



Tirzepatide Preparation and Purification

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Overview

Tirzepatide is a Glucagon-like peptide-1 (GLP-1) agonist and is considered one of the most promising diabetes drugs on the market. As a next-generation GLP-1 drug, it not only stimulates insulin release in the body effectively controlling blood sugar levels, but also demonstrates advantages for decreasing appetite, inducing weight loss, and decreasing chances for other diabetes-related complications. This application note establishes a method for the HPLC purification of crude Tirzepatide using the Octopus Plus preparative HPLC system coupled with a Luna 10 μm -PREP C18(3) HPLC column. The purity of the Tirzepatide was tested using the Agilent 1100 HPLC system and a high-pressure preparation system coupled with a Luna 5 μm C18(2) HPLC column. Crude Tirzepatide sample was provided by a customer in China.

Sample Preparation

Purification on the Luna 10 μm -PREP C18(3) column

Dissolve 120 mg of Tirzepatide sample in 5 mL of Dimethyl Sulfoxide. Dilute this solution with 5 mL of Water / Acetonitrile (1:1, v/v).

Analytical purity testing on the Luna 5 μm C18(2) column

Fractions were collected and injected onto the Agilent 1100 HPLC system for confirmation of purity.

Table 1. Tirzepatide Structural Information.

Name	Molecular Weight (Da)	Structural Formula
Tirzepatide	4813.45	$\text{C}_{225}\text{H}_{348}\text{N}_{48}\text{O}_{68}$

Purification LC Conditions

Column: Luna 10 μm -PREP C18(3)

Dimensions: 250 x 10 mm

Part No.: [00G-4616-NO](#)

Mobile Phase: A: 10 mM Ammonium Acetate in Water
B: Acetonitrile / 10 mM Ammonium Acetate in Water (80:20, v/v)

Gradient:	Time (min)	%B
	0	40
	50	47

Flow Rate: 5.0 mL/min

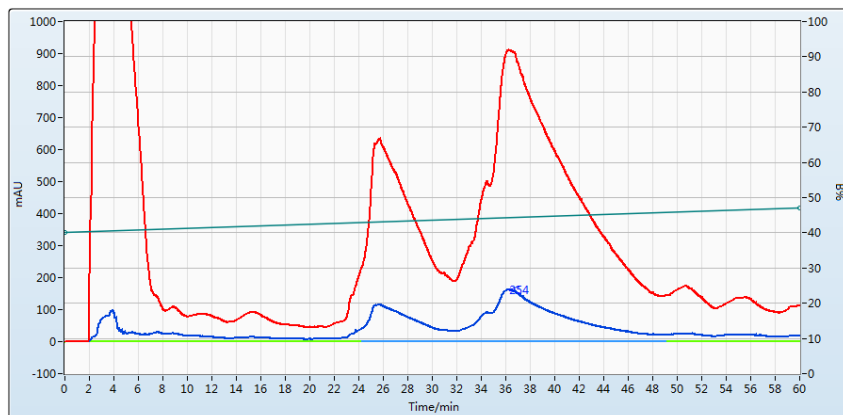
Injection Volume: 10 mL

Temperature: Ambient

Detection: UV @ 220 nm

System: Octopus Plus (Agela)

Figure 1. Preparative Chromatogram.



Analytical Purity Testing LC Conditions

Column: Luna™ 5 µm C18(2)

Dimensions: 250 x 4.6 mm

Part No.: [00G-4252-E0](#)

Mobile Phase: A: 0.1 % Trifluoroacetic Acid in Water

B: 0.1 % Trifluoroacetic Acid in Acetonitrile

Gradient:	Time (min)	%B
	0	40
	30	55

Flow Rate: 1.0 mL/min

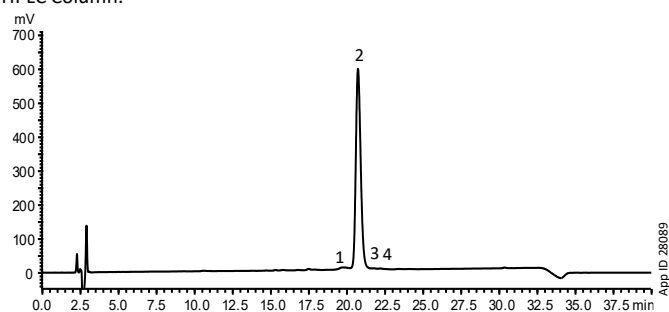
Injection Volume: 30 µL

Temperature: 30 °C

Detection: UV @ 220 nm

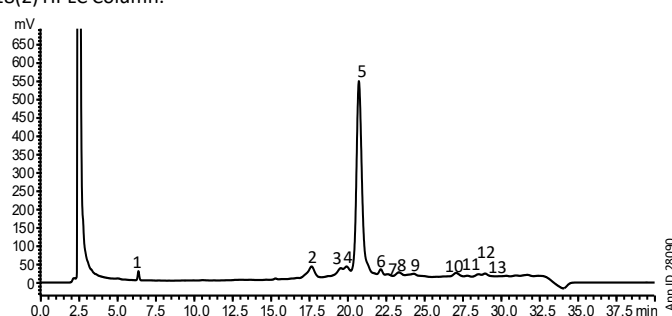
LC System: Agilent® 1100 Series

Figure 2. 36.0 – 48.1 min Fraction Detection Using the Luna 5 µm C18(2) HPLC Column.



Peak No.	Analyte	Retention Time (min)	Area	Tailing Factor	Resolution (USP)	% Area
1	Tirzepatide Impurity	19.74	249828	-	-	1.83
2	Tirzepatide	20.74	13296084	1.223	1.06	97.43
3	Tirzepatide Impurity	21.76	56531	-	0.82	0.41
4	Tirzepatide Impurity	22.19	44235	-	0.34	0.33
Total			13646677			100.00

Figure 3. Detection Pattern of Crude Tirzepatide Using the Luna 5 µm C18(2) HPLC Column.



Peak No.	Analyte	Retention Time (min)	Area	Tailing Factor	Resolution (USP)	% Area
1	Tirzepatide Impurity	3.38	174399	1.048	-	0.99
2	Tirzepatide Impurity	17.66	939924	0.854	23.18	5.36
3	Tirzepatide Impurity	19.56	624074	-	1.70	3.56
4	Tirzepatide Impurity	19.94	676844	-	0.30	3.86
5	Tirzepatide	20.74	13652063	1.299	0.92	77.79
6	Tirzepatide Impurity	22.16	342276	-	2.56	1.95
7	Tirzepatide Impurity	22.62	93869	-	0.70	0.54
8	Tirzepatide Impurity	23.35	227951	-	1.01	1.30
9	Tirzepatide Impurity	24.31	251029	-	1.28	1.43
10	Tirzepatide Impurity	27.07	204392	1.033	3.69	1.16
11	Tirzepatide Impurity	27.80	31126	1.106	1.38	0.18
12	Tirzepatide Impurity	28.54	141083	-	1.18	0.80
13	Tirzepatide Impurity	28.96	190274	-	0.55	1.08
Total			1759303			100.00



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