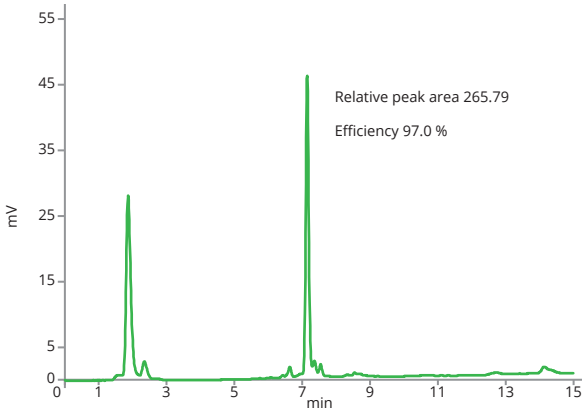
APPLICATION LIST #472

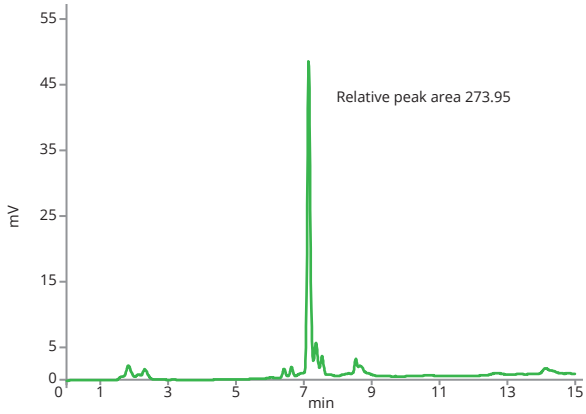
Prepurification of adipokinetic hormones

Adipokinetic hormones (AKHs) are insect anti-stress hormones that maintain the biochemical and physiological homeostasis of the insect body (Kodrík, 2008). AKHs are octa, nona- or decapeptides with both termini blocked: the N-terminus by a pyroglutamate residue and the C-terminus by an amide. Typically, a specific antibody and ELISA method are used for their quantification in the insect central nervous system and haemolymph. For the latter, prior to executing the ELISA test, several pre-purification steps are required; they also involve a solid phase extraction cartridge.

In the test, the AKH from the firebug Pyrrhocoris apterus known as Pyrap-AKH was employed. Its structure is: pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-NH2 (Kodrík et al., 2000).



Sample Separation – 160 pmol-Pyrap-AKH



Control Sample – 160 pmol-Pyrap-AKH

References

Kodrík D. (2008) Adipokinetic hormone functions that are not associated with insect flight. Physiol. Entomol. 33: 171-180.

Kodrík D., Socha R., Šimek P., Zemek R. and Goldsworthy G.J. (2000) A new member of the AKH/RPCH family that stimulates locomotory activity in the firebug, Pyrrhocoris apterus (Heteroptera), Insect Biochem. Mol. Biol. 30: 489-498.

APPLICATION LIST #472

Prepurification of adipokinetic hormones

MSPE Method

MSP column	Micro Spin SpeExtra™ column MSPE C18P 0.22 µm Nylon membrane, 15 mg, 0.7 mL
Solution A	0.11% trifluoroacetic acid (TFA) in water
Solution B	0.1% TFA in 60% acetonitrile
Centrifugation	2000 rev/min
MSPE steps	**1. Solution B, 0.6 mL** **2. Solution A, 0.6 mL** **3. Pyrap-AKH 160 pmol in Solution A, 0.6 mL** **4. The eluate applied again on the cartridge, 0.6 mL** **5. Solution A, 0.6 mL** **6. Elution with 0.3 mL Solution B and 0.3 mL 100% acetonitrile**

HPLC method

Column	Chromolith Performance RP-18e column 150 × 4.6 mm (Merck)
Mobile phase	A = 0.11% TFA in water, B = 0.1% TFA in 60% acetonitrile
Gradient	0–2 min 30 % B, 2–12 min 30–90 % B, 12–15 min 90 % B
Flow rate	1.5 mL/min
Detection	Fluorescence, wavelength Ex 280 and Em 348 nm (Waters model 2475)

This application was developed by the Biology centre CAS, České Budějovice, The Czech Republic

BIOLOGY CENTRE CAS