Separation of Insulin Degradation Products with Jupiter™ Proteo

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Introduction

Insulin was the first protein to be sequenced and is still one of the most widely used recombinant protein pharmaceutical in the world.[1] Insulin is the key protein involved in glucose metabolism and remains the primary treatment for type 1 diabetes patients worldwide.[2] Although insulin is a fairly small protein and reasonably hydrophobic, performing analytical and preparative purification of insulin can prove challenging due to the similar structure of prominent breakdown products. Common post-translational modification like deamidation can produce minor contaminants that can be difficult to separate from the native insulin protein.

> "purification can prove challenging due to the similar structure of prominent breakdown products"

In this study, efforts were undertaken to evaluate reversed phase separation of human insulin from common breakdown products on Jupiter Proteo, a column specifically designed for improved small protein and peptide separations.

Figure 1.
Chromatogram of human insulin injected on Jupiter Proteo

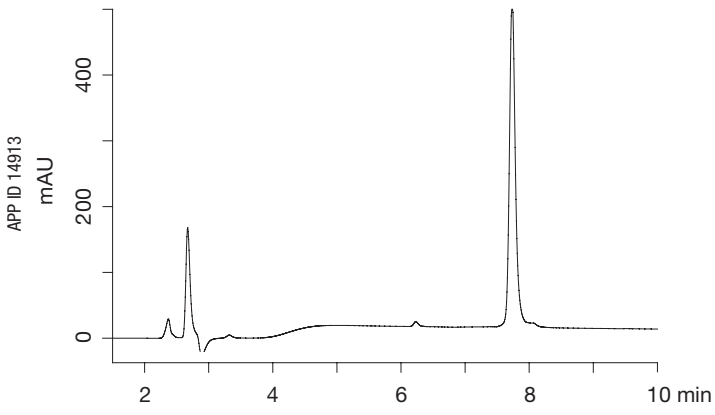

Figure 1: Reversed phase separation of human insulin on a Jupiter Proteo column. Note the excellent shape and symmetry of the insulin peak around eight (8) minutes.

Materials and Methods

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump and UV detector, and using HP Chemstation software (Version A.08.03) for data analysis. The HPLC column used for the analysis was Jupiter 4µ Proteo 250 x 4.6 mm (Phenomenex, Torrance, CA, USA). Recombinant human insulin and trifluoroacetic acid (TFA) were purchased from Sigma Chemicals (St. Louis, MO, USA). Solvents were purchased from Fisher Scientific (Fairlawn, NJ, USA).

For all separations an aliquot of 5µg of human insulin was injected on the Jupiter Proteo column running at a flow rate of 1.0 mL/min. Mobile phase A was 0.1% trifluoroacetic acid in water, mobile phase B was 95% acetonitrile/ 0.85% trifluoroacetic acid in water. A gradient from 20%B to 75%B over 15 minutes was used for all separations. Insulin deamidation and degradation products were generated by incubating 2.5 mg of Insulin in 25 mM tris/ 50 mM sodium citrate, pH 9.2 at 60° C. Aliquots were removed at different timepoints and analyzed by HPLC to ascertain changes in levels of degradation.

Results

Efforts were undertaken to optimize conditions for rapid separation of insulin from common breakdown products. Figure 1 demonstrates a typical chromatogram for human insulin. Insulin elutes as a well-defined peak around 8 minutes retention time (approximately 45% organic). In order to assess the ability of the Jupiter Proteo column to selectively separate insulin from prominent breakdown products, sample was incubated under basic conditions at 60°C; conditions that have been well established for generating protein deamidation.[3] Samples were removed at 15, 30, and 60 minute timepoints and then injected on the Jupiter Proteo column to ascertain the ability of the Jupiter Proteo material to resolve these common breakdown products from native insulin.

Figure 2 shows chromatograms of three different timepoints from a high-temperature incubation of insulin under basic conditions. As expected, increasing time in basic conditions results in a substantial increase in the amounts of deamidated insulin and other breakdown products being observed as additional peaks in the chromatograms. Although these degradation products are chemically very similar to insulin, the Jupiter Proteo column provides near-baseline separation of these minor components. Such powerful selectivity demonstrates the ability of Jupiter Proteo to be used for both analytical and preparative insulin applications.

Figure 2. HPLC Chromatograms of insulin under accelerated degradation conditions

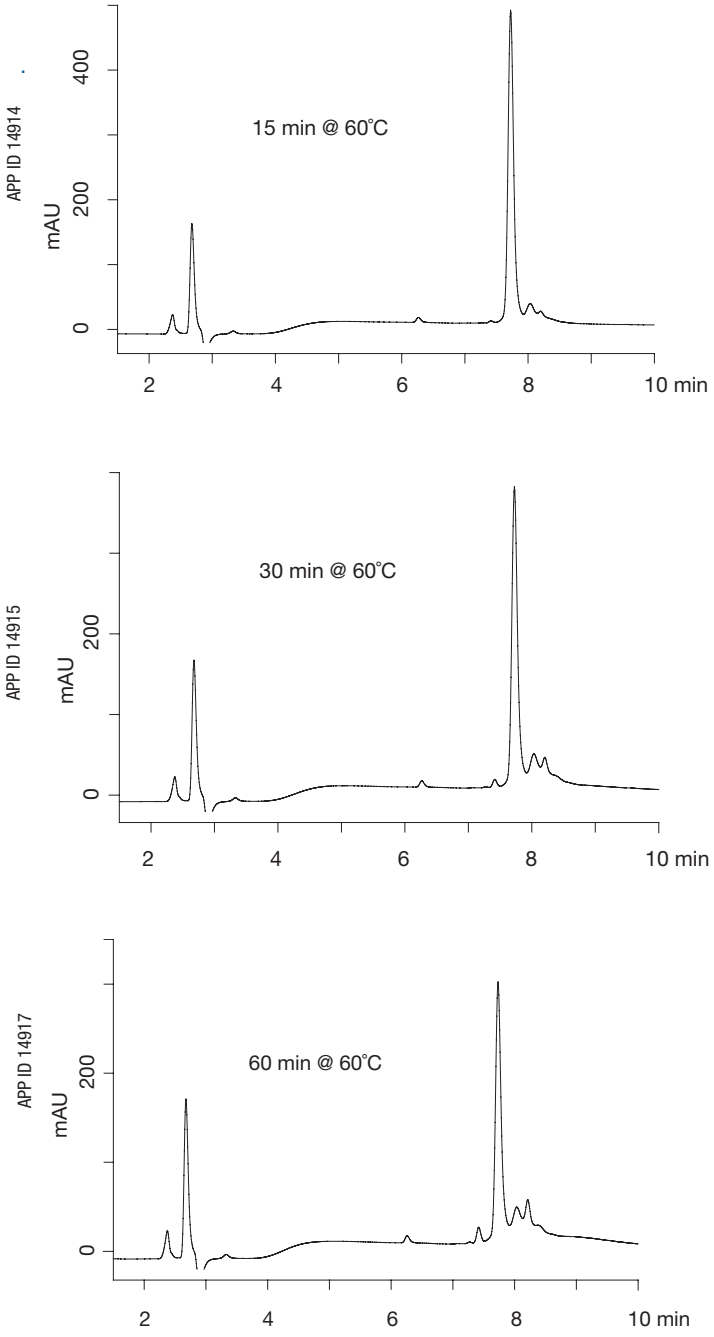

Figure 2: HPLC Chromatograms of different timepoints of insulin incubated under basic conditions at 60°C. Timepoints were taken at 15, 30, and 60 minutes. Note the increase of numerous degradation products with extended exposure at elevated temperature.

References

1. Sanger, F. (1959) *Science* 129, 1340-1344
2. Close, K. (2004) *Diabetis Close up*, V3 #6 www.closeconcerns.com
3. Hvass, A.; Hach, M.; Jars, M.V. (2003) *American Biotechnology Laboratory* 2, 8-12

If you would like more information on these columns or any of the applications listed, please contact Phenomenex. Also, if you are new to protein and peptide HPLC or are doing method development work call us today to reserve your ***FREE*** copy of our 75-page ***A User's Guide- Introduction to Peptide and Protein HPLC.***

ORDERING INFORMATION

Order Number	Description
00G-4167-E0-TN	Jupiter Proteo 4µ 250 x 4.6 mm

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