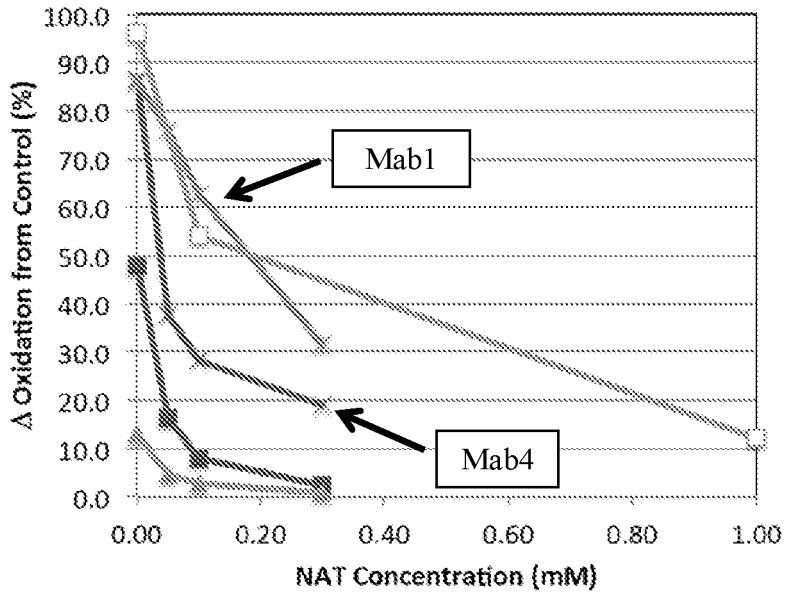
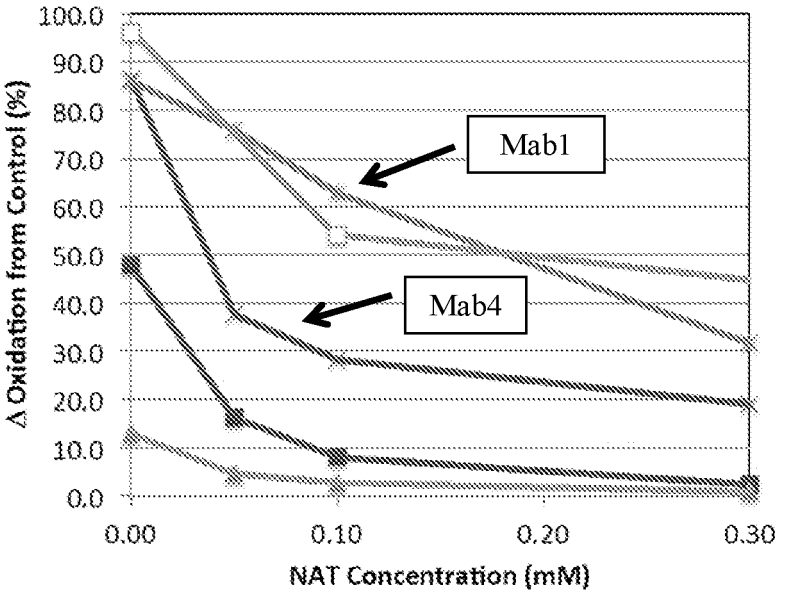
FIG. 1





	Molecule	Residue	SASA
	Mab2	HC53	169
	Mab2	HC106	48
	Mab4	HC52	63
	Mab1	HC103	114
	Mab6	HC103-104	117

FIG. 2A

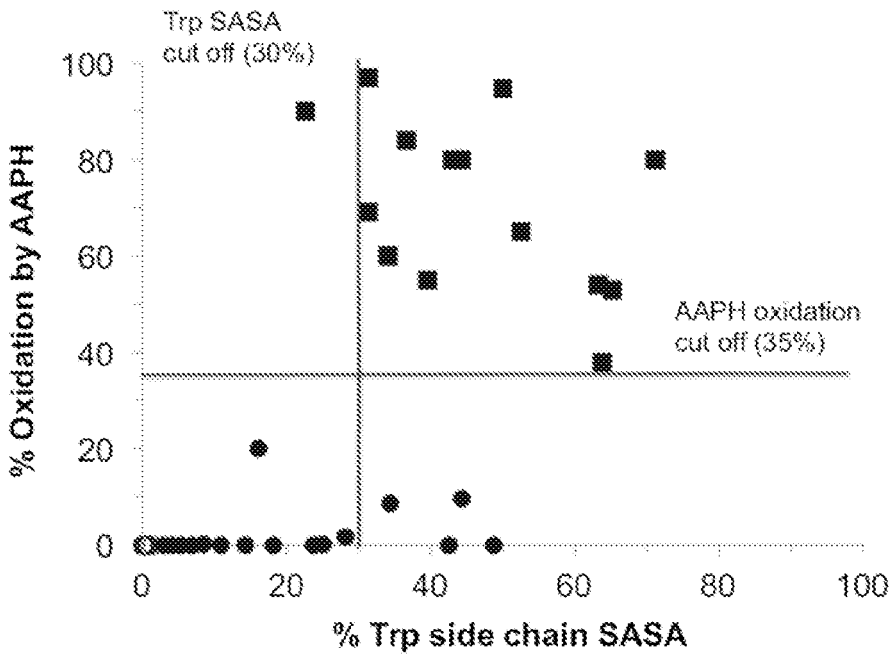

Trp SASA
cut off (30%)
AAPH oxidation
cut off (35%)
% Oxidation by AAPH
% Trp side chain SASA
0
20
40
60
80
100

FIG. 2B

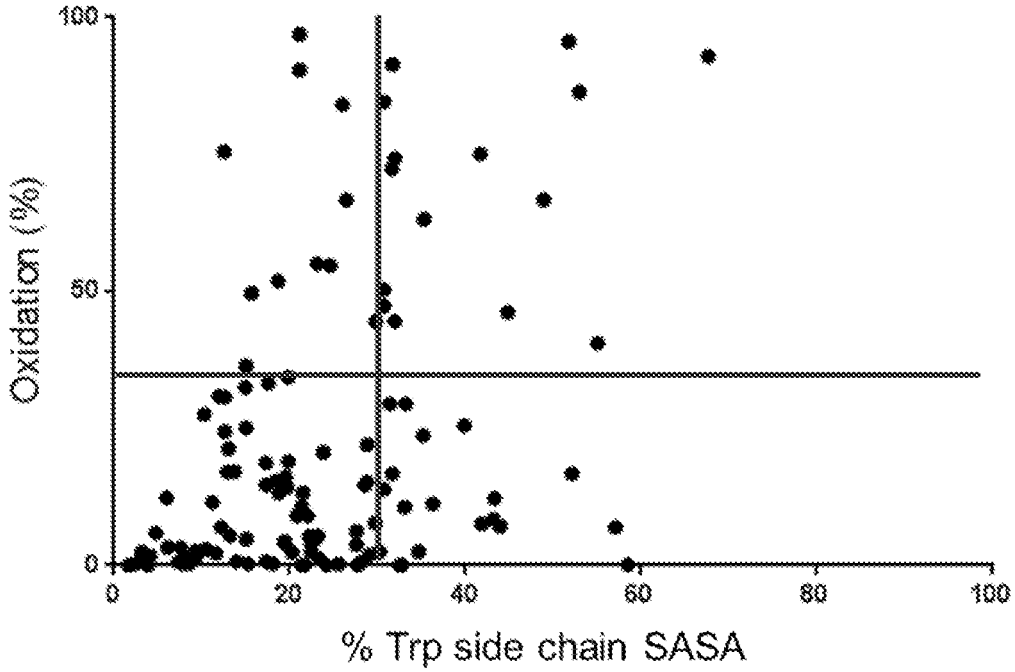

Oxidation (%)
% Trp side chain SASA
0
50
100
0
20
40
60
80
100

FIG. 3

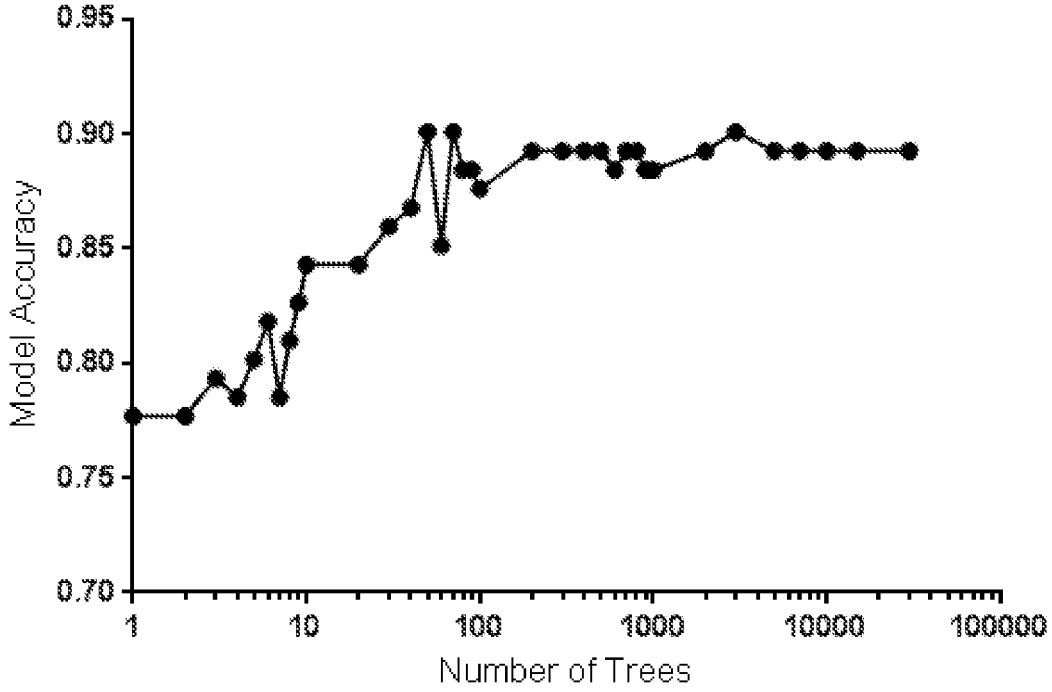

0.95
0.90
0.85
0.80
0.75
0.70
Model Accuracy
1
10
100
1000
10000
100000
Number of Trees

FIG. 4

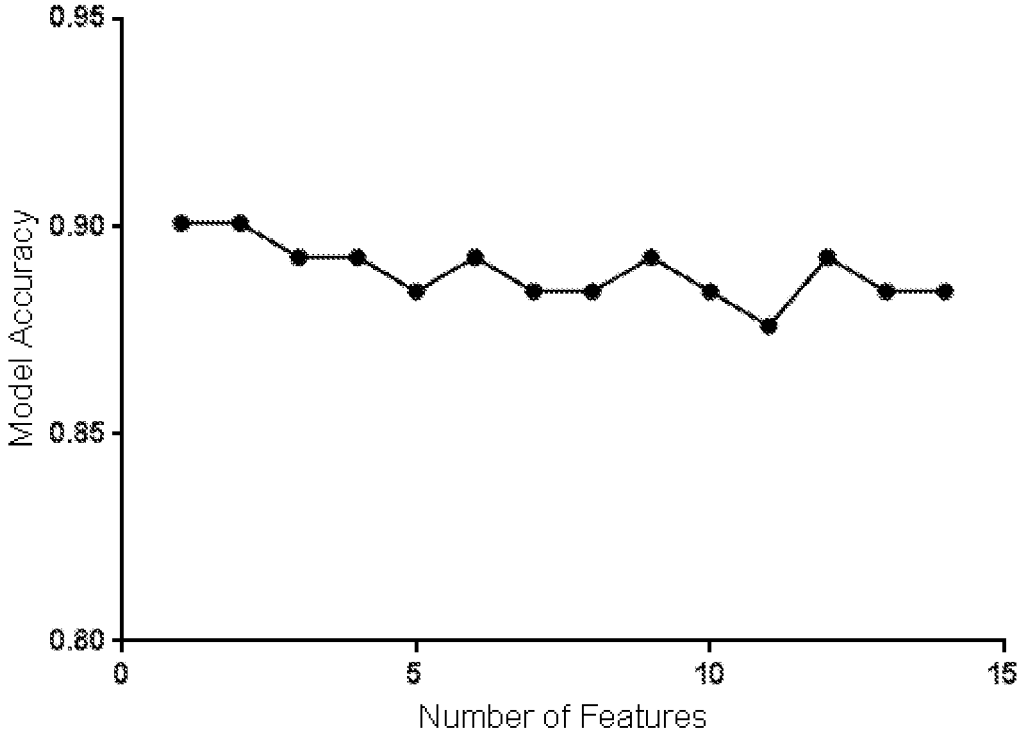


FIG. 5

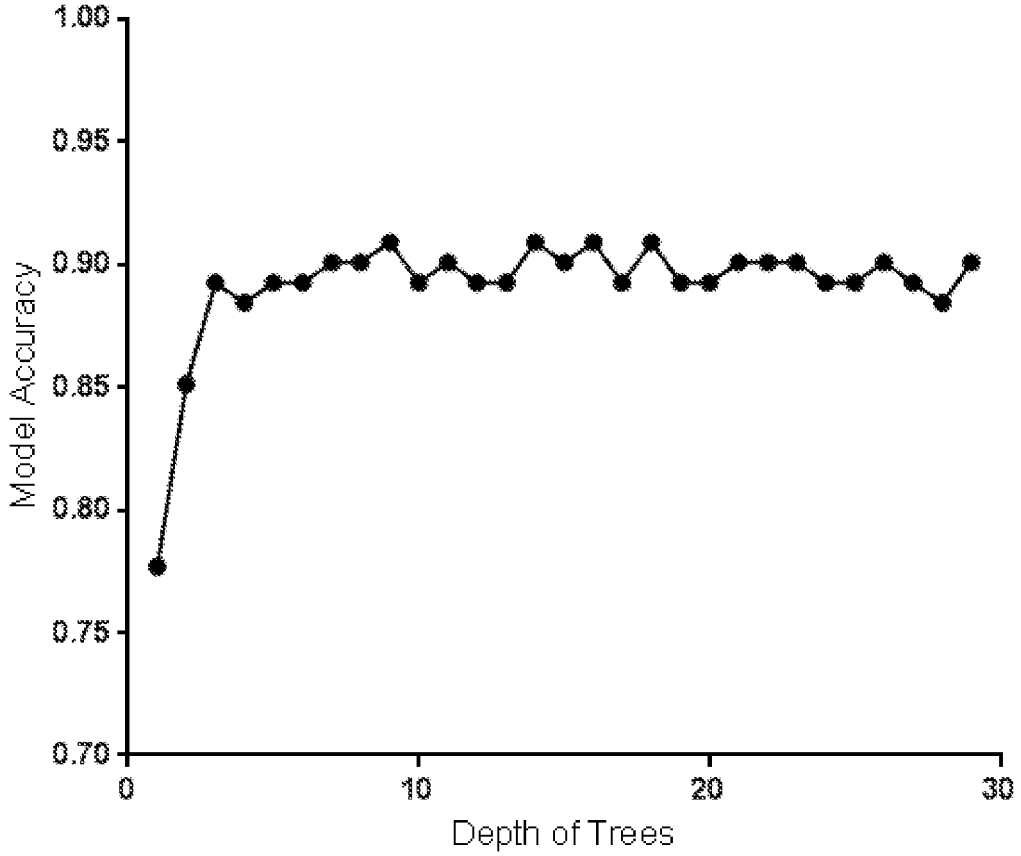

1.00
0.95
0.90
0.85
0.80
0.75
0.70
Model Accuracy
0
10
20
30
Depth of Trees

FIG. 6

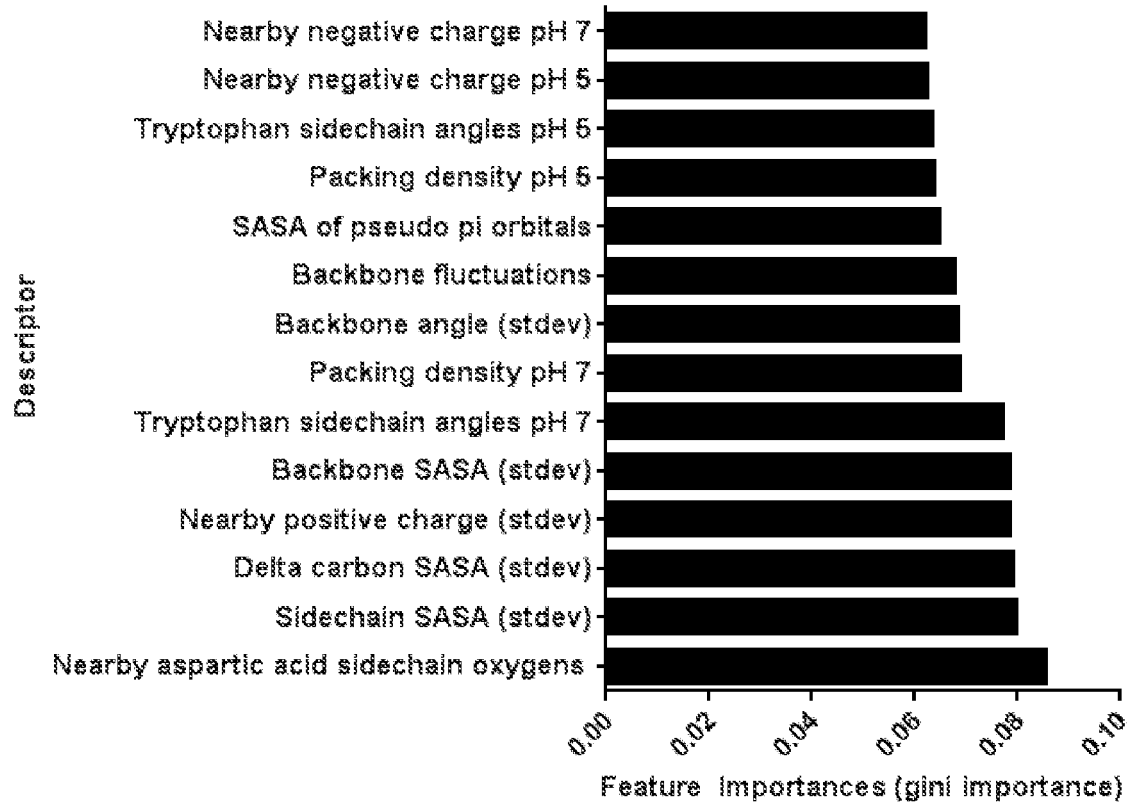
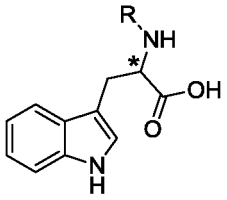
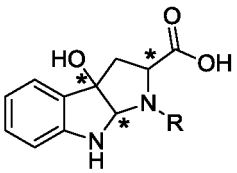

Nearby negative charge pH 7
Nearby negative charge pH 5
Tryptophan sidechain angles pH 5
Packing density pH 5
SASA of pseudo pi orbitals
Backbone fluctuations
Backbone angle (stdev)
Packing density pH 7
Tryptophan sidechain angles pH 7
Backbone SASA (stdev)
Nearby positive charge (stdev)
Delta carbon SASA (stdev)
Sidechain SASA (stdev)
Nearby aspartic acid sidechain oxygens
Descriptor
0.00
0.02
0.04
0.06
0.08
0.10
Feature importances (gini importance)

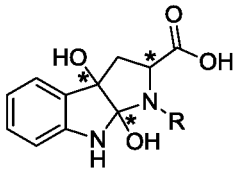
FIG. 7



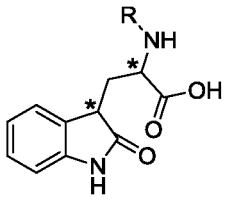
1a, R = H, tryptophan(Trp)
1b, R = Ac, N-Ac-tryptophan (NAT)



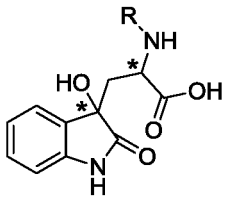
2a, R = H, 1,2,3,3a,8,8a-hexahydro-3a-hydroxypyrrolo [2,3-b]-indole-2-carboxylic acid (PIC)
2b, R = Ac, N-Ac-PIC



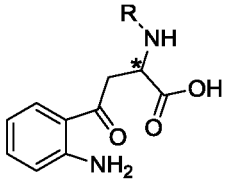
3a, R = H, 3a, 8a-dihydroxy-PIC
3b, R = Ac, N-Ac-3a, 8a, dihydroxy-PIC



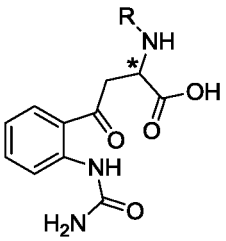
4a, R = H, oxindolylalanine (Oia)
4b, R = Ac, N-Ac-Oia



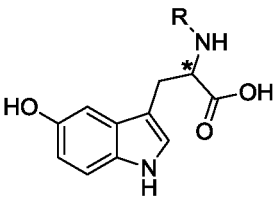
5a, R = H, dioxindolylalanine (DiOia)
5b, R = Ac, N-Ac-DiOia



6a, R = H, Kynurenine (Kyn)
6b, R = Ac, N-Ac-Kyn

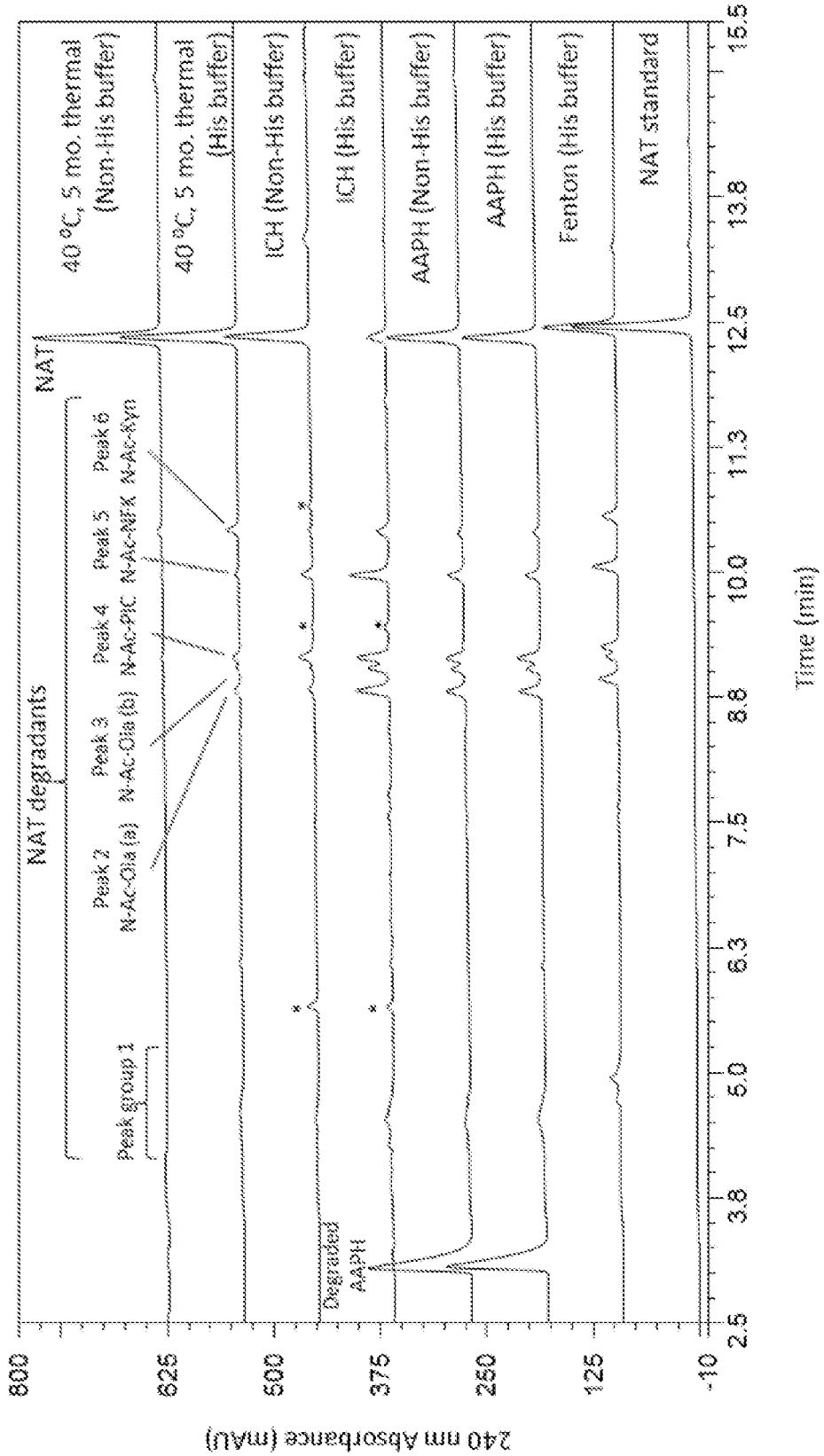


7a, R = H, N-formylkynurenine (NFK)
7b, R = Ac, N-Ac-NFK



8a, R = H, 5-hydroxy-tryptophan (5-OH-Trp)
8b, R = Ac, N-Ac-5-OH-Trp

FIG. 8A


NAT degradants
NAT
Peak group 1
Peak 2
N-Ac-Dia (a)
Peak 3
N-Ac-Dia (b)
Peak 4
N-Ac-PIC
Peak 5
N-Ac-NFK
Peak 6
N-Ac-Kyn
40 °C, 5 mo. thermal (Non-His buffer)
40 °C, 5 mo. thermal (His buffer)
ICH (Non-His buffer)
ICH (His buffer)
AAPH (Non-His buffer)
AAPH (His buffer)
Fenton (His buffer)
NAT standard
Degraded AAPH
240 nm Absorbance (mAU)
800
625
500
375
250
125
-10
2.5
3.8
5.0
6.3
7.5
8.8
10.0
11.3
12.5
13.8
15.5
Time (min)

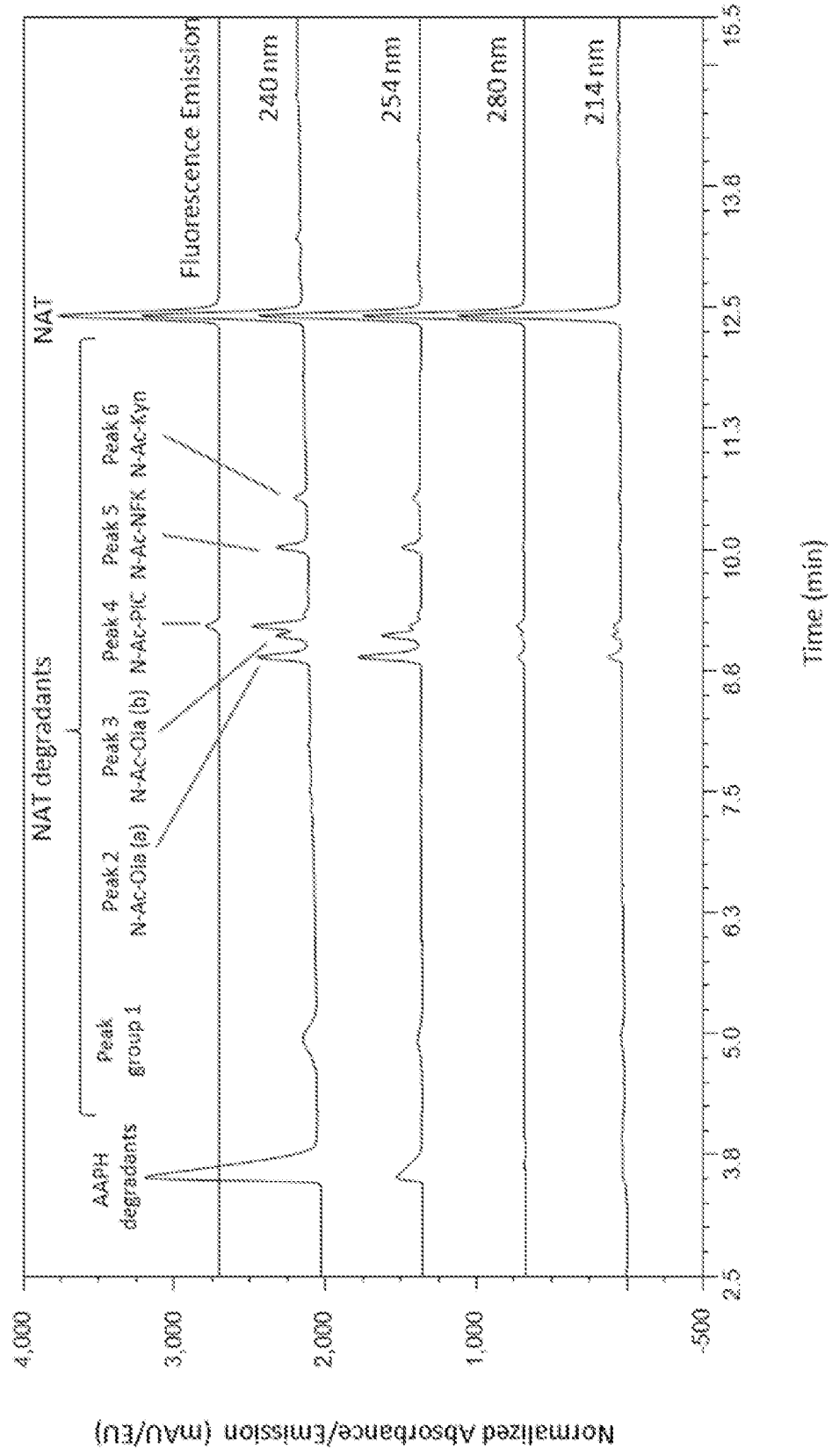

NAT degradants
AAPH degradants
Peak group 1
Peak 2 N-Ac-Oia (a)
Peak 3 N-Ac-Oia (b)
Peak 4 N-Ac-PIC
Peak 5 N-Ac-NFK
Peak 6 N-Ac-Kyn
NAT
Fluorescence Emission
240 nm
254 nm
280 nm
214 nm
Normalized Absorbance/Emission (mAU/EU)
Time (min)
4,000
3,000
2,000
1,000
-500
2.5
3.8
5.0
6.3
7.5
8.8
10.0
11.3
12.5
13.8
15.5

FIG. 8B

FIG. 9

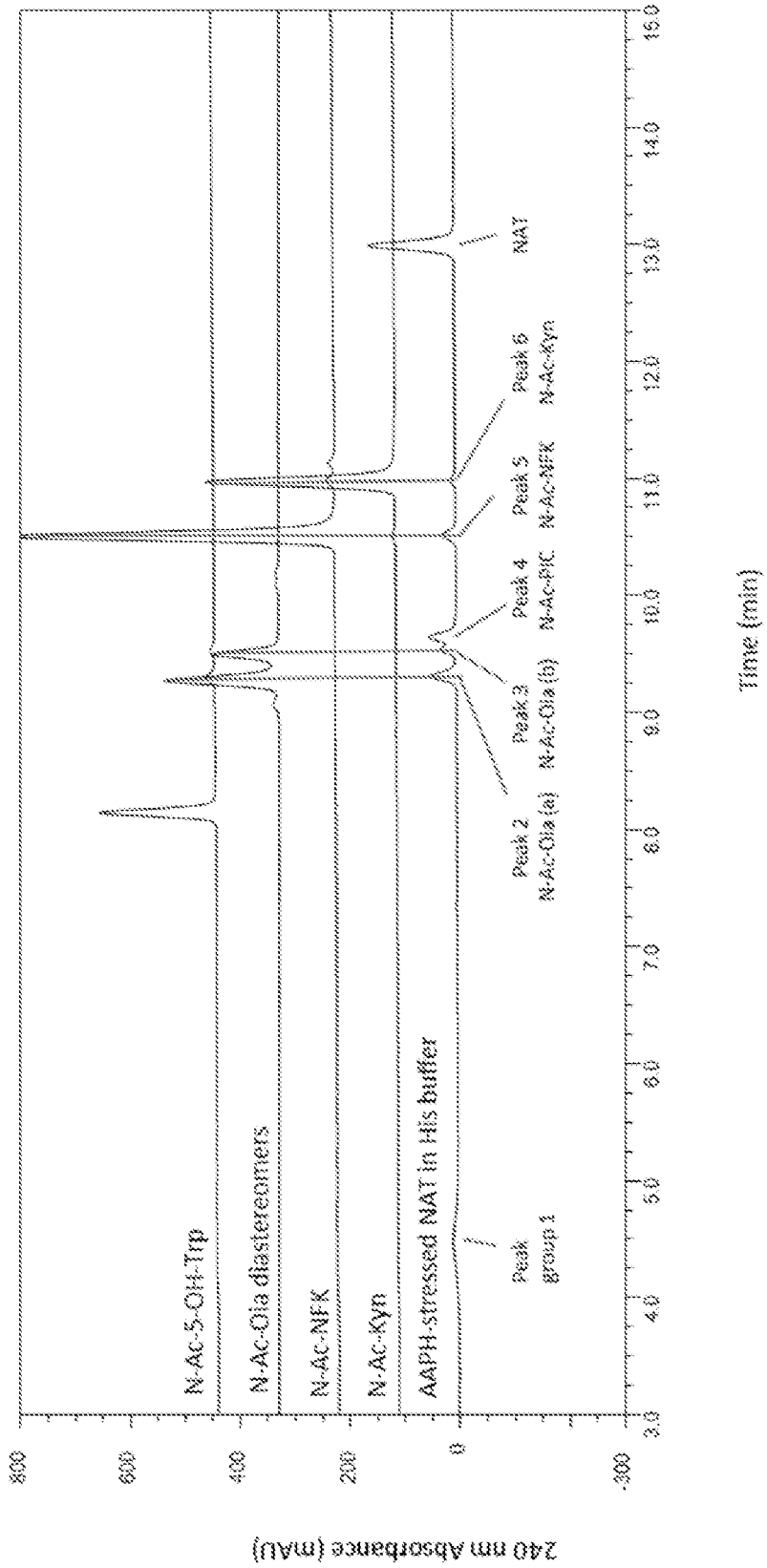

240 nm Absorbance (mAU)
800
600
400
200
0
-300
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Time (min)
N-Ac-5-OH-Trp
N-Ac-Dia diastereomers
N-Ac-NFK
N-Ac-Kyn
AAPH-stressed NAT in His buffer
Peak group 1
Peak 2 N-Ac-Dia (a)
Peak 3 N-Ac-Dia (b)
Peak 4 N-Ac-PIC
Peak 5 N-Ac-NFK
Peak 6 N-Ac-Kyn
NAT

FIG. 10

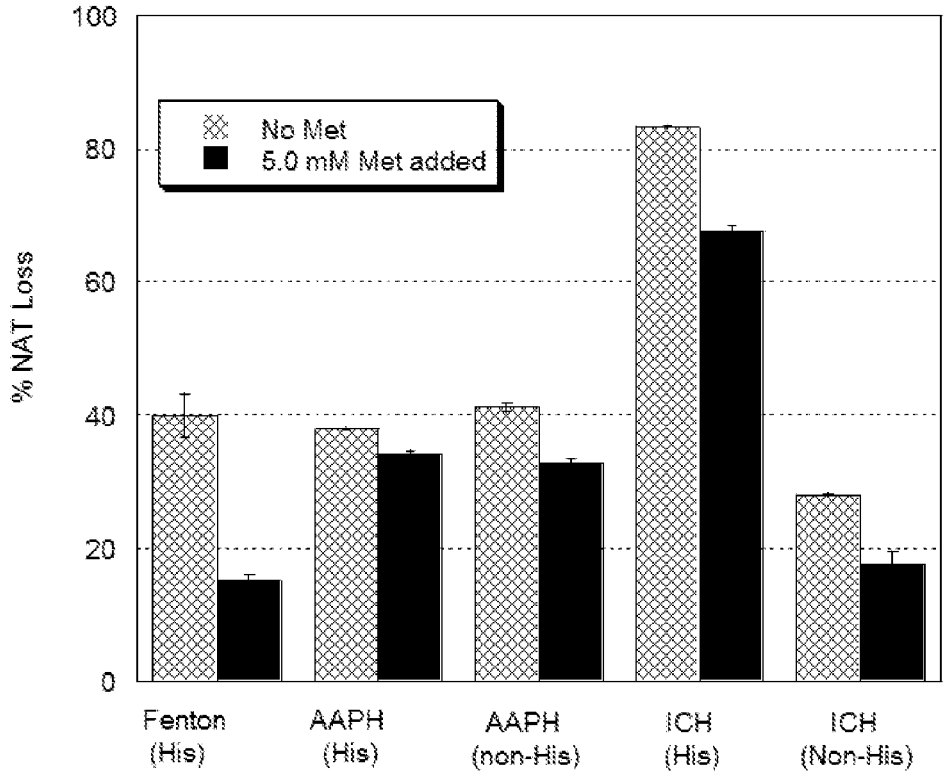

100
80
60
40
20
0
% NAT Loss
No Met
5.0 mM Met added
Fenton (His)
AAPH (His)
AAPH (non-His)
ICH (His)
ICH (Non-His)

FIG. 11

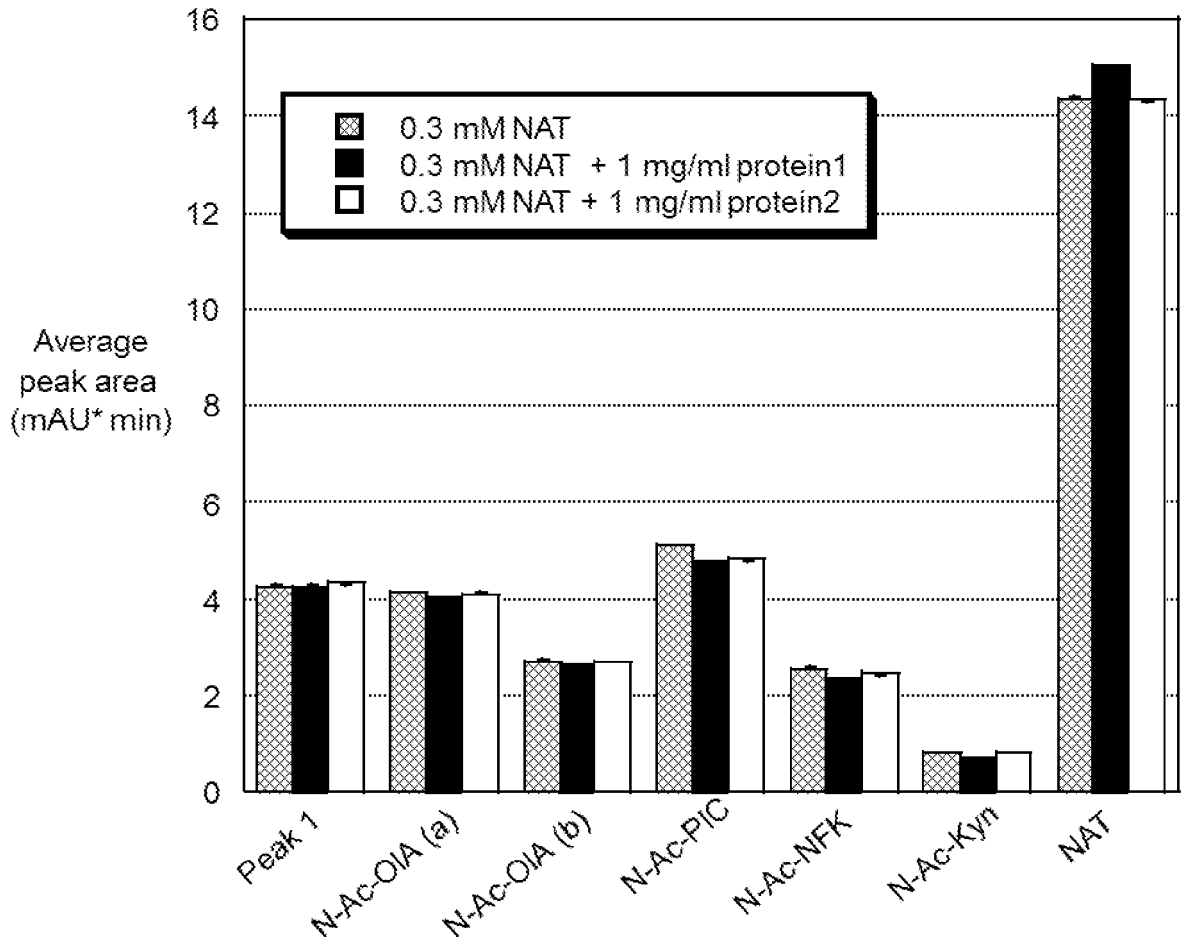

16
14
12
10
8
6
4
2
0
Average peak area (mAU* min)
0.3 mM NAT
0.3 mM NAT + 1 mg/ml protein1
0.3 mM NAT + 1 mg/ml protein2
Peak 1
N-Ac-OIA (a)
N-Ac-OIA (b)
N-Ac-PIC
N-Ac-NFK
N-Ac-Kyn
NAT

FIG. 12

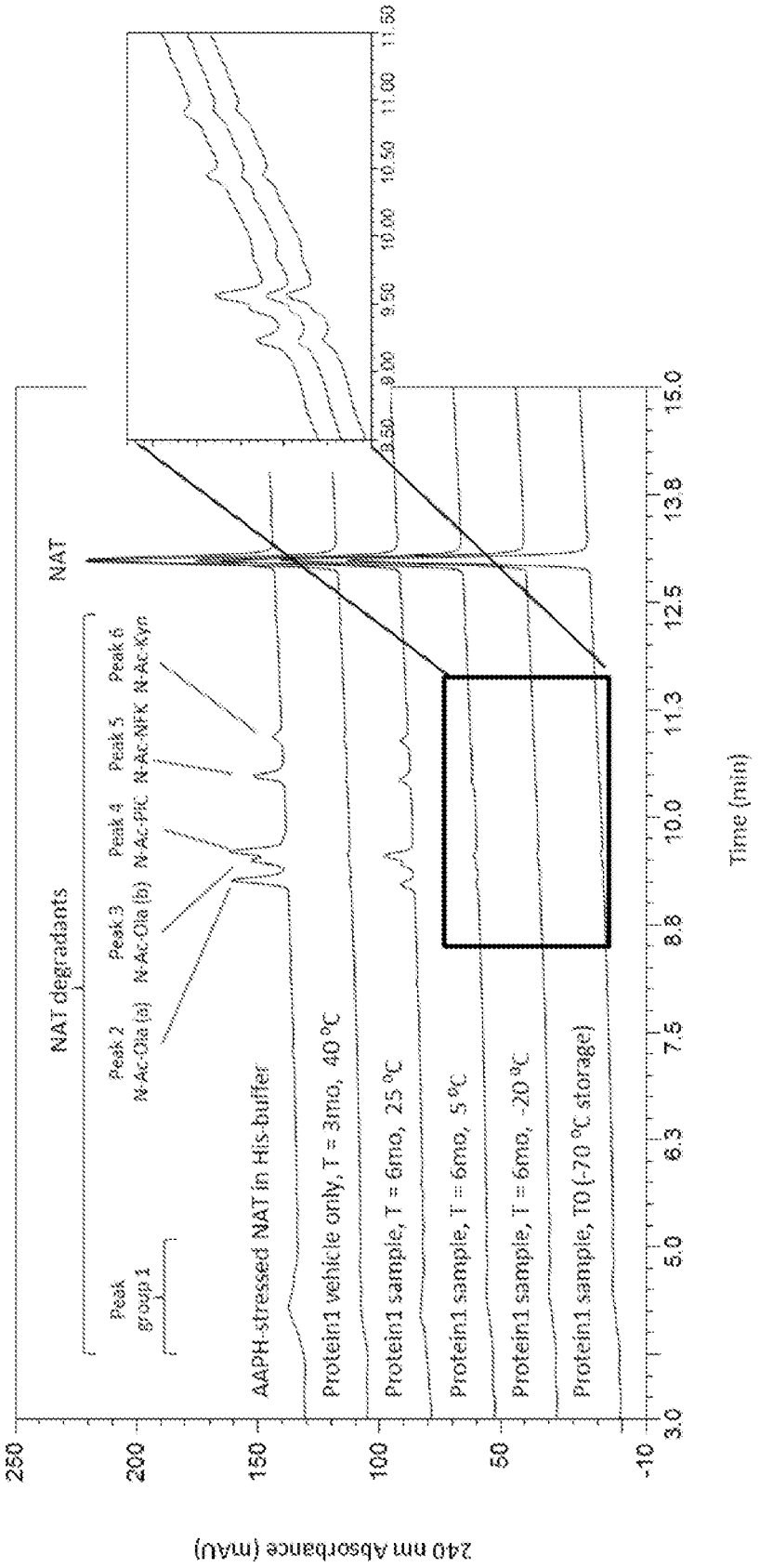

240 nm Absorbance (mAU)
250
200
150
100
50
-10
3.0
5.0
6.3
7.5
8.8
10.0
11.3
12.5
13.8
15.0
Time (min)
NAT
NAT degradants
Peak group 1
Peak 2 N-Ac-Dia (a)
Peak 3 N-Ac-Dia (b)
Peak 4 N-Ac-FK
Peak 5 N-Ac-NFK
Peak 6 N-Ac-Kyn
AAPH-stressed NAT in His-buffer
Protein1 vehicle only, T = 3mo, 40 °C
Protein1 sample, T = 6mo, 25 °C
Protein1 sample, T = 6mo, 5 °C
Protein1 sample, T = 6mo, -20 °C
Protein1 sample, T0 (-70 °C storage)
8.50
9.00
9.50
10.00
10.50
11.00
11.50

FIG. 13

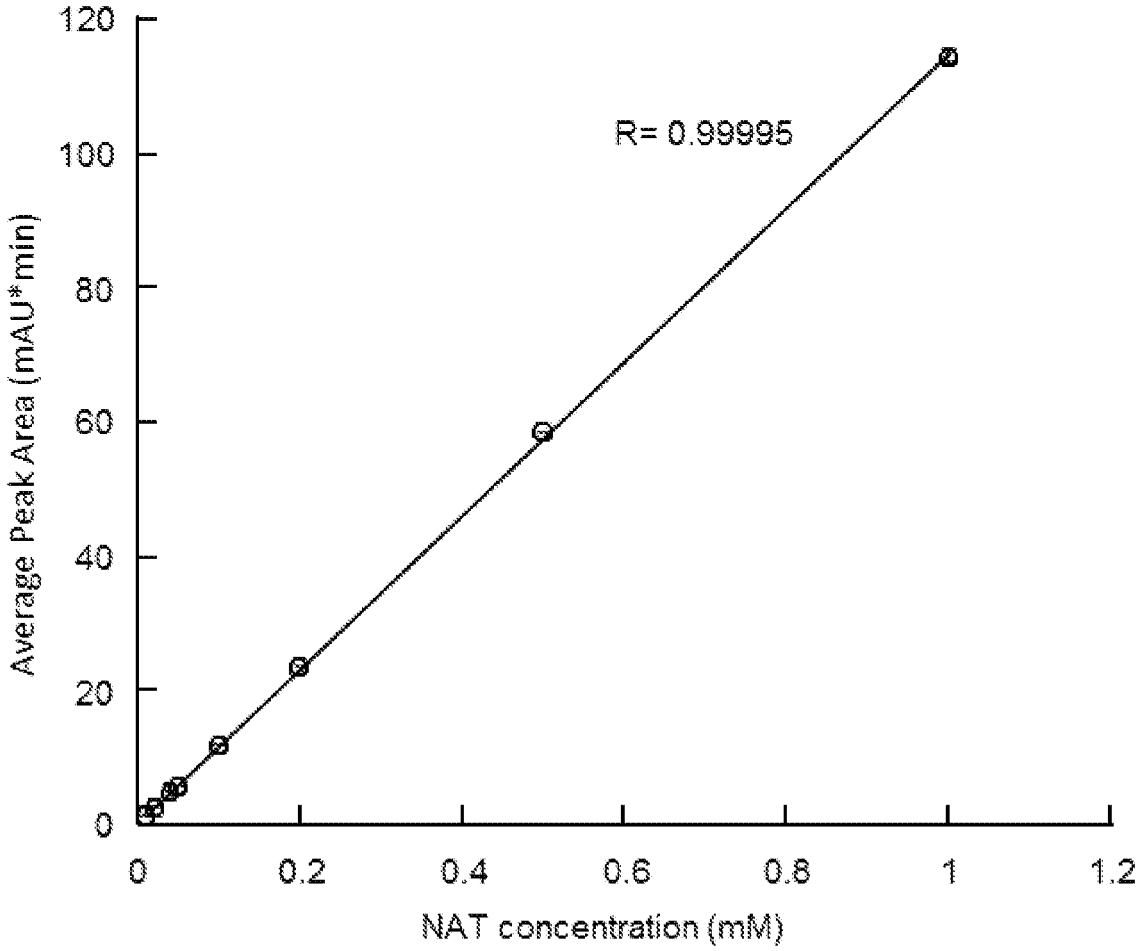
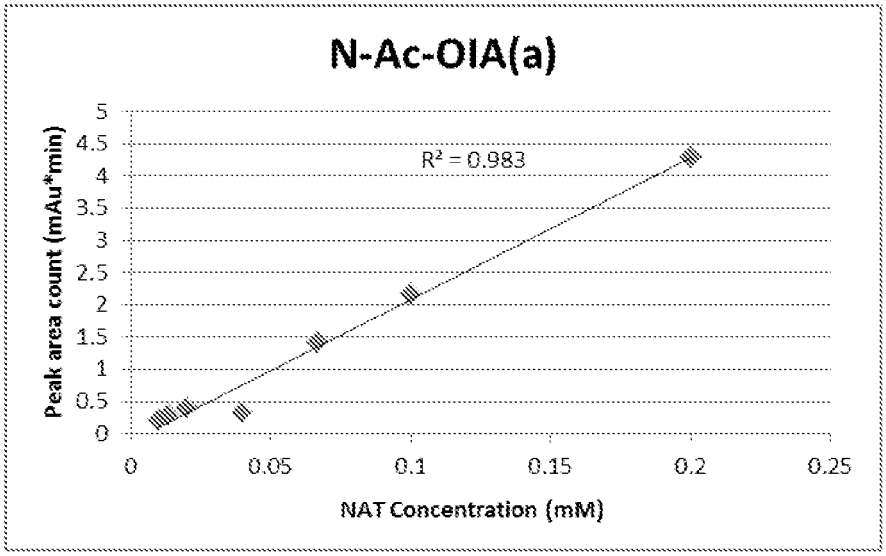
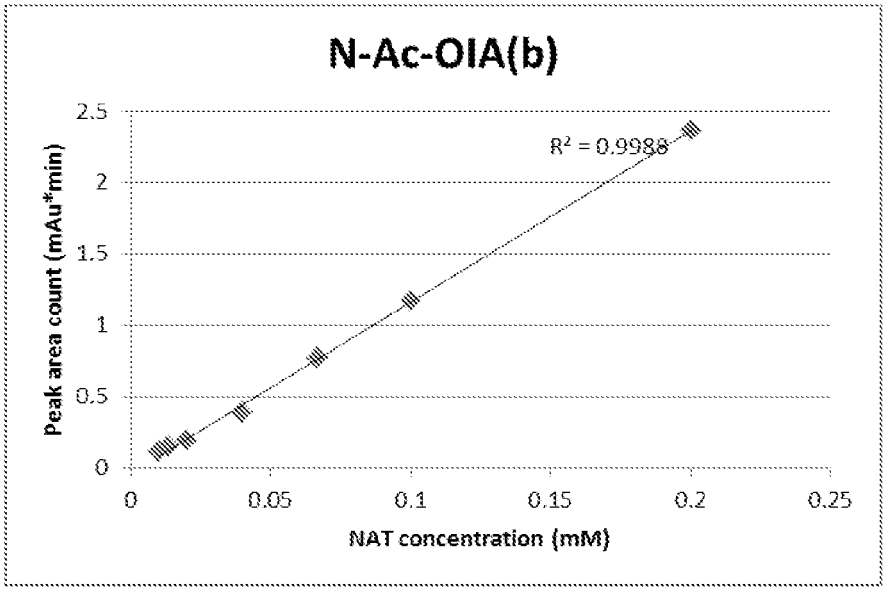

120
100
80
60
40
20
0
Average Peak Area (mAU*min)
R= 0.99995
0
0.2
0.4
0.6
0.8
1
1.2
NAT concentration (mM)

FIG. 14


N-Ac-OIA(a)
Peak area count (mAu*min)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
R² = 0.983
0
0.05
0.1
0.15
0.2
0.25
NAT Concentration (mM)


N-Ac-OIA(b)
Peak area count (mAu*min)
2.5
2
1.5
1
0.5
0
R² = 0.9988
0
0.05
0.1
0.15
0.2
0.25
NAT concentration (mM)

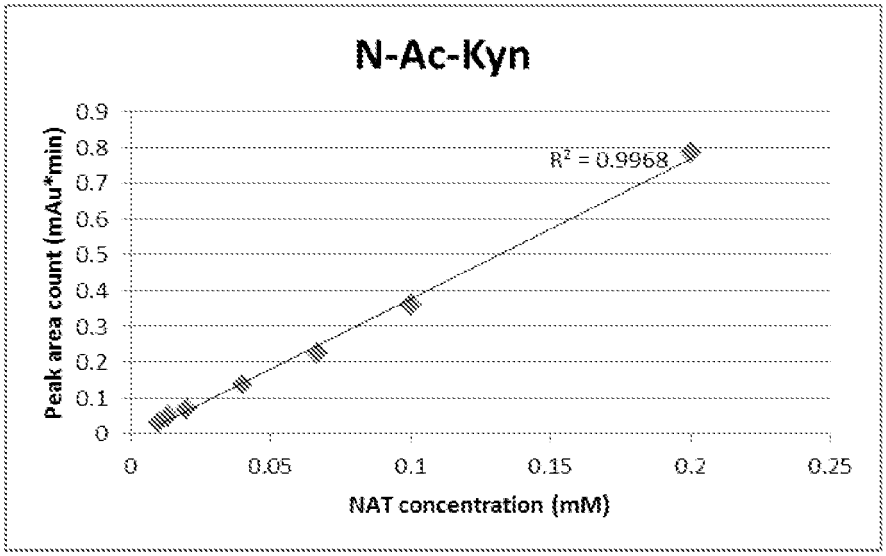
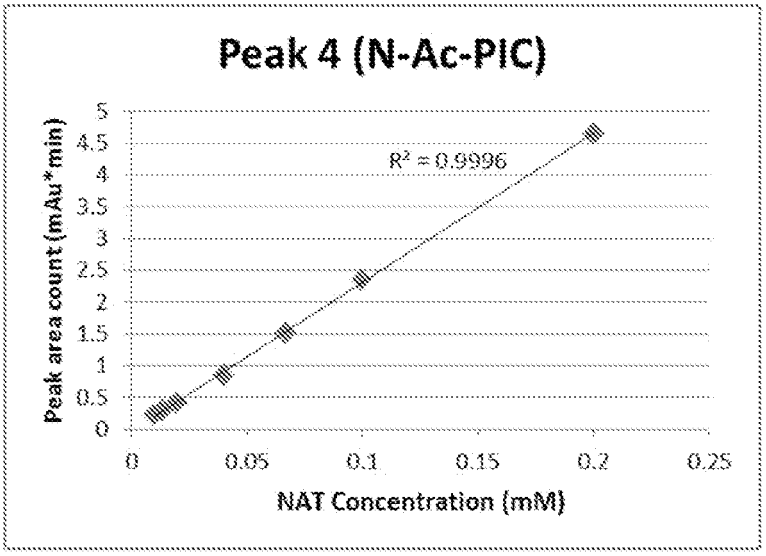
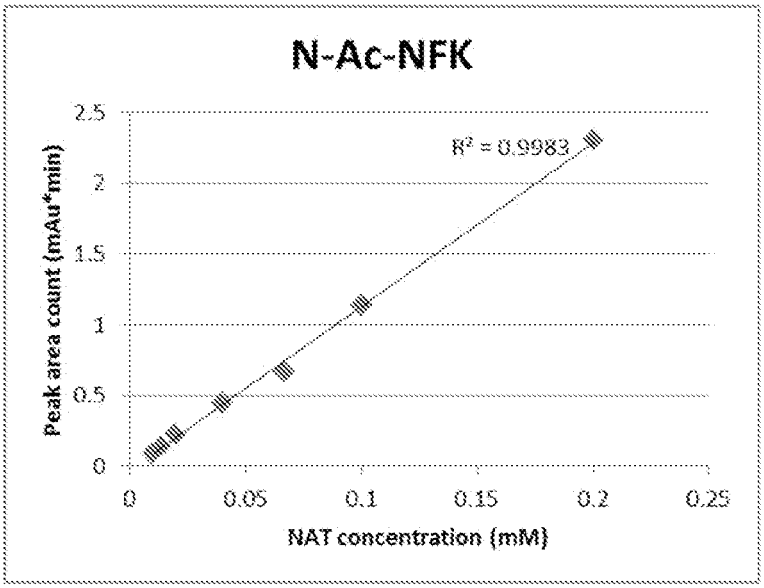

N-Ac-Kyn
Peak area count (mAu*min)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
R² = 0.9968
0
0.05
0.1
0.15
0.2
0.25
NAT concentration (mM)

FIG. 14 (continued)





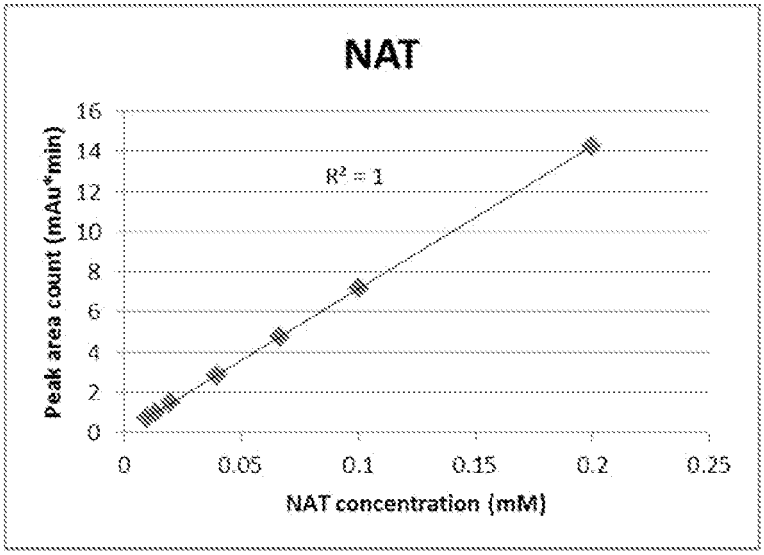


FIG. 15A

Peak 2 and 3: N-Ac-Oia diastereomers

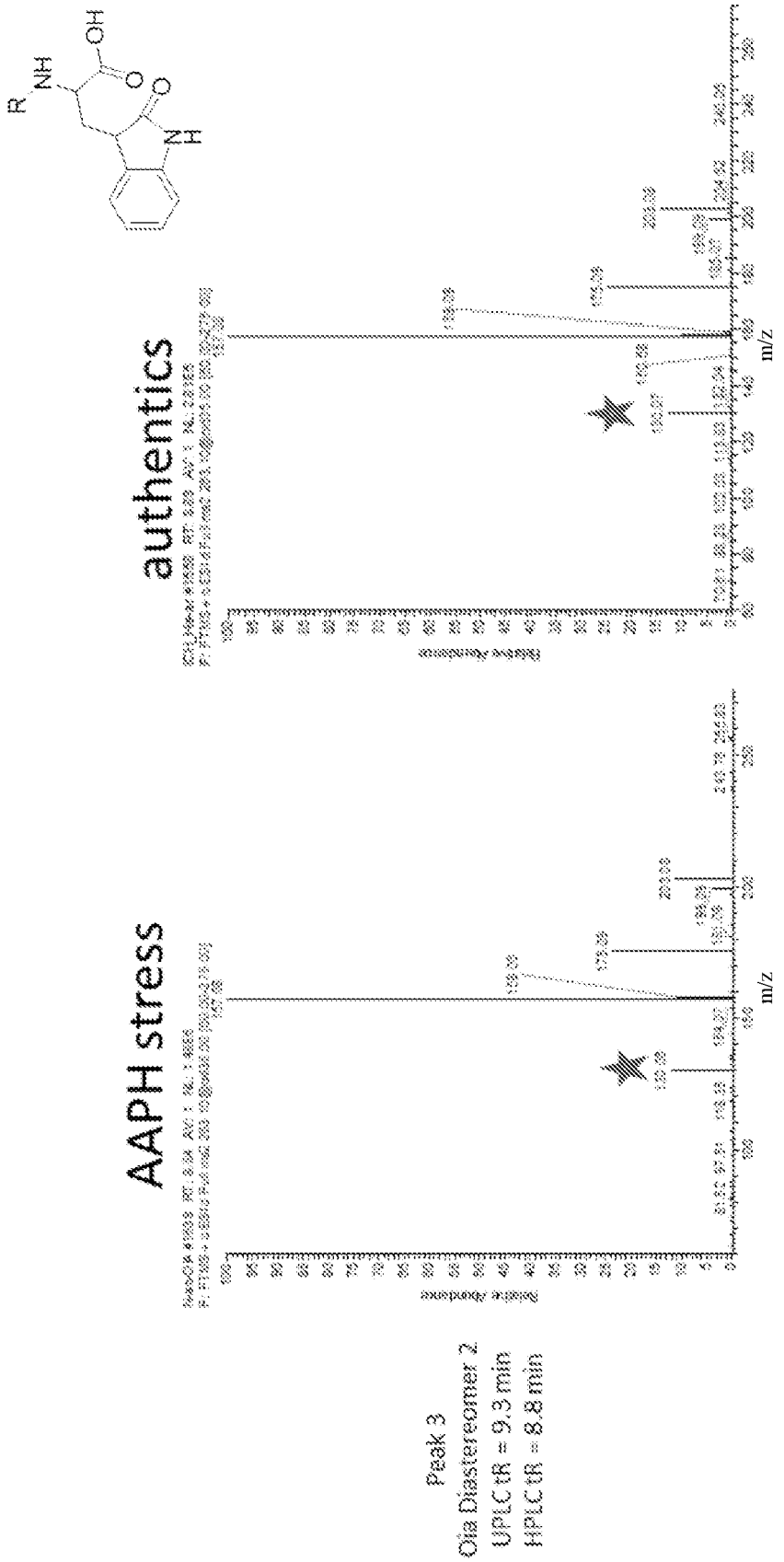


FIG. 15A (continued)

Peak 2 and 3: N-Ac-Oia diastereomers

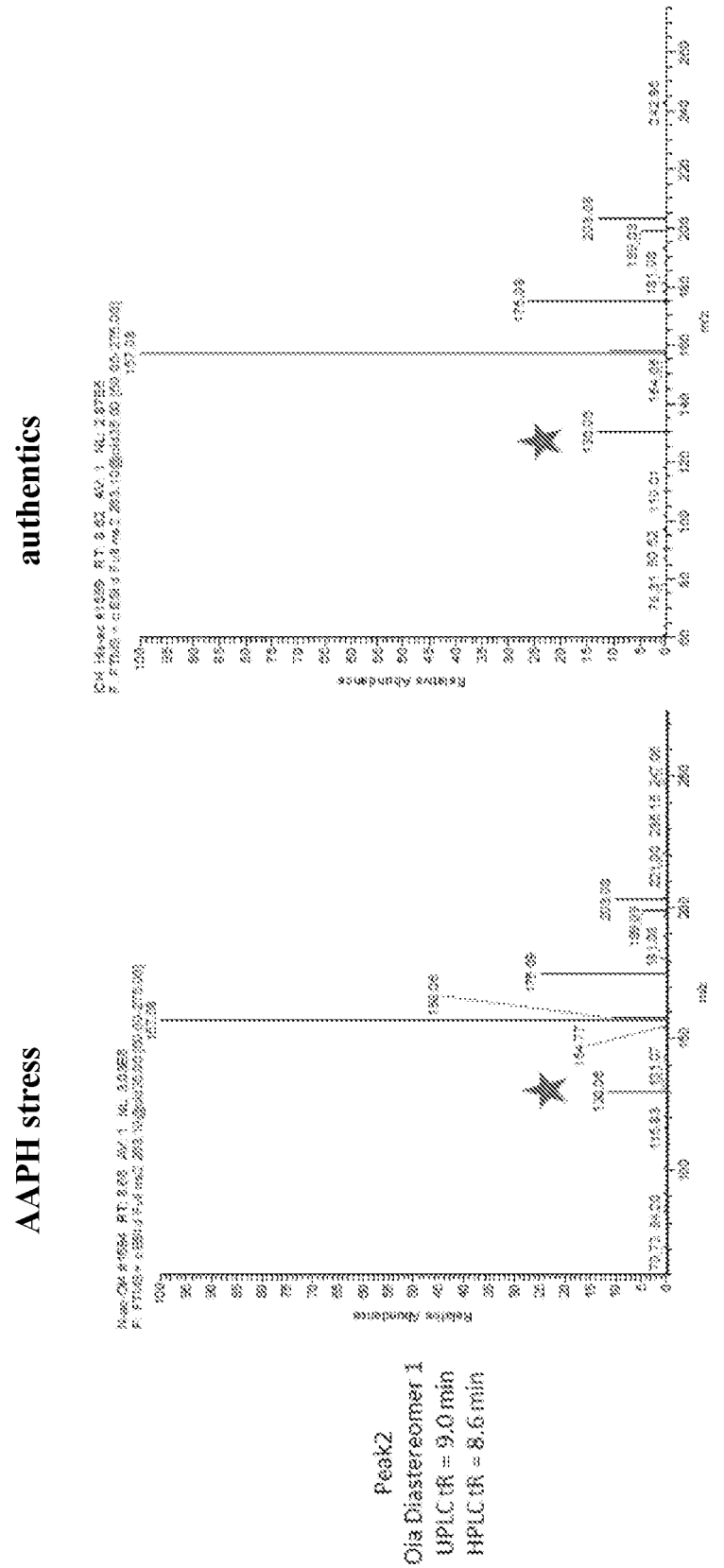
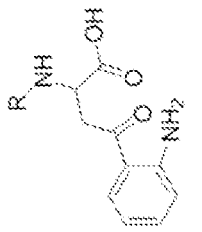
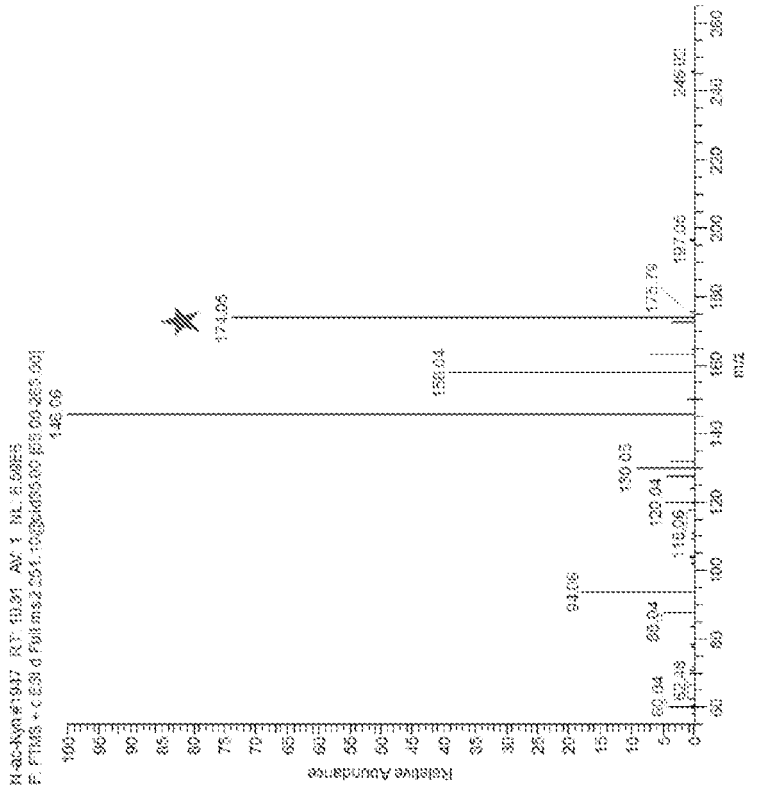


FIG. 15B

Peak 6: N-Ac-Kyn



authentic



AAPH stress

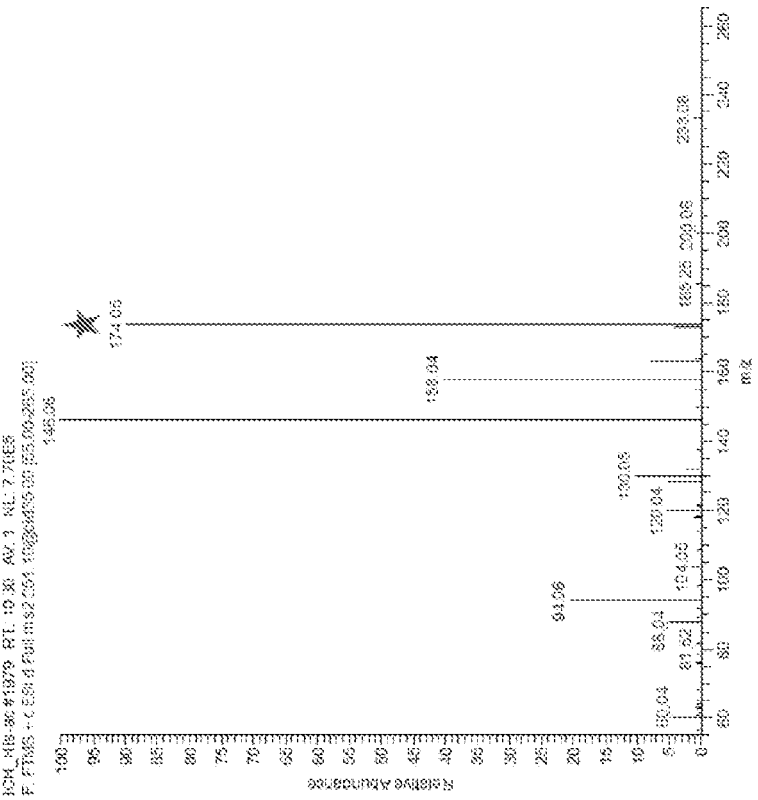
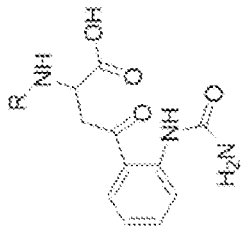


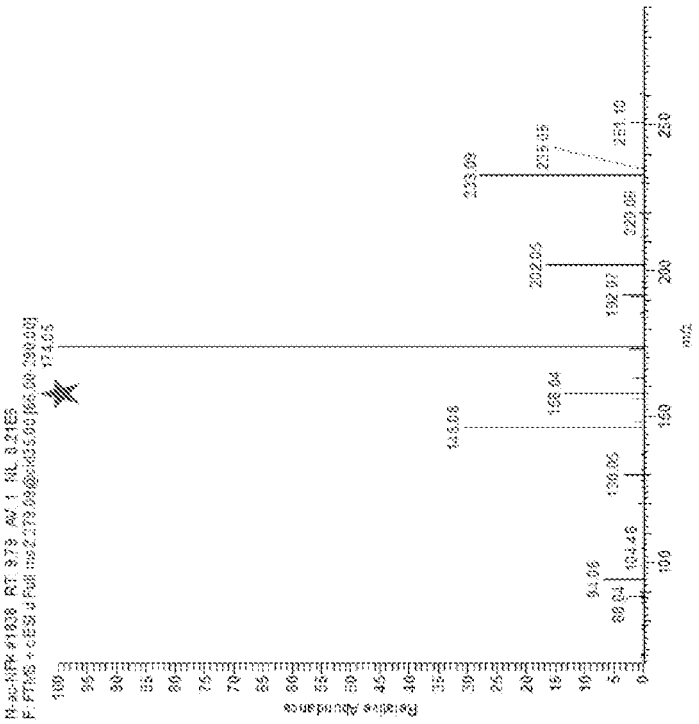
FIG. 15C

Peak 5: N-Ac-NFK

AAPH stress

authentic





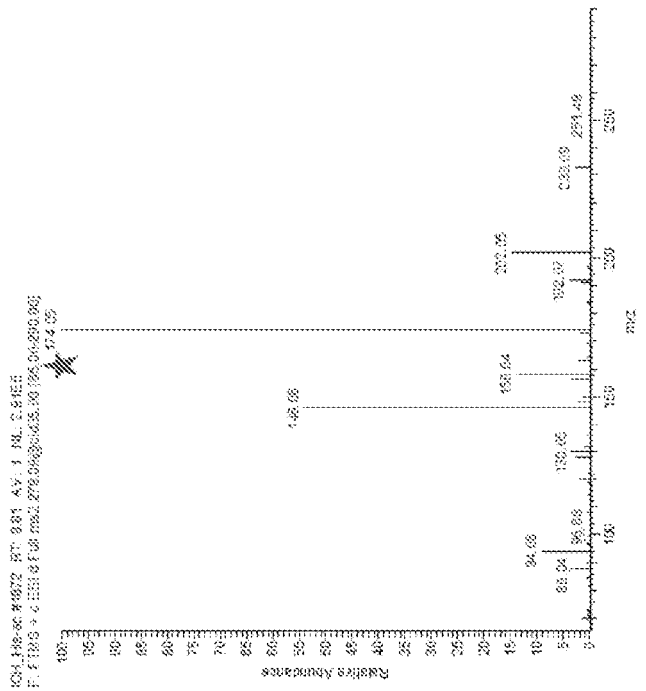


FIG. 15D

MS1 = 263.1 in Peak Group 1 and Peak 4 are not N-Ac-5HTP

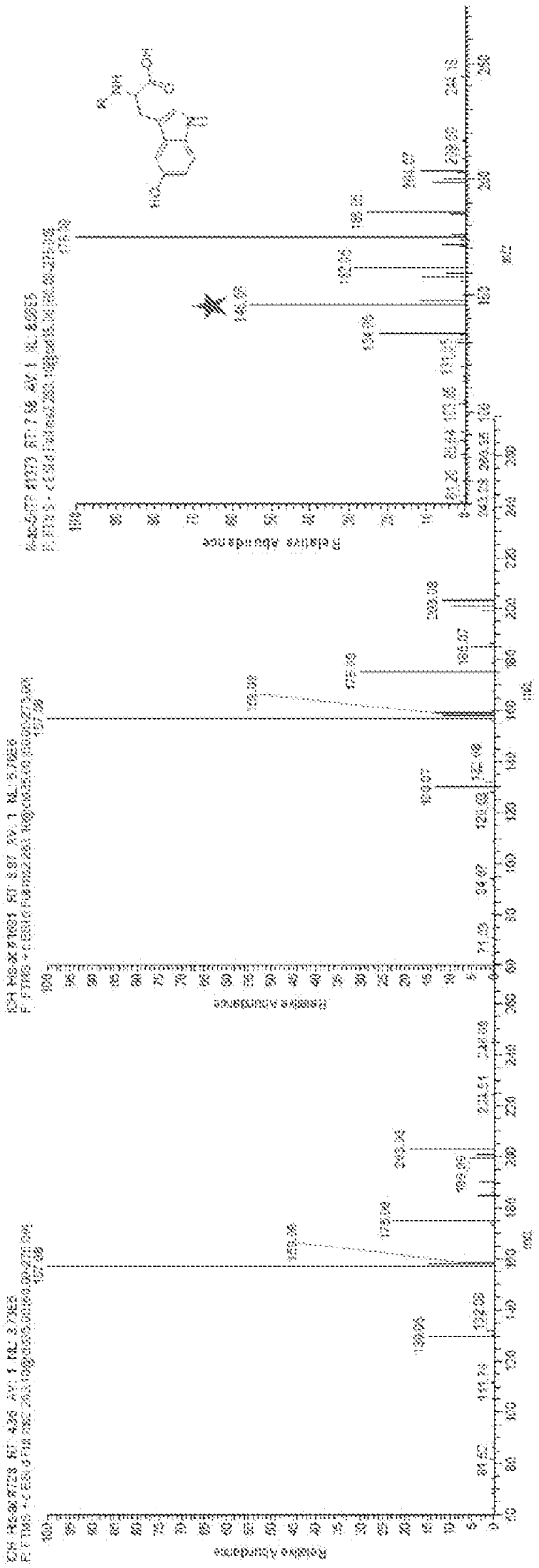


FIG. 15E **One or both unknown 263.1 peaks could contain stereoisomers of N-Ac-PIC**

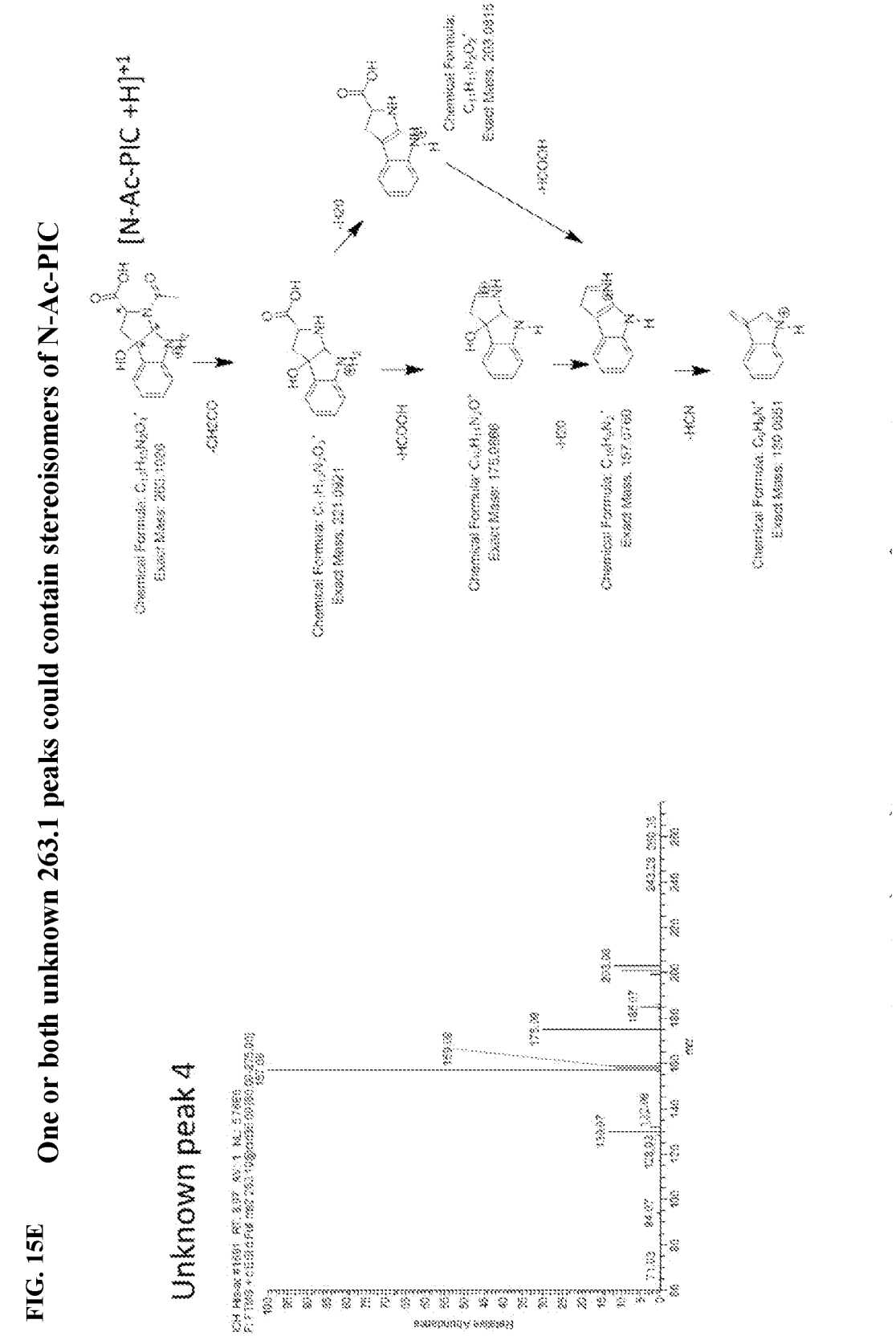


FIG. 15F

MS1 = 279.1 in Peak Group 1 are likely diastereomers of N-Ac-DiOia or N-Ac-3a, 8a-dihydroxy-PIC

