Oxytocin Injection

General Notices

Details for the public consultation of this monograph are as follows:

<table>
<thead>
<tr>
<th>EAG BIO</th>
<th>Biological and Biotechnological Products</th>
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</table>
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| Notes: | Revised monograph  
The Related substances and Assay methods have been updated |

Action and use

Oxytocic.

DEFINITION

Oxytocin Injection is a sterile solution of Oxytocin or a sterile dilution of Oxytocin Concentrated Solution in Water for Injections.

*The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.*

Content of oxytocin, C₄₃H₆₆N₁₂O₁₂S₂

90.0 to 110.0% of the stated amount of the peptide.

CHARACTERISTICS

A clear, colourless liquid.

IDENTIFICATION

A. Carry out the method for thin-layer chromatography, Appendix III A, using a high performance silica gel G precoated plate (Merck 5631 plates are suitable) and a mixture of 1 volume of glacial acetic acid, 6 volumes of water, 30 volumes of methanol and 70 volumes of dichloromethane as the mobile phase but allowing the solvent front to ascend 8 cm above the line of application. Prepare a solution containing 17 µg per mL of oxytocin EP CRS by dissolving the contents of one vial in the requisite volume of a 1.56% w/v solution of sodium dihydrogen
orthophosphate (solution A). For solution (1) evaporate 5 mL of the injection on a rotary evaporator at 30° and
dissolve the residue in 0.5 mL of water.

For injections containing 10 IU per mL prepare solution (2) by evaporating 5 mL of solution A on a rotary evaporator
at 30° and dissolving the residue in 0.5 mL of water. Apply separately to the plate 1 µL of solutions (1) and (2), dry in
a stream of cold air and develop immediately.

For injections containing 5 IU per mL prepare solution (2) by evaporating 2.5 mL of solution A on a rotary evaporator
at 30° and dissolving the residue in 0.5 mL of water. Apply separately to the plate 2 µL of solutions (1) and (2), dry in
a stream of cold air and develop immediately.

For injections containing 1 IU per mL use solution A as solution (2). Apply separately to the plate 10 µL of solutions
(1) and (2), dry in a stream of cold air and develop immediately.

After removal of the plate, allow it to dry in a stream of cold air for 10 minutes and develop again in a tank,
previously equilibrated for 30 minutes with fresh mobile phase. After removal of the plate, allow it to dry in a stream
of warm air for 10 minutes, cool and spray with phosphomolybdotungstic reagent until the plate appears translucent.
Immediately place the plate in a tank, previously equilibrated for 30 minutes with 13.5M ammonia, for 5 minutes,
remove and dry in a stream of warm air. The principal spot in the chromatogram obtained with solution (1)
corresponds in position, size and intensity to that in the chromatogram obtained with solution (2).

B. In the Assay, the chromatogram obtained with solution (1) exhibits a peak with the same retention time as the
principal peak in the chromatogram obtained with solution (2).

TESTS

Acidity

pH, 3.5 to 4.5, Appendix V L.

Related substances

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

(1) Dilute a volume of the injection, if necessary, with sufficient water to produce a solution containing the
equivalent of 5 IU per mL of Oxytocin.
(2) Dilute 1 volume of solution (1) to 50 volumes with water.
(3) Dissolve 16 mg of oxytocin/desmopressin validation mixture EPCRS in 10 mL of water.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 4.6 mm) packed with end-capped octadecysilyl silica gel for
chromatography (5 µm) (Waters Symmetry C18 is suitable is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1.0 mL per minute.
(d) Use a column temperature of 25°.
(e) Use a detection wavelength of 220 nm.
(f) Inject 200 µL of each solution.

MOBILE PHASE
**Mobile phase A** 1.56% w/v of sodium dihydrogen orthophosphate in water, filtered through a membrane filter with a pore size of 0.22 µm.

**Mobile phase B** 1 volume of acetonitrile and 1 volume of water.

<table>
<thead>
<tr>
<th>Time (Minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>70→40</td>
<td>30→60</td>
<td>linear gradient</td>
</tr>
<tr>
<td>30-30.1</td>
<td>40→70</td>
<td>60→30</td>
<td>linear gradient</td>
</tr>
<tr>
<td>30.1-45</td>
<td>70</td>
<td>30</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>

When the chromatograms are recorded under the prescribed conditions, the relative retention times with reference to oxytocin (retention time about 10 min) are: impurity 1, about 0.90; impurity 2, about 1.5; impurity 3, about 1.55; impurity 4, about 1.65.

**SYSTEM SUITABILITY**

The test is not valid unless, in the chromatogram obtained with solution (3), the resolution between the peaks due to oxytocin and desmopressin is at least 5.0.

**LIMITS**

In the chromatogram obtained with solution (1):

the area of any secondary peak is not greater than 0.75 times the area of the principal peak in the chromatogram obtained with solution (2) (1.5%);

the sum of the areas of any secondary peaks is not greater than 2.5 times the principal peak in the chromatogram obtained with solution (2) (5%).

Disregard any peak with an area less than 0.15 times the area of the principal peak in the chromatogram obtained with solution (2) (0.3%).

**ASSAY**

Carry out the method for liquid chromatography, Appendix III D, using the following solutions.

1. Dilute a volume of the injection, if necessary, with sufficient water to produce a solution containing the equivalent of 5 IU per mL of Oxytocin.
2. 0.0008% w/v of oxytocin EPCRS in water.
3. Dissolve 16 mg of oxytocin/desmopressin validation mixture EPCRS in 10 mL of water.

**CHROMATOGRAPHIC CONDITIONS**

The chromatographic conditions described under Related substances may be used with an injection volume of 20 µL.

**DETERMINATION OF CONTENT**

Calculate the content of oxytocin, C43H66N12 O12S2, in the injection from the chromatograms obtained and using the declared content of C43H66N12O12S2, in oxytocin EPCRS.
LABELLING

The strength is stated as the number of IU (Units) per mL. The label also states the equivalent number of micrograms of oxytocin per mL.

IMPURITIES

The impurities limited by the requirements of this monograph include:

1. Carbamidooxytocin
2. α-Oxytocin dimer
3. Acetyloxytocin
4. β-Oxytocin dimer