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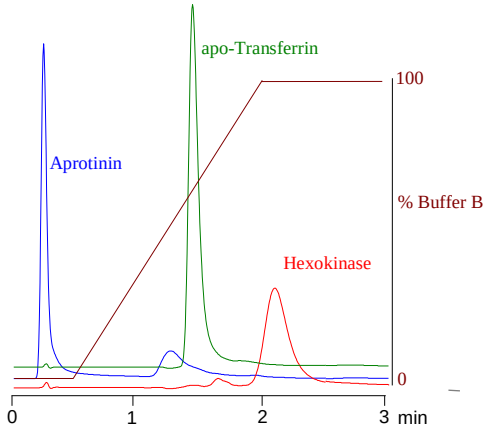
# APPLICATION NOTE

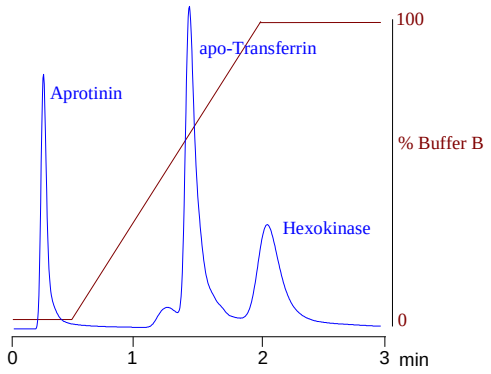
## Separation of APROTININ from apo-Transferrin and Hexokinase on Polymeric Hard Gel Anion-Exchange Columns.

The quest for stable rigid macroporous polymeric media has yielded to a number of stationary phases with similar mechanical stability than Silica. The chemical stability, however, far exceeds that of its Silica counterpart.
In this application note we have shown the use of high capacity (80 mg/ml BSA. Dynamic) **STYROS™ DEAE** column to separate three proteins with important commercial use.
Using high flow rates (1,100 cm/hr of linear velocity) it is possible to find the optimum conditions for the separation in a short time.
The following comparative chromatograms show the separation of the tree proteins during 3 minute runs.



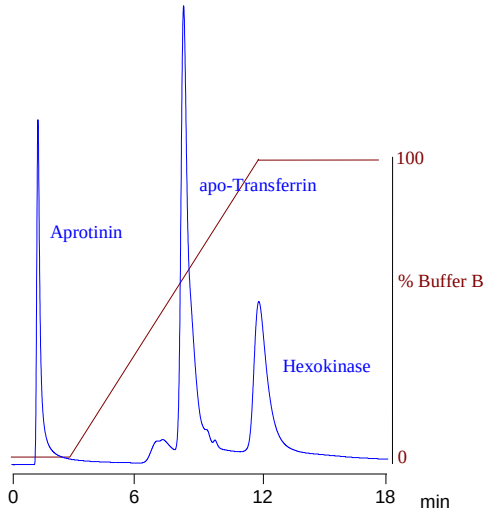

Chromatograms of Aprotinin, apo-Transferrin and Hexokinase, run at 3 ml/min (1,100 cm/hr) on a 4.6 x 50 mm **STYROS™ DEAE** column.

The high mechanical stability contrasts sharply with soft gel media that lacks the rigidity required to support high back pressures resulting from increased flow rates.
The media's capacity does also play an important role during the scale up from analytical to semi preparative and even full production.
In the present application, the retained proteins (apo-Transferrin and Hexokinase) are eluted with high salt concentrations. This gives substantial control to the operator in separating each of the mixture's components without being concerned over the displacement of the low binding eluents by the high binding ones.
The mixture of the three proteins is separated at the same flow rate of 1,100 cm/hr.
The baseline separation only takes 3 minutes. The column does not reach its pressure limit during the run.




Mixture of Aprotinin, apo-Transferrin and Hexokinase, run at 3 ml/min (1,100 cm/hr) on a 4.6 x 50 mm **STYROS™ DEAE** column

At soft gel flow rates of 180 cm/hr, the separation remains the same; the time however is increased to 18 minutes that is without taking into consideration the time required to recondition the column.




**Operating parameters for the chromatograms.**

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|---|---|
| HPLC System | Agilent 1100, Standard Cell |
| Columns | **STYROS™ DEAE/XP** 4.6 x 50 mm. |
| Buffer A | 50 mM TRIS, pH = 8 |
| Buffer B | A + 1 M NaCl, pH = 8 |
| Detection: | 285 nm |
| Flow rate: | 3 and 0.5 ml/min |
| Temperature | 20 °C |
| Injection volume | 20 µ l |
| Sample: | Aprotinin, apo-Transferrin, Hexokinase 2mg/ml each in buffer A. |

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