OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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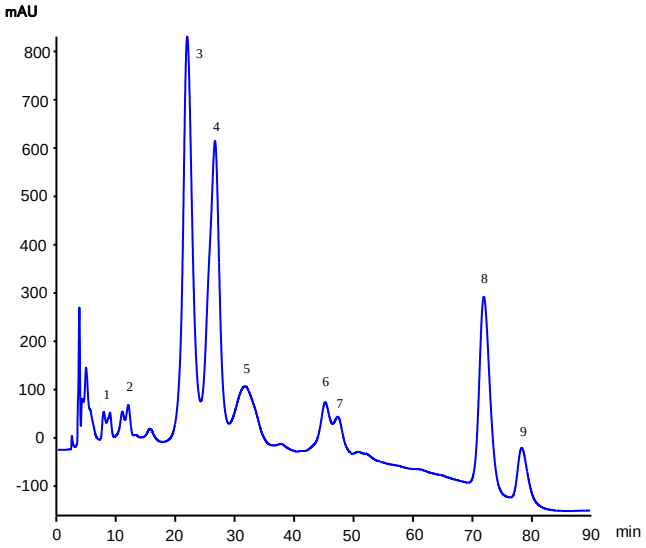
APPLICATION NOTE

Hydrophobic Interaction Chromatography: Separation of Various Snake Venom

Small variations of proteins and peptides can be seen during the separation of Snake Venom on HIC mode.
Here we have chosen an HIC-Ether column of 150 mm of length and 4.6 mm of diameter to separate 4 different venom of different species.
With only 20 µl of sample the chromatogram shows a sensitivity of up to 800 mAU and therefore small quantities of eluents can be seen.
In the first chromatogram of Malay Cobra up to 9 peaks are seen.
It is important to note that the linear velocity for the separation is 180 cm/hr.
The column volume is low: 2.5 ml as opposed to above 3 ml. This allows smaller sample quantities to be run and still get high sensitivities.

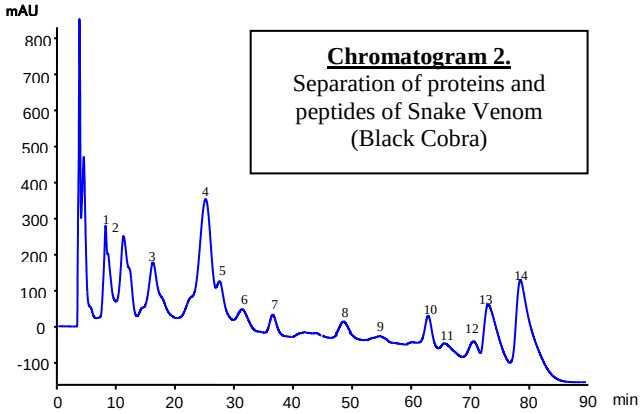


Chromatogram 1.
Separation of proteins and peptides of Snake Venom
(Malay Cobra)

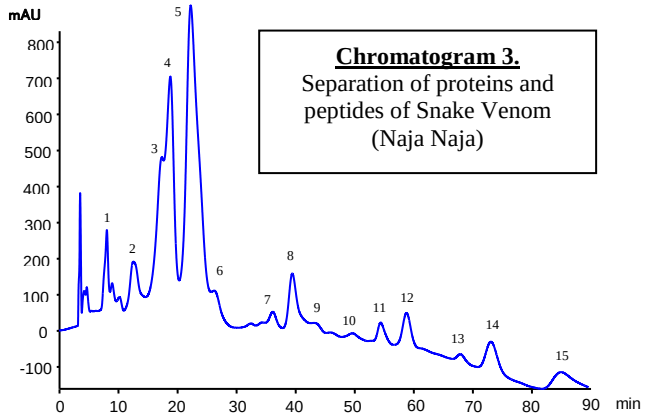
The columns are run at volumetric flow rates of 0.5 ml/min. The linear flow rate is 180 cm/hr.
The chromatograms show distinctly different entities.

Table 1. Operating parameters.

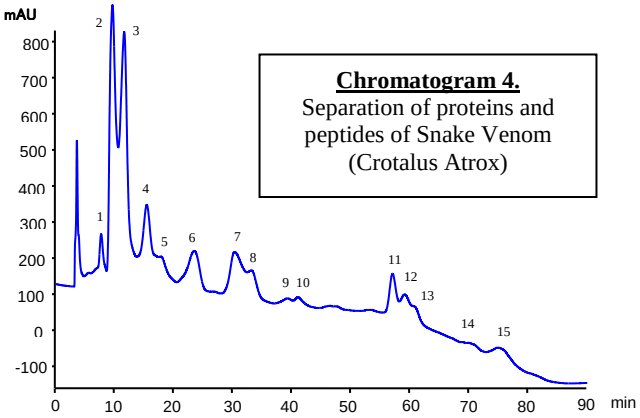
HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ HIC-Ether/XH** 4.6 X 150 mm(v=2.49ml)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 2.1 M SO4(NH4)2, pH=7
Flow rate	0.5 ml/min (180 cm/hr)
Gradient	90 % B for 2 min to 0 % B in 90 min (18 cv)
Temperature	30°C
Detection	214 nm
Injection volume	20 µl
Sample:	10 mg/ml each in buffer A.



Chromatogram 2.
Separation of proteins and peptides of Snake Venom
(Black Cobra)



Chromatogram 3.
Separation of proteins and peptides of Snake Venom
(Naja Naja)



Chromatogram 4.
Separation of proteins and peptides of Snake Venom
(Crotalus Atrox)

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