Colorimetric Assay of Epinephrine

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Abstract □ A new colorimetric assay method for epinephrine, based on the reaction with thiosemicarbazide in alkaline medium, is presented. Beer's law is followed in the range of 25–300 μg. Data on precision, accuracy, and specificity are included. A special calculation is adopted in the presence of norepinephrine. The method, because of its simplicity, is particularly suited for routine analysis of official preparations of epinephrine.

Keyphrases □ Epinephrine—colorimetric assay, reaction with thiosemicarbazide □ Thiosemicarbazide—reagent in colorimetric assay of epinephrine □ Colorimetry—analysis, epinephrine, reaction with thiosemicarbazide

Several methods for the quantitative determination of epinephrine in pharmaceutical preparations are available. The official methods (1–3) have not proved completely satisfactory because they lack sensitivity to microquantities of the hormone and are time consuming.

In view of these disadvantages, several spectrophotofluorometric methods based on the oxidation of epinephrine to "adrenochrome" have been developed (4–7). These methods show high sensitivity, but the presence of certain antioxidants interferes with the development of the fluorescence (7). Other available methods either require previous separation from interfering substances, especially norepinephrine (8–10), or are based on color reactions given by the phenolic function of the molecule (11–17). Hence, the development of a simple and timesaving colorimetric method specific for the catecholic function is desirable.

EXPERIMENTAL[1]

Reagents—The following were used (all of analytical grade): epinephrine bitartrate, thiosemicarbazide solution (0.10%), and standard sodium hydroxide solution.

Assay Procedure—*Calibration Curve*—Pipet several aliquots of exactly 0.5–3 ml of epinephrine bitartrate solution (180 μg/ml is equivalent to 100 μg pure epinephrine/ml) into 25-ml volumetric flasks. Dilute each to 10 ml with water and then add, in order, 1.0 ml of thiosemicarbazide solution and 1.0 ml of 0.2 *N* NaOH. Mix well and let stand for 30 min. Dilute to volume with water and measure the absorption at 395 and 485 nm against a blank prepared under the same conditions using 1.0 ml of water in place of the thiosemicarbazide reagent.

Sample Preparation and Assay—Measure an aliquot of the epinephrine preparation containing 1.0 mg of epinephrine into a 10-ml volumetric flask and dilute to volume with water. Proceed as described for the calibration curve using 2.0 ml of this solution. Calculate the amount of epinephrine from the calibration curves.

Factors Affecting Color Formation—The absorption spectra were studied following the assay procedure for samples of 200 μg of epinephrine.

Interference—The effect of norepinephrine was determined by adding different amounts (by weight) to an epinephrine solution and performing the analysis according to the assay procedure.

The possible effect of the presence of adrenochrome as an oxidation product was studied. Samples of epinephrine solution were determined before and after oxidation with iodine (7).

The possible interference from other ingredients of epinephrine preparations was studied by adding the specific quantities found in official preparations.

Specificity to Catecholic Function—The procedure was also applied to solutions of other phenols and phenol-ethers with different numbers of hydroxy groups (carbolic acid, catechol, resorcinol, phloroglucinol, cresol, pyrogallol, and guaiacol).

Accuracy—Recovery trials were performed by adding known quantities of epinephrine bitartrate solution to different samples of epinephrine preparations. Analyses were made before and after addition.

[1] The absorption spectra were recorded on a Beckman DU-2 instrument.

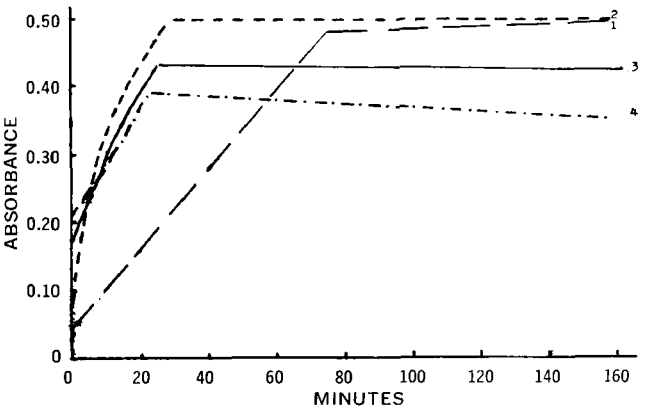

Figure 1—*Influence of pH on color formation. Key: 1, pH 11.0; 2, pH 11.3; 3, pH 11.5; and 4, pH 11.7. These values correspond in the assay to 1.0 ml of sodium hydroxide of 0.1, 0.2, 0.3, and 0.5* N, *respectively.*

RESULTS AND DISCUSSION

A characteristic orange-yellow color with an absorption maximum at 395 nm develops when epinephrine reacts with thiosemicarbazide in alkaline aqueous medium. A standard curve was plotted for various volumes of epinephrine solution. Beer's law was followed over a concentration range of 25–300 μg of pure epinephrine, and the color reached its maximum after 30 min at room temperature (25°) and remained stable for an additional 2 hr.

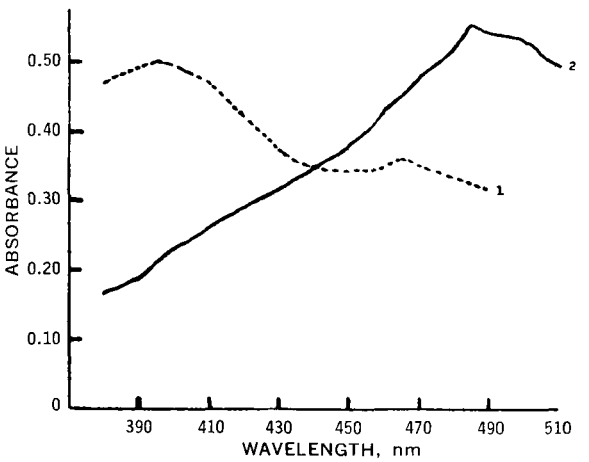

Figure 2—*Absorption spectra of the colors of (1) epinephrine and (2) norepinephrine.*

Table I—Determination of Epinephrine in Presence of Various Amounts of Norepinephrine

Epinephrine, μg	Norepinephrine, μg	Absorbance 395 nm	Absorbance 485 nm	Epinephrine, % Found	Deviation
200	0.00	0.500	0.330	100	0.0
180	20.0	0.480	0.350	90.5	+0.55
160	40.0	0.470	0.385	80.6	+0.75
120	60.0	0.425	0.465	60.3	+0.50

Table II—Colors Produced by Different Phenols and Phenol-Ethers with Thiosemicarbazide Reagent

Compound	Color Produced	Compound	Color Produced
Carbolic acid	None	Cresol	None
Catechol	Orange-yellow	Pyrogallol	None
Resorcinol	None	Guaiacol	None
Phloroglucinol	None		

The effects of temperature, the alkali concentration, and the presence of other chemicals on the color development were studied. Change of temperature had an insignificant effect on chromophore formation. Figure 1 shows that 0.2 *N* NaOH (pH of solution 11.3) is the optimum concentration for the color intensity and stability. The presence of sodium chloride, chlorobutanol, and sodium metabisulfite in the concentrations used in the official preparations produced no effect on color development, intensity, or stability. Adrenochrome produced no color under the experimental conditions. This result is to be expected, since adrenochrome lacks the catecholic function. But norepinephrine, when similarly treated, gave a red color with an absorption maximum at 485 nm and reached its maximum after 30 min. To determine the amount of intact epinephrine in the presence of its decomposition product norepinephrine, the absorption was measured at two wavelengths, 395 and 485 nm, the maxima for epinephrine and norepinephrine chromophores, respectively (Fig. 2). The amount of intact epinephrine was then deduced from the formula:

$$\% \text{ epinephrine} = \left(\frac{a_1 \times b_2}{a_2 \times b_1}\right) \times 100 \qquad \text{(Eq. 1)}$$

where a_1 = absorbance of sample at 395 nm, a_2 = absorbance of sample at 485 nm, b_1 = absorbance of standard epinephrine (200 μg) at 395 nm, and b_2 = absorbance of standard epinephrine (200 μg) at 485 nm.

The method was applied to various concentrations of epinephrine and norepinephrine mixtures (Table I).

It was found (Table II) that mono- and trihydroxybenzene derivatives do not produce any color under the experimental conditions. Moreover, dihydroxy compounds with the hydroxy substituent in the *meta*- or *para*-position fail to give the reaction.

Methylation of one or both hydroxy groups in the catechol molecule also prevents color development. Samples of aqueous epinephrine preparations were analyzed before and after the addition of known quantities of epinephrine solution (Table III).

Table III—Recovery of Epinephrine

Preparation	Epinephrine Added, μg	Absorbance Values Before Addition	Absorbance Values After Addition	Recovery, %	Deviation, %
Injection Solution	100	0.235	0.480	100	0.0
	50	0.230	0.350	100	0.0
Inhalation	100	0.245	0.490	100	0.0
Ophthalmic solution	50	0.245	0.362	97.5	2.5

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