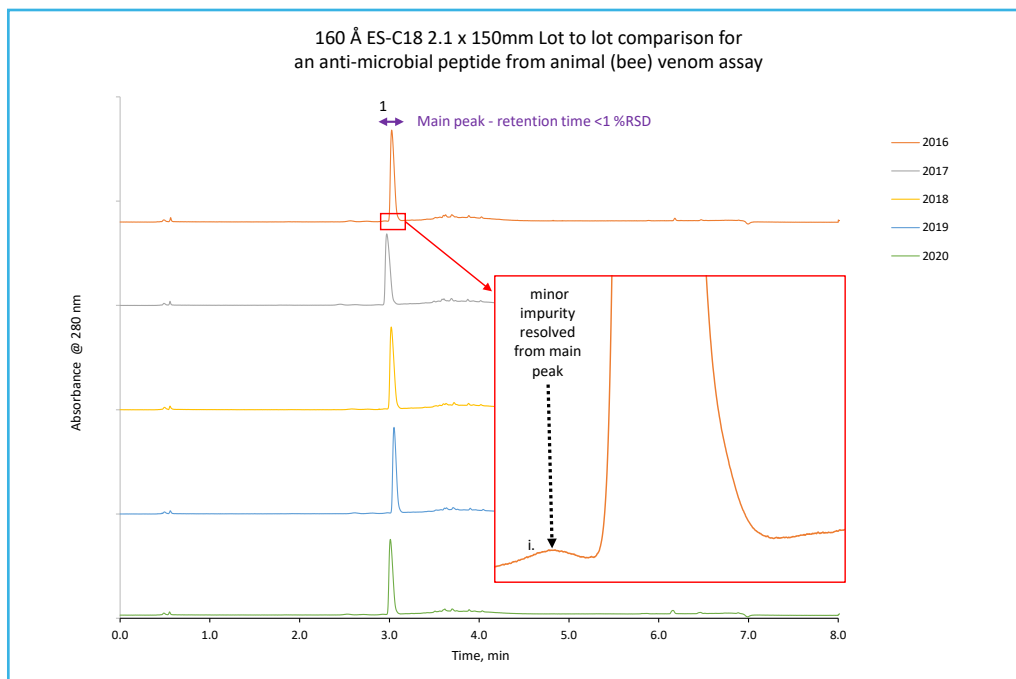




Peptide Analysis of Bee Venom Assay for Antimicrobial Properties Using HALO® Peptide

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PEAK IDENTITIES:

1. Melittin
- i. Impurity of a honey bee venom standard

TEST CONDITIONS:

Column: HALO 160 Å C18, 2.7 µm, 2.1x150mm

Part Number: 92122-702

Mobile Phase: **A:** Water/0.1% TFA
B: ACN/0.1% TFA

Gradient:	Time	%B
	0.0	40
	2.0	40
	6.0	100
	6.1	100
	6.2	40
	7.0	40

Flow Rate: 0.6 mL/min

Pressure: 408 bar

Temperature: 60 °C

Detection: 280 nm

Injection Volume: 1 µL

Sample Solvent: Water/ 0.1% TFA

Data Rate: 100 Hz

Response Time: 0.025 sec

Flow Cell: 1 µL

LC System: Shimadzu Nexera

Antimicrobial peptides in animal venom (vAMPs) are natural antibiotics of emerging interest. As resistance over conventional antibiotics has become an area of concern, vAMPs are key alternative early drug discovery candidates. An assay of melittin from honey bee venom completed in <10 min (total analysis time) was demonstrated on five different manufactured HALO® 160 Å ES-C18 lots (2016, 2017, 2018, 2019, and 2020) illustrating the separation profile reproducibility over a five-year period.

The main active vAMP component in honey bee venom was resolved from minor related impurity peaks (unidentified) with a retention time reproducibility of <1% RSD. Furthermore, a closely related low abundant impurity peak could be separated. Critical aspects are achieved with HALO® column technology to develop reliable assays to support biomedical, and drug development research of vAMPs' physiological role in human diseases, as well as microbial and parasitic infections.

