# Test Report

## 1. Method and Conditions

| Sample Information | | | |
|---|---|---|---|
| Sample | Retatrutide | | |
| Trait | Solid | | |
| Requirements | Develop a method to separate Retatrutide from impurities. | | |
| Reference Method | / | | |
| Instrument | Agilent 1260 Infinity | | |
| **Conditions** | | | |
| Column | Ultisil® LP-C18 (4.6×250 mm, 5 µm) | | |
| Mobile Phase | A: 80 mmol/L ammonium dihydrogen phosphate (pH 6.5)<br>B: acetonitrile | | |
| Flow Rate | 1.0 mL/min | | |
| Injection Volume | 20 µL | | |
| Column Temperature | 45 °C | | |
| Detector | UV | | |
| Detector Wavelength | 214 nm | | |
| Gradient Profile | Time (min) | Phase A (%) | Phase B (%) |
| | 0 | 75 | 25 |
| | 5 | 75 | 25 |
| | 50 | 30 | 70 |
| | 51 | 75 | 25 |
| | 60 | 75 | 25 |

**Preparation/Pretreatment**:

1) Mobile Phases:

A: Weigh 9.2 g of ammonium dihydrogen phosphate ($NH_4H_2PO_4$), dissolve in 1000 mL water, then adjust the pH to 6.5 with diluted ammonia.

B: HPLC-grade acetonitrile.

2) Diluent:

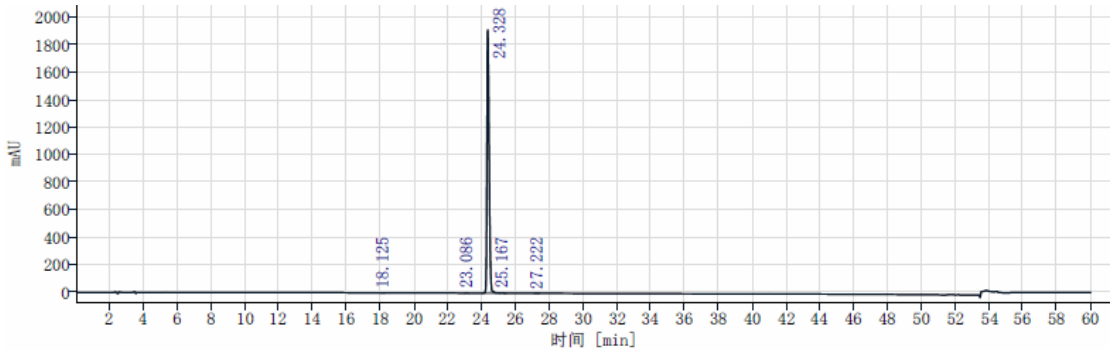
Weigh 1.90 g of sodium phosphate dodecahydrate ($Na_2PO_4 \cdot 12H_2O$) and 8.18 g of NaCl, dissolve in 1 L water and mix well. Adjust the pH to 7.0.

3) Test Solution:

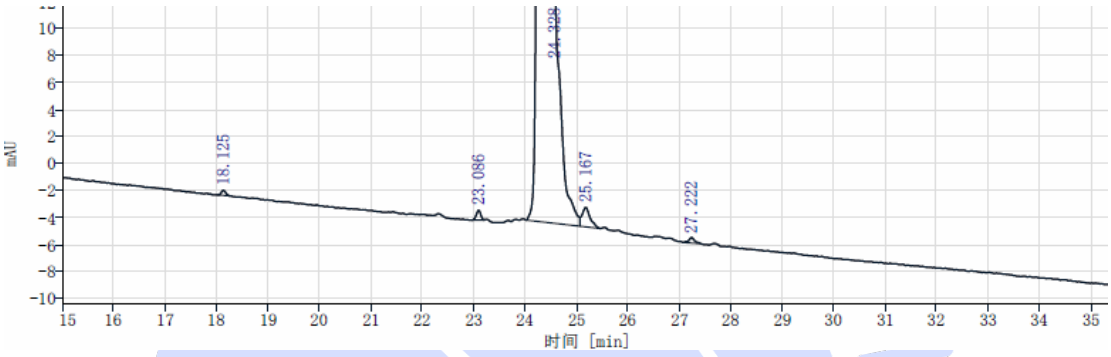
Dissolve an aliquot of Retatratide in the Diluent to a concentration of 1 mg/mL.

## 2. Chromatograms and Data

1) Test Solution:




*Full-range*




*Zoomed-in*

| Ret. Time (min) | Height | Peak Area | Area % | Res. | Tailing | Plates |
|---|---|---|---|---|---|---|
| 18.125 | 0.37 | 2.23 | 0.01 | - | 0.82 | 205387 |
| 23.086 | 0.73 | 4.35 | 0.03 | 30.73 | 0.93 | 321913 |
| 24.328 | 1919.65 | 17152.58 | 99.85 | 6.21 | 1.46 | 169583 |
| 25.167 | 1.44 | 15.70 | 0.09 | 3.15 | 1.38 | 115216 |
| 27.222 | 0.41 | 3.21 | 0.02 | 8.41 | 0.94 | 322636 |

**3. Conclusion**

Using the Welch Ultisil® LP-C18 (4.6×250 mm, 5 μm) under the specified conditions, the requirement of the test can be met.