DRAFT REVISIONS FOR COMMENTS

These draft amendment contains revised text of monographs for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in/ biologics-ipc@gov.in **before the last date for comments.**

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

Erythropoietin Injection. Page 4597

Identification
B. Determine by polyacrylamide gel electrophoresis (SDS-PAGE) followed by Immunoblotting (2.2.14)
Change **from:**

Test solution. Concentrate or dilute, if necessary, the injection under examination to give a solution containing 2000 IU per ml in water and then add 1 volume of SDS-PAGE sample buffer (concentrated) or 100-150 ng.

Reference solution (a). Dissolve the contents of a vial of *erythropoietin IPRS* in water to give a solution containing 3012 IU per ml and then add 1 volume of SDS-PAGE sample buffer (concentrated).

to:

Test solution. Concentrate or dilute, if necessary, the injection under examination to give a solution containing 20 IU per ml or 200ng (loading concentration) in water and then add 1 volume of SDS-PAGE sample buffer (concentrated)

Reference solution (a). Dissolve the contents of a vial of *erythropoietin IPRS* in water to give a solution containing 20 IU per ml or 200ng (loading concentration) and then add 1 volume of SDS-PAGE sample buffer (concentrated).

Follicle Stimulating Hormone Injection. Page 4623

Tests

Free Subunits. Determine by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12) under non-reducing conditions.

Change **from:**

Gel Dimensions. 1.5 mm thick……..In the electropherogram obtained, the content of *free subunits* is not more than 3 per cent.

to:

Gel Dimensions. 1.5 mm thick.

Resolving gel. 15 per cent acrylamide.

Sample buffer. Concentrated (5X) SDS-PAGE sample buffer.

Test solution. Dissolve the preparation under examination in water to obtain a concentration of 4.4 µg. To 90 µl of the solution add 20 µl of the sample buffer. Allow to stand for 4 hours at room temperature.

Reference solution. Dilute the reference preparation, for example if concentration of 0.61mg per ml is used, then the reference preparation is as follows:

Preparation of 1:10 dilution of reference solution. Add 10 µl of reference solution to 90 µl of *water* to make up volume to 100 µl.

Preparation of 1:100 dilution of reference solution. Take 10 µl of 1:10 dilution of reference solution to 90 µl of *water* to make up volume to 100 µl.

Reference solutions	Loading Amount (µg)	Volume to be taken (µl)	Water (µl)	Volume of 5X Sample Buffer (µl)	Total Volume (µl)
Reference solution (a). (l00 per cent)	2.20	3.6	48.4	13	65
Reference solution (b). 0.5 per cent of 4.4µg from 1:100 v/v dilution of reference solution (a)	0.02	3.3	48.7	13	65
Reference solution (c).1 per cent of 4.4µg from 1:100 dilution of reference solution (a)	0.04	6.6	45.4	13	65
Reference solution (d). 2.5 per cent of 4.4µg from 1:100 dilution of reference solution (a)	0.11	18.0	34.0	13	65
Reference solution (e): (5 per cent of 4.4µg from 1:10 dilution of reference solution (a)	0.22	3.6	48.4	13	65
Reference solution (f): 10 per cent of 4.4µg from 1:10 dilution of reference solution (a)	0.44	7.2	44.8	13	65

Sample treatment. Test solution, Reference solution (a), Reference solution (b), Reference solution (c), Reference solution (d), reference solution (e) and reference solution (f) are allowed to stand for 4 hours at room temperature then boil the Reference solution (d), Reference solution (e) and Reference solution (f) for 5 minutes to generate dissociated subunits.

Molecular weight marker. Take molecular weight marker with suitable range of 10 to 260 kDa.

Application. Load molecular weight marker, *Reference solution (a), Reference solution (b), Reference solution (c), Reference solution (d), Reference solution (e), Reference solution (f)*, test solution in sequence on a 15 per cent or equivalent and analyze using a suitable SDS-PAGE apparatus.

Detection. By silver staining.

The test is not valid unless (i) the last molecular weight marker band must be migrated to 75 per cent length of the gel, (ii) major bands of the molecular weight marker proteins should be visible in the gel, (iii) molecular weight

markers are resolved on the gel into discrete bands with a linear relationship between distance migrated and logarithm10 of the molecular mass, (iv) The graph obtained by plotting the $\log_{10}$ of molecular weight of 260 kDa to l0 kDa protein band in the marker *vs* respective migration distance is linear with r^2 0.90.

In the electropherogram obtained with the test solution, the content of *free subunits* is not more than 3 per cent.

Oxidised follitropin. Determine the oxidised follitropin content by chromatographic separation using Liquid chromatography (2.4.14)

Change **from:**

Solution (a). Dissolve about……The total oxidized forms are not more than 6 per cent.

to:

Diluent. Mix 100g of *saline* solution with 100mg *Tween 20* w/w in 10 ml then volume make up to 100ml with *saline*.

*Test solution***.** Use the Drug Product as such

Reference solution (a). Prepare 0.05 mg per ml solution using diluent.

Reference solution (b). Dilute 0.1 ml of strong *hydrogen peroxide* solution to 30 ml with *water*. Dissolve the contents of a vial of *follitropin IPRS* in this solution to obtain a concentration of 0.05 mg per ml. Incubate for 30 to 45 minutes. Add 1.5 mg of *L-methionine* to stop the reaction and inject the solution immediately.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm packed with butylsilyl silica gel (5 µm)
- column temperature: 30º
- mobile phase A: A. 0.2 M *phosphate buffer* solution adjusted to pH 2.5,
  B. 50 volumes of 0.2M *phosphate buffer*, pH2.5; 10 volumes of *water*; 40 volumes of *acetonitrile*
- flow rate: 1ml per minute
- a gradient programme using the conditions given below,
- spectrophotometer set at 210 nm
- sampler temperature: 4°
- injection volume: 100µl
- run time: 35 min

Time (in minute)	Mobile phase A per cent (v/v)	Mobile phase B per cent (v/v)
0	67	33
15.00	20	80
20.00	20	80
20.10	0	100
25.00	0	100
25.10	67	33
35.00	67	33

When the standard is tested in three times, Retention time of alpha and beta peaks is within ±0.5 minutes.

Inject reference solution (b). the test is not valid unless (i) the relative standard deviation for replicate injections is not more than per cent for follitropin-α and follitropin-β peaks separately in the chromatogram obtained with reference solution, (ii) the retention time of each of follitropin-α and follitropin-β peaks for replicate injections is within ±0.5 minutes.

Calculate the percentage of oxidation of the follitropin subunits using the following expression:

$$\frac{(A\alpha - ox) + (A\beta - ox)}{(A\alpha)+(A\alpha - ox)+(A\beta)+(A\beta - ox)} X100$$

Where, A_{α} : area for α-subunit;
$A_{\alpha\text{-}ox}$: amount of area for α-oxidation;
A_{β} : area for β-subunit;
$A_{\beta -ox}$: amount of area for β-oxidation

The total oxidized forms are not more than 6 per cent.

Rituximab. Page 4669

Tests

pH (2.4.24)

Change **from:**

pH (2.4.24). 6.3 to 6.7

to:

pH (2.4.24). 6.2 to 6.8

Rituximab Injection. Page 4676

Tests

pH (2.4.24)

Change **from:**

pH (2.4.24). 6.3 to 6.7

to:

pH (2.4.24). 6.2 to 6.8

Teriparatide. Page 4690

Identification

D. Determine by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12) under non-reducing conditions

Change **from:**

Gel dimensions. 1.5 mm thick……obtained with the reference solution (b).

to:

Gel dimensions. 1.5 mm thick.

Resolving gel. 20 per cent acrylamide.

Sample buffer. Concentrated (5X) sample buffer

Test solution. Dilute the preparation under examination with water to obtain a concentration of 1 mg per ml. Add20 µl of sample buffer to 80 µl of test solution. Incubate at room temperature for 10 minutes. Load 2.5 µl of sample into well which is equivalent to ~2 µg concentration.

Reference solution. Prepare in same manner as test solution.

Molecular weight marker. Take molecular weight marker with suitable range of 4 kDa to 200 kDa.

Load molecular weight marker, test and reference solutionin sequence on a 20 per cent *TRIS-glycine* gel or equivalent and analyze using a suitable SDS-PAGE apparatus.

Detection. By silver staining

All of the bands of the molecular weight standard must be detected and clearly separated. The lowest molecular weight marker of 4kDa band should run at least 80 per cent of the length of the gel.
The electropherogram obtained with the test solution shows a single broad band corresponding in position and intensity to the single broad band obtained with the reference solution.

Teriparatide Injection. Page 4699
Identification
C. Determine by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12) under non-reducing conditions

Change **from:**

Gel dimensions. 1.5 mm thick……obtained with the reference solution (b).

to:

Gel dimensions. 1.5 mm thick.

Resolving gel. 20 per cent acrylamide.

Sample buffer. Concentrated (5X) sample buffer
Test solution. Dilute the preparation under examination with water to obtain a concentration of 1 mg per ml. Add 20 µl of sample buffer to 80 µl of test solution. Incubate at room temperature for 10 minutes. Load 2.5 µl of sample into well which is equivalent to ~2 µg concentration.

Reference solution. Prepare in same manner as test solution.

Molecular weight marker. Take molecular weight marker with suitable range of 4 kDa to 200 kDa.

Load molecular weight marker, test and reference solution in sequence on a 20 per cent *TRIS-glycine* gel or equivalent and analyze using a suitable SDS-PAGE apparatus.

Detection. By silver staining

All of the bands of the molecular weight standard must be detected and clearly separated. The lowest molecular weight marker of 4 kDa band should run at least 80 per cent of the length of the gel.
The electropherogram obtained with the test solution shows a single broad band corresponding in position and intensity to the single broad band obtained with the reference solution.