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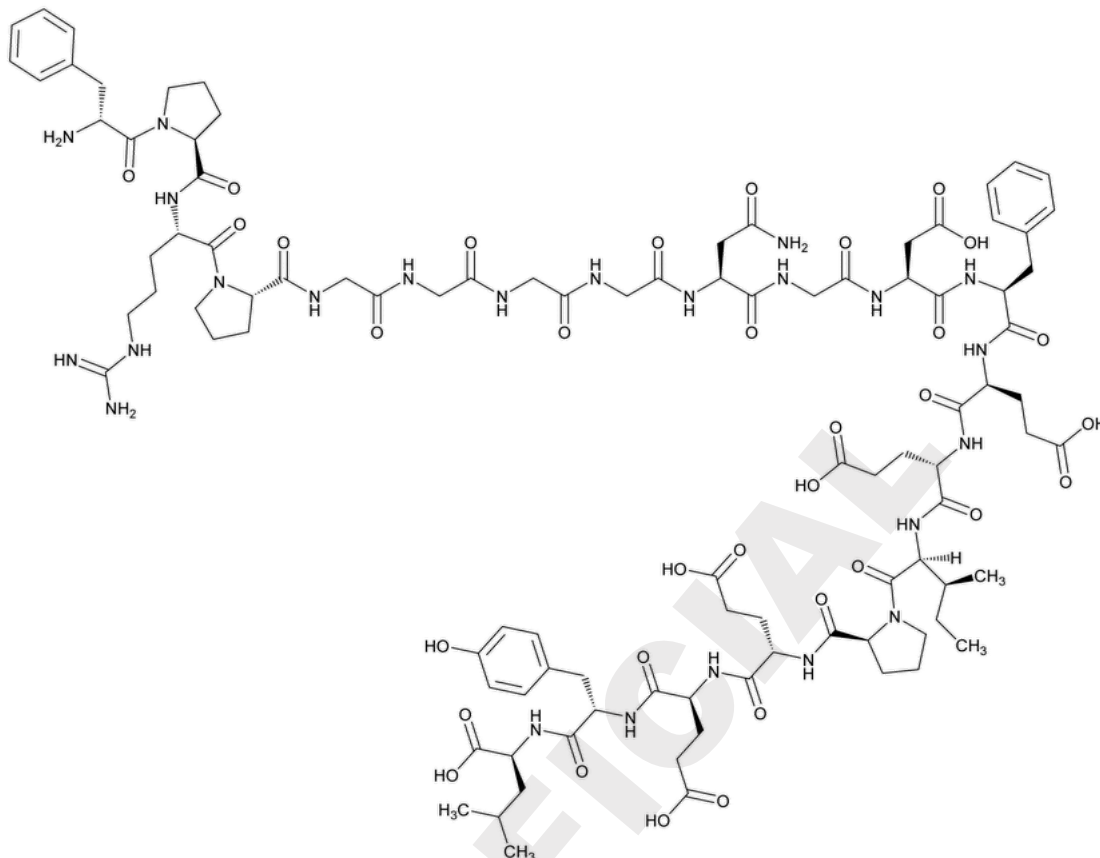
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Add the following:

▲Bivalirudin



DFPRPGGGGNG DFEEIPEEYL

 $C_{98}H_{138}N_{24}O_{33}$

2180.32

L-Leucine, D-phenylalanyl-L-prolyl-L-arginyl-L-prolylglycylglycylglycylglycyl-L-asparaginylglycyl-L-α-aspartyl-L-phenylalanyl-L-α-glutamyl-L-α-glutamyl-L-isoleucyl-L-prolyl-L-α-glutamyl-L-α-glutamyl-L-tyrosyl-;
 D-Phenylalanyl-L-prolyl-L-arginyl-L-prolylglycylglycylglycylglycyl-L-asparaginylglycyl-L-α-aspartyl-L-phenylalanyl-L-α-glutamyl-L-α-glutamyl-L-isoleucyl-L-prolyl-L-α-glutamyl-L-α-glutamyl-L-tyrosyl-L-leucine CAS RN®: 128270-60-0.

DEFINITION

Bivalirudin is a synthetic 20 amino acid peptide, which is a specific and reversible direct thrombin inhibitor. It contains NLT 96.0% and NMT 103.0% of bivalirudin ($C_{98}H_{138}N_{24}O_{33}$), calculated on the anhydrous, counter ion-free basis.

IDENTIFICATION

• A.

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution A, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.

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

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

• **B.** The monoisotopic mass by [Mass Spectrometry \(736\)](#) is 2179.0 ± 1.0 mass units.

• **C. AMINO ACID CONTENT**

For further discussion of the theory and applications, see [Biotechnology-Derived Articles—Amino Acid Analysis \(1052\)](#). [NOTE—Use a suitable, validated hydrolysis, separation, and calculation procedure, including amino acids used in the calculation.]

Standard solution: Standardize the instrument with a mixture containing equal molar per volume amounts of glycine and the L-form of the following amino acids: lysine, threonine, alanine, leucine, histidine, serine, valine, tyrosine, arginine, glutamic acid, methionine, phenylalanine, aspartic acid, proline, isoleucine, and tryptophan, and half the molar per volume amount of the L-form of cystine.

Sample solution: Accurately weigh out 1.0 mg of bivalirudin in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis Solution* containing 4% [phenol](#), freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 18 h. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter of 0.45-µm pore size.

Analysis

Samples: *Standard solution* and *Sample solution*

First record and measure the peak responses for each amino acid in the *Standard solution*. Express the content of each amino acid in moles.

Calculate the mean nmol of the amino acids in the *Sample solution*:

Result = (nmol found in the *Sample solution* for arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, phenylalanine, proline, and tyrosine)/20

Divide the nmol of each amino acid by the Result to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: See [Table 1](#).

Table 1

Name	Acceptance Criteria
Glycine	Between 4.5 and 5.9
Aspartic acid	Between 1.6 and 2.4
Glutamic acid	Between 3.6 and 4.4
Proline	Between 2.6 and 3.4
Leucine, isoleucine, arginine, and tyrosine	Between 0.7 and 1.3
Phenylalanine	Between 1.6 and 2.4

ASSAY

• PROCEDURE

Buffer solution: Dissolve 13.6 g of [sodium acetate trihydrate](#) 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

Standard solution A: 1.5 mg/mL of [USP Bivalirudin RS](#) in [water](#)

Standard solution B: 0.16 mg/mL of [USP \[Asp⁹\]-Bivalirudin RS](#) in [water](#)

System suitability solution: *Standard solution A* and *Standard solution B* (1:1)

Sample solution: 1.5 mg/mL of Bivalirudin in [water](#). Prepare in triplicate.

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: *Standard solution A* and *System suitability solution*

[NOTE—The relative retention times for [Asp⁹]-bivalirudin and bivalirudin are 0.93 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between the bivalirudin and [Asp⁹]-bivalirudin peaks, *System suitability solution*

Column efficiency: NLT 15,000 theoretical plates, *Standard solution A*

Relative standard deviation: NMT 1.5% for 3 replicate injections, *Standard solution A*

Analysis

Samples: *Standard solution A* and *Sample solution*

Calculate the percentage of bivalirudin (C₉₈H₁₃₈N₂₄O₃₃) in the portion of Bivalirudin 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 *Standard solution A*

C_S = concentration of [USP Bivalirudin RS](#) in *Standard solution A* (mg/mL)

C_U = concentration of Bivalirudin in the *Sample solution* (mg/mL)

Take the percentage of Bivalirudin for each *Sample solution*. Average the results to report the acceptance criteria.

Acceptance criteria: 96.0%–103.0% on the anhydrous, counter ion-free basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• PROCEDURE 1

[NOTE—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak equal to or above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Buffer solution, Solution A, Solution B, Mobile phase, and Chromatographic system: Proceed as directed in the Assay.

System suitability solution: Prepare a solution containing 2.5 mg/mL of [USP Bivalirudin RS](#) spiked with 0.05 mg/mL of [USP \[Asp⁹\]-Bivalirudin RS](#) and 0.05 mg/mL of [USP \[des-Glu¹³\]-Bivalirudin RS](#) in [water](#).

Sample solution: 2.5 mg/mL of Bivalirudin in [water](#)

Blank: [Water](#)

System suitability

Sample: *System suitability solution*

[NOTE—See [Table 3](#) for the relative retention times.]

Suitability requirements

Resolution: NLT 2.5 between [Asp⁹]-bivalirudin and bivalirudin

Column efficiency: NLT 12,000 theoretical plates for the bivalirudin peak

Analysis

Sample: *Sample solution*

Record the chromatograms and measure the response of each peak from the *Sample solution* using the drop-down integration method 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.

Calculate the percentage of each impurity in the portion of Bivalirudin taken:

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

r_U = peak response of each impurity

r_T = sum of all the peak responses