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When Validated Collection Procedures Differ from Clinical Sample Processing: A Lesson in Adaptability for the Determination of Rifampin

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OVERVIEW

Presentation of a case study detailing the establishment of stability conditions for rifampin clinical study samples collected without preservative when the bioanalytical method was originally validated with a preservative.

INTRODUCTION

A pharmacokinetic drug interaction study was contracted for the validation of a method to quantitate rifampin in human plasma for samples received from another clinical site.

Based upon existing literature and in-house experimental data, it has been determined necessary to maintain the integrity of rifampin plasma samples via addition of the antioxidant ascorbic acid. This sample processing information was provided by the bioanalytical laboratory to the clinical site, however, given the complexity of the clinical trial and timeliness of multiple stakeholders, study samples were collected without the addition of antioxidant.

Therefore, in order to provide accurate rifampin sample concentrations within the expectations of the regulatory guidelines for bioanalysis, it was necessary to determine and validate new stability conditions reflecting the timeframe for the processing of plasma samples. The case study for rifampin study sample collection represents a situation which can impact sample integrity, as recently discussed in the 11th GCC closed forum white paper.

METHOD

A calibration curve of rifampin was prepared from 1.00 - 1,000 ng/mL in human plasma (K2EDTA) treated with 10% (v/v) of 1.0 M ascorbic acid.

Rifampin and its deuterated internal standard (D8-rifampin) were extracted by protein precipitation using methanol. Chromatography was performed on a C18 column with a gradient of ammonium bicarbonate (pH 10) and acetonitrile. Data was acquired using a SCIEX API 5000 operated in positive ESI mode for the MRM transitions *m/z* 823.4 > 791.4 (rifampin) and *m/z* 831.5 > 799.4 (D8-rifampin).

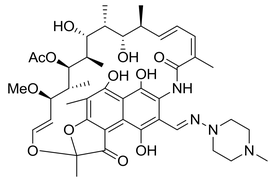


Figure 1. Chemical Structure of Rifampin

RESULTS

As part of method development and prior to sample collection, Rifampin stability in human whole blood (K2EDTA) was first established for a duration of 2h at a temperature of 22ºC (Table 1) as well as 4ºC (not shown as similar to 22ºC), followed by stability in plasma with and without the addition of ascorbic acid (Tables 2 and 3).

Table 1. Whole Blood Stability of Rifampin at 22ºC

Low QC (3.00 ng/mL)		High QC (750 ng/mL)	
Time (minutes)	Deviation (%)	Time (minutes)	Deviation (%)
0	NAP	0	NAP
15	1.90	15	2.20
30	-4.90	30	-5.30
60	-5.70	60	1.40
120	-0.50	120	-5.50

Table 2. Plasma Stability of Rifampin at 4ºC Without Ascorbic Acid

Low QC (3.00 ng/mL)		High QC (750 ng/mL)	
Time (minutes)	Deviation (%)	Time (minutes)	Deviation (%)
0	NAP	0	NAP
15	-7.6	15	-10.4
30	5.1	30	-12.6
60	-4.9	60	-10.2
90	-7.1	90	-4.7
120	-5.4	120	-10.4

Table 3. Plasma Stability of Rifampin at 4ºC with Ascorbic Acid

Low QC (3.00 ng/mL)		High QC (750 ng/mL)	
Time (minutes)	Deviation (%)	Time (minutes)	Deviation (%)
0	NAP	0	NAP
15	7.3	15	5.0
30	-1.3	30	1.7
60	-0.2	60	-2.6
90	-5.9	90	7.0
120	5.2	120	-6.2

Results demonstrated that although plasma stability was within the acceptance limits with and without ascorbic acid, better deviation are obtained with the addition of ascorbic acid to plasma to prevent rifampin oxidation. These optimal conditions were subsequently validated.

To address the impact of the omission of ascorbic acid to the study samples, long-term, short-term as well as freeze-thaw stabilities without ascorbic acid were performed.

Long-term stabilities were successfully validated for 97 days and 83 days, with and without ascorbic acid, respectively, demonstrating that the absence of antioxidant had minimal impact on long-term sample storage (Tables 4 and 5).

Table 4. Long-Term Stability of Rifampin in Human Plasma with Ascorbic Acid at -80ºC Nominal for 97 Days

	Low QC (3.00 ng/mL)	High QC (750 ng/mL)
	2.91	712.71
	3.15	704.40
	3.12	738.33
	2.96	754.76
	2.83	692.22
	2.99	729.24
Mean	*2.99*	*721.94*
S.D.	*0.12*	*23.13*
N	*6*	*6*
% C.V.	*4.1*	*3.2*
% Nominal	*99.8*	*96.3*

Table 5. Long-Term Stability of Rifampin in Human Plasma without Ascorbic Acid at -80ºC Nominal for 83 Days

	Low QC (3.00 ng/mL)	High QC (750 ng/mL)
	2.82	719.92
	2.91	737.12
	2.90	732.30
	2.78	770.27
	2.64	758.98
	2.77	761.38
Mean	*2.80*	*746.66*
S.D.	*0.10*	*19.69*
N	*6*	*6*
% C.V.	*3.5*	*2.6*
% Nominal	*93.4*	*99.6*

The next stage in evaluating study sample integrity was to obtain detailed feedback regarding collection timing and processing from the clinical site, so as to define the scope within which short-term stability would be re-assessed. A short-term stability of 45 minutes at 22ºC without ascorbic acid was evaluated, in addition to combined stability defined as 1 freeze/thaw cycle with 4h at room temperature and 8 days at -80ºC (Tables 6 and 7). All stability acceptance criteria could be met under these constrained conditions.

Table 6. Short-Term Stability of Rifampin in Human Plasma Without Ascorbic Acid for 45 minutes at 22ºC Nominal

	Low QC (3.00 ng/mL)	High QC (750 ng/mL)
	2.81	672.06
	3.00	706.92
	2.96	741.54
	2.71	709.33
	2.86	692.71
	2.62	777.27
Mean	*2.83*	*716.64*
S.D.	*0.15*	*37.43*
N	*6*	*6*
% C.V.	*5.1*	*5.2*
% Nominal	*94.2*	*95.6*

Table 7. Stabilities of Rifampin in Human Plasma Without Ascorbic Acid, Long-Term Stability at -80ºC Nominal for 8 days, Short Term Stability at 4ºC Nominal for 4.0 Hours and Freeze-Thaw Stability for 1 Freeze Thaw

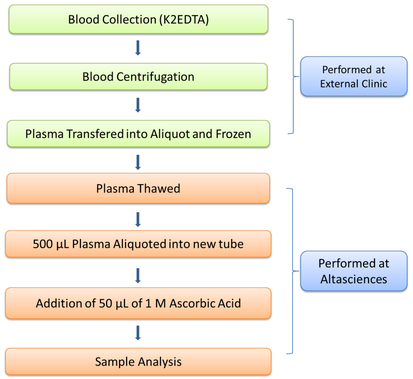
	Low QC (3.00 ng/mL)	High QC (750 ng/mL)
	2.88	875.44
	3.22	852.09
	3.19	879.98
	3.22	865.01
	2.74	879.73
	3.09	773.56
Mean	*3.06*	*854.30*
S.D.	*0.20*	*40.97*
N	*6*	*6*
% C.V.	*6.6*	*4.8*
% Nominal	*101.9*	*113.9*

Finally, to prove that the precision and accuracy of the method was not impacted by the addition of preservative after the plasma was fortified with rifampin, a within-batch precision and accuracy was performed at low and high QC concentration (Table 8).

Table 8. Precision and Accuracy for Rifampin Without Ascorbic Acid

	Low QC (3.00 ng/mL)	High QC (750 ng/mL)
	2.62	686.69
	2.56	688.59
	2.61	689.84
	2.56	687.00
	2.62	681.85
	2.61	677.30
Mean	*2.60*	*685.21*
S.D.	*0.03*	*4.74*
N	*6*	*6*
% C.V.	*1.1*	*0.7*
% Nominal	*86.6*	*91.4*

As the stabilities without the addition of ascorbic acid were proven acceptable, the analysis of the untreated samples was performed. In order to reflect the validated method, upon first thaw, study samples collected without the addition of the preservative were supplemented with 10% ascorbic acid prior to the extraction as per the following procedure:



Following analysis, treated samples were stored at -80ºC.

INCURRED SAMPLE REANALYSIS (ISR)

The analysis of ISR samples was performed using samples treated with 1M ascorbic acid following the first sample thaw. Data demonstrated the reliability and robustness of the assay with the adapted handling conditions since 100% of the ISR samples met acceptance criteria (i.e. re-analyzed samples were ≤ ±20% of the initially measured concentration).

CONCLUSION

The case study for rifampin clearly demonstrates the adjustments which bioanalytical laboratories might be forced to implement when clinical samples fail to be collected and processed under the optimal conditions established during method development and validation.