

⟨351⟩ ASSAY FOR STEROIDS

The following procedure is applicable for determination of those Pharmacopeial steroids that possess reducing functional groups such as α -ketols.

STANDARD PREPARATION

Dissolve in alcohol a suitable quantity of the USP Reference Standard specified in the individual monograph, previously dried under the conditions specified in the individual monograph and accurately weighed, and dilute quantitatively and stepwise with alcohol to obtain a solution having a concentration of about 10 μg per mL. Pipet 20 mL of this solution into a glass-stoppered, 50-mL conical flask.

ASSAY PREPARATION

Prepare as directed in the individual monograph.

PROCEDURE

To each of the two flasks containing the *Assay Preparation* and the *Standard Preparation*, respectively, and to a similar flask containing 20.0 mL of alcohol to serve as the blank, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of methanol, and mix. Then to each flask add 2.0 mL of a mixture of alcohol and tetramethylammonium hydroxide TS (9:1), mix, and allow to stand in the dark for 90 minutes. Without delay, concomitantly determine the absorbances of the solutions from the *Assay Preparation* and the *Standard Preparation* at about 525 nm, with a suitable spectrophotometer, against the blank. Calculate the result by the formula given in the individual monograph, in which C is the concentration, in μg per mL, of the Reference Standard in the *Standard Preparation*; and A_u and A_s are the absorbances of the solutions from the *Assay Preparation* and the *Standard Preparation*, respectively.