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ORIGINAL ARTICLE

Spectrophotometric Determination of Methyldopa and Levodopa by Oxidative Coupling Reactions using the *synthesis reagent* (2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole) and Investigation of Biological Activity

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KEYWORDS

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Levo-dopa

ABSTRACT: Based on oxidative coupling reactions utilizing the synthesized organic reagent 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole in acidic media, and with potassium dichromate as an oxidizing agent, a spectrophotometric method has been established for determining methyldopa and levodopa. For these drugs, the resultant complex exhibits peak absorption at 401.5 nm and 415 nm, respectively. The method adheres to Beer's law in the ranges of 1-55 µg ml^{-1} and 2.5-170 µg ml^{-1}, with molar absorption coefficients of 0.24 x 10^4 and 0.116 x 10^4 L mol^{-1} cm^{-1}, respectively. The quantification limit (LOQ) is set at 1.5769 µg ml^{-1} for methyldopa and 3.0616 µg ml^{-1} for levodopa, yielding recovery rates of 100.14% and 100.34%, and relative standard deviation rates of 2.748% and 0.779%. The nature of the resulting complex was examined using the continuous variation method (Job's method) and molar ratios, revealing a 1:2 ratio (reagent to drug compound) for both drugs. This method has been successfully applied to pharmaceutical formulations.

INTRODUCTION

The most prevalent heterocyclic atoms in aromatic organic compounds are oxygen, nitrogen, sulfur, and phosphorus [1]. Heterocyclic compounds are characterized by having one or more different atoms within their structure. Thiadiazol compounds are particularly noteworthy, especially in treating blood disorders and atherosclerosis [2]. These compounds demonstrate significant biological efficacy against certain bacteria [3] and effectively treat tuberculosis in the lungs. They also exhibit anti-cancer activities by inhibiting specific cancer cell types [4, 5]. Additionally, thiadiazol compounds are present in pharmaceuticals like the antibiotic Ceftezole [6]. The synthesized reagent, containing two aromatic amine groups Thiadiazol Figure 1, can react with drug compounds to form colored products.

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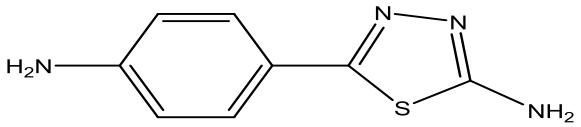

Figure 1. Thiadiazol.

Thiazole compounds are prominent in medical applications [7, 8]. The substituted amine groups on thiazoles, undergoing oxidative coupling [9, 10], can also transform into diazonium salts [11, 12], serving as effective reagents in pharmacology [13]. Methyldopa, a catecholamine, acts as an amino acid antagonist and an antihypertensive agent affecting the central nervous system. It antagonizes alpha-adrenergic receptors and stimulates alpha-2 receptors, leading to vasodilation and blood pressure reduction [14]. Despite its reduced usage due to the availability of drugs with fewer side effects, methyldopa remains vital for treating hypertension in developing countries, owing to its cost-effectiveness. However, it has side effects like drowsiness and gastrointestinal issues [15,16]. Methyldopa's chemical structure is characterized by an alkylamine chain linked to a benzene ring with a hydroxyl group (catechol) Figure 2.

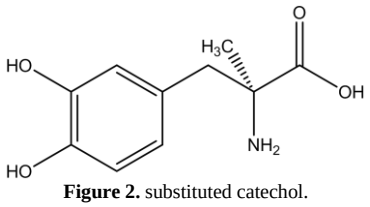

Figure 2. substituted catechol.

Levodopa, a natural amino acid, plays a crucial role in transmitting nerve impulses within the body and is used to treat Parkinson's disease, which arises from dopamine depletion in the brain. Unlike dopamine, which cannot cross the blood-brain barrier due to its lack of fat solubility, levodopa can cross this barrier. Once in the brain, levodopa undergoes metabolic transformation into dopamine, particularly in the basal ganglia, through the removal of the carboxyl group Figure 3. [17]. This process makes it effective in treating Parkinson's disease symptoms.

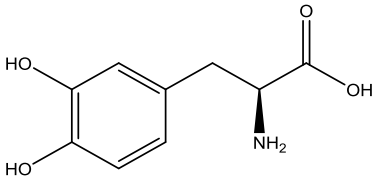

Figure 3. Substituted catechol.

Various methodologies have been employed to estimate methyldopa and levodopa concentrations, including spectral methods, chromatography, and electrochemical techniques. These diverse analytical approaches, encompassing spectroscopy [18-28], chromatographic techniques [29-33], and electrochemical methods [34-37], offer a range of accuracy and sensitivity levels suitable for different applications in pharmaceutical analysis.

MATERIALS AND METHODS

A spectrophotometer Double beam-Spectrophotometer Shimadzu UV-1800 PC, UV-Visible was used to conduct some experiments in the current study.

Synthesis of reagent 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole

To synthesize a specific compound, a detailed procedure was followed involving the dissolution of ρ-aminobenzoic acid in concentrated sulfuric acid, the addition of thiosemicarbazide, and subsequent heating. After cooling the mixture and adding ice, sodium hydroxide was used for neutralization. The resulting precipitate was then isolated and purified through

recrystallization using absolute ethanol. This process, detailed in the methodology, is essential for the successful synthesis of the compound [38], as indicated by the following reaction Figure 4. The constants and physical properties are shown in Table 1.

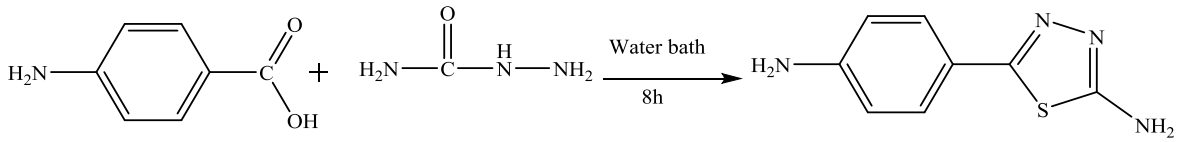

Figure 4. Synthesis of substituted thiadiazol ring.

Table 1. The physical constants and properties of the synthesized reagent.

Molecular formula	Molecular weight	M.P.(℃)	Yield (%)	Color
$C_8N_4SH_8$	192	166-169	98%	Pale yellow

MTT experiment

The mechanism of thiadiazol-substituted pyridazine pigment reversion is typically associated with NAD (P) H-dependent oxidoreductase enzymes in the cell's cytosol. The rate of this reversion is dependent on cellular metabolic activity, influenced by NAD (P) H levels. Cells with lower metabolic rates, like thymocytes and spleen cells, show minimal pigment reversion, while rapidly dividing cells exhibit higher rates. Test conditions can affect metabolic activity and, consequently, pigment reversion, without necessarily impacting cell viability. Additionally, the method of pigment reversion assessment whether intracellular (MTT, MTS) or extracellular (WST-1) influences the measured output. Evidence suggests spontaneous MTT upregulation in certain cell structures and lipid compartments, indicating that the MTT assay reflects cellular retroviral capacity, crucial for understanding cell processes and viability.

Chemical solutions

The preparation of solutions for Methyldopa, Levodopa, 2-Amino-5-(para-aminophenyl)-1,3,4-thiadiazole, Potassium dichromate, and Phosphoric acid involves precise measurements and dissolution in distilled water. Methyldopa and Levodopa solutions were synthesized at concentrations of 400 and 600 micrograms ml^{-1}, respectively, by dissolving specific amounts of the substances in 100 ml of distilled water. The thiadiazol compound is prepared at a $1x10^{-3}$ M concentration, while Potassium dichromate is prepared at $1x10^{-2}$ M. Phosphoric acid solution is prepared at 0.1 M concentration using a defined volume of concentrated acid diluted to 100 ml with distilled water. These preparations are essential for ensuring accurate concentrations for further experimental procedures.

MTT assay

In the MTT assay to assess the effects of thiadiazol, HdFn (Primary human dermal fibroblasts from newborn foreskin) and MCF-7 (breast cancer cells) were used. The cells were cultivated and incubated in a CO_2 environment at 37 °C. Serial dilutions of thiadiazol were synthesized and applied to MCF-7 cells, followed by incubation. MTT solution was then added to the wells, incubated further, and after medium removal, a solubilizing solution was used to dissolve formazan crystals. Absorbance was measured using an ELISA reader at 575nm (Bio-rad, Germany), and the results were analyzed statistically using Graph Pad Prism.

RESULTS AND DISCUSSION

The Figure 5. was infrared spectroscopy of the synthesized reagent Figure 6. reveals distinct bands at 3458, 3379, 3360, 3230 cm^{-1} corresponding to different structural groups, with specific frequencies observed for aromatic NH_2 stretching, aromatic C-H stretching at 3050 cm^{-1}, and two bending aromatic C-H bands at 925.8,887 cm^{-1}, structural vibration beams at 1597,1517.9,1440.8,1419.6cm^{-1}, two beams at 1658.7 cm^{-1}, two beams at 1170.7,1128cm^{-1}. The H^1NMR

spectrum shows characteristic signals at 6.24 of NH on the heterocyclic ring, aromatic NH on the benzene ring, and a dimeric aromatization pattern as described in Figure 7. The C_{13} spectrum displays signals at 168.05 for C_1, a signal of 153.51 for C_2, a double signal for C_3, and C_6, a double signal of 117.47 for C_4, C_8, and a double signal of 113.03 for C_5, C_7 as shown in Figure 8.

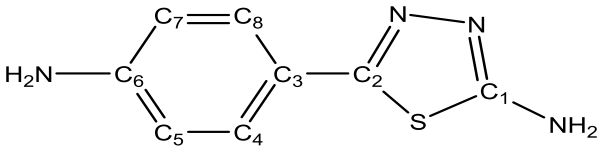

Figure 5. Reagent prepared .

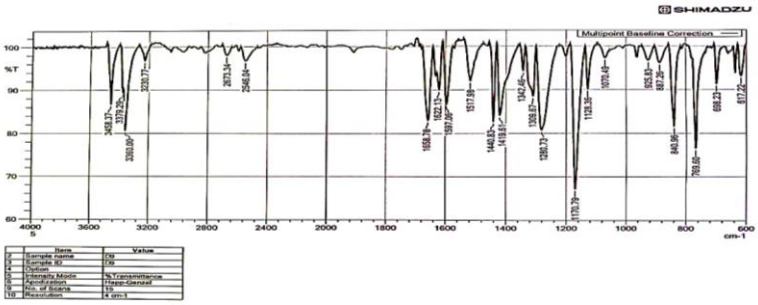

Figure 6. Infrared radiation of the synthesized reagent.

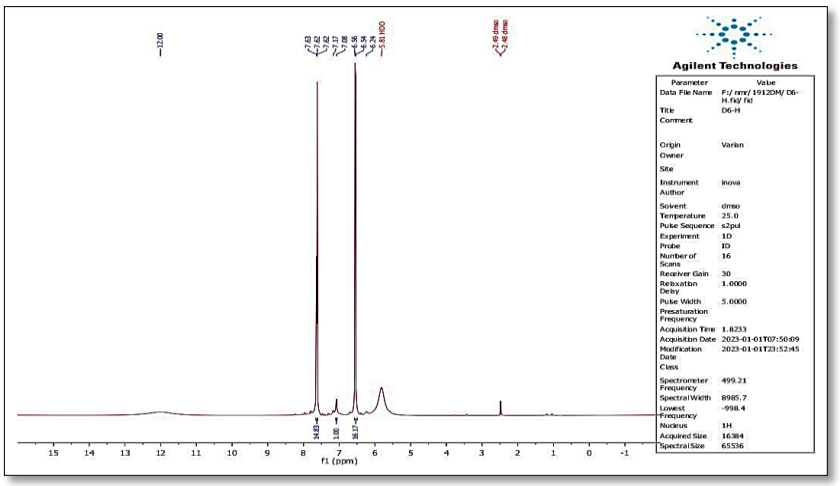

Figure 7. NMR spectrum of the synthesized reagent.

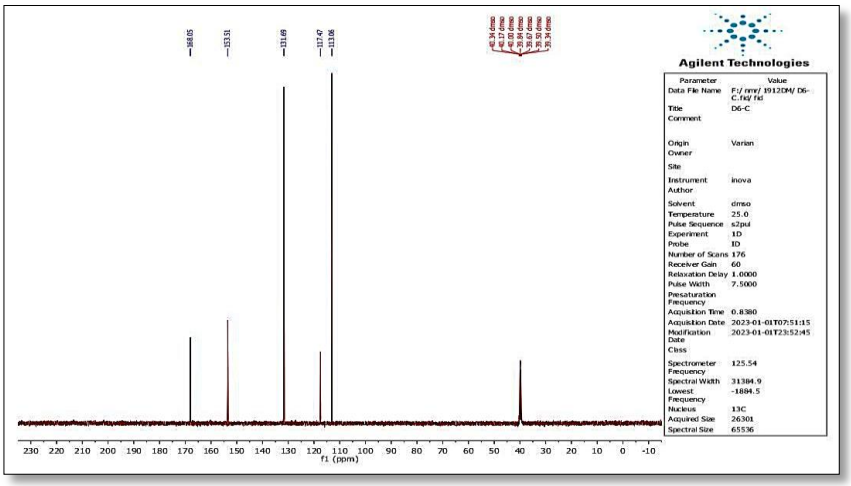

Figure 8. C^{13} spectrum of the synthesized reagent.

Set optimal conditions

Subsequent studies were carried out using concentrations of 40, and 60 µg ml^{-1} each of methyldopa and levodopa, respectively, by withdrawing 1 ml of 400, and 600 µg ml^{-1} of methyldopa and levodopa solutions, respectively, and adding 1 ml of thiadiazol reagent and 1 ml of potassium dichromate and 1 ml of hydrochloric acid in a final volume of 10 ml, and the adsorption of the product formed was measured at 401.5 and 415 nm for methyldopa and levodopa, respectively, against the mock solution.

Study the effect of reagent size

The effect of adding different volumes of the synthesized reagent (2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole) on the absorption of the complex formed against the mock solution of both methyldopa and levodopa, was studied. Table 2 below shows the best volume of the reagent is 1.25 and 1.0 ml for methyldopa and levodopa, respectively, it was adopted in the subsequent studies.

Table 2. Examination of the effect of volume reagent conc. 1×10^{-3}M with Absorbance of methyldopa and levodopa

Volume (ml) of Reagent 1×10^{-3}M	Absorbance L mol^{-1} cm^{-1}	
	Methyldopa	Levodopa
0.5	0.11	0.202
0.75	0.2	0.215
1.0	0.215	0.264
1.25	0.238	0.251
1.5	0.218	0.236

Study the effect of the type of oxidizing agent

In the study exploring various oxidizing agents for methyldopa and levodopa, it was determined that potassium dichromate, at a concentration of 0.01 M, yielded the highest absorption for both substances at the longest wavelength. Consequently, potassium dichromate was selected as the oxidizing agent for further studies. This finding, as illustrated in Figure 9, underscores the efficacy of potassium dichromate in enhancing the absorption characteristics of these compounds.

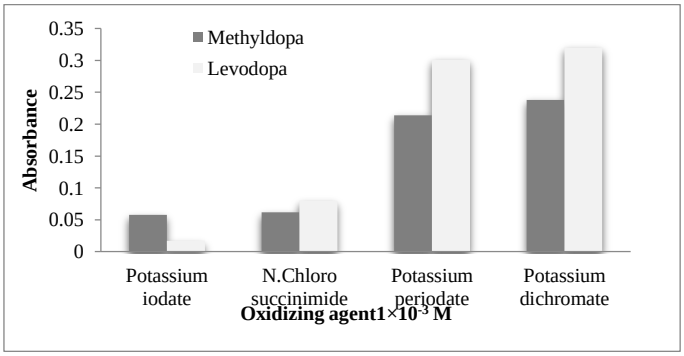

Figure 9. The effect of the type of oxidizing agent.

Study the effect of different volumes of potassium dichromate

After selecting potassium dichromate as the oxidizing agent, research was conducted to ascertain the ideal quantity required for optimal results. The study involved incrementally increasing the amounts of potassium dichromate added. According to the results presented in Table 3, the most effective amounts were determined to be 0.2 ml for methyldopa and 1.0 ml for levodopa. These quantities were then utilized in subsequent studies for

consistent and optimal outcomes.

Table 3. Study of the amount of oxidizing agent with Absorbance of methyldopa and levodopa

Volume (ml) of $K_2Cr_2O_7$ (1×10^{-2} M)	Absorbance L mol^{-1} cm^{-1}	
	Methyldopa	Levodopa
0.1	0.242	0.023
0.2	0.294	0.037
0.5	0.223	0.098
0.75	0.198	0.169
1.0	0.238	0.264
1.25	0.157	0.201
1.5	0.131	0.175

Study the effect of acid-type

In this study, the objective was to identify the most suitable acid by adding 1 ml of various acids at a concentration of 0.1 M to enhance sensitivity. The results, depicted in Figure 10, indicated that phosphoric acid led to the highest absorption for both methyldopa and levodopa. Based on these findings, phosphoric acid was selected for use in further studies.

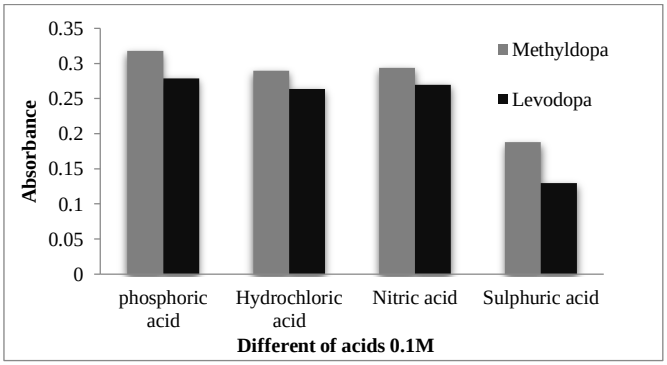

Figure 10. Study of the type of different acid.

Study the effect of different volumes of acid

In the study to determine the optimal amount of phosphoric acid for use in assays involving methyldopa and levodopa, varying volumes of acid were tested. As per Table 4, the ideal volume was found to be 0.75 ml for methyldopa and 1.0 ml for levodopa. These quantities were then standardized for use in subsequent experiments.

Table 4. study of the amount of acid with Absorbance of methyldopa and levodopa.

Volume (ml) of H_3PO_4 (0.1M)	Absorbance L mol^{-1} cm^{-1}	
	Methyldopa	levodopa
0.5	0.289	0.241
0.75	0.332	0.263
1.0	0.321	0.279
1.25	0.324	0.257
1.5	0.301	0.240

Study the effect of temperature

This study investigated how different temperatures affect the absorption of the complex formed with methyldopa

and levodopa. Figure 11 illustrates that the most effective temperature for oxidation and conjugation, resulting in the highest absorption at the longest wavelength, differs for the two compounds. Methyldopa showed high stability for up to two hours at this optimal temperature, whereas levodopa demonstrated less stability, maintaining its stability for about 20 minutes only.

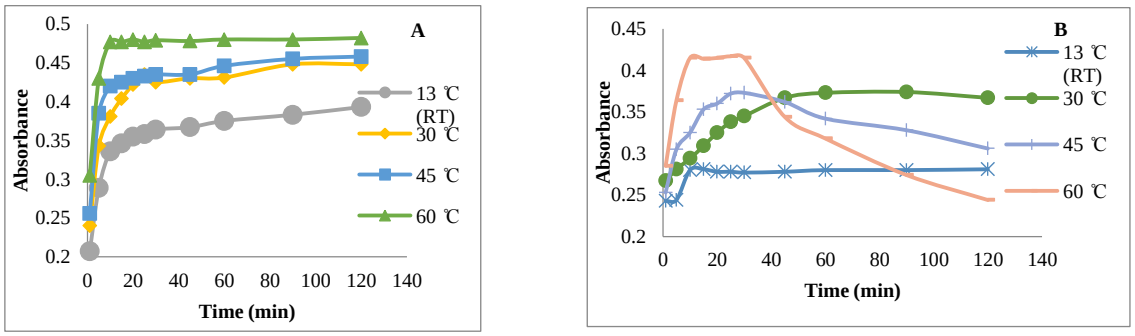

Figure 11. Study of the effect of temperature A: methyldopa, B: levodopa

Study the effect of the addition sequence

The study assessed how the sequence of adding components affects the absorption values of the complex formed. The results, as detailed in Table 5, indicated that the arrangement previously adopted in studies yielded the highest absorption. Consequently, this specific arrangement was confirmed for use in subsequent research.

Table 5. study of the effect of the addition sequence

Order Number	Reaction component	Absorbance L mol^{-1} cm^{-1}	
		Methyldopa	Levodopa
1	D+R+O+A	0482	0.416
2	D+R+A+O	0.438	0.316
3	D+O+A+R	0.309	----------
4	D+O+R+A	0.44	0.398
5	D+A+R+O	0.451	0.344
6	D+A+O+R	0.35	0.282
7	R+O+D+A	0.434	----------
8	R+O+A+D	0.462	----------
9	R+A+D+O	0.432	----------
10	R+A+O+D	0.377	----------

D: Levodopa;Methyldopa , R:reagent, O: $K_2Cr_2O_7$,A:H_3PO_4

Study the effect of surface tensile factors

In this study, the effect of adding 1 ml of various surfactant agents (positive, negative, and neutral) on the absorption of the formed methyldopa and levodopa complexes was examined. The results, as shown in Table 6, indicated that the surfactants did not significantly impact the absorption of either complex. Therefore, surfactants were excluded from further consideration in subsequent studies.

Table 6.. study of the effect of surface tensile factors

1ml of 0.1% Surfactant	Methyldopa		Levodopa	
	Absorbance L mol^{-1} cm^{-1}	λ_{max}(nm)	Absorbance L mol^{-1} cm^{-1}	λ_{max}(nm)
Without	0.482	401.5	0.416	**415**
CPC	0.191	411	Turbid	**Turbid**
SDS	Turbid	Turbid	Turbid	**Turbid**
Tween-20	0.324	402.6	0.255	**419**

Final absorption spectrum

Upon establishing the optimal conditions for methyldopa and levodopa, the absorption spectra of both compounds were plotted against their respective mock solutions. The results showed that the highest absorption for methyldopa occurred at 401.5 nm, and for levodopa at 415 nm, as illustrated in Figure 12. This finding was crucial for confirming the specific absorption characteristics of each compound under the determined optimal conditions.

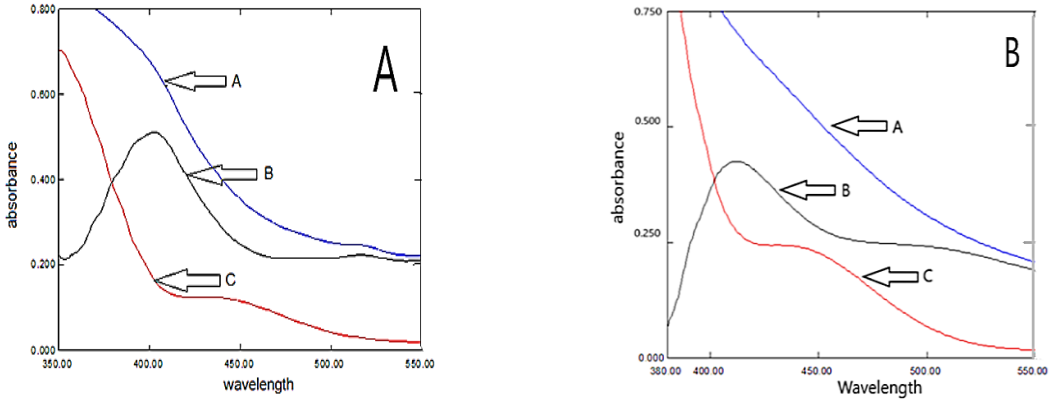

Figure 12. Final absorption spectrum

A: Methyldopa complex (40 µg ml^{-1}), B: Levodopa complex (60 µg ml^{-1})

A: complex solution of drug compound versus distilled water, B: complex solution of drug compound versus solution blank, C: blank versus distilled water

Standard curve

Applying the optimal conditions listed in Table 7, the standard curve was drawn for the determination of each methyldopa and levodopa in the aqueous solution by taking different concentrations ranging between (1-55) and (2.5-170) µg ml^{-1} of methyldopa and levodopa, respectively. As shown in Figure 13.

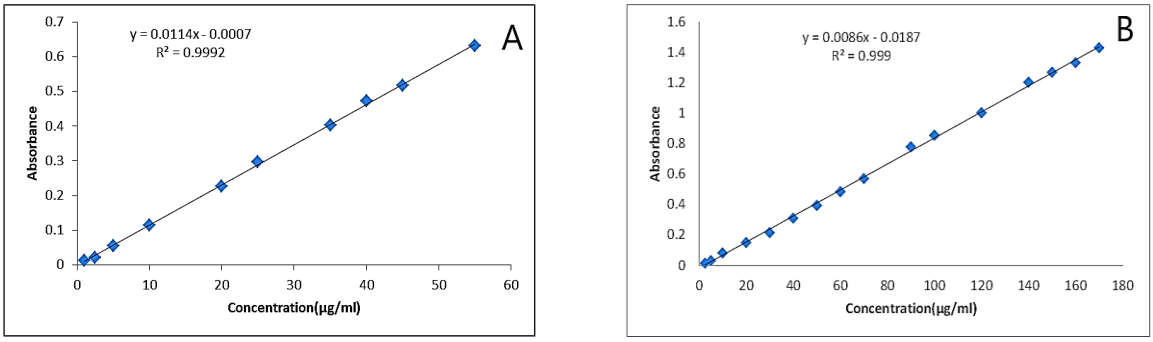

Figure 13. Standard curve: A for methyldopa: B for levodopa

Table 7. Summary of optimal conditions

Experimental conditions	Methyldopa	Levodopa
λ_{Max}(nm)	401.5	**415**
2-amino-5-(ρ-aminophenyl)-1,3,4-thiadiazol 1×10^{-3}M (ml)	1.25	**1**
$K_2Cr_2O_7$ 1×10^{-2}M (ml)	0.2	**1**
H_3PO_4 0.1 M (ml)	0.75	**1**
Temperature (°C)	60	**60**
Development Time (min.)	10	**10**
Stability period (min.)	≤110	**20**

Method accuracy and compatibility

To evaluate the method's accuracy and compatibility for methyldopa, five readings at four different concentrations were analyzed. The results, as presented in Table 8, demonstrated that the method possesses good accuracy and compatibility, indicating its reliability for measuring methyldopa concentrations.

Table 8. Accuracy and compatibility of the method.

Compound	Amount added(µg ml^{-1})		Recovery* (%)	Average Recovery (%)	RSD*(%)
	Taken	Found			
Methyldopa	2.5	2.43	97.2	100.14	3.076
	5	4.913	98.26		3.885
	20	20.29	101.49		2.831
	45	46.65	103.66		1.20
Levodopa	5	5.07	101.4	100.34	2.559
	50	49.87	99.74		0.324
	100	100.082	100.08		0.1477
	150	150.22	100.15		0.087

* Average of Five determinations

Study the nature of the resulting product

In the study, Job's method and molar ratios were utilized to investigate the nature of the complex formed from the interaction between methyldopa and levodopa with the synthesized thiazole reagent. This approach helped in understanding the stoichiometry and chemical behavior of the complex in the reaction.

Continuous changes method

The Job method was applied to diluted solutions to determine the synthetic molar ratios. The total volume of the drug compound and the reagent in the mixture was maintained at 3 ml, with a final volume of 10 ml. Both the drug compound and the reagent had equal concentrations, with 2×10^{-3} M for methyldopa and 5×10^{-4} M for levodopa. The results, as depicted in Figure 14, revealed that the complex's ratio is 1:2 between the drug compound and the reagent 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole for both drugs.

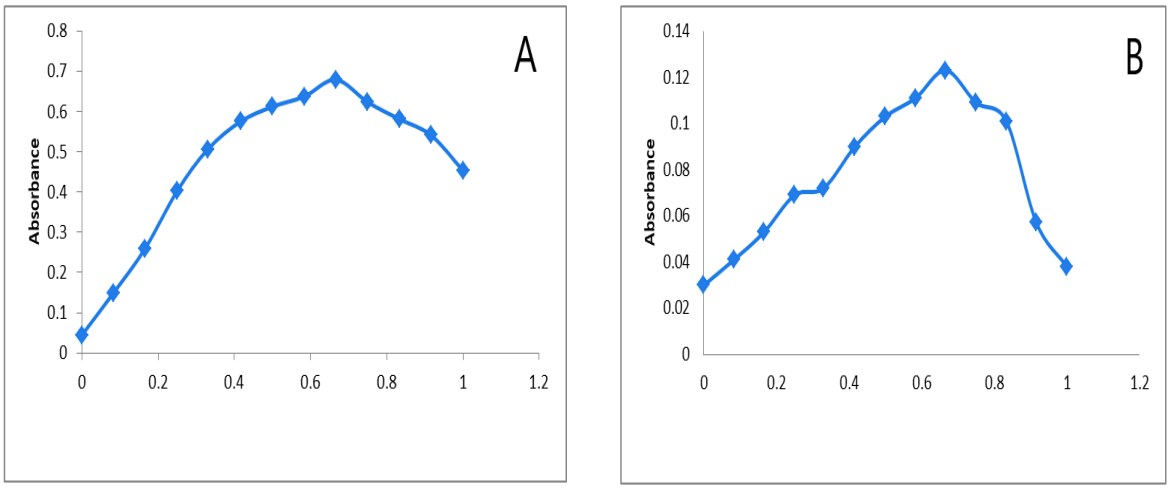

Figure 14. Method of continuous changes A: for methyldopa: B for levodopa.

Molar ratio method

To corroborate the findings from Job's method, the molar ratio method was employed. This involved adding increasing volumes (0-3 milliliters) of the drug compound to a fixed 1-milliliter volume of the reagent 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole. Both substances were at equal concentrations in a final volume of 10 ml, specifically 0.00189 M for methyldopa and 0.0005 M for levodopa. The results, shown in Figure 15, confirmed the accuracy of the percentages derived from Job's method.

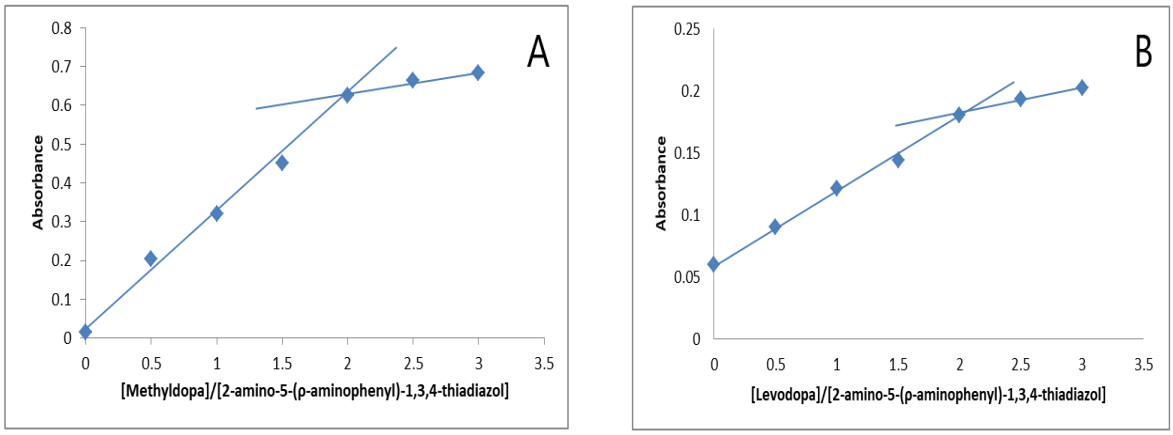

Figure 15. Molar ratio method A: for methyldopa B: for levodopa.

Proposed chemical reaction for methyldopa and levodopa

In the reaction with 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole, both methyldopa and levodopa form complexes in a 1:2 ratio Figure 16. Methyldopa results in a light brown complex, while levodopa forms a brown complex. This reaction occurs in the presence of potassium dichromate as an oxidizing agent within an acidic medium. The specific mechanism proposed for this reaction helps in understanding the complex formation process.

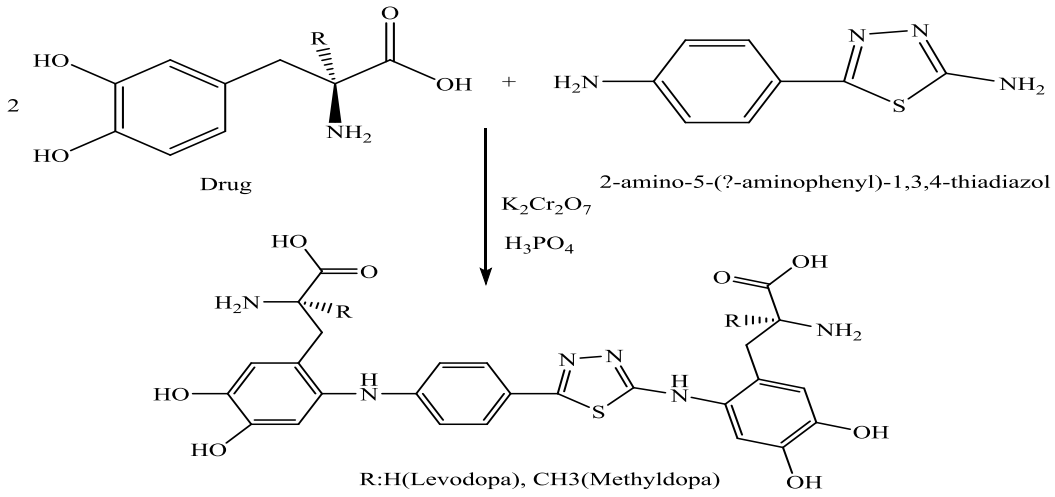

Figure 16. Reaction of the reagent with drug.

Calculate the stability constant for the products formed

The stability constant of the complexes formed between both methyldopa and levodopa and the reagent 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole was calculated. The ratio for the formation of these complexes was determined to be 1:2. As indicated in Table 9, the stability constant for the complexes is notably high, suggesting a strong interaction between the drug compounds and the reagent in the formed complexes.

Table 9. The stability constant of the two complexes formed

Compound	Conc.(mol l^{-1})	Absorbance L mol^{-1} cm^{-1}		α	Average K_{st} (l^2 mol^{-2})
		As	Am		
Methyldopa	9.5×10^{-5}	0.129	0.197	0.347	**$1.66x10^9$**
	1.9×10^{-4}	0.423	0.487	0.130	
	2.8×10^{-4}	0.545	0.622	0.124	
Levodopa	2.5×10^{-5}	0.0236	0.059	0.5989	**$4.84x10^9$**
	5×10^{-5}	0.0546	0.086	0.3643	
	7.6×10^{-5}	0.0873	0.1023	0.1466	

Applying the developed method to pharmaceutical preparations

Analysis of methyldopa tablets

Using the standard curve of methyldopa in its pure form, the concentration of methyldopa in the tablets was found. Table 10 shows the obtained results.

Table 10. Determination of methyldopa in pharmaceutical preparations.

Pharmaceutical Preparation	Certified Value(mg)	Amount Present(μg ml^{-1})		Drug content found* mg	Recovery (%)*	Average Recovery (%)
		Taken	Found			
Tablets Bristol Laboratories ltd	250	2.5	2.58	258.0	103.2	**101.01**
		5	4.930	246.5	98.60	
		20	19.747	246.85	98.74	
		45	46.53	258.5	103.40	
Tablets S.D.I.-IRAQ	250	2.5	2.513	251.30	100.52	**100.225**
		5	4.913	245.65	98.26	
		20	19.857	248.23	99.29	
		45	46.28	257.1	102.84	

From Table 10, it can be concluded that the developed method for the determination of methyldopa in its pharmaceutical formulation is of high accuracy.

Evaluation of the results of the proposed method with the standard addition method

The efficiency of the developed method for estimating methyldopa was validated using the standard addition method. The agreement of the results obtained, as displayed in Figure 17 and Table 11, with those predicted by the proposed method indicates its high selectivity. This congruence reinforces the method's reliability and effectiveness in selectively determining methyldopa.

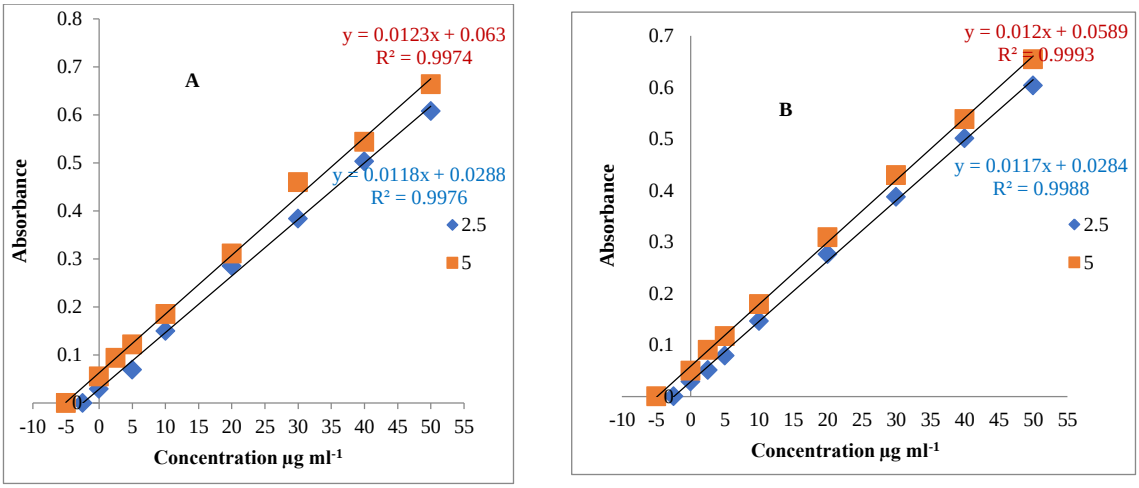

Figure 17. Standard addition curve for the determination of methyldopa in pharmaceutical.

Table 11. Determination of methyldopa by the standard and suggested addition methods

Pharmaceutical preparation	Certified value (mg)	Amount present (µg ml^{-1})	Drug content found (mg)		Recovery (%) of standard addition procedure
			Present method*	Standard addition procedure	
Tablets	250	2.5	258.00	244.07	97.63
Bristol Laboratories ltd		5.0	246.5	256.10	102.44
Tablets	250	2.5	251.30	242.725	97.09
S.D.I.-IRAQ		5	245.65	245.416	98.166

Results of the MTT Assay

The relationship between viability and concentration of thiadiazol, as shown in Figure 18 and Table 12, reveals its significant IC_{50} value for normal cells and anti-tumor efficacy against the MCF-7 cell line. The activity of 2-amino-5-(p-aminophenyl)-1,3,4-thiadiazole against breast cancer cells increases with concentration, peaking at 200 µg mL^{-1}, significantly reducing cancer cell viability. In contrast, lower doses exhibit minimal effects on normal HdFn cells. This indicates that 2-amino-5-(p-aminophenyl)-1,3,4-thiadiazole is potent against MCF-7 cells at higher doses while having a minimal impact on normal cells across all tested concentrations.

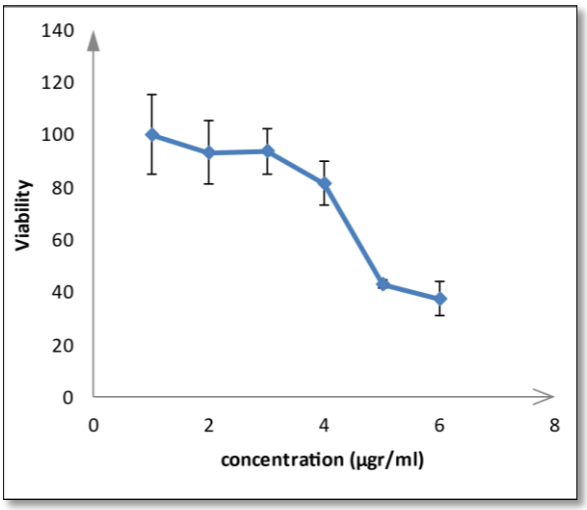

Figure 18. The relationship between viability and concentration.

Table 12. The relationship between viability and concentration.

Concen.	MCF-7		HdFn	
	Mean	SD	Mean	SD
200.00	65.93	2.79	72.30	3.55
100.00	72.44	4.19	83.72	1.39
50.00	77.62	2.70	94.52	0.71
25.00	91.60	3.43	95.45	0.64
12.50	94.17	3.44	95.06	1.24

DISCUSSION

The synthesis of thiadiazoles as potential cancer treatments has been a focus of research over the past 20 years, yielding several significant compounds targeting various cellular and enzymatic pathways. For example, Amgen's development of AMG 900 1, a potent pan-aurora kinase inhibitor, has shown effectiveness against taxane-resistant tumor cell lines [39]. An AZD1152-resistant HCT116 variant cell line that carries the aurora B mutation (W221L) [40] was responsive to AMG 900 1. After that, two selective and orally accessible thiadiazole derivatives aurora kinase inhibitors were found by Cee et al. [41]. Vatalanib (PTK787) 2 [42] (Figure 12) has an IC_{50} of 380 and 20 nM, respectively, and inhibits both VEGFR-1 and VEGFR-2. At both the preclinical and clinical levels [43], PARP inhibitors are also being explored in cancer treatment, with 2-amino-5-(p-aminophenyl)-1,3,4-thiadiazole derivatives showing promise as orally accessible agents [44-45].

It was clear from the data that the produced 2-amino-5-(p-aminophenyl)-1,3,4-thiadiazole had good to mild growth-inhibitory efficacy against the cancer cell lines under study. MCF-7 was shown to be the cell line that was most susceptible to the novel compounds' cytotoxic effect, according to research. Additionally, due to its exceptional pharmacological qualities, thiadiazole Core has constantly attracted researchers' attention and has grown into a thriving area of research 35–38. It is known as "privileged scaffolds". The thiadiazole core, recognized as a "privileged scaffold," continues to attract research for its high affinity in drug development, particularly in cancer therapy [46-51]. The thiazole ring is regarded as a primary scaffold present in different bioactive compounds because of the aforementioned wide range of structural properties. These derivatives have emerged as gifted cores that need additional development to produce more potent and selective cytotoxic medicines. The pyridine ring-containing thiadiazole derivatives exhibit anticancer activity, and interesting outcomes were found with halogens, methoxy, and dimethylamino groups effective against breast cancer cell lines [52].

Biological efficacy

Antibacterial testing were carried out using the agar diffusion technique. Antimicrobial activity were evaluated by measuring the diameter of the zone of inhibition against test organisms after incubation at 37°C for a 24-hour period, following the observed zone of inhibition value [53]. The compounds discussed in this manuscript demonstrated effectiveness against both gram-positive and gram-negative bacteria. This activity is detailed in Table 13, where the measured biological activities of the synthesized compounds are presented. The data showcases the compounds' potential in addressing bacterial infections. Pathogenic microorganisms cause serious and deadly infectious illnesses. Heterocyclic rings are crucial in organic compounds with biological activity used as medications in human and veterinary medicine or insecticides and pesticides in agriculture. Thiadiazoles, nitrogen–sulfur heterocycles, are widely used as biologically active molecular structural units and medicinal chemistry intermediates. Many 2-amino-1,3,4-thiadiazole derivatives can be used to synthesize pharmaceuticals, and several have shown stronger antibacterial efficacy than traditional medications[54].

Table 13. shows the biological activity of the 2-amino-5-(p-aminophenyl)-1,3,4-thiodiazole.

Comp No.	*Staphylococcus aureus*		*Escherichia Coli*	
	10 (mg ml^{-1})	**1 (mg ml^{-1})**	**10 (mg ml^{-1})**	**1 (mg ml^{-1})**
2-amino-5-(p-aminophenyl)-1,3,4-thiadiazole	20	24	17	**19**
Control (DMSO)	-	-	-	**-**
Amoxicillin 25mg	25		**-**	
Ciprofloxacin 5 mg	**-**		**15**	

CONCLUSIONS

A spectrophotometric method has been developed to determine microgram quantities of methyldopa and levodopa using the reagent 2-amino-5-(para-aminophenyl)-1,3,4-thiadiazole. The method, based on oxidative coupling, exhibits good accuracy and compatibility, with specific wavelengths of 401.5 nm for methyldopa and 415 nm for levodopa. It has been successfully applied in pharmaceutical tablets for methyldopa and pharmaceutical preparations for Levodopa. The method's simplicity and sensitivity are noteworthy, and it operates effectively in an aqueous medium. The method's results align well with those from the standard addition method.

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Conflict of interest

There are no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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