THE COLORIMETRIC DETERMINATION OF ANEURINE BY AUERBACH'S METHOD

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Auerbach's method[1] for the colorimetric determination of aneurine in pharmaceutical preparations, like that adopted in the United States Pharmacopœia XII, is based on that of Melnick and Field[2] but is simpler and more rapid than either. Auerbach accelerated the colour development by heating at 60°C. for from 3 to 15 minutes, and for colourless simple solutions of aneurine be diluted with isopropyl alcohol instead of extracing the pigment with xylene, claiming an accuracy of ±3 per cent. Recently Elvidge[3] reported that the method was not reproducible and quoted errors of up to 13 per cent. in determinations without the use of standards; however, even using standards he obtained low and erratic results on tablets, the maximum deviations from the mean being +8 per cent. and −5 per cent. On the other hand Brown *et al.*[4] reported good agreement between the Melnick and Field colorimetric method and the thiochrome method and pointed out that the former was the more reliable whereas the latter, being more sensitive, was better for samples of low potency. The high sensitivity of the thiochrome method was acknowledged also by Adamson and Handisyde[5] who reported that in ordinary routine work with this method a greater precision than ±5 per cent. could not be relied upon. The present paper describes experiments carried out to establish a modified Auerbach's method which was used for control purposes before the thiochrome method became official in the British Pharmacopœia.

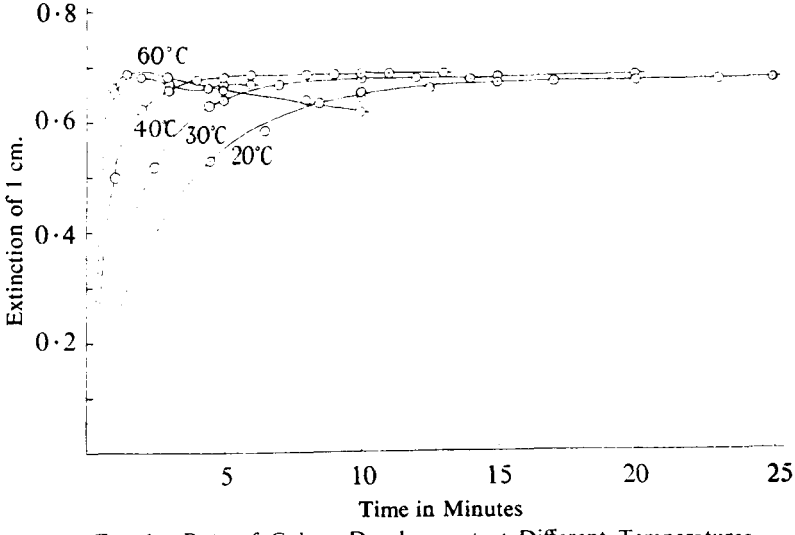

Fig. 1. Rate of Colour Development at Different Temperatures.

Modifications to the Original Method

The original method requires only small volumes of solution and reagents, for example 1 ml. and 2 ml., and an initial modification to increase precision, was to use 5 times the original amounts. However, difficulties in securing uniform heating of the larger volumes were encountered and hence consideration was given to determining conditions under which rapid coupling occurred at room temperature. Figure 1 shows the effect of temperature on the rate of development of colour, 20 to 25 minutes being required for complete coupling at room temperature.

Auerbach specifies the use of 20 to 25 per cent. ethyl alcohol or methyl alcohol as the solvent during coupling, and experiments showed that lower results were obtained with methyl alcohol and isopropyl alcohol but that constant maximum values were obtained with 20 to 25 per cent. ethyl alcohol. For simple solutions Auerbach uses a less alkaline diazotate reagent than that used for an elixir and tablets whereas Allport[6] recommends the more alkaline reagent for both. Experiments showed that the degree of alkalinity was important, as indeed was expected because of the importance of *p*H in coupling reactions, and an optimum was chosen. Slightly low results were obtained when the reagent was used immediately after preparation but constant values resulted when the reagent was used between 2 and 3 minutes after adding the alkali. The rate of colour development increased with increase in concentration of the diazotate reagent and, using 0·06 per cent., maximum colour was obtained in 10 minutes. Acid added after coupling slightly decreased the colour intensity, but when added before coupling was complete it arrested the coupling reaction and this effect was used in determining colour development curves.

Solutions of Aneurine

Although according to Auerbach many substances affect the intensity and shade of the colour produced, it was found that traces of inorganic salts and a relatively large proportion of dextrose in the preparations examined made no difference. The reproducibility and precision of the final method were tested by carrying out determinations on different days with freshly prepared reagents (Table I).

TABLE I

Reproducibility of proposed method

Day	Aneurine Solution	Diazotate Reagent	Extinction Value
1	1	A	1·042 1·040 1·044 1·040
2	2	B	1·030 1·033
		C	1·040 1·045
3	2	D	1·041 1·042
		E	1·038 1·041

From these results it was concluded that the modified method, unlike Auerbach's original method, was sufficiently reproducible to permit the use of a calibration curve. The proposed method for simple solutions is described below and calibration curves obtained with International Standard Vitamin B_1 and with a sample of aneurine hydrochloride B.P. are shown in Figure 2.

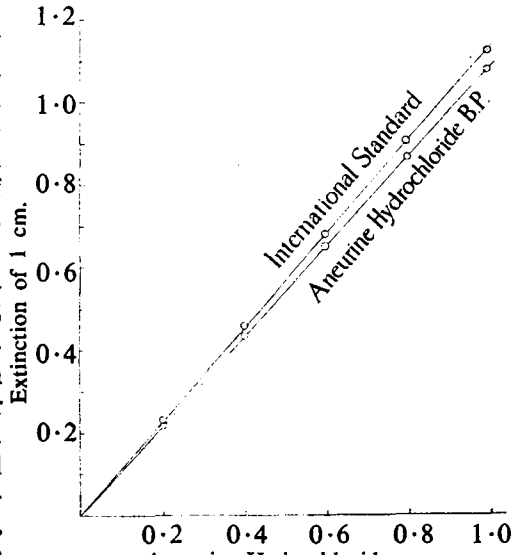

FIG. 2. Calibration curve.

Reagents. (1) *p*-Aminoacetophenone Solution, 0·06 per cent. in 0·2N hydrochloric acid. (2) Sodium nitrite Solution, 0·2 per cent. (freshly prepared). (3) Diazotate reagent, cool 10 ml. of *p*-aminoacetophenone solution to 5°C., add 3 ml. of sodium nitrite solution and mix. After 3 minutes add 3 ml. of 2N sodium hydroxide and mix by shaking. Use between 2 and 5 minutes after preparation.

Method. Prepare a dilution of the sample to contain about 0·1 mg. of aneurine hydrochloride /ml. and having an acid concentration equivalent to 0·01N. Transfer 5 ml. to a 50 ml. graduated flask, add 10 ml. of ethyl alcohol (50 per cent. v/v), mix, add 5 ml. of diazotate reagent and again mix. Place in a water-bath at 20°C. for 12 minutes and then dilute to 50 ml. with isopropyl alcohol. Determine the extinction in a 1 cm. cell using an Ilford 604 filter, subtract the value of a reagent blank and read off the amount of aneurine from a calibration curve.

TABLETS OF ANEURINE

Auerbach extracted aneurine from powdered tablets with alcohol (50 per cent.) by heating at 60°C. for 10 minutes. Using this method very low results were obtained (Table II) and this may account for Elvidge's results on tablets which, despite the use of standards, were on an average 20 per cent. low.

TABLE II
EXTRACTION OF ANEURINE FROM TABLETS

Method		Aneurine Hydrochloride found per cent.
Auerbach		1·66, 1·52, 1·54
Alcohol (50 per cent.) at room temperature	hours 19	1·88, 1·83
	46	2·05, 2·00
	146	1·81, 1·76

Modifications (Table II) gave higher results, but none was considered entirely satisfactory. The United States Pharmacopœia XII "dissolves" tablets in 0·01N hydrochloric acid whilst Wokes[7] heats for 10 minutes with a mixture of 15 ml. concentrated hydrochloric acid and 25 ml. of water. For other materials various methods have been used, for example, boiling with 1 per cent. hydrochloric acid[8] or for 10 minutes with 0·005N hydrochloric acid[9] and boiling for 1 hour with 0·4N sulphuric acid.[10] Recovery experiments made with the particular tablets under examination showed that the following method was satisfactory.

Method. Finely powder 20 tablets and weigh accurately an amount of powder expected to contain about 10 mg. of aneurine into a 100-ml. conical flask. Add exactly 5 ml. of dilute hydrochloric acid and 10 ml. of water, heat to boiling and boil gently for 4 minutes. Cool, add (x − 1) ml. of N sodium hydroxide, transfer to a 100 ml. graduated flask and dilute to a 100 ml. with water. (The value of x is the number of ml. of N sodium hydroxide required to neutralise the hydrochloric acid in a control determination, after cooling). Use 5 ml. of this solution for a determination as described for simple solutions.

SUMMARY

1. Auerbach's method for the colorimetric determination of aneurine has been modified. The modified method is of greater precision, is reproducible and permits the use of a calibration curve.
2. The inapplicability of Auerbach's method to certain tablets has been shown and a more satisfactory method is described.

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