⟨226⟩ 4-EPIANHYDROTETRACYCLINE

This chromatographic procedure is provided to demonstrate that the content of 4-epianhydrotetracycline, a degradation product of tetracycline, does not exceed the limit given in the individual monograph.

PROCEDURE

EDTA buffer: Dissolve 37.2 g of edetate disodium in 800 mL of water, adjust with ammonium hydroxide to a pH of 7.8, dilute with water to 1000 mL, and mix.

Support phase: Add 5 mL of *EDTA buffer* to 10 g of acid-washed chromatographic siliceous earth for column chromatography, and mix until the siliceous earth is uniformly moistened.

Test solution: Prepare as directed in the individual monograph.

Procedure: Prepare a 15-mm × 170-mm chromatographic tube with a 4-mm × 50-mm outlet by packing it, in increments, with *Support phase*, firmly tamping down each increment, until the tube is filled to a height of about 10 cm. In a beaker, prepare a mixture of 1 g of acid-washed chromatographic siliceous earth for column chromatography and 1 mL of *Test solution.* Transfer the mixture to the top of the column. Dry-wash the beaker with *Support phase*, and transfer to the column to provide an additional 1-cm layer on top of the mixture containing the *Test solution.* Within 30 minutes, pass chloroform through the column, and collect successive fractions of 5.0 mL, 5.0 mL, 10.0 mL, 10.0 mL, and 5.0 mL. Observe the column during elution, and note the appearance of two separate yellow bands. The fraction or fractions containing the first yellow band contain the anhydrotetracyclines. Discard these fractions. The fractions after the first yellow band contain the 4-epianhydrotetracycline. Determine the absorbance of each 4-epianhydrotetracycline fraction at the wavelength of maximum absorbance at about 438 nm, with a suitable spectrophotometer, diluting each fraction, if necessary, with chloroform, and using chloroform as the blank. Calculate the quantity, in mg, of 4-epianhydrotetracycline in each fraction by the formula:

$$\text{Result} = A_U VD/A_S$$

A_U = absorbance of the fraction taken
V = volume of the fraction taken (mL)
D = dilution factor, if the fraction was diluted
A_S = absorptivity of 4-epianhydrotetracycline at 438 nm, 20.08

From the sum of the quantities of 4-epianhydrotetracycline found in the fractions, calculate the percentage of 4-epianhydrotetracycline in relation to the tetracycline hydrochloride equivalent contained in the *Test solution.*