Oxytocin

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Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Manufacturers are also invited to submit samples of their products to the IPC to ensure that the proposed revised monograph adequately controls the quality of the product(s) they manufacture. Comments and samples 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, with a copy to Dr. Gaurav Pratap Singh (email: gpsingh.ipc@gov.in) before the last date for comments.

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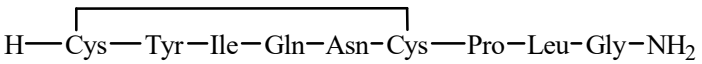
Oxytocin. Page 3167
Change **to**: **Oxytocin**



$C_{43}H_{66}N_{12}O_{12}S_2$ Mol. Wt.1007.2

Oxytocin is $S^{3.1}$,$S^{3.6'}$:$S^{3.6}$-Cyclo(L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide)].

Synthetic cyclic nonapeptide having the structure of the hormone produced by the posterior lobe of the pituitary gland that stimulates contraction of the uterus and milk ejection in receptive mammals. It is available in the freeze-dried form as an acetate.

Oxytocin contains not less than 93.0 per cent and not more than 102.0 per cent of oxytocin, $C_{43}H_{66}N_{12}O_{12}S_2$, calculated on the anhydrous and acetic acid-free basis.

Category. Uterine stimulant

Description. A white or almost white, hygroscopic powder.

Identification

Test B may be omitted if tests A and C are carried out. Test C may be omitted if tests A and B are carried out.

A. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with the reference solution.

B. Amino acid analysis (2.2.19). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids, taking 1/6 of the sum of the number of moles of aspartic acid, glutamic acid, proline, glycine, isoleucine and leucine as equal to 1. The values fall within the following limits: aspartic acid: 0.90 to 1.10; glutamic acid: 0.90 to 1.10; proline: 0.90 to 1.10; glycine: 0.90 to 1.10; leucine: 0.90 to 1.10; isoleucine: 0.90 to 1.10; tyrosine: 0.7 to 1.05; half-cystine: 1.4 to 2.1. Not more than traces of other amino acids are present.

C. Determine by Nuclear Magnetic Resonance Spectrometry (2.4.34).

NOTE—Concentrations of Oxytocin in both the reference solution and the test solution must be the same (within 5 per cent of each other) but can be adjusted based on the quality of the spectrum obtained. The spectra must be acquired under the same conditions for both the reference solution and the test solution. The spectra obtained are of sufficient quality to allow quantification of the integrals of the resonances specified below to be obtained. Integrals and spectra of both the reference solution and the test solution can be repeated and averaged.

Buffer solution. Dissolve 27.6 g of *sodium dihydrogen phosphate* in 900 ml of *water*, adjusted to pH 5.0 with *orthophosphoric acid* or *10 M sodium hydroxide* and dilute to 1000 ml with *water* and mix.

Test solution. A 1.0 per cent w/v solution of the substance under examination in the buffer solution. Proceed as directed under reference solution.

Reference solution. A 1.0 per cent w/v solution of *oxytocin IPRS* in the buffer solution. Lyophilize 1 ml of the solution to dryness, redissolve in *deuterium oxide*, lyophilize again, redissolve in *deuterium oxide*, and lyophilize once again (to replace exchangeable hydrogens with deuterium). Dissolve in 1 ml of *deuterium oxide* containing 0.5 per cent v/v solution of *(2,2,3,3-(d4)-3-(trimethylsilyl) propionic acid sodium salt* (TSP) as a chemical shift reference.

Examine the H NMR spectrum from 0 to 8 ppm. The H NMR spectrum obtained is qualitatively similar to the H NMR spectrum obtained with oxytocin IPRS.

Tests

pH (2.4.24) 3.0 to 6.0, determined on 2.0 per cent w/v solution in *carbon dioxide-free water*.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 12.5 mg of the substance under examination in mobile phase A and dilute to 50.0 ml with mobile phase A.

Reference solution (a). Dissolve the contents of a vial of *oxytocin for peak identification IPRS* (containing impurities B, D, E and I) in 1.0 ml of mobile phase A.

Reference solution (b). Dissolve the contents of a vial of *oxytocin impurity F IPRS* in 1.0 ml of mobile phase A.

Reference solution (c). Dissolve the contents of one vial of *oxytocin IPRS* in mobile phase A to obtain a solution having similar concentration as that of the test solution.

Chromatographic system

- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3 µm),
- mobile phase: A. a 1.56 per cent w/v solution of *sodium dihydrogen phosphate* in *water*,
  B. a mixture of equal volumes of *acetonitrile* and *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 50 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	70	30
30	40	60
30.1	70	30
45	70	30

Name	Relative retention time
Oxytocin impurity I[1]	0.9
Oxytocin impurity D[2]	0.95
Oxytocin (Retention time: about 10 minutes)	1.0
Oxytocin impurity E[3]	1.4
Oxytocin impurity F[4]	1.4
Oxytocin impurity B[5]	1.7

[1](5-aspartic acid)oxytocin,
[2](3-leucine)oxytocin,
[3]$N^{2.1}$-acetyloxytocin,
[4][5-(3-cyanoalanine)]oxytocin,
[5]$S^{3.1}$,$S^{3.6'}$:$S^{3.6}$,$S^{3.1'}$-cyclo[bis(L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide)] (oxytocin antiparallel dimer).

Inject reference solution (a) and (b) to identify the peaks due to oxytocin impurity B, D, E, I and oxytocin impurity F, respectively.

Inject reference solution (a). The test is not valid unless the peak-to-valley ratio is minimum 2.0, where H_p is height above the baseline of the peak due to impurity D and H_V is the height above the baseline of the lowest point of the curve separating this peak from the peak due to oxytocin.

Inject the test solution. The area of any peak due to oxytocin impurity B and oxytocin impurity I, each of, is not more than 1.0 per cent, the sum of the areas of oxytocin impurity E and F is not more than 1.5 per cent, the area of any other secondary peak is not more than 1.0 per cent and the sum of the areas of all the secondary peaks is not more than 5.0 per cent, calculated by area normalization. Ignore any peak with an area less than 0.1 per cent.

Acetic acid content. 6.0 per cent to 10.0 per cent.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 15.0 mg of the substance under examination in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Reference solution. A 0.01 per cent w/v solution of *glacial acetic acid* in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. dilute 0.7 ml of *orthophosphoric acid* to 1000 ml with *water*; adjusted to pH 3.0 with *5 M sodium hydroxide*,
  B. *methanol*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
5	95	5
10	50	50
20	50	50
22	95	5
30	95	5

The retention time of acetic acid is about 4 minutes.

Note- The baseline presents a steep rise after the start of the linear gradient, which corresponds to the elution of the peptide from the column.

Inject the reference solution and the test solution.

Calculate the content of acetic acid.

Water (2.3.43). Not more than 5.0 per cent, determined on 50 mg.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

- injection volume: 25 µl.

Inject reference solution (c) and the test solution.

Calculate the content of $C_{43}H_{66}N_{12}O_{12}S_2$.

Oxytocin intended for use in the manufacture of parenteral preparations without a further procedure for the removal of bacterial endotoxins complies with the following additional requirement.

Bacterial endotoxins (2.2.3). Not more than 0.5 Endotoxin Unit per unit of oxytocin.

Oxytocin intended for use in the manufacture of parenteral preparations without a further sterilisation procedure complies with the following additional requirement.

Sterility (2.2.11). Complies with the test for sterility.

Storage. Store protected from light and moisture, at a temperature between 2° to 8°. If the substance is sterile, store in sterile airtight, tamper-proof container.

Labelling. The label states (1) the oxytocic activity in terms of number of oxytocin units (IU) per mg; (2) whether or not the contents are intended for use in the manufacture of parenteral preparations.

2.4.26. Solubility.

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Change **to**: **Oxytocin**. Very soluble in *water*. It dissolves in dilute solutions of *acetic acid* and of *ethanol (95 per cent)*.