

10288 COMPETITIVE PROBLEMS IN THE DRUG INDUSTRY

6505-290-0022 (P. D. No. 7)

Reagents and Solutions.

Standard Solution. Prepare a solution of U.S.P. Reserpine Reference Standard containing a known final concentration of 1.0 mg per ml in chloroform. Prepare this solution prior to use.

Sample Solution. Prepare a sample Reserpine solution containing a known final concentration of 1.0 mg per ml in chloroform. Prepare this solution prior to use.

Solvent System.

Immobilized Phase. Dilute 70 ml of formamide (99%) to 100 ml with methanol.

Mobile Phase. One part by volume of benzene and one part by volume of cyclohexane equilibrated with formamide.

Procedure:

Line standard chromatography jars which have been set up for descending chromatography with 12-1/4 by 22-1/2 inch Whatman No. 1 chromatography paper. Fill the solvent troughs with mobile phase solvent, and place sufficient amount of the same solvent in the chamber to allow the liner to dip into the solvent. Allow the chamber to equilibrate 12 hours.

Cut Whatman No. 1 chromatography paper into strips 5 inches by 18 inches and make a line 2-1/2 inches from one end. Mark application points at one inch intervals along this line. Immerse the paper in the immobile phase solvent and remove the excess solvent by pressing between two dry chromatography sheets and passing through a hand wringer. Spot 20.0 ul of standard and sample solutions, respectively, at separate application points, using a micropipet. Place the chromatogram in the chamber and allow it to develop for approximately 5 hours, or until the solvent front is one inch from the bottom of the paper. Remove the chromatograms, mark the solvent front, and air dry for a few minutes to remove the excess solvent. Observe, while still wet, under ultraviolet light at 360 mμ. Note the spots, then completely dry in a 90° C. oven for 10 to 15 minutes. Again observe under the ultraviolet light. The chromatogram may be sprayed lightly with glacial acetic acid to intensify the fluorescence. The R_f value for Reserpine shall be 0.34 ± 10 percent.