Egypt. J. Chem. **Vol. 67**, No. 8. pp. 451 - 457 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Spectrophotometric Estimation of Methyldopa Drug in pure and pharmaceutical formulations

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Abstract

A simple, new, and sensitive spectrophotometric technique for the determination of methyldopa was presented in this research article. The suggested technique includes reacting metoclopramide with $NaNO_2$ in the presence of hydrochloric acid to produce diazonium salt, and then the drug methyldopa reacts with the diazonium salt to produce a yellow azo dye. The maximum wavelength of the dye was 458 nm. This method is effectively used for the determination of methyldopa in different pharmaceutical formulations. It has been found that there are no significant interactions between common excipients and pure methyldopa. The results were processed statistically, and compared with those obtained from officially approved methods, they were found to be reasonable.

Keywords: Methyldopa, Spectrophotometry, Sodium nitrite, Metoclopramide, Sulfamic acid.

1. Introduction

A simple, new, and sensitive spectrophotometric technique for the determination of methyldopa was presented in this research article. In 1960, MTD was discovered [1]. The IUPAC name of the methyldopa drug is (2*S*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid. This drug is used to treat excessive blood pressure and is offered under the name Aldomet and other products [2]. It is one of the most commonly used therapies for high blood pressure in pregnant women. Various drugs are often recommended for other forms of high blood pressure, such as very high blood pressure that causes symptoms. It can be taken orally or by venous injection. The effects begin to appear after around 5 hours and remain for roughly a day. Drowsiness is a common adverse effect. Red blood cell destruction, liver issues, and allergic reactions are among the more serious side effects. MTD belongs to the alpha-2 adrenergic receptor agonist class of drugs. It works by activating the brain to reduce sympathetic nervous system activity. Spectrophotometric determination is an uncomplicated, speedy, and sensitive analytical technique for quantitative analysis that offers realistic and substantial cost-effective benefits as compared with other methods. Consequently, they have numerous options for pharmacological analyses [3]. Numerous analytical approaches have been proposed for determining MTD. In the literature, many techniques have been presented for the assay of MTD in diverse categories of samples involving pharmacological preparations, like chromatographic [4–8], flow-injection [9–12], electroanalytical techniques [13–15], and spectrophotometric [16–22]. The goal of this paper is to find simple spectrophotometric analytical approaches for determining MTD in pharmaceutical formulation.

2. EXPERIMENTAL

2.1. Apparatus

A spectrophotometer model CE-7200 UV-Vis with a cuvette of 1.0 cm has been employed to investigate electronic spectral measurements.

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Received date 14 November 2023; revised date 14 January 2024; accepted date 07 February 2024
DOI: 10.21608/EJCHEM.2024.248561.8873

2.2. CHEMICALS AND REAGENTS

2.2.1. Materials

Every employed chemical has been within analytical reagent grade and MTD was obtained from the Iraqi Government's State Company for Drugs Industry and Medical Appliances in Samarra (SDI).

1. **A standard stock solution of 200 µg/ml MTD** has been freshly prepared by dissolving 0.02 g of MTD in 10 mL of distilled water. Then, it was diluted to a mark with a similar solvent in a 100-ml volumetric flask.
2. **Sodium nitrite $NaNO_2$ 1.0 %** has been prepared by dissolving 1.0 g in distilled water and diluted to the mark with the identical solvent in the volumetric flask of 100 ml.
3. **Hydrochloric acid solution (HCl 1.0 M)** has been prepared by transferring 4.25 ml of concentrated acid and diluting it up to a mark with purified water in a volumetric flask of 50 ml.
4. **Sulfamic acid H_3NSO_3 (0.5%)** has been prepared by dissolving 0.5 g of sulfamic acid in distilled water and completing a mark in a volumetric flask of 100 mL with purified water.
5. **Sodium Hydroxide NaOH (1.0 M)** has been prepared by dissolving 4.0 g NaOH in distilled water and diluting up to a mark in a volumetric flask of 100 mL with a similar solvent.
6. **Glucose, Sucrose, Maltose, Lactose (100 µg/mL)** done by weighing 0.01 g of each sugar and dissolving them in distilled water, transferring them to a flask of 100 ml, and diluting to the mark with distilled water.
7. **Starch (100 µg/mL)** Triturate a soluble starch 0.01 g using slight cold water into a thin paste. Then, insert 100 mL of boiled water. Boil up to a clear solution can be gotten for 5 min. This solution must be as newly organized as necessary [23].

2.2.2. Preparation of MTD pharmacological in tablet form

The 10-tablet content has been grinded and mixed appropriately. A definite quantity of the fine powder has been precisely weighted for a proportional 250 mg per tablet, dissolved in 50 mL of distilled water, swirled, and left to stand for 5 min. Then, it was diluted to 100 mL in the flask with purified water. The solution has been processed via Whatman filter paper No.41 to avoid undissolved and suspended materials before use. The primary filtrate portion has been rejected.

2.3. Determination of Wavelength Maximum (λ_{max})

To determine the λ_{max}, 1.25 mL of (0.5%) MCP has been conveyed to a 10 mL volumetric flask. After that, 1 mL of HCl (1.0 M) and 1 mL of (0.1%) $NaNO_2$ solution have been inserted. The contents have been mixed well and left to stand for 3 minutes. After that, 1 mL of H_3NSO_3 (0.5%) was carefully inserted to neutralize excess nitrous acid, then 1 mL of NaOH (1 M) was added, and 0.5 mL of 200 µg/mL of the drug MTD was added. The absorbance of the colored product was read in contradiction to the reagent blank within the 400–600 nm range. The extreme absorbance wavelength for colored products has been 458 nm, based on Figure 1. All reagent blanks have a trivial absorbance at the corresponding λ_{max} under the tentative conditions.

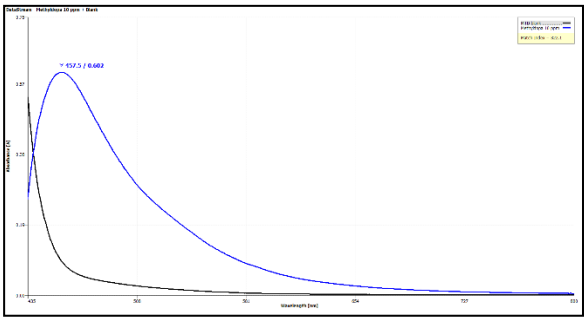


Figure 1: Absorption spectra of the colored reacted product (MTD 10 µg/mL) in contradiction of the reagent blank.

3. RESULTS AND DISCUSSION

3.1. Optimizing reaction variables

To establish optimal investigational conditions for swift and measurable development of a colored product with exceptional stability and sensitivity, as a result of a variety of circumstances such as:

- Volumes of MCP
- Volumes of HCl
- Volumes of $NaNO_2$
- First reaction time
- Volumes of H_3NSO_3
- Second reaction time
- Volumes of NaOH
- Order of Addition

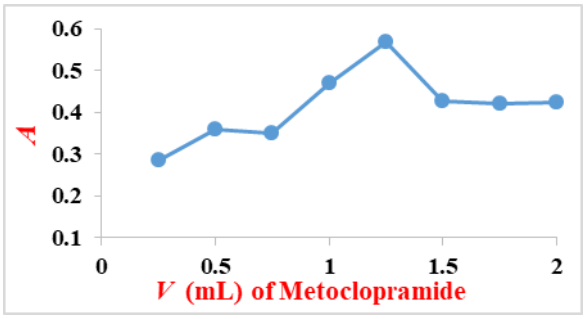
3.2. Optimization of the Experimental Conditions

The finest experimentation conditions have been started by changing single parameters and perceiving their influence on the absorbance of colored species.

3.2.1. Volumes of MCP

The effect of MCP volume on the formation of the colored product has been examined. Varying volumes of 0.5% MCP solutions in the range (0.25–2) mL were added and measured for the solutions' absorbance. The test has shown that 1.25 mL of MCP solution gave maximum absorbance. Figure 2 illustrates the influence of different acids on the reaction.

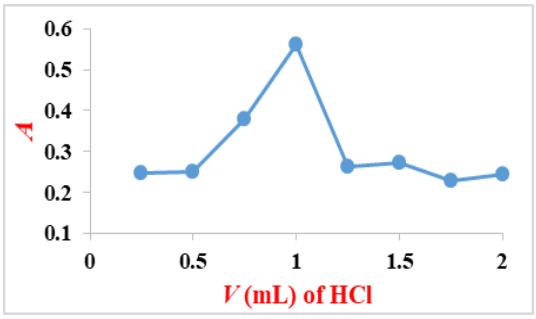
Figure 2: Influence of the volume of MCP (0.5%) on the absorbance of the reaction product



3.2.2. Volumes of HCl

The consequence of the HCl volume on the colored product has been examined. Varying volumes of standard (1.0 M) HCl solutions in the range (0.25–2) mL were added and measured for the solutions' absorbance. The investigation showed that 1 mL of HCl solution gave the highest absorbance, as depicted in Figure 3.

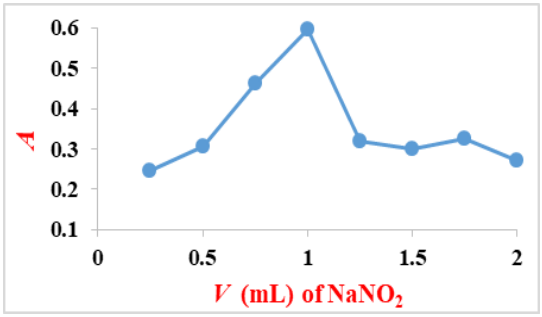
Figure 3: Influence of Volume of HCl (1.0 M) on the absorbance of the reaction product



3.2.3. Volumes of $NaNO_2$

The effect of $NaNO_2$ volume on the formation of the colored product has been examined. Varying volumes of standard (0.1%) $NaNO_2$ solutions in the range (0.25–2) mL were added and measured for the solutions' absorbance. The test has shown in Figure 4 that 1 mL of $NaNO_2$ solution gave maximum absorbance.

Figure 4: Influence of Volume of $NaNO_2$ (1.0%) on the absorbance of the reaction product



3.2.4. First reaction time

The consequence of time on the color intensity of the reaction at different times (0–15 min) was studied by measuring the absorbance at room temperature (25±1°C). It was found that the reaction got maximum absorbance at 3 min, and the value started to decrease gradually when the reaction time was raised above 3 min, as stated in Table 1.

Table 1: The consequence of the first time in the reaction product.

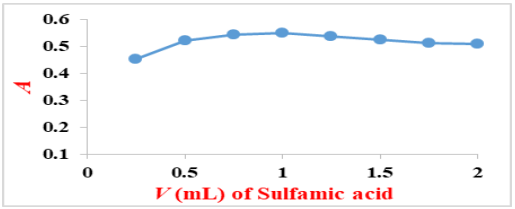
Time (min)	Absorbance
0	0.475
1	0.490
3	0.545
5	0.436
7	0.495
10	0.480
15	0.467

3.2.5. Volumes of H_3NSO_3

The consequence of H_3NSO_3 volume on the formation-colored product was studied.

Varying volumes of standard 0.5% H_3NSO_3 solutions in the range (0.25–2) mL were added and measured for the solutions' absorbance. The investigation showed in Figure 5 that 1 mL of H_3NSO_3 solution had the highest absorbance.

Figure 5: Consequence of volume of H_3NSO_3 (0.5%) in the absorbance of the reaction product



3.2.6. Second reaction time

The consequence of the second time on the color intensity of the reaction at different times (0–15) min was studied by measuring the absorbance at room temperature (25±1°C). It was found that the reaction got maximum absorbance at 0 min, and the value starts to decrease gradually when reaction time is raised above 0 min, as stated in Table 2.

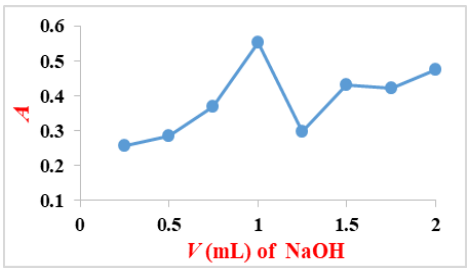
Table 2: The consequence of the second time on the reaction product.

Time (min)	Absorbance
0	0.560
1	0.535
3	0.357
5	0.353
7	0.313
10	0.341
15	0.315

3.2.7. Volumes of NaOH

The consequence of NaOH *volume* on the formation-colored product has been tested. Varying volumes of standard (1.0 M) NaOH solutions in the range (0.25-2) mL were added and measured for the solutions' absorbance. The 1 mL NaOH solution has the greatest absorbance as detected by Figure 6

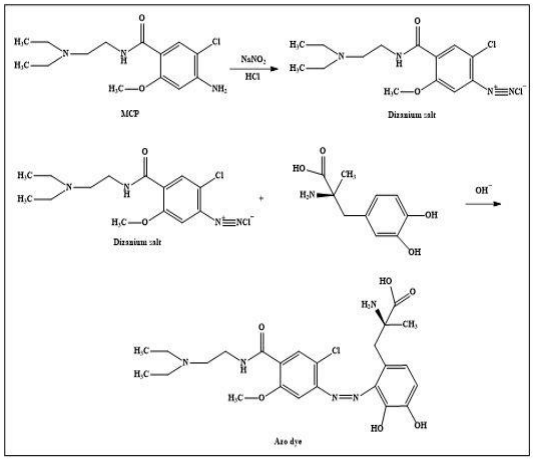
Figure 6: Consequence of Volume of NaOH (1.0 M) in the absorbance of the reaction product



3.3. The proposed mechanism

The yellow-colored product is obtained after reacting MCP with $NaNO_2$ in the presence of HCl to produce diazonium salt, and then the MTD reacts with the diazonium salt to produce a yellow azo dye. The following mechanism explains the reaction, as shown in Scheme 1.

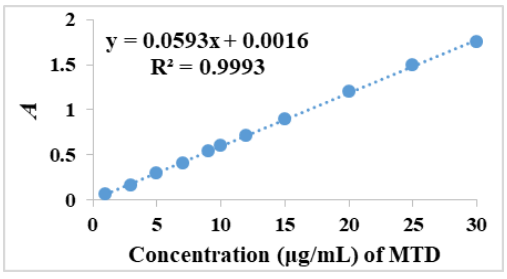
Scheme (1): The steps of reaction in the proposed method



3.4. General Procedure and Calibration Graph

By adopting 10 mL volumetric flasks, 1.25 mL of (0.5%) MCP has been added. After that, 1 mL of HCl (1.0 M) and 1 mL of (0.1%) $NaNO_2$solution has been inserted. The contents have been mixed well and left to stand for 3 minutes. After that, 1 mL of H_3NSO_3 (0.5%) has been inserted carefully to neutralize excess nitrous acid, then 1 mL of NaOH (1 M) was added and an increasing volume (0.05–1.5) mL of 200 µg/mL of the drug MTD was added. The volume has been achieved up to the mark with purified water, and the resultant solution has been determined at 458 nm in contradiction of the reagent blank treated in the same way. The calibration curves for MTD showed excellent linearity at concentration ranges of 1–30 µg/mL, based on Figure 7.

Figure 7: Calibration result of MTD of the resulting product



3.5. Spectral Features of the Suggested Method

Molar absorptivity, Beer's Law, and Sandell's sensitivities for MTD are given in Table 3 based on the described experimental conditions.

Table 3: The Optical features and statistical information for determining Methyldopa.

Parameter	Value
λ_{max} (nm)	458
Color	Yellow
Linearity range (µg/mL)	1 – 30
Molar absorptivity (L/mol.cm)	12525.05
Regression equation	y= 0.0593x + 0.0016
Calibration Sensitivity (L/ mg)	0.0593
Sandal's Sensitivity (µg/cm^2)	0.0346
Correlation of Linearity (R^2)	0.9993
Correlation coefficient (r)	0.9996
Detection limit LOD (µg/mL)	0.2200
Quantification limit LOQ (µg/mL)	0.7332

3.6. Precision and Accuracy of the Suggested Technique

The precision of the planned approach has been determined based on replicate analysis of three sample solutions at three concentration levels [24]. The relative standard deviations (RSD %) have been 0.1848–1.4488 %; on the other hand, the relative error percentage (R.E. percent) was used to assess the accuracy of the offered techniques (Table 4). The consequences indicated worthy method accuracy at each concentration level.

Table 4: Precision and Accuracy for the suggested approach

MTD Conc. (µg/mL)		RE%	S.D	R.S.D.* %
Taken	Found*			
3	2.9185	-2.7169	0.0025	1.4408
9	9.0961	1.0680	0.0010	0.1848
15	15.0658	0.4384	0.0053	0.5912

**Average of three determinations.*

3.7. Interference Investigation

The fallouts of the interference investigation have shown that no interference has existed from the excipients studied: sucrose, starch, lactose, glucose, and maltose [25]. The recovery of MTD ranged (from 99.22–101.25%). (Table 5) specifies the non-appearance of interferences for these excipients.

Table 5: The effect of the existence of (100 µg/mL) from the excipients on determining the MTD 10 µg/mL based on the suggested method.

Excipients	Conc. of Interferences µg/mL	Con. Found µg/mL	RE%	% Recovery
Sucrose	5	5.12	1.2479	101.25
	10	10.09	0.9106	100.91
starch	5	5.11	1.0793	101.08
	10	10.01	0.0675	100.07
lactose	5	4.94	-0.6071	99.39
	10	9.91	-0.9444	99.06
glucose	5	5.02	0.2361	100.24
	10	9.96	-0.4384	99.56
maltose	5	5.92	-0.7757	99.22
	10	9.99	-0.1012	99.90

3.8. Application of the Suggested Technique for Analyzing MTD in pharmaceutical Formulation

To increase the insurance, the suggested spectrophotometric process has been employed for determining MTD in pharmaceutical preparation samples. (Table 6) shows the result of accuracy based on relative error percent and reveals that the process has been reasonably accurate.

Table 6: The results of the determination of MTD in pharmaceuticals formulation by the suggested technique

.Industrial application	Conc. of MTD (µg/mL)		Rec %	S.D*	R.S.D* %
	Present	Found			
Aldomet tablet 250 mg, Lebanon	5	5.05	100.9781	0.1039	2.0578
	10	10.14	101.4165	0.0902	0.8894

Methyldopa tablet 250 mg, England	5	4.90	97.94266	0.0799	1.6316
	10	10.04	100.4047	0.3015	3.0028

**Average of three determinations*

4. CONCLUSION

The projected approach for determining MTD experimentally and pharmacologically has been uncomplicated, inexpensive, sensitive, and speedy. The recovery investigation data and the statistical considerations noticeably specify the accuracy and reproducibility of the technique. The suggested process is appropriate for the assay and drug evaluation in pharmacological productions to guarantee a huge quality control standard.

Conflicts of interest

There are no conflicts to declare.

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