Determination of Methyldopa in Pharmaceutical Dosage Forms and Biological Fluids Based on Oxidation at the Tubular Carbon Electrode

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Abstract □ A method for the determination of methyldopa in pharmaceutical dosage forms based on electrochemical oxidation at the tubular carbon electrode is presented. The method is highly specific and may be used to analyze methyldopa in the presence of other types of pharmaceuticals. A comparison with two other colorimetric procedures of analysis shows the new method to have fewer manipulative steps with comparable precision and accuracy. In addition, the utility of the method in determining methyldopa in urine, plasma, and whole blood is demonstrated.

Keyphrases □ Methyldopa—method for determination in pharmaceutical dosage forms, urine, plasma, and whole blood, electrochemical oxidation, tubular carbon electrode, compared to compendial and modified colorimetric procedures □ Oxidation, electrochemical—use of tubular carbon electrode for determination of methyldopa in pharmaceutical dosage forms, urine, plasma, and whole blood □ Tubular carbon electrode—determination of methyldopa in pharmaceutical dosage forms and biological fluids

Various chemical methods for the determination of methyldopa are available. Paper chromatography, potentiometric titrimetry in a nonaqueous medium, UV spectrophotometry, colorimetry, and fluorescence have been applied to the determination of methyldopa in dosage forms and biological fluids (1-7). Although highly sensitive and specific, these methods involve separation and other manipulative steps including controlled reactions for color or fluorescence formations (2-4, 6, 7). A possible alternative method for methyldopa may be an electrochemical method similar to the ones presented for ascorbic acid and levodopa (8, 9).

The methods for ascorbic acid and levodopa are based on continuous analysis in flowing streams by oxidation of the drugs at the tubular carbon electrode (TCE) (10). This electrochemical method has the advantages over other commonly employed methods of being extremely fast and simple without any significant loss in accuracy and precision. Studies (9, 11) of the electrochemical oxidation of levodopa and catecholamines suggested that methyldopa may be assayed in this manner. Thus, an investigation of the oxidation of methyldopa at the TCE was undertaken to develop an analytical method. In this paper a method for the determination of methyldopa in pharmaceutical dosage forms is presented and compared to the official USP method and a colorimetric procedure. In addition, the utility of the new electrochemical method in determining methyldopa in urine, plasma, and whole blood is demonstrated.

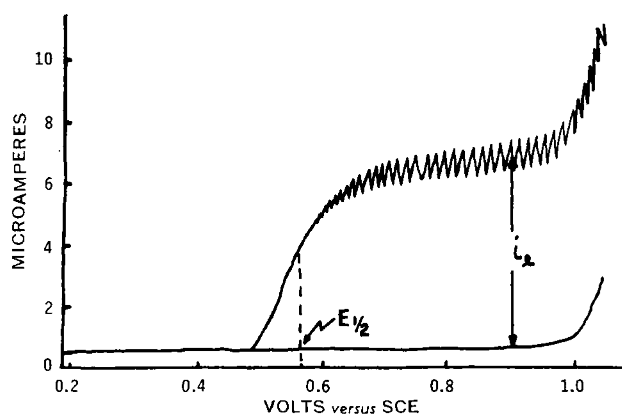

Figure 1—*Methyldopa current–voltage curve using a 10^{-4}* M *solution in 1* N *hydrochloric acid, a flow rate of 7 ml/min, and a scan rate of 0.2 v/min.*

EXPERIMENTAL

Apparatus—The electrode assembly, flow system, pump, and polarography system were the same as reported previously (8).

Chemicals—All chemicals were commercially available and were utilized as received. Standard solutions of methyldopa[1] were prepared by dissolving weighed amounts of the powder in aqueous 1 *N* hydrochloric acid.

Procedures for Determining Methyldopa—Current-voltage curves were determined using a TCE which had previously been cleaned with ethyl acetate. A volume flow rate of 7 ml/min was commonly employed and was controlled to ±1%. The voltage was scanned anodically from zero volt *versus* the saturated calomel electrode (SCE) at 0.20 v/min. Calibration plots of limiting current *versus* methyldopa concentrations were prepared and used directly to calculate drug concentration in unknown samples.

Method A: Analysis of Solid Dosage Form—Tablets were weighed and powdered, and a weighed aliquot was dissolved in 1 *N* hydrochloric acid to give approximately a 10^{-4} *M* solution. This solution was filtered, if necessary, and then pumped directly to the TCE for analysis.

Method B: Analysis in Urine—A 10-ml sample of urine containing 10^{-4} *M* methyldopa was diluted to 100 ml with distilled water. The pH of the solution was adjusted to 2.5 with concentrated hydrochloric acid and passed through a column containing 5 ml of cation-exchange resin[2] at a flow rate of 1.5 ml/min. The resin was previously prepared for use by washing with 100 ml of 4 *N* hydrochloric acid, followed by washing with distilled water until neutral to litmus. Methyldopa was then eluted from the column by the addition of 250 ml of 1 *N* hydrochloric acid solution. The acid solution was pumped directly to the TCE for analysis. A blank urine sample was also prepared by this method.

Method C: Analysis in Plasma—A 1-ml sample of plasma containing 10^{-4} *M* methyldopa was diluted to 100 ml with 1 *N* hydrochloric acid solution. The solution was pumped directly to the TCE for assay. An appropriate blank solution was also prepared.

Method D: Analysis in Whole Blood—A 2-ml sample of rabbit whole blood containing 10^{-4} *M* methyldopa was centrifuged for 30 min. One milliliter of the plasma was diluted to 100 ml with 1 *N* hydrochloric acid and pumped directly to the TCE for analysis. An appropriate blank sample was also prepared.

[1] Merck Sharp and Dohme Laboratories, Rahway, N.J.
[2] Amberlite IR-120, 20-50 mesh, hydrogen form, Mallinckrodt Chemical Works, St. Louis, Mo.

Table I—Methyldopa Analysis

Product	Amount Declared per Sample, mg	Percent Recovery ± SD[a]		
		TCE Method[b]	Colorimetric Method[c]	Colorimetric Method[d]
A[e]	25.0	100.26 ± 0.60	98.77 ± 1.07	98.00 ± 2.90
B[f]	25.0	98.73 ± 1.55	98.15 ± 3.20	98.53 ± 1.38
C[g]	25.0	98.73 ± 1.55	99.08 ± 1.30	98.70 ± 1.83

[a] Calculated on basis of three assays of a single aliquot. [b] TCE method with 1 N HCl as medium. [c] Ref. 7. [d] Ref. 12. [e] Commercial tablet containing methyldopa. [f] Commercial tablet containing methyldopa plus hydrochlorothiazide. [g] Commercial tablet containing methyldopa plus chlorothiazide.

To determine if other antihypertensive drugs or compounds commonly found in dosage forms with methyldopa alter its current-voltage curve or are oxidized at the TCE, the following studies were performed. Solutions of guanethidine monosulfate, hydrochlorothiazide, chlorothiazide, triamterene, chlorthalidone, hydralazine hydrochloride, reserpine, and phenobarbital (10^{-4} M) were prepared with and without methyldopa. Each solution was pumped through the TCE, and the voltage was scanned from 0 to 1.0 v *versus* the SCE.

RESULTS AND DISCUSSION

A typical current-voltage curve for the oxidation of methyldopa at the TCE is shown in Fig. 1. The limiting current was linear with respect to concentration over the 10^{-3}–10^{-5} M range. With the volume flow rate at 7 ml/min, a typical calibration plot has a slope of 6.2 μamp/10^{-4} M with a zero intercept. A plot of the logarithm limiting current *versus* logarithm flow rate has a slope of 0.30, which agrees with theoretical considerations of the tubular electrode geometry (11).

Current-voltage scans on 10^{-4} M solutions of the compounds listed under *Experimental* showed that only hydralazine hydrochloride and reserpine interfere with the analysis of methyldopa. The other compounds listed did not show an oxidation wave between 0 and 1 v *versus* the SCE.

The accuracy of the TCE method in determining methyldopa content of solid dosage forms is shown in Table I. The new method gives data in agreement with existing colorimetric procedures (7, 12) with comparable precision. The sensitivity of the TCE method as well as the colorimetric procedures is in the range of 10^{-4}–10^{-5} M. In comparison with the colorimetric methods, the TCE procedure was faster and simpler. Once the sample solutions were prepared, 25–30 determinations could be run each hour. The reproducibility of the TCE, as determined by repeatedly assaying a single sample dilution, is indicated by a deviation of the mean of 0.95% for five determinations.

A study of the analysis of methyldopa in urine, plasma, and whole blood was performed utilizing the new TCE method. In the determination of urine samples, a cation-exchange resin procedure (13) was employed to separate methyldopa from oxidizable components present in urine and gave polarographic waves between 0 and 1.0 v that interfered with the analysis of the drug. The separation was achieved by adjusting the pH of the urine sample containing methyldopa to 2.5 and passing the solution through the resin. Subsequent elution with 1 N hydrochloric acid gave an analytical solution that was free of the interfering species. A blank urine sample prepared by the same method gave identical results. The use of 1 N hydrochloric acid solution was ideal in many ways; not only was it satisfactory for the elution of methyldopa from the resin, but it also provided the necessary electrolyte and pH for the successful analysis of the drug as predetermined in the development of the TCE method. Percent recovery of methyldopa from urine samples was 89.86 ± 0.62%.

A resin cleanup procedure was not required for plasma and whole blood determinations since background current for blank samples did not reveal any interferences from components present in the biological fluids. Quantities of methyldopa (10^{-4} M) were added to plasma and whole blood samples. In whole blood determinations, samples were centrifuged and analyzed. Percent recoveries of drug from plasma and whole blood samples were 99.67 ± 0.40 and 79.57 ± 0.40%, respectively. The differences in recovery percentages between plasma and whole blood suggest that there may be some binding of methyldopa to components present in whole blood. Pretreatment of whole blood samples to cause hemolysis of blood cells and/or the use of a protein denaturant should increase the percent recovery of the drug.

The preparation of samples is rapid for plasma and whole blood and somewhat slower for urine due to passage of the sample through the cation-exchange column. Nevertheless, the analysis of methyldopa in these biological samples by the TCE method is convenient, fast, and simple with acceptable precision and accuracy.

Although automation was not employed in this study, the method may readily be incorporated into automated or semiautomated systems because it employs continuous analysis in a flowing stream of sample.

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