Millennial Scientific

APPLICATION NOTE

# Simultaneous Determination of Insulin Aspart and its phenolic preservatives using NanoPak-C All Carbon HPLC columns

**Background.** Insulin aspart is a type of rapid-acting insulin medication used to manage diabetes mellitus, both type 1 and type 2. It is a man-made 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 contribute to improved 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 metacresol 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.

HPLC analysis is an essential tool for ensuring the quality, safety, and efficacy of insulin aspart medications. By providing detailed information about the composition and stability of the formulation, HPLC analysis safeguards patients and supports the development of improved insulin aspart therapies. Separating insulin aspart from phenol and meta-Cresol using HPLC requires careful consideration of column selection, mobile phase optimization, and potential challenges due to their similar properties.

NanoPak- C All carbon columns offer a unique surface chemistry with a stronger affinity for hydrophobic compounds such as insulin aspart and phenolic preservatives. This translates to better separation between these compounds and potential interfering substances present in the sample matrix. It also leads to cleaner peaks and more accurate quantification. These features allow enhanced selectivity, improved sensitivity, and potentially faster analysis times.

## Probe Analytes

Insulin Aspart (NOVOLOG clinical formulation): 2mg/mL in 0.01M HCL
Phenol: 0.86 mg/ml
Meta cresol: 1mg/ml

## Instrumentation

| ***HPLC Conditions*** | |
|---|---|
| Column | Nanopak-C All Carbon 150 x 4.6 mm, 8um |
| Mobile phase (Acidic) | Mobile Phase A: 0.1% TFA in water (pH 2)<br>Mobile Phase B: 0.1% TFA in Acetonitrile<br>Gradient: Time %B<br>0 20<br>10 65 |
| Injection volume | 10ul |
| Flow | 1ml/min |
| UV detection | 220nm |

## Results

Baseline separation between insulin aspart, phenol, and meta-cresol peaks is critical for accurate quantification of insulin. **Figure 1** shows a representative chromatogram of Insulin aspart, Phenol and meta cresol indicating a clear baseline separation of the peaks within 10 minutes. The results demonstrate that the optimized HPLC method is suitable for routine analysis and quality control of insulin aspart in clinical formulations.

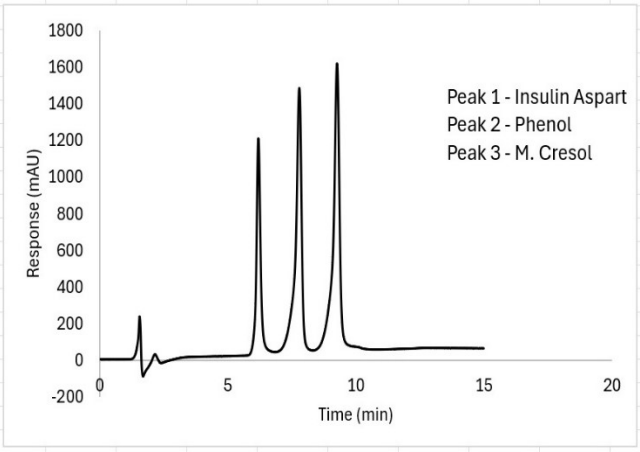


***Figure 1 shows a representative chromatogram of Insulin aspart, Phenol, m-cresol***