Rapid bioanalysis of pomalidomide at sub-ng/mL levels in human plasma

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Immunomodulatory drugs (IMiD) are a novel group of orally available chemotherapy agents. Pomalidomide is an IMiD that acts as a molecular glue and is administered in oral capsules alone or in combination with other drugs for treating multiple myeloma in humans.[1,2] Alternative formulations are needed for patients with dysphagia and pediatric patients who cannot swallow the drug.[2]

Challenges, such as low bioavailability, arise when developing alternative formulations. Therefore, sensitive and selective assays for high-confidence detection and quantitative performance in biological matrices are needed to ensure the safety and efficacy of promising IMiDs.

The presented method demonstrates a sensitive quantitation assay for pomalidomide using 25 µL of human plasma and a rapid protein precipitation method (Figure 1).

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

- **Sub-ng/mL level quantitation of an IMiD:** Achieve a 0.1 ng/mL LLOQ for pomalidomide in human plasma using the SCIEX 7500 system
- **Ideal analytical performance:** Achieve accurate quantitative performance with %CV <10% at all concentration levels across a linear dynamic range (LDR) spanning 3.6 orders of magnitude
- **Fast analysis with negligible carryover:** Perform rapid LC-MS/MS analysis of pomalidomide in matrix with little to no carryover
- **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
- **Streamlined data management:** Data acquisition and processing are integrated into SCIEX OS software, a 21 CFR Part 11 compliance-ready platform

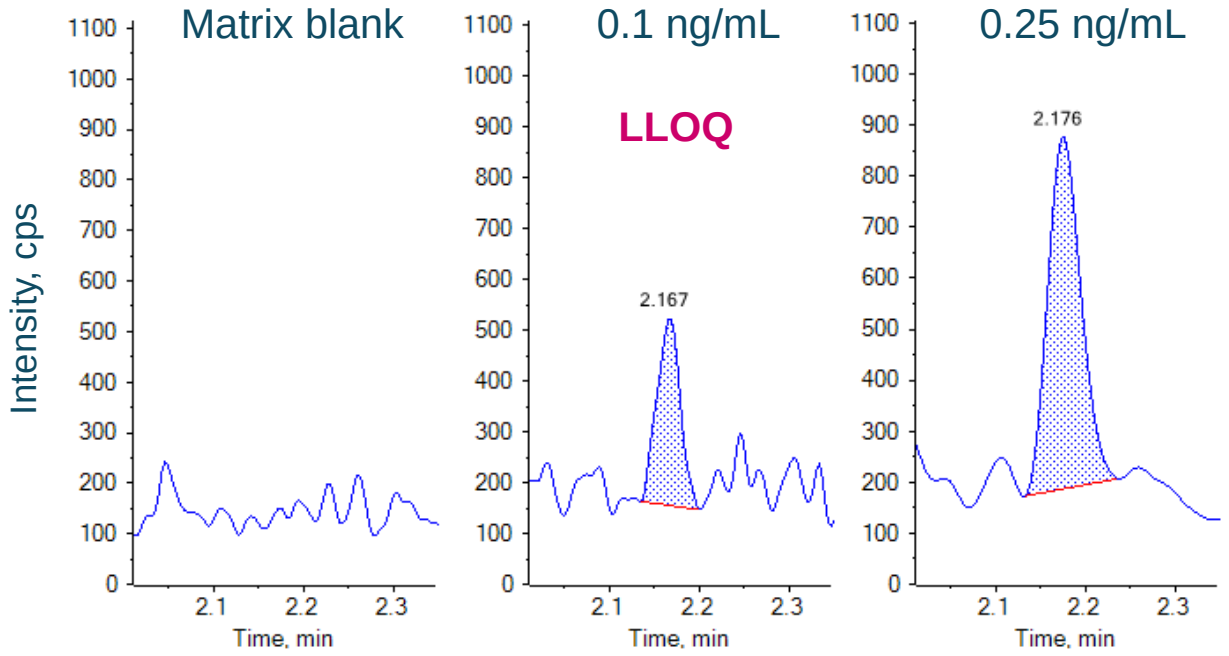

Figure 1. Representative extracted ion chromatograms (XICs) of pomalidomide. A lower limit of quantitation (LLOQ) of 0.1 ng/mL was achieved for pomalidomide in human plasma. No matrix interference was observed at the retention time of the analyte.

Introduction

Pomalidomide is one of the orally available IMiDs with potent antimyeloma activity used to treat patients who are exhausted from the frontline IMiD treatment or experiencing a relapse.[1] It exerts its potent antimyeloma and immune-stimulating effects by binding to its target E3 ligase, cereblon. Upon binding, endogenous proteasomal degradation is activated for the targeted removal of the transcription factors, Ikaros and Aiolos.[3]

For patients that require alternative formulations, different drug combinations with pomalidomide and non-invasive routes of drug administration are tested to achieve improved clinical outcomes. With molecular glue degrader therapeutics on the rise, researchers are seeking analytical methods that can provide rapid quantitative answers to maintain the pace of drug development.

LC-MS/MS methods are increasingly applied for the quantitation of therapeutics as they offer the most sensitive and selective platforms for bioanalysis. Here, an LC-MS/MS quantitation assay for pomalidomide in human plasma is presented.

Methods

Samples and reagents: Pomalidomide was purchased from Tocris Biosciences and was reconstituted in dimethyl sulfoxide (DMSO).

Sample preparation: A working stock was prepared by diluting pomalidomide in acetonitrile. Individual concentrations were prepared using serial dilution of pomalidomide in acetonitrile. A 1.25 µL aliquot of the individual concentrations was spiked into 25 µL of human plasma to make a calibration range from 0.1 ng/mL to 400 ng/mL. Protein precipitation was performed with 75 µL of acetonitrile. Samples were vortexed for 30 seconds and centrifuged at 12000 rcf for 8 minutes at room temperature.

Table 1. Chromatographic gradient for pomalidomide.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.0	*95*	*5*
0.2	*95*	*5*
3.5	*10*	*90*
4.0	*10*	*90*
4.1	*95*	*5*
5.0	*95*	*5*

Finally, 50 µL of supernatant was transferred to injection vials for LC-MS/MS analysis.

Chromatography: Sample separation was performed using an ExionLC system at a 0.3 mL/min flow rate 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 and an injection volume of 10 µL was used for analysis. A mixture of 1:1:1 (v/v/v) acetonitrile:methanol:water was used as a needle wash solvent.

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	*45 psi*
Source temperature	*550°C*
Ion spray voltage	*2500 V*
CAD gas	*9*

Table 3. MRM parameters used for quantitation.

ID	Precursor ion (*m/z*)	Fragment ion (*m/z*)	CE (V)	CXP (V)
*Pomalidomide 1**	*274.1*	*200.9*	*30*	*15*
Pomalidomide 2	*274.1*	*163.1*	*40*	*15*

**Transition was used for quantitation*

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 sub-ng/mL level quantitation assay of pomalidomide using 25 µL of human plasma on the SCIEX 7500 system. Low solubility and stability in an aqueous mixture are common analytical challenges experienced during

the detection of pomalidomide. The method was optimized for a sensitive quantitation assay from sample extraction to chromatography and MS detection.

The calibration curve ranged from 0.1 ng/mL to 400 ng/mL and was prepared as described in the sample preparation section.

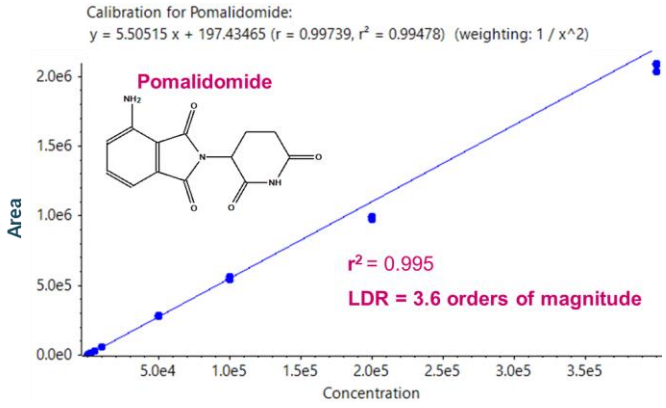

Figure 2. Calibration curve and structure of pomalidomide. Linearity was achieved between 0.1 ng/mL and 400 ng/mL and spanned an LDR of 3.6 orders of magnitude with an r^2 of 0.995. Each concentration level was run in triplicate.

Individual concentrations were run in triplicate. Linearity was achieved between 0.1 ng/mL and 400 ng/mL with an r^2 of 0.995 (Figure 2).

Analytical performance was evaluated for accuracy and precision. The accuracy of the calculated mean must 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 must be <20% at the LLOQ and <15% at higher concentrations.

Accuracy was within ±11% of the nominal concentration and the %CV was <10% for pomalidomide (Figure 3). Calculated accuracy and %CV values met the acceptance criteria at each concentration level.

Carryover was assessed by injecting a matrix blank sample after the upper limit of quantitation (ULOQ) at 400 ng/mL. No visible peak was observed at the retention time of the analyte (2.176 min) in the post-ULOQ matrix blank (Figure 4).

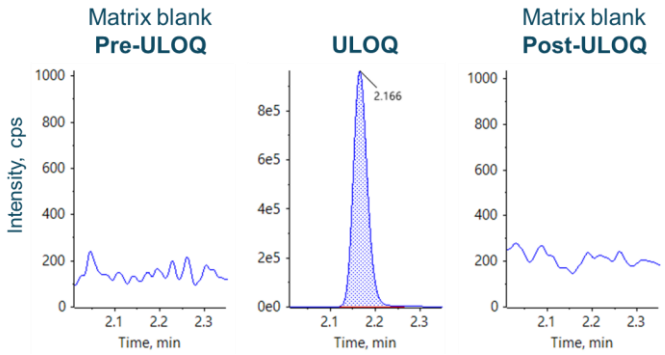

Figure 4. XICs of the pre-ULOQ matrix blank, ULOQ at 400 ng/mL and post-ULOQ are shown. No visible carryover was observed after the batch.

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
Pomalidomide 241.11 > 200.95	0.10	3 of 3	9.622e-2	8.765e-3	9.11	96.22
Pomalidomide 241.11 > 200.95	0.25	3 of 3	2.616e-1	2.290e-2	8.75	104.65
Pomalidomide 241.11 > 200.95	0.50	3 of 3	5.420e-1	2.456e-2	4.53	108.39
Pomalidomide 241.11 > 200.95	1.00	3 of 3	1.021e0	6.678e-2	6.54	102.06
Pomalidomide 241.11 > 200.95	2.50	3 of 3	2.514e0	5.908e-2	2.35	100.57
Pomalidomide 241.11 > 200.95	5.00	3 of 3	4.964e0	2.509e-2	0.51	99.27
Pomalidomide 241.11 > 200.95	10.00	3 of 3	1.032e1	1.530e-1	1.48	103.16
Pomalidomide 241.11 > 200.95	50.00	3 of 3	5.102e1	1.242e0	2.43	102.05
Pomalidomide 241.11 > 200.95	100.00	3 of 3	1.002e2	1.634e0	1.63	100.25
Pomalidomide 241.11 > 200.95	200.00	3 of 3	1.788e2	2.187e0	1.22	89.40
Pomalidomide 241.11 > 200.95	400.00	3 of 3	3.760e2	5.938e0	1.58	93.99

Figure 3. Quantitative performance for pomalidomide 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.

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 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.

Conclusions

- An LLOQ of 0.1 ng/mL was reached for the quantitation of pomalidomide in human plasma
- Efficient 5-minute bioanalysis of pomalidomide was demonstrated using a rapid sample preparation, small sample volume (25 µL) and without visible carryover
- Linearity was achieved between 0.1 ng/mL and 400 ng/mL, generating an LDR spanning 3.6 orders of magnitude with an r^2 of 0.995
- The method demonstrated accurate and highly reproducible (%CV <10%) 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 a nominal mass spectrometer to support data acquisition, processing and management on a single platform

References

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