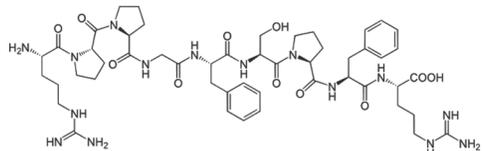
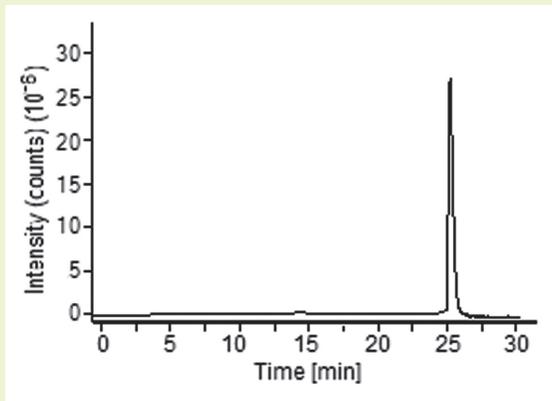


Bradykinin (BK)

Peptide analyzed in saliva sample



Bradykinin

Note: Bradykinin is a highly potent bioactive peptide. This peptide exhibits hypotensive actions (reduces blood pressure). BK has been implicated also in various shock syndromes. The peptide can be synthesized or obtained from snakes, wasps' venom, and similar sources. After the synthesis or extraction there is a need for analytical methods to assess purity of the obtained product.

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Mobile Phase: A: DI H₂O / 0.1% formic acid (v/v)
B: Acetonitrile / 0.1% formic acid (v/v)

Gradient:	time (min.)	%B
	0.0	90
	5.0	90
	10.0	70
	20.0	60
	20.1	30
	30.0	30
	30.1	90

Post Time: 3 min

Injection vol.: 2µL

Flow rate: 0.4 mL/min

Detection: LC-ESI/MS was performed using a Thermo Finnigan SpectraSystem HPLC

Sample: Saliva sample was prepared according to Vickers *et al.* [1], with one modification: Instead of 0.1M orthophosphoric acid, 0.1% formic acid was used to stabilize BK.

Peak: 1. Bradykinin: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, 3+ charge

t₀: 0.9 min

Discussion

A simple, sensitive, and specific gradient method was developed for the quantification of Bradykinin in saliva. In addition to the intact peptide, the gradient applied in the developed method was designed to analyze its impurities and metabolites.

Robustness of the presented analysis against small modifications in pH, flow rate and percentage of the mobile phase composition was investigated. It was determined that none of the factors studied had a significant effect (at $\alpha=0.05$ level) on the retention of BK in saliva samples.

[1] "High-performance liquid chromatographic determination of bradykinin in saliva: a critical review and a new method", E.R. Vickers, C. Goebel, L.E. Mather, L. Mackay, R.J. Wells, J. of Chromatography B: Biomedical Sciences and Applications, Volume 755, Issues 1-2, 5 May 2001, Pages 101-110.