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Cosyntropin

SYSMEHFRWG KPVGKKRRPV KVYP

$C_{136}H_{210}N_{40}O_{31}S$ 2933

$\alpha^{1\text{-}24}$-Corticotropin CAS RN®: 16960-16-0; UNII: 72YY86EA29.

DEFINITION

Cosyntropin is a synthetic peptide whose sequence is identical to the first 24 amino acids of human adrenocorticotropic hormone (ACTH).

Cosyntropin contains NLT 90% and NMT 102% of cosyntropin ($C_{136}H_{210}N_{40}O_{31}S$), calculated on the anhydrous, acetic acid-free basis.

IDENTIFICATION

- **A.** The retention time of the cosyntropin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. Amino Acid Analysis**

Hydrolysis solution: 12 N hydrochloric acid containing a small crystal (0.1%–1.0%) of phenol

Sample hydrolysate preparation: Transfer a portion of cosyntropin into a vial, weigh it, and dissolve in water to a concentration of 3 mg/mL. Transfer 70 mg of this solution to a vacuum hydrolysis tube. Add 70 µL of *Hydrolysis solution,* seal the tube, and heat for 24 h at 110°. Cool the tube, and remove the solvents at reduced pressure. Resuspend the hydrolysate residue in 1 mL of 0.020 M hydrochloric acid, and filter.

Solution A: Prepare a solution having a composition of 140 mM sodium acetate and 17 mM triethylamine, and adjust with phosphoric acid to a pH of 5.02.[1]

Solution B: Acetonitrile and water (60:40)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
0.5	99	1
18	95	5
19	91	9
29.5	77	23
40	77	23
40.01	40	60
50	40	60

Sample solution: Add 140 µL of 0.2 M borate buffer (pH 8.8) to 20 µL of *Sample hydrolysate preparation.* Add 40 µL of 10 mM 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). Incubate for 2 min at room temperature. Transfer to an insert in an HPLC vial, seal with a silicone cap, and incubate for 10 min at 55°.

Standard solution: Prepare a solution having known equimolar amounts of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine with half the

equimolar amount of L-cystine. [NOTE—Suitable concentrations are 0.1 mM and 0.05 mM, respectively.] Derivatize with AQC as outlined for *Sample solution*.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 3.9-mm × 15-cm; 4-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Samples: *Sample solution* and *Standard solution*

Suitability requirements: All 17 amino acid peaks must be visible in the *Standard solution*.

Resolution: NLT 1.5 between each of the 17 amino acid peaks in the *Standard solution*. [NOTE—In those cases where the peak does not recover to the baseline, the following criteria will be applied: $(hp - hv)/hp \times 100 \pm 90\%$, where hp is the height of the minor peak, and hv is the height of the valley between the peaks.]

Analysis

Samples: *Sample solution* and *Standard solution*

First record and measure the responses for each amino acid peak in the *Standard solution*. Express the content of each amino acid in moles.

Calculate the mean nmol of the amino acids:

$$\text{Result} = (\text{nmol found in the } \textit{Analysis} \text{ for Glu, Gly, Val, Phe, Lys, Arg, Pro})/17$$

Divide the nmol of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: 3.5–4.7 of lysine; 0.9–1.1 of histidine; 2.7–3.3 of arginine; 1.1–2.2 of serine; 0.9–1.1 of glutamic acid; 2.5–3.5 of proline; 1.8–2.2 of glycine; 0.9–1.1 of methionine; 1.7–2.2 of tyrosine; 0.9–1.1 of phenylalanine; 2.7–3.3 of valine

ASSAY

• **PROCEDURE**

Mobile phase: 365 mL of acetonitrile, 10 mL of glacial acetic acid, and 10 g of ammonium sulfate. Dilute with water to 2 L, and a pH of about 3.3.

Standard solution: 1.0 mg/mL of USP Cosyntropin Acetate RS

Sample solution: 1.0 mg/mL of Cosyntropin

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.0 mL/min

Injection volume: 50 µL

Run time: 50 min

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.0 between the main cosyntropin peak and the reduced Trp-cosyntropin peak in the *Standard solution*

Retention time: 16–20 min for the cosyntropin peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the cosyntropin peak from three replicate injections of the *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of cosyntropin ($C_{136}H_{210}N_{40}O_{31}S$) in the portion of Cosyntropin taken:

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

r_U = peak response of cosyntropin from the *Sample solution*

r_S = peak response of cosyntropin from the *Standard solution*

C_S = concentration of cosyntropin in each vial of USP Cosyntropin Acetate RS in the *Standard solution* (mg/mL)

C_U = concentration of Cosyntropin, calculated on the anhydrous, acetic acid-free basis, in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–102.0% of cosyntropin on the anhydrous, acetic acid-free basis

IMPURITIES

• **Organic Impurities, Related Peptides**

Mobile phase, Standard solution, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay.*

Peak identification solution: 1.0 mg/mL of cosyntropin in 1% (v/v) glacial acetic acid. Add 50 µL of 30% hydrogen peroxide:water (1:999). Let stand for 2 h to produce the main impurity, cosyntropin sulfoxide.

System suitability

Samples: *Standard solution* and *Peak identification solution*

Suitability requirements

Resolution: NLT 1.0 between the main cosyntropin peak and the reduced Trp-cosyntropin peak in the *Standard solution*

Retention time: 16–20 min for the cosyntropin peak, *Standard solution*

Relative retention time of main impurity in *Peak identification solution*: About 0.4

Relative standard deviation: NMT 2.0% for the cosyntropin peak from three replicate injections of the *Standard solution*

Analysis

Sample: *Sample solution*

Integrate the chromatogram using the normalization procedure.

Calculate the percentage of cosyntropin-related impurities in the portion of Cosyntropin taken:

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

r_i = peak response of any individual impurity from the *Sample solution*

r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria

Individual impurities: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Cosyntropin sulfoxide	0.4	2
Reduced Trp in cosyntropin	0.9	2
Any other individual impurity	—	1
Total impurities	—	5

OTHER COMPONENTS

• **Acetic Acid in Peptides ⟨503⟩:** 8%–13% using a 1-mg/mL *Test Solution* prepared by dissolving 10 mg of Cosyntropin in 10 mL of *Solution A* and *Solution B* (95:5)

• **Trifluoroacetic Acid Content:** Perform the method contained in ⟨503⟩, substituting a suitable quantity of trifluoroacetic acid for the *Standard solution.* The portion of Cosyntropin taken shall contain NMT 0.5% trifluoroacetic acid.

SPECIFIC TESTS

• **UV Absorption Spectrophotometry**

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Wavelength range: 240–280 nm

Sample solution: 0.2 mg/mL in 0.1 M hydrochloric acid

Acceptance criteria: Absorptivity of 0.51–0.61, calculated on the anhydrous and acetic acid-free basis, at the maximum wavelength of 276 nm

Ratio: A276/A248, 2.4–2.9

- **Bacterial Endotoxins Test ⟨85⟩:** It contains NMT 10 USP Endotoxin Units/mg of cosyntropin.
- **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count is less than 100 cfu/g.
- **Water Determination, *Method 1c*⟨921⟩:** NMT 10.0%

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight, light-resistant containers at 2°–8°.
- **USP Reference Standards ⟨11⟩**
  USP Cosyntropin Acetate RS

[1] A suitable substitute is Eluant A from Waters Corporation, catalog number WAT052890.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
COSYNTROPIN	Ying Han Associate Science & Standards Liaison	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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