

Supplementary data for the article:

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Supplementary Data

Article title: Digestion by pepsin releases biologically active chromopeptides from C-phycocyanin, a blue-colored biliprotein of microalga *Spirulina*

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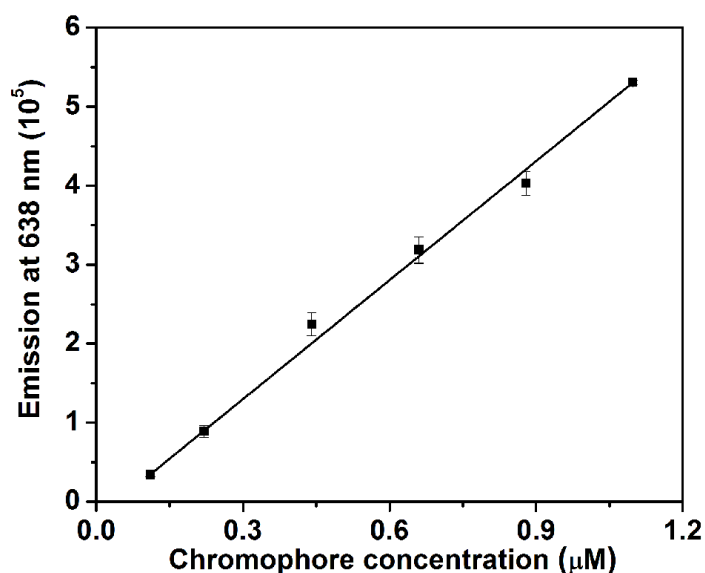


Figure S0. Standard curve for determination of chromopeptides concentration (excitation wavelength was 578 nm). Values are shown as means \pm standard deviations.

Table S1A. MS spectrum of fraction I, after 24h digestion. PCB is bound to cysteine residue of peptide.

*Glutamine residue deamidation (0.98476).

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
505.9951	4	2019.9491	2018.9632	0.9859*	AADQRGKDKCARD-PCB
674.3265	3	2019.9734	2018.9632	1.0102*	AADQRGKDKCARD-PCB
404.9977	5	2019.9494	2018.9632	0.9861*	AADQRGKDKCARD-PCB
587.2895	1	586.2817	586.2791	0.0026	PCB

Table S1B. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 506.00 (z=4) from MS spectrum of fraction I. *Glutamine residue deamidation (0.98476).

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
478.5677	3	1432.6796	1432.6841	-0.0045	AADQRGKDKCARD
650.6492	3	1948.9241	1947.9256	0.9985*	ADQRGKDKCARD-PCB
626.9698	3	1877.88591	1876.8885	0.9974*	DQRGKDKCARD-PCB
519.5899	3	1555.74621	1555.7856	-0.0394	AADQRGKDKC-PCB
587.2854	1	586.2776	586.279136	-0.0016	PCB
646.3108	2	1290.6216	1290.5990	0.0226	DQRGKDKCARD

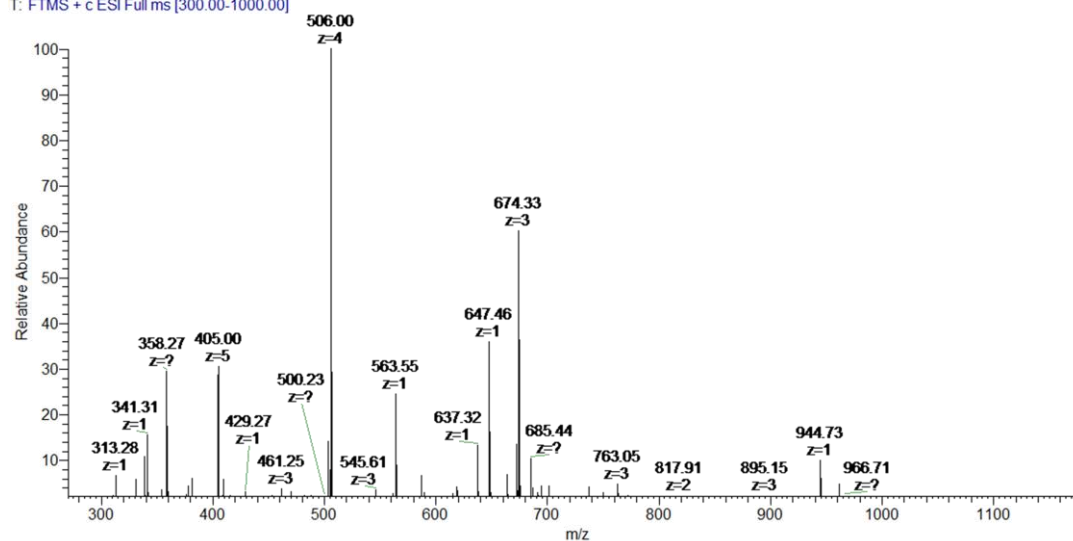
Table S1C. Fragmentation (MS3 spectrum) of ion with m/z ratio 478.57 (z=3) from MS2 spectrum of ion with m/z ratio 506.00 (z=4), representing AADQRGKDKCARD sequence without PCB (MS2 506.00, MS3 478.57).

m/z	z	experimental mass	theoretical mass	Δ mass	ion
143.081	1	142.0732	142.0737	-0.0005	B2 1+
258.1076	1	257.0998	257.1006	-0.0008	B3 1+
386.1915	1	385.1837	385.1592	0.0245	B4 1+
542.2661	1	541.2583	541.2603	-0.0020	B5 1+
599.2872	1	598.2794	598.2818	-0.0024	B6 1+
727.382	1	726.3742	726.3768	-0.0026	B7 1+
842.4087	1	841.4009	841.4037	-0.0028	B8 1+
364.1946	2	726.3735	726.3761	-0.0026	B7 2+
421.7078	2	841.3999	841.4031	-0.0032	B8 2+
485.7549	2	969.4941	969.4981	-0.0040	B9 2+
537.2594	2	1072.503	1072.507	-0.0042	B10 2+
434.2212	3	1299.6401	1299.6449	-0.0048	B12 3+
707.3116	1	706.3038	706.3063	-0.0025	Y6 1+
592.2849	1	591.2771	591.2794	-0.0023	Y5 1+
464.1905	1	463.1827	463.1844	-0.0017	Y4 1+
361.1817	1	360.1739	360.1752	-0.0013	Y3 1+
681.8283	2	1361.641	1361.646	-0.0050	Y12 2+
646.3099	2	1290.604	1290.609	-0.0046	Y11 2+
588.7965	2	1175.577	1175.582	-0.0044	Y10 2+
524.7675	2	1047.519	1047.523	-0.0040	Y9 2+
446.7172	2	891.4187	891.4221	-0.0034	Y8 2+
418.2066	2	834.3975	834.4007	-0.0032	Y7 2+
354.1594	2	706.3031	706.3057	-0.0026	Y6 2+
296.6461	2	591.2765	591.2787	-0.0022	Y5 2+
454.8878	3	1361.64	1361.645	-0.0054	Y12 3+
431.2090	3	1290.604	1290.608	-0.0048	Y11 3+
392.8667	3	1175.577	1175.581	-0.0048	Y10 3+

Table S1D. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 506.00 (z=4) (MS2 506.00, MS3 587.29).

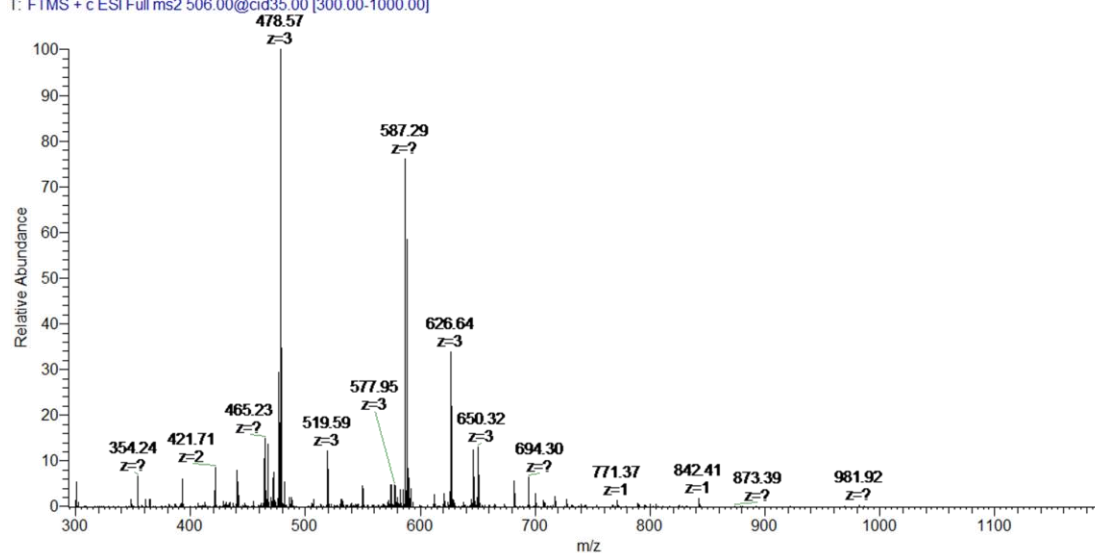
m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2160	1	464.2160	464.2185	-0.0025	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.1379	1	299.1379	299.1396	-0.0017	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)
271.1435	1	271.1435	271.1447	0.0012	C ₁₆ H ₁₉ N ₂ O ₂ without CO group

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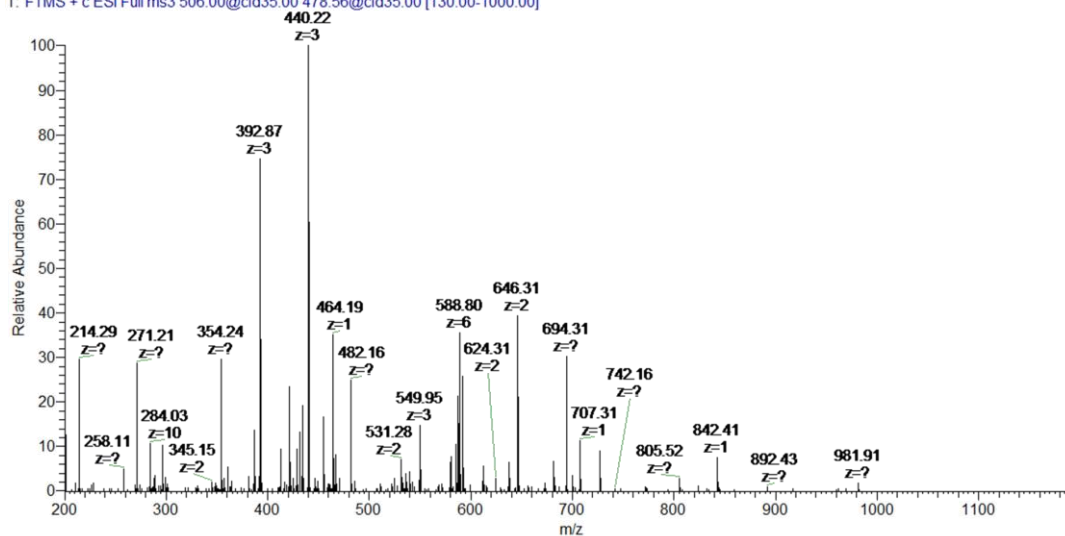
A

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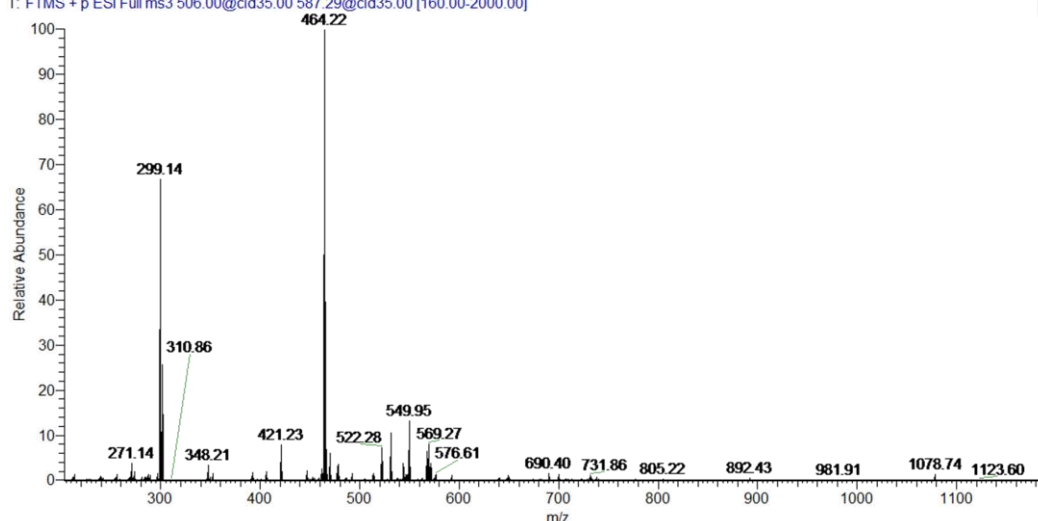
B

OB3465 #282 RT: 3.98 AV: 1 NL: 1.18E6
T: FTMS + c ESI Full ms3 506.00@cid35.00 478.56@cid35.00 [130.00-1000.00]



C

OB03437 #1.67 RT: 0.01-0.81 AV: 67 NL: 7.95E6
T: FTMS + p ESI Full ms3 506.00@cid35.00 587.29@cid35.00 [160.00-2000.00]



D

Figure S1. (A) MS spectrum of fraction I, after 24 h digestion. Peaks with m/z ratios 405.00, 506.00 and 474.33 arise from AADQRGKDKCARD chromopeptide. (B) MS2 spectrum of molecular ion with m/z ratio 506.00 ($z=4$) from MS spectrum of fraction I. (C) Fragmentation (MS3 spectrum) of ion with m/z ratio 478.57 ($z=3$) from MS2 spectrum of ion with m/z ratio 506.00 ($z=4$), representing AADQRGKDKCARD sequence without PCB. (D) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 ($z=1$) from MS2 spectrum of ion with m/z ratio 506.00 ($z=4$), representing PCB.

Table S2A. MS spectrum of fraction II, after 24h digestion. PCB is bound to cysteine residue of peptide.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
412.2027	3	1233.5846	1233.5852	-0.0006	AACLR D-PCB
617.7999	2	1233.5841	1233.5852	-0.0011	AACLR D-PCB
546.7628	2	1091.5099	1091.511036	-0.0011	CLR D-PCB
587.2869	1	586.2791	586.2791	0.0000	PCB

Table S2B. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 617.80 (z=2) from MS spectrum of fraction II, representing AACLRD-PCB.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
648.3123	1	647.30447	647.3061	-0.0016	AACLRD
587.2855	1	586.2778	586.2791	-0.0015	PCB
464.2175	1	463.2175	464.2185	-0.0010	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.13	1	298.1387	299.1396	-0.0009	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)

Table S2C. Fragmentation (MS3 spectrum) of ion with m/z ratio 648.31 (z=1) from MS2 spectrum of ion with m/z ratio 617.80 (z=2), representing AACLRD sequence without PCB (MS2 617.80, MS3 648.31).

m/z	z	experimental mass	theoretical mass	Δ mass	ion
246.0896	1	245.0818	245.0829	-0.0011	b3 1+
359.1733	1	358.1655	358.167	-0.0015	b4 1+
515.2737	1	514.2659	514.2681	-0.0022	b5 1+
577.2739	1	576.2661	576.2685	-0.0024	y5 1+
506.2372	1	505.2294	505.2313	-0.0019	y4 1+
403.2283	1	402.2205	402.2222	-0.0017	y3 1+
290.1447	1	289.1369	289.1381	-0.0012	y2 1+

Table S2D. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 617.80 (z=2), which represents PCB (MS2 617.80, MS3 587.29).

m/z	z	experimental mass	theoretical mass	Delta mass	Structure
464.2162	1	464.2162	464.2185	-0.0023	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.1381	1	299.1381	299.1396	-0.0015	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)
271.1433	1	271.1433	271.1447	-0.0014	C ₁₆ H ₁₉ N ₂ O ₂ without CO group

Table S2E. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 546.76 (z=2) from MS spectrum of fraction II, representing CLRD-PCB.

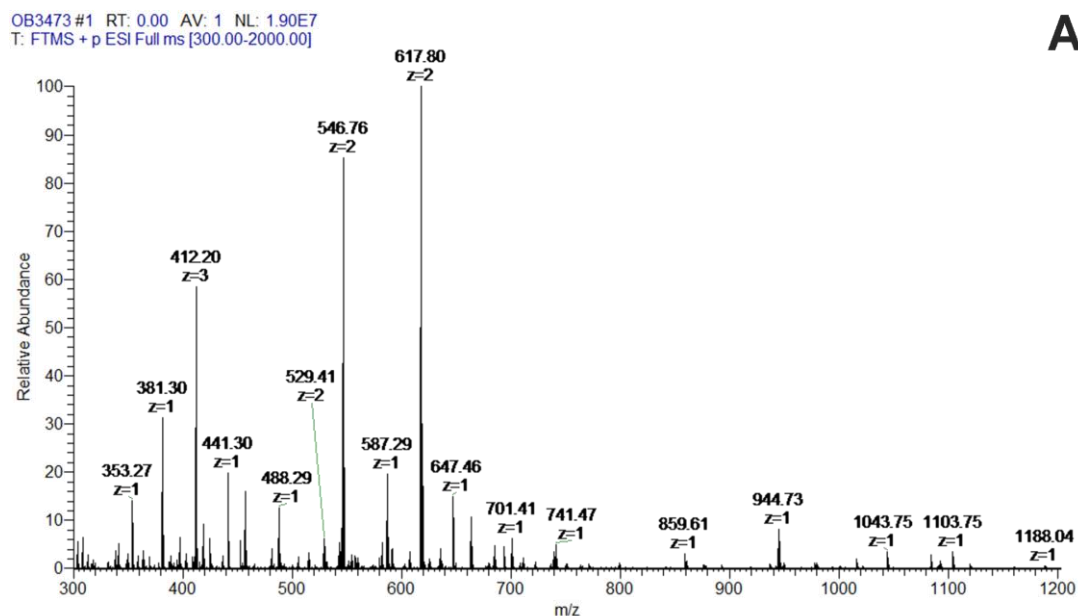
m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
506.2383	1	505.2305	505.2319	-0.0014	CLRD
587.2853	1	586.2775	586.2791	-0.0017	PCB

Table S2F. Fragmentation (MS3 spectrum) of ion with m/z ratio 506.23 (z=1) from MS2 spectrum of ion with m/z ratio 546.76 (z=2), representing CLRD sequence without PCB (MS2 546.76, MS3 506.23).

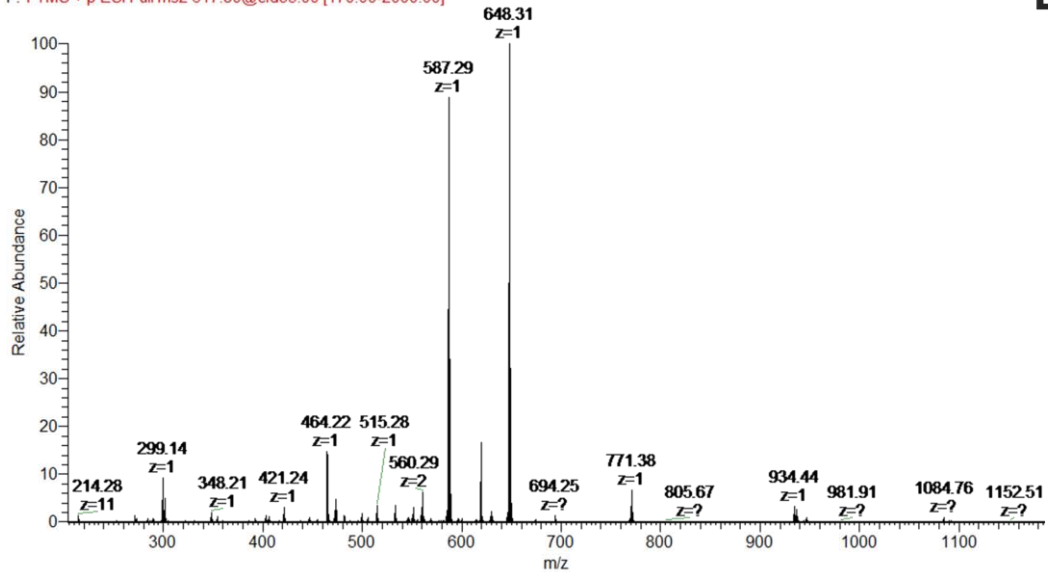
m/z	z	experimental mass	theoretical mass	Δ	ion
217.0997	1	216.0919	216.0927	-0.0008	b2 1+
373.2001	1	372.1923	372.1938	-0.0015	b3 1+
403.2283	1	402.2205	402.2222	-0.0017	y3 1+
290.1448	1	289.137	289.1381	-0.0011	Y2 1+

Table S2G. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 546.76 (z=2), which represents PCB (MS2 546.76, MS3 587.29).

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2159	1	464.2159	464.2185	-0.0026	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.1379	1	299.1379	299.1396	-0.0017	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)
271.1432	1	271.1432	271.1447	-0.0015	C ₁₆ H ₁₉ N ₂ O ₂ without CO group

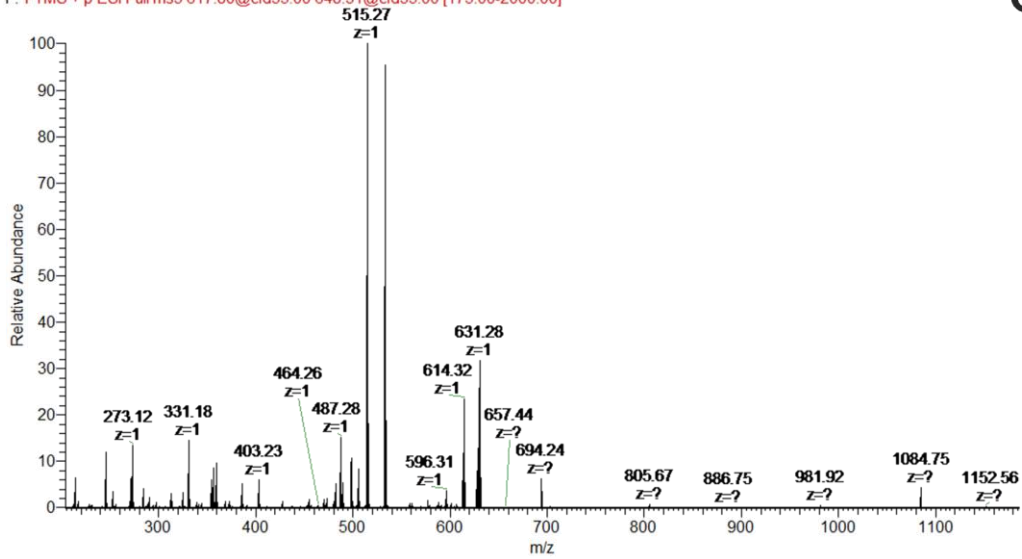


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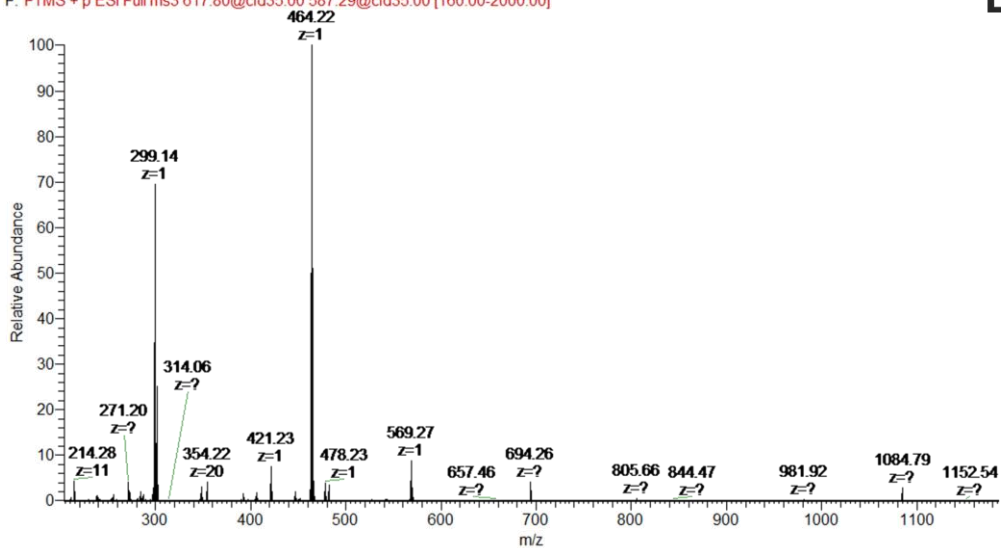
B

OB3474 #489-700 RT: 9.97-10.55 AV: 32 NL: 1.62E6
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C

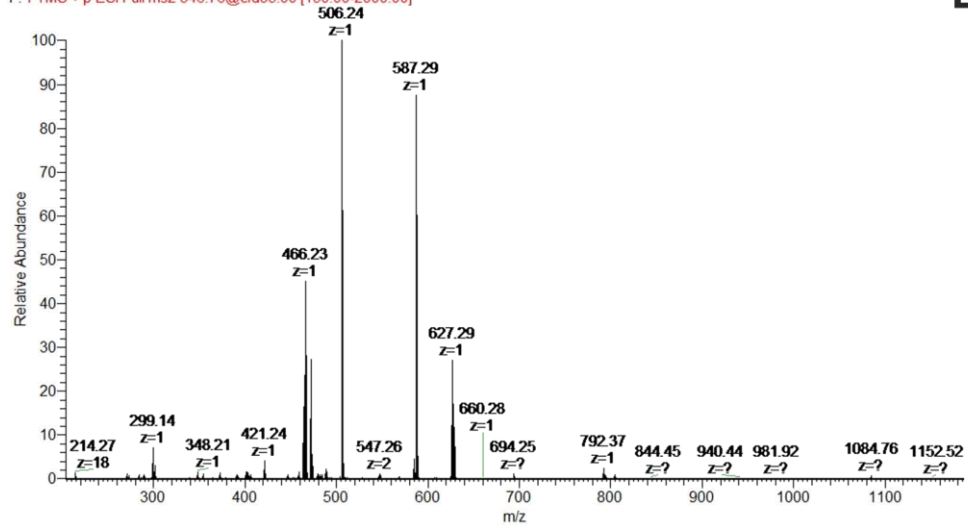
OB3474 #518-700 RT: 9.46-9.94 AV: 27 NL: 3.31E6
F: FTMS + p ESI Full ms3 617.80@cid35.00 587.29@cid35.00 [160.00-2000.00]



D

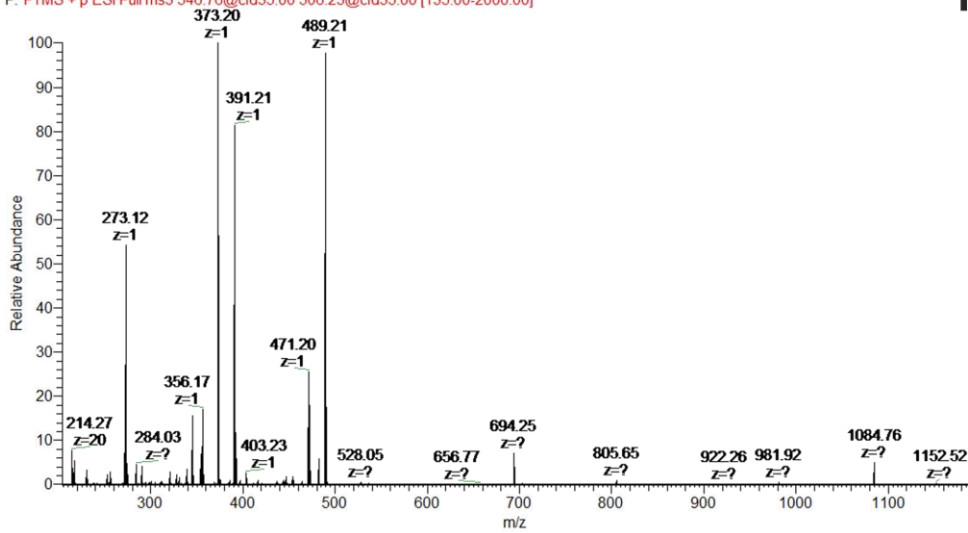
OB3474 #206-680 RT: 4.79-6.06 AV: 76 NL: 6.97E6
F: FTMS + p ESI Full ms2 546.76@cid35.00 [150.00-2000.00]

E



OB3474 #334-700 RT: 6.08-7.78 AV: 92 NL: 1.46E6
F: FTMS + p ESI Full ms3 546.76@cid35.00 506.23@cid35.00 [135.00-2000.00]

F



OB3474 #526-700 RT: 9.46-9.94 AV: 27 NL: 3.31E6
F: FTMS + p ESI Full ms3 617.80@cid35.00 587.29@cid35.00 [160.00-2000.00]

G

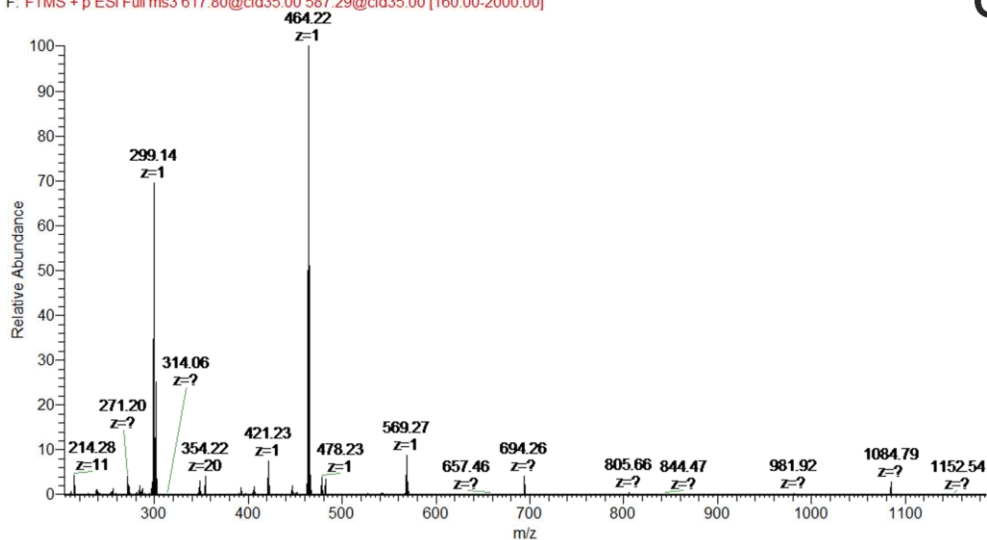


Figure S2. (A) MS spectrum of fraction II, after 24 h digestion. Peaks with m/z ratios 412.2 ($z=3$) and 617.80 ($z=2$) arise from AACLRD chromopeptide, while peak with m/z ratio 546.76 ($z=2$) represents CLRD chromopeptide (B) MS2 spectrum of molecular ion with m/z ratio 617.80 ($z=2$) from MS spectrum of fraction II. (C) Fragmentation (MS3 spectrum) of ion with m/z ratio 648.31 ($z=1$) from MS 2 spectrum of ion with m/z ratio 617.80 ($z=2$), representing AACLRD sequence without PCB. (D) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 ($z=1$) from MS 2 spectrum of ion with m/z ratio 617.80 ($z=2$), representing PCB. (E) MS2 spectrum of molecular ion with m/z ratio 546.76 ($z=2$) from MS spectrum of fraction II. (F) Fragmentation (MS3 spectrum) of ion with m/z ratio 506.23 ($z=1$) from MS 2 spectrum of ion with m/z ratio 546.76 ($z=2$), representing CLRD sequence without PCB. (G) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 ($z=1$) from MS2 spectrum of ion with m/z ratio 546.76 ($z=2$), representing PCB.

Table S3A. MS spectrum of fraction III after 24h digestion. PCB is bound to cysteine residue of peptide.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
979.4609	1	978.4531	978.4521	0.0009	CSAL-PCB
490.2352	2	978.4517	978.4521	-0.0004	CSAL-PCB
587.2866	1	586.2788	586.2791	-0.0004	PCB

Table S3B. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 490.23 ($z=2$) from MS spectrum of fraction III.

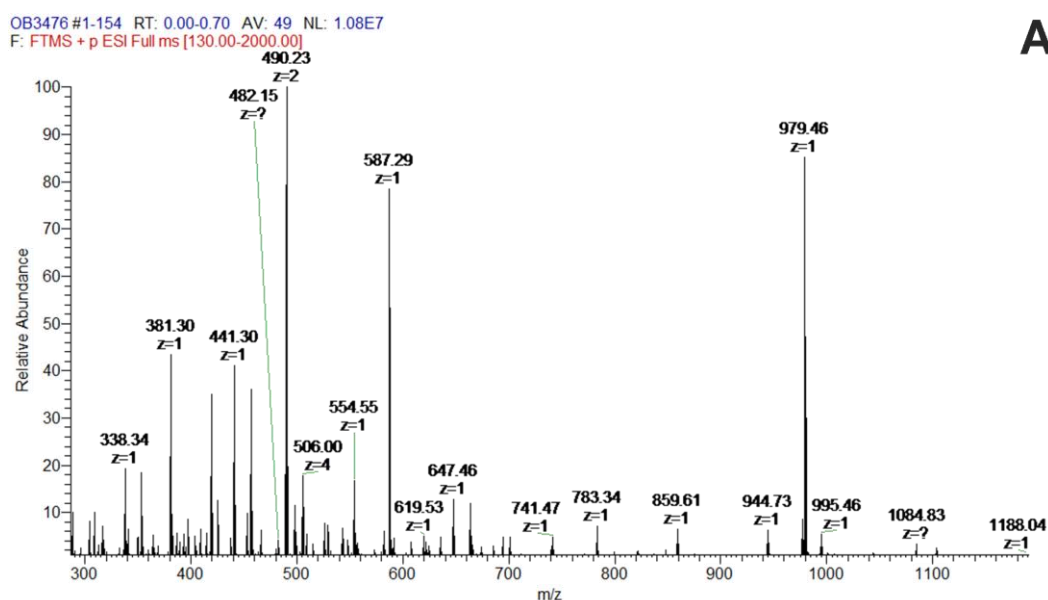
m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
393.1797	1	392.1719	392.1730	-0.0011	CSAL
375.1693	1	374.1615	374.1624	-0.0010	CSAL dehydrated
848.3638	1	847.3560	847.3569	-0.0009	CSA-PCB
244.0747	1	243.0669	243.0672	-0.0003	CSA dehydrated
777.3270	1	776.3192	776.3198	-0.0006	CS-PCB
587.2853	1	586.2775	586.2791	-0.0017	PCB

Table S3C. Fragmentation (MS3 spectrum) of ion with m/z ratio 393.18 (z=1) from MS2 spectrum of ion with m/z ratio 490.23 (z=2). This ion represents CSAL sequence without PCB (MS2 490.23 MS3 393.18).

m/z	z	experimental mass	theoretical mass	Δ mass	ion
191.0478	1	190.04	190.0407	-0.0007	b2 1+
262.0848	1	261.077	261.0778	-0.0008	b3 1+
290.1701	1	289.1623	289.1632	-0.0009	y13 1+
203.1384	1	202.1306	202.1312	-0.0006	y2 1+
132.1014	1	131.0936	131.0941	-0.0005	y1 1+

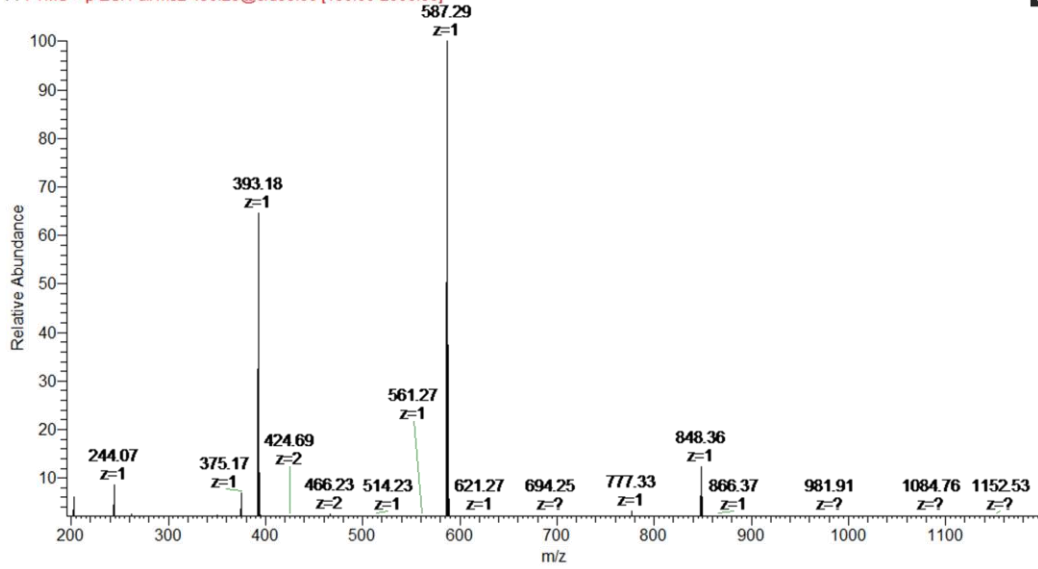
Table S3D. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 490.23 (z=2). This ion represents PCB (MS2 490.23, MS3 587.29).

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2163	1	464.2163	464.2185	-0.0022	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.1381	1	299.1381	299.1396	-0.0015	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)
271.1433	1	271.1433	271.1447	-0.0014	C ₁₆ H ₁₉ N ₂ O ₂ without CO group



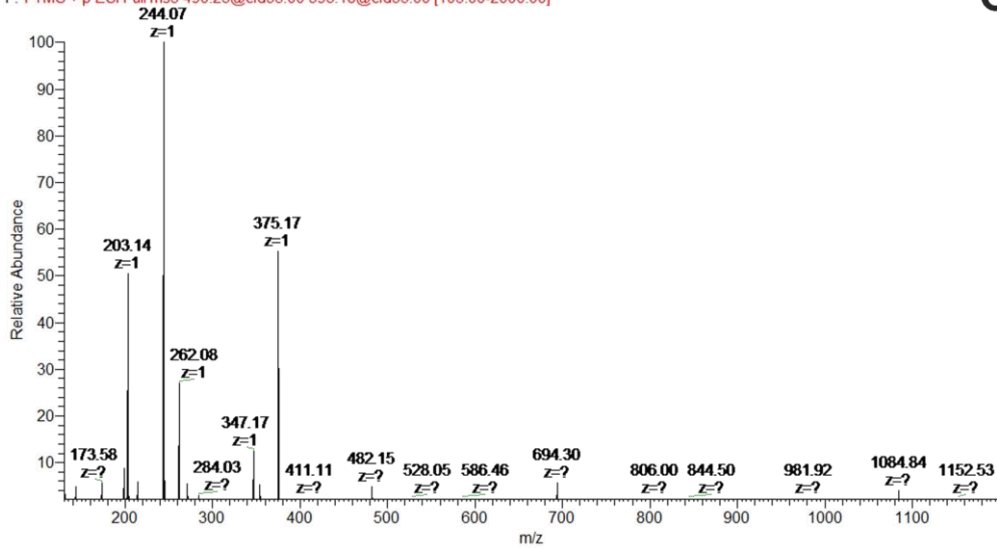
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B



OB3476 #55-271 RT: 1.26-2.32 AV: 57 NL: 2.06E6
F: FTMS + p ESI Full ms3 490.23@cid35.00 393.18@cid35.00 [105.00-2000.00]

C



OB3476 #85-459 RT: 2.34-2.89 AV: 30 NL: 3.27E6
F: FTMS + p ESI Full ms3 490.23@cid35.00 587.29@cid35.00 [160.00-2000.00]

D

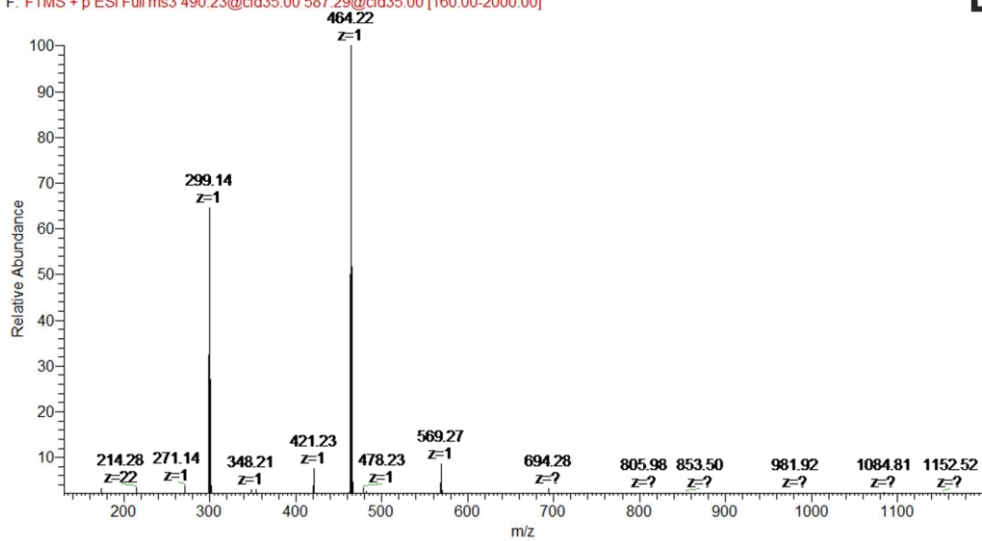


Figure S3. (A) MS spectrum of fraction III, after 24 h digestion. Peak with m/z ratios 490.23 (z=2) and 979.46 (z=1) arise from CSAL chromopeptide; (B) MS2 spectrum of molecular ion with m/z ratio 490.23 (z=2) from MS spectrum of fraction IV; (C) Fragmentation (MS3 spectrum) of ion with m/z ratio 393.18 (z=1) from MS2 spectrum of 490.23 (z=2), representing CSAL sequence, without PCB; (D) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of 490.23 (z=2), representing PCB.

Table S4A. MS spectrum of fraction IV, after 24h digestion. PCB is bound to cysteine residue of peptide.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
482.2383	2	962.4609	962.4571	0.0038	AACL-PCB
963.4694	1	962.4616	962.4571	0.0044	AACL-PCB
497.2428	2	992.4699	992.4677	0.0022	ATCL-PCB
993.48	1	992.4722	992.4677	0.0044	ATCL-PCB
547.7669	2	1093.5181	1093.5154	0.0027	TATCL-PCB
1094.5282	1	1093.5204	1093.5154	0.0049	TATCL-PCB
587.2886	1	586.28077	586.2791	0.0016	PCB

Table S4B. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 482.24 (z=2) from MS spectrum of fraction IV.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
377.1845	1	376.1767	376.178	-0.0013	AACL
892.4259	1	891.4181	891.4195	-0.0014	ACL-PCB
416.6876	2	831.3595	831.3620	0.0025	AAC-PCB
832.3681	1	831.3603	831.3620	-0.0017	AAC-PCB
821.3919	1	820.3809	820.3824	-0.0015	CL-PCB
235.1106	1	234.1028	234.1033	-0.0005	CL
587.2850	1	586.2772	586.2791	-0.0020	PCB

Figure S4C. Fragmentation (MS3 spectrum) of ion with m/z ratio 377.18 (z=1) from MS2 spectrum of ion with m/z ratio 482.24 (z=2), representing AAACL sequence without PCB (MS2 482.24, MS3 377.18).

m/z	z	experimental mass	theoretical mass	Δ mass	ion
246.0898	1	245.082	245.0829	-0.0009	b3 1+
235.1103	1	234.1025	234.1033	-0.0008	y2 1+

Table S4D. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 482.24 (z=2), which represents PCB (MS2 482.24, MS3 587.29).

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2160	1	464.2160	464.2185	-0.0025	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.1380	1	299.1380	299.1396	-0.0016	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)
271.2304	1	271.2304	271.1447	0.0857	C ₁₆ H ₁₉ N ₂ O ₂ without CO group

Table S4E. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 497.24 (z=2) from MS spectrum of fraction IV.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
407.1951	1	406.1873	406.1886	-0.0013	ATCL
488.2347	2	974.4537	974.4572	-0.0034	ATCL-PCB dehydrated
862.3792	1	861.3714	861.3726	-0.0012	ATC-PCB
821.3889	1	820.3811	820.3824	-0.0013	CL-PCB
235.1106	1	234.1028	234.1033	-0.0005	CL
587.2852	1	586.2774	586.2791	-0.0018	PCB
461.7217	2	921.4434	921.4301	0.0133	TCL-PCB

Table S4F. Fragmentation (MS3 spectrum) of ion with m/z ratio 407.20 (z=1) from MS2 spectrum of ion with m/z ratio 497.24 (z=2), representing ATCL sequence without PCB (MS2 497.24, MS3 407.20).

m/z	z	experimental mass	theoretical mass	Δ mass	ion
173.0915	1	172.0837	172.0843	-0.0006	b2 1+
235.1104	1	234.1026	234.1033	-0.0007	y2 1+

Table S4G. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 497.24 (z=2), which represents PCB (MS2 497.24, MS3 587.29).

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2163	1	464.2163	464.2185	-0.0022	C ₂₆ H ₃₀ N ₃ O ₅ (PCB with one pyrrole less)
299.1381	1	299.1381	299.1396	-0.0015	C ₁₇ H ₁₉ N ₂ O ₃ (PCB half)
271.1826	1	271.1826	271.1447	0.0379	C ₁₆ H ₁₉ N ₂ O ₂ without CO group

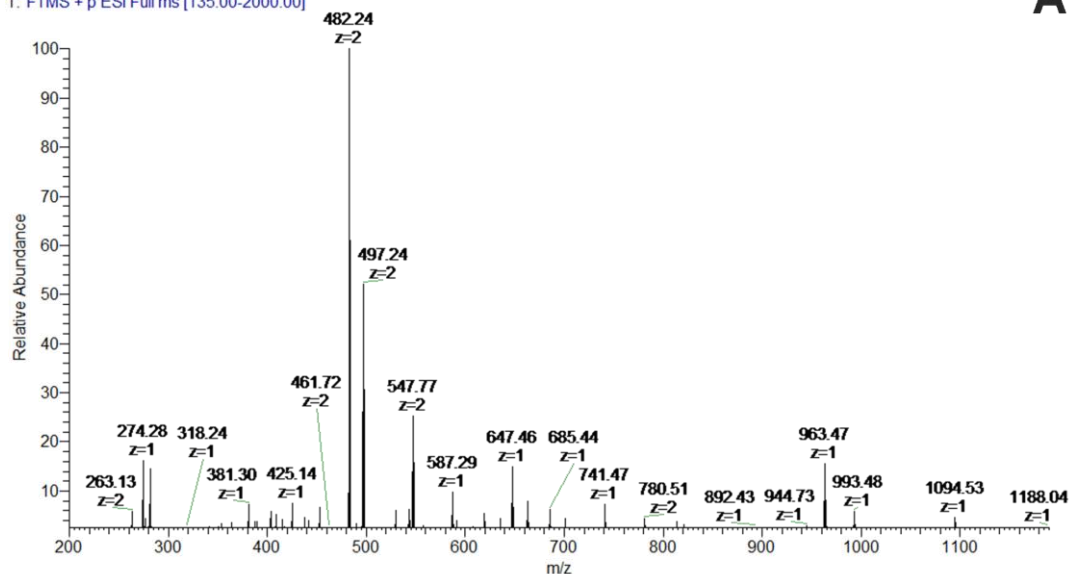
Table S4H. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 547.77 (z=2) from MS spectrum of fraction IV.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
538.7582	2	1075.5007	1075.5049	-0.0041	TATCL-PCB dehydrated
490.2319	1	489.2241	489.2257	-0.0017	TATCL dehydrated
922.436	1	921.4282	921.4301	-0.0019	TCL-PCB
452.7163	2	903.4169	903.4195	-0.0026	TCL-PCB dehydrated
821.3886	1	820.3808	820.3824	-0.0016	CL-PCB
587.2850	1	586.2772	586.2791	-0.0020	PCB
461.7215	2	921.4430	921.4301	0.0129	TCL-PCB
497.2399	2	992.4798	992.4677	0.0121	ATCL-PCB
508.2322	1	507.2322	507.2250	0.0072	TATCL

Table S4I. Fragmentation (MS3 spectrum) of ion with m/z ratio 508.23 (z=1) from MS2 spectrum of ion with m/z ratio 547.77 (z=2), representing TATCL sequence without PCB. Data were obtained after recording MS3 spectrum of this ion (MS2 547.76, MS3 508.23).

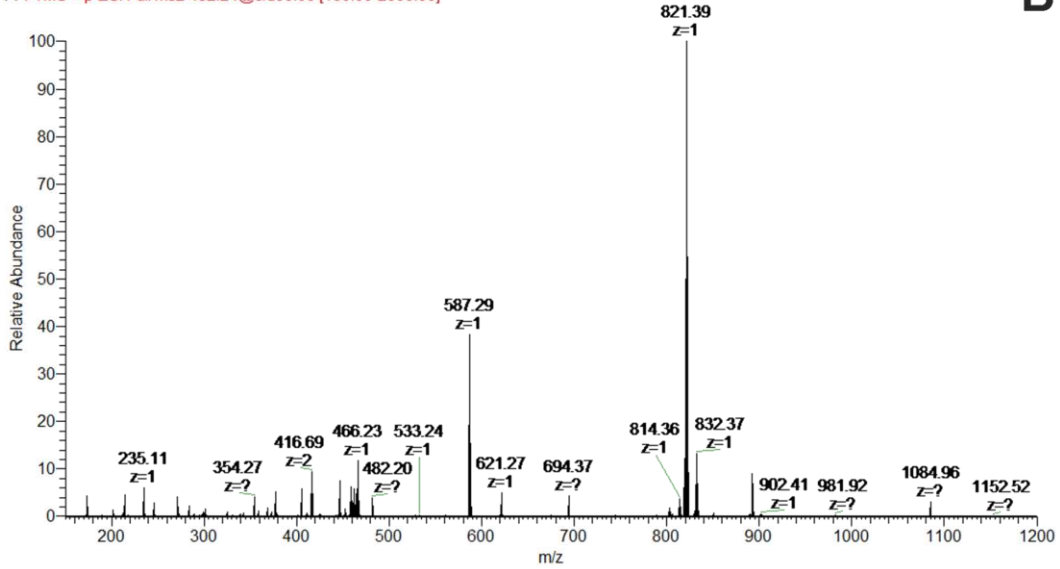
m/z	z	experimental mass	theoretical mass	delta	ion
173.113	1	172.1052	172.0843	0.0209	b2 1+
274.1396	1	273.1318	273.1319	-0.0001	b3 1+
377.1479	1	376.1401	376.1411	-0.001	b4 1+
336.1578	1	335.1500	335.151	-0.001	y3 1+

OB03438 #1 RT: 0.00 AV: 1 NL: 5.22E7
T: FTMS + p ESI Full ms [135.00-2000.00]

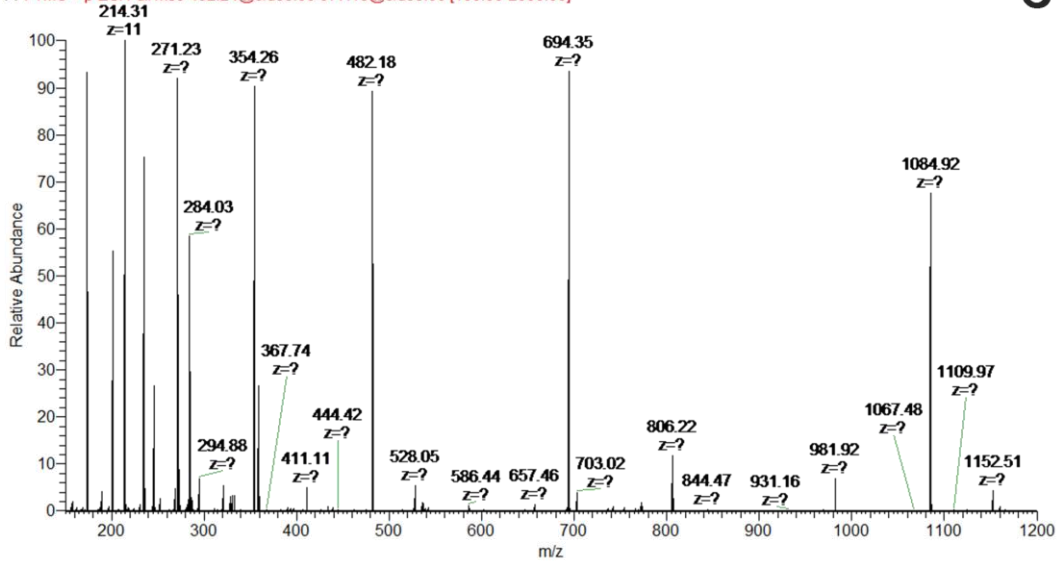


A

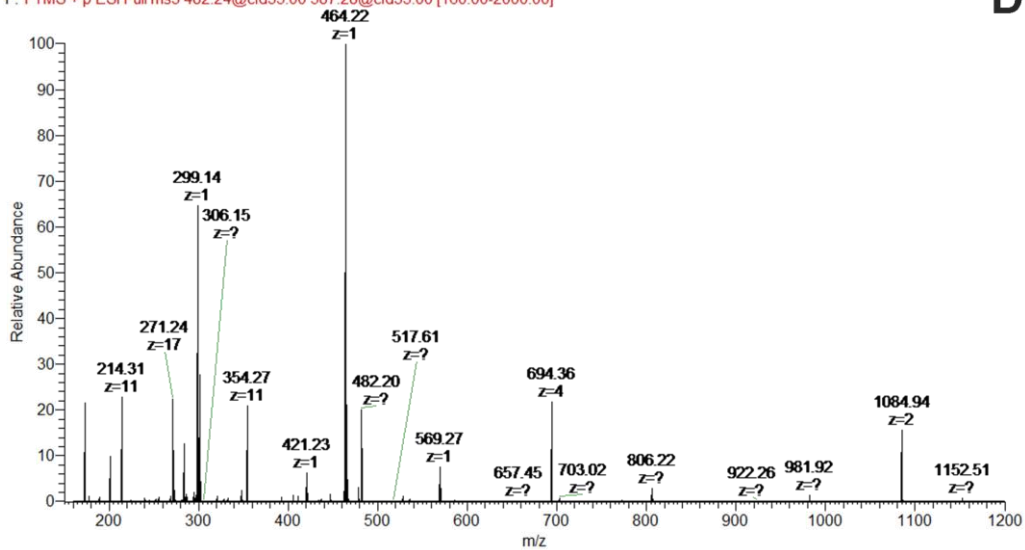
OB3479 #129-368 RT: 2.35-3.15 AV: 48 NL: 2.68E6
F: FTMS + p ESI Full ms2 482.24@cid35.00 [130.00-2000.00]



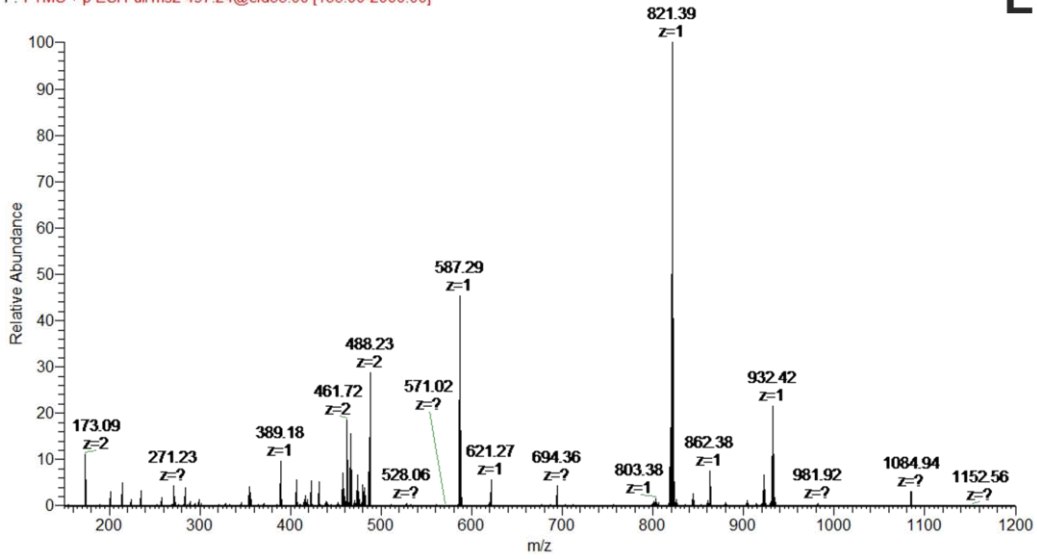
OB3479 #81-428 RT: 3.17-3.58 AV: 23 NL: 1.10E5
F: FTMS + p ESI Full ms3 482.24@cid35.00 377.18@cid35.00 [100.00-2000.00]



OB3479 #159-319 RT: 3.61-4.08 AV: 26 NL: 4.71E5
F: FTMS + p ESI Full ms3 482.24@cid35.00 587.28@cid35.00 [160.00-2000.00]

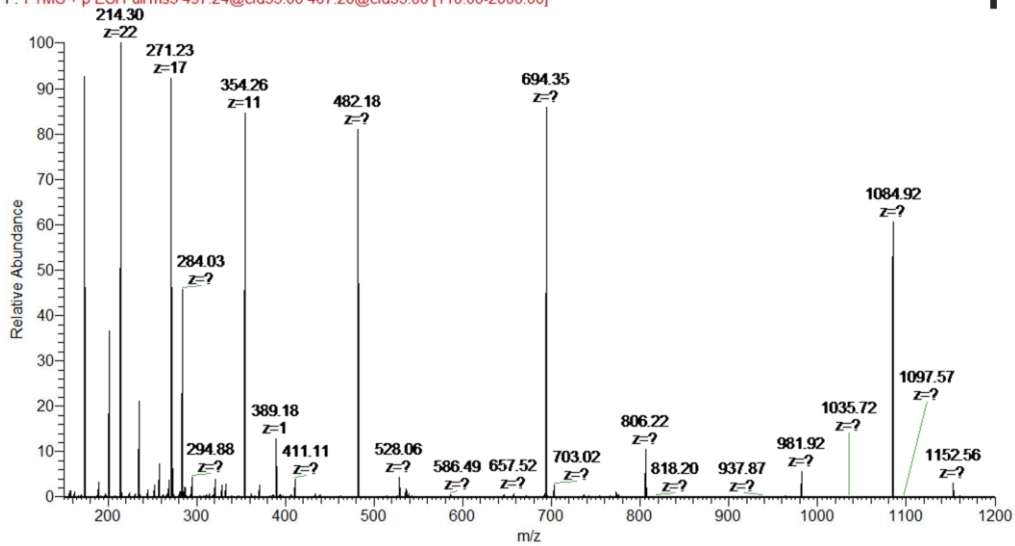


OB3479 #262-392 RT: 4.65-5.79 AV: 68 NL: 1.52E6
F: FTMS + p ESI Full ms2 497.24@cid35.00 [135.00-2000.00]



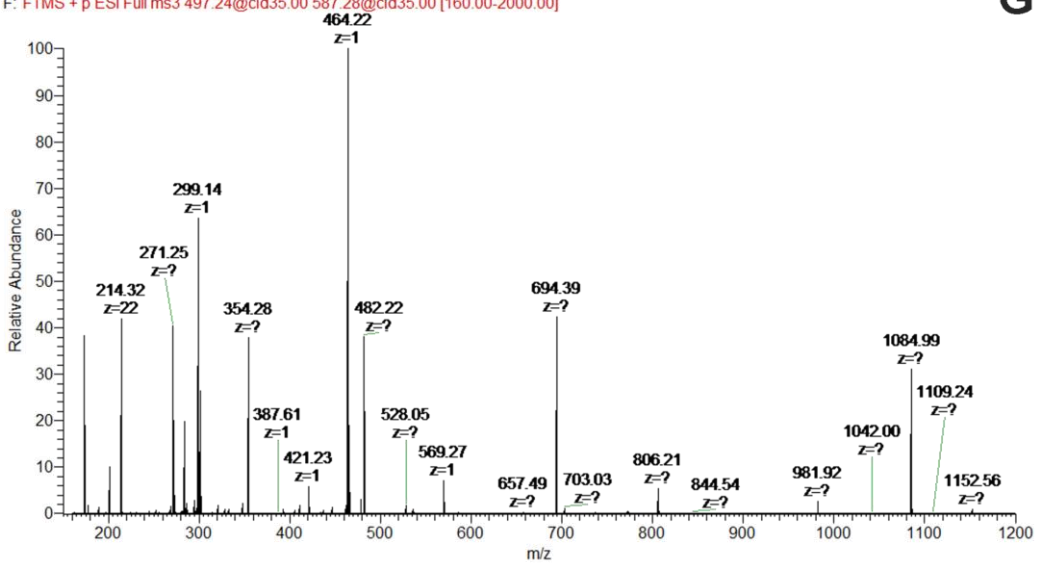
E

OB3479 #305-714 RT: 6.83-7.34 AV: 28 NL: 1.37E5
F: FTMS + p ESI Full ms3 497.24@cid35.00 407.20@cid35.00 [110.00-2000.00]



F

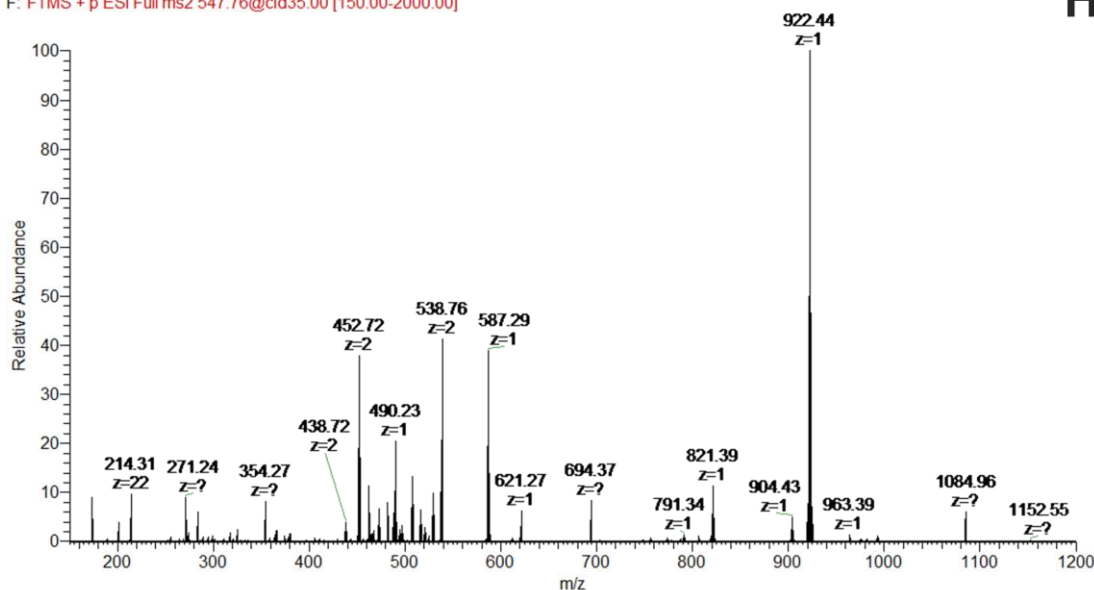
OB3479 #274-451 RT: 5.81-6.30 AV: 27 NL: 3.00E5
F: FTMS + p ESI Full ms3 497.24@cid35.00 587.28@cid35.00 [160.00-2000.00]



G

OB3479 #386-599 RT: 7.36-8.20 AV: 50 NL: 9.97E5
F: FTMS + p ESI Full ms2 547.76@cid35.00 [150.00-2000.00]

H



OB3479 #536-639 RT: 9.74-9.96 AV: 13 NL: 1.39E5
F: FTMS + p ESI Full ms3 547.76@cid35.00 508.23@cid35.00 [135.00-2000.00]

I

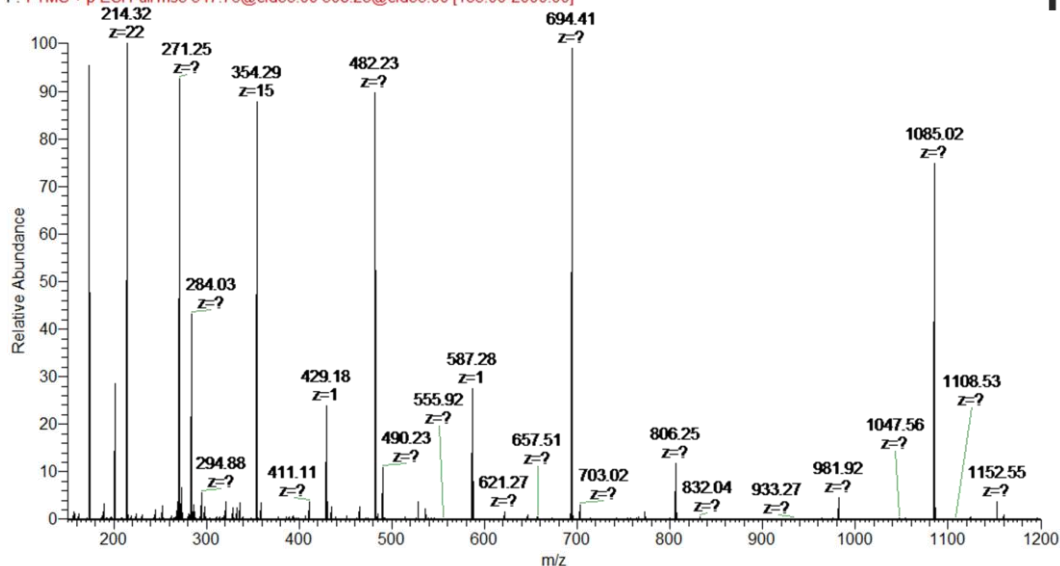


Figure S4. (A) MS spectrum of fraction IV, after 24 h digestion. Peaks with m/z ratios 482.24 ($z=2$), 497.24 ($z=2$) and 547.77 ($z=2$) arise from AACL, ATCL and TATCL chromopeptides, respectively; (B) MS2 spectrum of molecular ion with m/z ratio 482.24 ($z=2$) from MS spectrum of fraction IV; (C) Fragmentation (MS3 spectrum) of ion with m/z ratio 377.18 ($z=1$) from MS2 spectrum of ion with m/z ratio 482.24, representing AACL sequence without PCB; (D) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 ($z=1$) from MS2 spectrum of ion with m/z ratio 482.24, representing PCB; (E) MS2 spectrum of molecular ion with m/z ratio 497.24 ($z=2$) from MS spectrum of fraction IV; (F) Fragmentation (MS3 spectrum) of ion with m/z ratio 407.20 ($z=1$) from MS2 spectrum of ion with m/z ratio 497.24 ($z=2$), representing ATCL sequence without PCB; (G) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 ($z=1$) from MS2 spectrum of ion with m/z ratio 497.24 ($z=2$), representing PCB; (H) MS2 spectrum of molecular ion with m/z ratio 547.76 ($z=2$) from MS spectrum of fraction IV; (I) Fragmentation (MS3 spectrum) of ion with m/z ratio 508.23 ($z=1$) from MS2 spectrum of ion with m/z ratio 547.76 ($z=2$), representing TATCL sequence without PCB.

Table S5A. MS spectrum of fraction V, after 24h digestion. PCB is bound to cysteine residue of peptide.

m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
411.1983	2	820.3809	820.3829	-0.0020	CL-PCB
821.3898	1	820.3820	820.3829	-0.0010	CL-PCB
587.2858	1	586.27797	586.279136	-0.0012	PCB

Table S5B. Fragmentation (MS2 spectrum) of molecular ion with m/z ratio 411.20 (z=2) from MS spectrum of fraction V.

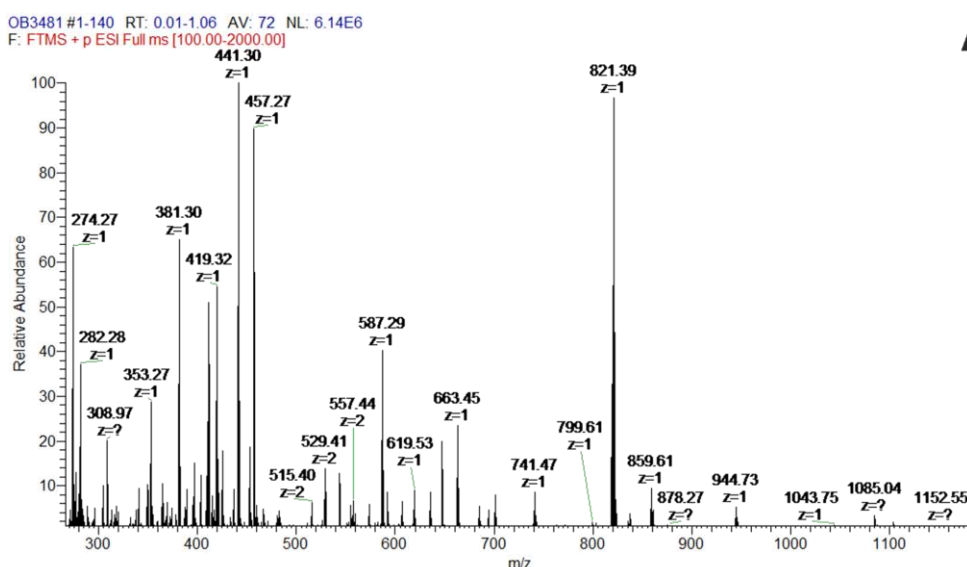
m/z	z	experimental mass	theoretical mass	Δ mass	Sequence
235.1106	1	234.1028	234.1038	-0.0010	CL
587.2849	1	586.2771	586.2791	-0.0021	PCB

Table S5C. Fragmentation (MS3 spectrum) of ion with m/z ratio 235.11 (z=1) from MS2 spectrum of ion with m/z ratio 411.20 (z=2), representing CL sequence without PCB (MS2 411.20, MS3 235.11).

m/z	z	experimental mass	theoretical mass	Δ mass	ion
132.1015	1	131.0937	131.0941	-0.0004	y1 1+

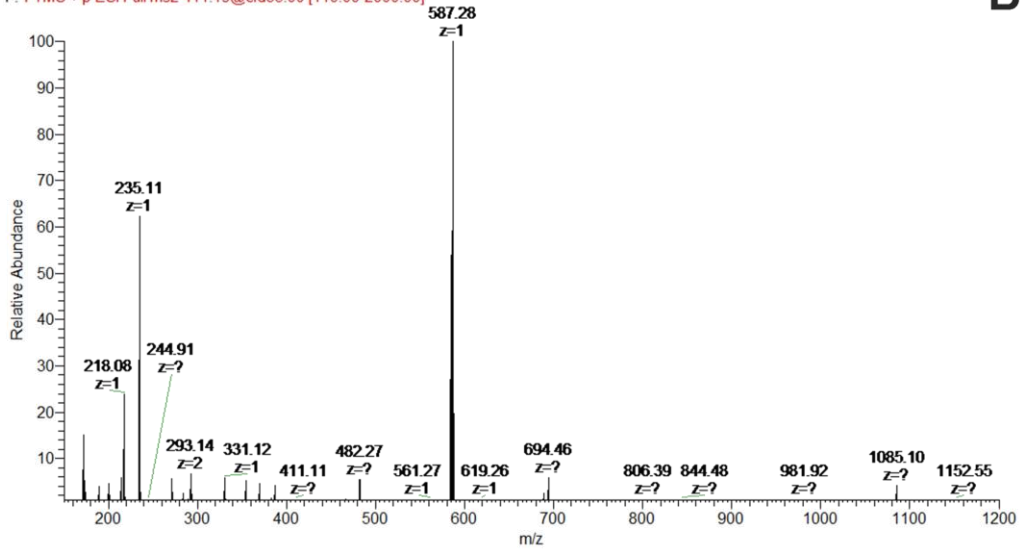
Table S5D. Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1), which represents PCB and was obtained from MS2 spectrum of molecular ion with m/z ratio 411.20 (z=2) (MS2 411.20, MS3 587.29).

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2164	1	464.2164	464.2185	-0.0021	$C_{26}H_{30}N_3O_5$ (PCB with one pyrrole less)
299.1382	1	299.1382	299.1396	-0.0014	$C_{17}H_{19}N_2O_3$ (PCB half)
271.1070	1	271.1070	271.1447	-0.0377	$C_{16}H_{19}N_2O_2$ without CO group



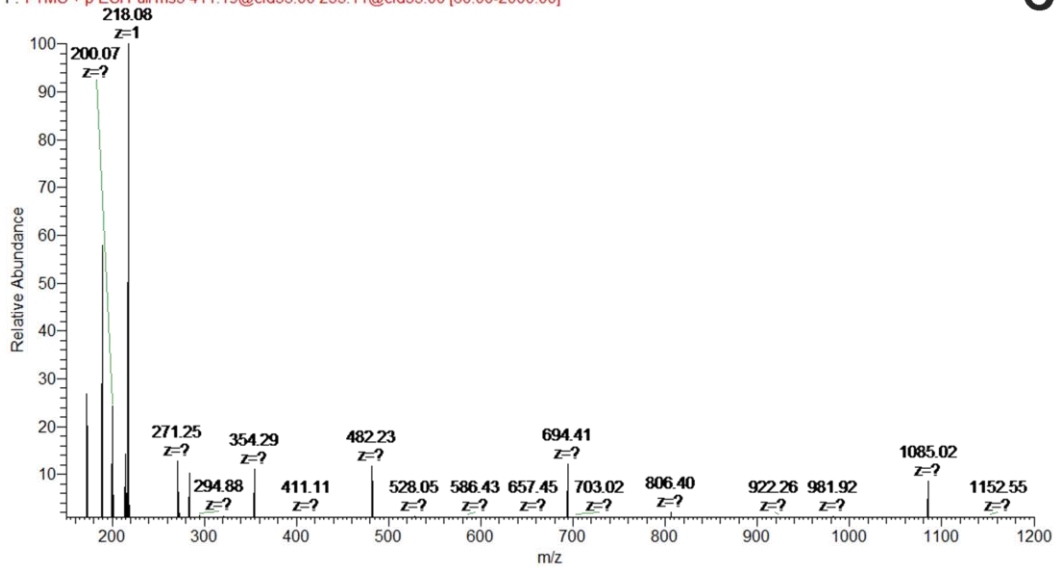
OB3481 #15-257 RT: 1.08-1.53 AV: 28 NL: 2.45E6
F: FTMS + p ESI Full ms2 411.19@cid35.00 [110.00-2000.00]

B



OB3481 #86-198 RT: 1.55-2.08 AV: 29 NL: 5.97E5
F: FTMS + p ESI Full ms3 411.19@cid35.00 235.11@cid35.00 [60.00-2000.00]

C



OB3481 #107-274 RT: 2.11-2.68 AV: 31 NL: 9.37E5
F: FTMS + p ESI Full ms3 411.19@cid35.00 587.29@cid35.00 [160.00-2000.00]

D

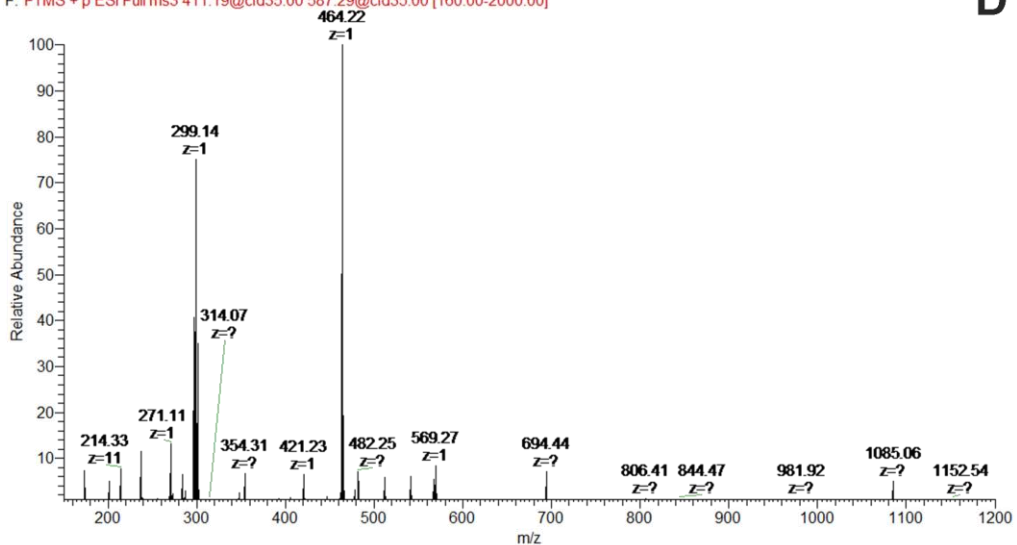


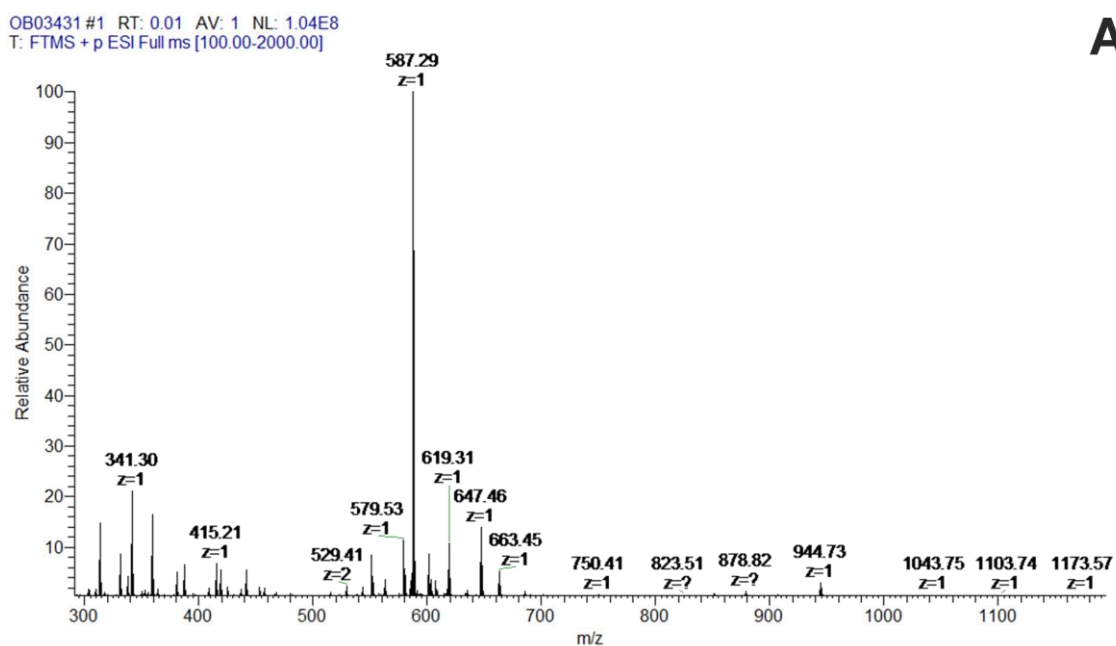
Figure S5. (A) MS spectrum of fraction V, after 24 h digestion. Peaks with m/z ratios 411.20 and 821.39 arise from CL chromopeptide; (B) MS2 spectrum of molecular ion with m/z ratio 411.19 (z=2) from MS spectrum of fraction V; (C) Fragmentation (MS3 spectrum) of ion with m/z ratio 478.57 (z=3) from MS2 spectrum of ion with m/z ratio 411.19 (z=2), representing CL sequence without PCB; (D) Fragmentation (MS3 spectrum) of ion with m/z ratio 587.29 (z=1) from MS2 spectrum of ion with m/z ratio 411.19 (z=2), representing PCB.

Table S6A. Molecular mass of phycocyanobilin, determined after recording MS spectrum of phycocyanobilin preparation.

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
587.2857	1	586.2779	586.2791	-0.0013	PCB

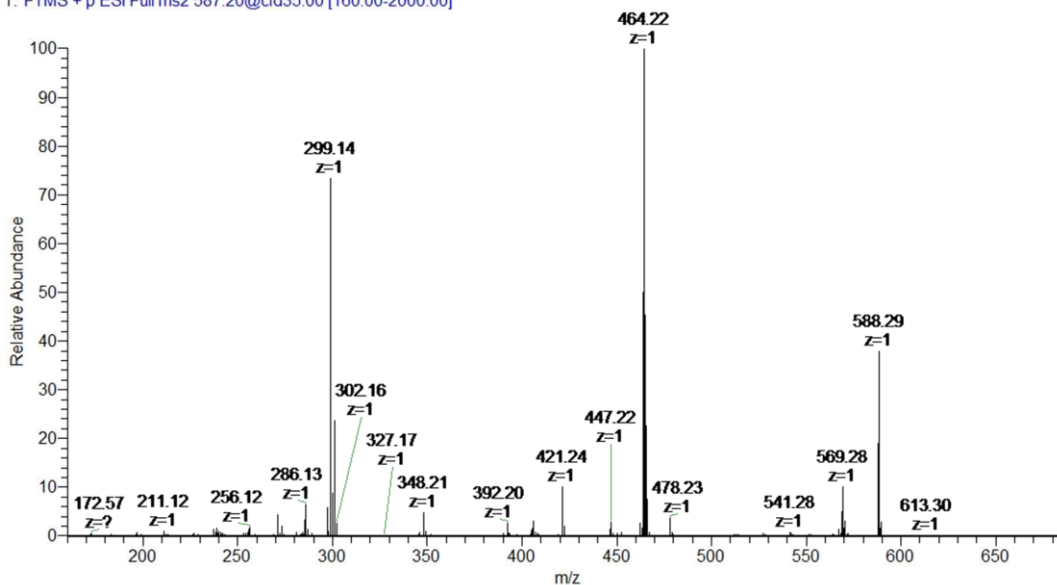
Table S6B. Fragmentation of molecular ion with m/z ratio 587.29 (z=1) from MS spectrum of phycocyanobilin. Data were obtained after recording MS2 spectrum of this ion.

m/z	z	experimental mass	theoretical mass	Δ mass	Structure
464.2178	1	464.2178	464.2185	-0.0007	$C_{26}H_{30}N_3O_5$ (PCB with one pyrrole less)
299.1391	1	299.1391	299.1396	-0.0005	$C_{17}H_{19}N_2O_3$ (PCB half)
271.1443	1	271.1443	271.1447	-0.0004	$C_{16}H_{19}N_2O_2$ without CO group



OB03432 #1 RT: 0.00 AV: 1 NL: 6.12E7
T: FTMS + p ESI Full ms2 587.20@cid35.00 [160.00-2000.00]

B



OB03434 #1 RT: 0.00 AV: 1 NL: 1.60E7
T: FTMS + p ESI Full ms3 587.20@cid35.00 299.14@cid35.00 [80.00-2000.00]

C

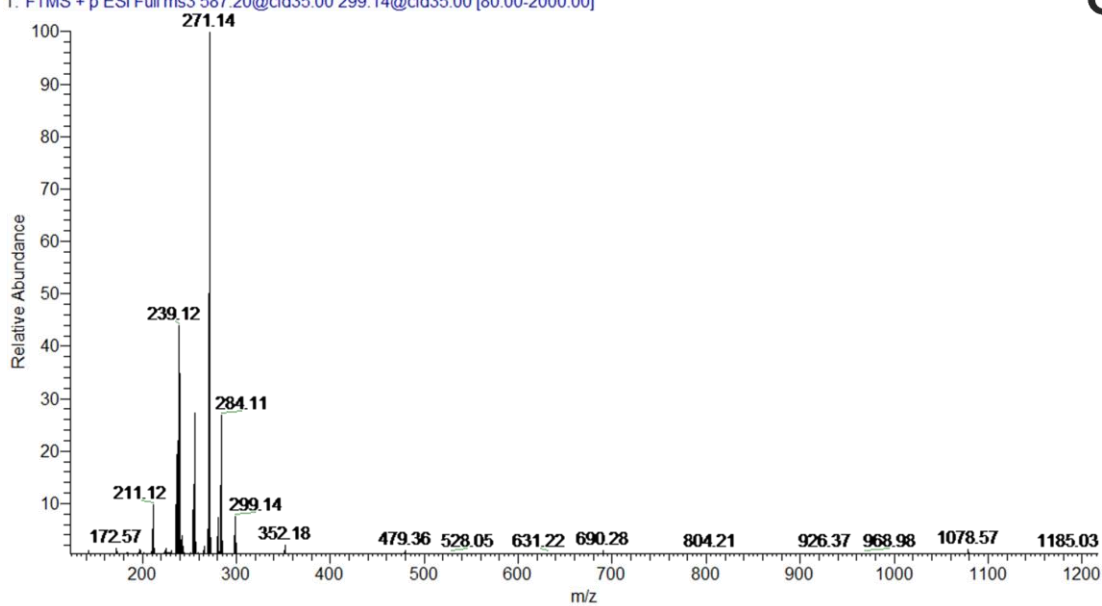


Figure S6. (A) MS spectrum of phycocyanobilin. Peak with m/z ratio 587.29 (z=1) arises from PCB; (B) MS2 spectrum of molecular ion with m/z ratio 587.29 (z=1) from MS spectrum of PCB; (C) MS3 spectrum of molecular ion with m/z ratio 299.14 (z=1) from MS2 spectrum of ion with m/z ratio 587.29 (z=1).

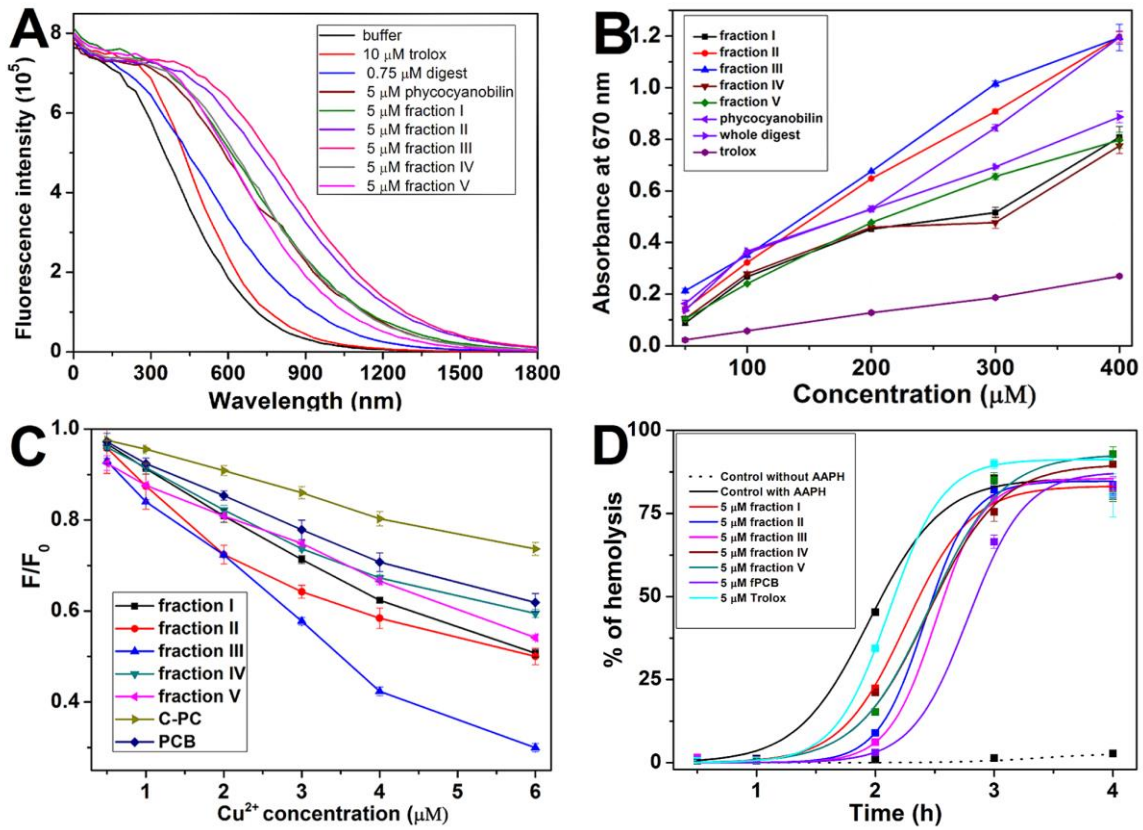


Figure S7. Results of ORAC test (A), reducing power test (B) and hemolysis inhibition (D) of chromopeptides, PCB and C-PC digest in comparison to Trolox as positive control. Figure C shows quenching of chromophore fluorescence (emission and excitation wavelengths were 638 and 578 nm, respectively) in chromopeptides, C-PC and PCB by Cu²⁺ ions. F₀ and F represents fluorescence of chromophore in absence and presence of different concentrations of Cu²⁺ (0.5, 1, 2, 3, 4, and 6 μM), respectively. Concentration of chromophore was 1 μM. In figures B, C and D, values are shown as means ± standard deviations.

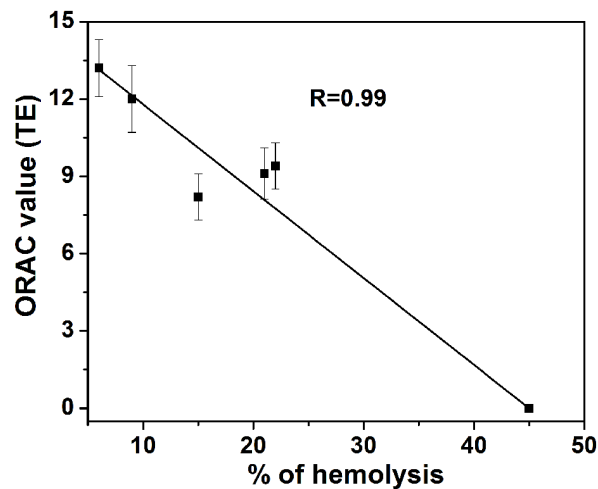


Figure S8. Correlation between effects of chromopeptides on erythrocytes hemolysis and ORAC values. Values are shown as means ± standard deviations.

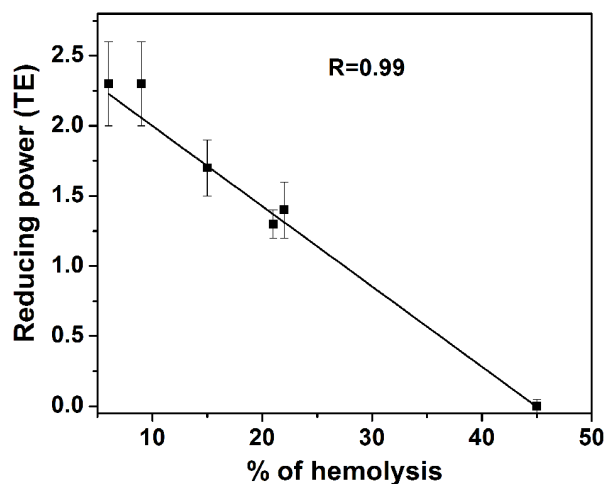


Figure S9. Correlation between effects of chromopeptides on erythrocytes hemolysis and reducing power. Values are shown as means \pm standard deviations

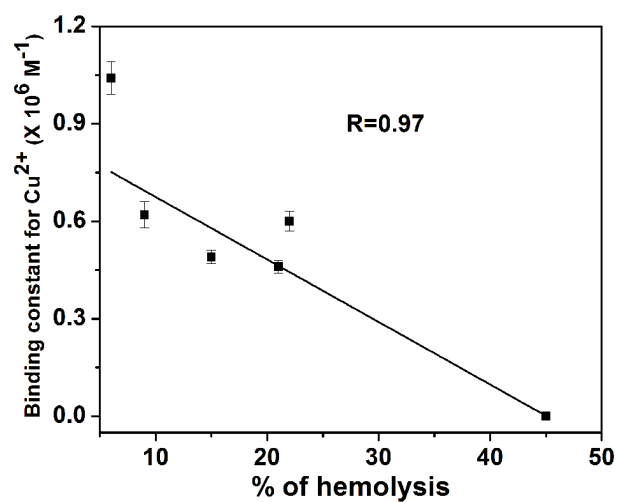


Figure S10. Correlation between effects of chromopeptides on erythrocytes hemolysis and binding constants for Cu²⁺. Values are shown as means \pm standard deviations.

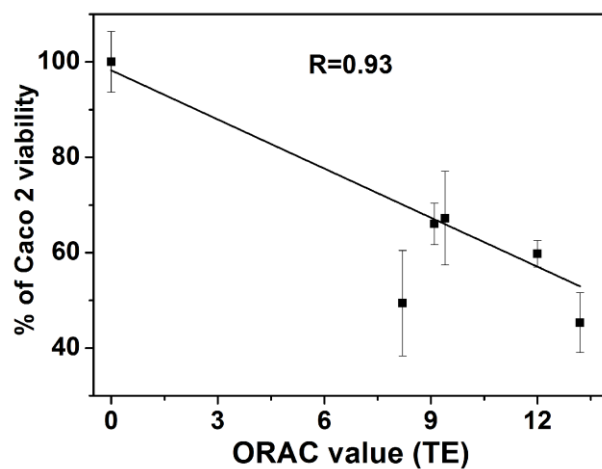


Figure S11. Correlation between cytotoxicity of chromopeptides on Caco-2 cell and ORAC values. Values are shown as means \pm standard deviations.

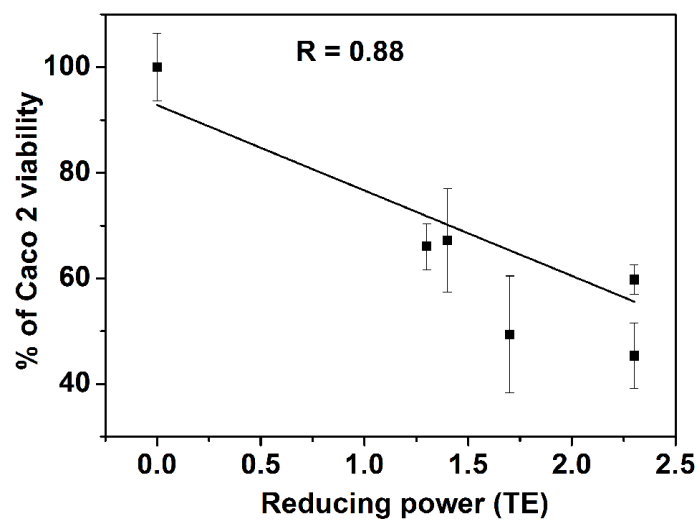


Figure S12. Correlation between cytotoxicity of chromopeptides on Caco-2 cell and reducing power. Values are shown as means \pm standard deviations.

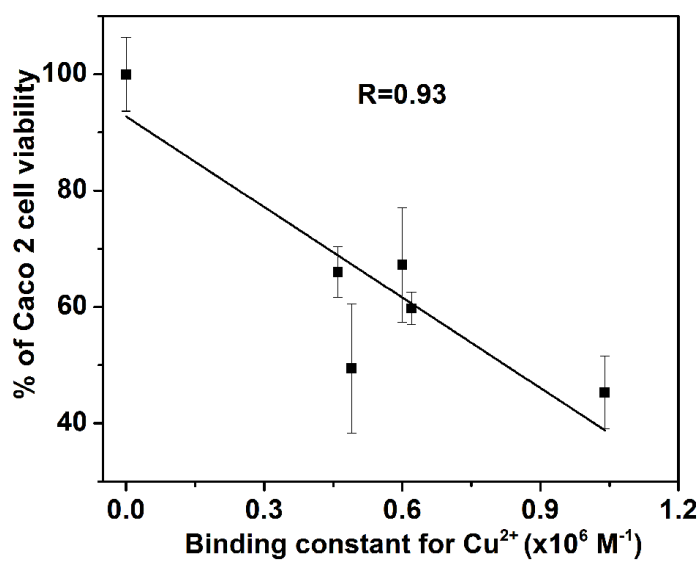


Figure S13. Correlation between cytotoxicity of chromopeptides on Caco-2 cell and binding constants for Cu²⁺. Values are shown as means \pm standard deviations

Rechromatography of chromopeptide fractions

Rechromatography of chromopeptide fractions was conducted on HPLC Agilent 1260 system (Agilent, USA). Separation was performed by analytical Zorbax Eclipse Plus C-18 column (4.6 mm x 100 mm, 3.5 μ m particles; Agilent, USA) connected with HPLC system. 200 μ L of 10 μ M chromopeptides were applied on column. Elution was done with less steep gradients in comparison to chromatography of pepsin digest in order to achieve better resolution. Gradient conditions for each fraction were given below appropriate rechromatograms (**Figures S14-S18**). Relative purity of chromopeptides in comparison to other peptides (absence of peak at 615 nm) was estimated using absorbance at 215 nm. The flow rate was 1 mL/min with simultaneous detection at 215, 280 and 615 nm.

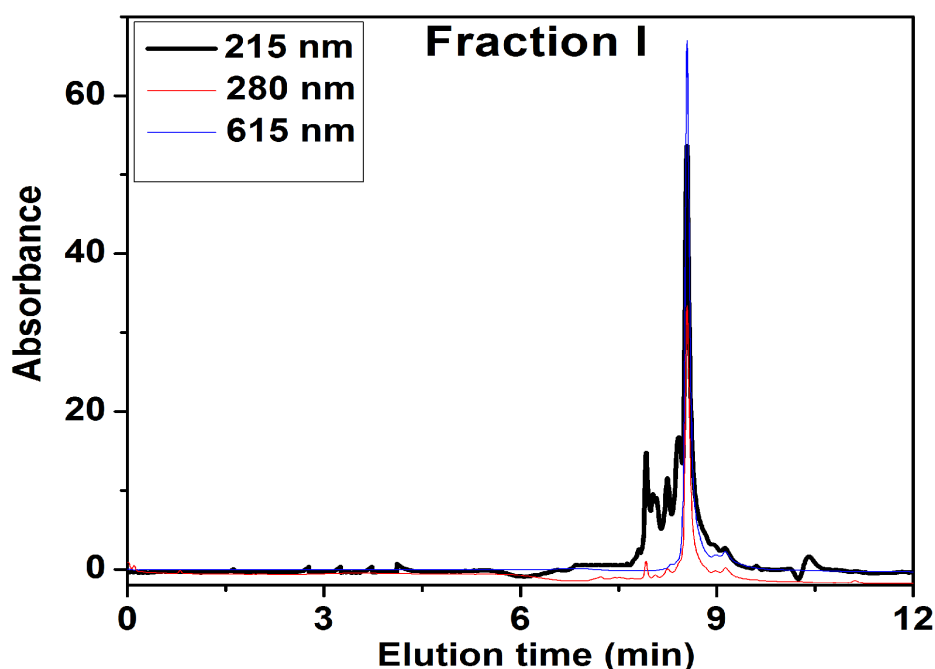


Figure S14. Rechromatogram of fraction I; Elution conditions: 100% solvent A (0.1% formic acid) - one column volume, gradient from 100% solvent A to 20% solvent B (0.1% formic acid in acetonitrile) - one column volume, gradient from 80% solvent A to 25% solvent B - five column volumes. Relative purity 72%.

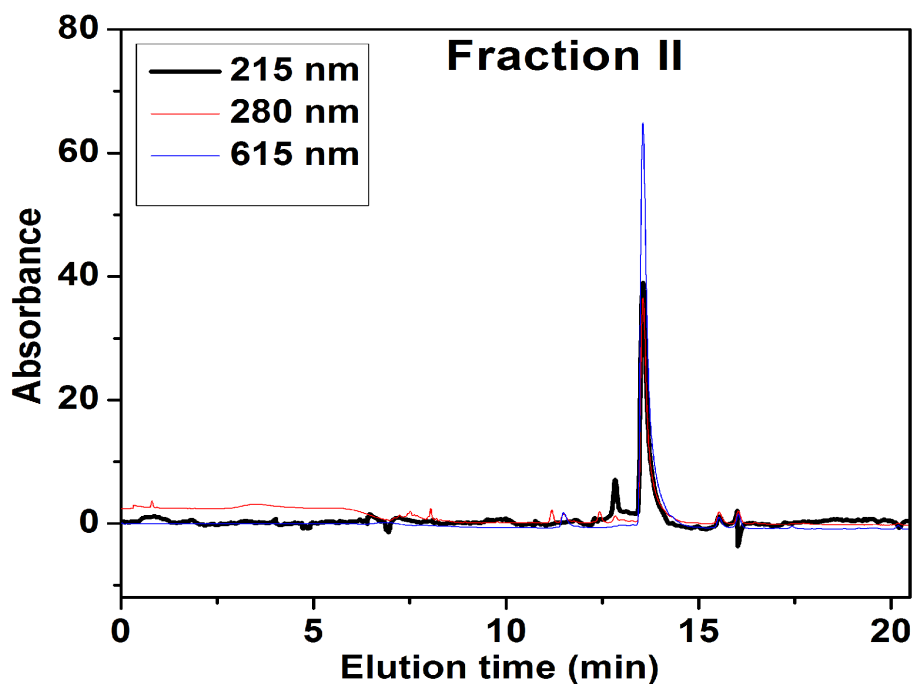


Figure S15. Rechromatogram of fraction II; Elution conditions: 100% solvent A (0.1% formic acid) - one column volume, gradient from 100% solvent A to 20% solvent B (0.1% formic acid in acetonitrile) - one column volume, gradient from 80% solvent A to 30% solvent B - ten column volumes. Relative purity 91%.

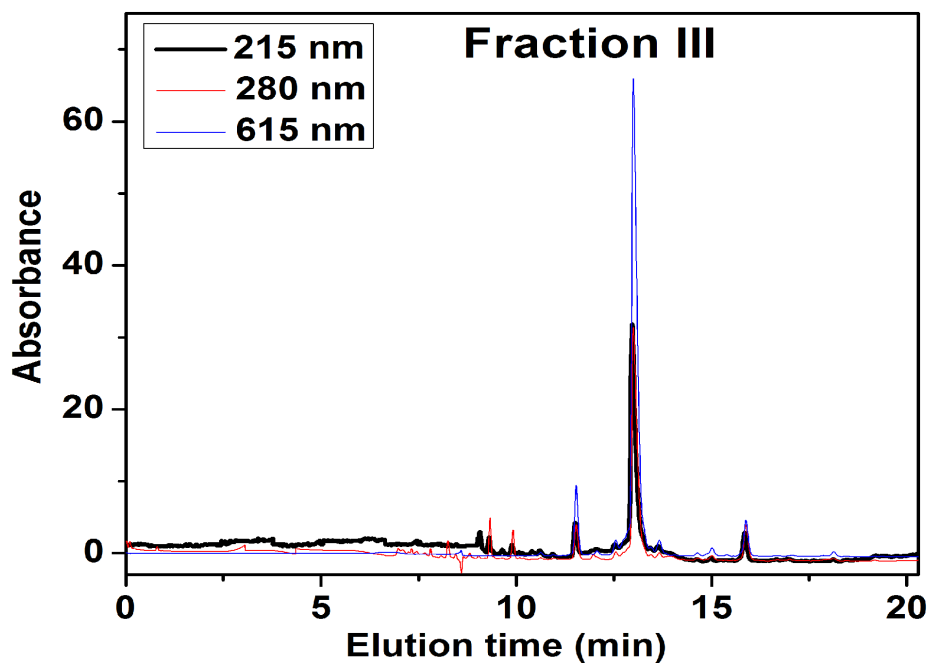


Figure S16. Rechromatogram of fraction III; Elution conditions: 100% solvent A (0.1% formic acid) - one column volume, gradient from 100% solvent A to 25% solvent B (0.1% formic acid in acetonitrile) - one column volume, gradient from 75% solvent A to 35% solvent B - ten column volumes. Relative purity 95%.

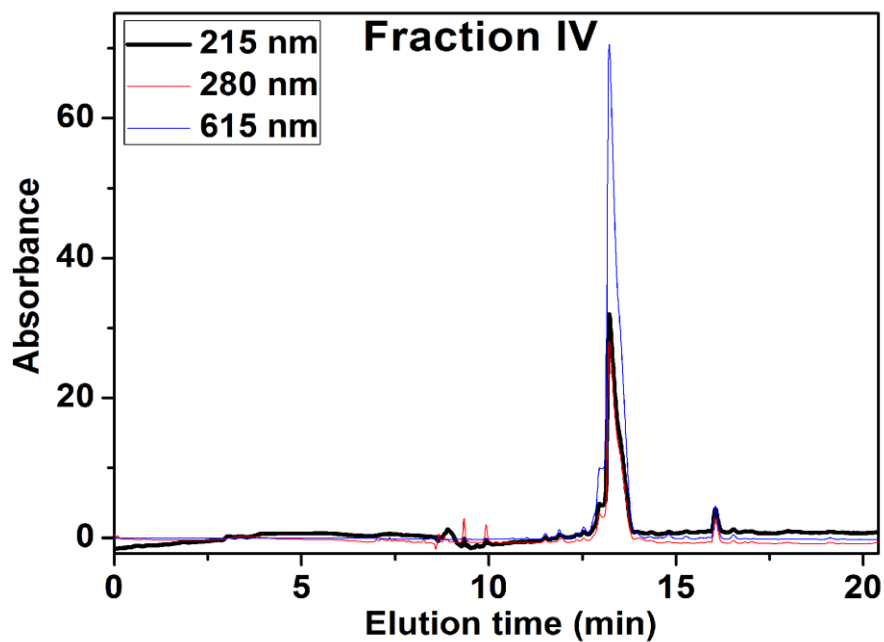


Figure S17. Rechromatogram of fraction IV; Elution conditions: 100% solvent A (0.1% formic acid) - one column volume, gradient from 100% solvent A to 25% solvent B (0.1% formic acid in acetonitrile) - one column volume, gradient from 75% solvent A to 35% solvent B - ten column volumes. Relative purity 98%.

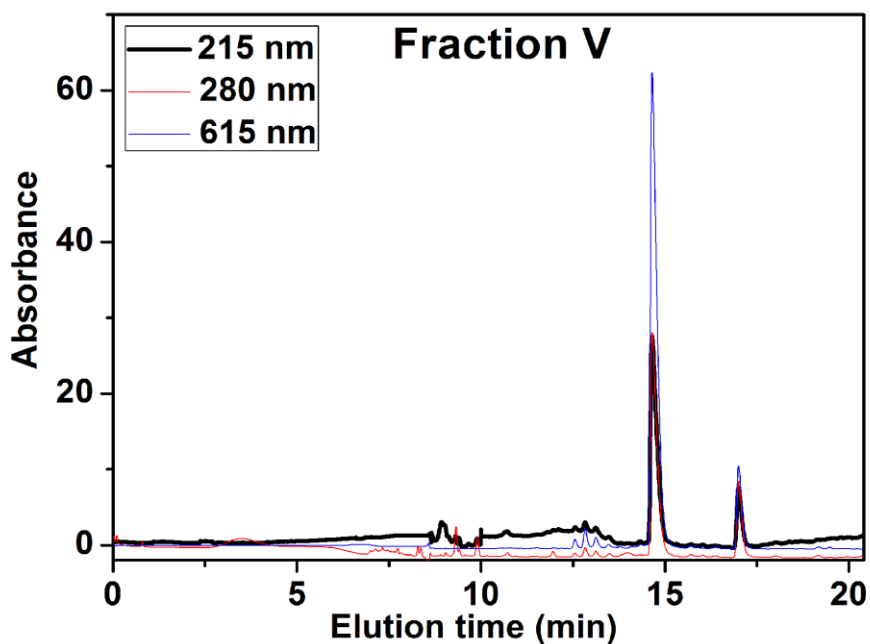


Figure S18. Rechromatogram of fraction V; Elution conditions: 100% solvent A (0.1% formic acid) - one column volume, gradient from 100% solvent A to 25% solvent B (0.1% formic acid in acetonitrile) - one column volume, gradient from 75% solvent A to 35% solvent B - ten column volumes. Relative purity 92%.