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Bendamustine Hydrochloride for Injection

DEFINITION

Bendamustine Hydrochloride for Injection is a sterile, lyophilized mixture of Bendamustine Hydrochloride and Mannitol. It contains NLT 90.0% and NMT 110.0% of the labeled amount of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$).

IDENTIFICATION

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

Change to read:

• **B.** ▲**Spectroscopic Identification Tests** ⟨197⟩, *Ultraviolet-Visible Spectroscopy*: **197U**▲ (CN 1-May-2020)

Sample solution: Nominally equivalent to 5 µg/mL of bendamustine hydrochloride, from Bendamustine Hydrochloride for Injection, in water

Acceptance criteria: Meets the requirements

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: Nominally equivalent to 4.2 mg/mL of bendamustine hydrochloride in *Diluent*, from Bendamustine Hydrochloride for Injection

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

Sample: *Standard solution*

Suitability requirements

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

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bendamustine hydrochloride ($C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$) in the portion of Bendamustine Hydrochloride for Injection 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 = nominal concentration of bendamustine hydrochloride in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements

IMPURITIES

• **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 B RS, USP Bendamustine Related Compound C RS, USP Bendamustine Related Compound D RS, USP Bendamustine Related Compound E RS, USP Bendamustine Related Compound F 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 for Injection 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 0.1%.

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bendamustine related compound A	0.25	0.76	0.3

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Bendamustine related compound B[a]	0.57	0.84	0.2
Bendamustine related compound C[b]	0.60	0.83	—
Bendamustine related compound D	0.69	0.93	0.6
Bendamustine related compound E	0.73	1.2	1.5
Bendamustine related compound F	0.88	0.61	0.5
Bendamustine related compound G[b]	0.90	3.1	—
Bendamustine	1.0	—	—
Bendamustine related compound H	1.15	0.98	0.9
Bendamustine related compound I[b]	1.20	1.1	—
Any individual unspecified impurity	—	1.0	0.2
Total impurities	—	—	3.5

[a] It is a free base of USP Bendamustine Related Compound B RS: 4-(1-Methyl-5-morpholino-1*H*-benzimidazol-2-yl)butanoic acid.

[b] This process impurity is controlled in the drug substance monograph. It is included in the table for identification only, and it is not to be reported in the total impurities.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** Meets the requirements
- **Sterility Tests ⟨71⟩:** Meets the requirements
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections
- **pH ⟨791⟩:** 2.5–3.5 in a constituted solution prepared as directed in the labeling
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described in *Packaging and Storage Requirements ⟨659⟩, Injection Packaging*. Store at controlled room temperature.
- **Labeling ⟨7⟩:** Meets the requirements
- **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 B RS

4-(1-Methyl-5-morpholino-1*H*-benzimidazol-2-yl)butanoic acid hydrochloride.

$C_{16}H_{21}N_3O_3 \cdot x$HCl

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 F RS

Mannitol-1-yl 4-{5-[bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoate.

$C_{22}H_{33}Cl_2N_3O_7$ 522.42

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 FOR INJECTION	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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