

## Analysis of Insulin by HPLC-UV

### Method 1: Insulin Impurity Profile

Column: [Waters Inertsil ODS-2 .5µm 4.6\\*150mm](#) or [Grace 218TP C18 Column 150\\*4.6mm 5um HPLC Column](#) (Ctrl + Click to follow link)

Elution Type: Gradient

Elution A: 20mM Phosphate buffer pH 2.7

Elution B: Acetonitrile

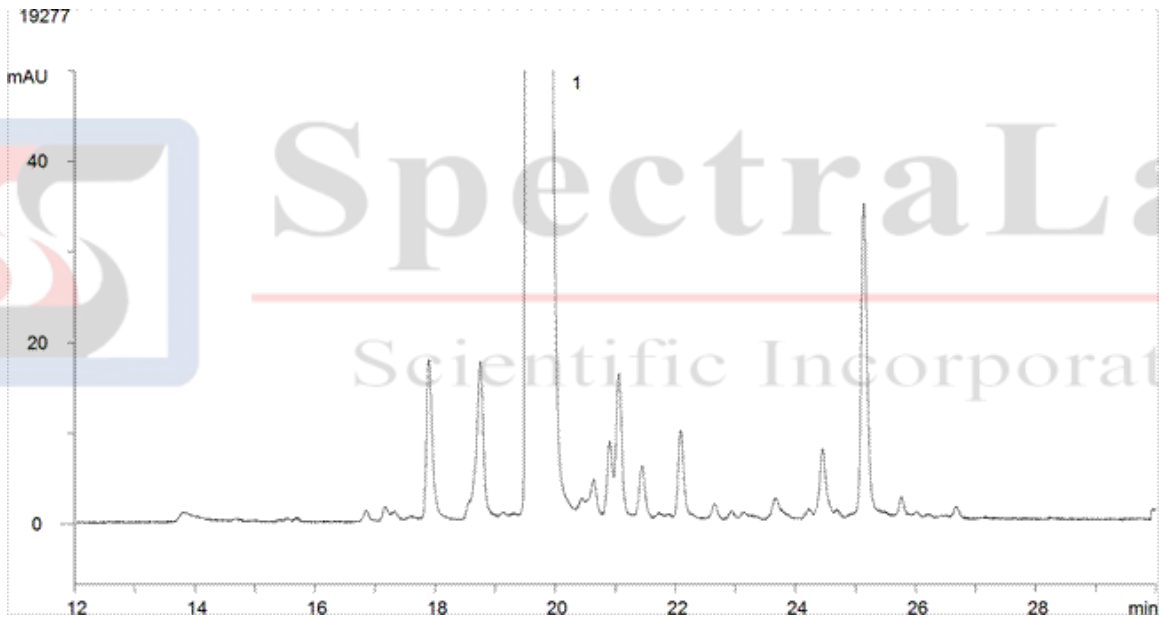
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	78	22
2	30	66	34

Flow Rate: 1 mL/min

Col. Temp: 40 °C

Detection: [UV-Vis Abs.-Diode Array \(PDA\)](#) @ (22°C) (Ctrl + Click to follow link)



1: Insulin

### Method 2: Insulin Human and Animal Mixture

Column: [Waters Inertsil ODS-2 .5µm 4.6\\*150mm](#) or [Grace 218TP C18 Column 150\\*4.6mm 5um HPLC Column](#) (Ctrl + Click to follow link)

Elution Type: Gradient

Elution A: 0.1% TFA and 5% Acetonitrile in Water

Elution B: 0.08% TFA , 90% Acetonitrile in Water

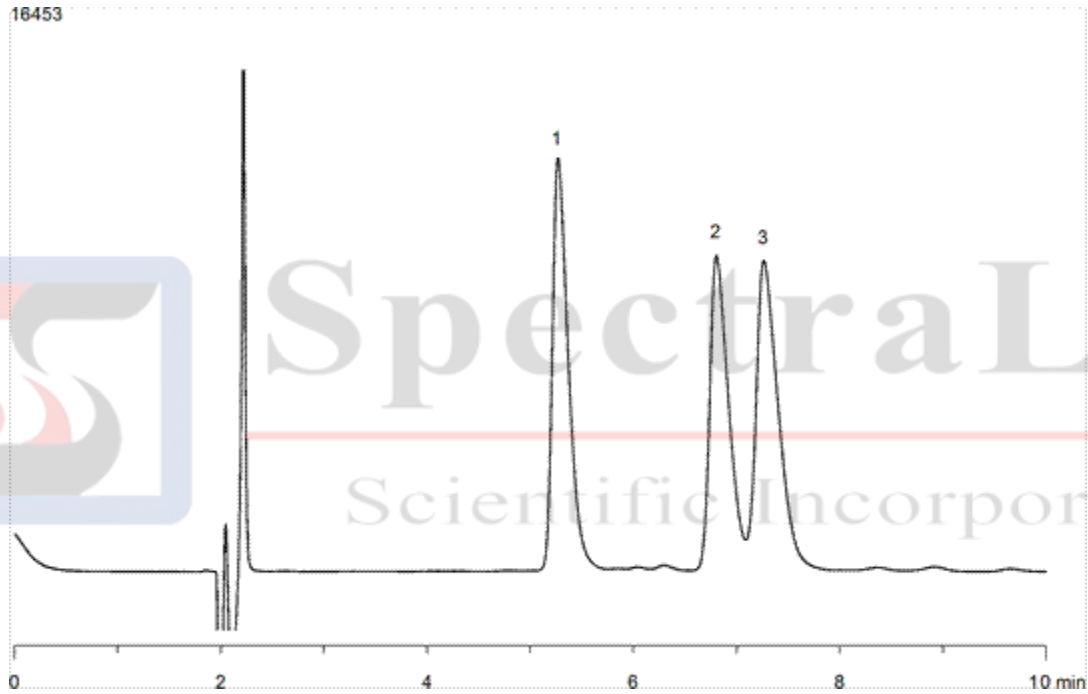
**Gradient Profile:**

Step No.	Time (min)	Pct A	Pct B
1	0	70	30
2	15	68	32

**Flow Rate:** 1 mL/min

**Col. Temp:** 25 °C

**Detection:** [UV-Vis Abs.-Diode Array \(PDA\)](#) @ 214 nm (25°C) [\(Ctrl + Click to follow link\)](#)



- 1: Bovine Insulin
- 2: Human Insulin
- 3: Porcine Insulin

**Reference:** <http://www.phenomenex.com/Application/Detail>