Bendamustine Hydrochloride

Type of Posting  Revision Bulletin
Posting Date  22–Feb–2019
Official Date  01–Mar–2019
Expert Committee  Chemical Medicines Monographs 3
Reason for Revision  Compliance

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 3 Expert Committee has revised the Bendamustine Hydrochloride monograph. The purpose for the revision is to widen the acceptance criteria for Bendamustine related compound D in the test for Organic Impurities from NMT 0.10% to NMT 0.15% to be consistent with the FDA-approved specification.

The Bendamustine Hydrochloride Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Feiwen Mao, Senior Scientific Liaison (301-816-8320 or fm@usp.org).
Add the following:

**Bendamustine Hydrochloride**

\[ \text{C}_9\text{H}_8\text{Cl}_2\text{N}_2\text{O}_3 \cdot \text{HCl} 394.72 \]

1H-Benzimidazole-2-butanoic acid, 5-{bis(2-chloroethyl)amino}-1-methyl-1H-benzimidazole-2-yl)butanoic acid monohydrochloride

**DEFINITION**

Bendamustine Hydrochloride is anhydrous or contains one molecule of hydration. The anhydrous form contains NLT 98.0% and NMT 102.0% of bendamustine hydrochloride \((\text{C}_9\text{H}_8\text{Cl}_2\text{N}_2\text{O}_3 \cdot \text{HCl})\), calculated on the as-is basis. The monohydrate form contains NLT 98.0% and NMT 102.0% of bendamustine hydrochloride \((\text{C}_9\text{H}_8\text{Cl}_2\text{N}_2\text{O}_3 \cdot \text{HCl} \cdot \text{H}_2\text{O})\), calculated on the anhydrous and solvent-free basis.

**IDENTIFICATION**

- **A. INFRARED ABSORPTION** (197): [NOTE—Methods described in (197K) or (197A) may be used.]

- **B.** The retention time of the major peak of the Sample solution corresponds to that of the Standard solution, as obtained in the Assay.

- **C. IDENTIFICATION TESTS—GENERAL** (191), Chemical Identification Tests, Chloride

**ASSAY**

- **PROCEDURE**
  Solution A: 0.1% (v/v) trifluoroacetic acid in water
  Solution B: 0.1% (v/v) trifluoroacetic acid in acetonitrile

Mobile phase: See Table 1.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>73</td>
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<tr>
<td>16</td>
<td>73</td>
<td>27</td>
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<tr>
<td>25</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>26</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>31</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>40</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>45</td>
<td>93</td>
<td>7</td>
</tr>
</tbody>
</table>

Diluent: 1-Methyl-2-pyrrolidone and Solution A (1:1)

**Sample solution:** 4.2 mg/mL of USP Bendamustine Hydrochloride in Diluent

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 254 nm

**Column:** 4.6-mm x 15-cm; 5-µm packing L60

**Temperatures**

- **Autosampler:** 2°C–8°C
- **Column:** 30°C

**Flow rate:** 1 mL/min

**Injection volume:** 2 µL

**Analysis time:** 25 min

**System suitability**

- [NOTE—The slower syringe draw rate and higher detector sampling rate can be applied in order to improve the precision.]

**Sample:** Standard solution

**Suitability requirements**

- **Tailing factor:** NMT 2.0
- **Relative standard deviation:** NMT 1.0%

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of bendamustine hydrochloride \((\text{C}_9\text{H}_8\text{Cl}_2\text{N}_2\text{O}_3 \cdot \text{HCl})\) in the portion of Bendamustine Hydrochloride taken:

\[
\text{Result} = \left( \frac{r_0}{r_s} \right) \times \left( \frac{C_s}{C_u} \right) \times 100
\]

- **r_0** = peak response from the Sample solution
- **r_s** = peak response from the Standard solution
- **C_s** = concentration of USP Bendamustine Hydrochloride RS in the Standard solution (mg/mL)
- **C_u** = concentration of Bendamustine Hydrochloride in the Sample solution (mg/mL)

**Acceptance criteria:** 98.0%–102.0% on the as-is basis for the anhydrous form; 98.0%–102.0% on the anhydrous and solvent-free basis for the monohydrate form

**IMPURITIES**

- **Residue on Ignition** (281): NMT 0.1%

Change to read:

**ORGANIC IMPURITIES**

Mobile phase, Diluent, Standard solution, Sample solution, and Chromatographic system: Proceed as directed in the Assay.

**System suitability solution:** 4.2 mg/mL of USP Bendamustine Hydrochloride RS, and 0.02 mg/mL each of USP Bendamustine Related Compound A RS, USP Bendamustine Related Compound C RS, USP Bendamustine Related Compound D RS, USP Bendamustine Related Compound E RS, USP Bendamustine Related Compound G RS, USP Bendamustine Related Compound H RS, and USP Bendamustine Related Compound I RS in Diluent

**Sensitivity solution:** 2 µg/mL of USP Bendamustine Hydrochloride RS in Diluent, from the Standard solution

**System suitability**

**Samples:** System suitability solution and Sensitivity solution

**Suitability requirements**

- **Resolution:** NLT 5 between the bendamustine related compound G and bendamustine peaks; NLT 4 between the bendamustine related compound H and bendamustine related compound I peaks, System suitability solution

**Signal-to-noise ratio:** NLT 10, Sensitivity solution
Analysis

Sample: Sample solution

Calculate the percentage of each impurity in the portion of Bendamustine Hydrochloride taken:

\[
\text{Result} = \left( \frac{r_u}{\sum [r_u \times (1/F)] + r_s} \right) \times \left( \frac{1}{F} \right) \times 100
\]

- \( r_u \) = peak area of each impurity from the Sample solution
- \( F \) = relative response factor for each impurity (see Table 2)
- \( r_s \) = peak area of bendamustine from the Sample solution

Acceptance criteria: See Table 2. The reporting threshold is 0.05%.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bendamustine related compound A</td>
<td>0.25</td>
<td>0.76</td>
<td>0.25</td>
</tr>
<tr>
<td>Bendamustine related compound C</td>
<td>0.60</td>
<td>0.83</td>
<td>0.20</td>
</tr>
<tr>
<td>Bendamustine related compound D</td>
<td>0.69</td>
<td>0.93</td>
<td>0.15* (USP 1-Mar-2019)</td>
</tr>
<tr>
<td>Bendamustine related compound E</td>
<td>0.73</td>
<td>1.2</td>
<td>0.45</td>
</tr>
<tr>
<td>Bendamustine related compound G</td>
<td>0.90</td>
<td>3.1</td>
<td>0.35</td>
</tr>
<tr>
<td>Bendamustine</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bendamustine related compound H</td>
<td>1.15</td>
<td>0.98</td>
<td>0.30</td>
</tr>
<tr>
<td>Bendamustine related compound I</td>
<td>1.20</td>
<td>1.1</td>
<td>0.40</td>
</tr>
<tr>
<td>Any individual unspecified impurity</td>
<td>—</td>
<td>1.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Total impurities</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Bacterial Endotoxins Test (85): Meets the requirements

Microbial Enumeration Tests (62) and Tests for Specified Microorganisms (62): The total aerobic microbial count is NMT 10³ cfu/g. The total combined molds and yeasts count is NMT 10² cfu/g.

Additional Requirements

Packaging and Storage: Preserve in well-closed containers. Store at room temperature.

USP Reference Standards (11)

- USP Bendamustine Hydrochloride RS
- USP Bendamustine Related Compound A RS
- USP Bendamustine Related Compound C RS
- USP Bendamustine Related Compound D RS
- USP Bendamustine Related Compound E RS
- USP Bendamustine Related Compound G RS
- USP Bendamustine Related Compound H RS
- USP Bendamustine Related Compound I RS

Specific Tests

- Water Determination (921), Method I, Method Ia: NMT 1.0% for the anhydrous form; 3.0%–5.5% for the monohydrate form