Agilent Anion-Exchange Media for Proteins

Technical Overview

Introduction

Agilent PL-SAX combines rigid macroporous polystyrene/divinylbenzene (PS/DVB) polymer matrix and chemically stable quaternized polyethyleneimine coating that allows the analysis of biomolecules over a wide range of mobile phase conditions and pH. The physical stability of the media permits their use with high eluent flow rates and high speed gradients for very rapid separations. The excellent stability of PL-SAX ensures rapid equilibration between separations using aggressive cleanup procedures employing high salt, NaOH, mineral and organic acids, and a wide range of organic solvents. Some examples of the use of PL-SAX, with 1000Å and 4000Å pore sizes, are given here.

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Fast protein fingerprinting of snake venoms on PL-SAX 1000Å

The characterization of elution profiles of complex aqueous biological matrices such as snake venom is accomplished in minutes using the open pore structure of PL-SAX columns. Sample preparation of venom often involves only dilution and filtration. These crude samples from the monocellate (Thailand) cobra (*Naja naja kaouthia*) (Figure 1) and western diamondback rattlesnake (*Crotalus atrox*) (Figure 2) were chromatographed to produce very distinct protein elution profiles. It is possible using this 40 min gradient to fractionate the sample and identify biologically active components in each of the venom samples.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.35 M NaCl, pH 8
Gradient: Linear 0-100% B in 40 min
Flow Rate: 1 mL/min
Detection: UV, 280 nm

The venom samples were diluted with Eluent A and filtered prior to the analysis.

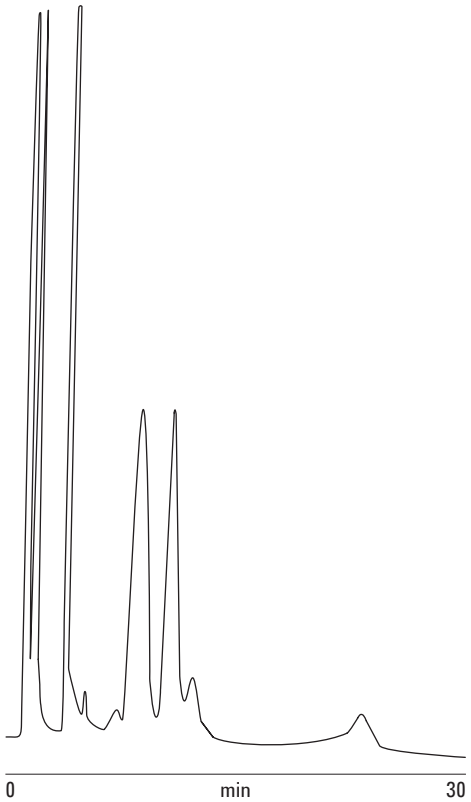

Figure 1. Raw data chromatogram of a cobra venom on Agilent PL-SAX 1000Å.

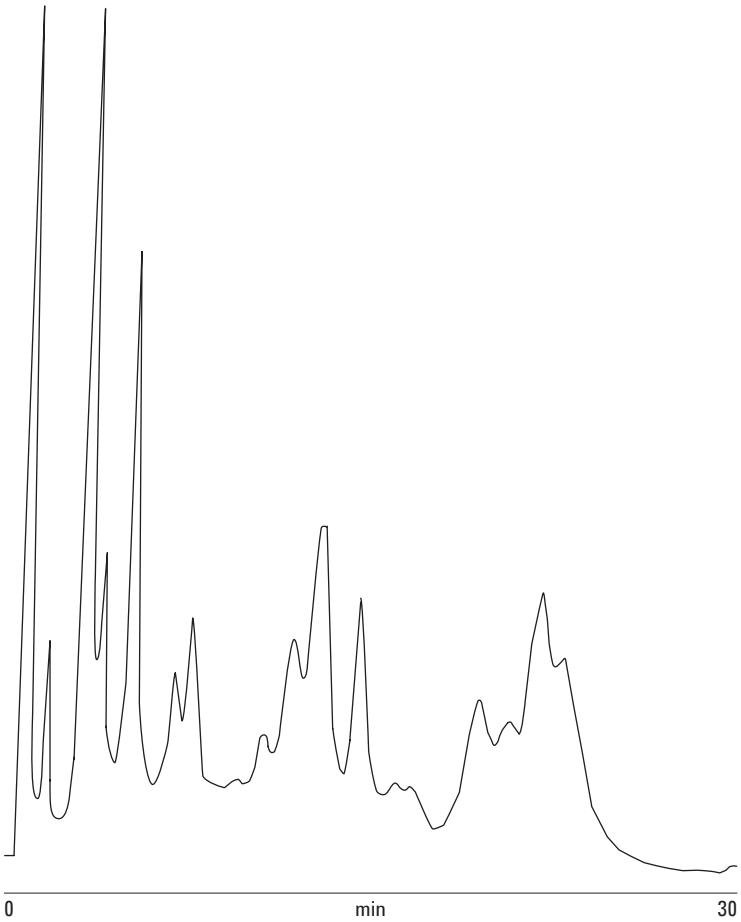

Figure 2. Raw data chromatogram of a rattlesnake venom on Agilent PL-SAX 1000Å.

Basic proteins

The quaternary amine functionality and pH stability of the PL-SAX adsorbent enables anion-exchange separations to be carried out at high pH. For the analysis of trypsinogen (pI 9.3) and α-chymotrypsinogen (pI 8.3), an eluent pH of 11 is used to ensure that the trypsinogen has a net negative charge.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.01 M Tris HCl, pH 11
Eluent B: A + 0.35 M NaCl, pH 11
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

Peak Identification

1. Trypsinogen
2. α-chymotrypsinogen

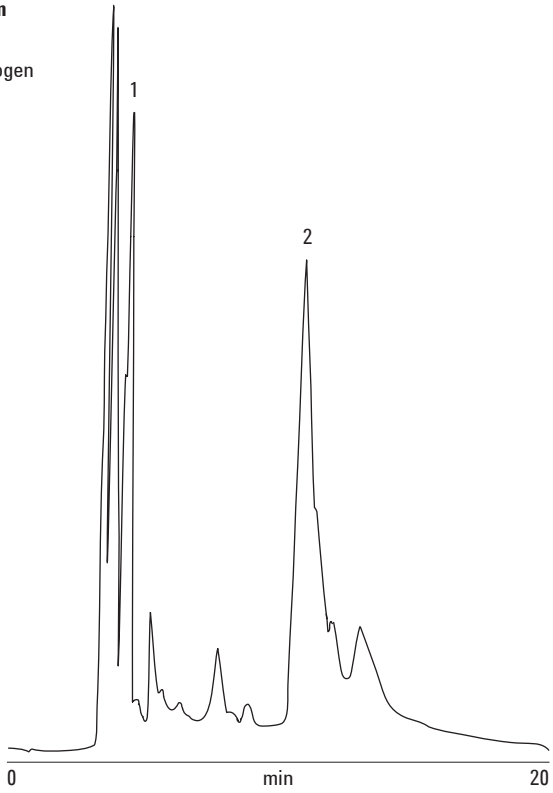

Figure 3. Analysis of trypsinogen and α-chymotrypsinogen on Agilent PL-SAX 1000Å.

Gastric juice pepsins

The strong ion-exchange functionality PL-SAX is ideally suited to the analysis of gastric juices at low pH. In this trial, 250 µL of gastric juice was dialyzed against 0.05 M sodium acetate at pH 4.1 and passed through a 0.45 µm filter. Pepsins 1, 5, 3a, 3b and 3c were well resolved for the identification of peptic ulcer disease in clinical research.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: Sodium acetate, pH 4.1
Eluent B: A + 1 M NaCl
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

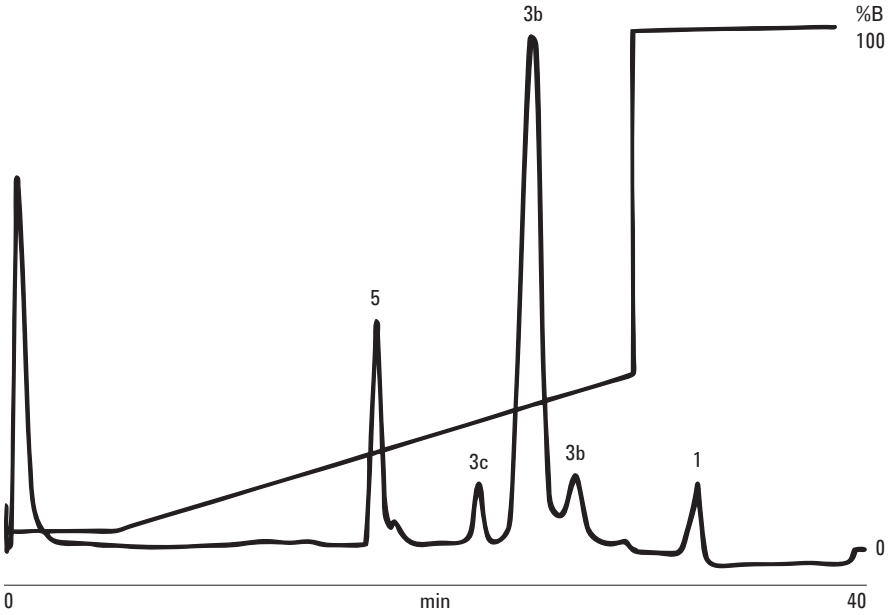

Figure 4. Analysis of gastric juice Agilent PL-SAX 1000Å (separation courtesy of N B Roberts, Royal Liverpool Hospital, UK).

Maintaining the bioactivity of ß-galactosidase

Ion-exchange uses aqueous eluents and so is considered a technique that does not denature proteins. PL-SAX is used to purify enzymes when it is a requirement to maintain the biological activity of enzymes such as ß-galactosidase. Partially purified ß-galactosidase from *Escherichia coli* was dialyzed against eluent A overnight, prior to analysis.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.01 M Tris HCl, pH 7.5
Eluent B: A + 1 M NaCl, pH 7.5
Gradient: Linear 0-100% B in 40 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

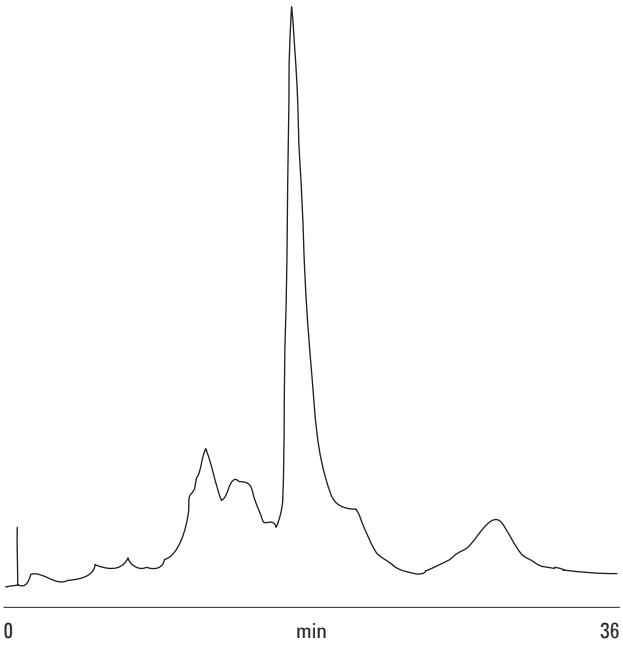

Figure 5. Analysis of ß-galactosidase on Agilent PL-SAX 1000Å (separation courtesy of Mary Anne Rounds, Purdue University, USA).

Analysis of liver cytosol

For the analysis of cytosol from liver, 10 g of tissue was homogenized with 50 mL buffer and centrifuged at 100,000 g to separate the membrane proteins from the cytosol (the portion containing the water soluble proteins). The cytosol contained 57 mg/mL protein by Bradford Assay and 0.15 M NaCl, and was diluted with water (1:9) and passed through a 0.45 µm syringe filter.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.01 M Tris HCl, pH 8
Eluent B: A + 0.5 M NaCl, pH 8
Gradient: Linear 0-40% B in 30 min
Flow Rate: 0.01 mL/min
Detection: UV, 280 nm

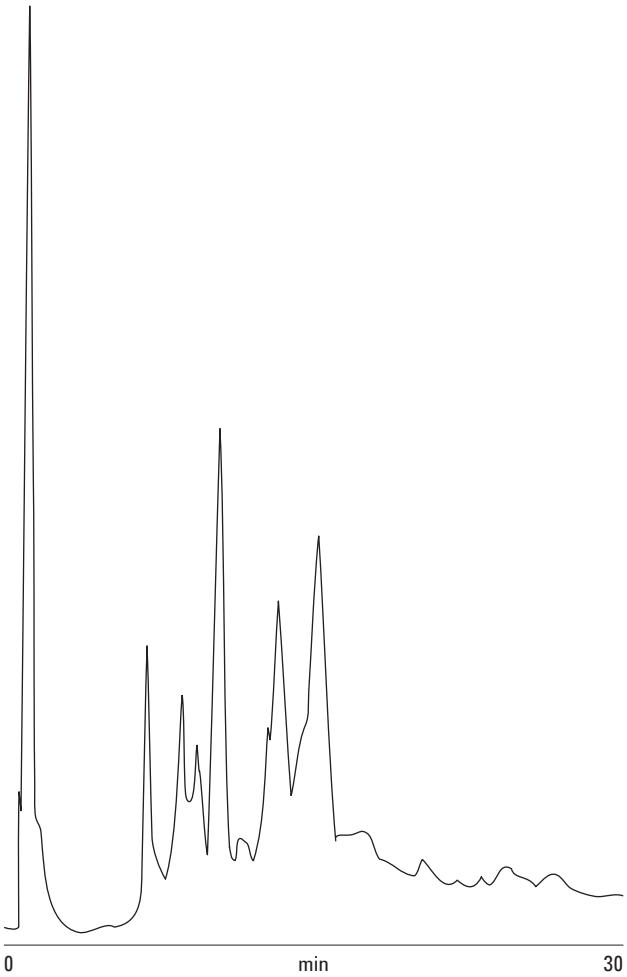

Figure 6. Raw data chromatogram of liver cytosol on Agilent PL-SAX 1000Å (separation courtesy of Mary Ann Rounds, Purdue University, USA).

Analysis of choline kinase on PL-SAX 4000Å

PL-SAX 4000Å material has an open pore structure for high resolution and high speed applications or for the separation of very large molecules. The capability of the media is evident in the analysis of choline kinase. A sample of 75 mL of partially purified choline kinase from a liver cytosol contained approximately 1 mg of the enzyme at molecular weight 160,000.

Conditions

Column:	PL-SAX 4000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1803)	
Eluent A:	20 mM Tris 5% ethylene glycol, pH 7.5	
	1.0 mM Ethylene glycol tetraacetic acid	} required to retain
	2.0 mM ß-Mercaptoethanol	} enzyme activity
	0.2 mM Phenylmethylsulfonyl fluoride	}
Eluent B:	A + 1 M KCl	
Flow Rate:	3.0 mL/min	
Detection:	UV, 280 nm	

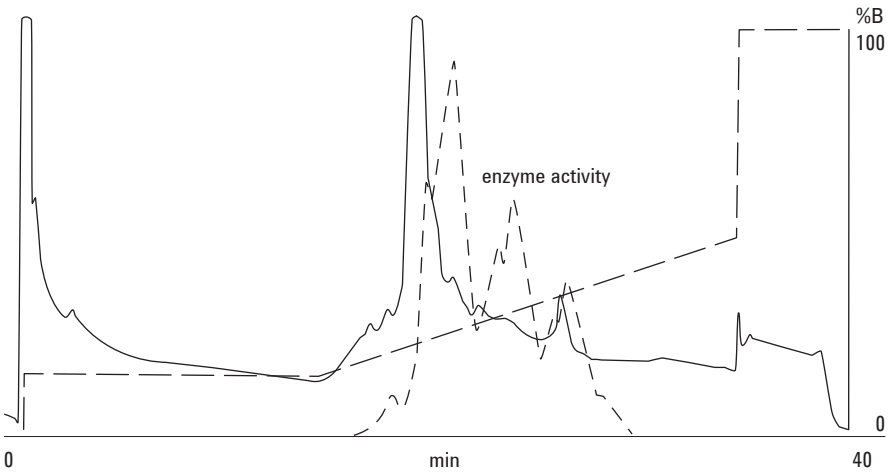

Figure 7. Raw data chromatogram of liver choline kinase Agilent PL-SAX 4000Å (separation courtesy of T Porter, Purdue University, USA).

Agilent PL-SAX Strong Anion-Exchange Columns

PL-SAX is designed for anion-exchange HPLC separations of proteins and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable polymer, extends the operating pH range. What's more, anion-exchange capacity is independent of pH.

These data represent typical results. For further information, contact your local Agilent Sales Office.

www.agilent.com/chem

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