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Add the following:

▲Bivalirudin for Injection

DEFINITION

Bivalirudin for Injection is a sterile, lyophilized powder or cake for reconstitution. Bivalirudin used in the manufacture of Bivalirudin for Injection complies with the compendial requirements stated in the Bivalirudin monograph. It contains the equivalent of NLT 90.0% and NMT 110% of the labeled amount of bivalirudin ($C_{98}H_{138}N_{24}O_{33}$).

IDENTIFICATION

• **A.**

Standard solution and **Sample solution:** Prepare as directed in the *Assay*.

Identity sample solution: Mix equal volumes of the *Standard solution* and *Sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*. The major peaks of the *Identity sample solution* co-elute.

• **B. Bioidentity**

Thrombin inhibition activity

Buffer solution: 50 mM tris(hydroxymethyl)aminomethane hydrochloride and 120 mM sodium chloride in water. Adjust to a pH of 7.40 ± 0.04. Add bovine serum albumin to this solution to obtain a 1-mg/mL concentration and pass through a filter of 0.45-µm pore size.

Stop solution: Glacial acetic acid

Chromogenic substrate solution: 5 mM solution of *H*-D-cyclohexylalanyl-Ala-Arg-*p*-nitroanilide diacetate salt in water

Human thrombin solution: 10 µg/mL of human thrombin[1] in *Buffer solution*

Diluted human thrombin solution: Dilute *Human thrombin solution* with *Buffer solution* to obtain a 0.5-µg/mL solution. Thrombin from alternate sources must be standardized in the substrate reaction. To perform the standardization, prepare 6 replicates by adding 910 µL of *Buffer solution* to each sample tube followed by 30 µL of *Chromogenic substrate solution*. Add 60 µL of *Diluted human thrombin solution*, mix on a vortex mixer, and begin the 20-min incubation, as described below. Determine the absorbance at 405 nm for each replicate. Use water to auto-zero.

Calculate the standardized volume (E_C) of *Diluted human thrombin solution* from the mean absorbance (A_M) as follows:

$$E_C = (0.45 \times 60\ \mu L)/A_M$$

Standard solution: Prepare a 0.6-mg/mL solution of USP Bivalirudin RS in water. Dilute with *Buffer solution* to obtain a 5-µg/mL solution.

Sample solution: Prepare a 0.6-mg/mL solution of Bivalirudin for Injection in water. Make 3 independent preparations of this solution.

Measure the absorbance of each of the 0.6-mg/mL solutions at 275 nm (A_{275}), using water to auto-zero.

Calculate the concentration of bivalirudin ($C_{98}H_{138}N_{24}O_{33}$), C_B, in mg/mL, in the *Sample solution*:

$$C_B = A_{275}/0.62$$

From this concentration, dilute with *Buffer solution* to obtain a 5-µg/mL solution. Prepare triplicate dilutions from each independently prepared 0.6-mg/mL solution.

Blank, Control test solution, Sample test solution, and **Standard test solution**

Analysis: In each sample tube, add the *Buffer solution* first, then 30 µL of *Chromogenic substrate solution*, and then the *Standard solution* or *Sample solution* (if using). Mix on a vortex mixer and incubate for 10 min at 37° in a water bath. Add the appropriate volume (E_C) of *Diluted human thrombin solution* to give a final concentration of 0.095 NIH Units/mL and activate the chronometer immediately. Mix on a vortex mixer for a few seconds and heat to 37° for 20 min ± 15 s in a water bath. Stop the reaction by adding 100 µL of *Stop solution*. Measure the absorbance of the 6 solutions at 405 nm using water to auto-zero.

Prepare the solutions for the analysis as indicated in *Table 1*.

Table 1

	Blank	Control Test Solution	Sample Test Solution	Standard Test Solution
Number of replicates	1	6	3	3

	Blank	Control Test Solution	Sample Test Solution	Standard Test Solution
Total number of UV readings	1	6	9	3
Chromogenic substrate solution	30 µL	30 µL	30 µL	30 µL
Bivalirudin	100 µL (*Standard solution*)	0	100 µL (*Sample solution*)	100 µL (*Standard solution*)
Diluted human thrombin solution	0	E_C	E_C	E_C
Buffer solution	1000 µL − 30 µL − 100 µL	1000 µL − 30 µL− E_C	1000 µL − 30 µL − 100 µL − E_C	1000 µL − 30 µL − 100 µL − E_C

System suitability

Samples: *Control test solution* and *Standard test solution*

Suitability requirements

Relative standard deviation: NMT 5% for 6 replicates, *Control test solution*

Mean absorbance: Between 0.428 and 0.473, *Control test solution*

Average inhibition: 44%–50%, *Standard test solution*

Analysis

Samples: *Control test solution* and *Sample test solution*

Calculate the percentage of thrombin inhibition for each of the 9 sample readings of the sample tested:

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

r_U = absorbance response from the *Sample test solution*

r_S = average absorbance response from the 6 readings of the *Control test solution*

The final percentage inhibition result is given as the average of these 9 values.

Acceptance criteria

Average inhibition: 42%–52%

Inhibition of each single Sample test solution: 41%–53%

Relative standard deviation of the inhibition of the Sample test solutions: NMT 10%, 9 readings

ASSAY

• **Procedure**

Buffer solution: Dissolve 8.2 g of sodium acetate in 900 mL of water. Adjust with glacial acetic acid to a pH of 6.5 ± 0.1. Dilute with water to 1000 mL and pass through a filter of 0.2-µm pore size.

Solution A: *Buffer solution* and water (1:1)

Solution B: *Buffer solution* and acetonitrile (1:1)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
30	65	35
35	65	35
35.1	90	10
40	90	10

System suitability solution: 275 µg/mL of USP Bivalirudin RS and 3 µg/mL of USP [Asp^9]-Bivalirudin RS in water

Standard solution: 275 µg/mL of USP Bivalirudin RS in water

Sample solution: Transfer the entire contents of a vial (250 mg) to a 100-mL volumetric flask. Dissolve in and dilute with water to volume or to 275 µg/mL, nominally.

Chromatographic system

(See *Chromatography (621), System Suitability*.)

Mode: LC

Detector: UV 215 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Temperatures

Autosampler: 2°–8°

Column: 40°

Flow rate: 1.2 mL/min

Injection volume: 40 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

[NOTE—The relative retention times for [Asp^9]-bivalirudin and bivalirudin are about 0.93 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the bivalirudin and [Asp^9]-bivalirudin peaks, *System suitability solution*

Column efficiency: NLT 12,000 theoretical plates, *Standard solution*

Relative standard deviation: NMT 1.0% for 3 replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of bivalirudin ($C_{98}H_{138}N_{24}O_{33}$) in the portion of Bivalirudin for Injection taken:

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

r_U = peak response of bivalirudin from the *Sample solution*

r_S = mean peak response of bivalirudin from the *Standard solution*

C_S = concentration of USP Bivalirudin RS in the *Standard solution* (µg/mL)

C_U = nominal concentration of bivalirudin in the *Sample solution* (µg/mL)

Acceptance criteria: 90.0%–110.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **PROCEDURE**

Buffer solution: Dissolve 27.2 g of sodium acetate trihydrate in 1800 mL of water, and adjust with glacial acetic acid to a pH of 6.5 ± 0.1. Dilute with water to 2000 mL and pass through a filter of 0.2-µm pore size.

Solution A: *Buffer solution* and water (1:1)

Solution B: *Buffer solution* and acetonitrile (1:1)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	90	10
5	85	15
30	65	35
35	65	35
35.1	90	10
40	90	10

System suitability solution: Prepare a solution containing 2.5 mg/mL of USP Bivalirudin RS spiked with 0.025 mg/mL of USP [Asp^9]-Bivalirudin RS in water.

Sample solution 1 (high concentration): Transfer the entire contents of a vial (250 mg) to a 100-mL volumetric flask. Dissolve in and dilute with water to volume.

Sample solution 2 (low concentration): Dilute 2.0 mL of *Sample solution 1* with water to 100 mL.

Blank: Water

Chromatographic system: Proceed as directed in the *Assay*.

System suitability

Sample: *System suitability solution*

[NOTE—See *Table 4* for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between the bivalirudin and [Asp^9]-bivalirudin peaks

Column efficiency: NLT 10,000 theoretical plates

Analysis

Samples: *Sample solution 1* and *Sample solution 2*, single injection

Record the chromatograms, and measure each peak area from *Sample solution 2* using the drop-down method of integration with respect to the baseline. Exclude from the integration the peaks present in the blank. Among all the integrated peaks, only those with a signal-to-noise ratio higher than 10 shall be used for the calculation. Report the area of all peaks of the chromatogram of *Sample solution 1*. To calculate the corrected bivalirudin peak area for *Sample solution 2*, report the area of the bivalirudin peak of the *Sample solution 2* chromatogram.

Calculate the corrected total peak area (r_{CT}) in *Sample solution 1*:

$$r_{CT} = r_T - r_H + r_L \times D$$

r_T = total peak area from *Sample solution 1*

r_H = bivalirudin peak area from *Sample solution 1*

r_L = bivalirudin peak area from *Sample solution 2*

D = dilution factor, 50

Calculate the percentage of each impurity in the portion of Bivalirudin for Injection taken by using the corrected total peak area:

$$\text{Result} = (r_U/r_{CT}) \times 100$$

r_U = peak response of each impurity from *Sample solution 1*

r_{CT} = corrected total peak area

Acceptance criteria: See *Table 4*.

Table 4

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Fragment [1–11]	0.49	0.7
Fragment [12–20]	0.60	1.0
Total fragments[a]	0.44–0.65	1.8
[Asp^9]-bivalirudin	0.93	1.0
Bivalirudin	1.00	—
Unspecified impurities	—	1.0
Total impurities	—	6

[a] Peptide fragment peaks resulting from degradation.

PERFORMANCE TESTS

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

SPECIFIC TESTS

• **Completeness of Solution** ⟨641⟩

Sample solution: Reconstitute 1 vial of Bivalirudin for Injection with 5 mL of carbon dioxide-free water.

Acceptance criteria: After 3 min, the solution is clear and free from undissolved solids.

• **Constituted Solution:** At the time of use, it meets the requirements in *Injections and Implanted Drug Products* ⟨1⟩*, Product Quality Tests Common to Parenteral Dosage Forms, Specific Tests, Completeness and Clarity of Solutions*.

• **Bacterial Endotoxins Test** ⟨85⟩**:** Meets the requirements

• **Sterility Tests** ⟨71⟩**:** Meets the requirements

• **Water Determination** ⟨921⟩**,** *Method I, Method Ic***:** NMT 4.0%

• **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections

• **pH** ⟨791⟩

Sample solution: Use the *Sample solution* prepared in the test for *Completeness of Solution*.

Acceptance criteria: 5.0–6.0

• **Other Requirements:** Meets the requirements in *Labeling* ⟨7⟩

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass. Store at controlled room temperature.

• **Labeling:** Label it to indicate its synthetic origin.

• **USP Reference Standards** ⟨11⟩

USP Bivalirudin RS

USP [Asp^{9}]-Bivalirudin RS▲ (USP 1-Dec-2023)

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[1] A suitable human alpha-thrombin is available from Sigma-Aldrich T6884.

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

Topic/Question	Contact	Expert Committee
BIVALIRUDIN FOR INJECTION	Kishan Chandra Senior Scientist I, Documentary Standards	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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