Purity assessment of melittin isolated from honeybee venom using a YMC-Triart Bio C18 column

Melittin is the major bioactive peptide in honeybee venom and is responsible for many of its reported anti-inflammatory, antimicrobial, and anticancer properties.

However, crude bee venom also contains allergenic components such as phospholipase A2 (PLA-2) and apamin, making high-purity melittin essential for pharmaceutical research and therapeutic applications.

This Application Note, based on the work of Tabesh et al. [1], demonstrates the analytical purity assessment of melittin purified from honeybee venom using green organic mobile phase modifiers. The analysis was performed using a wide-pore YMC-Triart Bio C18 column, providing efficient separation of melittin from the major allergenic impurities.

The analysis of crude honeybee venom reveals melittin as the major peptide component, alongside several impurities, including the allergens apamin and PLA-2 (Figure 1). The chromatographic profile highlights the complexity of the crude venom matrix, with an initial melittin purity of approximately 60% [1].

Table 1: Chromatographic conditions

Column:	YMC Triart Bio C18 (5 µm, 30 nm) 150 x 4.6 mm ID
Part No.:	TA30S05-1546WT
Eluents:	A) water + 0.1% TFA*
	B) acetonitrile + 0.1% TFA*
Gradient:	20-80%B (0-10 min), 100%B (10-14 min), 20%B (14-19 min)
Flow rate:	1 mL/min
Injection:	3 µL (200 ng/µL)
Detection:	UV at 214 nm
Sample:	bee venom feed (1 g/L)

* TFA: trifluoroacetic acid

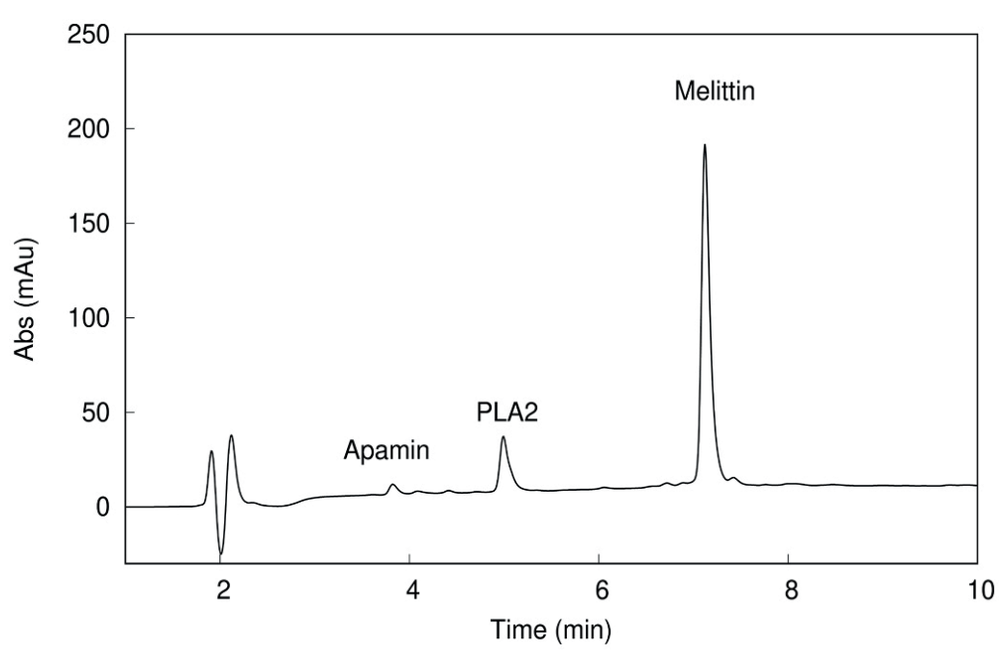

Figure 1: RP-HPLC analysis of crude honeybee venom (1 g/L).

Following purification using a green mobile phase composed of dimethyl carbonate (DMC) and isopropanol (IPA) [2], the major allergenic impurities were effectively removed (Figure 2). Reliable purity assessment and effective monitoring of the purification process were enabled by the excellent selectivity of the YMC-Triart Bio C18 column, which provided baseline separation of melittin from the remaining venom components.

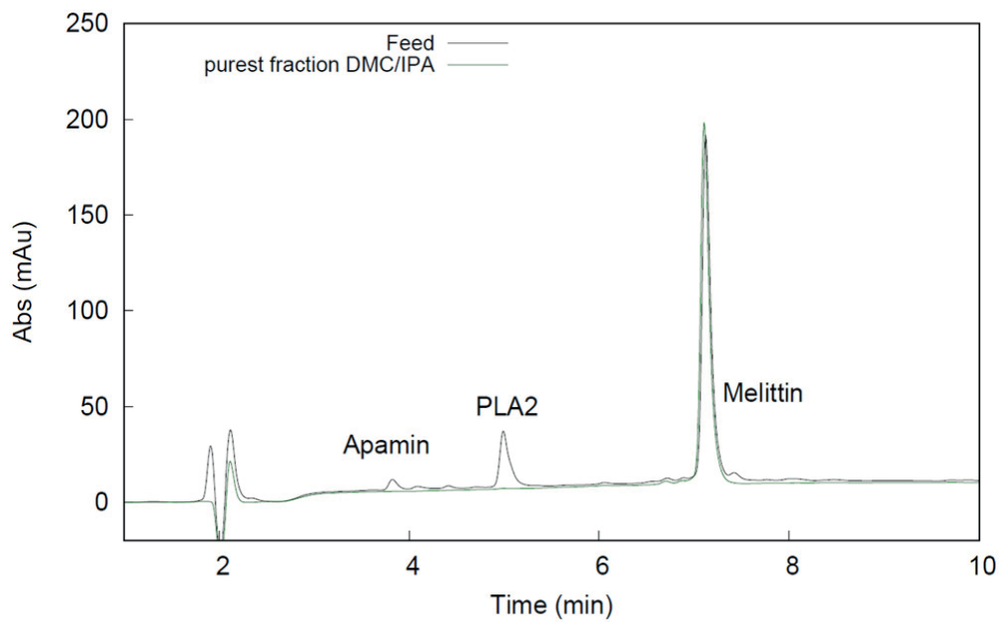

Figure 2: RP-HPLC comparison of crude honeybee venom feed (black) and melittin purified using a green DMC/IPA-based purification method (green).

References

[1] Tabesh, A., De Luca, C., Faraji Shovey, A., Canton, R., Catani, M., Cavazzini, A., Rezadoost, H., Nosengo, C., & Felletti, S., Expanding the use of green solvents for the isolation of melittin from honeybee venom. J. Chromatogr. A, 2025, 1763, 466460

[2] YMC Application Note: Increasing focus on greener eluents in preparative chromatography

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