OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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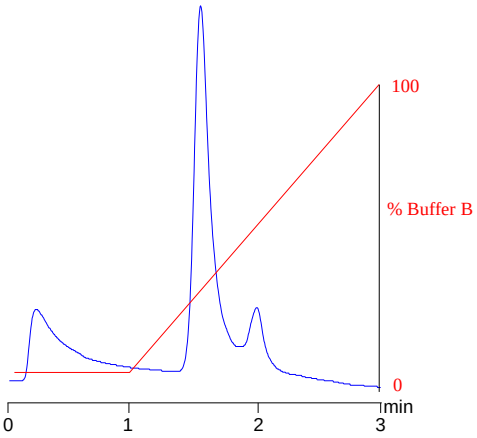
APPLICATION NOTE

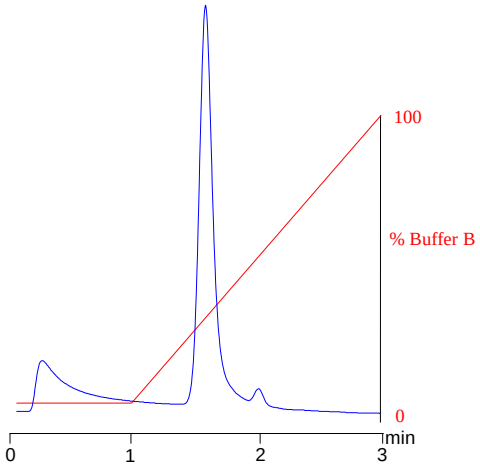
Fast Separation of IgG on STYROS™ HQ: *High Capacity Polymeric Gigaporous Strong Anion Exchanger.*

The use of Immunoglobulins preparations made by cold ethanol fractionation has been associated with vasoactive reactions that range from pain at the injection site to flushing, anxiety, and even hypotension.
Additional purification steps must be implemented in order to increase the purity of the products.
The present example provides an enhanced testing procedure to gage the effectiveness of the purification method, by rapidly identifying the end products using STYROS™ HQ liquid chromatography column.
A sample of fraction II + III W of the cold-ethanol fractionation of plasma is run using a short 5 cm strong anion exchanger column with high dynamic capacity (100 mg/ml BSA).



Chromatogram 1
STYROS™ HQ/XH 4.6 X 50 mm
Fraction II + III W from cold ethanol fractionation of plasma.

The above fraction is subjected to a number of additional purification steps to yield the final product.
The same chromatographic method could compare the outcome of the process during a 3 minute run.
Although the purity of the monomeric form of IgG (major peak) has increased from 60 % to 70 %, the presence of up to 30 % of polymers and dimers as well as other fragments is still noticeable.
The chromatographic conditions are summarized in table1.



Chromatogram 2
STYROS™ HQ/XH 4.6 X 50 mm
Purified Immunoglobulin sample.

Table 1. Operating parameters for the chromatograms.

HPLC System.	HP 1100
Column	STYROS™ HQ/XH 4.6 X 50 mm
Mobile Phase	A: 20 mM TRIS, pH = 8 B: 20 mM TRIS, 1 M NaCl, pH = 8
Flow rate	5 ml/min (1,800 cm/hr)
Gradient	As indicated.
Temperature	30°C
Detection	280 nm
Injection volume	5 µl
Sample:	As indicated.

A viable alternative to the cold ethanol precipitation or slow batch chromatographic separation with soft gel, is the use of hard gel polymeric media to run continuous chromatographic techniques such as *Simulated Moving Bed chromatography.*
STYROS™ media is well suited for such continuous processes. High dynamic capacity as well as high resolution helps minimize the size of the bed and therefore the number of columns required for the process.
The mechanical stability of the media allows faster flow rates and shorter separation time. The chemical stability within the full pH range provides unrestricted use of solvents and buffer solution, as well as CIP and sanitization procedure.