HILIC: A Complementary Chromatographic Approach for Studying Small Molecules in Bothrops jararaca Venom

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Snake venoms are widely studied due to snakebite incidents and are classified by the WHO as neglected diseases. While the proteins in venoms have been well studied and characterized, low-mass molecules are still relatively unexplored, primarily due to their low abundance, shorter role in envenomation, and limitations of analytical techniques. However, studying these small molecules can provide valuable insights into the molecular structure, functions, and evolutionary relationships of venom toxins. This study aimed to investigate low-mass molecules (<10 kDa) in *Bothrops jararaca* venom by comparing two chromatographic methods. The venom was processed using ultrafiltration (<10 kDa) to isolate the small molecules, which were then analyzed using reverse-phase chromatography (RP-HPLC), hydrophilic interaction liquid chromatography (HILIC), and mass spectrometry (MALDI-TOF and LC-MS/MS). The analyses were conducted analytically, using the same sample concentration, the same running method, and the same column specifications for the comparison of the two chromatographic approaches. The chromatographic profiles revealed a greater diversity of peaks with HILIC compared to RP-HPLC. MALDI-TOF analysis of the low-mass material showed molecules ranging from 1000 to 1500 Da. In LC-MS/MS, HILIC demonstrated better ionization of the molecules and identified approximately twice as many molecules as RP-HPLC. Additionally, HILIC revealed families of BPPs molecules and metallopeptidase fragments, while RP-HPLC detected BPPs and L-amino-acid oxidase fragments. De novo analyses showed a significant difference in the number of molecules identified: RP-HPLC found 29 molecules, while HILIC identified 59 sequences. In summary, although RP-HPLC is the most traditional approach for studying molecules in snake venoms, HILIC demonstrated superior performance in the analysis of low-mass molecules in *B. jararaca*, showing that these molecules have amphiphilic characteristics and a better tendency for hydrophilic separation. This study suggests that HILIC can be a valuable tool for the discovery of low-mass molecules, contributing to the isolation and characterization of these molecules and, consequently, to the discovery of new biological activities.

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