

Sepax Technologies, Inc.
Better Surface Chemistry for Better Separation

Application Data



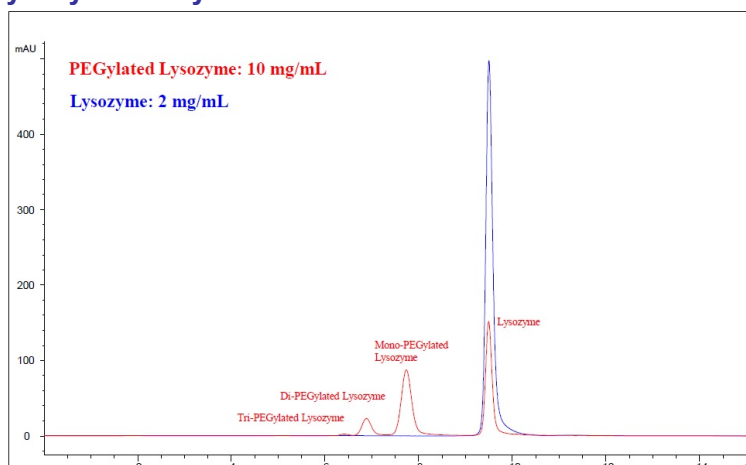
PEGylated Lysozyme Separation– Sepax SEC, RP, IEX, and Mixed Mode

PEGylation to protein is widely used in chemical modification to improve the pharmacokinetics, its half-life and stability of biotherapeutic drugs. Different chromatography methods are being used to analyze the polymeric modification of protein samples. In this application, Sepax studied PEGylated Lysozyme as an example by a variety of HPLC Columns including, Size Exclusion, Reversed Phase, Ion Exchange, and Mixed Mode for analytical characterization.

Size Exclusion Chromatography (SEC)

SEC: Mono-, Di-, and Tri-PEGylated Lysozyme which have different molecular sizes were well separated by the Zenix-C SEC 300A column in this study. Zenix-C SEC has a laydown monolayer that eliminates secondary interactions that can be seen with the long linear chains of PEG once conjugated to a protein that can get stuck in traditional SEC coatings.

Lysozyme PEGylation Monitor on Zenix-C SEC-300 Column

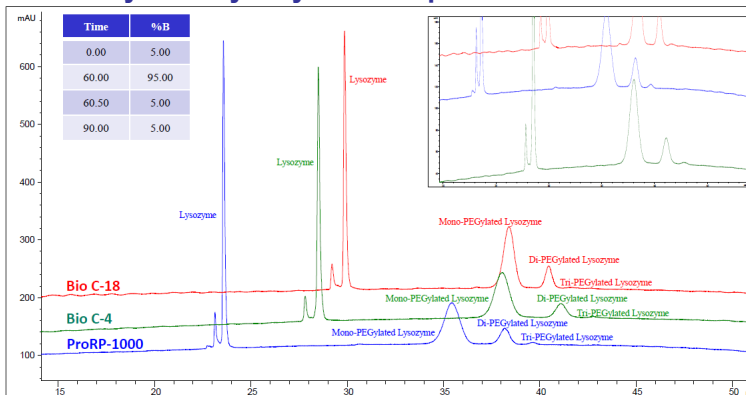


Column: Zenix-C SEC-300 7.8 x 300 mm; Mobile Phase: 150 mM sodium phosphate, pH 7.0. Isocratic, 15 min; Flow Rate: 1.00 mL/min; Detector: 280 nm; Column Temperature: 25 °C; Sample: 10 mg/mL PEGylated lysozyme, 2 mg/mL Lysozyme; Injection Amount: 50 µg

Reversed Phase (RP)

In this study, Proteomix RP-1000 gives a wider retention window range between PEGylated and unPEGylated protein. While Bio-C4 gives a better resolution when the focus of separation is between Mono-PEGylated protein and Di-PEGylated protein.

PEGylated Lysozyme- Comparison RP Columns

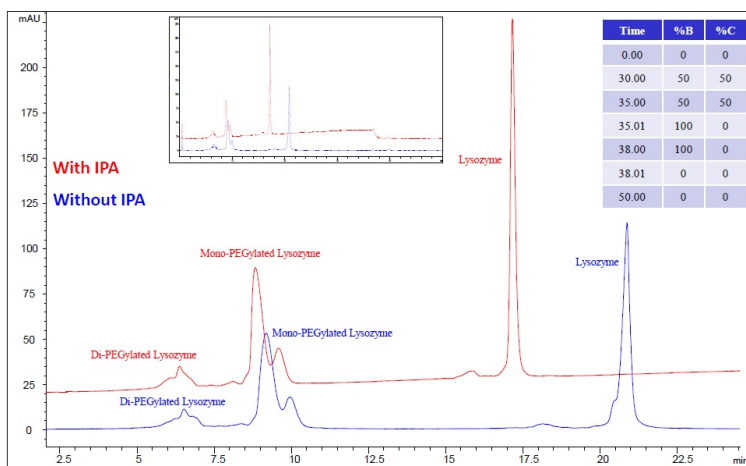


Column: Bio-C4, Bio-C18, 5 µm, 4.6 x 250 mm & Proteomix RP-1000, 5 µm, 4.6 x 100 mm; Mobile Phase: A: Water + 0.1% TFA, B: Acetonitrile + 0.1% TFA; Flow Rate: 0.5 mL/min; Detector: 214 nm; Column Temperature: 60, 80 °C; Sample: 2 mg/mL PEGylated Lysozyme; Injection Amount: 10 µg

Ion Exchange Chromatography (IEX)

In this study, introducing IPA in the running conditions on a Sepax Proteomix column helps tighten the peak shape of both PEGylated and unPEGylated protein peaks. At a low concentration of IPA, the method is under still under non-denaturing conditions. Due to high pI of Lysozyme (11.1), cation exchange chromatography was used to analyze PEGylated Lysozyme sample.

PEGylated Lysozyme -Proteomix SCX-NP5 Gradient 2 –With and Without IPA

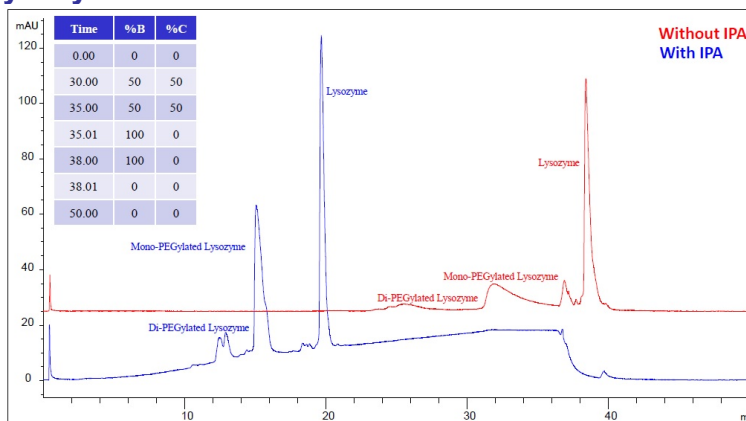


Column: Proteomix SCX-NP5, 5 ?m, NP, 4.6 x50 mm; Mobile Phase: A: 20 mM Sodium Phosphate, pH 6, B: A + 1.0 M NaCl, pH 6, C: IPA; Flow Rate: 0.75 mL/min; Detector: 280 nm; Column Temperature: 25 °C; Sample: 10 mg/mL PEGylated Lysozyme; Injection Amount: 60 ?g

?Mixed Mode (MM)

Sepax offers two brand new products: Mixed mode SCX and Mixed mode SAX. Due to the high PI of Lysozyme (11.1), mixed mode SCX was used to evaluate the PEGylated protein. Under IEX testing conditions, while Proteomix SCX provided an enhanced separation of isoforms of Mono-PEGylated Lysozyme, MMSCX column gave a better resolution of isoforms of Di-PEGylated Lysozyme. Under HIC conditions, MMSCX column was able to show better separation between Di-PEGylated and Tri-PEGylated Lysozyme.

PEGylated Lysozyme – Mixed Mode SCX-IEX Condition With and Without IPA



Column: Mixmode SCX, 5 ?m, NP, 4.6 x50 mm; Mobile Phase: A: 20 mM Sodium Phosphate, pH 6, B:A + 1.0 M NaCl, pH 6, C:IPA; Flow Rate: 0.75 mL/min; Detector: 280 nm; Column Temperature: 25 °C; Sample: 10 mg/mL PEGylated Lysozyme; Injection Amount: 60 ?g

[View the Application Note here.](#)

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Separation Method	Sepax Column	Part Number
SEC	Zenix SEC C-300 3 μm , 300 \AA , 7.8 x 300 mm	233300-7830
RP- Silicia Based	Bio-C4 5 μm , 300 \AA , 4.6 x 250 mm	106185-4625
RP- Silicia Based	Bio-C18 5 μm , 300 \AA , 4.6 x 250 mm	106185-4625
RP- PSDVB Based	Proteomix RP-1000 5 μm , 1000 \AA , 4.6 x 100 mm	465950-4610
IEX	Proteomix SCX-NP5 5 μm , NP, 4.5 x 50 mm	401NP5-4605
Mixed Mode	Mixed Mode SCX 5 μm , NP, 4.6 x 50 mm	410105-4605

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