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Synthesis of New Azo Compounds and Their Application for a Simple Spectrophotometric Determination of Methyldopa Drug Using Anthranilic Acid and 2-Aminopyrimidine as Reagents

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Abstract: The goal of the current work is to synthesize methyldopa derivatives. Based on these reactions, two easy, speedy, accurate, inexpensive, and sensitive spectrophotometric approaches have been established for determining methyldopa (MED) in both pure and pharmaceutical forms. The proposed azo-coupling method depends on forming an azo compound between methyldopa drug and 2-AMPY or ANTH to produce two compounds of MED-2AMPY and MED-ANTH in the alkaline medium. The characterization of synthesized compounds utilizing UV-Visible and FT-IR spectra. FT-IR spectra of 2AMPY-MED confirmed the existence of OH, C-H_{or}, C-H_{al}, NH, N=N, C=O, and C=C vibration at 3455, 3059, 2973, 3100, 1476,1692, and 1560 cm^{-1}, and FT-IR spectra of ANTH-MED confirmed the existence of OH, C-H_{or}, NH, C=O and N=N vibration at 3490, 3050, 3100, 1701 and 1462 cm^{-1}, correspondingly. The obtained color of azo compounds was spectrophotometrically measured for the previously mentioned azo compounds at 450 and 455 nm, respectively. Under perfect conditions, the azo compound solutions exhibited molar absorptivities of 1563.0058 and 2091.0285 $L.mol^{-1}.cm^{-1}$, Sandell's sensitivity of 0.135 and 0.10 $\mu g.cm^{-1,}$ and Beer-Lambert's law are obeyed over the ranges 6.25- 62.5 mg. L^{-1} for the two developed procedures, respectively.

Keywords: 2-Aminopyrimidine, Anthranilic acid, Spectrophotometry, Methyldopa, Pharmaceutical formulations.

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1. INTRODUCTION

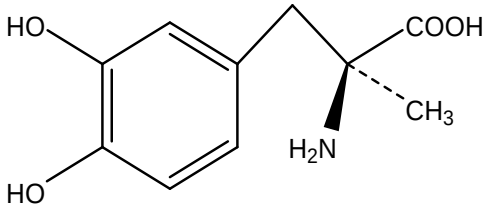
Methyldopa (MED), IUPAC name was α-methyl-3,4-dihydroxyphenylalalnine, whose structure is shown in Scheme 1. Methyldopa is a catecholamine derivative commonly used to treat mild to moderate arterial hypertension. Methyldopa is classified as a pro-drug since it works chiefly due to its metabolism in the central nervous system to α-methyl norepinephrine, an α2-adrenergic agonist(1,2). Several methods for quantifying methyldopa in pharmaceutical formulations have been proposed, including HPLC (3-5), polarography (6), flow injection analysis(7-10), titrimetry(11), potentiometry(12), and spectrophotometry methods(13-19). Aromatic amines were previously identified using the diazotization reaction. It is based on the reaction of a chromogenic reagent with a free primary amine to produce a diazonium salt. The technique includes using sulfamic acid or urea to remove excess nitrous acid, the stability of an intermediate diazonium salt at low temperatures, and the ejection of nitrogen bubbles (20-22). The

need for a simple, fast, low-cost, and selective method for determining methyldopa is evident based on the foregoing considerations. The technique described in this paper is depened on the reaction of methyldopa drug with 2-aminopyrimidine and anthranilic acid to produce orange color azo compounds (λ_{max} = 450 and 455 nm), respectively. Also, the reaction conditions were investigated by experimental design approaches in order to optimize the analytical response. When compared to other publications (23-25), the analytical results obtained by using the proposed method are reliable.

2. EXPERIMENTAL

2.1. Apparatus

The new approach and the standard method used a portable UV-Vis spectrophotometer single beam (160) that used 1 cm quartz cells to measure absorbance with a wavelength range between 200 and 800 nm. The pH solutions were recorded using a Metlar pH meter. A digital Sartorius balance was used for the weighing process.



Scheme 1: The structure of methyldopa (26).

2.2. Reagents and Solutions

All of the chemicals utilized were of the highest quality. The purity of methyldopa (99.8%) was obtained from SAMARRA, FT-IR AQ, (SDI), 2-aminopyrimidine, and anthranilic acid from the Sigma-Aldrich company. Stock methyldopa drug solution (250 mg L^{-1}) was prepared by dissolving 25 mg in D.W. and diluting it in the 100 mL volumetric flask to the mark. A stock 2-aminopyrimidine and anthranilic acid solution (250 mg L^{-1}) were prepared by dissolving 25 mg in D.W. and diluting it in the volumetric flask (100 mL). 25% sodium hydroxide, 4% urea solution, and (1.0 %) $NaNO_2$ solution.

2.3. General Procedure for Pharmaceutical Preparations

Tablets 250 mg of Aldomet (Lebanon) and Aldosam (SDI) were carefully weighted, and the average dosage weight was calculated. The distilled water was used to dissolve the entire weight. The solution was then diluted in a volumetric flask (100 mL) and filtered to achieve complete solubility.

2.4. Synthesis of MED Azo Compound (23)

To 2-AMPY or ANTH (3.0 mmol) ice, conc. HCl (1.0 mL), and a (3.3 mmol) solution of $NaNO_2$ in H_2O (9 mL) were subsequently added, and the mixture was stirred at 0-10 ^{0}C for 8 minutes to produce a diazonium salt ($RN_3^+Cl^-$). To a methyldopa drug (3.0 mmol) solution in D.W (15 mL) 10% aq. sodium hydroxide (3 mL) was added. As well as the diazonium salt solution was subsequently added at 0-10 °C. The orange compound (2AMPY-MED) was produced by filtering the resulting product, washing it with small amounts of cold water, and drying it at 70 °C. Formula: $C_{14}H_{15}N_5O_4$; Mwt: 317.3 g/mol; Yield: 82%; m.p: 243-245 ^{0}C; FTFT-IR (cm^{-1}): OH (3455), CH_{or}(3059), CH_{al}(2973), NH(3100),C=O(1692), C=C(1560), N=N(1476) and orange compound (ANTH-MED). Formula: $C_{17}H_{17}N_3O_6$; Mwt: 359.35 g/mol; Yield: 87%; m.p: 221-224 ^{0}C; FT-IR (cm^{-1}): OH (3490), CH_{or}(3050), NH(3100),C=O(1701), C=C(1592), N=N(1462). The purity of these compounds were checked by TLC using ethylacetate-n-hexane as eluent (27).

2.5. A General Method of Diazotization

The most efficient method was to produce an azo coupling solution by adding 1 mL of methyldopa 250 mg L^{-1} to a volumetric flask (10 mL) soaked in an ice bath (0-10 ^{0}C), 1 mL of hydrochloric acid (1:1), and 1 mL of (1%) $NaNO_2$ solution step by step. After 20 minutes, the mixture was prepared to use. Also, add (1.25 mL) of 4% urea solution with stirring to remove the excess nitrite, followed by adding 1.5 of 2-aminopyrimidine or 2.0 mL of anthranilic acid 250 mg L^{-1}. For 2AMPY or ANTH, add sodium hydroxide (1.0 or 1.75 mL, 25%), then dilute the mixture to 10 mL with D.W. The azo dye solution appears orange, and the absorption wavelengths for azo-2AMPY and azo-ANTH are 450 nm and 455 nm, respectively (28).

3. RESULTS AND DISCUSSION

2- amino pyrimidine and anthranilic acid has been used as chromogenic reagents to evaluate methyldopa drug. This procedure is based on a reaction between MED drug and reagents using azo-coupling reaction and producing an intensely colored azo dye solution (Scheme 2).

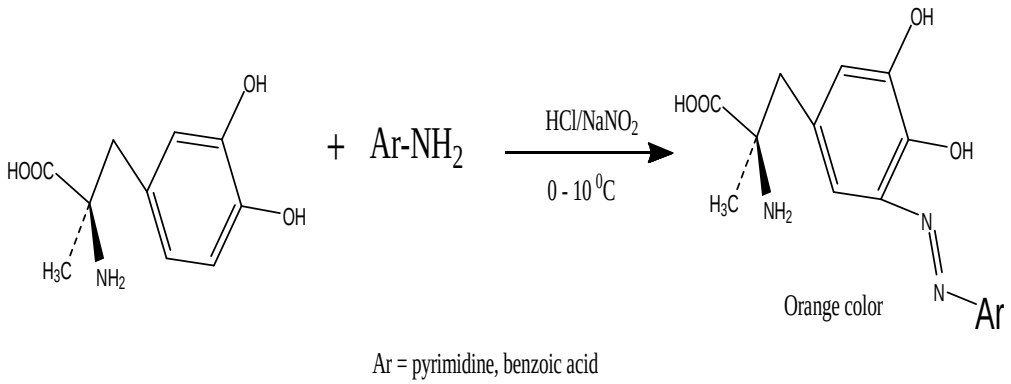
Absorption spectra of azo compounds MED–2AMPY and MED-ANTH system against a blank in an alkaline medium were produced orange-colored products which absorb maximally at 450 and 455 nm, as revealed in Figure 1 and Figure 2.

3.1. Synthesis and Characterization of MED Azo Compounds

By converting 2-AMPY and ANTH reagents to diazonium salt using HCl concentrated solution and sodium nitrate, followed by coupling with methyldopa, we synthesized novel methyldopa azo derivatives. FT-IR spectra of 2AMPY-MED confirm the existence of OH, C-H_{or}, C-H_{al}, NH, N=N, C=O, and C=C vibration at 3455, 3059, 2973, 3100, 1476,1692 and 1560 cm^{-1}, and FT-IR spectra of ANTH-MED confirm the existence of OH, C-H_{or}, NH, C=O and N=N vibration at 3490, 3050, 3110, 1701 and 1462 cm^{-1} (28), respectively.

3.2. Optimization of the Experimental Conditions

Parameters affected the absorption intensity of colored azo compounds, such as volume and type of acid, $NaNO_2$ volume, and reaction time. The influence of various acids was achieved for the formation of the diazonium salt solution, and the results are recorded in Table 1. The perfect acid volume was 1.0 and 0.25 mL for MED-2AMPY and MED-ANTH, respectively (Figure 5).



Scheme 2: Azo-coupling reaction (27).

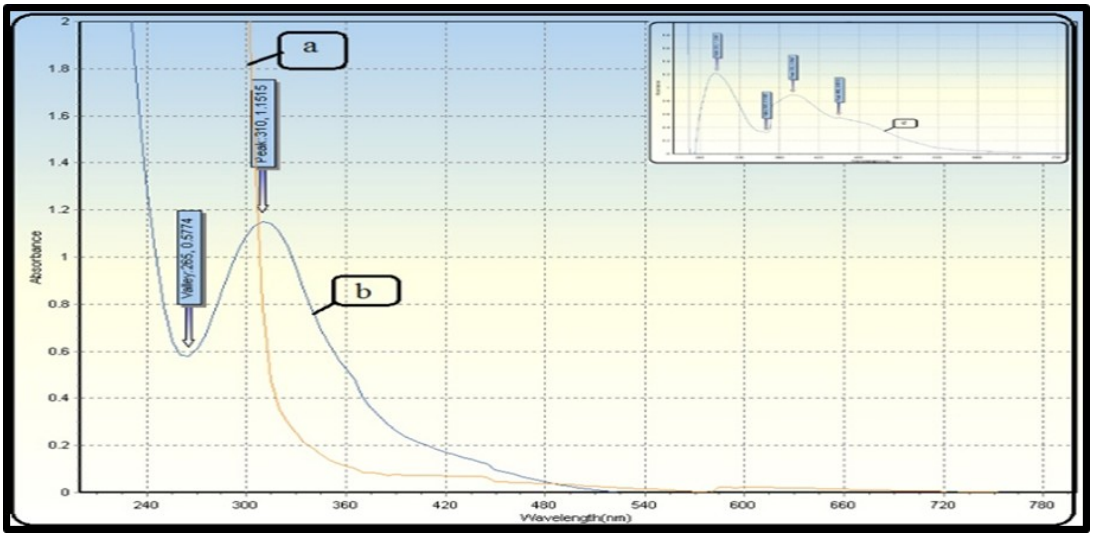


Figure 1: Absorption spectrum of (25µg.mL^{-1}) for (a- 2-Amino. Reag., b-MED drug) versus the blank solution (D.W), and c- MED- 2-Amino. azo comp. versus the blank solution.

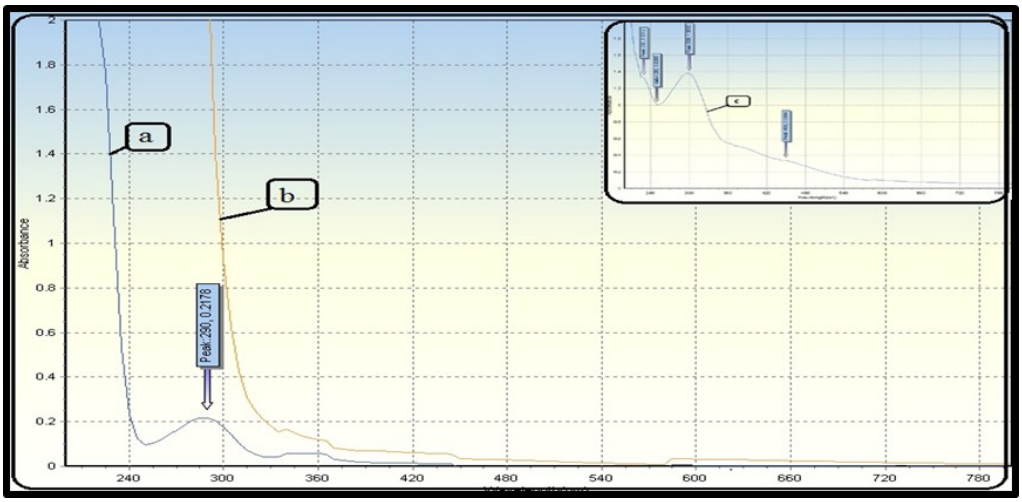


Figure 2: Molecular absorption spectrum of (25 µg/mL) for (a-MED drug, b-Anthranilic acid. Reag.) versus the blank solution (D.W), and c- MED- 2-Amino. azo comp. versus the blank of solution.

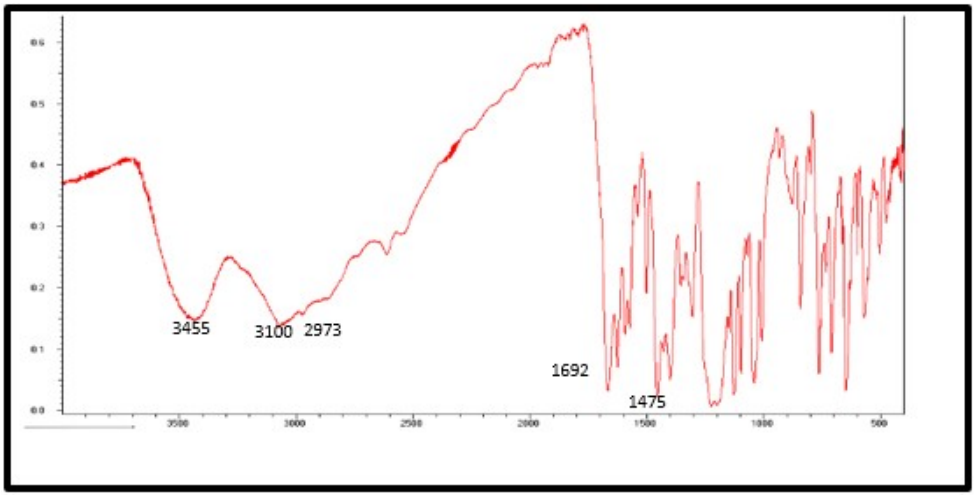


Figure 3: FT-IR of 2AMPY-MED azo compound.

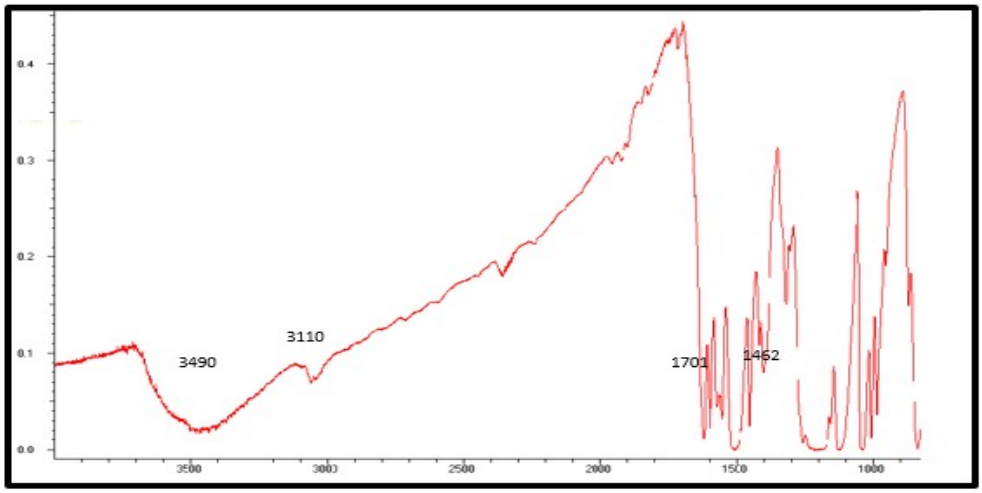


Figure 4: FT-IR of ANTH-MED azo compound.

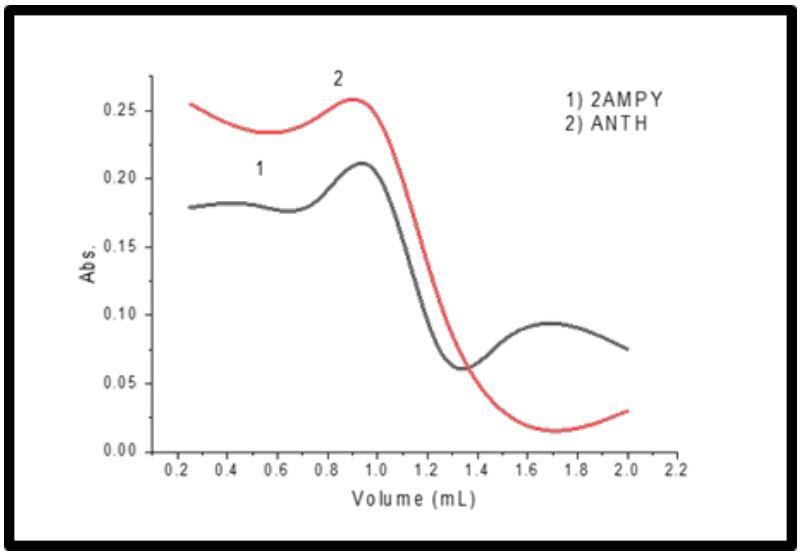


Figure 5: Effect of the volume of acid.

By experimenting with various volumes between the range of (0.2- 2.0 mL), the effect of the volume of sodium nitrite (1.0%) was examined. It showed that 1.0 mL for MED-2AMPY and MED-ANTH produced the best absorption intensity, as shown in Figure 6. To remove and extract the excess nitrous acid, varying volumes (0.25- 2.0 mL) from 4% urea solution were utilized (Figure 7).

Table 2 studied the effect of various bases on forming azo derivative (25%) of NaOH, KOH, and NH3 solution. The findings indicate that the ideal base was NaOH solution. The different volumes of NaOH (25%) from (0.25 to 2.0 mL) were examined. The best absorbance appeared by adding 1.0 mL and 1.7 mL for MED-2AMPY and MED-ANTH, respectively, as in Figure 8.

1.5 and 2.0 mL from reagent (2AMPY or ANTH) gave the greatest absorbance and was formed with high sensitivity, as shown in Figure 11. Under the perfect conditions (type and volume of acid, NaNO2 volume, and type of base), the reaction's stoichiometry between MED and 2-AMPY or ANTH was studied with continuous variation methods (29). The stoichiometric ratio between 2AMPY or ANTH with MED was 1:1, Figures 9 and 10.

3.3. Calibration Curve

The calibration graph for MED pure form through azo-coupling reaction with 2AMPY or ANTH showed excellent linearity at concentration ranges of 6.25 – 62.5 mg L^{-1}. The results are shown in Figure 12.

3.4. Comparison with Literature Studies

The results of the suggested method were contrasted with those of the previously published ones. Table 5 compares the performance of the suggested process with that of other methods in evaluating MED drugs for a variety of samples.

Table 1. Effect of the type of acid.

Type of acid	Abs. of MED-2AMPY (450 nm)	Abs. of MED-ANTH (455 nm)
HCl	0.205	**0.245**
CH_3COOH	0.115	**0.175**
HNO_3	0.080	**0.080**
H_2SO_4	0.072	**0.061**

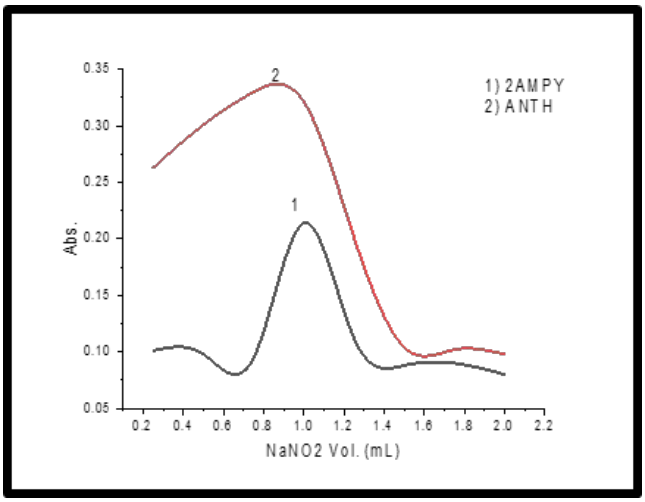


Figure 6: Effect of sodium nitrite (1%).

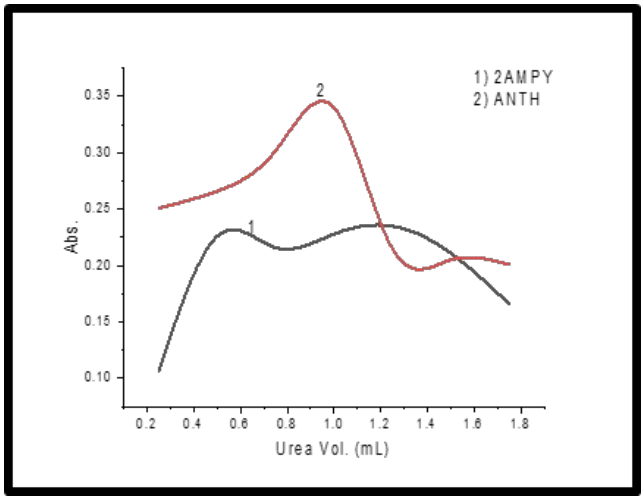


Figure 7: Effect of the volume of urea.

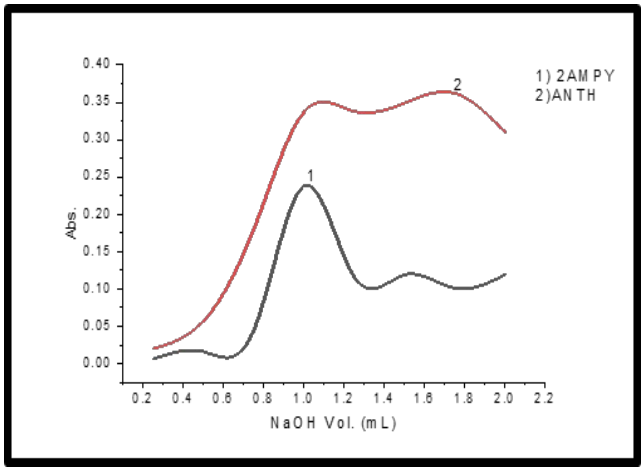


Figure 8: Effect of volume of NaOH.

Table 2: Effect of the type of base.

Type of base	Abs. of MED-2AMPY (450 nm).	Abs. of MED-ANTH (455 nm)
NaOH	0.238	**0.342**
KOH	0.090	**0.173**
NH_3	0.084	**0.060**

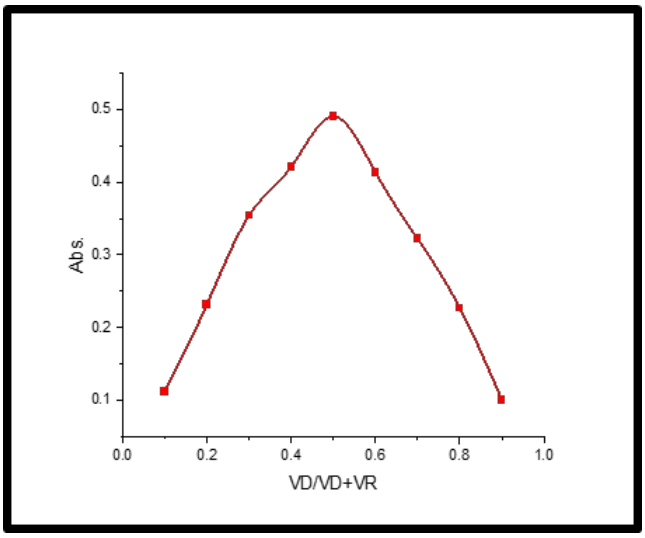


Figure 9: Continuous variation method of 2AMPY-MED azo compound.

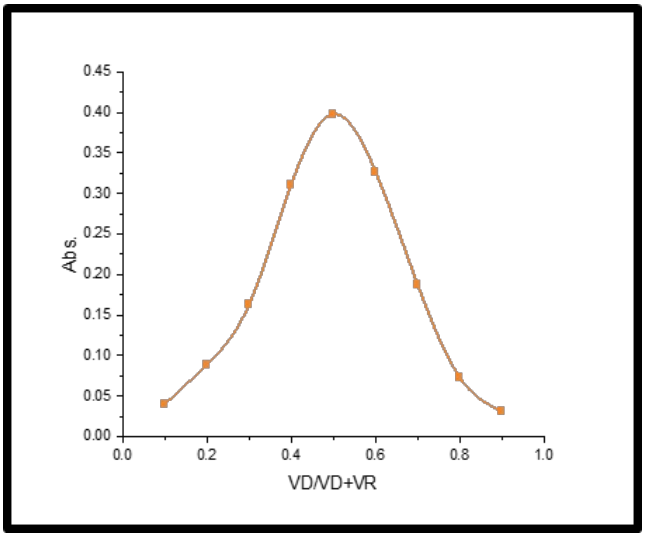


Figure 10: Continuous variation method of ANTH-MED azo compound.

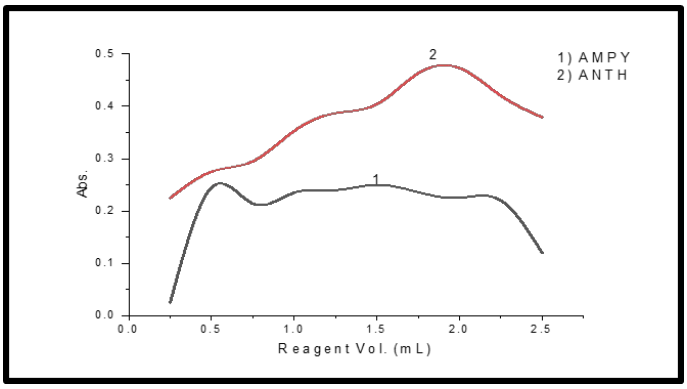


Figure 11: Effect of reagent.

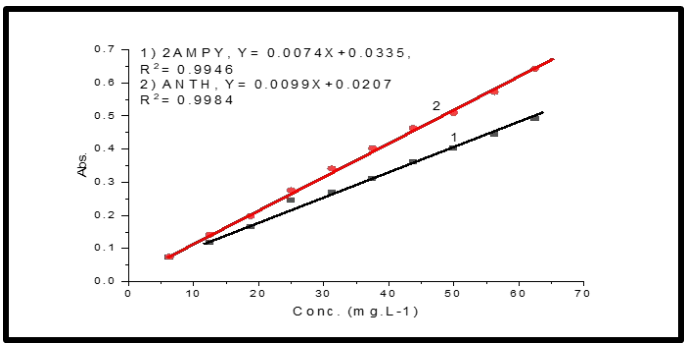


Figure 12: Calibration graph of MED-2AMPY and MED-ANTH (resulting product).

Table 3: Optical characteristics of the calibration graph for determination of MED by 2AMPY and ANTH reagent.

Parameters	**MED-2AMPY**	**MED-ANTH**
λ_{max} (nm)	450	455
Color	Orange	
Regression equation	Y=0.0074X+0.0335	Y=0.0099X+0.0207
Linearity range(mg L-1)	6.25-62.5	6.25-62.5
Correlation Cofficient (R^2)	0.9946	0.9984
ε($L.mol^{-1}.cm^{-1}$)	1563.0058	2091.0285
Sandell's sensivity ,µg . cm^{-2}	0.135	0.10
Slope (b)	0.0074	0.0099
Intercept(a)	0.0335	0.0207
LOD(mg L^{-1})	1.77	1.32
LOQ(mg L^{-1})	5.80	4.37
C.L.for the slope($b\pm ts_b$), 95%	$0.0335\pm9.25 \times10^{-5}$	$0.0099\pm 2.2 \times10^{-3}$
C.L.for the intercept($a\pm ts_a$), 95%	$0.0074\pm 3.5\times10^{-3}$	$0.0207\pm 6.6\times 10^{-5}$
Standard error for regression line ,$S_{y/x}$	0.01	0.0088
*C.L for Conc.(X_1) mg L^{-1} at 95%	25.31± 0.99	24±0.77
*C.L for Conc.(X_2) mg L^{-1} at 95%	35.62±0.59	35±0.49
*C.L for Conc.(X_3) mg L^{-1} at 95%	48.95±1.17	51±1.20

*MED-2AMPY (X_1=25, X_2=35, X_3=50) and MED-ANTH (X_1= 25, X_2= 35, X_3=50)

Table 4. Evaluation of MED drug in commercial tablets by spectrophotometric technique.

Drug	Conc. of drug mg L^{-1}		Relative Error%	Recov. %	Average Recov.%	RSD% (n=3)
	Taken	Found				
			MED-2AMPY			
Methyldopa (Aldomet)	12.50	12.13	-3.05	97.04	100.90	5.10
	25.00	25.94	3.76	103.76		4.31
	37.50	38.22	1.92	101.92		2.02
Methyldopa (Aldosam)	12.50	12.80	2.4	102.5	101.66	4.20
	25.00	26.00	4	104		3.62
	37.50	36.92	-1.5	98.5		2.44
			MED-ANTH			
Methyldopa (Aldomet)	12.50	11.93	-4.56	95.44	99.63	4.70
	25.00	26.18	4.72	104.72		3.99
	37.50	37.03	-1.25	98.75		1.92
Methyldopa (Aldosam)	12.50	12.40	-0.8	99.2	97.00	3.83
	25.00	24.01	-3.96	96.04		4.56
	37.50	35.92	-4.21	95.78		1.09

Table 5: Comparing the suggested method's LOD and LOQ values to those of other methyldopa evaluation techniques reported in the literature.

Method	LOD	LOQ	Ref.
HPLC	0.027 mg L^{-1}	-	(30)
Spectrophotometric method	0.152 mg L^{-1}	0.460 mg L^{-1}	(31)
electrochemical sensor	9.0 nM	-	(32)
Flow injection method	0.769 mg L^{-1}	-	(33)
Colorimetric method	0.38 mg L^{-1}	-	(34)
Nanostructured TiO_2 Carbon Paste Based Sensor	1µM	-	(35)
Electrochemical method	0.01 mg L^{-1}	-	(36)
Electrochemical method	8 µM	-	(37)
HPLC	-	2 ng/mL	(38)
Spectrophotometric method	1.77 mg L^{-1} 1.32 mg L^{-1}	5.80 mg L^{-1} 4.37 mg L^{-1}	Present work

4. CONCLUSION

The suggested method for evaluating methyldopa in bulk and pharmaceutical dosage forms is straightforward, precise, accurate, and selective. Unlike the chromatographic technique, this one is quick, inexpensive, and requires no expensive tools. As a result, it may be successfully used for routine evaluation of methyldopa medication in bulk and commercial formulation.

5. CONFLICT OF INTEREST

The researchers affirm that there are no conflicts of interest.

6. ACKNOWLEDGMENT

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