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Time (min)	Solution A (%)	Solution B (%)
29	12	88
30	12	88
31	61	39
45	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 of 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](#) in 0.01 N [hydrochloric acid](#) to obtain a solution having a concentration of about 0.5 mg/mL. Let stand at 50° for 48 h. Prolong the incubation period if necessary. At least 7% total of all four desamido glucagons [(Glu³)-glucagon, (Asp²⁸)-glucagon, (Glu²⁴)-glucagon, and (Glu²⁰)-glucagon] should be present in the solution.

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

Sample solution: 0.5 mg/mL of Glucagon in 0.01 N [hydrochloric acid](#)

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 3-mm × 15-cm; 3-μm packing [L1](#)

Temperatures

Autosampler: 2°–8°

Column: 45°

Flow rate: 0.5 mL/min

Injection volume: 15 μL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: Four peaks eluting after the glucagon peak that correspond to the desamido glucagons [(Glu³)-glucagon, (Asp²⁸)-glucagon, (Glu²⁴)-glucagon, and (Glu²⁰)-glucagon] are clearly visible. The resolution between the main peak and the first eluting desamido peak [(Glu³)-glucagon] is NLT 1.5, *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 (C₁₅₃H₂₂₅N₄₃O₄₉S) in the portion of Glucagon 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

For glucagon of recombinant DNA origin: 90%–105% on the anhydrous basis

For glucagon of synthetic origin: 93.0%–105.0% on the anhydrous, acetic acid-, ammonium-, and chloride-free basis

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• PROCEDURE

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the Assay.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Glucagon taken:

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

r_U = peak response for each impurity

r_T = sum of the responses of all peaks

Acceptance criteria

See [Table 2](#) for glucagon of recombinant DNA origin and [Table 3](#) for glucagon of synthetic origin. The reporting threshold is 0.05%.

Table 2

Name	Acceptance Criteria, NMT (%)
Total of all four desamido glucagons ^a	2.0
Total impurities and related compounds	6.0

^a These are (Glu³)-glucagon, (Asp²⁸)-glucagon, (Glu²⁴)-glucagon, and (Glu²⁰)-glucagon.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Sum of Met(O ²⁷)-glucagon peaks	0.25 and 0.27 (diastereomers of the sulfoxides)	0.5
(Glu ³)-glucagon	1.09	0.5
(Asp ²⁸)-glucagon	1.15	0.5
(Glu ²⁴)-glucagon	1.34	0.5
(Glu ²⁰)-glucagon	1.39	0.5
Each unspecified impurity	—	0.5
Total impurities	—	2.0

[NOTE—The manufacturer should determine the suitability of the monograph method for their process-related impurities, in particular for an unknown peak eluting at a relative retention time of 0.97, for (D-His¹)-glucagon and (des-Thr⁵)-glucagon.]

PROCESS-RELATED IMPURITIES AND OTHER COMPONENTS

[NOTE—These tests only need to be performed for glucagon of synthetic origin.]

Change to read:

• ACETIC ACID IN PEPTIDES

Mobile phase: 40 mM [sodium hydroxide](#) in [water](#)

Standard stock solution: 1.6 mg/mL of potassium acetate (equivalent to 1 mg/mL of acetic acid) in [water](#)



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