In-depth Bioanalytical Investigation and Root Cause Analysis of Lamotrigine Severe Degradation in Hemolyzed Plasma Samples by LC-MS/MS

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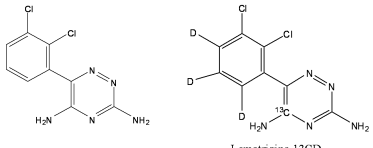
OVERVIEW

- **Purpose**
  - Overcoming degradation of lamotrigine in hemolyzed plasma due to the presence of organic solvent.
- **Method**
  - Calibrants and QC samples were spiked in human plasma with a range of 5.00 to 1500.00 ng/mL.
  - Hemolyzed QC samples were prepared at 1% and 5% hemolysis, with and without the addition of 0.4% H_3PO_4 as preservative, and storage conditions at -20°C and -80°C.
  - Additionally, one set of QCs had the spiking solutions evaporated and reconstituted with 5% hemolyzed plasma before storage at -20°C and -80°C.
- **Results**
  - The results demonstrate that at 1% hemolyzed plasma, lamotrigine was stable at -20°C for a storage period up to 71 days. However, 5% hemolysis showed degradation up to 31%.
  - The addition of 0.4% H_3PO_4 showed degradation up to 21% for 47 days at -20°C
  - The QC samples without the organic solvent (spiking solution evaporated and reconstituted with matrix) showed a bias less than 10% for a period up to 71 days, both at -20°C and -80°C.
  - The hemolyzed samples stored at -80°C showed a bias less than 10% for 71 days for both hemolysis levels. The rate of degradation was controlled by storing the samples at a lower temperature.

INTRODUCTION

Regulatory agencies may require that hemolyzed samples be tested during the course of method development and validation of a bioanalytical method. Hemolyzed plasma has been demonstrated to pose bioanalytical issues for certain drugs and/or metabolites due to analyte degradation, recovery issues, matrix effect or adsorption to blood component(s). Stability of analytes in hemolyzed plasma can be overcome by storage temperature, addition of preservatives or the modification of different extraction conditions. In the present work, an in-depth bioanalytical investigation and root cause analysis of lamotrigine (**Figure 1**) stability in hemolyzed plasma was performed.

Figure 1: Lamotrigine and Lamotrigine-$^{13}CD_3$



LAMOTRIGINE
Chemical Formula: $C_9H_7Cl_2N_5$
Exact Mass: 255.01
Molecular Weight: 256.09

Lamotrigine-13CD3
Chemical Formula: $C_8{}^{13}CH_4D_3Cl_2N_5$
Exact Mass: 259.03
Molecular Weight: 260.10

METHODS

SOLUTION PREPARATION

Stock and spiking solutions (Lamotrigine and Lamotrigine-$^{13}CD_3$ 250.00 µg/mL and 100.00 µg/mL respectively) were prepared in ACN: H_2O 50:50% (v/v)

SAMPLE EXTRACTION

- Analytical range: 5.00 to 1500.00 ng/mL
- Lamotrigine and Lamotrigine-$^{13}CD_3$ were extracted from human plasma using a liquid-liquid extraction.

CHROMATOGRAPHY

- HPLC System: HPLC System: Agilent Technologies Series 1100
- Column: XBridge C18 50 X 2.1 mm, 5 µm
- Mobile phase: 10µM K_2EDTA, 10mM ammonium bicarbonate pH 8.0 in H_2O and MeOH

DETECTION

- Mass Spectrometer: AB Sciex API3000
- Electrospray positive ionization
- Lamotrigine: m/z 256.1 / 211.0
- Lamotrigine-$^{13}CD_3$: m/z 262.0 / 217.0

RESULTS

Hemolyzed plasma can impact analytes stability during storage. In this study, the stability of lamotrigine in hemolyzed plasma was investigated under four different conditions. The stability of lamotrigine was evaluated over time at 1% and 5% hemolyzed plasma. The use of a stable-labeled internal standard ensured that the observed stabilities where not due to ion suppression/enhancement from matrix, but the actual degradation is seen in hemolyzed samples. The hemolyzed plasma quality controls samples used for stability evaluations were compared to freshly prepared quality control in hemolyzed plasma.

The stability of lamotrigine in hemolyzed plasma was evaluated under the four following conditions:

- Storage temperature: -20ºC and -80ºC
- Preservative: 0.4% H_3PO_4 and stored at -20ºC
- Removal of organic solvent used to fortify (spike) samples: evaporation of spiking solution and reconstitution with hemolyzed plasma

STORAGE TEMPERATURE AT -20 °C

The results demonstrated that at 1% hemolysis, lamotrigine was stable at -20°C for a storage period up to 71 days (**Table 1**). However, 5% of hemolysis showed biases of -16% to -31% for 21 and 71 days, which is greater than the acceptance criteria of 15% (**Table 2**).

Table 1: Stability of Lamotrigine in 1% Hemolyzed Samples Stored at -20°C for 71 Days

	Concentration (ng/mL) 71 Days			
	15.00		1125.00	
	Comparison	Stability	Comparison	Stability
	16.5	14.79	1104.62	1061.22
	15.3	14.83	1117.27	1045.99
	15.7	14.69	1147.05	1040.17
Mean	15.84	14.77	1122.98	1049.13
S.D.	0.58	0.07	21.79	10.87
N	3	3	3	3
% C.V.	3.6	0.5	1.9	1.0
% Nominal	105.6	98.5	99.8	93.3
% Deviation	-6.8		-6.6	

Table 2: Stability of Lamotrigine in 5% Hemolyzed Samples Stored at -20ºC for 21 and 71 Days

	Concentrations (ng/mL) 21 Days				Concentrations (ng/mL) 71 Days			
	15.00		1125.00		15.00		1125.00	
	Comparison	Stability	Comparison	Stability	Comparison	Stability	Comparison	Stability
	14.4	12.14	1132.35	905.23	16.5	10.73	1104.62	821.40
	14.3	11.98	1129.03	931.69	15.3	11.05	1117.27	850.76
	15.3	12.13	1056.88	927.66	15.7	11.02	1147.05	851.82
Mean	14.68	12.08	1106.09	921.53	15.84	10.94	1122.98	841.33
S.D.	0.51	0.09	42.65	14.26	0.58	0.18	21.79	17.26
N	3	3	3	3	3	3	3	3
% C.V.	3.5	0.7	3.9	1.5	3.6	1.6	1.9	2.1
% Nominal	97.9	80.5	98.3	81.9	105.6	72.9	99.8	74.8
% Deviation	-17.7		-18.7		-31.0		-25.1	

IN PRESENCE OF PRESERVATIVE

Lamotrigine stabilities in hemolyzed samples containing 0.4% H_3PO_4 as preservative were within the acceptance criteria at the two hemolysis levels for 21 days (data not shown). However, after 47 days of storage, a bias between -10.6 to -21.1% was observed. The addition of acid as preservative could be a solution for a short period, but over time stability was not controlled (**Table 3**).

Table 3: Stability of Lamotrigine in 1% and 5% Hemolyzed Samples Containing 0.4% H_3PO_4 Stored at -20°C for 47 Days

	1% Hemolyzed Plasma Concentration (ng/mL)				5% Hemolyzed Plasma Concentration (ng/mL)			
	Comparison	Stability	Comparison	Stability	Comparison	Stability	Comparison	Stability
	15.7	12.84	1094.66	1000.10	15.7	12.59	1094.66	1005.81
	16.6	13.98	1169.22	1026.15	16.6	12.78	1169.22	1022.38
	15.8	11.73	1159.25	1002.11	15.8	12.63	1159.25	1033.20
Mean	16.05	12.84	1141.05	1009.45	16.05	12.67	1141.05	1020.40
S.D.	0.46	1.11	40.48	14.49	0.46	0.10	40.48	13.90
N	3	3	3	3	3	3	3	3
% C.V.	2.9	8.7	3.5	1.4	2.9	0.8	3.5	1.4
% Nominal	107.0	85.6	101.4	89.7	107.0	84.4	101.4	90.7
% Deviation	-20.0		-11.5		-21.1		-10.6	

REMOVAL OF ORGANIC SOLVENT

The set of hemolyzed samples, without organic solvent (spiking solution evaporated and reconstituted with matrix), showed a bias less than 10% for a period up to 71 days at -20°C and -80°C. The root cause analysis showed that the organic content present in lamotrigine spiking solutions was greatly impacting the stability of lamotrigine in hemolyzed samples. This test is more reflective of incurred samples since there is no presence of organic solvent or additive in incurred hemolyzed samples. The data of the stability performed at -20ºC is presented in **Table 4** and at -80ºC in **Table 5**.

Table 4: Stability of Lamotrigine in 5% Hemolyzed Samples Without Organic Solvent Stored at -20ºC for 71 Days

	Concentration (ng/mL)			
	Low QC 15.00		High QC 1125.00	
	Comparison	Stability	Comparison	Stability
	16.5	15.38	1104.62	1122.98
	15.3	16.13	1117.27	1134.79
	15.7	16.20	1147.05	1139.74
Mean	15.84	15.90	1122.98	1132.50
S.D.	0.58	0.45	21.79	8.61
N	3	3	3	3
% C.V.	3.6	2.8	1.9	0.8
% Nominal	105.6	106.0	99.8	100.7
% Deviation	0.4		0.8	

Table 5: Stability of Lamotrigine in 5% Hemolyzed Samples Without Organic Solvent Stored at -80ºC for 71 Days

	Concentration (ng/mL)			
	Low QC 15.00		High QC 1125.00	
	Comparison	Stability	Comparison	Stability
	15.7	14.82	1165.77	1029.18
	14.1	14.95	1098.87	1178.85
	15.2	14.42	1136.33	1109.40
Mean	14.98	14.73	1133.66	1105.81
S.D.	0.78	0.27	33.53	74.90
N	3	3	3	3
% C.V.	5.2	1.9	3.0	6.8
% Nominal	99.9	98.2	100.8	98.3
% Deviation	-1.7		-2.5	

STORAGE TEMPERATURE AT -80°C

The hemolyzed samples stored at -80°C had a bias less than 10% for 71 days for both hemolysis levels. The rate of degradation was controlled by storing the samples at a lower temperature (**Table 6**).

Table 6: Stability of Lamotrigine in 1% and 5% Hemolyzed Samples Stored at -80ºC for 71 Days

	1% Hemolysis stored at -80°C for 71 days Concentration (ng/mL)				5% Hemolysis stored at -80°C for 71 days Concentration (ng/mL)			
	Comparison	Stability	Comparison	Stability	Comparison	Stability	Comparison	Stability
	15.7	15.82	1165.77	1148.25	15.7	15.75	1165.77	1084.06
	14.1	15.02	1098.87	1120.56	14.1	15.21	1098.87	1151.59
	15.2	15.90	1136.33	1127.63	15.2	16.12	1136.33	1145.34
Mean	14.98	15.58	1133.66	1132.15	14.98	15.69	1133.66	1127.00
S.D.	0.78	0.49	33.53	14.38	0.78	0.46	33.53	37.32
N	3	3	3	3	3	3	3	3
% C.V.	5.2	3.1	3.0	1.3	5.2	2.9	3.0	3.3
% Nominal	99.9	103.9	100.8	100.6	99.9	104.6	100.8	100.2
% Deviation	4.0		-0.1		4.8		-0.6	

CONCLUSION

In conclusion, hemolysis can impact the stability of an analyte over time. Lamotrigine was shown to be stable in hemolyzed plasma by removing the organic solvent in the matrix or by storing the samples at -80°C.

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