Low-ng/mL quantitation of glucagon-like peptide-1 (GLP-1) analog in rat plasma

Sensitive quantitative analysis of semaglutide in rat plasma using the SCIEX 7500 system

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This technical note demonstrates a sensitive method to quantify a glucagon-like peptide-1 (GLP-1) analog, semaglutide, in rat plasma on a high-end triple quadrupole mass spectrometer. A lower limit of quantitation (LLOQ) of 0.2 ng/mL was determined using a 10-minute LC-MS/MS method (Figure 1).

GLP-1 is an incretin hormone that stimulates glucose-dependent insulin release in humans.[1] GLP-1 analogs have attracted attention in drug development because they help regulate systemic homeostasis. Several GLP-1 analogs, including orally available semaglutide, are clinically approved and are used to treat type 2 diabetes.[1]

Some GLP-1 analogs have low oral availability and are administered via subcutaneous injection in a frequent dosing regimen, leading to a treatment burden on patients.[2] When developing alternative formulations (non-injectables and long-acting analogs), challenges arise, such as low bioavailability of the analogs in blood and limited sample availability. Such limitations impact the performance of quantitative assays used to assess pharmacodynamics throughout drug development. Therefore, sensitive and selective assays for high-confidence detection and quantitative performance in biological matrices are needed to ensure the safety and efficacy of the promising analogs.

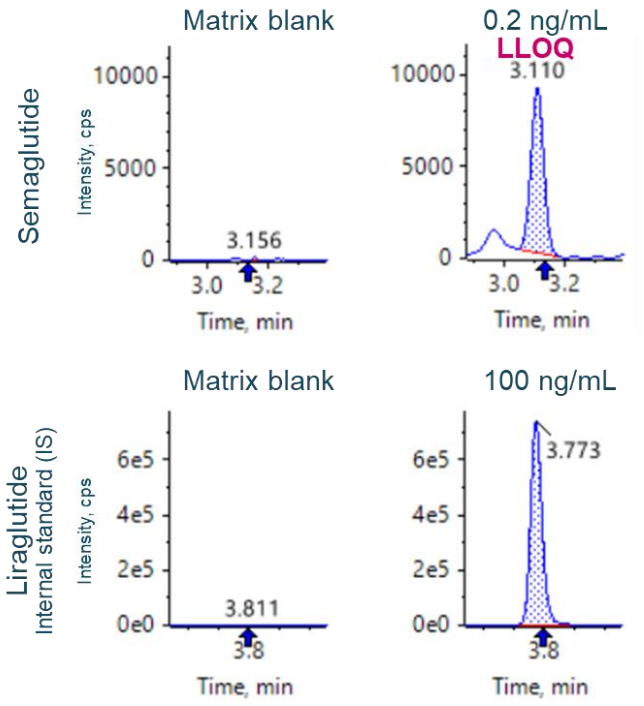

Figure 1. Representative extracted ion chromatograms (XICs) of semaglutide and liraglutide (IS) at the LLOQ level. An LLOQ of 0.2 ng/mL was achieved for semaglutide in rat plasma. No matrix interference was observed at the retention time of the analyte.

The presented method demonstrates a sensitive quantitative method for a commercially available GLP-1 analog, semaglutide, in rat plasma. Sensitivity at the low-ng/mL level was achieved in rat plasma using the SCIEX 7500 system. Front-end enhancements on the instrument facilitated improved overall ion generation, capture and transmission, providing optimal quantitative sensitivity.

Key features of the quantitation of semaglutide using the SCIEX 7500 system

- **Low-ng/mL level quantitation of a high-potency GLP-1 analog:** Achieve a 0.2 ng/mL LLOQ for semaglutide in rat plasma on the SCIEX 7500 system
- **Ideal analytical performance:** Achieve accurate quantitative performance with %CV <13% at all concentration levels across a linear dynamic range (LDR) spanning 3 orders of magnitude
- **Enhanced sensitivity unlocked:** Improved front-end technology with the D Jet ion guide, OptiFlow Pro ion source and E Lens probe enhanced the ion generation, capture and transmission, enabling users to reach desired quantitative sensitivity[3]
- **Streamlined data management:** Data acquisition and processing are integrated into SCIEX OS software, a 21 CFR Part 11 compliance-ready platform

Methods

Sample preparation: The commercially available GLP-1 analogs, semaglutide (Figure 2A) and liraglutide (Figure 2B), were reconstituted in 6% formic acid in methanol and 3% formic acid in methanol, respectively. Semaglutide was spiked into 100 µL of rat plasma at concentrations ranging from 0.2 ng/mL to 200 ng/mL. Liraglutide was used as an internal standard (IS) and spiked at 10 ng. Protein precipitation was performed with 300 µL of methanol. Samples were vortexed for 30 seconds and centrifuged at 12000 rcf for 12 minutes at room temperature. The supernatant was transferred to a new Eppendorf tube. A 300 µL aliquot of 10% aqueous ammonia was added and gently mixed and 600 µL of the sample volume was transferred to an anion exchange Oasis MAX µ-Elution plate. Samples were washed with 5% ammonia in 1:1 (v/v) methanol/water. A second wash was performed using 1:2:2 (v/v/v) water:methanol:acetonitrile. Finally, elution was performed using 6% formic acid in 1:2:2 (v/v/v) water:methanol:acetonitrile mixture. The final elution volume was 100 µL.

Chromatography: Sample separation was performed using an ExionLC system at a flow rate of 0.4 mL/min on a Phenomenex Kinetex C18 (2.1 x 50 mm, 2.6 µm, 100 Å) column. A 10-minute gradient was run using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B (Table 1). The column temperature was maintained at 50°C. An injection volume of 5 µL was used for analysis. A mixture of 1:1:1 (v/v/v) acetonitrile:methanol:water was used as a needle wash solvent.

Table 1. Chromatographic gradient for semaglutide and liraglutide.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	*70*	*30*
0.2	*70*	*30*
3.0	*55*	*45*
5.0	*40*	*60*
5.1	*5*	*95*
7.1	*5*	*95*
7.2	*70*	*30*
10.0	*70*	*30*

Mass spectrometry: The optimized source and gas parameters are listed in Table 2 and the optimized analyte-dependent MRM parameters are included in Table 3.

Table 2. Source and gas parameters.

Parameter	Value
Polarity	*Positive*
Ion source gas 1	*50 psi*
Ion source gas 2	*60 psi*
Curtain gas	*42 psi*
Source temperature	*550°C*
Ion spray voltage	*5000 V*
CAD gas	*9*

Table 3. MRM parameters used for quantitation.

ID	Precursor ion (*m/z*)	Fragment ion (*m/z*)	CE (V)	CXP (V)
Semaglutide	*1029*	*1109.5*	*40*	*15*
*Liraglutide**	*939*	*1064.0*	*40*	*20*

**Liraglutide was used as an IS*

Data processing: Data collection and analysis were performed in SCIEX OS software, version 3.0. Peaks were automatically integrated using the MQ4 algorithm and a weighting of $1/x^2$ was used for quantitation.

Quantitative performance

This technical note demonstrates a low-ng/mL level quantitation assay of semaglutide in rat plasma using the SCIEX 7500 system. Low solubility in an aqueous mixture and poor ionization of analogs are common challenges in the analytical detection of GLP-1 analogs. The method was optimized to achieve a sensitive quantitation assay from sample extraction to chromatography and MS detection.

The calibration curve ranged from 0.2 ng/mL to 200 ng/mL and

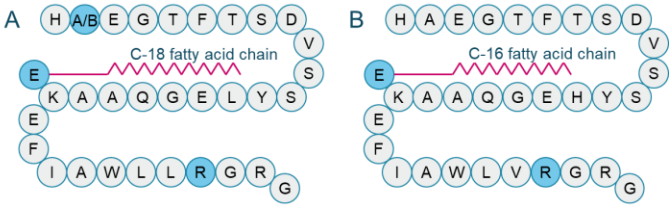

Figure 2. Structures of GLP-1 analogs. The left panel shows the structure of the target analyte, semaglutide (A) and the right panel shows the structure of the IS, liraglutide (B).

was prepared as described in the sample preparation section. Individual concentrations were run in triplicate.

Analytical performance was evaluated for accuracy and precision. The accuracy of the calculated mean was expected to be between 80% and 120% at the LLOQ and between 85% and 115% at higher concentrations. The %CV of the calculated mean for each concentration was expected to be <20% at the LLOQ and <15% at higher concentrations.

Accuracy was within ±11% of the nominal concentration and the %CV was <13% for semaglutide (Figure 3). Calculated accuracy and %CV values met the acceptance criteria at each concentration level.

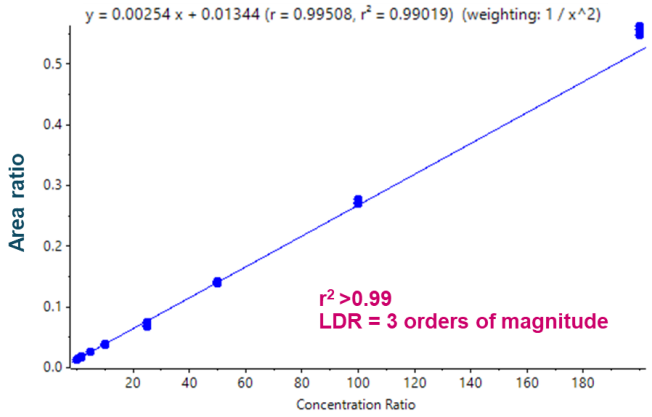

Figure 4. Calibration curve for semaglutide. The area ratio between semaglutide and liraglutide (IS) was used to generate a calibration curve. Each concentration level was run in triplicate. Linearity was achieved between 0.2 ng/mL and 200 ng/mL and spanned an LDR of 3 orders of magnitude with an r^2 >0.99.

An LLOQ of 0.2 ng/mL was achieved for semaglutide (Figure 1). No interferences were observed in the rat plasma matrix blank (Figure 1). Linearity was achieved between 0.2 ng/mL and 200 ng/mL with a coefficient of determination (r^2) >0.99 (Figure 4). An LDR spanning 3 orders of magnitude was achieved.

Compliance-ready SCIEX OS software

SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing workstations. Figure 5 illustrates the features of SCIEX OS software that are used for monitoring the audit trail, acquiring and processing data and configuring user access.

Actual Concentration	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates	Value #1	Value #2	Value #3
0.20	0.204	0.013	6.18	101.99	0.207	0.190	0.215
0.50	0.455	0.041	9.11	91.01	0.410	0.465	0.491
1.50	1.656	0.207	12.49	110.41	1.634	1.877	1.384
5.00	4.857	0.156	3.22	97.15	4.741	5.035	4.796
10.00	9.681	0.303	3.13	96.81	9.872	9.840	9.331
25.00	22.522	1.465	6.50	90.09	21.460	21.914	24.193
50.00	50.215	0.689	1.37	100.43	50.171	49.550	50.925
100.00	102.013	1.350	1.32	102.01	101.067	101.413	103.559
200.00	213.266	3.123	1.46	106.63	213.882	216.035	209.882

Figure 3. Quantitative performance for semaglutide analysis. Reproducibility and accuracy results were determined from the calibration curve across 3 replicates at each concentration. Statistical results were summarized using the Analytics module in SCIEX OS software.

Figure 5. Features of the SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and levels of access for the administrator, method developer, analyst and reviewer levels.

The audit trail feature enables users to audit critical user actions and locks in data integrity. The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards.

The configuration module allows users to assign roles and access as the administrator, method developer, analyst and reviewer.

Conclusions

- An LLOQ of 0.2 ng/mL was reached for the quantitation of semaglutide in rat plasma
- Linearity was achieved between 0.2 ng/mL and 200 ng/mL, generating an LDR spanning 3 orders of magnitude with an r^2 >0.99
- The method demonstrated accurate and highly reproducible (%CV <13%) quantitative performance at all concentrations
- Sensitivity was achieved on the SCIEX 7500 system with an improved front-end technology for better ion generation, capture and transmission
- SCIEX OS software is compliance-ready to support 21 CFR Part 11 and integrates with an accurate mass spectrometer to support data acquisition, processing and management on a single platform

References

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