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Change to read:

^Epoetin

APPRLICDSR VLERYLLEAK EAENITTGCA EHCSLNENIT VPDTKVNFYA
WKRMEVGQQA VEVWQGLALL SEAVLRGQAL LVNSSQPWEP LQLHVDKAVS
GLRSLTTLLR ALGAQKEAIS PPDAASAAPL RTITADTFRK LFRVYSNFLR
GKLKLYTGEA CRTGD

C₈₀₉H₁₃₀₁N₂₂₉O₂₄₀S₅ 18,236.06 Da (amino acid sequence)

DEFINITION

Epoetin is the recombinant form of human erythropoietin. It is a 165-amino acid glycoprotein manufactured using recombinant DNA technology. The presence of the impurities, host cell DNA, and host cell protein in Epoetin is process-specific, and is controlled through the purification process. The impurity levels are determined by validated methods and limits approved by the competent regulatory authority. It has a potency of NLT 140,000 and NMT 200,000 international units (IU) per absorbance unit (AU) at 280 nm.

IDENTIFICATION

- A. [ERYTHROPOIETIN BIOASSAYS \(124\)](#): Meets the requirements
- B. **PEPTIDE MAPPING**

Solution A: Trifluoroacetic acid and water (1.5:1000)
Solution B: Acetonitrile, trifluoroacetic acid, and water (900:1.2:100)
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30.0	78	22
110.0	58	42
130.0	35	65
130.1	10	90
140.0	10	90
140.1	90	10

Phosphate buffer saline: 137 mM sodium chloride, 2.7 mM potassium chloride, 8.1 mM disodium hydrogen phosphate, and 1.47 mM potassium dihydrogen phosphate

Buffer A: 20 mM sodium citrate and 100 mM sodium chloride, pH 6.9, prepared as follows. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water, and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2-µm pore size.

Digest stop solution: 8 M guanidine hydrochloride

Digest buffer stock: 1.0 M tris(hydroxymethyl) aminomethane solution. Adjust with hydrochloric acid to a pH of 7.3.

Lys-C stock solution: Dissolve lysyl endopeptidase (Lys-C), containing approximately 4.5 AU/mg, in *Digest buffer stock* to a final concentration of 2 mg/mL. Expiry of reconstituted Lys-C is 2 years at -70 ± 10°, and 1 day at 5 ± 3°.

Lys-C digest solution: Add 20 µL of *Lys-C stock solution* to 180 µL of *Digest buffer stock*. Expiry is 1 day stored on ice or at 5 ± 3°.

Standard solution: 100 µg of [USP Erythropoietin RS](#) in 50 µL of water. Buffer exchange by a suitable method into *Buffer A*. Add 5 µL of *Lys-C digest solution* to 50 µL of buffer exchanged [USP Erythropoietin RS](#). Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Sample solution: 100 µg of Epoetin in 50 µL of *Phosphate buffer saline*. If necessary, buffer exchange by a suitable method into *Buffer A*. Add 5 µL of *Lys-C digest solution*. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Blank solution: 50 µL of *Phosphate buffered saline*. Add 5 µL of *Lys-C digest solution*. Incubate at 37° for 30 min. Add 50 µL of *Digest stop solution* and mix.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 214 nm

Column: 3.0-mm × 25-cm; 5-µm packing L7

Column temperature: 30°

Flow rate: 0.2 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements: 10 major peaks must be present as illustrated in the reference chromatogram provided with the lot of [USP Erythropoietin RS](#) being used. The change in retention times for each of peaks 5, 8, and 9 is NMT 1.2 min when compared to the first injection for three replicate injections. The ratio of relative heights percentage (RRH%) for each peak 8 and peak 9 in the *Standard solution* injections must be within 94%–106%.

Signal-to-noise ratio: NLT 3 for peak 6

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank solution*

Follow each injection of *Standard solution* with a *Blank solution* injection. Record the retention time and response (peak height) of each of the 10 major peaks for both the *Standard solution* and *Sample solution*.

Calculate the relative peak height percentage (RH%) of peak 8 and peak 9 as compared to peak 5 for both the initial *Standard solution* injection and *Sample solution* taken:

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

r_U = peak height of peak 8 or 9

r_S = peak height of peak 5

Calculate the ratio of RRH% for peak 8 and peak 9:

$$\text{Result} = (RH_x/RH_S) \times 100$$

RH_x = RH% of peak 8 or 9 for the *Standard solution*

RH_S = RH% of peak 8 or 9 for the *Sample solution*

Acceptance criteria: The chromatographic profile of the initial *Sample solution* injection is similar to that of the *Standard solution*. The absolute difference between the retention times of peaks 5, 8, and 9 in the *Sample solution* must be within 1.2 min when compared to the initial *Standard solution* injection. The RRH% for each peak 8 and peak 9 in the *Sample solution* must be within 94%–106% when compared to the initial *Standard solution*. There must be no new peaks greater than peak 6 in height in the *Sample solution* that are not present in the *Standard solution* injection.

ASSAY

• [ERYTHROPOIETIN BIOASSAYS \(124\)](#)

Acceptance criteria: Meets the requirements. It has a potency of NLT 140,000 and NMT 200,000 IU/AU at 280 nm.

IMPURITIES

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Mobile phase: 20 mM sodium citrate and 100 mM sodium chloride, pH 6.9, prepared as follows. Dissolve 5.80 g of sodium citrate dihydrate, 0.057 g of citric acid, and 5.84 g of sodium chloride in 800 mL of water and mix. Adjust with hydrochloric acid to a pH of 6.9 and dilute with water to 1 L. Pass through a nylon filter of 0.2-µm pore size.

System suitability solution: 2 mg/mL of [USP Erythropoietin RS](#) in *Mobile phase*. Heat at 80° for 30 min, and use immediately.

Sample solution: 2 mg/mL of Epoetin

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 230 nm

Column: 7.8-mm × 30-cm; packing L20

Temperatures

Autosampler: 2°–8°