Improved LC-MRM workflow for quantification of glucagon-like peptide-1 analogues

Featuring the SCIEX 7500 system, powered by SCIEX OS software

John R. Thorup, Eshani Nandita, Zoe Zhang
SCIEX, Redwood City, USA

In this technical note, a robust and sensitive LC-MRM method was developed for the quantification of liraglutide, a clinically approved glucagon-like peptide-1 (GLP-1) analogue. Excellent sensitivity was achieved for the quantification of liraglutide at an LLOQ of 0.5 ng/mL in rat plasma with outstanding reproducibility, accuracy, and linearity. Solid quantification at low-level concentrations is highly advantageous for pharmacokinetic and pharmacodynamic studies of peptide therapeutics.

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.

This technical note describes the quantification of the GLP-1 analogue, liraglutide in rat plasma using a MRM method on the SCIEX 7500 System.

Key features of GLP-1 analogue quantification using the SCIEX 7500 system

- Quantify GLP-1 analogues in complex biological matrix with outstanding reproducibility, precision, accuracy, and linearity
- Achieve improved sensitivity through hardware improvements including:
  - D Jet ion guide—increased capture and transmission of analyte ions[1]
  - OptiFlow Pro ion source—latest generation ion source with maximum flexibility and robustness[1]
  - E Lens probe—increased field strength improves desolvation and ion generation[1]
- Employ a single platform for streamlined data acquisition, processing, and management using SCIEX OS software
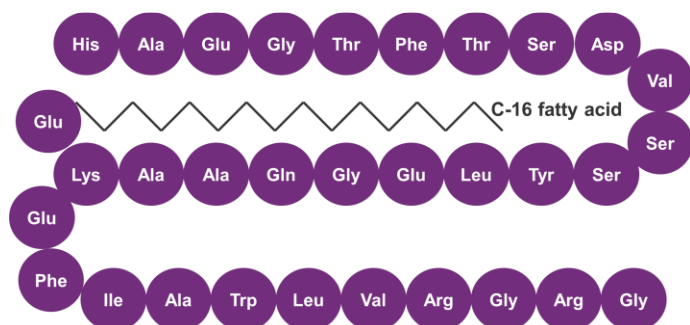

Figure 1. Structure of GLP-1 analogue, liraglutide. It is composed of 31 amino acids with a C-16 free fatty acid derivative bound to a glutamoyl spacer.

Methods

Samples and reagents: Liraglutide, LC-MS grade acetonitrile and methanol, and ammonium acetate were purchased from Sigma Aldrich. Insulin lispro was purchased from USP. Rat plasma (Sprague Dawley, K2 EDTA) was purchased from BioIVT.

Sample preparation: Liraglutide and insulin lispro (internal standard, IS) were spiked into the extracted rat 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.

Chromatography: 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 flowrate 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. Mobile phase gradient is summarized in Table 1. Operating column temperature was 40 °C. Injection volume was 10 µL.

Table 1. LC method.

Time (min)	Flow rate (µL/min)	%A	%B
0.0	5	90	10
1.0	5	90	10
3.0	5	60	40
5.0	5	60	40
5.1	5	10	90
7.0	5	10	90
7.1	5	90	10
8.5	5	90	10

Mass spectrometry: MS analysis was performed on a SCIEX 7500 system. The optimized MRM parameters are summarized in Table 2.

Table 2. 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

Monitored MRM transitions for liraglutide and the IS are summarized in Table 3 below. For all the MRM transitions, an entrance potential (EP) of 10 V was used.

Table 3. MRM method parameters.

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

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

Quantification 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.
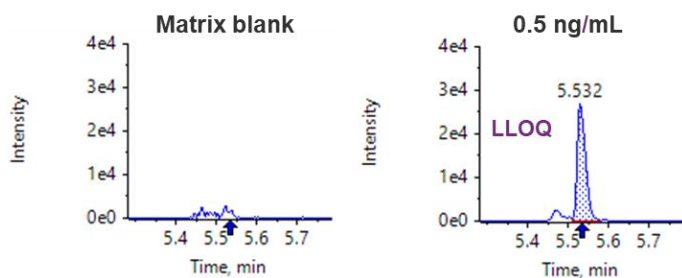
Figure 2. XICs displaying the MRM transition of liraglutide. Matrix blank from extracted plasma (left) and LLOQ of liraglutide (right) are shown. No noticeable interference was observed in the matrix blank.

Calibration curves were analyzed in triplicates. 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)

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 3. No significant interference was observed in the matrix blank.

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.

Table 4. Accuracy and precision results at each concentration level. Each concentration point was measured in triplicate.

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

A highly robust and sensitive method for the quantification of liraglutide in a complex biological matrix was demonstrated.

Calibration for Liraglutide1: $y = 50.72883 x + 0.78083$ ($r = 0.99626$, $r^2 = 0.99253$) (weighting: $1/x$)
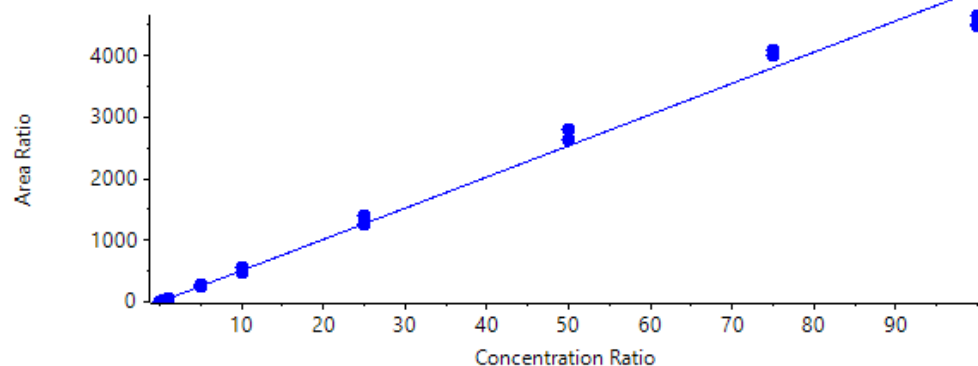
Figure 3. Calibration curve of liraglutide from 0.5 to 1000 ng/mL. LDR of 3.3 orders of magnitude was achieved.

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

References

1. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-A.

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
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