J. Pharm. Pharmac., 1974, **26**, 972–974 *Received March* 13, 1974

A new colorimetric method for the determination of methyldopa

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A rapid and convenient method is described for the determination of methyldopa in pharmaceutical preparations. This is based on measuring the intensity of the orange colour, developed on reacting methyldopa with thiosemicarbazide in alkaline medium, at 490 nm. The colour obeys Beer's law in a concentration range of 2·0–10 μg ml^{-1}. Since the catecholic function with free adjacent positions is required for the development of the colour, the method is highly specific.

Methyldopa (L-α-methyl-3,4-dihydroxyphenylalanine) has become a valuable selective antihypertensive agent. Sourkes (1954) recognized that it is a competitive inhibitor of dopa decarboxylase. It has been reported that the antihypertensive properties of methyldopa result from its ability to act as a substrate for the decarboxylase enzyme (Cass & Spriggs, 1961; Iversen, 1967).

The quantitative determination of methyldopa (B.P., 1973, U.S.P. XVIII), is based upon the colour produced when it is treated with iron salts in the presence of alkaline buffer. The official methods have the disadvantage that the colour developed is based on the phenolic function of the compound. Furthermore, the methods lack sensitivity to microquantities, require an elaborate buffer solution, and are time consuming.

Direct spectrophotometry does not allow for the unequivocal determination of the drug, as it is usually found in pharmaceutical preparations in association with other hypotensive, or diuretic drugs. Hence, the development of a simple, rapid, and specific method is highly desirable.

MATERIALS AND METHODS

Materials and reagents

Anhydrous methyldopa was obtained from Koch Light, methyldopa tablets and methyldopa with hydrochlorothiazide tablets were obtained from Charles E. Frosst & Co.

All the chemicals used were of analytical grade. Thiosemicarbazide (BDH), and standard sodium hydroxide solutions.

Thiosemicarbazide reagent. Dissolve 100 mg thiosemicarbazide in 75 ml of water by the aid of gentle heat, cool to room temperature and dilute to 100 ml with water.

General procedure—calibration curve

Aliquots of 0·2—1·0 ml of a methyldopa solution in 0·1N sulphuric acid (100 mg anhydrous methyldopa/100 ml) are pipetted into 100 ml volumetric flasks and the volume made up to 25 ml with water. To the flasks are then added, in order 4·0 ml

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of thiosemicarbazide reagent, and 6·0 ml of N sodium hydroxide solution with mixing. After allowing to stand for 60 min the flasks are made up to volume with water and the intensity of the colour measured at 490 nm against a blank prepared under the same conditions using 0·5 ml of water in place of the thiosemicarbazide reagent.

Sample preparation and assay

Weight and powder 20 tablets. Dissolve an accurately weighed quantity of the powder equivalent to about 100 mg of anhydrous methyldopa in sufficient 0·1N sulphuric acid to produce 100 ml. Filter and reject the first 20 ml of the filtrate. Proceed as described for the calibration curve using 0·5 ml of this solution. Calculate the amount of methyldopa from the calibration curve.

RESULTS AND DISCUSSION

A characteristic orange colour with absorption maximum at 490 nm develops when methyldopa reacts with thiosemicarbazide in alkaline aqueous medium. A standard curve was plotted for various concentrations of methyldopa. The colour was found to obey Beer's law over a concentration range of 2·0–10 μg ml^{-1}.

The orange colour produced can be explained on the basis of the reaction of 6-hydroxydopamine (Powell & Heacock, 1973) and that of adrenaline (Prasad, Ricci & others, 1973). The aminochrome derivative produced on oxidation of methyldopa then reacts forming a stable thiosemicarbazone.

Factors affecting colour formation

The effect of temperature, the alkali concentration, and the presence of other chemicals on the colour formation and stability was examined. A colour development time of 60 min at 20° was found to be necessary for a linear relation between absorbance and concentration, whereas this time is only 35 min at 25° and 25 min at 30°. The colour in each case is stable for an additional 2 h. Hence a development time of 60 min was selected to allow for complete colour development and a linear relation between absorbance and concentration at temperatures between 20° and 30°. 6·0 ml of N sodium hydroxide solution was found to be the optimum concentration for the colour intensity and stability.

Table 1. *Comparative analysis of methyldopa preparations.*

	Amount of methyldopa in mg*			
		Found		
Compound	Labelled	B.P.	U.S.P.	Thiosemi-carbazide
Methyldopa tablets	250	257·5	255	252·5
Methyldopa + hydro-chlorothiazide tablets ..	250	—	—	250·0

* Average of three determinations.

Specificity to catecholic function was also studied. The method was carried out on solutions of phenols and phenol-ethers (phenol, catechol, resorcinol, guiacol, pyrogallol, phloroglucinol). It was found that the catecholic function with free

adjacent positions is essential for the colour formation. Moreover, methylation of one or both hydroxyl groups in the catechol molecule prevents the colour from being developed.

Comparison between the suggested method and the B.P. and the U.S.P. methods was carried out on methyldopa tablets and methyldopa with hydrochlorothiazide tablets. The results are tabulated in Table 1.

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