Reenvisioning peptide LC-MS bioanalysis: combining quantitative flexibility with reliable performance

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This technical note demonstrates a sensitive, accurate, and highly reproducible method for quantitation of a peptide therapeutic using the novus V55 system. A lower limit of quantitation (LLOQ) of 25 pg/mL was achieved for the analysis of insulin degludec in rat plasma (Figure 1). The novus V55 system delivers reliable and uncompromised quantitative performance in a compact design.

Peptide therapeutics have had an incremental impact on drug development over the past decade. Compared with small-molecule therapeutics, peptides exhibit greater specificity and efficacy, making them ideal candidates for the treatment of critical diseases.[1] Given the immense potential of peptide therapeutics, sensitive and selective LC-MS assays are needed for accurate quantitation in complex biological matrices. Furthermore, because a wide range of charge states is encountered in peptide quantitation, flexibility is critical to achieve optimal sensitivity and selectivity. Here, quantitation of a model peptide therapeutic, insulin degludec, in rat plasma is demonstrated, combining excellent quantitative performance with a sustainable platform, the novus V55 system.[2]

Key benefits for analysis of insulin degludec using the novus V55 system

- **Low-pg/mL level of quantitation:** Achieve 25 pg/mL LLOQ for the quantitation of insulin degludec in rat plasma.
- **Robust analytical performance:** Achieve accurate quantitative performance with %CV <3 at all concentration levels across a linear dynamic range (LDR) of 3 orders of magnitude.
- **Extended mass range:** Gain flexibility for quantitation of therapeutic peptides with a broader mass range on the novus V55 system, enabling more options for m/z selection for optimal sensitivity and selectivity.
- **Small footprint without compromising quantitative fidelity:** Reach optimal bioanalytical quantitative performance using the most compact triple quadrupole mass spectrometer in its class.

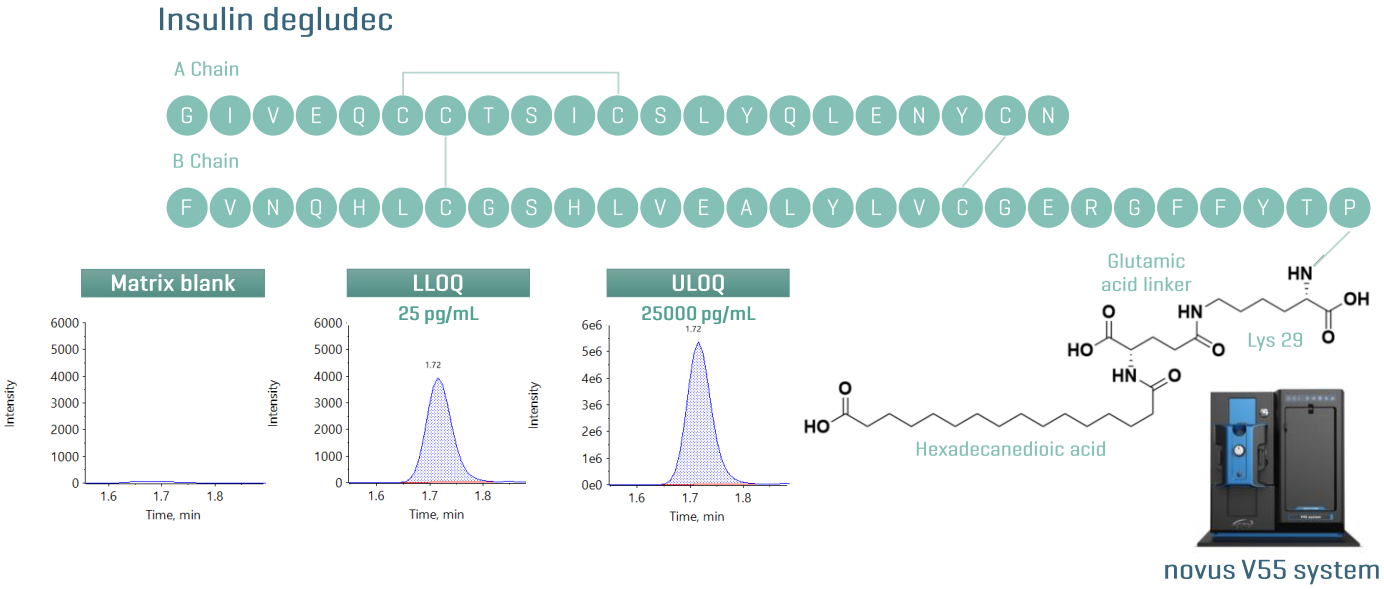

Figure 1. Representative extracted ion chromatograms (XICs) and structure of insulin degludec. An LLOQ of 25 pg/mL was achieved for insulin degludec in rat plasma. No interference was observed at the retention time of the analyte in the matrix blank. An upper limit of quantitation (ULOQ) of 25000 pg/mL was reached.

- **Streamlined data management:** Easily acquire, manage, and process data using SCIEX OS software, a 21 CFR Part 11-compliant platform.

Introduction

LC-MS has been widely adopted for bioanalytical work in the development of peptide therapeutics, largely attributed to the sensitivity and selectivity it provides. When performing peptide quantitation, bioanalytical labs are often challenged with choosing the best combination of m/z values that provides optimal selectivity and sensitivity. Beyond quantitative performance, as bioanalytical labs continue to grow and expand, the question of how to meet sustainability goals becomes more prominent.

In this study, insulin degludec was used as a model peptide therapeutic and analyzed in rat plasma. Analysis was performed on the novus V55 system, offering a wider quadrupole mass range, which allows for more quantitative flexibility for optimal selectivity and sensitivity. Additionally, the novus V55 system delivers highly reliable quantitative performance within a compact design, empowering bioanalytical laboratories with enhanced energy efficiency solutions.

Methods

Sample preparation: The commercially available insulin degludec (Figure 1) was reconstituted in dimethyl sulfoxide. Insulin degludec was diluted in dilution solvent containing 1% formic acid in a 75:25 (v/v) acetonitrile/water mixture. Individual concentrations were spiked into 100 µL of rat plasma at concentrations ranging from 10 pg/mL to 25000 pg/mL. Protein precipitation was performed with 100 µL of 4% phosphoric acid in methanol. Samples were vortexed for 30 seconds and centrifuged at 12000 rcf for 10 minutes at room temperature. The supernatant was transferred to a new Eppendorf tube containing 800 µL water, and samples were briefly vortexed and centrifuged. Then, 500 µL of each sample was loaded twice on a Phenomenex Strata-X-A microelution plate operated under positive pressure. Samples were consecutively washed with 5% aqueous ammonia and 20% acetonitrile in water. Finally, elution was performed using 50 µL of 1% trifluoroacetic acid in a solution containing 70:20:10 (v/v) acetonitrile/water/acetic acid. 50 µL of water was added to bring the final volume to 100 µL.

Chromatography: Sample separation was performed using an Agilent 1290 Infinity II LC System at a 0.6 mL/min flow rate on a Phenomenex Kinetex XB C18 (2.1 x 50 mm, 1.7 µm, 100 Å) column. A 6-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 55°C. An injection volume of 10 µL was used for analysis. A mixture of equal parts by volume of acetonitrile, methanol and water was used as a needle wash solvent.

Mass spectrometry: Analysis was performed on the novus V55 system (SCIEX). The optimized source and gas parameters are listed in Table 2, and the MRM parameters are included in Table 3.

Table 1. LC gradient conditions.

Time (Min)	Mobile phase A (%)	Mobile Phase B (%)
0.0	90	10
0.2	90	10
1	70	30
3.0	55	45
3.1	2	98
4.1	2	98
4.2	90	10
6	90	10

Table 2. Source and gas parameters.

Parameter	Value
Polarity	Positive
Ionization mode	ESI
Ion source gas 1	50 psi
Ion source gas 2	60 psi
Curtain gas	40 psi
Source temperature	500°C
Spray voltage	5000 V
CAD gas	9
Dwell time	100 ms

Table 3. MRM parameters used for quantitation on the novus V55 system.

ID	Precursor ion (m/z)	Fragment ion (m/z)	CE (V)	CXP (V)	DP (V)
Insulin degludec_1	1527.0	641.4	70	25	20
Insulin degludec_2	1527.0	1183.8	70	25	20

Data processing: Data collection and analysis were performed using SCIEX OS software, version 4.0. Peaks were integrated using the MQ4 algorithm, and a weighting of $1/x^2$ was used for quantitation of insulin degludec.

Automated compound optimization for peptide quantitation

The automated compound optimization feature in SCIEX OS software enables MRM optimization, allowing parameters such as DP, CE, and CXP voltages and ion source parameters to be optimized for unknown targets or known transitions (Figure 2).

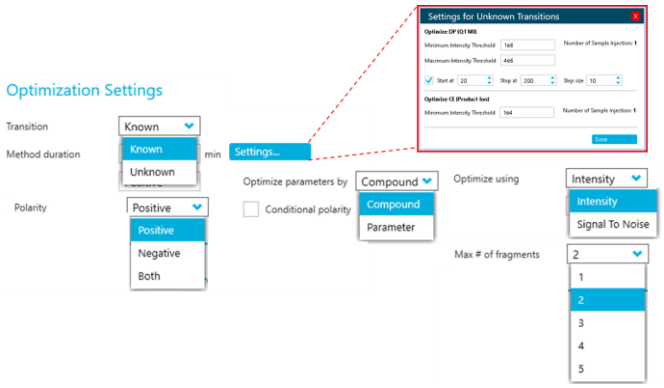

Figure 2. Automated compound optimization feature on SCIEX OS software. Easily optimize source and MRM conditions for optimal quantitation conditions for known and unknown targets.

During automated compound optimization, users can choose the operating polarity, select the number of target fragments for optimization, and whether MS/MS assessments should be made based on intensity or signal-to-noise (S/N).

Results from automated compound optimization can be stored in a compound database within SCIEX OS software, providing users with additional flexibility to easily access optimal settings for future use.

Quantitative performance on the novus V55 system

This technical note demonstrates a low-pg/mL level quantitation assay of insulin degludec in rat plasma using the novus V55 system.

A precursor ion at m/z 1527.0 (charge state 4) and a fragment ion at m/z 641.4 (charge state 5) were selected for quantitation of insulin degludec. The selection of the overall MRM transition was based on prioritizing the optimal sensitivity and selectivity of the assay. An LLOQ of 25 pg/mL was achieved for insulin degludec (Figure 1). No interferences were observed in the rat plasma matrix blank (Figure 1).

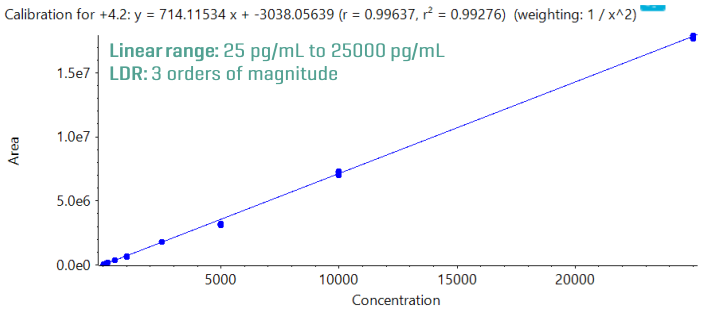

Figure 3. Calibration curve for quantitation of insulin degludec using the quantifier ion (1527.0 -> 641.4). The calibration curve was generated using a weighing factor of $1/x^2$.

The calibration curve ranged from 25 pg/mL to 25000 pg/mL and was prepared as described in the sample preparation section. Individual concentrations were run in triplicate.

Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
25.0	3 of 3	23.4	0.374	1.60	93.6
50.0	3 of 3	55.0	0.660	1.20	110.
100.	3 of 3	101.	1.06	1.05	101.
150.	3 of 3	146.	1.63	1.11	97.6
200.	3 of 3	226.	3.79	1.67	113.
500.	3 of 3	522.	7.57	1.45	104.
1000.	3 of 3	909.	11.2	1.23	90.9
2500.	3 of 3	2520.	22.3	0.885	101.
5000.	3 of 3	4450.	95.7	2.15	89.0
10000.	3 of 3	10000.	208.	2.07	100.
25000.	3 of 3	24800.	220.	0.887	99.4

Figure 4. Quantitative performance of insulin degludec. Reproducibility and accuracy results were determined from the calibration curve standards across 3 replicates at each concentration. Statistical results were summarized using the Analytics module in SCIEX OS software.

Linearity was achieved between 25 pg/mL and 25000 pg/mL with a coefficient of determination (r^2) of 0.993 (Figure 3). An LDR of 3 orders of magnitude was achieved, enabling measurement across a wide range of concentrations.

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.[3]

The assay accuracy was within ±13% of the actual concentration, and the %CV was less than 3%. The calculated percentage accuracy and %CV values were within the acceptance criteria at each concentration level (Figure 4).

Compliance-ready SCIEX OS software

Equivalent SCIEX OS software capabilities for regulated bioanalysis can be executed on the novus V55 system, ensuring high fidelity when performing method transfers while retaining critical compliance features.

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 used to monitor the audit trail, acquire and process data, and configure user access. The audit trail feature enables users to audit critical user actions and locks in data integrity.

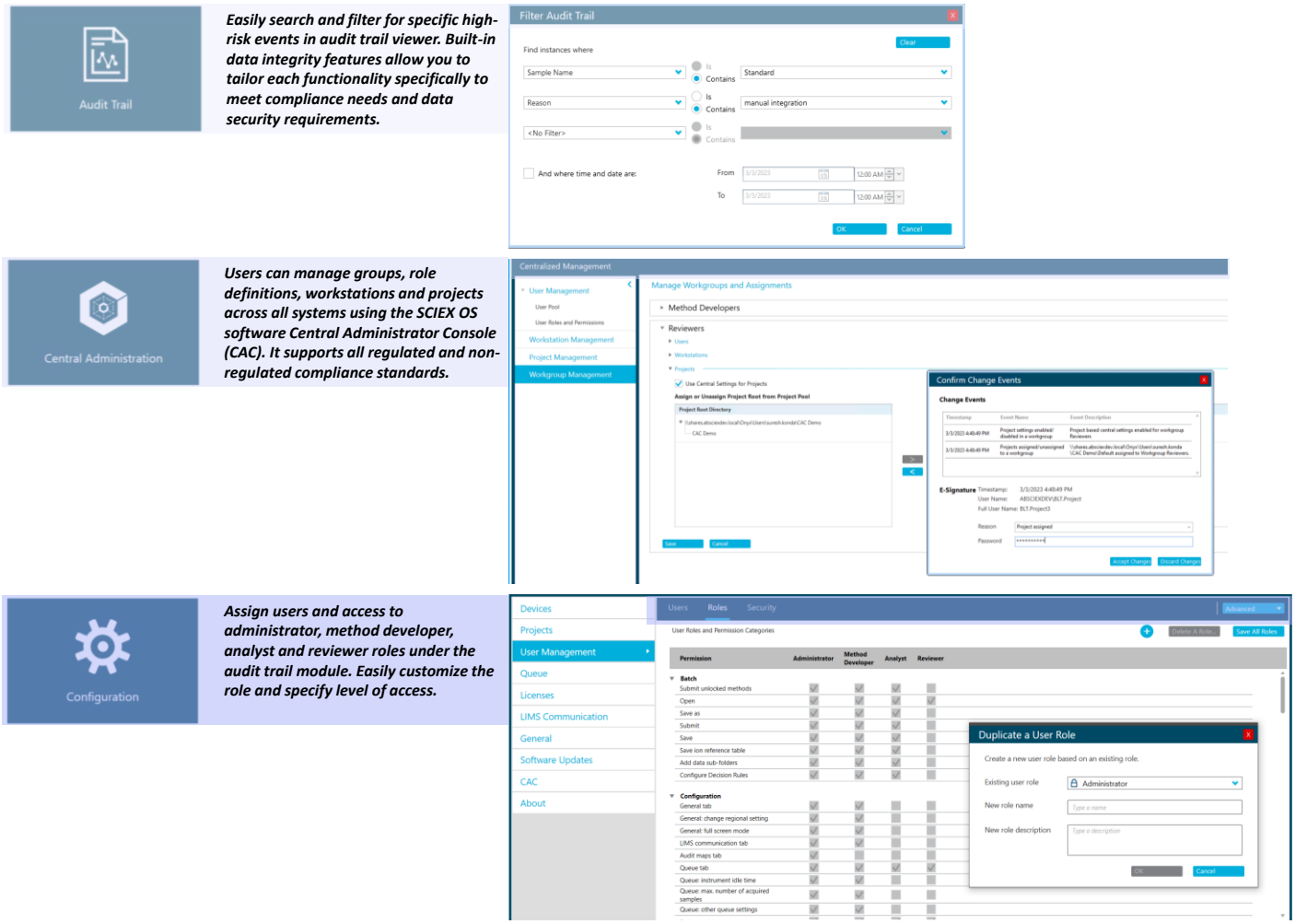

Figure 5. Features of the SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) 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 access levels for the administrator, method developer, analyst, and reviewer.

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

- A LLOQ of 25 pg/mL was achieved for the quantitation of insulin degludec in rat plasma.
- Increase quantitative flexibility for the analysis of peptide therapeutics with the wide mass range on the quadrupoles with the novus V55 system.
- Good quantitative performance was demonstrated with accurate and highly reproducible (%CV <3%) results on the novus V55 system.
- Linearity was achieved at concentrations ranging from 25 pg/mL to 25000 pg/mL with an r^2 >0.993, resulting in an LDR of 3 orders of magnitude.
- Maintain quantitative rigor while reducing operating costs with the novus V55 system, the most compact triple quadrupole mass spectrometer in its class
- Data management and compliance-readiness (21 CFR Part 11) features were shown using the SCIEX OS software to support peptide quantitation on the novus V55 system.

References

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