

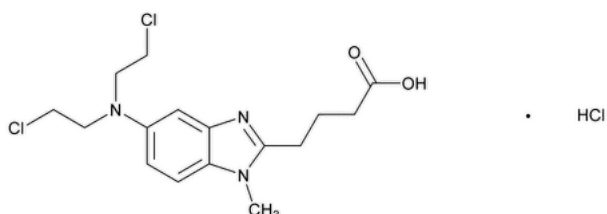
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Bendamustine Hydrochloride

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<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	

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