Sensitive and high-throughput quantitation of mezigdomide in rat plasma

Sub-ng/mL quantitation of molecular glue degraders in rat plasma using the SCIEX Triple Quad 6500+ system

Chao Zhou[1], Zhimin Long[1] and Eshani Nandita[2]
[1]SCIEX, China; [2]SCIEX, USA

This technical note demonstrates a sensitive method to quantify a molecular glue degrader, mezigdomide, in rat plasma using the SCIEX Triple Quad 6500+ system. A lower limit of quantitation (LLOQ) of 0.01 ng/mL was determined using a 5-minute LC-MS/MS method (Figure 1).

Molecular glue degraders are a class of small molecules that can induce the interaction between E3 ubiquitin ligase substrate receptors and target proteins. As a result, the target proteins are degraded by proteasomes following the initiation of ubiquitination. Molecular glue degraders are a class of protein degraders without the linker linkage. Unlike proteolysis targeting chimeras (PROTACs), molecular glue degraders have a low molecular weight. Therefore, the structures of molecular glue degraders are more similar to small molecule therapeutics. The size of the molecular glue degraders contributes to advantages in membrane permeability, bioavailability and higher druggability.[1]

Molecular glue degraders have gained significant interest for drug development pipelines due to their high potency in nanomolar drug concentrations and selectivity in protein targeting.[1] However, low circulating drug levels in complex matrices with limited sample volumes present analytical challenges. Therefore, sensitive and selective assays for the high-confidence detection and quantitation of molecular glue degraders in complex matrices using minimal sample extraction methods are needed to ensure the safety and efficacy in the drug development pipeline.

Mezigdomide is a novel E3 ubiquitin ligase cereblon (CRBN) regulator with immunomodulating, antiproliferative and pro-apoptic effects.[2] In this method, mezigdomide was used as a model standard for the development of a quantitative LC-MS/MS assay for molecular glues.

Key features of the quantitation of mezigdomide using the SCIEX Triple Quad 6500+ system

- **Sub-ng/mL level quantitation of molecular glue degrader:** Achieve a 0.01 ng/mL LLOQ for mezigdomide in rat plasma on the SCIEX Triple Quad 6500+ system
- **Ideal analytical performance:** Achieve accurate quantitative performance with %CV <10% at all concentrations across a linear dynamic range (LDR) spanning 4 orders of magnitude
- **Streamlined data management:** Data acquisition and processing are integrated into SCIEX OS software, a 21 CFR Part 11 compliance-ready platform

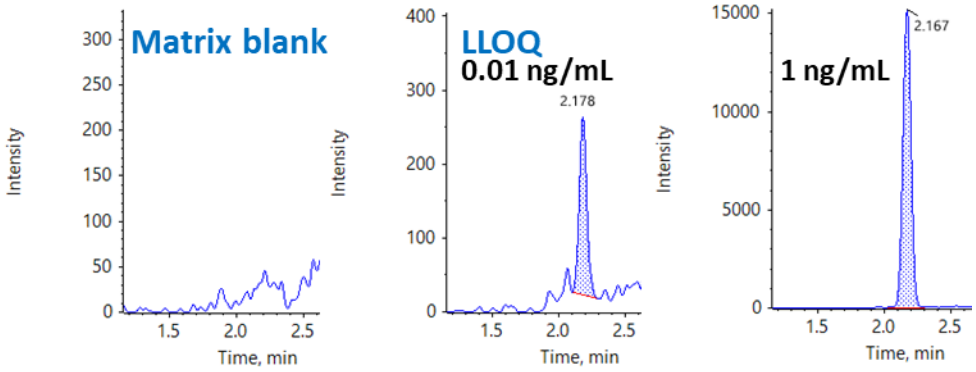

Figure 1. Representative extracted ion chromatograms (XICs) of the matrix blank and of mezigdomide at the LLOQ and at 1 ng/mL in rat plasma. An LLOQ of 0.01 ng/mL was achieved for mezigdomide in rat plasma. No matrix interference was observed at the retention time of the analyte.

Methods

Sample preparation: Mezigdomide was spiked into 100 µL of rat plasma at concentrations ranging from 0.01 to 100 ng/mL. Protein precipitation was performed with 300 µL of acetonitrile. Samples were vortexed for 1 minute and centrifuged at 4°C for 10 minutes at 15000 rpm. The supernatant was transferred to HPLC vials for LC-MS/MS analysis.

Chromatography: Sample separation was performed using an ExionLC AD system at a flow rate of 0.3 mL/min on an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm, 130 Å). A 5-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 40°C. An injection volume of 10 µL was used for analysis.

Table 1. Chromatographic gradient for mezigdomide.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	*80*	*20*
0.5	*80*	*20*
2.5	*10*	*90*
3.0	*10*	*90*
3.1	*80*	*20*
5.0	*80*	*20*

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	*50 psi*
Curtain gas	*35 psi*
Source temperature	*600°C*
Ion spray voltage	*5500 V*
CAD gas	*Medium*

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.

Table 3. MRM parameters used for quantitation.

ID	Precursor ion (*m/z*)	Fragment ion (*m/z*)	CE (V)	DP (V)
Mezigdomide 1	*568.3*	*252.2*	*44*	*130*
Mezigdomide 2	*568.3*	*363.1*	*35*	*130*

Mezigdomide 1 transition was used for quantitation.

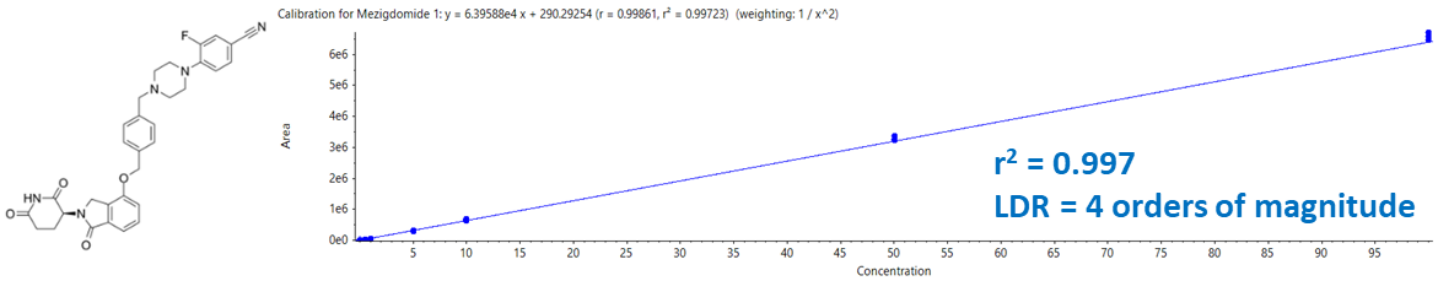

Figure 2. Structure and calibration curve for mezigdomide. The structure of mezigdomide is displayed in the left panel. The peak area of mezigdomide was used to generate a calibration curve (right panel). Each concentration was run in triplicate. Linearity was achieved between 0.01 ng/mL and 100 ng/mL and spanned an LDR of 4 orders of magnitude with an r^2 of 0.997.

Table 4. Calculated concentration, precision and accuracy for quantitation. Each concentration was measured in triplicate.

Concentration (ng/mL)	Accuracy (%)	CV (%)
0.01	*100*	*9.09*
0.1	*96.3*	*7.20*
0.5	*96.1*	*3.91*
1	*101*	*1.15*
5	*97.2*	*3.93*
10	*103*	*3.66*
50	*103*	*2.20*
100	*103*	*1.91*

Quantitative performance

This technical note demonstrates a low-ng/mL level quantitation assay of mezigdomide in rat plasma using the SCIEX Triple Quad 6500+ system. The method was optimized to achieve a sensitive quantitation assay from sample extraction to chromatography separation and MS detection. The calibration curve ranged from 0.01 ng/mL to 100 ng/mL and 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 ±5% of the nominal concentration and the %CV was <10% for mezigdomide (Table 4). Calculated accuracy and %CV values met the acceptance criteria at each concentration level.

An LLOQ of 0.01 ng/mL was achieved for mezigdomide (Figure 1). No interferences were observed in the rat plasma matrix blank (Figure 1). Linearity was achieved between 0.01 ng/mL and 100 ng/mL with a coefficient of determination (r^2) of 0.997 (Figure 2). An LDR spanning 4 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 3 illustrates the features of SCIEX OS software that are used for monitoring the audit trail, acquiring and processing data and configuring user access.

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.

Figure 3. 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.

Conclusions

- An LLOQ of 0.01 ng/mL was reached for the quantitation of mezigdomide in rat plasma
- Linearity was achieved between 0.01 ng/mL and 100 ng/mL, generating an LDR spanning 4 orders of magnitude with an r^2 value of 0.997
- The method demonstrated accurate and highly reproducible (%CV <10%) quantitative performance at all concentrations
- 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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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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