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

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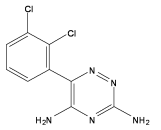
## OVERVIEW

- **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.

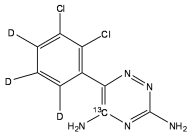
## INTRODUCTION

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 ºC

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 -20ºC 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 | 621.40 |
| | 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 | | -16.7 | | -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 | 13.60 |
| 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 | | -10.8 | |

## 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 -80ºC

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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