FIG. 1

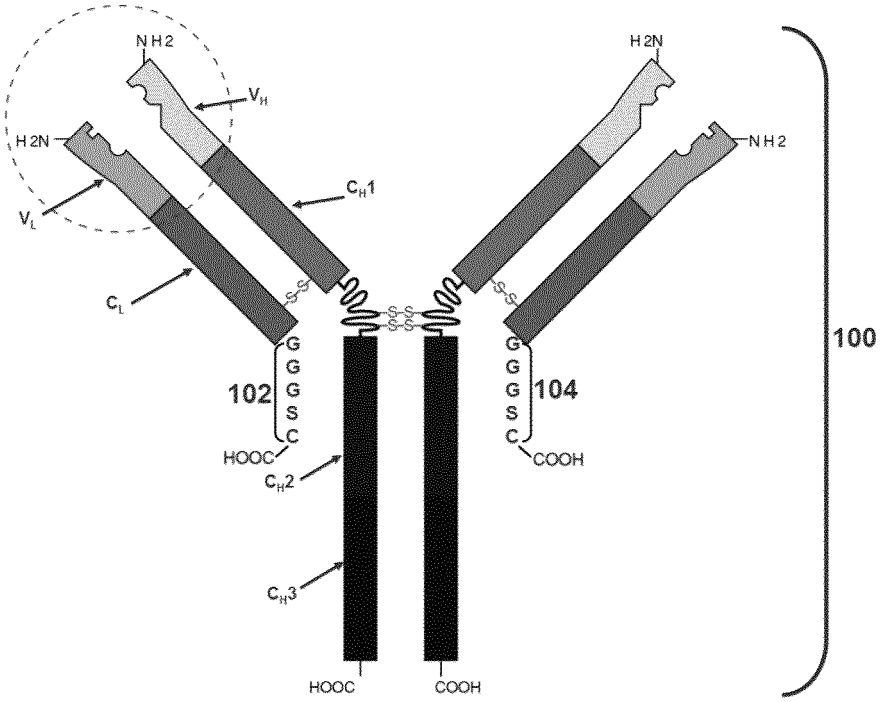

NH2
VH
H2N
VL
CH1
CL
G
G
G
S
C
102
HOOC
CH2
CH3
HOOC
COOH
H2N
NH2
G
G
G
S
C
104
COOH
100

FIG. 2

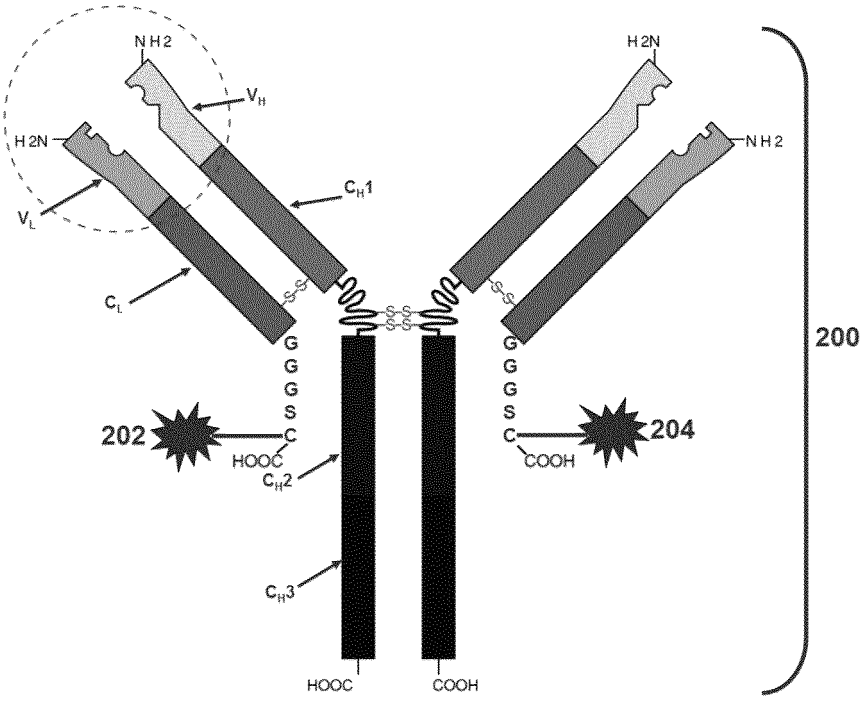

NH2
VH
H2N
VL
CH1
CL
G
G
G
S
C
202
HOOC
CH2
CH3
HOOC
COOH
H2N
NH2
G
G
G
S
C
204
COOH
200

FIG. 3

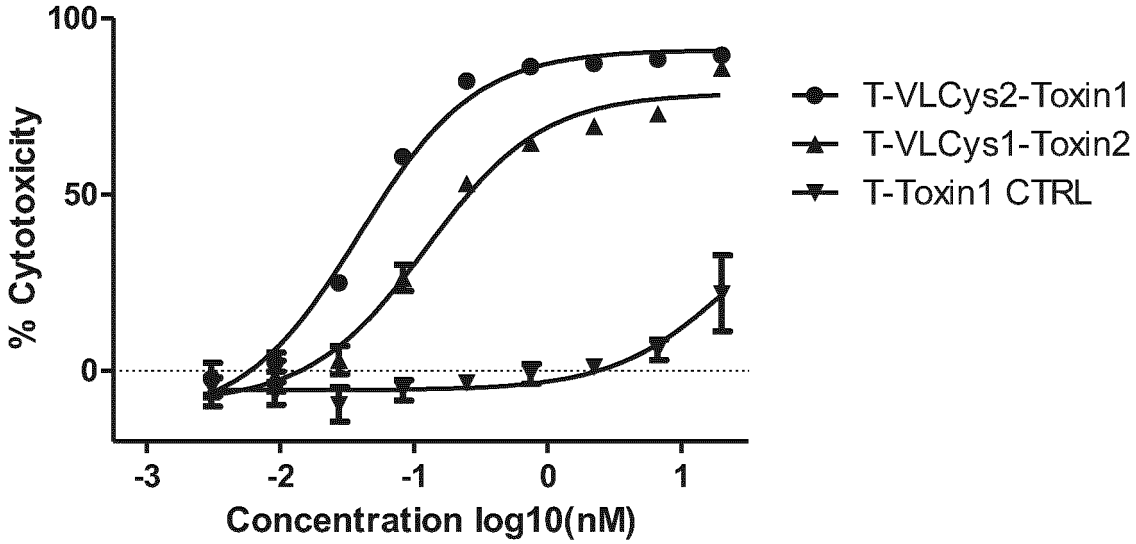
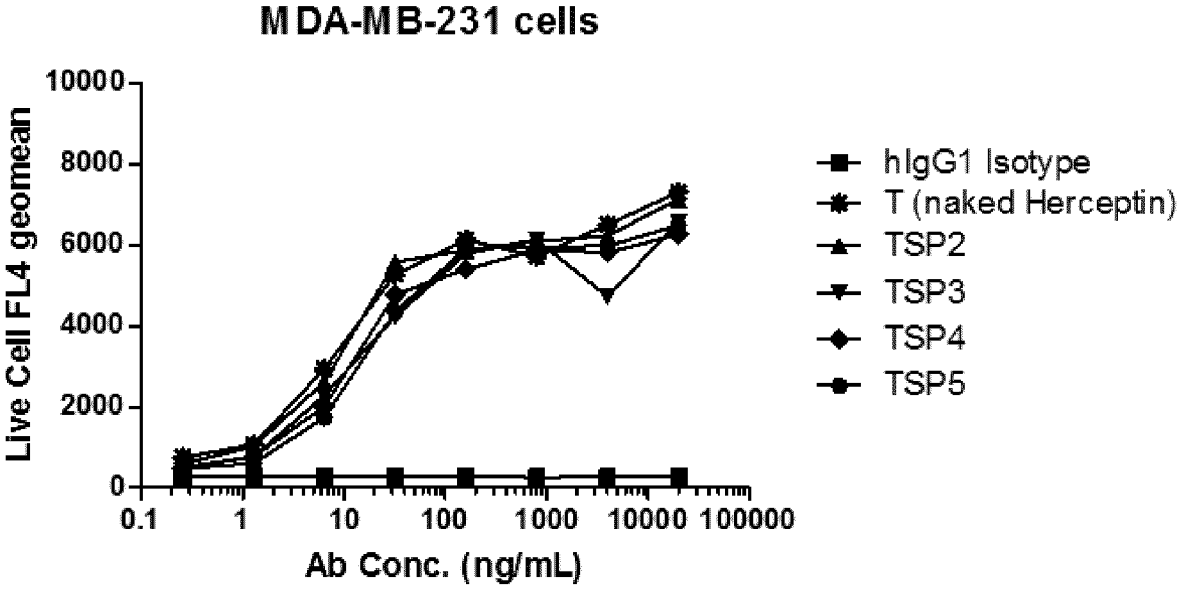

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration log10(nM)
T-VLCys2-Toxin1
T-VLCys1-Toxin2
T-Toxin1 CTRL

FIG. 4

A


MDA-MB-231 cells
Live Cell FL4 geomean
10000
8000
6000
4000
2000
0
0.1
1
10
100
1000
10000
100000
Ab Conc. (ng/mL)
hIgG1 Isotype
T (naked Herceptin)
TSP2
TSP3
TSP4
TSP5

B

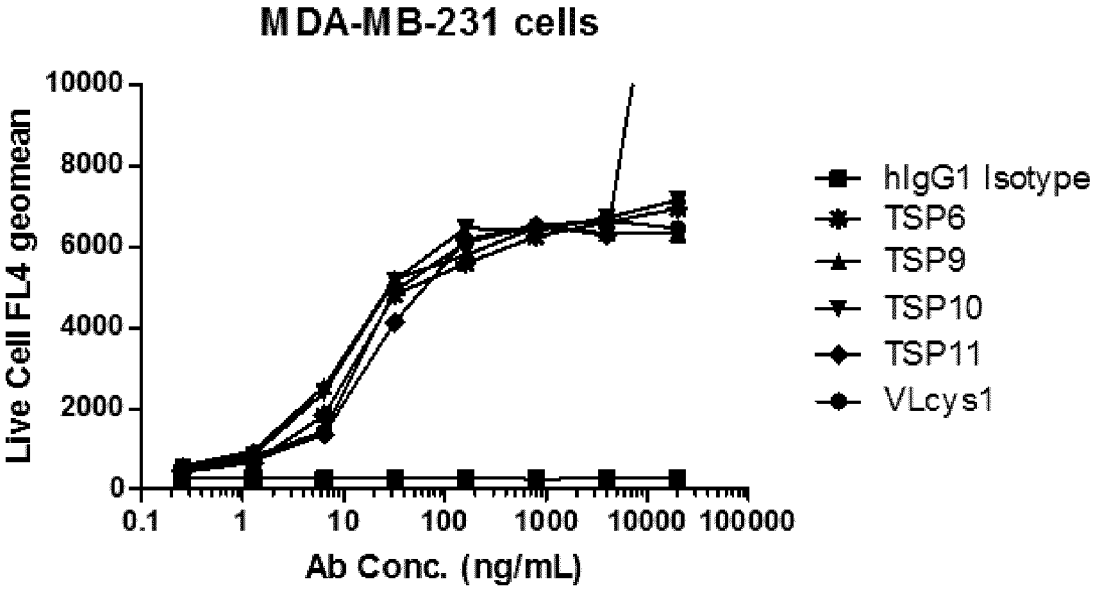

MDA-MB-231 cells
Live Cell FL4 geomean
10000
8000
6000
4000
2000
0
0.1
1
10
100
1000
10000
100000
Ab Conc. (ng/mL)
hIgG1 Isotype
TSP6
TSP9
TSP10
TSP11
VLcys1

FIG. 5

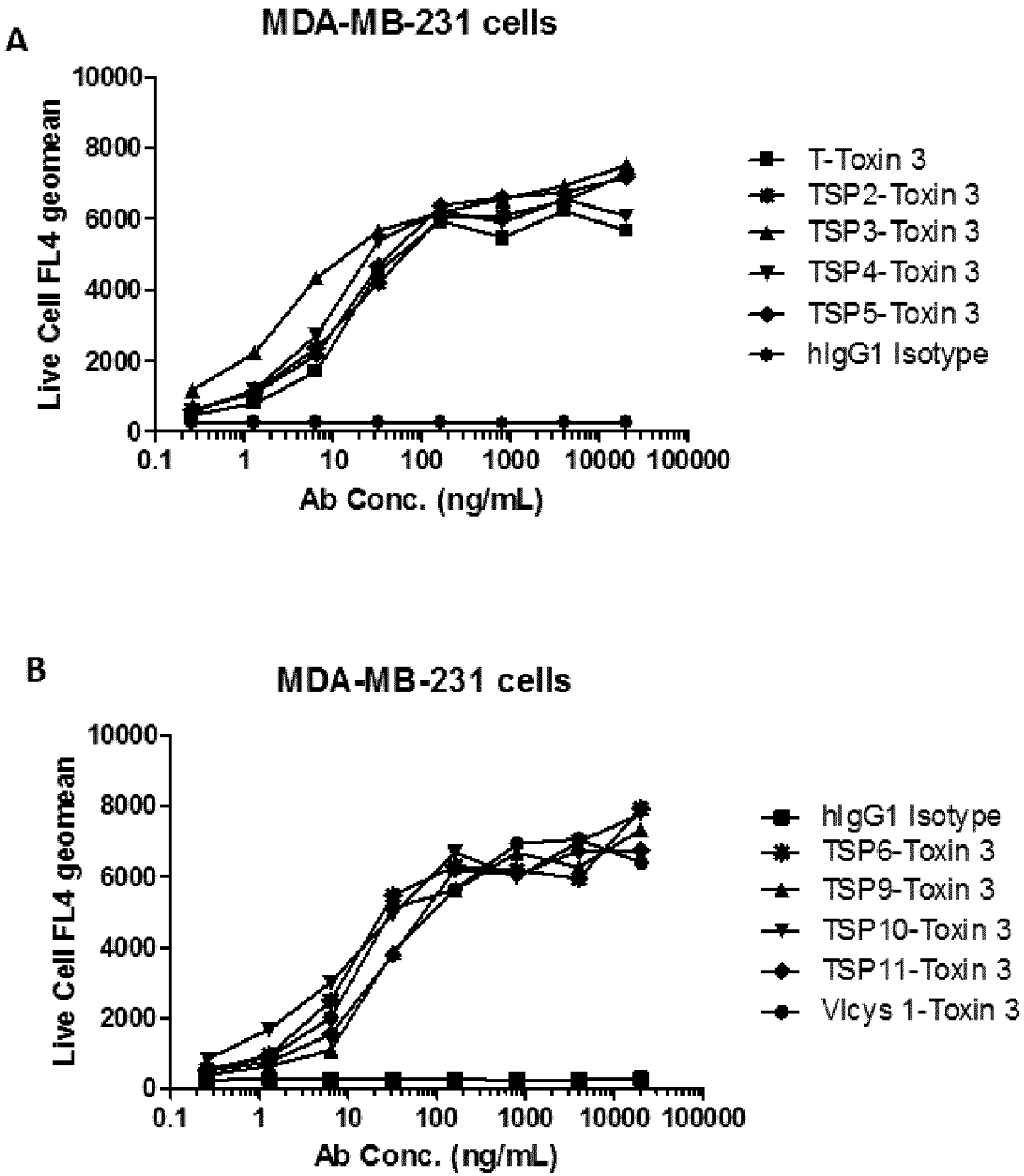

A
MDA-MB-231 cells
Live Cell FL4 geomean
10000
8000
6000
4000
2000
0
0.1
1
10
100
1000
10000
100000
Ab Conc. (ng/mL)
T-Toxin 3
TSP2-Toxin 3
TSP3-Toxin 3
TSP4-Toxin 3
TSP5-Toxin 3
hIgG1 Isotype
B
MDA-MB-231 cells
Live Cell FL4 geomean
10000
8000
6000
4000
2000
0
0.1
1
10
100
1000
10000
100000
Ab Conc. (ng/mL)
hIgG1 Isotype
TSP6-Toxin 3
TSP9-Toxin 3
TSP10-Toxin 3
TSP11-Toxin 3
VIcys 1-Toxin 3

FIG. 6

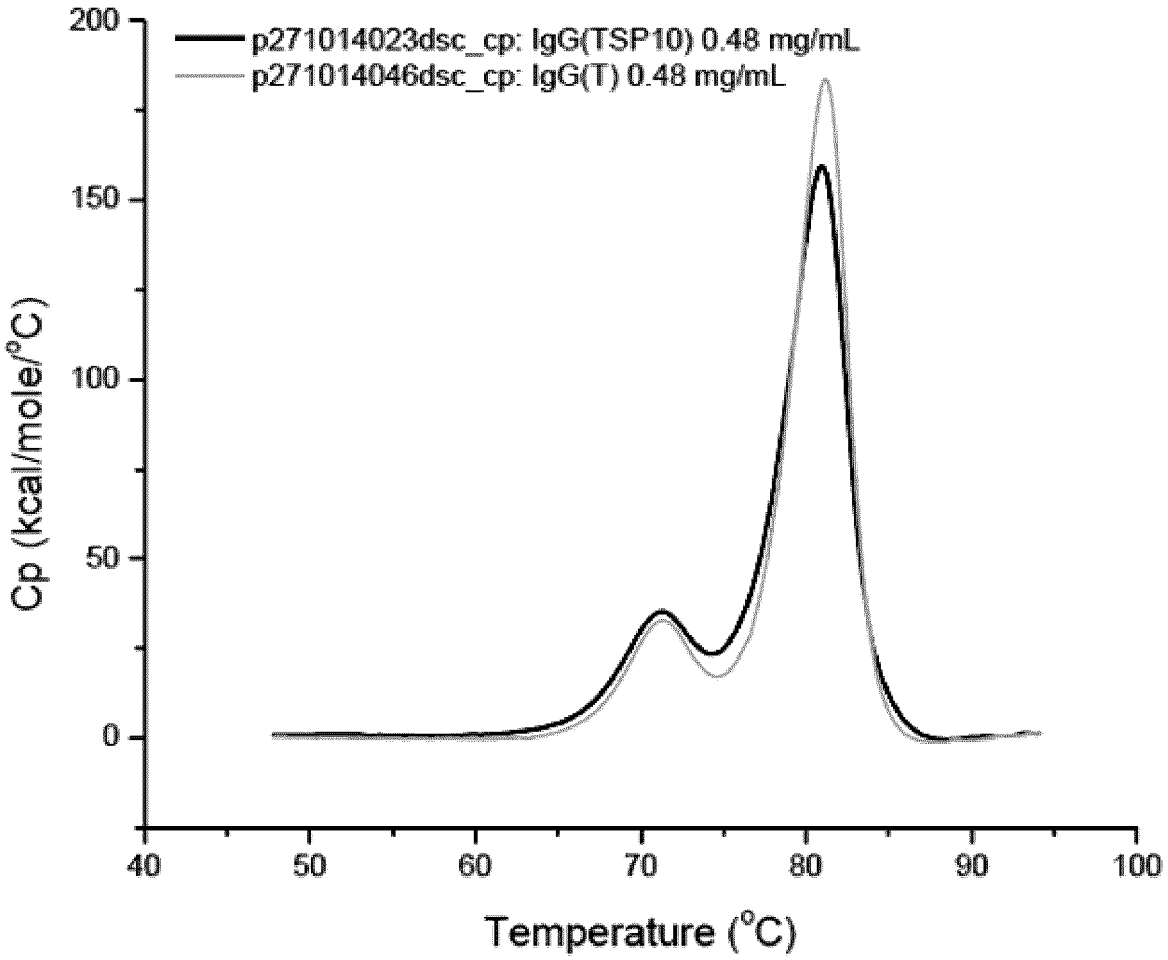

p271014023dsc_cp: IgG(TSP10) 0.48 mg/mL
p271014046dsc_cp: IgG(T) 0.48 mg/mL
Cp (kcal/mole/°C)
200
150
100
50
0
40
50
60
70
80
90
100
Temperature (°C)

FIG. 7

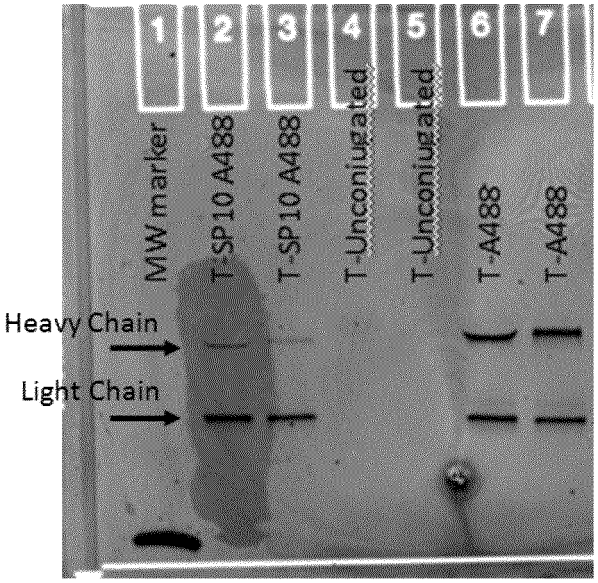

1
2
3
4
5
6
7
MW marker
T-SP10 A488
T-SP10 A488
T-Unconjugated
T-Unconjugated
T-A488
T-A488
Heavy Chain
Light Chain

FIG. 8

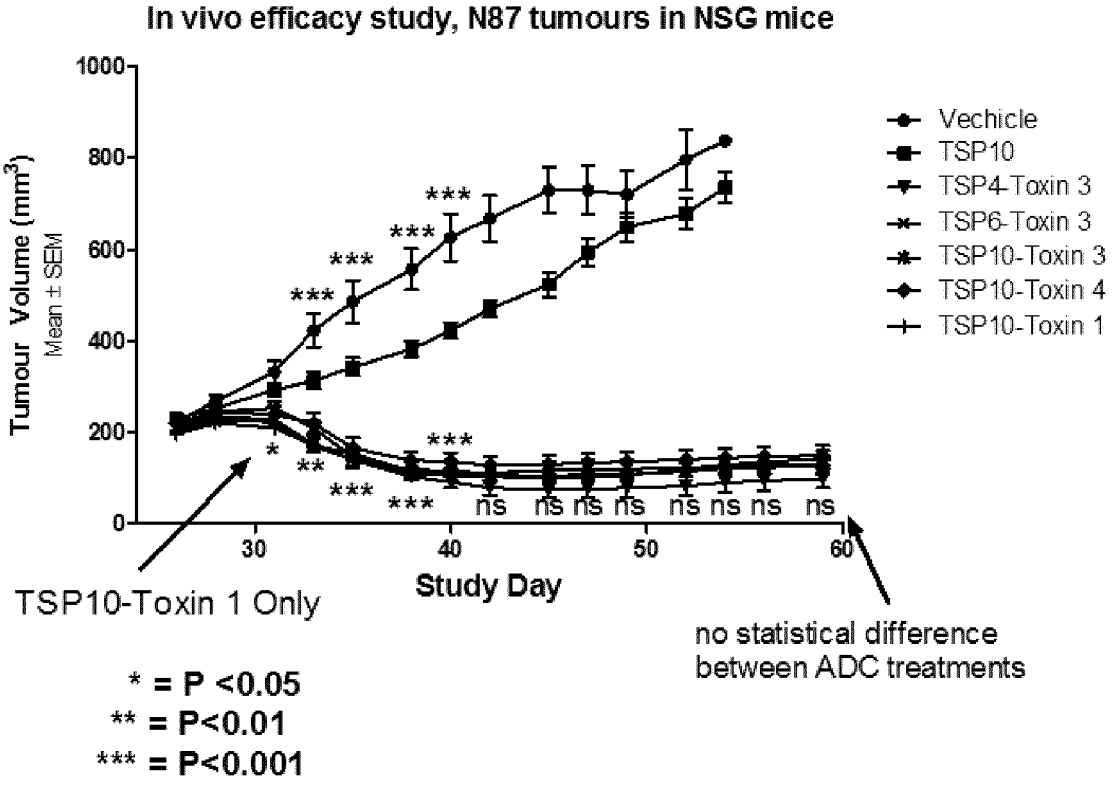

In vivo efficacy study, N87 tumours in NSG mice
Tumour Volume (mm3)
Mean ± SEM
1000
800
600
400
200
0
30
40
50
60
Study Day
Vechicle
TSP10
TSP4-Toxin 3
TSP6-Toxin 3
TSP10-Toxin 3
TSP10-Toxin 4
TSP10-Toxin 1
TSP10-Toxin 1 Only
no statistical difference
between ADC treatments
* = P <0.05
** = P<0.01
*** = P<0.001

FIG. 9

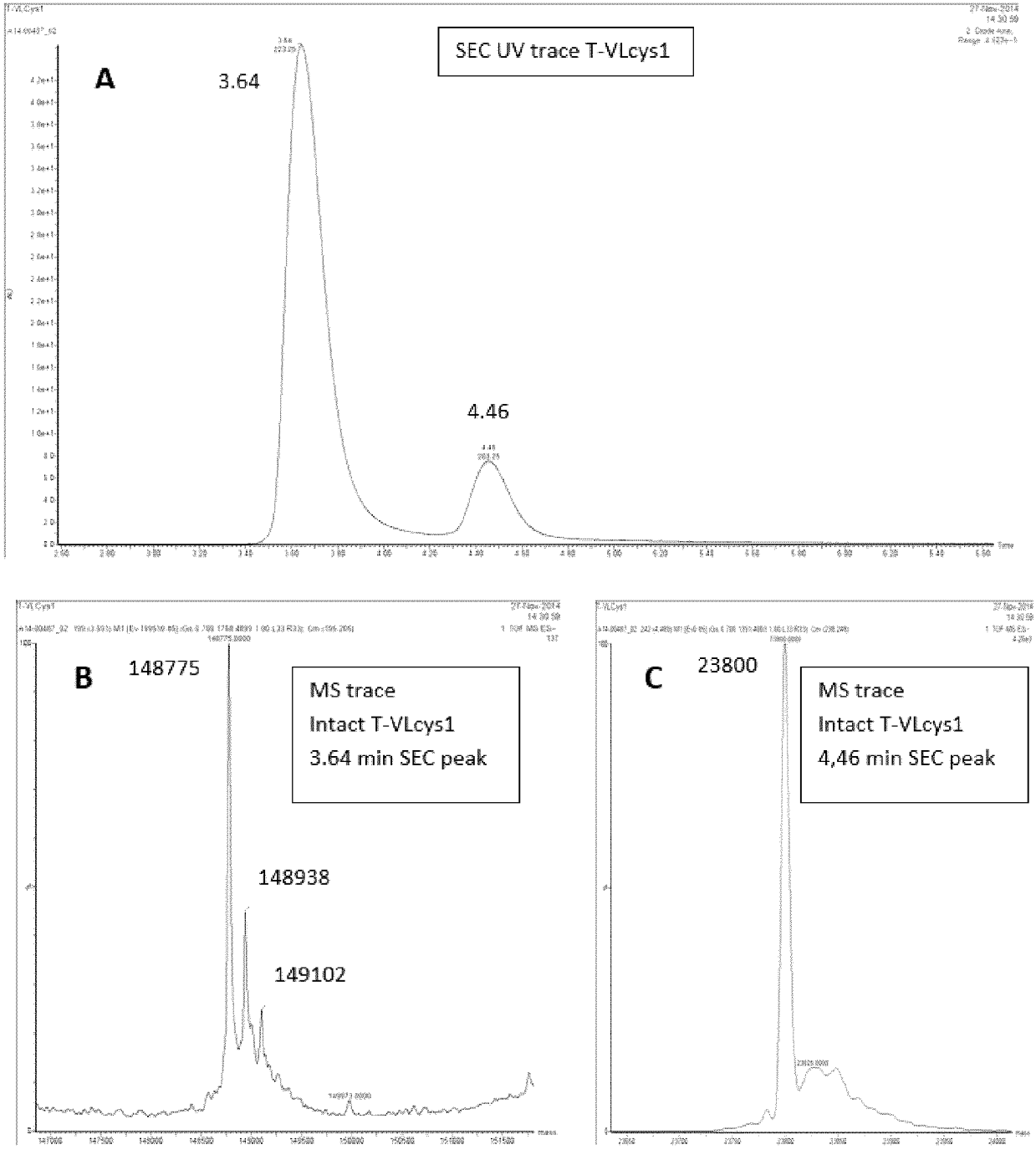

A
3.64
4.46
SEC UV trace T-VLcys1
B
148775
148938
149102
MS trace
Intact T-VLcys1
3.64 min SEC peak
C
23800
MS trace
Intact T-VLcys1
4,46 min SEC peak

FIG. 10

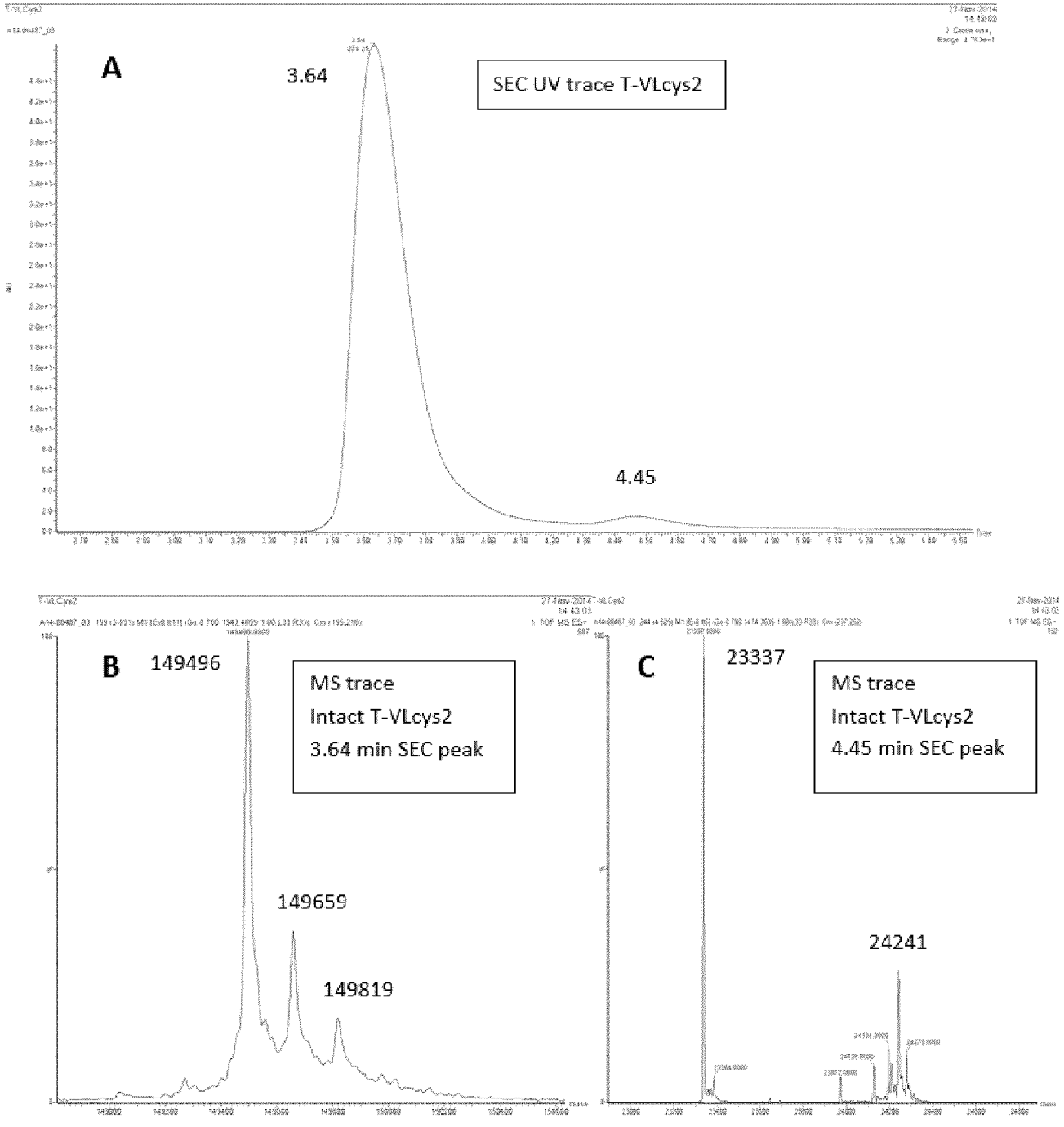

A
3.64
4.45
SEC UV trace T-VLcys2
B
149496
149659
149819
MS trace
Intact T-VLcys2
3.64 min SEC peak
C
23337
24241
MS trace
Intact T-VLcys2
4.45 min SEC peak

FIG. 11

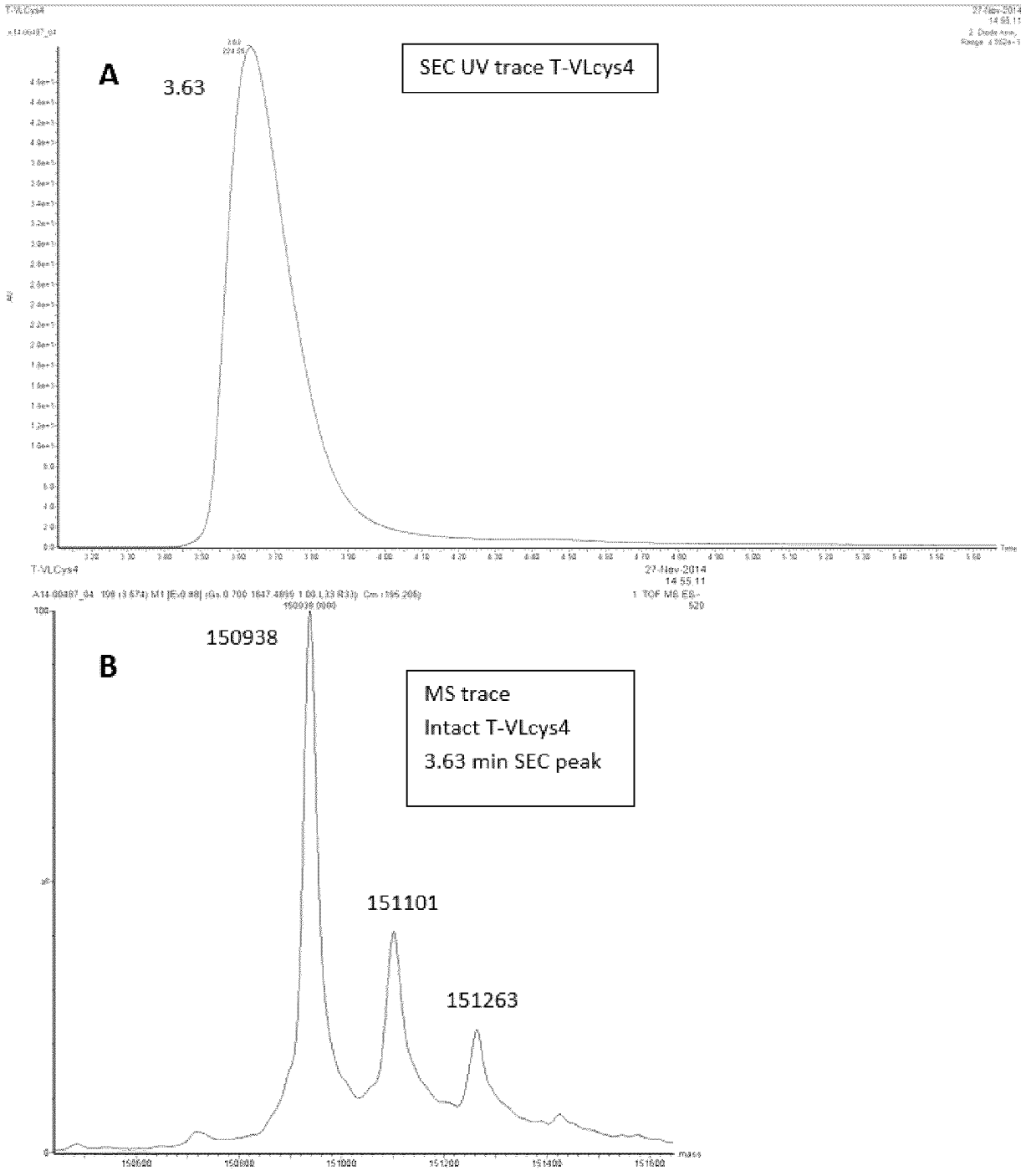
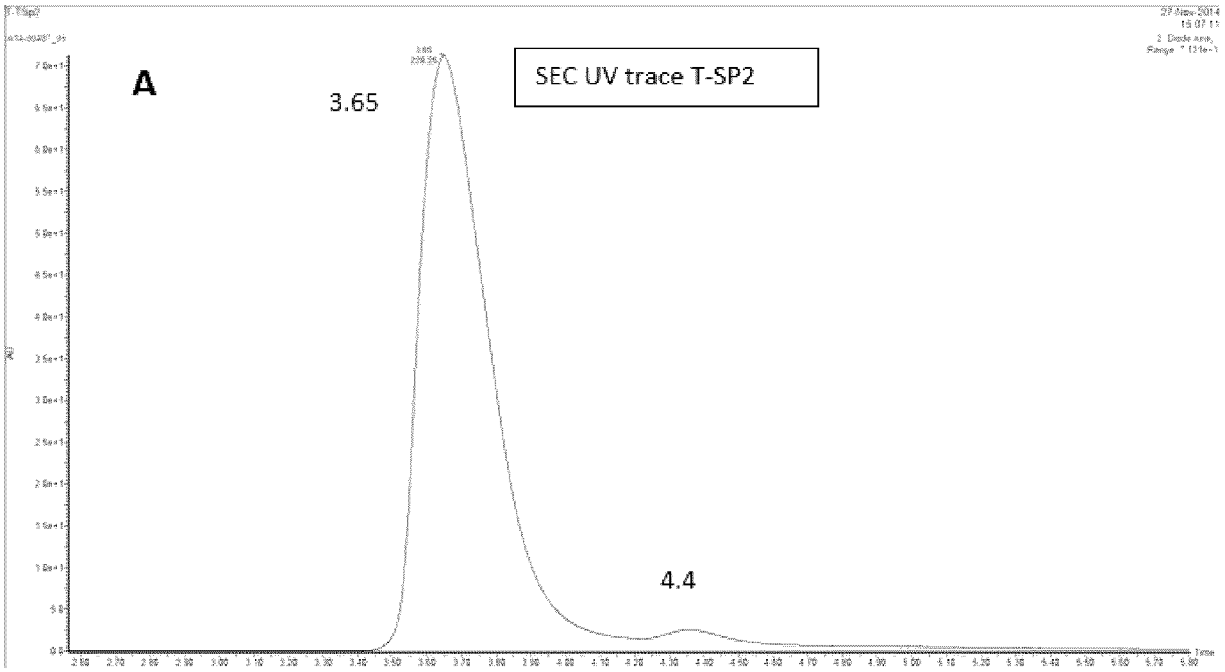

A
3.63
SEC UV trace T-VLcys4
B
150938
MS trace
Intact T-VLcys4
3.63 min SEC peak
151101
151263

FIG. 12


A
3.65
4.4
SEC UV trace T-SP2

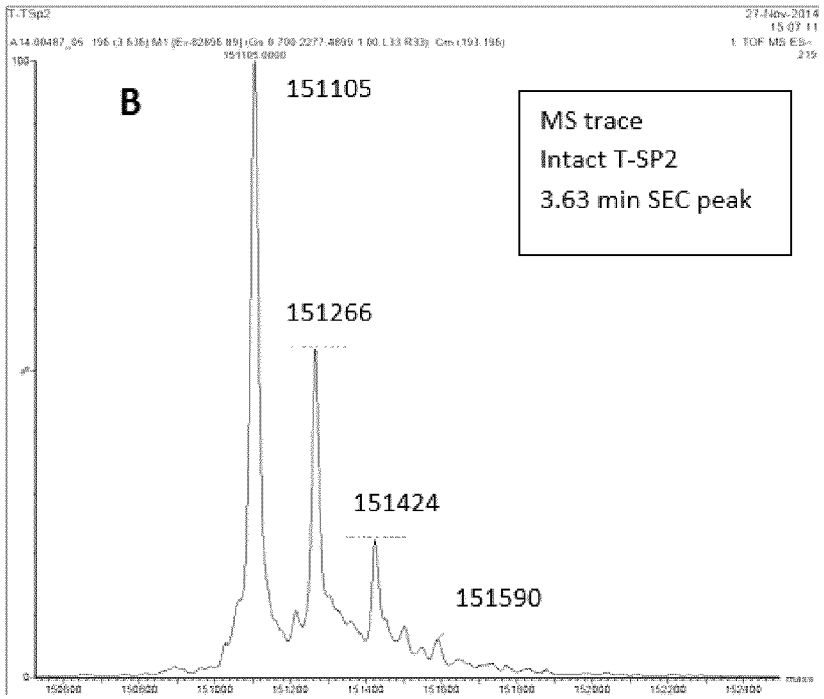

B
151105
151266
151424
151590
MS trace
Intact T-SP2
3.63 min SEC peak

FIG. 13

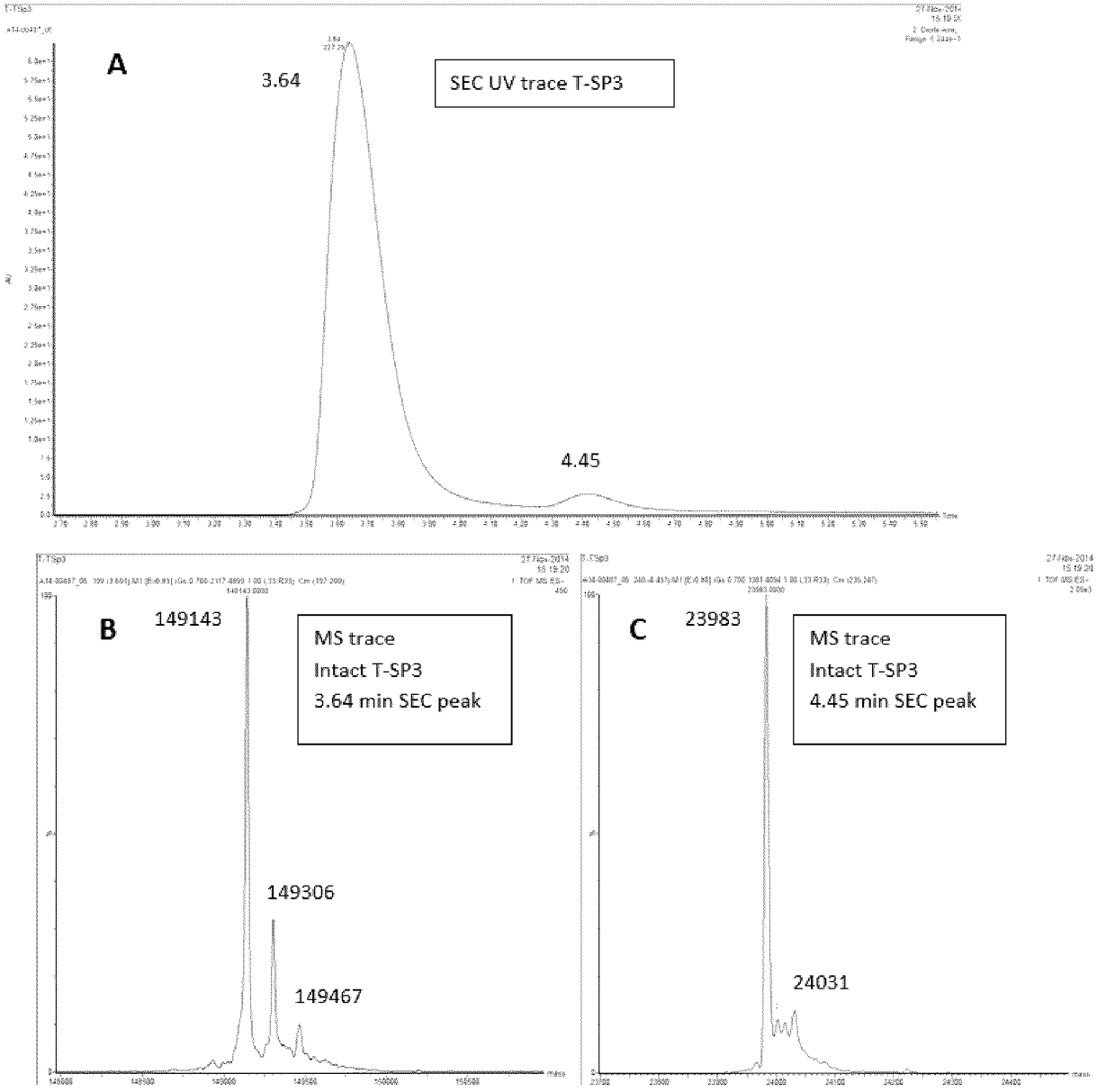

A
3.64
4.45
SEC UV trace T-SP3
B
149143
149306
149467
MS trace
Intact T-SP3
3.64 min SEC peak
C
23983
24031
MS trace
Intact T-SP3
4.45 min SEC peak

FIG. 14

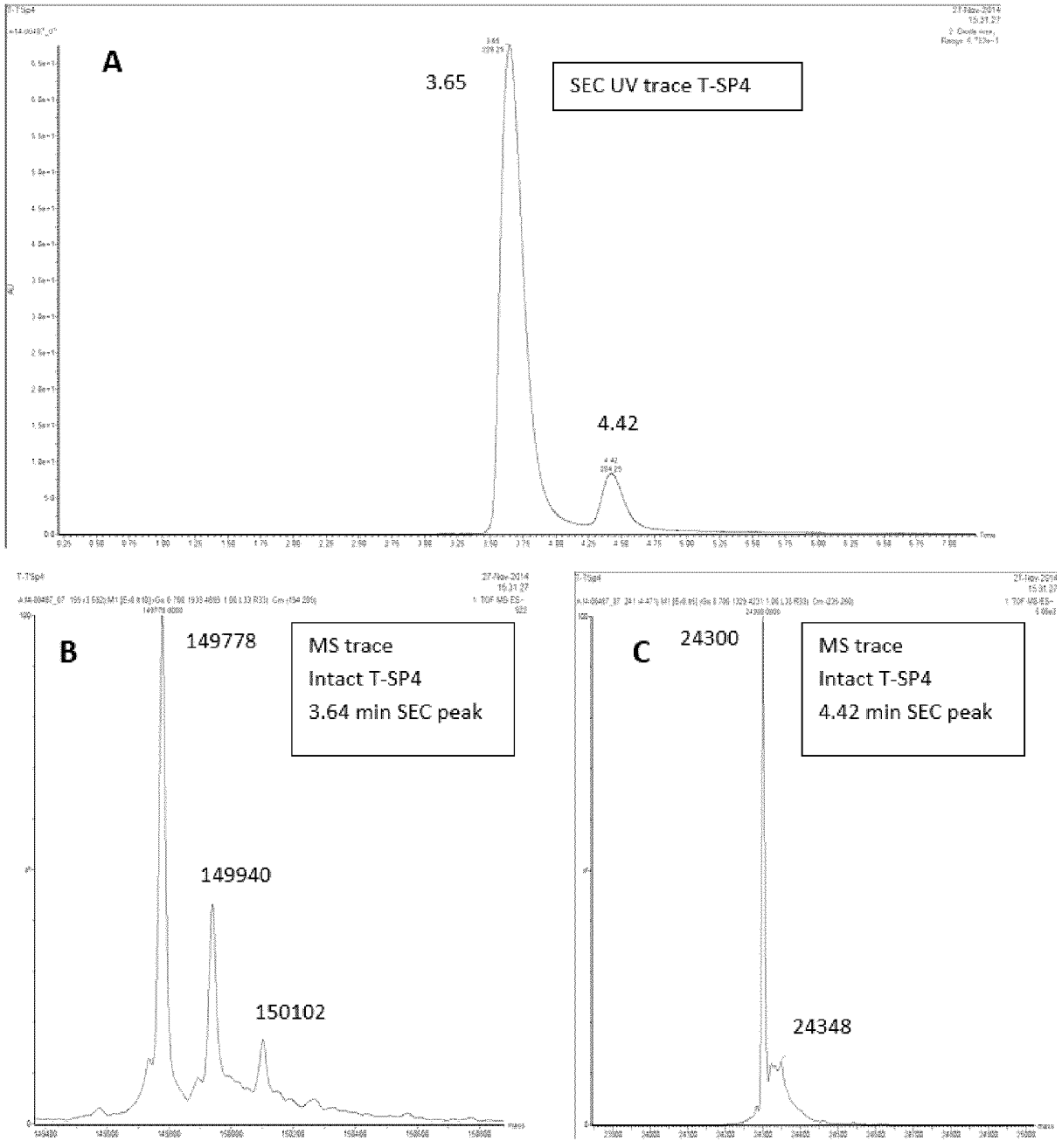
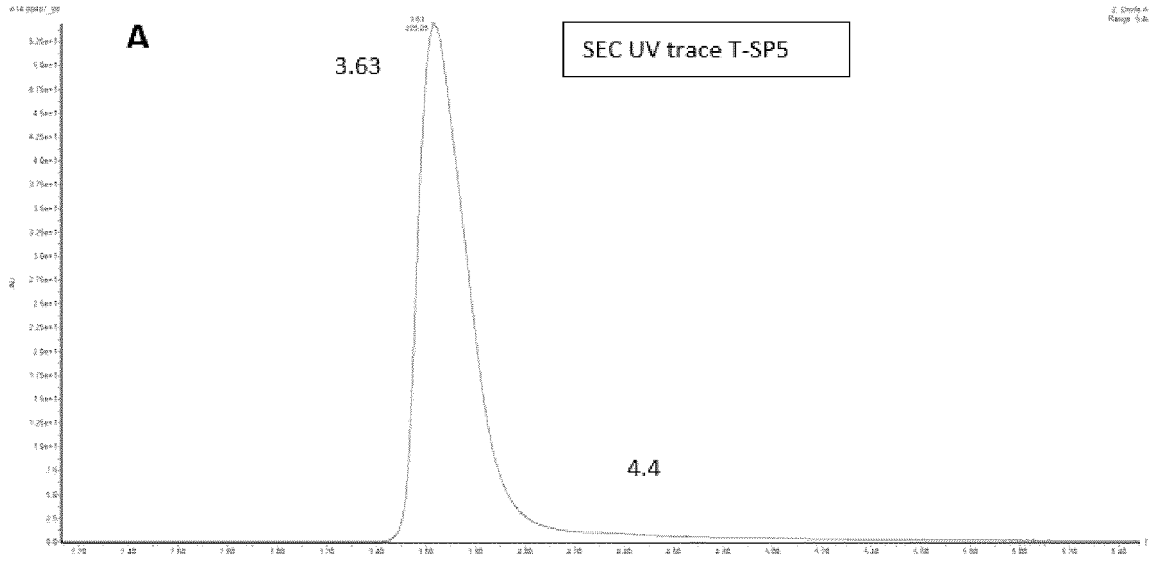

A
3.65
4.42
SEC UV trace T-SP4
B
149778
149940
150102
MS trace
Intact T-SP4
3.64 min SEC peak
C
24300
24348
MS trace
Intact T-SP4
4.42 min SEC peak

FIG. 15


A
3.63
4.4
SEC UV trace T-SP5

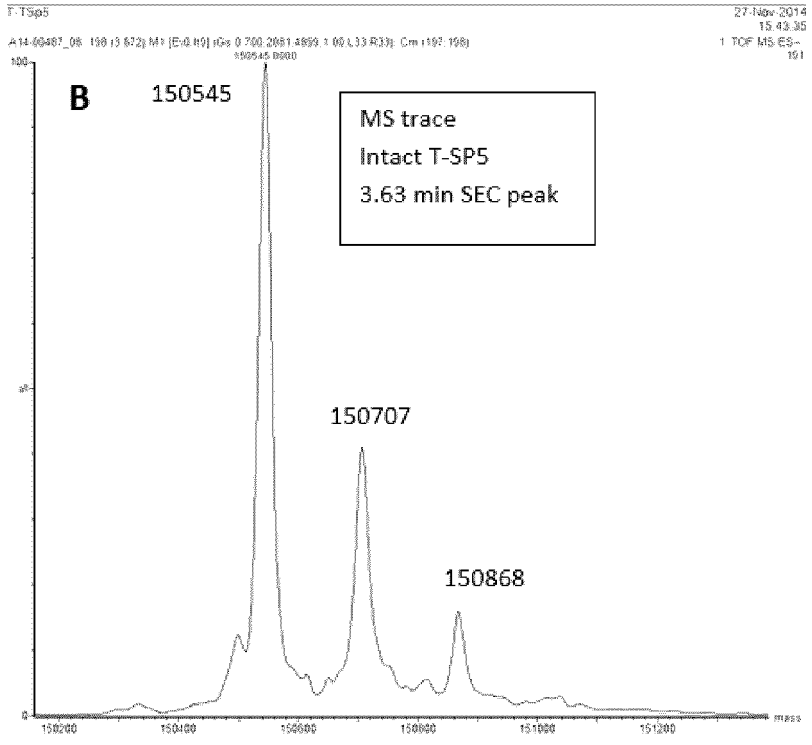

B
150545
150707
150868
MS trace
Intact T-SP5
3.63 min SEC peak

FIG. 16

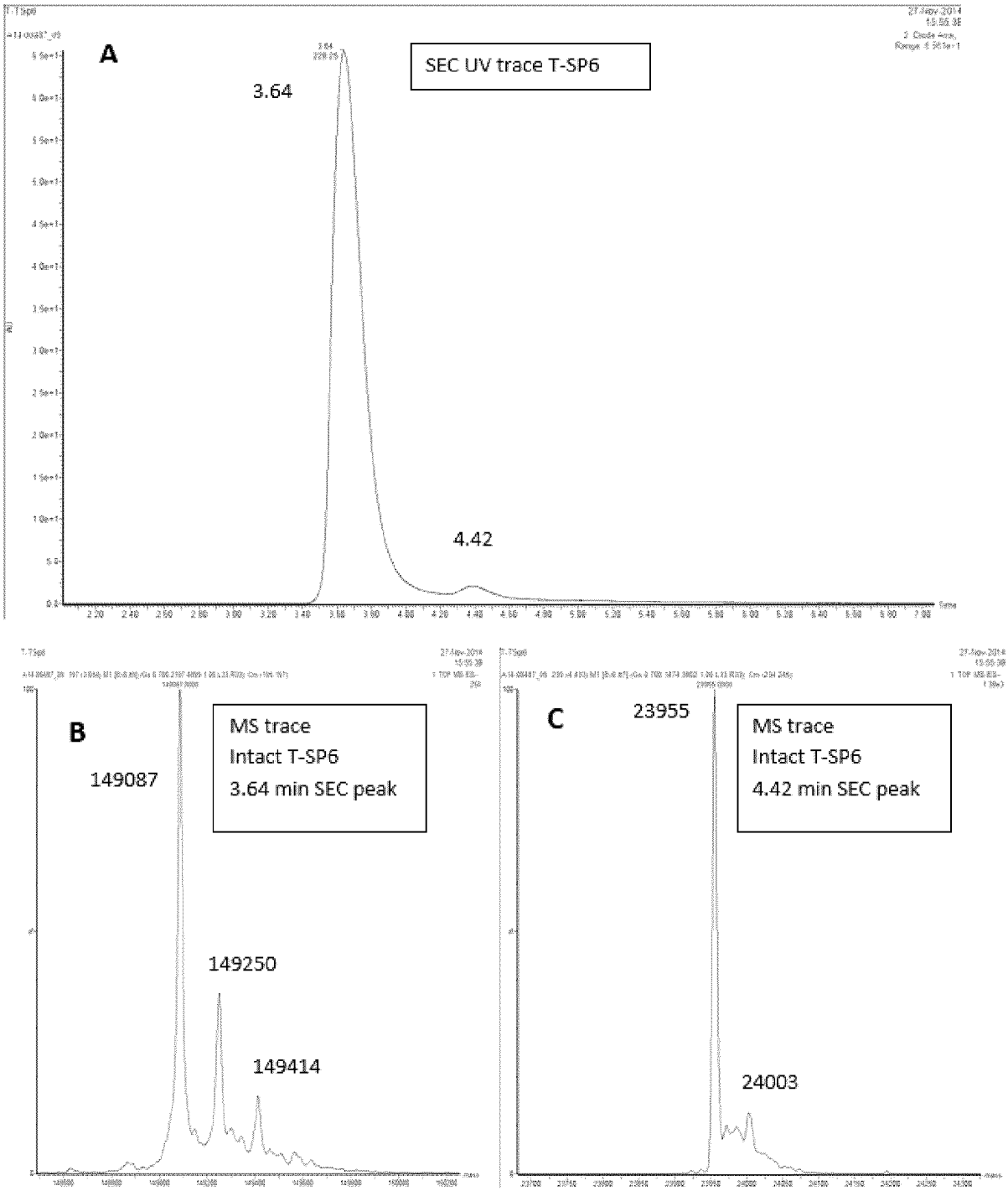

A
SEC UV trace T-SP6
3.64
4.42
B
MS trace
Intact T-SP6
3.64 min SEC peak
149087
149250
149414
C
MS trace
Intact T-SP6
4.42 min SEC peak
23955
24003

FIG. 17

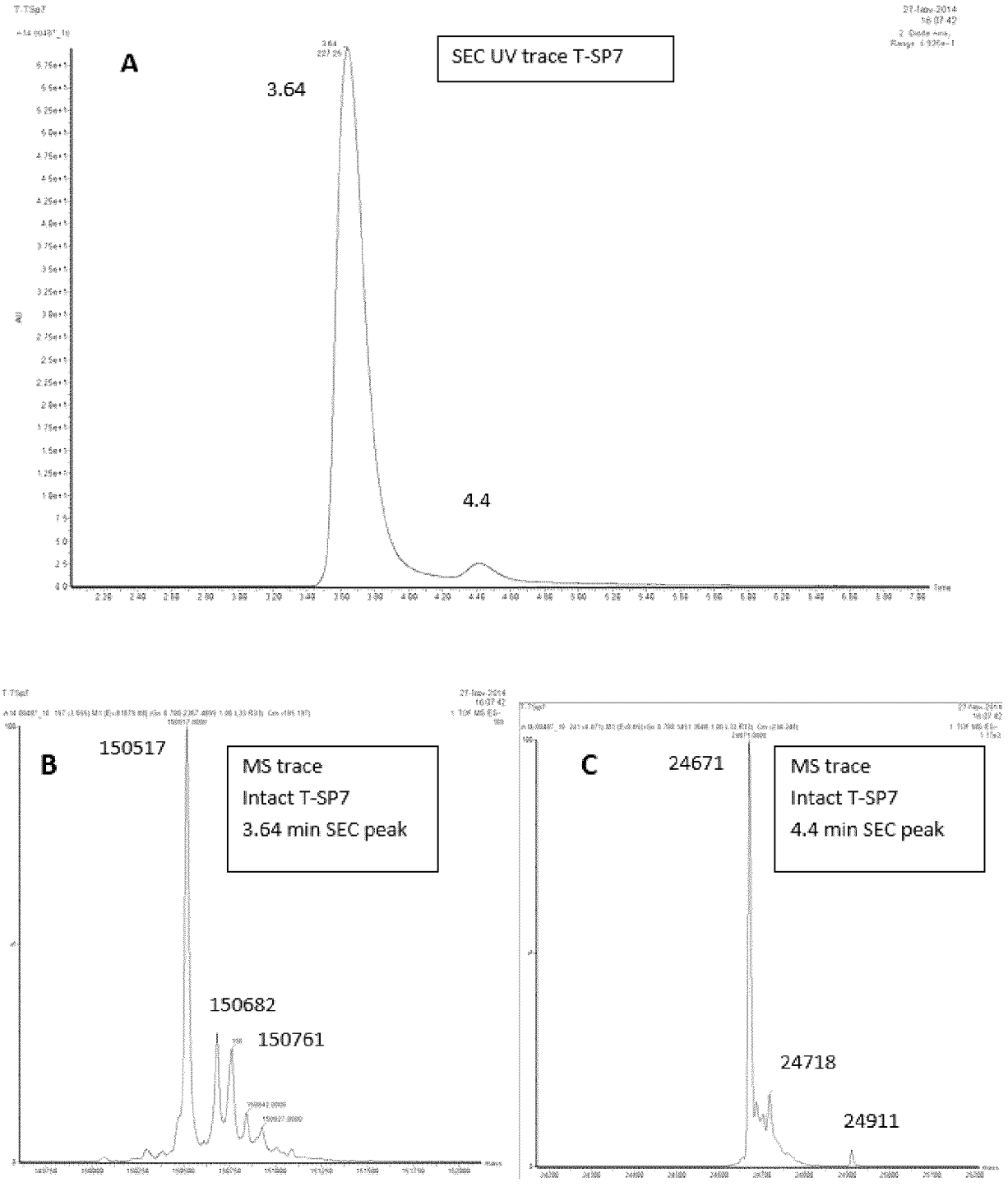

A
SEC UV trace T-SP7
3.64
4.4
B
150517
MS trace
Intact T-SP7
3.64 min SEC peak
150682
150761
C
24671
MS trace
Intact T-SP7
4.4 min SEC peak
24718
24911

FIG. 18

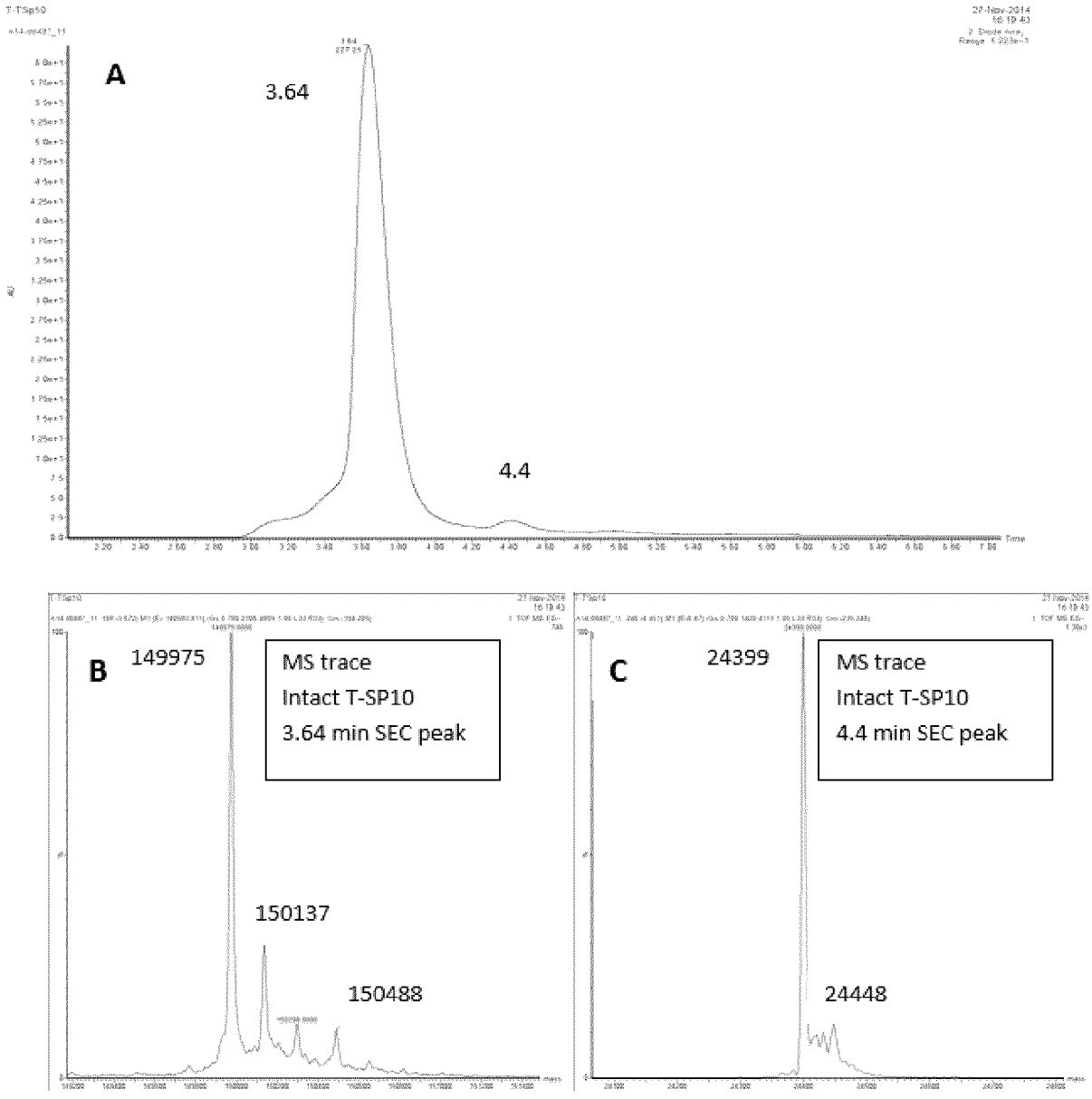
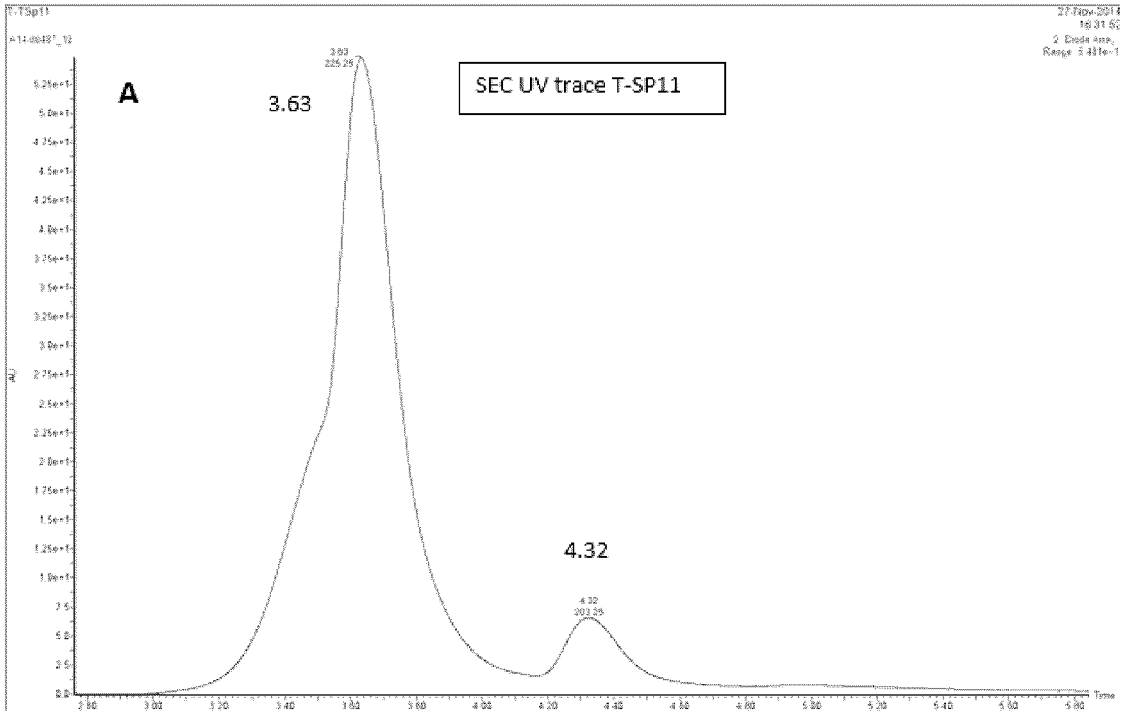
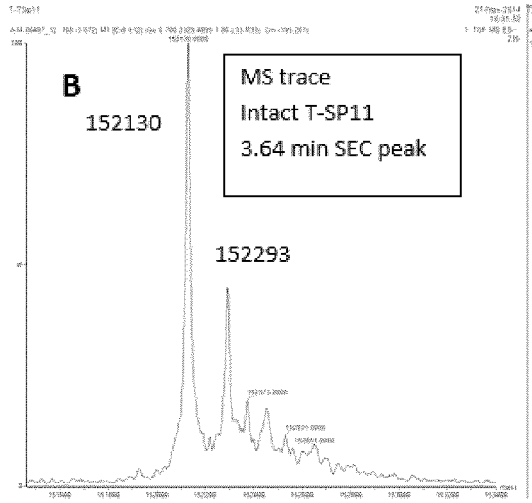

A
3.64
4.4
B
149975
150137
150488
MS trace
Intact T-SP10
3.64 min SEC peak
C
24399
24448
MS trace
Intact T-SP10
4.4 min SEC peak

FIG. 19


A
3.63
SEC UV trace T-SP11
4.32


B
152130
MS trace
Intact T-SP11
3.64 min SEC peak
152293

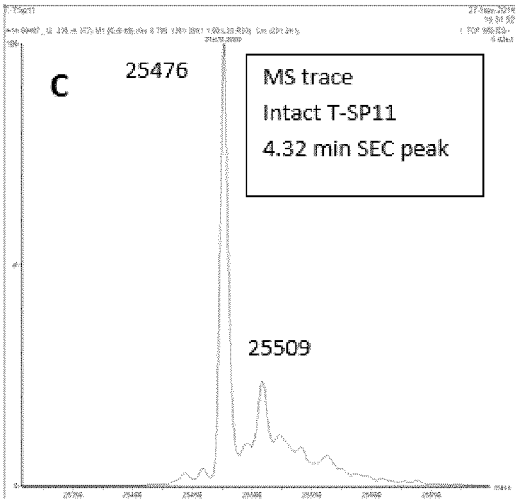

C
25476
MS trace
Intact T-SP11
4.32 min SEC peak
25509

FIG. 20

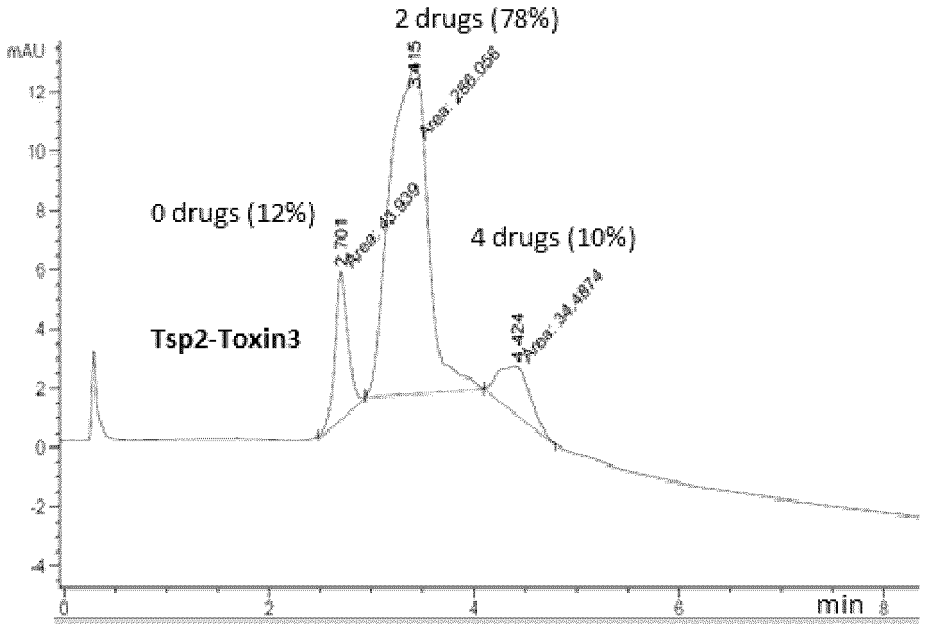

2 drugs (78%)
mAU
12
10
8
6
4
2
0
-2
-4
3.415
Area: 288.056
0 drugs (12%)
2.701
Area: 45.839
4 drugs (10%)
4.424
Area: 34.4874
Tsp2-Toxin3
0
2
4
6
min
8

FIG. 21

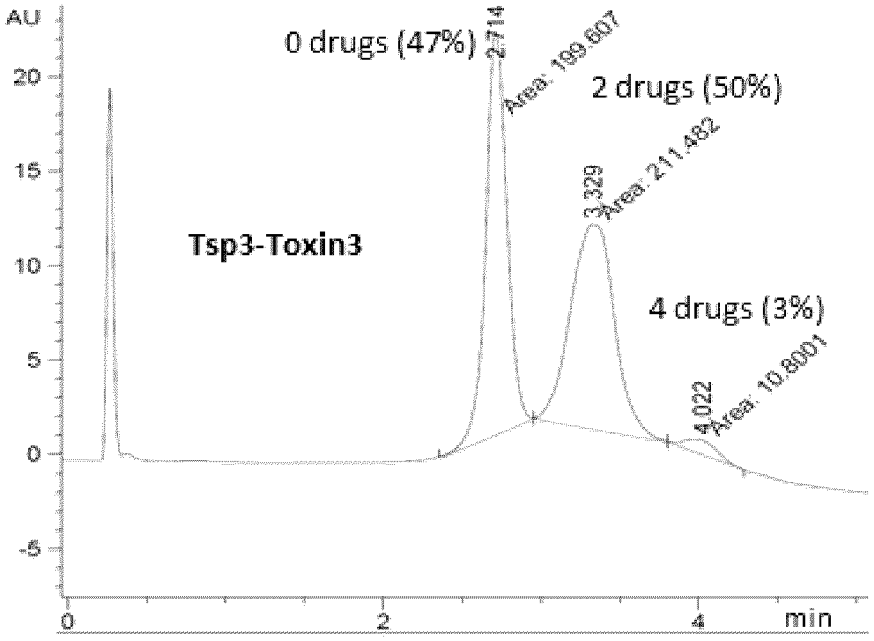

AU
20
15
10
5
0
-5
0 drugs (47%)
2.714
Area: 199.607
2 drugs (50%)
3.329
Area: 211.482
Tsp3-Toxin3
4 drugs (3%)
4.022
Area: 10.8001
0
2
4
min

FIG. 22

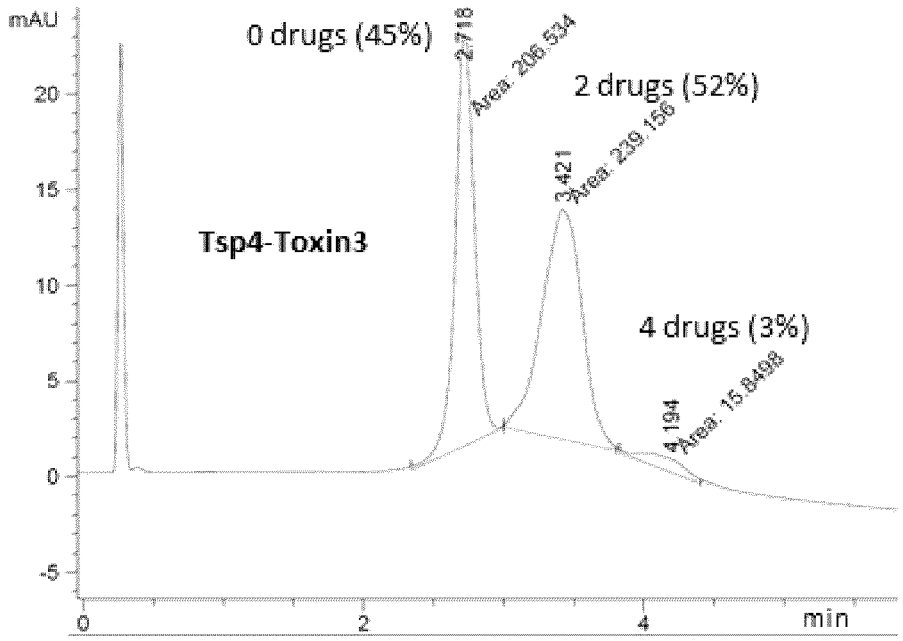

mAU
0 drugs (45%)
2.718
Area: 206.534
2 drugs (52%)
3.421
Area: 239.156
Tsp4-Toxin3
4 drugs (3%)
4.194
Area: 15.8498
20
15
10
5
0
-5
0
2
4
min

FIG. 23

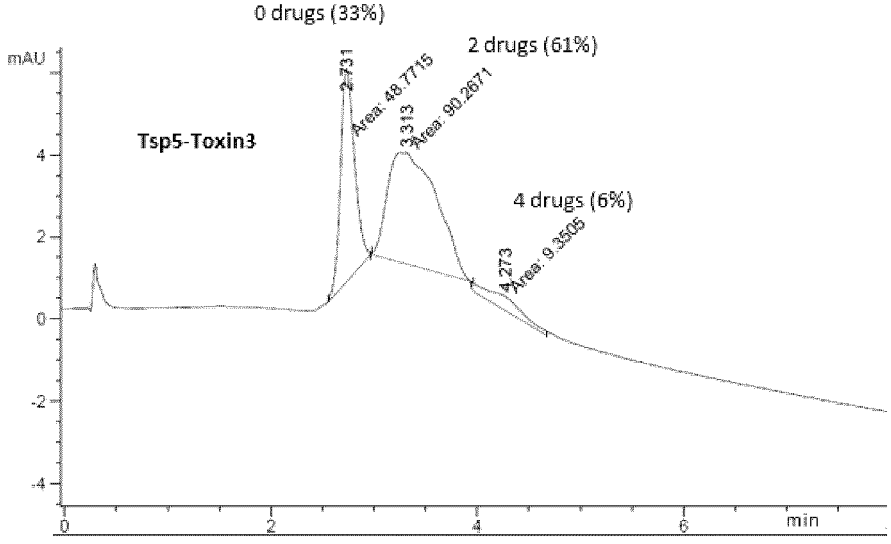

0 drugs (33%)
2 drugs (61%)
mAU
2.731
Area: 48.7715
3.313
Area: 90.2671
Tsp5-Toxin3
4 drugs (6%)
4.273
Area: 9.3505
4
2
0
-2
-4
0
2
4
6
min

FIG. 24

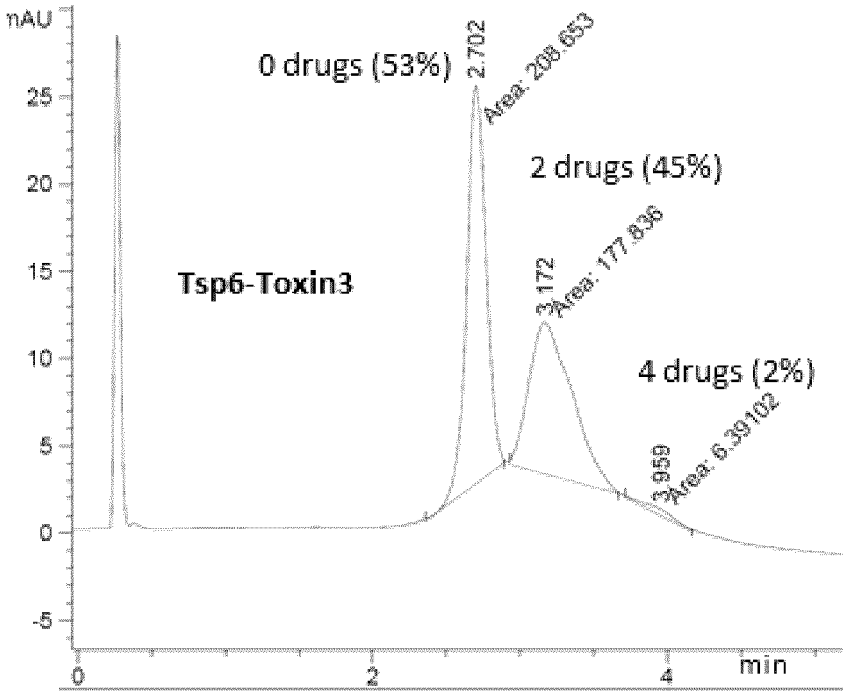

nAU
25
20
15
10
5
0
-5
0 drugs (53%)
2.702
Area: 208.653
2 drugs (45%)
3.172
Area: 177.836
Tsp6-Toxin3
4 drugs (2%)
3.959
Area: 6.39102
0
2
4
min

FIG. 25

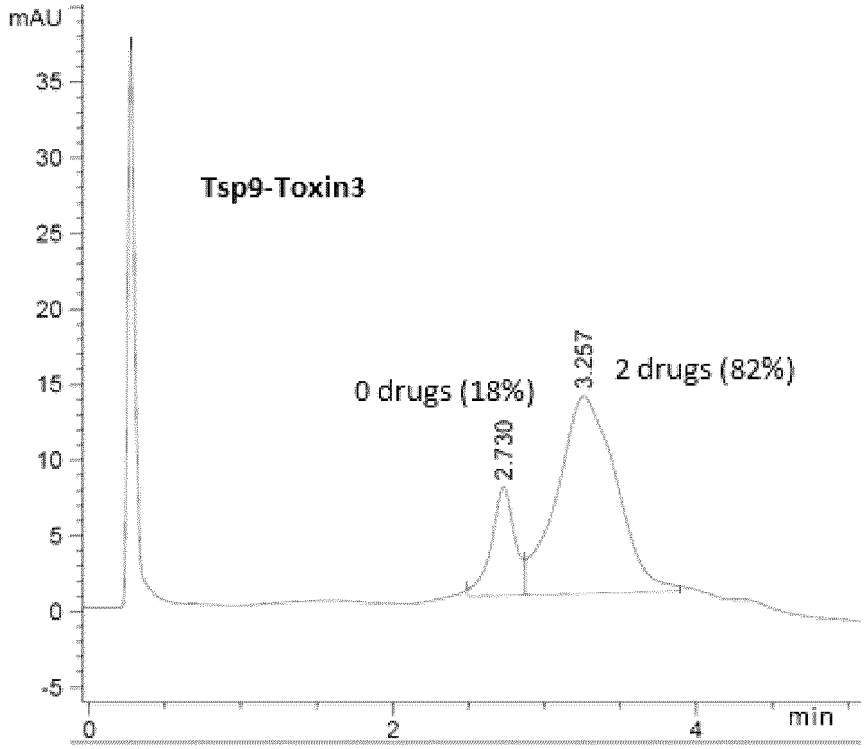

mAU
35
30
25
20
15
10
5
0
-5
Tsp9-Toxin3
0 drugs (18%)
2.730
3.257
2 drugs (82%)
0
2
4
min

FIG. 26

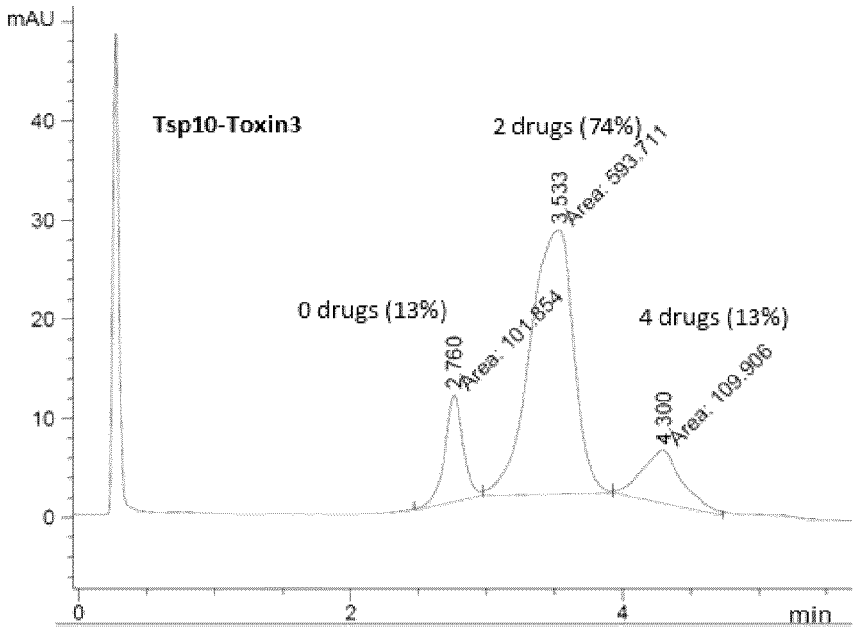

mAU
40
30
20
10
0
Tsp10-Toxin3
2 drugs (74%)
3.533
Area: 593.711
0 drugs (13%)
2.760
Area: 101.854
4 drugs (13%)
4.300
Area: 109.906
0
2
4
min

FIG. 27

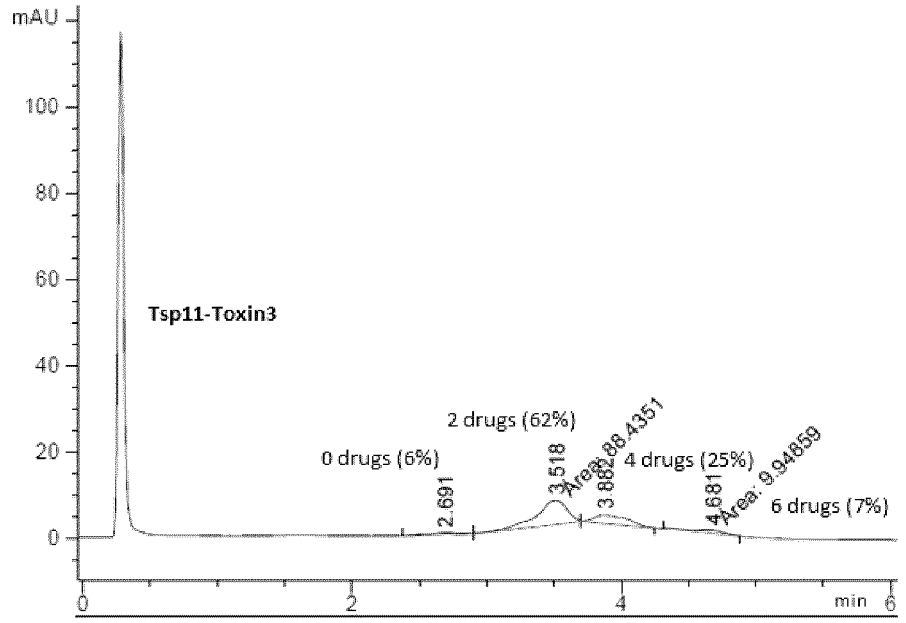

mAU
100
80
60
40
20
0
Tsp11-Toxin3
2 drugs (62%)
0 drugs (6%)
3.518
Area: 88.4351
3.882
4 drugs (25%)
4.681
Area: 9.94859
6 drugs (7%)
2.691
0
2
4
min
6

FIG. 28

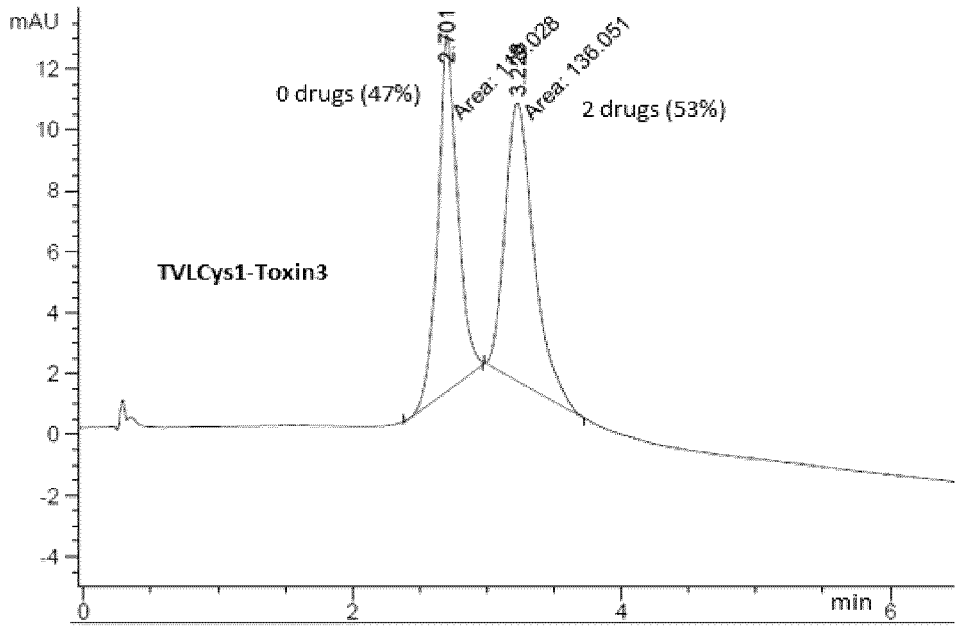

mAU
12
10
8
6
4
2
0
-2
-4
0 drugs (47%)
2.701
Area: 135.051
2 drugs (53%)
TVLCys1-Toxin3
0
2
4
min
6

FIG. 29

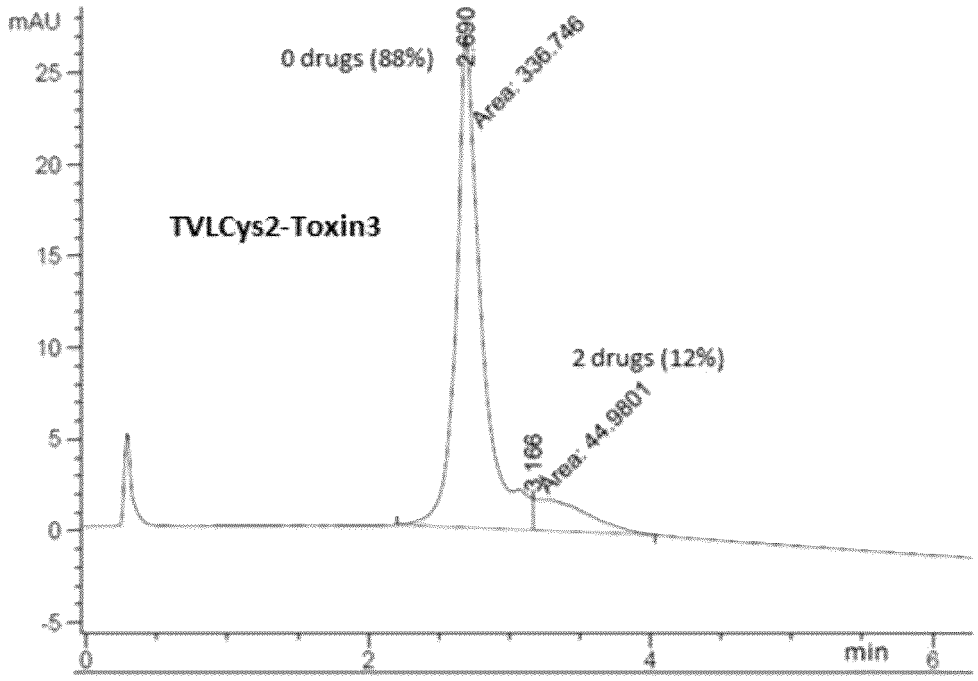

mAU
25
20
15
10
5
0
-5
0 drugs (88%)
2.690
Area: 336.746
TVLCys2-Toxin3
2 drugs (12%)
Area: 44.9801
0
2
4
min
6

FIG. 30

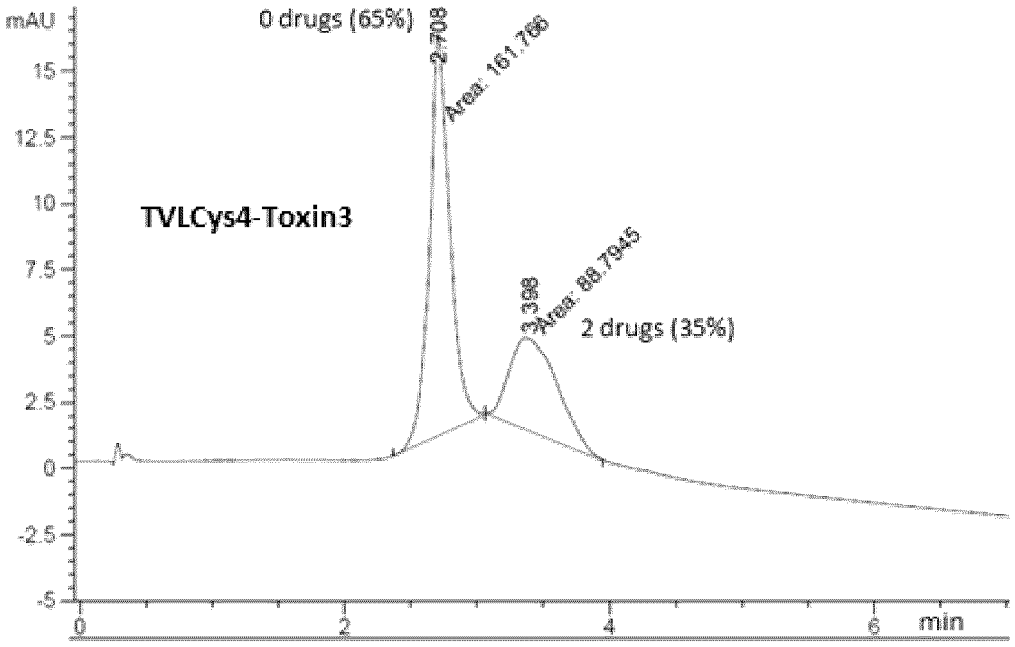

mAU
15
12.5
10
7.5
5
2.5
0
-2.5
-5
0 drugs (65%)
2.708
Area: 161.786
TVLCys4-Toxin3
3.398
Area: 88.7945
2 drugs (35%)
0
2
4
6
min

FIG. 31

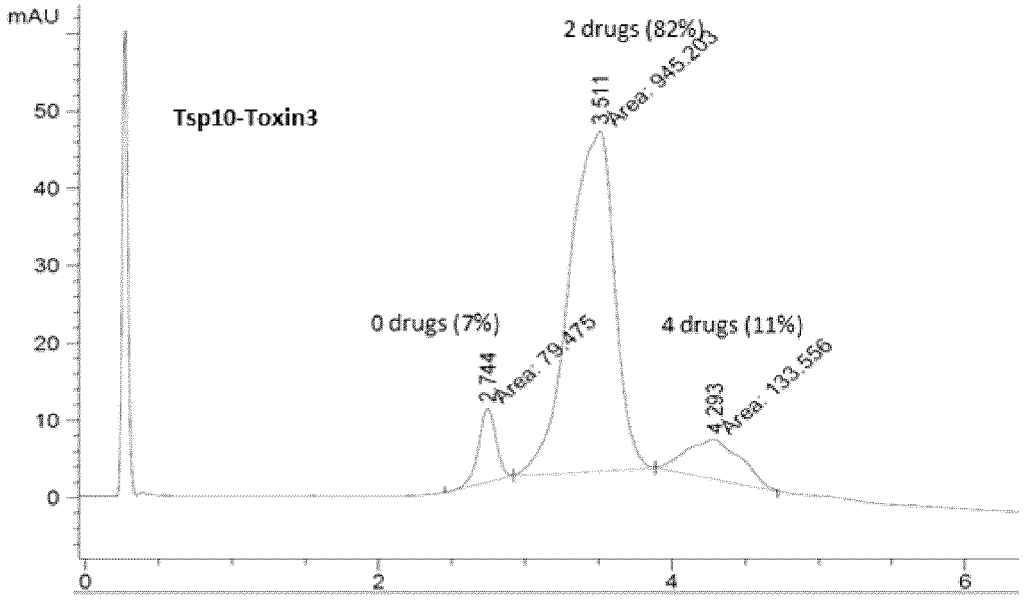

mAU
50
40
30
20
10
0
2 drugs (82%)
3.511
Area: 945.203
Tsp10-Toxin3
0 drugs (7%)
2.744
Area: 79.475
4 drugs (11%)
4.293
Area: 133.556
0
2
4
6

FIG. 32

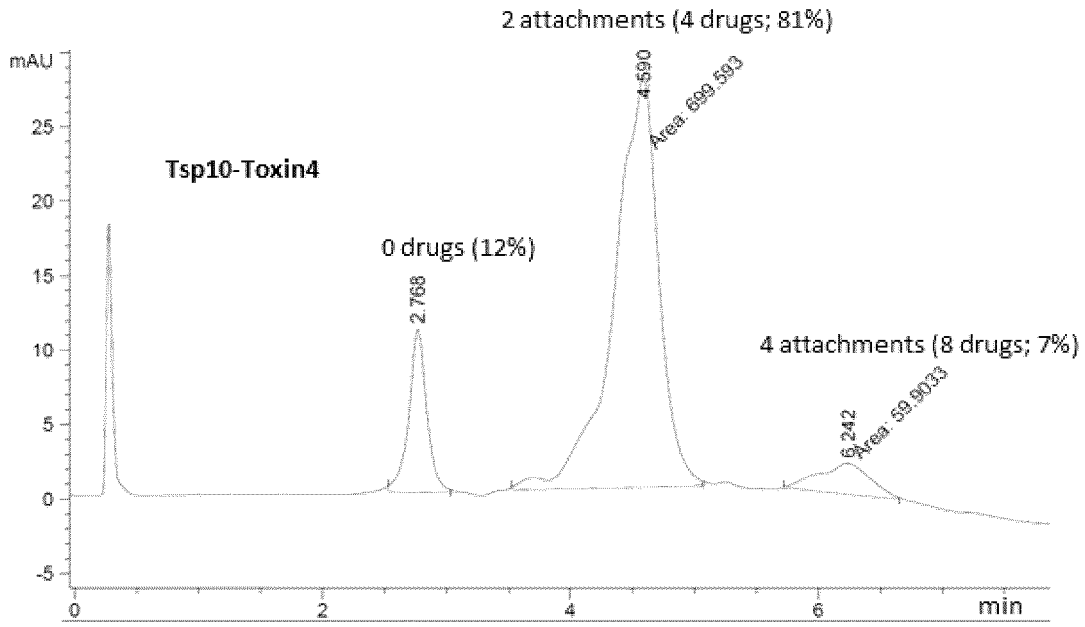

2 attachments (4 drugs; 81%)
mAU
25
20
15
10
5
0
-5
Tsp10-Toxin4
0 drugs (12%)
2.768
4.590
Area: 699.593
4 attachments (8 drugs; 7%)
6.242
Area: 59.9033
0
2
4
6
min

FIG. 33

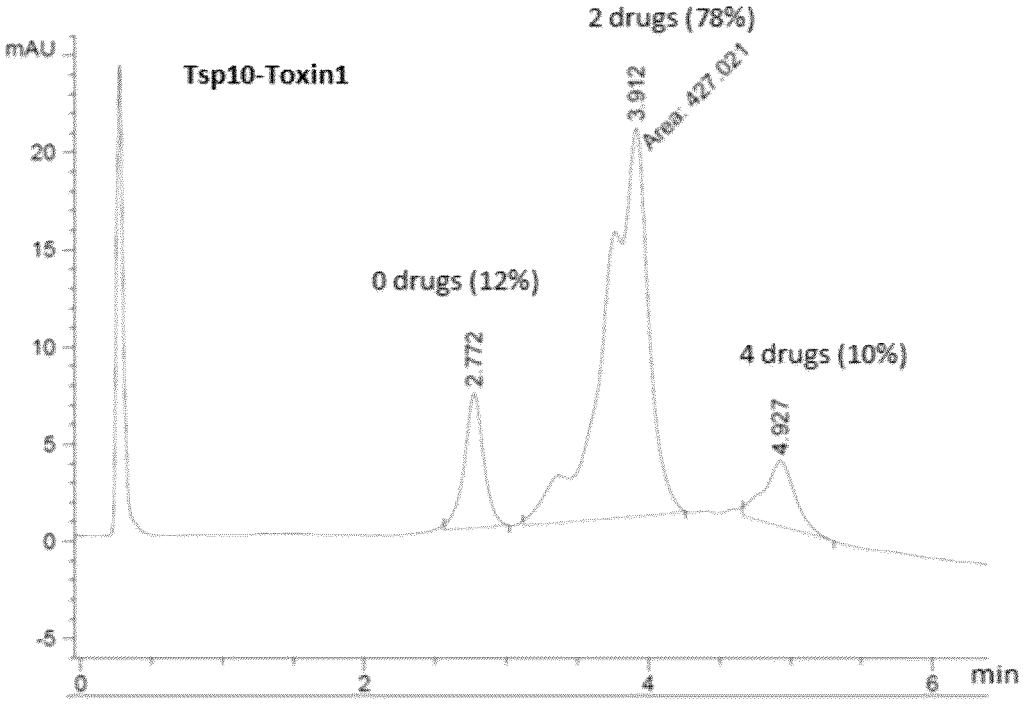

2 drugs (78%)
mAU
Tsp10-Toxin1
3.912
Area: 427.021
20
15
10
5
0
-5
0 drugs (12%)
2.772
4 drugs (10%)
4.927
0
2
4
6
min

FIG. 34

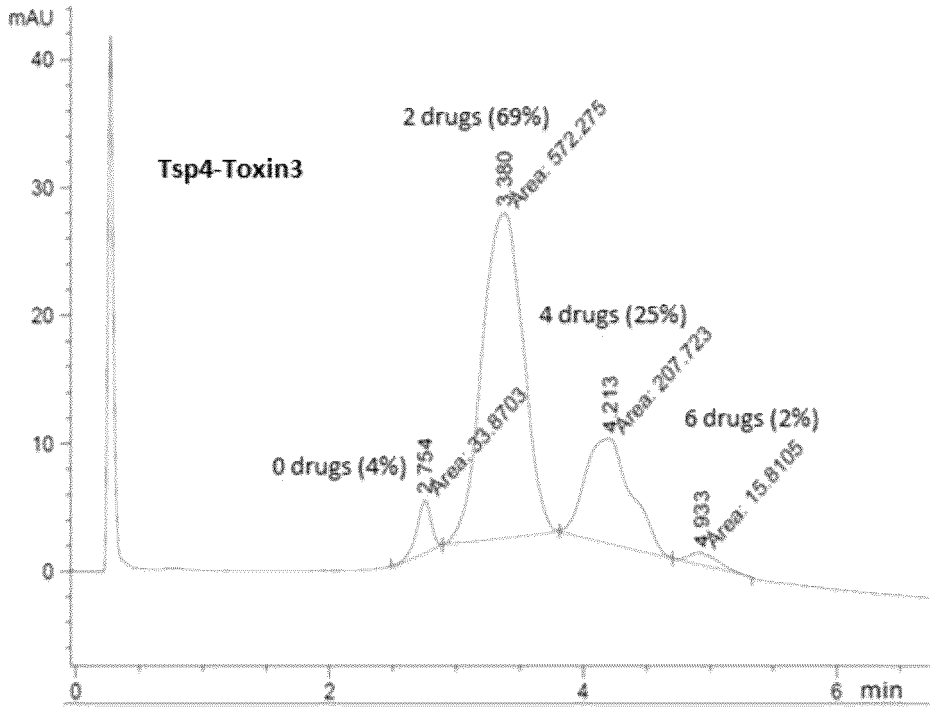

mAU
40
30
20
10
0
Tsp4-Toxin3
2 drugs (69%)
3.380
Area: 572.275
4 drugs (25%)
4.213
Area: 207.723
6 drugs (2%)
4.933
Area: 15.8105
0 drugs (4%)
2.754
Area: 33.8703
0
2
4
6 min

FIG. 35

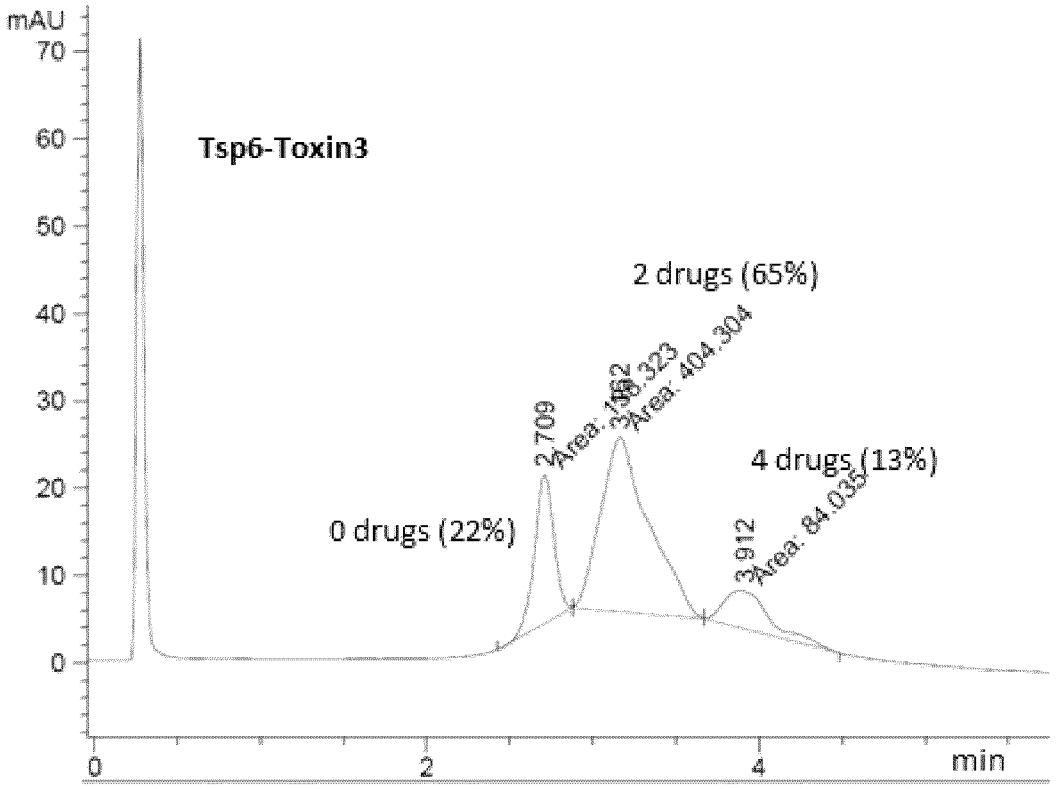

mAU
70
60
50
40
30
20
10
0
Tsp6-Toxin3
2 drugs (65%)
2.709
Area: 404.304
4 drugs (13%)
3.912
Area: 84.0351
0 drugs (22%)
0
2
4
min

FIG. 36

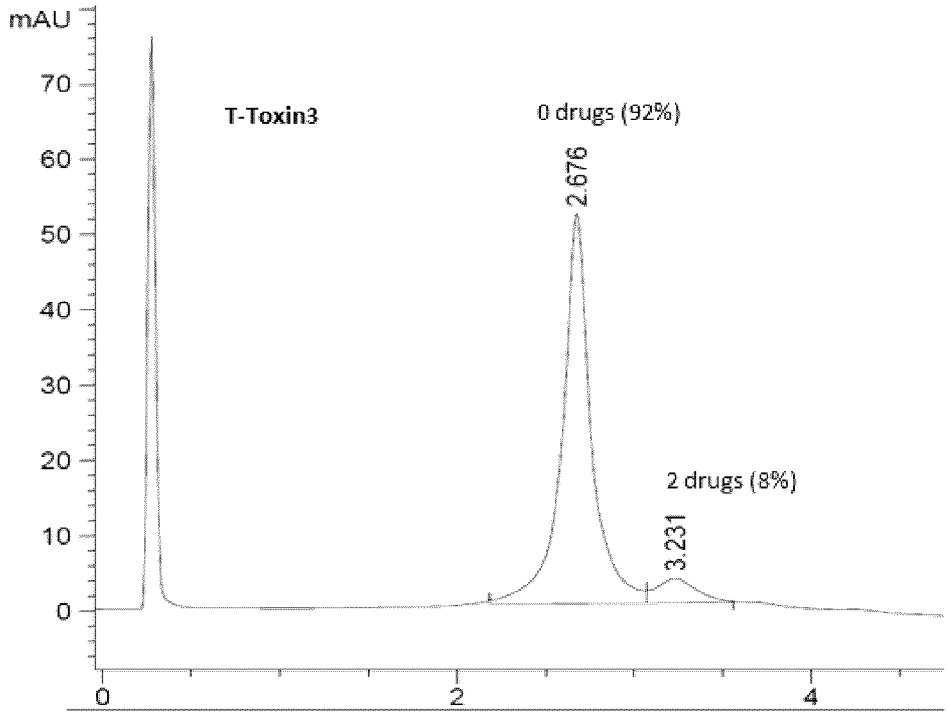

mAU
70
60
50
40
30
20
10
0
T-Toxin3
0 drugs (92%)
2.676
2 drugs (8%)
3.231
0
2
4

FIG. 37

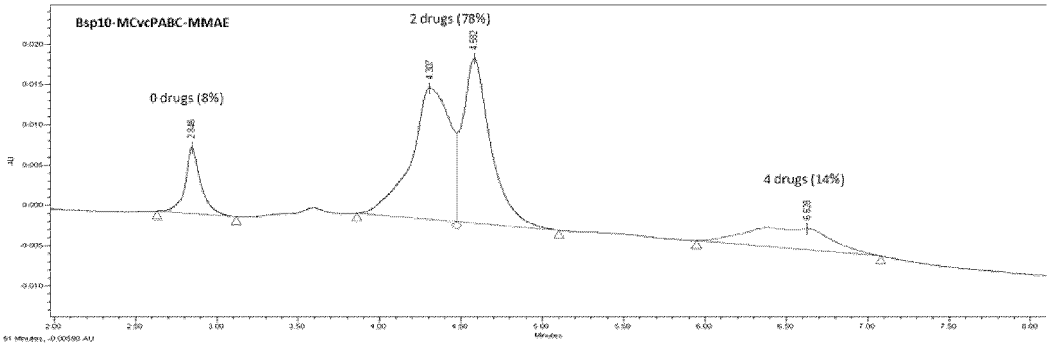

Bsp10-MCvcPABC-MMAE
0 drugs (8%)
2 drugs (78%)
4 drugs (14%)

FIG. 38

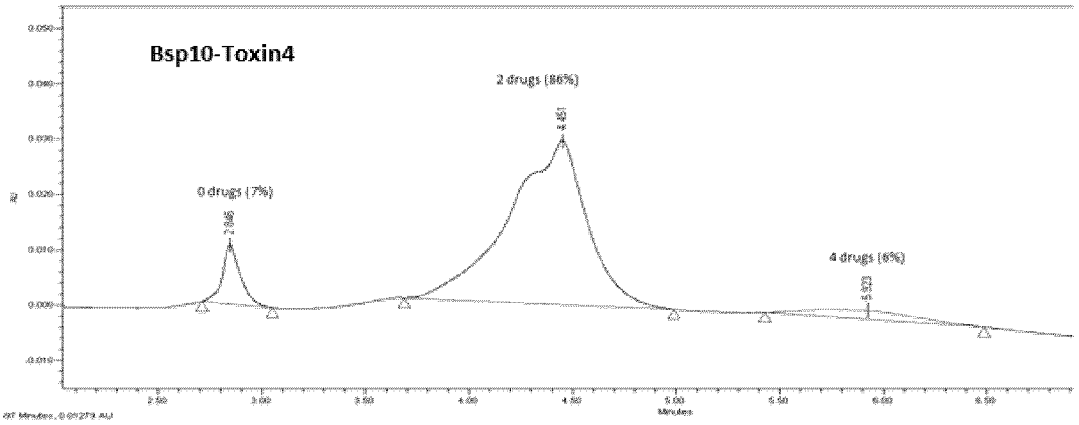


FIG. 39

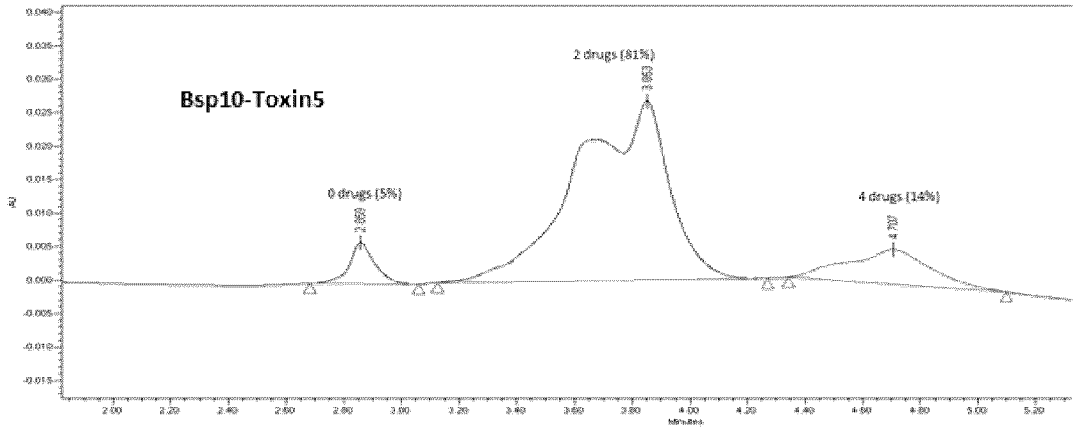


FIG. 40

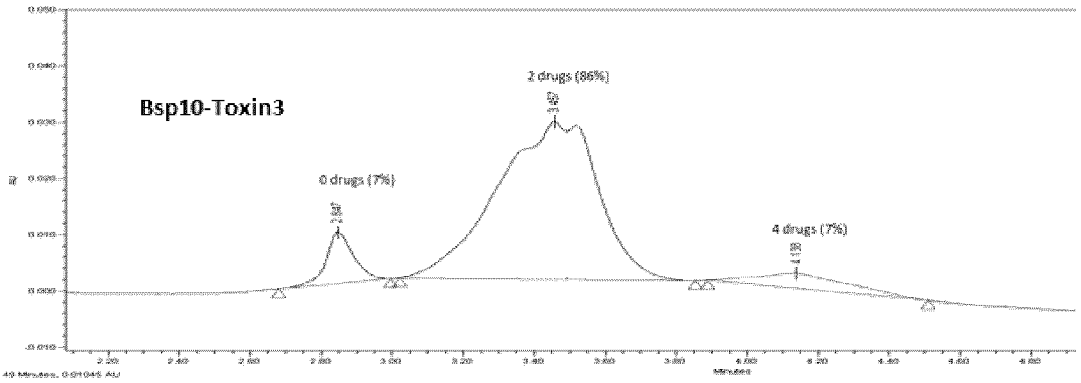


FIG. 41

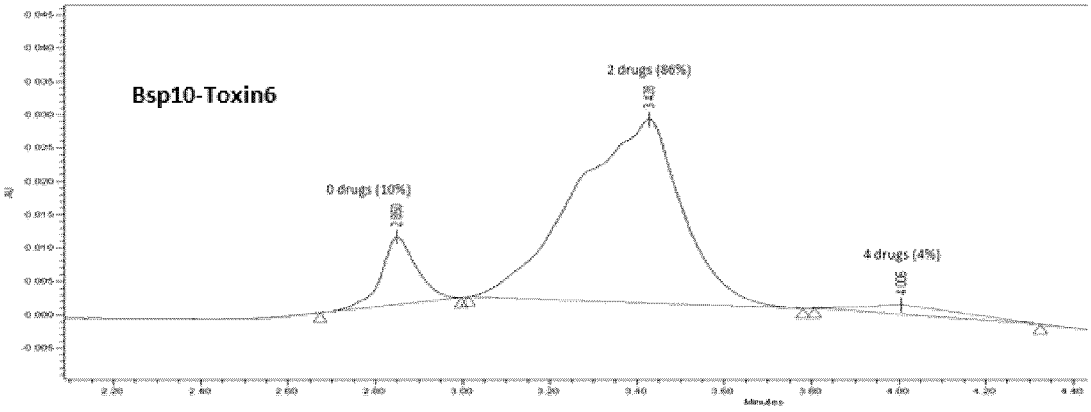

Bsp10-Toxin6
0 drugs (10%)
2 drugs (86%)
4 drugs (4%)

FIG. 42

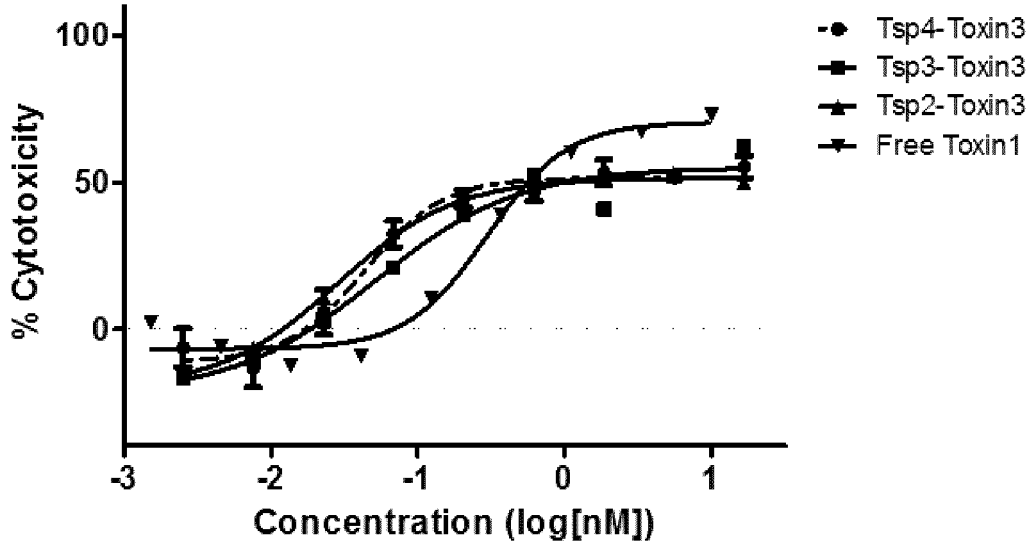

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
Tsp4-Toxin3
Tsp3-Toxin3
Tsp2-Toxin3
Free Toxin1

FIG. 43

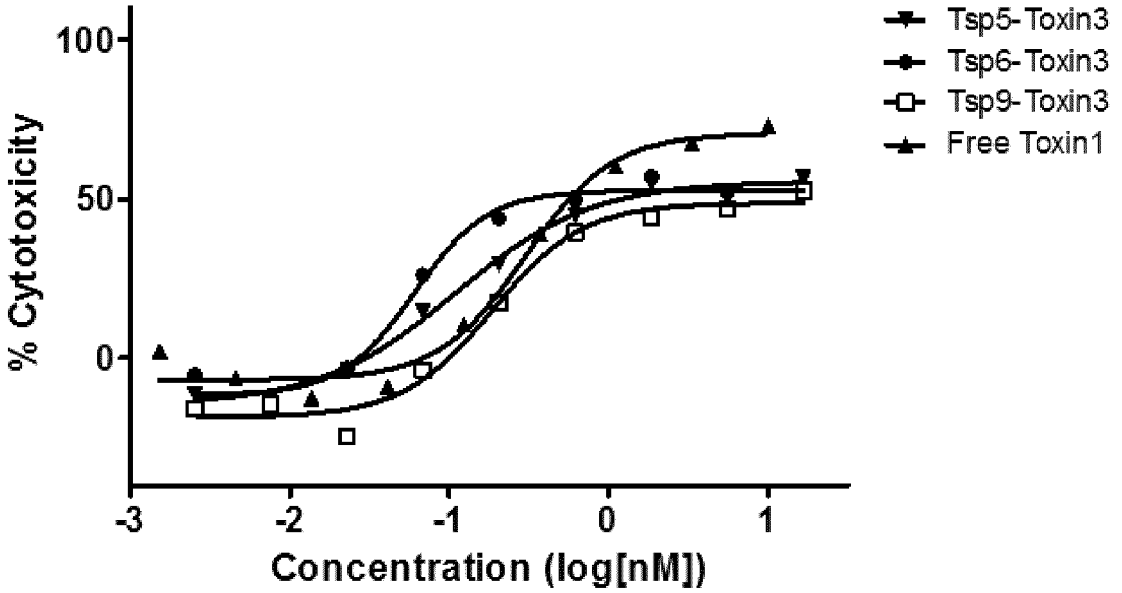

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
Tsp5-Toxin3
Tsp6-Toxin3
Tsp9-Toxin3
Free Toxin1

FIG. 44

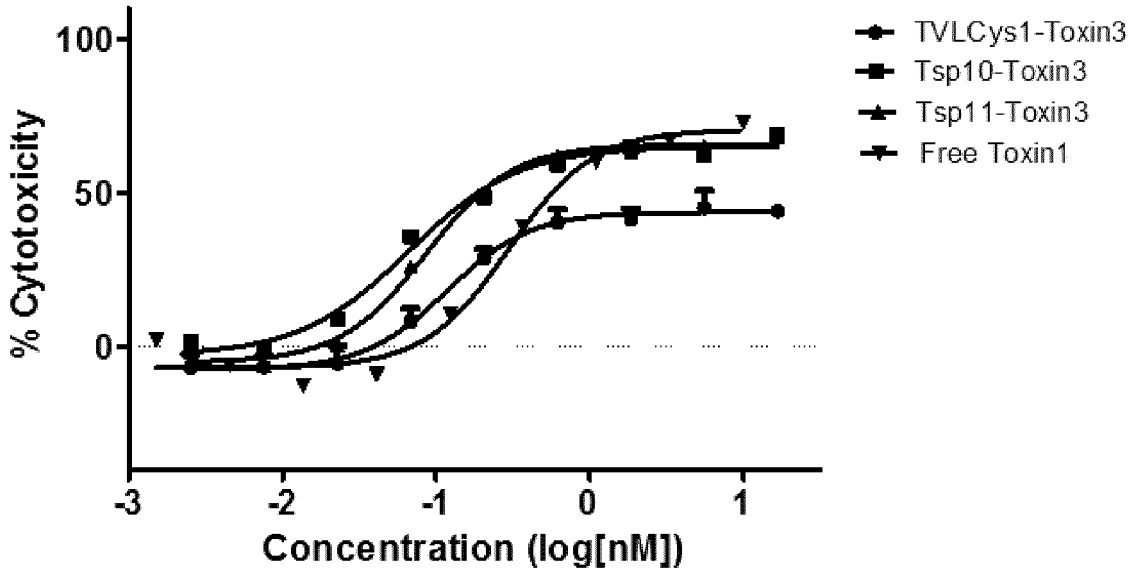

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
TVLCys1-Toxin3
Tsp10-Toxin3
Tsp11-Toxin3
Free Toxin1

FIG. 45

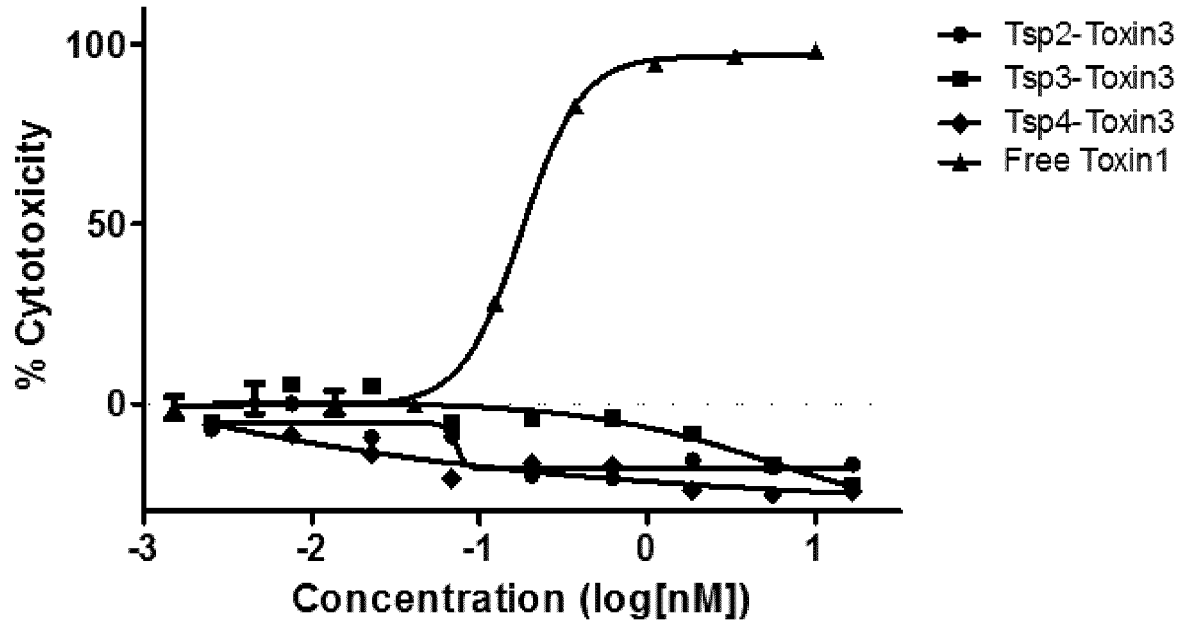

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
Tsp2-Toxin3
Tsp3-Toxin3
Tsp4-Toxin3
Free Toxin1

FIG. 46

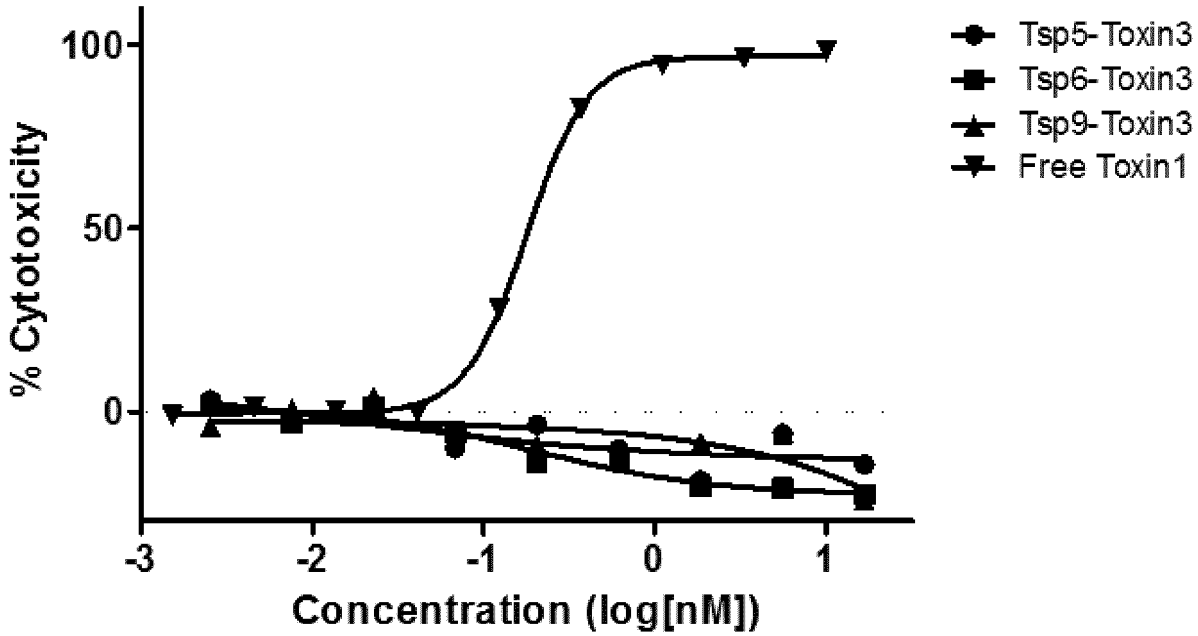

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
Tsp5-Toxin3
Tsp6-Toxin3
Tsp9-Toxin3
Free Toxin1

FIG. 47

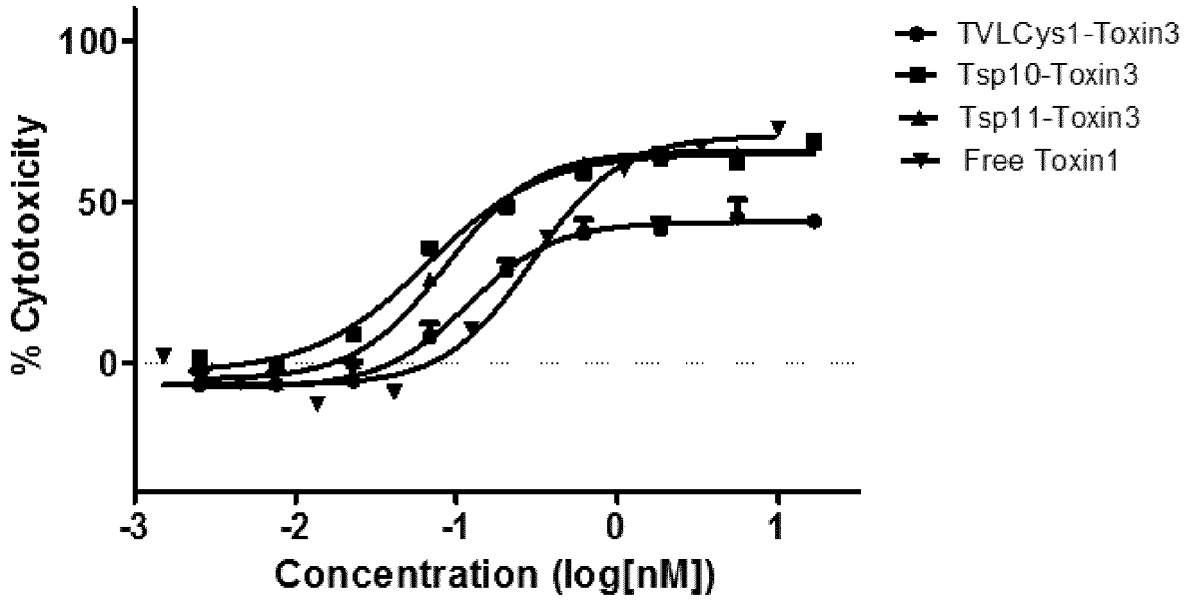

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
TVLCys1-Toxin3
Tsp10-Toxin3
Tsp11-Toxin3
Free Toxin1

FIG. 48

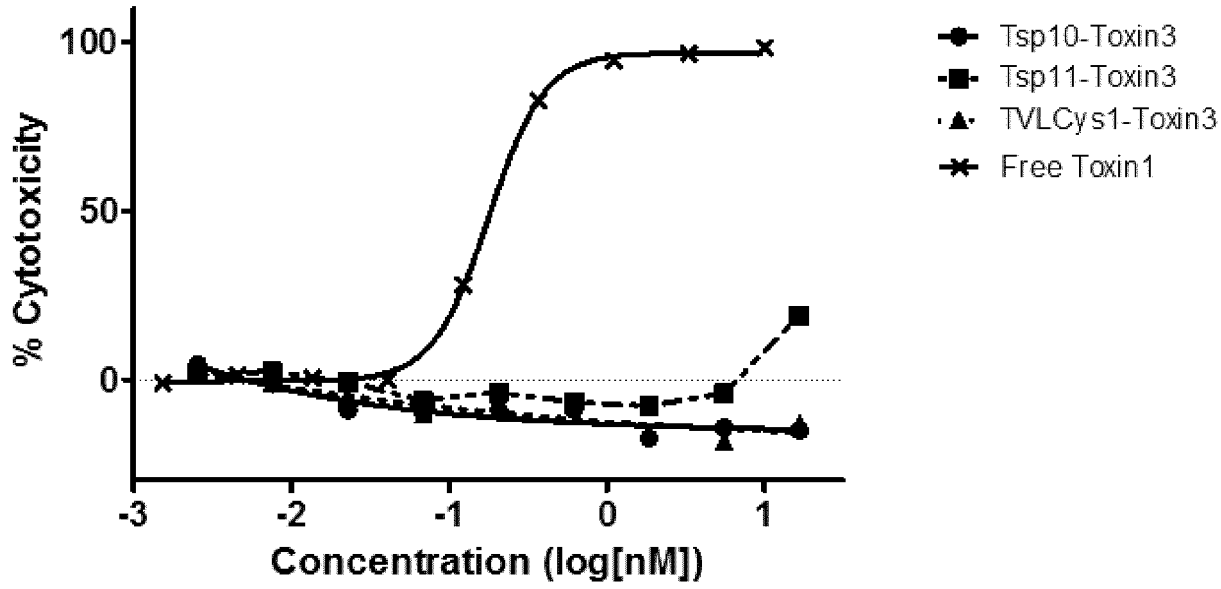

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
Concentration (log[nM])
Tsp10-Toxin3
Tsp11-Toxin3
TVLCys1-Toxin3
Free Toxin1

FIG. 49

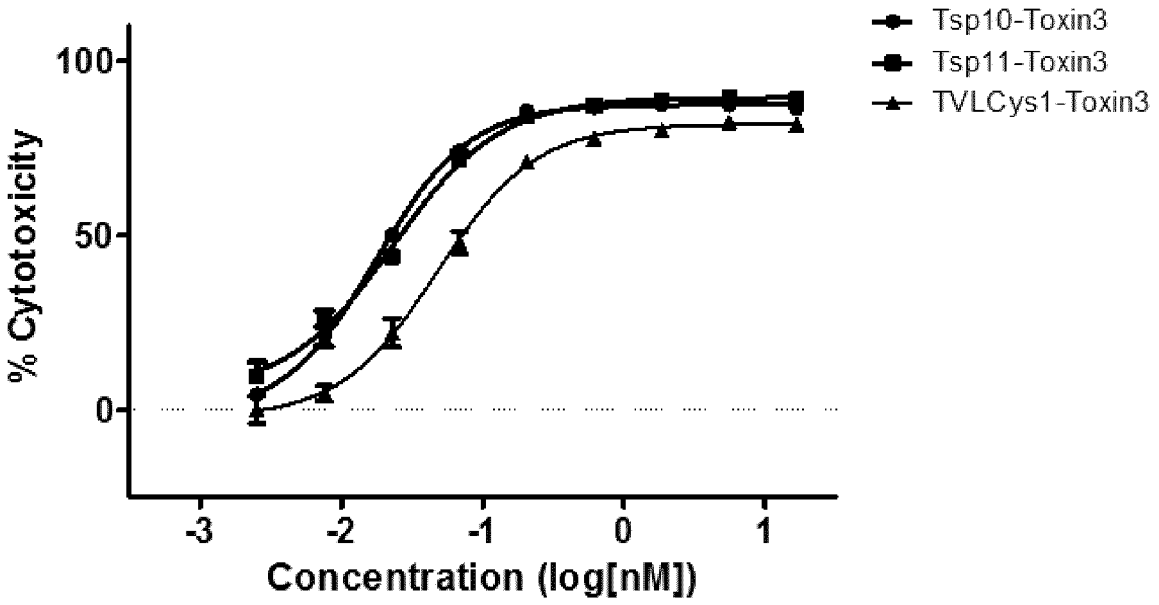

Tsp10-Toxin3
Tsp11-Toxin3
TVLCys1-Toxin3
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
Concentration (log[nM])

FIG. 50

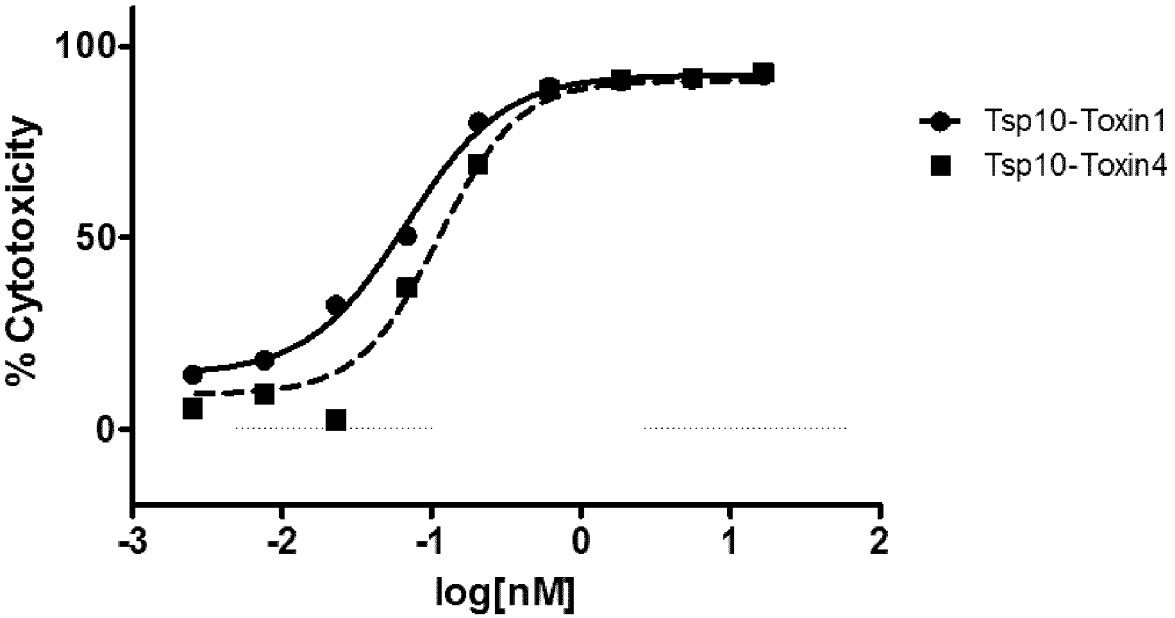

Tsp10-Toxin1
Tsp10-Toxin4
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
2
log[nM]

FIG. 51

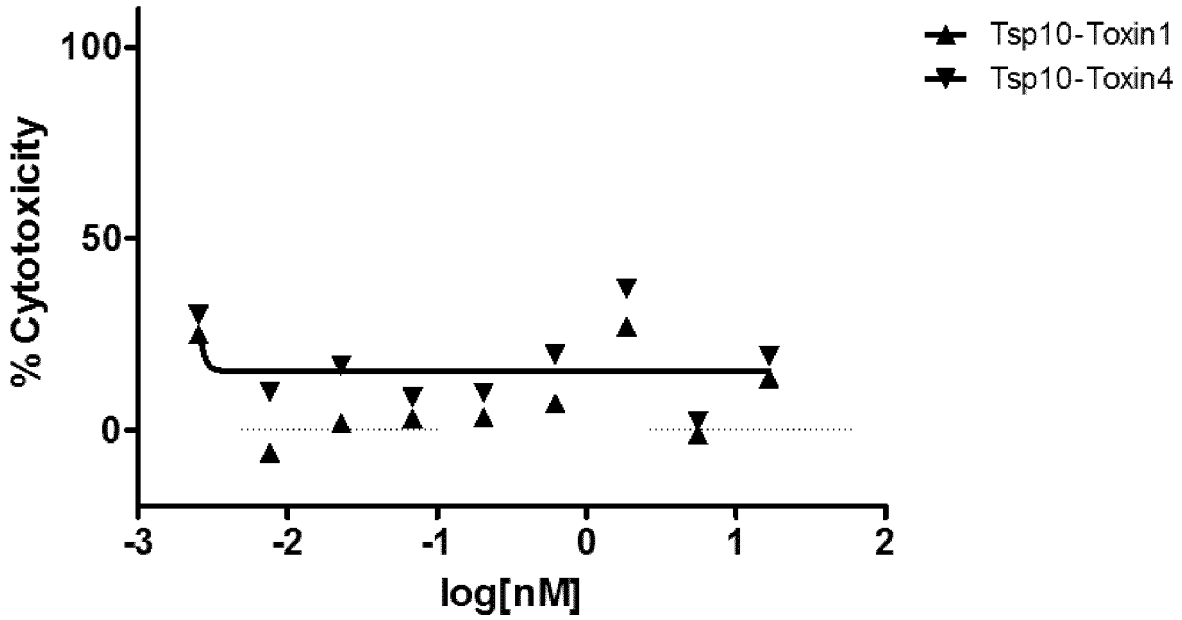

Tsp10-Toxin1
Tsp10-Toxin4
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
2
log[nM]

FIG. 52

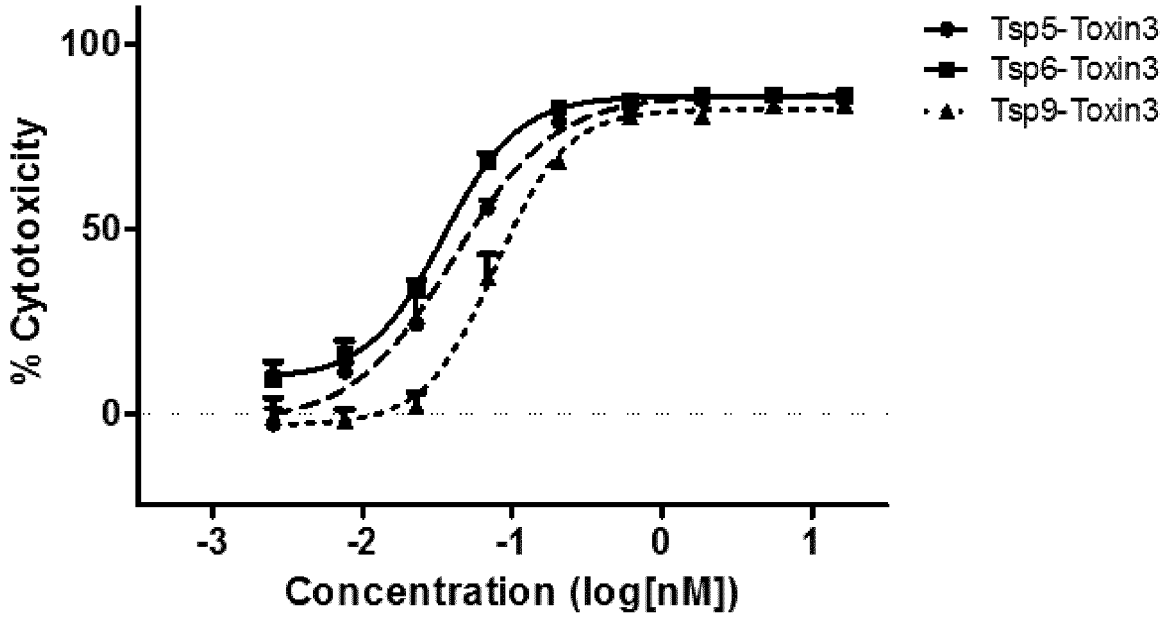

Tsp5-Toxin3
Tsp6-Toxin3
Tsp9-Toxin3
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
Concentration (log[nM])

FIG. 53

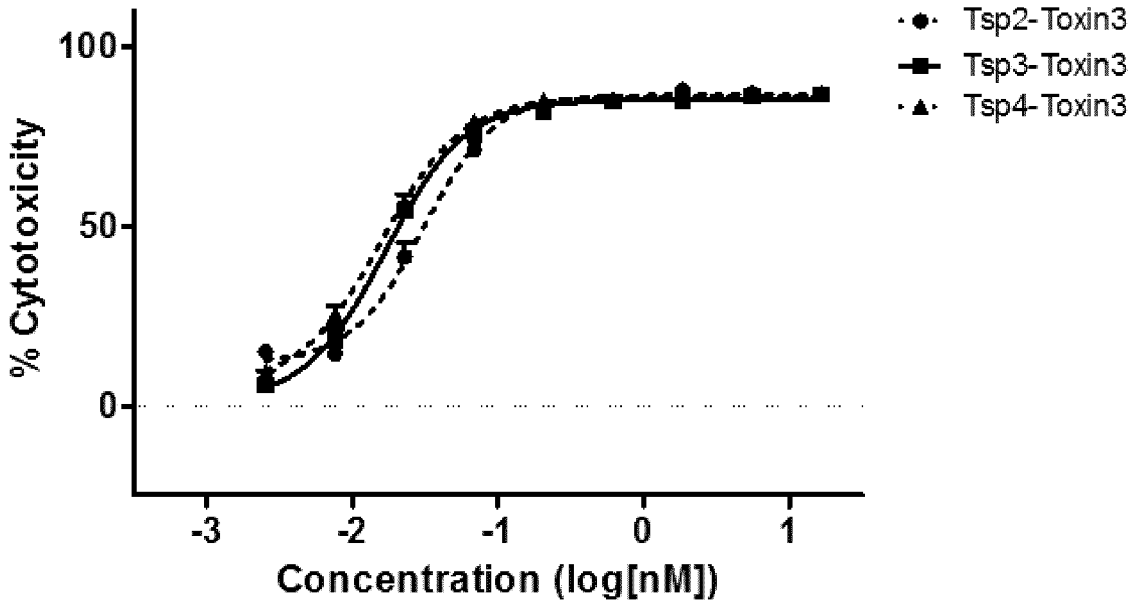

Tsp2-Toxin3
Tsp3-Toxin3
Tsp4-Toxin3
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
Concentration (log[nM])

FIG. 54

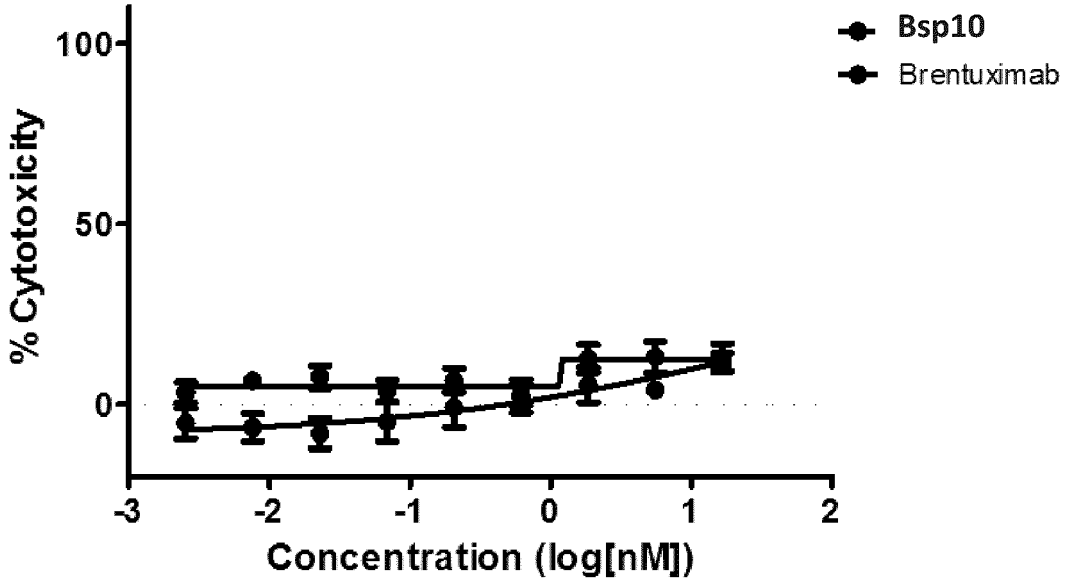

Bsp10
Brentuximab
% Cytotoxicity
100
50
0
-3
-2
-1
0
1
2
Concentration (log[nM])

FIG. 55

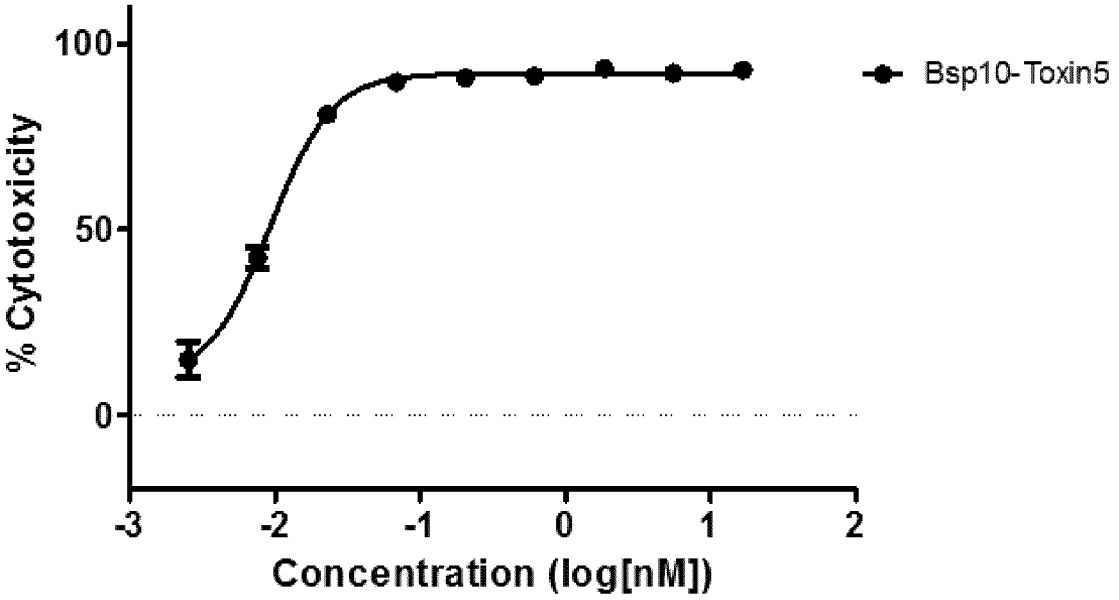

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
2
Concentration (log[nM])
Bsp10-Toxin5

FIG. 56

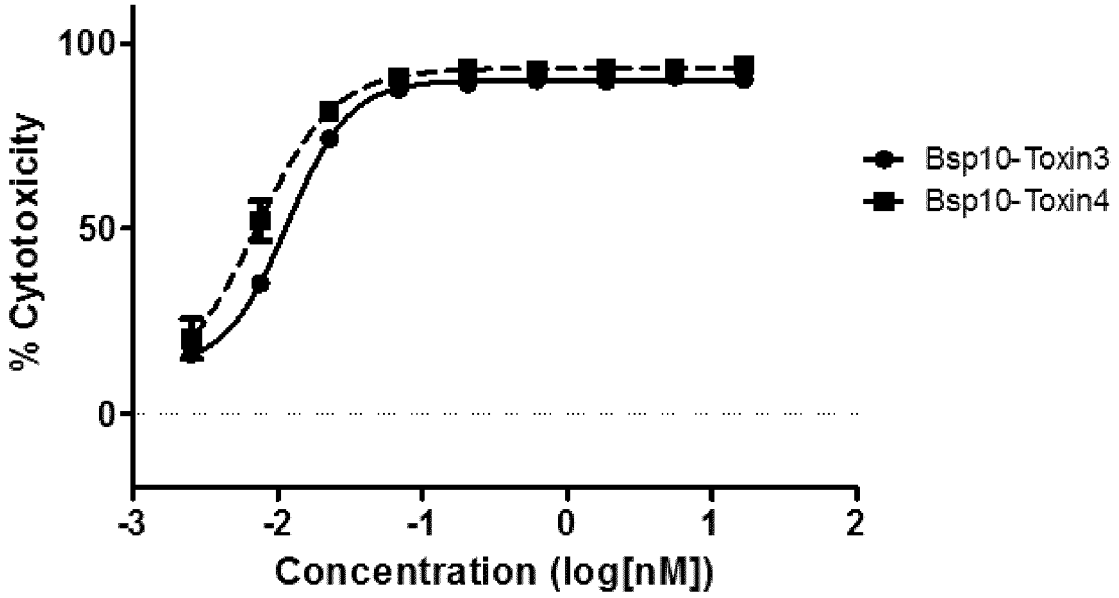

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
2
Concentration (log[nM])
Bsp10-Toxin3
Bsp10-Toxin4

FIG. 57

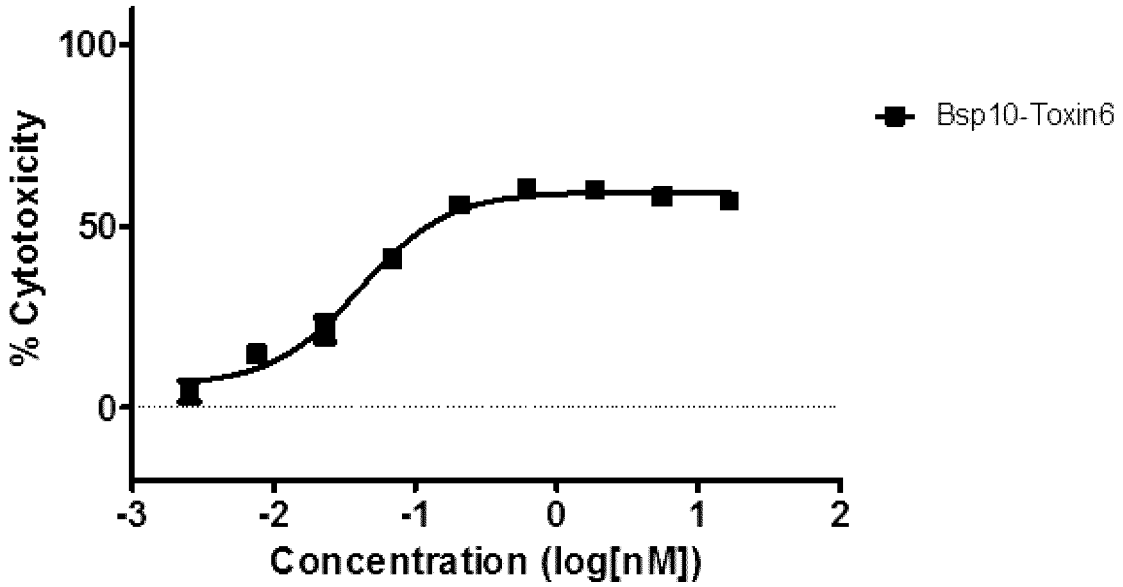

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
2
Concentration (log[nM])
Bsp10-Toxin6

FIG. 58

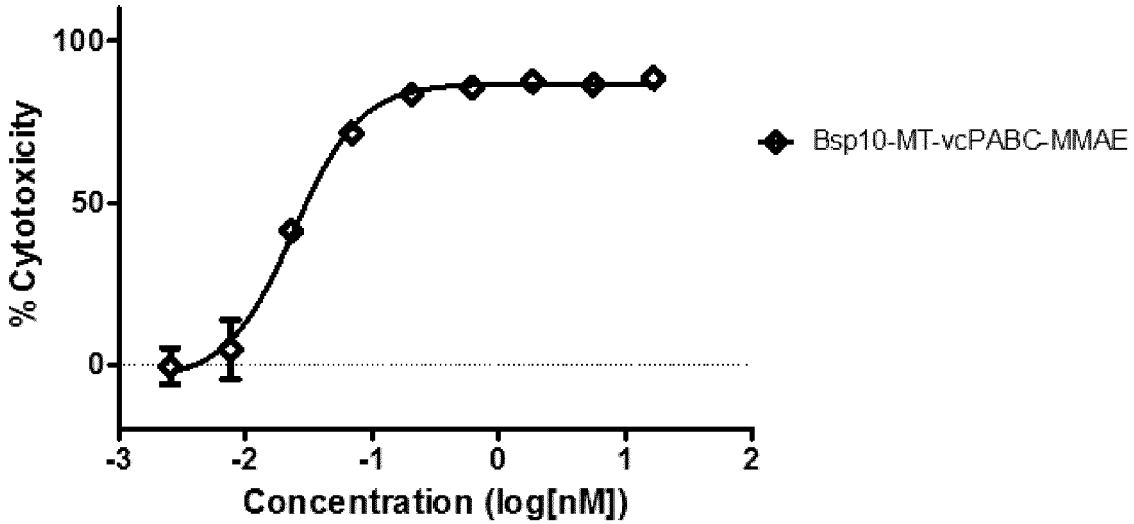

100
50
0
% Cytotoxicity
-3
-2
-1
0
1
2
Concentration (log[nM])
Bsp10-MT-vcPABC-MMAE

FIG. 59

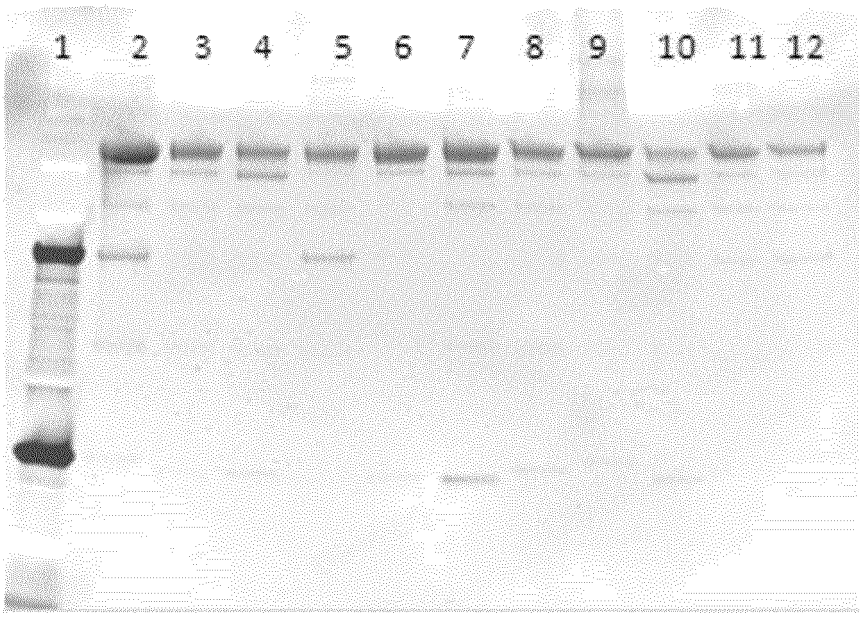


FIG. 60

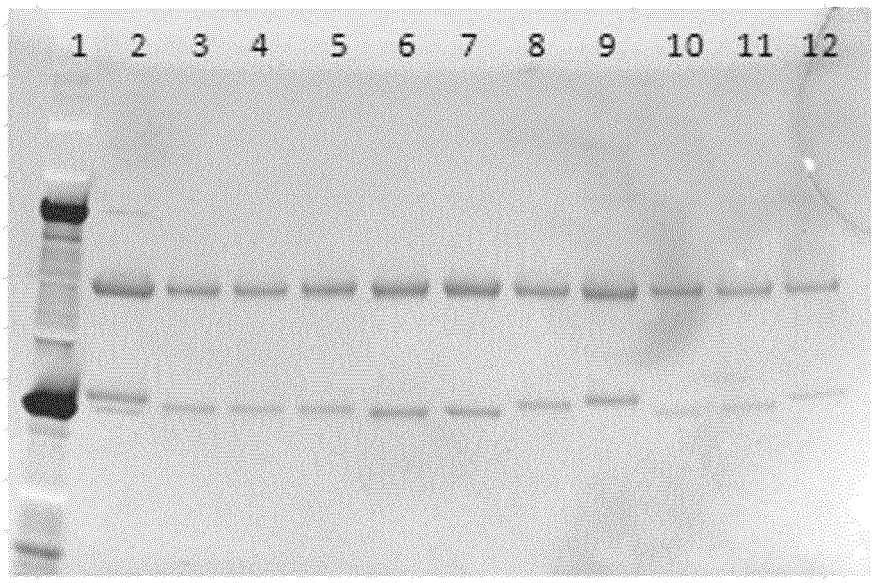


FIG. 61

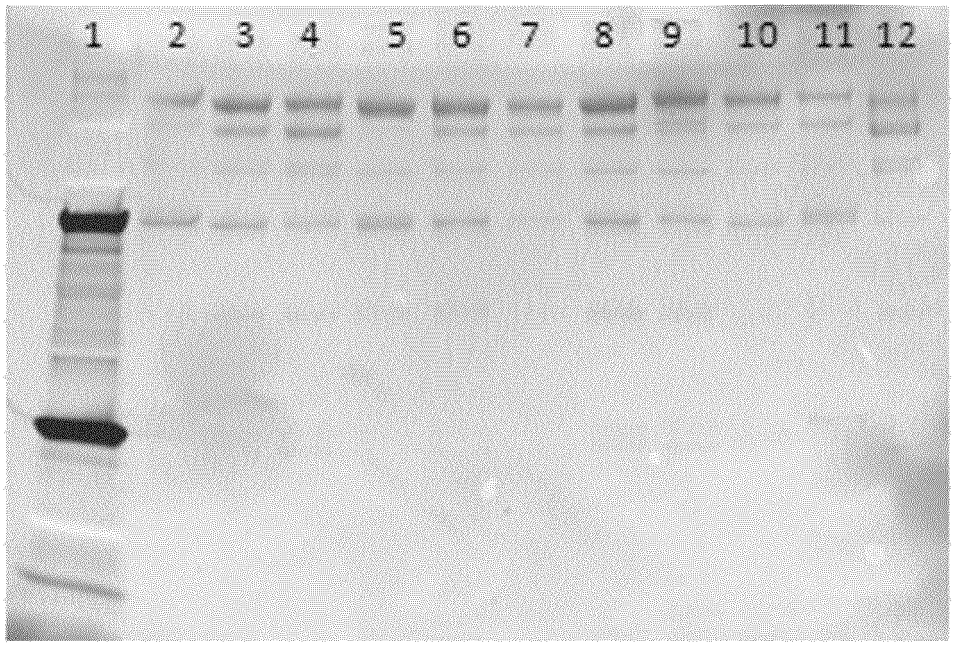


FIG. 62

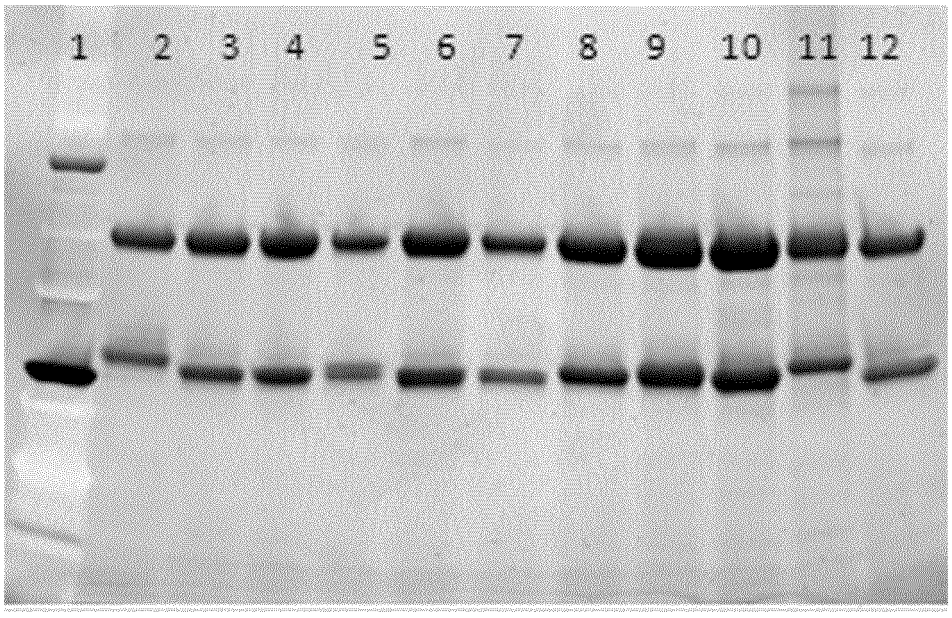
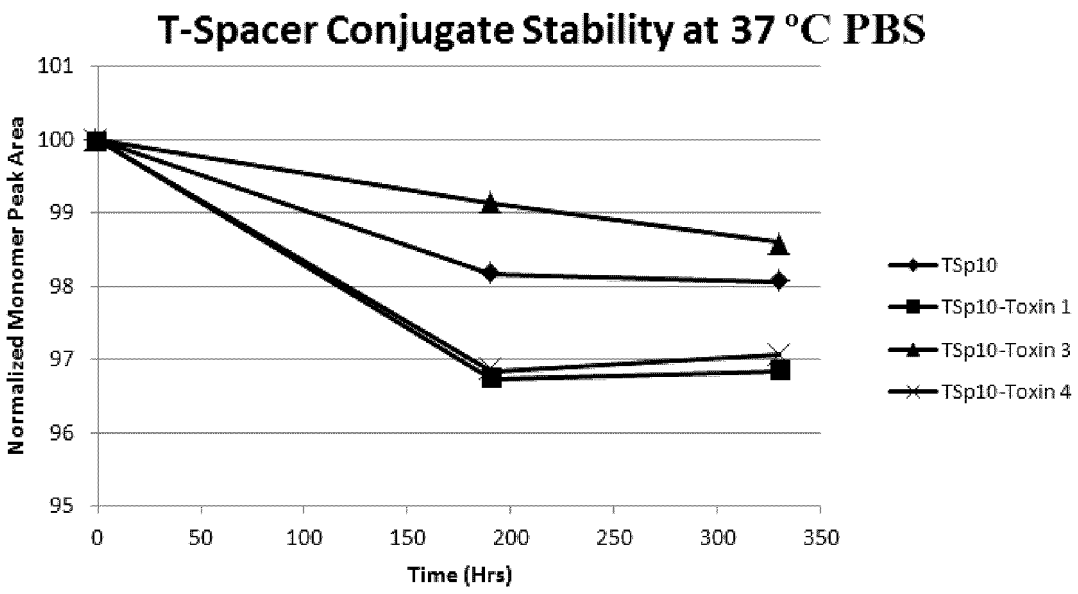
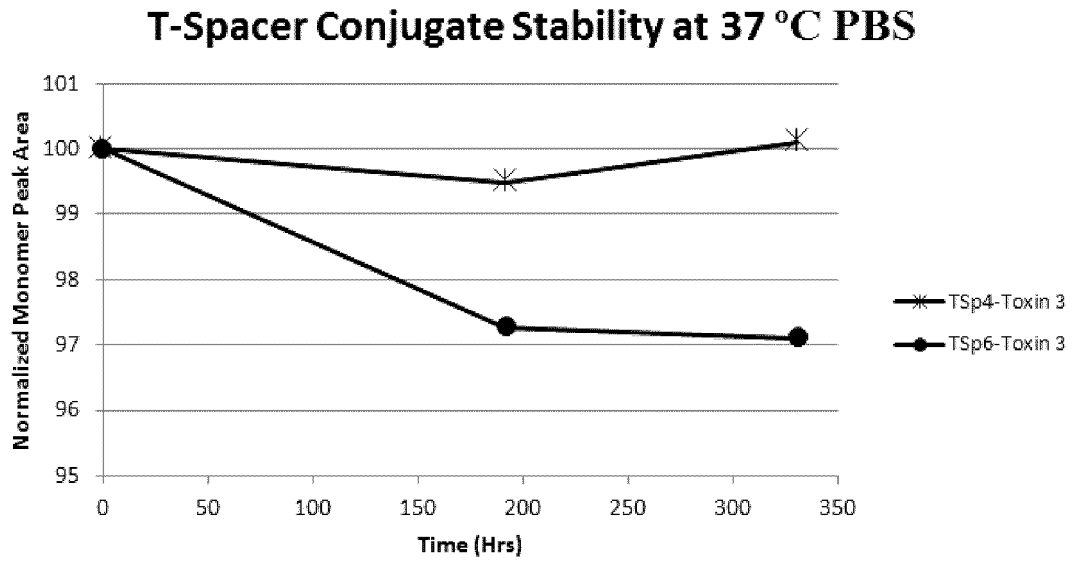


FIG. 63

A


T-Spacer Conjugate Stability at 37 °C PBS
Normalized Monomer Peak Area
101
100
99
98
97
96
95
0
50
100
150
200
250
300
350
Time (Hrs)
TSp10
TSp10-Toxin 1
TSp10-Toxin 3
TSp10-Toxin 4

B


T-Spacer Conjugate Stability at 37 °C PBS
Normalized Monomer Peak Area
101
100
99
98
97
96
95
0
50
100
150
200
250
300
350
Time (Hrs)
TSp4-Toxin 3
TSp6-Toxin 3