

Supplementary Materials: LC-HRMS Screening and Identification of Novel Peptide Markers of Ricin Based on Multiple Protease Digestion Strategies

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Table S1. The specific ricin tryptic peptides under digestion with denaturation and reduction.

T#&chain	Amino acid sequence	(M + H) ⁺	(M + 2H) ²⁺	(M + 3H) ³⁺	(M + 4H) ⁴⁺
T2A-glyc*	QYPIINFTTAGATVQSYTNFIR	3675.6946	1838.3509	1225.9031	919.6791
T7A	VGLPINQR	896.5312	448.7692	299.5152	-
T8A	FILVELSNHAELSVTLALDVTNA YVVGYS	3206.7095	1603.8584	1069.5747	802.4328
T9A	AGNSAYFFHPDNQEDAEATHL FTDVQNR	3307.5038	1654.2555	1103.1728	827.6314
T10A	YTFAFGGNYDR	1310.5800	655.7936	437.5315	-
T11A	LEQLAGNLR	1013.5738	507.2905	338.5294	-
T12A	ENIELGNPGLPEEASALYYSTGG TQLPTLAR	3440.7219	1720.8646	1147.5788	860.9359
T13A	SFIICQMISEAAR	1581.8127	791.4100	527.9424	-
T23A	FSVYDVSILIPILALMVYR	2212.2450	1106.6261	738.0865	553.8167
T3B	NGLCVDVR	875.4403	438.2238	292.4850	-
T5B	FHNGNAIQL WPCK	1584.7740	792.8906	509.9223	396.9494
T6B	SNTDANQLWTLK	1390.6961	695.8517	464.2369	-
T10B	CLTTYGYSPGVYVMYDCNTAA TDATR	2949.3103	1475.1588	983.7750	737.5791
T11B-glyc*	WQIWDNGTIINPR	2991.2987	1496.1530	997.7710	748.5801
T12B	SSLVLAATSGNSGTTLTVQTNIY AVSQGWLPNTNTQPFVTTIVGL YGLCLQANSQGVWIEDCSSEK	6933.4237	3467.2155	2311.8127	1734.1119
T14B	DNCLTSDSNIR	1237.5477	619.2775	413.1874	-
T18B	NDGTILNLYSGLVLDVR	1862.0018	931.5045	621.3388	-
T20B	QIILYPLHGDPNQIWLPLF	2277.2430	1139.1251	759.7525	570.0667

Trypsin digest peptides numbered from the amino terminal of the polypeptide chain. * Glycopeptide. The LC-MS observed ions were indicated in bold.

Table S2. The specific ricin tryptic peptides under direct digestion.

T#&chain	Amino acid sequence	(M + H) ⁺	(M + 2H) ²⁺	(M + 3H) ³⁺	(M + 4H) ⁴⁺
T2A-glyc*	QYPIINFTTAGATVQSYTNFIR	3675.6946	1838.3509	1225.9031	919.6791
T7A	VGLPINQR	896.5312	448.7692	299.5152	-
T8A	FILVELSNHAELSVTLALDVTN AYVVGYS	3206.7095	1603.8584	1069.5747	802.4328
T9A	AGNSAYFFHPDNQEDAEATH LFTDVQNR	3307.5038	1654.2555	1103.1728	827.6314
T10A	YTFAFGGNYDR	1310.5800	655.7936	437.5315	-
T11A	LEQLAGNLR	1013.5738	507.2905	338.5294	-
T12A	ENIELGNPGLPEEASALYYSTG GTQLPTLAR	3440.7219	1720.8646	1147.5788	860.9359
T13A	SFIICQMISEAAR	1581.8127	791.4100	527.9424	-
T23A	FSVYDVSILIPILALMVYR	2212.2450	1106.6261	738.0865	553.8169
T24A-ss- T1B ^a	CAPPPSSQF-ss- ADVCMDEPIVR	2275.0196	1138.0138	759.0118	569.5108

T3B-ss-	NGLCVDVR-ss-				
T5B ^a	FHNGNAIQLWPCK	2400.1699	1200.5886	800.7281	600.7979
T6B	SNTDANQLWTLK	1390.6961	695.8517	464.2369	-
T10B ^a	CLTTYGYSPGVYVMIYDCNTAA TDATR	2947.2947	1474.1510	983.1031	737.5791
T11B-gly*	WQIWDNGTIINPR	2991.2987	1496.1530	997.7710	748.5801
T12B	SSLVLAATSGNSGTTLTVQTNIIY AVSQGWLPNTNTQPFVTTIVGL YGLCLQANSQGVWIEDCSSEK	6933.4237	3467.2155	2311.8127	1734.1119
T14B-ss-	DNCLTSDSNIR-ss-				
T16B ^a	ILSCGPASSGQR	2410.1085	1205.5579	804.0410	603.2826
T18B	NDGTILNLYSGLVLDVR	1862.0018	931.5045	621.3388	466.2564
T20B	QIILYPLHGDPNQIWLPLF	2277.2430	1139.1251	759.7525	570.0667

Trypsin digest peptides numbered from the amino terminal of the polypeptide chain. a: Disulfide bond peptide. * Glycopeptide. The LC-MS observed ions were indicated in bold.

Table S3. Theoretical mass to charge values of ricin digested peptides from Glu-C after trypsin.

T#&chain	Amino acid sequence	(M + H) ⁺	(M + 2H) ²⁺	(M + 3H) ³⁺	(M + 4H) ⁴⁺
TG1A	IFPK	504.3180	252.6626	-	-
TG2A-glyc*	QYPIINFITAGATVQSYTNFIR	3675.6946	1838.3509	1225.9031	919.6791
TG3A	AVR	345.2245	-	-	-
TG4A	GR	232.1404	-	-	-
TG5A	LTGAD	577.2828	289.1450	-	-
TG6A	VR	274.1873	-	-	-
TG7A	HE	285.1193	-	-	-
TG8A	IPVLPNR	808.5039	404.7556	270.1728	-
TG9A	VGLPINQR	896.5312	448.7692	299.5152	-
TG10A	FILVE	620.3654	310.6863	-	-
TG11A	LSNHAE	670.3155	355.6614	-	-
TG12A	LSVTLALD	831.4822	416.2447	277.8322	-
TG13A	VTNAYVVGYR	1141.6000	571.3036	381.2048	-
TG14A	AGNSAYFFHPD	1225.5272	613.2672	409.1805	307.1372
TG15A	NQE	390.1619	-	-	-
TG16A	D	134.0448	-	-	-
TG17A	AE	219.0975	-	-	-
TG18A	AITHLFTD	917.4727	459.2340	306.4957	-
TG19A	VQNR	516.2889	258.6481	-	-
TG20A	YTFAFGGNYD	1154.4789	577.7431	385.4978	-
TG21A	R	175.1189	-	-	-
TG22A	LE	261.1445	-	-	-
TG23A	QLAGNLR	771.4471	359.2272	-	-
TG24A	E	148.0604	-	-	-
TG25A	NIE	375.1874	-	-	-
TG26A	LGNGPLE	699.3671	350.1872	233.7939	-
TG27A	E	148.0604	-	-	-
TG28A	AISALYYSTGGTQLPTLAR	2146.1179	1073.5626	716.0441	-
TG29A	SFIICQMISE	1283.6374	642.3223	428.5506	-
TG30A	AAR	317.1932	-	-	-
TG31A	FQYIE	699.3348	350.1710	-	-
TG32A	GE	205.0819	-	-	-
TG33A	MR	306.1594	-	-	-
TG34A	TR	276.1666	-	-	-
TG35A	IR	288.2030	-	-	-
TG36A	YNR	452.2252	-	-	-
TG37A	R	175.1189	-	-	-
TG38A	SAPD	389.1667	-	-	-
TG39A	PSVITLE	758.4294	379.7183	-	-

TG40A	NSWGR	619.2947	310.1510	-	-
TG41A	LSTAIQE	761.4039	381.2056	-	-
TG42A	SNQGAFASPIQLQR	1516.7866	758.8969	506.2670	-
TG43A	R	175.1189	-	-	-
TG44A	NGSK	405.2092	-	-	-
TG45A	FSVYD	630.2769	315.6420	-	-
TG46A	VSILIPILMVYR	1600.9858	800.9965	534.3334	-
TG47A-ss-TG2B ^a	CAPPPSSQF-ss-VCMD	1397.5535	699.2804	466.5226	-
TG1B	AD	205.0819	-	-	-
TG3B	PE	245.1132	-	-	-
TG4B	PIVR	484.3242	-	-	-
TG5B	IVGR	444.2929	-	-	-
TG6B-ss-TG9B ^a	NGLCVD-ss-FHNGNAIQLWPCK	2145.0003	1073.0038	715.7154	537.0555
TG7B	VR	274.1873	-	-	-
TG8B	DGR	347.1673	-	-	-
TG10B	SNTD	436.1674	-	-	-
TG11B	ANQLWTLK	973.5465	487.2769	325.1870	-
TG12B	R	175.1189	-	-	-
TG13B	DNTIR	618.3205	309.6639	-	-
TG14B	SNGK	405.2092	-	-	-
TG15B-ss-TG16B ^a	CLTTYGYSPGVYVMIYD-ss-CNTAATD	2637.1194	1319.0633	879.7113	660.0353
TG17B	ATR	347.2037	-	-	-
TG18B	WQIWD	747.3460	347.1766	-	-
TG19B	NGTIINPR	884.4948	442.7510	295.5031	-
TG20B	SSLVLAATSGNSGTTTLTVQTNI YAVSQGWLPTNNTQPFVTTIV GLYGLCLQANSQVWIED	6284.1859	3142.5966	2095.4002	1571.8024
TG21B	CSSE	425.1337	-	-	-
TG22B	K	147.1128	-	-	-
TG23B	AE	219.0975	-	-	-
TG24B	QQWALYAD	994.4628	497.7350	332.1591	-
TG25B	GSIRPQQNR	1055.5704	528.2888	352.5283	-
TG26B	D	134.0448	-	-	-
TG27B-ss-TG30B ^a	NCLTSD-ss-ILSCGPASSGQR	1824.7458	912.9094	608.9639	456.9912
TG28B	SNIR	489.2780	-	-	-
TG29B	ETVVK	575.3399	288.1736	-	-
TG31B	WMFK	611.3010	306.1541	-	-
TG32B	ND	248.0877	-	-	-
TG33B	GTILNLYSGLVLD	1377.7624	689.3848	459.9256	-
TG34B	VR	274.1873	-	-	-
TG35B	ASD	292.1139	-	-	-
TG36B	PSLK	444.2816	-	-	-
TG37B	QIILYPLHGD	1168.6360	584.8217	390.2169	-
TG38B	PNQIWLPLF	1127.6248	564.3160	376.5464	-

Trypsin/Glu-C tandem digest peptides numbered from the amino terminal of the polypeptide chain.

^a: Disulfide bond peptide. * Glycopeptide. Ricin specific peptide was indicated in red color. The LC-MS observed ions of ricin specific peptides were indicated in bold.

Table S4. The ricin specific peptides under pepsin digestion at pH 1~2.

P#&chain	Amino acid sequence	(M + H) ⁺	(M + 2H) ²⁺	(M + 3H) ³⁺	(M + 4H) ⁴⁺
P3A	TTAGATVQSYTNF	1360.6379	680.8226	454.2175	340.9154
P4A	IRAVRGRL	940.6163	470.8118	314.2103	-
P7A	PINQRF	774.4257	387.7165	258.8134	-
P13A	DVTNAYVVG YRAGNSAYF	1966.9293	983.9683	656.3146	492.4883
P17A	TDVQNRYTF	1143.5429	572.2751	381.8525	-

P19A	GGNYDRL	794.3791	397.6932	265.4646	-
P24A	EEAISAL	732.3774	366.6923	244.7973	-
P25A	YYYSTGGTQL	1152.5208	576.7640	384.8451	-
P28A	IICIQMISEAARF	1494.7807	747.8940	498.9317	374.4511
P34A	SVYDVSIL	895.4771	448.2422	299.1639	-
P36A-ss-	MVYRCAPPPSSQF-ss-				
P1B ^a	NADVCMDEPIVIRIVGRNGL	3533.7164	1767.3622	1178.5774	884.1850
P2B-ss-	CVDVRDGRF-ss-				
P4B ^a	WPCKSNTDANQL	2440.1136	1220.5608	814.0431	610.7843
P3B	HNGNAIQL	866.4479	433.7276	289.4875	-
P6B	KRDNTIRSNGKCL	1504.8012	752.9043	502.2719	376.9562
	TTYGYSPGVYVMIYDCNTAAT				
P7B	DATRWQIWD	4614.1704	2307.5888	1538.7283	1154.2985
	NGTIINPRSSL				
P9B	AATSGNSGTTL	979.4691	490.2382	327.1612	-
P15B	QANSGQVWIEDCSSEKAEQQ	2607.1780	1304.0926	869.7308	652.5504
	WAL				
P23B	DVRASDPSL	959.4792	480.2432	320.4979	-
P24B	KQIIL	614.4235	307.7154	205.4794	-
P26B	HGDPNQIWL	1079.5268	540.2671	360.5138	-

Pepsin digest peptides numbered from the amino terminal of the polypeptide chain. a: Disulfide bound peptide. The LC-MS observed ions were indicated in bold.

Table S5. The ricin specific peptides under pepsin digestion at pH 2~4.

P#&chain	Amino acid sequence	(M + H) ⁺	(M + 2H) ²⁺	(M + 3H) ³⁺	(M + 4H) ⁴⁺
P2B-ss-	DVCMDPE-ss-CVDVRDGRF	1871.7725	936.3902	624.5961	468.6991
P4B ^a					
P9B	PCKSNTDA	835.3614	418.1843	279.1253	-
P14B	KRDNTIRSNGKCL	1504.8012	752.9043	502.2719	-
P25B	DNGTIINPRSSL	1286.6699	643.8386	429.5615	322.4234
P29B	TSGNSGTTL	837.3948	419.2011	279.8031	-
P60B	TSDSNIRE	921.4272	461.2172	307.8139	-
P77B	HGDPNQ	667.2794	334.1433	223.0980	-

Pepsin digest peptides numbered from the amino terminal of the polypeptide chain. a: Disulfide bound peptide. The LC-MS observed ions were indicated in bold.

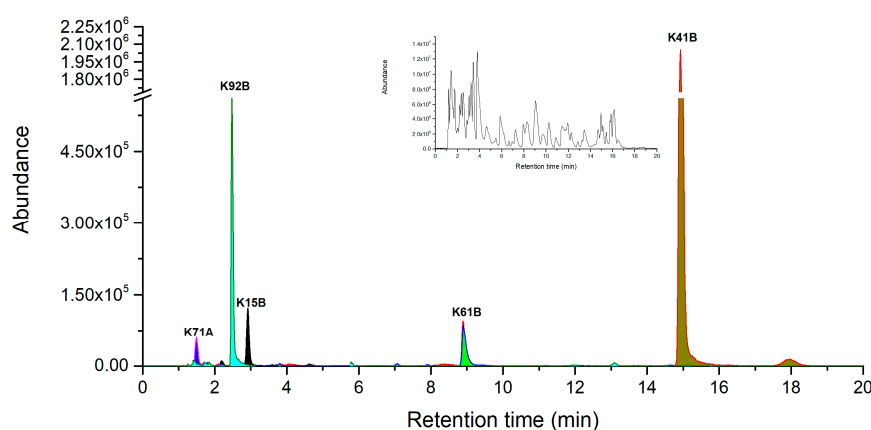


Figure S1. LC-high resolution accurate mass analysis of the proteinase K digest of 0.1 mg/mL of purified ricin. Base peak chromatogram overlaid on extracted ion chromatograms of ricin maker peptides. Total ion chromatography of ricin at top right corner window.

IFPKQYPIINFITAGATVQSYTNFIRAVRGRLITGADVREIPVLPNRVGLPINQRFILVELSN
 HAELSVTLALDVTNAYVVGYSRAGNSAYFFHPDNQEDAEAITHLFTDVQNRYSFAFGGNY
 DRLEQLAGNLRENIELGNGLPEEAISALYYSTGGTQLPTLARFSFIICQMISEAARFQYIEG
 EMRTRIRYNRRSAPDPSVITLENSWGRLSTAQESNQGAFAFASPIQLQRRNGSKFSVYDVSILI
 PIIALMVYRCAPPPSSQF
 ADVCMDPEPIVRIVGRNGLCVDVRDGRFHNGNAIQLWPCKSNTDANQLWTLKRDNTIRS
 NGKCLTTYGYSPGVYVMIYDCNTAATDATRWQIWDNGTIINPRSSLVLAATSGNSGTTLT
 QTNIAVSQGWLPNTNTQPFVTTIVGLYGLCLQANSQVWIEDCSSEKAEQQWALYADGS
 IRPQQNRDNCLTSDSNIRETVVKILSCGPASSGQRWMFKNDGTILNLYSGLVLDVRASDPS
 LKQIILYPLHGDPNQIWLPLF

Figure S2 shows peptide markers from trypsin/Glu-C tandem digestion. The markers are highlighted in yellow and labeled with orange boxes: TG13A, TG26A, TG28A, TG6B-ss-TG9B, TG11B, TG27B-ss-TG30B, and TG37B.

Figure S2. Peptide markers from trypsin/Glu-C tandem digestion.

IFPKQYPIINFITAGATVQSYTNFIRAVRGRLITGADVREIPVLPNRVGLPINQRFILVELSN
 HAELSVTLALDVTNAYVVGYSRAGNSAYFFHPDNQEDAEAITHLFDVQNRYSFAFGGNY
 DRLEQLAGNLRENIELGNGLPEEAISALYYSTGGTQLPTLARFSFIICQMISEAARFQYIEG
 EMRTRIRYNRRSAPDPSVITLENSWGRLSTAQESNQGAFAFASPIQLQRRNGSKFSVYDVSILI
 PIIALMVYRCAPPPSSQF
 ADVCMDPEPIVRIVGRNGLCVDVRDGRFHNGNAIQLWPCKSNTDANQLWTLKRDNTIRS
 NGKCLTTYGYSPGVYVMIYDCNTAATDATRWQIWDNGTIINPRSSLVLAATSGNSGTTLT
 QTNIAVSQGWLPNTNTQPFVTTIVGLYGLCLQANSQVWIEDCSSEKAEQQWALYADGS
 IRPQQNRDNCLTSDSNIRETVVKILSCGPASSGQRWMFKNDGTILNLYSGLVLDVRASDPS
 LKQIILYPLHGDPNQIWLPLF

Figure S3 shows peptide markers from chymotrypsin digestion. The markers are highlighted in yellow and labeled with orange boxes: C4A, C18A, C31A, C4B, C8B, C18B, C27B, and C31B.

Figure S3. Peptide markers from chymotrypsin digestion.

IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVGLPINQRFILVELSN
 HAELSVTLALDVTNAYVVG YRAGNSAYFFHPDNQEDAEAITHLFTDVQNRYTFAFGGNY
 DRLEQLAGNLRENIELGNGPLEE AISALYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEG
 EMRTRIRYNRRSAPDPSVITLENSWGRLSTAQESNQGAFASPIQLQRRNGSKFSVYDVSILI
 PIIALMVYRCAPPSSQF
 ADVCMDEPIVIRIVGRNGLCVDVRDGRFHNGNAIQLWPCKSNTDANQLWTLKRDNTIRS
 NGKCLTTYGYSPGVYVMYDCNTAATDATRWQIWDNGTIINPRSSLVLAATSGNSTTLTV
 QTNIAVSQGWLPNTNTQPFVTTIVGLYGLCLQANSQVWIEDCSSEKAEQQWALYADGS
 IRPQQNRDNCLTSDSNIRETVVKILSCGPASSGQRWMFKNDGTILNLYSGLVDVRASDPS
 LKQIILYPLHGDPNQIWLPLF

Figure S4. Peptide markers from pepsin digestion at pH 1–2.

IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVGLPINQRFILVELSN
 HAELSVTLALDVTNAYVVG YRAGNSAYFFHPDNQEDAEAITHLFTDVQNRYTFAFGGNY
 DRLEQLAGNLRENIELGNGPLEE AISALYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEG
 EMRTRIRYNRRSAPDPSVITLENSWGRLSTAQESNQGAFASPIQLQRRNGSKFSVYDVSILI
 PIIALMVYRCAPPSSQF
 ADVCMDEPIVIRIVGRNGLCVDVRDGRFHNGNAIQLWPCKSNTDANQLWTLKRDNTIRS
 NGKCLTTYGYSPGVYVMYDCNTAATDATRWQIWDNGTIINPRSSLVLAATSGNSTTLTV
 QTNIAVSQGWLPNTNTQPFVTTIVGLYGLCLQANSQVWIEDCSSEKAEQQWALYADGS
 IRPQQNRDNCLTSDSNIRETVVKILSCGPASSGQRWMFKNDGTILNLYSGLVDVRASDPS
 LKQIILYPLHGDPNQIWLPLF

Figure S5. Peptide markers from proteinase K digestion.

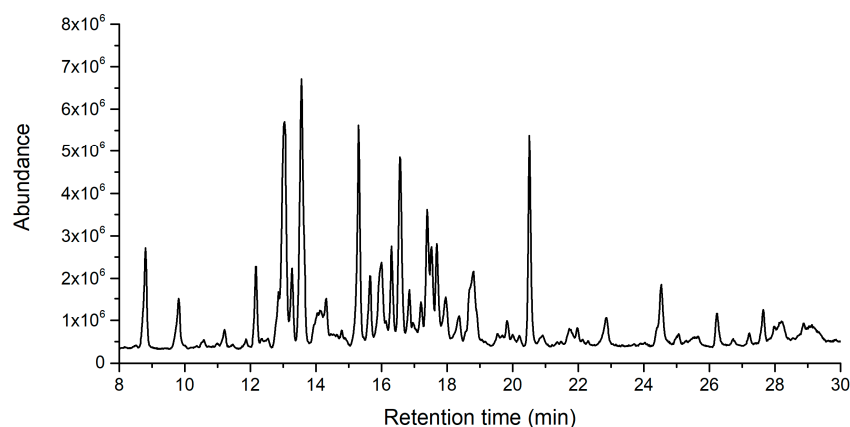


Figure S6. Total ion chromatography of LC-high resolution accurate mass analysis of the trypsin combined with Glu-C digest of 0.1 mg/mL of purified ricin.

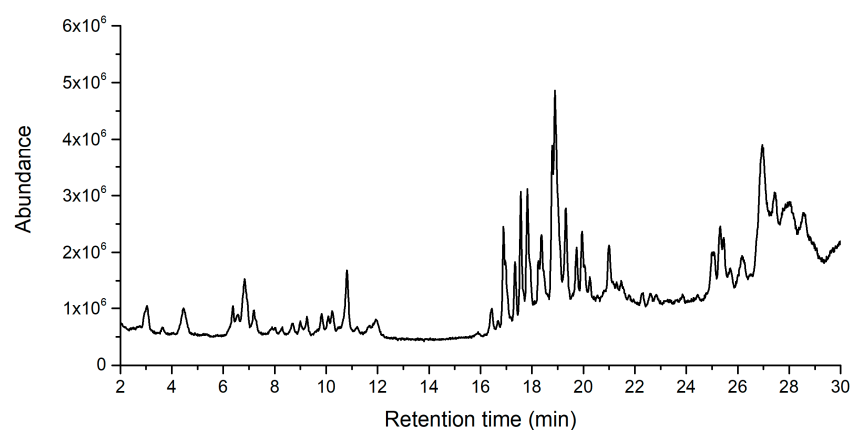


Figure S7. Total ion chromatography of LC-high resolution accurate mass analysis of the chymotrypsin digest of 0.1 mg/mL of purified ricin under denaturation and reduction.

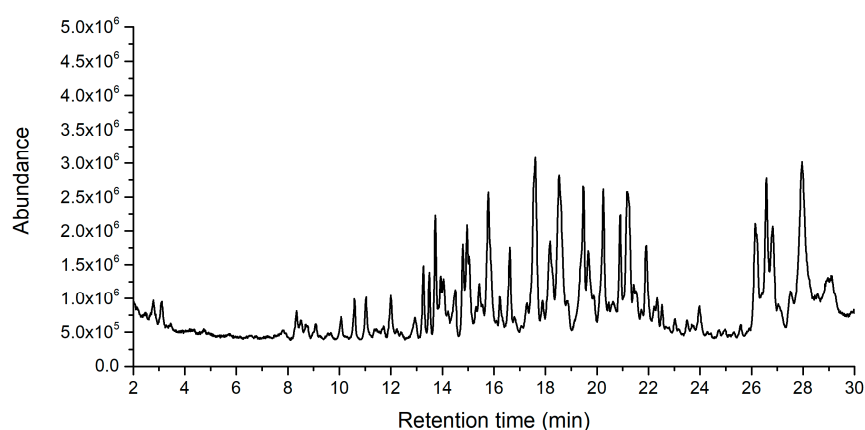


Figure S8. Total ion chromatography of LC-high resolution accurate mass analysis of the direct chymotrypsin digestion of 0.1 mg/mL of purified ricin.

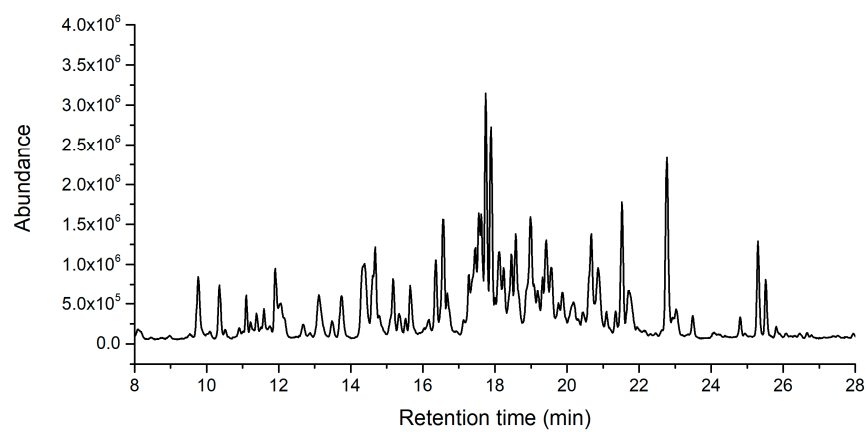


Figure S9. Total ion chromatography of LC-high resolution accurate mass analysis of the pepsin digestion of 0.1 mg/mL of purified ricin (pH 1~2).