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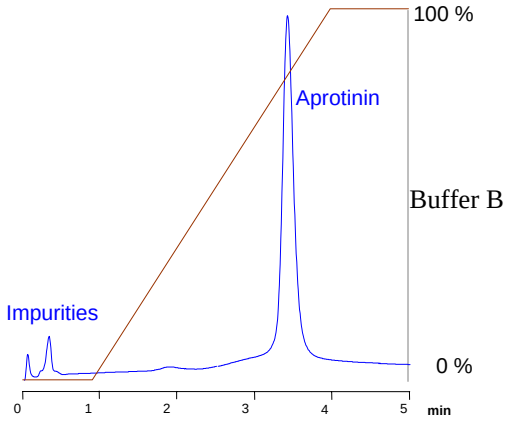
APPLICATION NOTE

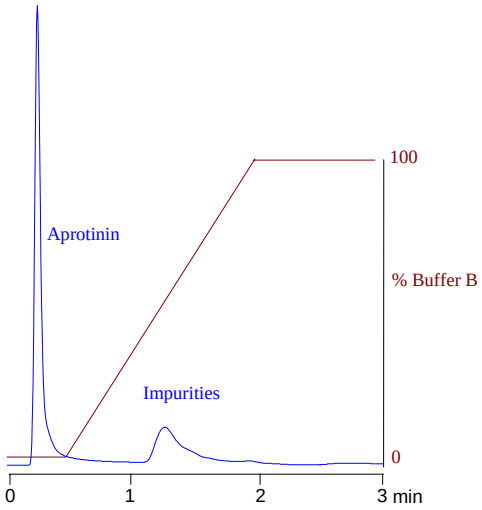
APROTININ Purity Test on STYROS™ Anion and Cation Exchanger. Polymeric Hard Gel Stationary Phase.

Often times the end user needs to assess the purity of a protein sample in a fast and reliable way.
In the present application we have used ion exchange stationary phases to test commercial samples of Aprotinin tested by the manufacturer as having trace impurities.
Ion exchange resins separate biomolecules on the basis of differences in charge characteristics: either cationic or anionic. Such charges are dependent on the pH of the buffer system as well as the isoelectric point (pI) of the biomolecules.
If the buffer pH is below the pI, the separation is done on a cation exchanger. If the buffer pH is above the pI, an anion exchanger is used.
Aprotinin has a pI of 10.5, it will therefore only be retained on cation exchanger with buffers having pH's below its isoelectric point.
The impurities however, can have different pI as is the case here.
Using STYROS™ SP/XH (strong macroporous polymeric high capacity cation exchanger) at pH 7, one can separate the bulk of the protein from its impurities.



Chromatogram of a commercial sample of Aprotinin, run at 2 ml/min (720 cm/hr) on a 4.6 x 50 mm STYROS™ SP/XH column.

The protein elutes in less than 4 minutes after only 8 column volume of salt gradient. To be certain the peaks labeled as impurities are not the result of the surface saturation, the resin should have high dynamic binding capacity. STYROS™ SP has 100 mg/ml of protein capacity measured with Lysozyme. It would therefore require substantial amount of binding protein to reach the saturation point.
This test can be double checked by using an affinity surface specific to Aprotinin. See Application 36 (AN120904) for the on line affinity capture of Aprotinin on StyrosZyme™ TPCK-Trypsin.
The use of an Anion exchanger provides a different profile. Here at pH 8 Aprotinin is not retained. The impurities however, are. The bulk of the protein of interest can therefore be isolated at the beginning of the run in a concentrated form and in absence of salt.
The issue of capacity is also important in this case. In contrast to soft gel with minimal capacity, STYROS™ DEAE provides up to 80 mg/ml of dynamic BSA capacity. This minimizes the contamination of the target protein by the impurities co-eluting with it as a result of the column saturation.



Chromatogram of a commercial sample of Aprotinin, run at 3 ml/min (1,100 cm/hr) on a 4.6 x 50 mm STYROS™ DEAE/XP column.

Operating parameters for the chromatograms.

HPLC System	Agilent 1100, Standard Cell
Cation exchanger: Anion exchanger:	STYROS™ SP/XH 4.6X50 mm, STYROS™ DEAE/XP 4.6X50 mm
Cation exchange buffer	A: 50 mM Phosphate, pH= 7 B: A + 1 M NaCl, pH= 7
Anion exchange buffer:	A: 50 mM Tris, pH= 8 B: A + 1 M NaCl, pH= 8
Detection:	285 nm
Flow rate:	As indicated
Temperature	20 °C
Injection volume	20 µl
Sample:	Commercial Aprotinin