Optimization and application of several assays screening for PLA2 in snake venom
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Abstract

Nanofractionation is a relatively new technique where a solution is separated by high-performance liquid chromatography (HPLC) and collected in small fractions of several microliters. In this study this technique was used to separate eight snake venoms. The fractions were collected in 384 well plate and freeze-dried. These plates were tested with an assay for the presence of PLA2s. Three assays were optimized and tested for their applicability on testing snake venoms for PLA2s, two colorimetric assays with bromothymolblue or cresol red as indicator and one fluorescent assay with fluorescein as indicator. The bromothymol blue assay deemed to be unsuited as the substrate, phosphatidilcholine, forms salvation complexes with the bromothymolblue interfering with the absorption of bromothymolblue. The cresol red assay gave better results for higher venom concentrations and the fluorescein assay for lower concentrations. Cresol red was chosen to test the venoms with which produced repeatable bioprofiles that prove that this method can be used to screen snake venoms for PLA2s.