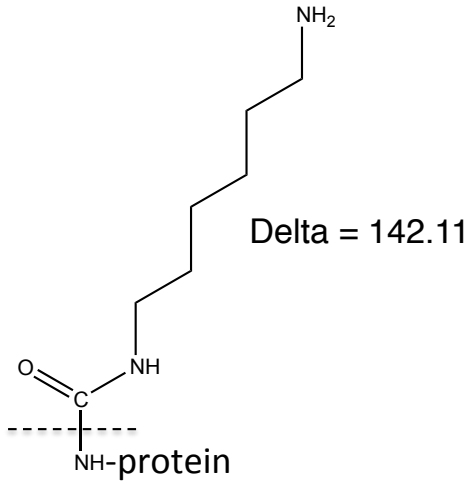
Supplemental Material for

Novel reaction products of glutathione with vapors of an aliphatic (hexamethylene) diisocyanate by *Adam V. Wisnewski, Morgen Mhike, Justin M. Hettick, Jian Liu, and Paul D. Siegel*

TABLE OF CONTENTS Page #

Figure E1. Expected modifications via GSH-HDI reaction products

A.



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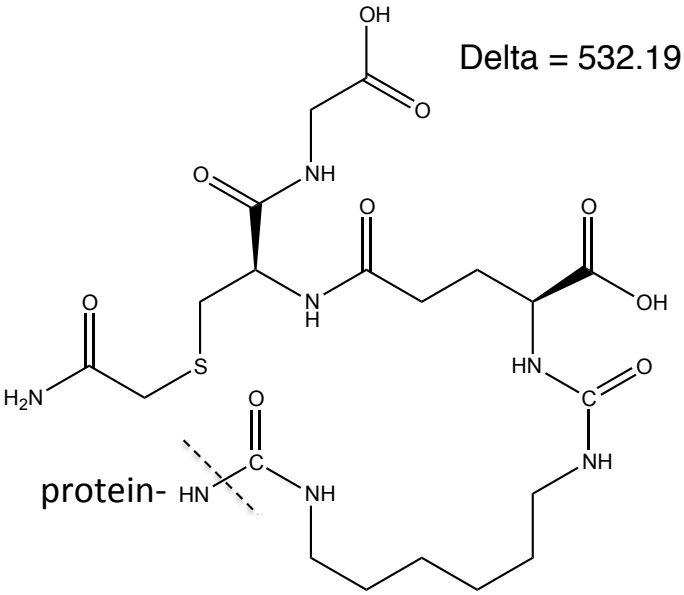

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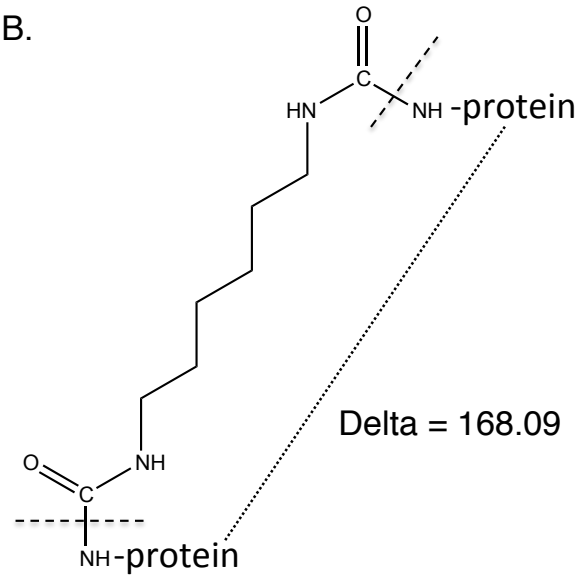

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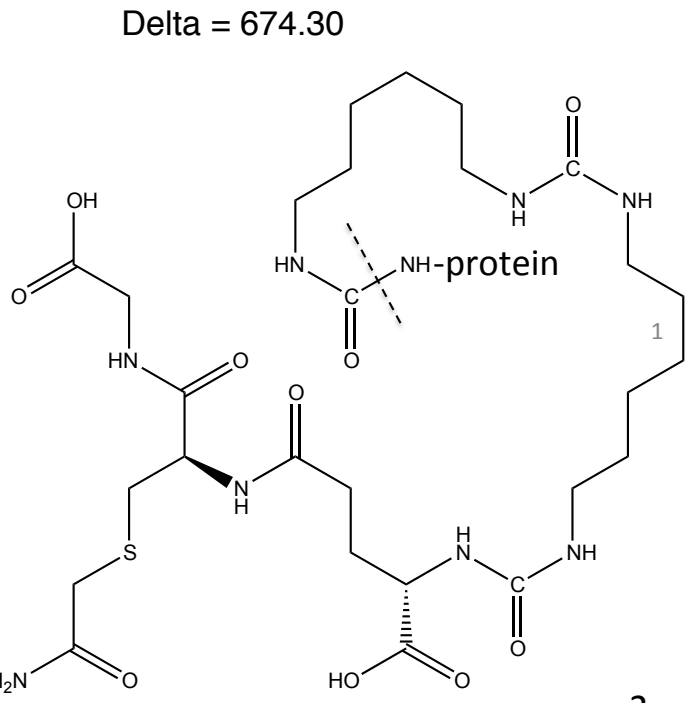


Figure E2. MS/MS of 783.26 m/z product

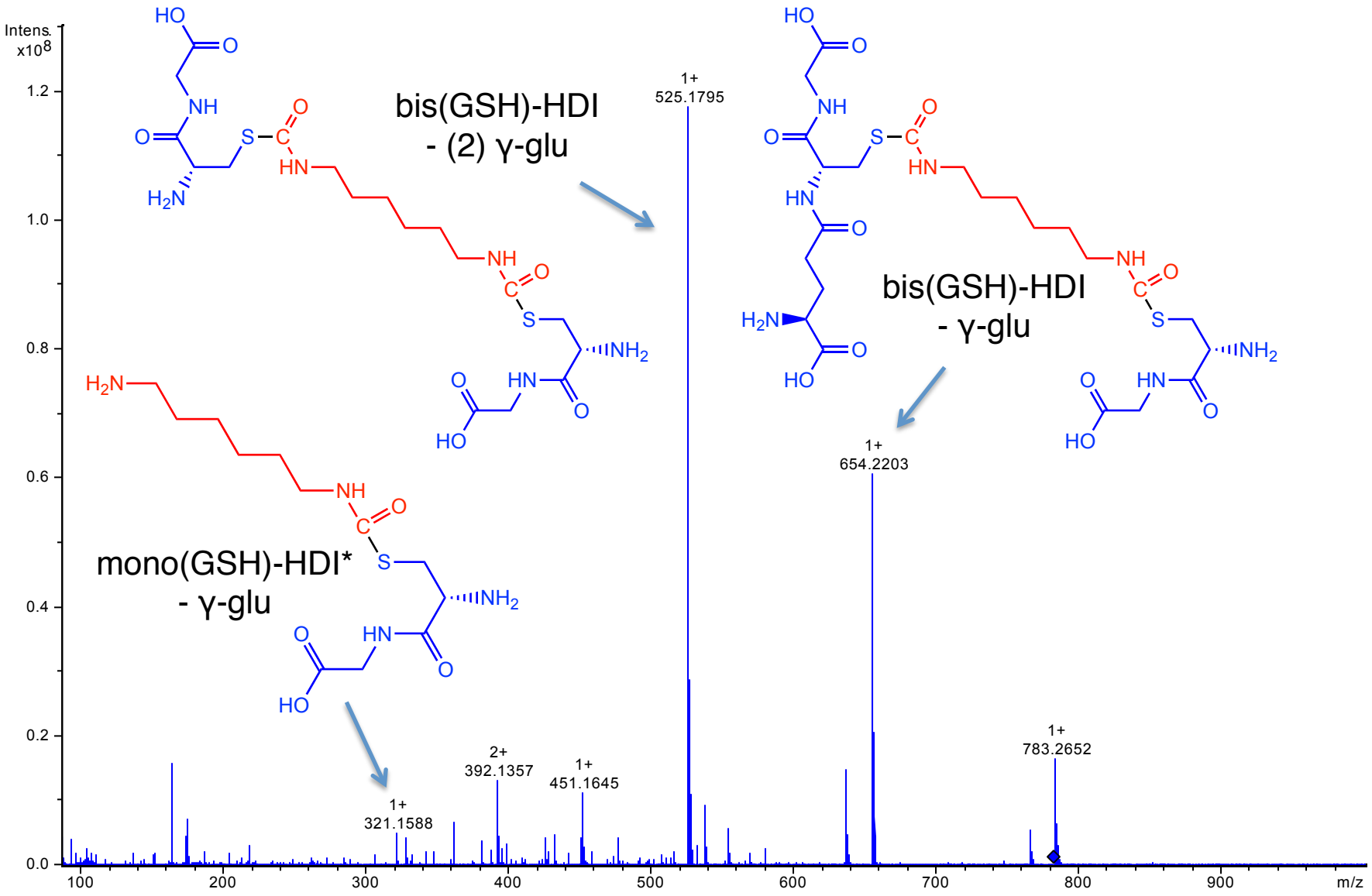


Figure E3. MS/MS of 450.19 m/z product

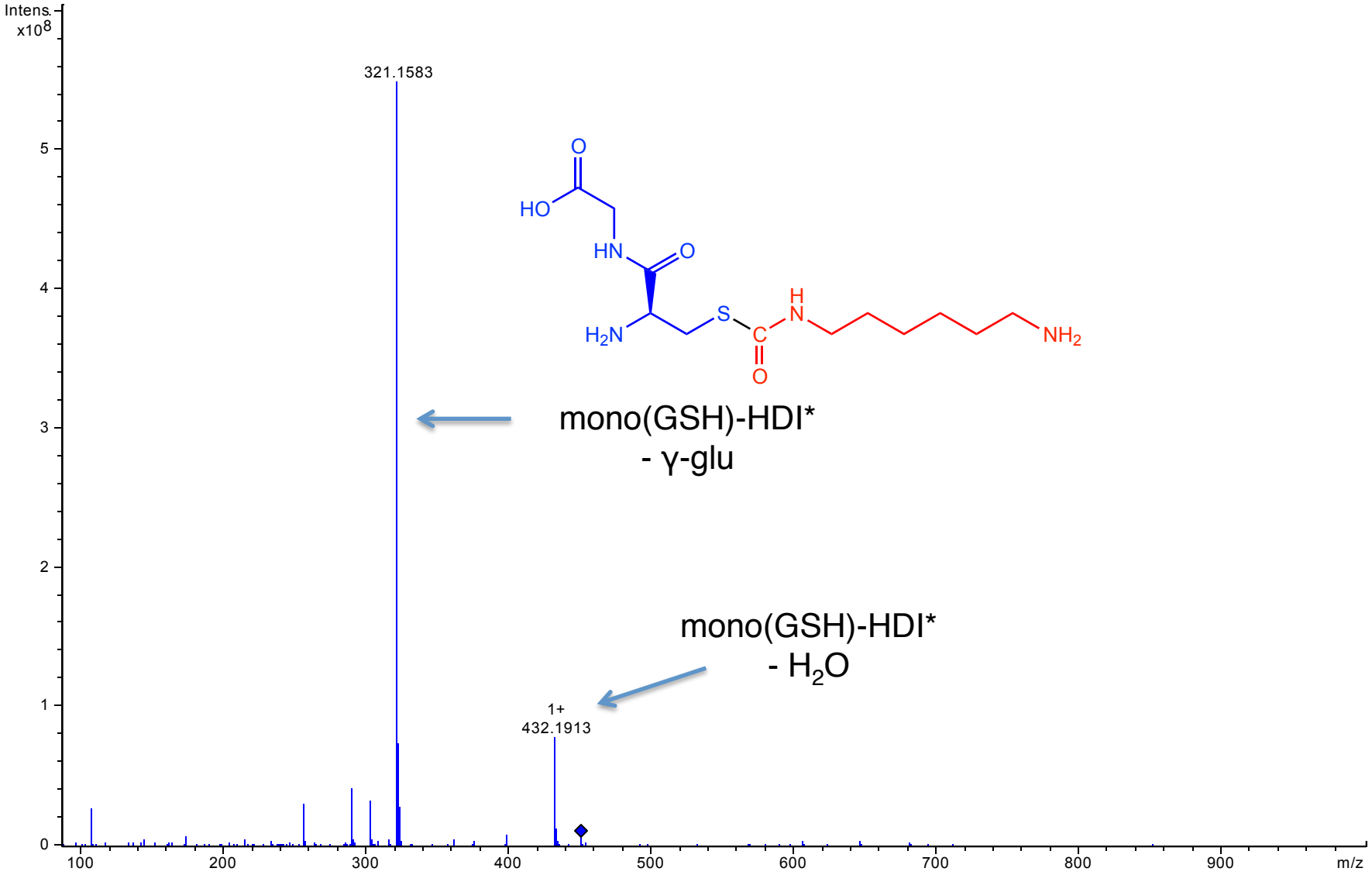


Figure E4. MS/MS of 476.17 m/z product

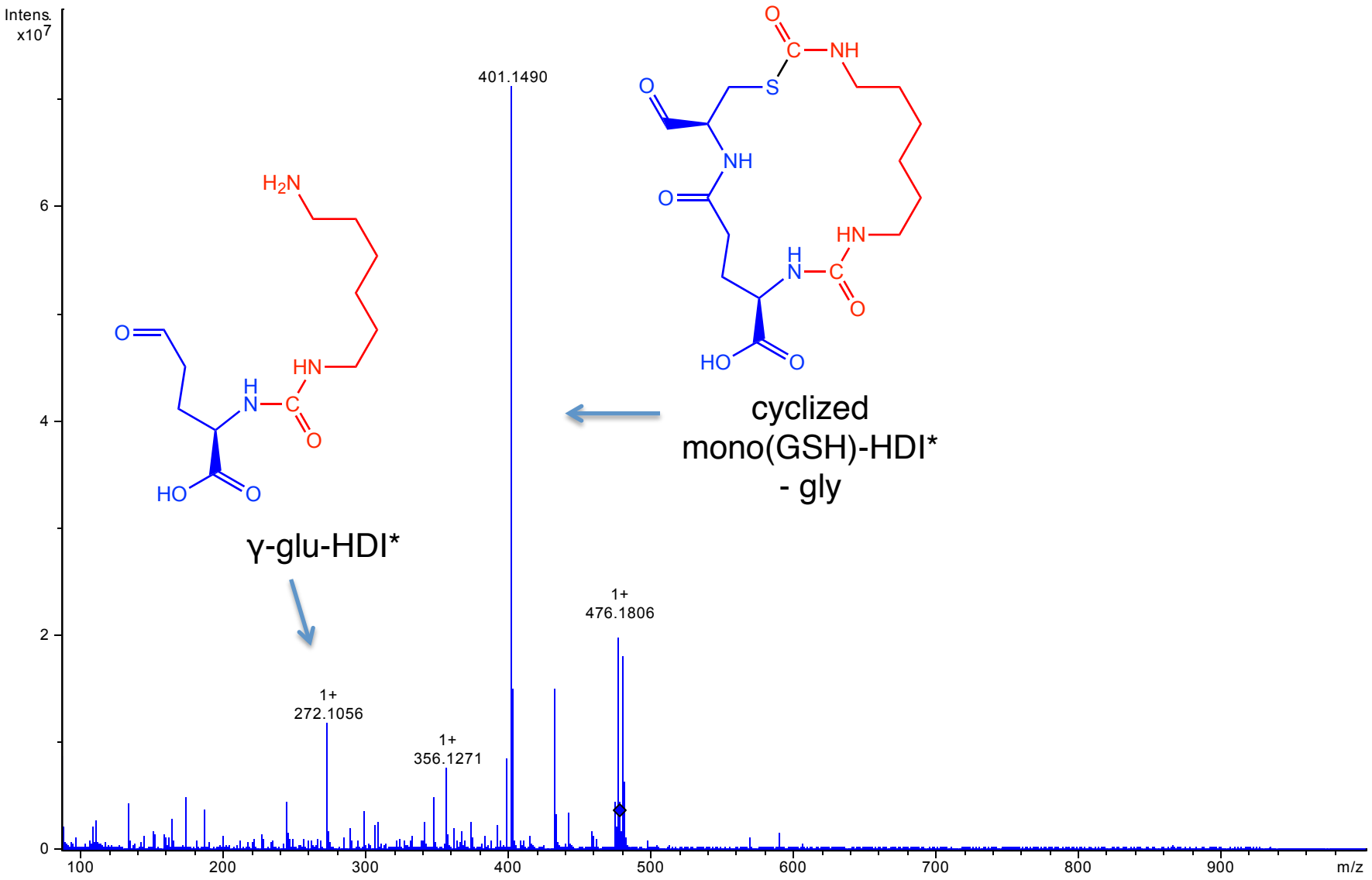


Figure E5. MS/MS of 618.28 m/z product

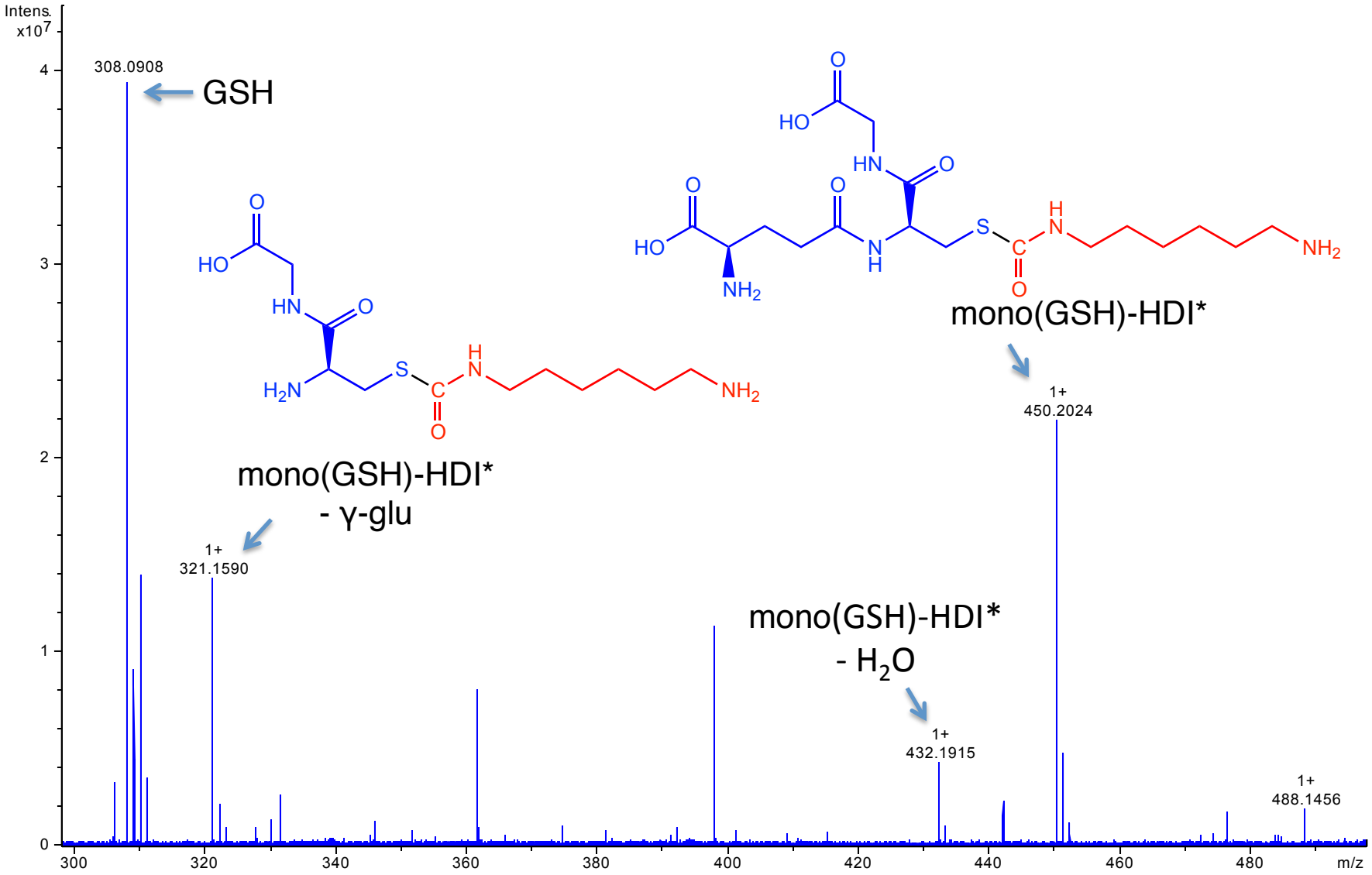
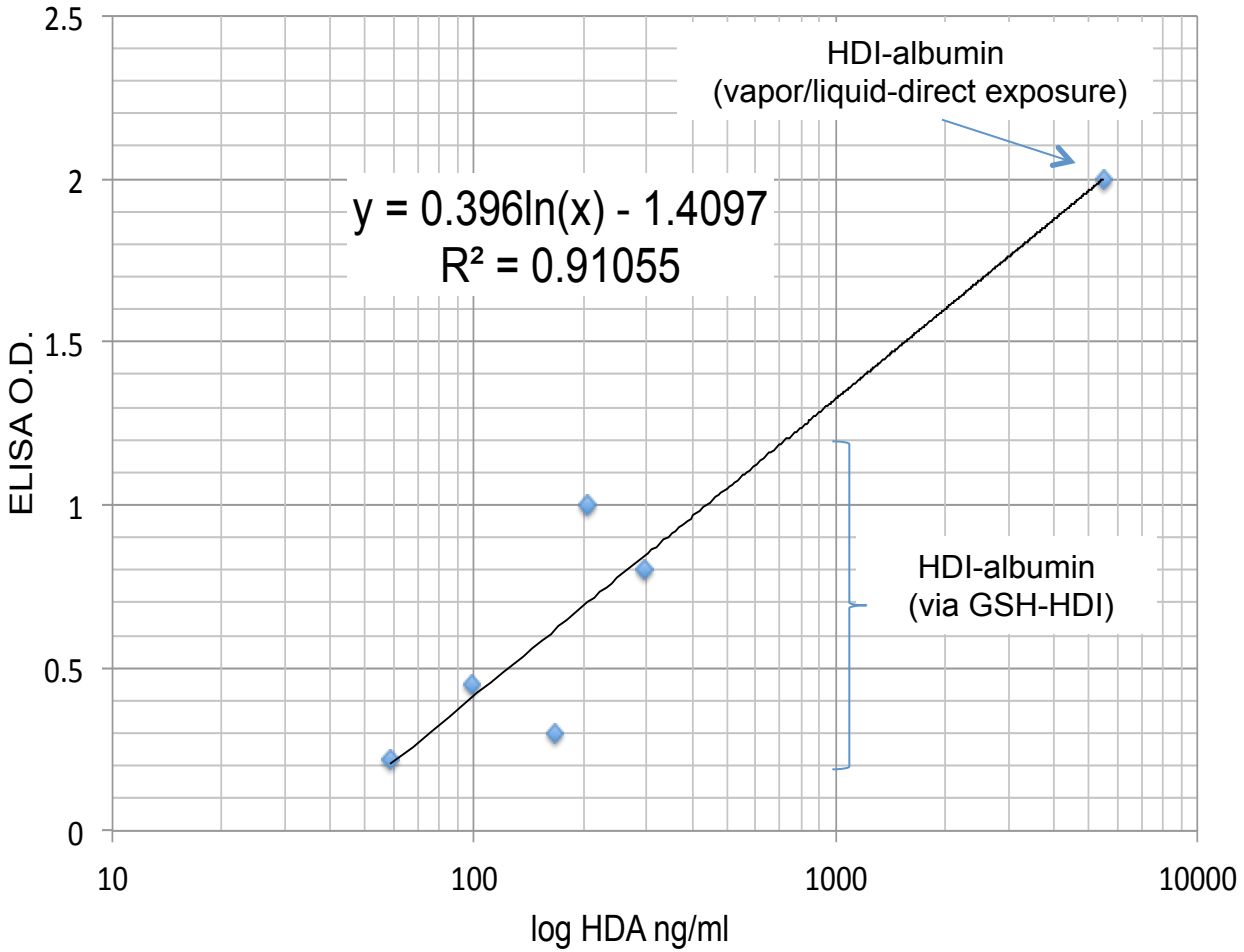


Figure E6. Correlation between anti-HDI ELISA O.D. and amount of acid hydrolyzable HDA



Scatter plot of data for individual samples of human albumin incubated with GSH-HDI at 37°C for 3 hours at pH 9.0, dialyzed against PBS and then analyzed by ELISA with anti-HDI rabbit serum and by HPLC, to quantitate the amount of HDA released upon acid hydrolysis.

Figure E7. MS/MS of novel albumin peptide cross-linked to GSH via HDI. GSH was acetylated during workup

K.VFDEFKPLVEEPQNLIKQNCELFEQLGEYK.F + Carbamidomethyl (C); HDI_532 (K)

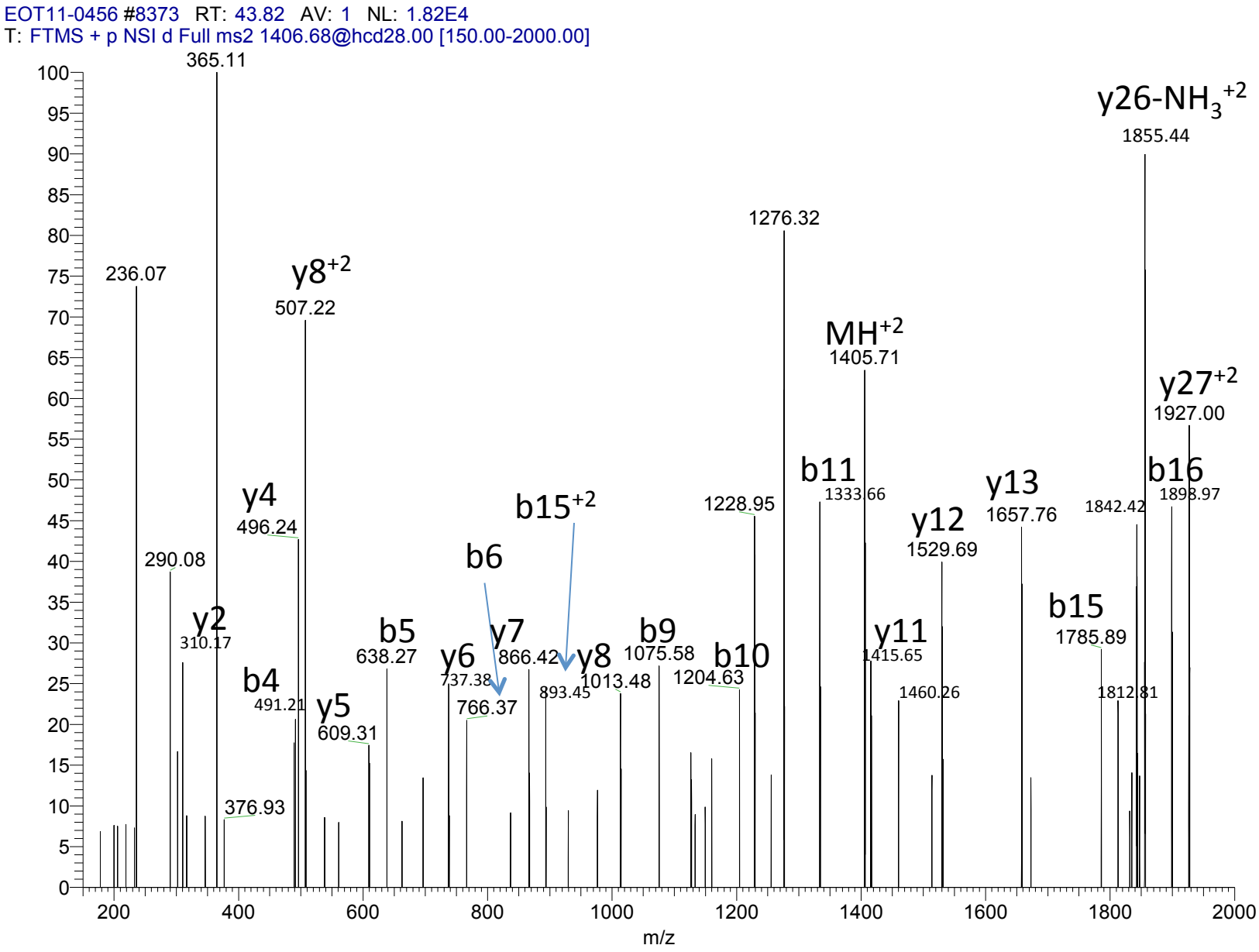


Figure E8. Expected MS/MS data of novel albumin peptide cross-linked to GSH via HDI. GSH was acetylated during workup

K.VFDEFKPLVEEPQNLIKQNCELFEQLGEYK.
F + Carbamidomethyl (C); HDI_532 (K)

Red boxed fragment Ions were observed in MS/MS spectra

u=HDI-GSH (532) plus Lys

User AA Formula 1: **C26 H46 N9 O9 S1**
Elemental Composition: C188 H290 N46 O60 S2

MH^{+1}(av)	MH^{+1}(mono)	MH^{+2}(av)	MH^{+2}(mono)	MH^{+3}(av)	MH^{+3}(mono)
4218.6358	4216.0491	2109.8216	2108.5282	1406.8835	1406.0212

[–] **Main Sequence Ions**

b	b^{+2}				y	y^{+2}
---	---	1	V	30	---	---
247.1441	---	2	F	29	4116.9807	2058.9940
362.1710	---	3	D	28	3969.9123	1985.4598
491.2136	---	4	E	27	3854.8854	1927.9463
638.2821	---	5	F	26	3725.8428	1863.4250
766.3770	383.6921	6	K	25	3578.7744	1789.8908
863.4298	432.2185	7	P	24	3450.6794	1725.8433
976.5138	488.7606	8	L	23	3353.6266	1677.3170
1075.5823	538.2948	9	V	22	3240.5426	1620.7749
1204.6249	602.8161	10	E	21	3141.4742	1571.2407
1333.6674	667.3374	11	E	20	3012.4316	1506.7194
1430.7202	715.8637	12	P	19	2883.3890	1442.1981
1558.7788	779.8930	13	Q	18	2786.3362	1393.6717
1672.8217	836.9145	14	N	17	2658.2776	1329.6424
1785.9058	893.4565	15	L	16	2544.2347	1272.6210
1898.9898	949.9986	16	I	15	2431.1506	1216.0790
2559.3038	1280.1555	17	u	14	2318.0666	1159.5369
2687.3623	1344.1848	18	Q	13	1657.7526	829.3800
2801.4053	1401.2063	19	N	12	1529.6941	765.3507
2961.4359	1481.2216	20	C(Carbamidomethyl)	11	1415.6511	708.3292
3090.4785	1545.7429	21	E	10	1255.6205	628.3139
3203.5626	1602.2849	22	L	9	1126.5779	563.7926
3350.6310	1675.8191	23	F	8	1013.4938	507.2506
3479.6736	1740.3404	24	E	7	866.4254	433.7164
3607.7322	1804.3697	25	Q	6	737.3828	369.1951
3720.8162	1860.9117	26	L	5	609.3243	305.1658
3777.8377	1889.4225	27	G	4	496.2402	248.6237
3906.8803	1953.9438	28	E	3	439.2187	220.1130
4069.9436	2035.4754	29	Y	2	310.1761	155.5917
---	---	30	K	1	147.1128	74.0600

Table E1. MS/MS data identifying HDI modified albumin peptides- pH 7.0 / 18 hr carbamoylation

m/z	*charge*	*rel. mol. mass*	*AA start*	*AA end*	*Score*	*E-value*	*Sequence*	*Modifications*	*Matched ions*
323.6773	4	1290.68	1	10	53.31	0.0007	DAHKSEVAHR	HDI 142 (K)	6
684.8679	2	1367.7213	11	20	44.92	0.0033	FKDLGEENFK	HDI 142 (K)	13
1188.9539	3	3563.8399	13	41	111.24	5.30E-10	DLGEENFKALVLIAFAQYLQQCPFEDHVK	Carbamidomethyl (C); HDI 142 (K)	26
891.9687	4	3563.8458	13	41	47.38	0.0012	DLGEENFKALVLIAFAQYLQQCPFEDHVK	Carbamidomethyl (C); HDI 142 (K)	25
1255.012	3	3762.0142	21	51	29.06	0.043	ALVLIAFAQYLQQCPFEDHVKLVNEVTEFAK	Carbamidomethyl (C); HDI 142 (K)	7
1320.1179	2	2638.2212	52	73	48.9	0.0014	TCVADESAENCDKSLHTLFGDK	2 Carbamidomethyl (C); HDI 142 (K)	17
1037.5863	2	2073.158	65	81	68.29	0.0000067	SLHTLFGDKLCTVATLR	Carbamidomethyl (C); HDI 142 (K)	12
692.06	3	2073.1583	65	81	50.53	0.0004	SLHTLFGDKLCTVATLR	Carbamidomethyl (C); HDI 142 (K)	17
974.1687	3	2919.4843	115	137	47.45	0.002	LVRPEVDVMCTAFHDNEETFLKK	Carbamidomethyl (C); HDI 142 (K)	18
730.8728	4	2919.4623	115	137	46.17	0.0028	LVRPEVDVMCTAFHDNEETFLKK	Carbamidomethyl (C); HDI 142 (K)	25
399.9076	3	1196.7009	137	144	40.85	0.0028	KYLYEIAR	HDI 142 (K)	6
599.3584	2	1196.7022	137	144	40.71	0.0029	KYLYEIAR	HDI 142 (K)	7
602.2884	3	1803.8434	161	174	93.15	4.60E-08	YKAAFTECCQAADK	2 Carbamidomethyl (C); HDI 142 (K)	9
902.9288	2	1803.843	161	174	92.3	5.60E-08	YKAAFTECCQAADK	2 Carbamidomethyl (C); HDI 142 (K)	11
1134.0615	2	2266.1084	163	181	99.68	1.60E-08	AAFTECCQAADKAACLLPK	3 Carbamidomethyl (C); HDI 142 (K)	21
669.3591	2	1336.7037	200	209	47.49	0.0018	CASLQKFGER	Carbamidomethyl (C); HDI 142 (K)	8
697.8929	2	1393.7712	223	233	49.87	0.00063	FPKAEFAEVSK	HDI 142 (K)	9
695.3556	3	2083.0449	258	274	71.4	0.00001	ADLAKYICENQDSISSK	Carbamidomethyl (C); HDI 142 (K)	18
913.9767	2	1825.9388	263	276	55.26	0.00035	YICENQDSISSKLK	Carbamidomethyl (C); HDI 142 (K)	9
844.9639	2	1687.9133	275	286	55.09	0.00031	LKECCEKPLLEK	2 Carbamidomethyl (C); HDI 142 (K)	9
422.9859	4	1687.9145	275	286	37.29	0.018	LKECCEKPLLEK	2 Carbamidomethyl (C); HDI 142 (K)	8
1206.9059	3	3617.6959	287	317	84.96	3.00E-07	SHCIAEVENDEMPADLPSLAADFVESKDVCK	2 Carbamidomethyl (C); HDI 142 (K)	22
905.4322	4	3617.6995	287	317	45.78	0.0025	SHCIAEVENDEMPADLPSLAADFVESKDVCK	2 Carbamidomethyl (C); HDI 142 (K)	14
480.2804	3	1437.8194	349	359	48.04	0.0008	LAKTYETTLEK	HDI 142 (K)	7
719.9167	2	1437.8188	349	359	38.98	0.0065	LAKTYETTLEK	HDI 142 (K)	8
887.4049	3	2659.1929	352	372	71.12	0.0000056	TYETTLEKCCAAADPHECYAK	3 Carbamidomethyl (C); HDI 142 (K)	20
1330.6049	2	2659.1952	352	372	50.64	0.00064	TYETTLEKCCAAADPHECYAK	3 Carbamidomethyl (C); HDI 142 (K)	17
1275.9958	3	3824.9656	373	402	55.22	0.0002	VFDEFKPLVEEPQNLIKQNCELFEQLGEYK	Carbamidomethyl (C); HDI 142 (K)	16
957.2484	4	3824.9645	373	402	34.24	0.025	VFDEFKPLVEEPQNLIKQNCELFEQLGEYK	Carbamidomethyl (C); HDI 142 (K)	21
729.7465	3	2186.2177	373	389	54.18	0.00017	VFDEFKPLVEEPQNLIK	HDI 142 (K)	16
1094.1148	2	2186.215	373	389	48.49	0.00065	VFDEFKPLVEEPQNLIK	HDI 142 (K)	17
1371.2166	2	2740.4186	390	410	90.55	8.00E-08	QNCELFEQLGEYKFQNALLVR	Carbamidomethyl (C); HDI 142 (K)	14
914.4813	3	2740.422	390	410	81.81	5.80E-07	QNCELFEQLGEYKFQNALLVR	Carbamidomethyl (C); HDI 142 (K)	23
891.5346	2	1781.0546	414	428	67	0.0000032	KVPQVSTPTLVEVSR	HDI 142 (K)	8
1105.8968	3	3314.6686	446	472	49.08	0.0012	MPCAEDYLSVVLNQLCVLHEKTPVSDR	2 Carbamidomethyl (C); HDI 142 (K)	11
829.6758	4	3314.674	446	472	44.09	0.0037	MPCAEDYLSVVLNQLCVLHEKTPVSDR	2 Carbamidomethyl (C); HDI 142 (K)	20
536.9496	3	1607.8269	473	484	56.97	0.00027	VTKCCTESLVNR	2 Carbamidomethyl (C); HDI 142 (K)	9
804.9202	2	1607.8259	473	484	56.04	0.00033	VTKCCTESLVNR	2 Carbamidomethyl (C); HDI 142 (K)	10
896.4295	3	2686.2666	501	521	51.5	0.00079	EFNAETFTFHADICTLSEKER	Carbamidomethyl (C); HDI 142 (K)	22
424.278	3	1269.8121	525	534	61.48	0.0000063	KQTALVELVK	HDI 142 (K)	7
635.9132	2	1269.8119	525	534	58.82	0.000012	KQTALVELVK	HDI 142 (K)	8
661.6858	3	1982.0357	542	557	85.38	3.40E-07	EQLKAVMDDFAAFVEK	HDI 142 (K)	11
667.0174	3	1998.0304	542	557	62.94	0.000063	EQLKAVMDDFAAFVEK	HDI 142 (K); Oxidation (M)	10
1000.0233	2	1998.032	542	557	53.32	0.00057	EQLKAVMDDFAAFVEK	HDI 142 (K); Oxidation (M)	20
821.3824	2	1640.7502	561	573	49.14	0.0011	ADDKETCFAEEGK	Carbamidomethyl (C); HDI 142 (K)	7
642.4114	2	1282.8083	574	585	76.68	2.20E-07	KLVAASQAALGL	HDI 142 (K)	8

Table E2. MS/MS data identifying HDI modified albumin peptides- pH 9.0 / 3 hr carbamoylation

m/z	*charge*	*rel. mol. mass*	*AA start*	*AA end*	*Score*	*E-value*	*Sequence*	*Modifications*	*matched ions*
323.6772	4	1290.6798	1	10	36.64	0.036	DAHKSEVAHR	HDI 142 (K)	5
684.8612	2	1367.7078	11	20	42.23	0.0064	FKDLGEENFK	HDI 142 (K)	7
456.91	3	1367.7082	11	20	45.56	0.003	FKDLGEENFK	HDI 142 (K)	7
891.9611	4	3563.8153	13	41	57.1	0.00016	DLGEENFKALVLIAFAQYLQQCPFEDHVK	Carbamidomethyl (C); HDI 142 (K)	28
1188.9442	3	3563.8108	13	41	101.83	5.50E-09	DLGEENFKALVLIAFAQYLQQCPFEDHVK	Carbamidomethyl (C); HDI 142 (K)	25
941.5031	4	3761.9831	21	51	40.3	0.0043	ALVLIAFAQYLQQCPFEDHVKLVNEVTEFAK	Carbamidomethyl (C); HDI 142 (K)	13
1255.0014	3	3761.9824	21	51	65.13	0.000014	ALVLIAFAQYLQQCPFEDHVKLVNEVTEFAK	Carbamidomethyl (C); HDI 142 (K)	26
880.4066	3	2638.1979	52	73	42.93	0.0045	TCVADESAENCDKSLHTLFGDK	2 Carbamidomethyl (C); HDI 142 (K)	11

Table E2. MS/MS data identifying HDI modified albumin peptides- pH 9.0 / 3 hr carbamoylation

m/z	*charge*	*rel. mol. mass*	*AA start*	*AA end*	*Score*	*E-value*	*Sequence*	*Modifications*	*matched ions*
1320.1064	2	2638.1982	52	73	56.81	0.00018	TCVADESAENCDKSLHTLFGDK	2 Carbamidomethyl (C); HDI 142 (K)	17
519.292	4	2073.1391	65	81	40.09	0.0062	SLHTLFGDKLCTVATLR	Carbamidomethyl (C); HDI 142 (K)	12
692.0533	3	2073.138	65	81	43.29	0.003	SLHTLFGDKLCTVATLR	Carbamidomethyl (C); HDI 142 (K)	15
1037.577	2	2073.1394	65	81	79.1	7.80E-07	SLHTLFGDKLCTVATLR	Carbamidomethyl (C); HDI 142 (K)	13
1108.4725	2	2214.9304	82	98	34.68	0.0091	ETYGEMADCCAKQEPER	2 Carbamidomethyl (C); HDI 142 (K)	17
713.6849	3	2138.0329	99	114	35.82	0.036	NECFLQHKDDNPNLPR	Carbamidomethyl (C); HDI 142 (K)	16
584.8986	5	2919.4566	115	137	39.45	0.014	LVRPEVDVMCTAFHDNEETFLKK	Carbamidomethyl (C); HDI 142 (K)	20
974.1595	3	2919.4566	115	137	45.71	0.0032	LVRPEVDVMCTAFHDNEETFLKK	Carbamidomethyl (C); HDI 142 (K)	12
730.8723	4	2919.4603	115	137	47.36	0.0022	LVRPEVDVMCTAFHDNEETFLKK	Carbamidomethyl (C); HDI 142 (K)	20
734.871	4	2935.4548	115	137	37.96	0.019	LVRPEVDVMCTAFHDNEETFLKK	Carbamidomethyl (C); HDI 142 (K); Oxidation (M)	18
599.3521	2	1196.6897	137	144	42.22	0.0025	KYLYEIAR	HDI 142 (K)	7
399.9045	3	1196.6917	137	144	54.75	0.00014	KYLYEIAR	HDI 142 (K)	7
902.9204	2	1803.8262	161	174	85.31	2.30E-07	YKAAFTECCQAADK	2 Carbamidomethyl (C); HDI 142 (K)	22
602.2827	3	1803.8264	161	174	93.16	3.80E-08	YKAAFTECCQAADK	2 Carbamidomethyl (C); HDI 142 (K)	9
1134.0509	2	2266.0872	163	181	76.33	0.0000032	AAFTECCQAADKAACLLPK	3 Carbamidomethyl (C); HDI 142 (K)	22
514.3006	3	1539.8799	175	186	36.46	0.0098	AACLLPKLDELR	Carbamidomethyl (C); HDI 142 (K)	13
770.9476	2	1539.8806	175	186	38.99	0.0055	AACLLPKLDELR	Carbamidomethyl (C); HDI 142 (K)	9
669.353	2	1336.6914	200	209	36.43	0.025	CASLQKFGER	Carbamidomethyl (C); HDI 142 (K)	7
581.348	2	1160.6815	210	218	33.61	0.039	AFKAWAVAR	HDI 142 (K)	7
697.8873	2	1393.76	223	233	48.26	0.0011	FPKAEFAEVSK	HDI 142 (K)	9
598.3397	3	1791.9974	226	240	49.93	0.00059	AEFAEVSKLVTDLTK	HDI 142 (K)	15
897.0058	2	1791.9971	226	240	73.48	0.0000026	AEFAEVSKLVTDLTK	HDI 142 (K)	12
1000.4707	3	2998.3903	234	257	36.51	0.024	LVTDLTKVHTECCHGDLLECADDR	3 Carbamidomethyl (C); HDI 142 (K)	17
1042.5186	2	2083.0226	258	274	74.91	0.0000047	ADLAKYICENQDSISSK	Carbamidomethyl (C); HDI 142 (K)	19
695.3488	3	2083.0247	258	274	94.41	5.20E-08	ADLAKYICENQDSISSK	Carbamidomethyl (C); HDI 142 (K)	18
913.968	2	1825.9214	263	276	43.67	0.0055	YICENQDSISSKLK	Carbamidomethyl (C); HDI 142 (K)	7
563.6403	3	1687.899	275	286	33.94	0.044	LKECCEKPLLEK	2 Carbamidomethyl (C); HDI 142 (K)	7
422.9822	4	1687.8996	275	286	42.3	0.0065	LKECCEKPLLEK	2 Carbamidomethyl (C); HDI 142 (K)	8
844.9566	2	1687.8986	275	286	48.71	0.0014	LKECCEKPLLEK	2 Carbamidomethyl (C); HDI 142 (K)	9
1468.3543	3	4402.0411	277	313	46.83	0.0014	ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK	3 Carbamidomethyl (C); HDI 142 (K)	10
1079.5165	4	4314.0369	277	313	34	0.029	ECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESK	Carbamidomethyl (C); HDI 168 (K)	11
905.4235	4	3617.6649	287	317	56.79	0.00017	SHCIAEVENDEMPADLPSLAADFVESKDVCK	2 Carbamidomethyl (C); HDI 142 (K)	20
1206.8946	3	3617.662	287	317	83.32	3.60E-07	SHCIAEVENDEMPADLPSLAADFVESKDVCK	2 Carbamidomethyl (C); HDI 142 (K)	24
1221.6112	2	2441.2078	318	336	73.22	0.0000063	NYAEAKDVFLGMFLYEYAR	HDI 142 (K)	17
719.9108	2	1437.8069	349	359	36.8	0.012	LAKTYETTLEK	HDI 142 (K)	8
480.2758	3	1437.8057	349	359	48.46	0.00085	LAKTYETTLEK	HDI 142 (K)	7
665.7999	4	2659.1707	352	372	34.45	0.02	TYETTLEKCCAAADPHECYAK	3 Carbamidomethyl (C); HDI 142 (K)	16
1330.5881	2	2659.1616	352	372	68.93	0.0000065	TYETTLEKCCAAADPHECYAK	3 Carbamidomethyl (C); HDI 142 (K)	22
1275.9847	3	3824.9323	373	402	62.64	0.000042	VFDEFKPLVEEPQNLIKQNCELFEQLGEYK	Carbamidomethyl (C); HDI 142 (K)	18
957.24	4	3824.9307	373	402	71.87	0.0000051	VFDEFKPLVEEPQNLIKQNCELFEQLGEYK	Carbamidomethyl (C); HDI 142 (K)	25
1406.0119	3	4215.0139	373	402	58.66	0.0001	VFDEFKPLVEEPQNLIKQNCELFEQLGEYK	Carbamidomethyl (C); HDI 532 (K)	18
729.7402	3	2186.1987	373	389	51.61	0.0004	VFDEFKPLVEEPQNLIK	HDI 142 (K)	16
1094.106	2	2186.1974	373	389	54.55	0.00021	VFDEFKPLVEEPQNLIK	HDI 142 (K)	19
914.4733	3	2740.398	390	410	83.7	4.50E-07	QNCELFEQLGEYKFQNALLVR	Carbamidomethyl (C); HDI 142 (K)	22
1371.2047	2	2740.3948	390	410	91.82	7.10E-08	QNCELFEQLGEYKFQNALLVR	Carbamidomethyl (C); HDI 142 (K)	14
891.5279	2	1781.0412	414	428	63.66	0.0000092	KVPQVSTPTLVEVSR	HDI 142 (K)	8
594.6872	3	1781.0399	414	428	69.53	0.0000024	KVPQVSTPTLVEVSR	HDI 142 (K)	8
829.669	4	3314.6468	446	472	34.23	0.04	MPCAEDYLSVVLNQLCVLHEKTPVSDR	2 Carbamidomethyl (C); HDI 142 (K)	22
1105.8879	3	3314.6419	446	472	52.99	0.00054	MPCAEDYLSVVLNQLCVLHEKTPVSDR	2 Carbamidomethyl (C); HDI 142 (K)	13
804.9125	2	1607.8104	473	484	58.4	0.00019	VTKCCTESLVNR	2 Carbamidomethyl (C); HDI 142 (K)	10
536.9433	3	1607.808	473	484	61.87	0.000089	VTKCCTESLVNR	2 Carbamidomethyl (C); HDI 142 (K)	9
1074.2654	4	4293.0325	485	519	43.55	0.0032	RPCFSALEVDETYVPKEFNAETFTFHADICTLSEK	2 Carbamidomethyl (C); HDI 142 (K)	23
896.4286	3	2686.2639	501	521	49.46	0.0013	EFNAETFTFHADICTLSEKER	Carbamidomethyl (C); HDI 142 (K)	8
635.9079	2	1269.8013	525	534	54.81	0.000039	KQTALVELVK	HDI 142 (K)	8
424.2742	3	1269.8006	525	534	55.92	0.00003	KQTALVELVK	HDI 142 (K)	7
661.6806	3	1982.0198	542	557	75.65	0.0000036	EQLKAVMDDFAAFVEK	HDI 142 (K)	11
644.9713	3	1931.8919	546	560	39.73	0.01	AVMDDFAAFVEKCCK	2 Carbamidomethyl (C); HDI 142 (K)	15

Table E2. MS/MS data identifying HDI modified albumin peptides- pH 9.0 / 3 hr carbamoylation

m/z	*charge*	*rel. mol. mass*	*AA start*	*AA end*	*Score*	*E-value*	*Sequence*	*Modifications*	*matched ions*
966.9536	2	1931.8926	546	560	66.62	0.000021	AVMDDFAAFVEKCCK	2 Carbamidomethyl (C); HDI 142 (K)	12
821.3742	2	1640.7339	561	573	53.11	0.00029	ADDKETCFAEEGK	Carbamidomethyl (C); HDI 142 (K)	10
547.9185	3	1640.7336	561	573	54.72	0.0002	ADDKETCFAEEGK	Carbamidomethyl (C); HDI 142 (K)	8
642.4056	2	1282.7967	574	585	75.41	3.90E-07	KLVAASQAALGL	HDI 142 (K)	7