Sensitive quantification of human glucagon-like peptide 1 (GLP-1) analogue in plasma using LC-MS/MS

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ABSTRACT

GLP-1 analogues are responsible for the regulation of insulin and glucagon secretion. Recent advancements towards the development of next-generation insulin therapeutics have generated a need for more sensitive MS technologies. Analysis of GLP-1 analogs such as liraglutide can often be challenging as it is faced with poor ionization and fragmentation due to the inherent large peptide structure. Additionally, peptide therapeutics such as liraglutide are highly potent, and oral administration is often at low bioavailability. Therefore, a highly sensitive and robust platform is essential for the quantification of GLP-1 analogs. Herein, an ultra-sensitive LC-MS/MS method was developed for the quantification of liraglutide in plasma.

INTRODUCTION

- GLP-1 analogues are altered forms of native GLP-1 that offer an improved ability to reduce and maintain normal blood glucose levels via regulation of insulin and glucagon secretion. The analogues exhibit improved resistance to enzymatic degradation and are well tolerated due to high sequence homology with native GLP-1.
- Due to the high potency and low dosages administered, sensitive quantitative measurements are essential when examining the pharmacokinetic and pharmacodynamic profiles of peptide therapeutics. This, along with an increase in research and development of novel GLP-1 analogues, has generated a high demand for sensitive and robust bioanalytical workflows.
- However, developing sensitive LC-MRM methods for peptide therapeutics, such as liraglutide, is often challenging, due to the large structure that results in poor ionization and fragmentation.
- Herein, a highly sensitive workflow for quantification of the GLP-1 analogue, liraglutide, in rat plasma using a novel triple quadrupole mass spectrometer is presented.

MATERIALS AND METHODS

Sample preparation:
Liraglutide (Sigma Aldrich) is a GLP-1 analogue in the form of a lipopeptide. Liraglutide was spiked into plasma at various concentration levels (0.5 - 500 ng/mL). Insulin lispro was used as an internal standard and was also spiked into plasma. Stock solutions of liraglutide and the IS were prepared with acetonitrile/5mM ammonium acetate (70:30, v/v) and a serial dilution was performed to prepare working solutions.

Rat plasma was extracted using protein precipitation with acetonitrile/methanol (70:30, v/v). The resulting supernatant was diluted and used as the biological matrix for this assay.

LC conditions:
Analytes were separated using a Halo ES-C18 column (2.1 mm × 50 mm, 2.7 µm, 160 Å) on an ExionLC system. Total method time was 8.5 min at a flow rate of 0.5 mL/min. Mobile phase A was composed of 0.1% formic acid in water while mobile phase B was composed of 0.1% formic acid in acetonitrile. Operating column temperature was 40 ºC. Injection volume was 10 µL.

MS conditions:
MS analysis was performed on a SCIEX 7500 system. Sample analysis was performed in positive MRM mode. Optimal MS and source conditions are shown in Table 1. Monitored MRM transitions for liraglutide and the IS are summarized in Table 2.

Data processing:
MRM data was processed with SCIEX OS software 2.0 using the MQ4 integration algorithm. Linear regression with 1/x weighting was applied for the quantification of liraglutide.

Table 1. MS and source conditions.

Parameter	Setting
Polarity	Positive
Curtain gas	35 psi
Gas 1	20 psi
Gas 2	80 psi
CAD gas	11
Ion spray voltage	3500 V
Source temperature	300 °C

- Positive polarity was applied for the LC-MRM analysis
- The optimal curtain gas, CAD gas, ion spray voltage, and source temperature were 35 psi, 11, 3500 V, and 300 ºC, respectively
- The optimal ion source gas 1 and 2 were 20 psi and 80 psi, respectively

- For all the MRM transitions, including the IS, CE and CXP were optimized
- For liraglutide MRM transitions, the CE was 45 V while the CXP was 15 V
- For the insulin lispro MRM transition, optimal CE was achieved at 46 V while CXP was optimized to 18 V for best quantitative sensitivity

Table 2. MRM^{HR} parameters and fragments for quantification.

ID	Q1 mass (*m/z*)	Q3 mass (*m/z*)	CE (V)	CXP (V)
Liraglutide 1	939.06	1064.3	45	15
Liraglutide 2	939.06	1128.6	45	15
Insulin lispro*	1162.0	217.1	46	18

**MRM transition for internal standard*

Table 3. LC conditions.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	0.5	90	10
1.0	0.5	90	10
3.0	0.5	60	40
5.0	0.5	60	40
5.1	0.5	10	90
7.0	0.5	10	90
7.1	0.5	90	10
8.5	0.5	90	10

- An ExionLC system was used for analytical separation
- A flow rate of 0.5 mL/min was applied
- With the developed method, the retention time of the liraglutide analyte was at 5.53 min

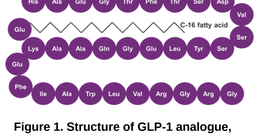


Figure 1. Structure of GLP-1 analogue, liraglutide.

- Quantification of complex structures such as GLP-1 analogues was evaluated using a model analyte, liraglutide
- It is composed of 31 amino acids with a C-16 free fatty acid derivative bound to a glutamoyl spacer
- The quantitative assay was developed using an LC-MRM method

RESULTS

In this workflow, a sensitive LC-MRM method was developed for the quantification of GLP-1 analogue, liraglutide, in rat plasma. All method parameters were carefully optimized to ensure the best sensitivity. Quantification was performed in positive ion MRM mode.

The 4+ multiply charged precursor ion for liraglutide, *m/z* 939.06, displayed the highest signal. Two fragment ions of the precursor ion of *m/z* 939.06 were monitored including *m/z* 1064.3 and *m/z* 1128.6.

The y-ion fragment at *m/z* 1064.3 produced the most sensitive signal and was used for quantification.

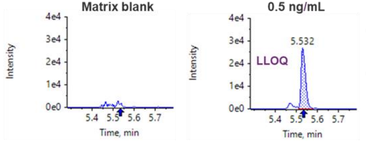


Figure 2. XICs displaying the MRM transition of liraglutide.
Matrix blank from extracted plasma (left) and LLOQ of liraglutide (right) are shown.

- No noticeable interference was observed in the matrix blank (Figure 2)
- An LLOQ of 0.5 ng/mL for liraglutide quantification was achieved

The LLOQ was determined based on the requirements that the %CV of the average of the concentration is below 20% and accuracy is between 80% and 120%. For the concentrations above the LLOQ, the %CV of the mean of the calculated concentration was required to be below 15% while accuracy was required within the range from 85% to 115%.

The LLOQ for liraglutide was determined to be 0.5 ng/mL as shown in Figure 2. No significant interference was observed in the matrix blank.

Table 4. Quantitative summary of liraglutide.

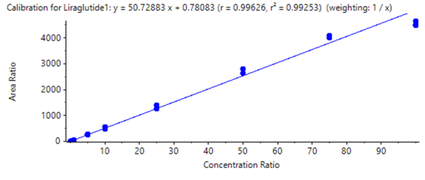
Concentration (ng/mL)	Accuracy (%)	CV (%)
0.5	95.07	6.88
1	89.38	4.91
5	94.39	6.37
10	101.35	4.70
50	107.08	3.27
100	105.41	7.72
250	103.31	6.48
500	108.47	3.29
750	105.94	1.12
1000	89.61	2.00

- Each of the concentration measurements were performed in triplicate
- At the LLOQ of 0.5 ng/mL, the accuracy was within ±10% of the nominal concentration, demonstrating high accuracy of the LC-MRM assay at low-level concentrations
- At the ULOQ of 1000 ng/mL, the accuracy was within ±11% of the nominal concentration, demonstrating high accuracy of the LC-MRM assay at the higher-end concentrations

Exceptional accuracy and precision were achieved for liraglutide across the concentration range of 0.5 ng/mL to 1000 ng/mL (Table 4).

Overall, the %CV was less than 8% for all measured concentrations, demonstrating high reproducibility.

Calibration curves were analyzed in triplicate. The linear range was determined to be between 0.5 ng/mL and 1000 ng/mL.



A linear dynamic range (LDR) of 3.3 orders of magnitude was achieved (Figure 3)

Figure 3. Calibration curve of liraglutide.

CONCLUSIONS

- An ultra-sensitive MRM based GLP-1 analogue quantification workflow using SCIEX 7500 system was developed
- Liraglutide in rat plasma was quantified at 0.5 ng/mL in rat plasma with outstanding reproducibility, accuracy, and linearity with a LDR of 3.3 orders of magnitude
- Low-levels of quantification suggest applications in pharmacokinetics and pharmacodynamics studies of peptide therapeutics
- A cumulative gain in sensitivity was observed for GLP-1 analogue assays in complex matrices as a result of the combined improvements in front-end technology including the OptiFlow Pro ion source, E Lens probe, and D Jet ion guide

TRADEMARKS/LICENSING