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Sensitive Quantitation of Glucagon-like peptide-1 (GLP-1) Analog Tirzepatide in Plasma Using Automated LC-MS/MS workflow

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Introduction

In recent years, research on and the use of glucagon-like peptide-1 receptor (GLP-1R) agonists have surged due to their effectiveness in treating obesity and type 2 diabetes. Among the notable advancements is the peptide tirzepatide, which is sold under the brand name Zepbound for weight loss, and Mounjaro for diabetes[1, 2]. Development of sensitive bioanalytical assay to determine pharmacokinetics and toxicokinetics is critical to advance these medicines. Here we utilized AssayMAP automated sample preparation to extract tirzepatide from human plasma. Our findings indicate that utilizing AssayMAP RP-S cartridges for sample cleanup can enhance assay sensitivity by 5 times compared to using traditional organic solvent precipitation alone.

Experimental

All materials (Acetonitrile, Tirzepatide, semaglutide, formic acid, 96-well LoBind plates and AssayMAP RP-S cartridge) were purchased from commercial sources,.

A mixture of 150 µL acetonitrile (ACN) and150 µL methanol (MeOH) was added to 100 µL of human plasma aliquot fortified with different concentration of tirzepatide, semaglutide was used as internal standard. Vortex for 2 min and then spun down at 16, 000 g for 10 min. 350 µL of each supernatant was transferred to a 96-well plate and dried down under nitrogen gas with heating.110 µL of water was added to each well to reconstitute. The solution was further purified by AssayMAP RP-S cartridges.

RP-S cartridges were conditioned with 80% ACN and equilibrated with water. 100 µL of above reconstitute samples were loaded onto RP-S cartridges at 3 µL/min, cartridges were washed once with water and once with 10% ACN 10 mM ammonium formate buffer. The final elution step was carried out by loading 30 µL of 5% formic acid (FA) in 80% ACN to the cartridge at 3 µL/min. 30 µL of water with 5% formic acid was added to the final elution and 5 µL was injected into LC/MS for analysis.

All data were collected with 1290 Infinity II Bio LC coupled to 6495D LC/TQ system (Table 1 and Table 2). Tirzepatide MRM transitions were optimized and 1204.4→396.2 was chosen as quantifier and 1204.4→299.2 was chosen as qualifier with optimal collision energy. Semaglutide MRM transition (1029.4→1238.1) was also optimized.

Experimental

Figure 1. AssayMAP Bravo Platform and 6495D LC/TQ system.

Table 1. UHPLC gradient and conditions

LC Conditions	
Column	AdvanceBio Peptide Mapping 120Å, 2.1 x 150 mm, 2.7 µm
Column temperature	80 ºC
Injection volume	5 µL
Autosampler temp	4 ºC
Needle wash	5 seconds in wash port (50:50/water: MeOH)
Mobile phase	A = Water + 0.1% FA B = ACN + 0.1% FA
Flow rate	0.5 mL/min
Gradient program	Time %B 0 30 3.0 45 5.0 60 5.1 95 8.0 95 8.2 30 10 30
Stop time	10 min

Table 2. AdvanceBio 6495D TQ MS conditions.

MS Conditions	
Gas temperature	290 ºC
Drying gas flow	15 L/min
Nebulizer gas	30 psi
Sheath gas temperature	400 ºC
Sheath gas flow	11 L/min
Capillary voltage	6000V
Nozzle voltage	2000V

Results and Discussion

All sample preparation steps were evaluated to achieve the best sensitivity for tirzepatide quantification in human plasma.

- First, organic solvent composition to precipitate proteins was evaluated, ACN : MeOH (1:1) was found out to be the best for tirzepatide and semaglutide extraction.
- Second, loading buffer including water, water with 0.1% FA, 10%, 20% and 40% acetonitrile were evaluated, pure water turned out to be the best loading buffer for GLP-1 peptides.
- Third, washing buffer was evaluated, including water, water with 0.1% FA, water with 1% FA, and 10 mM NH_4COOH pH 10 buffer with/without 10% acetonitrile, the combination of water and 10% acetonitrile in 10 mM NH_4COOH pH 10 buffer turned out to be the best washing solution.
- Last, elution buffer were evaluated, including 10 mM NH_4COOH pH 10 buffer with 30% or 80% acetonitrile, 80% acetonitrile with 0.1%, 1% and 5% FA, 5% FA in 80% ACN produce the best results.

MassHunter Quant 12 was used to perform quantitative analysis of calibration curve and quality control samples. Blank human plasma and tirzepatide low limit of quantification (LLOQ) of 0.05 ng/mL as shown in Figure 2 (A), the calibration curve was linear up to 1000 ng/mL with quadratic fit and $1/x^2$ weight as shown in Figure 2 (B), and Table 3.

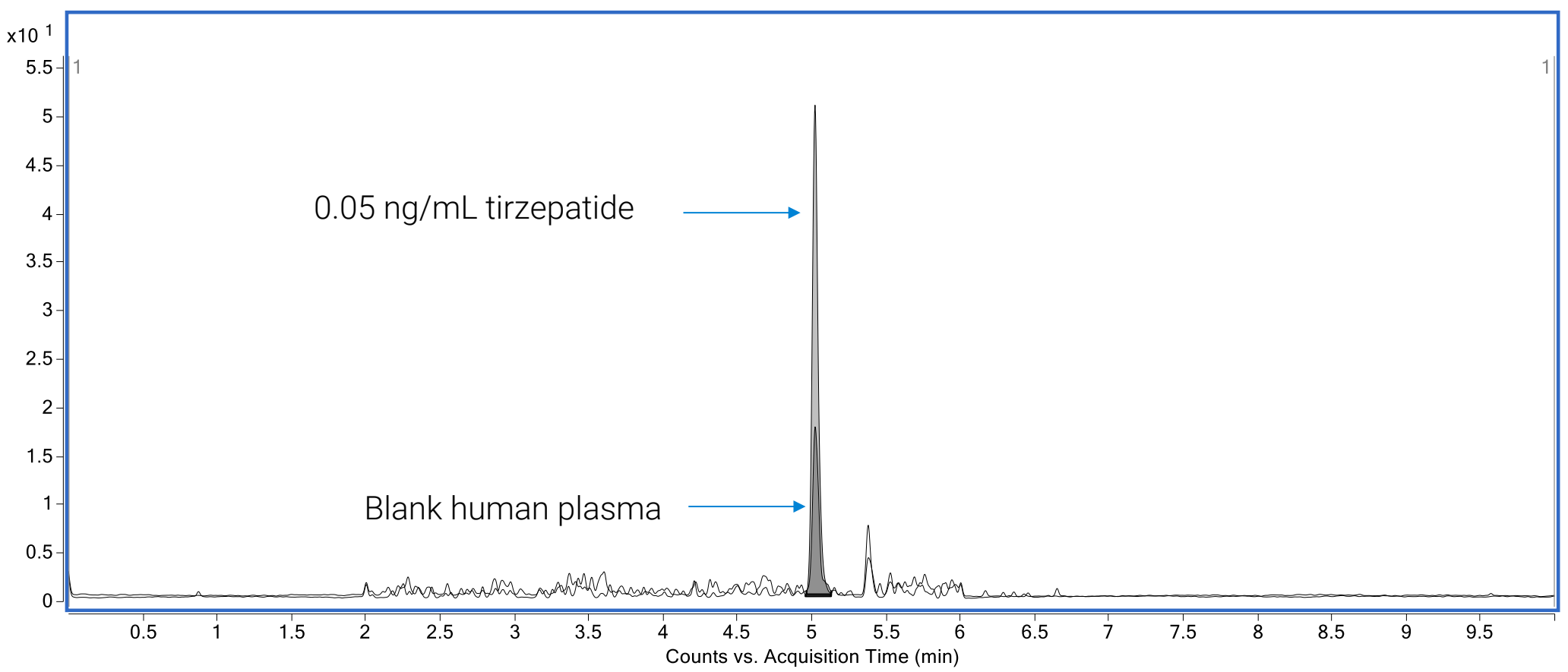

Figure 2. (A) EIC of blank human plasma lowest calibration point. (B) Calibration curve of tirzepatide in human plasma from 0.05 to 1000 ng/mL, insert is zoon in of the lowest five calibration points.

Results and Discussion

The intra-day and inter-day analytical precision and accuracy of quality control samples were determined from three independent runs performed over three days. The precision and accuracy results for tirzepatide in human plasma were shown in Table 4. All levels of quality control samples (n=4) met acceptance criteria of 20% as recommended by regulatory agency. The results demonstrated excellent assay performance by using automated sample preparation.

Table 3. Tirzepatide calibration curve (n=3) performance over 3 runs.

Calibration (ng/mL)	0.05	0.2	0.3	1	10	20	50	200	400	1000
Mean	0.0503	0.189	0.301	1.019	10.276	20.621	51.645	194.025	395.977	964.558
% Bias	0.5	-5.5	0.4	1.9	2.8	3.1	3.3	-3.0	-1.0	-3.5
% CV	2.9	7.4	1.1	8.0	0.8	3.5	2.3	3.0	2.6	5.2

Table 4. Tirzepatide quality control samples (n=4) precision and accuracy over 3 runs.

	QC conc. (ng/mL)	0.05 (LLOQ)	0.1 (low QC)	5.00 (mid QC)	800 (high QC)
Run 1	Mean	0.0507	0.0915	5.0159	794.3824
	% Bias	1.3	-8.5	0.3	-0.7
	% CV	9.2	5.3	2.2	1.0
Run 2	Mean	0.0506	0.0997	4.8651	821.4187
	% Bias	1.3	-0.3	-2.7	2.7
	% CV	5.1	5.2	3.2	7.2
Run 3	Mean	0.0518	0.0952	5.350	723.432
	% Bias	3.7	-4.8	7.0	-9.6
	% CV	12.0	8.8	6.5	0.4
Inter-day	Mean	0.0511	0.0955	5.077	779.744
	% Bias	2.1	-4.5	1.5	-2.5
	% CV	8.5	7.0	5.8	6.8

Conclusions

- An automated LCMS workflow was developed for tirzepatide quantification in human plasma. The lower limit of quantification is 0.05 ng/mL and linear up to 1000 ng/mL from 100 µL starting material , covering a dynamic range of 4.5 order of magnitude. These results are among the best reported in the literature.
- In three qualification runs, intra-day and inter-day QC sample's precision and accuracy all met regulatory acceptance criteria demonstrating excellent assay performance and reproductivity.

References

1. Mounjaro- tirzepatide injection, solution. DailyMed. 13 May 2022.
2. Zepbound- tirzepatide injection, solution. DailyMed. 9 Nov. 2023.

https://www.agilent.com/en/promotions/asms

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