Mass Spectrometric Characterization of 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine *N*-Oxidized Metabolites Bound at Cys^{34} of Human Serum Albumin

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FIGURE LEGENDS

Figure S-1. Product ion mass spectra of A) HS-PhIP, B) H[^{34}S]-PhIP and C) HS-[$^{2}H_5$]-PhIP.

Figure S-2. Reconstructed ion chromatograms of C*PF (C-desamino-PhIP), C*P (C-desamino-PhIP) and C-desamino-PhIP produced by pronase E digestion of NO_2-PhIP-modified human SA (left panel) and corresponding product ion mass spectra (right panel).

Figure S-3. Full scan mass spectrum of the acid hydrolysis products of the synthetic LQQC*PF (C-[S=O]-PhIP) sulfinamide. The protonated PhIP is observed at *m/z* 225.2 and the singly charged LQQC(SO_2H)PF is observed at *m/z* 767.4.

Figure S-4. Product ion mass spectrum of C*PF (C-[S=O]-PhIP).

Figure S-5. Comparison of trypsin/chymotrypsin digestion of NO-PhIP-modified human SA using different denaturing agents. The digestion efficiency was assessed through the measurement of the LQQC*PF (C-[S=O]-PhIP) sulfinamide adduct by LC-ESI/MS/MS. The NO-PhIP-modified SA was preincubated with denaturing reagents for 1 h at 37 ºC prior to enzymatic digestion. The SA samples in the CH_3CN/NH_4HCO_3 solvent mixtures were digested without dilution, whereas the SA samples in 6 M urea or 50% trifluoroethanol were diluted by 10-fold prior to enzyme digestion. Each bar graph represents the average and standard deviation of 3 experiments.

Figure S-1

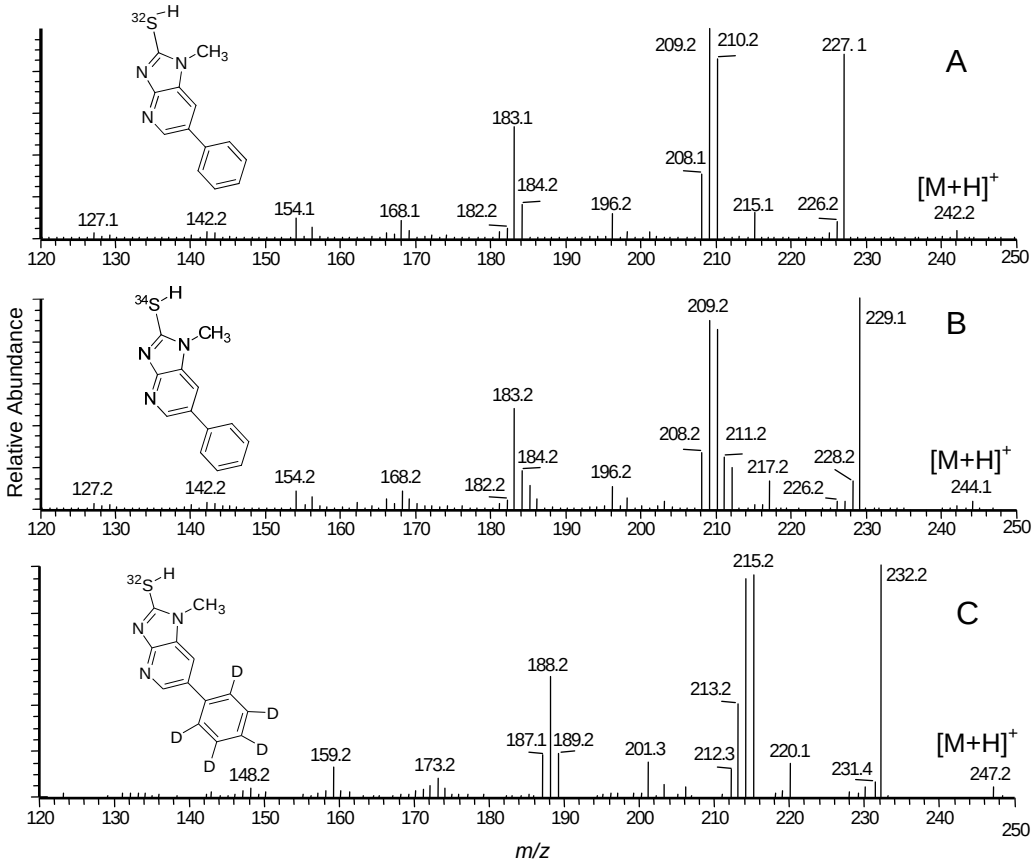

A
B
C
Relative Abundance
m/z
[M+H]+
242.2
244.1
247.2
209.2
210.2
227.1
183.1
208.1
184.2
196.2
215.1
226.2
127.1
142.2
154.1
168.1
182.2
229.1
183.2
208.2
211.2
217.2
228.2
127.2
142.2
154.2
168.2
182.2
196.2
226.2
215.2
232.2
188.2
213.2
187.1
189.2
201.3
212.3
220.1
231.4
159.2
173.2
148.2
D
120
130
140
150
160
170
180
190
200
210
220
230
240
250

Figure S-2

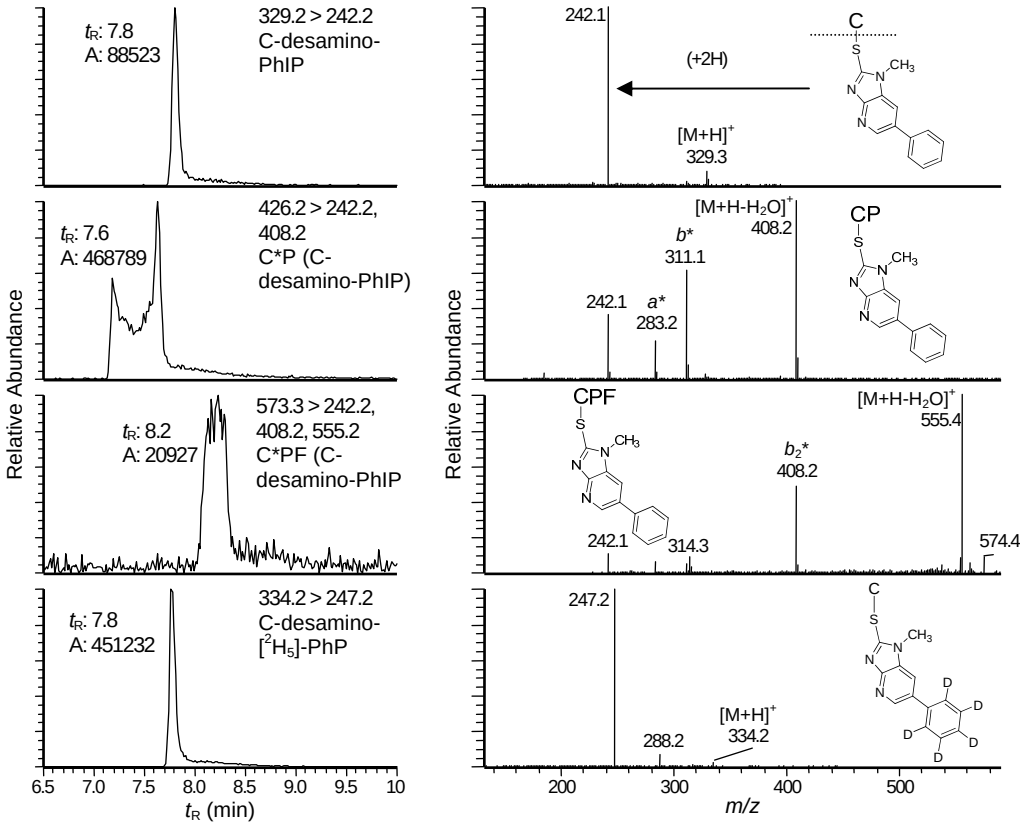

tR: 7.8
A: 88523
329.2 > 242.2
C-desamino-PhIP
tR: 7.6
A: 468789
426.2 > 242.2, 408.2
C*P (C-desamino-PhIP)
tR: 8.2
A: 20927
573.3 > 242.2, 408.2, 555.2
C*PF (C-desamino-PhIP
tR: 7.8
A: 451232
334.2 > 247.2
C-desamino-[2H5]-PhP
Relative Abundance
tR (min)
242.1
(+2H)
[M+H]+
329.3
[M+H-H2O]+
408.2
b*
311.1
242.1
a*
283.2
CP
CPF
b2*
408.2
555.4
242.1
314.3
574.4
247.2
288.2
[M+H]+
334.2
m/z

Figure S-3

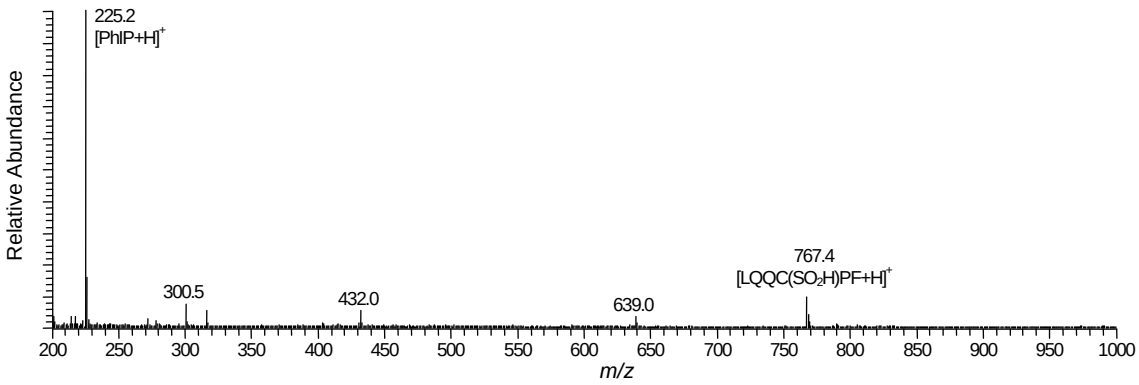

225.2
[PhIP+H]+
300.5
432.0
639.0
767.4
[LQQC(SO2H)PF+H]+
Relative Abundance
m/z
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000

Figure S-4

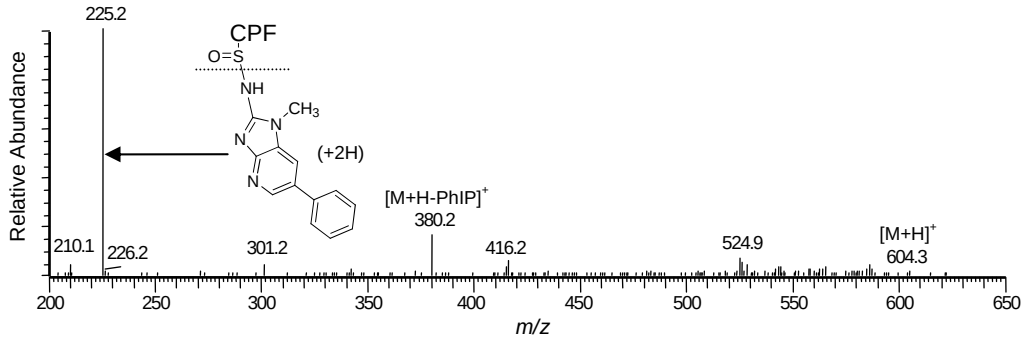
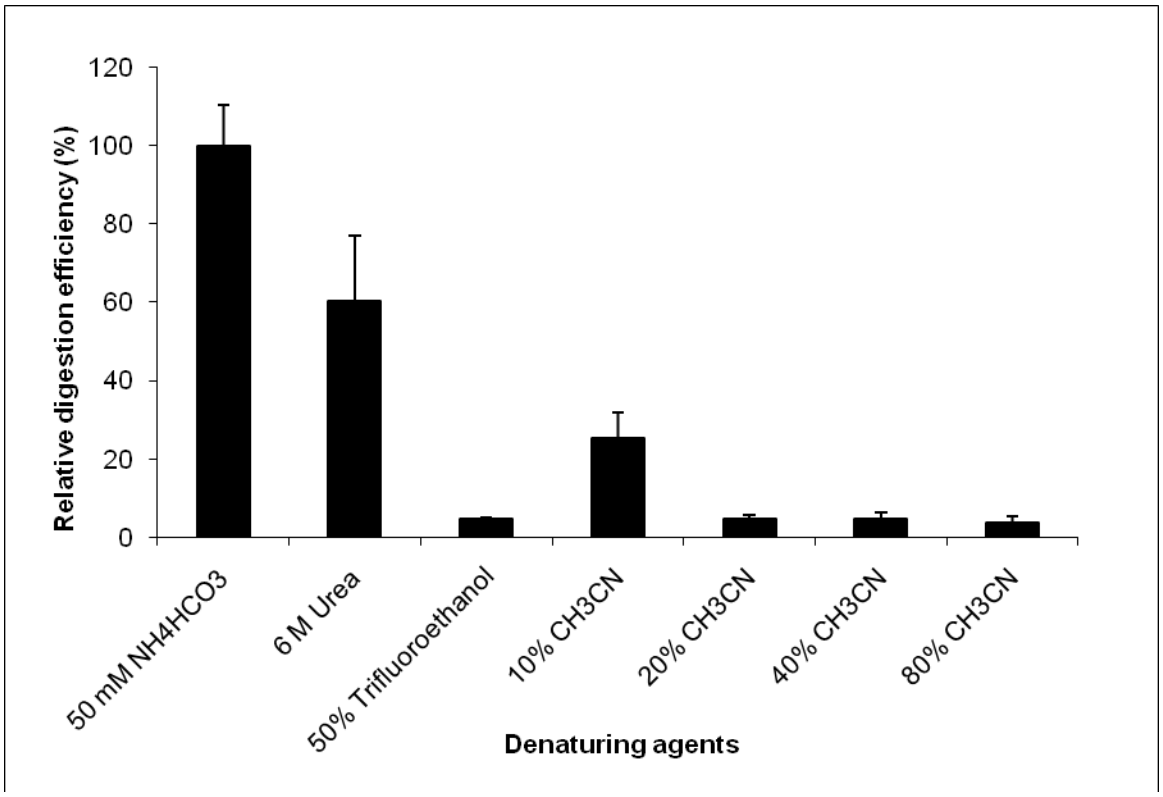

225.2
CPF
O=S
NH
CH3
N
N
N
(+2H)
[M+H-PhIP]+
380.2
210.1
226.2
301.2
416.2
524.9
[M+H]+
604.3
Relative Abundance
200
250
300
350
400
450
500
550
600
650
m/z

Figure S-5


Relative digestion efficiency (%)
120
100
80
60
40
20
0
50 mM NH4HCO3
6 M Urea
50% Trifluoroethanol
10% CH3CN
20% CH3CN
40% CH3CN
80% CH3CN
Denaturing agents