Detection and quantification of GLP-1 analogs in human plasma: A comprehensive analytical approach

Hao Yang[1], Cristina Jacob[1], Romain Huguet[1], Min Du[2]

[1] Thermo Fisher Scientific, San Jose, CA, United States; [2] Thermo Fisher Scientific, Lexington, MA, United States

Abstract

Glucagon-like peptide-1 (GLP-1) analogs have emerged as pivotal therapeutic agents in the management of type 2 diabetes mellitus 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[1]. Given their significant clinical utility, accurate detection and quantification of GLP-1 analogs in human plasma are essential for pharmacokinetic studies, therapeutic monitoring, and ensuring patient safety. Herein, we demonstrate a versatile LCMS solution, developed using a Thermo Scientific™ Stellar™ mass spectrometer coupled to a Thermo Scientific™ Vanquish™ UHPLC system, for the bioanalysis of semaglutide and liraglutide, two prominent GLP-1 analogs, in human plasma. With this solution, we can consistently achieve a lower quantitation limits (LOQ) of 50 pg/mL extracted from human plasma for both GLP-1 analogs using a multiple-fragment-ions method for quantitation.

Introduction

GLP-1 receptor analogs have emerged as a significant therapeutic class in the treatment of type 2 diabetes mellitus and obesity[1]. GLP-1 is an incretin hormone that plays a crucial role in glucose homeostasis; however, native GLP-1 has a very short half-life due to rapid degradation by dipeptidyl peptidase-4 and renal clearance, limiting its therapeutic potential[2]. To overcome these limitations, chemically synthesized GLP-1 receptor analogs have been developed. These analogs are designed to resist enzymatic degradation and prolong circulation time, thereby enhancing their efficacy and therapeutic benefit. Notable examples of GLP-1 receptor analogs include semaglutide and liraglutide, both manufactured by Novo Nordisk, for weight loss treatment and significant improvement in glycemic control. This study aims to develop a robust analytical method for the detection and quantification of semaglutide and liraglutide in human plasma. The ability to precisely measure GLP-1 analog levels will facilitate better understanding of their pharmacodynamics and pharmacokinetics, ultimately contributing to optimized clinical outcomes in patients receiving GLP-1-based therapies.

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 0.5 to 500 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.

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 Stellar MS.

Software

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

Materials and methods

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

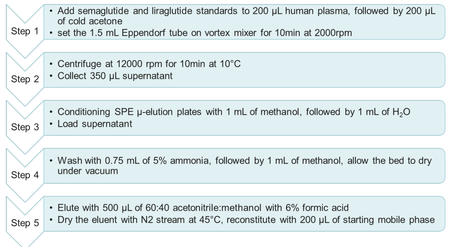


Table 1. LC and autosampler conditions for separation of GLP-1 analogs.

HPLC column	C4 column, 2.1 x 50mm, 1.7µm	
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	25
	5	50
	5.5	95
	7.5	95
	8	25
	10	25
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 – 10 minutes	

Table 2. MS source and scan settings for the analysis of GLP-1 analogs.

MS Source Settings	Stellar MS
Positive ion (V)	4100
Sheath Gas (Arb)	35
Aux Gas (Arb)	10
Sweep Gas (Arb)	1
Ion transfer tube temperature (°C)	200
Vaporizer temperature (°C)	225

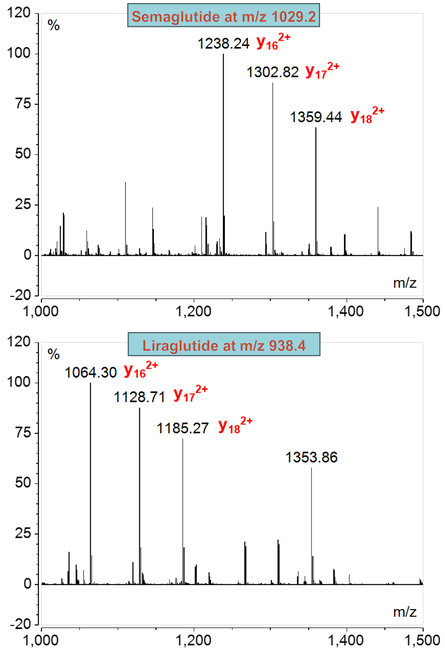
Scan Settings (tMS2 mode)	Stellar MS
Isolation window (*m/z*)	2
Collision energy (%)	30
Scan rate (kDa/sec)	125
Scan range (*m/z*)	1000-1500
RF lens (%)	70
Maximum injection time (ms)	100

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 tMS2 mode on Stellar MS. Figure 2 showed collected tMS2 spectra for both GLP-1 analogs. With HCD fragmentation, the method yielded multiple high intensity fragments (> 50% abundance), and the quantitation using a single ion (most intense fragment) and multiple ions (summing the XICs of top 3 intense fragment ions) were evaluated. Table 3 showed the list of quantitation ion(s) used for each method. Using the multiple-ions summing method, as shown in Figure 3a, we observed 2x improvement in sensitivity for GLP-1 analysis without compromising the selectivity.

Figure 2. tMS2 of semaglutide at *m/z* 1029.3 (top) and liraglutide at *m/z* 938.4 (bottom) using HCD fragmentation method. Only top 3 most intense fragments, which are doubly charged y_{16} to y_{18} ions, were labeled.



Results

LC-MS analysis of GLP-1 analogs in human plasma (continued)

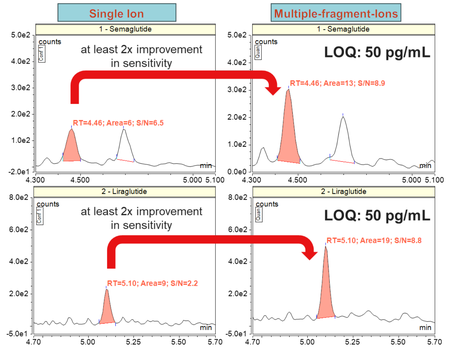
Figure 3b showed that we can achieve excellent precision (i.e., ≤15% RSD) and accuracy (i.e., ≤15% difference) across 3 orders of LDR for both GLP-1 analogs. The ability to accurately and consistently quantify these low levels of GLP-1 analogs will ensure optimal dosing of these therapeutics to patients diagnosed with type II diabetes mellitus and obesity for maximum efficacy while minimizing the adverse side effects.

Table 3. Quantitation ion(s) for the GLP-1 analogs using a single vs multiple-fragment ions data processing method.

Data Processing Method	Semaglutide	Liraglutide
Single ion	1238.0	1064.0
Multiple-fragment ions	1238.0, 1302.8, 1359.4	1064.0, 1128.8, 1185.4

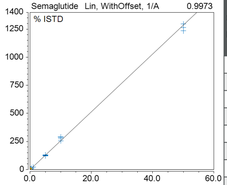
Figure 3. Quantitation performances for the analysis of semaglutide and liraglutide in human plasma. a) XICs of extracted samples at LOQ level (i.e., 50 pg/mL) processed using a single versus multiple-fragment-ions method. With the multiple-fragment-ions method, a 2x improvement in peak area without reducing the signal-over-noise ratio was observed for both GLP-1 analogs. b) Calibration plots, and precision and accuracy measurements for the analysis of GLP-1 analogs in human plasma. Using the multiple-fragment-ions method, excellent precision and accuracy across 3 orders of linear dynamic range were achieved for both GLP-1 analogs.

a) XICs of extracted samples at 50 pg/mL using single ion and multiple-fragment-ions method

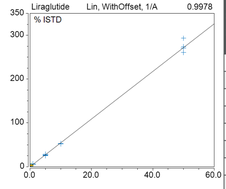


Results

b) Calibration plots, and precision and accuracy measurements for the analysis of GLP-1 analogs in human plasma



Concentration (ng/mL)	Semaglutide	
	Precision (%RSD)	Accuracy (% Diff)
0.05	9.5	6.6
0.1	13	3.6
0.2	7.7	3.4
0.5	4.6	6.8
1	1.8	12
5	1.2	1.8
10	5.6	7.9
50	13	1.7



Concentration (ng/mL)	Liraglutide	
	Precision (%RSD)	Accuracy (% Diff)
0.05	11	7
0.1	7.8	1.2
0.2	7.1	5.7
0.5	5.1	3.8
1	8.9	5.2
5	2.9	3.4
10	1.9	4.1
50	0.6	1

Conclusions

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

- Robust quantitation of semaglutide and liraglutide with a LOQ of 50 pg/mL using HCD fragmentation and multiple-fragment-ions method
- Excellent precision and accuracy across 3 orders of LDR for the analysis of GLP-1 analogs

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

General Laboratory Equipment – Not For Diagnostic Procedures.

Trademarks/licensing