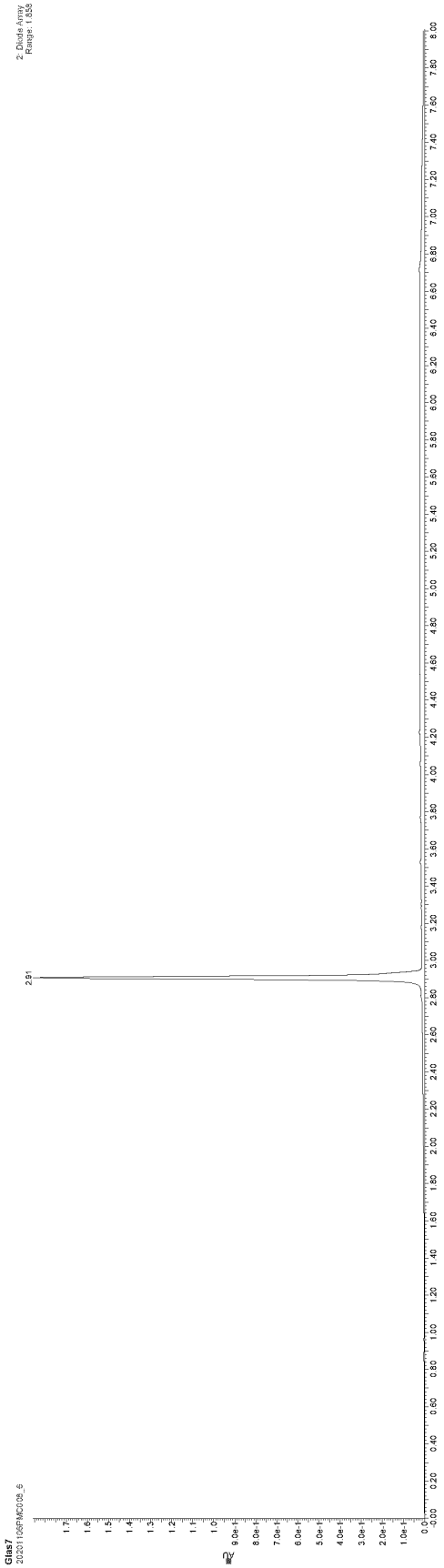


Figure 1

Figure 2

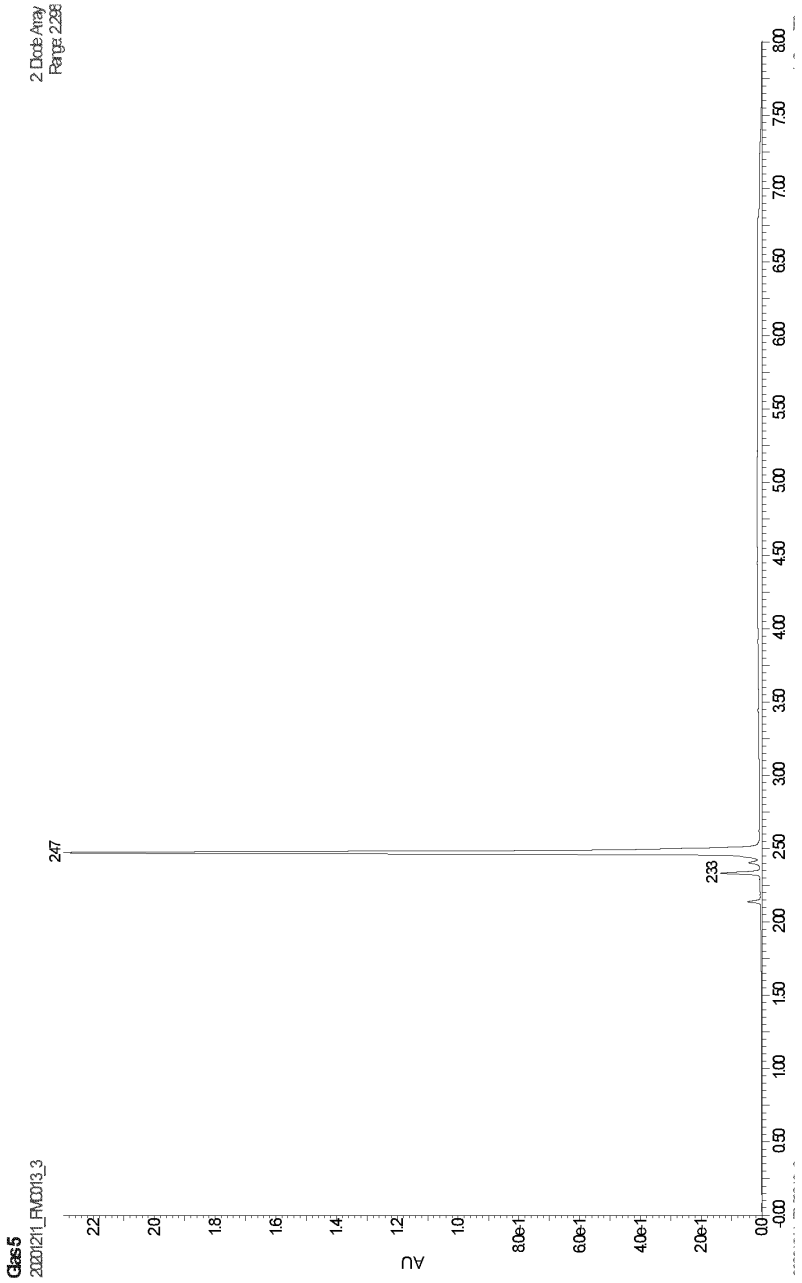


Figure 3

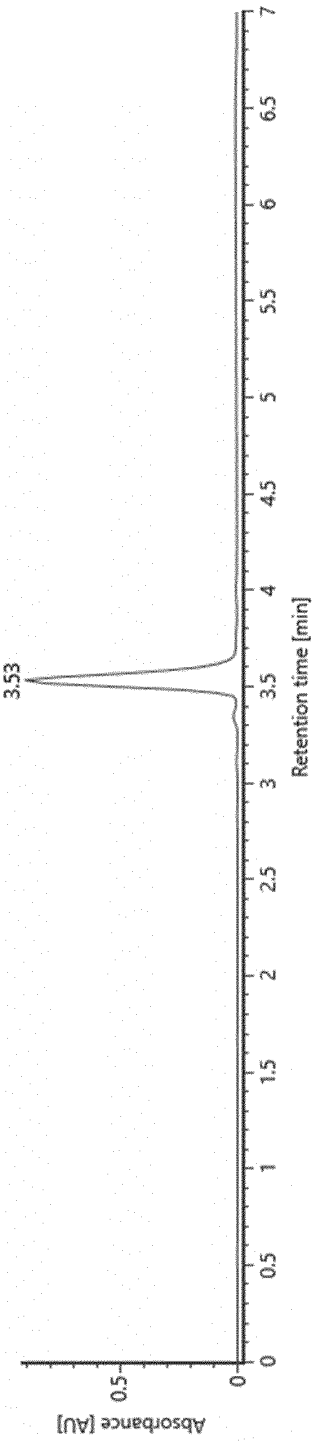


Figure 4

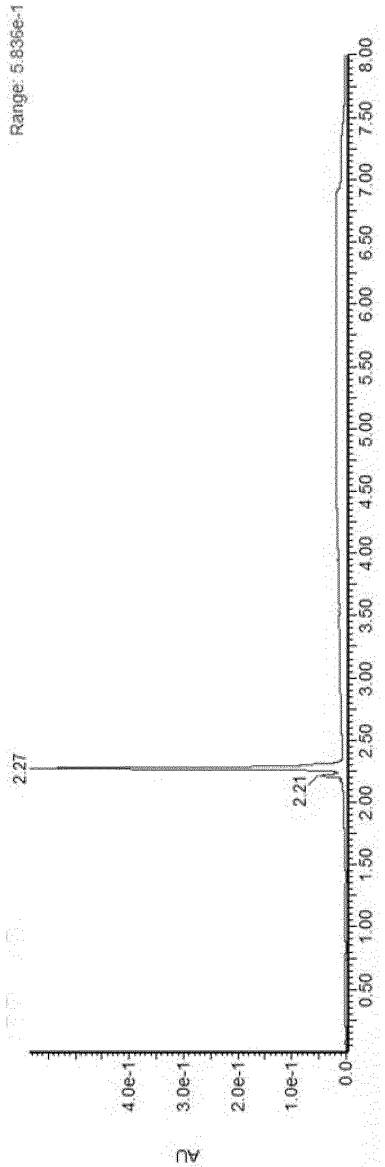


Figure 5

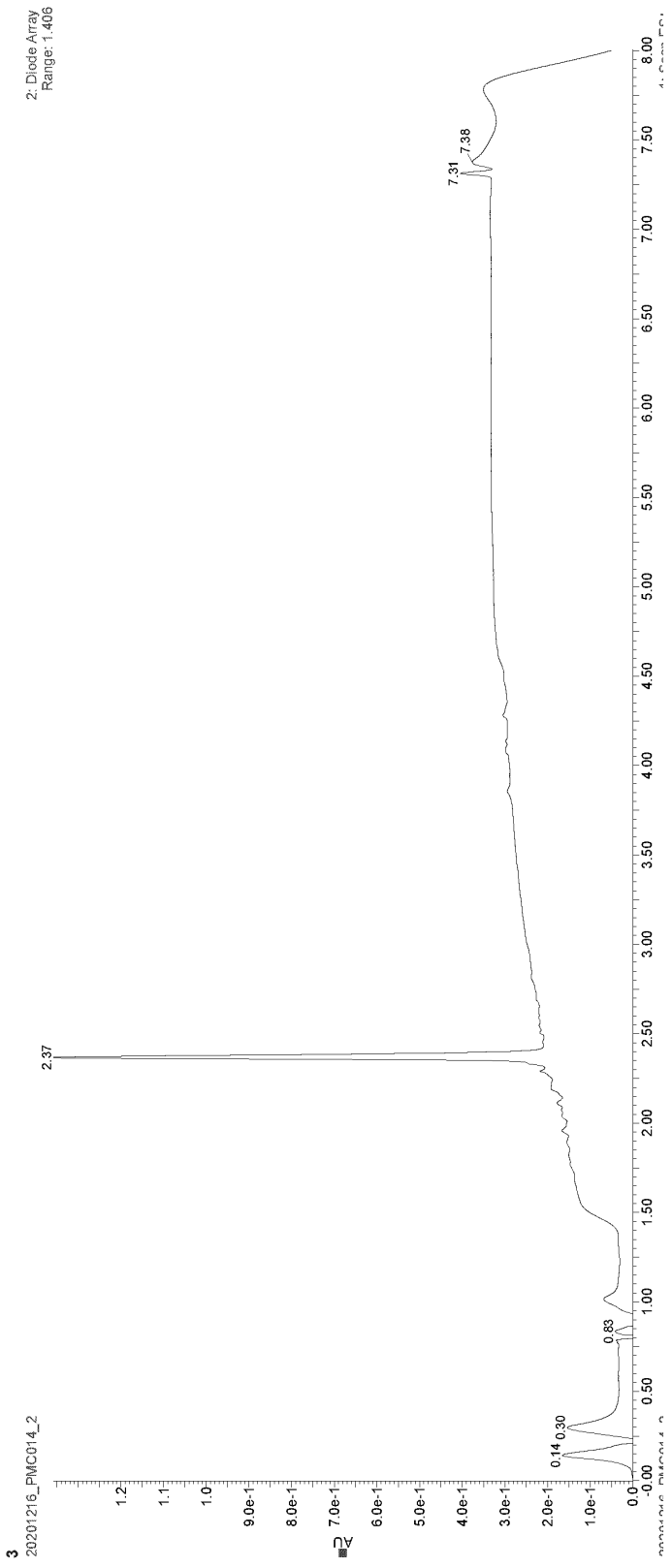

2: Diode Array
Range: 1.406
3
20201216_PMC014_2
AU
2.37
0.14
0.30
0.83
7.31
7.38

Figure 6

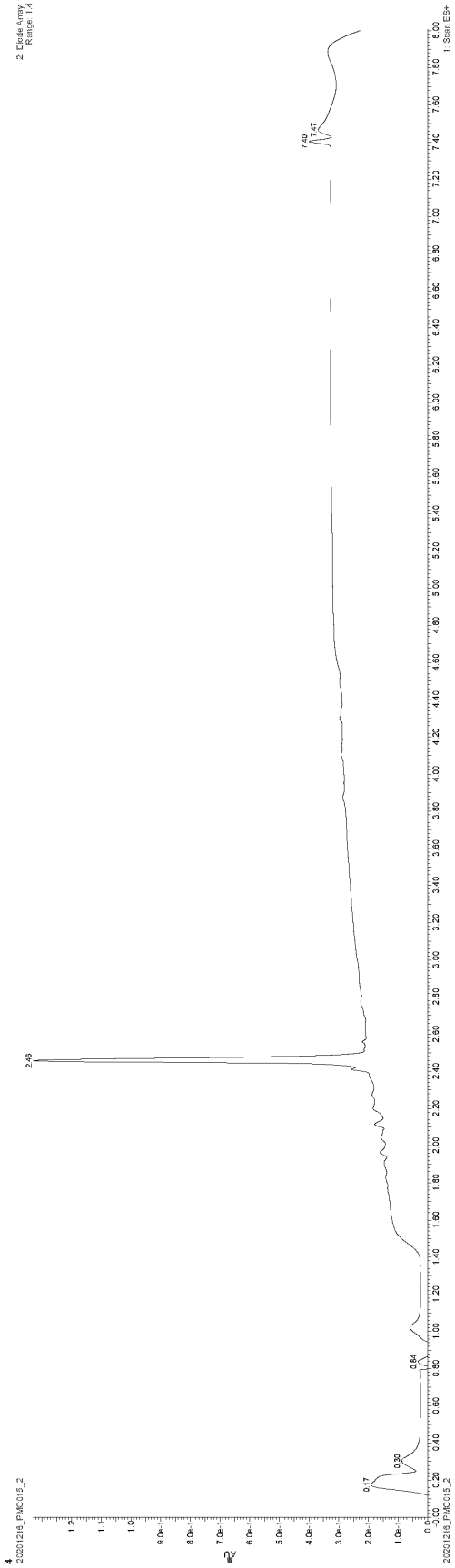

2: Diode Array
Range: 1.4
1: Scan ES+
TIC
2.46
7.40
7.47
0.84
0.30
0.17
20201216_FMC015_2
20201216_FMC015_2

Figure 7

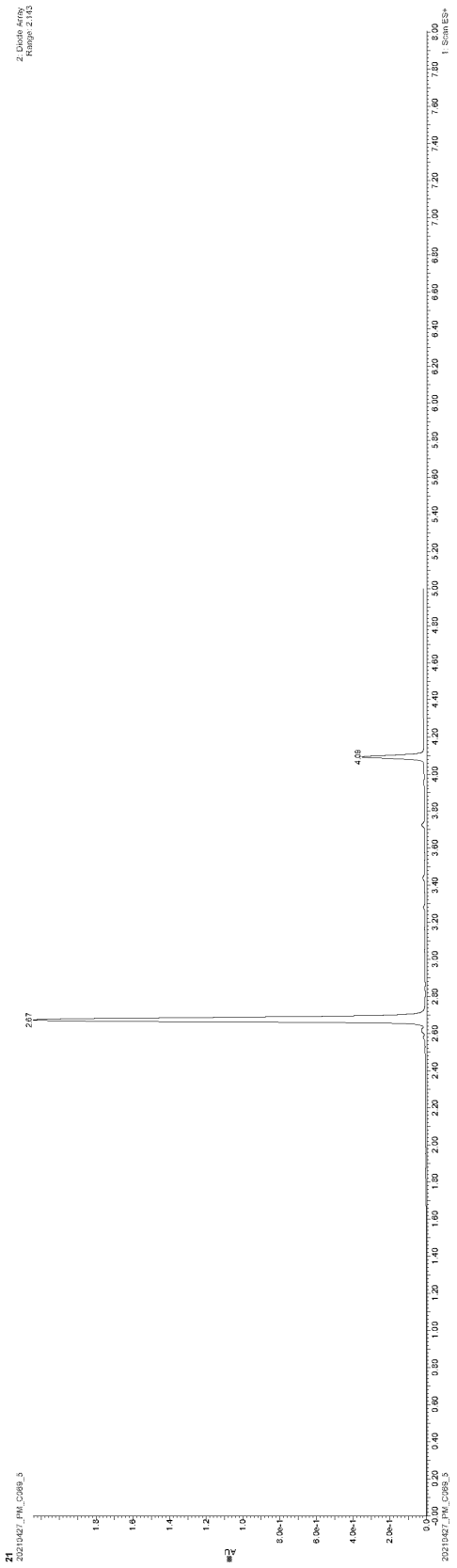

2: Diode Array
Range: 2.143
1: Scan ES+
2.67
4.08
20210427_PM_C068_3
20210427_PM_C068_3

Figure 8

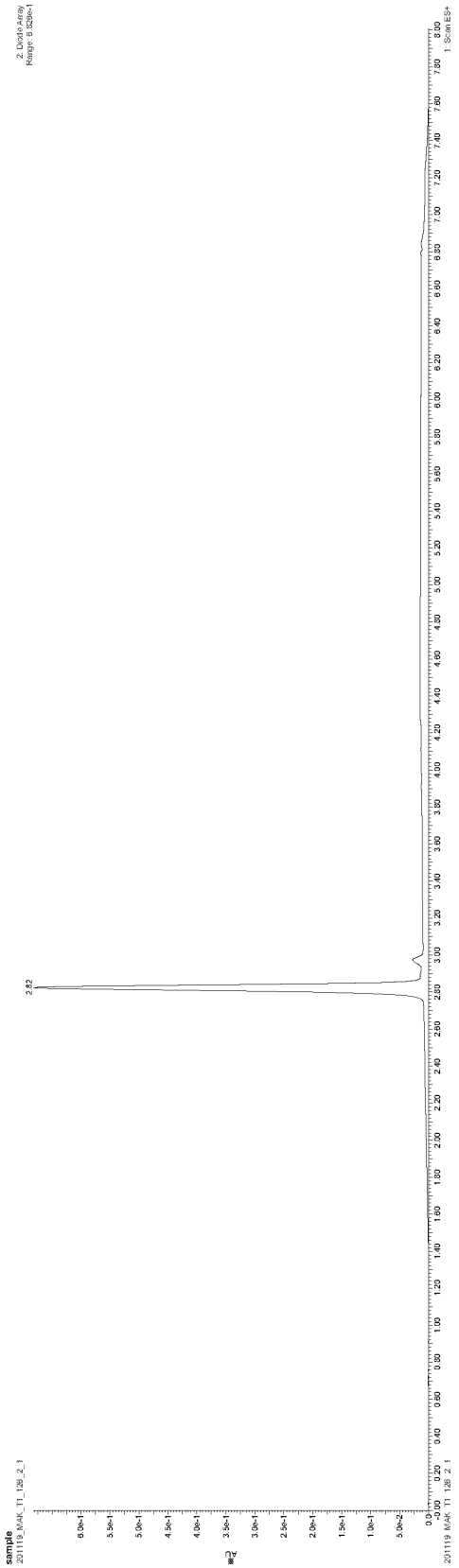

2: Diode Array
Range: 8.629e-1
1: Scan ES+
2.82
sample
20111 9_MAK_T1_129_2_1
AU
20111 9 MAK T1 129 2 1

Figure 9

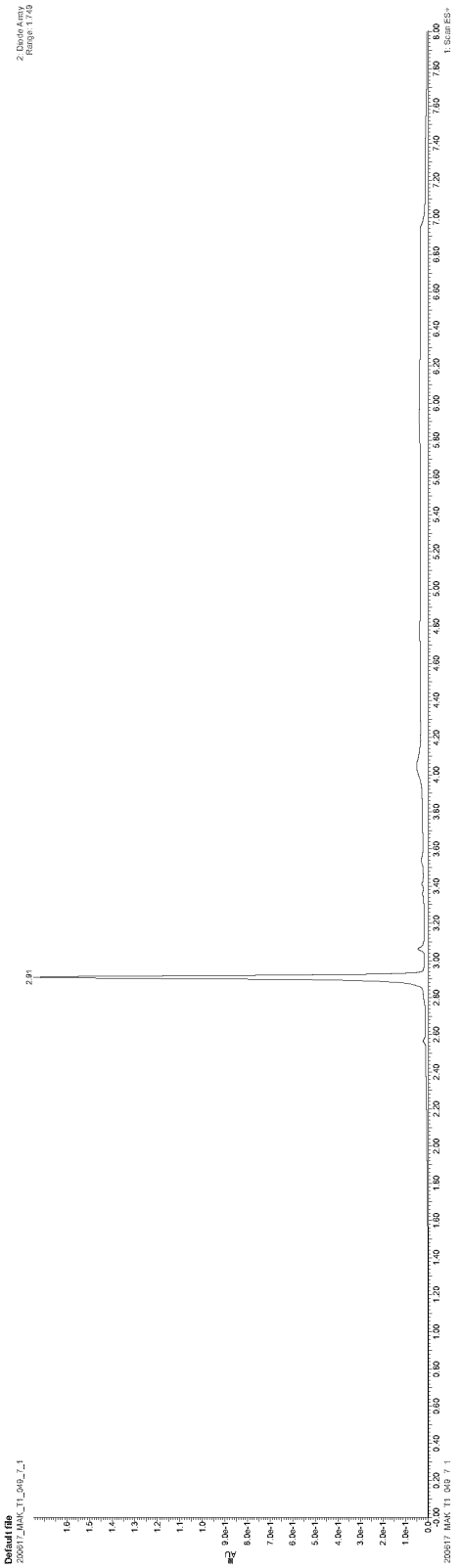

2: David Army
Range 1.749
1: Scan ES+
2.91
Default file
200617_MAR_T1_049_7_1
200617 MAR T1 049 7 1

Figure 10

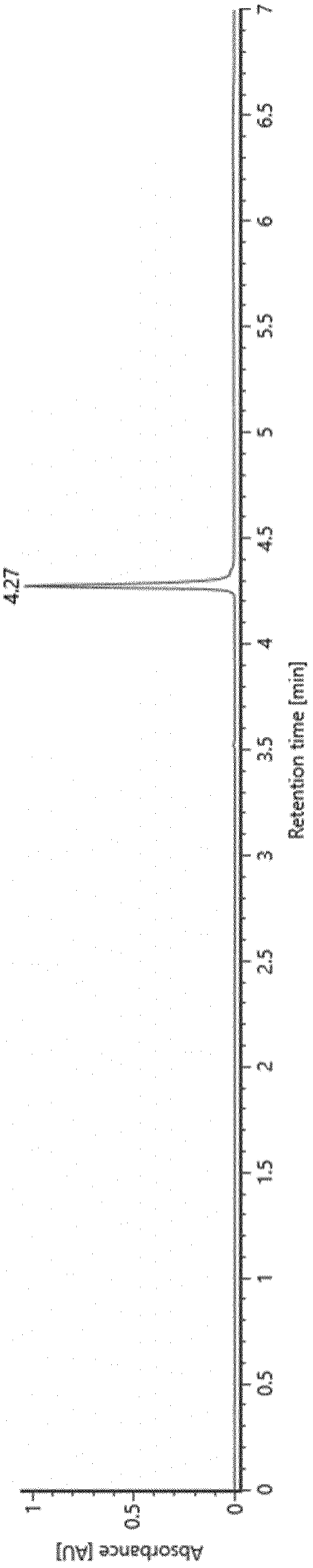

4.27
Absorbance [AU]
0
0.5
1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
Retention time [min]

Figure 11

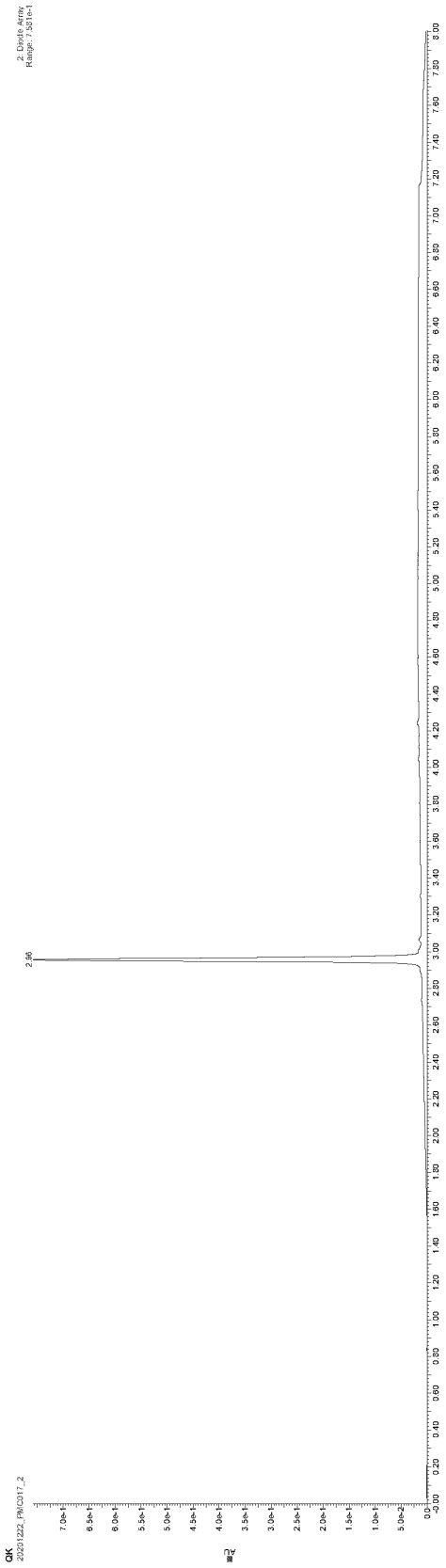


Figure 12

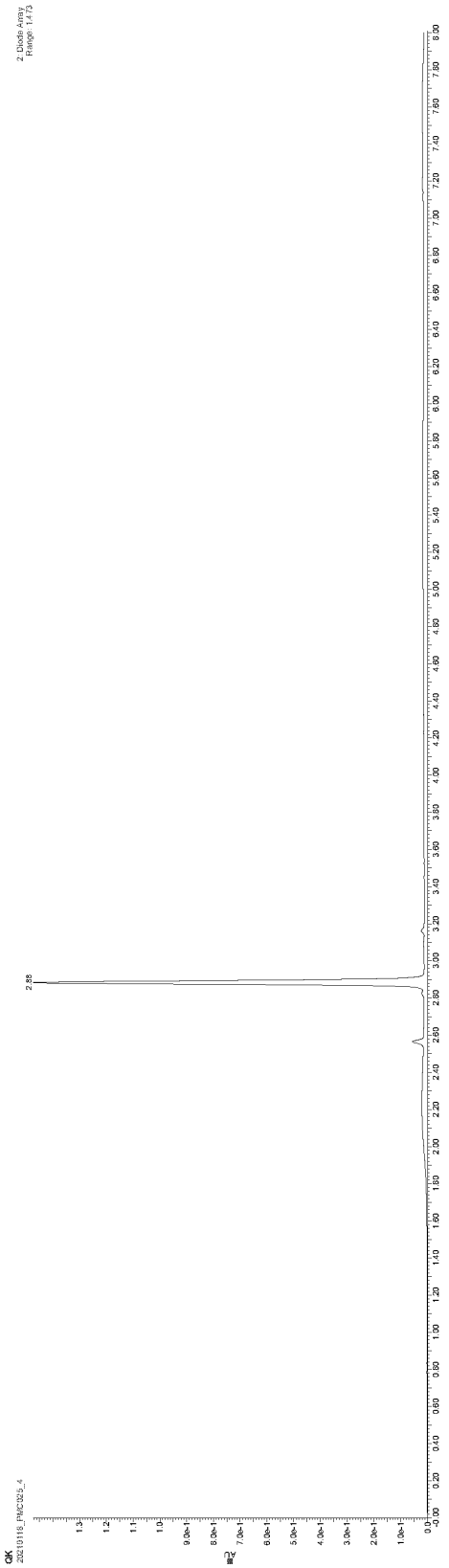


Figure 13

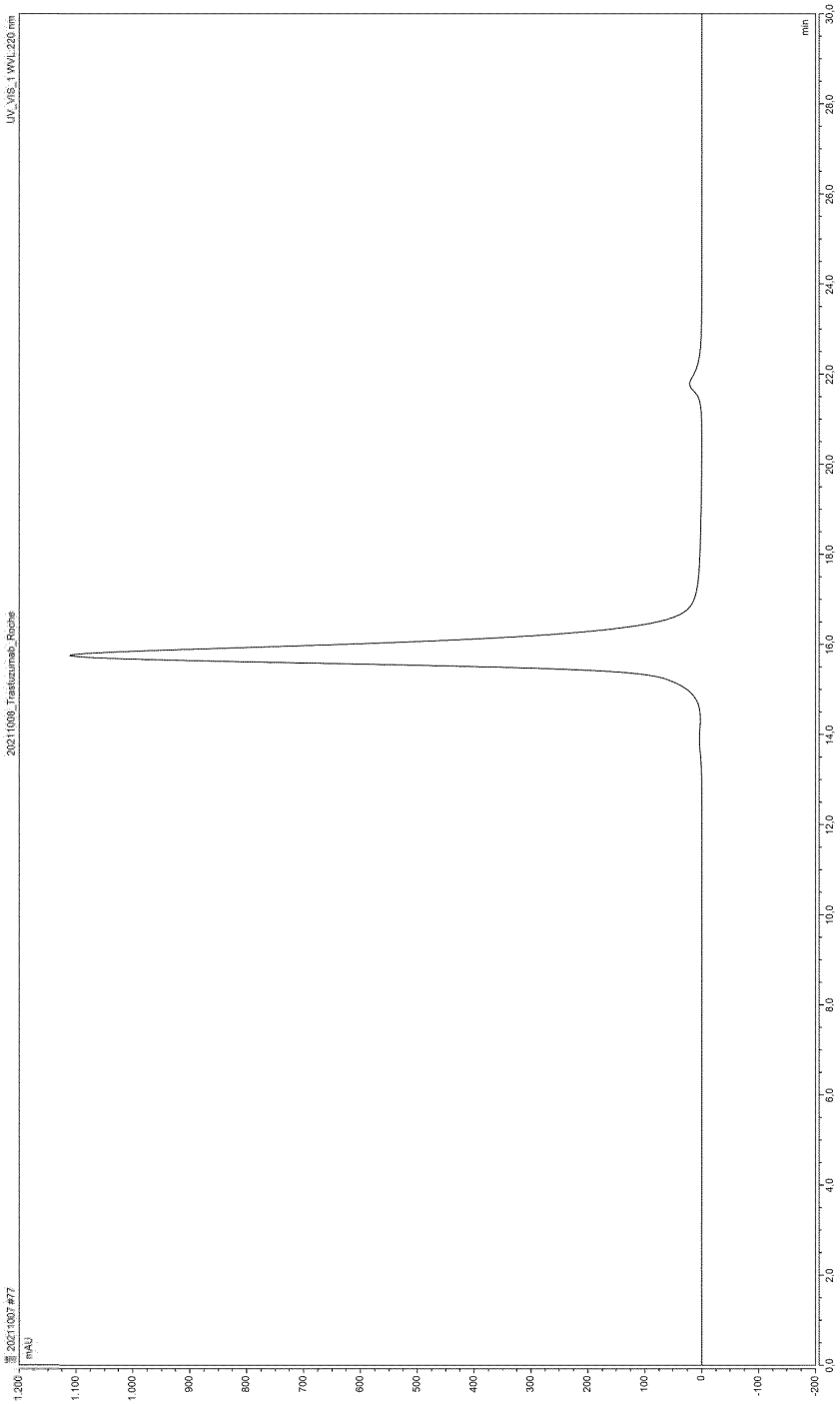

UV_VIS_1 WVL:220 nm
20211008_Trastuzumab_Roche
20211007 #77
mAU
min
1.200
1.100
1.000
900
800
700
600
500
400
300
200
100
0
-100
-200
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0

Figure 14

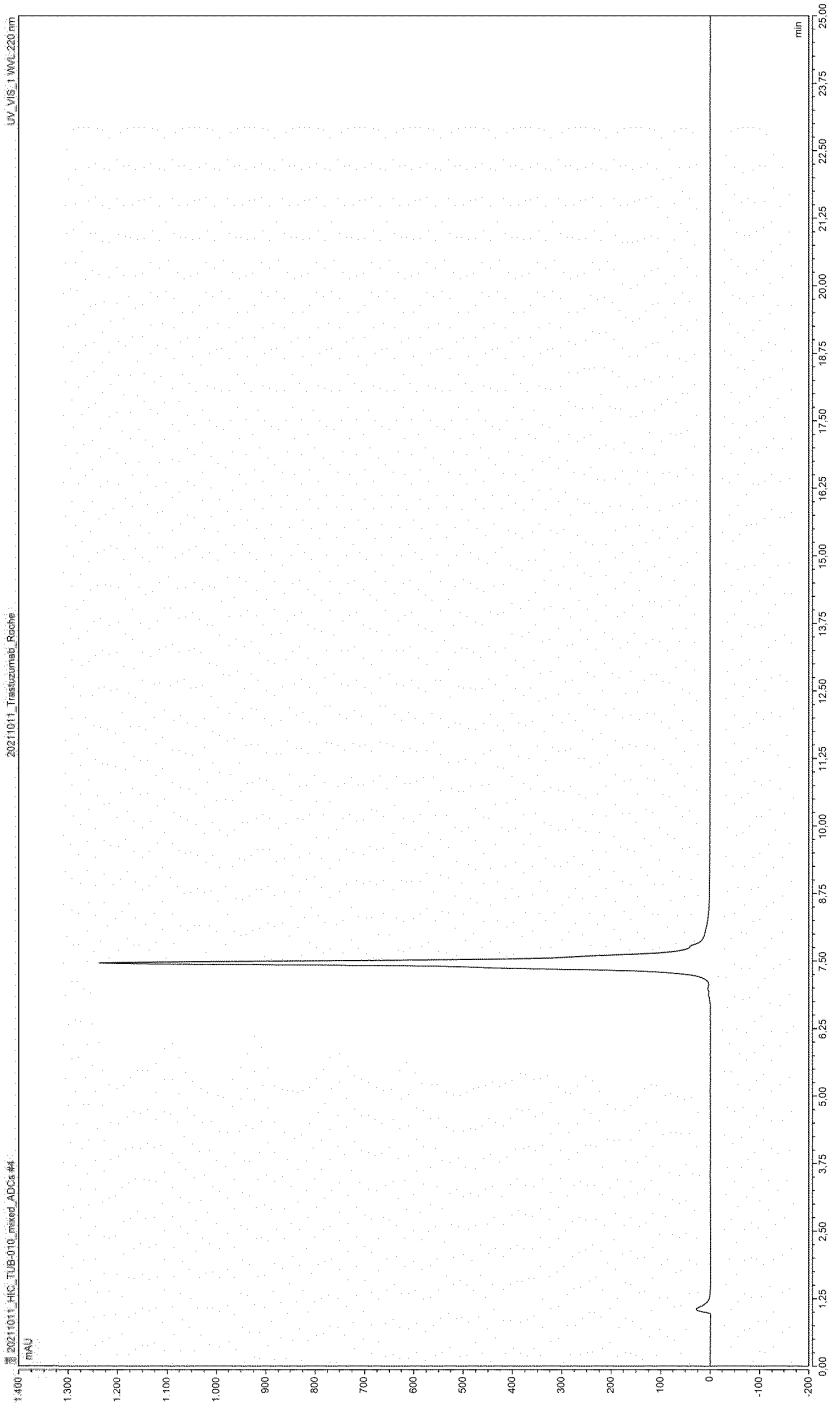

20211011_HIC_TUB-010_mixed_ADCs #4
UV_VIS_1 WvL:220 nm
20211011_Trastuzumab_Roche
mAU
min

Figure 15

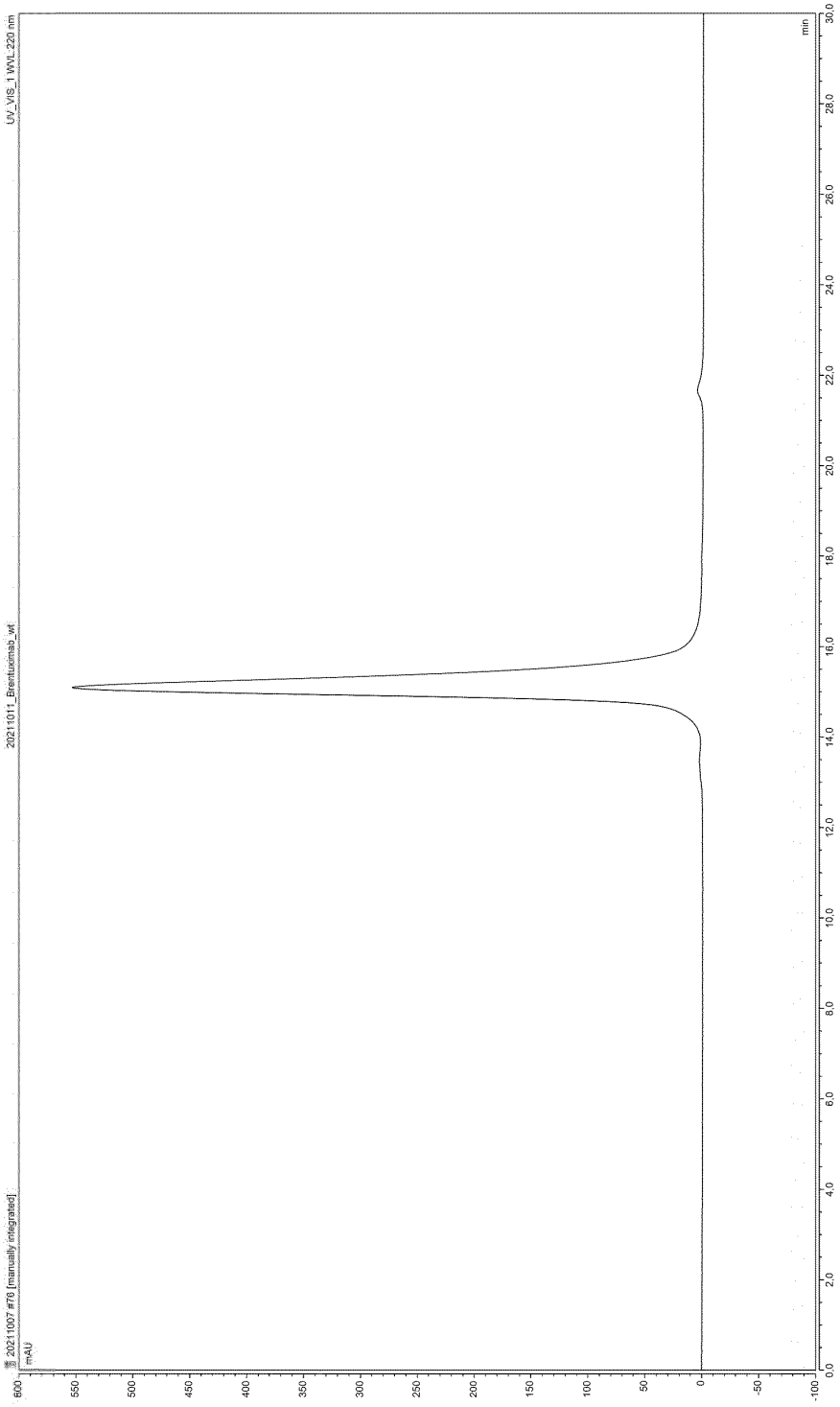

UV_VIS_1 WVL:220 nm
20211011_Brentuximab_wt
20211007 #76 [manually integrated]
mAU
min
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0

Figure 16

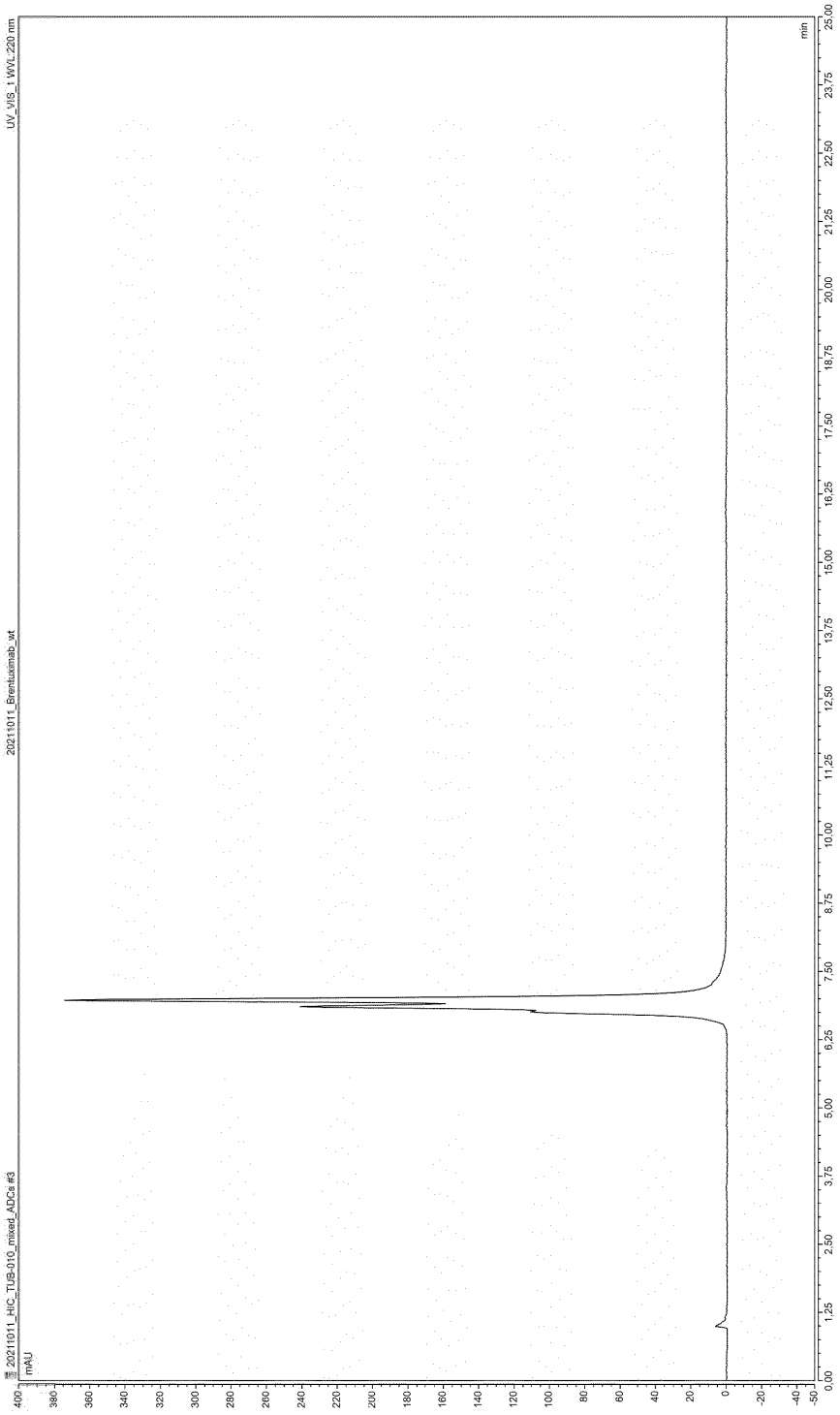

20211011_HIC_TU9-010_mixed_ADCs #3
mAU
20211011_Brentuximab_wt
UV_VIS_1 WVL:220 nm
min

Figure 17

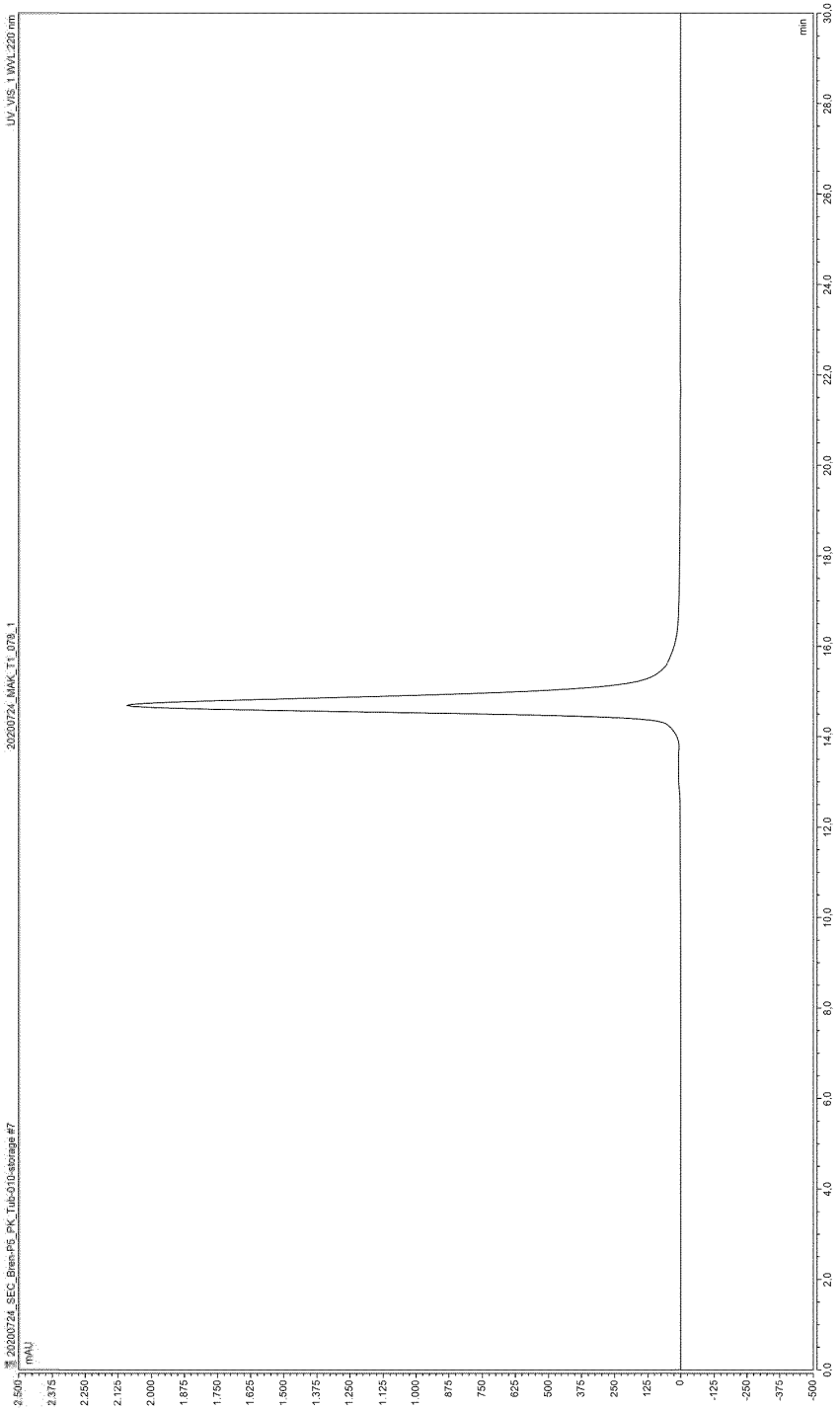

20220724_SEC_Brem-P5_PK_Tub-010-storage #7
mAU
20220724_MAK_T1_079_1
UV_VIS_1 WVL:220 nm
min

Figure 18

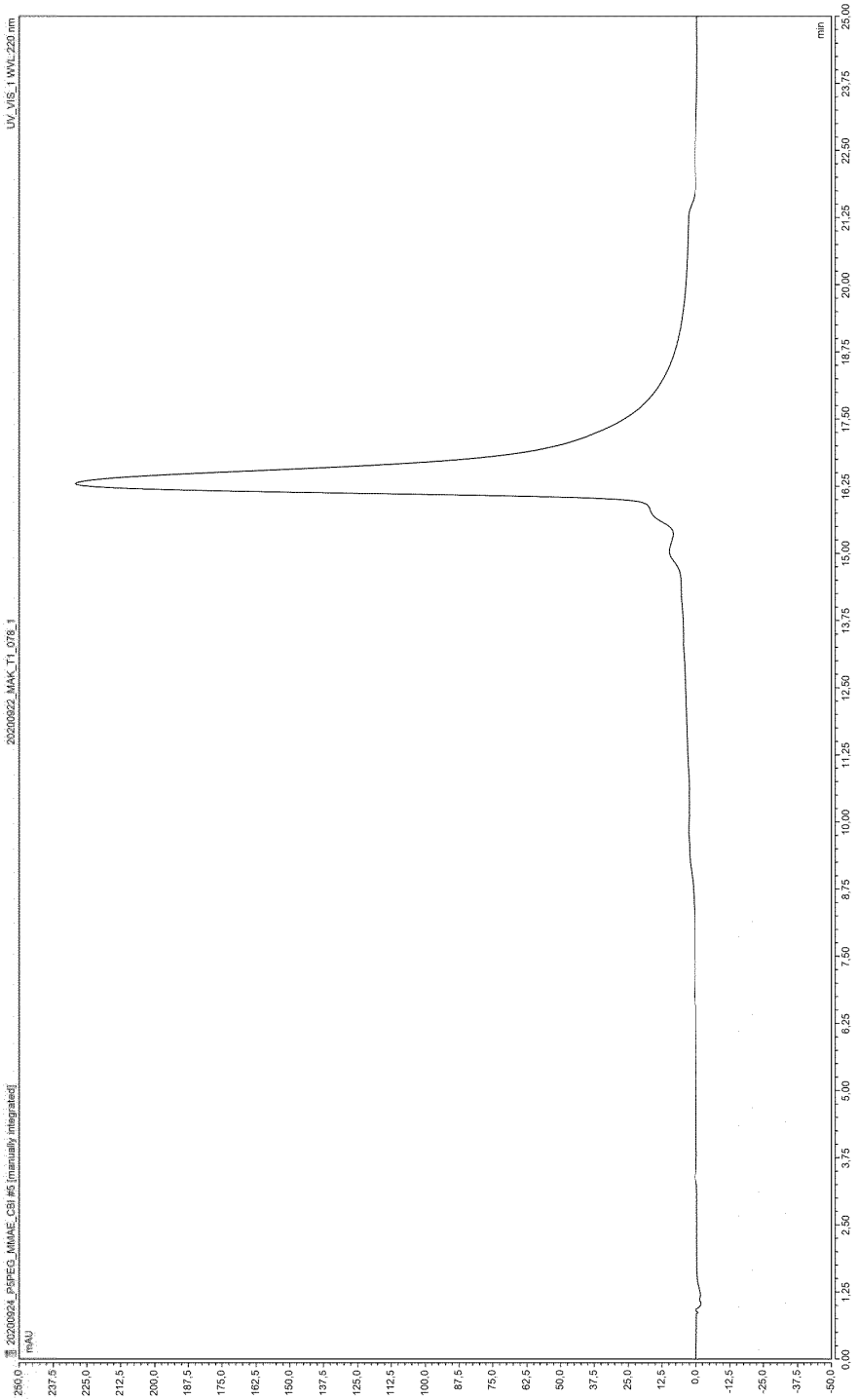

UV_VIS_1 WVL:220 nm
20220922_MAK_T1_078_1
20220924_PSPEG_MMAE_CSI #5 [manually integrated]
mAU
min

Figure 19

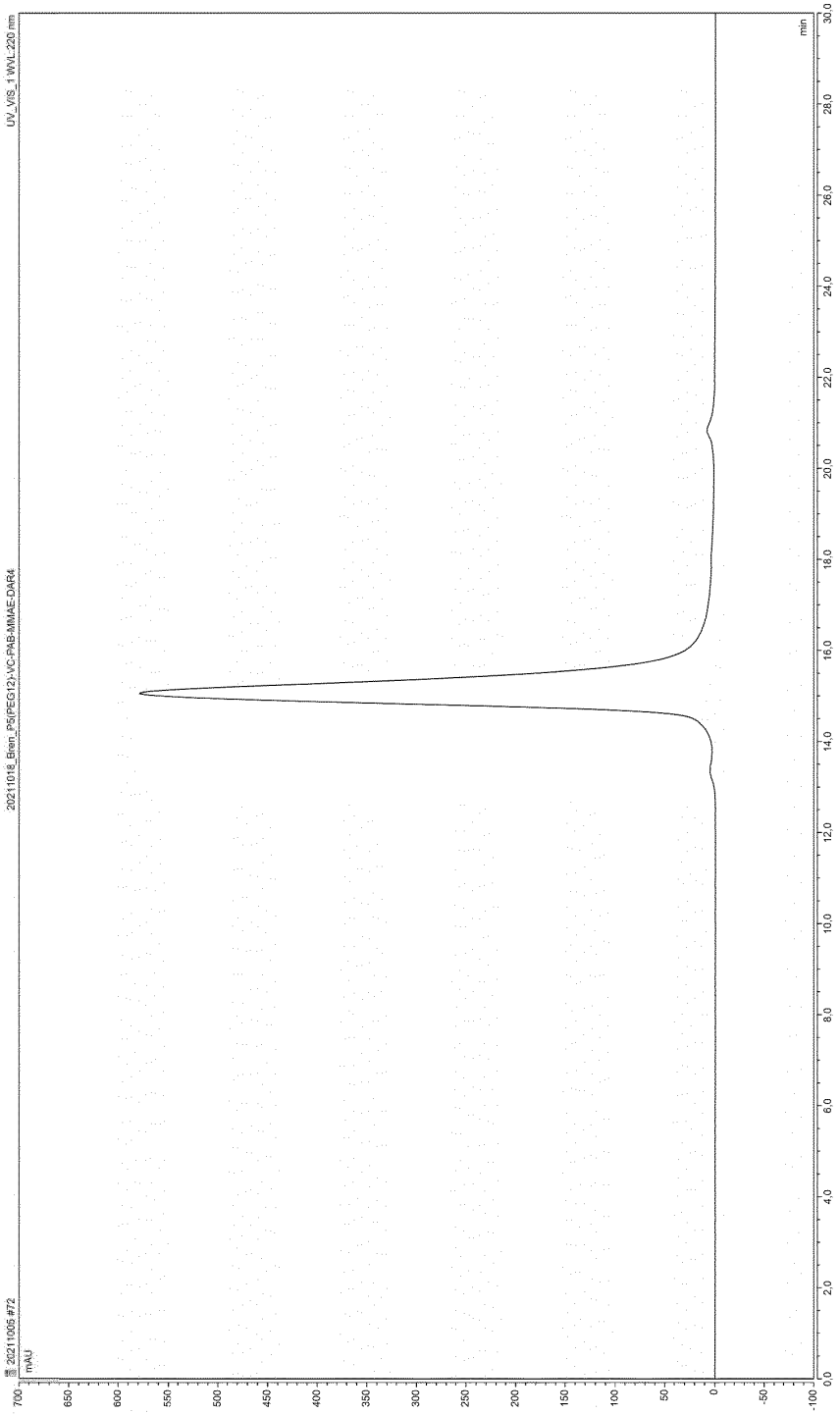

20211018_Brien_P5(PEG12)-VC-PAB-MMAE-DAR4
UV_VIS_1 WVL:220 nm
20211006 #72
mAU
min

Figure 20

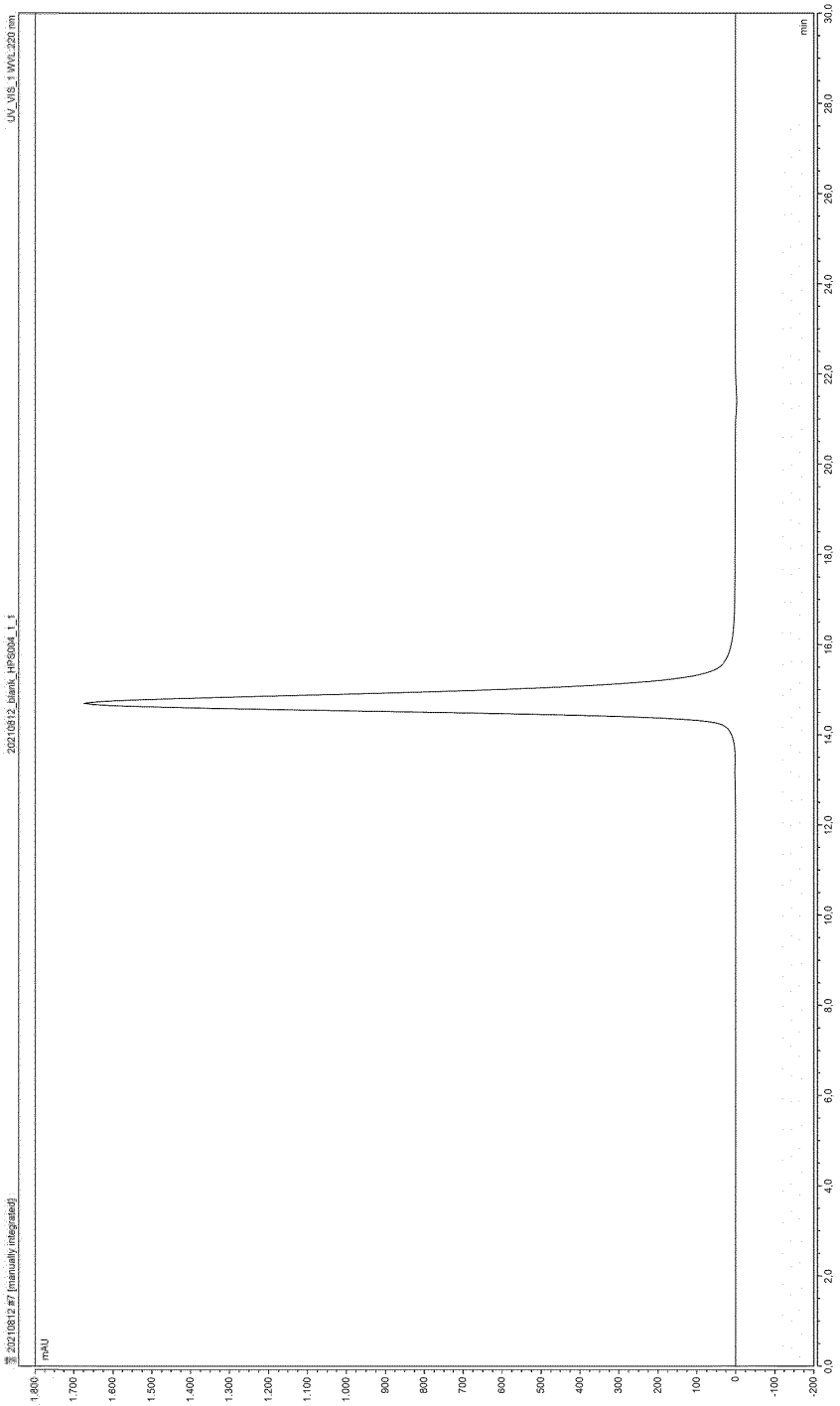

UV_VIS_1 WVL:220 nm
20210812_blank_HPS004_1_1
20210812 #7 [manually integrated]
mAU
min
1,800
1,700
1,600
1,500
1,400
1,300
1,200
1,100
1,000
900
800
700
600
500
400
300
200
100
0
-100
-200
0,0
2,0
4,0
6,0
8,0
10,0
12,0
14,0
16,0
18,0
20,0
22,0
24,0
26,0
28,0
30,0

Figure 21

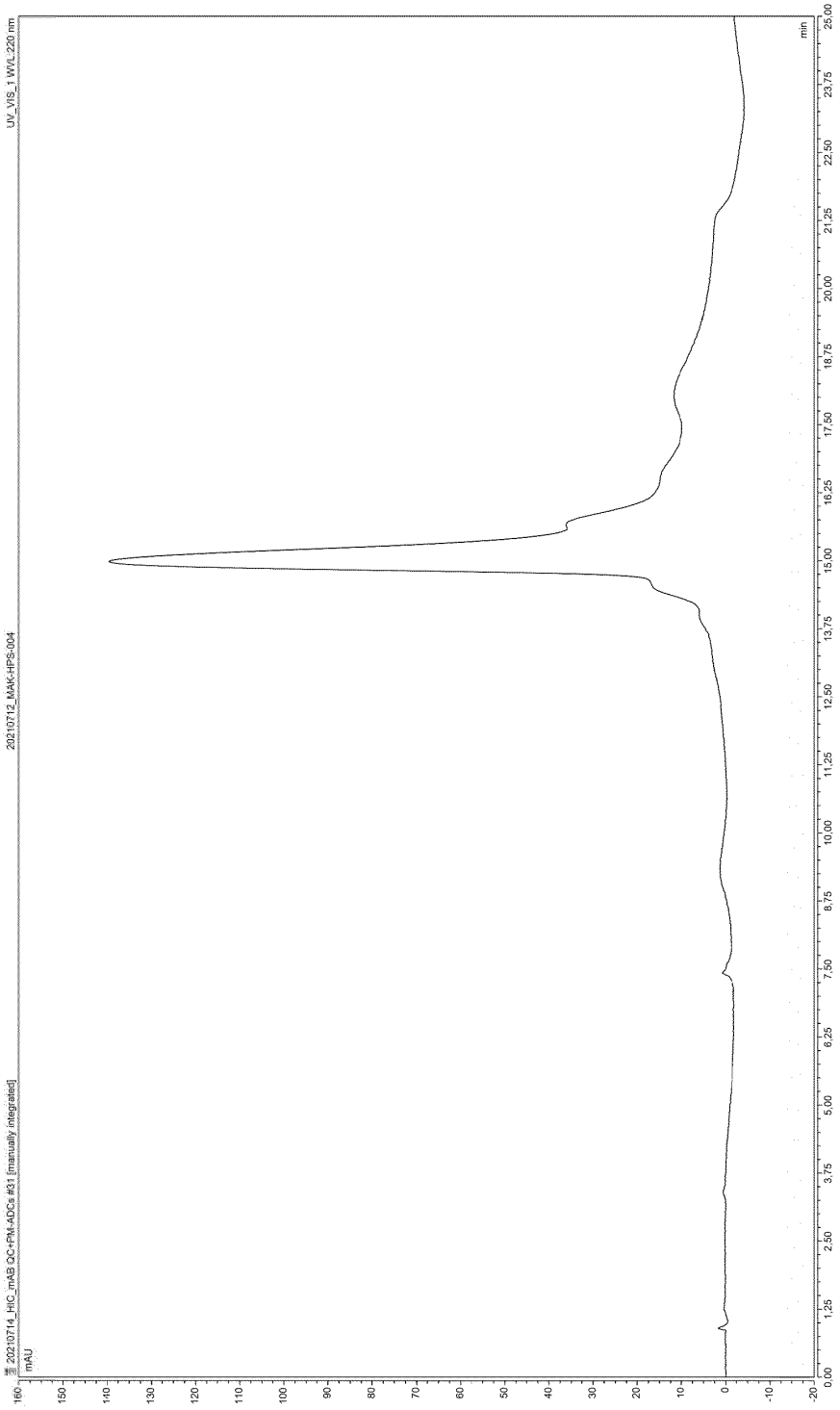

20210714_HIC_mAB QC+PNI-ADCs #31 [manually integrated]
mAU
20210712_MAK-HPS-004
UV_VIS_1 WVL:220 nm
min

Figure 22

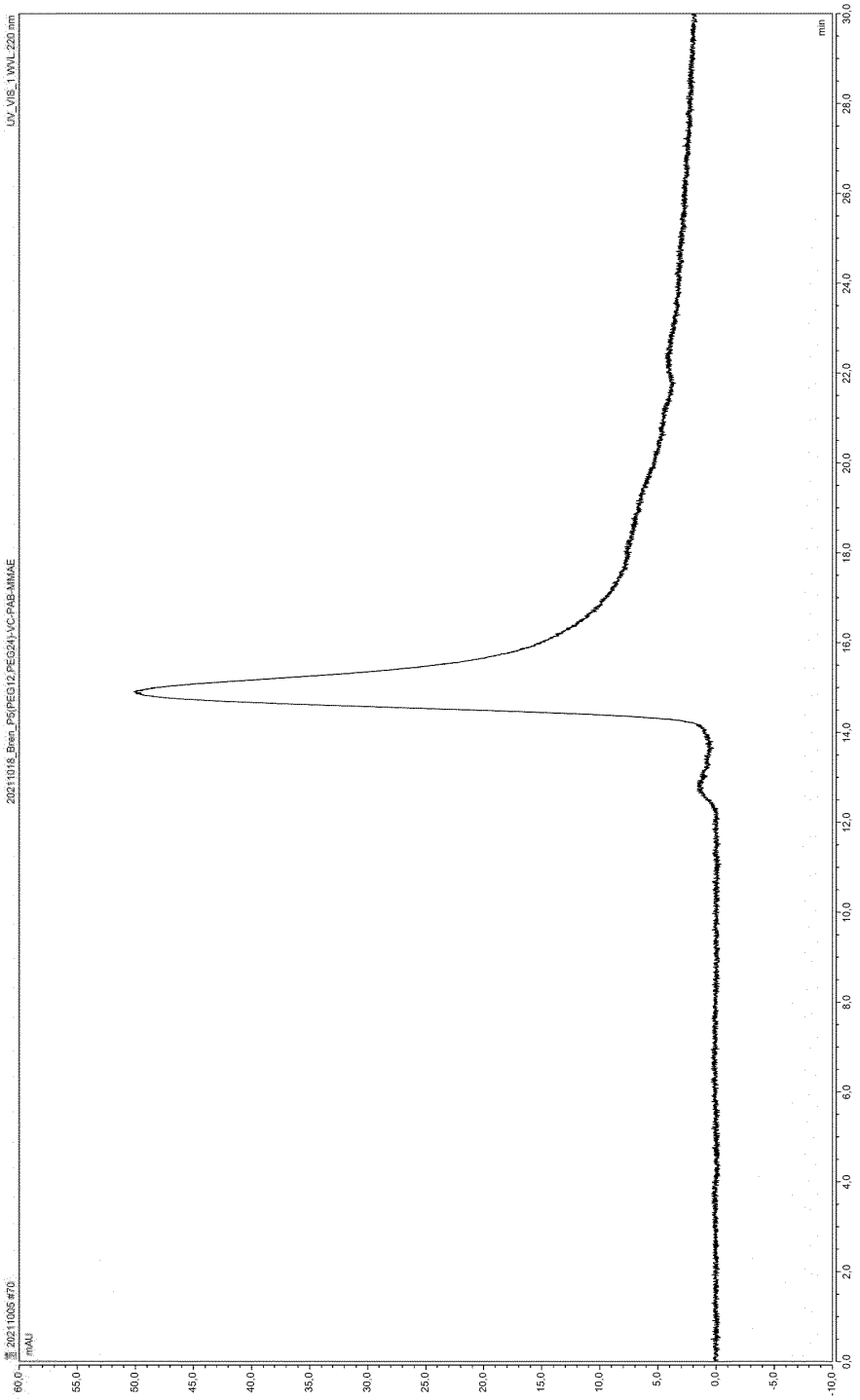

UV_VIS_1 WVL:220 nm
20211018_Brien_P5(PEG12,PEG24)-VC-PAB-MMAE
20211005 #70
mAU
min

Figure 23

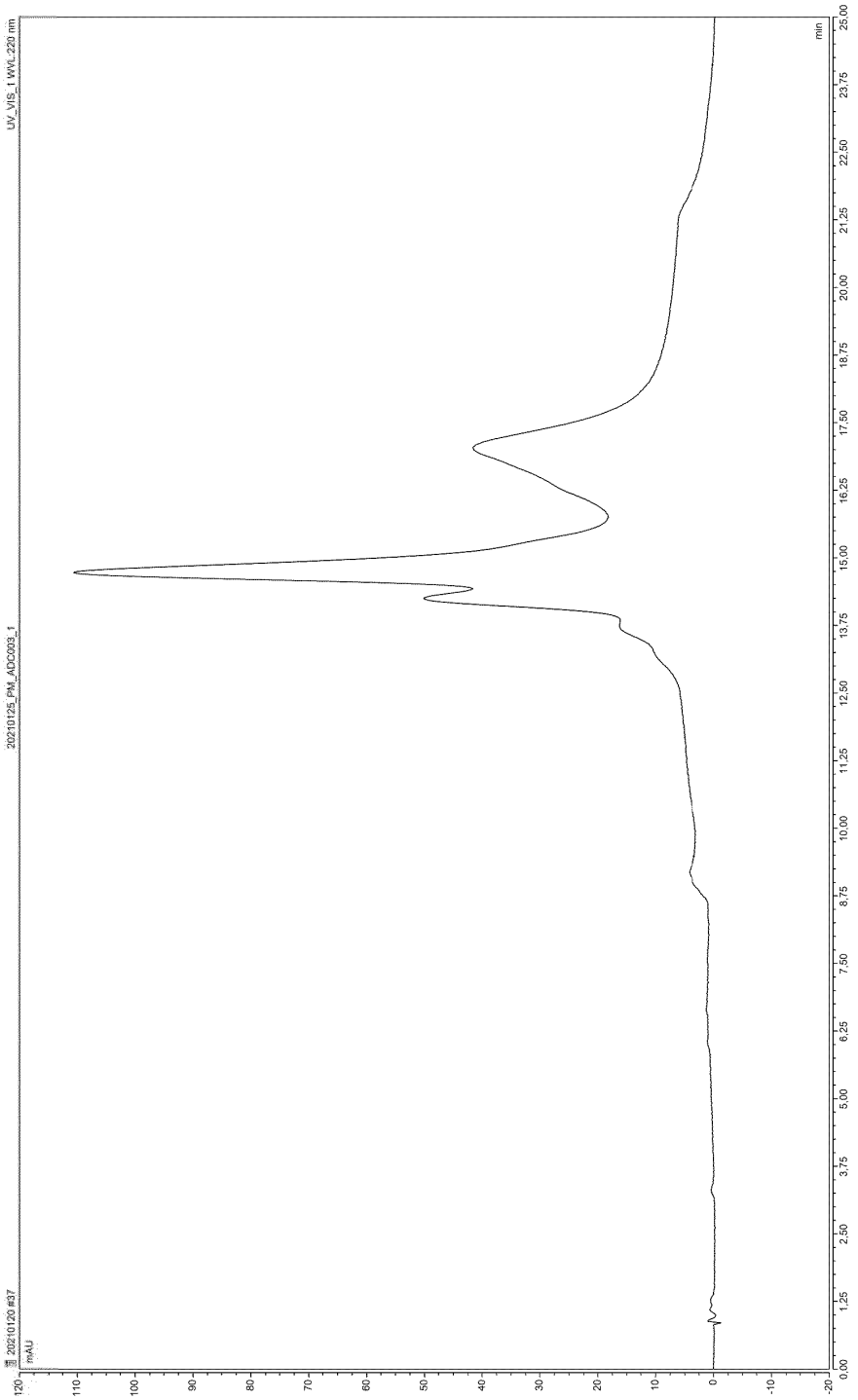

UV_VIS_1 WVL:220 nm
20210125_PM_ADC003_1
20210120 #37
mAU
min
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
0,00
1,25
2,50
3,75
5,00
6,25
7,50
8,75
10,00
11,25
12,50
13,75
15,00
16,25
17,50
18,75
20,00
21,25
22,50
23,75
25,00

Figure 24

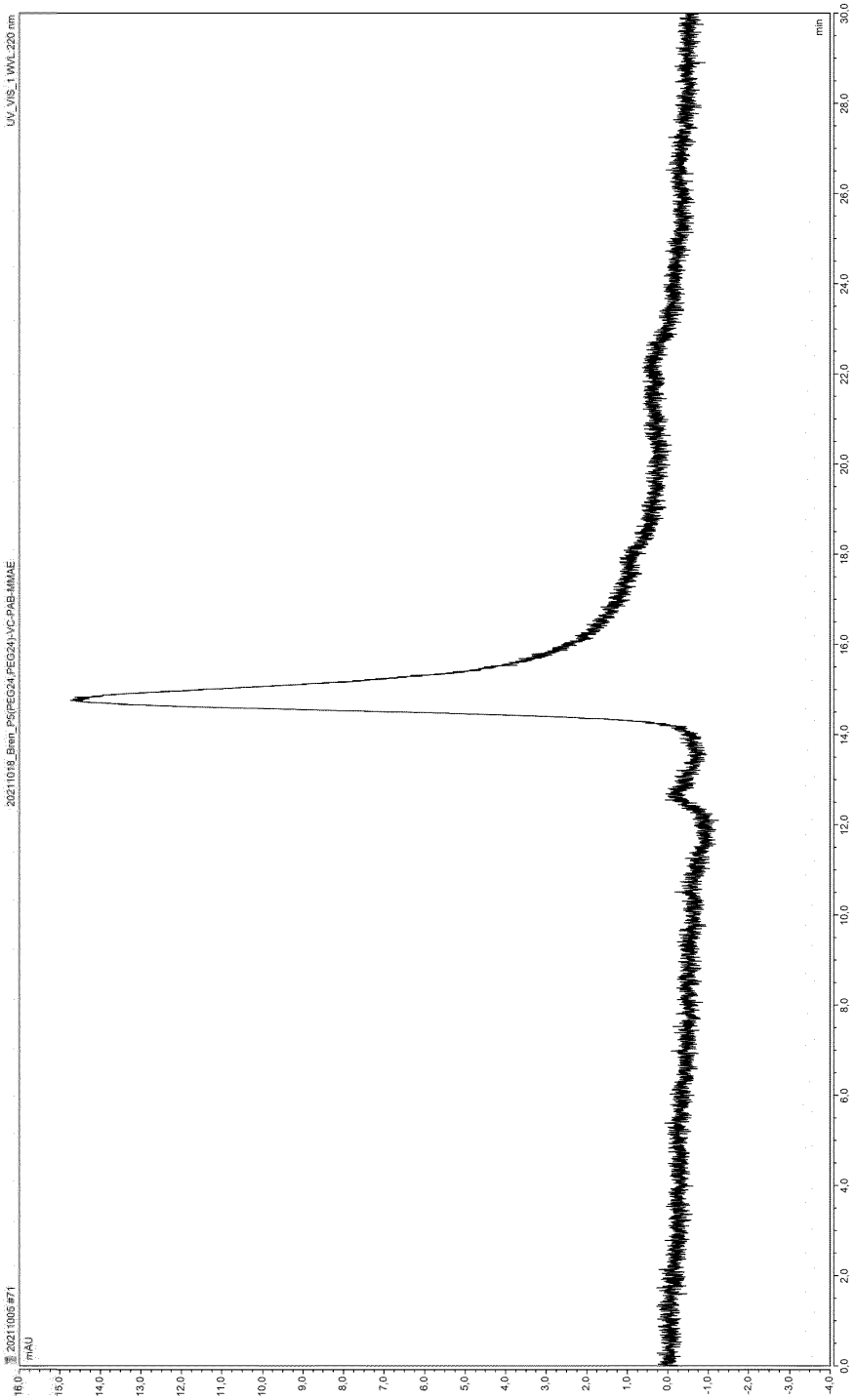

UV_VIS_1 WVL:220 nm
20211018_Brian_P5(PEG24-PEG24)-VC-PAB-MMAE
20211005 #71
mAU
min

Figure 25

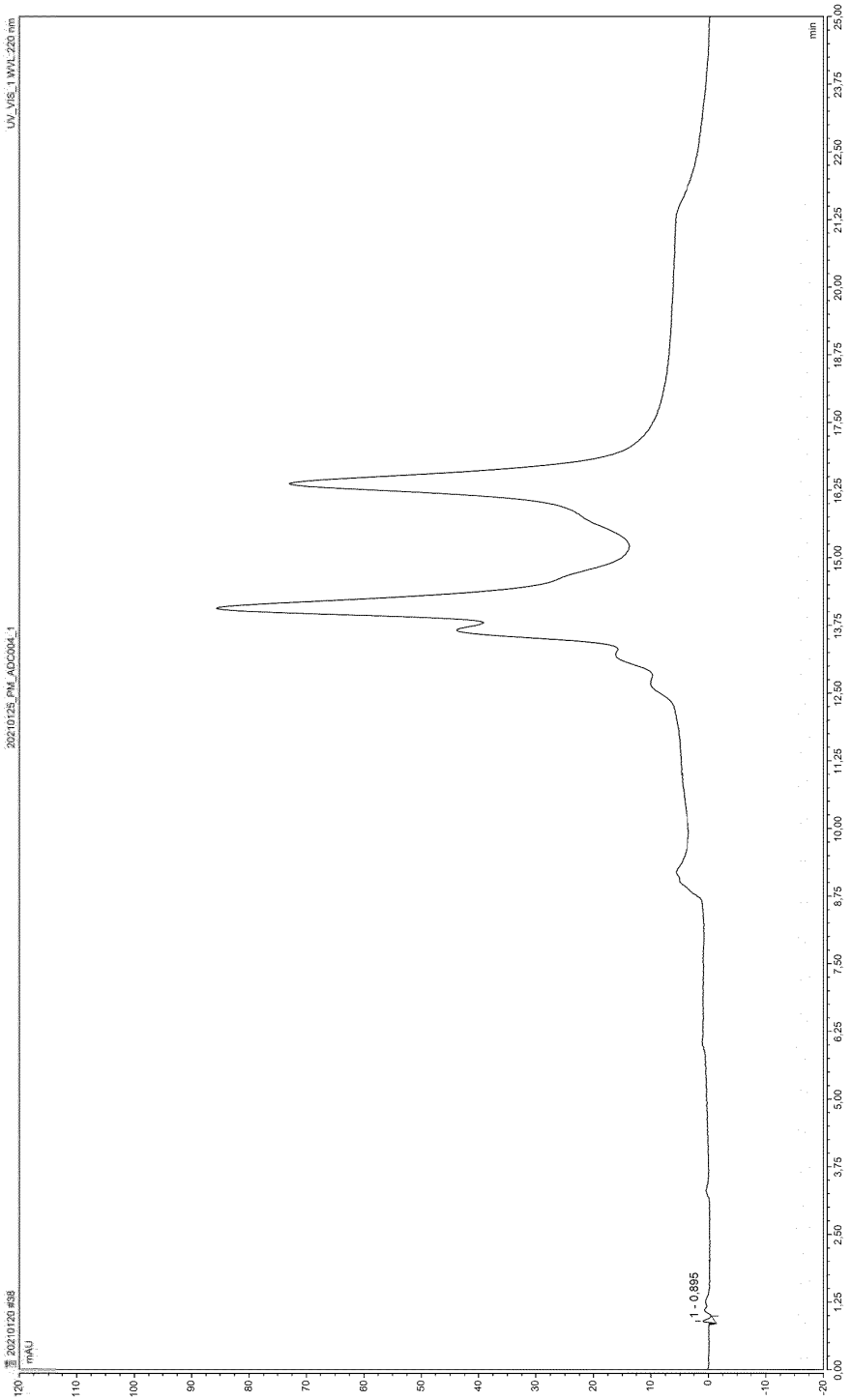

UV_VIS_1 WVL:220 nm
20210125_PM_AOC004_1
20210120 #38
mAU
min
1 - 0.895

Figure 26

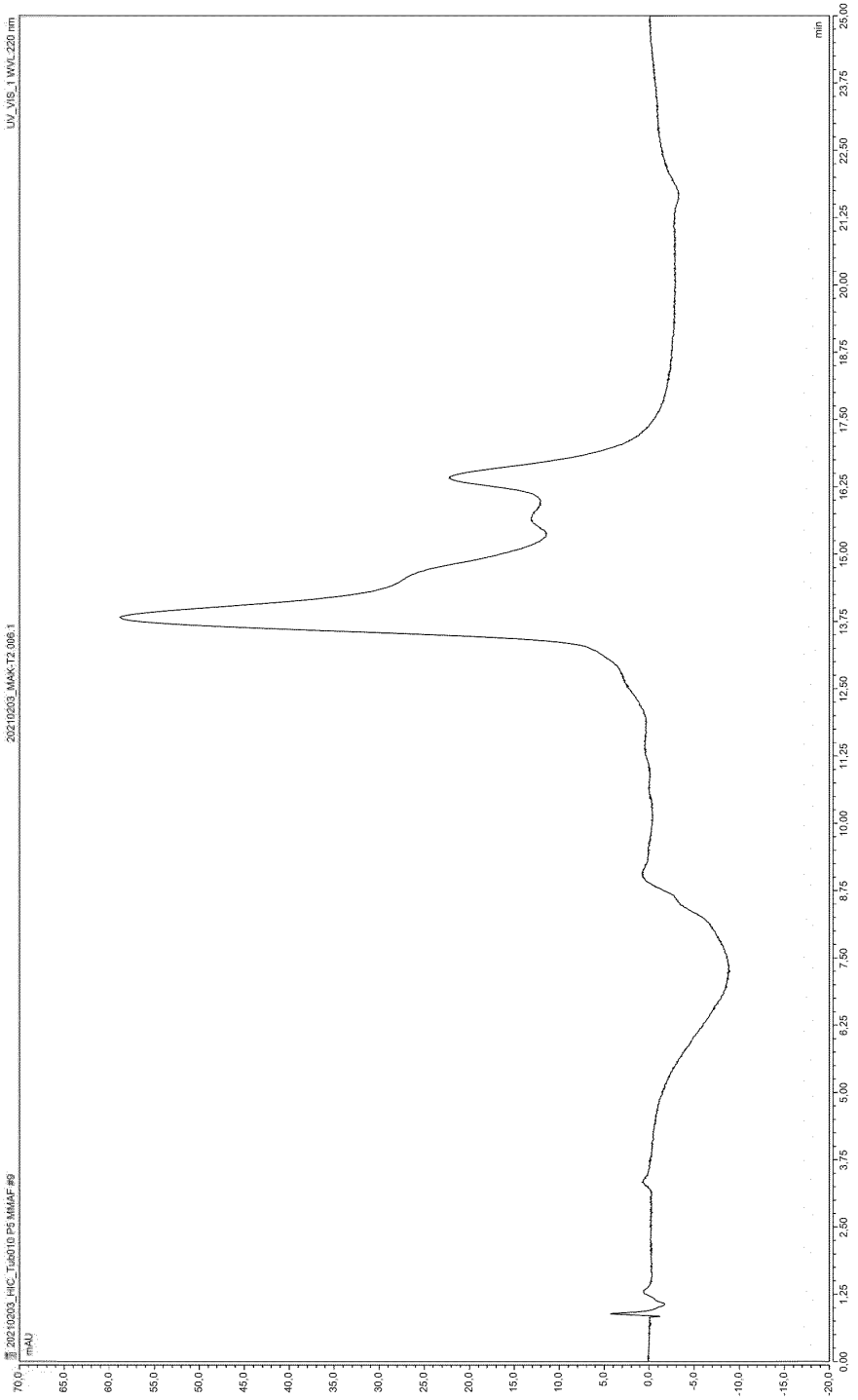

UV_VIS_1 WVL:220 nm
20210203_MAK-T2 006.1
20210203_HIC_Tab010 P5 MMAF #9
mAU
min
70,0
65,0
60,0
55,0
50,0
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
-5,0
-10,0
-15,0
-20,0
0,00
1,25
2,50
3,75
5,00
6,25
7,50
8,75
10,00
11,25
12,50
13,75
15,00
16,25
17,50
18,75
20,00
21,25
22,50
23,75
25,00

Figure 27

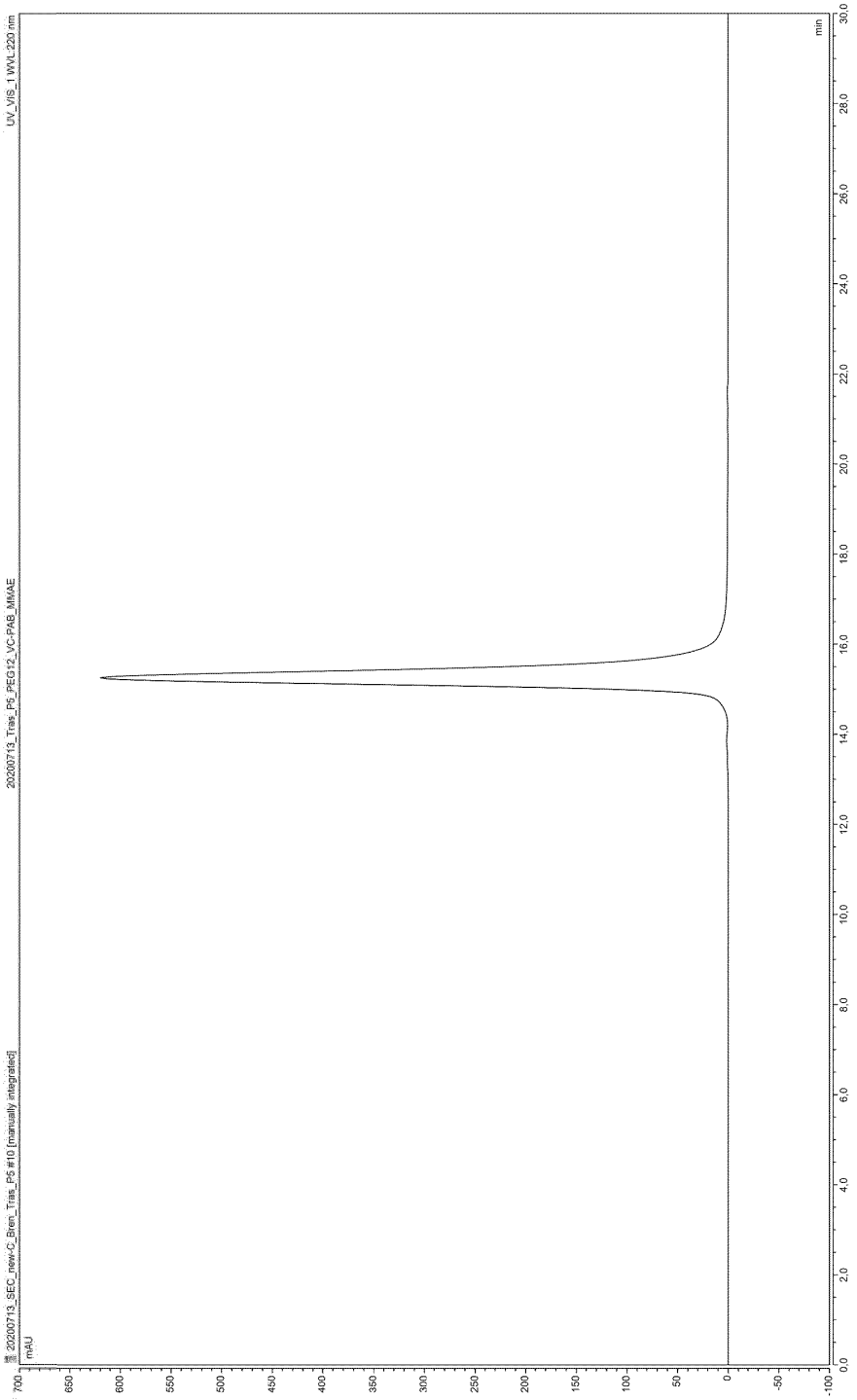

UV_VIS_1 WVL:220 nm
20220713_Trial_P5_PEG12_VC-PAB_MMAE
20220713_SEC_new-C_Brien_Trial_P5 #10 [manually integrated]
mAU
min

Figure 28

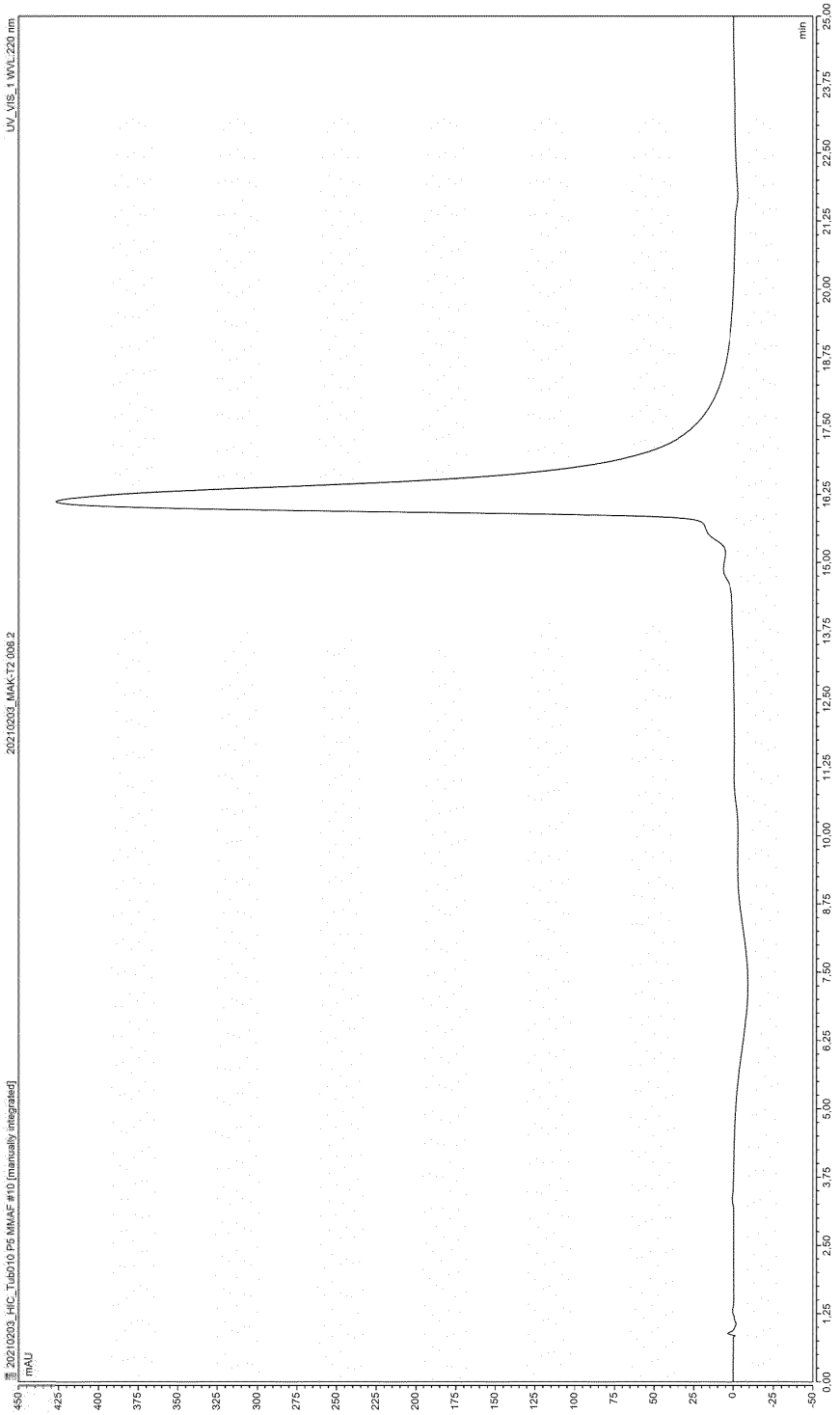

UV_VIS_1 WVL:220 nm
20210203_MAK-T2 008.2
20210203_HIC_Tub010 P5 MMAF #10 [manually integrated]
mAU
min

Figure 29

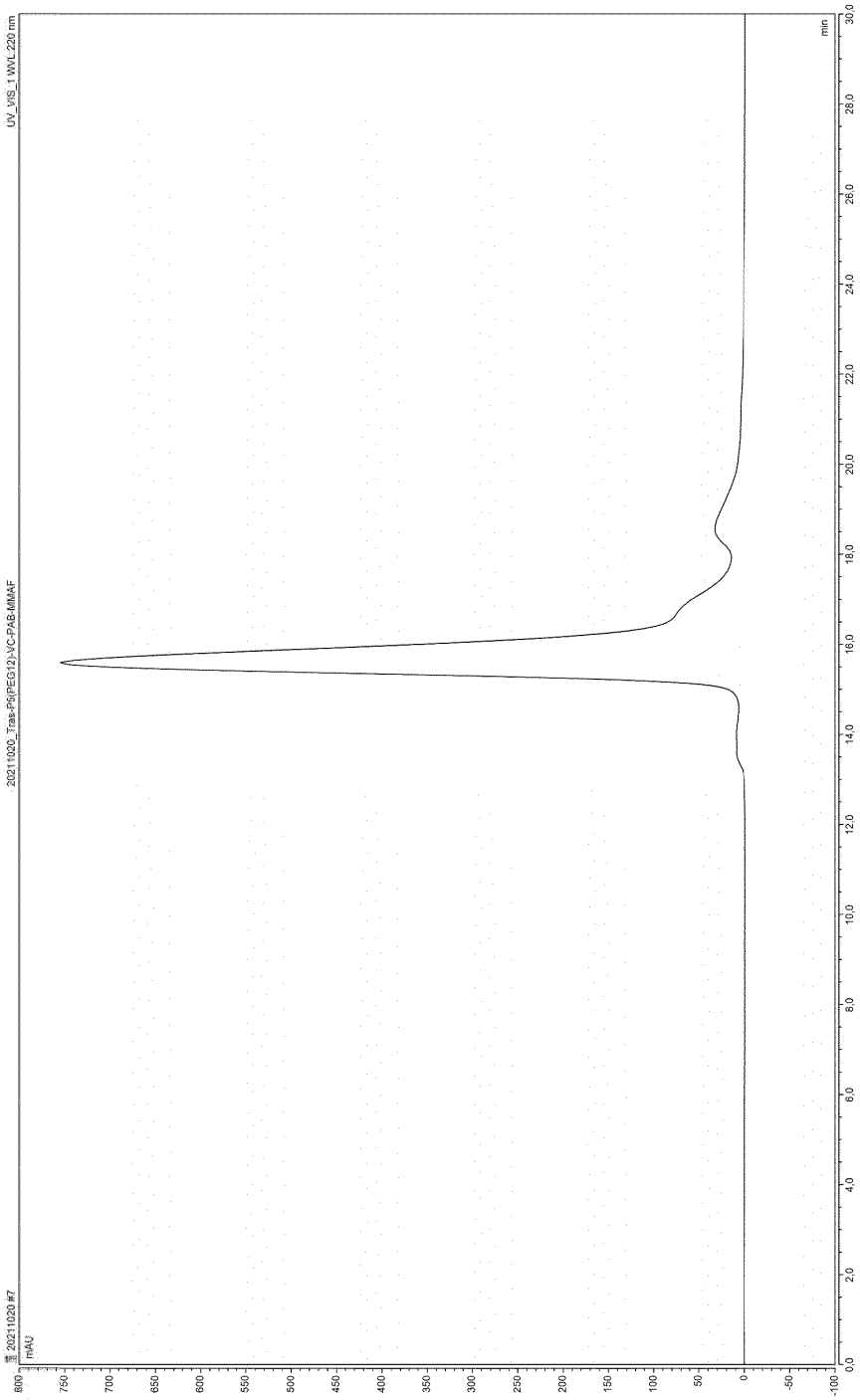

UV_VIS_1 WVL:220 nm
20211020_Trastu-P9(PEG12)-VC-PAB-MMAF
20211020 #7
mAU
min

Figure 30

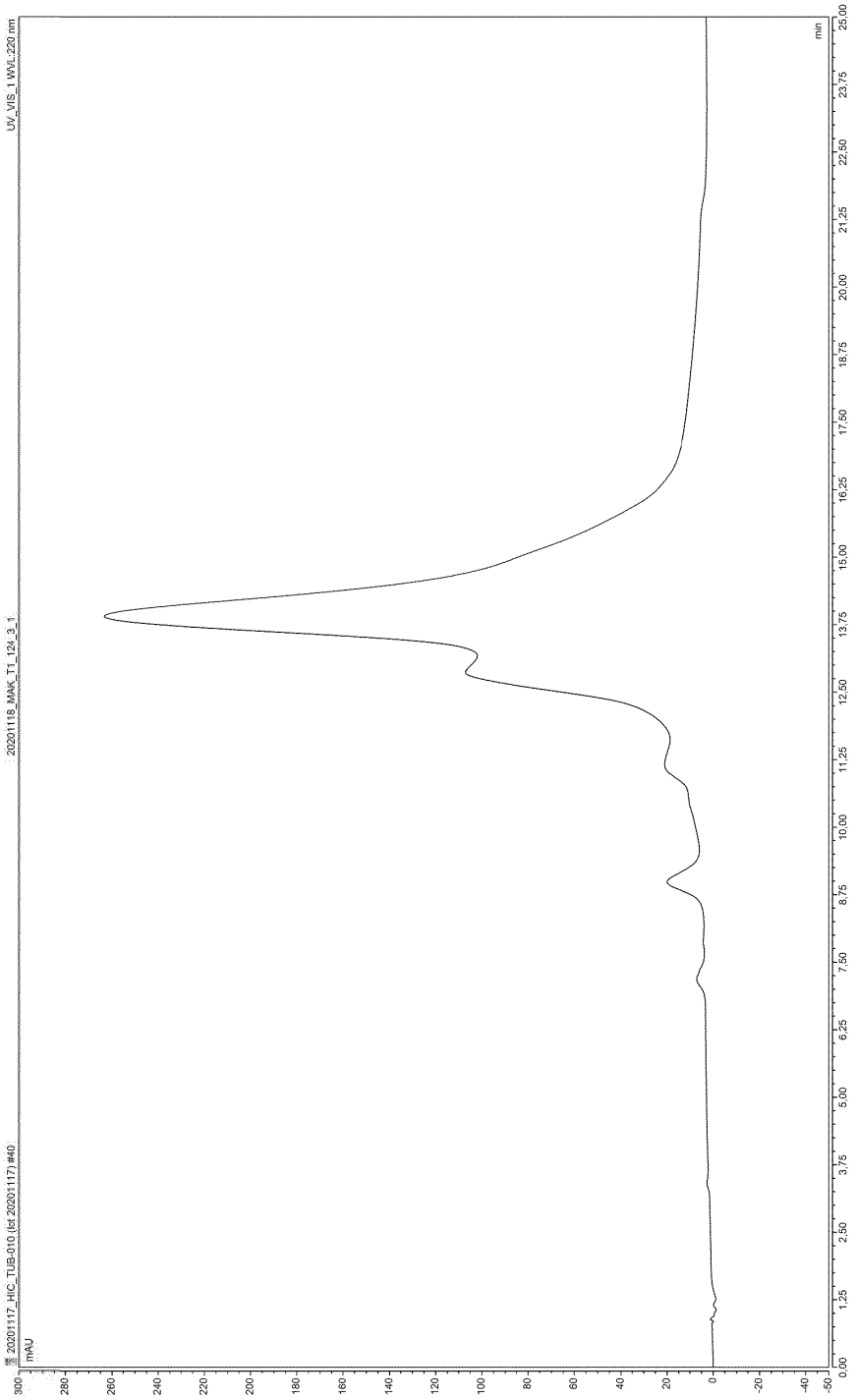

20201117_HIC_TUB-010 (ist 20201117) #40
mAU
20201118_MAK_T1_124_3_1
UV_VIS_1 WVL:220 nm
min

Figure 31

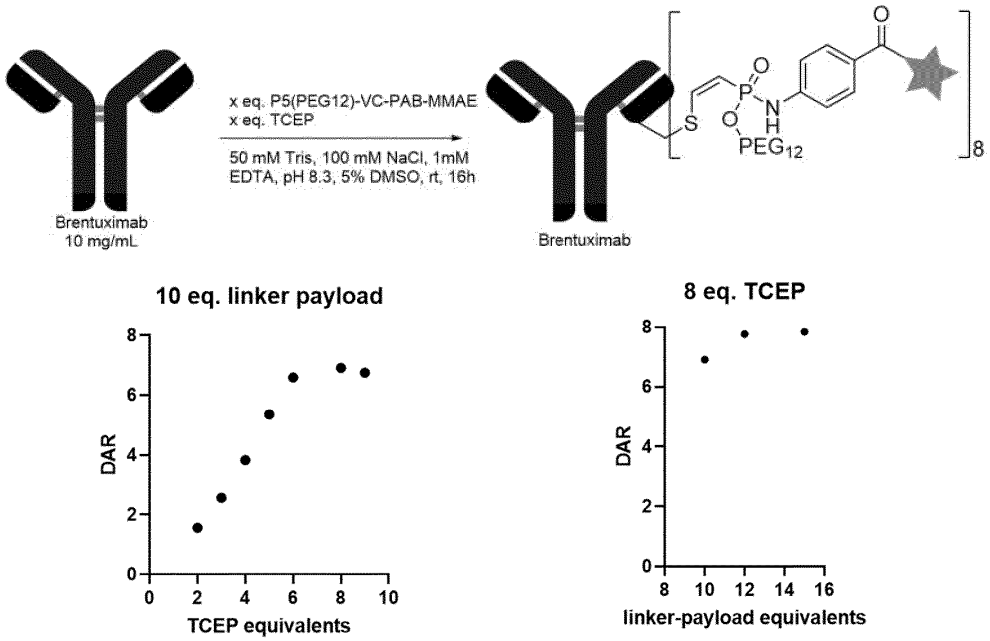


Figure 32

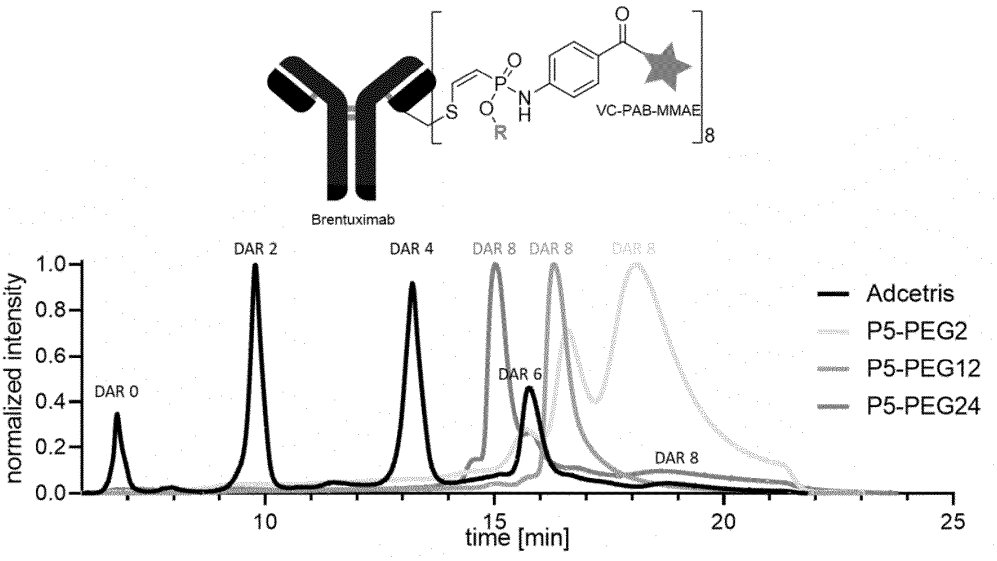


Figure 33

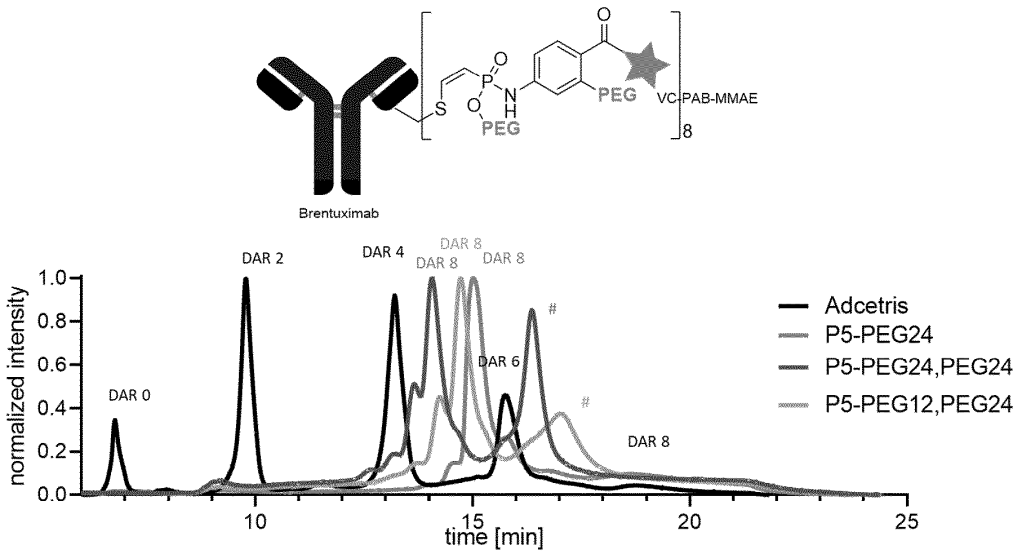


Figure 34

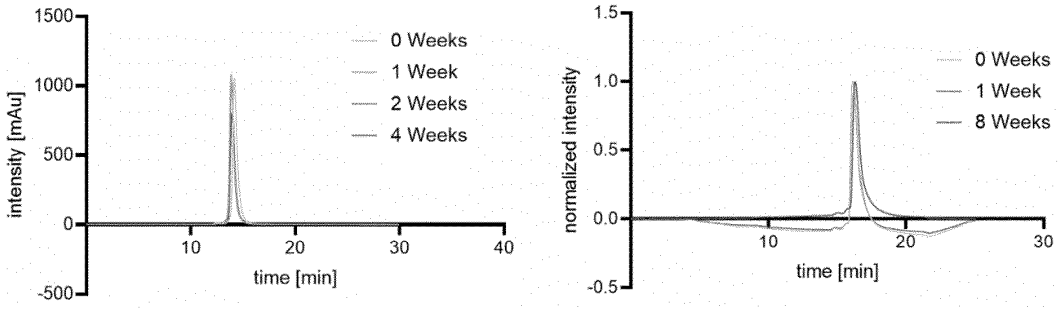


Figure 35

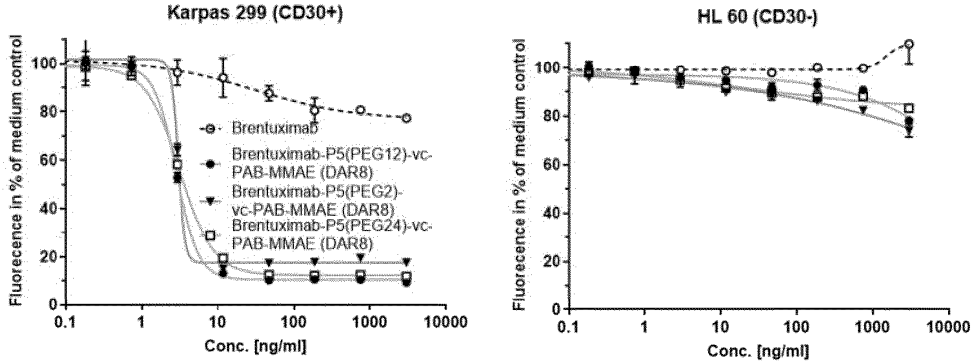


Figure 36

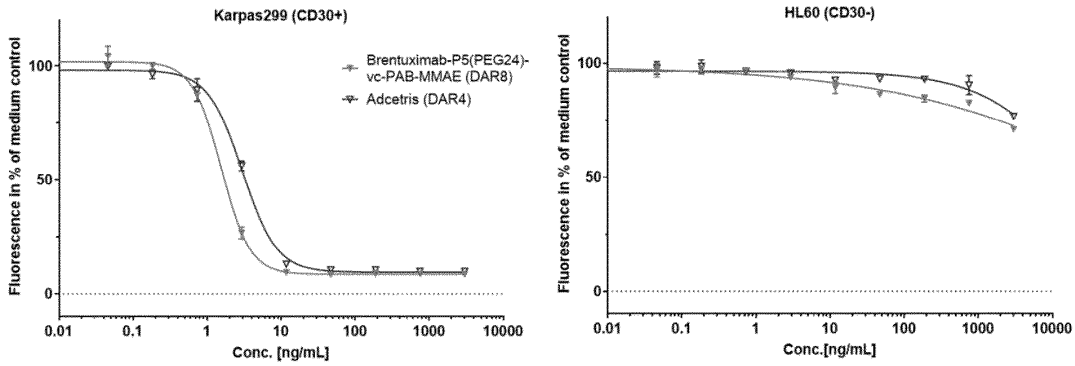


Figure 37

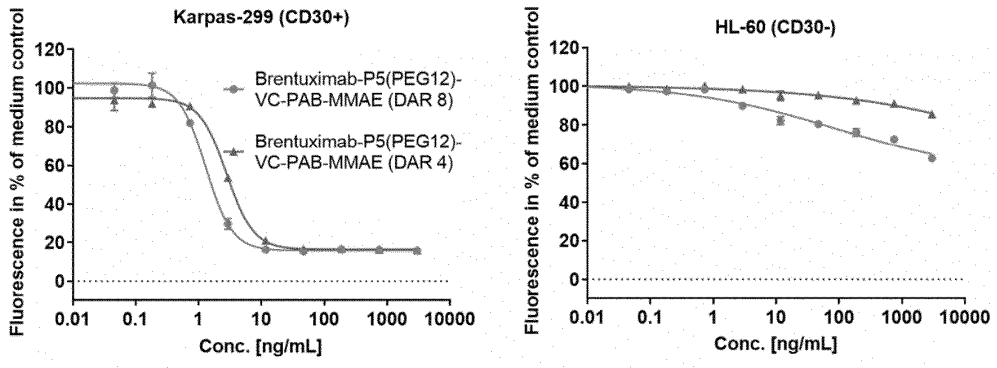


Figure 38

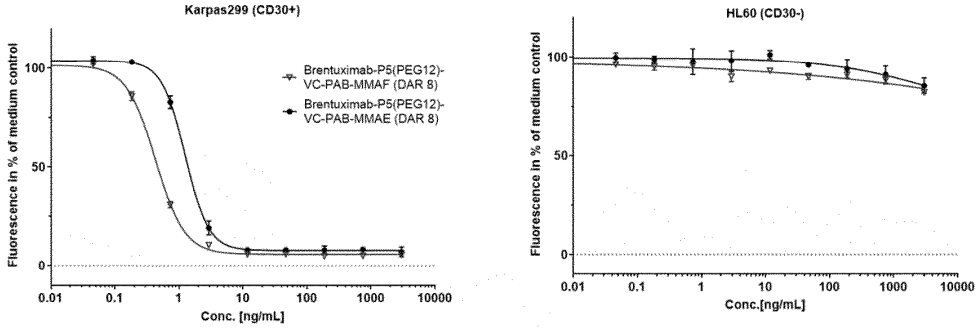


Figure 39

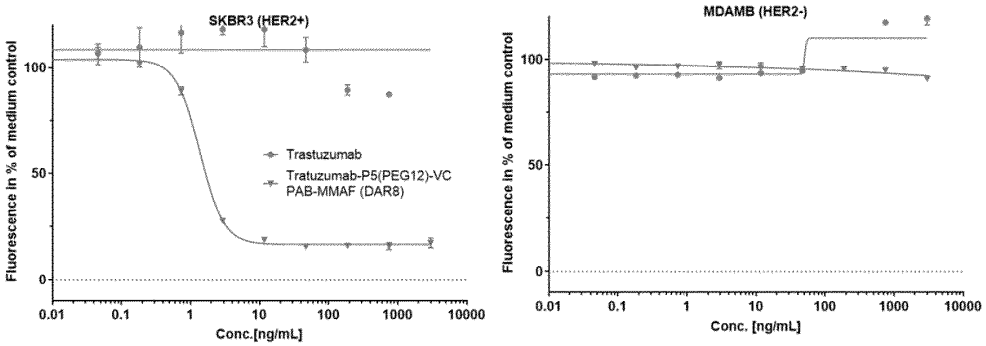


Figure 40

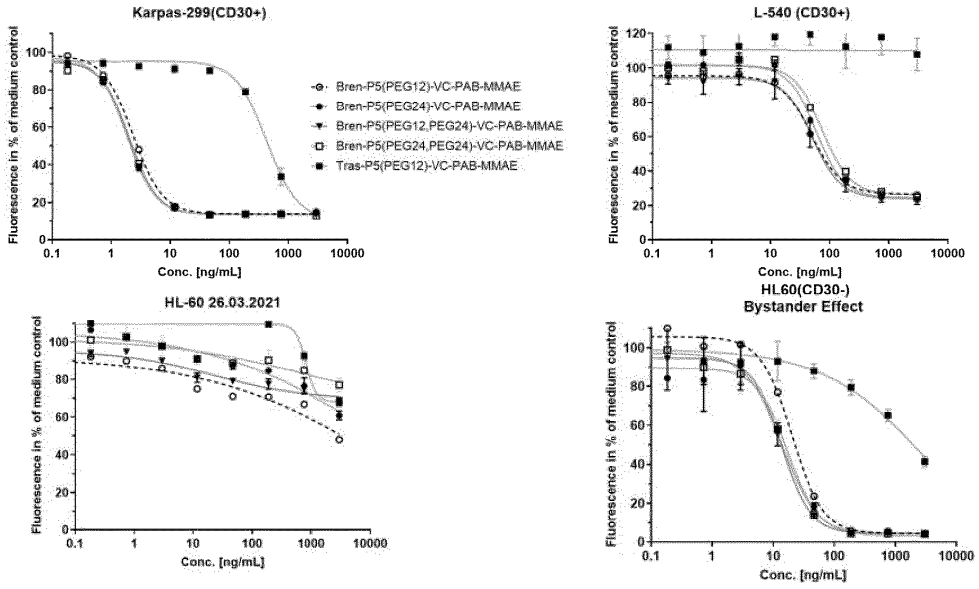


Figure 41

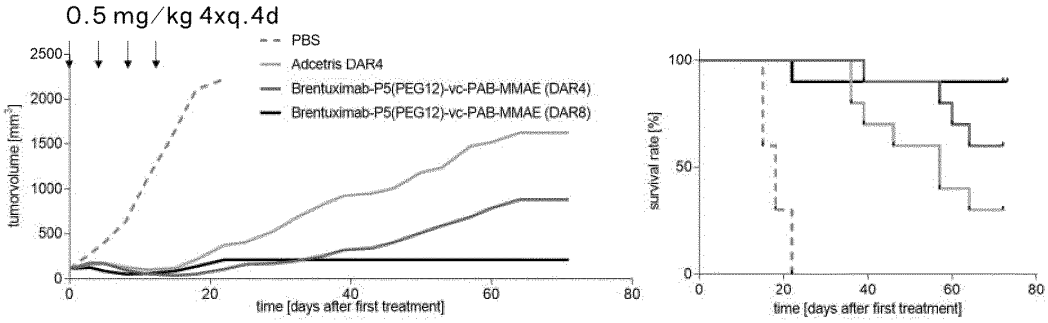


Figure 42

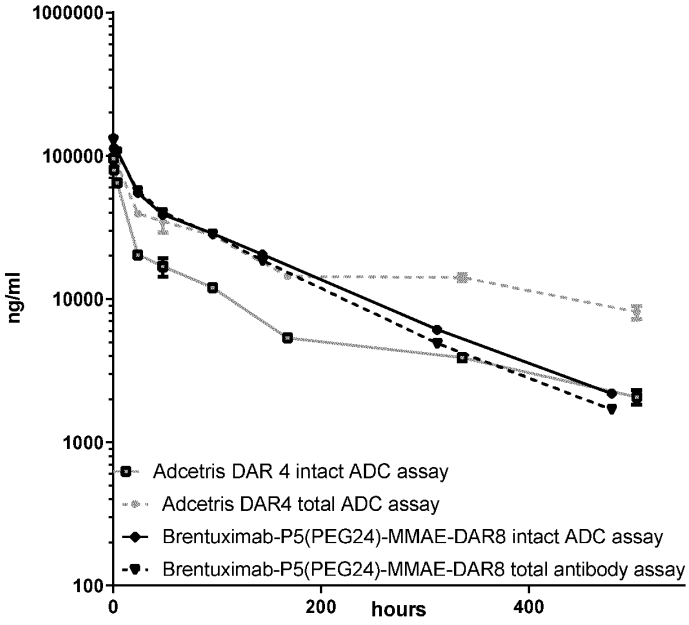

1000000
100000
10000
1000
100
ng/ml
0
200
400
hours
Adcetris DAR 4 intact ADC assay
Adcetris DAR4 total ADC assay
Brentuximab-P5(PEG24)-MMAE-DAR8 intact ADC assay
Brentuximab-P5(PEG24)-MMAE-DAR8 total antibody assay

Figure 43

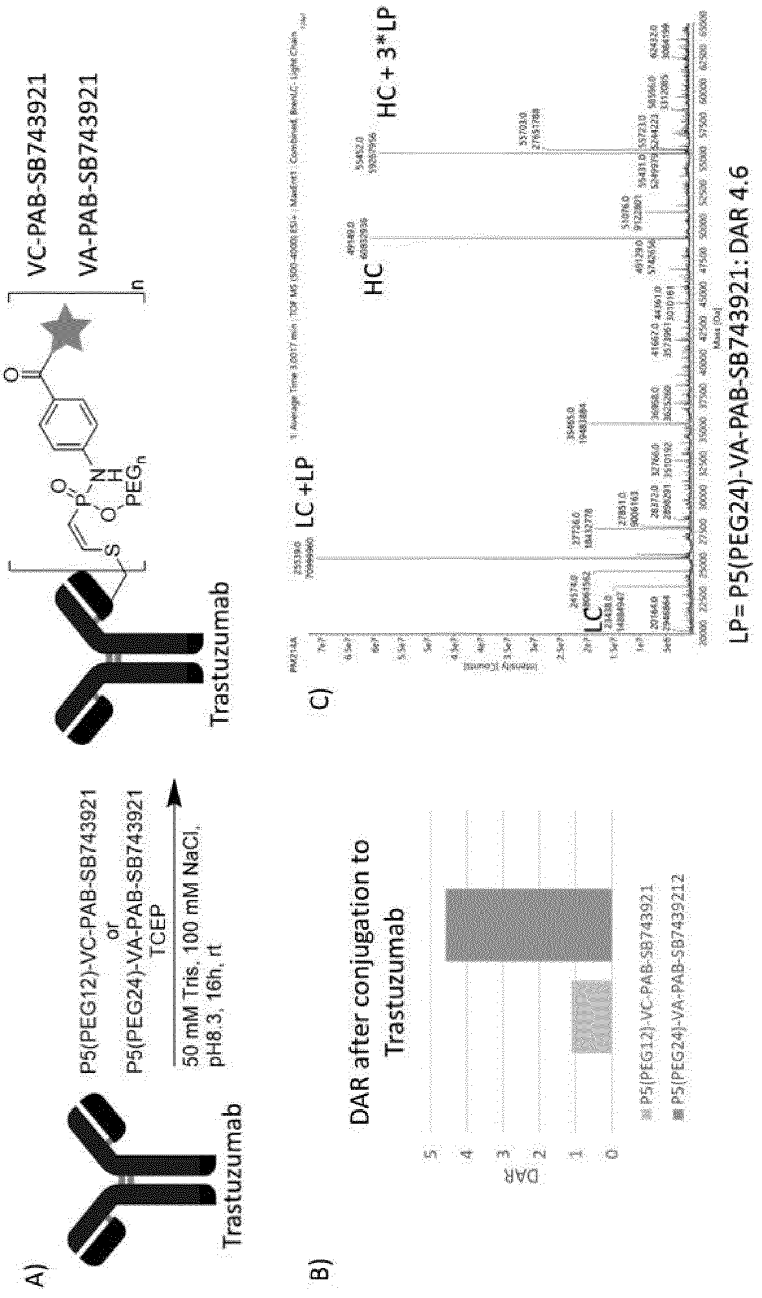

A)
Trastuzumab
P5(PEG12)-VC-PAB-SB743921
or
P5(PEG24)-VA-PAB-SB743921
TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Trastuzumab
VC-PAB-SB743921
VA-PAB-SB743921
n
B)
DAR after conjugation to
Trastuzumab
DAR
P5(PEG12)-VC-PAB-SB743921
P5(PEG24)-VA-PAB-SB743212
C)
LC +LP
LC
HC
HC + 3*LP
LP= P5(PEG24)-VA-PAB-SB743921: DAR 4.6

Figure 44

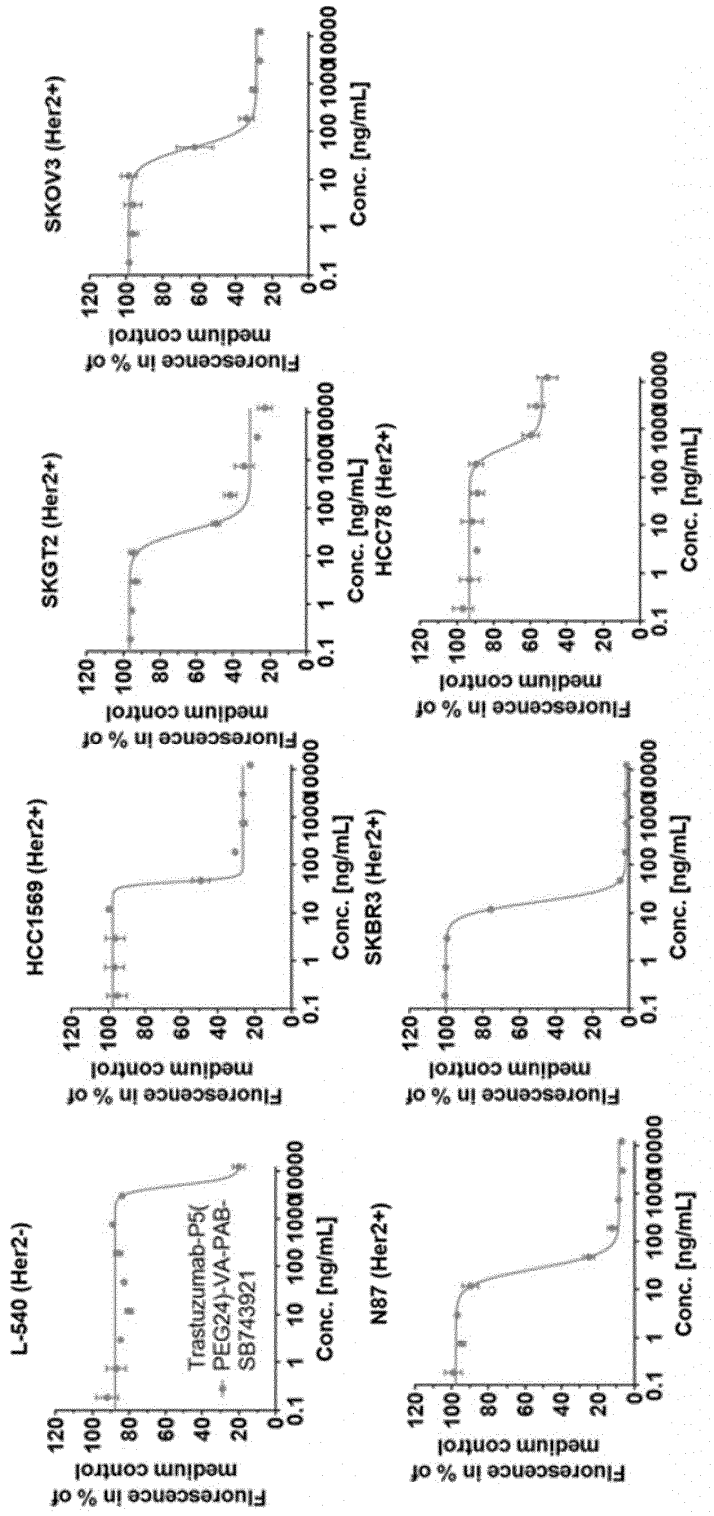

L-540 (Her2-)
HCC1569 (Her2+)
SKGT2 (Her2+)
SKOV3 (Her2+)
N87 (Her2+)
SKBR3 (Her2+)
HCC78 (Her2+)
Trastuzumab-P5(PEG24)-VA-PAB-SB743921
Fluorescence in % of medium control
Conc. [ng/mL]
0
20
40
60
80
100
120
0.1
1
10
100
1000
10000
100000

Figure 45

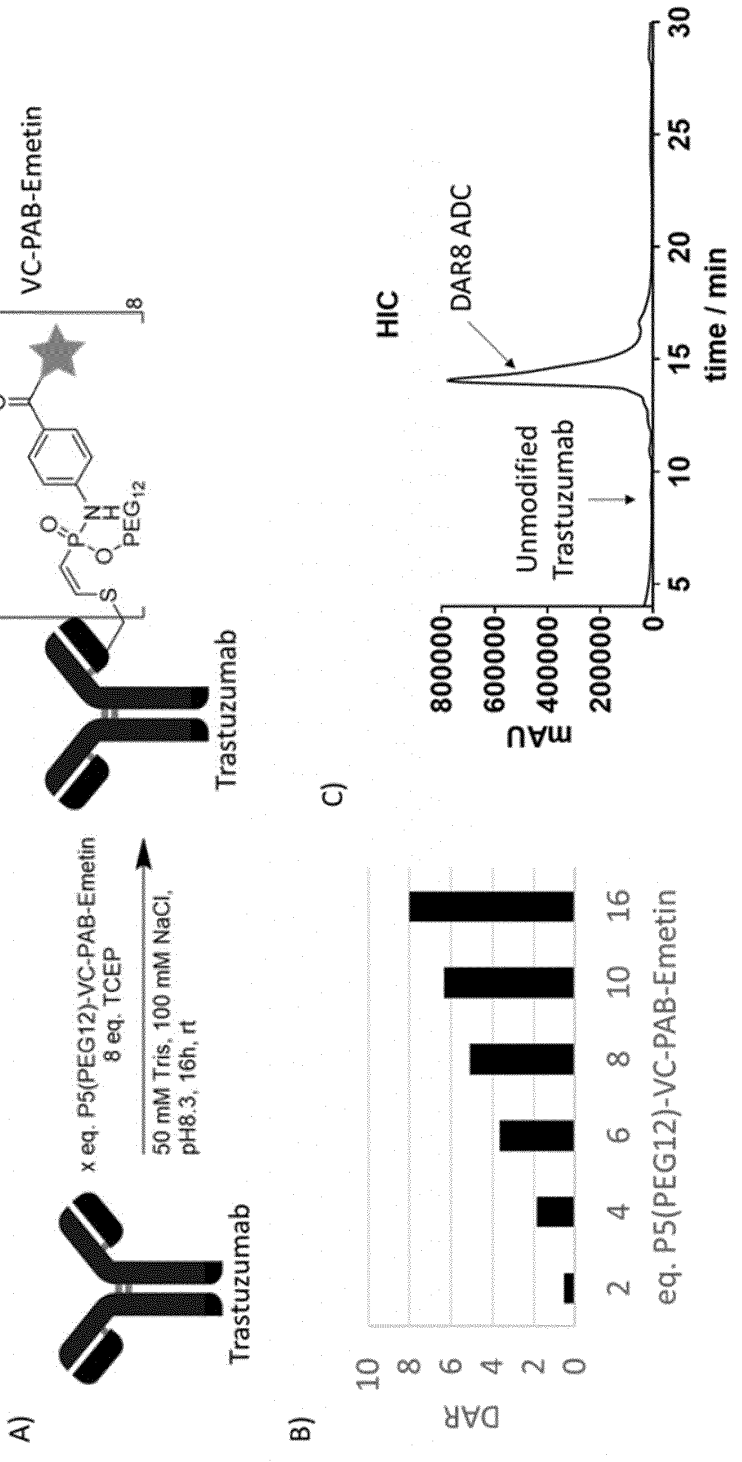

A)
Trastuzumab
x eq. P5(PEG12)-VC-PAB-Emetin
8 eq. TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Trastuzumab
VC-PAB-Emetin
8
PEG12
B)
DAR
0
2
4
6
8
10
2 4 6 8 10 16
eq. P5(PEG12)-VC-PAB-Emetin
C)
HIC
DAR8 ADC
Unmodified
Trastuzumab
mAU
800000
600000
400000
200000
0
5 10 15 20 25 30
time / min

Figure 46

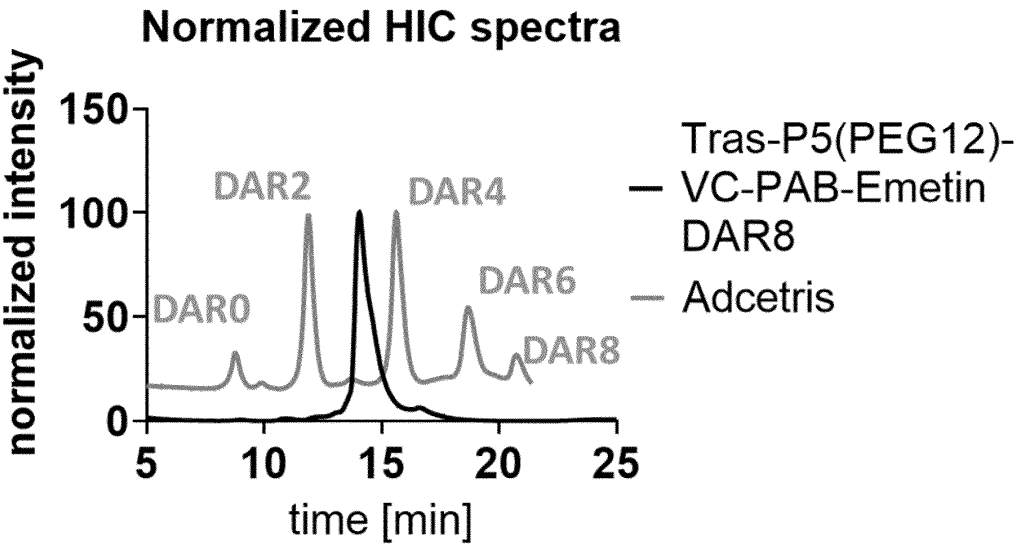

Normalized HIC spectra
normalized intensity
150
100
50
0
DAR0
DAR2
DAR4
DAR6
DAR8
5
10
15
20
25
time [min]
Tras-P5(PEG12)-VC-PAB-Emetin DAR8
Adcetris

Figure 47

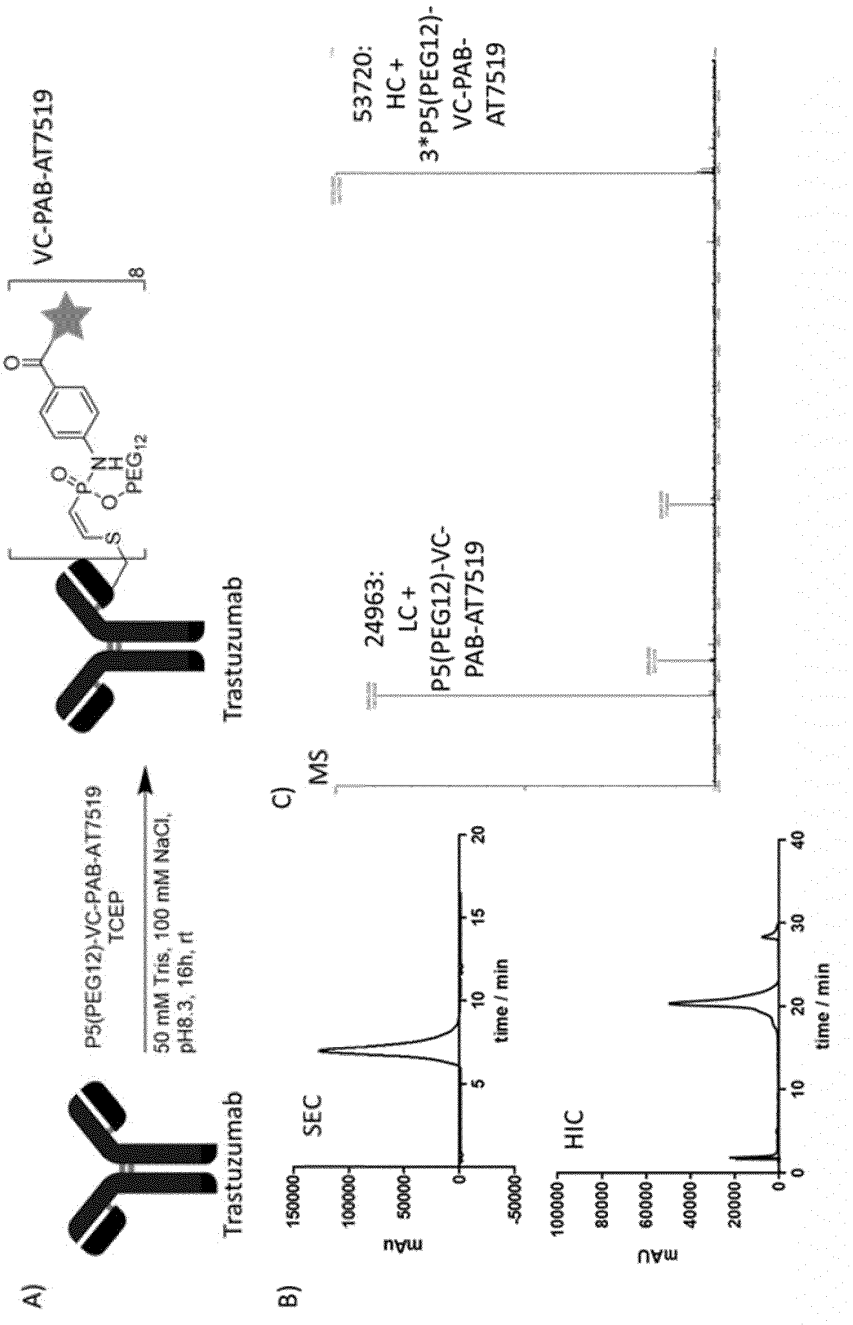

A)
Trastuzumab
P5(PEG12)-VC-PAB-AT7519
TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Trastuzumab
VC-PAB-AT7519
8
PEG12
B)
SEC
mAu
time / min
HIC
mAU
time / min
C)
MS
24963:
LC +
P5(PEG12)-VC-
PAB-AT7519
53720:
HC +
3*P5(PEG12)-
VC-PAB-
AT7519

Figure 48

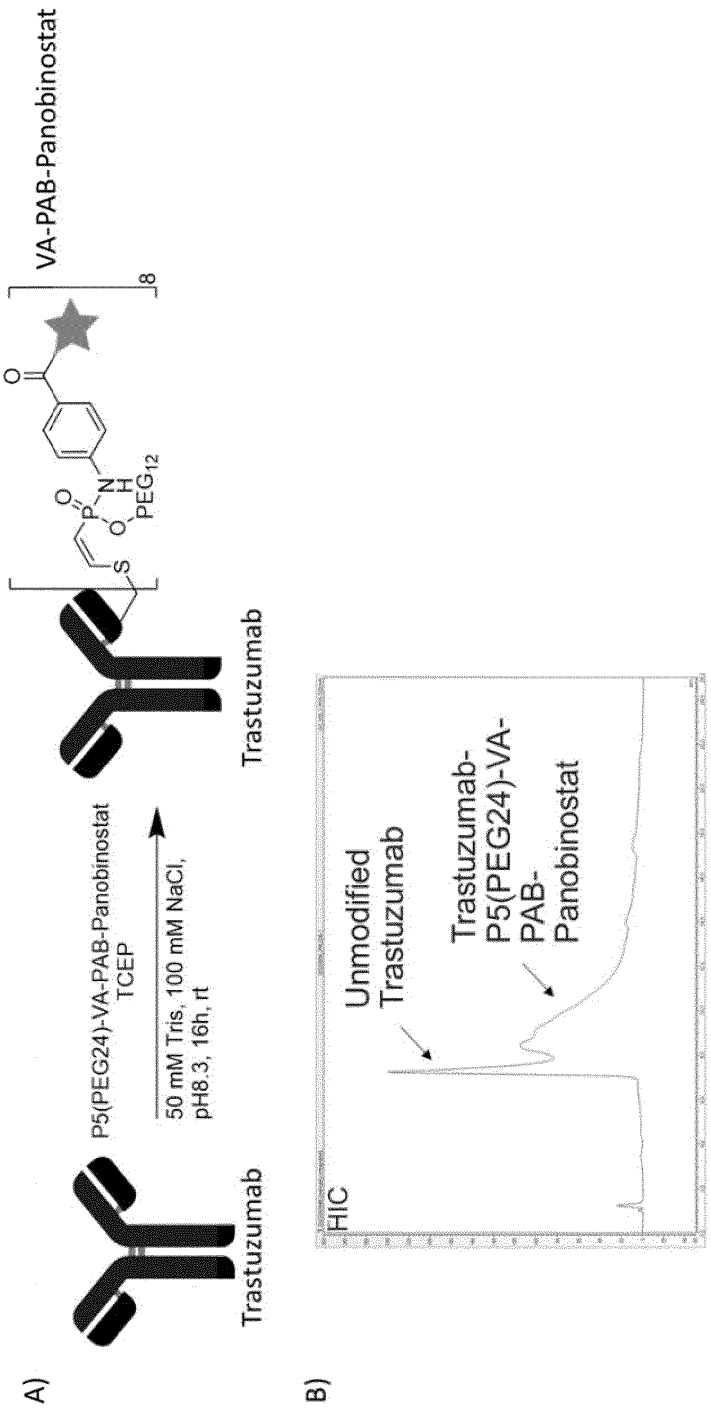

A)
Trastuzumab
P5(PEG24)-VA-PAB-Panobinostat
TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Trastuzumab
VA-PAB-Panobinostat
8
PEG12
B)
HIC
Unmodified
Trastuzumab
Trastuzumab-
P5(PEG24)-VA-
PAB-
Panobinostat

Figure 49

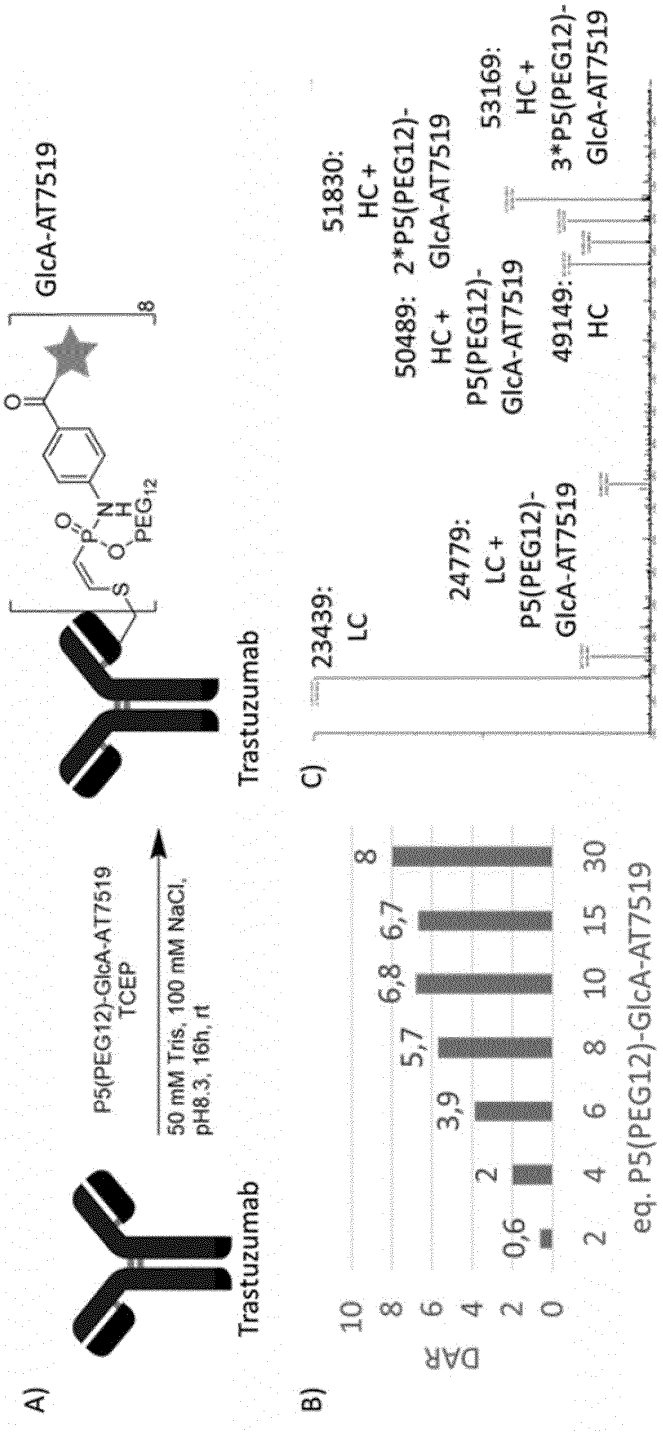

A)
Trastuzumab
P5(PEG12)-GlcA-AT7519
TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Trastuzumab
GlcA-AT7519
8
PEG12
B)
DAR
0
2
4
6
8
10
0,6
2
3,9
5,7
6,8
6,7
2 4 6 8 10 15 30
eq. P5(PEG12)-GlcA-AT7519
C)
23439:
LC
24779:
LC +
P5(PEG12)-
GlcA-AT7519
50489:
HC +
P5(PEG12)-
GlcA-AT7519
51830:
HC +
2*P5(PEG12)-
GlcA-AT7519
49149:
HC
53169:
HC +
3*P5(PEG12)-
GlcA-AT7519

Figure 50

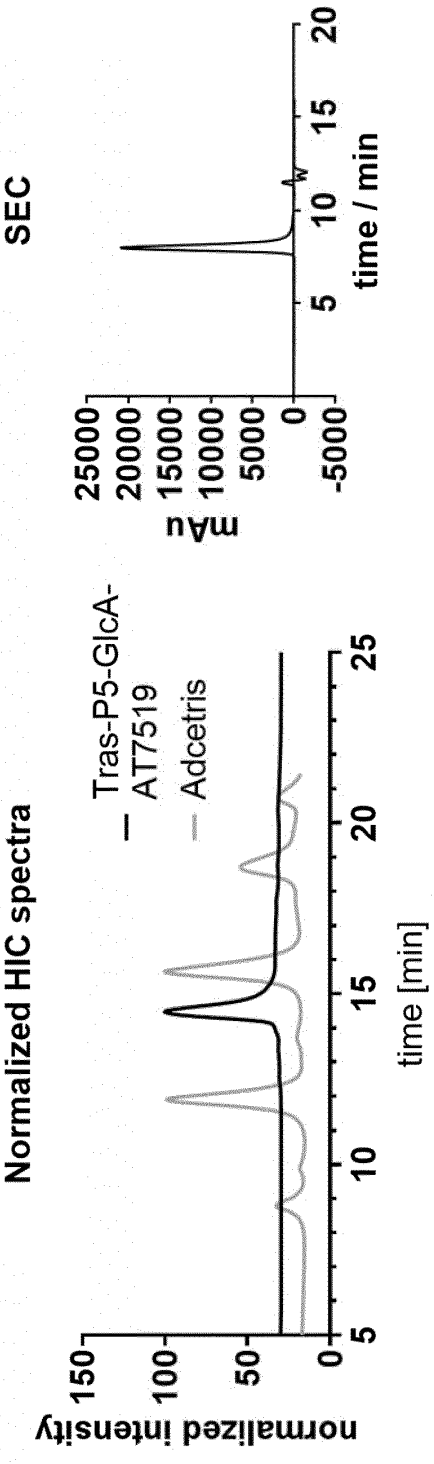

SEC
mAu
25000
20000
15000
10000
5000
0
-5000
5
10
15
20
time / min
Normalized HIC spectra
Tras-P5-GlcA-AT7519
Adcetris
normalized intensity
150
100
50
0
5
10
15
20
25
time [min]

Figure 51

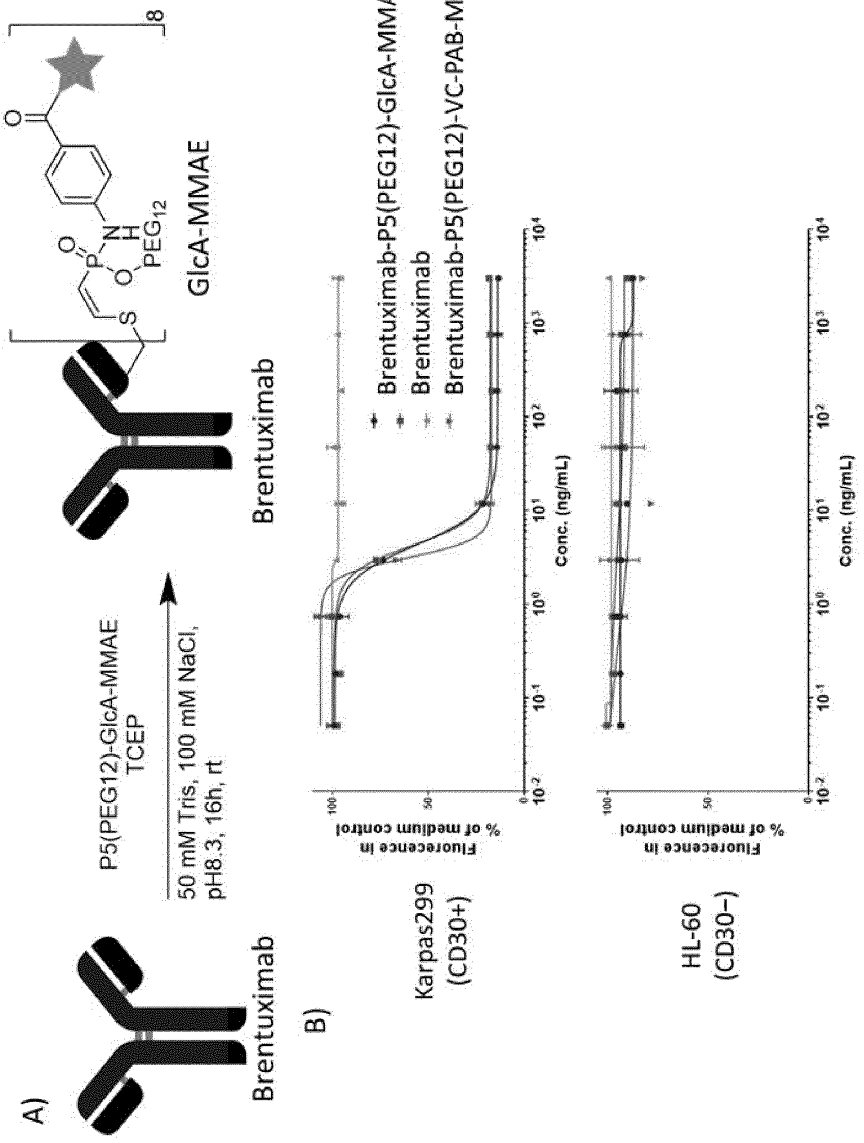

A)
Brentuximab
P5(PEG12)-GlcA-MMAE
TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Brentuximab
GlcA-MMAE
B)
Brentuximab-P5(PEG12)-GlcA-MMAE
Brentuximab
Brentuximab-P5(PEG12)-VC-PAB-MMAE
Fluorescence in % of medium control
Conc. (ng/mL)
Karpas299 (CD30+)
HL-60 (CD30−)

Figure 52

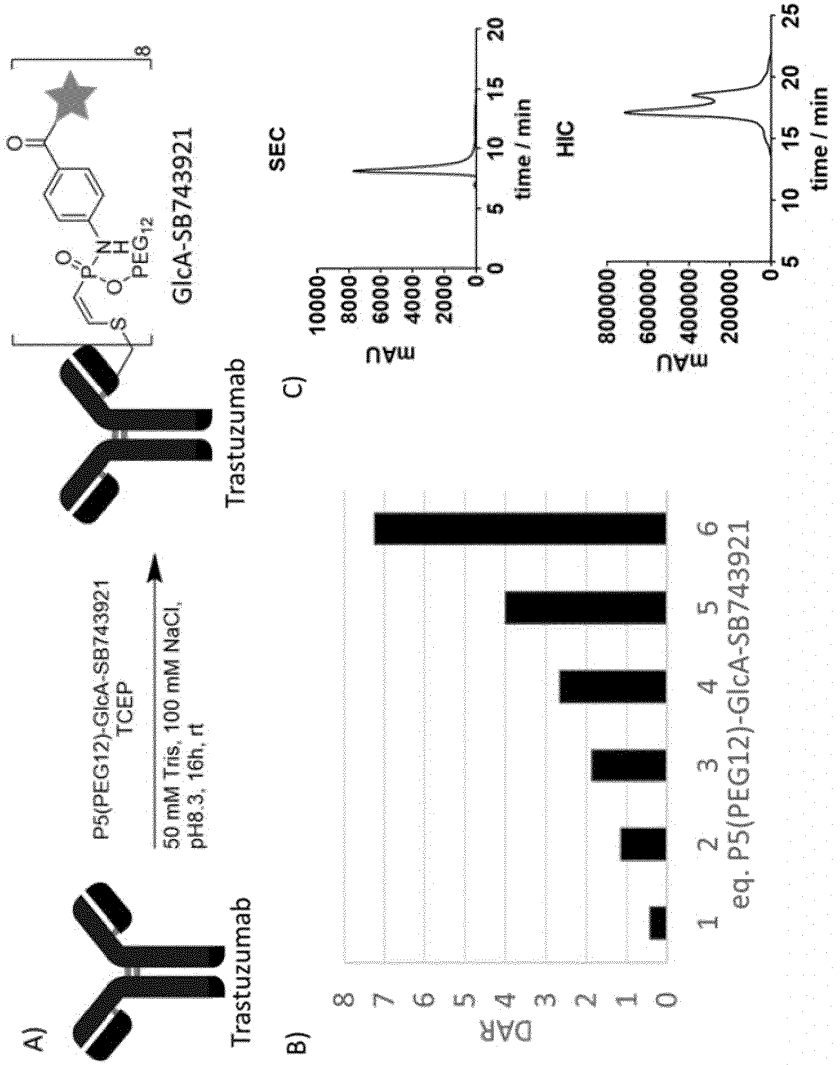
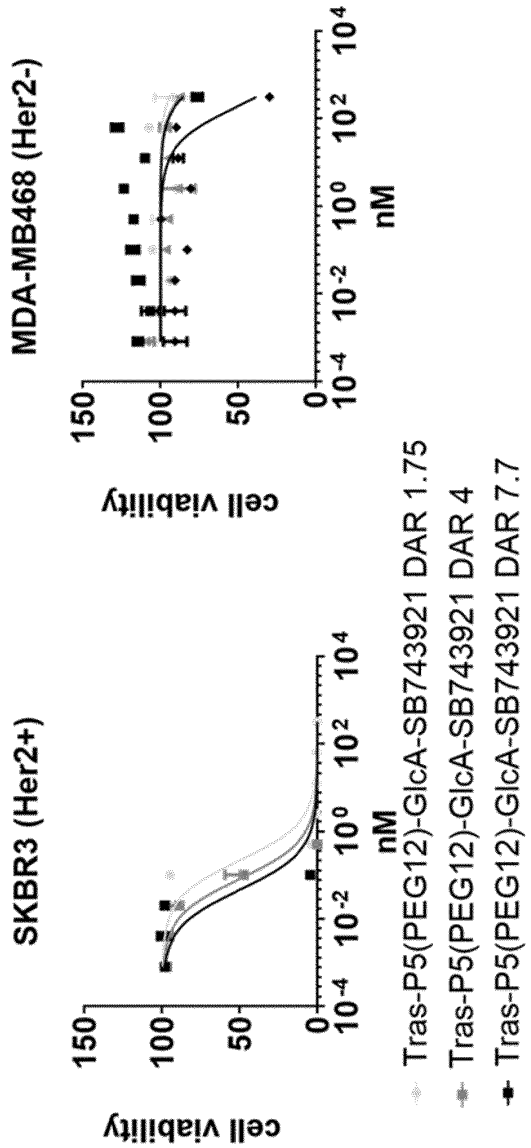

A)
Trastuzumab
P5(PEG12)-GlcA-SB743921
TCEP
50 mM Tris, 100 mM NaCl,
pH8.3, 16h, rt
Trastuzumab
GlcA-SB743921
B)
DAR
eq. P5(PEG12)-GlcA-SB743921
C)
SEC
mAU
time / min
HIC
mAU
time / min

Figure 53


SKBR3 (Her2+)
MDA-MB468 (Her2-)
cell viability
150
100
50
0
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
nM
Tras-P5(PEG12)-GlcA-SB743921 DAR 1.75
Tras-P5(PEG12)-GlcA-SB743921 DAR 4
Tras-P5(PEG12)-GlcA-SB743921 DAR 7.7