OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

Hydrophobic Interaction Chromatography: Desalting and Concentration.

Proteins have been desalted and concentrated in a fast mode and in an efficient way with high yield rather than using the lengthy methods of precipitation, centrifugation and dialysis.

The present chromatogram shows the separation of a Monoclonal Antibody (MAb) on a 4.6 x 100 mm HIC-Butyl column.

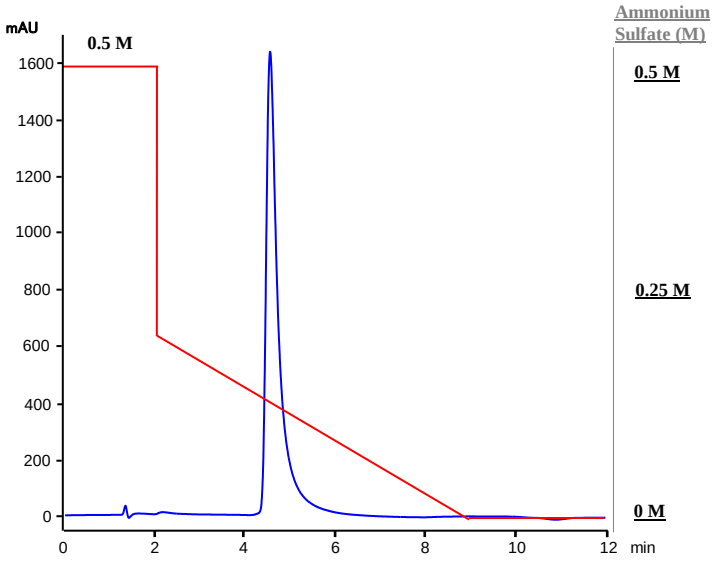

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ HIC-Butyl/XH 4.6 X 100 mm (v=1.66ml)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 0.5 M SO4(NH4)2, pH=7
Flow rate	1 ml/min (360 cm/hr)
Gradient	100 % B for 2 min. 30 to 0 % B to 9 min. 0 % B to 12 min.
Temperature	30°C
Detection	214 nm
Injection volume	5 µl (21µg)
Sample:	Human IgG from Sigma. (4.2 mg/ml in buffer A)

As noted in the chromatogram the IgG peak is sharp and elutes in less than one minutes registering 1600 mAU of sensitivity at 214 nm.
The amount of sample is 21 µg for the injection.
This allows for the batch that is processed to be desalted and at the same time concentrated very efficiently.
The starting buffer is of 0.5 molar concentration and the protein elutes between 0.2 and 0.1 M of salt that is considerably faster than dialysis.

It is to be noted that this process is only intended for concentration and desalting of the protein and would not be used to further separate the components of a mixture.

It is a mild process that does not involve organic solvents and therefore is not prone to denature the proteins.

Additionally the media has remarkably low back pressure and therefore can be a good candidate for Simulated Moving Bed separation making it a continuous process rather than a discontinuous one.

The polymeric hard gel nature of the product can also be taken advantage of to run it at flow rates of well above 180 cm/hr that is typical for a column of 4.6 mm diameter ID.