3.2.P.5.2 *Analytical Procedure—Attachment 1*

ATTACHMENT 1: Preparation and Analysis of Drug Product Samples (G1113)

Topic	Description	
Diluent	0.05 % trifluoroacetic acid (TFA) in 50:50 acetonitrile: water (v/v)	
Sample Preparation	Concentration	approximately 0.36 mg/mL Stelbat in Diluent
	Number of Preparations	2 (only 1 is required for identity)
	Instructions	Transfer 5 tablets into a volumetric flask. Add a portion of the diluent and shake on mechanical shaker until tablets are completely disintegrated. Dilute to volume with diluent. Do not exceed 2 mg/mL in the initial dilution. Filter or centrifuge.
	Shelf life:	4 days, at ambient or refrigerated conditions protected from light (may be extended with supporting data).
Sample Analysis	Number of Injections:	1 of each preparation
	Acceptance Criteria (Assay):	The %RSD between the two assay results must be ≤ 1.5%.
	Acceptance Criteria (Identity):	1. The retention time ratio of the sample peak to standard peak is within 0.95 to 1.05. 2. The UV spectrum of the sample peak compares favorably to the UV spectrum of the standard peak from 210 nm to 400 nm.

Item	Calculation/Results
Assay Sample	Determine the amount of Stelbat in each sample replicate as follows: $$\frac{mg}{tablet} in\ sample = \frac{A_{SPL}}{ANSR} \times \frac{DF}{N}$$ Where: A_{SPL} = Area of Stelbat in the sample ANSR = Average normalized standard response factor of standard injections DF = Overall sample dilution factor N = Number of tablets in sample preparation Determine the % label claim of the sample as follows: $$\%Label\ Claim = \frac{mg/tablet}{LC} \times 100$$ Where: LC = Label claim (mg/tablet) of tested sample

<table>
<tr><th>Item</th><th>Calculation/Results</th></tr>
<tr><td>Individual Degradation Products</td><td>
For unspecified degradation products, calculate the area% relative to the sum of all peaks (excluding blank peaks) according to the following equation.
$$\text{Area \% Unspecified degradation product} = \frac{\text{Area}_{\text{deg}}}{\text{Area}_{\text{Total}}} \times 100\%$$
Area_{deg} = Peak area of degradation product
$\text{Area}_{\text{Total}}$ = Total peak area in chromatogram (non blank)
100 = Conversion to percent

For specified degradation products, calculate the area% relative to the sum of all peaks (excluding blank peaks) according to the following equation.
$$\text{Area \% Specified degradation product} = \frac{\text{Area}_{\text{deg}}}{\text{Area}_{\text{Total}}} \times 100\% \times \text{RFC}$$
Area_{deg} = Peak area of impurity
$\text{Area}_{\text{Total}}$ = Total peak area in chromatogram (non blank)
RFC = Response factor correction (See table below)
100 = Conversion to percent

<table>
<tr><th>Compound</th><th>Approximate Retention Time (min)</th><th>Relative Retention Time</th><th>Relative Response Factor (RRF)</th><th>Response Factor Correction, RFC (1/RRF)</th></tr>
<tr><td>Stelbat</td><td>9.3</td><td>1.00</td><td>N/A</td><td>N/A</td></tr>
<tr><td>Impurity 1</td><td>7.9</td><td>0.85</td><td>0.964</td><td>1.000</td></tr>
<tr><td>Impurity 2</td><td>8.7</td><td>0.94</td><td>0.717</td><td>1.395</td></tr>
<tr><td>Impurity 3</td><td>9.8</td><td>1.05</td><td>1.323</td><td>0.756</td></tr>
</table>
Note: As the relative response factor for Impurity 1 is between 0.8 and 1.2, no response factor correction is required for this impurity.
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</table>

Item	Calculation/Results
Total Impurities	Calculate the total impurities: $Total\ Degradation\ products = \sum \%\ Individual\ degradation\ product$ Note: Only individual degradation products at or above 0.05% reporting limit should be included in the Total Degradation Products Result
Identity	Calculate the retention time ratio for the Stelbat peak in the sample compared to the standard. Compare the UV spectrum of the sample peak to the UV spectrum of the working standard from 210 nm to 400 nm.
Reporting Impurities	Report the area% of each individual specified named degradation product ≥ 0.05% area according to the specification document. Report the area% of any unspecified degradation product ≥ 0.05% area according to the specification document. For total degradation products add the unrounded results for all peaks ≥ 0.05% area (specified and unspecified) and report as per specification. If no individual peak ≥ 0.05% area, report results for total degradation products as < 0.05% area.
Reporting Assay	Determine the average assay for the duplicate sample replicates. Report final results in accordance with specifications.
Reporting Identity	If both conditions are met, the identification is met; report as “Pass.” If either of the conditions is not met, report identification as “Fail.”