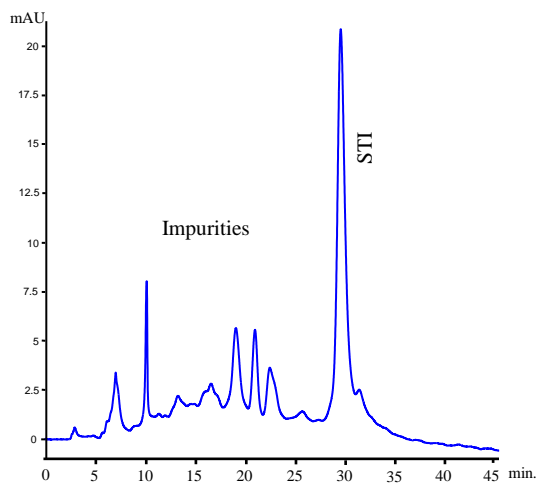


APPLICATION NOTE

Separation of Soybean Trypsin Inhibitor with STYROS™ HQ Simulated Monolith™ Polymeric.

The following chromatogram shows the separation of a pre-purified sample of Trypsin Inhibitor from Glycine max (soybean) on a **STYROS™ HQ/XH Simulated Monolith™** column.



Chromatogram 1
Pre-purified STI on **STYROS™ HQ/XH**
4 x 300 mm at 1 ml/min (478 cm/hr)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment and quaternary pump.
Columns	STYROS™ HQ/XH 4 X 300 mm (3.77 ml)
Mobile phase.	A: 20 mM Tris, pH=8.2 B: A + 1 M NaCl, pH= 8.2
Flow rates	1 ml/min (478 cm/hr of linear velocity)
Gradient	0 to 40 % B in 12 cv
Temperature	30°C
Detection	280 nm
Injection volume	30 µl
Pressure Drop	22 bar (320 psi)
Sample:	5 mg/ml of pre-purified STI in buffer A.

The back pressure of the 300 mm column is similar to a 50 mm Monolith. The resolving power however is substantially higher.

Such low back pressures make these columns very appropriate for process scale pumps.

The sizes of the columns are not limited either. Unlike Monoliths with high back pressures the Simulated Monolith™ columns can be made in all sizes including preparative columns with low back pressures.

The Simulated Monolith™ columns are ideal for Simulated Moving Bed Chromatography to generate continuous separation processes.

The high capacity of the resin allows the use of salt at the start of the gradient and an overall lower salt for the complete elution while retaining the shape of the peak. No fronting or tailing is seen.

The benefits that Simulated Monolith™ columns can bring to the process can be summed up as follows:

- Absence of leachables
- High chemical stability
- High physical stability
- Availability in different sizes
- High resolution at low and high flow rates
- Low back pressures
- Tolerant to rapid changes of buffer
- High capacity
- Possibility of CIP
- Extended lifetime
- High pressure tolerance
- Availability in most chemistry
- A first step towards process scale separations

A direct comparison with of similar size Monolith columns of 4.6 x 50 mm provides a better picture.

	Simulated Monolith™	Monolith
<i>Maximum operating pressure</i>	3000 psi (21 MPa)	1200 psi (8.2 MPa)
<i>Maximum operating temperature</i>	70 °C	70 °C
<i>Recommended flow rate</i>	Up to 1,740 cm/hr	Up to 540 cm/hr
<i>Maximum flow rate</i>	1,800 cm/hr	720 cm/hr
<i>Typical back pressure at 1ml/min</i>	44 psi	290 psi
<i>Solvent compatibility</i>	All HPLC solvents	Most HPLC solvents
<i>pH range</i>	1-14	2-12
<i>Capacity (BSA)</i>	90 mg/ml	18 mg/ml
<i>Buffer changes</i>	No restrictions	Restricted

