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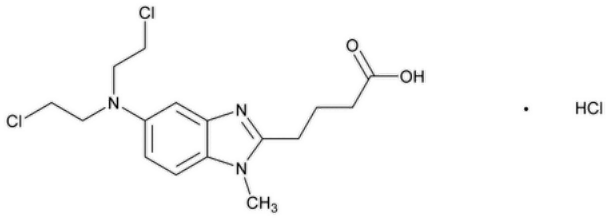
Bendamustine Hydrochloride

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-bendamustine-hcl-20190222



$C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$ 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 CAS RN®: 3543-75-7.

Bendamustine (free base)

$C_{16}H_{21}Cl_2N_3O_2$ 358.26 CAS RN®: 16506-27-7.

Monohydrate

$C_{16}H_{21}Cl_2N_3O_2 \cdot HCl \cdot H_2O$ 412.74 CAS RN®: 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

Change to read:

- **A.** ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197A or 197K▲ (CN 1-May-2020)
- **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

Time (min)	Solution A (%)	Solution B (%)
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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
BENDAMUSTINE HYDROCHLORIDE	Documentary Standards Support	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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