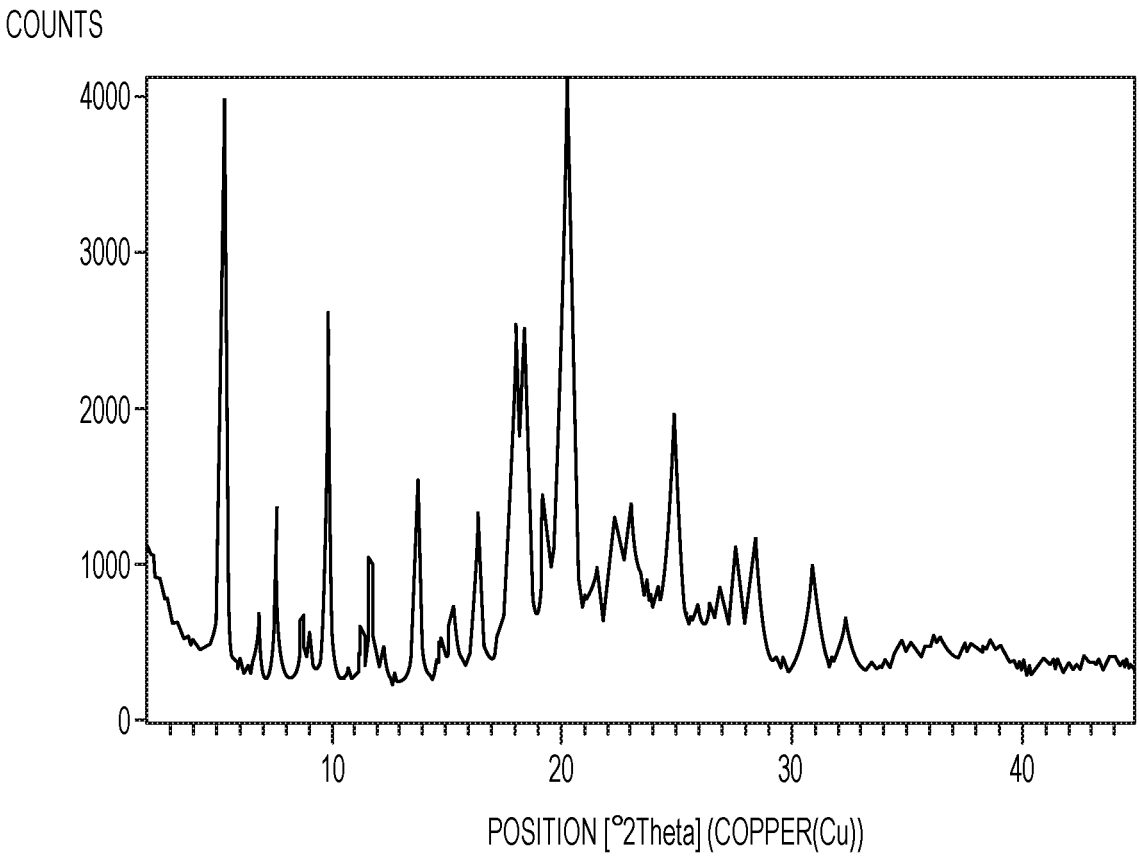

COUNTS
4000
3000
2000
1000
0
10
20
30
40
POSITION [°2Theta] (COPPER(Cu))

Figure 1

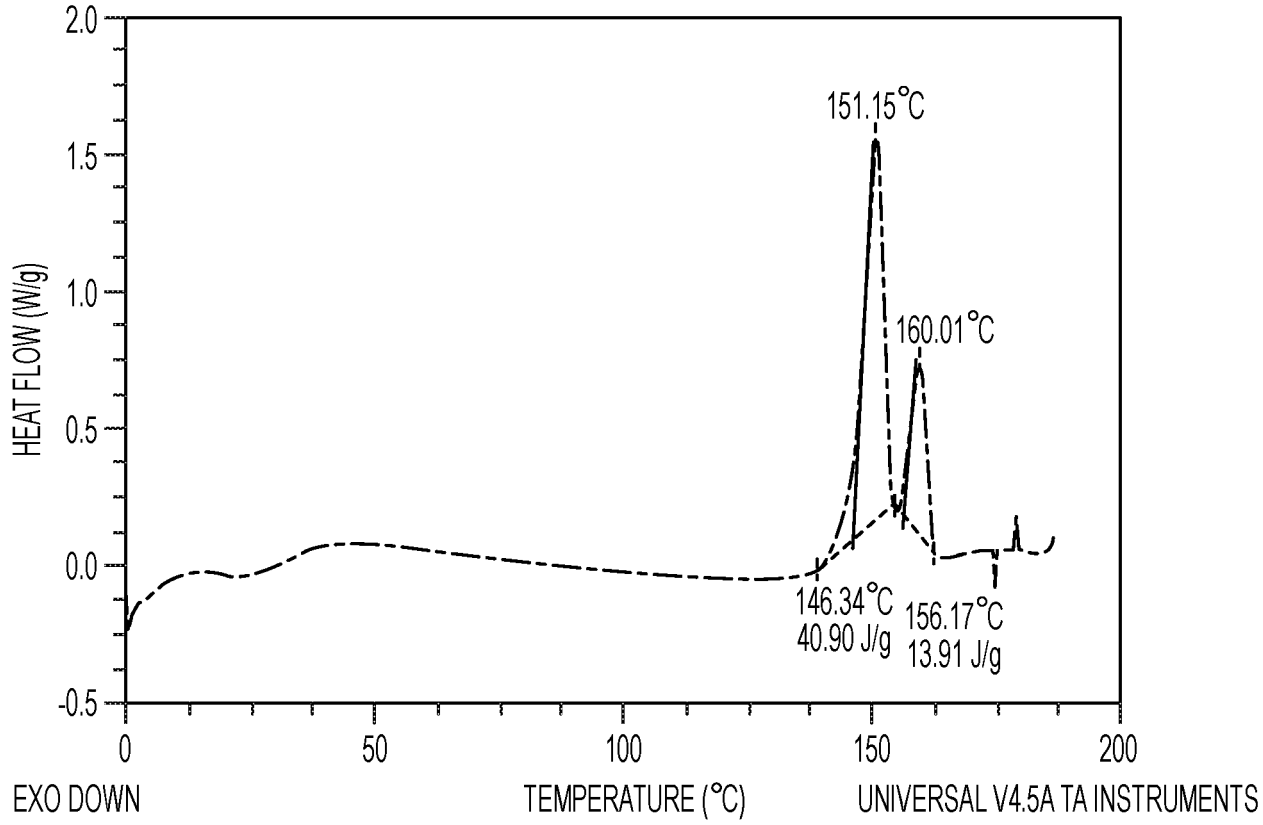

2.0
1.5
1.0
0.5
0.0
-0.5
HEAT FLOW (W/g)
151.15°C
160.01°C
146.34°C
40.90 J/g
156.17°C
13.91 J/g
0
50
100
150
200
EXO DOWN
TEMPERATURE (°C)
UNIVERSAL V4.5A TA INSTRUMENTS

Figure 2

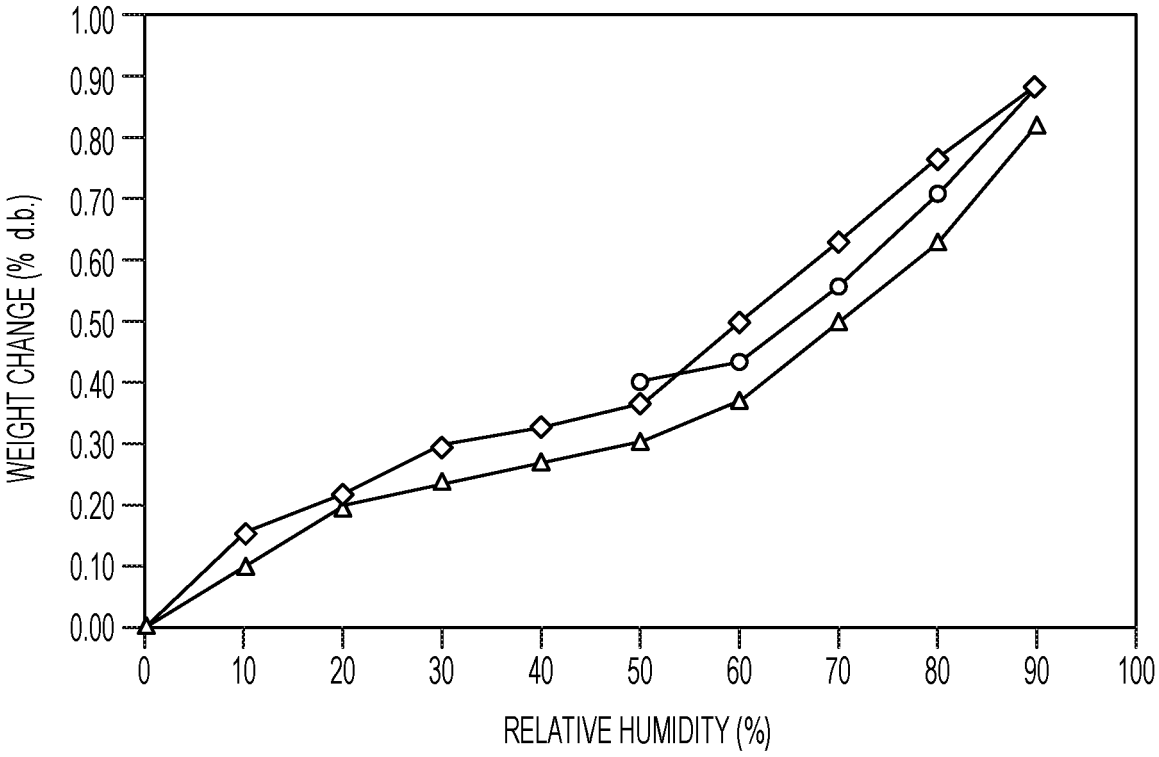

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
WEIGHT CHANGE (% d.b.)
0
10
20
30
40
50
60
70
80
90
100
RELATIVE HUMIDITY (%)

Figure 3

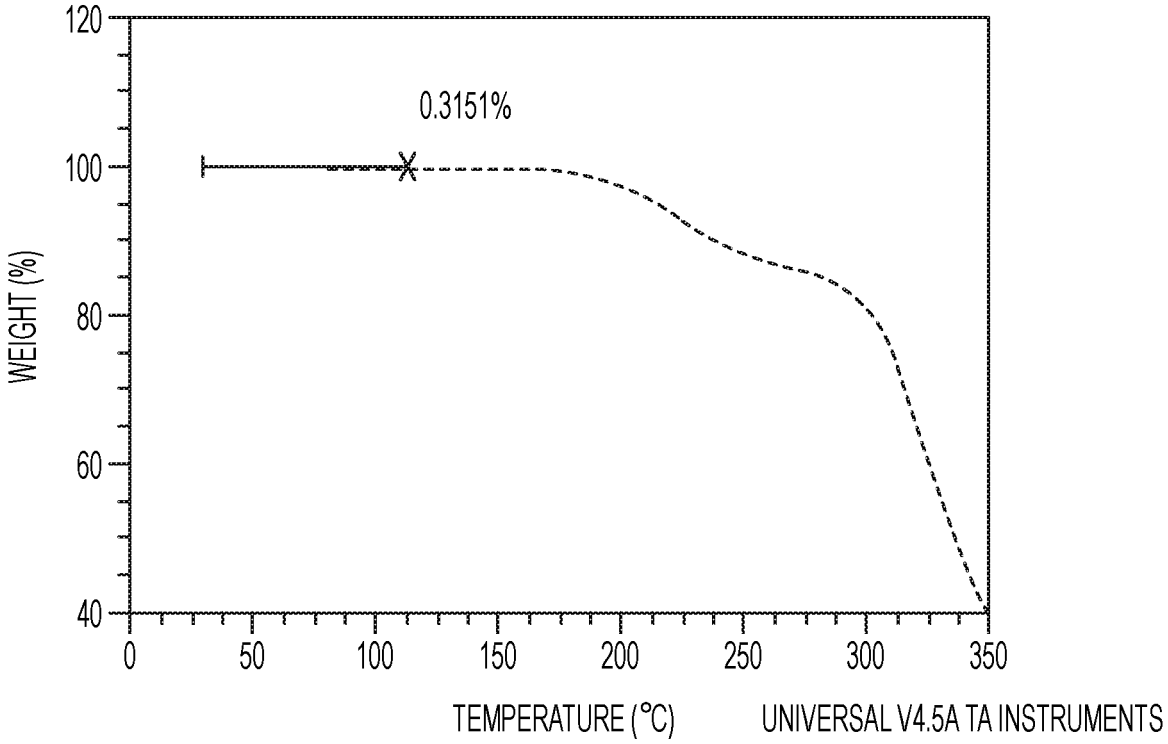

120
100
80
60
40
WEIGHT (%)
0.3151%
0
50
100
150
200
250
300
350
TEMPERATURE (°C)
UNIVERSAL V4.5A TA INSTRUMENTS

Figure 4

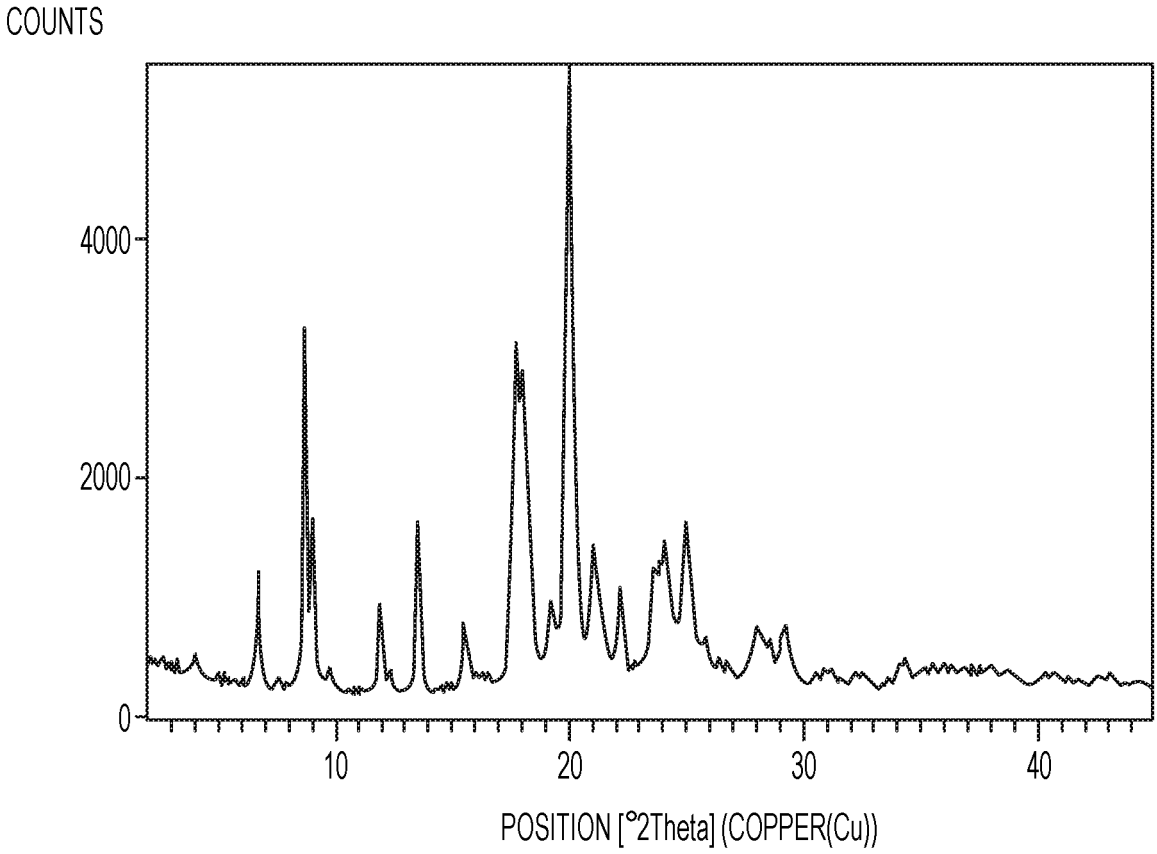


Figure 5

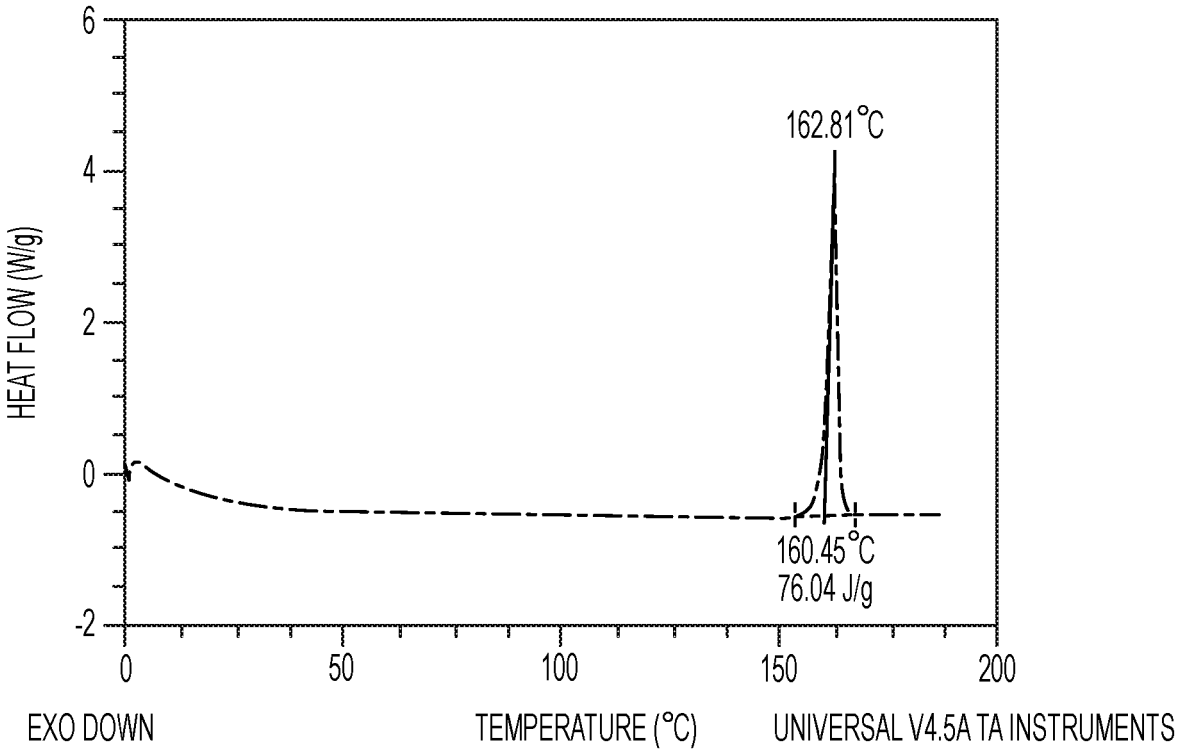

6
4
2
0
-2
HEAT FLOW (W/g)
162.81°C
160.45°C
76.04 J/g
0
50
100
150
200
EXO DOWN
TEMPERATURE (°C)
UNIVERSAL V4.5A TA INSTRUMENTS

Figure 6

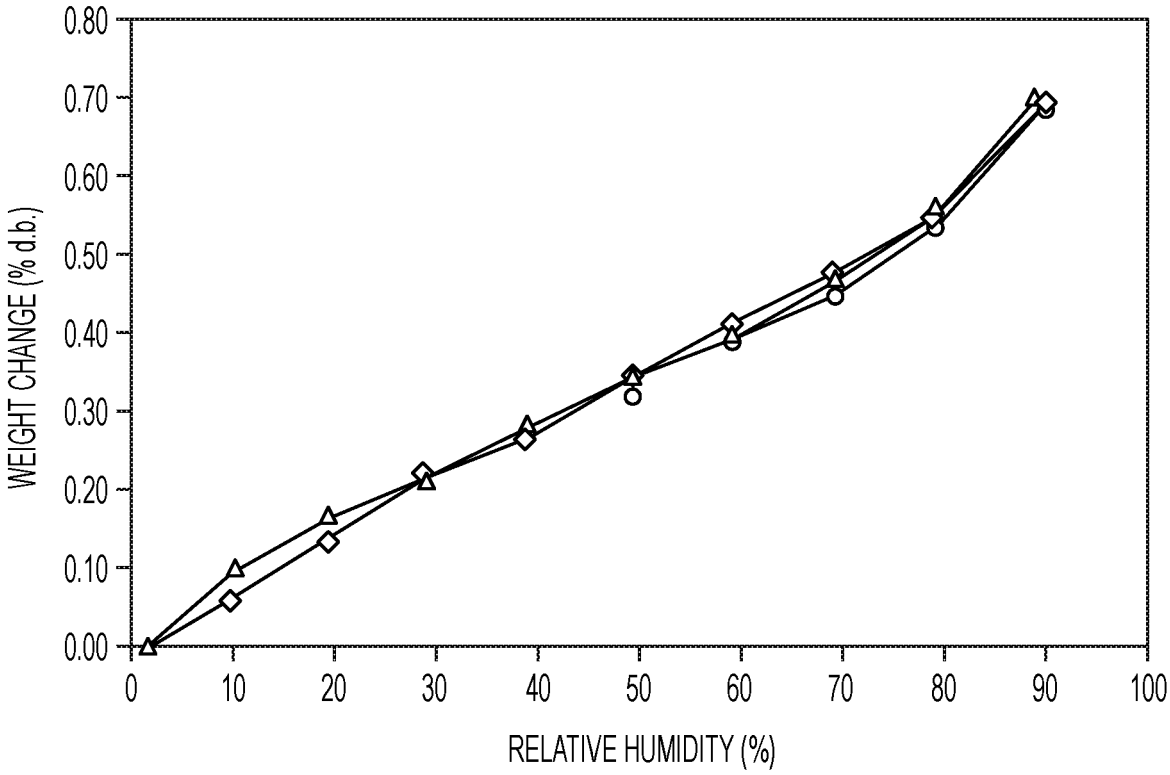

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
WEIGHT CHANGE (% d.b.)
0
10
20
30
40
50
60
70
80
90
100
RELATIVE HUMIDITY (%)

Figure 7

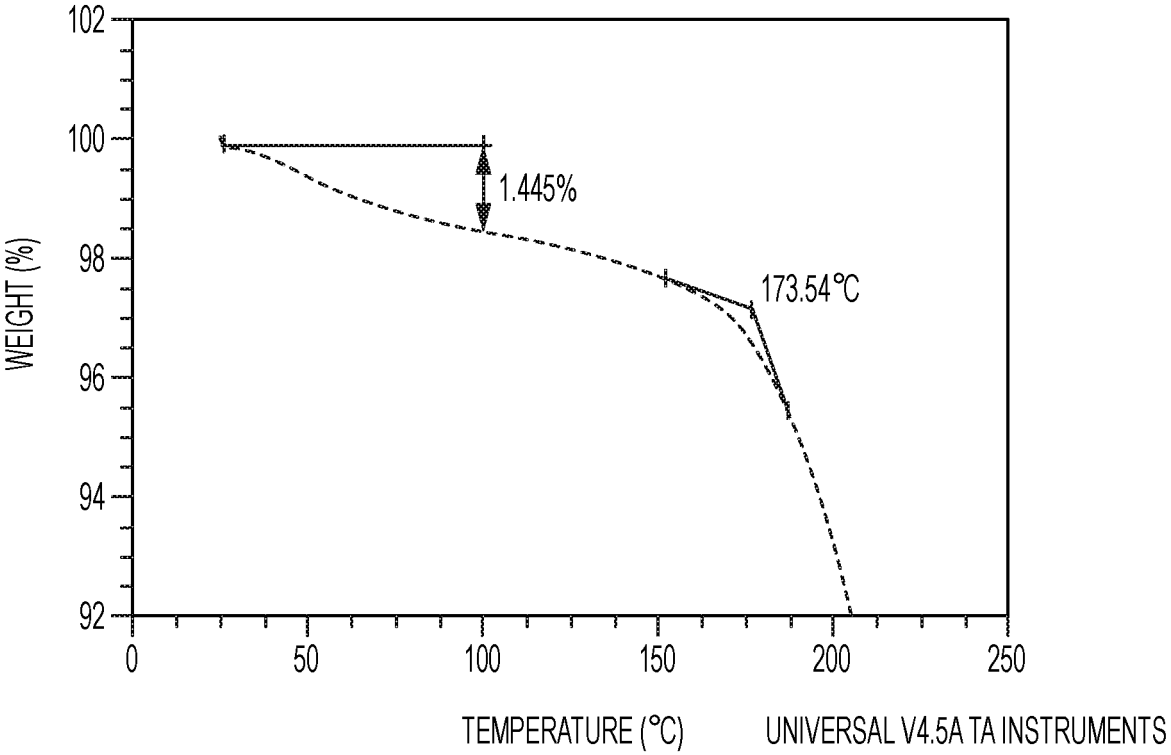

102
100
98
96
94
92
WEIGHT (%)
1.445%
173.54°C
0
50
100
150
200
250
TEMPERATURE (°C)
UNIVERSAL V4.5A TA INSTRUMENTS

Figure 8

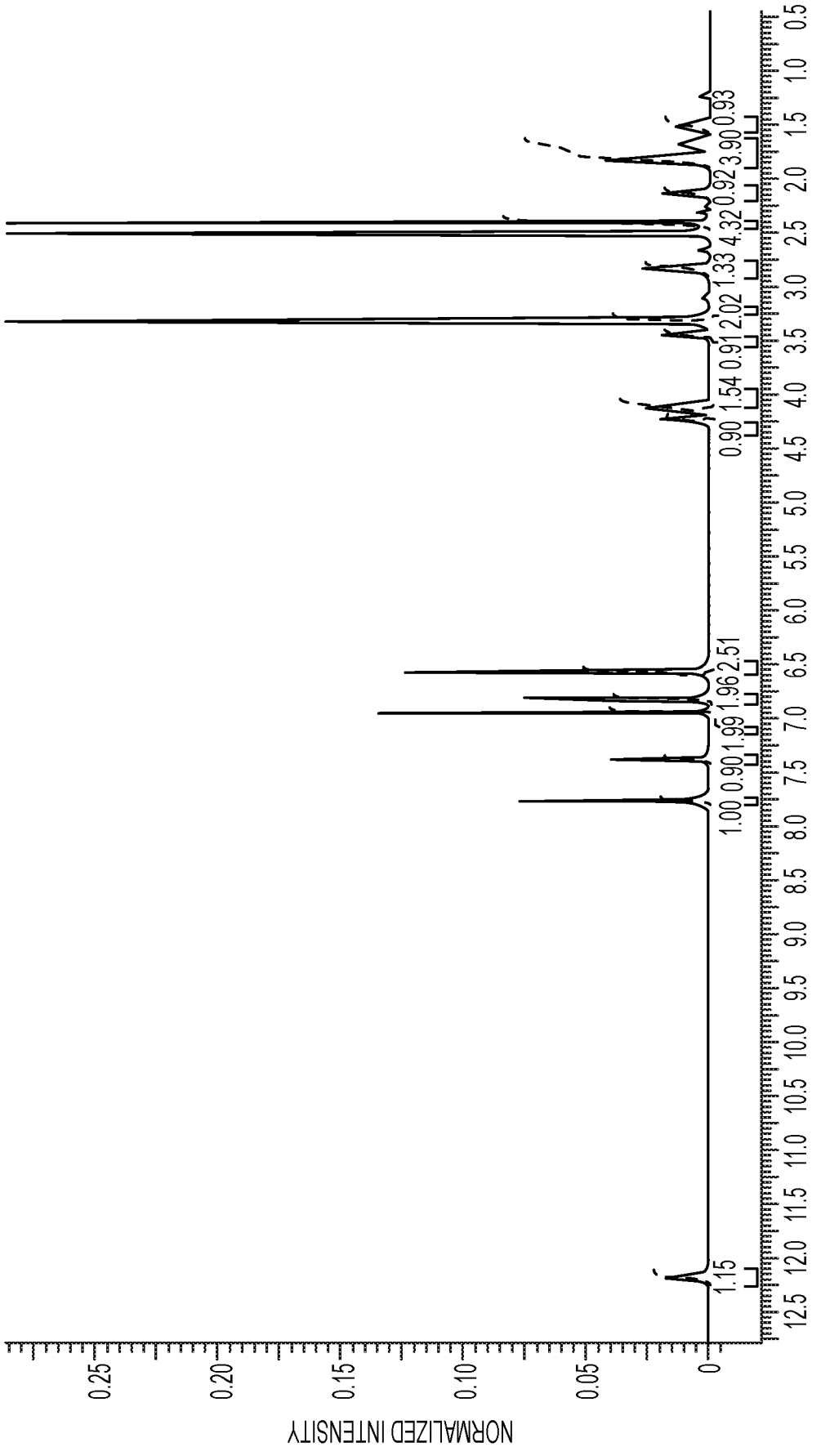

NORMALIZED INTENSITY
0.25
0.20
0.15
0.10
0.05
0
CHEMICAL SHIFT (ppm)
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1.15
1.00 0.90 1.99 1.96 2.51
0.90 1.54 0.91 2.02 1.33 4.32 0.92 3.90 0.93

Figure 9

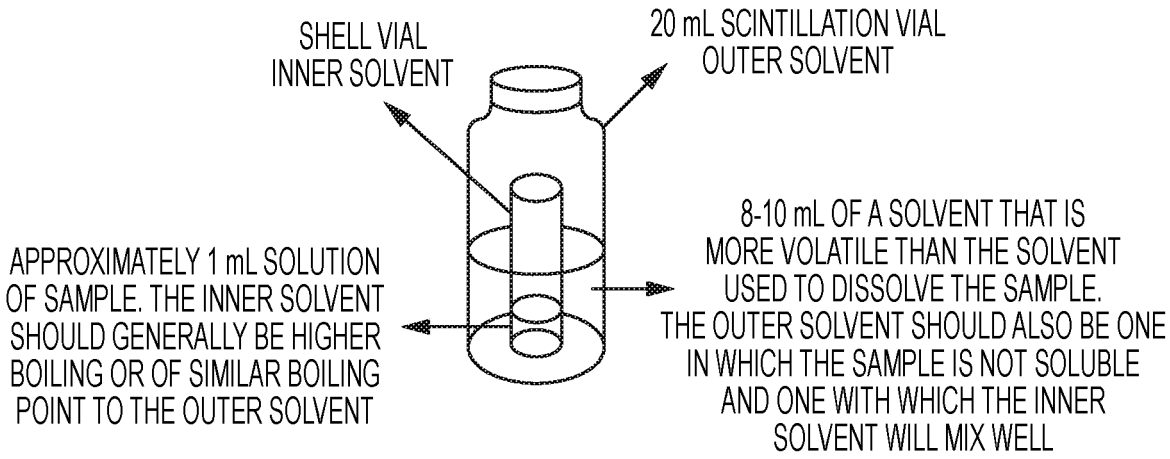
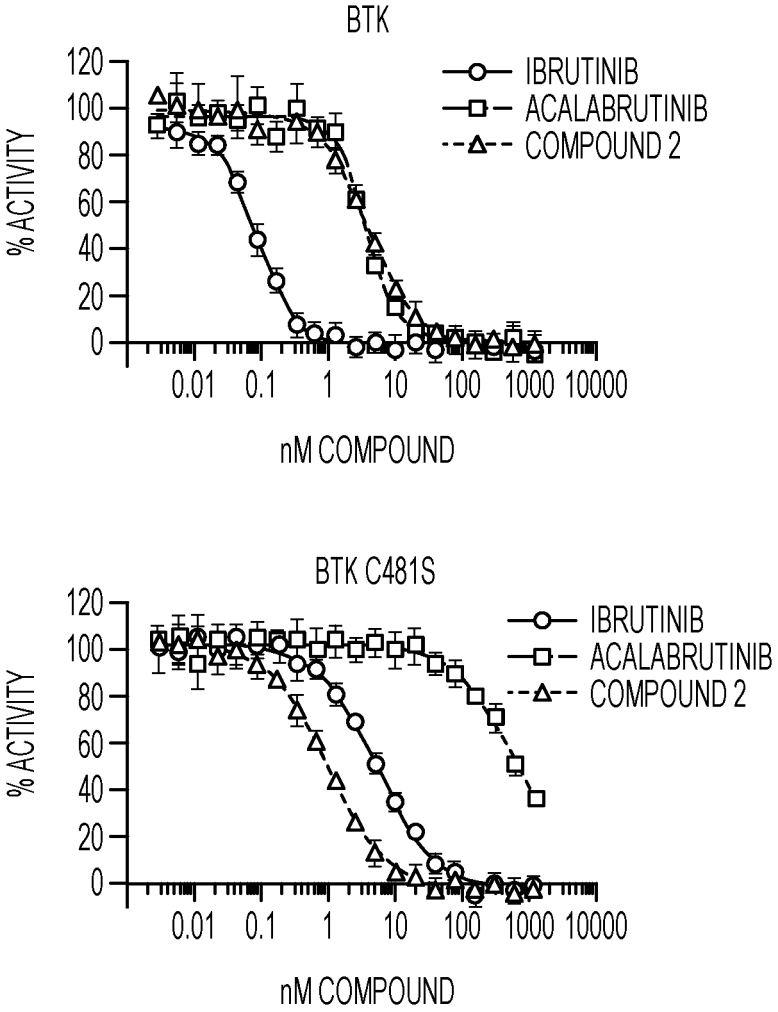

SHELL VIAL
INNER SOLVENT
20 mL SCINTILLATION VIAL
OUTER SOLVENT
APPROXIMATELY 1 mL SOLUTION OF SAMPLE. THE INNER SOLVENT SHOULD GENERALLY BE HIGHER BOILING OR OF SIMILAR BOILING POINT TO THE OUTER SOLVENT
8-10 mL OF A SOLVENT THAT IS MORE VOLATILE THAN THE SOLVENT USED TO DISSOLVE THE SAMPLE. THE OUTER SOLVENT SHOULD ALSO BE ONE IN WHICH THE SAMPLE IS NOT SOLUBLE AND ONE WITH WHICH THE INNER SOLVENT WILL MIX WELL

Figure 10

EFFECTS OF Cys481Ser ON THE ACTIVITY OF BTK INHIBITORS[a]



IC_{50} (nM), [ATP] = 50 μM	WT BTK	Cys481Ser BTK	FOLD CHANGE
COMPOUND 2	4.6	1.1	0.2
IBRUTINIB	0.1	6.6	66
ACALABRUTINIB	4.2	707	168

[a]DIRECT MEASUREMENT OF KINASE ACTIVITY OF HUMAN RECOMBINANT WT BTK OR Cys481Ser MUTANT BTK WAS PERFORMED IN A FRET KINASE ASSAY

Figure 11

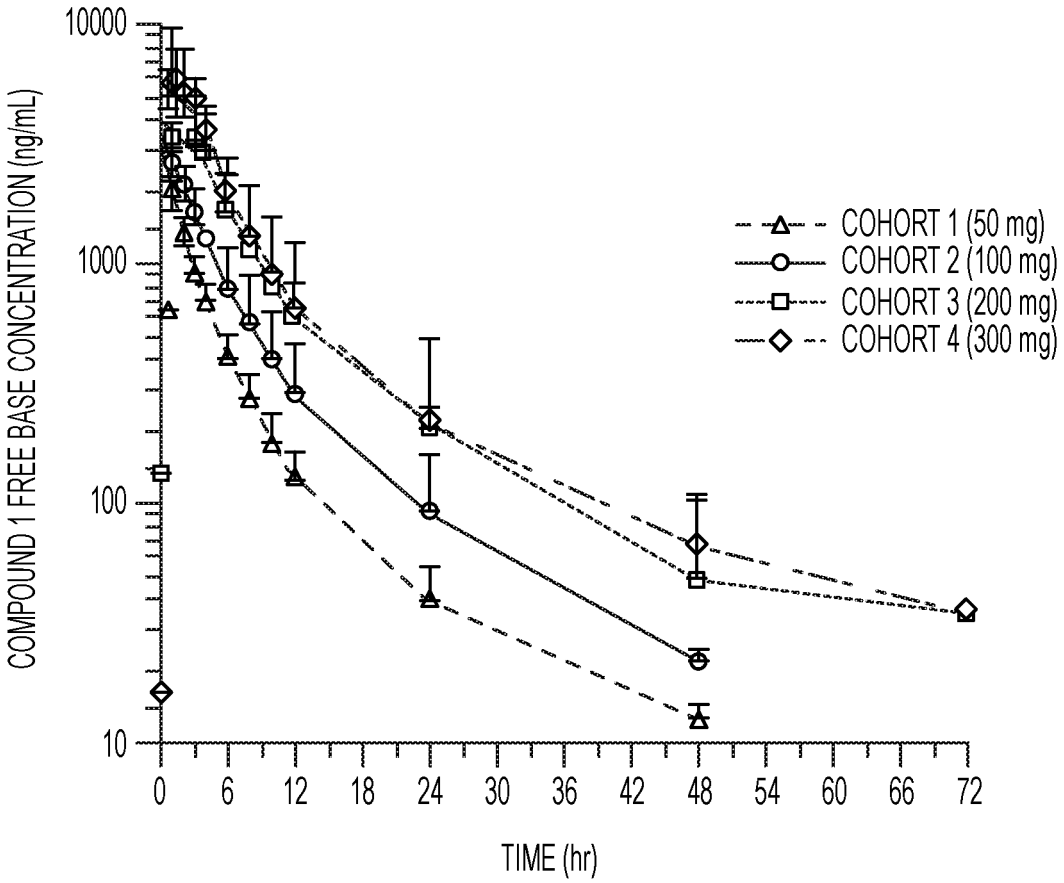
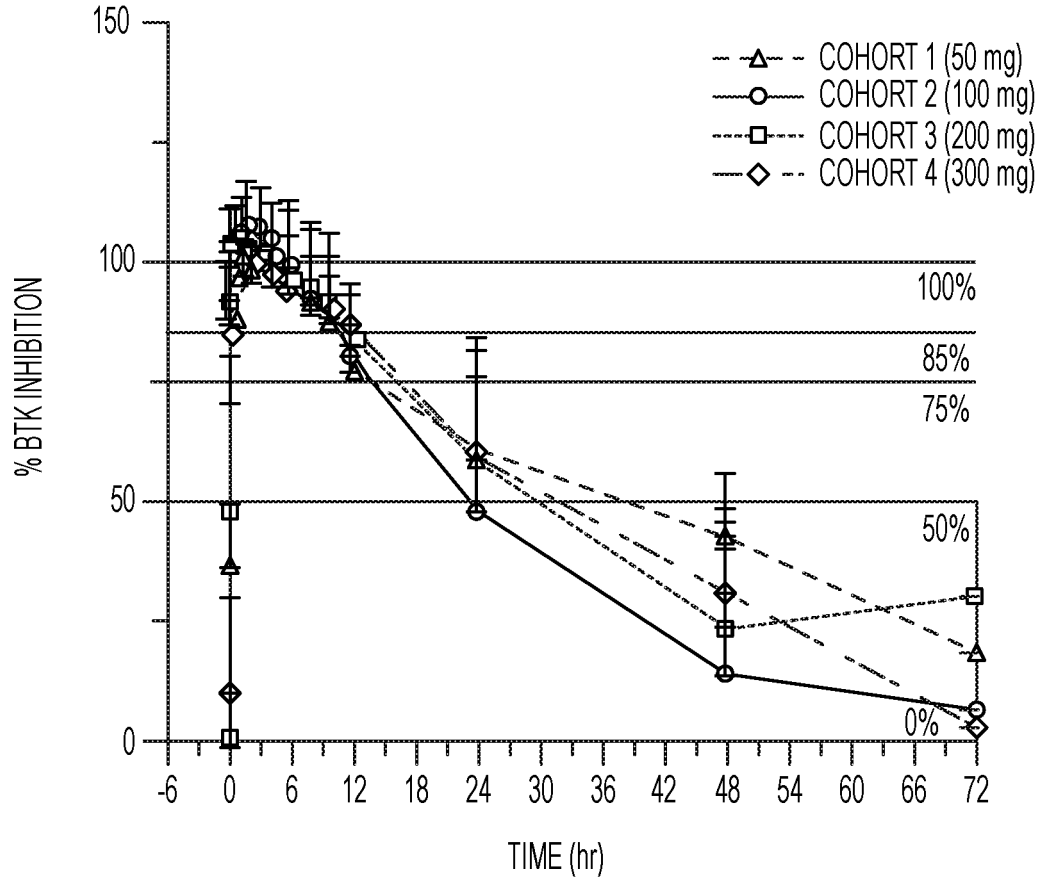

10000
1000
100
10
COMPOUND 1 FREE BASE CONCENTRATION (ng/mL)
0
6
12
18
24
30
36
42
48
54
60
66
72
TIME (hr)
COHORT 1 (50 mg)
COHORT 2 (100 mg)
COHORT 3 (200 mg)
COHORT 4 (300 mg)
DATA REPORTED AS MEAN, SD

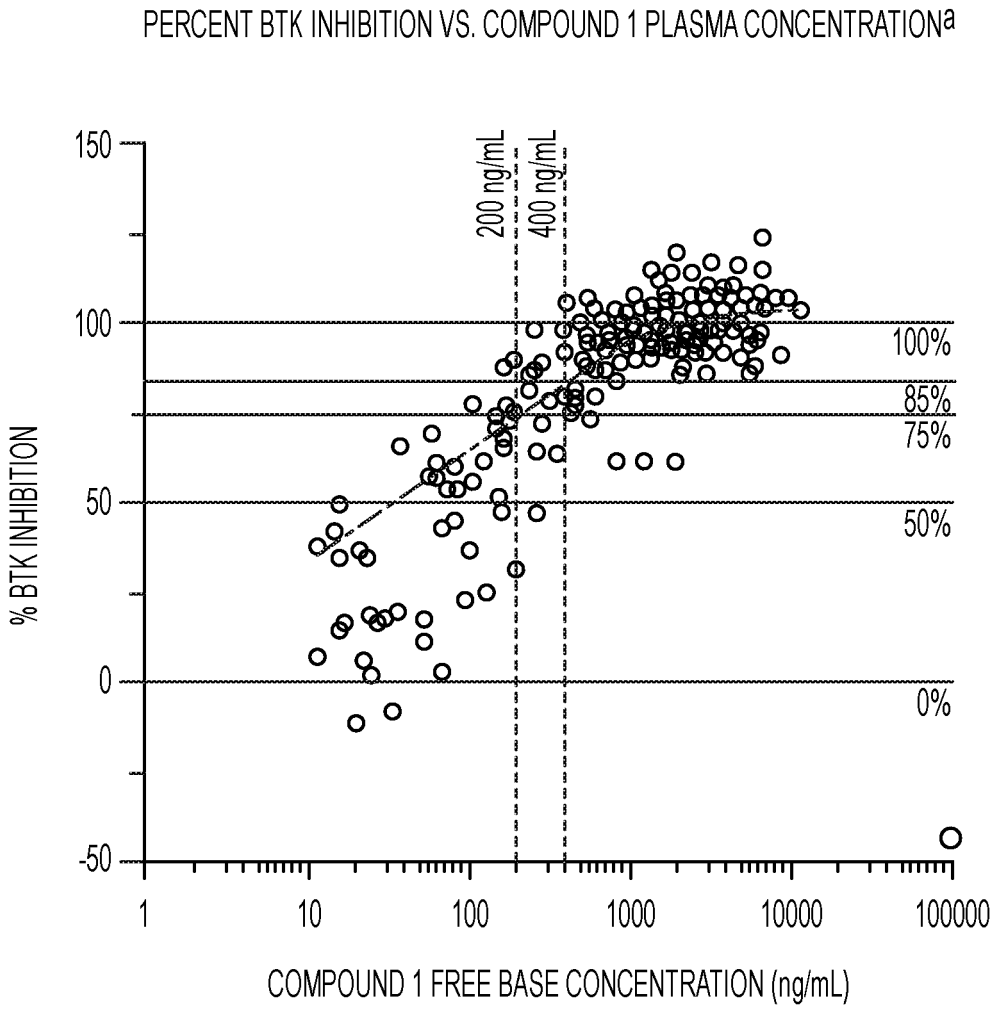
Figure 12

PERCENT BTK INHIBITION VS. TIME[a]



%BTK INHIBITION (MEAN, SD) DETERMINED BY ASSESSMENT OF pBTK AS DESCRIBED IN METHODS BTK, BRUTON'S TYROSINE KINASE; pBTK, PHOSPHORYLATED BTK.

Figure 13



%BTK INHIBITION DETERMINED BY ASSESSMENT OF pBTK AS DESCRIBED IN METHODS; COMPOUND 1 FREE BASE CONCENTRATION DETERMINED BY LC-MS/MS
BTK, BRUTON'S TYROSINE KINASE; pBTK, PHOSPHORYLATED BTK.

Figure 14

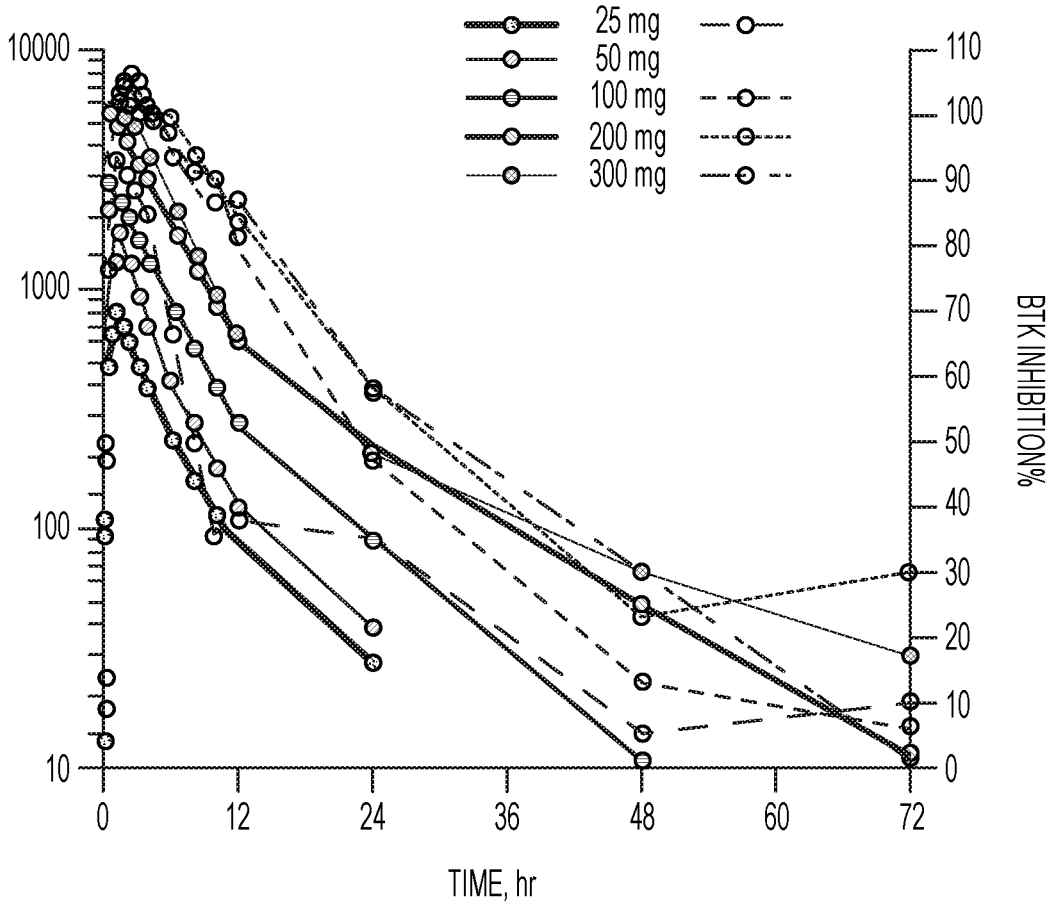

25 mg
50 mg
100 mg
200 mg
300 mg
10000
1000
100
10
110
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
TIME, hr
BTK INHIBITION%

Figure 15

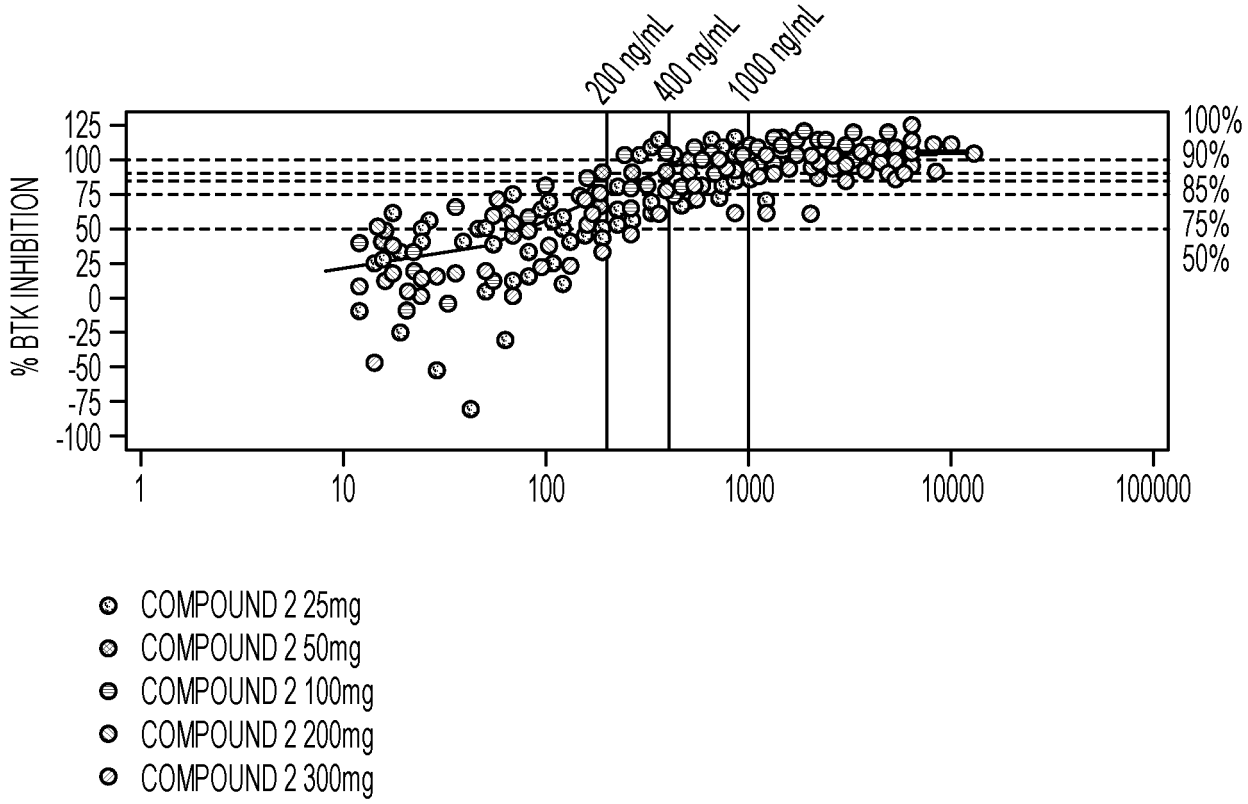

200 ng/mL
400 ng/mL
1000 ng/mL
% BTK INHIBITION
125
100
75
50
25
0
-25
-50
-75
-100
1
10
100
1000
10000
100000
100%
90%
85%
75%
50%
COMPOUND 2 25mg
COMPOUND 2 50mg
COMPOUND 2 100mg
COMPOUND 2 200mg
COMPOUND 2 300mg

Figure 16

COMPOUND	WT C481 BTK IC_{50} nM	C481S BTK IC_{50} nM	FOLD CHANGE [a]
COMPOUND 1	570	800	1.4
IBRUTINIB	16	1700	>100

[a]EXPRESSED AS IC_{50} OF COMPOUND AGAINST C481S-BTK DIVIDED BY IC_{50} OF COMPOUND AGAINST WT BTK.

Figure 17