A Rapid, Robust, Sensitive, and High-Throughput UPLC-MS/MS Assay to Quantitate Murine Double Minute 2 (MDM2)-p53 Inhibitor Alrizomadlin in Human Plasma

Yu Yang[1]; Yang Xu[2]; Ming Lu[2]; Yun Yang[2]; Aihua Liu[1]
[1]Alliance Pharma, Malvern, PA; [2]Ascentage Pharma Group Inc., Rockville, MD

OBJECTIVE

The objective of this study was to develop and validate a rapid, sensitive, and high-throughput UPLC-MS/MS method for the quantification of Murine Double Minute 2 (MDM2)-p53 inhibitor alrizomadlin (APG-115) in human plasma.

INTRODUCTION

Alrizomadlin (APG-115) is a novel, orally active small-molecule inhibitor of MDM2 that is often overexpressed in human cancers and is a negative regulator of the p53 tumor suppressor protein. Preclinical data suggest that alrizomadlin has therapeutic potential for the treatment of various solid tumors and hematologic malignancies. Alrizomadlin is being investigated in clinical trials both as a single agent and in combination with other classes of anticancer drugs such as lisaftoclax for the treatment of different types of cancer. Thus, a rapid and robust assay is needed to reliably quantitate alrizomadlin in human plasma in order to support clinical studies. We have hence successfully developed and validated a simple, rapid, robust, and sensitive UPLC-MS/MS assay to quantitate alrizomadlin in human plasma.

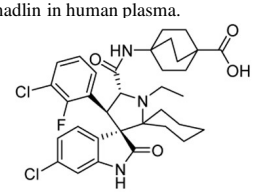

Figure 1: Chemical structure of alrizomadlin

METHOD

Sample Preparation

A 25.0-µL aliquot of human plasma (K_2 EDTA) containing APG-115 was extracted with internal standard (IS, APG-115-d5) by protein precipitation extraction (PPE) using acetonitrile (ACN). The extraction supernatant was further diluted with ACN/H_2O (50:50, *v/v*) before UPLC-MS/MS analysis.

LC-MS Conditions

HPLC: Shimadzu LC-30AD
Column: Waters ACQUITY UPLC™ BEH C18 column (130Å, 1.7 µm, 2.1 × 50 mm)
Column temperature: 40ºC
Mobile phase A: 0.1% formic acid in H_2O
Mobile phase B: 0.1% formic acid in ACN

METHOD (cont.)

Needle wash: ACN/Methanol/IPA/Acetone (1:1:1:1, *v/v/v/v*)
Flow rate: 0.6 mL/min
Gradient profile: Refer to Table 1.

Table 1: HPLC gradient profile

Time (min)	Events	Value
0.00	B conc.	50%
0.50	B conc.	50%
1.60	B conc.	95%
2.20	B conc.	95%
2.30	B conc.	50%
3.00	Stop	

SCIEX API 5500 LC-MS/MS system was used in electrospray ionization (ESI) mode with positive ions to monitor APG-115 and APG-115-d5 at ion transitions 642.2→352.4 and 647.3→352.4, respectively. A diversion valve was incorporated to reduce MS system contamination.

RESULTS

Carryover and Nonspecific Binding

To obviate the carryover issue, a mixture of ACN, methanol, isopropanol, and acetone was used as the needle wash solvent. To prevent the nonspecific-binding issue, a high percentage (≥50%) of organic solvent was used throughout the assay, including preparation of source and working solutions, final dilutions, and initial mobile-phase composition.

Linearity and Sensitivity

The quantitative assay was successfully developed and qualified within a range of 2.00 to 2000 ng/mL (Figures 2 and 3, Tables 2 and 3). The inter-assay accuracy (%) for the standard calibrators is 96.1% to 101.5%. The inter-assay precision (%CV) for the standard calibrators is 2.4% to 5.6% over 4 quantitative runs.

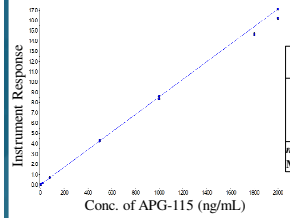

Figure 2: Typical APG-115 curve

Table 2: Curve parameters

Batch ID	Slope	Intercept	Correlation Coefficient (R)
01	7.76E-03	5.45E-04	0.9979
02	8.56E-03	8.90E-04	0.9991
04	8.90E-03	-9.44E-04	0.9994
06	7.33E-03	-9.27E-04	0.9982
n	4	4	4
Mean	8.14E-03	-1.09E-04	0.9987

RESULTS (cont.)

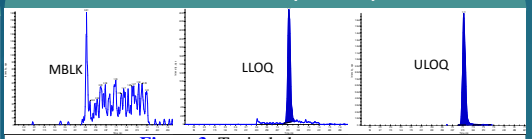

Figure 3: Typical chromatograms

Table 2: Precision and Accuracy for calibration standards

Batch ID	Concentration (ng/mL)							
	2.00	4.00	20.0	80.0	500	1000	1800	2000
01	1.82	4.45	20.5	86.7	518	1040	1710	2060
	2.08	3.94	19.1	77.2	480	1020	1670	1900
02	2.07	4.03	20.9	82.5	496	1010	1720	2000
	1.88	4.07	20.9	83.2	508	974	1700	1890
04	2.10	3.84	19.8	79.9	498	1010	1760	2030
	1.91	4.13	19.5	76.8	519	1040	1780	2010
06	2.07	3.93	20.7	80.7	490	1020	1790	1870
	1.99	3.86	17.9	82.2	480	1080	1740	2210
n	8	8	8	8	8	8	8	8
Mean	1.99	4.03	19.9	81.2	499	1020	1730	2000
SD	0.107	0.197	1.06	3.26	15.4	30.6	41.4	112
CV(%)	5.4	4.9	5.3	4.0	3.1	3.0	2.4	5.6
Accuracy(%)	99.5	100.8	99.5	101.5	99.8	102.0	96.1	100.0

Precision and Accuracy

Spiked quality control (QC) sample precision and accuracy were demonstrated at n=18 at the lower limit of quantification (LLOQ-QC, 2.00 ng/mL), at low QC (LQC, 6.00 ng/mL), medium QC (MQC, 120 ng/mL), and high QC (HQC, 1500 ng/mL) concentrations over three validation runs. The LLOQ and other QCs were within 20% and within 15%, respectively (Table 3).

Table 3: Inter-assay precision and accuracy for QC samples

QC (ng/mL)	n	Mean	S.D.	%CV	Accuracy
2.00	18	2.10	0.171	8.1	105.0
6.00	18	6.13	0.382	6.2	102.2
120	18	120	6.94	5.8	100.0
1500	18	1490	80.0	5.4	99.3

Internal Standard Response

As a reference for assay performance, the IS responses (peak areas) of the calibration standards and LLOQ-QC, LQC, MQC, and HQC samples from all of the inter-assay precision and accuracy batches were evaluated (Table 4).

Table 4: Internal standard response

Internal standard (IS):	APG-115-d5
IS response for accepted STDs and QCs (%CV):	3.4 to 19.6

Recovery

The recoveries of APG-115 and APG-115-d5 were deemed adequate to obtain precise and accurate determinations in the method- specified assay range (Table 5).

Table 5: Recoveries of APG-115 and APG-115-d5

Analyte:	APG-115	APG-115-d5
Overall Recovery(%):	99.2	105.0

RESULTS (cont.)

Selectivity and Specificity

Aliquots of 6 individual lots of blank human plasma were tested free from significant interference and spiked at the LLOQ level. The results showed that the method demonstrated acceptable selectivity for analyte APG-115 (Table 6).
Lisaftoclax (APG-2575) at 5000 ng/mL was tested to ensure that it did not contribute significant interference to APG-115 both at blank matrix and at LLOQ level (Table 7).

Table 6: Selectivity for APG-115

Batch ID	Blank Matrix	Concentration (ng/mL)	
		2.00	DEV (%)
01	BRH1608185	2.03	1.5
	BRH1608201	2.00	0.0
	BRH1608216	2.09	4.5
	BRH1608195	1.82	-9.0
	BRH1608160	2.25	12.5
	HMN500569	2.21	10.5
n		6	
Mean		2.07	
SD		0.156	
CV(%)		7.5	
Acc.(%)		103.5	

Table 7: Specificity for APG-115

Batch ID	APG-115			
	Specificity Matrix Blank Compared to LLOQ*(%)	Specificity Matrix Blank Spiked With Lisaftoclax (Peak Area)	Concentration in the Presence of Lisaftoclax	
			Specificity LLOQ (ng/mL) 2.00	DEV (%)
04 & 06	0	0	1.95	-2.5
	0	0	2.06	3.0
	0	0	1.93	-3.5
	0	0	2.26	13.0
	0	0	1.98	-1.0
	0	0	2.21	10.5
n	6	6	6	
Mean	0	0	2.07	
SD			0.140	
CV(%)			6.8	
Acc.(%)			103.5	

*Average LLOQ peak area of APG-115 = 1620

Established Stability of APG-115

Stock solution (1.00 mg/mL) stability: For 7 days at 2-8ºC and for 6 hours at room temperature (RT).
Working standard solution (50,000 ng/mL) stability: For 7 days at 2-8ºC.
Sample collection stability: For 4 hours on wet ice and at RT.
Short-term stability in human plasma: For 4 freeze/thaw cycles at −20ºC/RT and −70ºC/RT *w/* and *w/o* lisaftoclax.
Benchtop stability: For 19 hours at RT *w/* and *w/o* lisaftoclax.
Processed sample stability: For 51 hours at 2-8ºC.
Long-term stability: For 14 days at −20ºC and for 10 months at −70ºC *w/* and *w/o* lisaftoclax.

CONCLUSIONS

- A sensitive and fast LC-MS/MS method for the quantification of APG-115 was developed.
- The method was validated as linear, accurate, precise and reproducible. It can be used to determine the concentration of APG-115 as low as 2.00 ng/mL using only 25.0 µL of sample.