Sensitive quantitation of the proteolysis targeting chimera (PROTAC™), TL 13-112, in rat plasma using an LC-MS/MS workflow

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ABSTRACT

Proteolysis targeting chimeras (PROTACs) are endogenous protein degradation tools, capable of removing specific protein targets using a cell's own disposal machinery. PROTACs have evolved as a therapeutic modality, as several candidates have now moved into clinical trials. Sensitive and selective assays for high-confidence detection and quantitation of PROTACs are needed to ensure safety and efficacy in the drug development pipeline and because PROTACs have expressed high potency in nanomolar drug concentrations.

In this study, low-pg/mL quantitation for the PROTAC, TL 13-112, and its inactive control, TL 13-110, was achieved at a lower limit of quantitation (LLOQ) of 10 pg/mL using a highflow LC-MS/MS platform.

INTRODUCTION

The interest in targeted protein degradation has shifted from academia to industry after the therapeutic potential of a PROTAC was documented in 2001.[1] PROTACs have emerged as a therapeutic modality and several candidates have moved into clinical trials.[2] The potential of PROTACs is coded in their structure. A linker connects a protein of interest (POI) binding moiety to a ubiquitin E3 ligase recognition moiety (Figure 1A). The heterobifunctional structure enables PROTACs to bring the POI and E3 ligase closer in proximity. This induces the ubiquitination of the POI, which is then targeted by the disposal machinery of the cell.[2]

One of the many attractive hallmarks of PROTACs is their high potency in nanomolar drug concentrations.[3] While their potential is well-documented,[1] challenges remain for the analysis of PROTACs. Sensitive and selective assays for high-confidence detection and quantitation of PROTACs are needed to ensure the safety and efficacy in the drug development pipeline.

Here, a highly sensitive assay for the quantitation of PROTACs in a complex matrix was demonstrated. The quantitative performance of the assay was evaluated using the commercially available TL 13-112 (PROTAC) and TL 13-110 (inactive control) structures.

quantitation at low-pg/mL levels was achieved for both analytes in rat plasma using the SCIEX 7500 system. The front-end enhancements of the system facilitated greater sensitivity, which improved overall ion generation, capture and transmission.

MATERIALS AND METHODS

Sample preparation:
Commercially available individual PROTAC degrader (TL 13-112) and its inactive control (TL 13-110) were reconstituted in DMSO. PROTACs were spiked into 100 µL of rat plasma at concentrations ranging from 10 pg/mL to 15000 pg/mL. Protein precipitation was performed with 600 µL of 1:1 (v/v), acetonitrile/methanol.

Samples were vortexed for 30 seconds and then centrifuged at 13000 rpm for 12 minutes at room temperature. The supernatant was transferred to a new Eppendorf tube and dried under nitrogen flow. Samples were reconstituted using 200 µL of 1:1 (v/v), methanol/acetonitrile prior to analysis.

Chromatography:
Sample separation was performed using an ExionLC system at a flow rate of 0.3 mL/min using a Phenomenex Kinetex XB-C18 (2.1 x 50 mm, 1.7 µm, 100 Å) column. A 10-minute gradient was used for analysis (Table 1).

Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The column temperature was kept at 40ºC. An injection volume of 10 µL was used for analysis. A mixture with equal parts by volume of acetonitrile, methanol and water was used as the needle wash solvent.

Table 1. Chromatographic gradient.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0	*85*	*15*
0.2	*85*	*15*
5	*50*	*50*
5.5	*5*	*95*
8.5	*5*	*95*
8.6	*85*	*15*
10	*85*	*15*

Mass spectrometry:

- Samples were analyzed using the SCIEX 7500 system
- The optimized analyte-dependent MRM parameters are listed in Table 2
- The optimized source and gas parameters are listed in Table 3

Table 2. MRM parameters used for quantitation.

ID	Precursor ion (*m/z*)	Fragment ion (*m/z*)	CE (V)	CXP (V)
TL 13-112	*1002.2*	*584.1*	*70*	*15*
TL 13-110	*988.7*	*542.4*	*70*	*20*

Table 3. Source and gas parameters.

Parameter	Value
Polarity	*Positive*
Ion source gas 1	*55 psi*
Ion source gas 2	*65 psi*
Curtain gas	*45 psi*
Source temperature	*600ºC*
Ion spray voltage	*3000 V*
CAD gas	*7*

Data processing:
Data collection, analysis and quantitation were performed using 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.

RESULTS

- Given the high potency of PROTACs, sensitive and robust bioanalytical methods are needed for accurate quantitation to ensure proper safety and efficacy during pre-clinical evaluation
- This assay note demonstrates a low-pg/mL level quantitation assay of a PROTAC and its inactive control in rat plasma using the SCIEX 7500 system
- A calibration curve was prepared, as described in the sample preparation section, for concentrations ranging from 10 pg/mL to 15000 pg/mL. Individual concentrations were run in triplicate.
- An LLOQ of 10 pg/mL was achieved for both TL 13-112 and TL 13-110 (Figure 1B). No interferences were observed in the matrix blank (rat plasma) for either analyte (Figure 1B).
- Strong linearity was achieved for both analytes and the linear dynamic range (LDR) spanned 3.2 orders of magnitude (Figure 2)

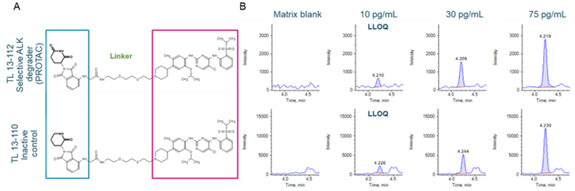

Figure 1. Low-pg/mL level quantitation was achieved for the PROTAC and its inactive control. The PROTAC used for this assay was TL 13-112 and the inactive control was TL 13-110.

- The structure of TL 13-112 contains an additional carbonyl oxygen on the pomalidomide group compared to the structure of TL 13-110 (see the part of structure in bold, Figure 1A)
- The blue rectangle highlights the POI binding moiety and the magenta rectangle highlights the E3 ligase binding moiety (Figure 1A)

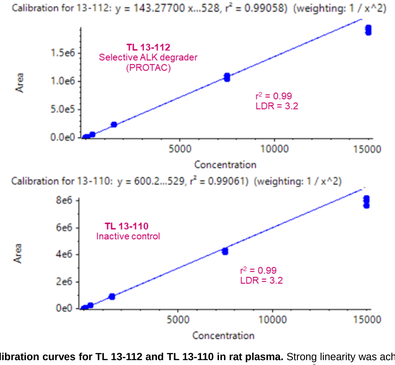

Figure 2. Calibration curves for TL 13-112 and TL 13-110 in rat plasma. Strong linearity was achieved for TL 13-112 (top panel) and TL 13-110 (bottom panel) in rat plasma, with a correlation coefficient (r^2) of 0.99 for both targets. Each concentration was run in triplicate.

Table 4. Accuracy and %CV at each concentration level measured for TL 13-112 and TL 13-110. Each concentration level was run in triplicate.

	TL 13-112		TL 13-110	
Concentration (pg/mL)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
10	103	8.76	102	7.34
15	93.6	3.74	108	9.44
30	102	9.33	107	2.02
75	98.3	5.75	108	2.19
375	103	5.94	98.1	1.70
1500	110	1.71	97.7	1.37
7500	100	3.07	95.0	1.26
15000	89.0	2.45	88.2	3.57

CONCLUSIONS

- An LLOQ of 10 pg/mL was reached for the quantitation of PROTACs in rat plasma with minimal sample preparation
- A highly sensitive assay for the quantitation of PROTACs was demonstrated on the SCIEX 7500 system with improved front-end technology for better ion generation, capture and transmission
- Excellent linearity, accuracy and precision were achieved for the concentrations analyzed, demonstrating exceptional quantitative performance
- Streamlined data acquisition, processing and management were performed using SCIEX OS software

REFERENCES

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