

A non-radioactive approach to investigate the metabolism of therapeutic peptides by tagging with ^{127}I and using ICP-MS analysis

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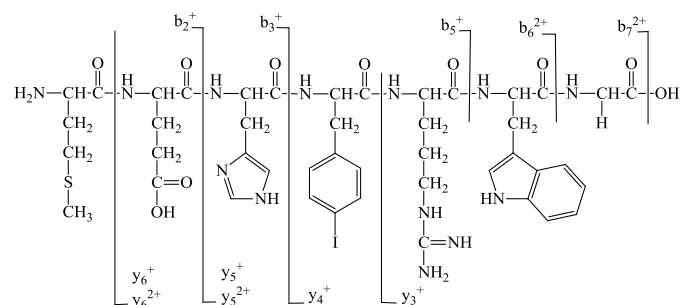
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Drug Metabolism and Disposition

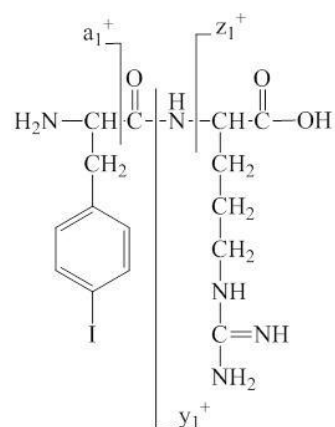
Supplemental Figure 1. The assignment of product ions from data-dependent multiple-stage mass analysis using chemical formulae from accurate mass measurements of A) ^{127}I -ACTH (4-10) and its metabolites B) M1, C) M2, D) M3, E) M4, F) M5, and G) M6.

A) Unchanged drug, ^{127}I -ACTH (4-10)

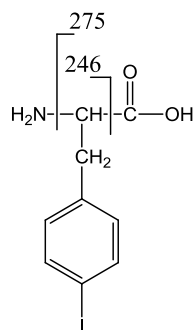


Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
$[\text{M}-\text{NH}_3-\text{H}_2\text{O}+2\text{H}]^{2+}$: 527.15039	$\text{C}_{44}\text{H}_{55}\text{O}_9\text{N}_{12}\text{IS}$, 2 3.0 RDB, 3.64 ppm
b_2^+ : 261.09121	$\text{C}_{10}\text{H}_{17}\text{O}_4\text{N}_2\text{S}$, 3.5 RDB, 3.28 ppm
b_3^+ : 398.15082	$\text{C}_{16}\text{H}_{24}\text{O}_5\text{N}_5\text{S}$, 7.5 RDB, 3.90 ppm
b_5^+ : 827.21610	$\text{C}_{31}\text{H}_{44}\text{O}_7\text{N}_{10}\text{IS}$, 14.5 RDB, 0.81 ppm
b_6^{2+} : 507.15106	$\text{C}_{42}\text{H}_{55}\text{O}_8\text{N}_{12}\text{IS}$, 21.0 RDB, 0.10 ppm
b_7^{2+} : 535.66382	$\text{C}_{44}\text{H}_{58}\text{O}_9\text{N}_{13}\text{IS}$, 22.0 RDB, 3.88 ppm
y_3^+ : 418.22046	$\text{C}_{19}\text{H}_{28}\text{O}_4\text{N}_7$, 9.5 RDB, 1.75 ppm
y_4^+ : 691.18630	$\text{C}_{28}\text{H}_{36}\text{O}_5\text{N}_8\text{I}$, 14.5 RDB, 2.19 ppm
y_5^+ : 828.24562	$\text{C}_{34}\text{H}_{43}\text{O}_6\text{N}_{11}\text{I}$, 18.5 RDB, 2.32 ppm
y_5^{2+} : 414.62626	$\text{C}_{34}\text{H}_{44}\text{O}_6\text{N}_{11}\text{I}$, 18.0 RDB, 1.86 ppm
y_6^+ : 957.28898	$\text{C}_{39}\text{H}_{50}\text{O}_9\text{N}_{12}\text{I}$, 20.5 RDB, 2.81 ppm
y_6^{2+} : 479.14689	$\text{C}_{39}\text{H}_{51}\text{O}_9\text{N}_{12}\text{I}$, 20.0 RDB, 0.22 ppm

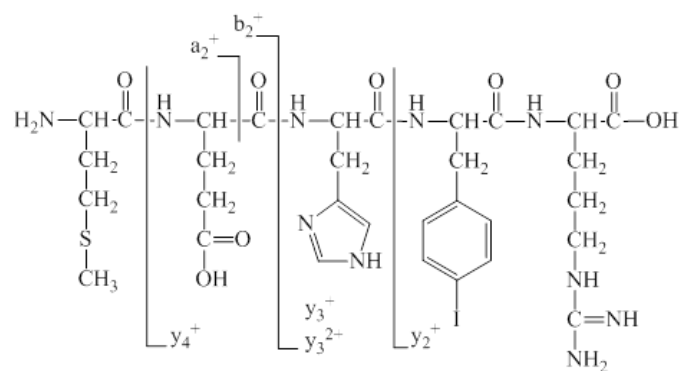
B) M1, ^{127}I -Phe-Arg



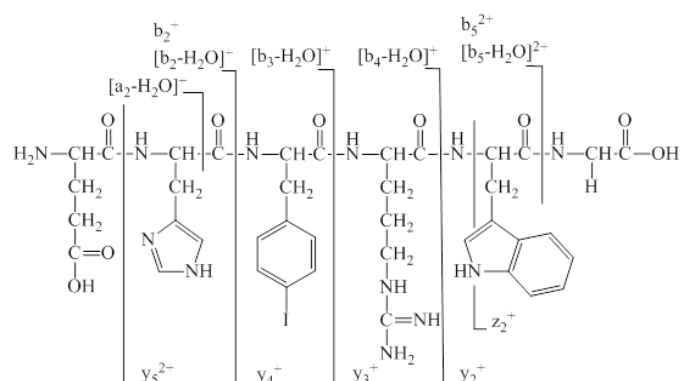
Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
$[M-NH_3+H]^+$: 431.05733	$C_{15}H_{20}O_3N_4I$, 7.5 RDB, -0.30 ppm
$[M-NH_3-H_2O+H]^+$: 413.04602	$C_{15}H_{18}O_2N_4I$, 8.5 RDB, -2.12 ppm
$[M-CH_5N_3+H]^+$: 389.03500	$C_{14}H_{18}O_3N_2I$, 6.5 RDB, -1.70 ppm
a_1^+ : 245.97706	C_8H_9NI , 4.5 RDB, -1.46 ppm
y_1^+ : 175.11873	$C_6H_{15}O_2N_4$, 1.5 RDB, -1.27 ppm
z_1^+ : 158.09222	$C_6H_{12}O_2N_3$, 2.5 RDB, -1.16 ppm

C) M2, ^{127}I -Phe

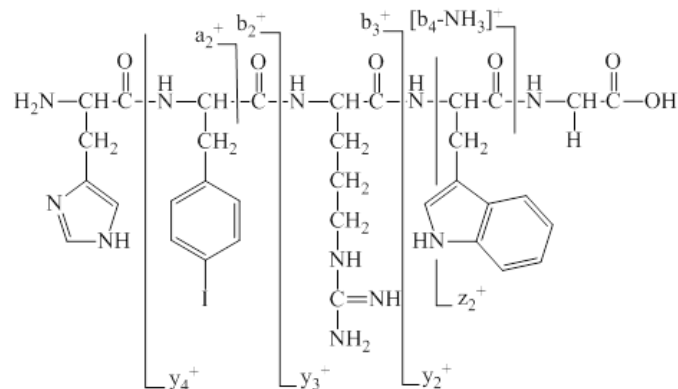
Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
245.97794	C_8H_9NI , 4.5 RDB, 2.12 ppm
274.95721	$C_9H_8O_2I$, 5.5 RDB, 3.13 ppm

D) M3, ^{127}I -ACTH (4-8)

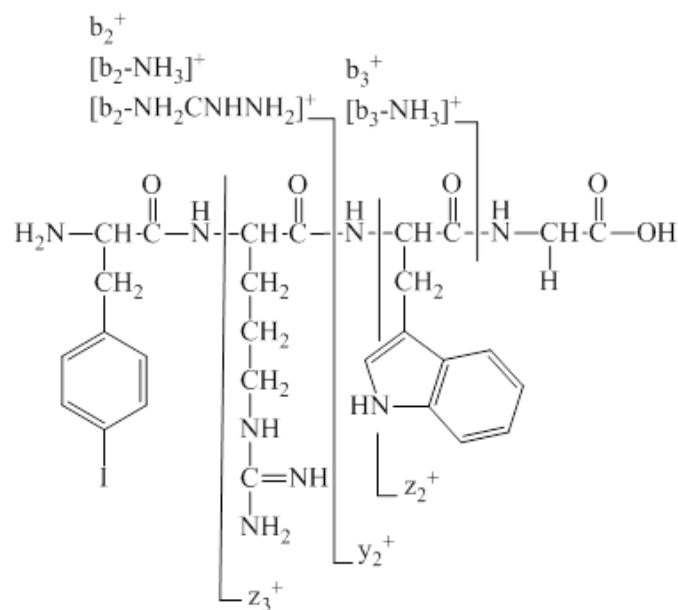
Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
$[M-NH_3-H_2O+2H]^{2+}$: 405.59729	$C_{31}H_{42}O_8N_9IS$, 15.0 RDB, -1.95 ppm
a_2^+ : 233.09491	$C_9H_{17}O_3N_2S$, 2.5 RDB, -2.27 ppm
b_2^+ : 261.09113	$C_{10}H_{17}O_4N_2S$, 3.5 RDB, 2.92 ppm
y_2^+ : 448.08521	$C_{15}H_{23}O_3N_5I$, 6.5 RDB, 2.68 ppm
y_3^+ : 585.14317	$C_{21}H_{30}O_4N_8I$, 10.5 RDB, 0.43 ppm
y_3^{2+} : 293.07512	$C_{21}H_{31}O_4N_8I$, 10.0 RDB, 0.07 ppm
y_4^+ : 714.18602	$C_{26}H_{37}O_7N_9I$, 12.5 RDB, 0.71 ppm

E) M4, ^{127}I -ACTH (5-10)

Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
$[M-H_2O+2H]^{2+}$: 470.14172	$C_{39}H_{49}O_8N_{12}I$, 21.0 RDB, 0.47 ppm
$[M-2H_2O+2H]^{2+}$: 461.13714	$C_{39}H_{47}O_7N_{12}I$, 22.0 RDB, 2.00 ppm
$[a_2-H_2O]^+$: 221.10338	$C_{10}H_{13}O_2N_4$, 6.5 RDB, 0.35 ppm
$[b_2-H_2O]^+$: 249.09847	$C_{11}H_{13}O_3N_4$, 7.5 RDB, 1.02 ppm
b_2^+ : 267.10919	$C_{11}H_{15}O_4N_4$, 6.5 RDB, 1.53 ppm
$[b_3-H_2O]^+$: 522.06336	$C_{20}H_{21}O_4N_5$, 12.5 RDB, 0.17 ppm
$[b_4-H_2O]^+$: 678.16512	$C_{26}H_{33}O_5N_9I$, 14.5 RDB, 1.08 ppm
b_5^{2+} : 441.63159	$C_{37}H_{46}O_7N_{11}I$, 20.0 RDB, 1.86 ppm
$[b_5-H_2O]^{2+}$: 432.62594	$C_{37}H_{44}O_6N_{11}I$, 21.0 RDB, 1.05 ppm
z_2^+ : 245.09227	$C_{13}H_{13}O_3N_2$, 8.5 RDB, 0.82 ppm
y_2^+ : 262.11889	$C_{13}H_{16}O_3N_3$, 7.5 RDB, 1.04 ppm
y_3^+ : 418.22046	$C_{19}H_{28}O_4N_7$, 9.5 RDB, 1.75 ppm
y_4^+ : 691.18490	$C_{28}H_{36}O_5N_8I$, 14.5 RDB, 0.17 ppm
y_5^{2+} : 414.62612	$C_{34}H_{44}O_6N_{11}I$, 18.0 RDB, 1.53 ppm

F) M5, ^{127}I -ACTH (6-10)

Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
a_2^+ : 383.03712	$\text{C}_{14}\text{H}_{16}\text{ON}_4\text{I}$, 8.5 RDB, 2.06 ppm
b_2^+ : 411.03141	$\text{C}_{15}\text{H}_{16}\text{O}_2\text{N}_4\text{I}$, 9.5 RDB, 0.40 ppm
b_3^+ : 567.13428	$\text{C}_{21}\text{H}_{28}\text{O}_3\text{N}_8\text{I}$, 11.5 RDB, 3.39 ppm
$[\text{b}_4\text{-NH}_3]^+$: 736.18661	$\text{C}_{32}\text{H}_{35}\text{O}_4\text{N}_9\text{I}$, 19.5 RDB, 2.02 ppm
z_2^+ : 245.09254	$\text{C}_{13}\text{H}_{13}\text{O}_3\text{N}_2$, 8.5 RDB, 1.92 ppm
y_2^+ : 262.11895	$\text{C}_{13}\text{H}_{16}\text{O}_3\text{N}_3$, 7.5 RDB, 1.27 ppm
y_3^+ : 418.22159	$\text{C}_{19}\text{H}_{28}\text{O}_4\text{N}_7$, 9.5 RDB, 4.45 ppm
y_4^+ : 691.18658	$\text{C}_{28}\text{H}_{36}\text{O}_5\text{N}_8\text{I}$, 14.5 RDB, 2.60 ppm

G) M6, ^{127}I -ACTH (7-10)

Product ion	Chemical formula, Ring plus Double Bonds (RDB), Mass Accuracy (ppm)
$[M-NH_3+H]^+$: 674.15976	$C_{28}H_{33}O_5N_7I$, 15.5 RDB, 2.26 ppm
$[M-NH_3-H_2O+H]^+$: 656.14785	$C_{28}H_{31}O_4N_7I$, 16.5 RDB, 0.27 ppm
b_2^+ : 430.07325	$C_{15}H_{21}O_2N_5I$, 7.5 RDB, -0.45 ppm
$[b_2-NH_2CNHNH_2]^+$: 371.02441	$C_{14}H_{16}O_2N_2I$, 7.5 RDB, -1.85 ppm
$[b_2-NH_3]^+$: 413.04647	$C_{15}H_{18}O_2N_4I$, 8.5 RDB, -1.03 ppm
b_3^+ : 616.15322	$C_{26}H_{31}O_3N_7I$, 14.5 RDB, 0.75 ppm
$[b_3-NH_3]^+$: 599.12713	$C_{26}H_{28}O_3N_6I$, 15.5 RDB, 1.54 ppm
z_2^+ : 245.09219	$C_{13}H_{13}O_3N_2$, 8.5 RDB, 0.50 ppm
y_2^+ : 262.11860	$C_{13}H_{16}O_3N_3$, 7.5 RDB, -0.07 ppm
Z_3^+ : 401.19310	$C_{19}H_{25}O_4N_6$, 10.5 RDB, -0.20 ppm

Supplemental Table 1. The % composition of ^{127}I -tagged metabolites from capillary UPLC-ICP-MS analysis of incubation of 10 μM of metabolite M3 [^{127}I -ACTH (4-8)], M4 [^{127}I -ACTH (5-10)] or M6 [^{127}I -ACTH (7-10)] in human liver S9 at 37°C for 15 min.

Metabolites	% Composition		
	Liver S9+M6	Liver S9+M4	Liver S9+M3
M6	0.45	1.33	ND
M5	ND	1.83	ND
M4	ND	0.14	ND
M3	ND	ND	8.24
M2	99.45	96.29	90.76
M1	0.09	0.42	1.01

Supplemental Table 2. The accurate m/z of singly and multiply charged ions of native ACTH (4-10) and its metabolites.

Metabolite	RRT	$[M+nH]^{n+}$: m/z (chemical formula, RDB, ppm)
UD, ACTH (4-10)	1.00	$[M+H]^+$: 962.43171 ($C_{44}H_{60}O_{10}N_{13}S$, 21.5 RDB, 1.64 ppm) $[M+2H]^{2+}$: *481.71879 ($C_{44}H_{61}O_{10}N_{13}S$, 21.0 RDB, 0.18 ppm) $[M+3H]^{3+}$: 321.48169 ($C_{44}H_{62}O_{10}N_{13}S$, 21.5 RDB, 0.40 ppm)
M6', ACTH (7-10)	0.94	$[M+H]^+$: *565.28796 ($C_{28}H_{37}O_5N_8$, 14.5 RDB, -0.32 ppm), $[M+2H]^{2+}$: 283.14764 ($C_{28}H_{38}O_5N_8$, 14.0 RDB, -0.25 ppm),
M5', ACTH (6-10)	0.93	$[M+H]^+$: 702.34762 ($C_{34}H_{44}O_6N_{11}$, 18.5 RDB, 0.81 ppm), $[M+2H]^{2+}$: *351.67727 ($C_{34}H_{45}O_6N_{11}$, 18.0 RDB, 0.30 ppm)
M4', ACTH (5-10)	0.93	$[M+H]^+$: 831.38951 ($C_{39}H_{51}O_9N_{12}$, 20.5 RDB, -0.17 ppm), $[M+2H]^{2+}$: *416.19831 ($C_{39}H_{52}O_9N_{12}$, 20.0 RDB, -0.37 ppm)
M3', ACTH (4-8)	0.71	$[M+2H]^{2+}$: *360.16820 ($C_{31}H_{48}O_8N_{10}S$, 13.0 RDB, -0.32 ppm)
M2', Phe	0.30	$[M+H]^+$: 166.08599 ($C_9H_{12}O_2N$, 4.5 RDB, -1.60 ppm)
M1', Phe-Arg	0.33	$[M+H]^+$: *322.18783 ($C_{15}H_{24}O_3N_5$, 6.5 RDB, 1.44 ppm)

*: base peak of intact molecule in the full scan mass spectrum