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Original Research Article

Spectrophotometric Method for Determination of Methyldopa in Bure and Pharmaceutical Formulation Based on Oxidative Coupling Reaction

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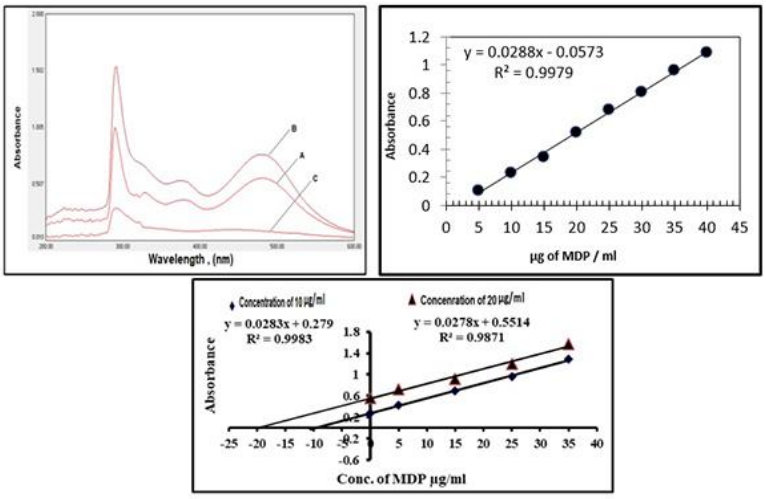
ABSTRACT

This study dealt with a simple, sensitive, and rapid spectrophotometric method to determine methyldopa (MDP) in bure form and it is pharmaceutical preparation (tablet) by using a new prepared organic reagent. The method is based on preparation a new organic reagent (Schiff's base) and using it in oxidative coupling reaction with MDP in acidic medium in the presence of potassium periodate to produce a stable, water-soluble orang complex with the maximum absorption signal at 481 nm, Beer's law is followed for standard MDP solutions in the range of 5.0-40 µg/mL with a negative deviation at concentrations higher than 40 µg/mL. The molar absorptivity and Sandell's sensitivity index values are 6.082×10^3 L.mol^{-1}.cm^{-1} and 0.0347µg /cm^2 with relative standard deviation of RSD% less than 5%, detection 1 limit of 0.8937 µg.ml^{-1} and correlation coefficient of 0.9979. Two approaches were applied to determine the MDP amount in its pharmaceutical formulation (tablets), the first depending on regression equation and the second on the standard addition method. The results were obtained with sufficient precision and accuracy, with characterization of the prepared organic reagents and the product of oxidative coupling reaction by FT-IR-spectroscopy.

GRAPHICAL ABSTRACT



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Introduction

Methyldopa MDP chemically known as 2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid ($C_{10}H_{13}NO_4$) (Scheme 1) [1], is a catecholamine derivative widely used in the control of moderate and severe arterial hypertension. Methyldopa is considered a prodrug since it acts mainly due to its metabolism in the central nervous system to a methyl norepinephrine [2, 3]. It works by relaxing the blood vessels so that blood can flow more easily through the body. It is one of the most preferred antihypertensive medications in pregnancy, particularly in complicated cases of pregnancy, and renal failure [4]. Methyldopa is an organic compound characterized as a white or colorless crystalline powder, very slightly soluble in alcohol, practically insoluble in ether [5].

Various analytical methods have been employed for the methyldopa analysis in pharmaceuticals formulations and/or biological specimens, including spectral method, where in assessing active chemicals in pharmaceutical formulations, UV-Vis spectrophotometric approaches are inexpensive, simple to use, and rapid to detect, involving only a few minutes in some instances [6-11], flow injection method [5], high-performance liquid chromatography method [12-14], electrochemical method [15], liquid chromatography method [16], and voltametric method [17] .

Materials and Methods

All the used analytical reagents and chemicals were obtained from Fluka and Sigma-Aldrich with a high purity. The following instruments were used for subsequent measurements: Fourier-transform infrared (FT-IR) Spectrophotometer NICOLT 100. UV-Vis Double-beam spectrophotometer-PG-92 and Vis spectrophotometer 722. Electro thermal melting point apparatus Stuart-SMP 11. HANNA2 pH 211, Microprossor pH meter. Stock solutions of Methyldopa (SDI-Iraq) 250 µg/mL were prepared by dissolving 0.025 g of pure Methyldopa (supplied by the P. M. E. Pharma. Industry Company, Iraq) in 100 mL distilled water. Hydrochloric acid solution of 2 M was prepared by diluting an appropriate volume of conc. HCl. Potassium periodate solution 10^{-2} M was prepared by dissolving 0.230 g of KIO_4 in 100 mL distilled water. Solutions of prepared organic reagents (1%) w/v were prepared by dissolving 1 g of prepared organic reagent in 30 mL of methyl alcohol, and then 100 mL was completed by using distilled water. Analysis of pharmaceutical formulation Tablet solutions of two brands of Aldosam (SDI, Samarra, Iraq) and Aldomet (Algorithm pharmaceutical Company, Lebanon) were used, each tablet containing 250 mg of Methyldopa. Each one is prepared by dissolved an accurately weighed amount of powder, equivalent to one tablet, in 20 mL distilled water, and filtered into 100 mL calibrated flask. Next, the solution was made to the volume with the distilled water. A suitable volume was diluted with distilled water and followed the recommended procedure.

General procedure

3 mL of organic reagent solutions (1% w/v) and 1.5 mL of potassium periodate were added to a series of 25 mL volumetric flask. The resulting oxidizing product was coupled with increasing volume from 250 µg/mL of MDP (5-40 µg/mL) followed by adding 2 mL of 2 M hydrochloric acid to each flask with shaking. After 10 min, the solution was diluted to the mark with distilled water, mixed well, and left to stand for 5 min at room temperature (25 °C). The absorbance was measured at 481 nm at room temperature against reagent blank.

Preparation of Schiff's base compounds (organic reagents)

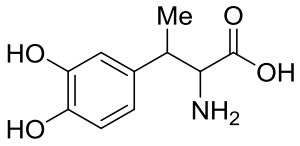
The compounds **1** and **2** indicated in Table 1 were prepared by grinding a mixture consisting of 0.001 mol of 2,6-diaminopyridene with 0.001 mol of each of anisaldehyde and salicylaldehyde separately in a ceramic basin. The mixture was placed in a beaker and 1-2 mL of absolute ethanol and 3-4 drops of glacial acetic acid was added to it, the mixture was irradiated in a microwave oven at medium temperature for 5 minutes. The solid product was washed by petroleum ether several times, followed by recrystallize it with ethanol.

Characterization of prepared organic reagents

The reaction was confirmed by the change in the physical properties such as melting point and color, the prepared compounds were characterized by infrared spectra as follow:

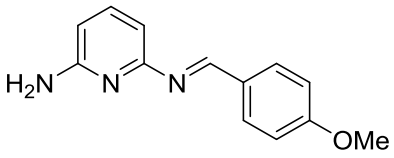
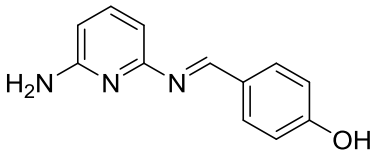
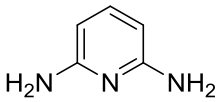
R_1 (Compound 1): Molecular Formula $C_{13}H_{13}N_3O$, yellow crystals, Yield: 84 %, m.p. (178-180 °C), IR (KBr): ύ = 3454 cm^{-1} (asy.), 3389 cm^{-1} (sy.) (Amin group -NH_2), 3164 cm^{-1} (C-H Aromatic), 1612 cm^{-1} (azomethine bond C=N), 1224 cm^{-1} (-OCH_3 group), and 1560-1582 Cm^{-1} (C=C aromatic).

R_2 (Compound 2): Molecular Formula $C_{12}H_{11}N_3O$, Orange crystals, Yield: 76 %, m.p. (167-169 °C), IR (KBr): ύ =3464 cm^{-1} (asy.), 3379 cm^{-1} (sy.) (Amin group -NH_2), 3169 cm^{-1} (C–H Aromatic), 1626 cm^{-1} (azomethine bond C=N), 3413 cm^{-1} (-OH group), and 1494-1572 Cm^{-1} (C=C aromatic).



Scheme 1: Structure of Methyldopa

Table 1: Selection of the best oxidizing reagent

Reagent symbol	Reagent (%)	λ_{max} (nm)	Abs.
R_1 (compound 1)		481	0.543
R_2 (compound 2)		433	0.142
R_3		408	0.218

Results and Discussion

500 µg (2 mL of solution was used at a concentration of 250 µg/mL) of methyldopa at final volume of 25 mL for subsequent experiences and absorbances were measured against the blank solution at 481 nm.

Optimum conditions for the reaction

In an acidic medium, several oxidizing reagents are used to react with MDP. The spectra were measured between 200 and 700 nm. As listed in Table 1, R_1 had the maximum absorption value when combined with MDP. Hence, R_1 was adopted as the best oxidizing reagent in subsequent experiments work.

Acid effect

The oxidative coupling reaction takes place in acidic medium. Various acids such as HCl, H_2SO_4, HNO_3, and CH_3COOH of 1 M have been tested to obtain high sensitivity. It was found that HCl is the best acid for the system (Figure 1) and different volumes (0.5-3.0 mL) of hydrochloric acid was added, the highest absorption of the colored output was given at 2 mL of hydrochloric acid which is recommended in this method (Figure 2).

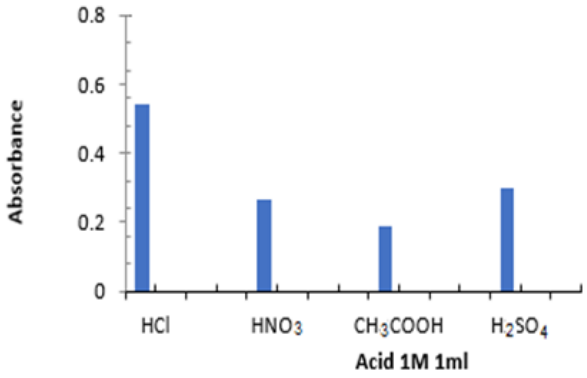


Figure 1: Various acids tested to obtain high sensitivity

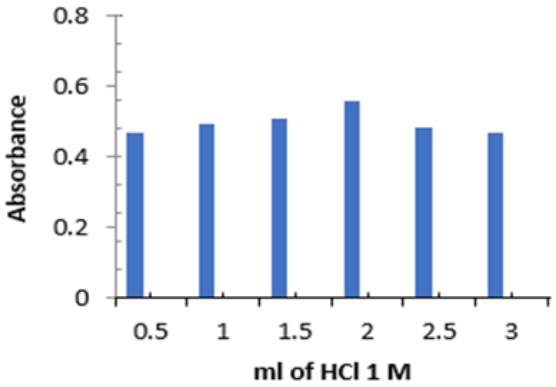


Figure 2: Effect of type and volume of acid on the sensitivity of the method

Effect of prepared organic reagent amount

This effect was investigated by using various quantities (1.0-4.0 mL) of R_1 solution (1%) in the presence of 2 mL of 1M HCl, and measuring the absorption of the solutions at 481 nm. It was found that the volume 2.5 mL of the reagent (R_1) gave the highest absorption value (Figure 3).

Effect of oxidant

Various oxidizing agents such as potassium chromate, potassium permanganate, potassium iodate, and potassium periodate with a concentration of 0.01 M have been tested in the presence of 2 mL of 1M HCl. It was found that KIO_4 is the best oxidant and 1.5 mL of KIO_4 gives the highest color intensity which is recommended in subsequent experiment (Figures 4 and 5).

Effect of coupling reaction time

This effect was studied by analyzing the effect of the reagent's coupling time with MDP (20.00 $\mu g.ml^{-1}$) at different time periods (0-20 minutes) before dilution. The results in Figure 6 show that 10 minutes is enough to complete the coupling process and obtain the colored output.

Effect of temperature

The effect of temperature on the color intensity of the product was studied in practice. The highest absorption was obtained when the colored product was developed at room temperature (25 °C), as displayed in Figure 7.

The Stability of product

The color intensity reached a maximum absorption after the reaction between methyl dopa (20.00 $\mu g.ml^{-1}$) with R_1 and KIO_4 at 5 min. Therefore, 5 min development time was chosen for further use. The obtained results are depicted in Figure 8.

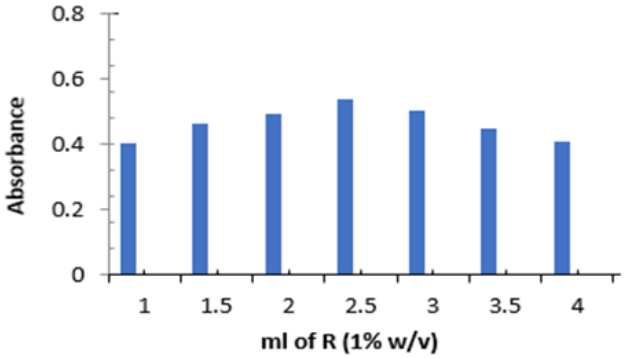


Figure 3: Effect of reagent amount on the sensitivity of the method

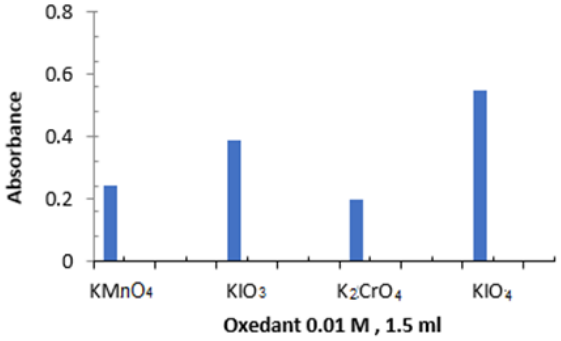


Figure 4: Effect the type of oxidant on absorption

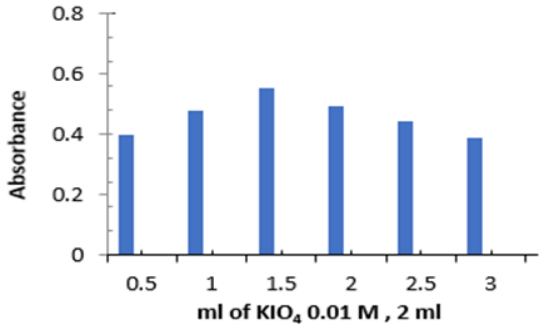


Figure 5: Effect of volume of oxidant on absorption

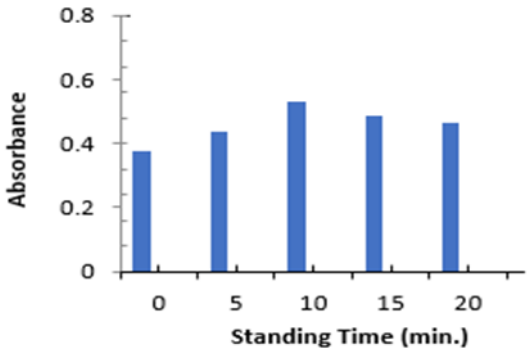


Figure 6: The effect of coupling time on absorption

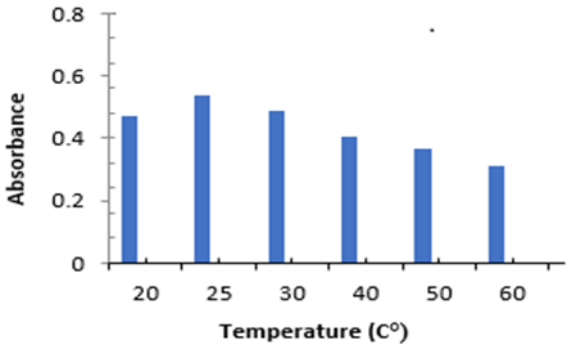


Figure 7: Effect of temperature on absorption

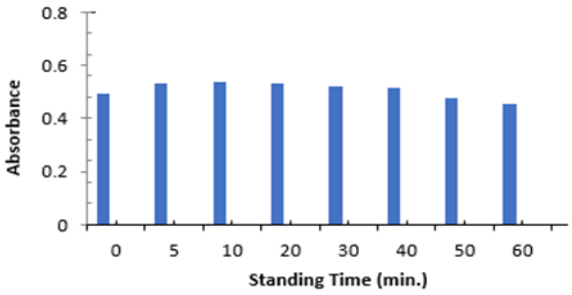


Figure 8: Effect of time on stability of product

Effect of sequence addition

As represented in Table 2, the best sequence that can be followed is (R: KIO_4: Drug: Acid) due to the higher absorption.

Final absorption spectrum

When adding the coupling reagents to a solution containing MDP in acidic medium with the presence of potassium periodate under optimal conditions experimentally a colored product is formed whose absorption spectrum is measured after 5 minutes of completion of additions and dilution. The final absorption spectrum in Figure 9 illustrates a maximum absorption at 481 nm against the blank solution.

Analytical data and the calibration curve

Under the optimum conditions, a linear calibration graph for the MDP determination was obtained over the concentration range of (5-40) $\mu g.ml^{-1}$. The linear regression equation is with correlation coefficient of 0.9979 and the molar absorptivity of the colored product was 6.082 x 10^3 $L.mol^{-1}.cm^{-1}$ and Sandell's index of 0.0347 μgcm^{-2}, which indicates that the method is highly sensitive. The linear calibration graph is demonstrated in Figure 10.

Quantitation

Under the described experimental conditions, standard calibration curves for MDP were drawn by plotting absorbance against concentration, as displayed in Figure 10. The relative standard deviation (RSD) and accuracy (average recovery %) for analyzing four replicates of each three different concentrations of MDP indicated that the method is precise and accurate (Table 3).
Limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the following equations:

$$LOD1= 3.3\sigma/b \text{ and } LOQ1= 10\sigma/b$$

Where, σ is the standard deviation and b is the slope of the calibration curve. The obtained results are in the accepted range below the lower limit of Beer's law range. The summary of optical characteristics and statistical data for the proposed method are presented in Table 4.

Pharmaceutical applications

The proposed method has been successfully applied in the assessment of pharmaceutical preparations containing MDP, which are Aldosam tablets 250 mg and Aldomet tablets 250 mg where three different concentrations of the drug were used with a concentration of 250 μg/mL and the same procedure steps were applied. The results in Table 5 revealed accuracy in determination of each drug.

Table 2: Effect of sequence addition

Addition Sequence	Absorbance at λ_{max}
R + KIO_4 + Drug + Acid	0.548
Drug + KIO_4 + R + Acid	0.097
R + Drug + KIO_4 + Acid	0.125
Acid + Drug + KIO_4 + R	0.218

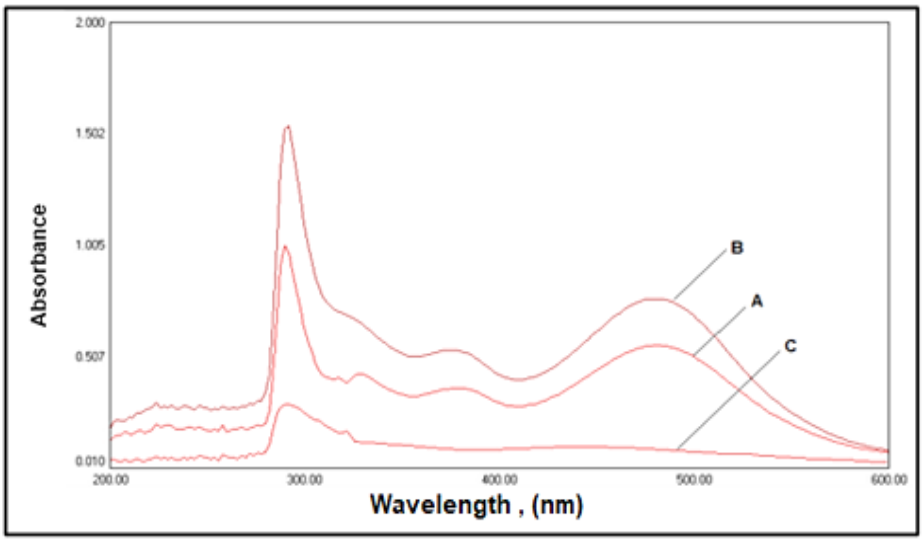


Figure 9: Absorption spectrum of 20 µg/Ml of MDP as measured: (A) vs. blank solution; (B) vs. distilled water, and (C) blank solution vs. distilled water

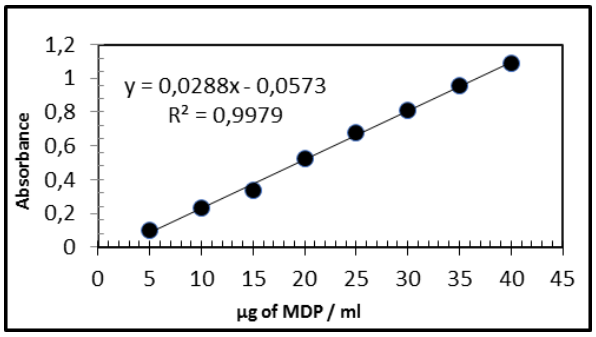


Figure 10: Calibration curve for MDP determination by using oxidative coupling reaction

Table 3: Accuracy and Precision

Drug Conc. µg/mL	RE (%)*	Recovery (%)*	Average Recovery (%)*	RSD (%)*
10	+ 1.51	101.51	101.02	1.534
20	+ 0.92	100.92		0.973
30	+ 0.64	100.64		0.517

**Average of four determinations*

Table 4: Summary of optical characteristics and statistical data for the proposed method

Analytical Parameter	Value
Linearity range (µg/mL)	5-40
Molar absorptivity (l.mol^{-1} . cm^{-1})	6.082 x 10^3
LOD (µg.ml^{-1})	0.8937
LOQ (µg.ml^{-1})	2.708
RSD	0.517-1.534
Sandell's index (µg .cm^{-2})	0.0347
Average recovery* (%)	101.02

**Average of four determinations*

Table 5: Determination of Methyldopa in pharmaceutical preparations by direct method

Drug	Name of drug	Pharmaceutical Type	Drug Conc., (µg/ml)	RE (%)*	Recovery (%)*	Average recovery (%)*
MDP	Aldosam (250 mg)	Tablet	5.0	1.29+	101.29	100.87
			20.0	0.85+	100.85	
			40.0	0.49+	100.49	
	Aldomet (250 mg)	Tablet	5.0	2.66+	102.66	101.38
			20.0	0.90+	100.90	
			40.0	0.58+	100.58	

**Average of six determinations*

Standard addition method

The standard addition method has been used to demonstrate that the proposed method is free of interference. When preparing the calibration curve, the solutions were handled with the same approved procedure, and their absorptions were measured at 481 nm. The results are represented in Figure 11 and Table 6.

Characterization of the product of oxidative coupling reaction by FT-IR spectra

The formed products have been injected in the liquid cell of the IR spectrophotometer and scanned from 4000 cm^{-1} to 400 cm^{-1}. While in the present method, the formed color was attributed to the formation of the product (Scheme 2) which as proved by the appearance of the -C=N function at 1658 cm^{-1}, phenolic group -OH at 3433 cm^{-1}, C=O function at 1607 cm^{-1}, and C=C aromatic at 1581-1484 cm^{-1}.

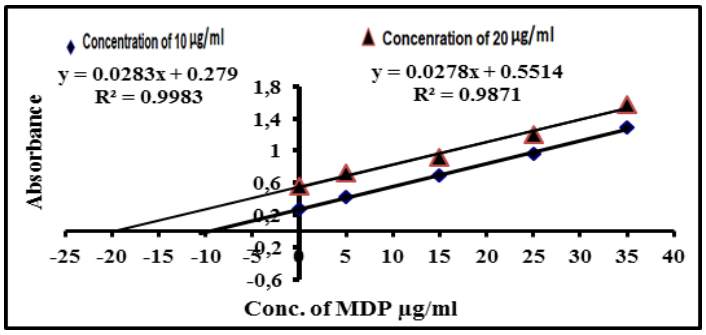
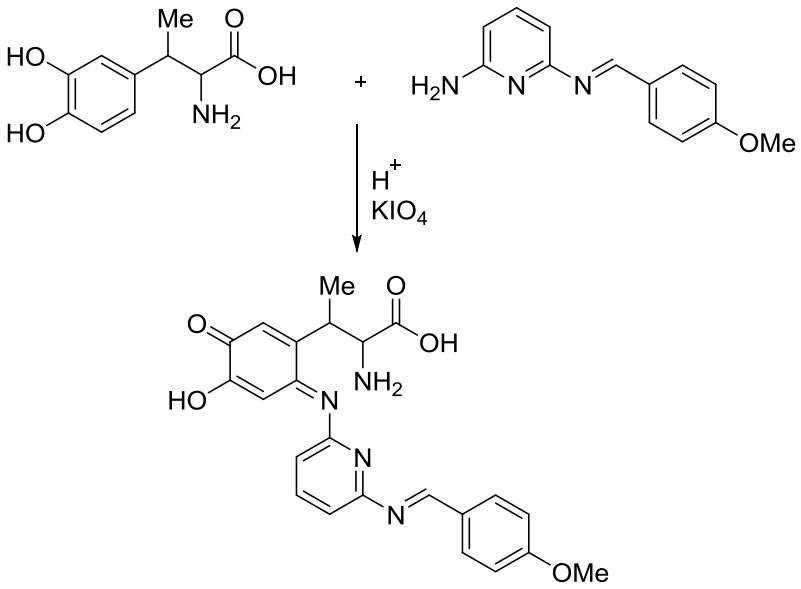


Figure 11: MDP determination by standard addition method in Aldosam tablet solution (250 mg)

Table 6: Standard addition method for estimating MDP in pharmaceutical formulation

Drug	Pharmaceutical Preparation	Present µg/ mL	Measured µg/mL	Recovery (%)
Aldosam (250 mg)	Tablet	10.00	9.85	98.5
		20.00	19.83	99.15



Scheme 2: Coupling between MDP and prepared organic reagent (R_1)

Conclusion

The proposed method offered clear advantages for the fast determination of MDP in pure form and in pharmaceutical preparation. The method was found to be simpler, faster, less expensive, more selective, and accurate than some of the previously published spectrophotometric methods. It offered a good linearity and precision and was applied for the MDP analysis in tablets by using new prepared compounds as coupling reagents.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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