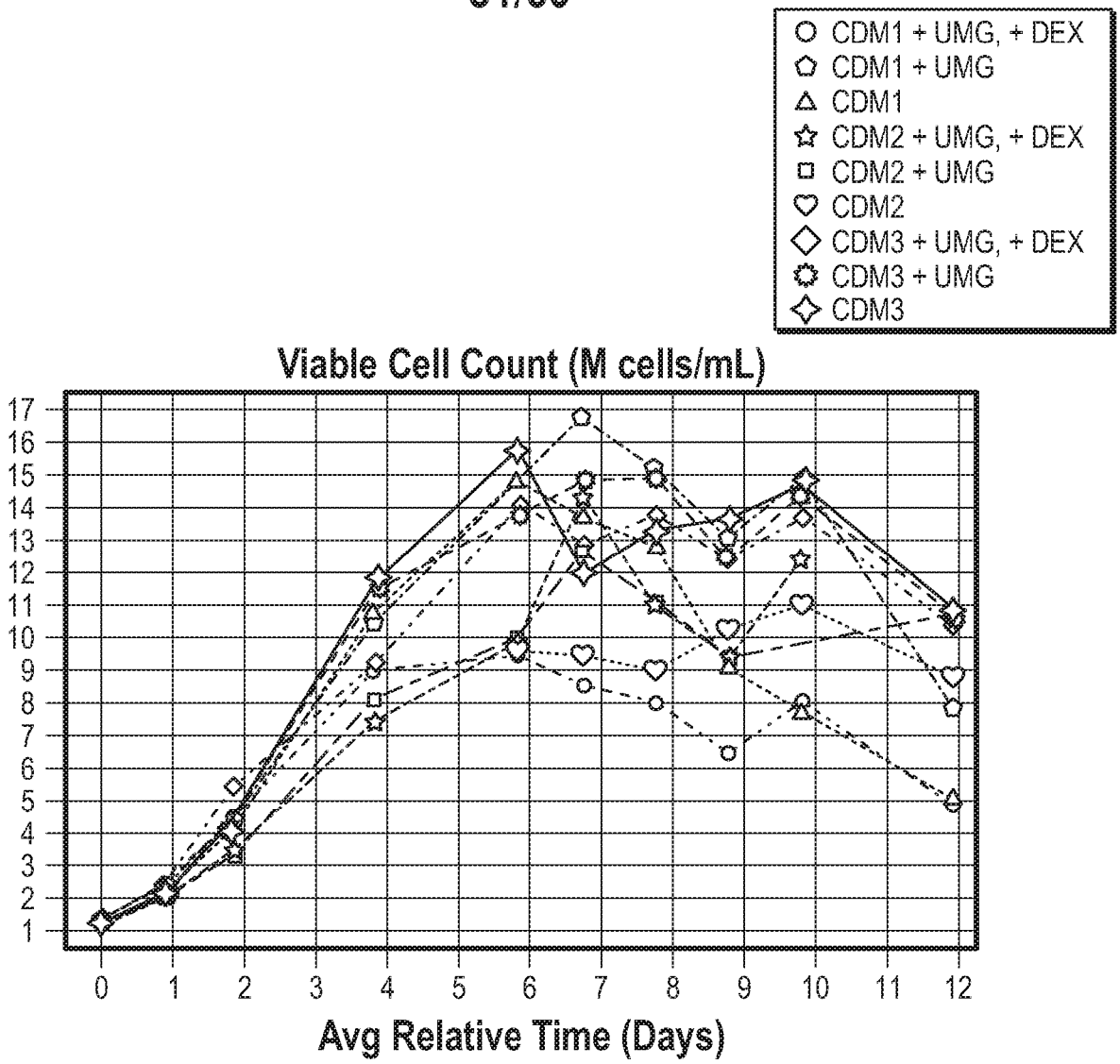

CDM1 + UMG, + DEX
CDM1 + UMG
CDM1
CDM2 + UMG, + DEX
CDM2 + UMG
CDM2
CDM3 + UMG, + DEX
CDM3 + UMG
CDM3
Viable Cell Count (M cells/mL)
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
Avg Relative Time (Days)

FIG. 31

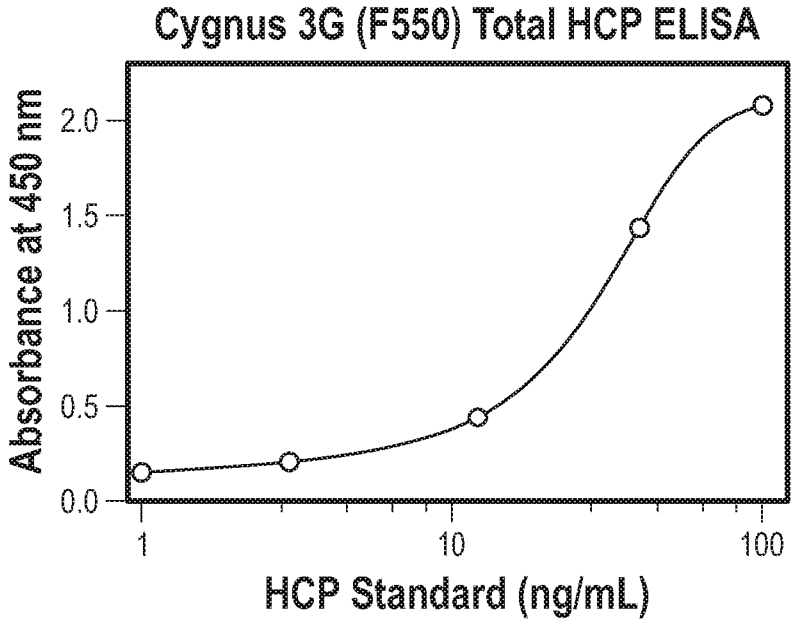

Cygnus 3G (F550) Total HCP ELISA
Absorbance at 450 nm
2.0
1.5
1.0
0.5
0.0
1
10
100
HCP Standard (ng/mL)

FIG. 32

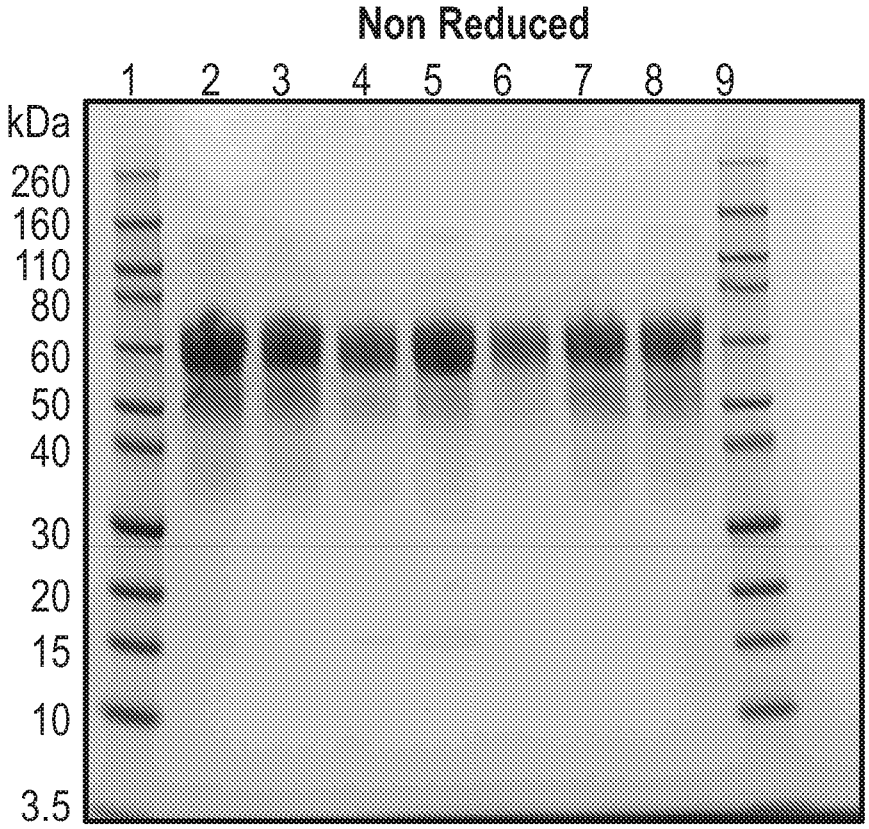

Non Reduced
1 2 3 4 5 6 7 8 9
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5

FIG. 33

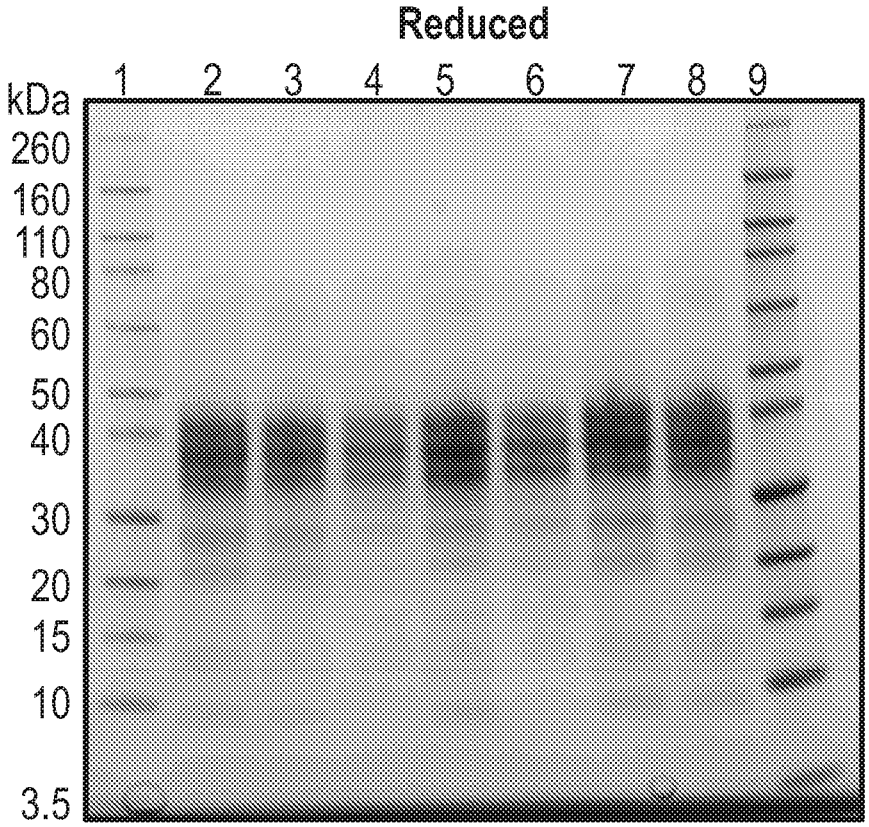

Reduced
1 2 3 4 5 6 7 8 9
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5

FIG. 34

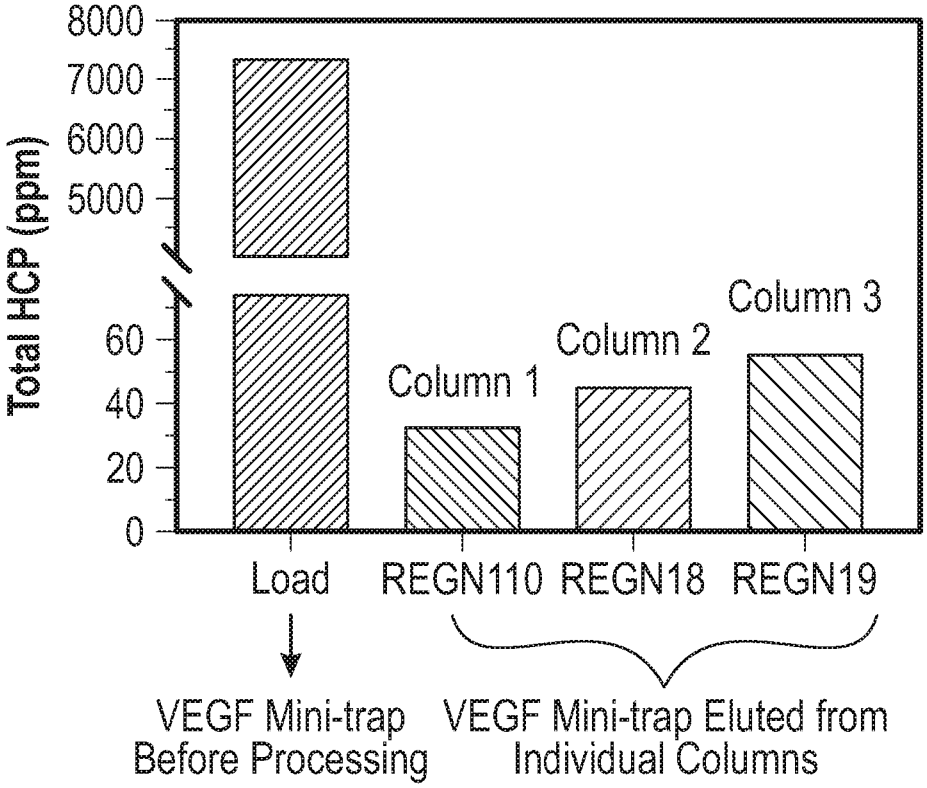


FIG. 35A

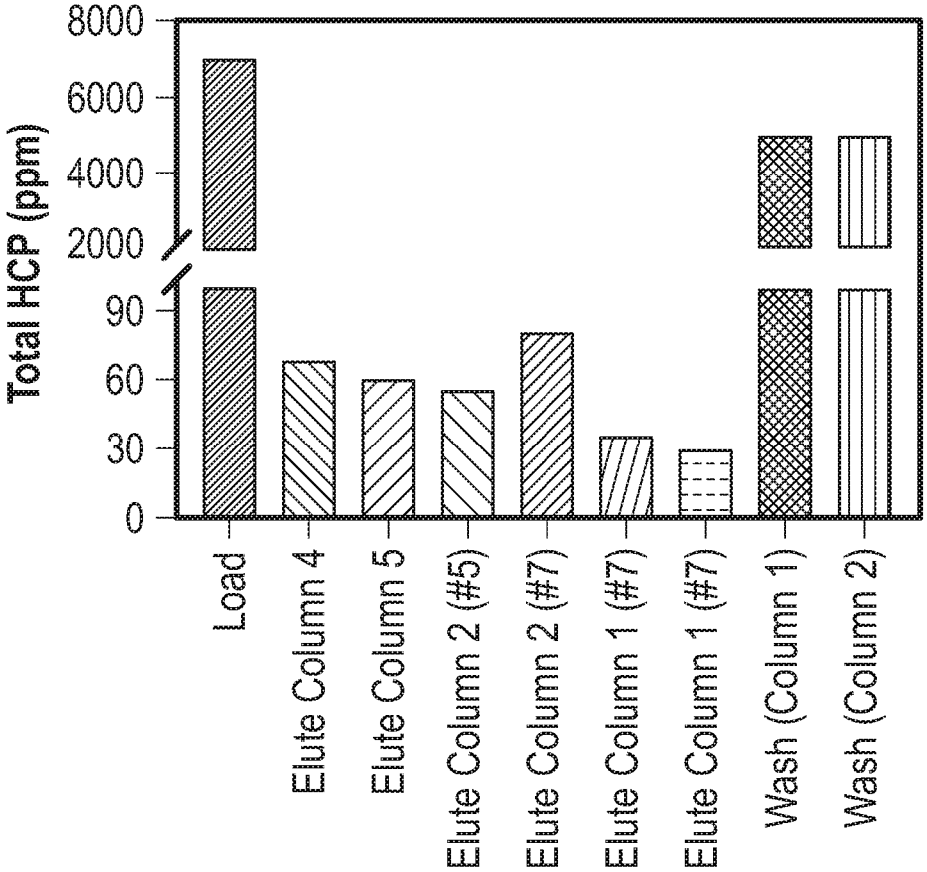


FIG. 35B

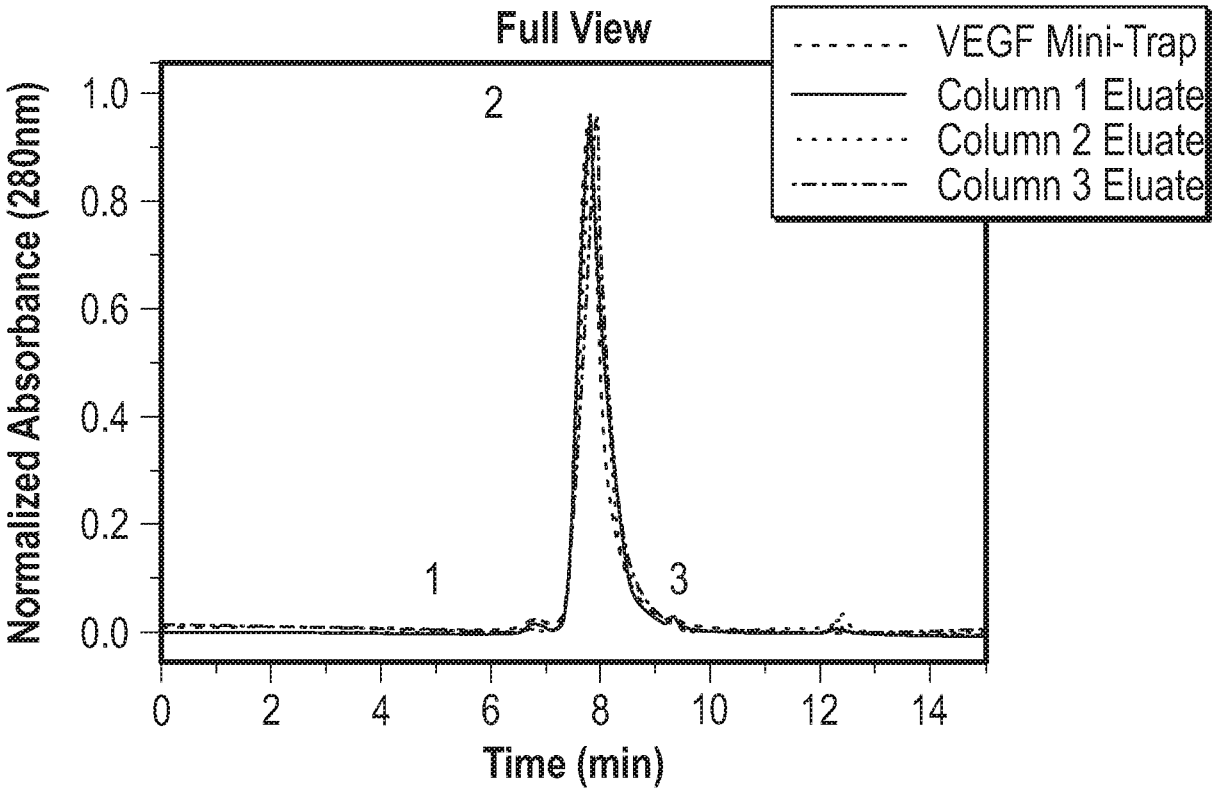


FIG. 36A

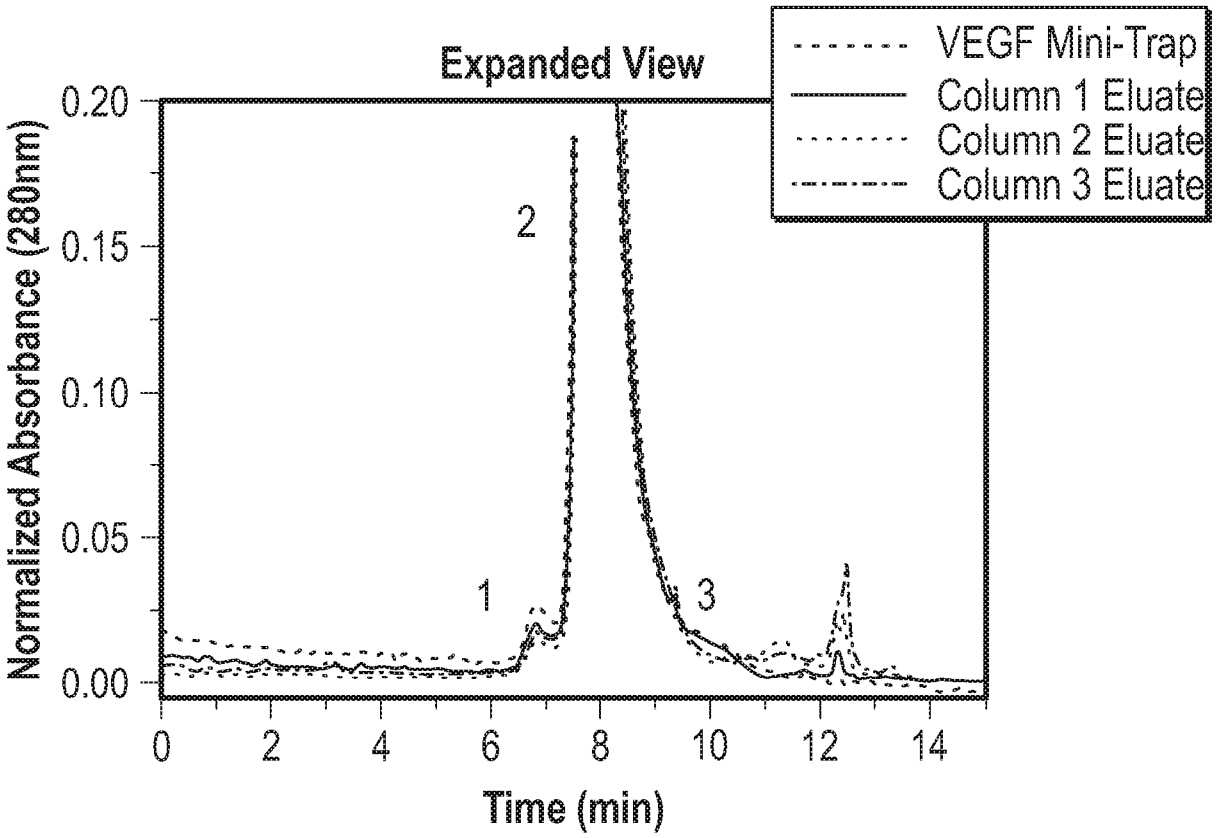


FIG. 36B

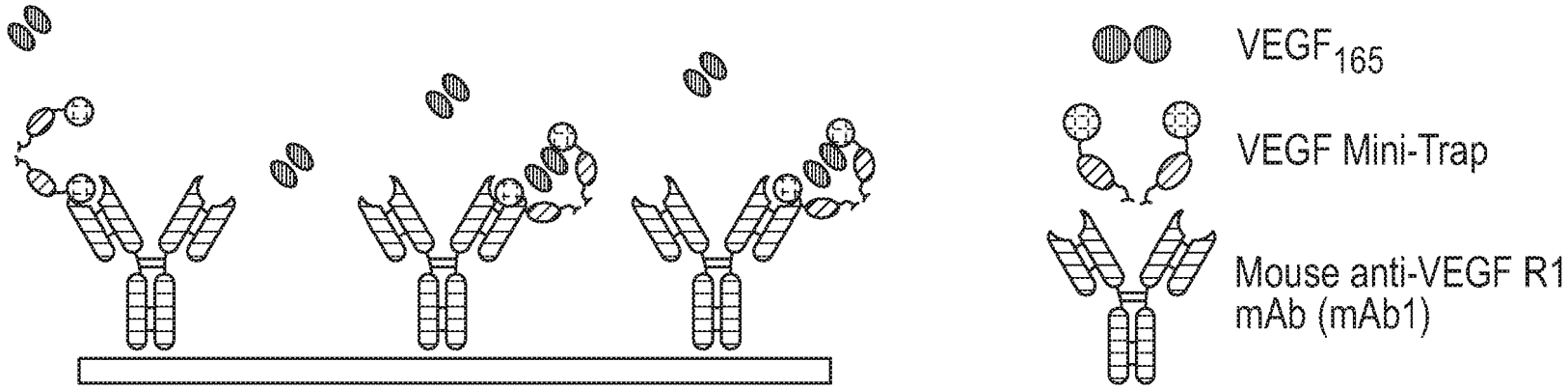


FIG. 37

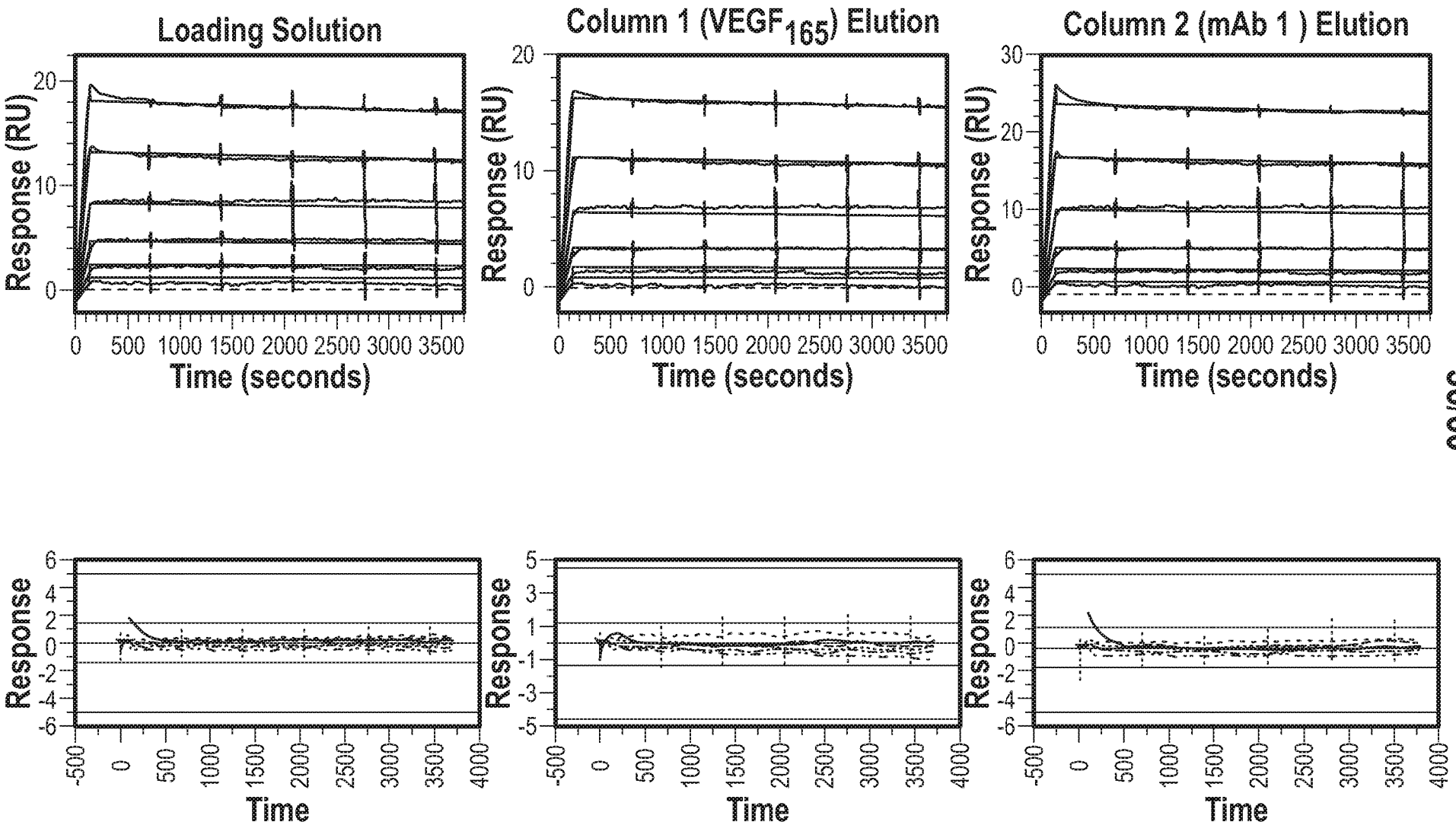

Loading Solution
Column 1 (VEGF165) Elution
Column 2 (mAb 1) Elution
Response (RU)
Time (seconds)
Response
Time

FIG. 38

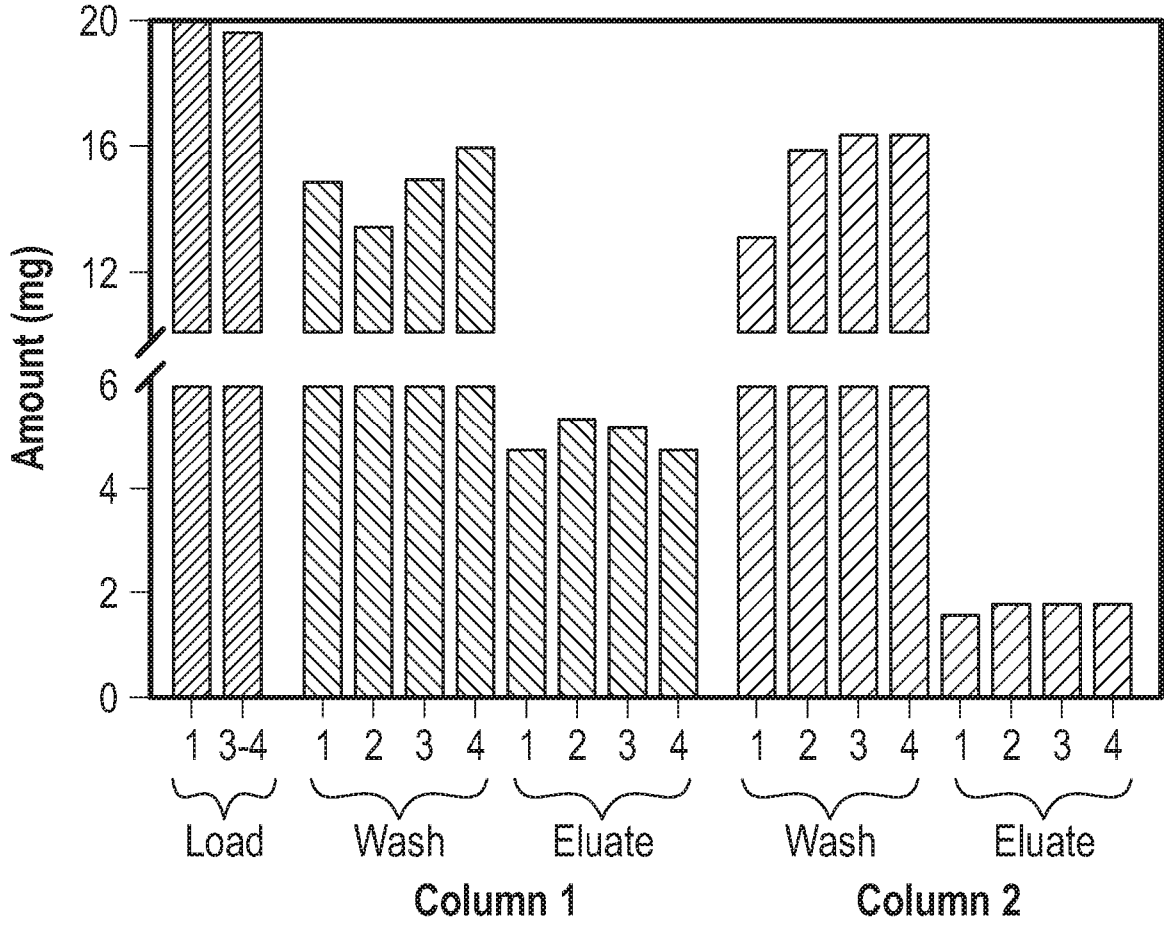

Amount (mg)
20
16
12
6
4
2
0
1 3-4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
Load
Wash
Eluate
Wash
Eluate
Column 1
Column 2

FIG. 39

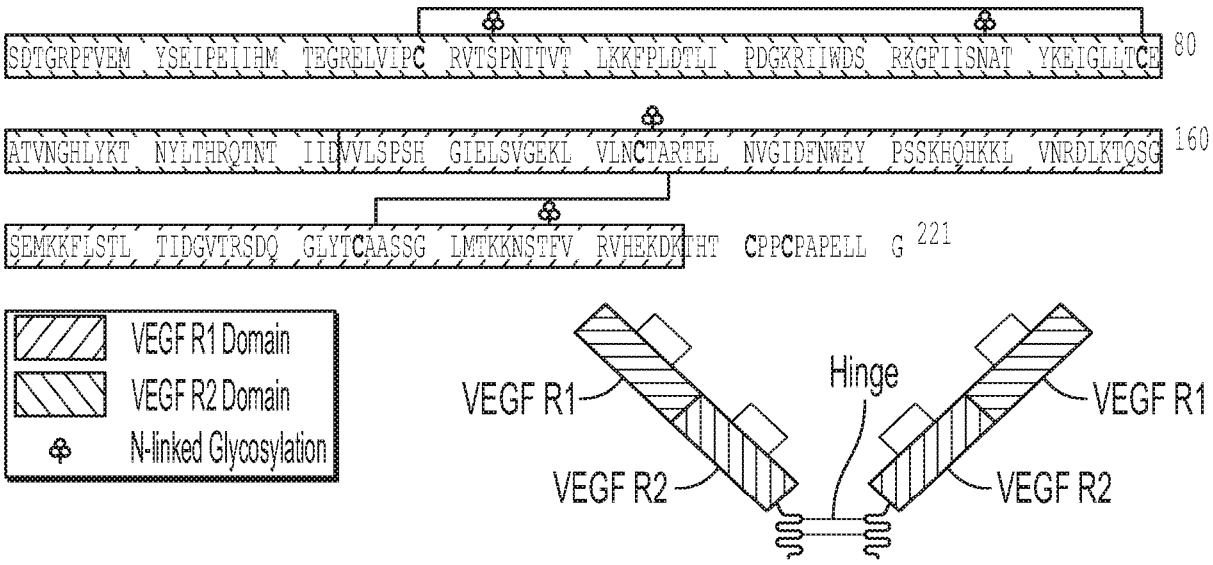

SDTGRPFVEM YSEIPEIIHM TEGRELVIPC RVTSPNITVT LKKFPLDTLI PDGKRIIWDS RKGFIISNAT YKEIGLLTCE 80
ATVNGHLYKT NYLTHRQTNT IIDVVLSPSH GIELSVGEKL VLNCTARTEL NVGIDFNWEY PSSKHQHKKL VNRDLKTQSG 160
SEMKKFLSTL TIDGVTRSDQ GLYTCAASSG LMTKKNSTFV RVHEKDKTHT CPPCPAPELL G 221
VEGF R1 Domain
VEGF R2 Domain
N-linked Glycosylation
VEGF R1
Hinge
VEGF R1
VEGF R2
VEGF R2

FIG. 40

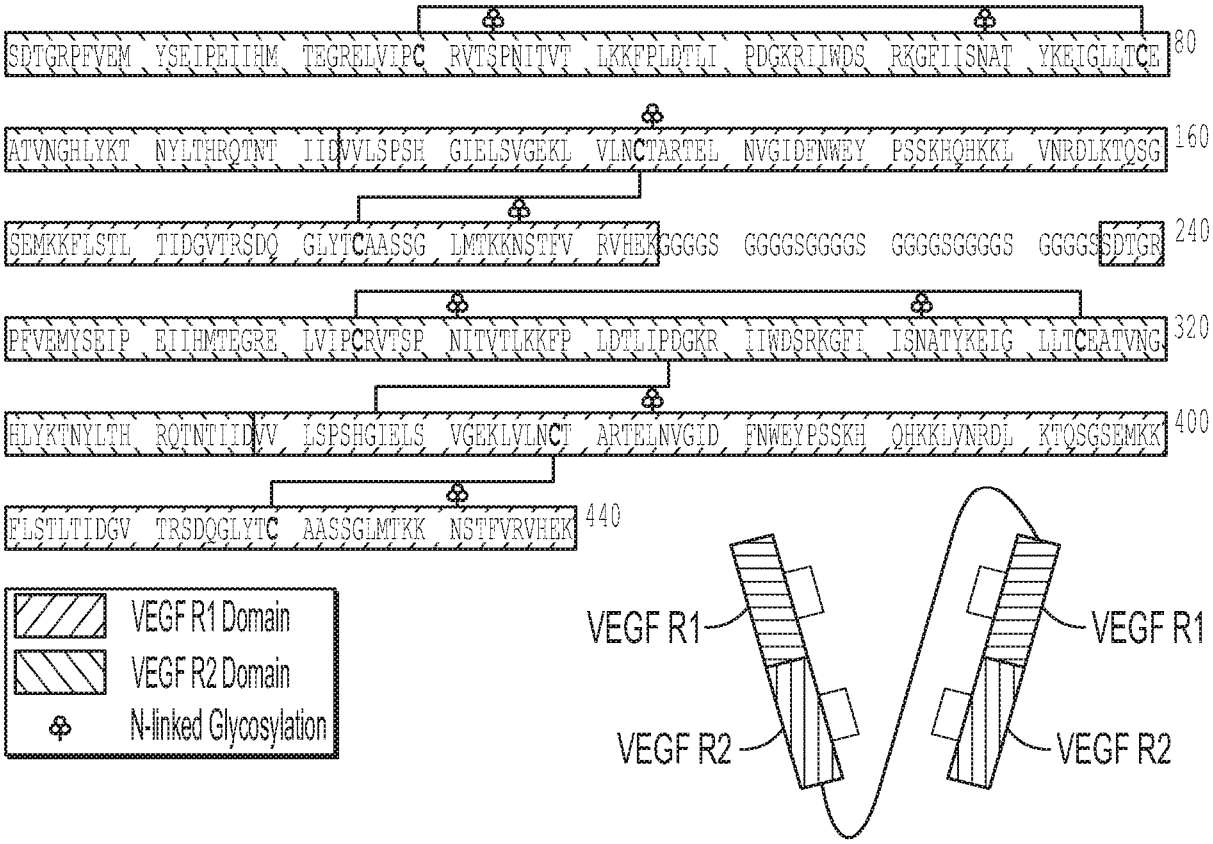

SDTGRPFVEM YSEIPEIIHM TEGRELVIPC RVTSPNITVT LKKFPLDTLI PDGKRIIWDS RKGFIISNAT YKEIGLLTCE 80
ATVNGHLYKT NYLTHRQTNT IIDVVLSPSH GIELSVGEKL VLNCTARTEL NVGIDFNWEY PSSKHQHKKL VNRDLKTQSG 160
SEMKKFLSTL TIDGVTRSDQ GLYTCAASSG LMTKKNSTFV RVHEKGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSDTGR 240
PFVEMYSEIP EIIHMTEGRE LVIPCRVTSP NITVTLKKFP LDTLIPDGKR IIWDSRKGFI ISNATYKEIG LLTCEATVNG 320
HLYKTNYLTH RQTNTIIDVV LSPSHGIELS VGEKLVLNCT ARTELNVGID FNWEYPSSKH QHKKLVNRDL KTQSGSEMKK 400
FLSTLTIDGV TRSDQGLYTC AASSGLMTKK NSTFVRVHEK 440
VEGF R1 Domain
VEGF R2 Domain
N-linked Glycosylation
VEGF R1
VEGF R1
VEGF R2
VEGF R2

FIG. 41

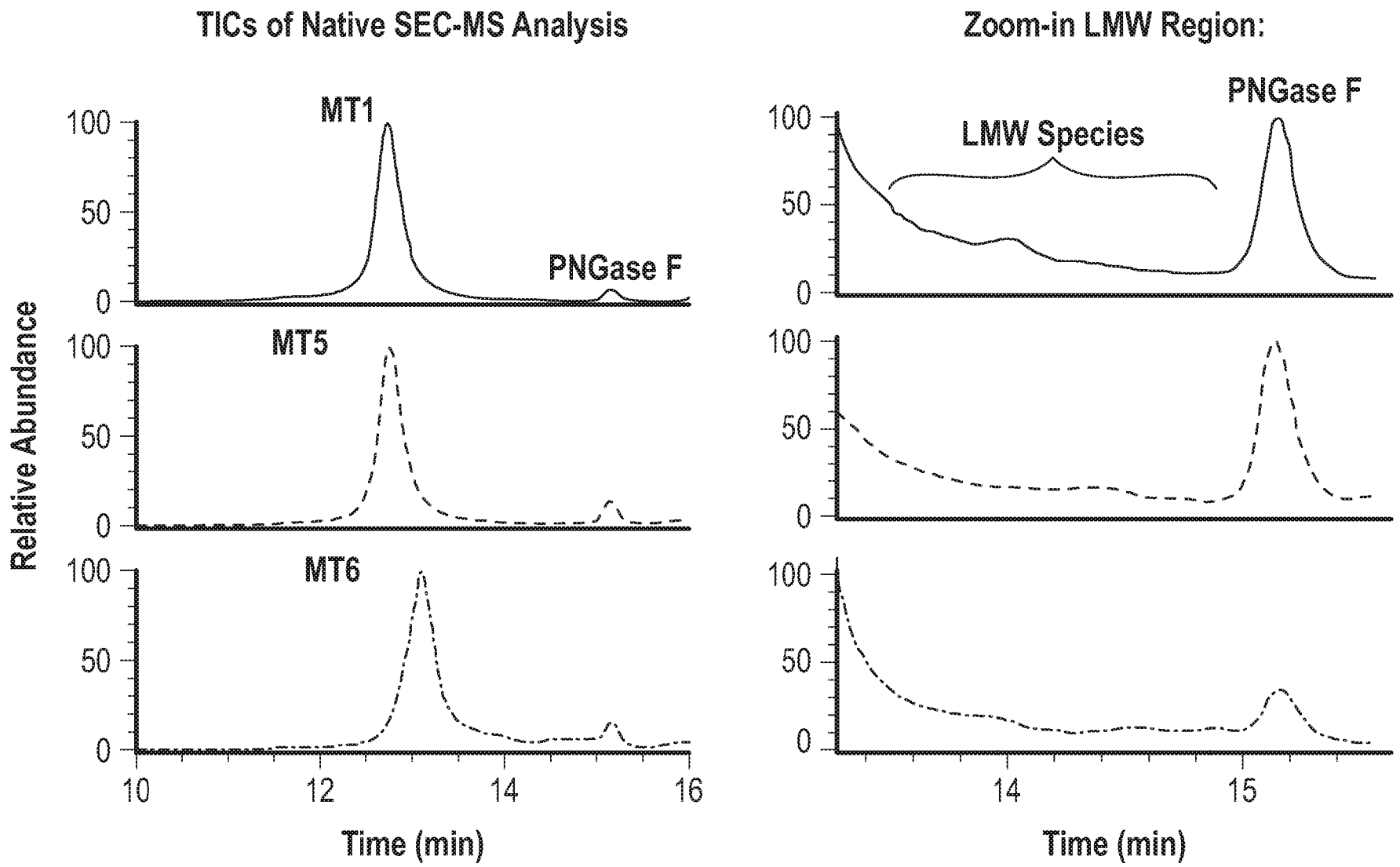

TICs of Native SEC-MS Analysis
Zoom-in LMW Region:
MT1
PNGase F
MT5
MT6
LMW Species
PNGase F
Relative Abundance
100
50
0
10
12
14
16
15
Time (min)
Time (min)

FIG. 42

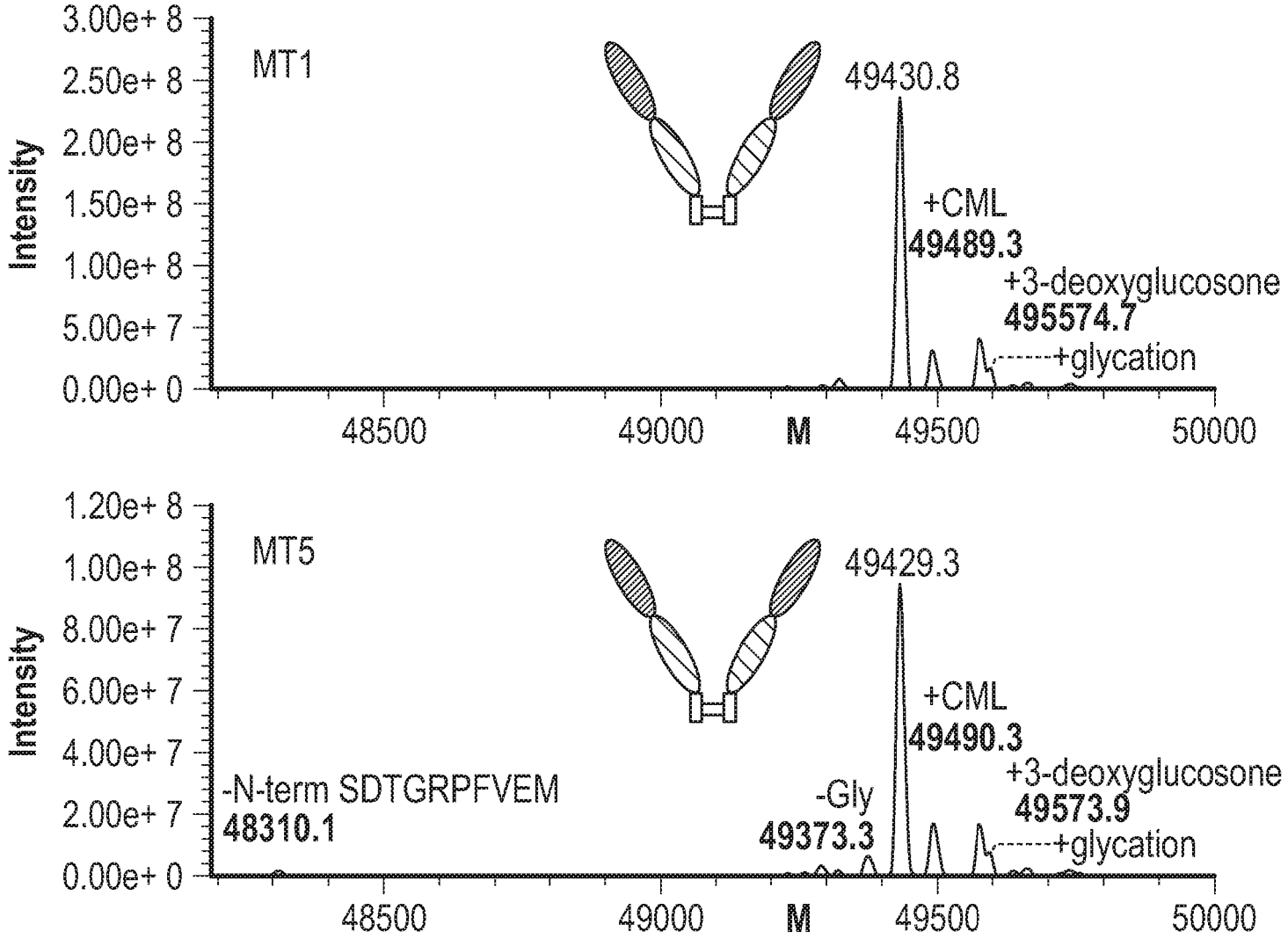

MT1
Intensity
3.00e+ 8
2.50e+ 8
2.00e+ 8
1.50e+ 8
1.00e+ 8
5.00e+ 7
0.00e+ 0
49430.8
+CML
49489.3
+3-deoxyglucosone
495574.7
+glycation
48500
49000
M
49500
50000
MT5
Intensity
1.20e+ 8
1.00e+ 8
8.00e+ 7
6.00e+ 7
4.00e+ 7
2.00e+ 7
0.00e+ 0
49429.3
+CML
49490.3
+3-deoxyglucosone
49573.9
+glycation
-N-term SDTGRPFVEM
48310.1
-Gly
49373.3
48500
49000
M
49500
50000

FIG. 43

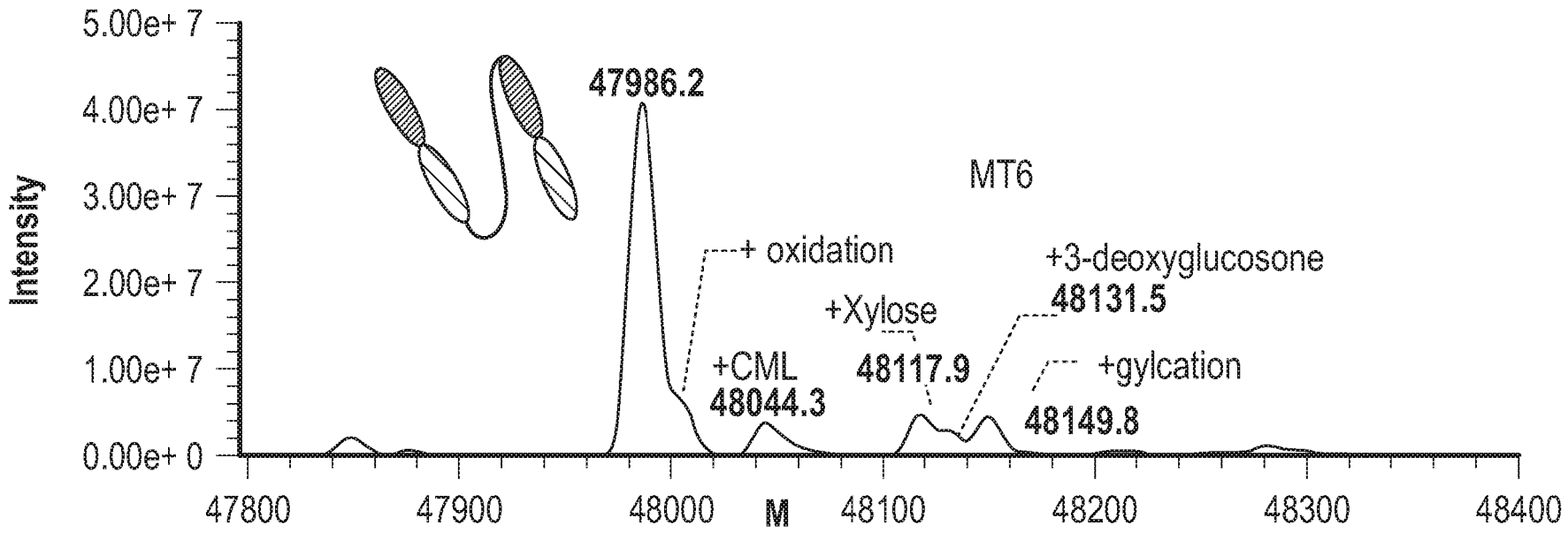

5.00e+ 7
4.00e+ 7
3.00e+ 7
2.00e+ 7
1.00e+ 7
0.00e+ 0
Intensity
47986.2
MT6
+ oxidation
+3-deoxyglucosone
48131.5
+Xylose
+CML
48044.3
48117.9
+gylcation
48149.8
47800
47900
48000
M
48100
48200
48300
48400

FIG. 44

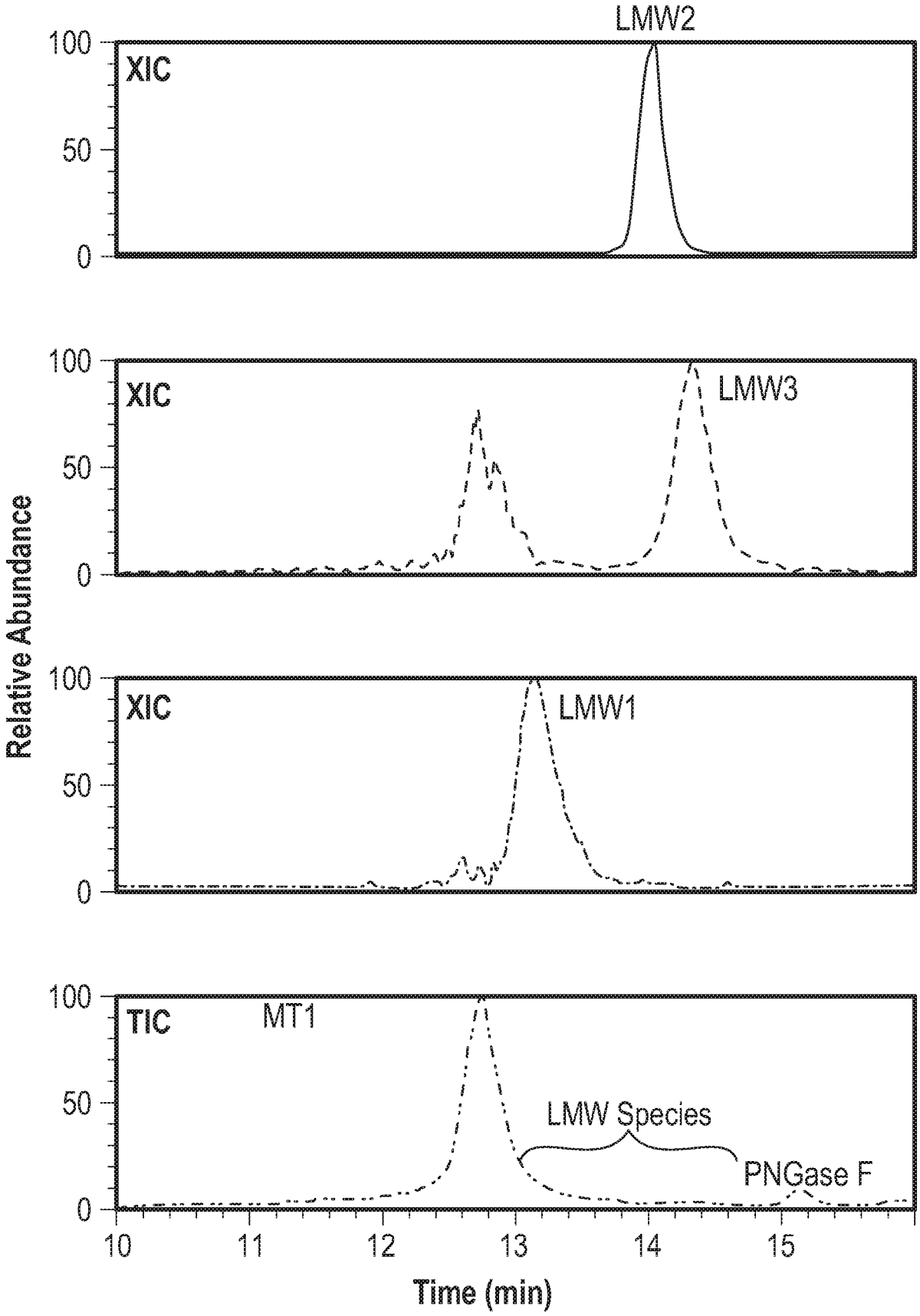

LMW2
XIC
100
50
0
XIC
LMW3
100
50
0
Relative Abundance
XIC
LMW1
100
50
0
TIC
MT1
LMW Species
PNGase F
100
50
0
10
11
12
13
14
15
Time (min)

FIG. 45A

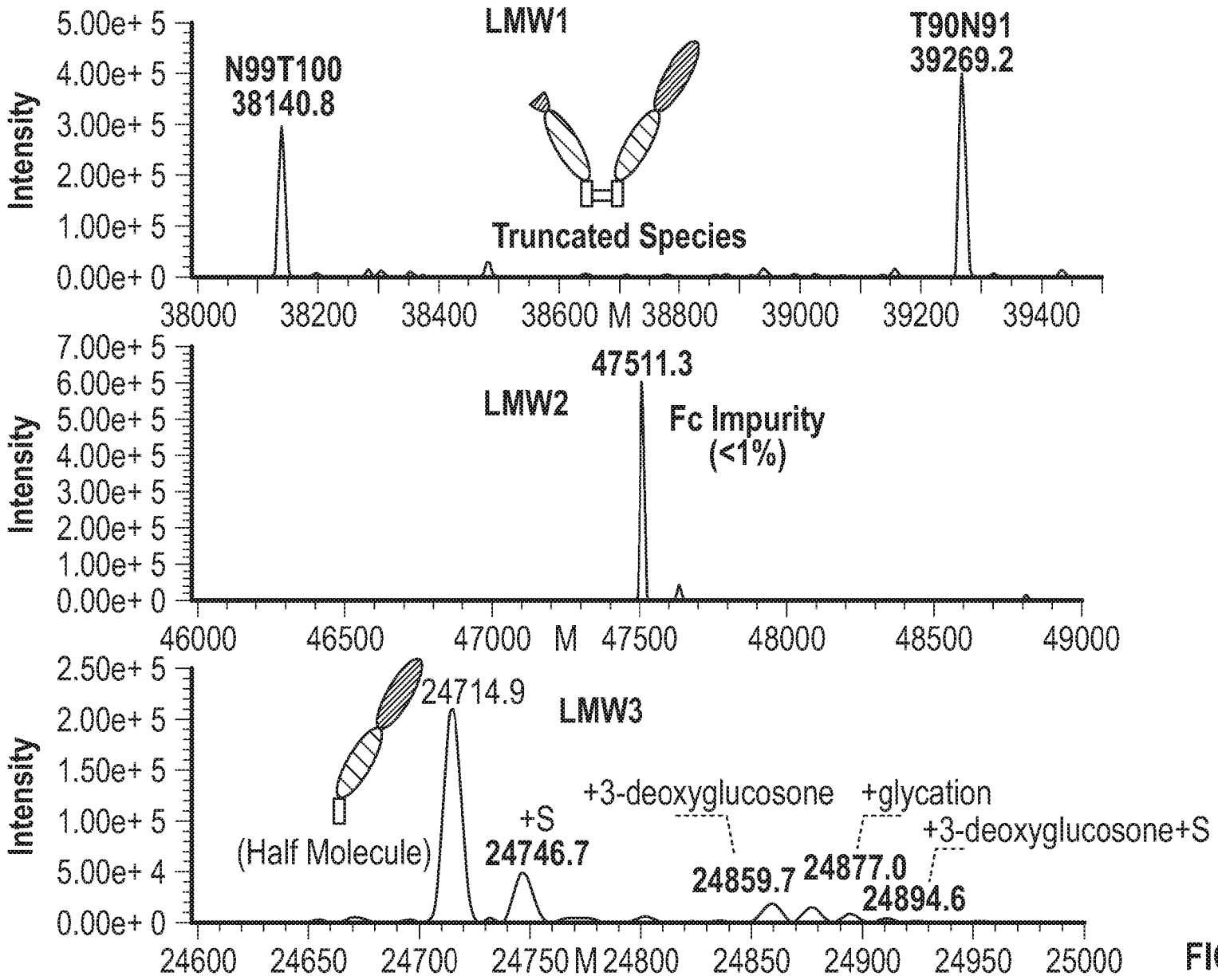

LMW1
N99T100
38140.8
T90N91
39269.2
Truncated Species
Intensity
5.00e+ 5
4.00e+ 5
3.00e+ 5
2.00e+ 5
1.00e+ 5
0.00e+ 0
38000
38200
38400
38600 M 38800
39000
39200
39400
47511.3
LMW2
Fc Impurity
(<1%)
7.00e+ 5
6.00e+ 5
46000
46500
47000 M 47500
48000
48500
49000
24714.9
LMW3
+3-deoxyglucosone
+glycation
+S
+3-deoxyglucosone+S
(Half Molecule)
24746.7
24859.7
24877.0
24894.6
2.50e+ 5
2.00e+ 5
1.50e+ 5
1.00e+ 5
5.00e+ 4
24600
24650
24700
24750 M 24800
24850
24900
24950
25000

FIG. 45B

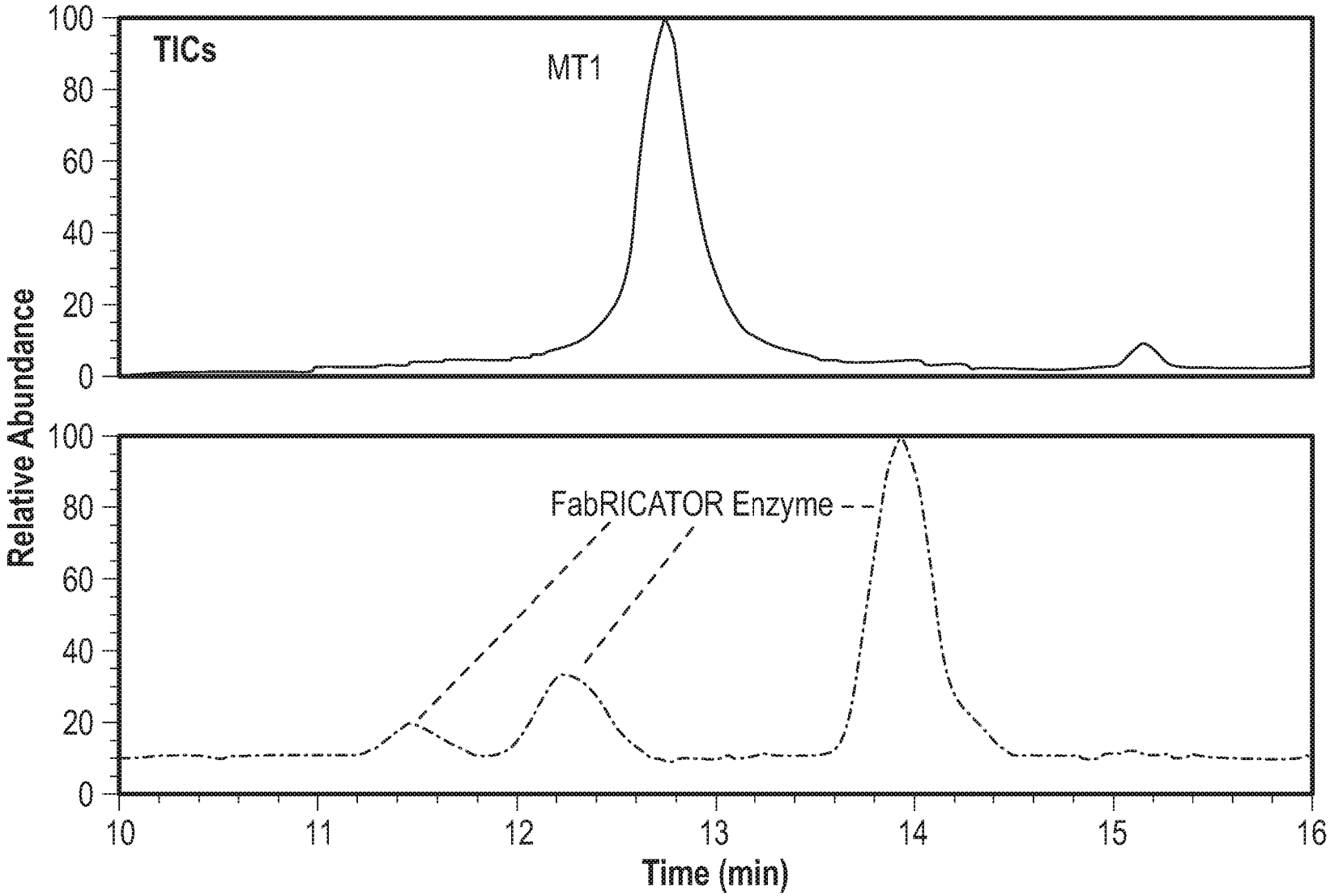

TICs
MT1
FabRICATOR Enzyme
Relative Abundance
Time (min)
100
80
60
40
20
0
10
11
12
13
14
15
16

FIG. 46

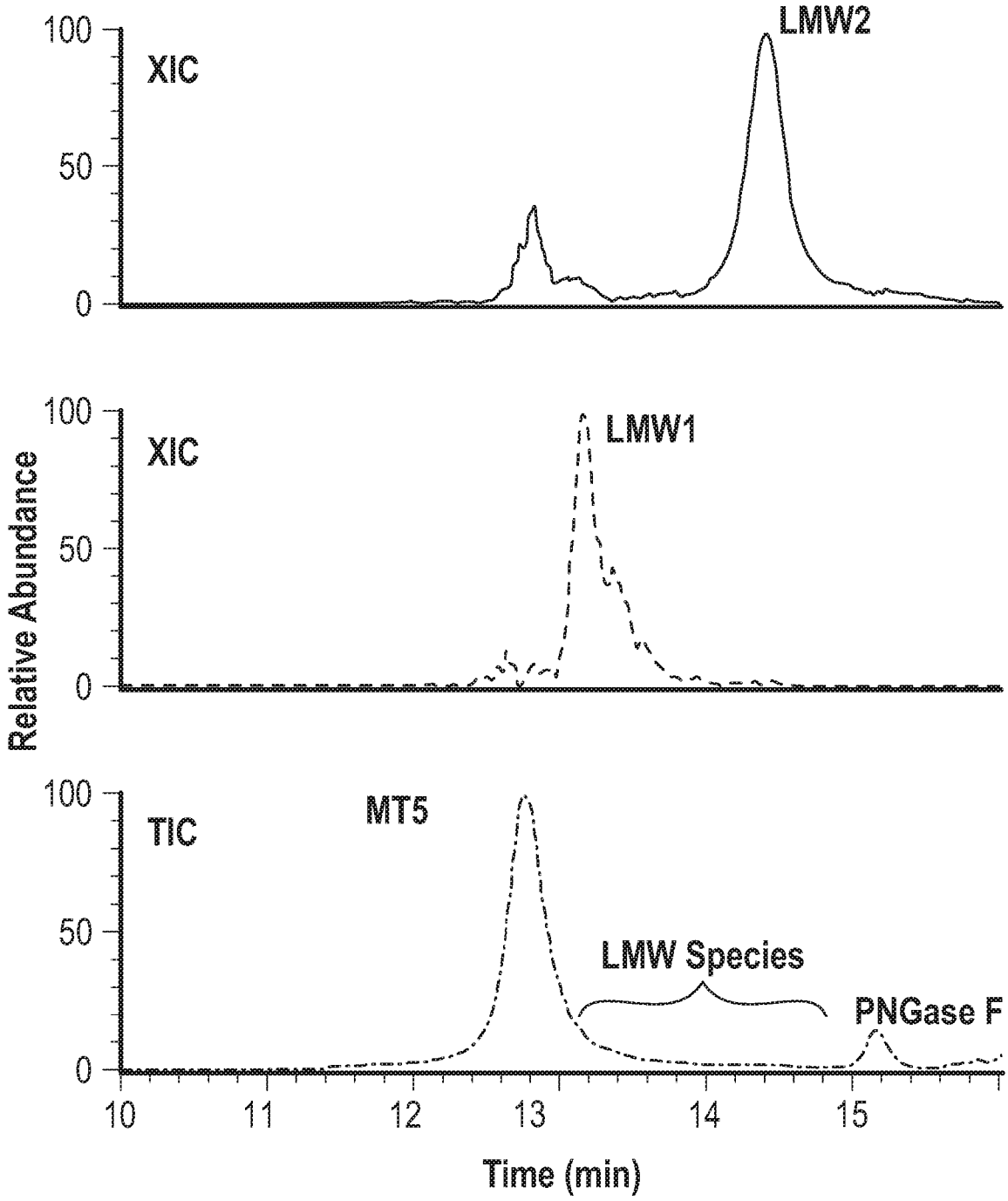

100
50
0
XIC
LMW2
100
50
0
XIC
LMW1
Relative Abundance
100
50
0
TIC
MT5
LMW Species
PNGase F
10
11
12
13
14
15
Time (min)

FIG. 47

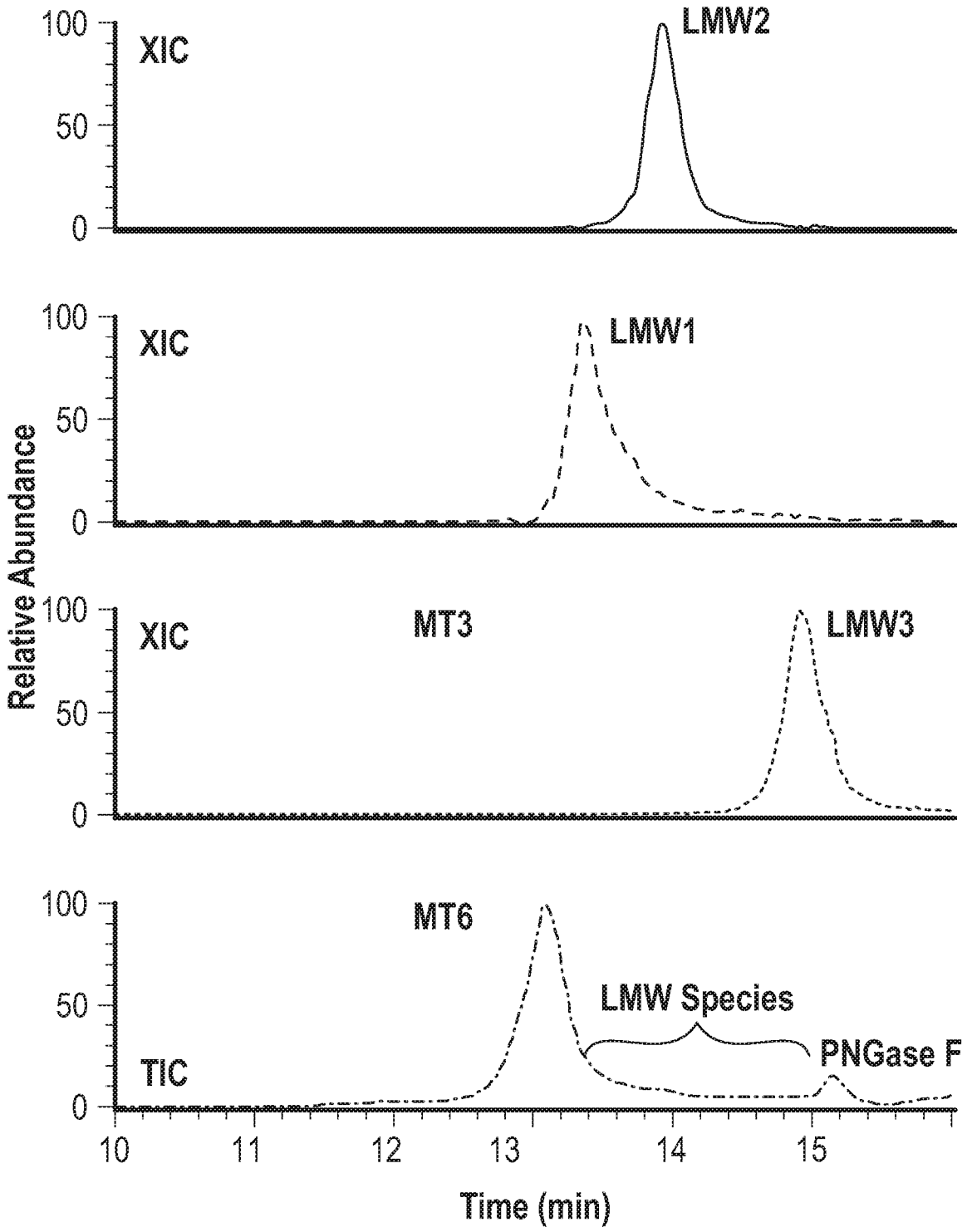

100
50
0
XIC
LMW2
XIC
LMW1
Relative Abundance
XIC
MT3
LMW3
MT6
LMW Species
PNGase F
TIC
10
11
12
13
14
15
Time (min)

FIG. 48

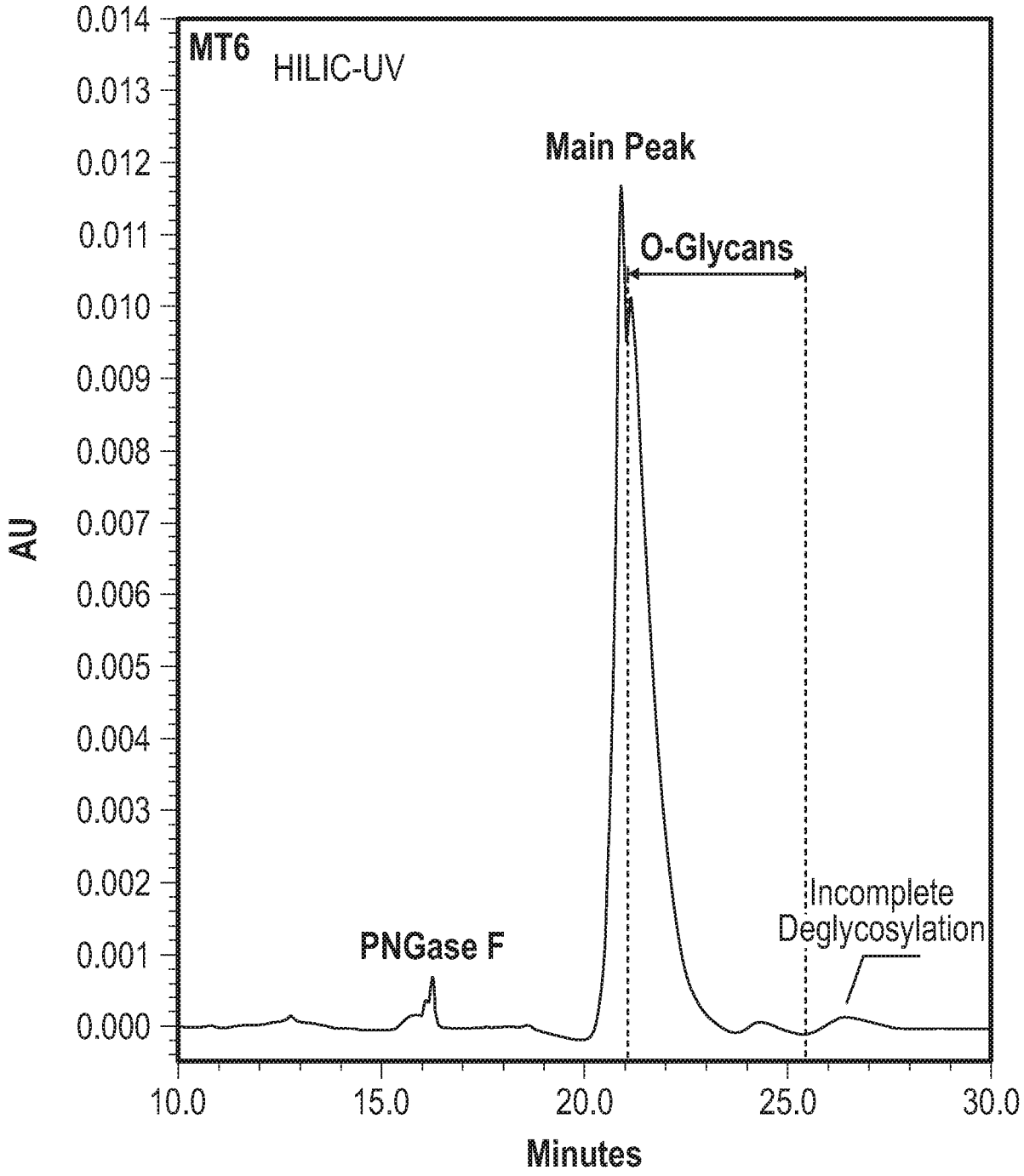

MT6
HILIC-UV
Main Peak
O-Glycans
PNGase F
Incomplete
Deglycosylation
AU
0.014
0.013
0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
10.0
15.0
20.0
25.0
30.0
Minutes

FIG. 49A

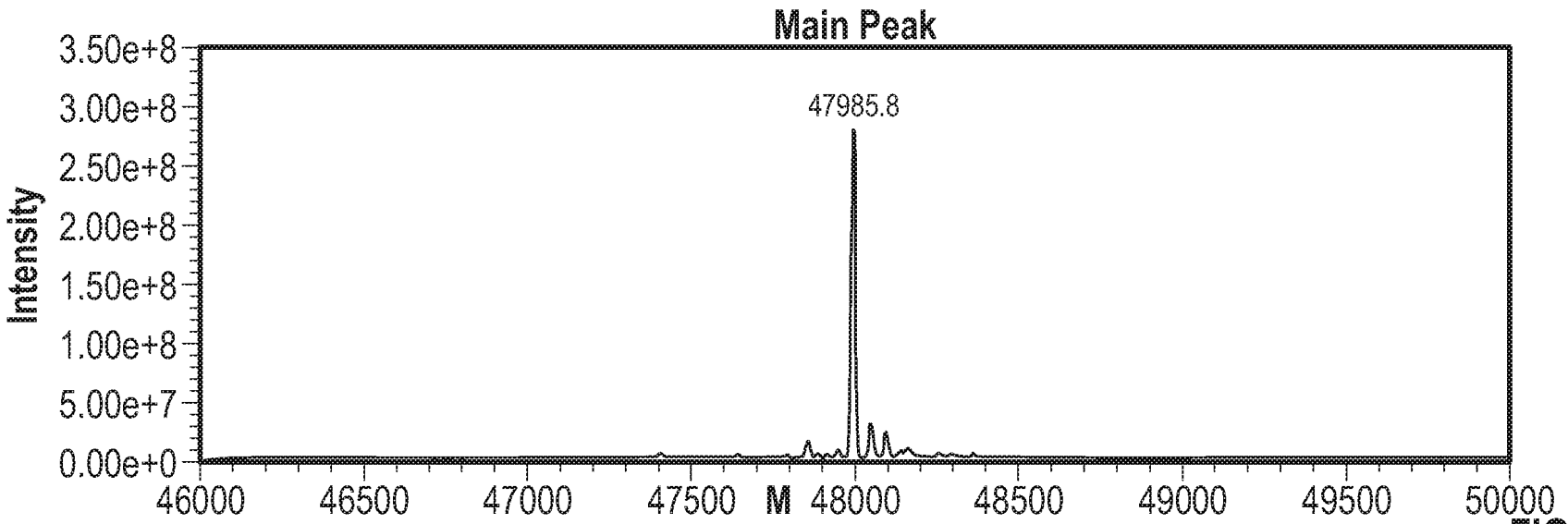


FIG. 49B

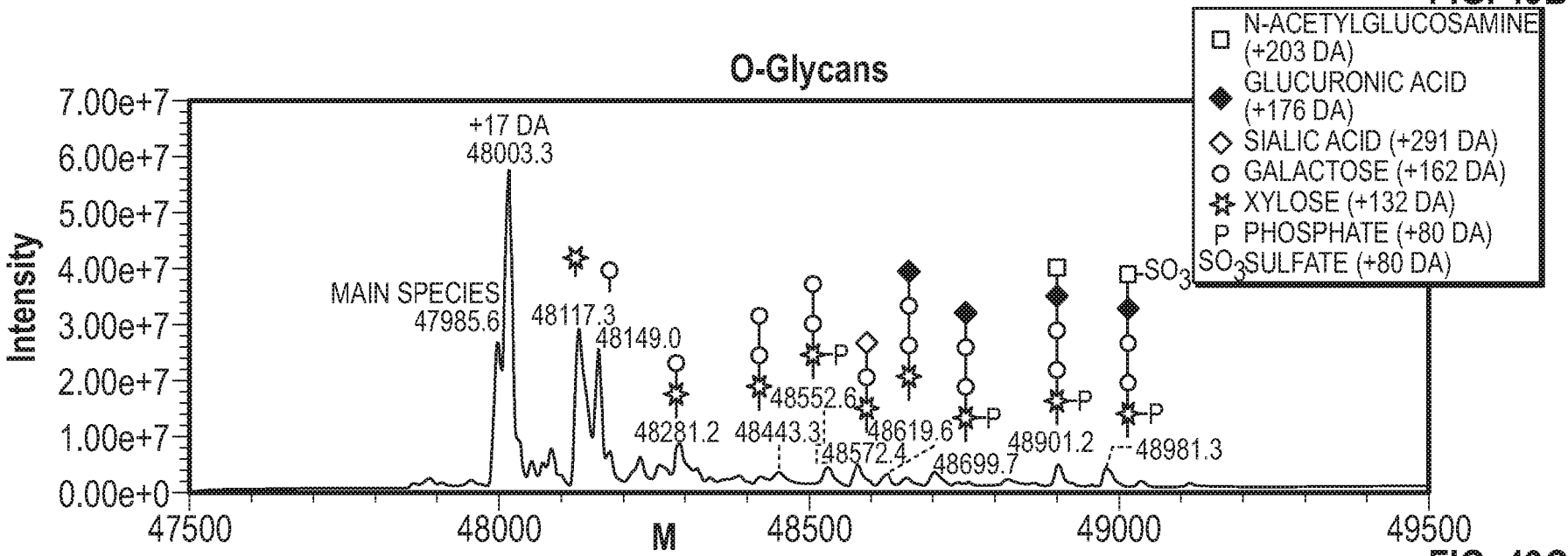
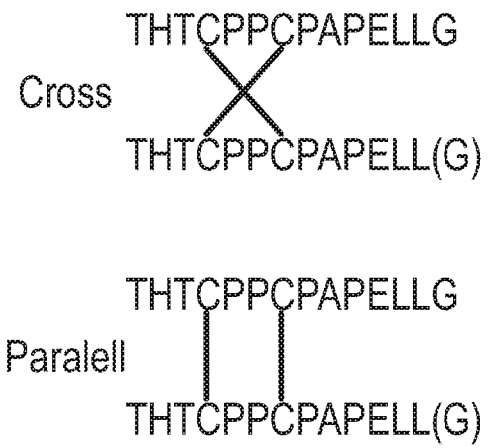


FIG. 49C


THTCPPCPAPELLG
Cross
THTCPPCPAPELL(G)
THTCPPCPAPELLG
Paralell
THTCPPCPAPELL(G)

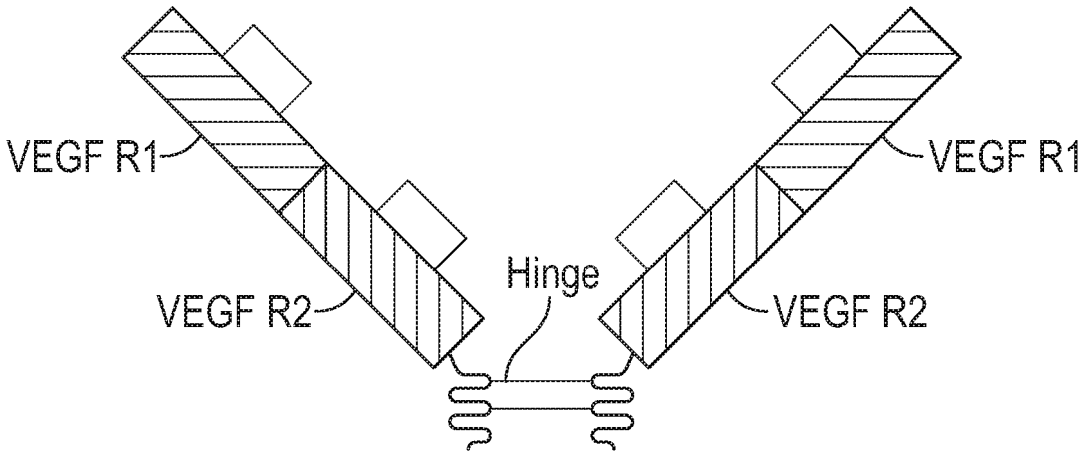

VEGF R1
VEGF R1
Hinge
VEGF R2
VEGF R2

FIG. 50

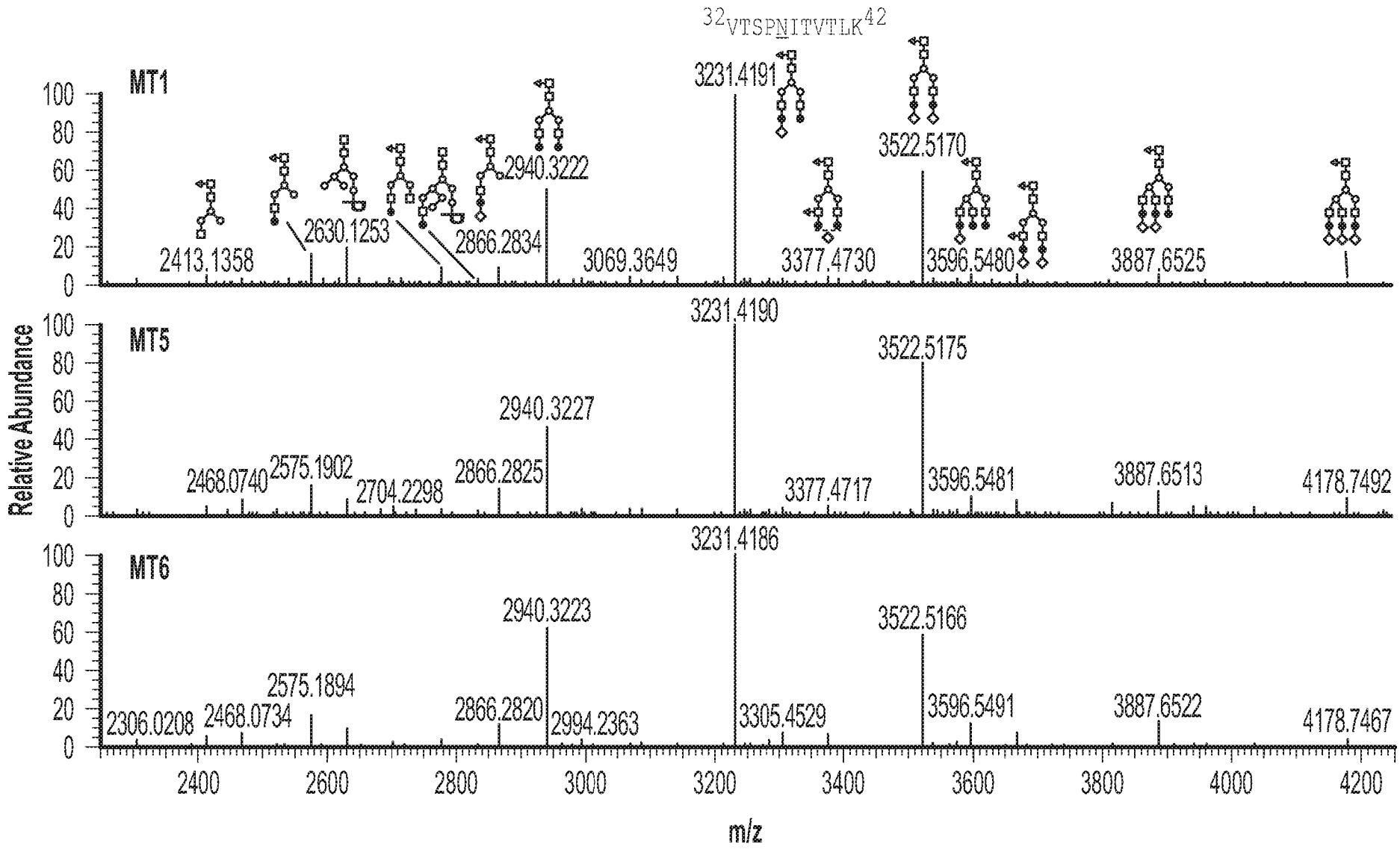

32VTSPNITVTLK42
MT1
MT5
MT6
Relative Abundance
m/z
2413.1358
2630.1253
2866.2834
2940.3222
3069.3649
3231.4191
3377.4730
3522.5170
3596.5480
3887.6525
3231.4190
3522.5175
2940.3227
2468.0740
2575.1902
2704.2298
2866.2825
3377.4717
3596.5481
3887.6513
4178.7492
3231.4186
2940.3223
3522.5166
2575.1894
2306.0208
2468.0734
2866.2820
2994.2363
3305.4529
3596.5491
3887.6522
4178.7467

FIG. 51

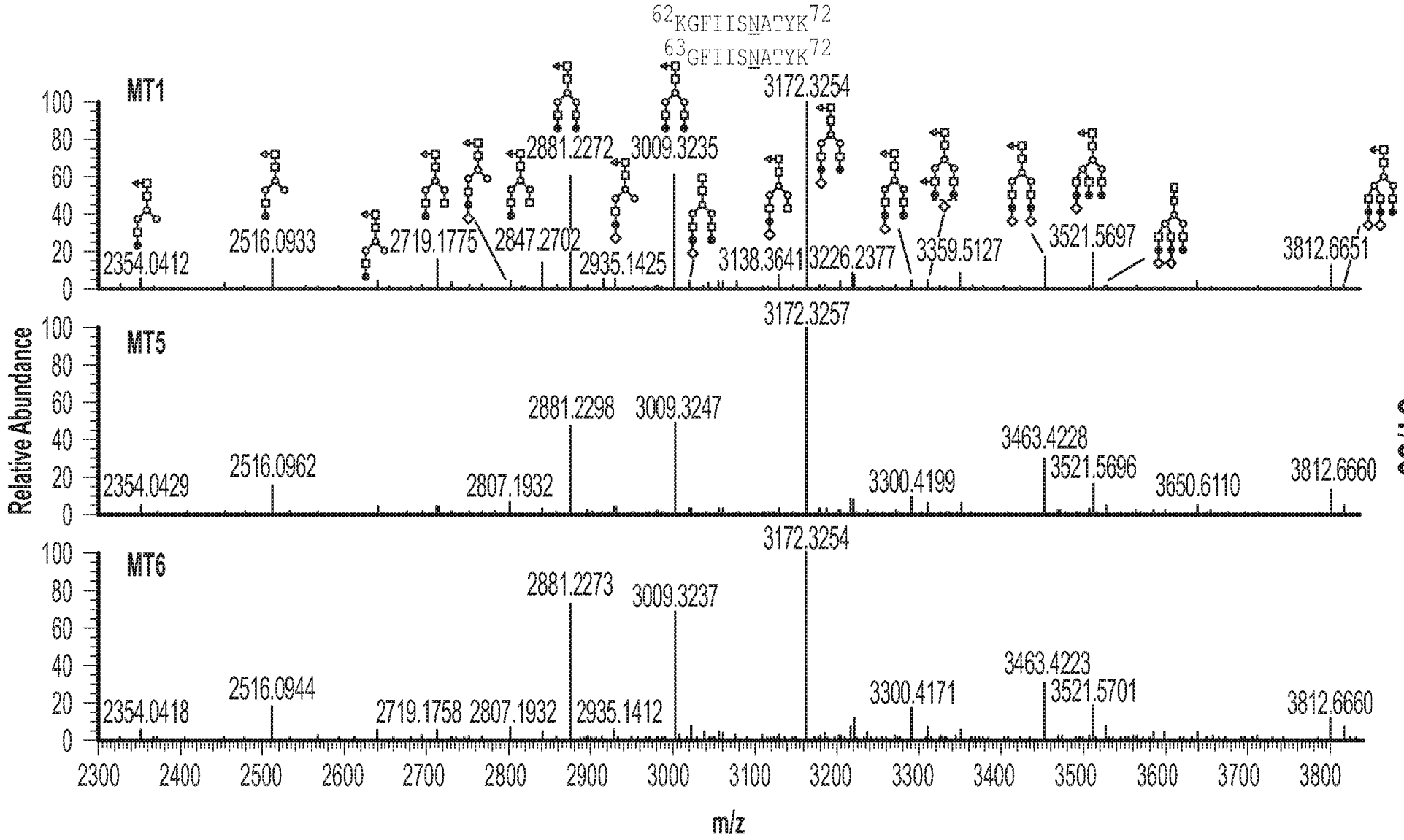

62KGFIISNATYK72
63GFIISNATYK72
MT1
MT5
MT6
Relative Abundance
m/z
3172.3254
2881.2272
3009.3235
2354.0412
2516.0933
2719.1775
2847.2702
2935.1425
3138.3641
3226.2377
3359.5127
3521.5697
3812.6651
3172.3257
2881.2298
3009.3247
2354.0429
2516.0962
2807.1932
3300.4199
3463.4228
3521.5696
3650.6110
3812.6660
3172.3254
2881.2273
3009.3237
2354.0418
2516.0944
2719.1758
2807.1932
2935.1412
3300.4171
3463.4223
3521.5701
3812.6660

FIG. 52

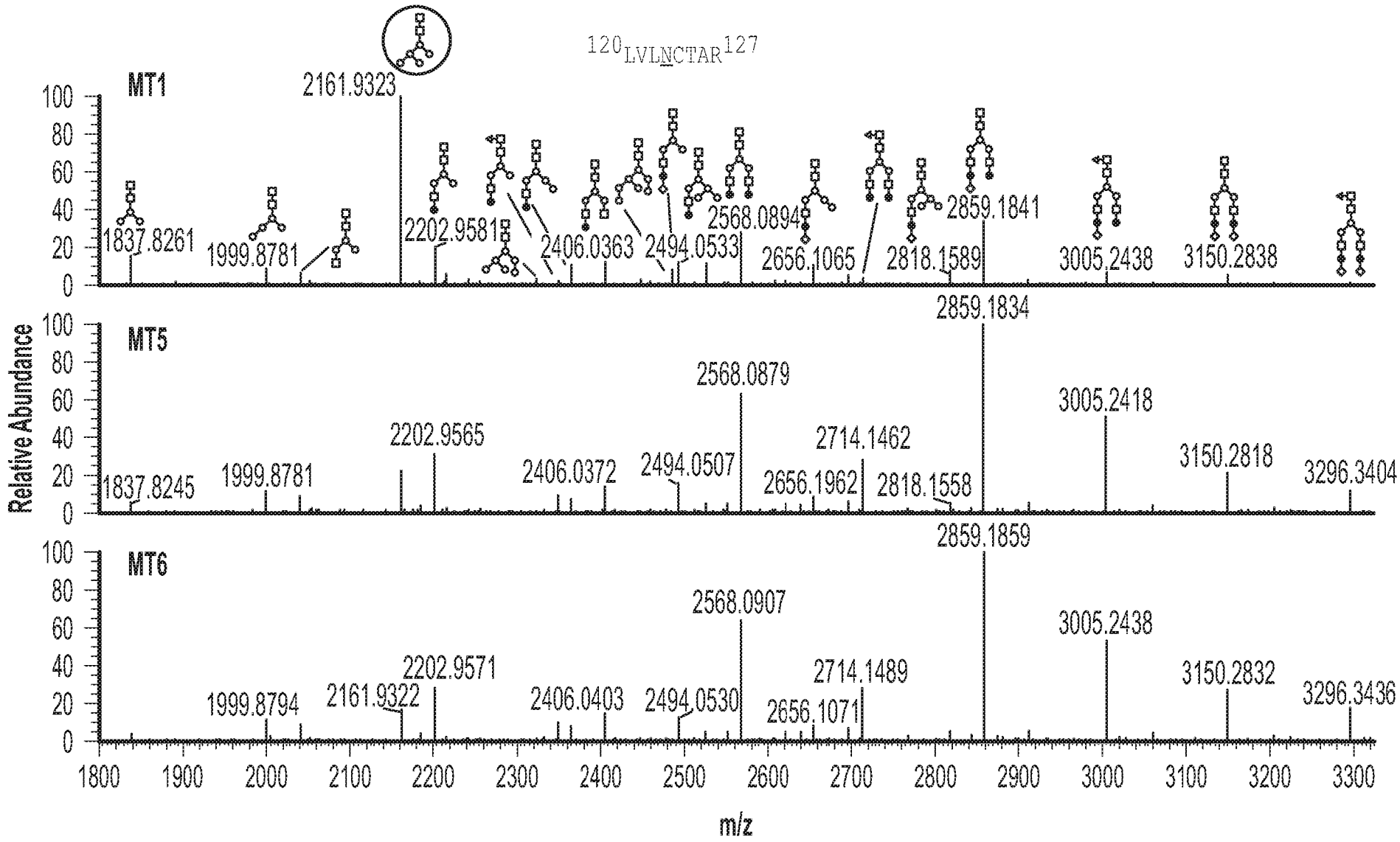

120LVLNCTAR127
MT1
2161.9323
1837.8261
1999.8781
2202.9581
2406.0363
2494.0533
2568.0894
2656.1065
2818.1589
2859.1841
3005.2438
3150.2838
MT5
2859.1834
2568.0879
3005.2418
2202.9565
2714.1462
3150.2818
3296.3404
1837.8245
1999.8781
2406.0372
2494.0507
2656.1962
2818.1558
MT6
2859.1859
2568.0907
3005.2438
2202.9571
2714.1489
3150.2832
3296.3436
1999.8794
2161.9322
2406.0403
2494.0530
2656.1071
Relative Abundance
m/z

FIG. 53

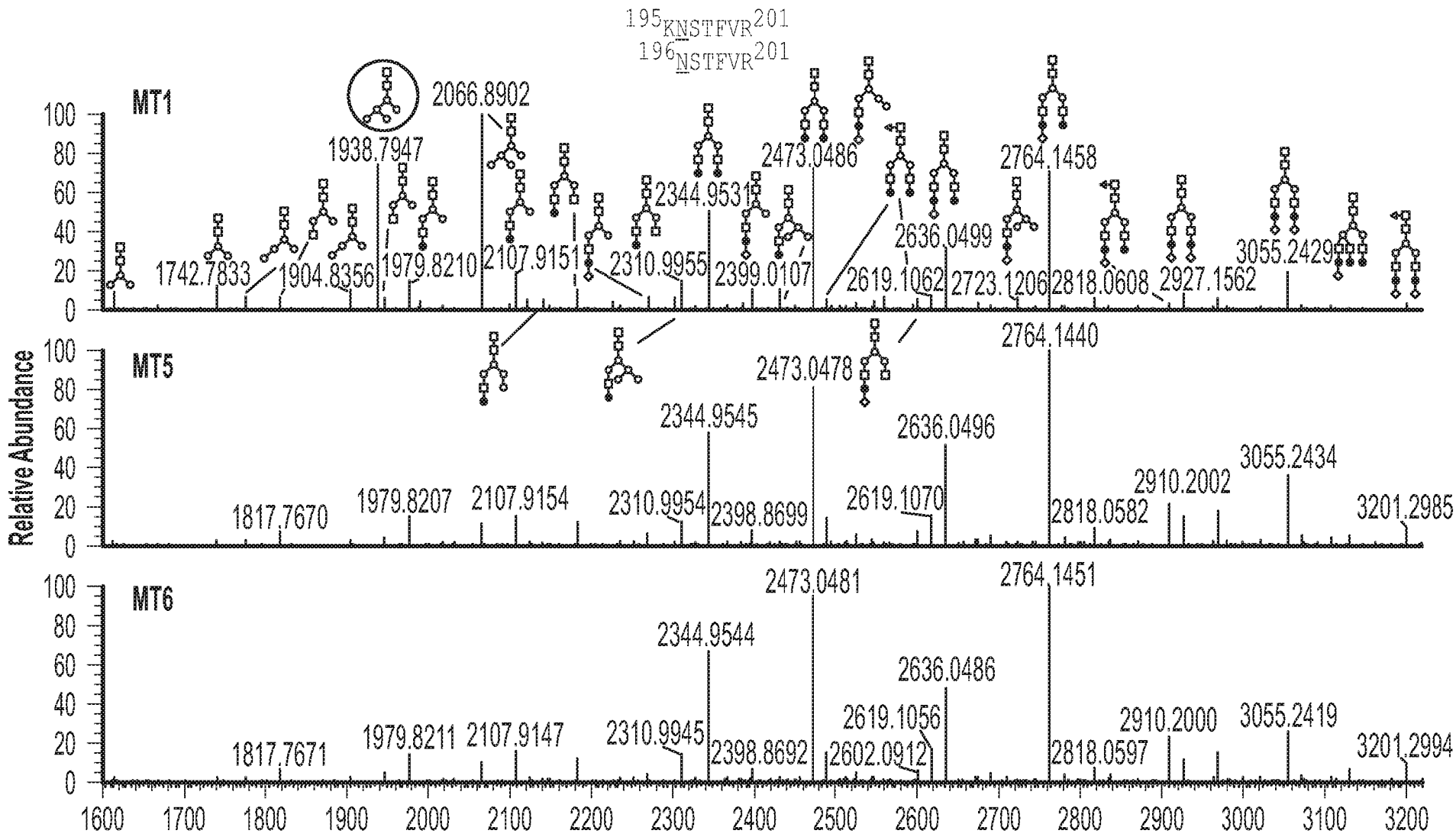

195KNSTFVR201
196NSTFVR201
MT1
MT5
MT6
Relative Abundance
2066.8902
1938.7947
2473.0486
2764.1458
2344.9531
2636.0499
1742.7833
1904.8356
1979.8210
2107.9151
2310.9955
2399.0107
2619.1062
2723.1206
2818.0608
2927.1562
3055.2429
2764.1440
2473.0478
2344.9545
2636.0496
3055.2434
2910.2002
1817.7670
1979.8207
2107.9154
2310.9954
2398.8699
2619.1070
2818.0582
3201.2985
2473.0481
2764.1451
2344.9544
2636.0486
2619.1056
2910.2000
3055.2419
1817.7671
1979.8211
2107.9147
2310.9945
2398.8692
2602.0912
2818.0597
3201.2994
1600
1700
1800
1900
2000
2100
2200
2300
2400
2500
2600
2700
2800
2900
3000
3100
3200

FIG. 54

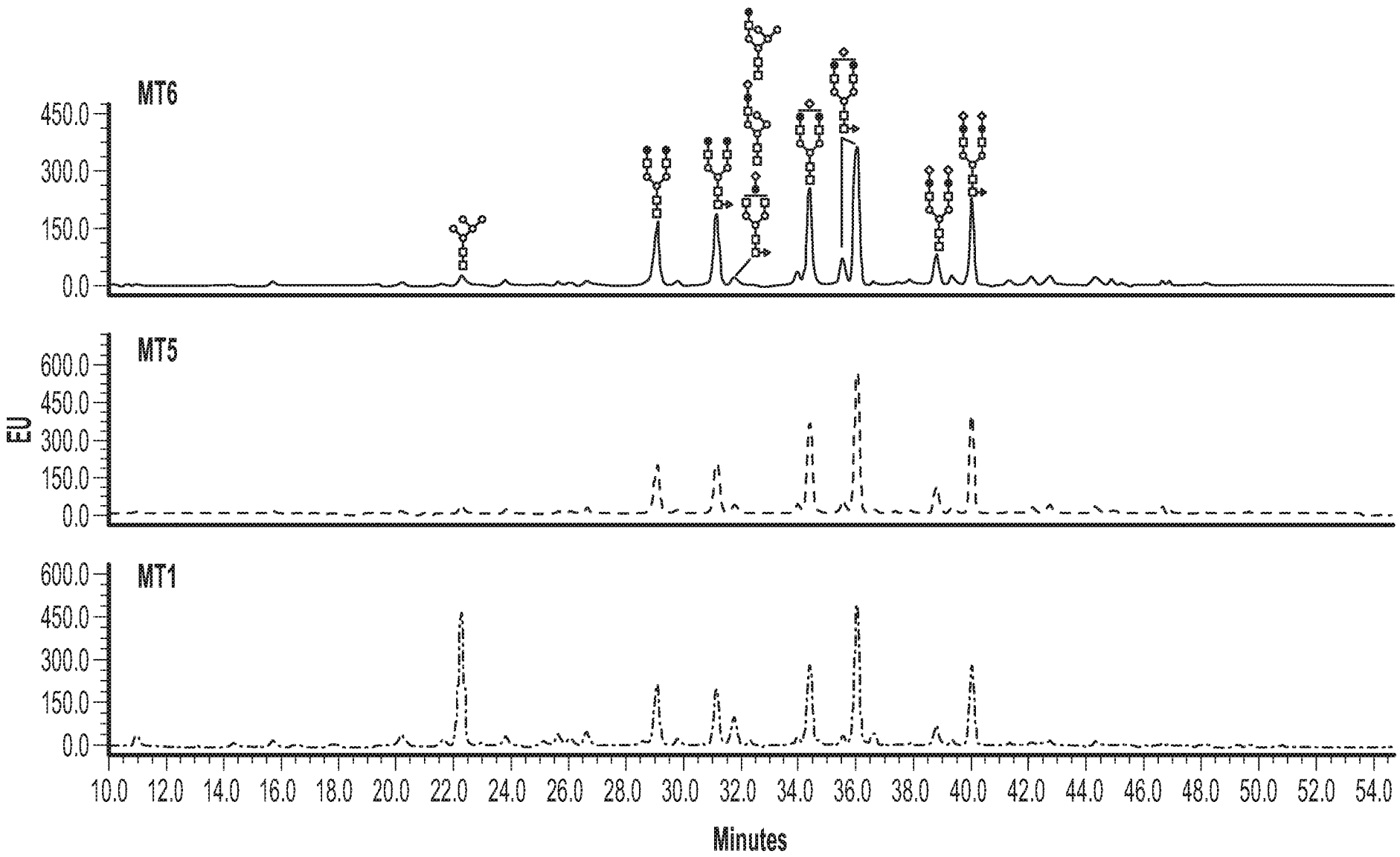

MT6
MT5
MT1
EU
450.0
300.0
150.0
0.0
600.0
10.0 12.0 14.0 16.0 18.0 20.0 22.0 24.0 26.0 28.0 30.0 32.0 34.0 36.0 38.0 40.0 42.0 44.0 46.0 48.0 50.0 52.0 54.0
Minutes

FIG. 55

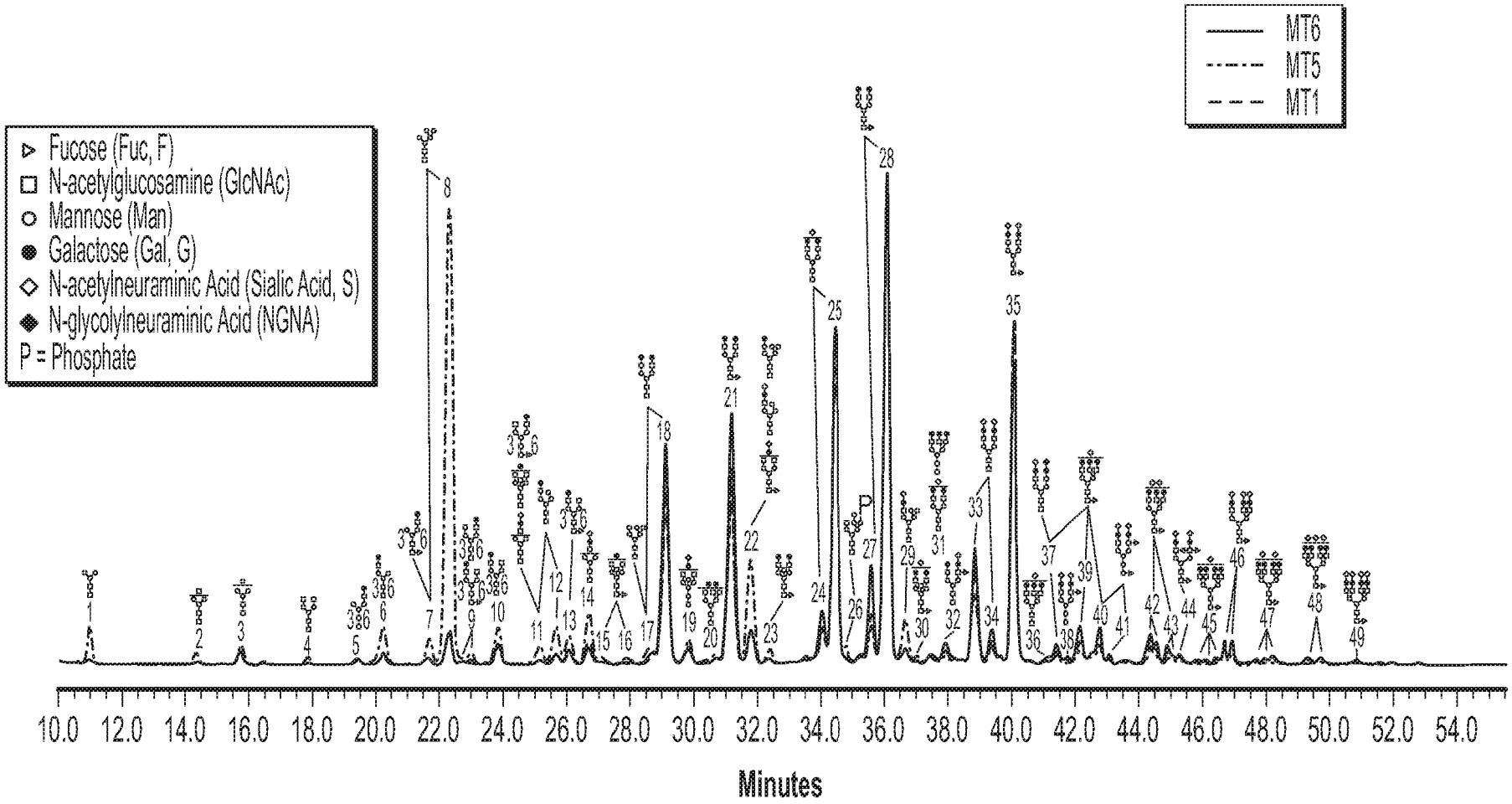

Fucose (Fuc, F)
N-acetylglucosamine (GlcNAc)
Mannose (Man)
Galactose (Gal, G)
N-acetylneuraminic Acid (Sialic Acid, S)
N-glycolylneuraminic Acid (NGNA)
P = Phosphate
MT6
MT5
MT1
10.0 12.0 14.0 16.0 18.0 20.0 22.0 24.0 26.0 28.0 30.0 32.0 34.0 36.0 38.0 40.0 42.0 44.0 46.0 48.0 50.0 52.0 54.0
Minutes

FIG. 56

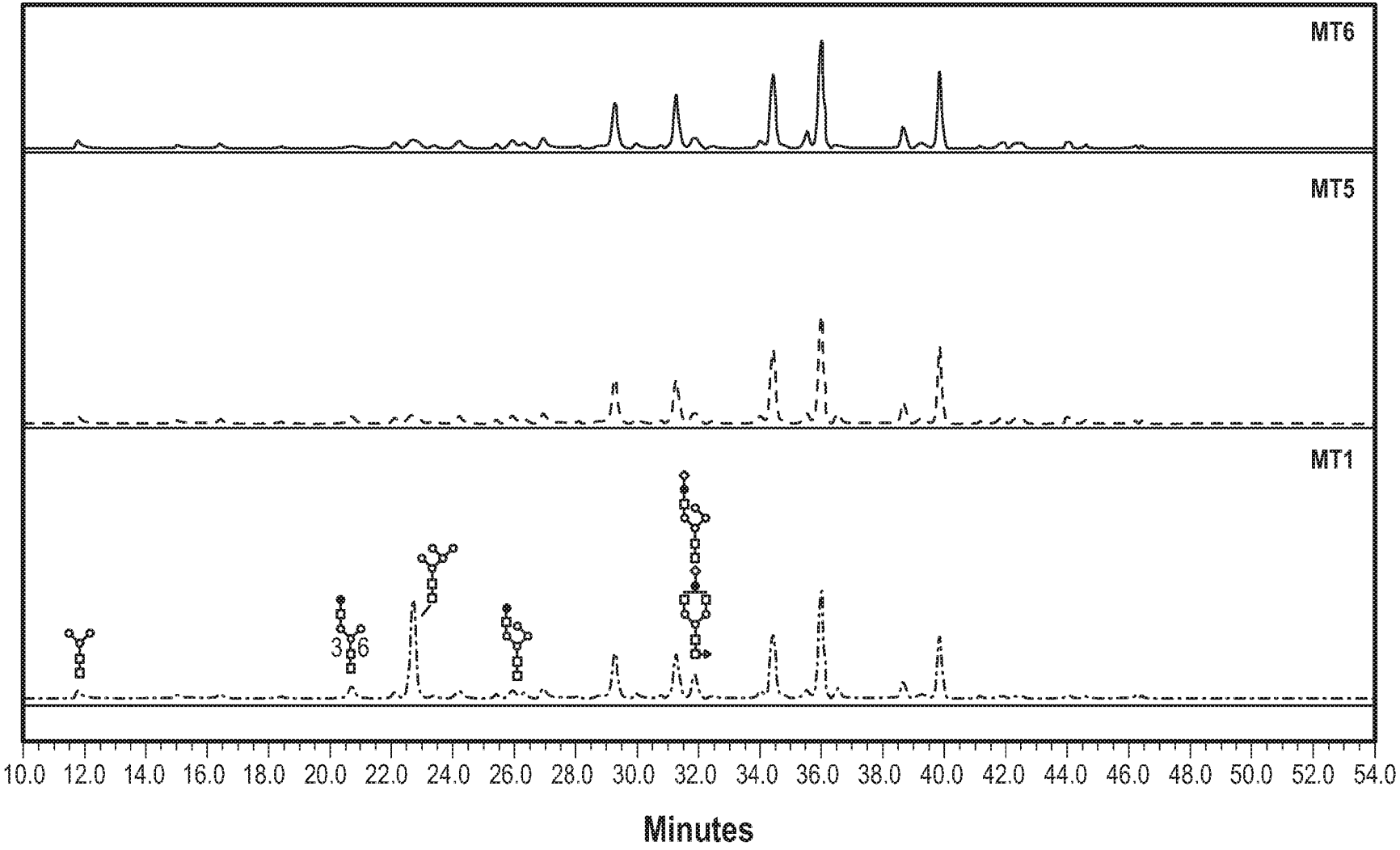

MT6
MT5
MT1
3
6
10.0 12.0 14.0 16.0 18.0 20.0 22.0 24.0 26.0 28.0 30.0 32.0 34.0 36.0 38.0 40.0 42.0 44.0 46.0 48.0 50.0 52.0 54.0
Minutes

FIG. 57

Peak No.	Abbreviation	Glycan Structure	% Peak Area			Theoretical Mass (Da)
			MT6	MT5	MT1	
1	G0-2GlcNAc		0.11	0.19	0.96	1221.50
2	G0-GlcNAc		0.09	0.11	0.34	1424.58
3	Man4		0.51	0.56	0.62	1383.56
4	G0		0.07	0.05	0.26	1627.66
5	G1-GlcNAc (1-6)		0.26	0.31	0.14	1586.63
6	G1-GlcNAc (1-3)		0.55	0.61	1.76	1586.63
7	G1F-GlcNAc (1-6)		0.34	0.37	0.92	1732.69
8	Man5		1.32	1.63	16.11	1545.61
9	G1F-GlcNAc (1-3)		0.35	0.26	0.45	1732.69
	G1 (1-6)					1789.71
10	G1 (1-3)		0.89	0.88	1.34	1789.71
11	bG1		0.26	0.22	0.78	1992.79
	G1S1-GlcNAc					1877.73
	G1F (1-6)					1935.77
12	Man4_G1		0.51	0.46	1.53	1748.69
13	G1F (1-3)		0.67	0.64	1.11	1935.77
14	G1S1-GlcNAc		0.73	1.20	1.86	1877.73
15	bG1F		0.00	0.00	0.32	2138.85

FIG. 58A

Peak No.	Abbreviation	Glycan Structure	% Peak Area			Theoretical Mass (Da)
			MT6	MT5	MT1	
16	bG1F		0.00	0.00	0.40	2138.85
17	Man6		0.51	0.41	0.85	1707.66
18	G2		9.95	8.85	7.96	1951.77
19	G1S1-GlcNAc		0.84	0.89	1.04	2080.81
20	G2+GlcNAc		0.28	0.28	0.57	2154.85
21	G2F		11.24	9.13	7.47	2097.82
22	Man5_G1		1.61	1.82	4.13	1910.74
	Man4_G1S1					2039.78
	G1FS1					2226.87
23	bG2F		0.27	0.32	0.66	2300.90
24	G2S1+Fuc		2.63	2.13	1.75	2242.86
25			13.42	14.46	9.50	
26	Man6_G0+Phos	P	0.00	0.00	0.53	1787.63
27	G2FS1		4.00	2.43	1.67	2388.92
28			18.85	21.19	15.94	2388.92
29	Man5_G1S1		0.51	0.62	1.70	2201.84
30	bG2FS1		0.00	0.00	0.27	2592.00
31	G2+NGNA		0.44	0.35	0.35	2258.86
	G3					2316.90

FIG. 58B

Peak No.	Abbreviation	Glycan Structure	% Peak Area			Theoretical Mass (Da)
			MT6	MT5	MT1	
32	G2S1+Fuc		0.85	0.59	0.38	2388.92
33	G2S2		4.56	4.36	2.57	2533.96
34			1.31	0.99	0.71	2533.96
35	G2FS2		10.76	13.67	8.33	2680.02
36	G3S1		0.20	0.14	0.11	2607.99
37	G2S1+NGNA		0.87	0.59	0.35	2549.95
	G3FS1					2754.05
38	G2S2+Fuc		0.23	0.13	0.07	2680.02
39	G3FS1		1.61	1.22	0.67	2754.05
40			1.77	1.77	0.95	2754.05
	G2FS2+Fuc					2826.07
41			0.24	0.24	0.15	
42	G3FS2		1.85	1.59	0.77	3045.15
43			0.78	0.77	0.32	
44	G2FS2+2Fuc		0.34	0.31	0.14	2972.13
45	G4FS1		0.57	0.29	0.12	3119.18
46	G3FS3		1.14	1.43	0.58	3336.24
47	G4FS2		0.89	0.72	0.24	3410.28
48	G4FS3		0.63	0.60	0.18	3701.38
49	G4FS4		0.22	0.23	0.07	3992.47

FIG. 58C

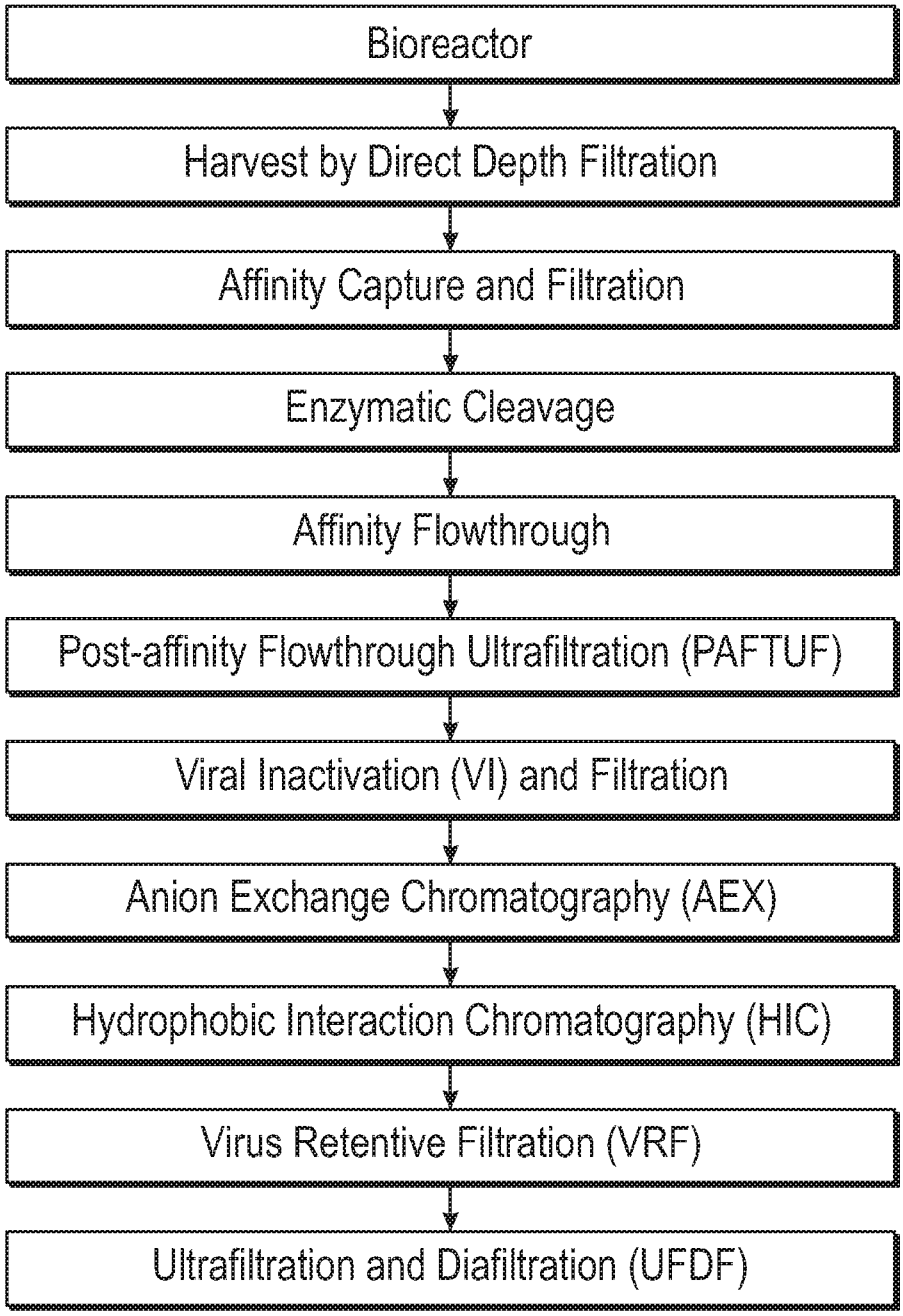

Bioreactor
Harvest by Direct Depth Filtration
Affinity Capture and Filtration
Enzymatic Cleavage
Affinity Flowthrough
Post-affinity Flowthrough Ultrafiltration (PAFTUF)
Viral Inactivation (VI) and Filtration
Anion Exchange Chromatography (AEX)
Hydrophobic Interaction Chromatography (HIC)
Virus Retentive Filtration (VRF)
Ultrafiltration and Diafiltration (UFDF)

FIG. 59