OraChrom, Inc.
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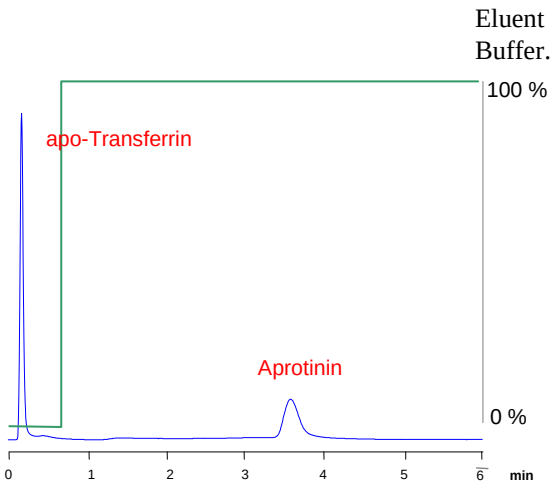
APPLICATION NOTE

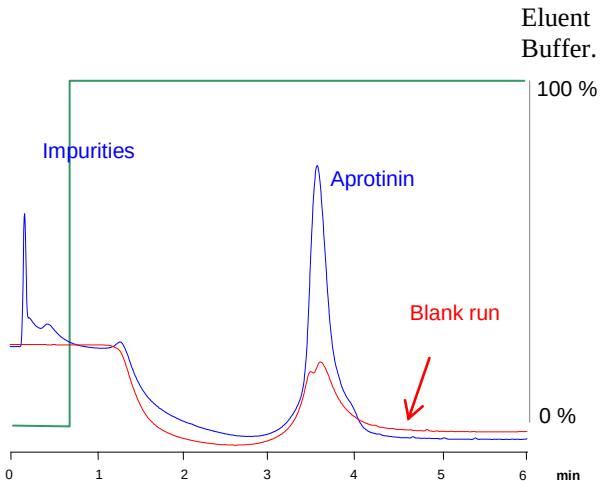
On Line Affinity capture of APROTININ on StyrosZyme™ TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Stationary Phase.

Aprotinin is a naturally occurring polypeptide found in tissues and blood. The highest concentrations are in bovine parotid gland, pancreas and lung. It consists of a single chain 58 amino-acid residue.
As a small and stable pancreatic serine protease inhibitor, the isoelectric point of Aprotinin is at pH 10.5. It is stable in neutral or acid media at high temperature. Irreversible changes occur in molecular structure at pH > 12.
Using **StyrosZyme™ TPCK-Trypsin**, a gigaporous polymeric hard gel stationary phase with immobilized Trypsin, we have developed an on line affinity method to specifically capture Aprotinin from a mixture in a simple capture-elution mode:



Capture of Aprotinin from a mixture of apo-Transferrin and Aprotinin, run at 2 ml/min (3,500 cm/hr) on a 2.1 x 50 mm **StyrosZyme™ TPCK-Trypsin** column.

It is to be noted that the elution occurs at pH 11.2 after 41 column volume wash with the elution buffer. The pH is prohibitive for Silica based stationary phase as is the pressure for soft gel media.
Similar separations can be achieved with commercial samples of Aprotinin in order to evaluate their purity.
The next chromatogram shows one such sample being evaluated by the present method.
Keeping the baseline of a blank run in the background, one can assess the amount of impurity the sample contains and eventually proceed in purifying it.
Considering the high capacity of the resin and its strong and specific binding, this method can be used in the bioseparations of biological fluids.



Capture of Aprotinin from a commercial sample of Aprotinin, run at 2 ml/min (3,500 cm/hr) on a 2.1 x 50 mm **StyrosZyme™ TPCK-Trypsin** column.

Operating parameters for the chromatograms.

HPLC System	Agilent 1100, Standard Cell
Columns	**StyrosZyme™ TPCK-Trypsin,** 2.1 x 50 mm
Binding buffer	50 mM Citrate, 50 mM NaCl, pH = 7
Eluent buffer:	50 mM Citrate, pH = 11.2
Detection:	285 nm
Flow rate:	2 ml/min (3,500 cm/hr)
Temperature	25 °C
Injection volume	20 µl
Samples:	As indicated

The availability of **StyrosZyme™** in small column formats allows to capture and effectively concentrate trace amounts of Aprotinin from any sample including biological fluids.
The strong specific binding of the media allows runs to be carried at linear velocities of 3,500 cm/hr and therefore results in short elution times.

Inc.