

Separation of Semaglutide and Liraglutide on NanoPak-C All Carbon HPLC columns

Background. Semaglutide and liraglutide are peptide drugs to treat diabetes and obesity. They are glucagon-like peptide-1 (GLP-1) analogs that mimic GLP-1 receptor agonists, a natural hormone that regulates blood sugar levels. Separation and purification of these peptides are essential to meet drug quality requirements. Their HPLC separation to meet regulatory identity and purity characterization could occur under acidic conditions. Their HPLC purification during scale-up manufacturing to ensure high purity, yield, and reproducibility could occur under alkaline conditions. Optimizing their separation and purification process involves selecting appropriate media, mobile phase composition, pH, flow rate, and temperature. NanoPak-C All carbon columns' features include pH and temperature stability, as well as tunable properties (selectivity, pore size, surface area). These features improve the separation and purification performance and reduce operational and maintenance costs. This note summarizes the optimal methods for the HPLC separation of semaglutide and liraglutide under acidic and alkaline conditions.

Probe Analytes

Semaglutide: BOC Sciences. CAS No. 910463-68-2

Liraglutide: BOC Sciences. CAS No. 204656-20-2.

Acidic: 1mg/mL in DI water pH 2.1

Alkaline: 1mg/mL in DI water pH 8.5

Instrumentation

HPLC Conditions			
Methods			
Column	Nanopak-C All Carbon 150 x 4.6 mm, 10 um		
Mobile phase (Acidic)	Mobile Phase A: 0.1% TFA in water Mobile Phase B: 0.1% TFA in Acetonitrile Gradient: Time %B 0 20 10 65		
Mobile phase (Alkaline)	Mobile Phase A: Ammonium Acetate Buffer (pH 8.5) Mobile Phase B: Acetonitrile Gradient: Time %B 0 20 10 50		
Injection volume	10ul		
UV detection	220nm		
Oven temperature	25deg C		

Results

Figures 1 and 2 show representative chromatograms under acidic and alkaline conditions of semaglutide and liraglutide, respectively. The results indicate that the acidic and alkaline conditions can significantly influence their retention times.

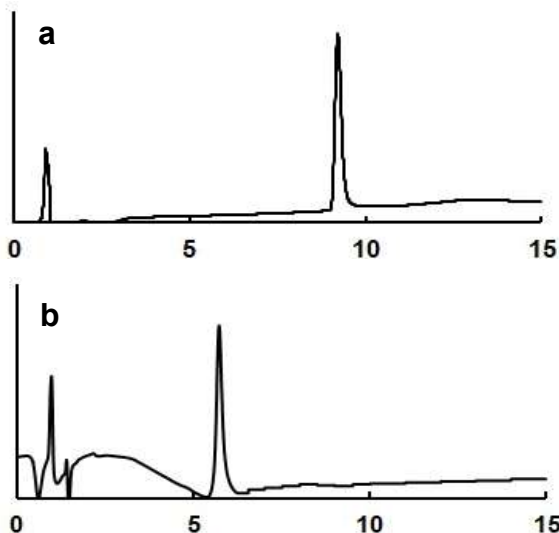


Figure 1. Representative chromatogram of Semaglutide under (a) acidic and (b) alkaline conditions.

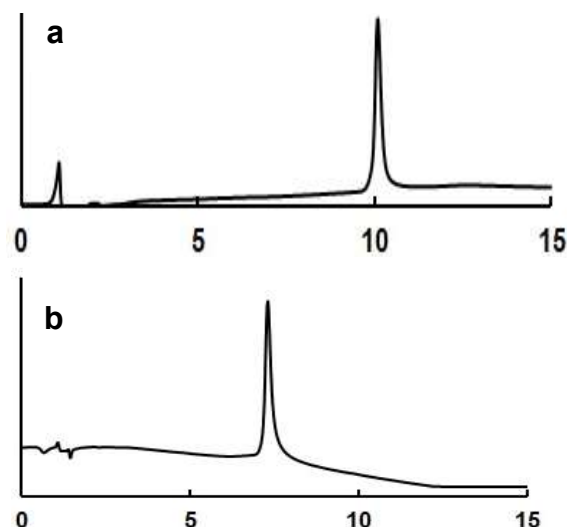


Figure 2. Representative chromatogram of Liraglutide under (a) acidic and (b) alkaline conditions.