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## Blue Tetrazolium

(3,3'-(3,3'-Dimethoxy[1,1'-biphenyl]-4,4'-diyl)bis[2,5-diphenyl-2H-tetrazolium]dichloride),  $C_{40}H_{32}Cl_2N_8O_2$  727.64 CAS RN®: 1871-22-3.

—Lemon-yellow crystals. Slightly soluble in water; freely soluble in chloroform and in methanol; insoluble in acetone and in ether.

**Solubility in Methanol:** Dissolve 1 g in 100 mL of methanol: complete solution results, and the solution is clear.

**Color:** Transfer a portion of the methanol solution obtained in the preceding test to a 1-cm cell, and determine its absorbance at 525 nm, against water as the blank: the absorbance does not exceed 0.20.

**ULTRAVIOLET-VISIBLE SPECTROSCOPY (857):** Its molar absorptivity in methanol, at 252 nm, is not less than 50,000.

### Suitability Test

**Standard preparation:** Dissolve in alcohol a suitable quantity of [USP Hydrocortisone RS](#), previously dried at 105° for 3 hours and accurately weighed, and prepare by stepwise dilution a solution containing about 10 µg per mL.

**Procedure:** Pipet 10-, 15-, and 20-mL portions of *Standard Preparation* into separate, glass-stoppered, 50-mL conical flasks. Add 10 mL and 5 mL, respectively, of alcohol to the flasks containing the 10- and 15-mL portions of *Standard preparation*, and swirl to mix. To each of the flasks, and to a fourth flask containing 20 mL of alcohol, add 2.0 mL of a solution prepared by dissolving 50 mg of blue tetrazolium in 10 mL of alcohol, mix, and then add 2.0 mL of a solution prepared by diluting 1 mL of tetramethylammonium hydroxide TS with alcohol to 10 mL. Mix, allow the flasks to stand in the dark for 90 minutes, and determine the absorbances of the three solutions of the steroid standard at 525 nm, with a suitable spectrophotometer, using the solution in the fourth flask as the blank. Plot the absorbances on the abscissa and the amount of hydrocortisone on the ordinate scale of arithmetic coordinate paper, and draw the curve of best fit: the absorbance of each solution is proportional to the concentration, and the absorbance of the solution containing 200 µg of hydrocortisone is not less than 0.50.

**Auxiliary Information** - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
BLUE TETRAZOLIUM	<a href="#">Documentary Standards Support</a> Associate Scientific Liaison.	HDQ Headquarters

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