

High-Resolution Cation-Exchange Chromatography for Analysis of Protein Purity and Microheterogeneity

Proteins produced for medicinal purposes must be shown to be consistently pure from lot to lot. A protein's polypeptide backbone is subject to a wide variety of post-translational modifications that are either enzymatically or chemically produced (phosphorylation, glycosylation, oxidation, deamidation, etc.) and lead to the creation of protein isoforms. Purity analysis and isoform analysis require a high-resolution separation technique. The reference below and Dionex Application Notes 125, 128, 129, and 213 show that ion-exchange chromatography (IEC) provides high-resolution separations of protein isoforms.

To demonstrate the high-resolving power of IEC, we separated a ribonuclease A (RA) standard that was certified as 75% pure by UV absorbance. RA has an isoelectric point of 9.3 suggesting it would best be separated by a cation-exchange column. Figures 1 and 2 display that both the ProPac® WCX and SCX columns (weak and strong cation-exchangers) show the impurity of the RA standard, but with distinct profiles and the WCX column producing more discrete peaks. Depending on the analysis goals, either column could be used.

Reference

Weitzhandler, M.; Farnan, D.; Horvath, J.; Rohrer, J. S.; Slingsby, R. W.; Avdalovic, N.; and Pohl, C. Protein Variant Separations by Cation-Exchange Chromatography on Tentacle-Type Polymeric Stationary Phases. *J. Chromatogr. A* **1998**, 828, 365–372.

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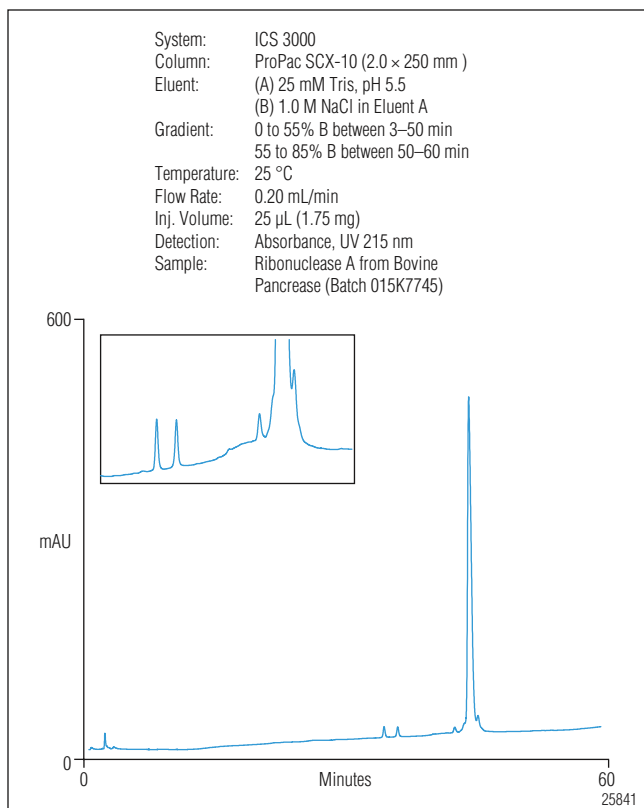


Figure 1. Separation of ribonuclease A from bovine pancreas using the ProPac SCX-10 column.

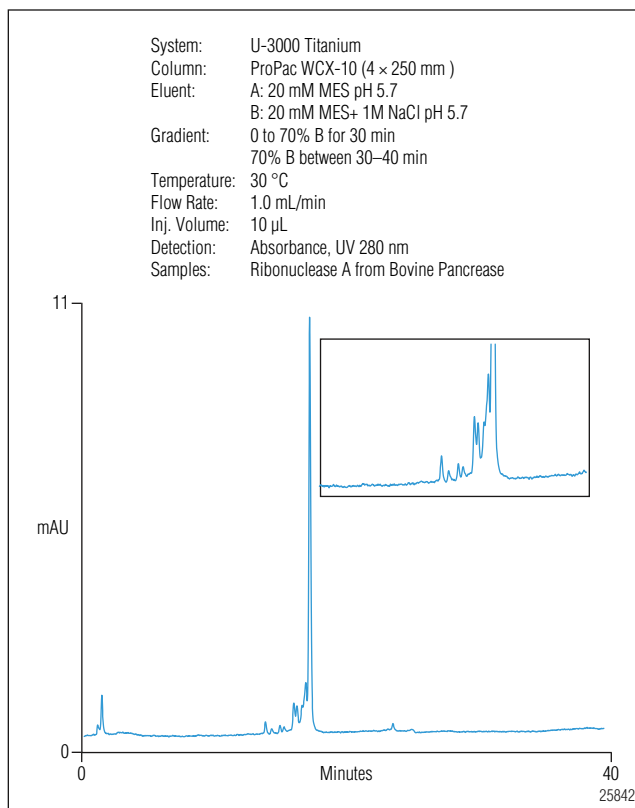


Figure 2. Separations of ribonuclease A from bovine pancreas using the ProPac WCX-10.

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