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

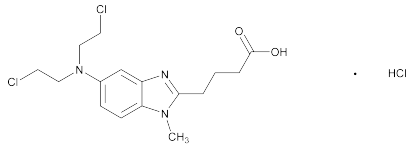
In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Chemical Medicines Monographs 3 Expert Committee has revised the Bendamustine Hydrochloride monograph. The purpose for the revision is to widen the acceptance criteria for Bendamustine related compound D in the test 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



$C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$ 394.72
1*H*-Benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl) amino]-1-methyl-, monohydrochloride;
4-{5-[Bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazole-2-yl}butanoic acid monohydrochloride [3543-75-7].
Bendamustine (free base)

$C_{16}H_{21}Cl_2N_3O_2$ 358.26
[16506-27-7].
Monohydrate

$C_{16}H_{21}Cl_2N_3O_2 \cdot HCl \cdot H_2O$ 412.74
[1374784-02-7].

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 ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$), calculated on the as-is basis. The monohydrate form contains NLT 98.0% and NMT 102.0% of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$), 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 1

Time (min)	Solution A (%)	Solution B (%)
0	93	7
5	93	7
13	73	27
16	73	27
25	43	57
26	10	90
31	10	90
40	93	7
45	93	7

Diluent: 1-Methyl-2-pyrrolidone and *Solution A* (1:1)
Standard solution: 4.2 mg/mL of USP Bendamustine Hydrochloride RS in *Diluent*
Sample solution: 4.2 mg/mL of Bendamustine Hydrochloride in *Diluent*
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 254 nm
Column: 4.6-mm × 15-cm; 5-µm packing L60
Temperatures
Autosampler: 2°–8°
Column: 30°
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 ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$) in the portion of Bendamustine Hydrochloride taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = 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:

$$\text{Result} = (r_U/\{\Sigma[r_U \times (1/F)] + r_S\}) \times (1/F) \times 100$$

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 2

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

SPECIFIC TESTS

- **WATER DETERMINATION** ⟨921⟩**,** *Method I, Method Ia***:** NMT 1.0% for the anhydrous form; 3.0%–5.5% for the monohydrate form
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** Meets the requirements
- **MICROBIAL ENUMERATION TESTS** ⟨61⟩ and **TESTS FOR SPECIFIED MICROORGANISMS** ⟨62⟩**:** The total aerobic microbial count is NMT 10^3 cfu/g. The total combined molds and yeasts count is NMT 10^2 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
4-{5-[Bis(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{16}H_{23}N_3O_4$ 321.38

USP Bendamustine Related Compound C RS
Ethyl 4-{5-[bis(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{18}H_{27}N_3O_4$ 349.43

USP Bendamustine Related Compound D RS
4-{5-[(2-Chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{14}H_{18}ClN_3O_2$ 295.77

USP Bendamustine Related Compound E RS
4-{5-[(2-Chloroethyl)(2-hydroxyethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid.
$C_{16}H_{22}ClN_3O_3$ 339.82

USP Bendamustine Related Compound G RS
4-[6-(2-Chloroethyl)-3,6,7,8-tetrahydro-3-methylimidazo[4,5-*h*][1,4]benzothiazin-2-yl]butanoic acid.
$C_{16}H_{20}ClN_3O_2S$ 353.86

USP Bendamustine Related Compound H RS
4-[5-({2-[(4-{5-[Bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoyl)oxy]ethyl}(2-chloroethyl)amino)-1-methyl-1*H*-benzimidazol-2-yl]butanoic acid.
$C_{32}H_{41}Cl_3N_6O_4$ 680.07

USP Bendamustine Related Compound I RS
Ethyl 4-{5-[bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.
$C_{18}H_{25}Cl_2N_3O_2$ 386.32▲ 2S (USP41)