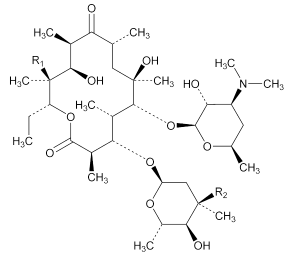
Erythromycin



Erythromycin	R_1	R_2
A	OH	OCH_3
B	H	OCH_3
C	H	OH

$C_{37}H_{67}NO_{13}$ 733.94

Erythromycin.

(3*R**,4*S**,5*S**,6*R**,7*R**,9*R**,11*R**,12*R**,13*S**,14*R**)-4-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione [114-07-8].

» Erythromycin consists primarily of erythromycin A ($C_{37}H_{67}NO_{13}$). The sum of the percentages of erythromycin A, erythromycin B, and erythromycin C is not less than 85.0 percent and not more than 100.5 percent, calculated on the anhydrous basis.

Packaging and storage—Preserve in tight containers.

USP Reference standards 〈11〉—

USP Erythromycin RS

USP Erythromycin B RS

USP Erythromycin C RS

USP Erythromycin Related Compound N RS

N-Demethylerythromycin A.

$C_{36}H_{65}NO_{13}$ 719.91

Identification—The IR absorption spectrum of a solution of it containing 50 mg per mL, previously dried at a pressure not exceeding 5 mm of mercury at 60° for 3 hours, in chloroform, determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Erythromycin RS, except in the region between 1980 cm^{-1} and 2050 cm^{-1}.

Specific rotation 〈781S〉: between −71° and −78°, determined after standing for 30 minutes.

Test solution: 20 mg per mL, in dehydrated alcohol.

Crystallinity 〈695〉: meets the requirements.

Water, *Method I* 〈921〉: not more than 10.0%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

Residue on ignition 〈281〉: not more than 0.2%.

Limit of thiocyanate—

Standard solutions—Transfer about 100 mg of potassium thiocyanate, previously dried at 105 ° for 1 hour, cooled, and accurately weighed, to each of two 50-mL volumetric flasks. Add about 20 mL of methanol to each flask, swirl to dissolve, dilute with methanol to volume, and mix. Transfer 5.0 mL of each of these stock solutions to separate 50-mL volumetric flasks, dilute with methanol to volume, and mix. Transfer 5.0 mL of each of these intermediate solutions to separate 50-mL low-actinic volumetric flasks. To each flask add 1.0 mL of ferric chloride TS, dilute with methanol to volume, and mix. [NOTE—Use these *Standard solutions* within 30 minutes.]

Test solution—Transfer about 100 mg of Erythromycin, accurately weighed, to a 50-mL low-actinic volumetric flask. Add 20 mL of methanol, and swirl to dissolve. Add 1.0 mL of ferric chloride TS, dilute with methanol to volume, and mix. [NOTE—Use this *Test solution* within 30 minutes.]

Blank solution—Transfer 1.0 mL of ferric chloride TS to a 50-mL low-actinic volumetric flask, dilute with methanol to volume, and mix. [NOTE—Use this *Blank solution* within 30 minutes.]

Procedure—Determine the absorbances of each *Standard solution* and the *Test solution* at the wavelength of maximum absorbance at about 492 nm with a spectrophotometer, using the *Blank solution* to zero the instrument. Calculate the suitability value, *S*, by the formula:

$$(A_1 / W_1)(W_2 / A_2)$$

in which A_1 and A_2 are the absorbance values obtained from the respective *Standard solutions;* and W_1 and W_2 are the weights, in mg, of the potassium thiocyanate taken to prepare the corresponding *Standard solutions*. In a suitable determination, the value, *S*, is not less than 0.985 and not more than 1.015. Calculate the percentage of thiocyanate in the Erythromycin taken by the formula:

$$(58.08/97.18)(A_U / W_U)(0.5)[(W_1 / A_1) + (W_2 / A_2)]$$

in which 58.08 and 97.18 are the molecular weights of the thiocyanate moiety and of potassium thiocyanate, respectively; A_U is the absorbance of the *Test solution;* W_U is the weight, in mg, of Erythromycin taken to prepare the *Test solution;* and the other terms are as defined above: not more than 0.3% is found.

Limit of related substances—Using the chromatograms of the *Assay preparation* and the *Diluted standard preparation* obtained in the *Assay*, calculate the percentage of any individual related substance observed having the greatest response, other than erythromycin A, erythromycin B, erythromycin C, and erythromycin A enol ether, in the Erythromycin taken by the formula:

$$25(CP / W)(r_i / r_S)$$

in which *C* is the concentration, in mg per mL, of USP Erythromycin RS in the *Diluted standard preparation; P* is the designated percentage of erythromycin A in the USP Erythromycin RS; *W* is the weight, in mg, of Erythromycin taken to prepare the *Assay preparation;* r_i is the peak response of an individual related substance, other than erythromycin A, erythromycin B, erythromycin C, or erythromycin A enol ether, observed in the chromatogram obtained from the *Assay preparation;* and r_S is the erythromycin A peak response in the chromatogram obtained from the *Diluted standard preparation:* not more than 3.0% of any individual related substance is found. Calculate the percentage of erythromycin A enol ether in the Erythromycin taken by the formula:

$$(25 / 11)(CP / W)(r_E / r_S)$$

in which 11 is the response factor for erythromycin A enol ether in relation to that of erythromycin A; r_E is the peak response of the erythromycin A enol ether peak observed in the chromatogram obtained from the *Assay preparation;* and the other terms are as defined above: not more than 3.0% of erythromycin A enol ether is found. The percentage of erythromycin B obtained in the *Assay* is not more than 12.0%; and the percentage of erythromycin C obtained in the *Assay* is not more than 5.0%.

Assay—

Solution A—Dissolve 1.75 g of dibasic potassium phosphate in 50 mL of water, adjust with diluted phosphoric acid (1 in 10) or 0.2 N sodium hydroxide to a pH of 9.0, add 400 mL of water, 165 mL of tertiary butyl alcohol, and 30 mL of acetonitrile. Dilute with water to 1000 mL, and mix.

Mobile phase—Prepare a mixture of *Solution A*, acetonitrile, and water (5:2:1). Make any necessary adjustments (see *System Suitability* under *Chromatography* 〈621〉).

Diluent—Prepare a mixture of pH 7.0 buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) and methanol (15:1).

pH 3.5 Buffer—Adjust 20 mL of pH 7.0 buffer (see under *Buffer Solutions* in the section *Reagents, Indicators, and Solutions*) with phosphoric acid to a pH of 3.5.

NOTE—Use the following solutions promptly, or within 1 day if stored in a refrigerator.

Standard preparation—Transfer about 100 mg of USP Erythromycin RS, accurately weighed, to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve, dilute with *Diluent* to volume, and mix.

Diluted standard preparation—Transfer 3.0 mL of the *Standard preparation* to a 100-mL volumetric flask, dilute with *Diluent* to volume, and mix. This solution contains about 0.12 mg of USP Erythromycin RS per mL.

Erythromycins B and C standard solution—Transfer about 5 mg each of USP Erythromycin B RS and USP Erythromycin C RS, both accurately weighed, to a 25-mL volumetric flask, add 5 mL of methanol, swirl to dissolve, dilute with *Diluent* to volume, and mix.

Resolution solution—Transfer about 2 mg of USP Erythromycin Related Compound N RS to a 10-mL volumetric flask, add 0.4 mL of *Standard preparation*, dilute with *Erythromycins B and C standard solution* to volume, and mix.

Erythromycin A enol ether retention time solution—Dissolve about 10 mg of USP Erythromycin RS in 2 mL of methanol. Add 10 mL of *pH 3.5 Buffer*, mix, and allow to stand for about 30 minutes.

Assay preparation—Transfer about 100 mg of Erythromycin, accurately weighed, to a 25-mL volumetric flask, add 5 mL of methanol, and swirl to dissolve. Dilute with *Diluent* to volume, and mix.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector and a 4.6-mm × 25-cm column that contains packing L21 (1000 Å) and is maintained at a constant temperature of about 65 °. The flow rate is about 2 mL per minute. Chromatograph the *Resolution solution*, and record the peak responses as directed for *Procedure:* the relative retention times are about 0.56 for erythromycin related compound N (N-demethylerythromycin A), 0.61 for erythromycin C, 1.0 for erythromycin A, and 1.6 for erythromycin B; and the resolution, *R,* between erythromycin related compound N and erythromycin C is not less than 0.8, and between erythromycin related compound N and erythromycin A not less than 5.5. Chromatograph the *Erythromycin A enol ether retention time solution*, and record the peak responses as directed for *Procedure:* the retention time of the erythromycin A enol ether peak is about 3.2 relative to that of the erythromycin A peak as observed in the chromatogram obtained from the *Resolution solution*. Chromatograph the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation for replicate injections is not more than 2.0%.

Procedure—Separately inject equal volumes (about 100 µL) of the *Standard preparation*, the *Diluted standard preparation*, the *Erythromycins B and C standard solution*, and the *Assay preparation* into the chromatograph, record the chromatograms for a period of time that is adequate to include the erythromycin A enol ether peak, if present, as determined in the chromatogram obtained from the *Erythromycin A enol ether retention time solution* (about five times the retention time of the main erythromycin A peak). Measure the areas of the peak responses. Calculate the percentage of erythromycin A in the portion of Erythromycin taken by the formula:

$$25(C_AP / W)(r_U / r_A)$$

in which C_A is the concentration, in mg per mL, of USP Erythromycin RS in the *Standard preparation; P* is the designated percentage of erythromycin A in USP Erythromycin RS; *W* is the quantity, in mg, of Erythromycin taken to prepare the *Assay preparation;* and r_U and r_A are the erythromycin A peak responses in the chromatograms obtained from the *Assay preparation* and the *Standard preparation*, respectively. Calculate the percentages of erythromycin B and erythromycin C in the portion of Erythromycin taken by the formula:

$$25(C_SP / W)(r_U / r_S)$$

in which C_S is the concentration, in mg per mL, of the relevant USP Reference Standard in the *Erythromycins B and C standard solution; P* is the designated percentage of erythromycin B or erythromycin C in the relevant USP Reference Standard; *W* is the quantity, in mg, of Erythromycin taken to prepare the *Assay preparation;* and r_U and r_S are the peak responses of the relevant analyte in the chromatograms obtained from the *Assay preparation* and the *Erythromycins B and C standard solution*, respectively.

Erythromycin Delayed-Release Capsules

» Erythromycin Delayed-Release Capsules contain not less than 90.0 per cent and not more than 115.0 percent of the labeled amount of erythromycin ($C_{37}H_{67}NO_{13}$).

Packaging and storage—Preserve in tight containers.

USP Reference standards ⟨11⟩—
USP Erythromycin RS

Identification—Prepare a test solution by mixing a quantity of finely ground Capsule contents with methanol to obtain a concentration of about 2.5 mg of erythromycin per mL. Prepare a Standard solution of USP Erythromycin RS in methanol containing 2.5 mg per mL. Apply separately 10 µL of each solution to a thin-layer chromatographic plate (see *Chromatography* ⟨621⟩) coated with a 0.25-mm layer of chromatographic silica gel. Place the plate in an unlined chromatographic chamber, and develop the chromatogram in a solvent system consisting of a mixture of methanol and chloroform (85:15) until the solvent front has moved about 7 cm. Remove the plate from the chamber, mark the solvent front, and allow the solvent to evaporate. Spray the plate with a mixture of alcohol, *p*-methoxybenzaldehyde, and sulfuric acid (90:5:5). Heat the plate at 100 ° for 10 minutes, and examine the chromatogram, in which erythromycin appears as a black-to-purple spot: the R_F value of the principal spot obtained from the test solution corresponds to that obtained from the Standard solution.

Dissolution ⟨711⟩—Proceed as directed for *Procedure* for *Method B* under *Apparatus 1 and Apparatus 2, Delayed-Release Dosage Forms.*

Apparatus 1: 50 rpm.

Times: 60 minutes for *Acid Stage;* 60 minutes for *Buffer Stage.*

Procedure—Transfer the contents of 1 Capsule to the apparatus. Proceed as directed for *Acid Stage*, 900 mL of 0.06 N hydrochloric acid being placed in the vessel instead of 1000 mL of 0.1 N hydrochloric acid, and the apparatus operated for 60 minutes instead of 2 hours. Do not perform an analysis at the end of the *Acid stage*. Continue as directed for *Buffer Stage*, 900 mL of the pH 6.8 phosphate buffer being used instead of 1000 mL. Determine the amount of $C_{37}H_{67}NO_{13}$ dissolved after 120 minutes by assaying a filtered portion of the solution under test as directed under *Antibiotics—Microbial Assays* ⟨81⟩.

Tolerances—Not less than 80% *(Q)* of the labeled amount of $C_{37}H_{67}NO_{13}$ is dissolved in 120 minutes.

Water, *Method I* ⟨921⟩: not more than 7.5%, 20 mL of methanol containing 10% of imidazole being used in place of methanol in the titration vessel.

Assay—Proceed as directed under *Antibiotics—Microbial Assays* ⟨81⟩, using not less than 5 Capsules blended for about 3 minutes in a high-speed glass blender jar containing 200 mL of methanol. Add 300 mL of *Buffer No. 3,* and blend again for about 3 minutes. Dilute an accurately measured volume of this