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

# Human Glucagon for Injection

General Notices

**Action and use**

Hormone; treatment of hyperglycaemia.

## DEFINITION

Human Glucagon for Injection is a sterile material consisting of freeze-dried Human Glucagon (produced by recombinant DNA technology) with or without excipients. It is supplied in a sealed container.

*The contents of the sealed container comply with the requirements for Powders for Injections stated under Parenteral Preparations and with the following requirements.*

**Content of glucagon, $C_{153}H_{225}N_{43}O_{49}S$**

75.0 to 104.0% of the stated amount.

## IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that of the principal peak in the chromatogram obtained with solution (2).

## TESTS

**Acidity**

pH of a 0.1% w/v solution, 2.5 to 3.5, Appendix V L.

**Related proteins and deamidated forms**

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the normalisation procedure.

(1) Dissolve the powder being examined in 0.01M *hydrochloric acid* to obtain the equivalent of 0.05% w/v of human glucagon. *Maintain the solution at 2° to 8°*.
(2) Dissolve the contents of a vial of *human glucagon EPCRS* in 0.01M *hydrochloric acid* to obtain a concentration of 0.05 % w/v. *Maintain the solution at 2° to 8°*.
(3) Dissolve the powder being examined in 0.01M *hydrochloric acid* to obtain a concentration of about 0.05% w/v human glucagon. Heat at 50° for 48 hours (generation of all 4 deamidated forms of glucagon at a total concentration of not less than 7%).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (15 cm × 3.0 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (ACE 3 C18 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 0.5 mL per minute.
(d) Use a column temperature of 45°.
(e) Use a detection wavelength of 214 nm.
(f) Inject 15 µL of each solution.

MOBILE PHASE

*Mobile phase A* 200 volumes of *acetonitrile for chromatography* and 800 volumes of the solution prepared as described below.

Dissolve 16.3 g of *potassium dihydrogen orthophosphate* in 800 mL of *water*, adjust to pH 2.7 with *orthophosphoric acid*.

*Mobile phase B* 400 volumes of *acetonitrile for chromatography* and 600 volumes of *water*.

Use the following gradient elution.

| Time (Minutes) | Mobile phase A (% v/v) | Mobile phase B (% v/v) | Comment |
|---|---|---|---|
| 0-25 | 61 | 39 | isocratic |
| 25-29 | 61→12 | 39→88 | linear gradient |
| 29-30 | 12 | 88 | isocratic |
| 30-31 | 12→61 | 88→39 | re-equilibration |

*Note*: The relative retention time with respect to glucagon (retention time about 21 minutes) of deamidated glucagon 1 is about 1.1. 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 (relative retention with reference to glucagon about 1.4).

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (3):

the *resolution factor* between the peak due to glucagon and the following peak due to deamidated glucagon 1 is at least 1.5;

the 4 peaks eluting after the principal peak, that correspond to the deamidated forms, are clearly visible;

and, in the chromatogram obtained with solution (2):

the *symmetry factor* of the peak due to glucagon is a maximum of 1.8;

the *repeatability* has a maximum relative standard deviation of 2.0% after 5 injections.

LIMITS

In the chromatogram obtained with solution (1):

the total area of all deamidated forms is not greater than 10.0%;

the total area of all *secondary peaks* is not greater than 15.0%.

## Water

Not more than 5.0%, Appendix IX C, Method III. Use the entire freeze dried contents of the sealed container (containing 1 mg of the powder).

## Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. Dissolve the contents of the sealed container in *water BET* to give a solution containing 1 mg of Human Glucagon per mL (solution A). The endotoxin limit concentration of solution A is

10 IU of endotoxin per mL.

## ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the method described under Related proteins and deamidated forms, injecting solutions (1) and (2).

DETERMINATION OF CONTENT

Calculate the content of human glucagon ($C_{153}H_{225}N_{43}O_{49}S$) from the chromatograms obtained and using the declared content of $C_{153}H_{225}N_{43}O_{49}S$ in *human glucagon EPCRS*.

## STORAGE

Human Glucagon for Injection should be protected from light and stored strictly in accordance with the manufacturer's instructions.