

Article

Mass Spectrometric Proof of Predicted Peptides: Novel Adipokinetic Hormones in Insects

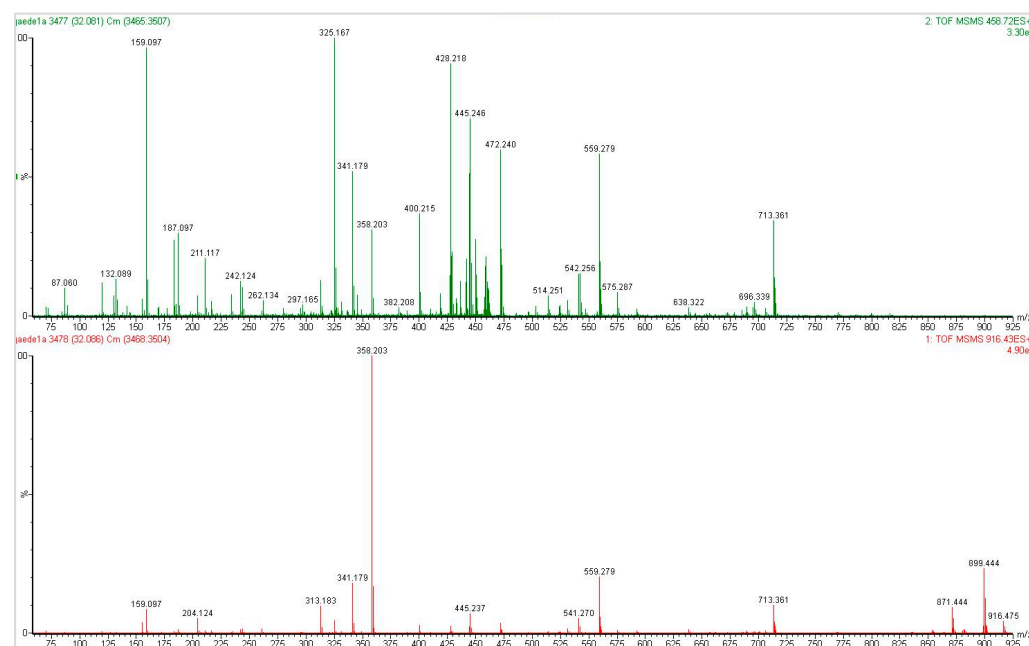
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b	211.108	325.151	472.220	559.252	656.304	713.326	–
i	72.081	87.056	120.081	60.045	70.066	30.034	159.092
b~	193.098	307.141	454.209	541.241	638.294	695.315	–
b*	194.082	308.125	455.193	542.225	639.278	696.299	–
	1	2	3	4	5	6	7
	Val	Asn	Phe	Ser	Pro	Gly	Trp
	7	6	5	4	3	2	1
y"	–	706.331	592.288	445.220	358.188	261.135	204.114
y~	–	688.321	574.278	427.209	340.177	243.125	186.103
y*	–	689.305	575.262	428.193	341.161	244.109	187.087

Figure S1. MS/MS spectra of the singly- and the doubly-charged ions of a peptide assigned to Manto-CC in *K. flavicollis* and the calculation of the expected fragment ions for sequence pQVNF-SPGWa using MassLynx (blocked termini: pQ and amidation).

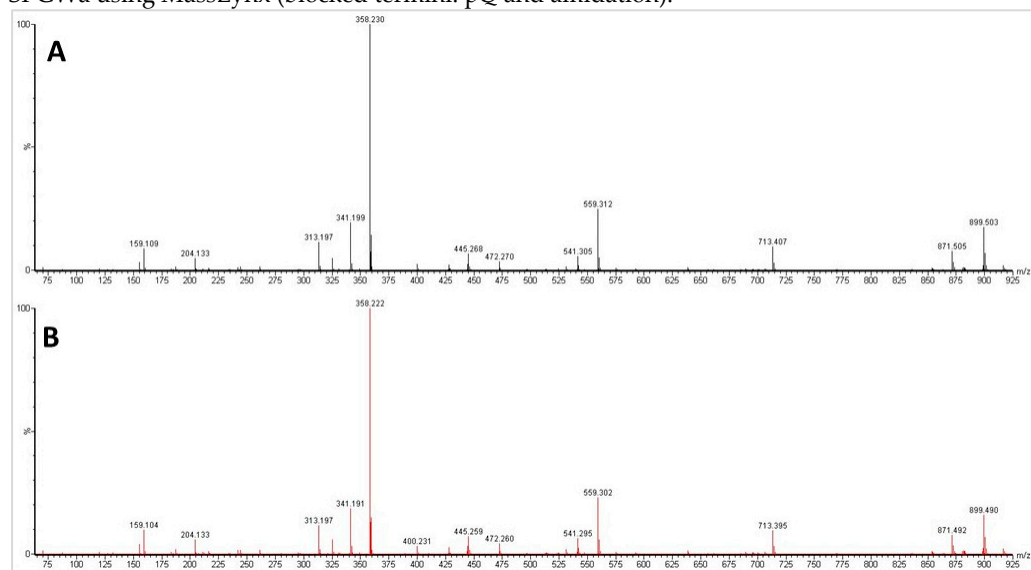
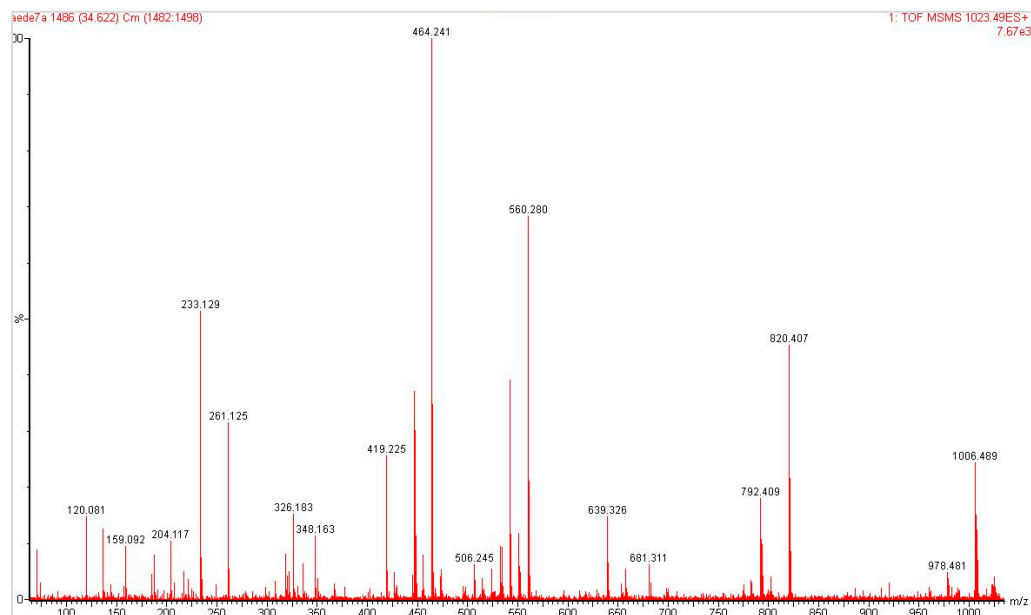


Figure S2. MS/MS spectra of the doubly-charged ions of the peptide assigned to Manto-CC in *K. flavicollis*. A) Standard peptide and B) CC extract were run with the same MS/MS method.



b	225.124	326.172	473.240	560.272	657.325	820.388	-
i	86.097	74.061	120.081	60.045	70.066	136.076	159.092
b~	207.113	308.161	455.229	542.262	639.314	802.378	-
b*	208.097	309.145	456.214	543.246	640.298	803.362	-
	1	2	3	4	5	6	7
	Leu	Thr	Phe	Ser	Pro	Tyr	Trp
	7	6	5	4	3	2	1
y*	-	799.378	698.330	551.262	464.230	367.177	204.114
y~	-	781.367	680.320	533.251	446.219	349.166	186.103
y*	-	782.351	681.304	534.235	447.203	350.150	187.087

Figure S3. MS/MS spectrum of the singly-charged ion of the peptide assigned to Volpe-CC in *P. tapulus* and the calculation of the expected fragment ions for sequence pQLTFSPYWa using Mass-Lynx (blocked termini: pQ and amidation).

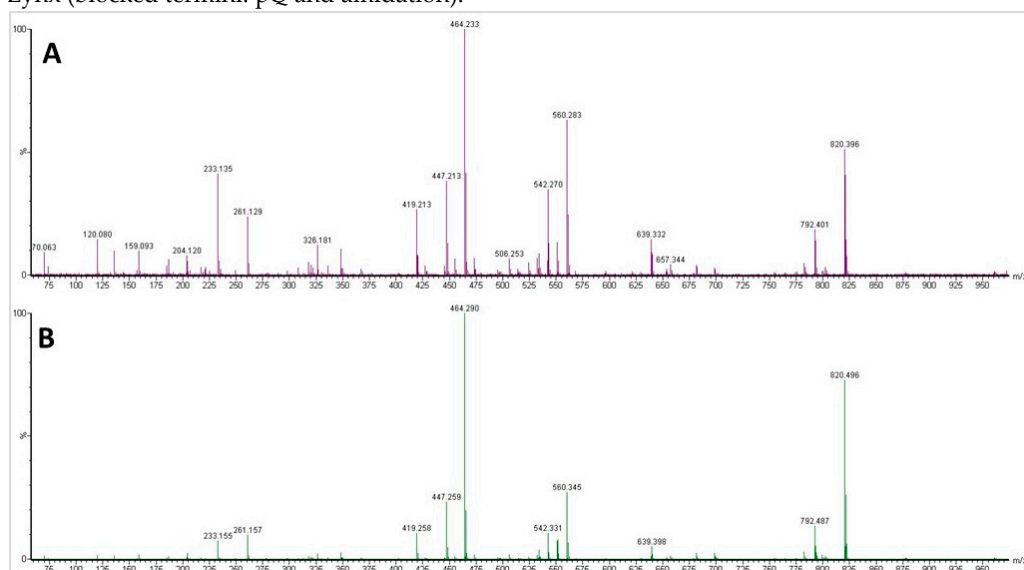
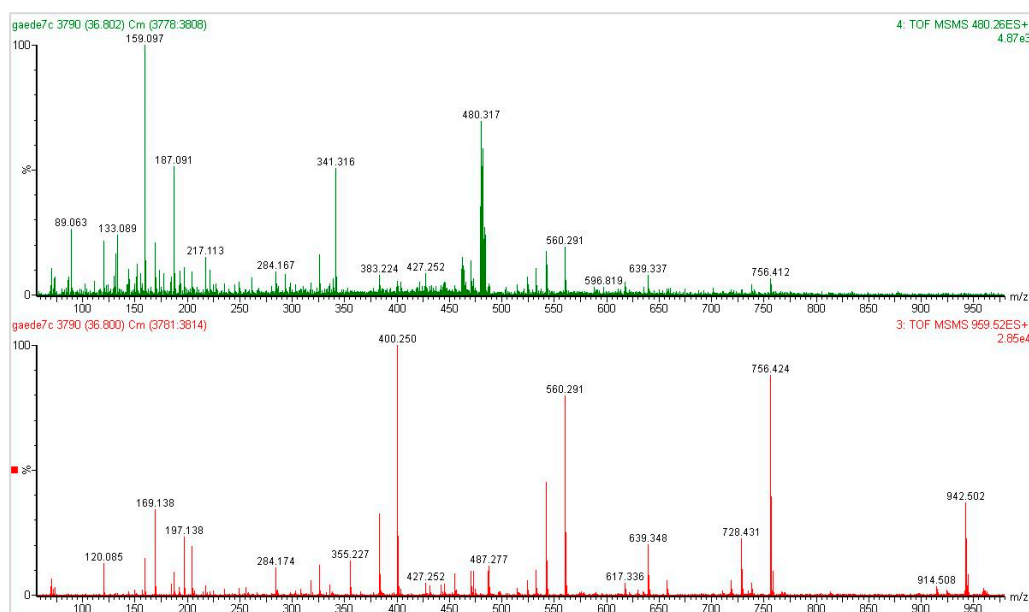


Figure S4. MS/MS spectra of the singly-charged ion of the peptide assigned to Volpe-CC in *P. tapulus*. A) CC extract and B) standard peptide were run with slightly different collision energy (17–26 eV and 17–25 eV), but the fragments are identical.



b	225.124	326.172	473.240	560.272	657.325	756.393	–
i	86.097	74.061	120.081	60.045	70.066	72.081	159.092
b~	207.113	308.161	455.229	542.262	639.314	738.383	–
b*	208.097	309.145	456.214	543.246	640.298	739.367	–
	1	2	3	4	5	6	7
	Leu	Thr	Phe	Ser	Pro	Val	Trp
	7	6	5	4	3	2	1
y"	–	735.383	634.335	487.267	400.235	303.182	204.114
y~	–	717.372	616.325	469.256	382.224	285.172	186.103
y*	–	718.356	617.309	470.240	383.208	286.156	187.087

Figure S5. MS/MS spectra of the singly- and doubly-charged ions of the peptide detected at m/z 959.52 tentatively assigned to sequence pQLTFSPVW in *P. tapulus* and the calculation of the expected fragment ions using MassLynx (blocked termini: pQ and amidation).

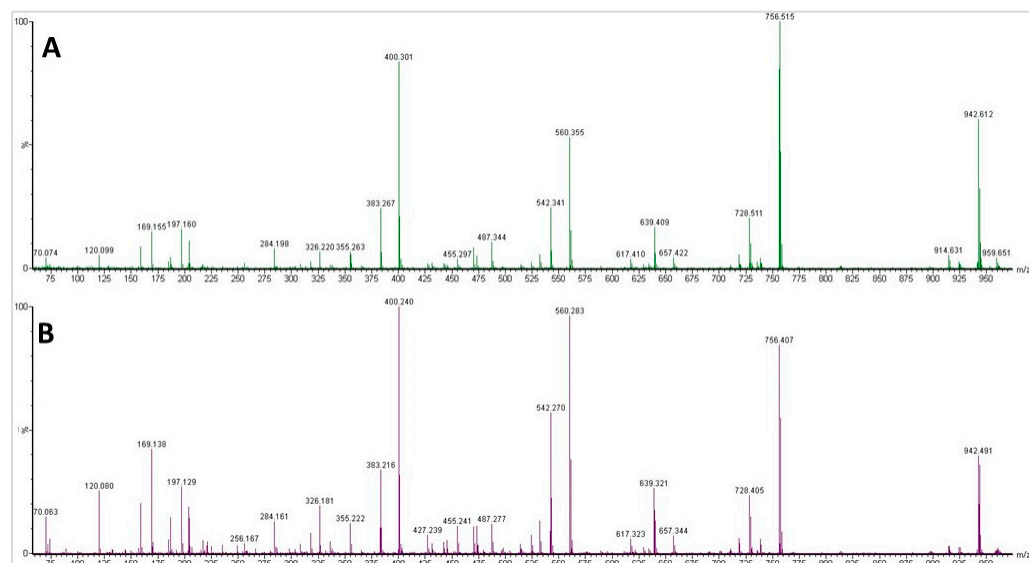
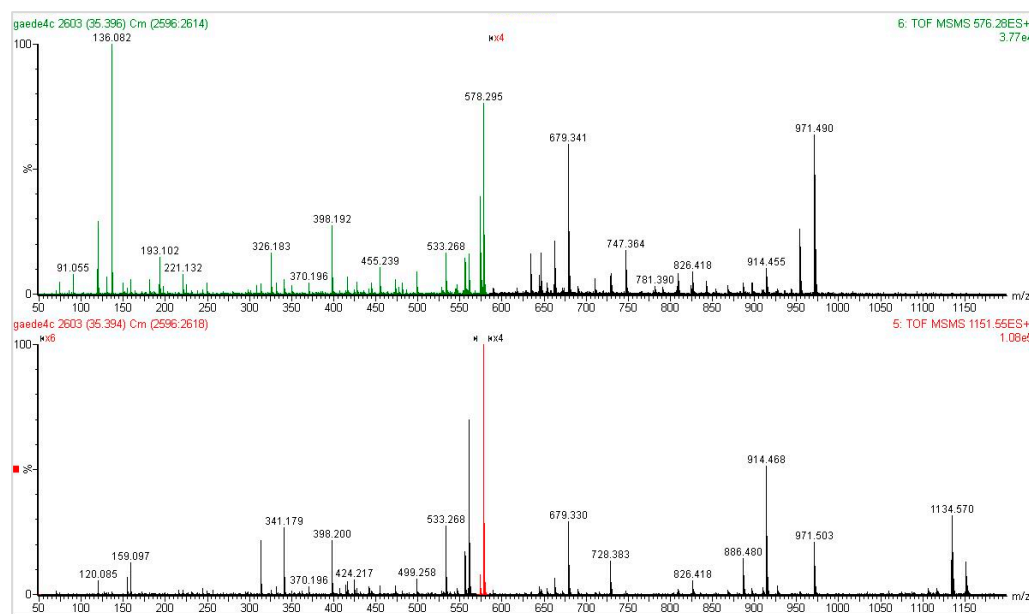


Figure S6. MS/MS spectra of the singly-charged ions of the peptide detected at m/z 959.52 as assigned to sequence pQLTFSPVW in *P. tapulus*, code-named Pegta-AKH. A) Standard peptide and B) CC extract were analyzed with the same MS/MS method and instrumentation.



b	225.124	326.172	473.240	574.288	671.341	728.362	914.441	971.463	-
i	86.097	74.061	120.081	74.061	70.066	30.034	159.092	30.034	136.076
b~	207.113	308.161	455.229	556.277	653.330	710.351	896.431	953.452	-
b*	208.097	309.145	456.214	557.261	654.314	711.335	897.415	954.436	-
	1	2	3	4	5	6	7	8	9
	Leu	Thr	Phe	Thr	Pro	Gly	Trp	Gly	Tyr
	9	8	7	6	5	4	3	2	1
y*	-	927.436	826.389	679.320	578.273	481.220	424.198	238.119	181.098
y~	-	909.426	808.378	661.310	560.262	463.209	406.188	220.109	163.087
y*	-	910.410	809.362	662.294	561.246	464.193	407.172	221.093	164.071

Figure S7. MS/MS spectra of the singly- and doubly-charged ions of the peptide detected at m/z 1151.55 assigned to sequence pQLTFTPGWGYa for Tabat-HoTH in *H. pluvialis* and the calculation of the expected fragment ions using MassLynx (blocked termini: pQ and amidation).

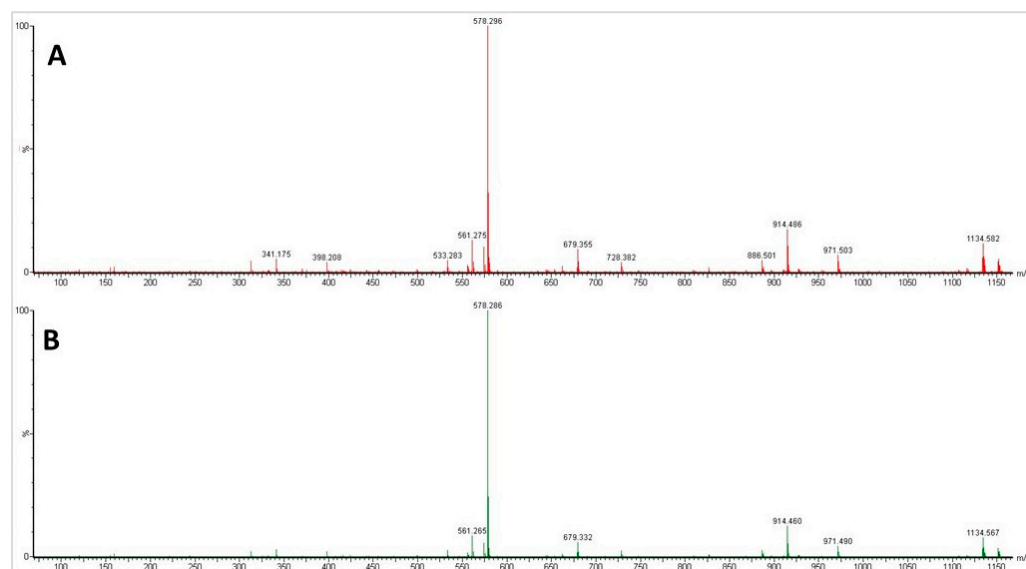
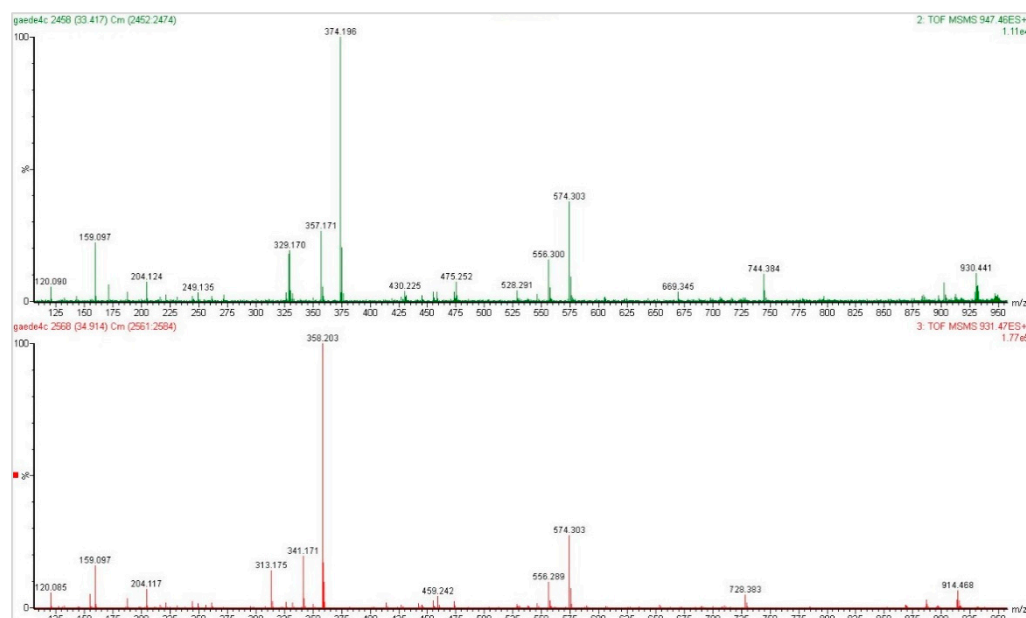


Figure S8. MS/MS spectra of the singly-charged ions of the peptide detected at m/z 1151.55 assigned to sequence pQLTFTPGWGYa for Tabat-HoTH in *H. pluvialis*. A) CC extract and B) stand-ard peptide were run with the same collision energy ramp.



b	225.124	326.172	473.240	574.288	687.335	744.357	-
i	86.097	74.061	120.081	74.061	86.061	30.034	159.092
b~	207.113	308.161	455.229	556.277	669.325	726.346	-
b*	208.097	309.145	456.214	557.261	670.309	727.330	-
	1	2	3	4	5	6	7
	Leu	Thr	Phe	Thr	Hyp	Gly	Trp
	7	6	5	4	3	2	1
y"	-	723.347	622.299	475.230	374.183	261.135	204.114
y~	-	705.336	604.288	457.220	356.172	243.125	186.103
y*	-	706.320	605.272	458.204	357.156	244.109	187.087

b	225.124	326.172	473.240	574.288	671.341	728.362	-
i	86.097	74.061	120.081	74.061	70.066	30.034	159.092
b~	207.113	308.161	455.229	556.277	653.330	710.351	-
b*	208.097	309.145	456.214	557.261	654.314	711.335	-
	1	2	3	4	5	6	7
	Leu	Thr	Phe	Thr	Pro	Gly	Trp
	7	6	5	4	3	2	1
y"	-	707.352	606.304	459.236	358.188	261.135	204.114
y~	-	689.341	588.293	441.225	340.177	243.125	186.103
y*	-	690.325	589.277	442.209	341.161	244.109	187.087

Figure S9. MS/MS spectra of the doubly-charged ions of the related peptides detected in *H. pluvialis* at m/z 931.47 and 947.46 assigned, respectively, to Tabat-AKH (pQLTFTPGWa) and its hydroxyproline derivative. The expected fragment ions for each of these peptide sequences were calculated using MassLynx (blocked termini: pQ and amidation).

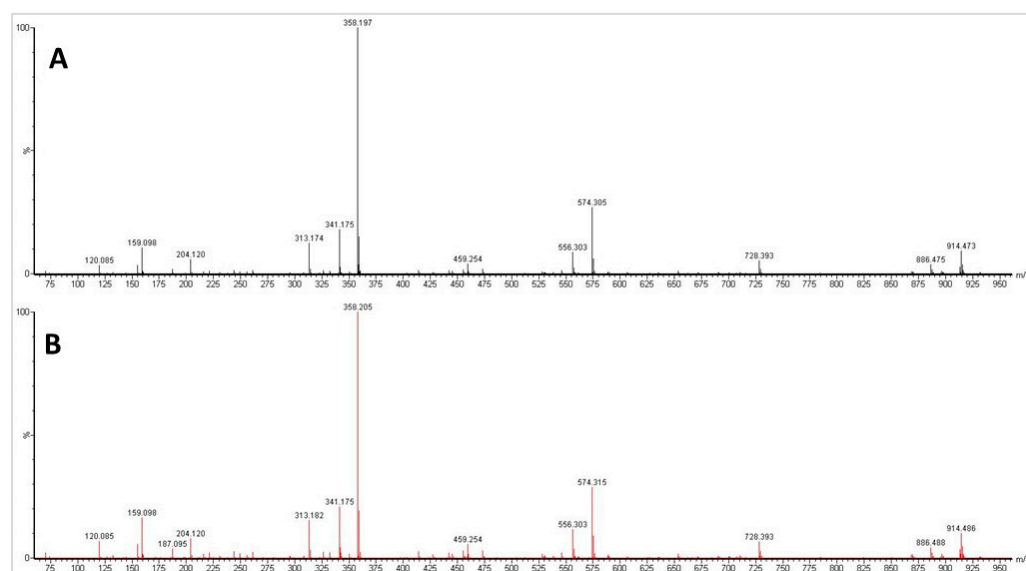


Figure S10. MS/MS spectra of the singly-charged ions of the peptide detected at m/z 931.47 assigned to sequence pQLTFTPGWa for Tabat-AKH in *H. pluvialis*. A) Standard peptide and B) CC extract were analyzed with the same MS/MS method.

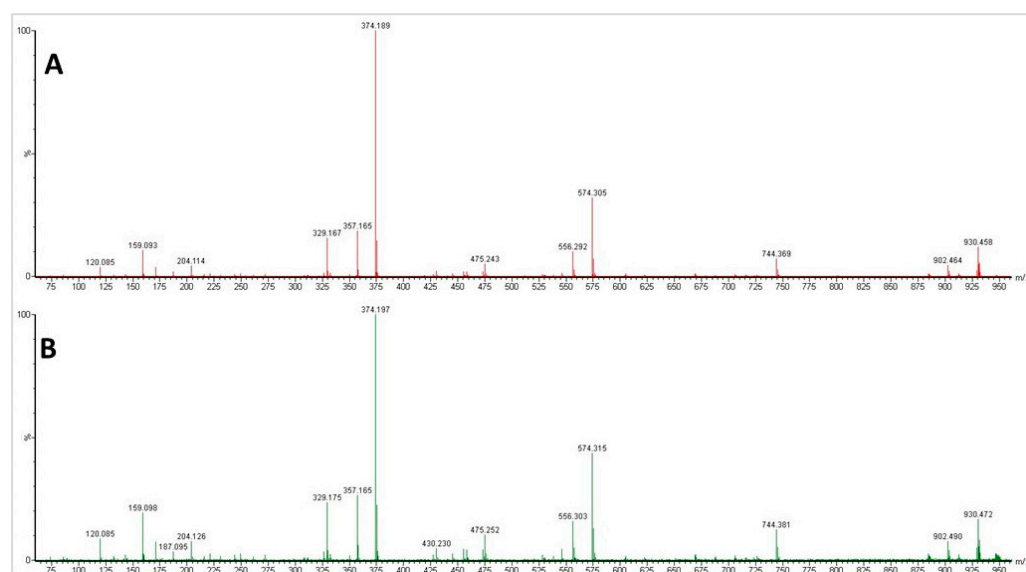


Figure S11. MS/MS spectra of the singly-charged ions of the peptide detected at 947.46 assigned to the hydroxyproline derivative of Tabat-AKH (HaepI-AKH) in *H. pluvialis*. A) Standard peptide (33.3 min) and B) CC extract (33.3 min) were analyzed with the same MS/MS method.

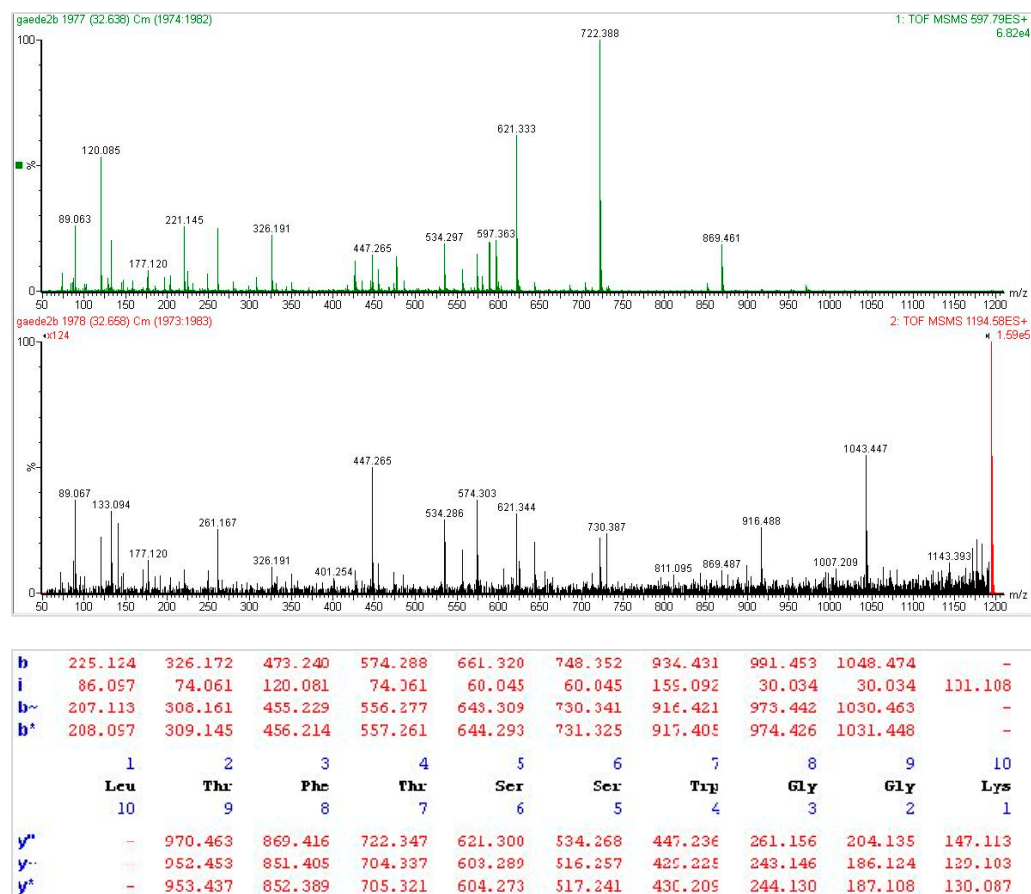


Figure S12. MS/MS spectra of the singly- and the doubly-charged ions of the peptide detected at m/z 1194.58 assigned to sequence pQLTFTSSWGK for Vanca-AKH in *O. nubilalis* and the calculation of the expected fragment ions using MassLynx (blocked terminus: pQ).

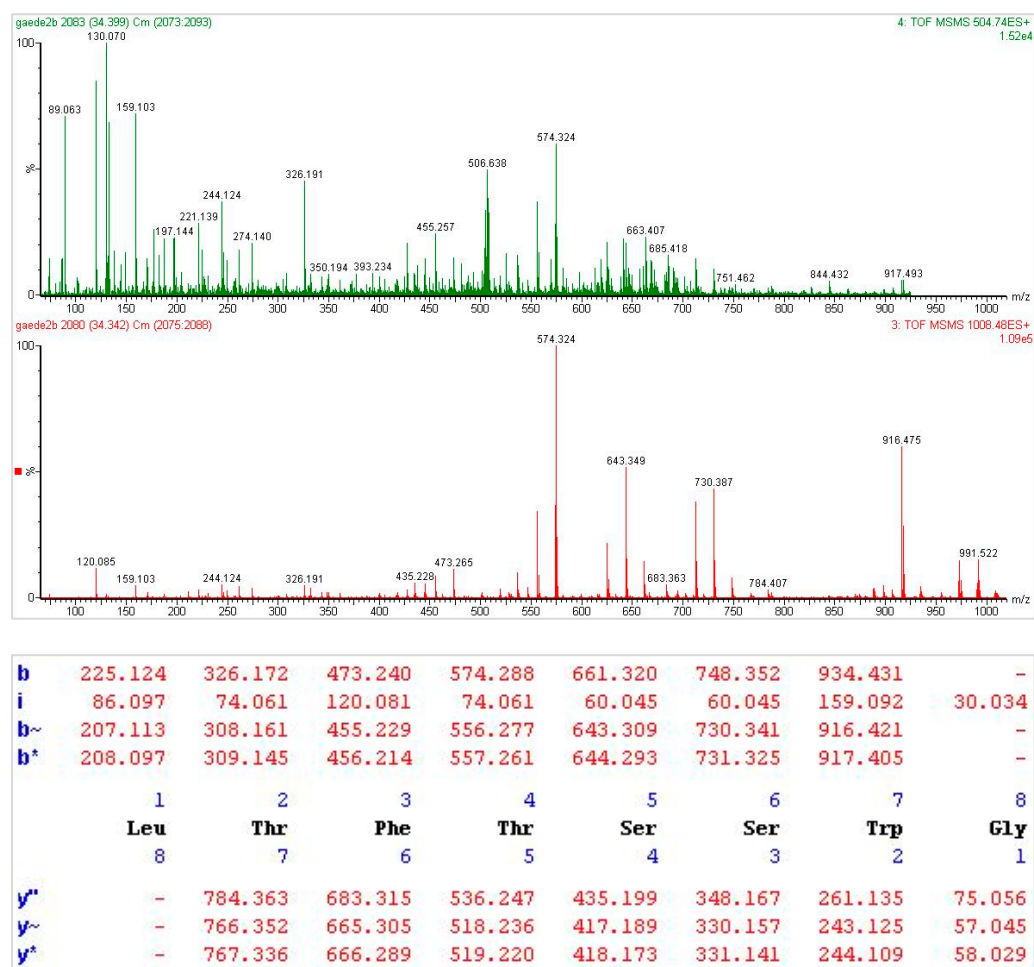


Figure S13. MS/MS spectra of the singly- and the doubly-charged ions of the peptide detected at m/z 1008.48 assigned to sequence pQLTFTSSWGa for Manse-AKH in *O. nubilalis* and the calculation of the expected fragment ions using MassLynx (blocked termini: pQ and amidation).

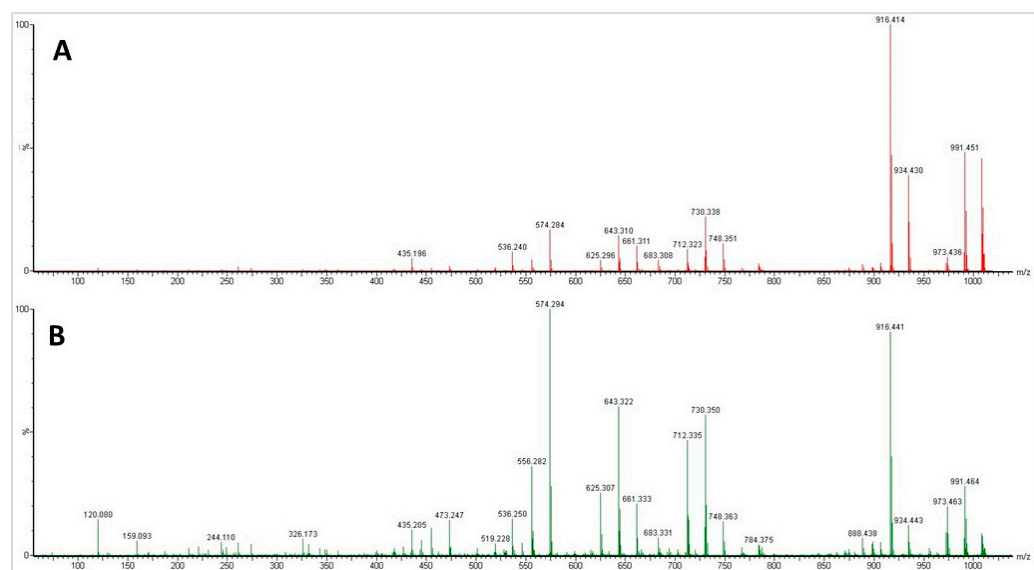


Figure S14. MS/MS spectra of the singly-charged ions of the peptide detected at m/z 1008.48 assigned to sequence pQLTFTSSWGa for Manse-AKH in *O. nubilalis*. A) Standard peptide (34.7 min) and B) CC extract (34.8 min) were with slightly different collision energy (15–20 eV and 17–26 eV), but the fragments are identical.

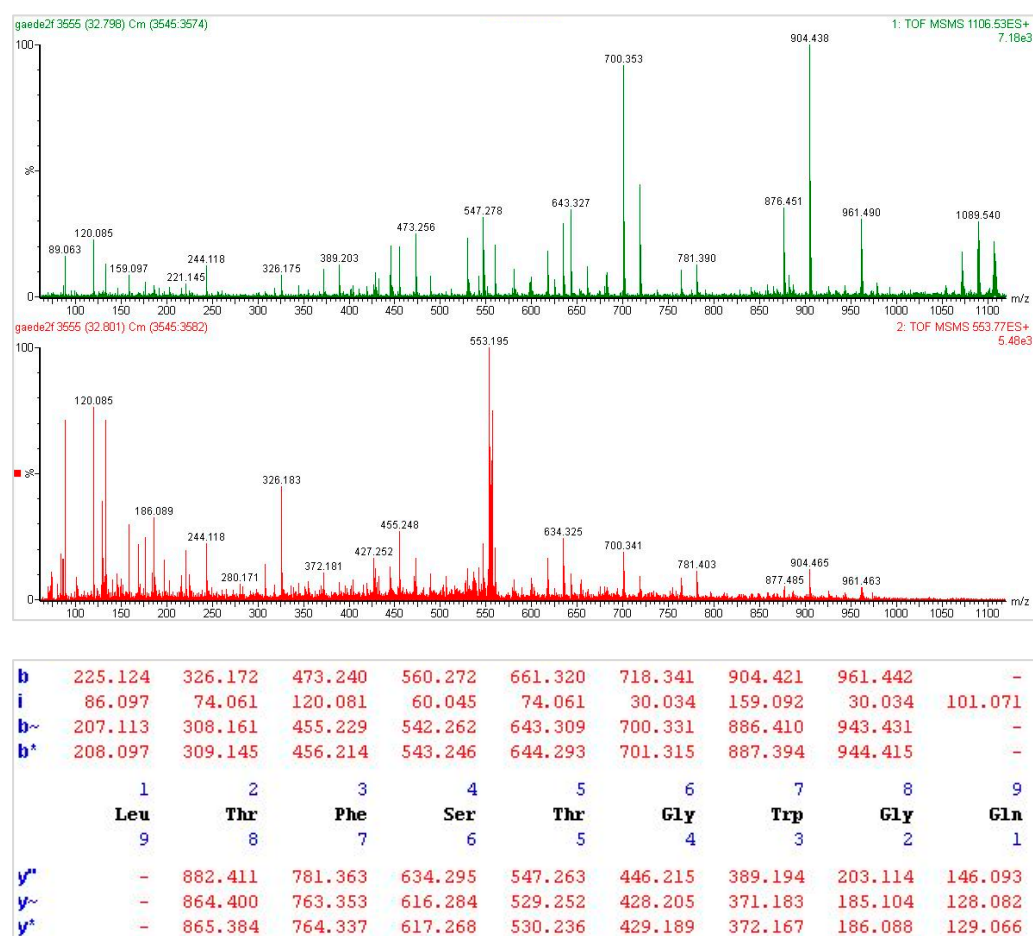


Figure S15. MS/MS spectra of the singly- and the doubly-charged ions of the peptide detected at m/z 1106.53 assigned to sequence pQLTFSTGWGQa for Ostnu-AKH in *O. nubilalis* and the calculation of the expected fragment ions using MassLynx (blocked termini: pQ and amidation).

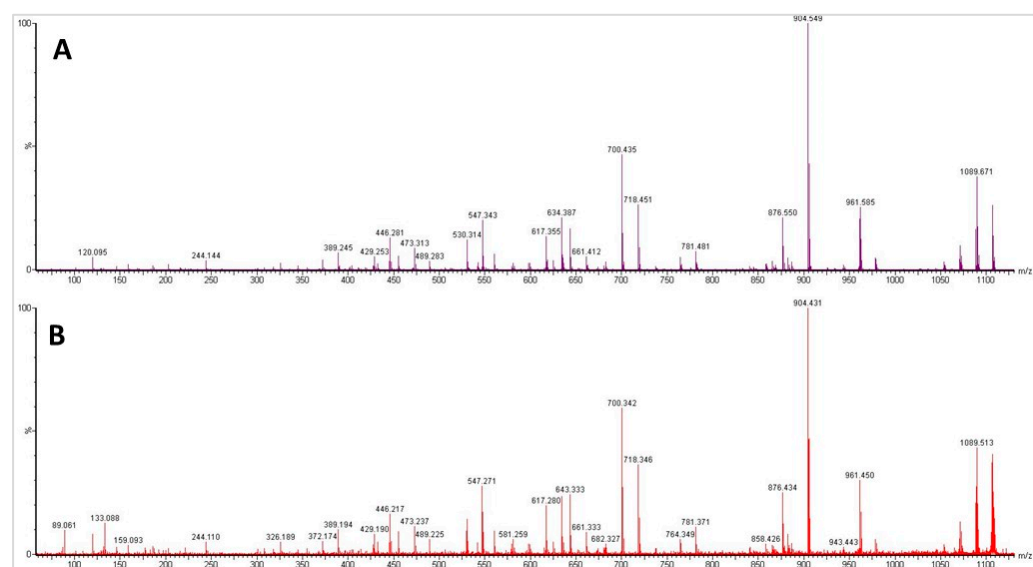


Figure S16. MS/MS spectra of the singly- and the doubly-charged ions of the peptide detected at m/z 1106.53 assigned to sequence pQLTFSTGWGQa for Ostnu-AKH in *O. nubilalis*. A) Standard peptide (34.2 min) and B) CC extract (34.3) were run with the same MS/MS method.

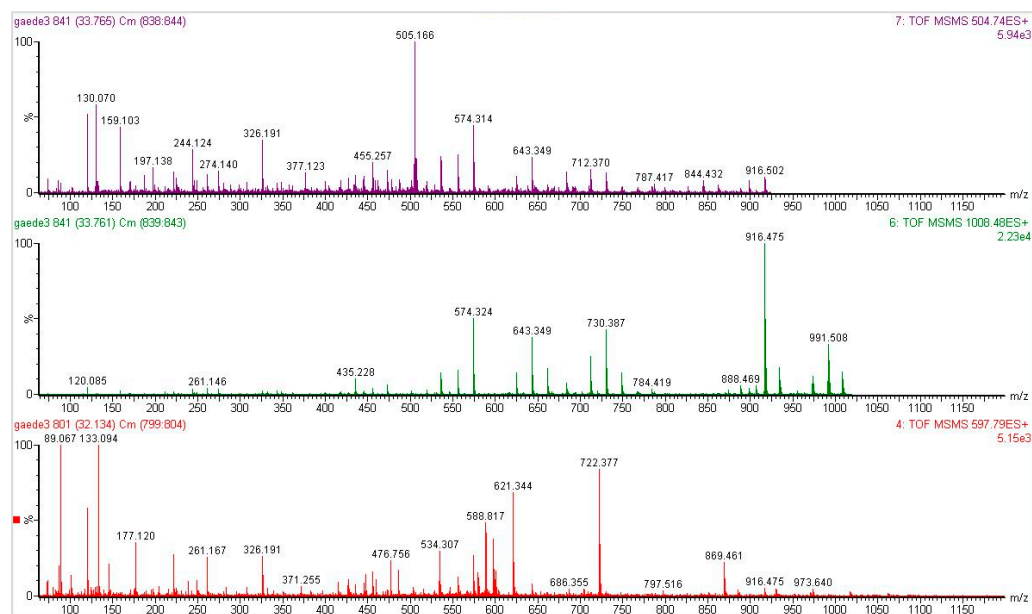


Figure S17. MS/MS spectra of the singly- and the doubly-charged ions of the peptides detected for Manse- and Vanca-AKH in *C. culmella*.

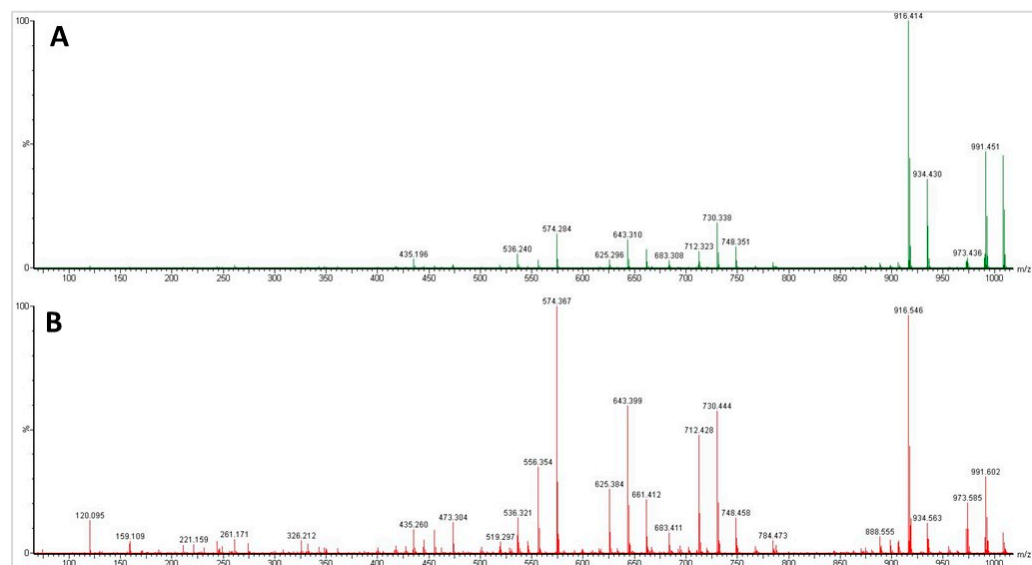


Figure S18. MS/MS spectra of the singly- and the doubly-charged ions of the peptides detected for Manse-AKH in *C. culmella*. A) Standard peptide and B) CC extract were run with slightly different collision energy (15–20 eV and 17–26 eV), but the fragments are identical.

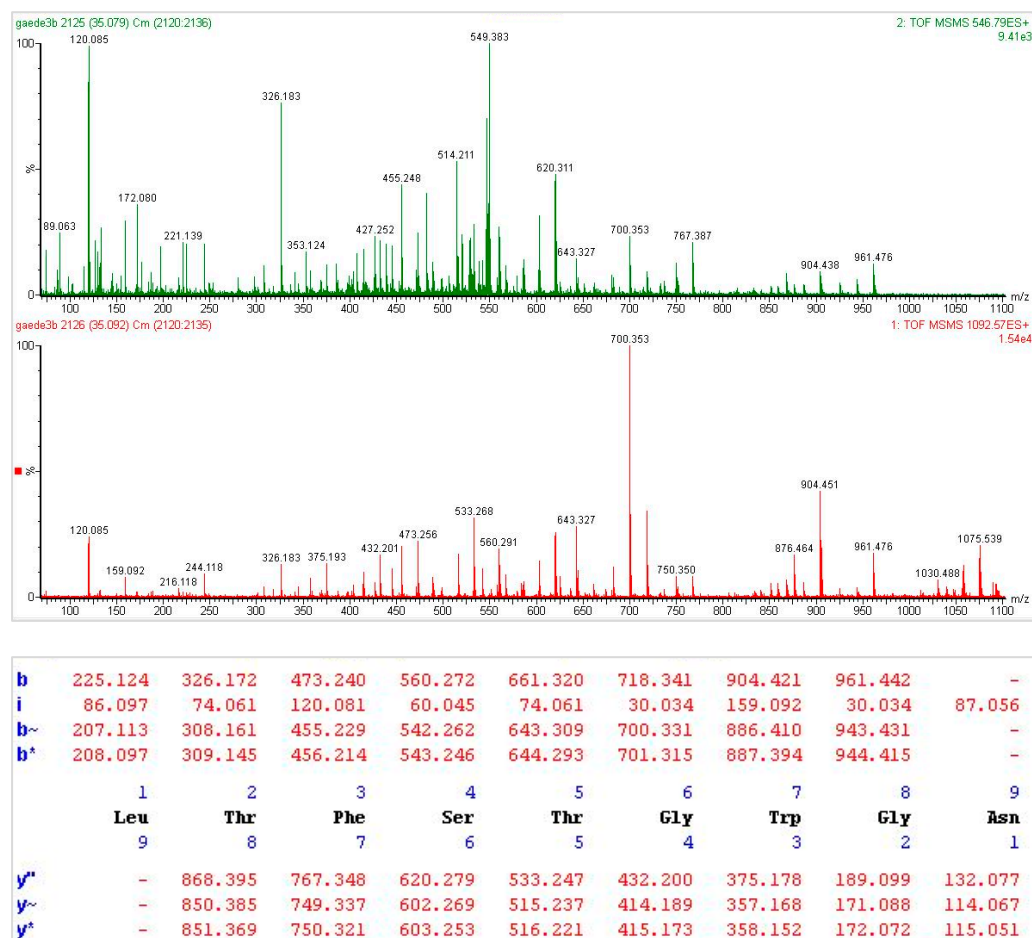


Figure S19. MS/MS spectra of the singly- and the doubly-charged ions of the peptide detected at m/z 1092.57 assigned to sequence pQLTFSTGWGN for Chipa-AKH in *C. culmella* and the calculation of the expected fragment ions using MassLynx (blocked termini: pQ and amidation).

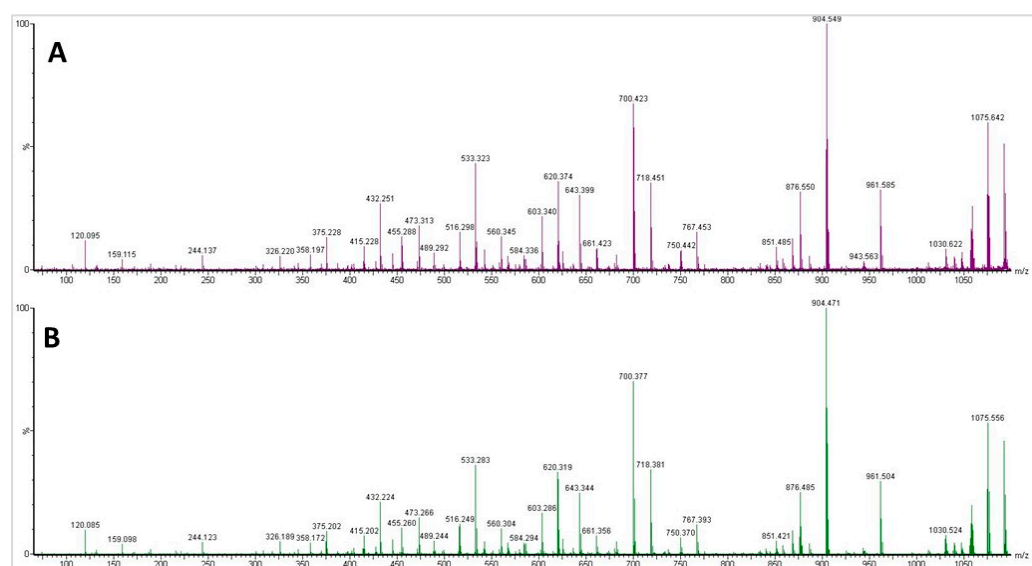


Figure S20. MS/MS spectra of the singly-charged ions of the peptide detected at m/z 1092.57 assigned to sequence pQLTFSTGWGN for Chipa-AKH in *C. culmella*. A) CC extract and B) standard peptide were analyzed with the same MS/MS method.

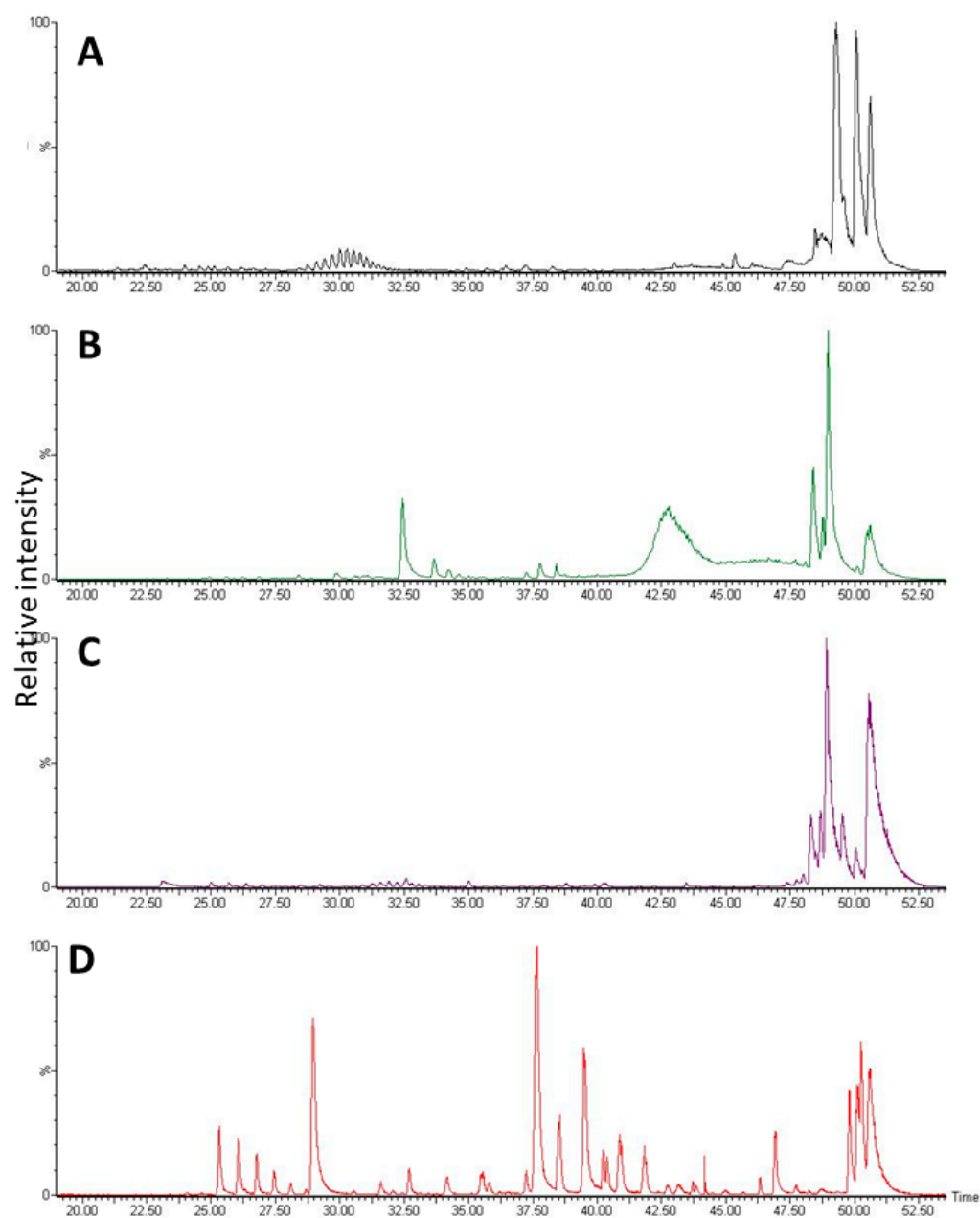


Figure S21. LC-chromatograms for the base peak in the overview scans for the CC extract of A) *P. tapulus*, B) *C. culmella* C) *O. nubilalis*, D) *K. flavicollis*.