Oxytocin
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-GlyNH₂

C₄₅H₆₆N₁₂O₁₂S₂ Mol. Wt.1007.2

Oxytocin is a cyclic nonapeptide hormone obtained by a process of fractionation from the posterior lobe of the pituitary gland of healthy oxen or other mammals or by synthesis that has the property of stimulating contraction of the uterus and milk ejection in receptive animals. It may be presented as a solid or as a solution in a solvent containing an appropriate antimicrobial preservative such as 0.2 per cent w/v solution of chlorbutol.

If it is derived from animal species, Oxytocin contains not less than 95.0 per cent and not more than 105.0 per cent of the stated number of Units of oxytocic activity. If it is a synthetic product presented as a solid, it contains not less than 560 Units per mg, calculated with reference to the peptide content and when presented as a liquid, it contains not less than 150 Units per ml.

Description. When presented as a solid, a white or almost white powder. When presented as a liquid, a clear colourless liquid.

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.

For hydrolysis:
Acid hydrolysis using hydrochloric acid containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

Hydrolysis solution. 6 M hydrochloric acid containing 0.1 per cent to 1.0 per cent of phenol.

Procedure

Liquid phase hydrolysis. Place the protein or peptide sample in a hydrolysis tube, and dry (the sample is dried so that water in the sample will not dilute the acid used for the hydrolysis). Add 200 µl of hydrolysis solution per 500 µg of lyophilised protein. Freeze the sample tube in a dry ice-acetone bath, and flame seal in vacuum. Samples are typically hydrolysed at 110° for 24 hours in vacuum or in an inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g. 48 hours and 72 hours) are investigated if there is a concern that the protein is not completely hydrolysed.

Vapour phase hydrolysis. This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available. Contamination of the sample from the acid reagent is also minimised by using vapour phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of hydrolysis solution. The hydrolysis solution does not come in contact with the test sample. Apply an inert atmosphere or vacuum (less than 200 µm of mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110° for a 24 hours hydrolysis time. Acid vapour hydrolys the dried sample. Any condensation of the acid in the sample vials is to be minimised. After hydrolysis, dry the test sample in vacuum to remove any residual acid.

For analysis:
Post-column ninhydrin derivatisation  
Ion-exchange chromatography with post-column ninhydrin derivatisation is one of the most common methods employed for quantitative amino acid analysis. As a rule, a lithium-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster sodium-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has a characteristic purple or yellow colour. Amino acids, except imino acid, give a purple colour, and show an absorption maximum at 570 nm. The imino acids such as proline give a yellow colour, and show an absorption maximum at 440 nm. The post-column reaction between ninhydrin and amino acids eluted from the column is monitored at 440 nm and 570 nm, and the chromatogram obtained is used for the determination of amino acid composition.

The detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for the proline derivative. Response linearity is obtained in the range of 20-500 pmol with correlation coefficients exceeding 0.999. To obtain good composition data, samples larger than 1 µg before hydrolysis are best suited for this amino acid analysis of protein/peptide.

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. For biological response, dissect out the uterus from a virgin female rat weighing between 120 to 200 g and in diestrus stage. Immediately before injection confirm the uterine stage of the rat by vaginal smear. Suspend one horn of the uterus in a organ bath containing 9.0 g of sodium chloride, 0.42 g of potassium chloride, 0.16 g of calcium chloride, 0.50 g of sodium bicarbonate, 0.25 g of dextrose, and 0.0053 g of magnesium chloride per litre of the solution. Maintain the bath temperature at 32º or any other suitable temperature at which spontaneous contractions of the uterus are abolished and the preparations maintain its sensitivity. Oxygenate the bath solution with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. Record the contractions of the uterine muscle on a recorder, using a suitable instrument producing linear response (for example an isotonic liver with a load of not exceeding 2 g or isotonic and linear transducer). Add to the bath two appropriate dilutions of the oxytocin reference solution, and record the contraction of the muscle following each dilution. The appropriate dilutions (doses) are those dilutions of the reference solution which produce clearly distinctive submaximal contractions. The required dilutions normally lies between 0.1 to 5 micro units per ml of the bath solution. When maximal contraction is reached, replace the bath solution by a fresh solution and wait until the muscle is relaxed completely and the pointer of the recorder returns to the base line. The doses of the different reference solutions should be added at regular intervals depending upon the rate of the recovery of the uterine muscle. Dissolve or dilute the preparation to be tested in a suitable diluent (preferably using bath solution) to obtain responses on the addition of two dilutions similar to the one used with the oxytocin reference solution. The two selected dilutions of the reference solution and preparation under examination should be applied according to a randomised block or Latin square design and at least three responses to each dilution should be recorded. The magnitude of contractions obtained with the reference solution is comparable to the contractions obtained with the test solution.

Tests

Peptide. 90.0 to 110.0 per cent of the stated amount of oxytocin, C_{43}H_{66}N_{12}O_{12}S_{2} expressed per mg for the solid, and in mg per ml for the liquid,

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 3.5 mg of the substance under examination in sufficient of a 1.56 per cent w/v solution of sodium dihydrogen phosphate to produce 10.0 ml or use the liquid preparation as appropriate.
Reference solution. Dissolve 3.5 mg of oxytocin RS in sufficient of a 1.56 per cent w/v solution of sodium dihydrogen phosphate to produce 10.0 ml.

Chromatographic system
- a stainless steel column 12 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 μm),
- mobile phase: appropriate proportions of a 1.56 per cent w/v solution of sodium dihydrogen phosphate (mobile phase A) and a mixture of equal volumes of acetonitrile and water (mobile phase B),
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 μl

Equilibrate the column with a mixture of 70 volumes of mobile phase A and 30 volumes of mobile phase B and record the chromatograms as follows. Operate by gradient elution increasing continuously and linearly the proportion of mobile phase B by 1.0 per cent v/v per minute for 30 minutes. Finally elute using the same mixture for 15 minutes to re-equilibrate the column.

Calculate the content of the peptide, C₄₃H₆₆N₁₂O₁₂S₂.

Assay. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve about 25 mg of the substance under examination in 100 ml of mobile phase A.

Reference solution. A 0.025 per cent w/v solution of oxytocin RS in mobile phase A.

Chromatographic system
- a stainless steel column 12.5 cm x 4.6 mm, packed with octadecysilane bonded to porous silica (5 μm),
- mobile phase: A. a 1.6 per cent w/v solution of sodium dihydrogen phosphate,
  B. a mixture of 50 volumes of acetonitrile and 50 volumes of water,
- a linear gradient programme using the conditions given below,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 25 μl.

<table>
<thead>
<tr>
<th>Time (in min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
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</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>70 - 40</td>
<td>30 - 60</td>
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<tr>
<td>30 – 30.1</td>
<td>40 - 70</td>
<td>60 – 30</td>
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<td>30.1 – 45</td>
<td>70</td>
<td>30</td>
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Inject the test solution and the reference solution.

Calculate the content of C₄₃H₆₆N₁₂O₁₂S₂.

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). Less than 0.5 Endotoxin Units 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 moisture. If the substance is intended for use in the manufacture of parenteral preparations, the container should be sterile, tamper-evident and sealed so as to exclude micro-organisms.

Labelling. The label states (1) the number of Units of oxytocic activity per mg (for solid) or per ml (for liquid); (2) either the animal species from which it is obtained or whether it is synthetic, as appropriate; (3) whether or not the contents are intended for use in the manufacture of parenteral preparations.
**Oxytocin Injection**

Oxytocin Injection is a sterile solution of Oxytocin in Water for Injections.

Oxytocin Injection contains not less than 90.0 per cent and not more than 110.0 per cent of the stated number of Units of oxytocin activity.

**Description.** A clear, colourless liquid.

**Identification**

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.

**Tests**

**pH** (2.4.24). 3.0 to 5.0

**Other Tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Bacterial endotoxins** (2.2.3). Less than 0.5 Endotoxin Units per Unit of oxytocin.

**Assay.** Determine by liquid chromatography (2.4.14).

**Test solution.** Use the injection under examination.

**Reference solution.** Dissolve the contents of one vial of oxytocin RS in a 1.65 per cent w/v solution of sodium dihydrogen orthophosphate to produce a solution containing the same concentration in µg of oxytocin as that stated on the label of the injection.

**Chromatographic system**

- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature. 40°C,
- mobile phase: a mixture of 85 volumes of a 0.2 per cent v/v solution of orthophosphoric acid and 15 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 50,000.

Inject the test solution and the reference solution.

Calculate the content of C₄₃H₆₆N₁₂O₁₂S₂ in the injection.

*Oxytocin Injection containing Oxytocin of natural origin obtained by extraction and purification complies with the following additional requirement.*

**Vasopressor activity.** Not more than 0.5 Unit per 20 Units of oxytocic activity, when assayed by the biological assay for vasopressor activity described below.

The vasopressor activity is estimated by comparing the activity of the preparation under examination with that of the Standard Preparation of arginine vasopressin under the conditions of a suitable method of assay.

**Standard Preparation**

The Standard Preparation is the 1st International Standard for Arginine vasopressin, established in 1978, consisting of freeze-dried synthetic arginine vasopressin peptide acetate with human albumin and citric acid (supplied in ampoules containing 8.20 Units), or any other suitable preparation the potency of which has been determined in relation to that of the International Standard.
Inject slowly into the tail vein of a male albino rat weighing about 300 g a solution of a suitable a-adrenoceptor blocking agent, for example 10 ml per kg of body weight of a solution prepared by dissolving 5 mg of phenoxybenzamine hydrochloride in 0.1 ml of ethanol (95 per cent), adding 0.05 ml of 1 M hydrochloric acid and diluting to 5 ml with saline solution. After 18 hours, anaesthetise the rat with an anaesthetic that will maintain a prolonged and uniform blood pressure. After 45 to 60 minutes, tie the rat on its back to the operating table by its hind legs. Cannulate the trachea with a short polyethylene tube of external diameter about 2.5 mm and dissect a carotid artery ready for cannulation. Then cannulate the femoral vein close to the inguinal ligament. Retract the abdominal muscles to expose the inguinal ligament. Cannulate the femoral vein to one side and dissect the femoral vein towards the inguinal ligament from the corresponding artery. When dissecting, a deep branch reaching the femoral vein must be found and tied off to prevent bleeding during cannulation. Tie a short polyethylene cannula of external diameter about 1 mm into the femoral vein by two ligatures and join by a short piece of flexible tubing to a 1-ml burette with an attached thistle funnel containing saline solution at about 37°. Firmly fix a wet absorbent cotton swab to the thigh so as to cover the incision and cannula. At this stage inject through the venous cannula 200 Units of heparin, dissolved in saline solution, per 100 g of body weight. Then tie in a carotid cannula of external diameter about 1 mm and connect by a column of saline solution containing heparin with a suitable pressure measuring device such as a mercury manometer of internal diameter about 2 to 3 mm.

The central and peripheral nervous system including both vagus and associated sympathetic nerves is left intact. No artificial respiration is necessary. Taking care that no air is injected, inject all solutions through the venous cannula by means of a 1-ml syringe graduated in 0.01 ml and wash in with 0.2 ml of saline solution from the burette.

Dilute the extract of the Standard Preparation and the preparation under examination with saline solution so that the volume to be injected is between 0.1 ml and 0.5 ml.

Choose two doses of the Standard Preparation such that the elevation of the blood pressure is about 4 kPa for the lower dose and about 7 kPa but always submaximal for the higher dose, the ratio of low to high dose being determined by the response and usually being 3 to 5. As an initial approximation doses of 3 and 5 milliUnits may be tried. Choose two doses of the preparation under examination with the same inter-dose ratio, matching the effects of the dose of the Standard Preparation as closely as possible. Inject doses at intervals of 10 to 15 minutes.

The two doses of the Standard Preparation and the two doses of the preparation under examination should be given in a randomised block or a Latin square design and four to five responses to each should be recorded.

Measure all the responses and calculate the result of the assay by standard statistical methods.

Storage. Store at temperature not exceeding 8°. Do not freeze.

Labelling. The label states (1) the number of Units of oxytocin activity per ml; (2) either the animal species from which it is obtained or whether it is synthetic, as appropriate.

Oxytocin Nasal Solution

Oxytocin Nasal Solution is a solution of Oxytocin in a suitable solvent containing an appropriate antimicrobial preservative.

Oxytocin Nasal Solution contains not less than 85.0 per cent and not more than 120.0 per cent of the stated number of Units of oxytocic activity.

Description. A clear, colourless solution.

Identification

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.

Tests

pH (2.4.24). 3.0 to 5.0

Other tests. Complies with the tests stated under Nasal Preparations.

Assay. Determine by liquid chromatography (2.4.14).
**Test solution.** Use the nasal solution under examination.

**Reference solution.** Dissolve the contents of one vial of oxytocin **RS** in a 1.65 per cent w/v solution of sodium dihydrogen orthophosphate to produce a solution containing the same concentration in µg of oxytocin as that stated on the label of the nasal solution.

Chromatographic system
- a stainless steel column 10 cm x 4.6 mm, packed with endcapped octadecylsilane bonded to porous silica (5 µm) (such as Nucleosil C18),
- column temperature. 40°,
- mobile phase: a mixture of 85 volumes of a 0.2 per cent v/v solution of orthophosphoric acid and 15 volumes of acetonitrile,
- flow rate. 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume. 200 µl.

Inject the reference solution. The test is not valid unless the theoretical plates is not less than 50,000.

Inject the test solution and the reference solution.

Calculate the content of C_{43}H_{66}N_{12}O_{12}S_{2} in the nasal solution.

**Oxytocin Nasal Solution containing Oxytocin of natural origin obtained by extraction and purification complies with the following additional requirement.**

**Vasopressor activity.** Not more than 0.5 Unit per 20 Units of oxytocic activity, when assayed by the biological assay for vasopressor activity described below.

The vasopressor activity is estimated by comparing the activity of the preparation under examination with that of the Standard Preparation of arginine vasopressin under the conditions of a suitable method of assay.

**Standard Preparation**

The Standard Preparation is the 1st International Standard for Arginine vasopressin, established in 1978, consisting of freeze-dried synthetic arginine vasopressin peptide acetate with human albumin and citric acid (supplied in ampoules containing 8.20 Units), or any other suitable preparation the potency of which has been determined in relation to that of the International Standard.

Inject slowly into the tail vein of a male albino rat weighing about 300 g a solution of a suitable a-adrenoceptor blocking agent, for example 10 ml per kg of body weight of a solution prepared by dissolving 5 mg of phenoxybenzamine hydrochloride in 0.1 ml of ethanol (95 per cent), adding 0.05 ml of 1 M hydrochloric acid and diluting to 5 ml with saline solution. After 18 hours, anaesthetise the rat with an anaesthetic that will maintain a prolonged and uniform blood pressure. After 45 to 60 minutes, tie the rat on its back to the operating table by its hind legs. Cannulate the trachea with a short polyethylene tube of external diameter about 2.5 mm and dissect a carotid artery ready for cannulation. Then cannulate the femoral vein close to the inguinal ligament. Retract the abdominal muscles to expose the inguinal ligament. Retract the superficial pudendal vein to one side and dissect the femoral vein towards the inguinal ligament from the corresponding artery. When dissecting, a deep branch reaching the femoral vein must be found and tied off to prevent bleeding during cannulation. Tie a short polyethylene cannula of external diameter about 1 mm into the femoral vein by two ligatures and join by a short piece of flexible tubing to a 1-ml burette with an attached thistle funnel containing saline solution at about 37°. Firmly fix a wet absorbent cotton swab to the thigh so as to cover the incision and cannula. At this stage inject through the venous cannula 200 Units of heparin, dissolved in saline solution, per 100 g of body weight. Then tie in a carotid cannula of external diameter about 1 mm and connect by a column of saline solution containing heparin with a suitable pressure measuring device such as a mercury manometer of internal diameter about 2 to 3 mm.
The central and peripheral nervous system including both vagus and associated sympathetic nerves is left intact. No artificial respiration is necessary. Taking care that no air is injected, inject all solutions through the venous cannula by means of a 1-ml syringe graduated in 0.01 ml and wash in with 0.2 ml of saline solution from the burette.

Dilute the extract of the Standard Preparation and the preparation under examination with saline solution so that the volume to be injected is between 0.1 ml and 0.5 ml.

Choose two doses of the Standard Preparation such that the elevation of the blood pressure is about 4 kPa for the lower dose and about 7 kPa but always submaximal for the higher dose, the ratio of low to high dose being determined by the response and usually being 3 to 5. As an initial approximation doses of 3 and 5 milliUnits may be tried. Choose two doses of the preparation under examination with the same inter-dose ratio, matching the effects of the dose of the Standard Preparation as closely as possible. Inject doses at intervals of 10 to 15 minutes.

The two doses of the Standard Preparation and the two doses of the preparation under examination should be given in a randomised block or a Latin square design and four to five responses to each should be recorded.

Measure all the responses and calculate the result of the assay by standard statistical methods.

Storage. Store at a temperature not exceeding 30º.

Labelling. The label states (1) the number of Units of oxytocic activity per ml; (2) either the animal species from which it is obtained or whether it is synthetic, as appropriate; (3) that the preparation is intended for intranasal administration only.