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Determination of Semaglutide and Tirzepatide in Plasma

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Introduction

Glucagon-like peptide-1 (GLP-1) agonists are considered one of the most promising classes of diabetes drugs on the market. Semaglutide and Tirzepatide, as next-generation GLP-1 drugs, not only stimulate insulin release in the body effectively controlling blood sugar levels, but also inhibit gastrointestinal motility and increase satiety. This technical note establishes a method for determining Semaglutide and Tirzepatide drugs in plasma, providing a scientific basis for further research on drug safety and therapeutic efficacy.

Sample Preparation

Step	Description
Sample Pre-treatment:	Combine 200 µL of EDTA anticoagulated bovine plasma and 400 µL of Methanol for protein precipitation. Centrifuge at 15,000 g for 5 minutes to obtain 500 µL supernatant. Add 400 µL of Water, then vortex and use SPE to further clean up the sample.
Condition:	Peptide-3-MW, 5 mg/1 mL 96-well plate (Agela) with 200 µL of Methanol, then 200 µL Water.
Load:	Pre-treated samples into wells.
Wash:	200 µL Water.
Elute:	50 µL elution solvent (5 % Formic Acid in Ethanol / Water (4:1, v/v)) twice. Vortex to mix.
Inject:	5 µL

LC Conditions

Column: Aeris™ 2.6 µm Peptide XB-C18
Dimensions: 100 x 2.1 mm
Part No.: 00D-4505-AN
Mobile Phase: A: 0.1 % Formic Acid
B: 0.1 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	30
0.5	30
3	65
3.5	65
4	98
5.5	98
5.6	30
7	30

Flow Rate: 0.3 mL/min
Injection Volume: 5 µL
Temperature: 40 °C
LC System: Shimadzu® LC-20AD
Detection: MRM
Detector: SCIEX® 6500 Triple Quad™

MS Conditions

Ion Source: ESI
Scan Mode: MRM Positive and Negative Mode
Source Temperature: 450 °C
GS1: 60 psi
GS2: 60 psi
CUR: 30 psi
CAD: High
IS: 5500 V

Table 1. Semaglutide and Tirzepatide Structural Information.

Name	Molecular Weight (Da)	Structural Formula
Semaglutide	4113.57	$C_{187}H_{291}N_{45}O_{59}$
Tirzepatide	4813.45	$C_{225}H_{348}N_{48}O_{68}$

Table 2. MRM Transitions and Parameters.

Name	Q1 (m/z)	Q3 (m/z)	DP (V)	CE (V)
Semaglutide	1029.3	1238.5	40	41
	1029.3	1110.3	40	39
	1029.3	690.2	40	39
	1029.3	960.5	40	51
Tirzepatide	1204.2	396.3	67	36
	1204.2	910.0	67	33
	1204.2	795.8	67	35

Results and Discussion

As shown in **Figure 1**, the linear range of the method for both compounds was 0.5 ng/mL to 200 ng/mL, with $R^2 > 0.995$. The limit of quantitation for both compounds was achievable at 0.5 ng/mL plasma concentration. **Figures 2** and **3** demonstrate well-defined peaks for both compounds with minimal residual carryover (**Figure 4**). Upon reaching the highest point on the curve at 200 ng/mL, the residual levels in the blank solution are below one-thousandth for Semaglutide and within five-ten-thousandths for Tirzepatide.

The spiked recovery and matrix effect were evaluated for plasma concentrations of 10 ng/mL and 100 ng/mL, respectively. The results are shown in **Table 4**. Both compounds exhibited absolute recoveries of over 80 %, however, they demonstrated some degree of matrix suppression effect.

Figure 1. Standard Curve for Semaglutide (Pink) and Tirzepatide (Blue).

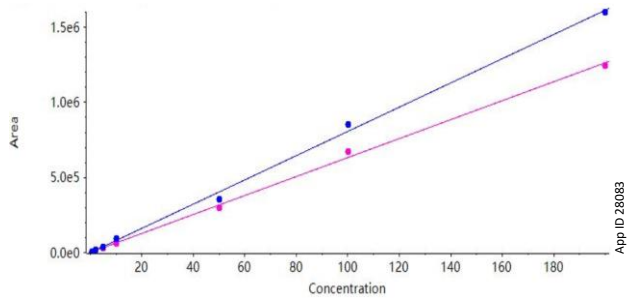


Figure 2. LQD – 0.5 ng/mL Matrix Standard Quantification Ion Chromatogram for Semaglutide (Left) and Tirzepatide (Right).

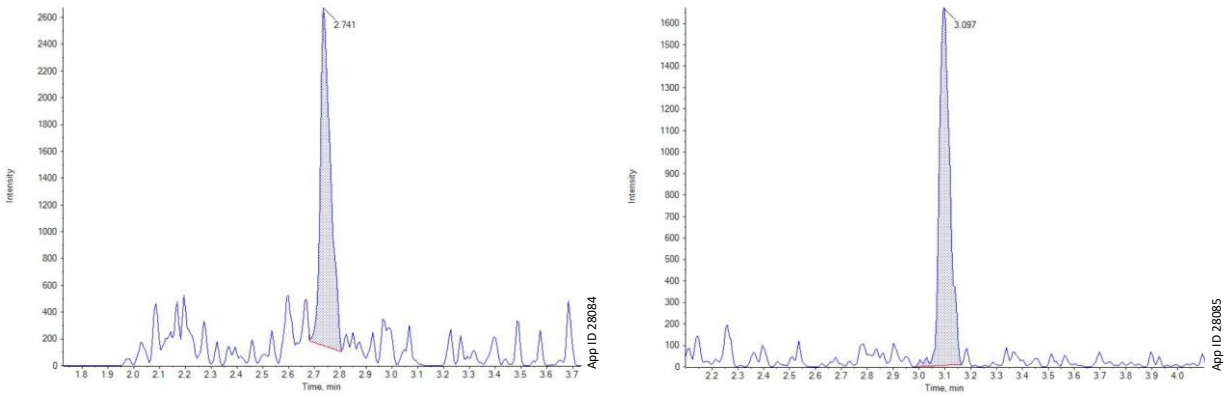
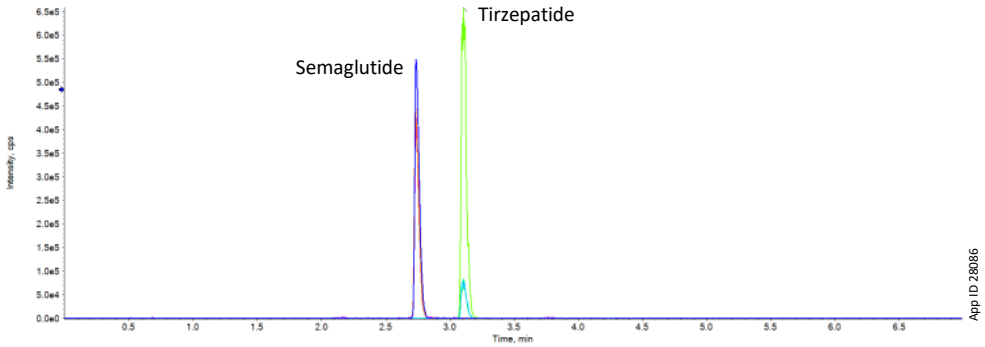


Figure 3. Chromatogram of Matrix Standard at the Highest Point of the Curve (200 ng/mL).



Have questions or want more details on implementing this method? We would love to help!
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Figure 4. Chromatogram of Blank Residuals after High Concentration Points on the Curve.

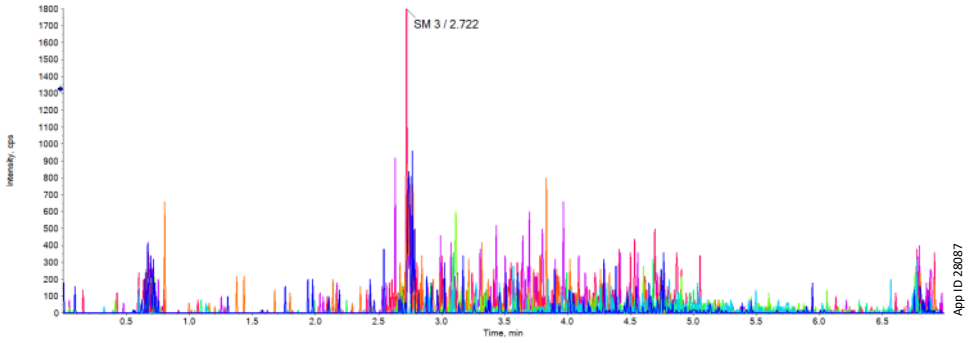

Table 3. Results of Spiked Recovery and Matrix Effect.

Compound	Semaglutide		Tirzepatide	
Standard Concentration	10 ng/mL	100 ng/mL	10 ng/mL	100 ng/mL
% Recovery	82.4	95.8	81.3	93.5
% Matrix Effect	76.6	87.0	60.6	58.8

Conclusions

This experiment established a quantitative method for Semaglutide and Tirzepatide in plasma. The limit of quantitation (LQD) achieved was 0.5 ng/mL, with a linear range of 0.5 ng/mL to 200 ng/mL. Sample recoveries were all greater than 80 % using the external standard method.

Aeris™ Ordering Information

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for 2.1 mm ID

*SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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