Millennial Scientific

APPLICATION NOTE

Development of a reliable HPLC method for the determination of Insulin variants using NanoPak-C All carbon columns

Background.

Insulin, a vital peptide hormone, plays a pivotal role in diabetes management by controlling blood sugar levels. Bovine insulin, as the name suggests, is insulin derived from cows. Its molecular structure is very similar to human insulin, differing by only three amino acids. As one of the first insulin preparations used, it is a baseline for comparative studies with newer insulin analogues. In some experimental models, bovine insulin might be used as a less expensive alternative to human insulin for preliminary studies.
Natural and genetically modified insulin types exist, with the latter designed for optimized drug delivery.

Insulin Aspart is a rapid-acting insulin medication used to manage diabetes mellitus, both type 1 and type 2. It is an artificial analog of human insulin. Unlike regular human insulin, insulin aspart works faster and has a shorter duration of action. This makes it ideal for managing blood sugar spikes that occur after meals. Proper use and monitoring can improve glycemic control and overall diabetes management. Phenol and meta-Cresol are phenolic preservatives commonly found in insulin aspart formulations (e.g., NovoLog produced by Novo Nordisk). Their primary function is to extend shelf life and maintain the sterility of the insulin solution. Phenol and meta cresol also help insulin aspart maintain its hexameric structure, which is crucial for proper function within the body. Some studies also suggest that aspart might degrade faster when the levels of these preservatives are depleted. On the other hand, higher concentrations of phenolic preservatives can cause localized allergic reactions at injection sites.

Insulin and its variants require detection and purity assessment for biotherapeutic and pharmaceutical applications. HPLC helps determine the purity of insulin preparations by detecting and quantifying impurities, degradation products, and related substances. Accurate quantification of insulin content is essential for ensuring the efficacy of insulin products.

NanoPak- C All carbon columns offer a unique hydrophobic surface chemistry for reverse phase separation of peptide compounds such as Insulin and its variants. The NanoPak-C (40um particle size) columns can be used as a cost-effective, robust, and efficient solution for pre-purifying Insulin and its variants.

This application note outlines a general approach to developing a simple and robust high-performance liquid chromatography (HPLC) method with 40um particle size column and diode array detection to analyze Insulin and its variants.

Instrumentation

HPLC Conditions	
Column	Nanopak-C All Carbon 250 x 4.6 mm, 40um
Mobile phase (Acidic)	Mobile Phase A: 0.1% TFA in water (pH) Mobile Phase B: 0.1% TFA in Acetonitrile Gradient: Time %B 0 20 10 65
Injection volume	10ul
Flow	1ml/min
UV detection	220nm

Probe Analytes

Human Insulin: 1 mg/mL in DI water pH 2.1 (0.05% TFA).
Bovine Insulin: 1 mg/mL in DI water pH 2.1 (0.05% TFA).
Insulin Aspart (NOVOLOG clinical formulation): 1mg/mL in Di water pH 2.1 (0.05% TFA).
Phenol: 0.86 mg/ml.
Meta cresol: 1mg/ml.

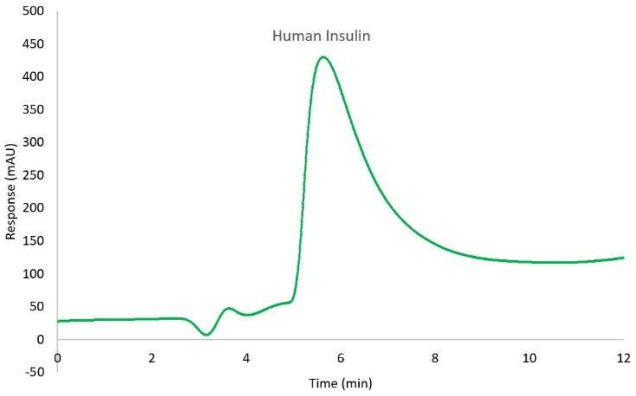

Figure 1 shows a representative chromatogram of Human Insulin.

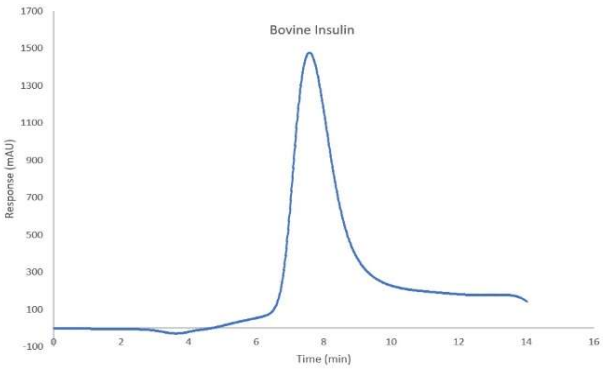

Figure 2 shows a representative chromatogram of Bovine Insulin.

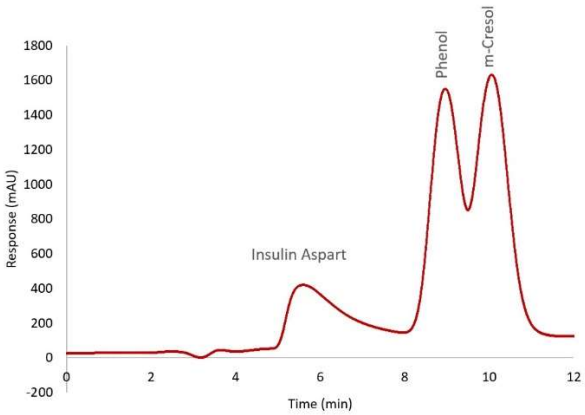

Figure 3 shows a representative chromatogram of Insulin aspart, phenol and m-cresol.

Results

Figures 1 and **2** show representative chromatograms of Human and Bovine Insulin respectively on NanoPak-C All carbon column (40um particle size).

Figure 3 shows a representative chromatogram of Insulin aspart and its phenolic preservatives.

The results indicate that these columns can be used as a standalone cleanup method for Insulin or as a pre-purification step for initially reducing sample complexity and removing a significant portion of impurities. This cleanup can be followed by final purification using preparative HPLC columns with 5-10um media.