⟨391⟩ EPINEPHRINE ASSAY

ASSAY

Ferro-citrate solution: On the day needed, dissolve 1.5 g of ferrous sulfate in 200 mL of water to which have been added 1.0 mL of dilute hydrochloric acid (1 in 12) and 1.0 g of sodium bisulfite. Dissolve 500 mg of sodium citrate in 10 mL of this solution, and mix.

Buffer solution: In a 50-mL volumetric flask mix 4.2 g of sodium bicarbonate, 5.0 g of potassium bicarbonate, and 18 mL of water (not all of the solids will dissolve at this stage). To another 18 mL of water add 3.75 g of aminoacetic acid and 1.7 mL of 6 N ammonium hydroxide, mix to dissolve, and transfer this solution to the 50-mL volumetric flask containing the other mixture. Dilute with water to volume, and mix until solution is complete.

Standard preparation: Transfer about 18 mg of USP Epinephrine Bitartrate RS, accurately weighed, to a 100-mL volumetric flask with the aid of 20 mL of sodium bisulfite solution (1 in 50), dilute with water to volume, and mix. Transfer 5.0 mL of this solution to a 50-mL volumetric flask, dilute with sodium bisulfite solution (1 in 500) to volume, and mix. [NOTE—Make the final dilution when the assay is carried out.] The concentration of USP Epinephrine Bitartrate RS in the *Standard preparation* is about 18 µg per mL.

Assay preparation: Transfer to a 50-mL volumetric flask an accurately measured volume of the Injection under assay, equivalent to about 500 µg of epinephrine, dilute with sodium bisulfite solution (1 in 500) to volume, if necessary, and mix. [NOTE—The final concentration of sodium bisulfite is in the range of 1 to 3 mg per mL, any bisulfite present in the Injection under assay being taken into consideration.]

Procedure: Into three 50-mL glass-stoppered conical flasks transfer, separately, 20.0-mL aliquots of the *Standard preparation*, the *Assay preparation*, and sodium bisulfite solution (1 in 500) to provide the blank. To each flask add 200 µL of *Ferro-citrate solution* and 2.0 mL of *Buffer solution*, mix, and allow the solutions to stand for 30 minutes. Determine the absorbances of the solutions in 5-cm cells at the wavelength of maximum absorbance at about 530 nm, with a suitable spectrophotometer, using the blank to set the instrument.

Calculate the quantity, in mg, of epinephrine ($C_9H_{13}NO_3$) in each mL of the Injection taken by the formula, in which 183.21 and 333.30 are the molecular weights of epinephrine and epinephrine bitartrate, respectively:

$$\text{Result} = (183.21/333.30)(0.05C/V)(A_U/A_S)$$

C = concentration of USP Epinephrine Bitartrate RS in the *Standard preparation* (µg/mL)
V = volume of Injection taken (mL)
A_U = absorbance of the *Assay preparation*
A_S = absorbance of the *Standard preparation*

ADDITIONAL REQUIREMENTS

• **USP REFERENCE STANDARDS** ⟨11⟩
USP Epinephrine Bitartrate RS