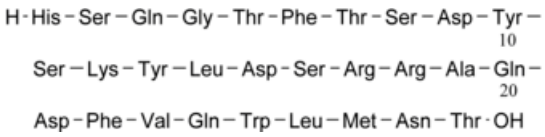
Edition: BP 2025 (Ph. Eur. 11.6 update)

Human Glucagon

General Notices

(Glucagon, Human, Ph. Eur. monograph 1635)



$C_{153}H_{225}N_{43}O_{49}S$ 3483

Action and use

Hormone; treatment of hypoglycaemia.

Preparation

Human Glucagon Injection

Ph Eur

DEFINITION

Polypeptide having the same structure (29 amino acids) as the hormone produced by the α-cells of the human pancreas, which increases the blood-glucose concentration by promoting rapid breakdown of liver glycogen.

Content

92.5 per cent to 105.0 per cent (anhydrous substance).

PRODUCTION

Human glucagon is produced by a method based on recombinant DNA (rDNA) technology. During the course of product development it must be demonstrated that the manufacturing process produces a product having a biological activity of not less than 1 IU/mg using a suitable validated bioassay.

Host-cell-derived proteins

The limit is approved by the competent authority.

Host-cell- and vector-derived DNA

The limit is approved by the competent authority.

CHARACTERS

Appearance

White or almost white powder.

Solubility

Practically insoluble in water and in most organic solvents. It is soluble in dilute mineral acids and in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Peptide mapping. Liquid chromatography (2.2.29).

Test solution Prepare a 5 mg/mL solution of the substance to be examined in *0.01 M hydrochloric acid*. Mix 200 µL of this solution with 800 µL of *0.1 M ammonium carbonate buffer solution pH 10.3 R* (diluted stock solution). Prepare a 2 mg/mL solution of *α-chymotrypsin for peptide mapping R* in *0.1 M ammonium carbonate buffer solution pH 10.3 R* and add 25 µL of this solution to the diluted stock solution. Place the solution in a closed vial at 37 °C for 2 h. Remove the vial and stop the reaction immediately by adding 120 µL of *glacial acetic acid R*.

Reference solution Prepare a 1 mg/mL solution of *human glucagon CRS* in *0.1 M ammonium carbonate buffer solution pH 10.3 R* (diluted stock solution) and continue as described for the test solution.

Column:

— *size*: *l* = 0.05 m, Ø = 4 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:

— *mobile phase A*: mix 500 µL of *trifluoroacetic acid R* and 1000 mL of *water R*;

— *mobile phase B*: mix 500 µL of *trifluoroacetic acid R* with 600 mL of *anhydrous ethanol R* and add 400 mL of *water R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 35	100 → 53	0 → 47
35 - 45	53 → 0	47 → 100
45 - 46	0 → 100	100 → 0
46 - 75	100	0

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Equilibration With mobile phase A for at least 15 min.

Injection 20 µL.

System suitability The chromatogram obtained with the reference solution is qualitatively similar to the chromatogram supplied with *human glucagon CRS*.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

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 to the principal peak in the chromatogram obtained with reference solution (a).

TESTS

Related proteins and deamidated forms

Liquid chromatography (2.2.29): use the normalisation procedure.

Test solution Dissolve the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of 0.5 mg/mL. *Maintain the solution at 2-8 °C.*

Reference solution (a) Dissolve the contents of a vial of *human glucagon CRS* in *0.01 M hydrochloric acid* to obtain a concentration of 0.5 mg/mL. *Maintain the solution at 2-8 °C.*

Reference solution (b) Dissolve the substance to be examined in *0.01 M hydrochloric acid* to obtain a concentration of about 0.5 mg/mL. Heat at 50 °C for 48 h (*in situ* preparation of all 4 deamidated forms of glucagon at a total concentration of not less than 7 per cent).

Column:

— *size*: *l* = 0.15 m, Ø = 3 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 45 °C.

Mobile phase:

— *mobile phase A*: dissolve 16.3 g of *potassium dihydrogen phosphate R* in 800 mL of *water R*, adjust to pH 2.7 with *phosphoric acid R* and add 200 mL of *acetonitrile for chromatography R*;

— *mobile phase B*: *acetonitrile for chromatography R*, *water R* (40:60 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 25	61	39
25 - 29	61 → 12	39 → 88
29 - 30	12	88
30 - 31	12 → 61	88 → 39

NOTE The end time of the isocratic elution may be adjusted so that the gradient begins after elution of the peak due to deamidated glucagon 4 (see relative retention below).

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 15 µL.

Relative retention With reference to glucagon (retention time = about 21 min): deamidated glucagon 1 = about 1.1; deamidated glucagon 4 = about 1.4.

System suitability:

— *resolution*: minimum 1.5 between the peaks due to glucagon and deamidated glucagon 1 in the chromatogram obtained with reference solution (b);

— *symmetry factor*: maximum 1.8 for the peak due to glucagon in the chromatogram obtained with reference solution (a);

— *repeatability*: maximum relative standard deviation of 2.0 per cent after 5 injections of reference solution (a);

— 4 peaks eluting after the principal peak, that correspond to the deamidated forms, are clearly visible in the chromatogram obtained with reference solution (b).

Limits:

— *deamidated forms*: maximum 0.8 per cent;

— *total*: maximum 3.0 per cent.

Water (*2.5.32*)

Maximum 10 per cent, determined on 50 mg.

Bacterial endotoxins (*2.6.14*)

Less than 10 IU/mg.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related proteins and deamidated forms with the following modification.

Injection Test solution and reference solution (a).

Calculate the percentage content of human glucagon ($C_{153}H_{225}N_{43}O_{49}S$) taking into account the assigned content of $C_{153}H_{225}N_{43}O_{49}S$ in *human glucagon CRS*.

STORAGE

In an airtight container, protected from light, at a temperature lower than -15 °C.

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