SUPPORTING INFORMATION

A sensitive quantification of the peptide apidaecin 1 isoforms in single bee tissues using a weak cation exchange pre-separation and nanocapillary liquid chromatography coupled with mass spectrometry

Jiří Danihlík[a,b], Marek Šebela[b], Marek Petřivalský[a], René Lenobel[b,*]

[a] Department of Biochemistry, Faculty of Science, Palacký University, Šlechtitelů 11, 78379 Olomouc, Czech Republic

[b] Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

Author e-mail addresses:

Jiri Danihlik, e-mail: danihlik@gmail.com; Marek Sebela, e-mail: marek.sebela@upol.cz;

Marek Petrivalsky, email: marek.petrivalsky@upol.cz; Rene Lenobel, e-mail: rene.lenobel@upol.cz

*To whom correspondence should be addressed:

Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 11, 783 71 Olomouc, Czech Republic; e-mail: rene.lenobel@upol.cz, tel.: +420585634872, fax: +420585634870.

Suplementary Table 1S: A summary of amino acid sequences of all apidaecin isoforms. Single amino acid differences are written in bold.

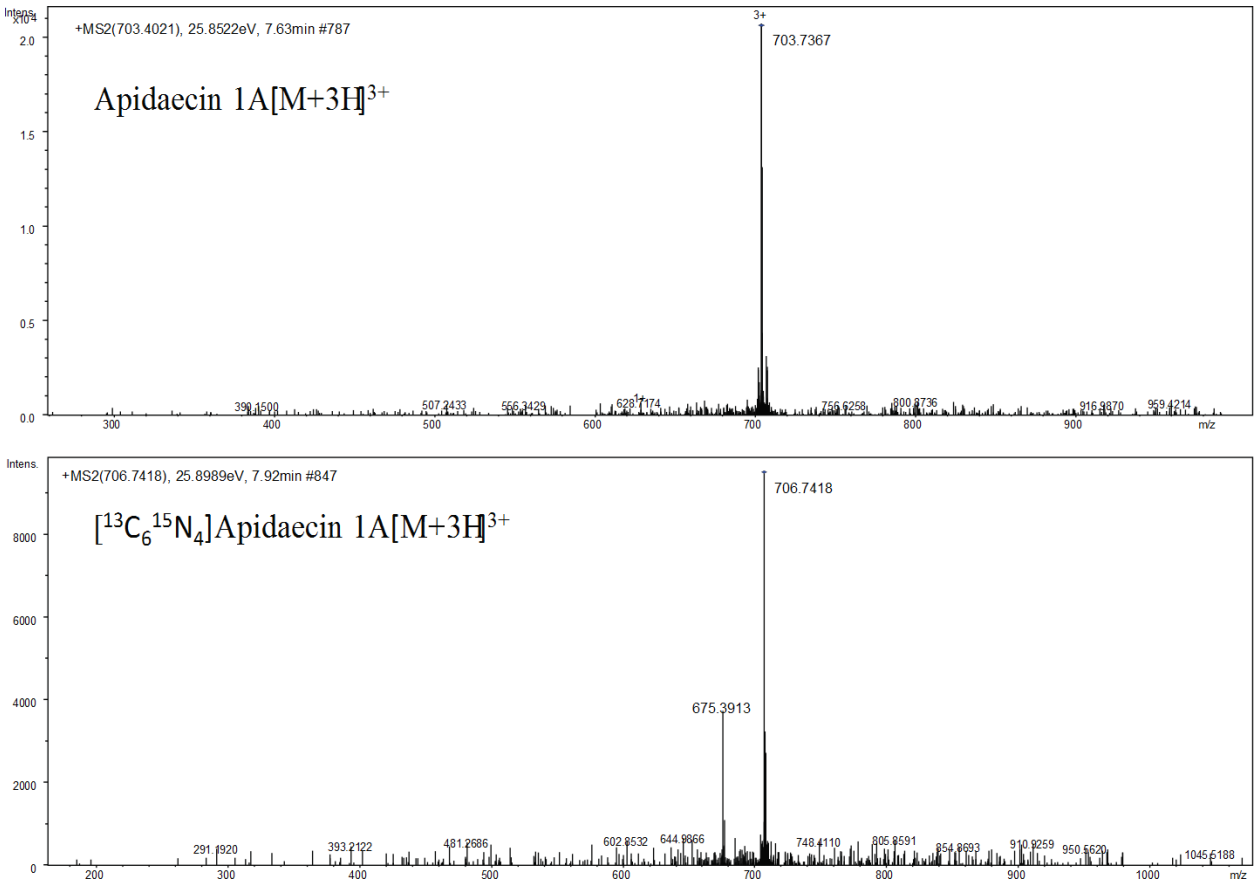
Peptide name	AA sequence	pI	Molecular weight (monoisotopic)	UniProt Entry
Apidaecin 1A	GNNRPVYIPQPRPPHPR**I**	11.71	2107.16	Q06601, P35581, Q06602
Apidaecin 1B	GNNRPVYIPQPRPPHPR**L**	11.71	2107.16	Q06601, P35581, Q06602
Apidaecin 2	GNNRP**I**YIPQPRPPHPRL	11.71	2121.17	Q06601
Apidaecin	GNNRPVYI**S**QPRPPHPRL	11.71	2210.22	Q06602

Supplementary Table 2S: Recovery achieved for the internal standard [$^{13}C_6{}^{15}N_4$]apidaecin 1A.*

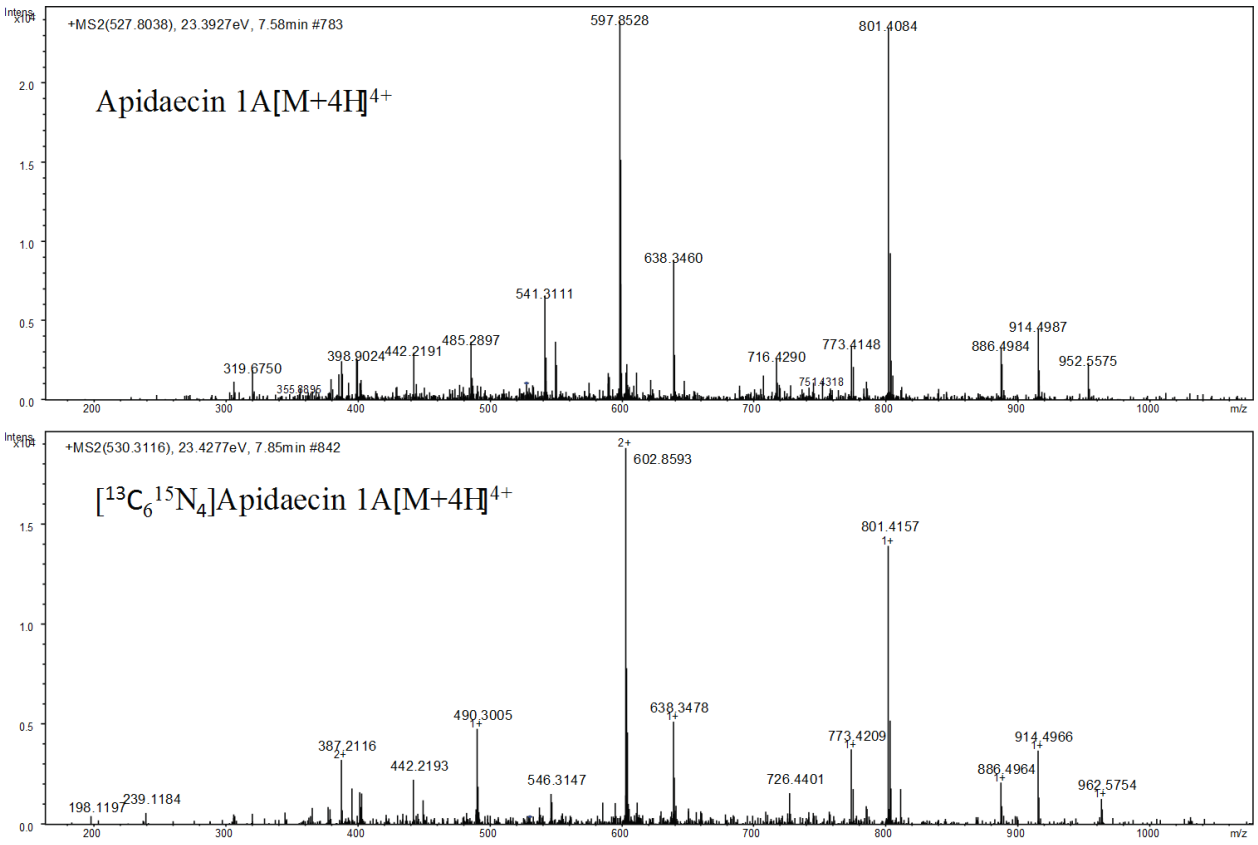
apidaecin 1A (pmol)	[$^{13}C_6{}^{15}N_4$]apidaecin 1A (pmol)	n	Recovery	
			Average (%)	SD
0.5	0.5	8	48	4.6
	1	8	41	3.0
	2.5	8	44	2.6
1	0.5	8	42	4.3
	1	8	46	9.2
	2.5	8	40	2.5
2.5	0.5	8	43	4.1
	1	8	43	3.9
	2.5	8	44	4.3

* SD is standard deviation calculated by in-build function of MS excel 2010.

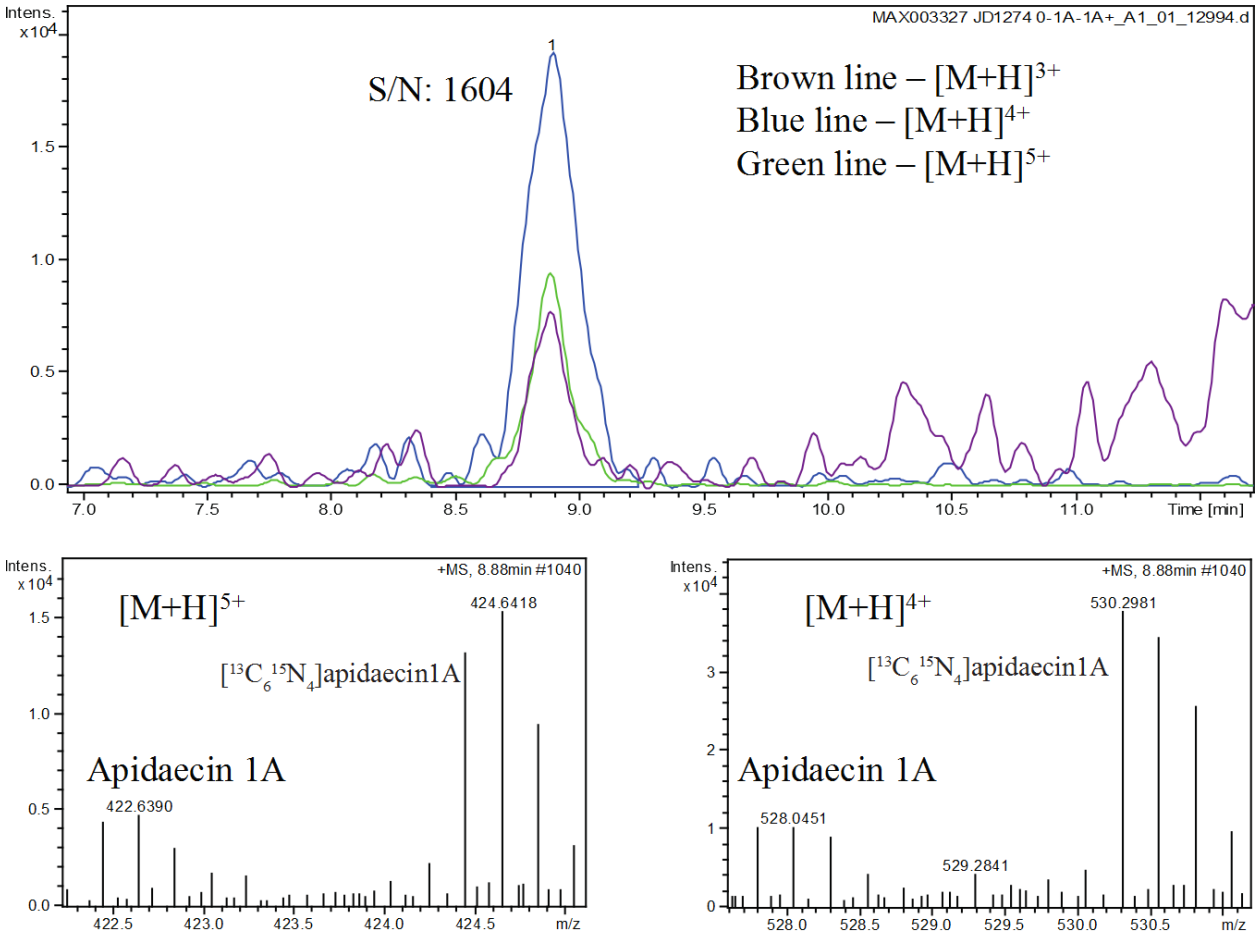
Supplementary Figure 1S: CID fragmentation spectra of triply-charged apidaecin 1A and internal standard [$^{13}C_6{}^{15}N_4$]apidaecin 1A.



Supplementary Figure 2S: CID fragmentation spectra of quadruply charged apidaecin 1A and internal standard [$^{13}C_6{}^{15}N_4$]apidaecin 1A.



Supplementary Figure 3S: A superposition of extracted ion chromatograms of three different charge states of detected apidaecin 1A at 0.1 pmol which was set up as limit of quantification. The chromatogram below shows that at least two or three different charge states of target analyte apidaecin 1A could be detected and extracted from collected MS spectra at LLOQ. The MS spectra representing intensities of $[M+4H]^{4+}$ and $[M+5H]^{5+}$ions of apidaecin 1A and internal standard $[^{13}C_6{}^{15}N_4]$apidaecin 1A at LLOQ are depicted at the bottom.



Supplementary Figure 4S: Extracted ion chromatograms and the corresponding mass spectra of target analyte apidaecin 1A at LLOQ of the described method. Chromatograms were extracted from two different nLC-MS analyses of hemolymph samples from two individual freshly emerged bees. The MS spectra of $[M+4H]^{4+}$ ions of detected apidaecin 1A are displayed on the right side of the figure.