⟨511⟩ SINGLE-STEROID ASSAY

In the following procedure, the steroid to be assayed is separated from related foreign steroids and excipients by thin-layer chromatography and determined following recovery from the chromatogram.

PREPARATION OF THE PLATE

Prepare a slurry from 30 g of chromatographic silica gel with a suitable fluorescing substance by the gradual addition, with mixing, of about 65 mL of a mixture of water and alcohol (5:2). Transfer the slurry to a clean, 20- × 20-cm plate, spread to make a uniform layer 250 µm thick, and allow to dry at room temperature for 15 minutes. Heat the plate at 105° for 1 hour, and store in a desiccator.

SOLVENT A

Mix methylene chloride with methanol (180:16).

SOLVENT B

Mix chloroform with acetone (4:1).

STANDARD PREPARATION

Dissolve in a mixture of equal volumes of chloroform and alcohol a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried as directed (see *USP Reference Standards* ⟨11⟩) and accurately weighed, to obtain a solution having a known concentration of about 2 mg per mL.

ASSAY PREPARATION

Prepare as directed in the individual monograph.

PROCEDURE

Divide the area of the chromatographic plate into three equal sections, the left and right sections to be used for the *Assay Preparation* and the *Standard Preparation,* respectively, and the center section for the blank. Apply 200 µL each of the *Assay Preparation* and the *Standard Preparation* as streaks 2.5 cm from the bottom of the appropriate section of the plate. Dry the solution as it is being applied, with the aid of a stream of air. Using the *Solvent* specified in the individual monograph, develop the chromatogram in a suitable chamber, previously equilibrated and lined with absorbent paper, until the solvent front has moved 15 cm above the initial streaks.

Remove the plate, evaporate the solvent, and locate the principal band occupied by the *Standard Preparation* by viewing under UV light. Mark this band, as well as corresponding bands in the *Assay Preparation* and blank sections of the plate. Remove the silica gel from each band separately, either by scraping onto glazed weighing papers or by using a suitable vacuum collecting device, and transfer it to a glass-stoppered, 50-mL centrifuge tube. To each tube add 25.0 mL of alcohol, and shake for not less than 2 minutes. Centrifuge the tubes for 5 minutes, pipet 20 mL of the supernatant from each tube into a glass-stoppered, 50-mL conical flask, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Proceed as directed for *Procedure* under *Assay for Steroids* ⟨351⟩, beginning with "Then to each flask."