FIG. 1A

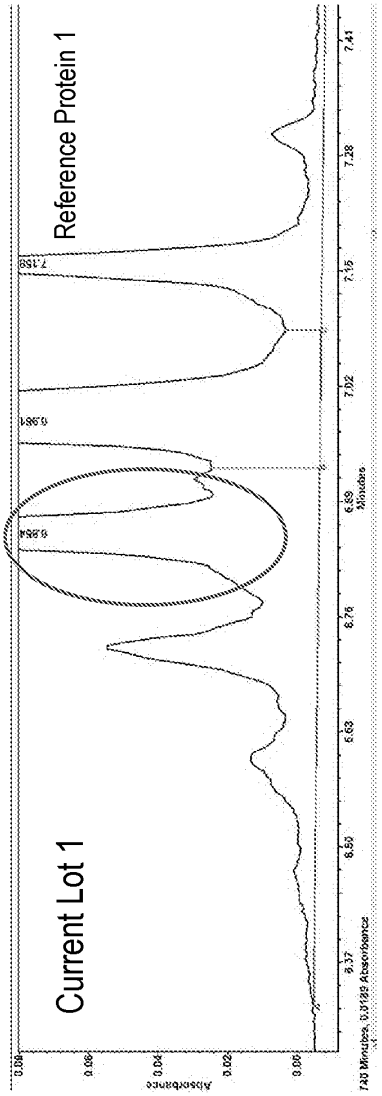
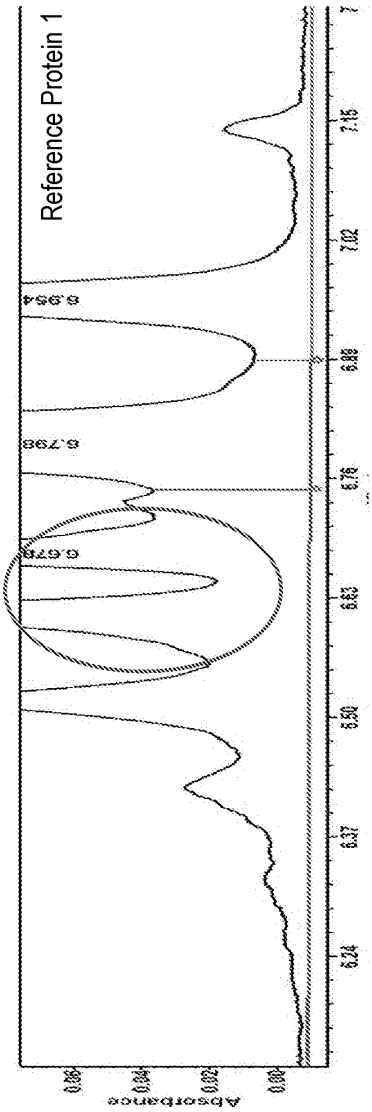
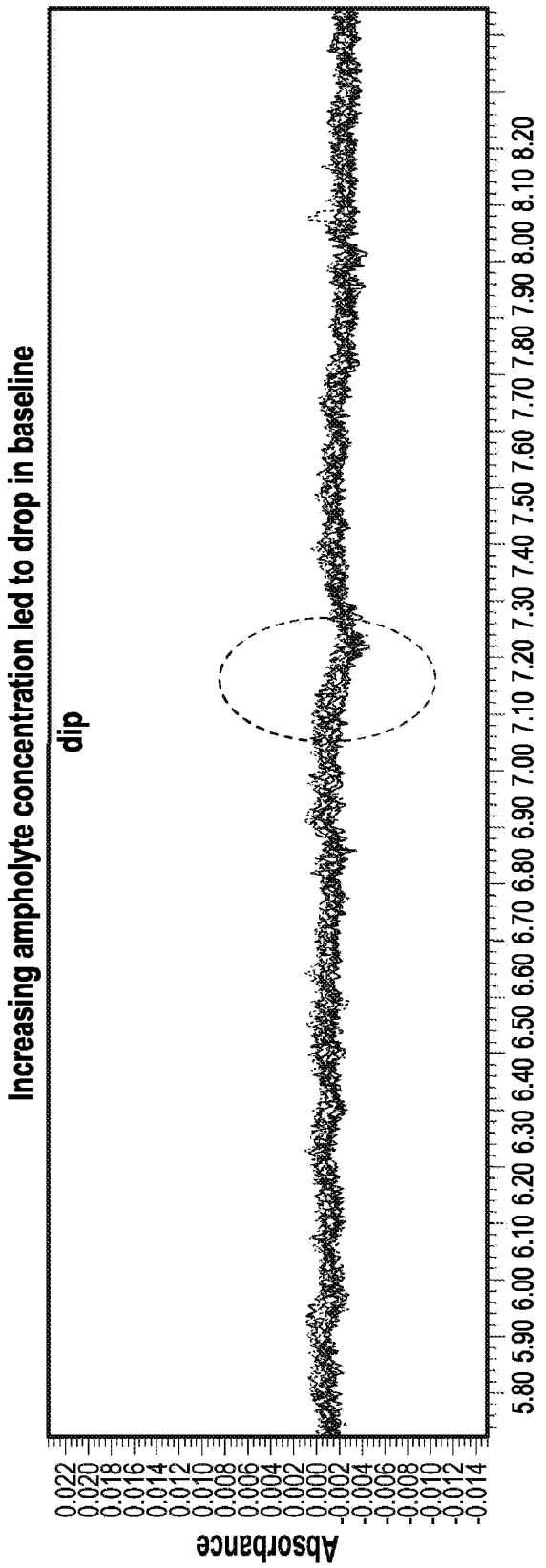

Reference Protein 1
Current Lot 1
Absorbance
Minutes

FIG. 1B


Reference Protein 1
Absorbance



Change in Peak Profile Due to Ampholytes not Proteins or Instrument

FIG. 2

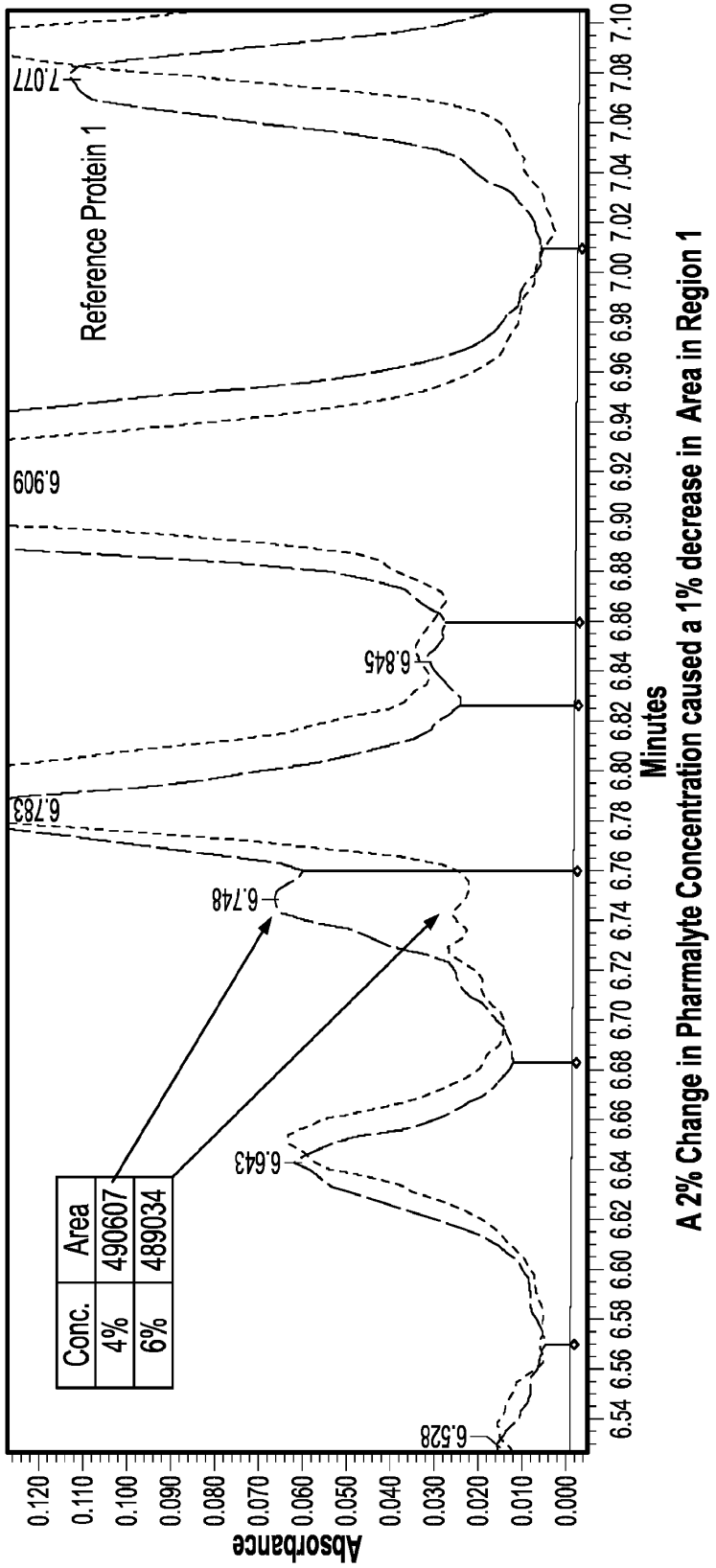

Reference Protein 1
7.077
6.909
6.845
6.783
6.748
6.643
6.528
Conc. | Area
4% | 490607
6% | 489034
Absorbance
Minutes
A 2% Change in Pharmalyte Concentration caused a 1% decrease in Area in Region 1

FIG. 3

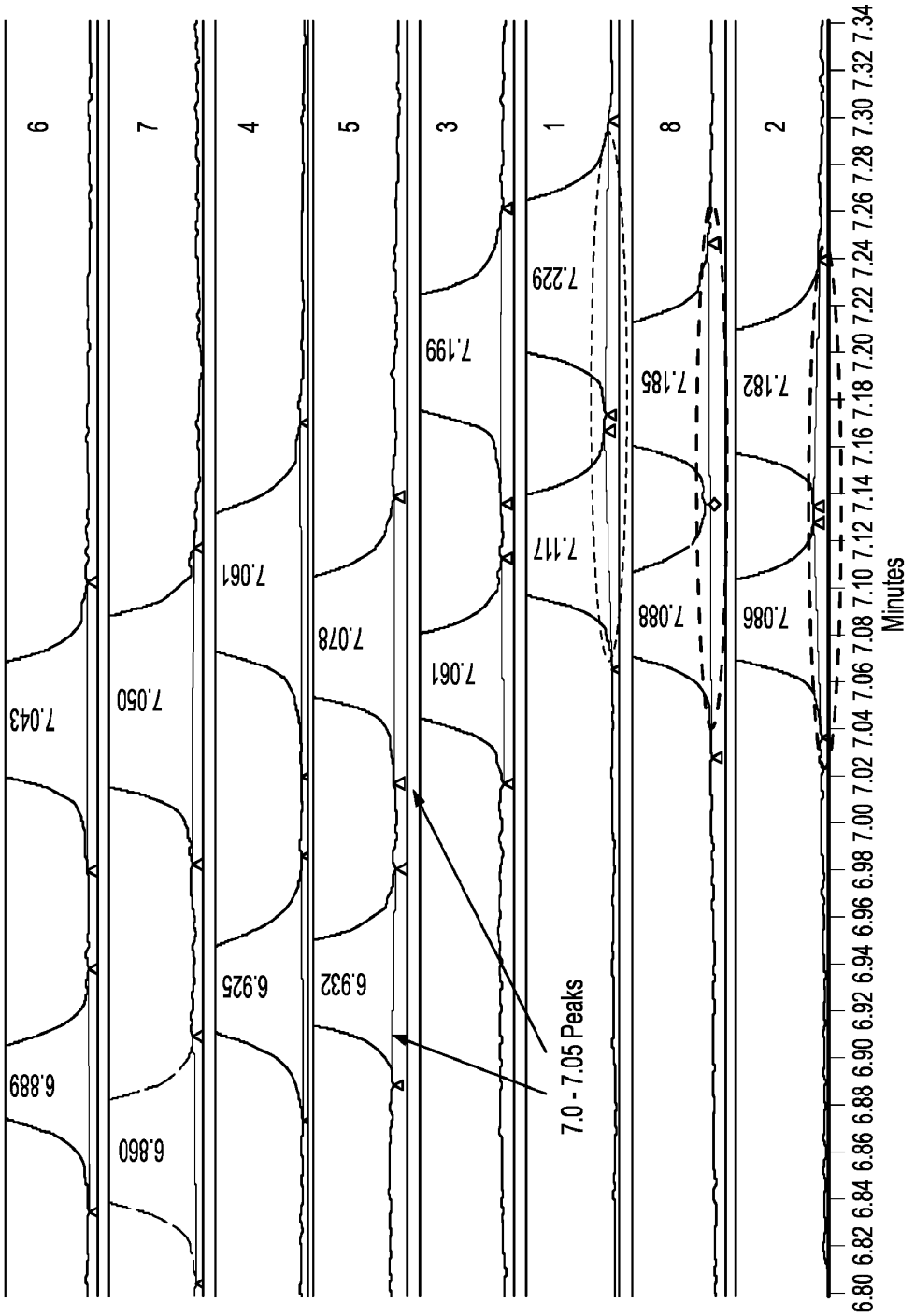

6
7
4
5
3
1
8
2
7.043
7.050
7.061
7.078
7.061
7.117
7.088
7.086
6.889
6.860
6.925
6.932
7.199
7.229
7.185
7.182
7.0 - 7.05 Peaks
6.80 6.82 6.84 6.86 6.88 6.90 6.92 6.94 6.96 6.98 7.00 7.02 7.04 7.06 7.08 7.10 7.12 7.14 7.16 7.18 7.20 7.22 7.24 7.26 7.28 7.30 7.32 7.34
Minutes

FIG. 4A

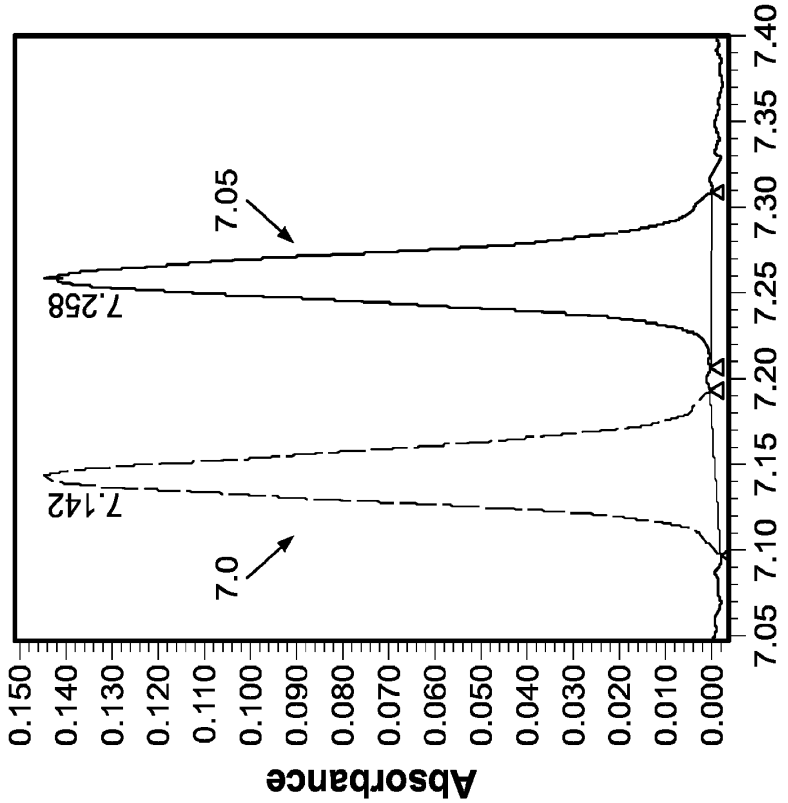


FIG. 5A

Ampholyte Lot	Resolution
6	2.808
7	2.729
4	2.530
5	2.651
3	2.069
1	***1.894***
8	***1.802***
2	1.731

FIG. 4B

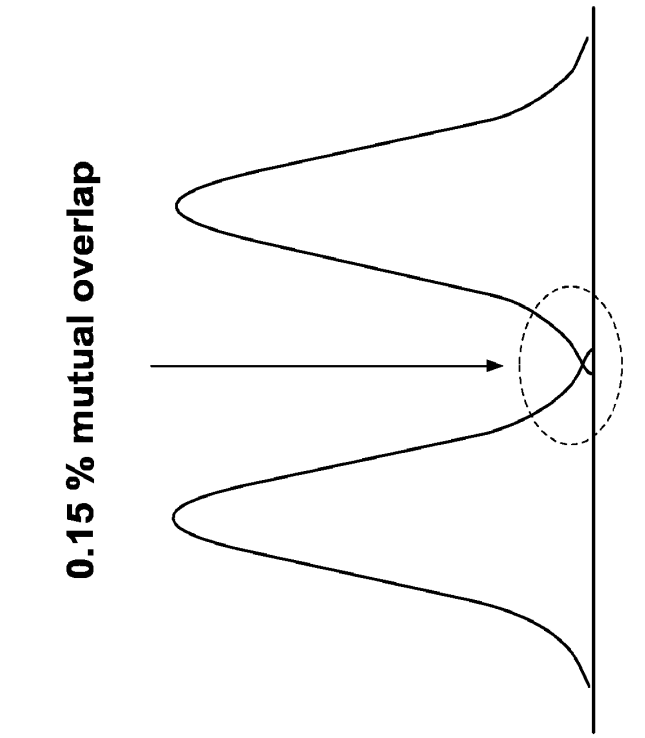

0.15 % mutual overlap
Resolution = 1.5
Chromatographic minimum criteria

FIG. 5C

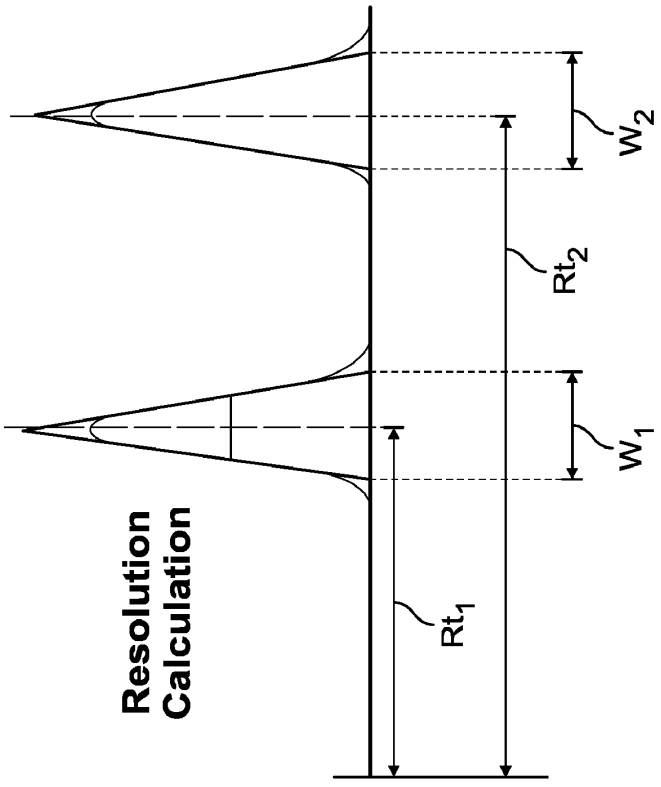

Resolution Calculation
Rt_1
Rt_2
W_1
W_2
$R = 2.0\ (Rt_2 - Rt_1)/(W_2+W_1)$

FIG. 5B

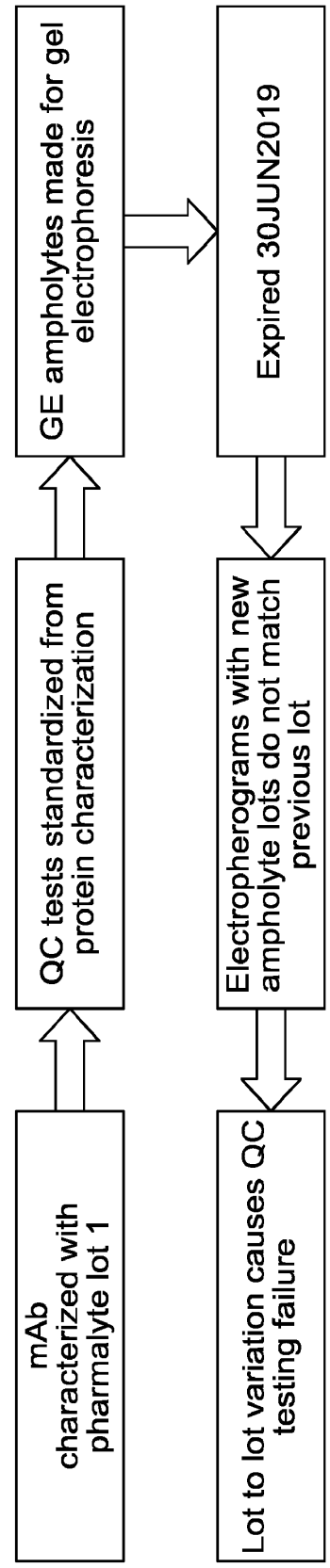
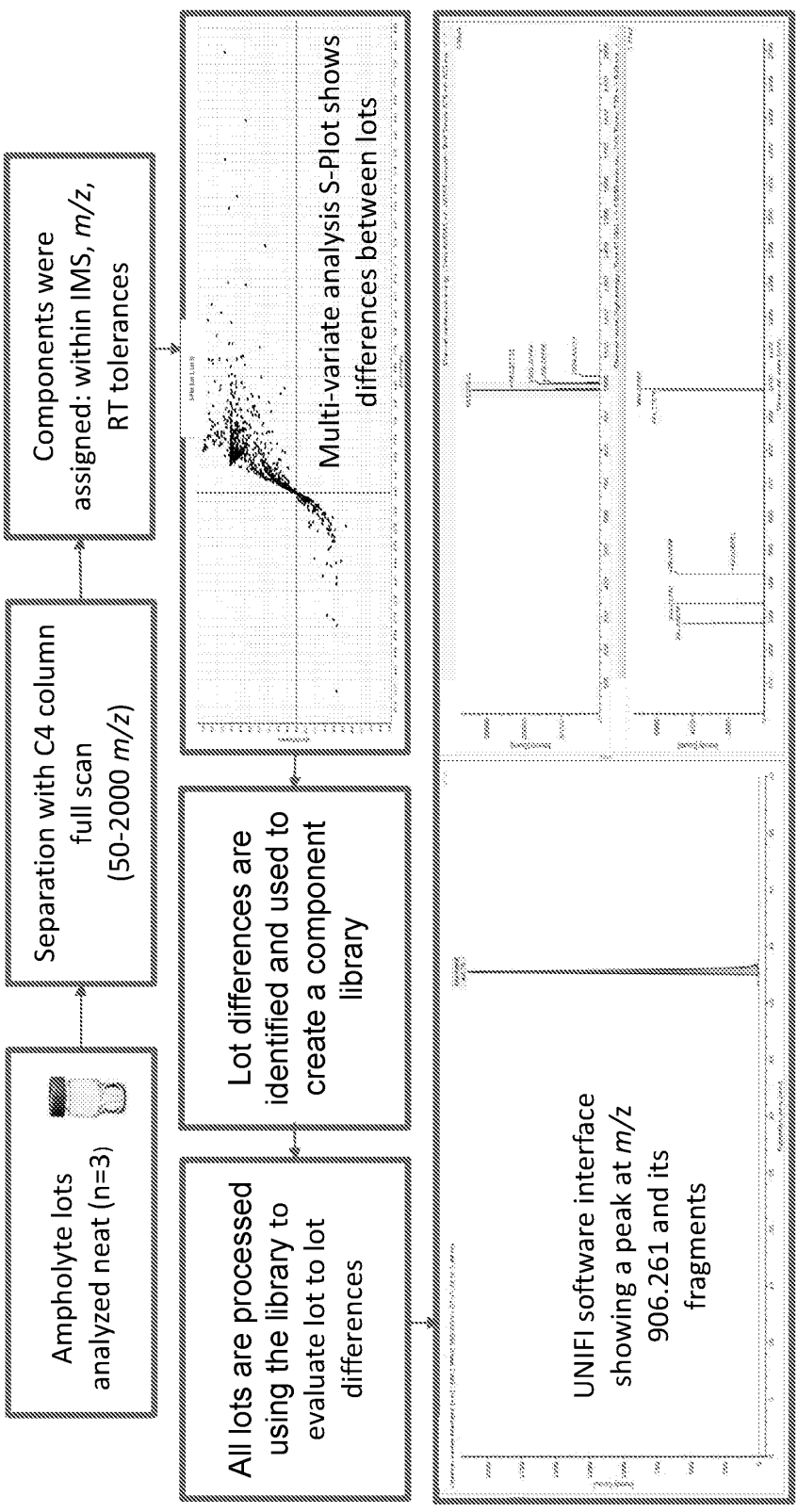

mAb characterized with pharmalyte lot 1
QC tests standardized from protein characterization
GE ampholytes made for gel electrophoresis
Expired 30JUN2019
Electropherograms with new ampholyte lots do not match previous lot
Lot to lot variation causes QC testing failure

FIG. 6

FIG. 7


Ampholyte lots analyzed neat (n=3)
Separation with C4 column full scan (50-2000 m/z)
Components were assigned: within IMS, m/z, RT tolerances
Multi-variate analysis S-Plot shows differences between lots
Lot differences are identified and used to create a component library
All lots are processed using the library to evaluate lot to lot differences
UNIFI software interface showing a peak at m/z 906.261 and its fragments

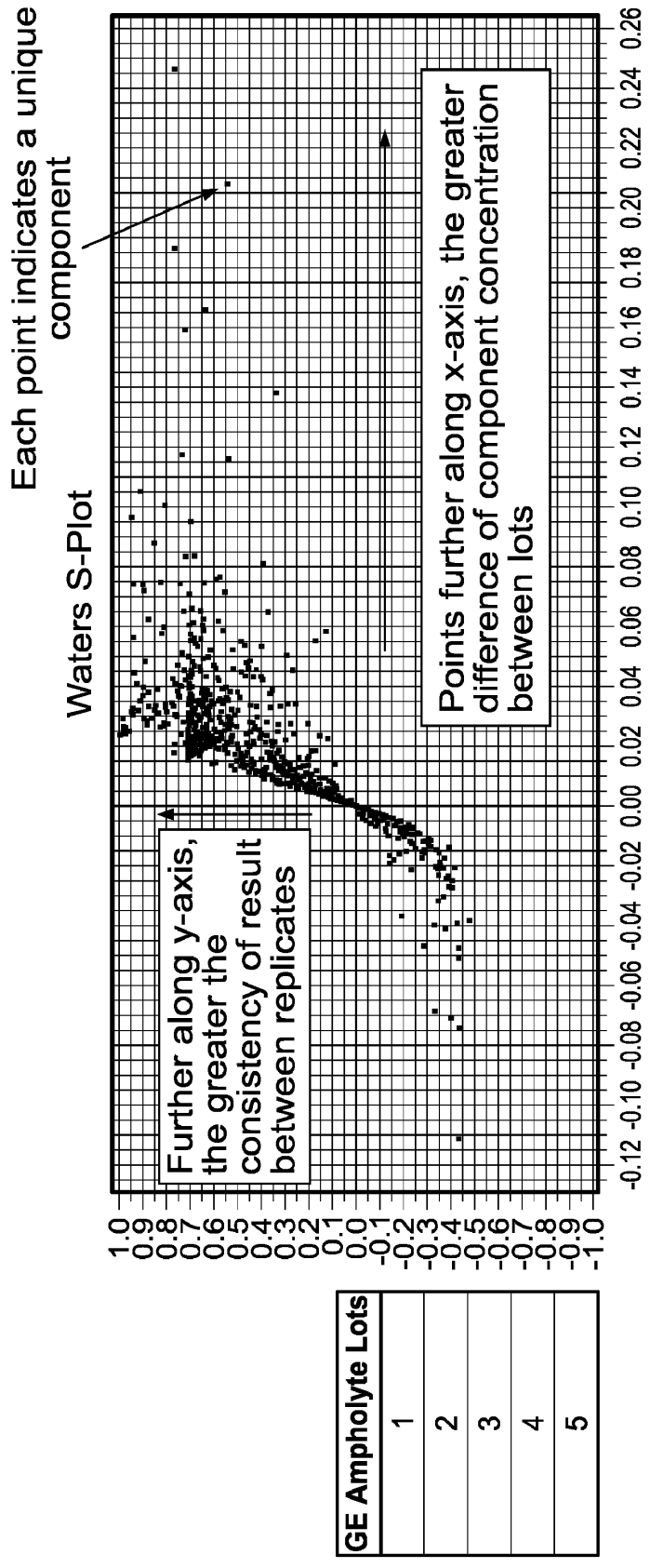

Each point indicates a unique component
Waters S-Plot
Further along y-axis, the greater the consistency of result between replicates
Points further along x-axis, the greater difference of component concentration between lots
1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0 -0.1 -0.2 -0.3 -0.4 -0.5 -0.6 -0.7 -0.8 -0.9 -1.0
-0.12 -0.10 -0.08 -0.06 -0.04 -0.02 0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18 0.20 0.22 0.24 0.26
GE Ampholyte Lots
1
2
3
4
5

FIG. 8B

FIG. 8A

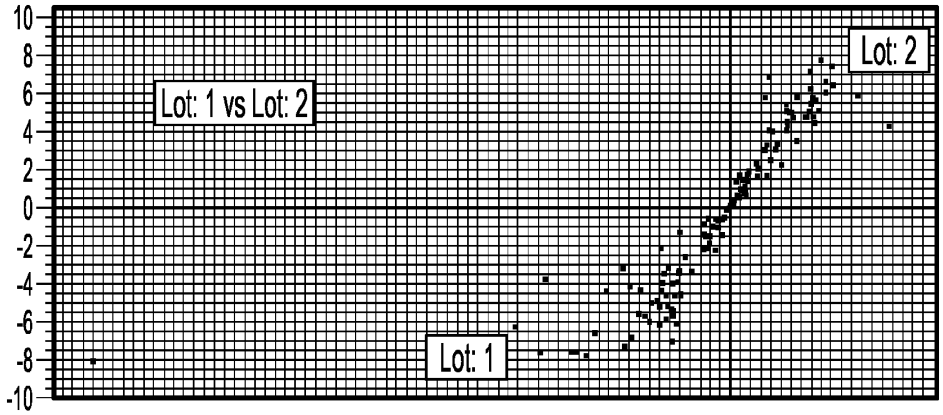


FIG. 9A

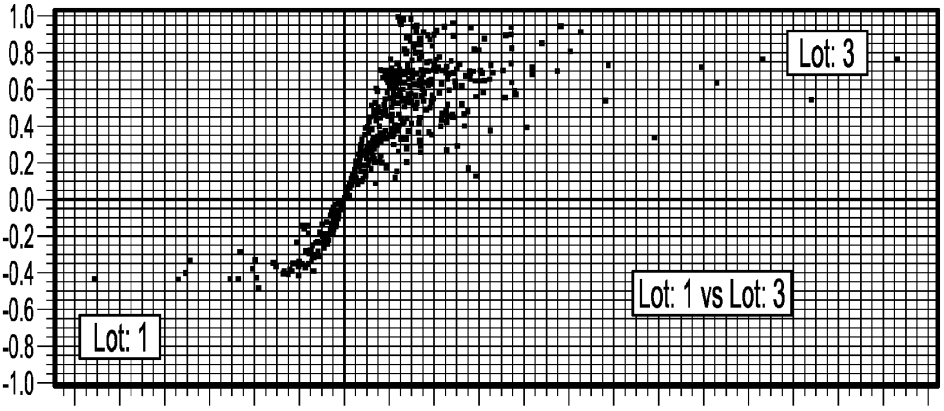


FIG. 9B

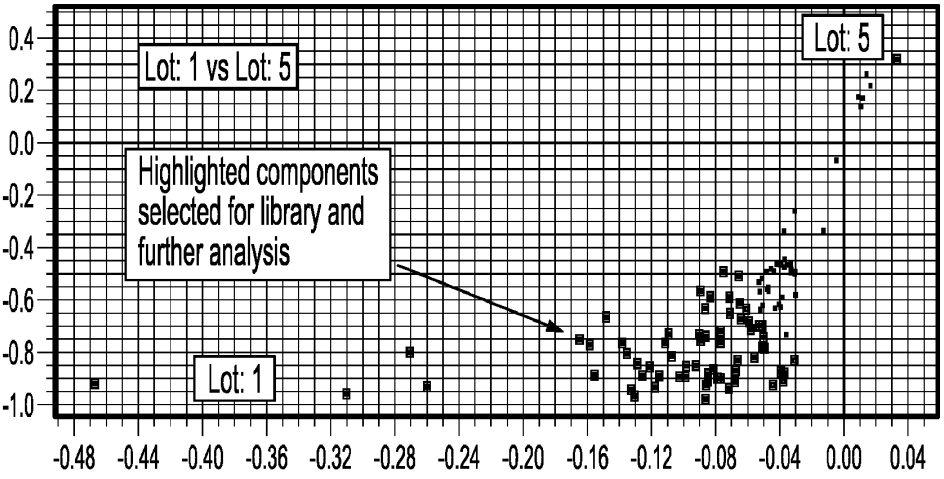


FIG. 9C

m/z value	Lot Number				
	1	2	3	4	5
280	100%	65%	35%	16%	24%
319	94%	100%	50%	59%	61%
329	100%	47%	40%	46%	43%
347	100%	67%	50%	31%	25%
373	90%	100%	85%	94%	60%
375	100%	85%	43%	41%	38%
376	100%	92%	50%	42%	25%
431	100%	65%	65%	53%	7%
504	100%	87%	73%	100%	67%
506	100%	100%	71%	85%	71%
508	68%	68%	62%	100%	49%
520	100%	80%	49%	54%	58%
534	100%	100%	59%	50%	40%
562	100%	79%	46%	40%	34%
906	24%	30%	28%	100%	18%
980	67%	94%	100%	33%	53%

FIG. 10A

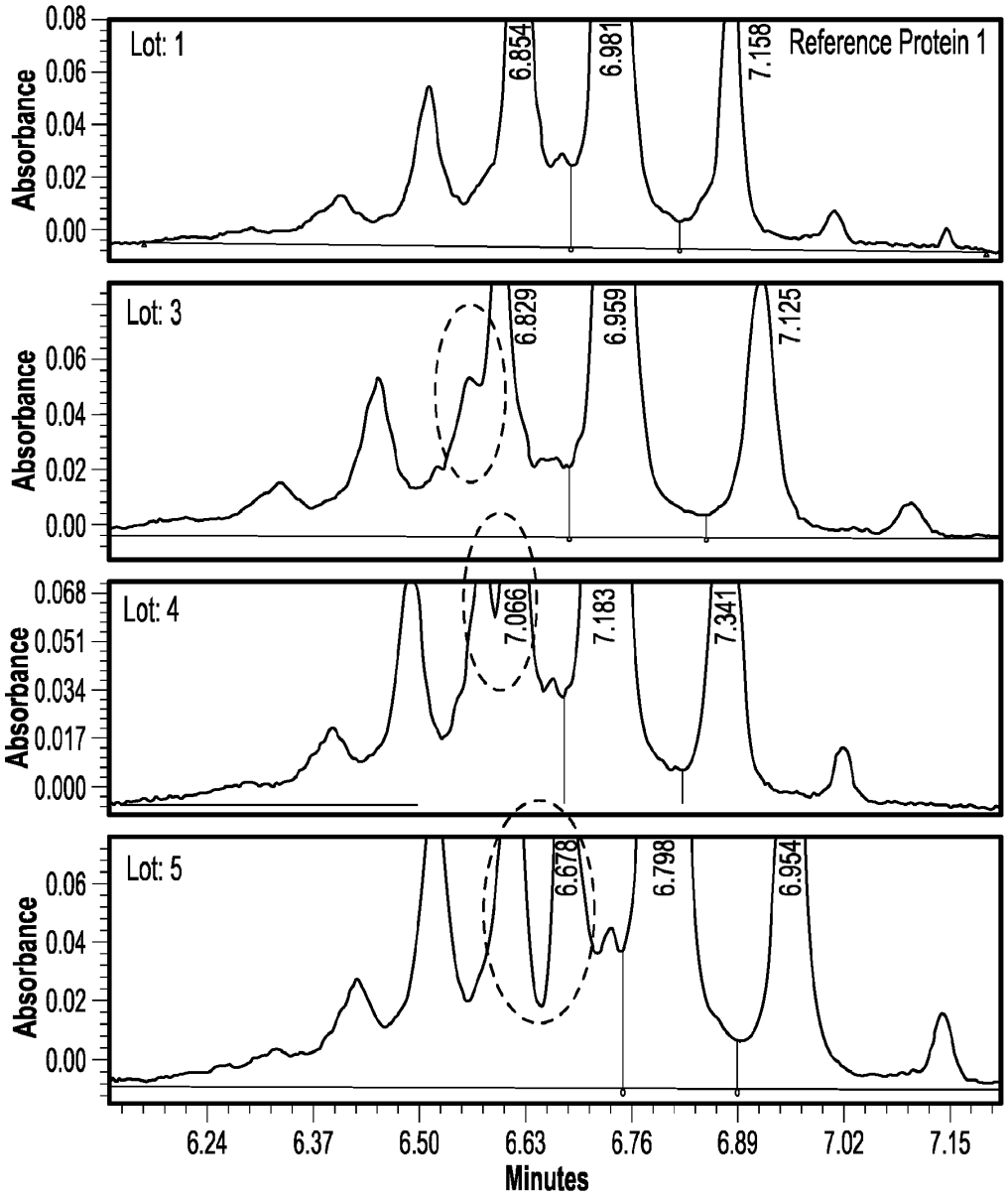

Lot: 1
Reference Protein 1
6.854
6.981
7.158
Lot: 3
6.829
6.959
7.125
Lot: 4
7.066
7.183
7.341
Lot: 5
6.678
6.798
6.954
Absorbance
0.08
0.06
0.04
0.02
0.00
0.068
0.051
0.034
0.017
0.000
6.24
6.37
6.50
6.63
6.76
6.89
7.02
7.15
Minutes

FIG. 10B

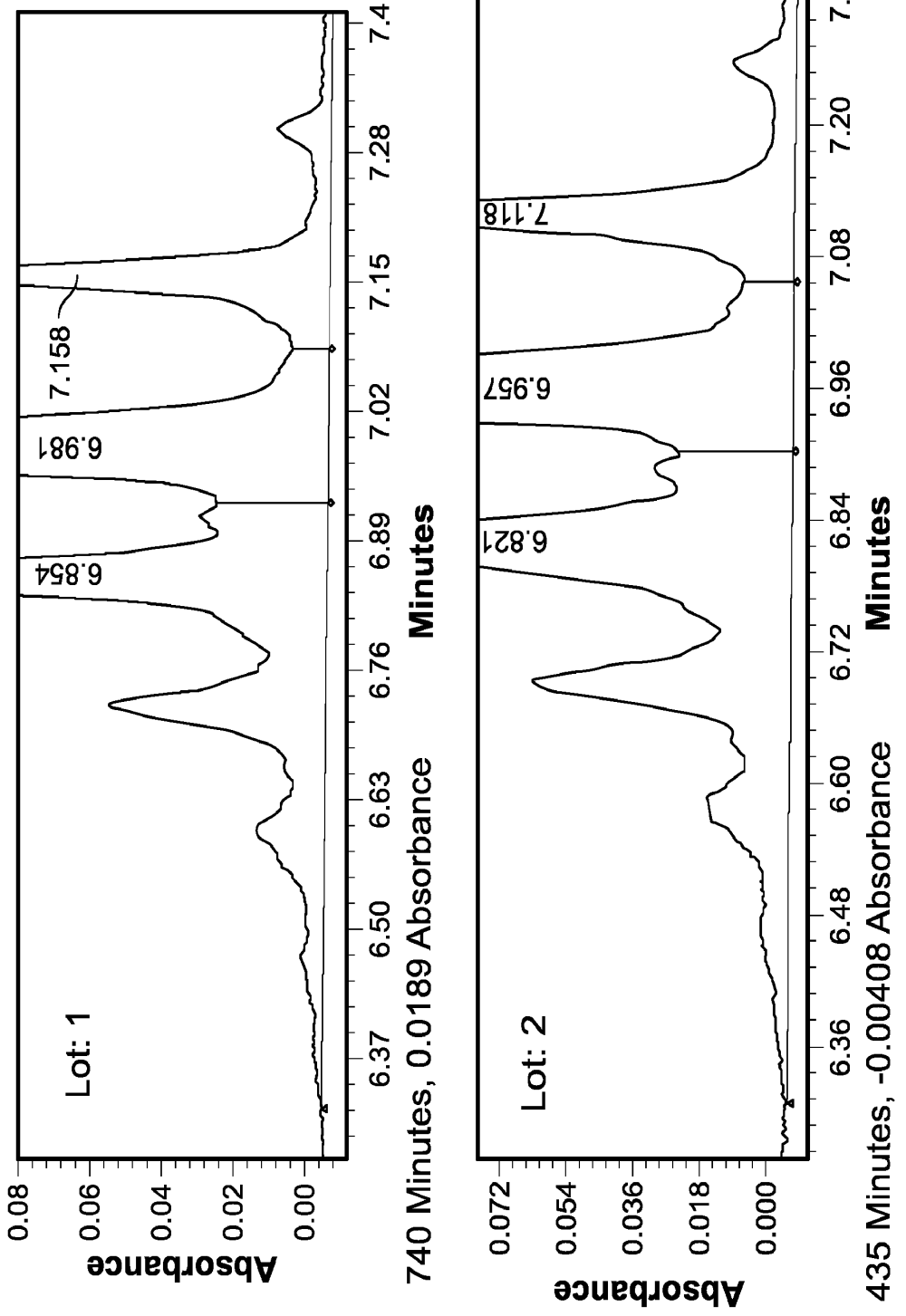

Lot: 1
Absorbance
0.08
0.06
0.04
0.02
0.00
6.854
6.981
7.158
6.37
6.50
6.63
6.76
6.89
7.02
7.15
7.28
7.4
740 Minutes, 0.0189 Absorbance
Minutes
Lot: 2
Absorbance
0.072
0.054
0.036
0.018
0.000
6.821
6.957
7.118
6.36
6.48
6.60
6.72
6.84
6.96
7.08
7.20
7.32
435 Minutes, -0.00408 Absorbance
Minutes

FIG. 10C

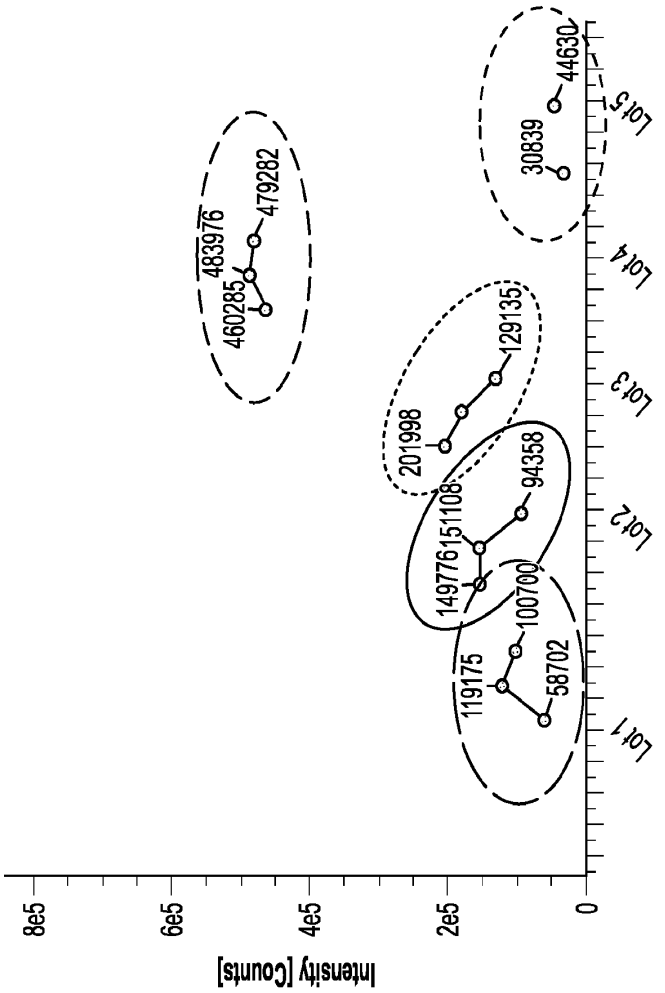


FIG. 11B

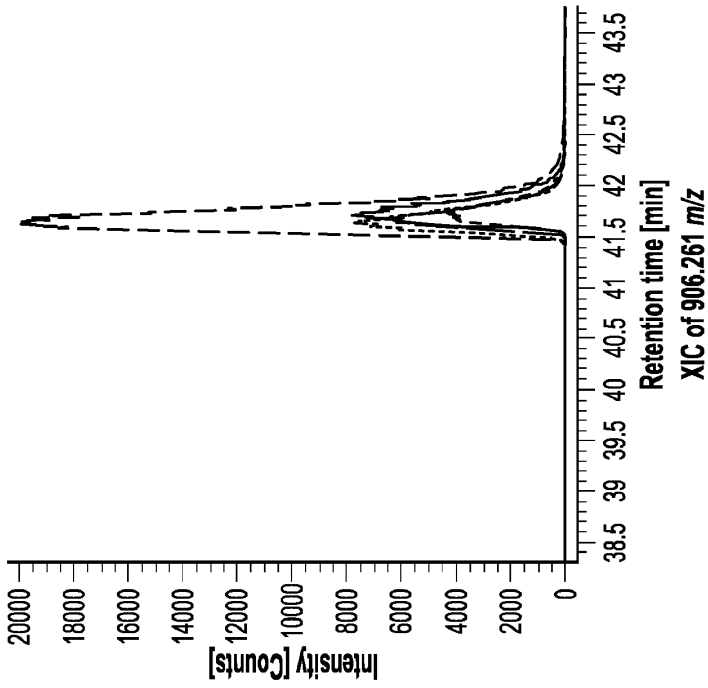


FIG. 11A

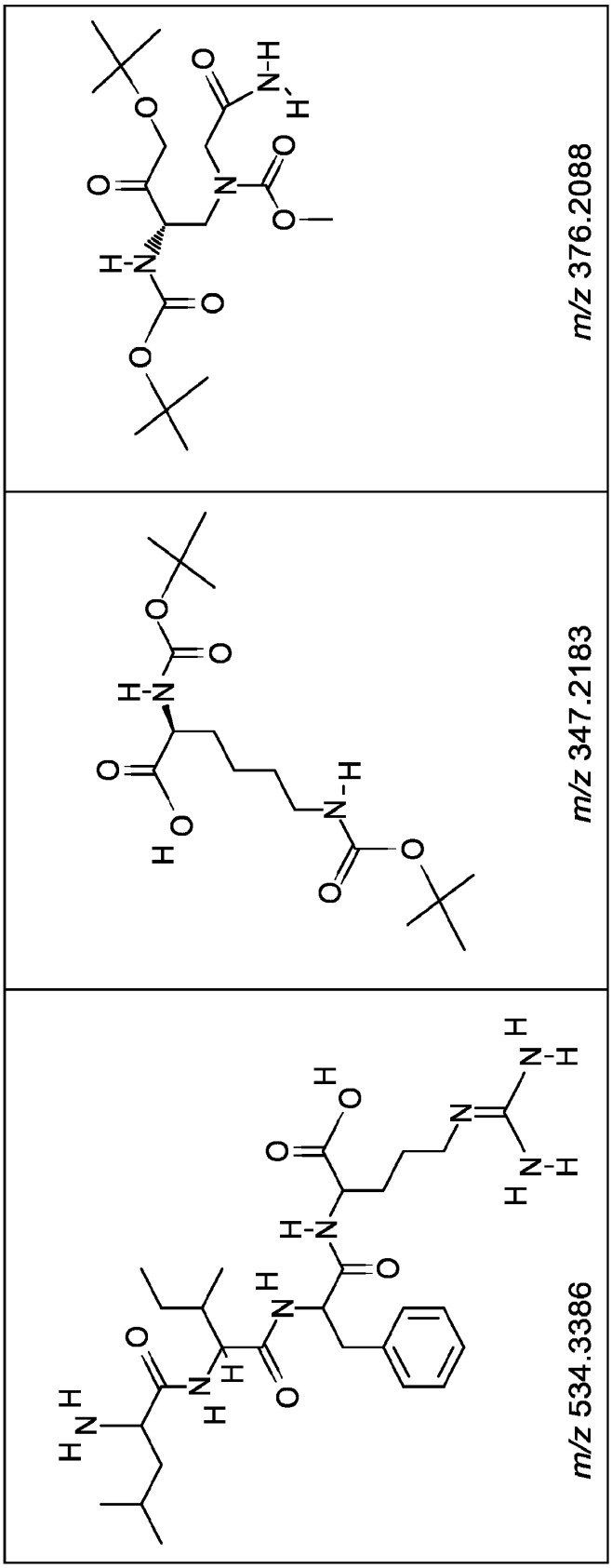


FIG. 12

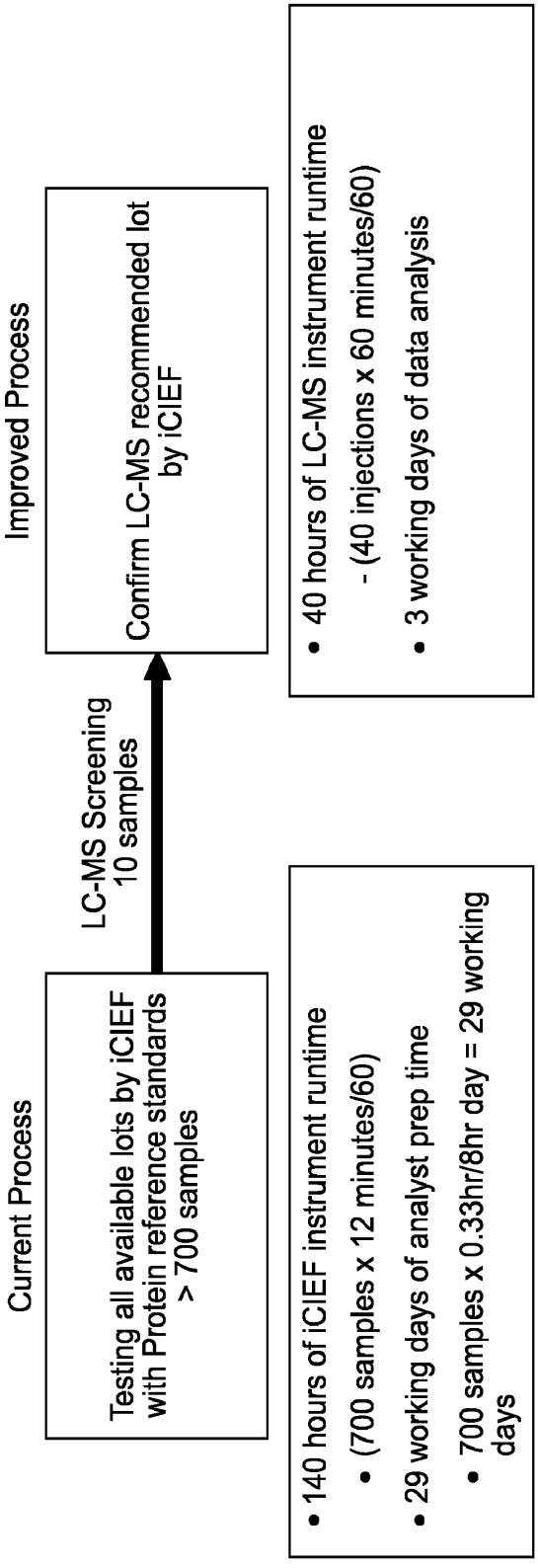

Current Process
Testing all available lots by iCIEF with Protein reference standards > 700 samples
LC-MS Screening 10 samples
Improved Process
Confirm LC-MS recommended lot by iCIEF
• 140 hours of iCIEF instrument runtime
• (700 samples x 12 minutes/60)
• 29 working days of analyst prep time
• 700 samples x 0.33hr/8hr day = 29 working days
• 40 hours of LC-MS instrument runtime
- (40 injections x 60 minutes/60)
• 3 working days of data analysis

FIG. 13