Sensitive LC-MS method for the quantitative analysis of semaglutide and liraglutide in human plasma

Hao Yang[1], Ke Ma[2], Min Du[3]
[1] Thermo Fisher Scientific, San Jose, CA, United States
[2] Thermo Fisher Scientific, Sunnyvale, CA, United States
[3] Thermo Fisher Scientific, Lexington, MA, United States

Abstract

Purpose: Demonstrate optimal detection of semaglutide and liraglutide, two Glucagon-like peptide-1 (GLP-1) analogs in human plasma.

Methods: A Thermo Scientific™ TSQ Altis™ Plus Triple Quadrupole Mass Spectrometer coupled with Thermo Scientific™ Horizon UHPLC system is used for the bioanalysis of semaglutide and liraglutide with a Thermo Scientific™ Hypersil GOLD™ Peptide Column.

Results: Sensitive detection and quantitation of 0.1 ng/mL semaglutide and liraglutide in human plasma with 3 orders of linear dynamic range were achieved. Carryover was below 15% of the LOQ for both GLP-1 analogs.

Introduction

GLP-1 analogs have emerged as pivotal therapeutic agents in the management of type 2 diabetes and obesity. These analogs mimic the physiological actions of endogenous GLP-1, including enhancing glucose-dependent insulin secretion, suppressing glucagon release, delaying gastric emptying, and promoting satiety. Given their significant clinical utilities, accurate detection and quantification of GLP-1 analogs in human plasma are essential for pharmacokinetic studies, therapeutic monitoring, and ensuring patient safety. This study aims to develop a sensitive LC-MS method for the detection and quantitation of semaglutide and liraglutide in human plasma.

Materials and methods

Sample Preparation

Semaglutide and liraglutide were obtained from Adipogen Life Sciences. 1 mg/mL stock solutions, and all calibration standards ranging from 1 to 1000 ng/mL were prepared in pure methanol. A 20 µL aliquot of standards were mixed with 200 µL of human plasma, then 200 µL cold acetone was added to precipitate the proteins. The mixture was vortexed and centrifuged at 12,000 rpm for 10 minutes. The supernatants were extracted using a workflow utilizing the solid phase extraction (SPE) µ-elution plate as shown in Figure 1. Liraglutide was used as an internal standard for the analysis of semaglutide, and vice versa. In both cases, internal standard was kept at 10 ng/mL.

Chromatography

See Table 1 for detailed LC and autosampler conditions used for the separation of GLP-1 analogs.

Mass Spectrometry

See Table 2 for detailed MS source and scan settings for the analysis GLP-1 analogs on TSQ Altis Plus MS.

Data Analysis

Thermo Scientific™ Enterprise compliance ready Chromeleon™ CDS 7.3.2 software was used for all instrument control, data acquisition, processing, and reporting.

Figure 1. Solid phase extraction (SPE) workflow for the extraction of GLP-1 analogs in 200 µL of human plasma.

- Step 1: Add semaglutide and liraglutide standards to 200 µL human plasma, followed by 200 µL of cold acetone; set the 1.5 mL Eppendorf tube on vortex mixer for 10min at 2000rpm
- Step 2: Centrifuge at 12000 rpm for 10min at 10°C; Collect 350 µL supernatant
- Step 3: Conditioning SPE µ-elution plates with 1 mL of methanol, followed by 1 mL of H_2O; Load supernatant
- Step 4: Wash with 0.75 mL of 5% ammonia, followed by 1 mL of methanol, allow the bed to dry under vacuum
- Step 5: Elute with 500 µL of 60:40 acetonitrile:methanol with 6% formic acid; Dry the eluent with N2 stream at 45°C, reconstitute with 200 µL of starting mobile phase

Table 1. MS source and scan settings for the analysis of GLP-1 analogs.

MS Source Settings	TSQ Altis Plus MS
Positive ion (V)	4100
Sheath Gas (Arb)	35
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
Ion transfer tube temperature (ºC)	200
Vaporizer temperature (ºC)	225

Table 2. LC and autosampler conditions for separation of GLP-1 analogs.

HPLC column	Hypersil Gold Peptide, 2.1 x 50mm, 1.9µm	
Flow Rate	0.25 mL/min	
Solvent A	0.4% formic acid in water (v/v)	
Solvent B	0.1% difluoro acetic acid in acetonitrile (v/v)	
Gradient	Time (min)	%B
	0	35
	5	60
	5.5	95
	7.5	95
	8	35
	10	35
Injection Volume	20 µL	
Needle Wash	After draw, 30 µL/s for 10s with 30% methanol	
Column Temperature	60°C	
Divert to Source	2.5 – 6.0 minutes	

Compound	Start Time (min)	End Time (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
Liraglutide	2.8	6	938.8	1064 (Quan)	35	150
Liraglutide	2.8	6	938.8	1128.8 (Conf1)	35	150
Liraglutide	2.8	6	938.8	1185.4 (Conf2)	35	150
Semaglutide	2.8	6	1029.4	1238.4 (Quan)	42	150
Semaglutide	2.8	6	1029.4	1302.8 (Conf1)	42	150
Semaglutide	2.8	6	1029.4	1359.4 (Conf2)	42	150

Results

LC-MS analysis of GLP-1 Analogs in Human Plasma

Semaglutide and liraglutide were extracted from human plasma via anionic exchange SPE technique utilizing the SPE µ-elution plate. All samples were evaluated using SRM mode on TSQ Altis Plus MS. Figure 2 showed collected full spectra for both GLP-1 analogs. With CID fragmentation, the method yielded multiple high intensity fragments (> 50% abundance), and the quantitation using a single ion (most intense fragment) was evaluated. Table 1 showed the list of quantitation and confirming ions used.

Figure 2. Full scan spectrum of semaglutide at m/z 1028.9 (left) and liraglutide at m/z 938.8 (right).

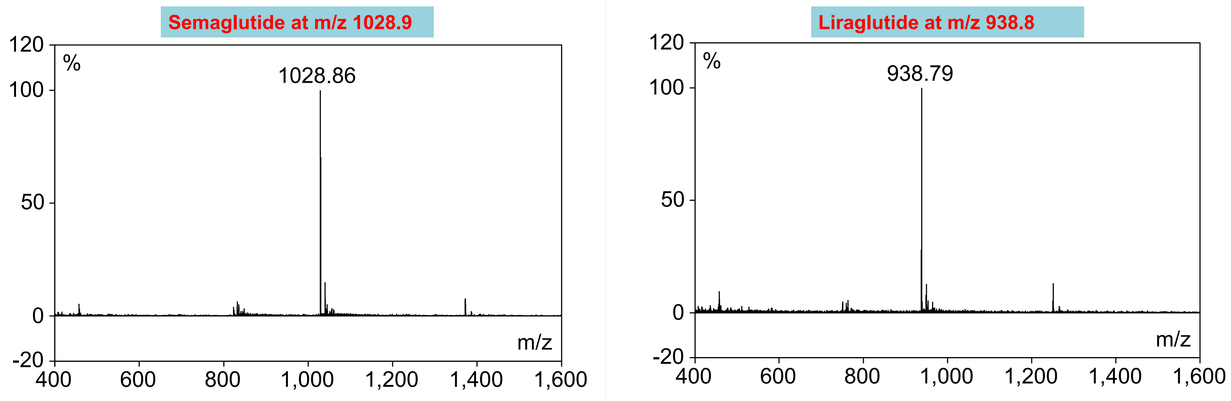
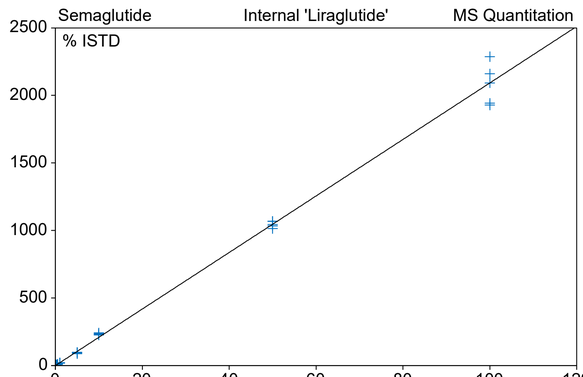


Figure 3 showed that we can achieve excellent precision (i.e., ≤10% RSD) and accuracy (i.e., ≤20% difference) across 3 orders of linear dynamic range (LDR) for semaglutide. The ability to accurately and consistently quantify these low levels of semaglutide will ensure optimal dosing of these therapeutics to patients diagnosed with type II diabetes mellitus and obesity for maximum efficacy while minimizing the adverse side effects.

Results

LC-MS analysis of GLP-1 Analogs in Human Plasma (continued)

Figure 3. Calibration plots, and precision and accuracy measurements for the analysis of semaglutide using liraglutide as an internal standard in human plasma. Using the SRM method, excellent precision and accuracy across 3 orders of linear dynamic range were achieved.



Concentration (ng/mL)	Semaglutide	
	Precision (%RSD)	Accuracy (% Diff)
0.1	9.7	7.9
0.2	10.0	17.0
0.25	2.0	-12.3
1	3.6	-8.7
5	4.5	-9.8
10	1.9	11.5
50	1.6	-0.1
100	6.7	-0.5

Figure 4. XICs of semaglutide samples at the LOQ (0.1 ng/mL, left), post-high concentration injection to assess carryover (following 100 ng/mL, middle), and matrix blank injection (Right). Observed carryover is less than 10% of the LOQ signal.

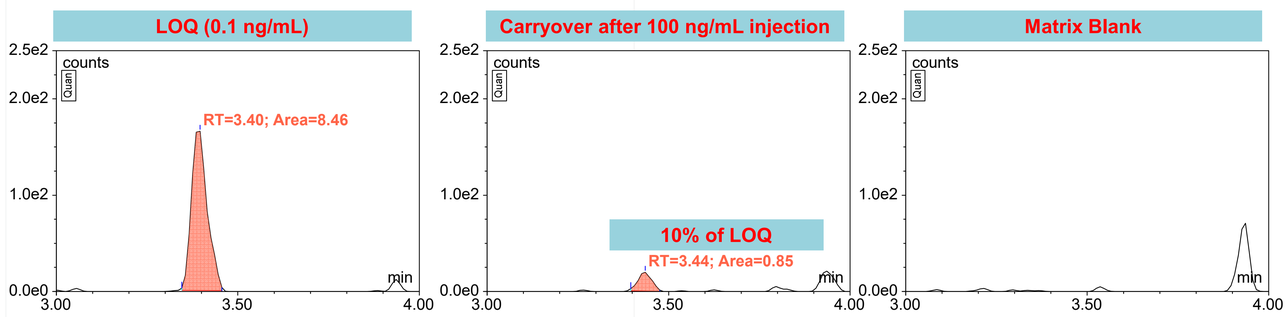
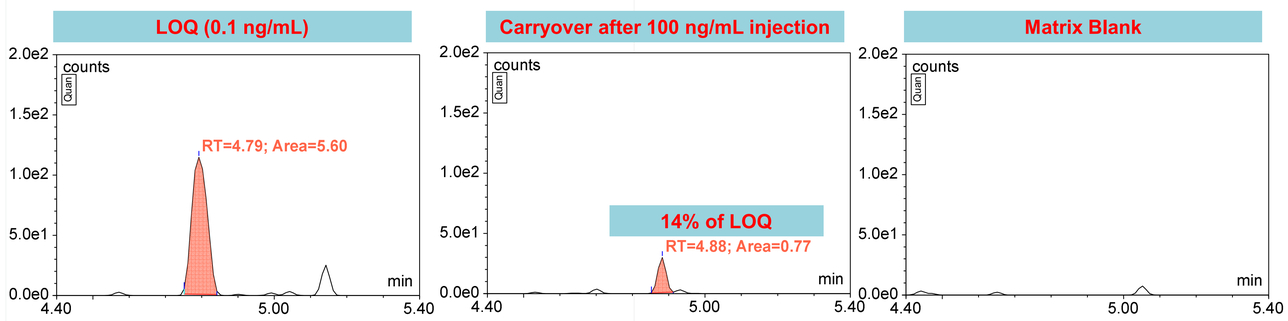


Figure 5. XICs of liraglutide samples at the LOQ (0.1 ng/mL, left), post-high concentration injection to assess carryover (following 100 ng/mL, middle), and matrix blank injection (Right). Observed carryover is less than 10% of the LOQ signal.



To evaluate and minimize carryover, blank plasma matrix was analyzed after injection of the highest concentration standard (100 ng/mL). As shown in Figure 4, carryover was minimal and below 10% of the LOQ (0.1 ng/mL). A “shark teeth” wash was embedded in the method, consisting of five cycles alternating between 25% and 95% MPB (0.5 minutes each). This wash cycle was repeated three times within a single injection method, providing effective needle and system cleaning without requiring multiple separate runs. The same procedure was applied to liraglutide, with results shown in Figure 5, where carryover was slightly higher at approximately 14% of the LOQ.

Conclusions

We demonstrated a comprehensive LCMS solution for highly sensitive quantitation of GLP-1 analogs in human plasma using the Vanquish UHPLC coupled to TSQ Altis Plus MS. The solution enables:

- Robust quantitation of semaglutide and liraglutide with a LOQ of 0.1 ng/mL
- Excellent precision and accuracy across 3 orders of LDR for the analysis of GLP-1 analogs
- Minimal carryover observied with post-injection signals remaining below 10% of the LOQ for semaglutide and below 14% for liraglutide following the highest concentration injection (100 ng/mL)

References

1. Drucker, D. J., Habener, J. F., & Holst, J. J. (2017). *Discovery, characterization, and clinical development of the glucagon-like peptides*. Journal of Clinical Investigation, 127(12), 4217-4227. https://doi.org/10.1172/JCI97233
2. Nauck, M. A., & Meier, J. J. (2018). *Incretin hormones: Their role in health and disease*. Diabetes, Obesity and Metabolism, 20(S1), 5-21. https://doi.org/10.1111/dom.13222

Trademarks/licensing