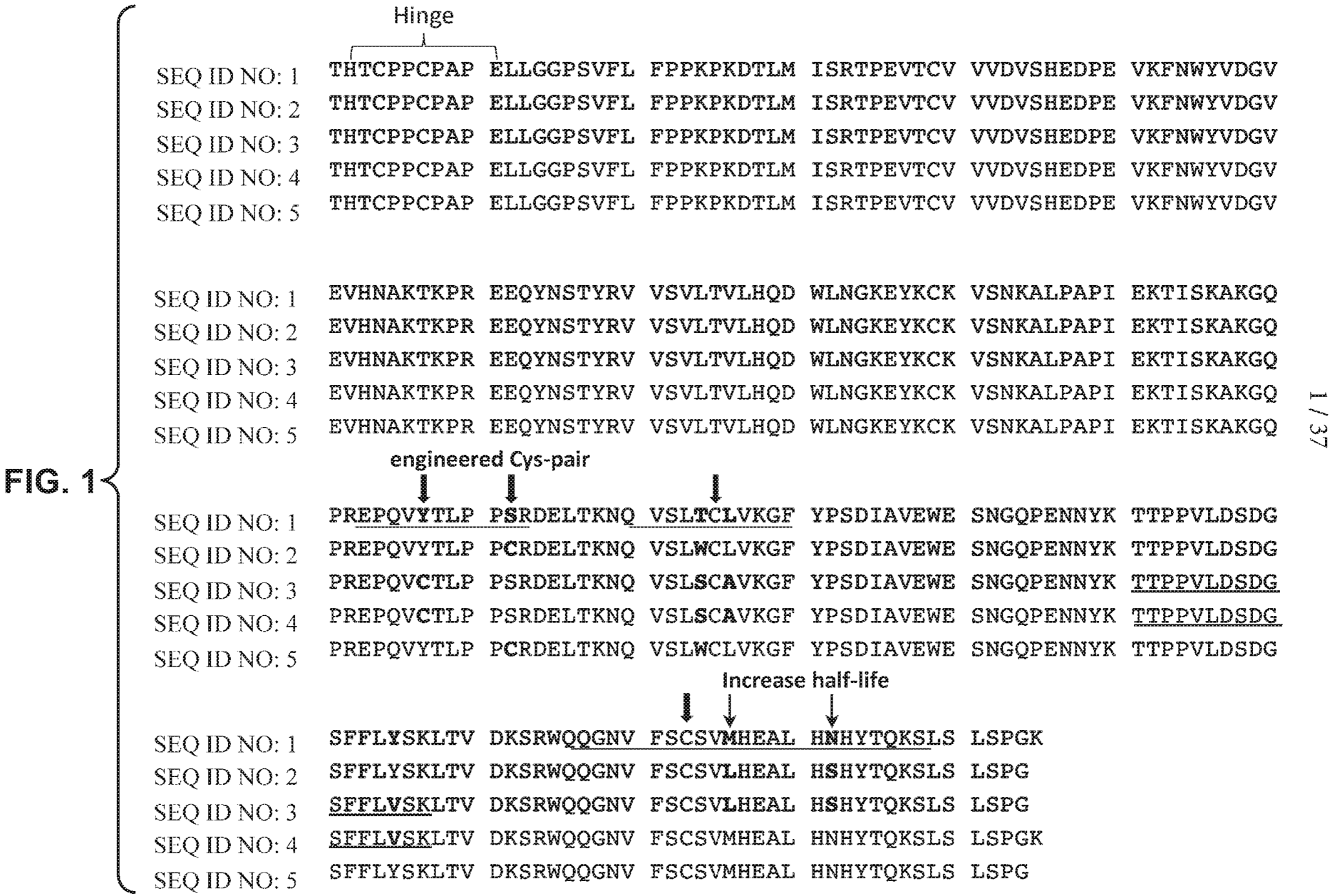

Hinge
SEQ ID NO: 1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
SEQ ID NO: 2 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
SEQ ID NO: 3 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
SEQ ID NO: 4 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
SEQ ID NO: 5 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV
SEQ ID NO: 1 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
SEQ ID NO: 2 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
SEQ ID NO: 3 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
SEQ ID NO: 4 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
SEQ ID NO: 5 EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ
engineered Cys-pair
SEQ ID NO: 1 PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
SEQ ID NO: 2 PREPQVYTLP PCRDELTKNQ VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
SEQ ID NO: 3 PREPQVCTLP PSRDELTKNQ VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
SEQ ID NO: 4 PREPQVCTLP PSRDELTKNQ VSLSCAVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
SEQ ID NO: 5 PREPQVYTLP PCRDELTKNQ VSLWCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
Increase half-life
SEQ ID NO: 1 SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
SEQ ID NO: 2 SFFLYSKLTV DKSRWQQGNV FSCSVLHEAL HSHYTQKSLS LSPG
SEQ ID NO: 3 SFFLVSKLTV DKSRWQQGNV FSCSVLHEAL HSHYTQKSLS LSPG
SEQ ID NO: 4 SFFLVSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
SEQ ID NO: 5 SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPG

FIG. 1

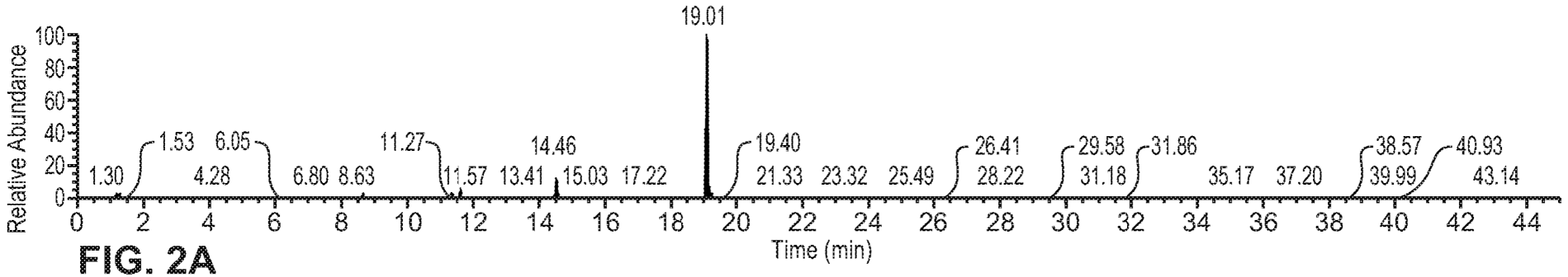


FIG. 2A

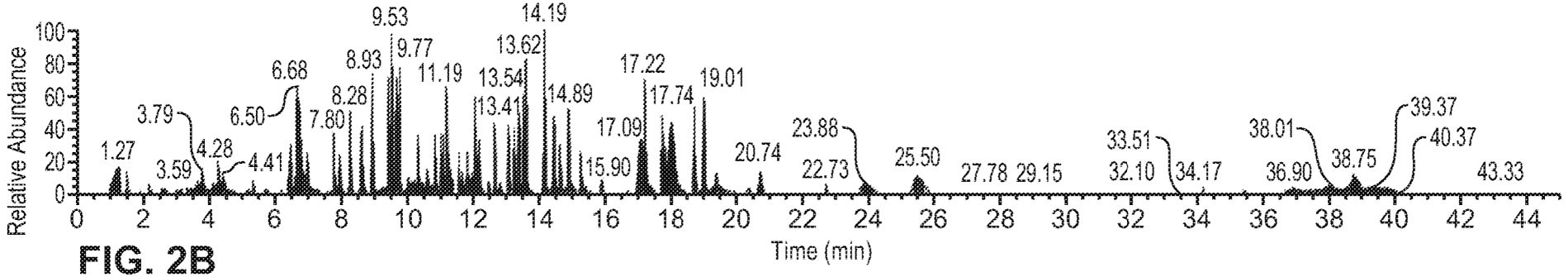


FIG. 2B

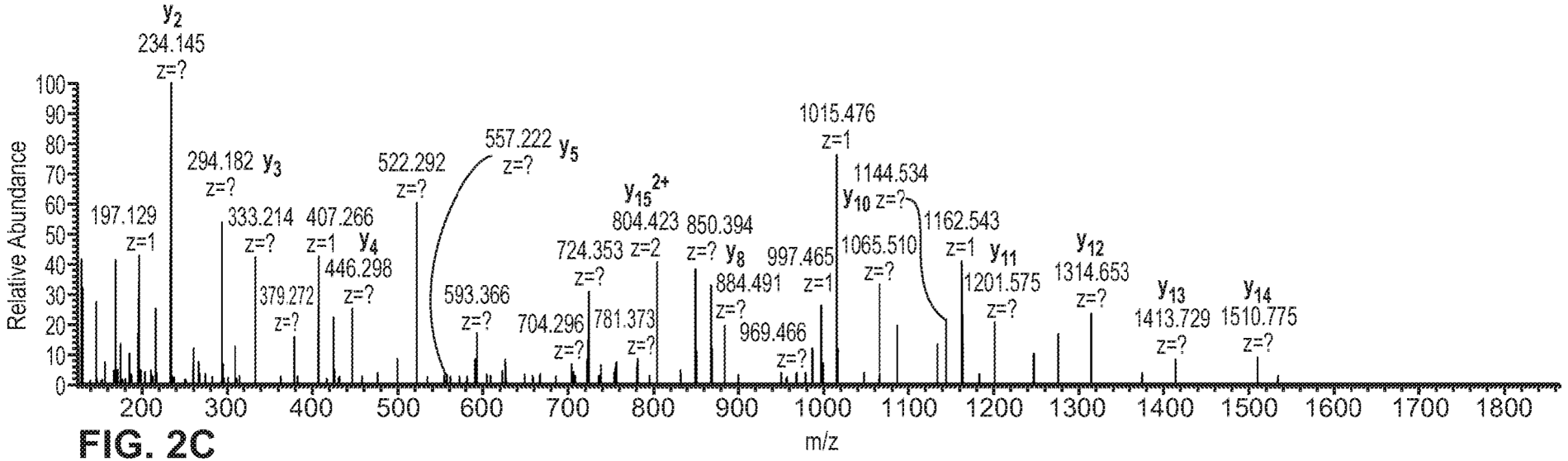


FIG. 2C

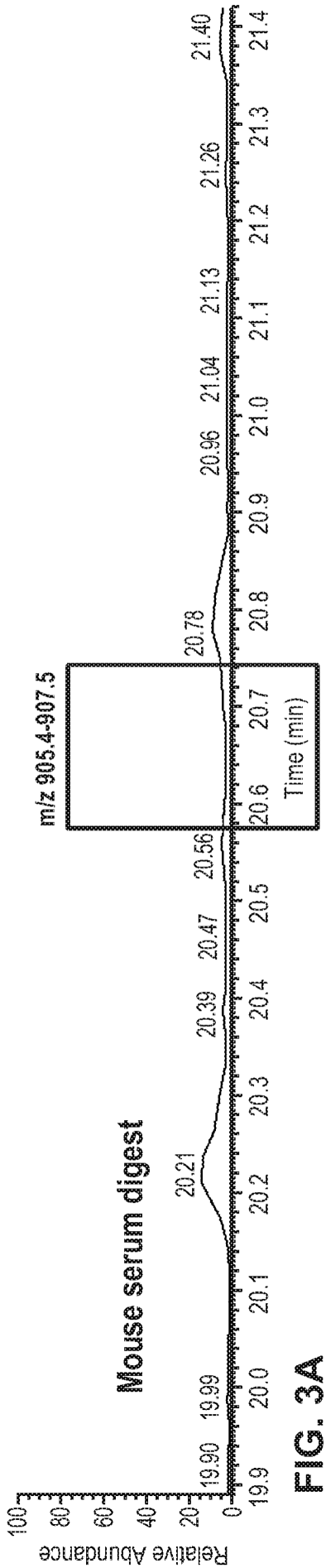

Mouse serum digest
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
19.9
19.90
19.99
20.0
20.1
20.21
20.2
20.3
20.39
20.4
20.47
20.5
20.56
20.6
20.7
20.78
20.8
20.96
20.9
21.04
21.0
21.13
21.1
21.2
21.26
21.3
21.40
21.4

FIG. 3A

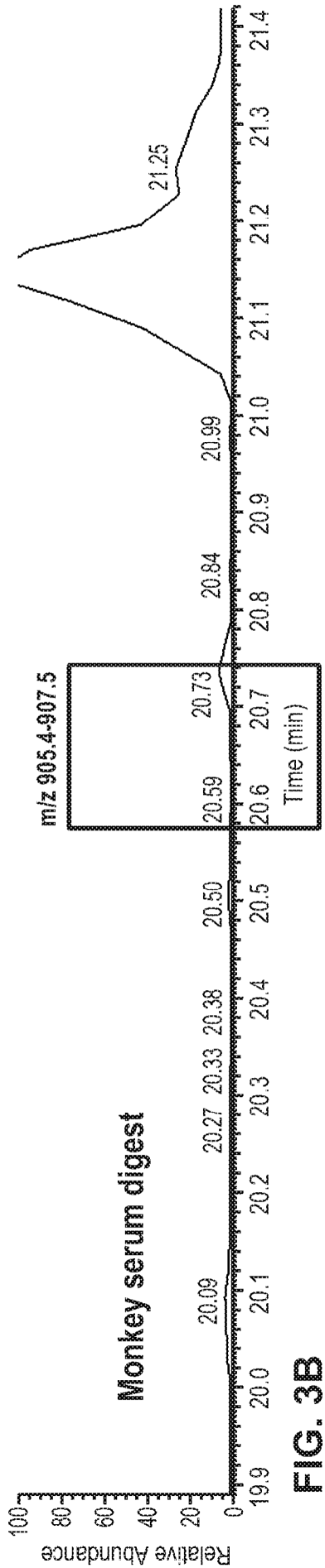

Monkey serum digest
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
19.9
20.0
20.09
20.1
20.2
20.27
20.3
20.33
20.38
20.4
20.50
20.5
20.59
20.6
20.7
20.73
20.8
20.84
20.9
20.99
21.0
21.1
21.2
21.25
21.3
21.4

FIG. 3B

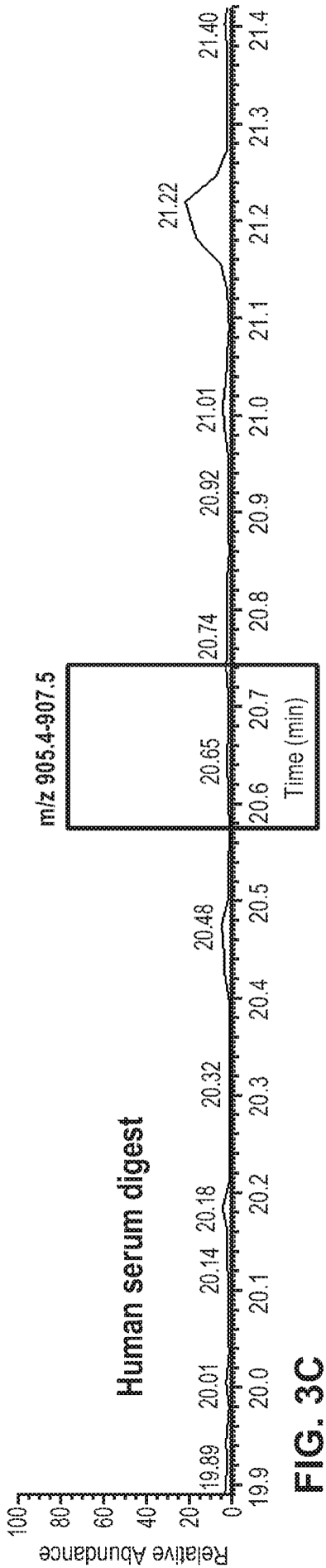

Human serum digest
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
19.89
20.01
20.0
20.14
20.1
20.18
20.2
20.32
20.3
20.48
20.4
20.5
20.65
20.6
20.7
20.74
20.8
20.92
20.9
21.01
21.0
21.1
21.22
21.2
21.3
21.40
21.4

FIG. 3C

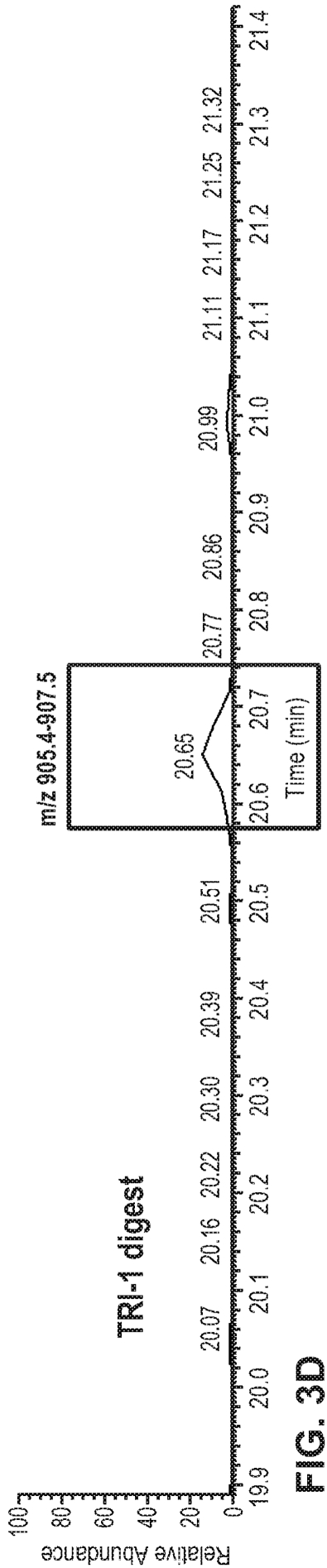

TRI-1 digest
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
19.9
20.0
20.07
20.1
20.16
20.2
20.22
20.3
20.30
20.39
20.4
20.51
20.5
20.6
20.65
20.7
20.77
20.8
20.86
20.9
20.99
21.0
21.11
21.1
21.17
21.2
21.25
21.3
21.32
21.4

FIG. 3D

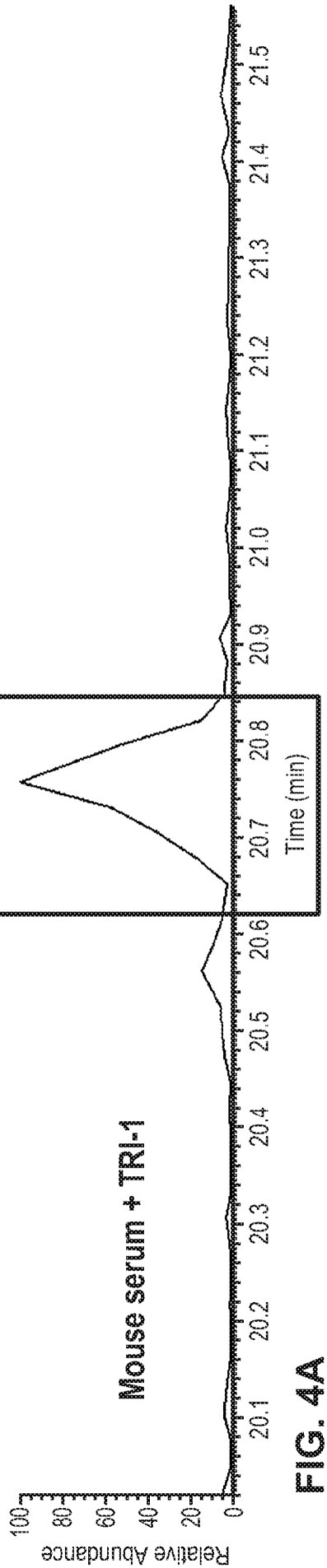

Mouse serum + TRI-1
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
20.1
20.2
20.3
20.4
20.5
20.6
20.7
20.8
20.9
21.0
21.1
21.2
21.3
21.4
21.5

FIG. 4A

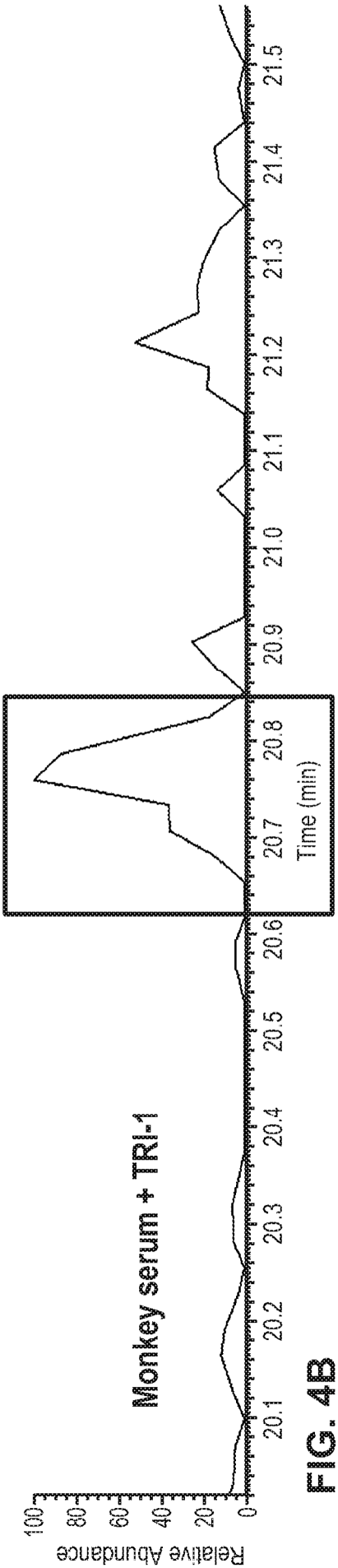

Monkey serum + TRI-1
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
20.1
20.2
20.3
20.4
20.5
20.6
20.7
20.8
20.9
21.0
21.1
21.2
21.3
21.4
21.5

FIG. 4B

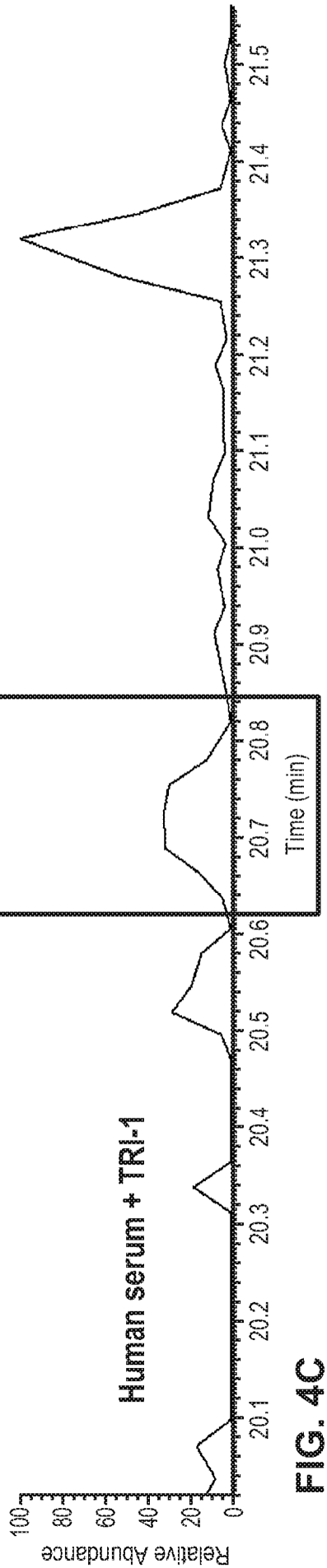

Human serum + TRI-1
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
20.1
20.2
20.3
20.4
20.5
20.6
20.7
20.8
20.9
21.0
21.1
21.2
21.3
21.4
21.5

FIG. 4C

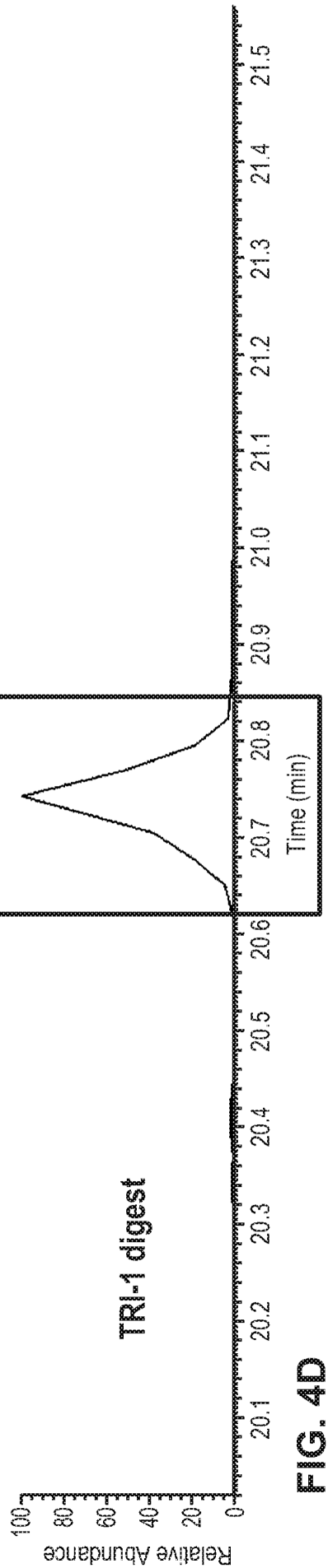

TRI-1 digest
m/z 905.4-907.5
Relative Abundance
Time (min)
100
80
60
40
20
0
20.1
20.2
20.3
20.4
20.5
20.6
20.7
20.8
20.9
21.0
21.1
21.2
21.3
21.4
21.5

FIG. 4D

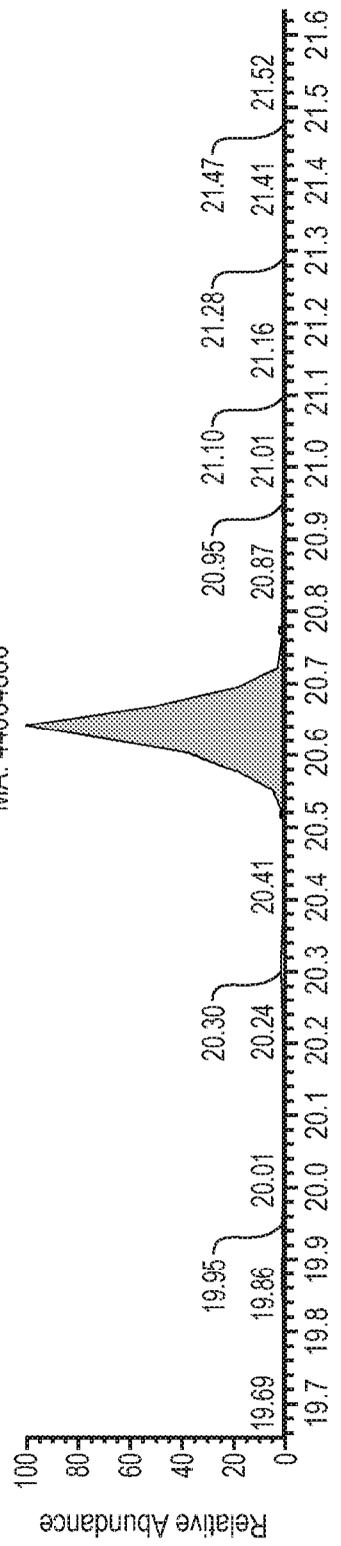


FIG. 5A

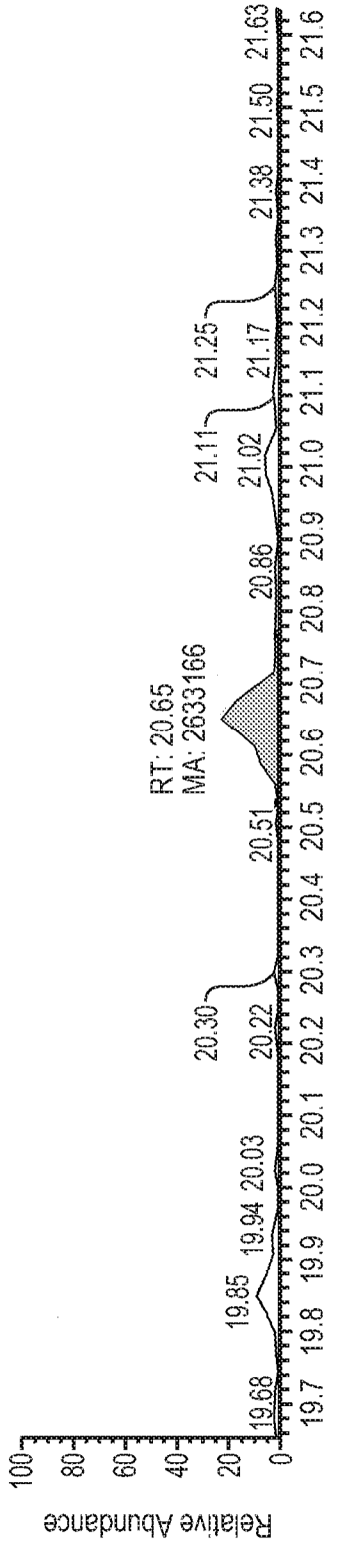


FIG. 5B

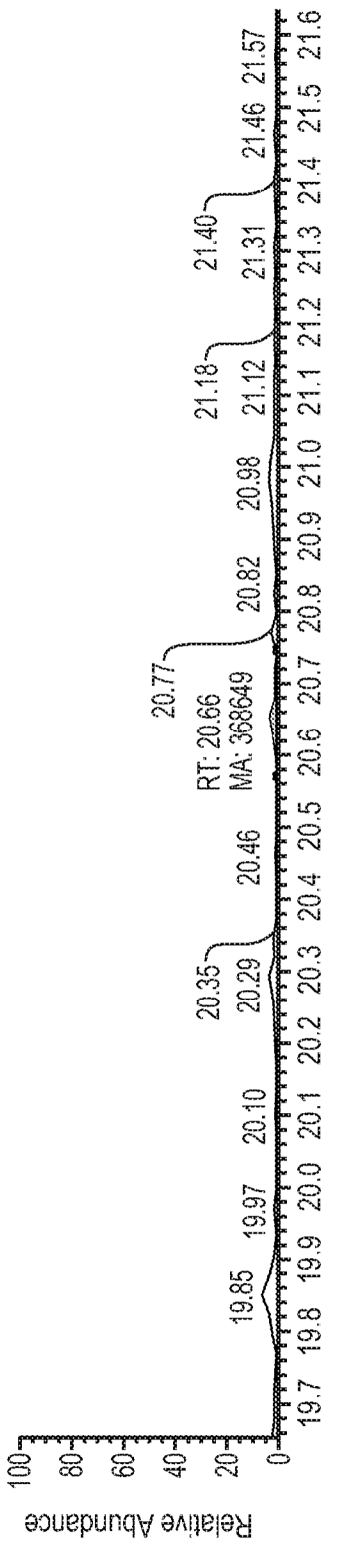


FIG. 5C

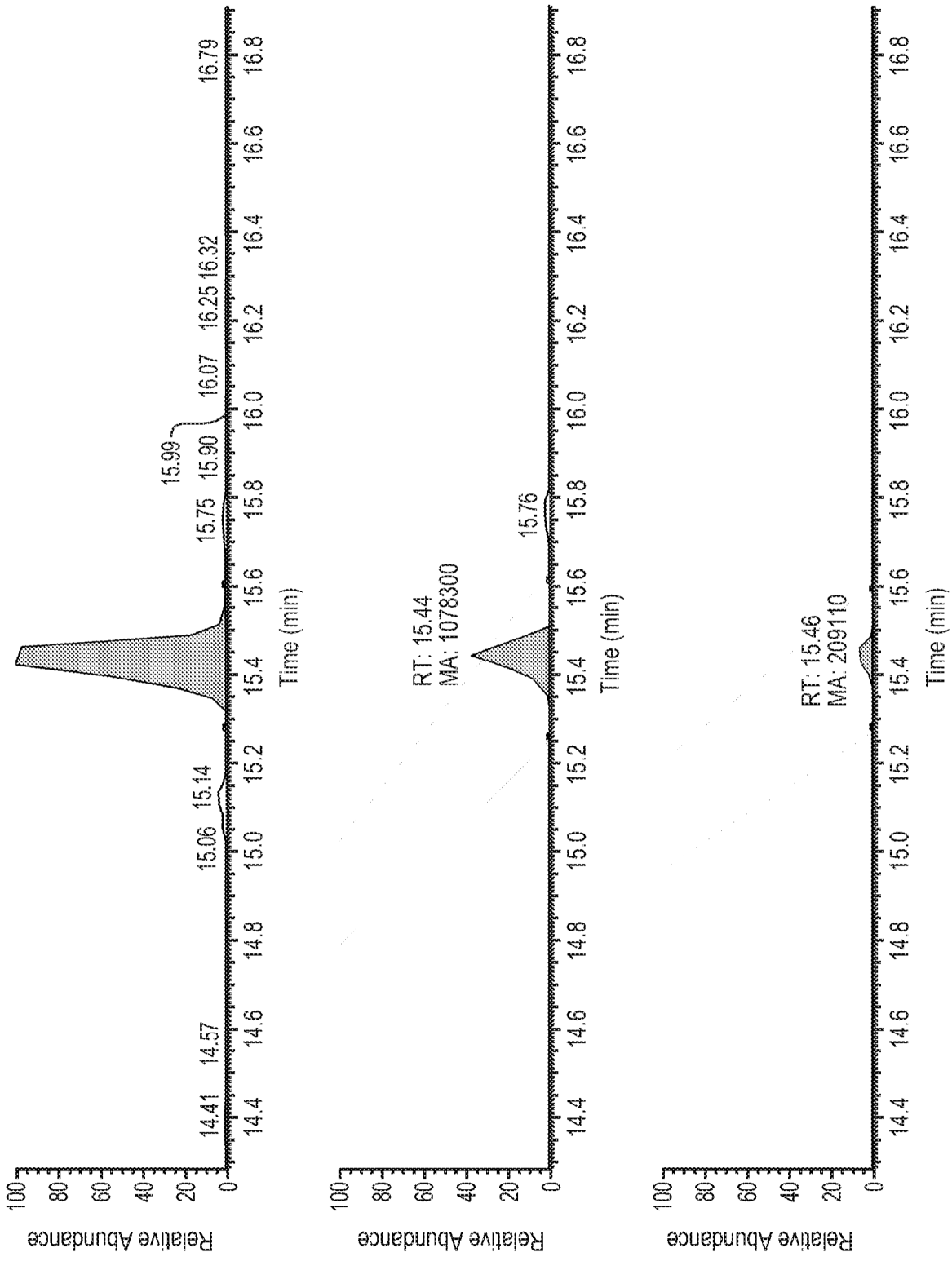


FIG. 6A

FIG. 6B

FIG. 6C

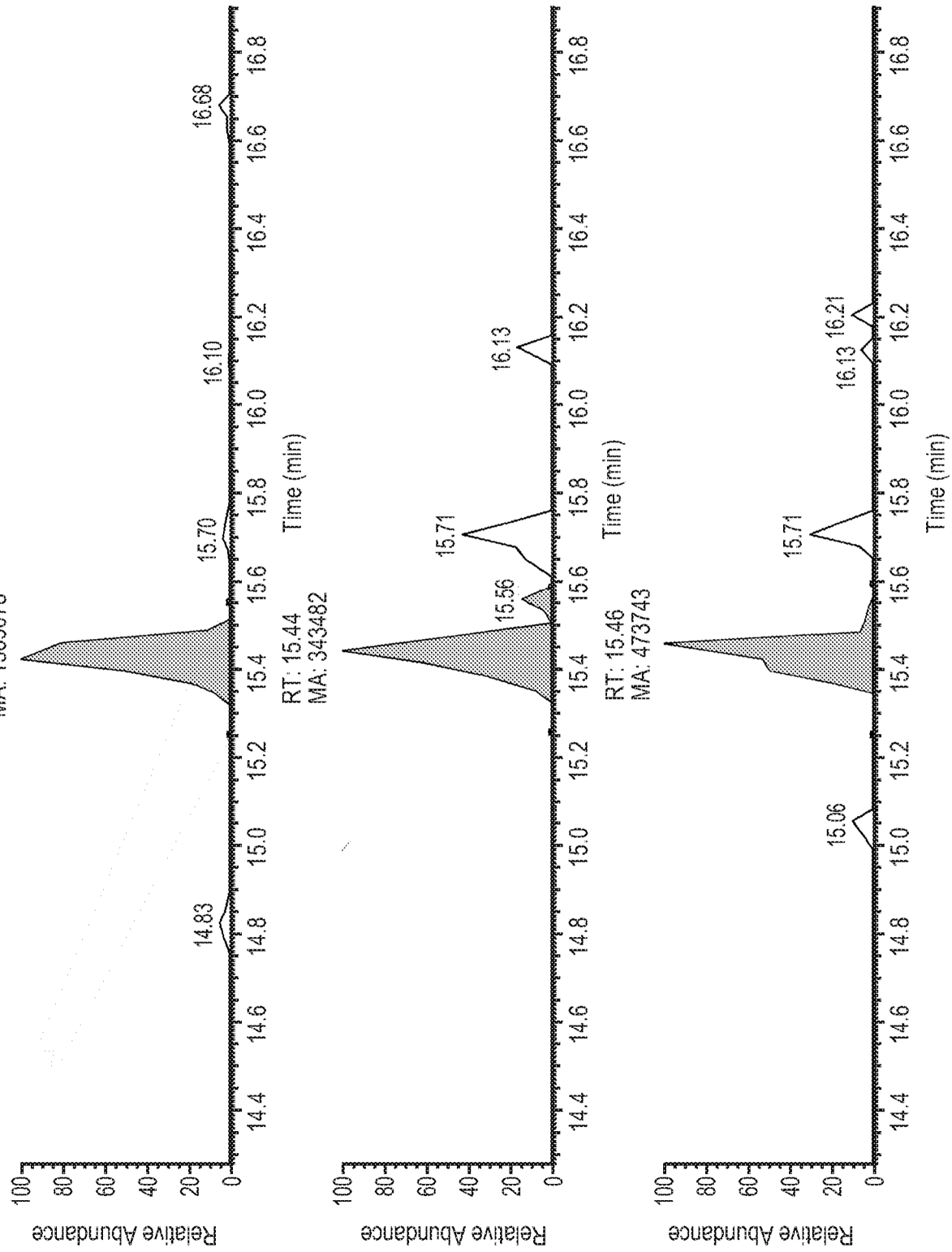


FIG. 7A

FIG. 7B

FIG. 7C

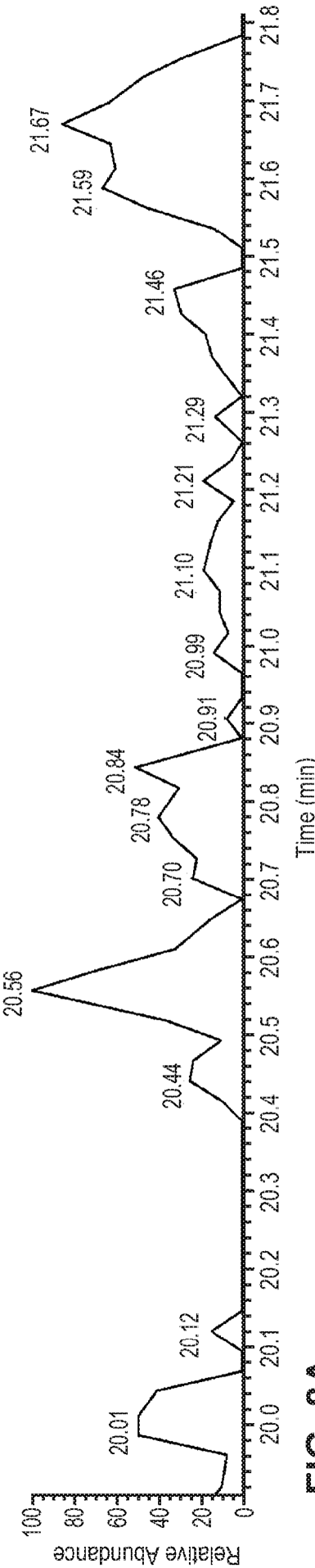

Relative Abundance
Time (min)
20.01
20.12
20.56
20.44
20.70
20.78
20.84
20.91
20.99
21.10
21.21
21.29
21.46
21.59
21.67

FIG. 8A

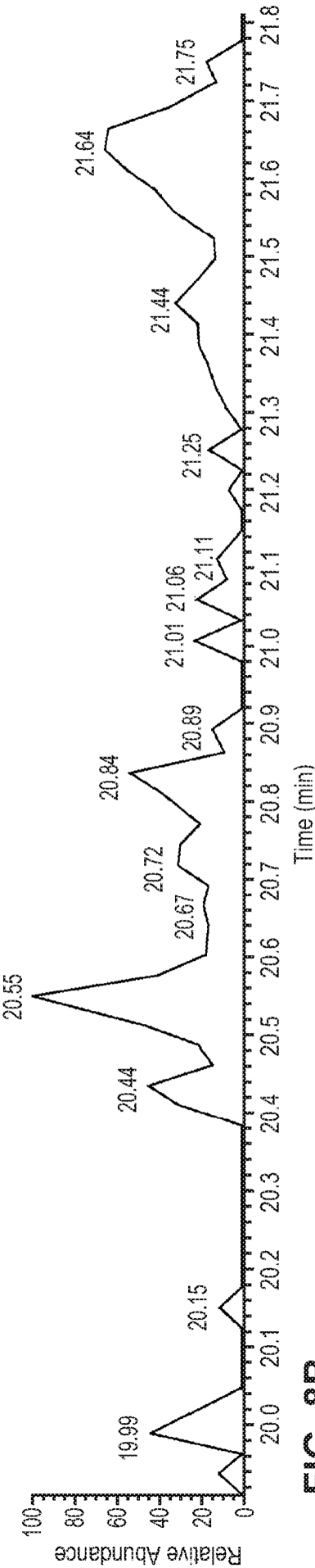

Relative Abundance
Time (min)
19.99
20.15
20.55
20.44
20.67
20.72
20.84
20.89
21.01
21.06
21.11
21.25
21.44
21.64
21.75

FIG. 8B

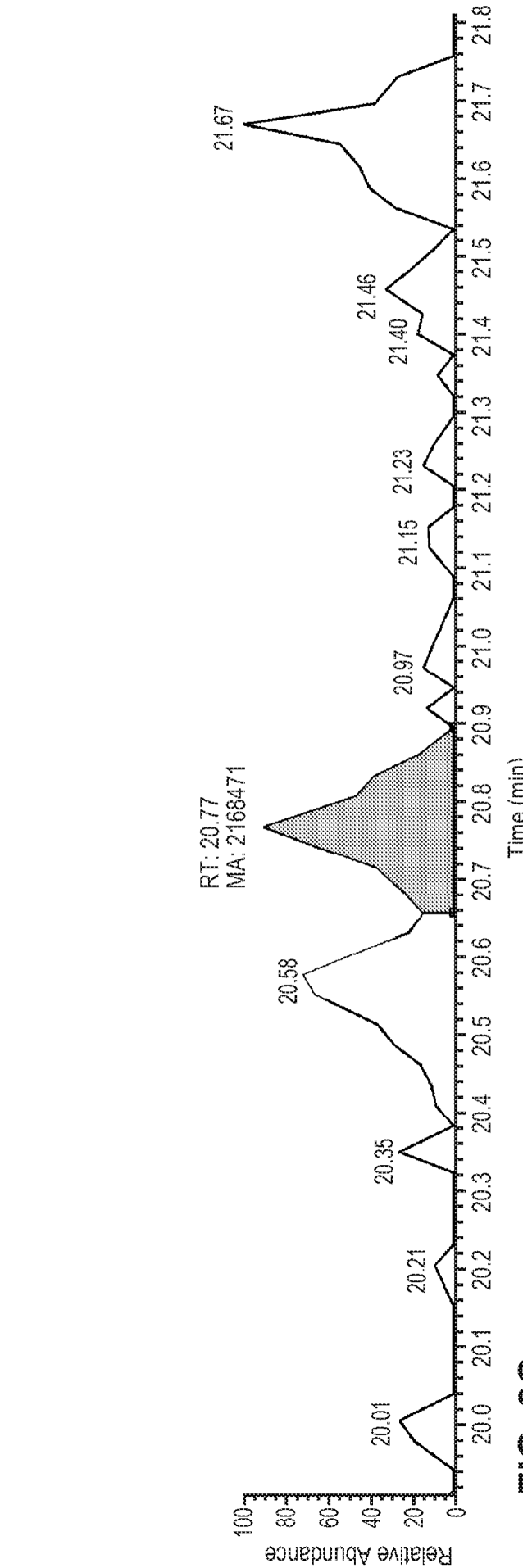

RT: 20.77
MA: 2168471
Relative Abundance
Time (min)
100
80
60
40
20
0
20.0
20.1
20.2
20.3
20.4
20.5
20.6
20.7
20.8
20.9
21.0
21.1
21.2
21.3
21.4
21.5
21.6
21.7
21.8
20.01
20.21
20.35
20.58
20.97
21.15
21.23
21.40
21.46
21.67

FIG. 8C

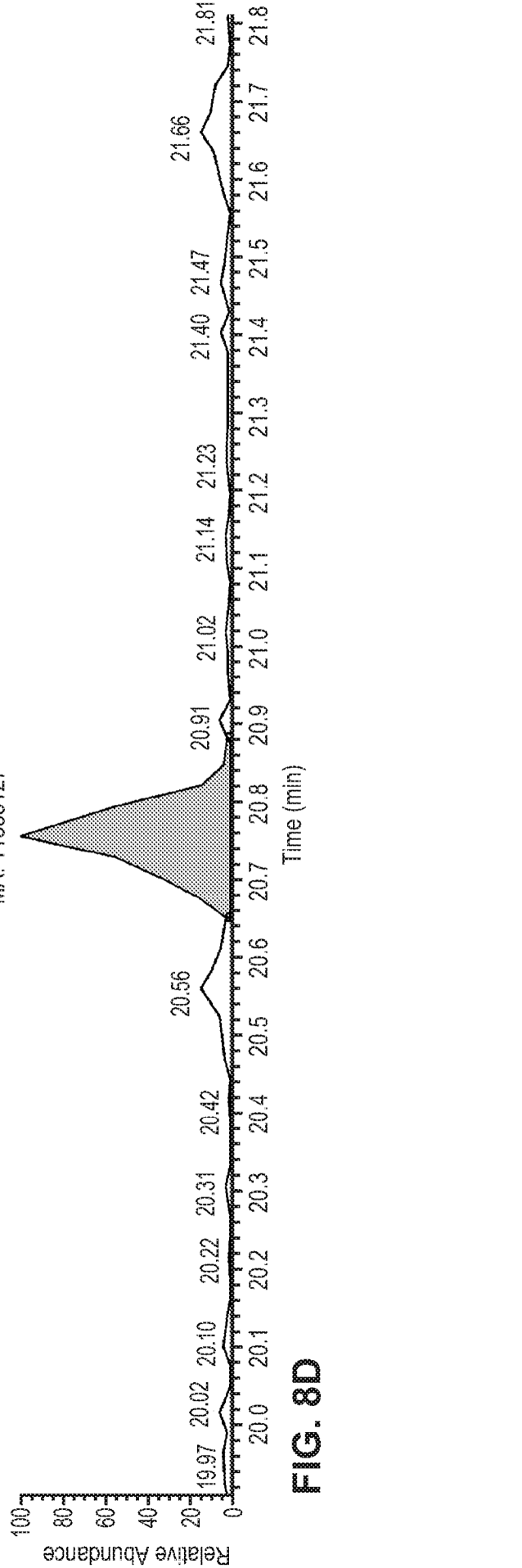

RT: 20.76
MA: 11833127
Relative Abundance
Time (min)
100
80
60
40
20
0
19.97
20.0
20.02
20.1
20.10
20.2
20.22
20.3
20.31
20.4
20.42
20.5
20.56
20.6
20.7
20.8
20.9
20.91
21.0
21.02
21.1
21.14
21.2
21.23
21.3
21.40
21.4
21.47
21.5
21.6
21.66
21.7
21.8
21.81

FIG. 8D

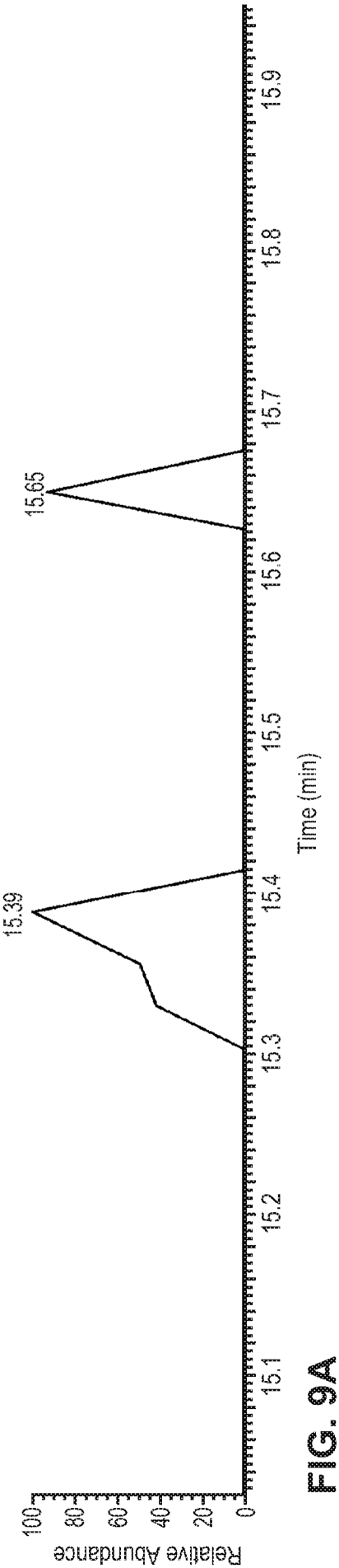

15.39
15.65
Relative Abundance
Time (min)
100
80
60
40
20
0
15.1
15.2
15.3
15.4
15.5
15.6
15.7
15.8
15.9

FIG. 9A

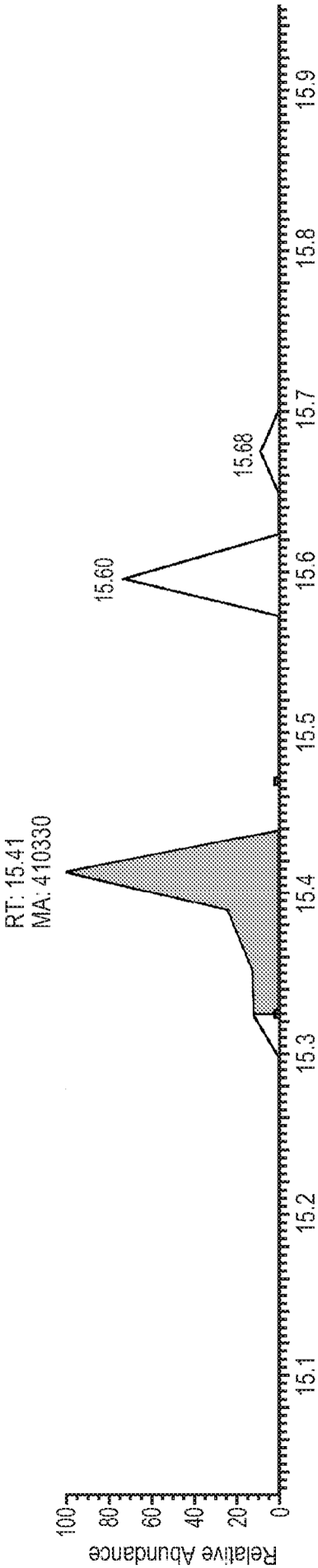

RT: 15.41
MA: 410330
15.60
15.68
Relative Abundance
Time (min)
100
80
60
40
20
0
15.1
15.2
15.3
15.4
15.5
15.6
15.7
15.8
15.9

FIG. 9B

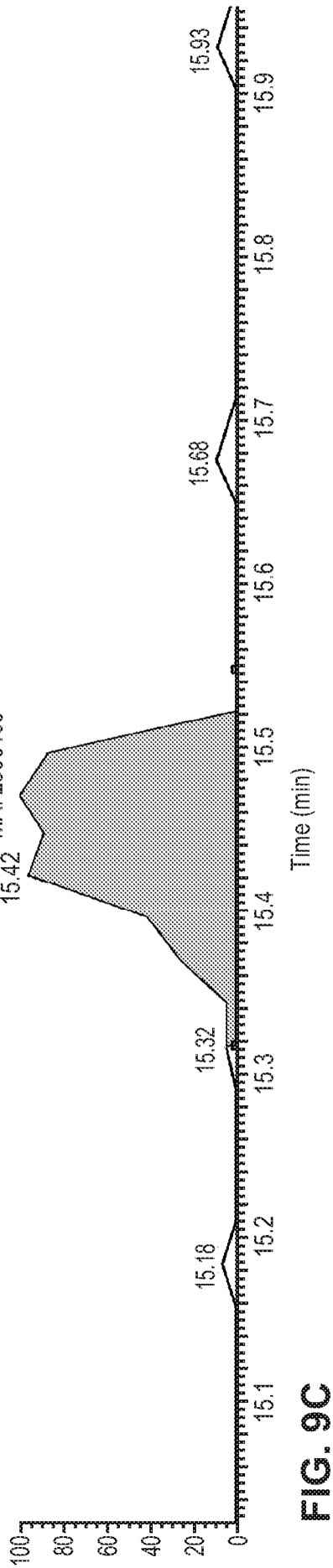

RT: 15.47
MA: 2500109
15.42
15.18
15.32
15.68
15.93
15.1
15.2
15.3
15.4
15.5
15.6
15.7
15.8
15.9
Time (min)
Relative Abundance
0
20
40
60
80
100

FIG. 9C

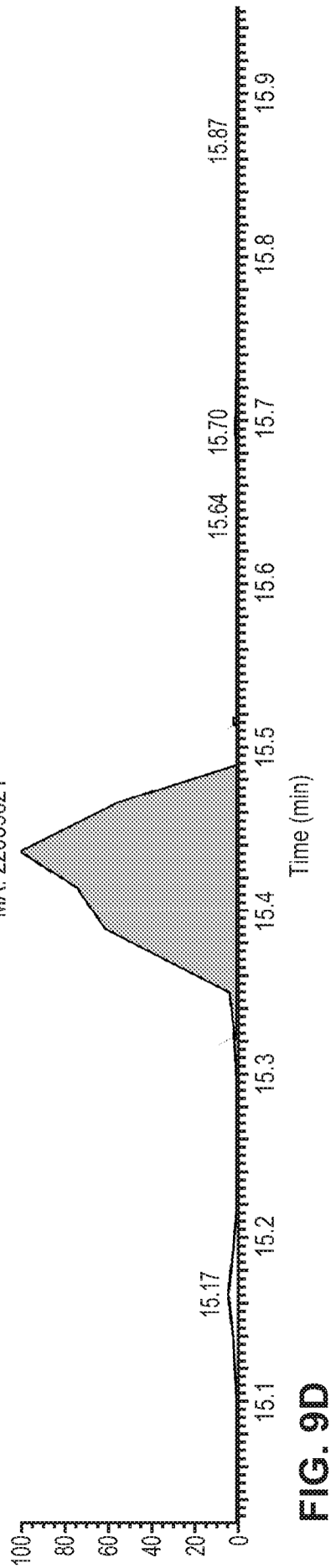

RT: 15.44
MA: 22039621
15.17
15.64
15.70
15.87
15.1
15.2
15.3
15.4
15.5
15.6
15.7
15.8
15.9
Time (min)
Relative Abundance
0
20
40
60
80
100

FIG. 9D

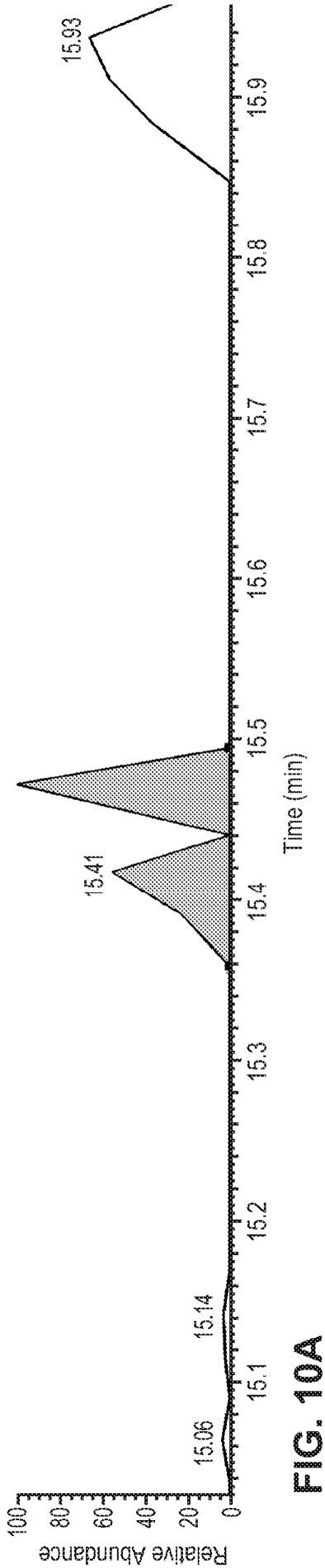


FIG. 10A

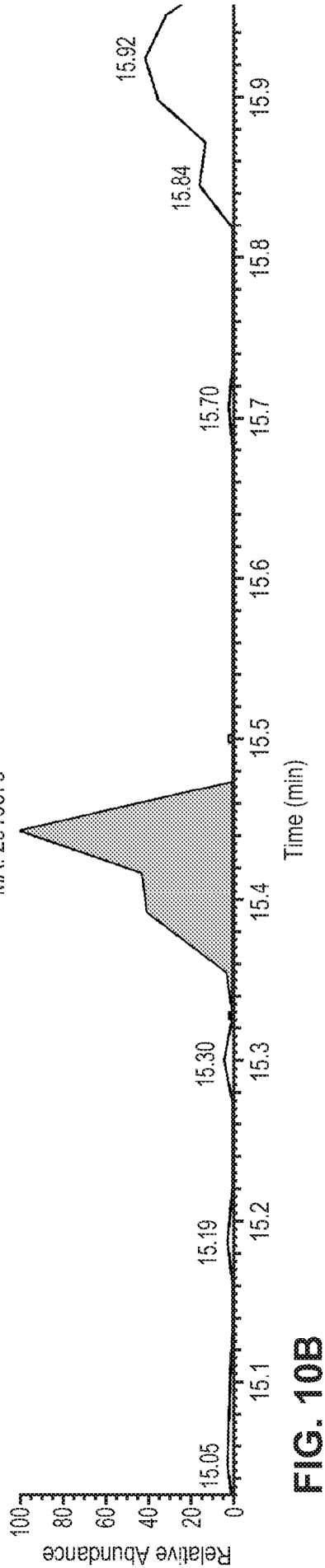


FIG. 10B

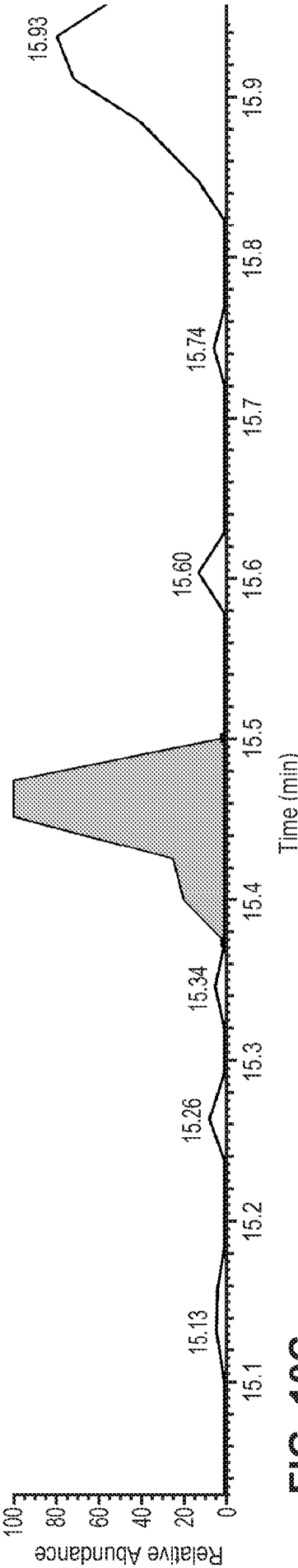


FIG. 10C

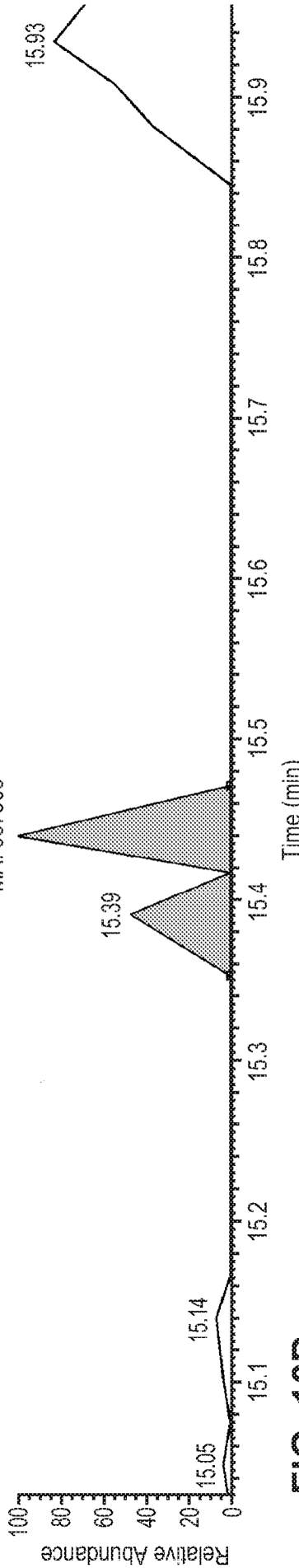


FIG. 10D

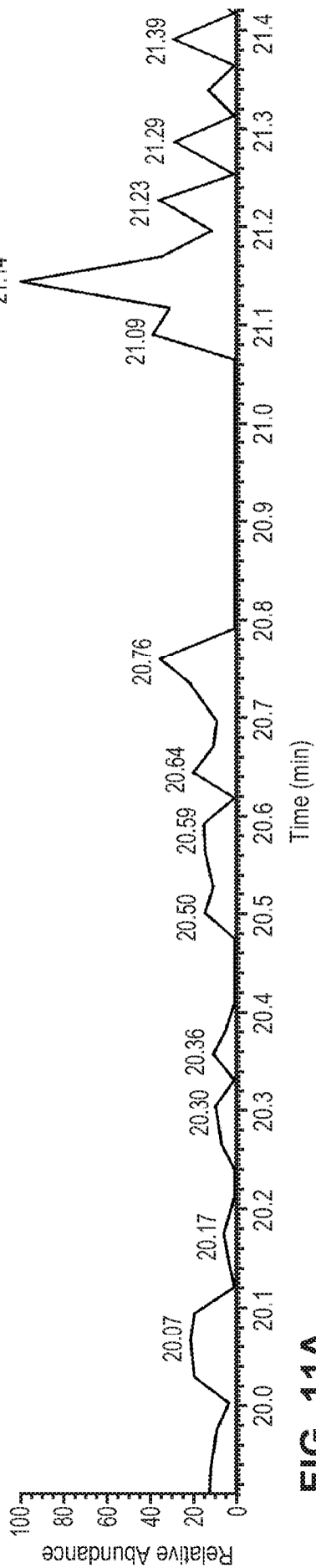


FIG. 11A

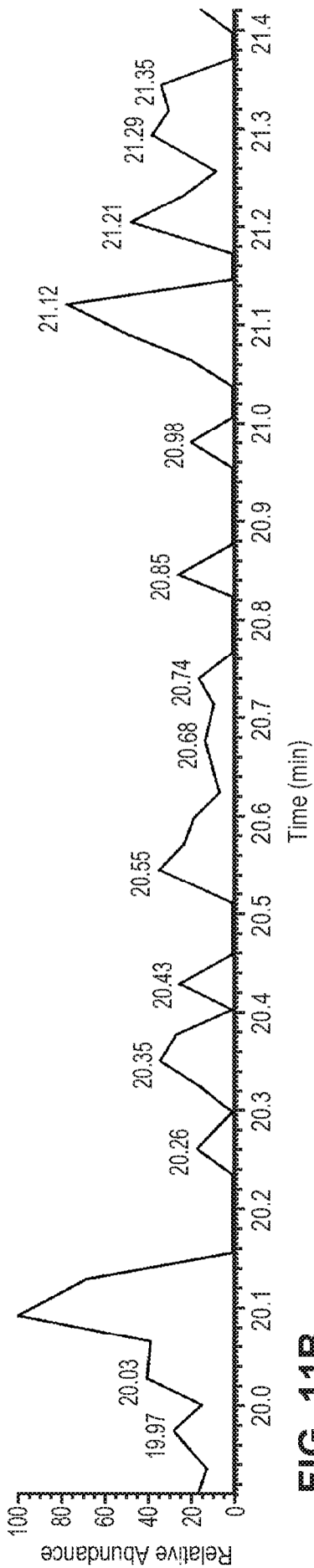


FIG. 11B

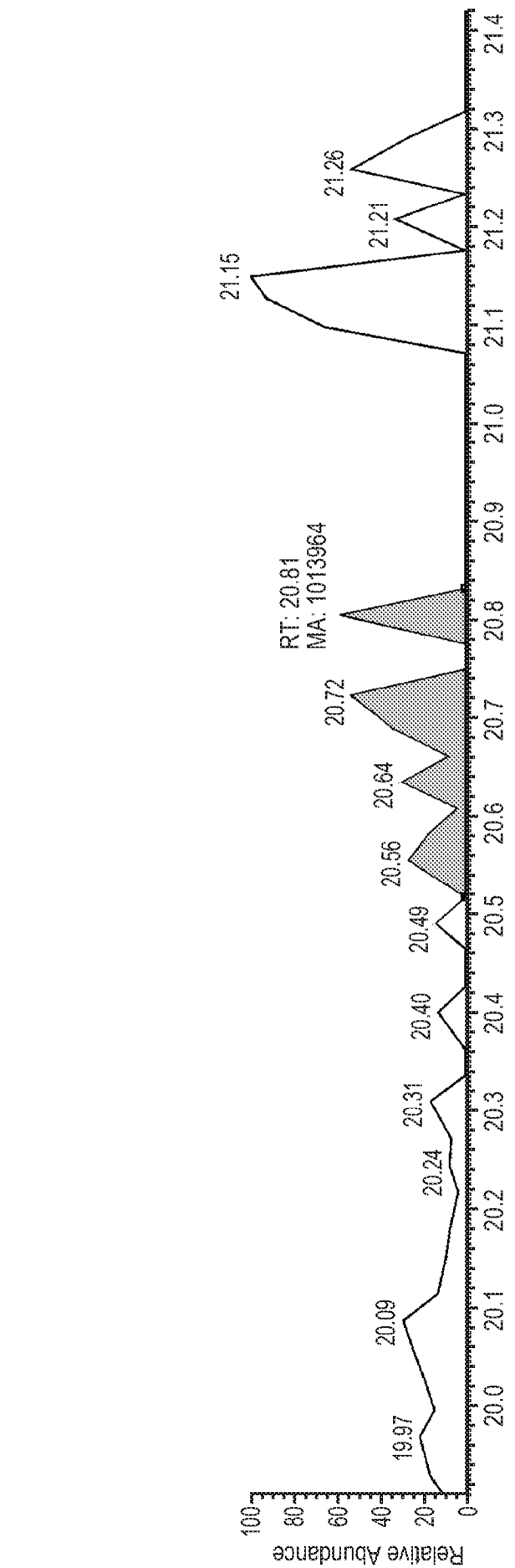


FIG. 11C

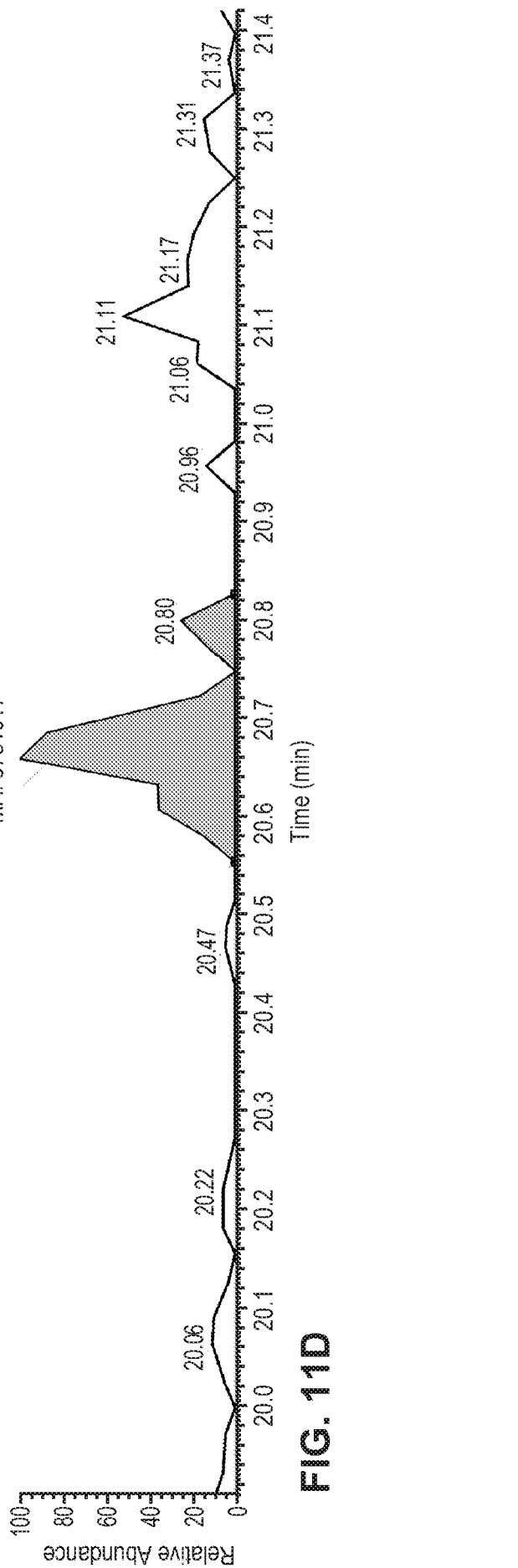


FIG. 11D

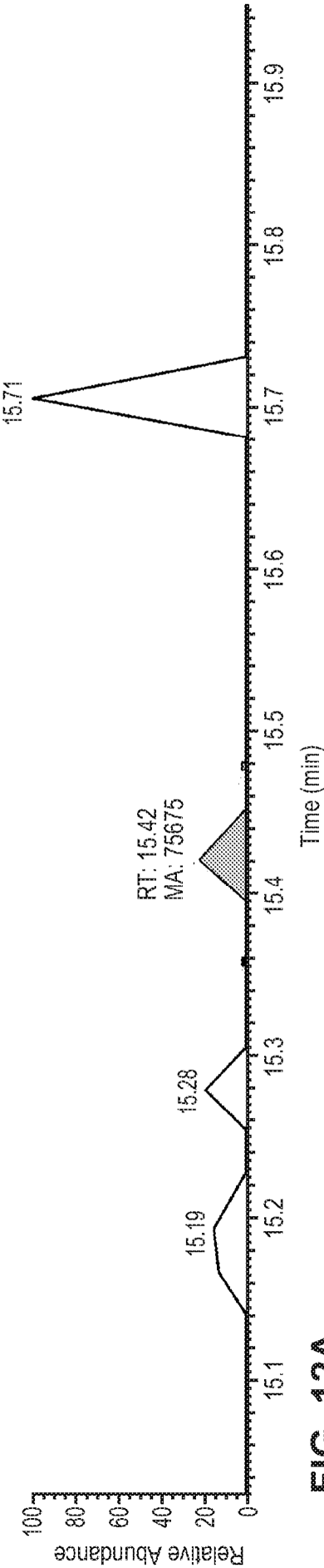


FIG. 12A

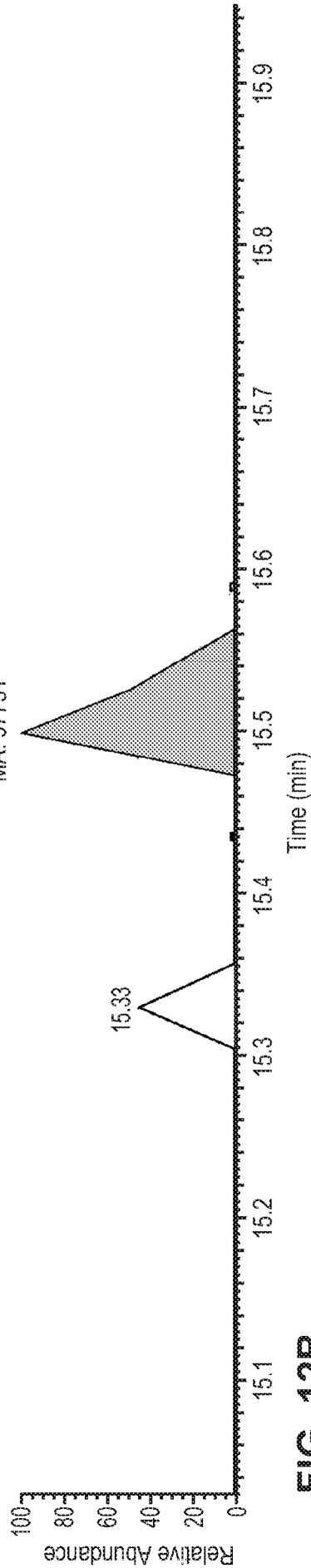


FIG. 12B

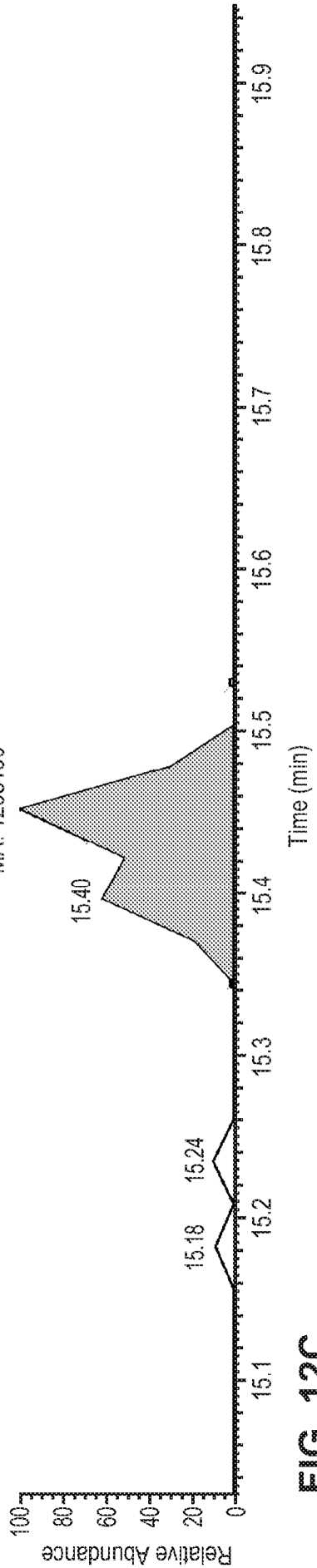


FIG. 12C

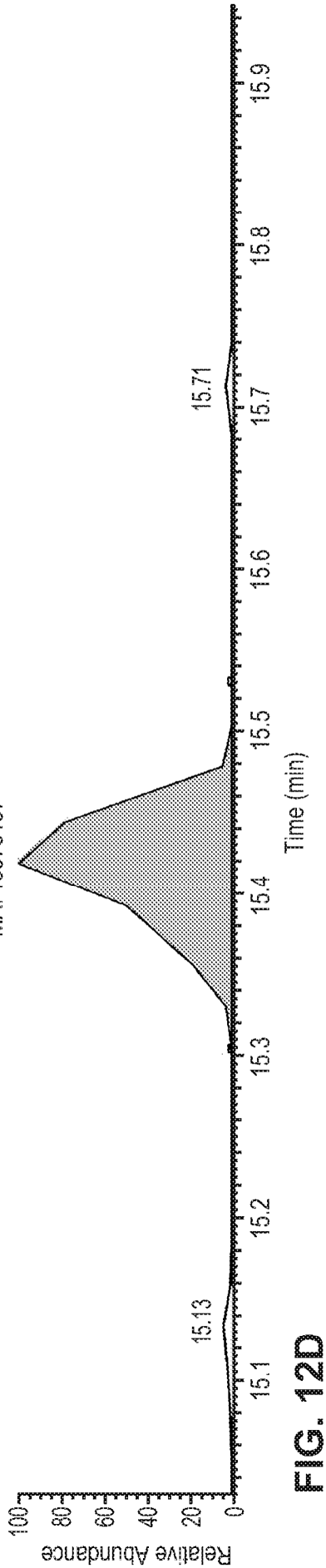


FIG. 12D

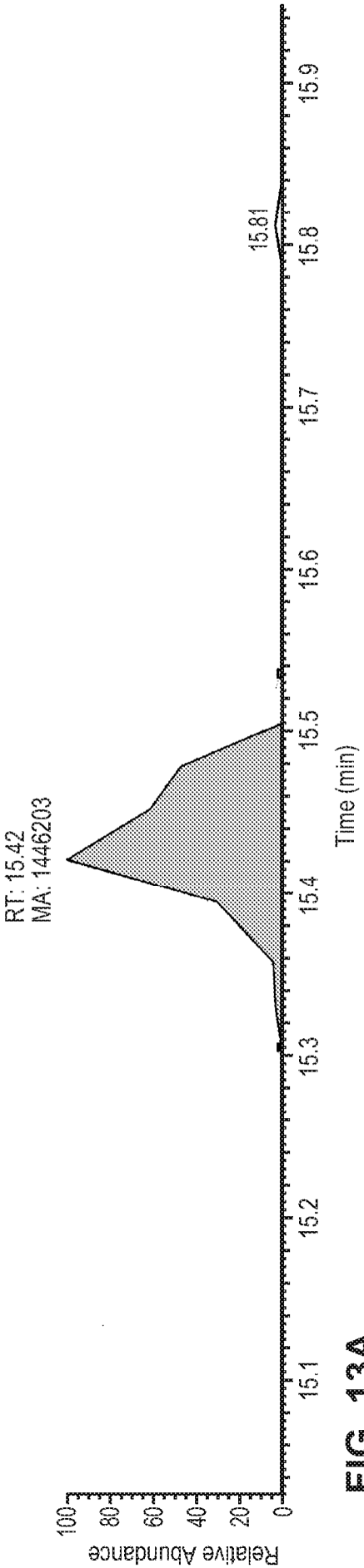


FIG. 13A

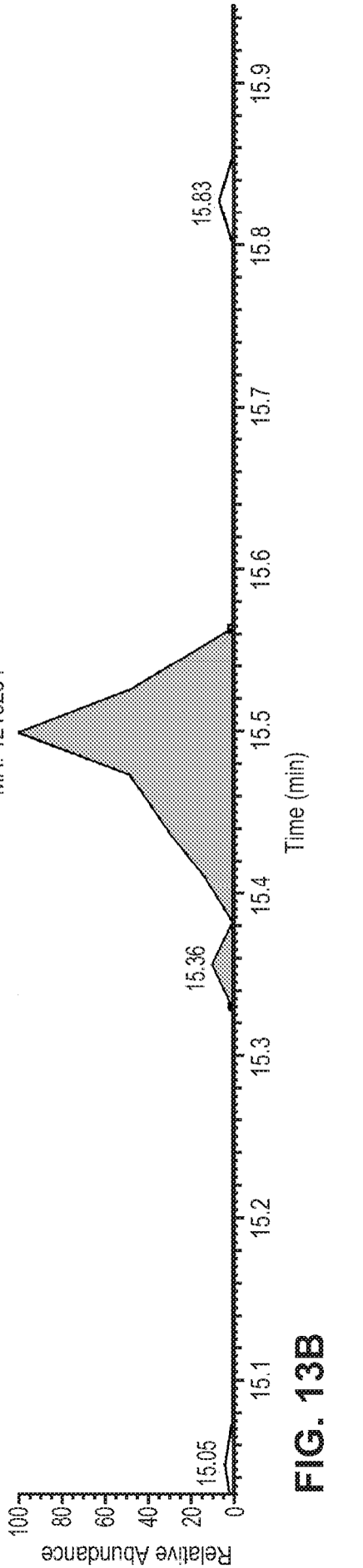


FIG. 13B

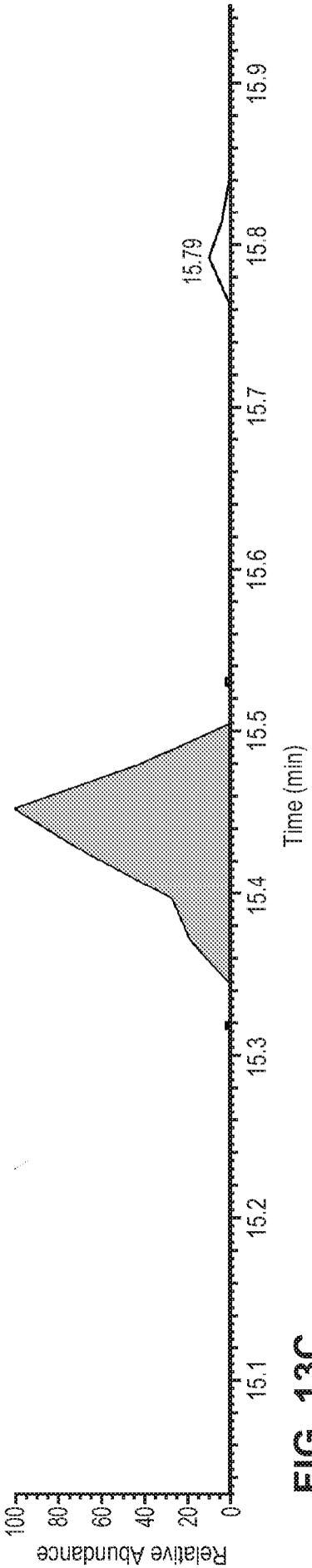


FIG. 13C

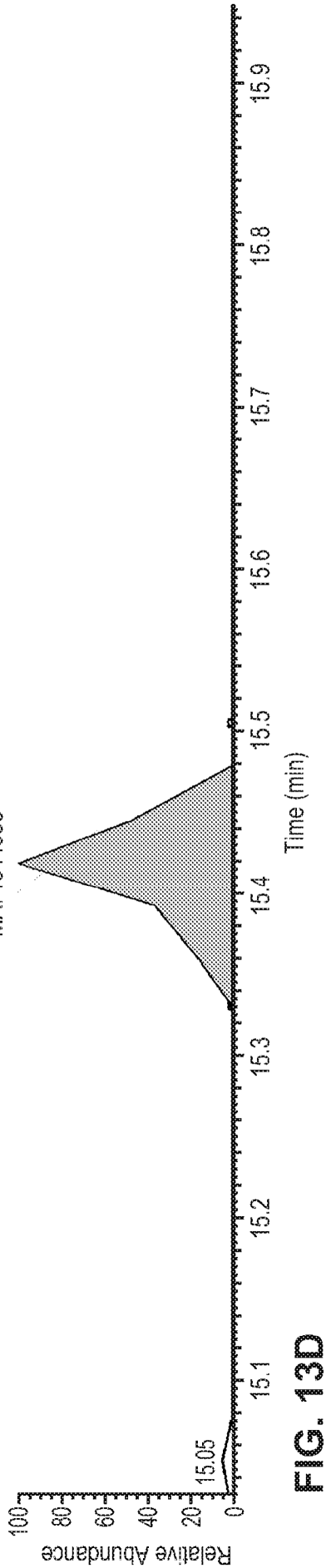


FIG. 13D

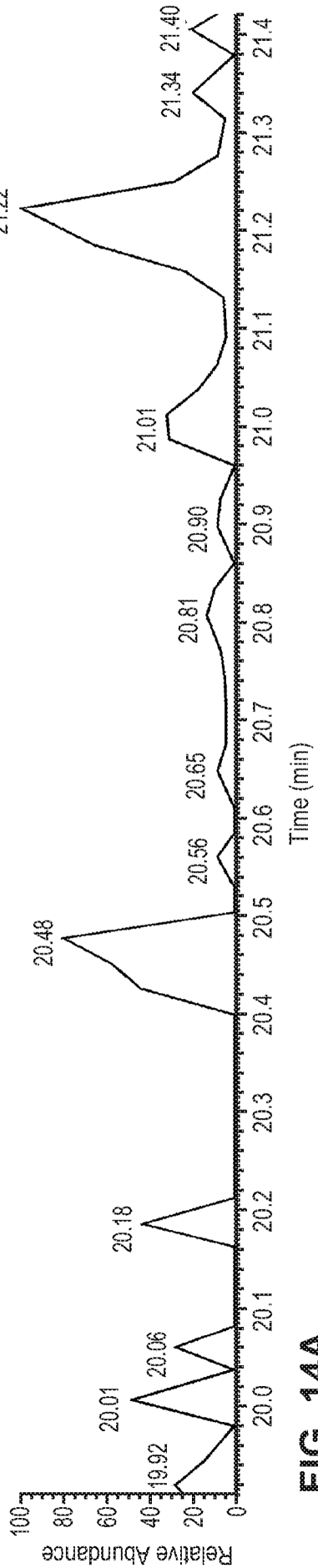


FIG. 14A

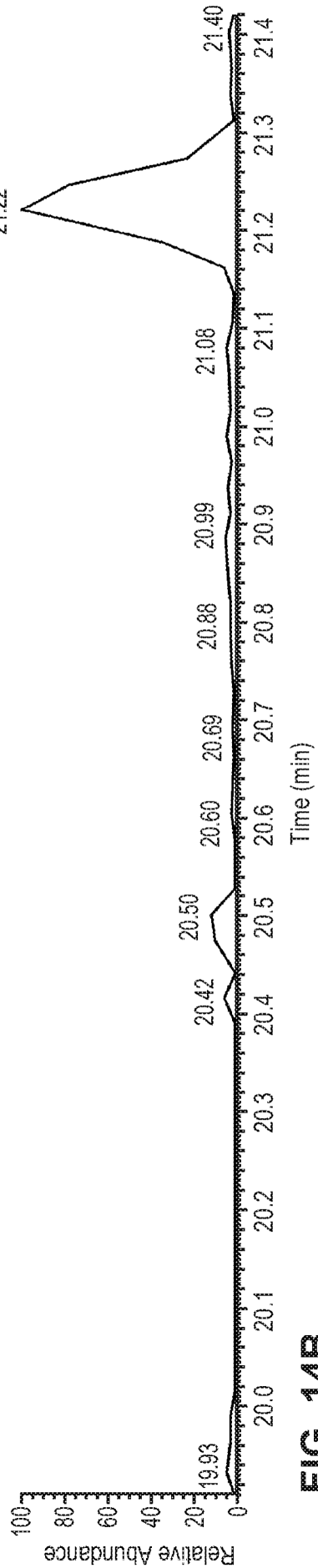


FIG. 14B

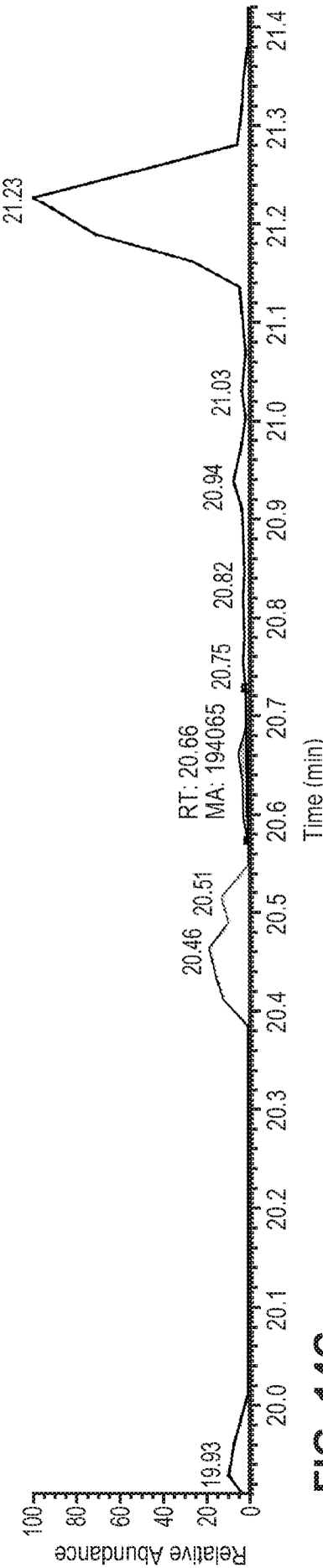


FIG. 14C

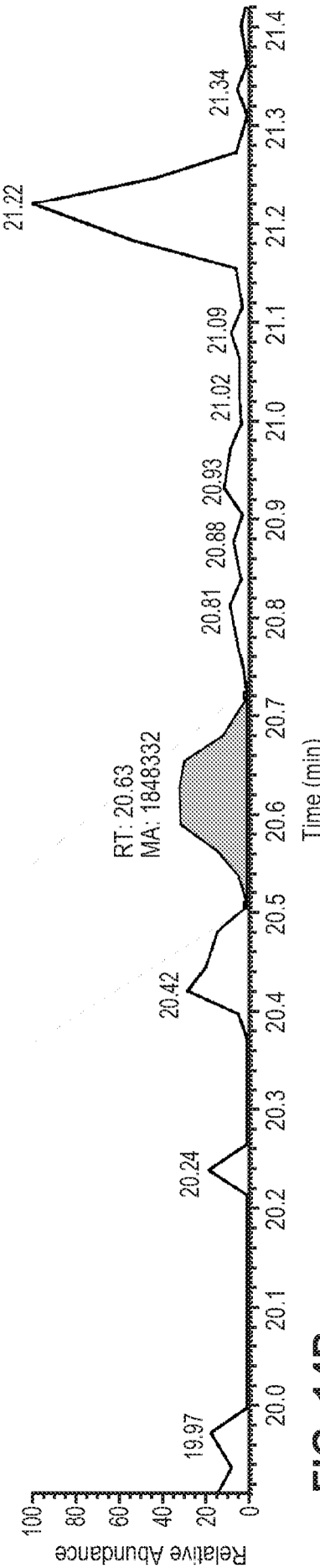


FIG. 14D

FIG. 15A

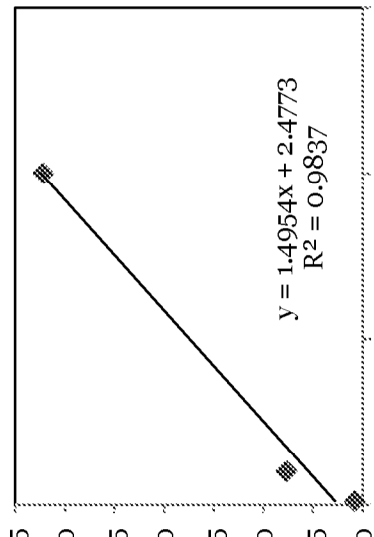


FIG. 15B

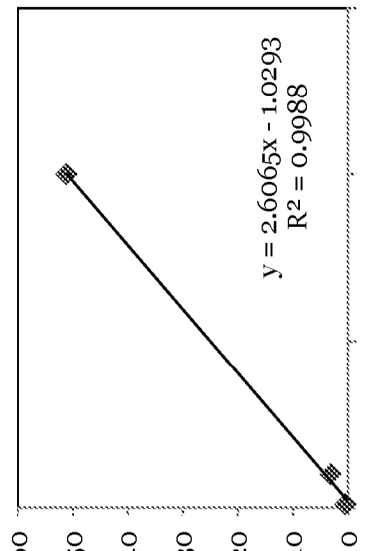


FIG. 15C

FIG. 15D

FIG. 16

Bispecific HC

EVRLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAESVKGRFTISRDNTKNTLYLEMNNVRTEDTGYYFCARTGK
YYDFWSGYPPGEEYFQDWGQGTLVIVSSDKTHTQVHLTQSGPEVRKPGTSVKVSCKAPGNTLKTYDLHWVRSVPGQGLQWMGWISHEGDKKVIVERFKAKVTI
DWDRSTNTAYLQLSGLTSGDTAVYYCAKGSKHRLRDYALYDDDGALNWAVDVDYLSNLEFWGQGTAVTVSSDKTHTASTKGPSVFPLAPSSKSTSGGTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYK**TTPPVLDSDGSFFLYS**KLTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPG **(SEQ
ID NO: 12)**

Bispecific LC

DFVLTQSPHSLSVTPGESASISCKSSHSLIHGDRNNYLAWYVQKPGRSPQLLIYLASSRASGVPDRFSGSGSDKDFTLKISRVETEDVGTYYCMQGR**ESPWTF
GQGTK**VDIKDKTHTASELTQDPAVSVALKQTVTITCRGDSLRSHYASWYQKKPGQAPVLLFYGKNNRPSGIPDRFSGSASGNRASLTITGAQAEDEADYYCSS
RDKSGSRLSVFGGGTKLTVLDKTHTRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK**DSTYSLSSTLTLSK**AD
YEKHKVYACEVTHQGLSSPVTKSFNRGEC **(SEQ ID NO: 13)**

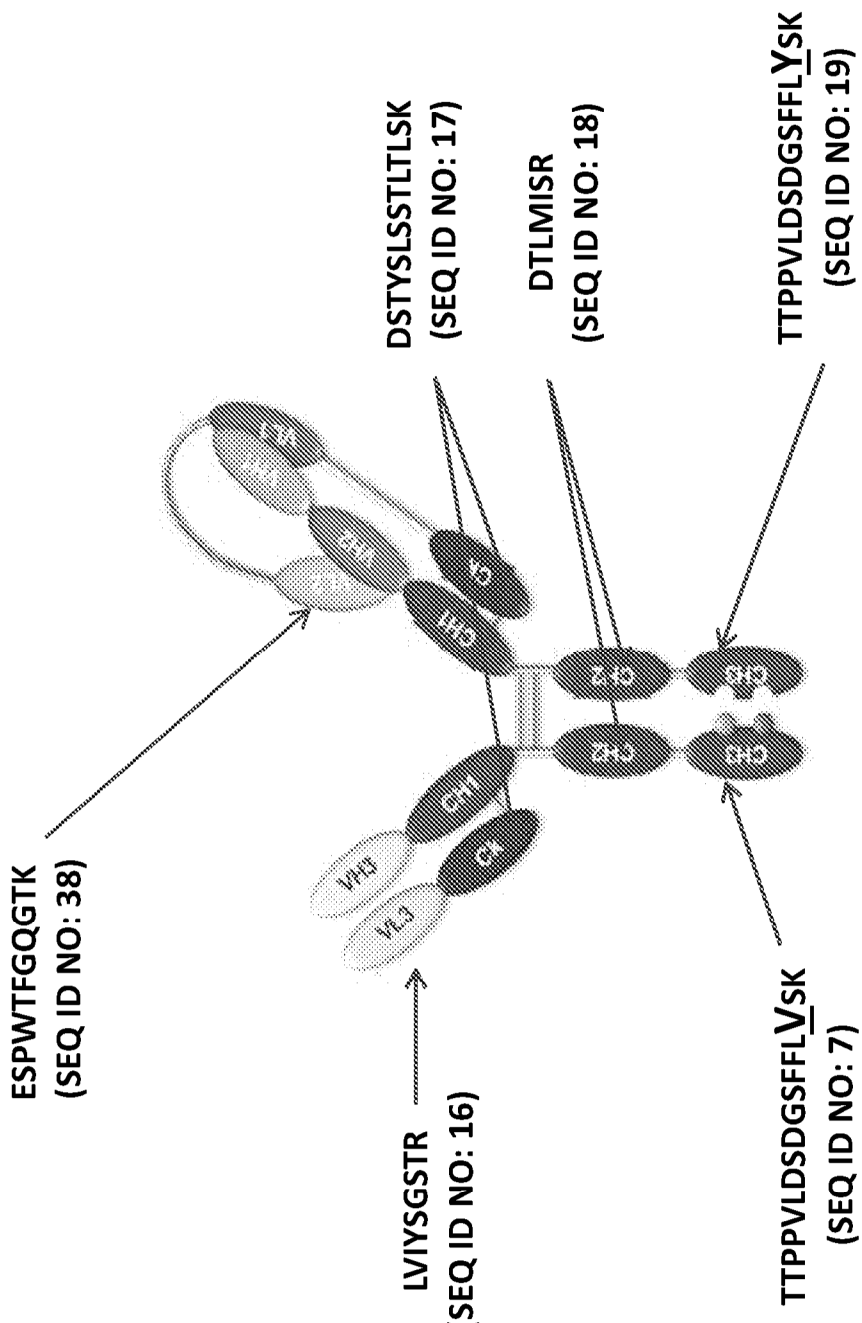
Monospecific HC

QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCD
YNWDFEHWGRGTPVIVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK**DTLMISR**TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYK**TTPPVLDSDGSFFLVS
K**LTVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPG **(SEQ ID NO: 14)**

Monospecific LC

EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPR**LVIYSGSTR**AAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIK
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC **(SEQ ID NO: 15)**

FIG. 17


ESPWTFGQGTK
(SEQ ID NO: 38)
LVIYSGSTR
(SEQ ID NO: 16)
DSTYSLSSTLTLSK
(SEQ ID NO: 17)
DTLMISR
(SEQ ID NO: 18)
TTPPVLDSDGSFFLVSK
(SEQ ID NO: 19)
TTPPVLDSDGSFFLVSK
(SEQ ID NO: 7)

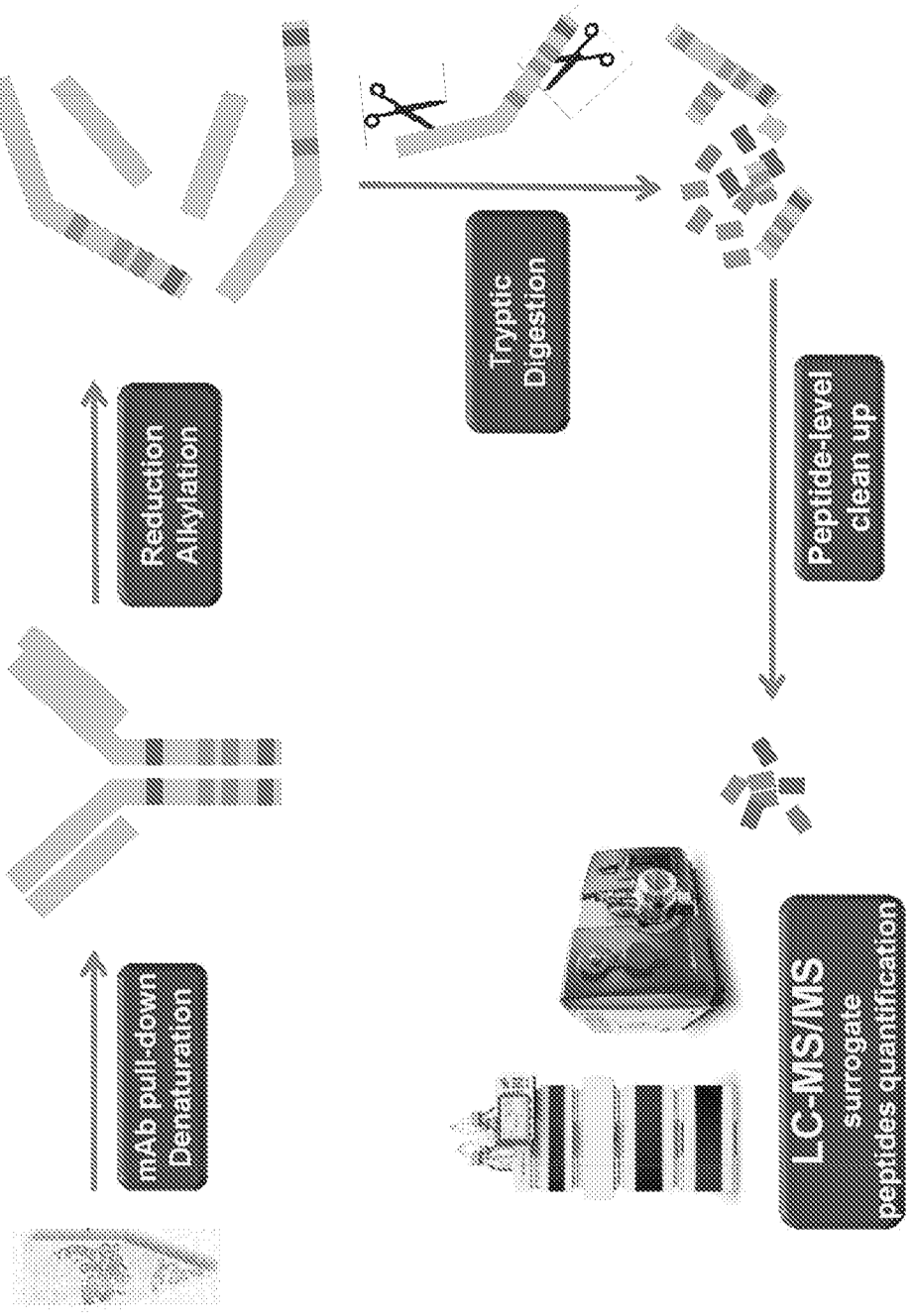

mAb pull-down
Denaturation
Reduction
Alkylation
Tryptic
Digestion
Peptide-level
clean up
LC-MS/MS
surrogate
peptides quantification

FIG. 18

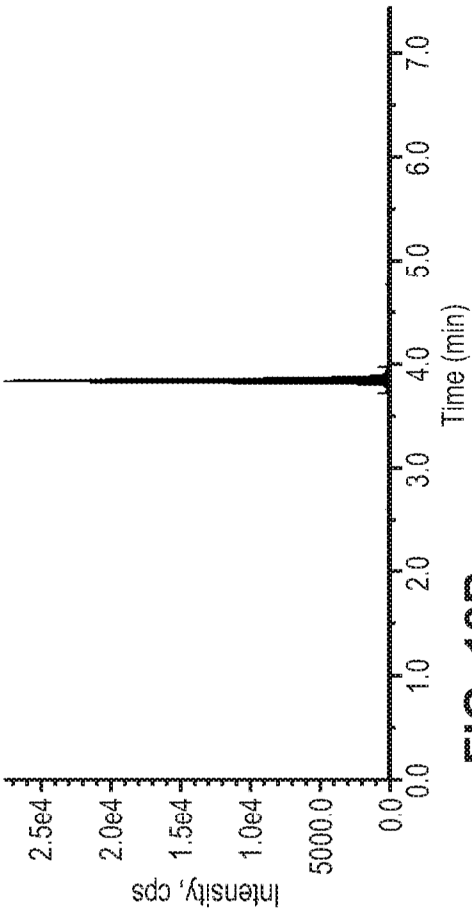


FIG. 19B

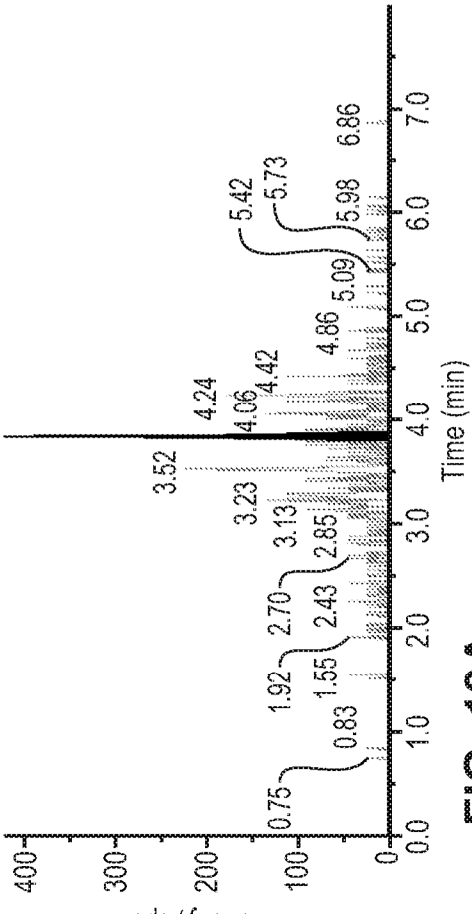


FIG. 19A

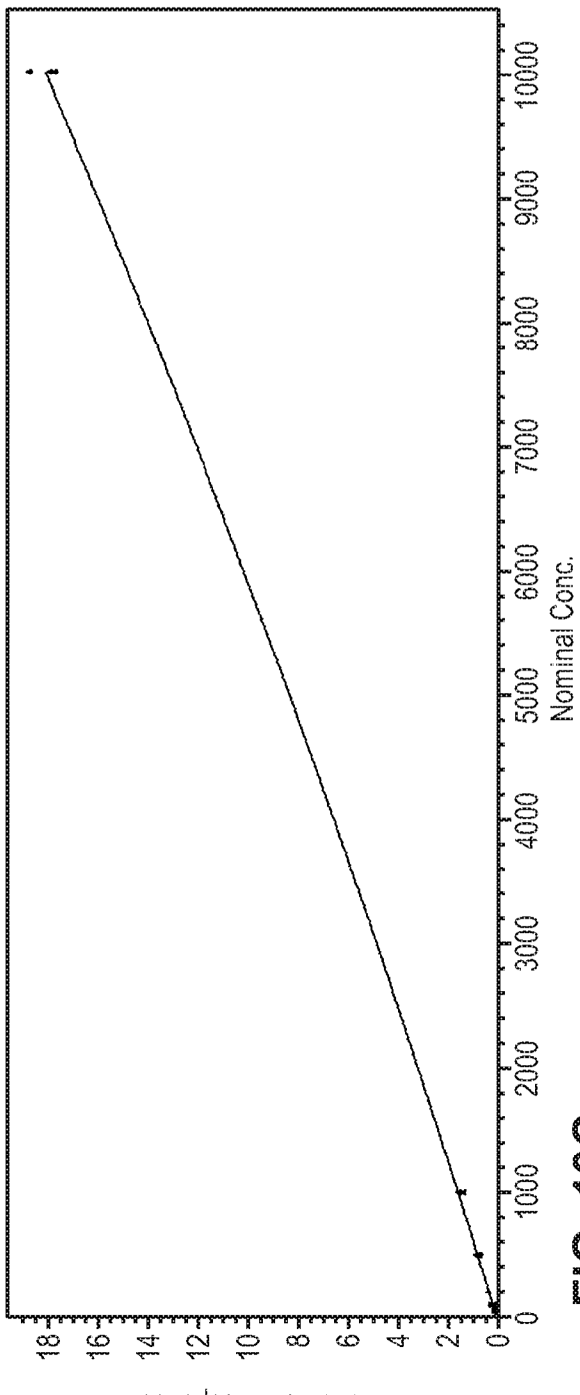
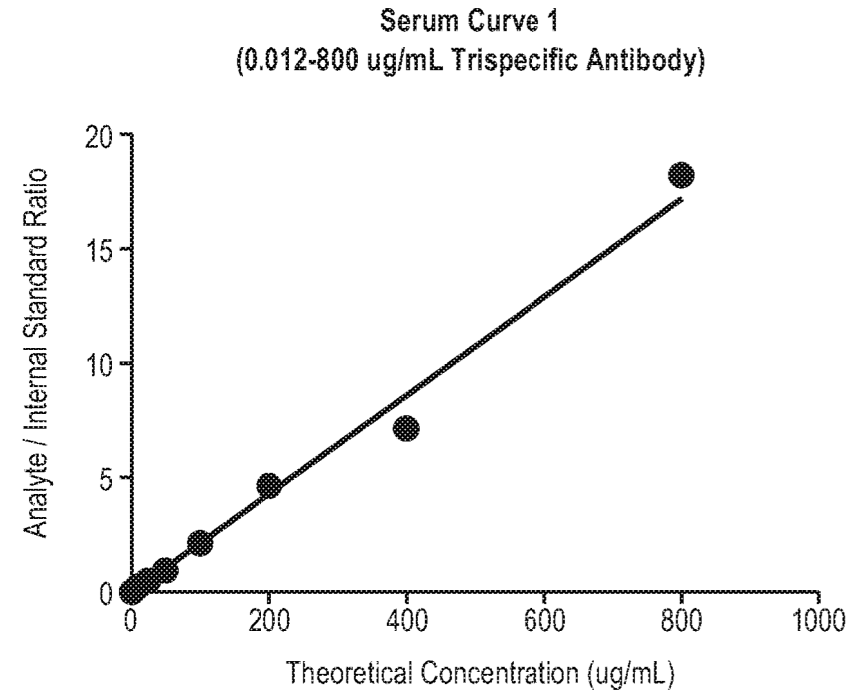
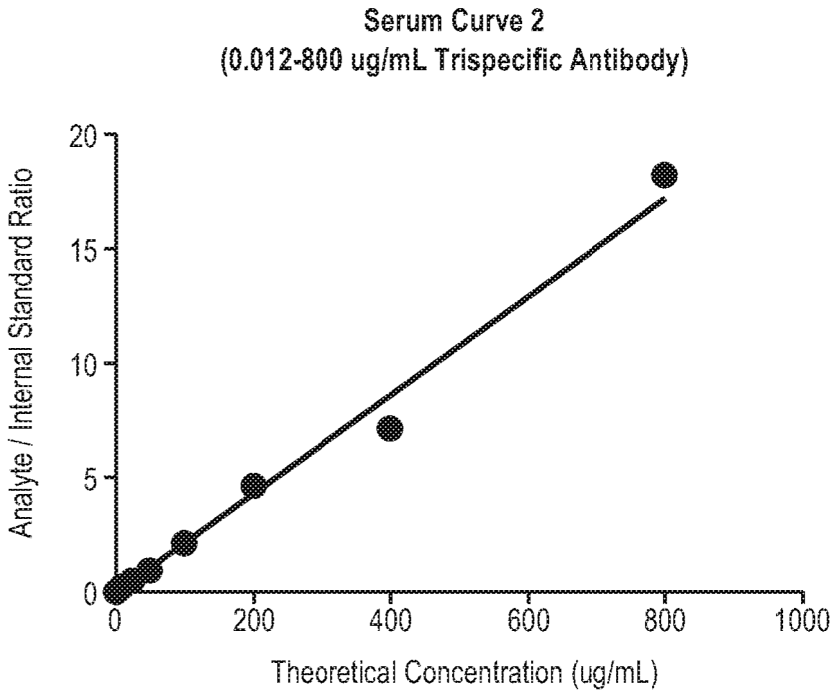


FIG. 19C



Y Intercept	0.001198
Slope	0.02149
R square (weighted)	0.9901

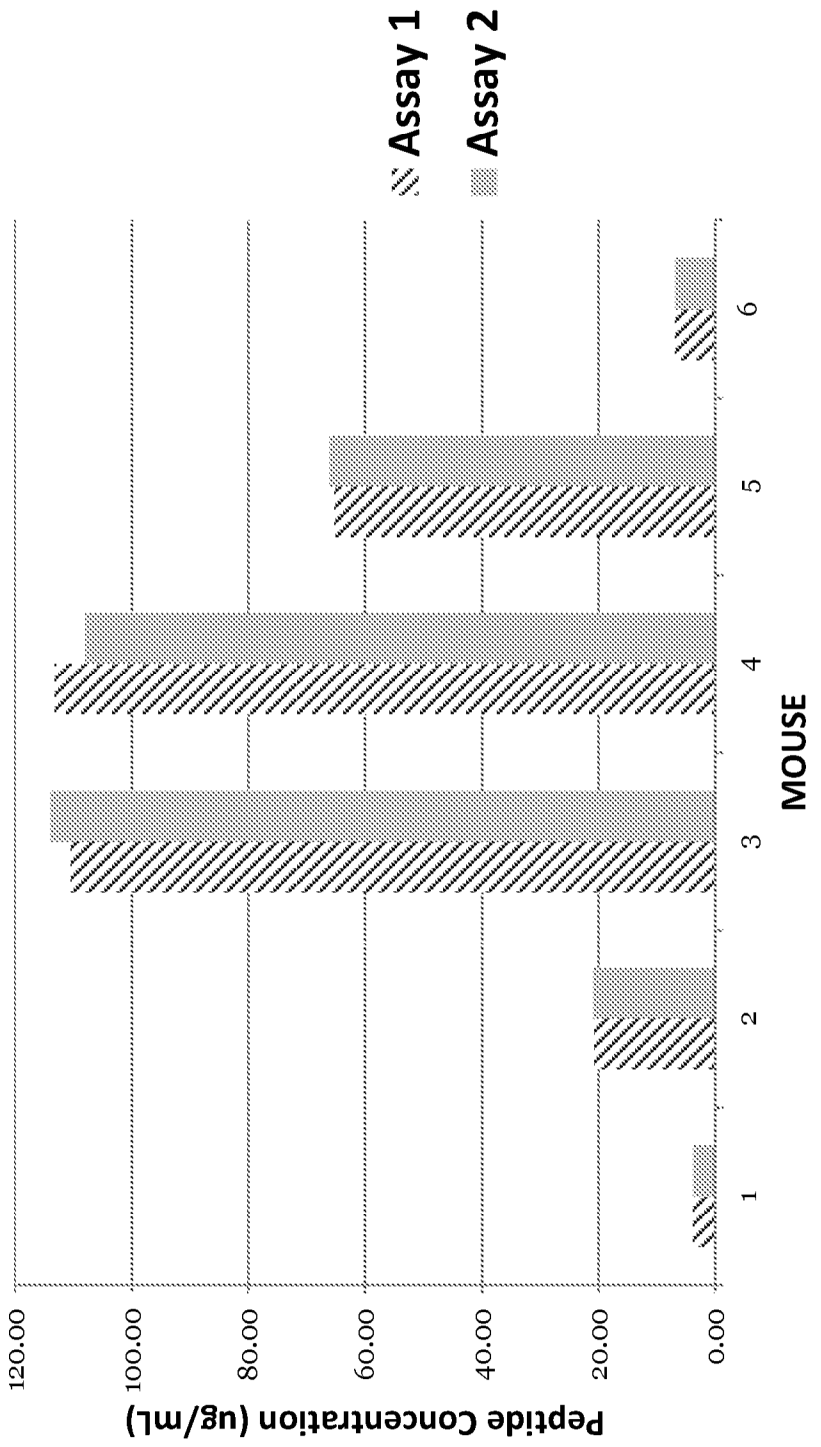
FIG. 20A



Y Intercept	0.0008343
Slope	0.02139
R square (weighted)	0.9899

FIG. 20B

FIG. 21A


LC-MS/MS Results
Assay 1
Assay 2
Peptide Concentration (ug/mL)
120.00
100.00
80.00
60.00
40.00
20.00
0.00
1
2
3
4
5
6
MOUSE

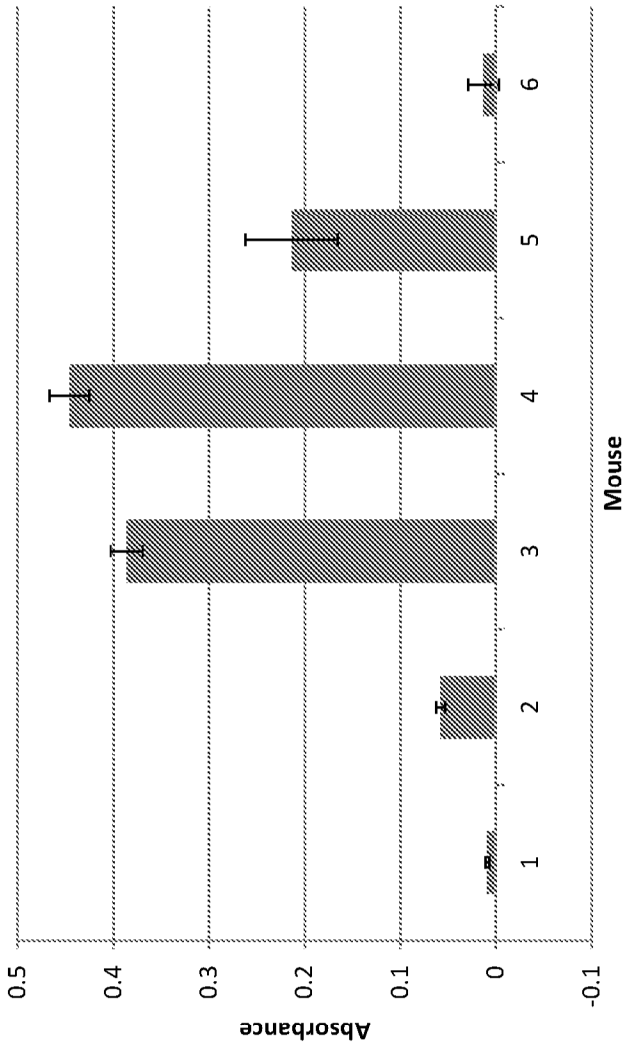


FIG. 21B

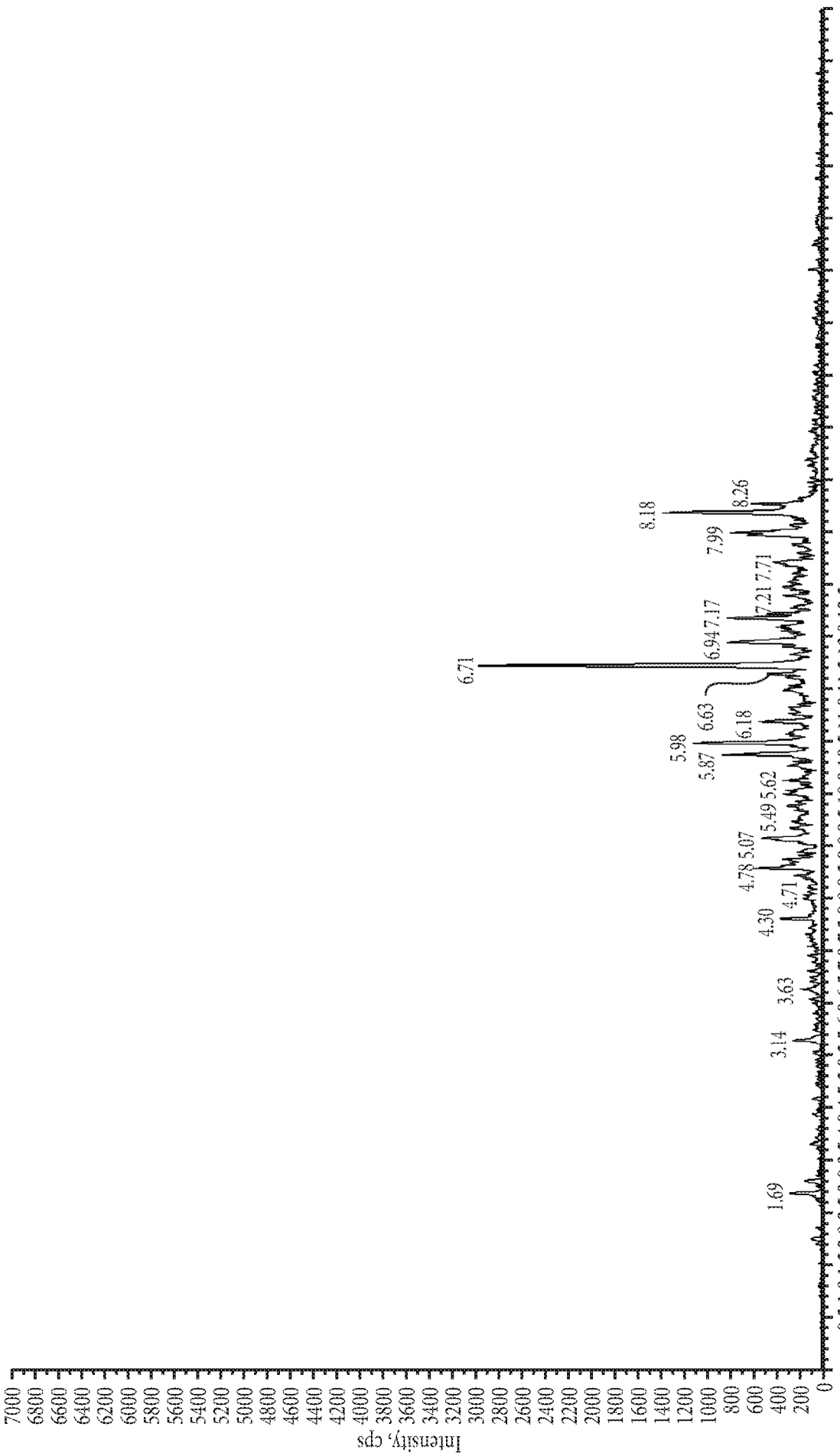

Intensity, cps
Time (min)
1.69
3.14
3.63
4.30
4.71
4.78
5.07
5.49
5.62
5.87
5.98
6.18
6.63
6.71
6.94
7.17
7.21
7.71
7.99
8.18
8.26

FIG. 22A

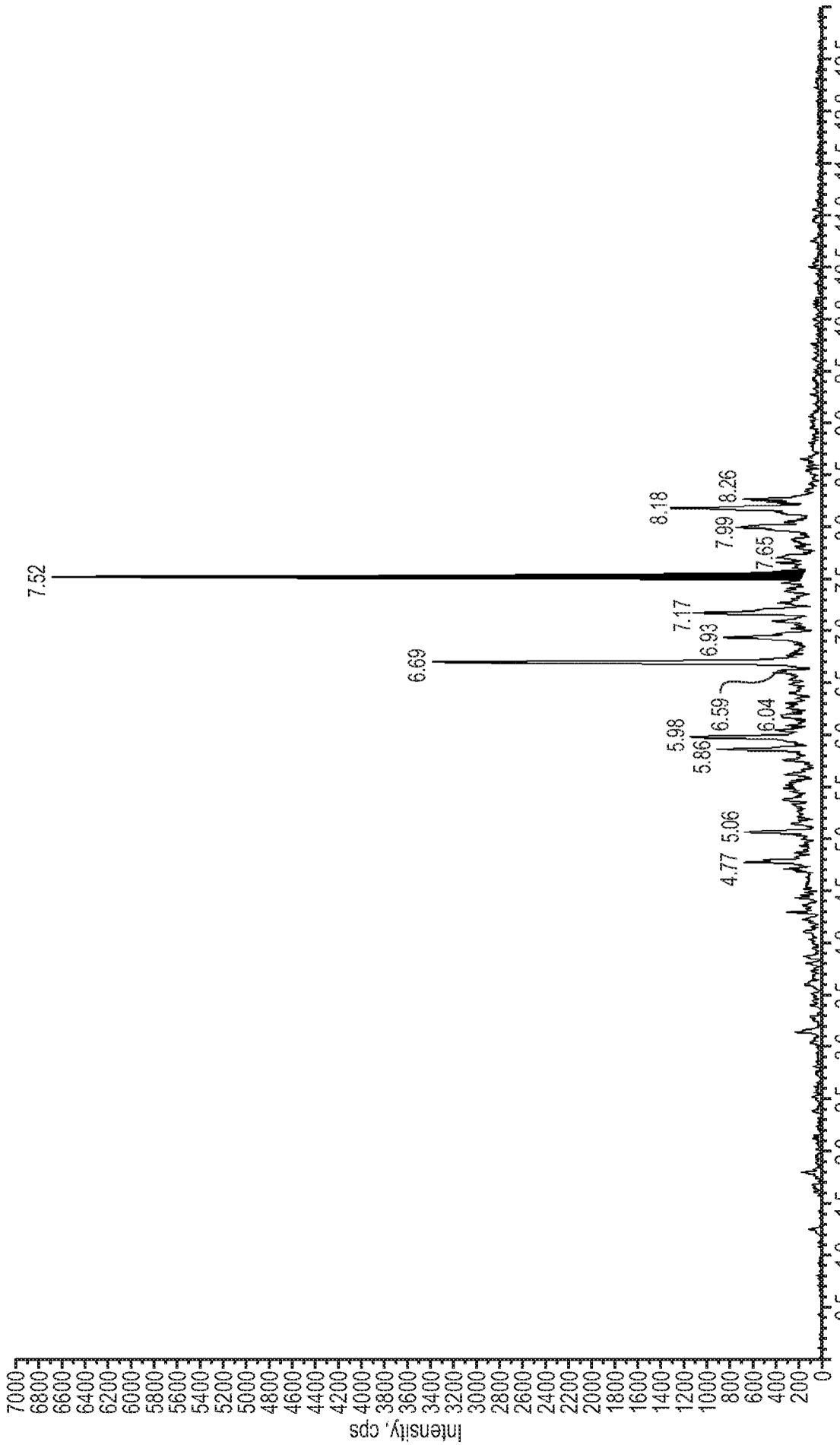

Intensity, cps
Time (min)
7.52
6.69
8.18
8.26
7.99
7.65
7.17
6.93
6.59
6.04
5.98
5.86
5.06
4.77

FIG. 22B

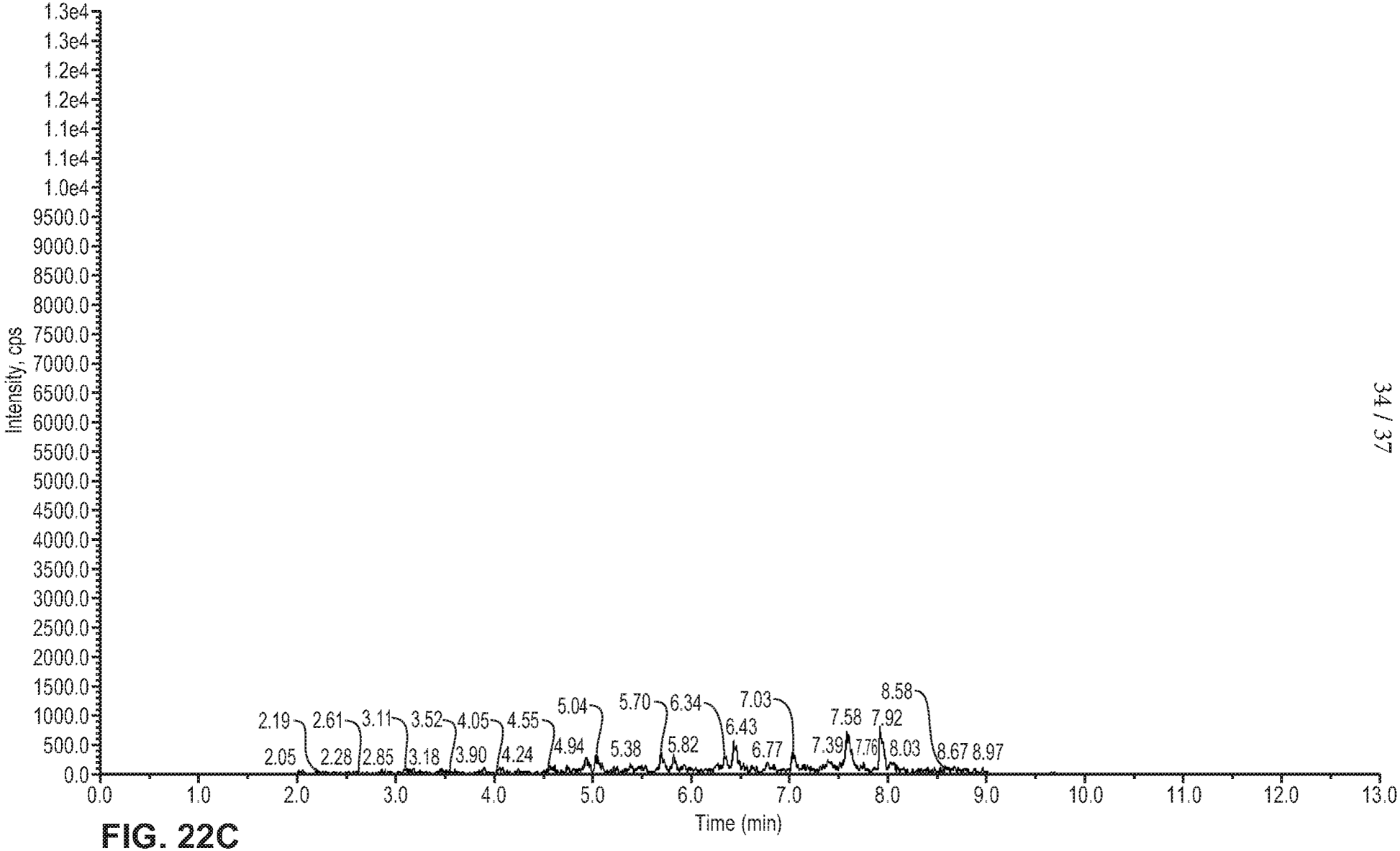

Intensity, cps
1.3e4
1.3e4
1.2e4
1.2e4
1.1e4
1.1e4
1.0e4
9500.0
9000.0
8500.0
8000.0
7500.0
7000.0
6500.0
6000.0
5500.0
5000.0
4500.0
4000.0
3500.0
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
2.19
2.05
2.61
2.28
3.11
2.85
3.52
3.18
4.05
3.90
4.55
4.24
5.04
4.94
5.70
5.38
6.34
5.82
6.43
7.03
6.77
7.39
7.58
7.76
7.92
8.03
8.58
8.67
8.97
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
Time (min)

FIG. 22C

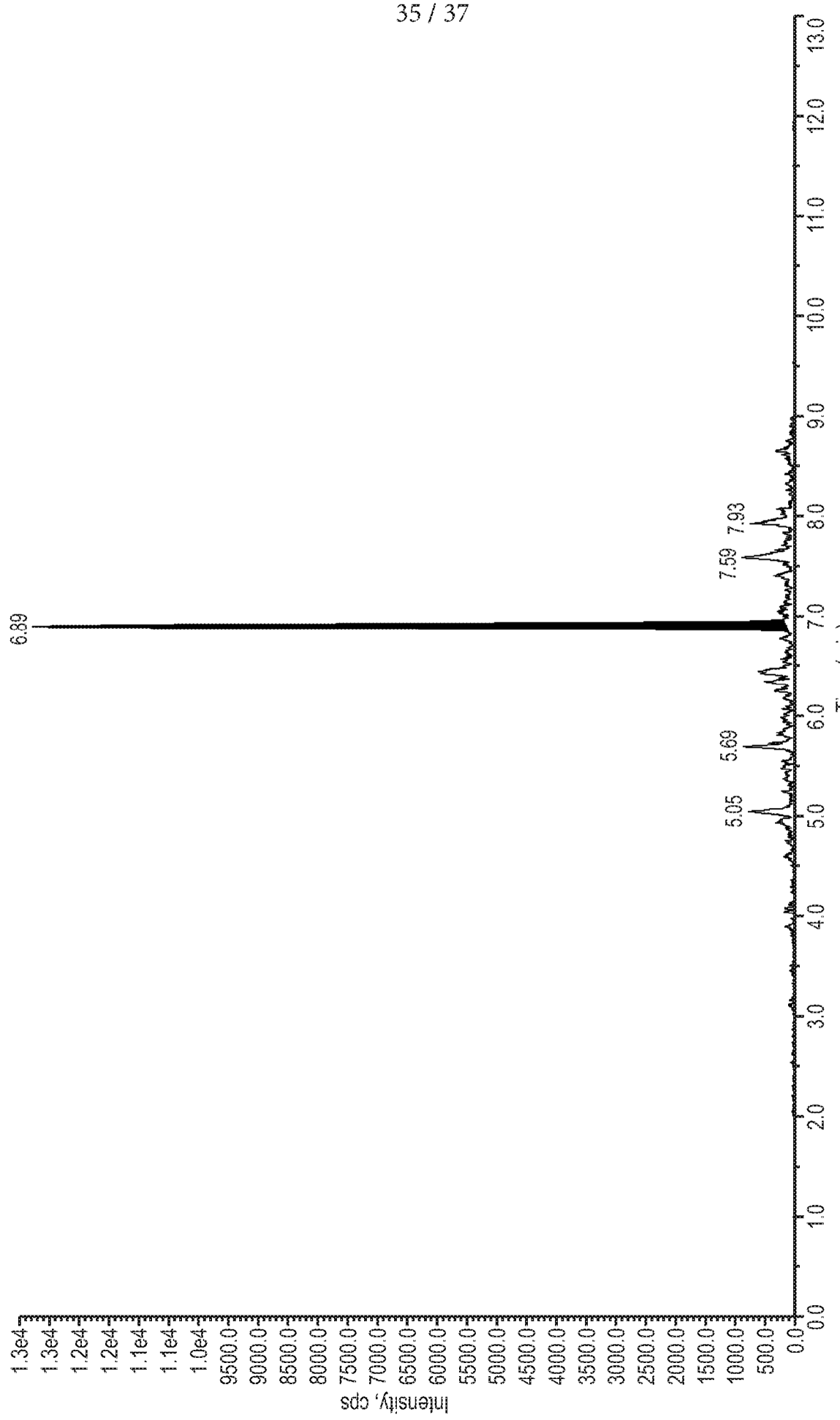

Intensity, cps
1.3e4
1.3e4
1.2e4
1.2e4
1.1e4
1.1e4
1.0e4
9500.0
9000.0
8500.0
8000.0
7500.0
7000.0
6500.0
6000.0
5500.0
5000.0
4500.0
4000.0
3500.0
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
Time (min)
6.89
5.05
5.69
7.59
7.93

FIG. 22D

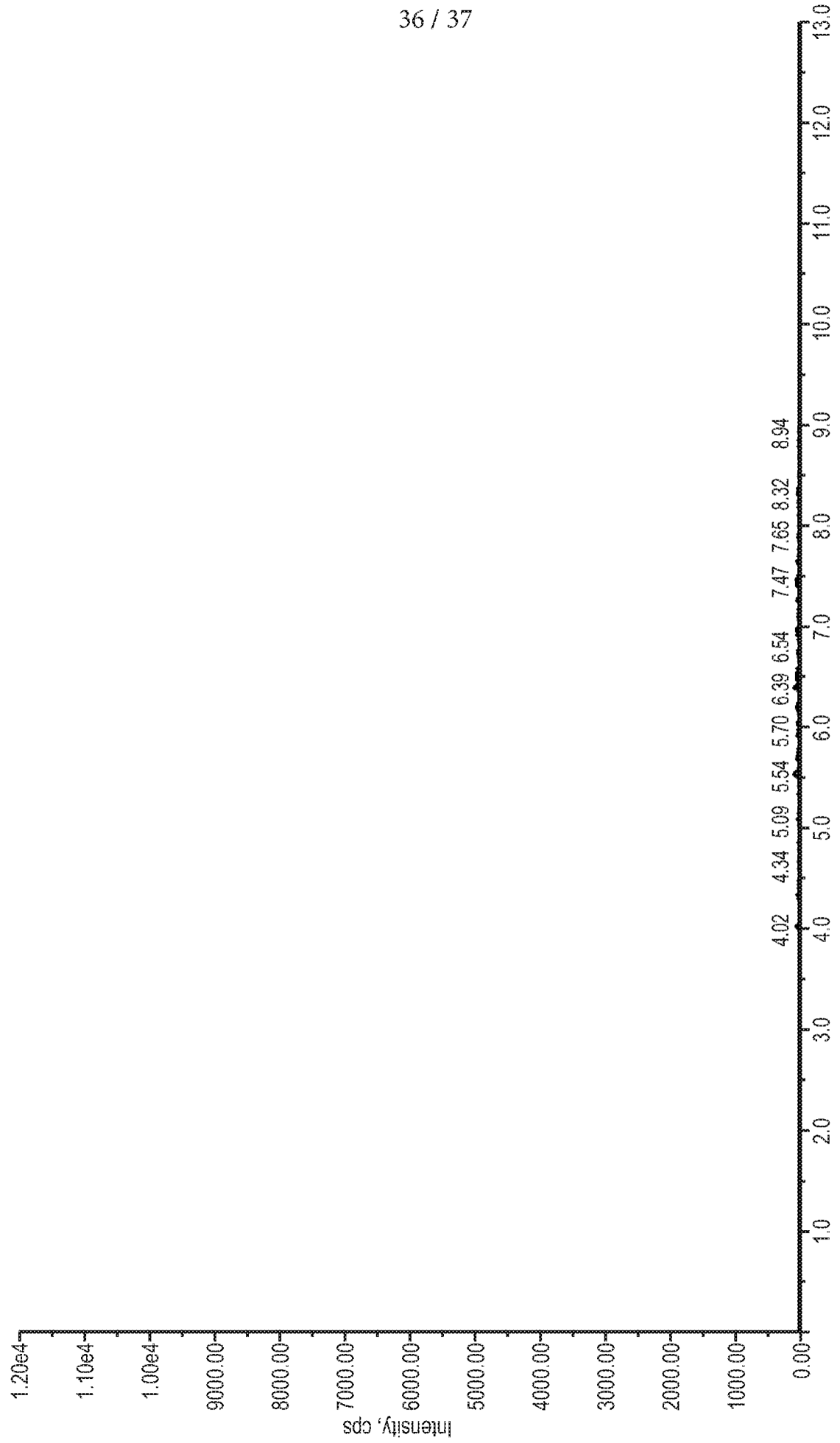

Intensity, cps
1.20e4
1.10e4
1.00e4
9000.00
8000.00
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
Time (min)
4.02
4.34
5.09
5.54
5.70
6.39
6.54
7.47
7.65
8.32
8.94

FIG. 22E

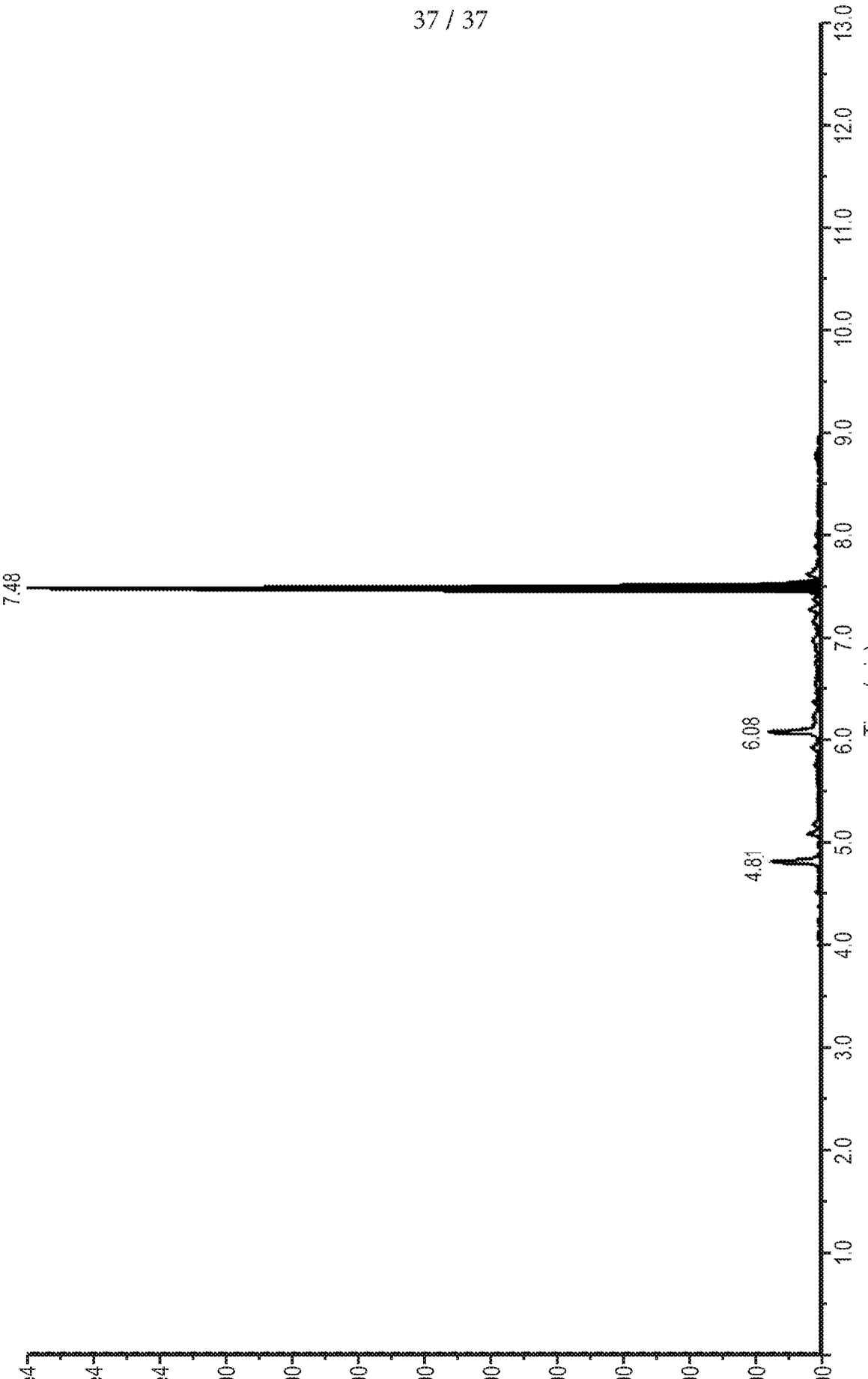

7.48
6.08
4.81
Intensity, cps
1.20e4
1.10e4
1.00e4
9000.00
8000.00
7000.00
6000.00
5000.00
4000.00
3000.00
2000.00
1000.00
0.00
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
Time (min)

FIG. 22F