



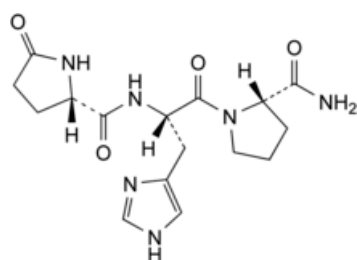
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Edition: BP 2025 (Ph. Eur. 11.6 update)

Protirelin

[General Notices](#)

(Ph. Eur. monograph 1144)



$C_{16}H_{22}N_6O_4$ 362.4 24305-27-9

Action and use

Thyrotrophin-releasing hormone.

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DEFINITION

5-Oxo-L-prolyl-L-histidyl-L-prolinamide.

Synthetic tripeptide with the same sequence of amino acids as the natural hypothalamic neurohormone, which stimulates the release and synthesis of thyrotropin.

Content

97.0 per cent to 102.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance

White or yellowish-white powder, hygroscopic.

Solubility

Very soluble in water, freely soluble in methanol.

IDENTIFICATION

A. Infrared absorption spectrophotometry ([2.2.24](#)).

Comparison [protirelin CRS](#).

B. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time and size to the principal peak in the chromatogram obtained with the reference solution.

TESTS

Appearance of solution

A 10 g/L solution is clear ([2.2.1](#)) and not more intensely coloured than reference solution Y₅ ([2.2.2, Method II](#)).

Specific optical rotation ([2.2.7](#))

-62 to -70 (anhydrous and acetic acid-free substance).

Dissolve 10 mg in 1.0 mL of [water R](#).

Related substances

Liquid chromatography ([2.2.29](#)).

Test solution Dissolve 5.0 mg of the substance to be examined in mobile phase A and dilute to 5.0 mL with mobile phase A.

Reference solution (a) Dissolve the contents of a vial of [D-His-protirelin CRS](#) in an appropriate volume of mobile phase A to obtain a concentration of 1 mg/mL. Mix equal volumes of this solution and the test solution.

Reference solution (b) Dilute 0.2 mL of the test solution to 10.0 mL with mobile phase A.

Column:

— size: $l = 0.25$ m, $\varnothing = 4.0$ mm,

— stationary phase: spherical [octadecylsilyl silica gel for chromatography R](#) (5 μ m) with a pore size of 12 nm.

Mobile phase:

— mobile phase A: a mixture of 100 mL of [acetonitrile for chromatography R](#), 1900 mL of [water R](#) and 2.0 g of [sodium octanesulfonate R](#), containing 2.5 mL/L of [tetraethylammonium hydroxide solution R](#); adjust to pH 3.5 with [phosphoric acid R](#),

— mobile phase B: a mixture of 300 mL of [acetonitrile for chromatography R](#), 1700 mL of [water R](#) and 2.0 g of [sodium octanesulfonate R](#), containing 2.5 mL/L of [tetraethylammonium hydroxide solution R](#); adjust to pH 3.5 with [phosphoric acid R](#),

Time (min)	Mobile phase A (per cent V/V)	Mobile phase B (per cent V/V)
0 - 30	74 → 41	26 → 59
30 - 35	41 → 74	59 → 26
35 - 50	74	26

Flow rate 1 mL/min.

Detection Spectrophotometer at 210 nm.

Injection 10 µL.

Relative retention With reference to protirelin (retention time = about 18 min): impurity C = about 0.2; impurity D = about 0.68; impurity A = about 0.91; impurity B = about 0.95; impurity E = about 1.08.

System suitability Reference solution (a):

- **resolution**: minimum 2.5 between the peaks due to impurity A and protirelin,
- **symmetry factor**: 0.9 to 1.2 for the peak due to protirelin.

Limits:

- **any impurity**: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent),
- **total**: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- **disregard limit**: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Acetic acid (2.5.34)

Maximum 2.0 per cent.

Test solution Dissolve 40.0 mg of the substance to be examined 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 solvents.

Water (2.5.12)

Maximum 7.0 per cent, determined on 0.200 g.

Bacterial endotoxins (2.6.14)

Less than 0.7 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

Liquid chromatography (2.2.29) as described in the test for related substances with the following modification.

Reference solution Dissolve the contents of a vial of *protirelin CRS* in an appropriate volume of mobile phase A to obtain a concentration of 1.0 mg/mL.

Calculate the content of protirelin ($C_{16}H_{22}N_6O_4$) using the peak areas of the chromatograms obtained with the test solution and the reference solution and the declared content of $C_{16}H_{22}N_6O_4$ in *protirelin CRS*.

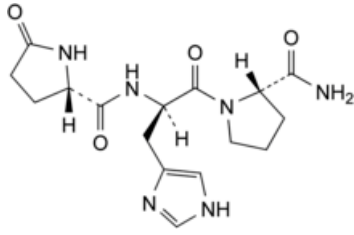
STORAGE

In an airtight container, protected from light at a temperature of 2 °C to 8 °C. If the substance is sterile, store in a sterile, airtight, tamper-evident container.

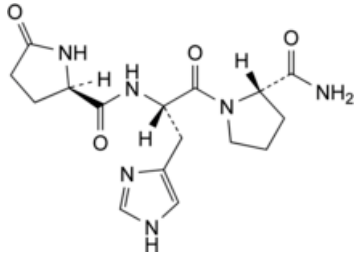
LABELLING

The label states the mass of peptide in the container.

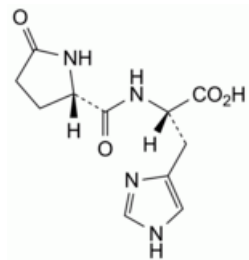
IMPURITIES



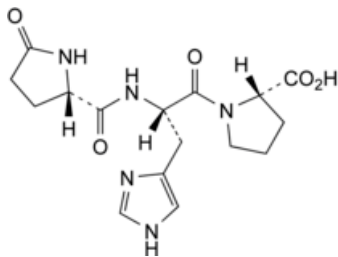
A. 5-oxo-L-prolyl-D-histidyl-L-prolinamide,



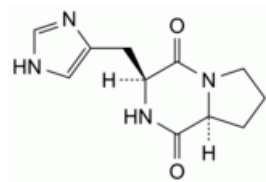
B. 5-oxo-D-prolyl-L-histidyl-L-prolinamide,



C. 5-oxo-L-prolyl-L-histidine,



D. 5-oxo-L-prolyl-L-histidyl-L-proline,



E. (3S,8aS)-3-(1H-imidazol-4-ylmethyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (cyclo(-L-histidyl-L-prolyl-)).

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