

Semaglutide Crude Analysis Using NanoPak-C All Carbon 4gm flash column

Mobile phase preparation:

1. Weigh 1.26gm ammonium formate (20mM) in 800ml DI (pH should come 5.7)
2. Adjust the pH to 8.5 with 25% Ammonia Solution. (Approximately 600ul required).
3. Increase the volume to 1Lit with DI water.
4. Check pH again, and confirm pH is 8.5

Semaglutide Sample Preparation:

1. Weigh 2mg of Semaglutide crude in 1ml micro-centrifuge tube and add 1ml of IPA: water (1:1) solution.
2. Sonicate the centrifuge tube till the sample gets dissolved completely.

Before analysis:

1. Attach the Flash column to the flash chromatography system. The column ends have Luer-Lok end fittings to create a leak-free seal. Ensure the column is securely attached to prevent any potential leakage during operation.
2. Flow 50ml of Ammonium formate pH 8.5 through the column to equilibrate before analysis.
3. Load the appropriate method parameters as mentioned below for the 4gm flash column on the instrument.
4. Set the gradient as shown below and start the run.
5. Inject 1ml of Semaglutide crude 2mg/ml sample onto the column using a Luer lock syringe.

Method parameters:

1. Instrument Details: - TELEDYNE ISCO Combi Flash Rf
2. Column Details: - 4gm NanoPak-C column.
3. GLP 1 source: - Shilpa life science.
4. Semaglutide crude conc.: - 2 mg/ml
5. Injection volume: - 1ml
6. Wavelength: - 214nm
7. Signal gain: - 5X
8. Mobile phase A: - 20mM ammonium formate pH 8.5
9. Mobile phase B: - Acetonitrile (ACN)
10. Equilibration volume: - 33.6ml

11. Flow rate: - 7 ml/min.
12. Pressure observed: - 7 PSI.
13. Gradient:

Time	%B
0	20
10	65
12	65
13	20
17	20

After analysis: -

1. Wash the column with acetonitrile (ACN); Approximately 100ml) with 20ml/min flow rate.
2. Very Important: Do not air dry the column. Store it in 100% methanol. If methanol is unavailable, ethanol or isopropyl alcohol will also be fine. If the column is air-dried or the bed dries up, significant time and effort will be needed to re-equilibrate and restore it to its original performance conditions.
3. Remove the column from the system and run warm water with the bypass tube.

Result chromatogram:

1. Semaglutide

