

Glucagon for Injection

DEFINITION
Glucagon for Injection is a sterile lyophilized mixture of the hydrochloride of glucagon with one or more suitable buffering and stabilizing agents. It contains NLT 65% and NMT 110% of the labeled amount of glucagon.

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the Assay.
- **B.** [GLUCAGON BIOIDENTITY TESTS \(123\)](#): Meets the requirements

ASSAY

Change to read:

• **PROCEDURE**

Solution A: Dissolve 16.3 g of [monobasic potassium phosphate](#) in 750 mL of [water](#), adjust with [phosphoric acid](#) to a pH of 2.7 (±0.05), add [water](#) to 800 mL, add 200 mL of [acetonitrile](#), and degas.

Solution B: Prepare a degassed solution of [acetonitrile](#) and [water](#) (4:6).

Mobile phase: See [Table 1](#). [NOTE—The ratio of *Solution A* to *Solution B* can be adjusted to obtain a retention time of about 21 min for the main peak.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	61	39
25 ^a	61	39
29	12	88
30	12	88
31	61	39
▲45▲ (USP 1-Aug-2022)	61	39

^a The end time of the isocratic elution can be adjusted so that the gradient begins after the fourth desamido peak elutes (relative retention time about 1.4). The rest of the program is then adjusted accordingly with this offset.

System suitability solution: Reconstitute a vial of ▲[USP Glucagon \(Human\) RS](#)▲ (USP 1-Aug-2022) in 0.01 N [hydrochloric acid](#) to obtain a solution with a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. At least 7% total of all four desamido glucagons should be present in the solution.

Standard solution: Reconstitute a vial of ▲[USP Glucagon \(Human\) RS](#)▲ (USP 1-Aug-2022) in 0.01 N [hydrochloric acid](#) to obtain a solution with a concentration of about 0.5 mg/mL.

Sample solution: Dissolve the substance to be examined in 0.01 N [hydrochloric acid](#) in order to obtain a concentration of 0.5 mg/mL of glucagon.

Chromatographic system
(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC**Detector:** UV 214 nm**Column:** 3-mm × 15-cm; 3-μm or less packing [L1](#)**Column temperature:** 45°**Flow rate:** 0.5 mL/min**Injection volume:** 15 μL**System suitability****Samples:** *System suitability solution* and *Standard solution***Suitability requirements****Resolution:** NLT 1.5 between the main peak and the first eluting desamido peak. Four peaks eluting after the glucagon peak that correspond to the desamido glucagons are clearly visible, *System suitability solution*.**Tailing factor:** NMT 1.8 for the glucagon peak, *Standard solution***Relative standard deviation:** NMT 2.0%, *Standard solution***Analysis****Samples:** *Standard solution* and *Sample solution*Calculate the percentage of glucagon ($C_{153}H_{225}N_{43}O_{49}S$) in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

 r_U = peak response from the *Sample solution* r_S = peak response from the *Standard solution* C_S = concentration of the *Standard solution* (mg/mL) C_U = concentration of the *Sample solution* (mg/mL)**Acceptance criteria:** 65%–110%**PERFORMANCE TESTS**

- [UNIFORMITY OF DOSAGE UNITS \(905\)](#): Meets the requirements

IMPURITIES**Change to read:**

- **ORGANIC IMPURITIES**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.**Sample solution:** Dissolve the substance to be examined in ▲0.01 N [hydrochloric acid](#)▲ (USP 1-Aug-2022) in order to obtain a concentration of 0.5 mg/mL of glucagon.**Analysis****Sample:** *Sample solution*

Calculate separately the percentage of each impurity in the portion of Glucagon for Injection taken:

$$\text{Result} = (r_U/r_T) \times 100$$

 r_U = peak response for each impurity r_T = sum of all the peak responses**Acceptance criteria:** NMT 14% total of all four desamido glucagons is found, and NMT 31% of total impurities and related compounds is found.**SPECIFIC TESTS**

- [WATER DETERMINATION \(921\)](#), [Method I](#), [Method Ic](#): NMT 4.0%

- **pH AND CLARITY OF SOLUTION:** Dissolve it in the solvent and in the concentration recommended in the labeling: the pH of the solution is between 1.7 and 3.5, and the solution is clear.

Change to read:

- [BACTERIAL ENDOTOXINS TEST \(85\)](#): ▲Meets the requirements▲ (USP 1-Aug-2022)