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SPECTROPHOTOMETRIC DETERMINATION OF THE METHYLDOPA AND LEVODOPA THROUGH OXIDATIVE COUPLING REACTIONS USING THE PREPARED REAGENT [2-AMINO-5-(PARA-AMINOPHENYL)-1,3,4-OXADIAZOLE], MOSUL, IRAQ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Ismail Younis Ismail KHALIL

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January 2024

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

SPECTROPHOTOMETRIC DETERMINATION OF THE METHYLDOPA AND LEVODOPA THROUGH OXIDATIVE COUPLING REACTIONS USING THE PREPARED REAGENT [2-AMINO-5-(PARA-AMINOPHENYL)-1,3,4-OXADIAZOLE], MOSUL, IRAQ

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Master of Science in Chemistry

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January 2024

This study involved the spectrometric determination of primary amine-containing pharmaceutical compounds. 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole was synthesized and used as a reagent for the analysis of the pharmaceutical compound's methyl-dopa and levodopa using the oxidative coupling method. The UV-spectra for methyl-dopa and levodopa give light brown and brown color in solution after treatment with oxidizing agents with λ_{Max} of 404 nm and 417 nm respectively. The optimal concentrations determined with the best reagent volume of 0.75 mL for methyl-dopa and 1.0 mL for levodopa was 40 µg/mL and 30 µg/mL, respectively. Moreover, Potassium dichromate was found to be the most effective oxidizing agent. In addition, the limit of detection (LOD) obtained for both drugs was1.021 µg/mL and 4.125 µg/mL, while the limit of quantitation (LOQ) obtained was 2.762 µg/mL and 8.143 µg/mL, respectively. The average percentage recovery for each drug was almost 99.663 and 100.06%, with relative standard deviations of (1.357, 1.246) %, respectively. The developed method was applied to pharmaceutical preparations with accuracy and high precision.

2024, 63 pages

Keywords: Oxidative coupling, Spectrophotometric determination, Oxadiazole, Methyl-dopa, Levodopa

ÖZET

HAZIRLANMIŞ REAKTİF [2-AMİNO-5-(PARA-AMİNOPHENYL) 1,3,4-OXADİAZOL] KULLANARAK METİLDOPA VE LEVODOPA'NIN OKSİDATİF BAĞLANTI REAKSİYONLARI İLE SPEKTROFOTOMETRİK TAYİNİ, MOSUL, IRAK

Ismail Younis Ismail KHALIL

Kimya, Yüksek Lisans

Tez Danışmanı: Doç. Dr. Ruken Esra DEMİRDÖĞEN

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Ocak 2024

Bu çalışma, birincil amin içeren farmasötik bileşiklerin spektrometrik olarak belirlenmesini içeriyordu. 2-amino-5-(para-aminofenil)-1,3,4-oksadiazol sentezlendi ve oksidatif birleştirme yöntemi kullanılarak farmasötik bileşiğin metil-dopa ve levodopa analizi için bir reaktif olarak kullanıldı. Metil-dopa ve levodopa için UV spektrumları, sırasıyla λ_{Max} 404 nm ve 417 nm olan oksitleyici maddelerle işlemden sonra çözeltide açık kahverengi ve kahverengi renk verdi. Metil-dopa için 0,75 mL ve levodopa için 1,0 mL'lik en iyi reaktif hacmiyle belirlenen optimal konsantrasyonlar sırasıyla 40 µg/mL ve 30 µg/mL idi. Ayrıca Potasyum dikromatın en etkili oksitleyici ajan olduğu bulunmuştur. Ek olarak, her iki ilaç için elde edilen tespit limiti (LOD) sırasıyla 1,021 µg/mL ve 4,125 µg/mL iken elde edilen kantitasyon limiti (LOQ) sırasıyla 2,762 µg/mL ve 8,143 µg/mL idi. Her bir ilaç için ortalama geri kazanım yüzdesi neredeyse %99.663 ve %100.06 olup göreceli standart sapmalar sırasıyla % (1.357, 1.246) olmuştur. Geliştirilen yöntem farmasötik preparatlara doğruluk ve yüksek hassasiyetle uygulandı.

2024, 63 sayfa

Anahtar Kelimeler: Oksidatif çiftleşme, Spektrofotometrik tayin, Oksadiazol, Metil-dopa, Levodopa

PREFACE AND ACKNOWLEDGEMENTS

I would like to express my gratitude towards my supervisor Assoc. Prof. Dr. Esra DEMIRDOGEN, and Dr. Mohanad AL-HAMAWEE for their continuous support, guidance, helpful suggestions, patience and motivation with their knowledge and experience in this study.

Ismail Younis Ismail KHALIL
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LIST OF SYMBOLS

°C	Celsius temperature
R^2	Correlation coefficient
g	Gram
h	Hour
ml	Milliliter
µg	Microgram
M	Molar concentration
nm	Nanometer
%	Percentage
ρ	Para - describes a molecule with substituents at the 1 and 4 positions on an aromatic compound
pH	Potential of hydrogen
ppm	Part per million
λ	Wavelength

LIST OF ABBREVIATIONS

DCQ	2,6-Dichloroquinone-4-chloroimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
EtOH	Ethanol alcohol
MtOH	Methanol alcohol
NC	Neocuproine reagent
OLED	Organic light-emitting diodes
R.T.	Room temperature
RSD %	Relative standard deviation
S.D.I.-Iraq	The state company for drugs industry and medical appliances in samarra, Iraq
THC	Thiamine Hydrochloride

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1. INTRODUCTION

Heterocyclic compounds are organic compounds that contain a ring structure composed of carbon atoms as well as heteroatoms include nitrogen (N), oxygen (O), sulfur (S), and others. These compounds play a significant role in the field of organic chemistry, medicinal chemistry, and drug discovery. Heterocyclic compounds are widely used in medicinal chemistry, and many drugs are based on or contain heterocyclic structures (Hendrickson *at el.* 1970). The incorporation of heterocycles into drug molecules can influence the biological activity, pharmacokinetics, and other properties of the compounds. In this study methyldopa and levodopa was investigated in various pharmaceutical tablets and capsules. Methyldopa is a medication used to treat high blood pressure or hypertension a common medical condition where the force of the blood against the walls of the arteries is consistently too high. It is also sometimes prescribed to manage certain conditions related to the nervous system such as Parkinson disease. Methyldopa is classified as an antihypertensive agent and is particularly useful in the treatment of hypertension during pregnancy (Molla *et al.* 2017). As a prodrug, it undergoes a chemical conversion in the body to its active form of alpha-methyl norepinephrine, which is a centrally acting alpha-2 adrenergic agonist. This active form of methyldopa stimulates alpha-2 receptors in the central nervous system, leading to a reduction in the sympathetic nervous system activity. The ultimate result is a decrease in the release of norepinephrine and other neurotransmitters, leading to vasodilation and a reduction in blood pressure (Pharmacopoeia, 2013).

On the other hand, levodopa or L-DOPA, is a medication commonly used in the treatment of Parkinson's disease. Parkinson's disease is a progressive neurological disorder that develops gradually over time and affects movement. It is characterized by a variety of motor and non-motor symptoms; however, the underlying cause of Parkinson's disease is not fully understood. The association of Parkinson's disease with degeneration of dopamine-producing neurons in the substantia nigra of brain was also studied. Moreover, dopamine as a neurotransmitter plays a key role in coordinating smooth and controlled movements in Parkinson's disease patients. Levodopa is a naturally occurring amino acid that crosses the blood-brain barrier and is converted into

dopamine in the brain. Dopamine as a neurotransmitter plays a crucial role in regulating movement and coordination in neurologically disorder patients. In Parkinson's disease, there is a depletion of dopamine-producing neurons in the brain, leading to motor symptoms such as tremors, rigidity, and bradykinesia (slowness of movement). By providing the levodopa as a precursor of dopamine to the brain, the level of dopamine in brain increases, this alleviation helps the symptoms associated with Parkinson's disease (Laurence and Bennett, 1990). There are different methods been developed to measure the exact quantity of the drugs in the medication.

A spectroscopic method was developed for the quantification of methyldopa by its reaction with sulfanilic acid in the presence of potassium chromate as a facilitating agent, this reaction produced a colored product with its maximum absorption observed at 495 nanometers, the method exhibited linearity within the Beer's law range of (1.5-40) µg/mL (Gowda *et al.* 2001). Similarly, previous studies have shown developed method for levodopa for determination in pharmaceutical preparation. A sensitive and simple spectroscopic method has been described for the determination of levodopa and methyldopa. The method involves in oxidizing these compounds using periodate's, followed by their coupling with 4-aminobenzoic acid in an acidic medium, the Beer's law range for the method was found to be (0.1-20) mg/L (Madrakian *et al.* 2006). In this study we developed a more sensitive method to accurately measure the quantity of the methyldopa and levodopa in pharmaceutical preparations.

1.1 The aim of study

- The aim of study is the development of economic, fast and reliable method for quantification of drugs using spectrometer.

- This study will provide the base for development of highly sensitive method for other active agents by applying colour producing oxidative reagents.

2. LITERATURE REVIEW

2.1 Heterocyclic Compounds

Unsaturated cyclic compounds are known as organic compounds containing one or more heteroatoms in their structure. The most common heteroatoms are oxygen, nitrogen, sulfur, and phosphorus in heterocyclic compounds (Hendrickson *at el.* 1970). Most unsaturated cyclic compounds contain free electron pairs that serve as catalysts. The presence of electron pairs in these heterocyclic compounds, provide high stability due to resonance and the electronegativity of the heteroatom. Moreover, the solubility of these compounds in both polar and nonpolar solvents increase due to these electron pairs, hence resulting charges from dissolution and occurrence of hydrogen bonding also increase compared to compounds lacking heteroatoms. These property enhances their effectiveness (Joule and Mills 2012).

Unsaturated cyclic compounds are widely found in nature, and their importance extends beyond their abundance. They hold significance in biological, chemical, agricultural, and industrial contexts. They are essential components of many biological systems, such as the nitrogenous bases of nucleic acids: purines (adenine and guanine) and pyrimidines (thymine, cytosine, and uracil), which play a fundamental role in reproduction. They also contribute in the formation of chlorophyll, a key component of photosynthesis in plants, as well as, hemoglobin in blood, which imparts its red color and is vital for oxygen transport in the bodies of humans and most vertebrate animals. Furthermore, they constitute essential components of the dietary system, including pyridoxine (Vitamin B6), niacin (Vitamin B3), riboflavin (Vitamin B2), thiamine (Vitamin B1), and ascorbic acid (Vitamin C). Among twenty amino acids found in various proteins, three are unsaturated cyclic compounds such as: histidine, proline, and tryptophan. Moreover, unsaturated cyclic compounds are also used in pharmaceutical preparations, such as amoxicillin, penicillin, cephalosporins, clavulanic acid, and others, due to their significant biological properties (Kunied and Mutsanga, 2002). Most natural products contain non-homogeneous rings in their structure, such as caffeine found in

coffee (Figure 1.1 a), tea (Figure 1.1 b), and cocoa (Figure 1.1 c) (Bath 2002, Al Falih 1999).

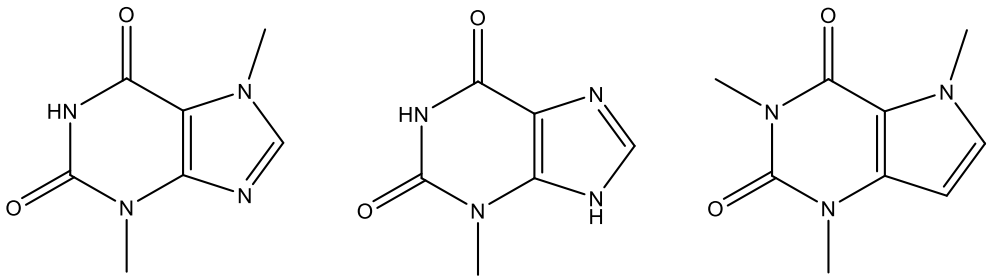


Figure 2.1 Structural representation of a) Coffee, b) Tea and c) cocoa

2.2 Hydrazide Compounds

Hydrazide compounds are considered crucial intermediates in the synthesis of non-homogeneous cyclic compounds, such as thiosemicarbazone derivatives, hydrazones, and oxadiazoles, in addition to their biological activity (Sen and Hajela, 1981), they are characterized by the following formula given in Figure 2.2:

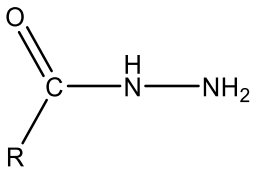


Figure 2.2 Structural formula of hydrazide

The non-homogeneous cyclic compounds are prepared by several methods, with one of the most important being the reaction of acid halides with hydrazine hydrate *(Narang et al.* 2012) as given in Figure 2.3:

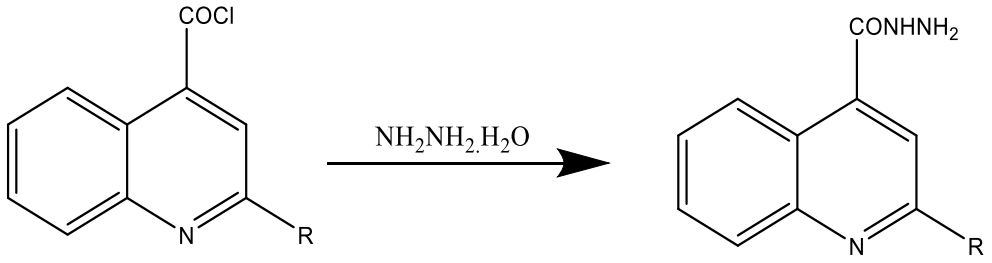
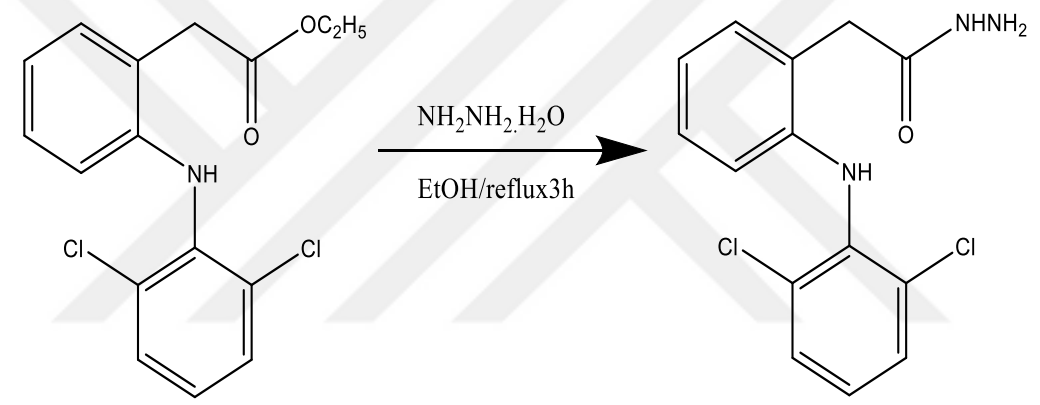


Figure 2.3 Synthesis of non-homogeneous cyclic compound using acid halide and hydrazine

Moreover, reaction of esters with hydrazine hydrate in the presence of ethanol took place, the final mixture of both reagents are heated for three hours continuously give non-homogeneous cyclic compounds as shown in Figure 2.4 (Amir and Shikha, 2004) :



Ethyl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate

2-(2-((2,6dichlorophenyl)amino)phenyl)acetohydrazide

Figure 2.4 Synthesis of non-homogeneous cyclic compound by refluxing hydrazine in ethanol

2.3 Thiosemicarbazone Compounds

Thiosemicarbazones are a class of organic compounds that contain the functional group R2C=N-NH-C(S)NH2, where R is typically a hydrogen or an organic group. These compounds are derived from the reaction of thiosemicarbazide with carbonyl compounds, such as aldehydes or ketones. Thiosemicarbazone derivatives are important intermediates for the preparation of thiazole, triazole, and oxadiazole derivatives, in

addition to their biological activity against certain types of bacteria. Therefore, these compounds have been studied for their diverse biological activities, including antimicrobial, antiviral, antitumor, and anti-inflammatory properties. These derivatives are synthesized using various methods (Bennur and VB, 1976), Thiosemicarbazones are found in two different isoforms: thiol form and thione form, given as follows in Figure 2.5:

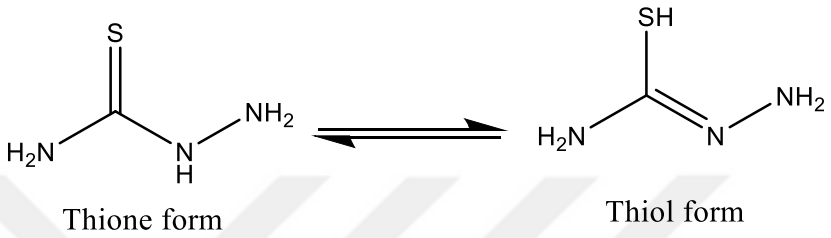


Figure 2.5 Isoform of thiosemicarbazones compounds

The synthesis of thiosemicarbazones compound was done by reacting hydrazide with isothiocyanates (trifluoromethyl) phenyl. The mixture was then heated for 40 minutes to yield a thiosemicarbazide substituted at(4,1) positions (Gümrükçüoğlu and Bekircan, 2021) as shown in Figure 2.6 .

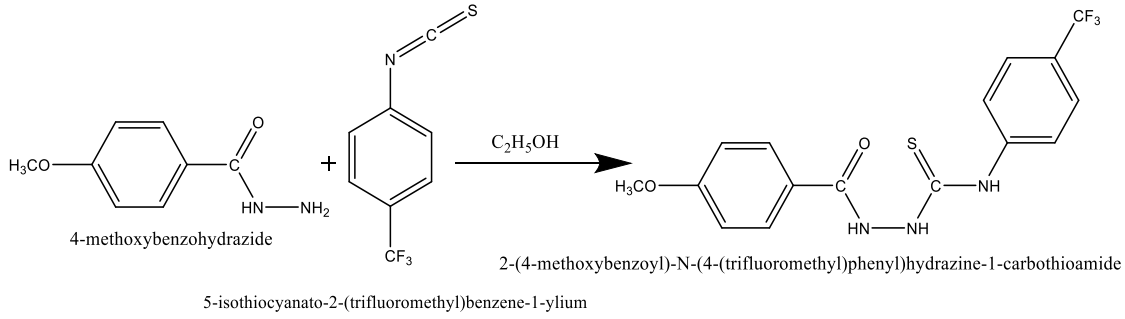


Figure 2.6 Synthesis of thiosemicarbazone compounds

These compounds often exhibit metal-chelating properties, forming complexes with various metal ions. This characteristic has led to the investigation of thiosemicarbazones as potential ligands for metal-based drugs and imaging agents. Thiosemicarbazones have been explored for their potential in cancer treatment due to their ability to inhibit

metal-dependent enzymes involved in cell proliferation. Some thiosemicarbazones have shown promising results in preclinical studies as potential anticancer agents (Bennur and VB, 1976).

2.4 Oxadiazole Compounds

Oxadiazole compounds are non-homogeneous cyclic organic compounds that contain the oxadiazole five-membered ring, consisting of three carbon atoms, one oxygen atom, and one nitrogen atom. The oxadiazole ring has various derivatives depending on the specific substitution pattern on the carbon and nitrogen atoms. The most common types are 1,2,4-oxadiazole and 1,3,4-oxadiazole (Nayak and Poojary, 2019). Moreover, 4,3,1-oxadiazole derivatives act as very promising bio-isosteres for esters and amides, they can significantly enhance drug activities by forming hydrogen bonds with receptors *(Sun et al.* 2013). Additionally, their high lipid affinity attributed to the azole group (-N=C-O) can access target sites through membrane permeation (Bajaj *et al.* 2015). These derivatives are mostly utilized in the production of electron transport layers in thin-film lighting devices, in the production of the light-emitting diodes (OLED) of oxadiazole polymers and photovoltaic cells (Homocianu and Airinei, 2016). These compounds also play a vital role in various pharmaceutical applications, such as many medications available in the market contain 4,3,1-oxadiazole rings in their structure. For instance, drugs like Raltegravir (an antiretroviral medication) and Zibotentan (anticancer agent), exert diverse biological activities (Nayak and Poojary, 2019), including antibacterial, antiviral, anti-tuberculosis, anti-asthma (Banik *et al.* 2021), anti-inflammatory, antidepressant, antimicrobial, analgesic, antidiabetic, and anticancer effects (Glomb and Świątek, 2021).

2.5 Azo Dyes

Azo dyes are considered among the most important known dyes, discovered by the scientist Greiss in 1860. The reason for their name, azo dyes, is attributed to the presence of the azo bridging group (-N=N-) as a chromophore, with SP_2 hybridization (Raafid *et al.* 2020). These dyes are widely used in the textile, food, pharmaceutical,

and cosmetic industries for coloring various materials, including fabrics, plastics, and paper. Azo compounds have good technical properties, including solvent resistance, stabile against environmental factors, water, and light. Therefore, they have been used in various fields, including coloring materials, plastic dyeing, textile fibers, and advanced applications in biomedical studies and organic synthesis (Moradi & Ghanadzadeh, 2019). Azo dyes hold biological significance as anti-inflammatory agents, antiseptics, diabetes treatment, and in phototherapy, which represents a new type of treatment for tumors and other diseases (Brown, 1981).

Azo dyes are prepared in two steps:

- Diazotization process:

The first step involves the diazotization process, discovered by the German scientist Peter Griess in 1858. It includes a protonation reaction to form the diazonium salt for primary aromatic amines. This reaction occurs with nitrous acid (HNO_2), prepared by the reaction of hydrochloric acid with sodium nitrite in low temperatures ranging from 0 to 5 ^oC.

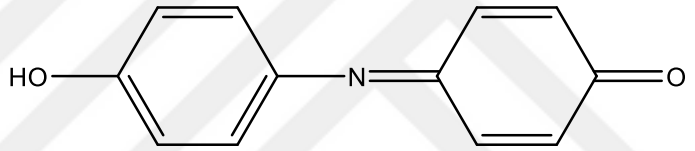
- Coupling Step:

The second step is the coupling step, where diazonium salts react with phenols or primary aromatic amines. This is a nucleophilic aromatic substitution reaction, where the diazonium ion is the electrophilic agent (Asniza *et al.* 2011). An orange-colored azo dye was prepared from the coupling reaction of para-methyl phenol with aniline in a basic medium (Sidney 1967).

2.6 Oxidative Coupling Reactions

Oxidative coupling reactions are among the most significant organic reactions with a broad scope in analytical chemistry. This method is easy, fast, and highly sensitive,

involving the coupling of two or more organic substances in the presence of a suitable oxidizing agent. The oxidation process yields efficient intermediate compounds (Sastry *et al.* 1981, Mcclain & Magidson, 1983).The resulting products serve as genuine chromogenic indicators (Sastry *et al.* 1985), reacting with each other to produce colored compounds that can be measured spectroscopically, polaro-graphically, or chromatographically (Bigley & Grob, 1985). Additionally, the technique of flow injection analysis has been applied in various analysis (Rodriguez *et al.* 1988). The first oxidative coupling reaction was the Berthelot reaction (also known as the endofenol reaction), discovered in 1859. It involves the reaction of phenol with ammonium ions under suitable oxidative conditions, resulting in the formation of the blue indophenol dye (Searle, 1984) shown in Figure 2.7.



4-((4-hydroxyphenyl)imino)cyclohexa-2,5-dien-1-one

Figure 2.7 Structural formula of blue indophenol dye

Temperature has a significant impact on oxidative coupling reactions and playing a crucial role in reaction activation, the temperature employed in some oxidative coupling reactions can reach up to 100°C to achieve high absorbance intensity. However, it has been observed that this intensity decreases over time (Al Falih, 1999). Moreover, while studying the mechanisms of oxidative coupling reactions of aromatic compounds, it was observed that some of these reactions rely on the formation of free radicals that enter the reaction in the presence of a catalyst (Sen and Hajela, 1981). While, the formation of intermediate compounds with the ability to undergo oxidative coupling with other compounds (Amir and Shikha, 2004). Further studies were conducted regarding the influence of the reaction medium. Moreover, it was found that oxidative coupling reactions can occur in neutral, acidic, or alkaline media, depending on the nature of the

reacting substances, reaction conditions, and the stability of the formed products in different reaction environments (Bennur and VB, 1976).

2.7 Diazotization and Coupling Reactions

Diazonium salts are among the most important compounds used in synthesizing many organic compounds, with azo dyes being a prominent example produced through the coupling of diazonium salts with other compounds. These salts possess electrophilic properties, allowing them to couple with compounds having high electron density. Consequently, they can be used to estimate many compounds through coupling with them. The azo group is typically attached to the para position concerning the hydroxyl or amine group. In the case of occupied para site, it prefers to attach to the ortho position. In addition, no reaction occurs when both sites become occupied. For compounds having two amino or hydroxyl groups, there binding become easier at the para position for one group and ortho for the other. Diazonium salts play a crucial role in dye chemistry, producing various colors when coupled with phenols, naphthols, and aniline (Maradiya & Patel, 2001). During diazotization reaction nitrous acid and primary aromatic amines reacted with each other, resulting in the formation of diazonium salts. This reaction requires a low temperature (0-5 degrees Celsius) to increase the stability of the resulting diazonium salt and enhance the solubility of nitrous acid (Bloom & Reenen, 2013). The diazotization and coupling reaction are exothermic because diazonium salts are unstable compounds (Saunders & Allen, 1985) as shown in Figure 2.8.

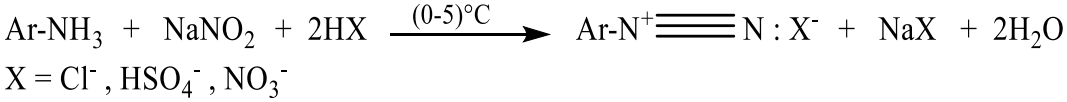


Figure 2.8 Diazotization reaction with primary aromatic amines

Nitrous acid is considered a weak acid, and as such, it is prepared in situ because it is not stable at room temperature. It is synthesized in aqueous solutions at relatively low temperature by treating the aqueous solution of sodium nitrite with a strong acid, such

as hydrochloric acid or nitric acid (Siggia & Hanna, 1963) as given in Figure 2.9. It is advisable to add an appropriate amount of sodium nitrite without exceeding its concentration, that can lead to the formation of contaminants in high proportions which ultimately affect the solutions that. However, to eliminate excess sodium nitrite, urea or sulfamic acid is added to the solution (Fahr and Lind 1966).

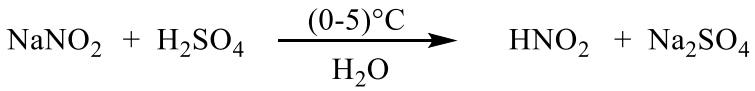
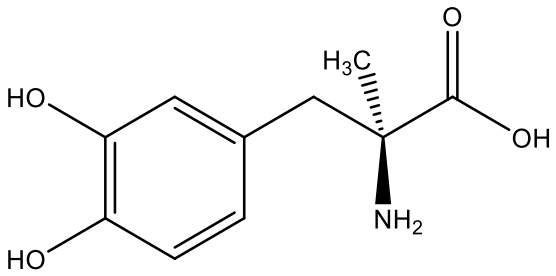


Figure 2.9 Synthesis reaction of nitrous acid

2.8 Study of Pharmaceutical Compounds

2.8.1 Methyldopa

Methyldopa is considered a blood pressure-lowering medication, its usage has decreased and it has been largely replaced by safer and more efficient alternatives, especially for patients with kidney function impairment, psychological disorders, or depression. Methyldopa typically exists as a odorless, white to off-white crystalline powder or as small white crystals with high water solubility and melting point between 275-280 ^{o}C. However, methyldopa has some side effects, including stomach pain, muscle sluggishness, pancreatitis, foot swelling, and liver inflammation, despite these side effects, it is still used to treat gestational hypertension and high blood pressure (Molla *et al.* 2017, Gasnier, 2013), the chemical structure of methyldopa is give below in Figure 2.10:



Methyldopa

3-(3,4-dihydroxyphenyl)-2-methyl-L-alanine

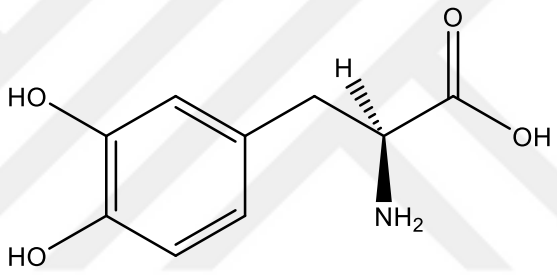
Figure 2.10 Structural Formula of methyldopa

Methyldopa is a prodrug that is metabolized in the body to its active form of alpha-methyl norepinephrine, which binds to and activates central alpha-2 adrenergic receptors. However, the half-life of methyldopa is relatively short, and it is usually administered multiple times per day. The pharmacokinetics study of methyldopa showed that, orally administered methyldopa is well-absorbed from the gastrointestinal tract. Moreover, its absorption is not affected by the presence of food. It binds to plasma proteins, primarily albumin and active metabolite, alpha-methyl norepinephrine (Pharmacopoeia, 2013), which can cross the blood-brain barrier. In addition, it metabolized in the liver through decarboxylation to form alpha-methyl norepinephrine, which is the pharmacologically active compound. By action, it helps in reducing sympathetic nervous system activity, methyldopa induces vasodilation, resulting in a decrease in peripheral resistance (Gasnier, 2013).

2.8.2 Levodopa

Levodopa a neurotransmitter, is one of the natural amino acids and precursor of dopamine, that functions within the body to transmit nerve impulses. It is used in the treatment of neurological disorders that result from Parkinson's disease, which is caused by a depletion of dopamine in the brain. Parkinson's disease is a neurodegenerative disorder characterized by a deficiency of dopamine, a neurotransmitter, in certain areas of the brain. Levodopa is administered orally and is absorbed from the small intestine. Once in the bloodstream, it crosses the blood-brain barrier and converted into dopamine

in the brain through the action of the enzyme aromatic L-amino acid decarboxylase (AADC) (Laurence and Bennett, 1990) as shown in Figure 2.9. This conversion primarily occurs in the dopaminergic neurons of the substantia nigra, a region of the brain affected in Parkinson's disease. In Parkinson's disease, there is a progressive loss of dopamine-producing neurons in the substantia nigra, leading to a deficiency of dopamine in the basal ganglia. Levodopa is effective in alleviating tremors in such cases, it is also a primary substance in the production of dopamine, and the latter is ineffective when directly used for treating Parkinson's disease due to its limited ability to cross the blood-brain barrier, because of its low-fat solubility. On the other hand, levodopa can cross the blood-brain barrier and is converted into dopamine in the basal ganglia by the removal of the carboxyl group (Laurence and Bennett, 1990).



Levodopa

3-(3,4-dihydroxyphenyl)-L-alanine

Figure 2.11 Structural Formula of levodopa

2.9 Oxadiazol Preparations

In a prior investigation, it was found feasible to synthesize oxadiazole compounds by reacting hydrazide with phenyl isocyanates in ethanol. Subsequently, the resulting semicarbazide compound was transformed using phosphorus oxychloride and subjected to thermal reflux for 5 hours to yield N-phenyl-5-(2-phenylquinolin-4-yl)-1,3,4-oxadiazol-2-amine (Xu *et al.* 2004). In another study, hydrazides were reacted with carboxylic acids in the presence of phosphorus oxychloride, resulting in the synthesis of various derivatives of oxadiazoles (Mansour *et al.* 2003).

In a comprehensive study, two highly effective techniques were developed and validated for the synthesis of 5-substituted-2-amino-1,3,4-oxadiazoles and their 2-aminosulfonylated derivatives, these methods harnessed the power of polymer-supported reagents, resulting in accelerated preparation and improved purification processes. As a result of these efforts, an extensive library of over 1500 unique compounds were successfully generated (Baxendale *et al.* 2005). A previous study unveiled an innovative borylated amidoxime reagent specifically designed for the direct synthesis of functionalized oxadiazole and quinazolinone derivatives. This reagent exhibits broad versatility in crafting a variety of biologically significant drug-like molecules. Its preparation is straightforward and cost-effective, allowing for large-scale production using readily available materials. Furthermore, the reagent can be readily converted into target compounds, thus enhancing its utility.

The synthesis of the amidoxime reagent involves the use of 4-(4,4,5,5-tetramethyl-1,2,3-dioxaborolan-2-yl) benzonitrile and hydroxylamine hydrochloride, with N, N-diisopropylethylamine serving as a base. All reagents were conducted reflux in ethanol. Notably, this approach offers distinct advantages, including a metal-free pathway for producing boronated oxadiazoles and quinazolinone derivatives. This streamlined synthesis process yields boron-rich pharmacophore compounds efficiently and cost-effectively, offering a significant advancement in drug development research (Das *et al.* 2022). Another study, about synthesis of novel heterocyclic compound was starting from p-phenylenediamine. The process began by converting p-diacetanilide and reacting it with the Vilsmeier-Haack reagent, resulting in the formation of (2,7-dichloropyrido[2,3-g] quinoline 3,8-dicarbaldehyde). The two carbonyl groups in the compound then underwent a reaction with two moles of 1-tetralone, leading to the formation of a chalcone compound. This chalcone compound was subsequently utilized as a reagent in the analysis of pharmaceutical compounds containing aromatic amino groups. This innovative approach offers potential applications in the field of pharmaceutical research and analysis (Thakafy *et al.* 2022).

2.10 Spectroscopic Determination using Oxidative Coupling Methods

In a previous study, a waste free synthesis of condensed heterocyclic compounds by Rhodium-catalyzed oxidative coupling of substituted arene or heteroarene carboxylic acids with alkynes were prepared. The direct oxidative coupling of 2-amino- and 2-hydroxybenzoic acids with internal alkynes proceeds efficiently in the presence of a rhodium/copper catalyst system under air to afford the corresponding 8-substituted isocoumarin derivatives. Some of which exhibit solid-state fluorescence. Depending on conditions, 4-ethenylcarbazoles can be synthesized selectively from 2-(arylamino)benzoic acids, the oxidative coupling reactions involving both heteroarene carboxylic acids and aromatic diacids in conjunction with an alkyne (Shimizu *et al.* 2009).

Gold complexes have emerged as catalysts for oxidative coupling reactions that create new C-C bonds when exposed to external oxidants. Recent research has unveiled cascade processes, where gold facilitates aryl C-H functionalization or nucleophilic addition before coupling occurs. These reactions harness gold's distinct reactivity to achieve C-C bond formation between partners that are challenging to connect using other catalysts. This concept paper delves into the advancements in gold-catalyzed oxidative coupling, which emphasizing its broad synthetic applicability for C-C bond formation (Hopkinson *et al.* 2011).

The oxidative coupling of enolates, enol silanes, and enamines offers a direct pathway for constructing valuable 1,4-dicarbonyl building blocks. Although this reaction was first reported in 1935 and saw significant progress in the 1970s, its development into a dependable methodology was somewhat restricted. However, recent years have witnessed numerous reports from various research teams highlighting advancements in neglected aspects of oxidative coupling. This micro-review offers a summary of these recent methodological breakthroughs and presents an overview of recent natural product synthesis that illustrate the potential of these transformations (Guo *et al.* 2012). A recent study utilized oxidative coupling reactions for the spectroscopic analysis of drugs, including Thiamine Hydrochloride (THC), Salbutamol, Phenylephrine, Folic Acid,

catecholamine, and Amoxicillin. The study introduced a straightforward, selective, and highly sensitive spectroscopic method for estimating multiple antibiotics. This method relied on the oxidative coupling reaction between the drug and a reagent within an oxidizing agent, resulting in the formation of a colored product, whose absorption could be detected at specific wavelengths (Aljeboree 2020).

In a previous study, porphyrins were synthesized through oxidative coupling reactions. The synthesis typically began with the preparation of initial materials through Suzuki coupling of intermediate bromoporphyrins or mixed aldehyde condensation. This was followed by an oxidative aromatic coupling, often utilizing high-valency metal oxidants like DDQ/Sc(OTf)3 and Fe(III) salts, among the most well-known. These porphyrins underwent oxidation not only with familiar aromatic hydrocarbons such as naphthalene and pyrene but also with more complex and heterogeneous ring fragments, including indole and carbazole and BODIPY. A precise relationship exists between the outcome of oxidative coupling within molecules and the nature of the second aromatic moiety, the cation in the porphyrin cavity, the oxidant, and the type of remaining intermediate substituent. Extending the chromophore of the porphyrin leads to significant changes in linear and nonlinear optical properties. The bathochromic shifts are very pronounced in absorption (reaching a maximum of 1.5-2 micrometers in some cases), and increases in cross-sections for two-photon absorption are typical for these functional dyes (Lewtak and Gryko 2012).

2.11 Spectrophotometric Determination Using Diazotization

In a previous study, a novel, rapid, and sensitive spectroscopic method was developed for the estimation of metronidazole. The method is based on the reduction of the nitro group (-NO2) of metronidazole to an amine group using zinc powder and concentrated hydrochloric acid in hot ethanol as the reducing agent. Then, diazonium ion was prepared from the reduced drug and coupled with para-hydroxybenzaldehyde (HB) to form a yellow-colored dye, that exhibited the highest absorbance at 410 nanometers. The method was successfully applied to the estimation of metronidazole in pure and pharmaceutical preparations (Alsamarrai, 2011).

Furthermore, a simple, fast, and sensitive spectroscopic method was developed for the estimation of sulfadiazine in some pharmaceutical formulations by a group of researchers. The method relies on the diazotization of sulfadiazine using sodium nitrite and hydrochloric acid, followed by its coupling with the detector γ-resorcylic acid (6,2-dihydroxybenzoic acid) in a basic medium of sodium hydroxide to form a stable, water-soluble yellow azo dye. This dye exhibits its highest absorbance at a wavelength of 458 nanometers. The method was successfully applied to the estimation of sulfadiazine in burn treatment creams and pharmaceutical tablets (Mohammed and Zebary, 2013).

In another study, a spectroscopic method for the estimation of paracetamol was developed, relying on the diazotization reaction and coupling with anthranilic acid in a basic medium to form a dense, yellow-colored, water-soluble, and stable azo dye. This dye exhibited its maximum absorbance at 421 nanometers. The method was successfully applied to the estimation of paracetamol in its pharmaceutical formulations (Abdul-Raheem and Alsamarrai, 2016).

In a recent study, a sensitive and accurate spectroscopic method was developed for the estimation of pyridoxine hydrochloride and phenylephrine hydrochloride drugs. The method relies on the diazotization and coupling reactions, combined in a standard addition method, in the form of a binary mixture in their pure aqueous solutions as well as in their pharmaceutical formulations. This is based on the difference between their respective maximum wavelengths (max λ). Pyridoxine hydrochloride was used as an analyte, and phenylephrine hydrochloride was used as an interferent, taking advantage of the wavelengths 276 and 306 nanometers as the most suitable pair due to the equal absorbance of phenylephrine hydrochloride at these wavelengths, while pyridoxine hydrochloride exhibits a significant difference in absorbance at these two wavelengths. The estimation was carried out in a basic medium of sodium hydroxide (0.1 M). The proposed method demonstrated good precision and accuracy, with recovery rates of 101.244% and 98.464% for both drugs, respectively. The relative standard deviation values did not exceed 3.14% and 1.68% for pyridoxine hydrochloride and phenylephrine hydrochloride, respectively. This method was successfully applied to pharmaceutical formulations in the form of tablets for pyridoxine hydrochloride and eye

drops for phenylephrine hydrochloride (Kamel and Abdoon, 2022). In another recent study, a sensitive spectroscopic method was developed for the estimation of Mesalazine through diazotization and the coupling reaction of Mesalazine with the Chalcone detector in a basic medium to form a stable, water-soluble, and colored azo dye. The product exhibits maximum absorbance at 450 nanometers. The recovery rate reached 101.48% with a relative standard deviation of ≥ 3.265%. The products of the Mesalazine and Chalcone detector were formed in a ratio of 2:1. The complete color development was described, and the proposed method was successfully applied for the determination of Mesalazine in pharmaceutical preparations (Thakafy *et al.* 2022).

2.12 Spectrophotometric methods for the determination of methyldopa

Methyldopa was quantified in its pure form and pharmaceutical preparations using various reactions, including oxidation and coupling reactions, methyldopa quantification was carried out by reacting it with brmamine-T in the presence of the oxidizing agent N-bromosuccinimide, using a phosphate buffered solution of 7.4 pH , this solution exhibited its highest absorption at a wavelength of 485 nanometers, the method's linearity was confirmed by Beer's law within the range of (4–65) µg/mL (Walash *et al.* 1982). Methyldopa was quantified using an advanced spectroscopic method involving its reaction with benzidine reagent in a basic medium in the presence of potassium periodate as a facilitating agent, this reaction exhibited its maximum absorption at a wavelength of 480 nanometers, the Beer's law range for the quantification of methyldopa was found to be between (0.8–72) µg/mL, the molar absorptivity coefficient was calculated to be 5×10^3 liter/mole.cm (Salih, 2000).

A spectroscopic method was newly introduced for the quantification of the drug methyldopa in its pure form and pharmaceutical preparations, this method involved its reaction with para-toluidine reagent in the presence of sodium periodate as an oxidizing agent, the reaction resulted in the highest absorption at a wavelength of 480 nanometers, the method's linearity was verified by Beer's law within the range of (2–50) µg/mL (Abdulrahman and Qaissy, 2005).

Quantities in the microgram range of (5-60) µg/mL of methyldopa were determined through its reaction with N, N-dimethylaniline reagent in the presence of potassium metaperiodate as an oxidizing agent. The reaction exhibits its highest absorption at 478 nanometers (Abachi and Da'amy, 2006). A spectroscopic method was described for the quantification of methyldopa in its pure form and pharmaceutical preparations. This method involves its reaction with hydrazide-2-furoic acid hydrazine as a reagent and sodium nitroprusside as an oxidizing agent in a basic medium, the reaction displayed its highest absorption at 487 nanometers, the method followed Beer's law within the range of (1-100) µg/mL (Al-Abachi *et al.* 2009). A refined spectroscopic method was also employed for the quantification of methyldopa by its reaction with thiamine hydrochloride reagent in the presence of an oxidizing agent from potassium periodate, the maximum absorption of the resulting product was measured at a wavelength of 480 nanometers, the method's linearity was within the range of (1-12) µg/mL, following the Beer's law. Moreover, the molar absorptivity calculated was to be 0.53×10^4 liter/mole.cm (Da and Moswi, 2013).

A novel spectroscopic method was developed for the quantification of methyldopa in its pure form and pharmaceutical preparations. This method involved its reaction with para-phenylenediamine reagent in the presence of an oxidizing agent from potassium periodate, the resulting product exhibited its maximum absorption at a wavelength of 494 nanometers, the method's linearity spanned the range of (1-10) µg/mL, following Beer's law. The calculated molar absorptivity was to be 0.61×10^5 liter/mol.cm (Da'amy and Moswi, 2014). Methyldopa could also be quantified using an advanced spectroscopic method that involved its reaction with N, N-dimethyl-para-phenylenediamine dihydrochloride reagent in the presence of ferricyanide-potassium cyanide oxidizing agent in a basic medium. This reaction produced the highest absorption for the resulting product at a wavelength of 556 nanometers, the method's linearity was observed within the range of (2-24) microgram/milliliter, following Beer's law. The molar absorptivity was calculated to be 1.06×10^4 liter/mole.cm (Humeidy, 2016).

A spectroscopic method was developed for the quantification of methyldopa by its reaction with 6,2-diaminopyridine reagent in the presence of potassium periodate as an oxidizing agent. The reaction displayed its highest absorption for the resulting product at a wavelength of 478 nanometers, the method's linearity spanned the range of (2-24) µg/mL, following Beer's law, the observed molar absorptivity calculated was to be 1.06×10^4 liter/mole.cm (HUMEIDY *et al.* 2020). A refined spectroscopic method was also utilized for the quantification of methyldopa by its reaction with 4,2-dinitrophenyl hydrazine reagent in the presence of potassium periodate as an oxidizing agent in an acidic medium, the reaction exhibited its highest absorption for the resulting product at a wavelength of 428 nanometers, the method's linearity was observed within the range of (1-30) µg/mL. Which is following Beer's law with the calculated molar absorptivity of 6589.908 liter/mole.cm (AL-ghanam and AL-Enizzi, 2022).

Various spectroscopic methods were also employed, relying on azo coupling reactions, for the quantification of methyldopa in its pure form and pharmaceutical preparations. A developed spectroscopic method used was 4-aminoacetophenone as azo reagent in an acidic medium to quantify methyldopa, this reaction exhibited its highest absorption for the resulting product at a wavelength of 560 nm, the method's linearity was observed within the range of (0.5-45) µg/mL (AbdulSattar, 2014). A developed spectroscopic method was also established for the quantification of methyldopa using 2-aminothiazole azo reagent in a basic medium, this reaction produced its maximum absorption at a wavelength of 565 nanometers, it was found that the method followed Beer's law within the range of (2.5-62) µg/mL, and the molar absorptivity was calculated to be 0.382×10^4 liter/mol.cm (Abood, 2020).

Complexation reactions involving charge-transfer complexes were also utilized for the quantification of methyldopa, it was quantified through its reaction with 3,2-dicyano-6,5-dichloro-4,1-benzoquinone (DDQ) reagent in an aqueous medium with a pH of 8.5, this reaction exhibited its maximum absorption at a wavelength of 422 nanometers, the method's linearity spanned between (0.1-10.4) µg/mL, following Beer's law (Salih, 2000).

A refined spectroscopic method was described for the quantification of methyldopa in its pure form and pharmaceutical preparations, this method relied on its reaction with para-bromanil reagent in an aqueous solution at a pH of 9 using a borate buffer, the complex formed displayed its maximum absorption at a wavelength of 350 nanometers, the method's linearity was observed within the range of (1-25) µg/mL, hence its following Beer's law with the molar absorptivity of 8075 liter/mole.cm (Al-Sharook, 2007).

A developed spectroscopic method was outlined for the quantification of methyldopa through its reaction with para-chloranil reagent, employing hydrogen peroxide, the resulting complex exhibited its maximum absorption at a wavelength of 535 nanometers (Gotardo *et al.* 2008). Another spectroscopic method was further developed for the quantification of methyldopa using para-dichloroquinone-4-chlorimide (DCQ) reagent in the presence of an acetate buffer with a pH of 8.0, the resulting complex exhibited its maximum absorption at a wavelength of 400 nanometers. With this method, it was possible to quantify drug quantities ranging from (4-20) µg/mL (Gadkariem *et al.* 2009). Other reactions were also utilized to quantify methyldopa through developed spectroscopic methods, including both direct and indirect reactions, methyldopa could be quantified by oxidizing it using sodium bismuthate in an acidic medium, the resulting product exhibited its highest absorption at a wavelength of 429 nm, the method's linearity was within the range of (8-130) µg/mL, following Beer's law (Sajjan *et al.* 2001).

Methyldopa could also be quantified by reacting it with sodium nitrite in an acidic medium, followed by the reaction of the resulting product with sodium hydroxide. The formed product exhibited its maximum absorption at a wavelength of 430 nm, within the range of (6.37-82.8) µg/mL, according to Beer's law (Ribeiro and Duarte, 2014). An indirect spectroscopic method was employed to quantify the compound methyldopa, this method relied on its oxidation, using a calculated excess of N-Bromo succinimide as an oxidizing agent, the surplus of N-Bromo succinimide led to the depletion of saffronin dye, causing a linear increase in dye absorption at 535 nm, the method's linearity ranged from (0.5-9) µg/mL, following Beer's law, the molar absorptivity was

calculated to be 5.32×10^4 liter/mole.cm (Al-Meshaekhy, 2017). Moreover, methyldopa was spectrophotometrically quantified indirectly through its reaction with copper(II) ions formed by the reaction of neocuproine (NC) reagent and copper(I) ions, this resulted in a complex with the highest absorption at a wavelength of 455 nm, the method's linearity was observed within the range of (0.4-3.6) µg/mL, following Beer's law, the molar absorptivity was calculated to be 7.7×10^4 liter/mole.cm (Abdel-Monem and Bahgat, 2018). In addition, methyldopa was also spectrophotometrically quantified in its pure form and pharmaceutical preparations by reacting it with molybdate ions, resulting in a colored product with its maximum absorption at 410 nanometers, the method's linearity was within the range of 6.30×10^{-3} - 1.89×10^{-2} liter/mole.cm (Ribeiro *et al.* 2006).

2.13 Spectrophotometric methods for the determination of levodopa

Developed spectroscopic methods for the determination of levodopa in its pure form and in pharmaceutical preparations was developed by using various reactions. Levodopa was successfully quantified using a modified spectroscopic method based on the oxidative coupling reaction, in which potassium dichromate was used as oxidizing agent, followed by its coupling with sulfanilic acid, the resulting complex exhibited the highest absorption at a wavelength of 495 nanometers, within the range of Beer's law (1-23) µg/mL. The molar absorptivity was determined to be 1.8×10^4 liter/mole.cm (Gowda *et al.* 2001). Another study showed the developed spectroscopic method for the determination of levodopa by oxidizing it with sodium nitroprusside, followed by coupling with hydrazide-2-furoic acid in a basic medium. This give a resultant a colored product with maximum absorption at 485 nm, falling within the Beer's law range of (0.1-22) µg/mL, the molar absorptivity was calculated to be 20600 liter/mol.cm (Esawati, 2002).

Levodopa could also be determined using an improved spectroscopic method employing the reagent 4-aminophenanthroline in a basic medium of sodium hydroxide, the resulting complex exhibits maximum absorbance at a wavelength of 519 nm, the method's linear range was found to be (0.2-30) µg/mL, According to Beer's law, with a

molar absorptivity of 0.58×10^4 liter/mol.cm (Hussein *et al.* 2013). Another spectroscopic method has been developed for the determination of levodopa, relying on its reaction with the reagent orthotolidine in the presence of potassium periodate, the resulting complex exhibits maximum absorbance at a wavelength of 629 nm, the method's linear range was found to be (0.1-28) µg/mL according to Beer's law, with a molar absorptivity of 2.76×10^4 liter/mol.cm (Hussein *et al.* 2013).

Levodopa was also quantified using the diazotization and coupling reaction approach, a spectroscopic method was developed for the determination of levodopa using the diazotized sulfanilamide reagent in the presence of sodium molybdate in an acidic medium, the resulting complex exhibited the highest absorbance at a wavelength of 500 nm. Moreover, the linear range of the method was within the limits of (0.1-2.8) µg/mL according to Beer's law (Nagaraja *et al.* 2001).

A spectroscopic method was developed for the determination of levodopa using diazotized sulfanilic acid, in this method, the diazotized sulfanilic acid reagent reacts with levodopa in a basic medium to form a complex that exhibits the highest absorbance at a wavelength of 475 nm. However, the linear range of the method was within the limits of (1.5-19.2) microgram/milliliter according to Beer's law, with a molar absorptivity of 0.709×10^4 liter/mol.cm (Shaikh *et al.* 2008). In addition to developed spectroscopic method, Sharook developed method for the determination of levodopa in its pure form and pharmaceutical preparations showed promising results. This method relies on its reaction with para-bromanyl in an aqueous solution at a pH of 9, using a borate buffer solution to form charge-transfer complexes, the maximum absorption of the formed complex was observed at a wavelength of 366 nm. Moreover, the method exhibited linearity within the range of (0.8-30) µg/mL, with a molar absorptivity of 8500 liter/mol.cm (Sharook, 2007).

By using alternative reactions levodopa was also determined spectrophotometrically, a developed spectroscopic method relied on the nitration of levodopa using sodium cobalt nitrite in acetic acid medium, the resulting product exhibited the highest absorption at a wavelength of 320 nm, within the range of (5-25) µg/mL. Which was following the

Beer's law, with the molar absorptivity of 0.7327×10^4 liter/mol.cm (Gamal *et al.* 2009). Quantification of levodopa by coupling it with the 4-aminoantipyrine reagent gives highest absorption at a wavelength of 530 nm. This developed method followed the Beer's law within the range of (2.5-20) µg/mL. However, the molar absorptivity for this method was calculated to be 0.597×10^4 liter/mol.cm (Gamal *et al.* 2009). Moreover, quantification of levodopa was studied by using the 4-aminoantipyrine reagent at pH 12. This yield the highest absorption at a wavelength of 454 nm within the Beer's law range of (49.30-221.8) µg/mL, the molar absorptivity for this method was determined to be 1.05×10^4 liter/mol.cm (Mohamed *et al.* 2009).

3. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Table 3.1 show the list of chemicals and ingredients used in this study. All the reagents were of analytical grade.

Table 3.1 List of chemical and reagent used in the study

NO	CHEMICALS	COMPANY
1	Sodium chloride	Scharlau
2	Chloroform	Sigma Aldrich
3	Absolute ethanol	Lobal chemia
5	Acetic acid	Scharlau
7	Ethanol	Lobal chemia
8	Ethyl acetate	Lobal chemia
9	Methanol	Lobal chemia
10	Ethyl ether	Scholar
11	H_2SO_4	Sigma Aldrich
12	HCl	Lobal chemia
13	NaOH	Lobal chemia
14	$KMnO_4$	Lobal chemia
15	HNO_2	Lobal chemia
16	H_2O	Lobal chemia
17	p-nitro phenol	Lobal chemia
18	acetaldehyde	Local chemia
19	Propanal	Local chemia
20	methylamine	Lobal chemia
21	Bodipy-dye	Turkiy
22	phenolphthalein	Lobal chemia
23	Iron filling	Lobal chemia
24	KIO3	Lobal chemia
25	Acetone	Lobal chemia

3.2 Instruments and Equipment's

Table 3.2 contains a list of the instruments along with the manufacturing companies that were used in this study.

Table 3.2 List of instruments and equipment's used in this study

No.	Instruments	Company
1.	Double Beam Spectrophotometer	Shimadzu UV-1800 PC
2.	Water Bath	Labtech
3.	Balanc	ae ADAM
6.	Fourier transform infrared spectrophotometer	Shimadzu
7.	The nuclear magnetic resonance (NMR)	Agilant Technologies
8.	sensitive balance	Sartorius
9.	UV-Visible Spectrophotometer	PG instruments T92
10.	pH meter	Inolab pH7110
11.	Microtube centrifuge	Stuart
12.	vortex mixer	M16
13.	Heating and magnetic stirrer	Velp scientific

3.3 Synthesis process of 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole reagent

For the synthesis of 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole reagent, following steps were followed. Step 1: Synthesis of the Hydrazide Para-amino benzoic acid was synthesized by Abood's developed method: A mixture consisting of 0.05 moles (6.85 g) of para-amino ethyl benzoate and 0.2 moles (10 ml) of (80%) aqueous hydrazine in 50 ml of ethanol was refluxed for 6 hours. The solvent was evaporated and yielded precipitate were filtered (Abood, 2020) and left it to dry at room temperature in the laboratory. Reagent was purified by recrystallizing them in ethanol, yield 81.2% of a pure product as shown in Figure 3.1.

Figure 3.1 Synthesis of the Hydrazide Para-amino benzoic acid

In Step 2: 4-(para-amino benzoic acid) thiosemicarbazid was synthesized by method developed by Salih. Mixture of 0.02 moles (3 g) of the hydrazide with 0.09 moles (6.84 g) of ammonium thiocyanate and 5 ml of concentrated hydrochloric acid (37%) was taken in a round-bottom flask containing 50 ml of ethanol. The reaction mixture was refluxed for 22 hours. The solvent was evaporated by half, and the solution was cooled by adding ice-cold water and left it for complete precipitation (Salih, 2000). The resulting precipitates were filtered and left to dry at room temperature. The product was recrystallized using ethanol which yield a product of 83.5% purity.

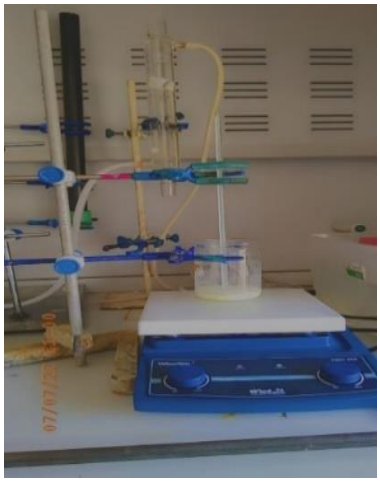


Figure 3.2 Synthesis of 4-(para-amino benzoic acid)thiosemicarbazid

In Step 3: 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole was synthesized by using method developed by (Al-Sharook, 2007): 0.002 moles (0.4 g) of thiosemicarbazid was

dissolved in 25 ml of methanol, and then 0.002 moles of mercury oxide (0.48 g) were added to the previous solution. The mixture was refluxed for (4-6) hours and then hot filtration was done. The solvent was evaporated and recrystallized using ethanol. The obtained yield of the formed precipitate was 75.1%. the mechanizum of reaction was shown in Figure 3.3.

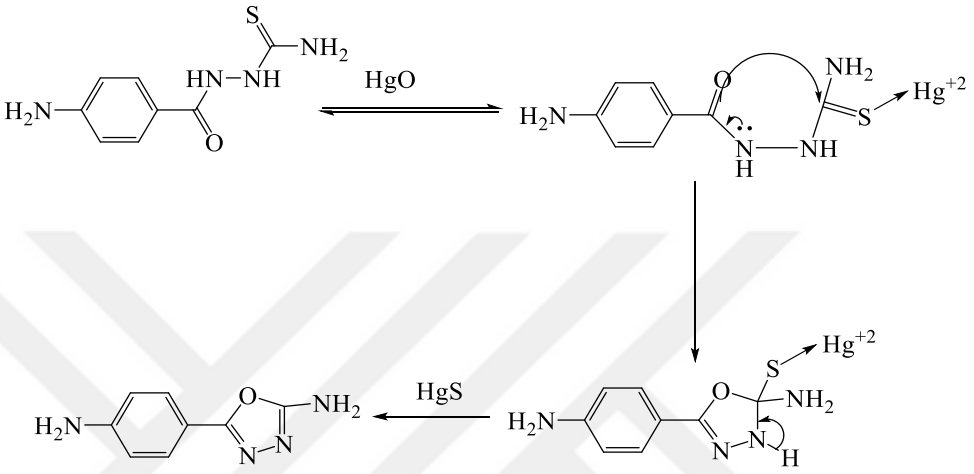


Figure 3.3 Reaction mechanism for the synthesis of 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole

3.4 Primary Drugs Tests with Prepared Reagent

When 1 ml of the methyldopa solution was mixed with 1 ml of already prepared oxadiazole reagent solution, and 1 ml of potassium dichromate solution was further added over it in an acidic medium. The potassium dichromate solution work as an oxidizing agent, while, addition of 1 ml of phosphoric acid in previous solutions mixture give a light brown-colored solution.

Similarly, 1 ml of the Levodopa solution was mixed with 1 ml of the prepared oxadiazole reagent solution. 1 ml of potassium dichromate solution was added over it as an oxidizing agent, along with 1 ml of hydrochloric acid. A colored solution was formed. Both the solution was further studied by spectrophotometer.

3.5 Optimization of Experimental Conditions

Subsequent studies were conducted using concentrations of 30 and 40 µg/ml for both Methyldopa and Levodopa, respectively. By withdrawing 1 ml from the 300 and 404 µg/ml solutions of Methyldopa and Levodopa, respectively, 1 ml of the oxadiazole reagent was added with further addition of 1 ml of potassium dichromate as an oxidizing agent and 1 ml of hydrochloric acid. Then, the final volume was adjusted to 10 ml by addition of deionize water up to the mark. The mixture was allowed to react for 10 minutes to complete the coupling reaction. The absorbance of the resulting product was then measured spectroscopically against the reference solution.

Moreover, effect of temperature, effect of reagent and its volume, effect of acid and its volume as well as order of addition of reagents were studied to determine the optimal conditions.

3.6 Study the Nature of the Resulting Product

The methods of Job and molar ratios were used to study the nature of the complex formed from the reaction of each of the methyldopa and the levodopa with the prepared oxadiazole reagent.

3.6.1 The continuous variation method (JOB's Method)

To determine the compositional molar ratios, the Job's method was applied to diluted solutions. The total volume of the drug compound and the reagent was 3 ml, with a final volume of 10 ml and equal concentrations of 2×10^{-3} M for both drugs and the reagents.

3.6.2 The molar ratio method

To validate the results obtained from the Job's method, the molar ratio method was applied. In this method, varying volumes (0-3 ml) of the drug compound were added to

a fixed volume of 1 ml of the reagent, 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole, both at equal concentrations. The total volume of the mixture was maintained at 10 ml, and the concentration of both compounds was 2 × 10-3 M.

3.7 The Proposed Chemical Reaction for Methyldopa

The proposed chemical reaction for both Methyldopa and levodopa with the 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole reagent occurs in a 2:1 ratio. Methyldopa forms a light brown complex, while levodopa forms a brown complex. This reaction takes place in an acidic environment with potassium dichromate acting as an oxidizing agent. The proposed mechanism for the reaction of methyldopa is as follows as shown if Figure

3.4:

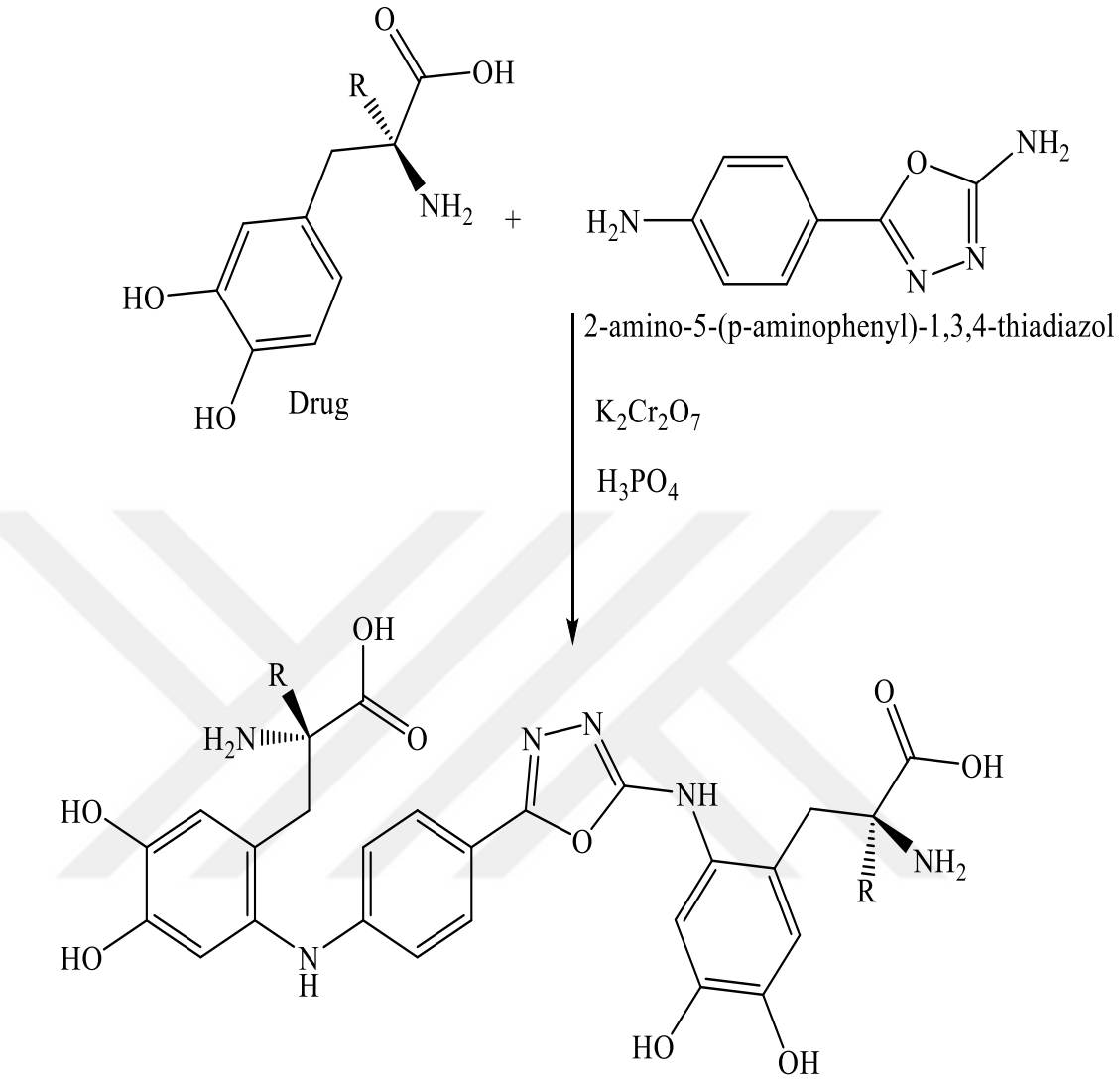


Figure 3.4 Schematic representation of Mechanisms of the diazotization reactions

3.8 Biological Study

The inhibitory effectivity of the prepared reagent (2-amino-5-(para-amino phenyl)-1,3,4-oxadiazole) was tested against two types of bacteria, one being Gram-negative Escherichia coli, and the other being Gram-positive Staphylococcus aureus. The Gram-negative and Gram-positive strains of bacteria were obtained from the Department of Life Sciences, College of Education, University of Mosul.

3.8.1 Inhibitory effectivity test

The inhibitory effectivity of the prepared reagent (2-amino-5-(para-amino phenyl)-4,3,1-oxadiazole) was assessed by following the method of Levne and its colleagues (Leven *et al.* 1979), and Vandepitte method (Vandepitte, 2003). In this procedure, individual colonies of the specified bacteria were separately injected into nutrient broth. The bacterial cultures were then incubated at 37°C for 18-24 hours of a duration. Subsequently, a series of dilutions were carried out using normal saline solution to achieve a concentration equivalent to 108 cells/cm^3, in comparison with the No.1 tube of the standard Macferland tubes. The bacterial suspension was then spread on the surface of regular nutrient agar plates using a sterilized glass spreader, and the plates were left in the incubator for 30 minutes for absorption.

For the purpose of studying the antibacterial activity of the prepared compounds, disks of filter paper with a diameter of 6 mm were prepared. Then disks were saturated with specific concentrations of the compounds dissolved in DMSO (Dimethyl sulfoxide) and affixed to the surface of agar plates using sterilized forceps. The plates were then incubated at 37°C for 18-24 hours. Afterward, the inhibition diameter was measured and compared with standard antibiotics such as Amoxicillin and Ciprofloxacin, used as control samples.

3.8.2 The (MTT) test

The impact of the prepared detector (2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole) was examined on strains of primary dermal fibroblasts isolated from the neonatal foreskin (HdFn natural cells,) and breast cancer cells (MCF-7) using the MTT test.

Initially, the cells under examination were cultured and then incubated at (37 °C) in an incubator containing 5% of carbon dioxide. After that, the concentration of the compounds to be tested on cancer cells was diluted to several known ratios. A dose of the prepared detector (2-amino-5-(para-aminophenyl)-4,3,1-oxadiazole) was then

introduced into (MCF-7) cells, and these cultured cells were incubated for a sufficient period of time at (37 °C) in the presence of (5%) carbon dioxide.

A solution of (10 microliters) of (MTT) was then injected into the 96 well plate and incubated again at (37 °C) with (5%) carbon dioxide. Afterward, the intermediate solutions were removed, and a reagent solution was added to each well to dissolve the formazan crystals. After completion of incubation in a humid atmosphere, the absorption of the prepared detector was measured at (575 nanometers) using a (Bio-Rad) ELISA reader, of German origin. Data were collected using statistical analysis (Graph Pad Prism).

4. RESULTS AND DISCUSSION

4.1 FTIR Analysis of 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol

Fourier Transform Infrared Spectroscopy is a technique used for analyzing the infrared absorption or emission spectra of a sample. Infrared spectroscopy involves the interaction of infrared radiation with matter, leading to the absorption, transmission, or reflection of energy. The resulting spectrum is a plot of the sample's absorption or transmission as a function of wavelength. It is a powerful tool for identifying functional groups in organic compounds, determining the composition of mixtures, and monitoring chemical reactions. In this study the prepared reagent 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol was as given in Figure 4.1 characterized by FTIR.

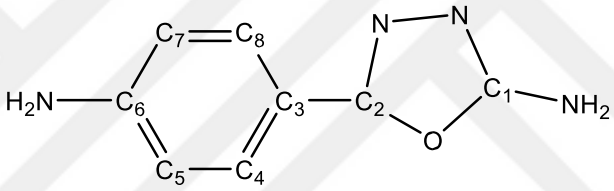


Figure 4.1 Structural representation of 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol

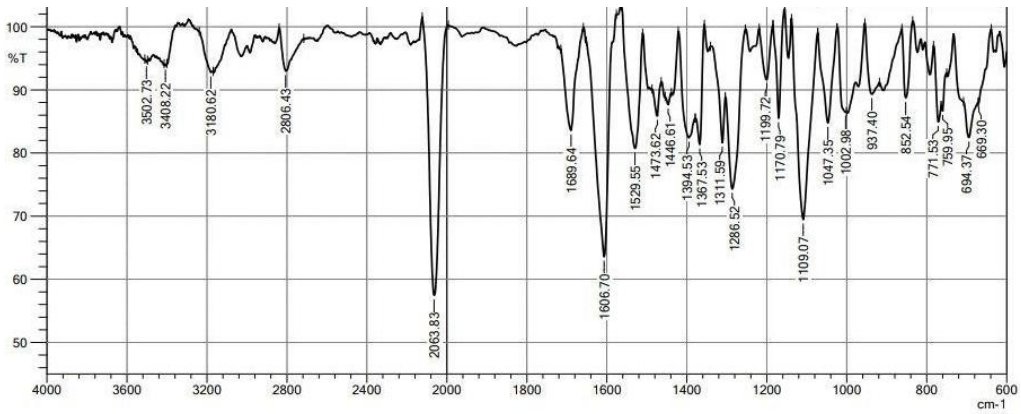


Figure 4.2 FTIR spectrum of 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol

The infrared spectroscopic analysis of the prepared reagent 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol as shown in Figure 4.2 shows three peaks at 3180.62,

3417.22, 3502.73 cm^{-1} indicating the presence of aromatic NH_2, and the C-H stretching of aromatic ring at 3030 cm^{-1}. In addition, two bending peaks of aromatic C-H at 852.54, 937.40 cm^{-1}, structural vibrational peaks of C-C at 1446.61, 1473.62, 1529.55, 1606.70 cm^{-1} and peak of C = N at 1689.64 cm^{-1} was observed. Moreover, two peaks of C-O-C was observed at 1109.07, 1286.52 cm^{-1}.

4.2 NMR Analysis

Nuclear Magnetic Resonance, is a powerful analytical technique used to study the magnetic properties of atomic nuclei. While proton NMR (^{1}H-NMR) specifically deals with the hydrogen nuclei, there are other types of NMR that involve different nuclei, such as carbon-13 NMR (^{13}C-NMR). In addition, ^{1}H-NMR, or proton nuclear magnetic resonance spectroscopy, is a powerful analytical technique used for determining the structure of organic compounds. It provides information about the hydrogen (proton) environment in a molecule, helping researchers identify and characterize different functional groups within the compound.

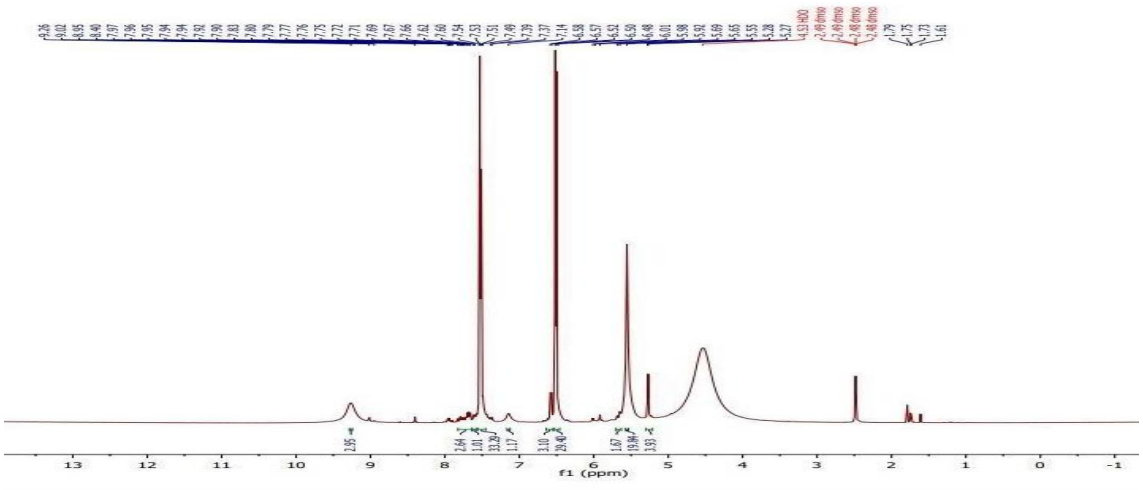


Figure 4.3 ^{1} H-NMR spectrum of *2-amino-5-(para-aminofenil)-4,3,1-oksadiazol*

In the (^{1}H-NMR) spectrum, a singlet signal at 6.52 ppm belong to the NH group on the heterocycle ring while, a doublets signal at 7.54, 7.49 ppm belongs to aromatic C-H.

Moreover, a singlet signal at 5.65 ppm belong to the aromatic NH on the benzene ring, as shown in Figure 4.3.

Carbon-13 nuclear magnetic resonance spectroscopy, is a technique used to study the nuclear magnetic properties of carbon-13 nuclei in a sample. While ^{13}C-NMR is less sensitive than ^{1}H-NMR (proton NMR) due to the lower natural abundance of ^{13}C nuclei. Moreover, it provides valuable information about the carbon environments in a molecule. ^{13}C-NMR is particularly useful for studying compounds where ^{1}H-NMR may not be informative, such as inorganic compounds or compounds with a low hydrogen content.

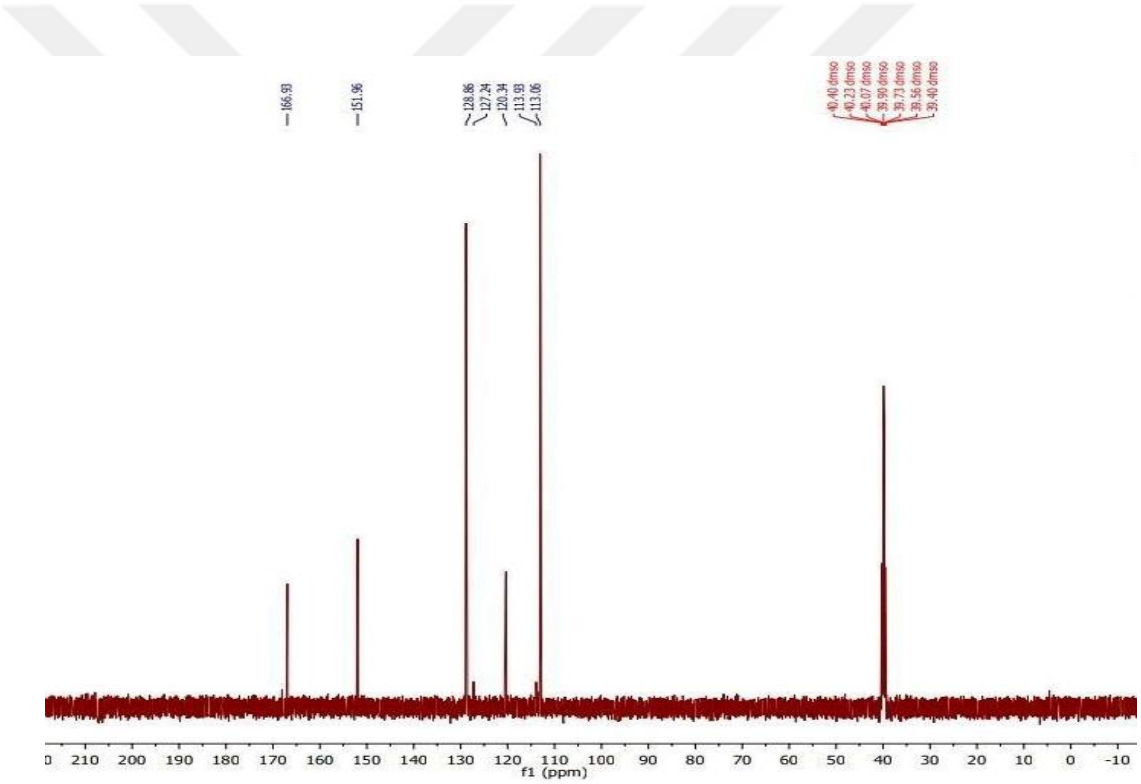


Figure 4.4 ^{13} C-NMR spectra of the *2-amino-5-(para-aminofenil)-4,3,1-oksadiazol*

The ^{13}C-NMR spectrum of 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol as shown in Figure 4.1shows a signal at 151.96 ppm belong to C1 as shown in Figure 4.3. in addition, a signal at 166.93 ppm, 128.86 ppm corresponding C2, and C3 respectively. However, a doublet signal at 127.24 ppm and 120.34 ppm was belong to C4, C5, C7

and C8 respectively. While a signal observed at 113.06 ppm correspond to C6 of the reagent molecule.

4.3 Primary Tests for Spectrophotometric Analysis of Methyldopa and Levodopa

UV-visible spectrometric studied of both drugs methyldopa and Levodopa reaction with 2-amino-5-(para-aminofenil)-4,3,1-oksadiazol in the presence of oxidizing agent in an acidic environment give maximum absorbance at 404 nm and 417 nm respectively.

4.4 Effect of Reagent Volume

The effect of adding different volumes of the reagent, (2-amino-5-(para-aminophenyl)-1,3,4oxadiazole), on the complex formation absorbance was studied for both Methyldopa and Levodopa. The table 4.1 below indicates that the optimal volume of the reagent is 1.0 and 0.75 ml for Methyldopa and Levodopa, respectively. Therefore, these volumes were adopted for subsequent studies.

Table 4.1 Study the effect of reagent volume

X ml of Reagent 1×10-3M	Absorbance	
	Methyldopa	Levodopa
0.25	0.097	0.181
0.50	0.124	0.196
0.75	0.169	0.245
1.00	0.224	0.230
1.25	0.211	0.218
1.50	0.198	0.193

4.5 Effect of Types of Oxidizing Agents

Different types of oxidizing agents were studied for Methyldopa and Levodopa by adding 1 ml of a 0.01 M solution. Figure 4.5 given below illustrates the best oxidizing agent chosen for this study with maximum absorbance for both Methyldopa and Levodopa at the specified wavelength.

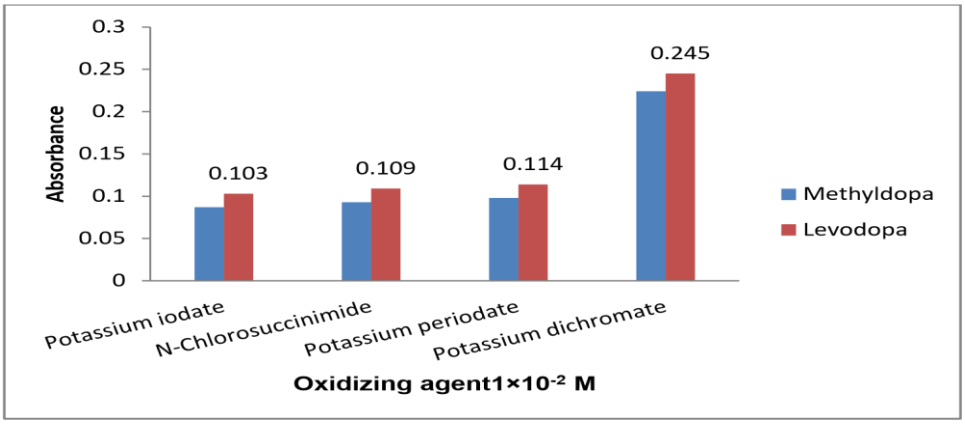


Figure 4.5 Graphical representation of effect of different types of oxidizing agent on absorption spectrum

Among potassium iodate, N-chlorosuccinimide, potassium periodate and potassium dichromate as an oxidizing agent, Potassium Dichromate give maximum absorbance. Therefore, it was selected for subsequent studies.

4.6 Effect of Different Volumes of Potassium Dichromate

After fixing the type of oxidizing agent, a study was conducted to determine the optimal quantity of potassium dichromate by increasing the amounts of the oxidizing agent in reaction. Table 4.2 demonstrates that the optimal quantity of the oxidizing agent was 0.3 ml for both drug compounds, hence, it was adopted for subsequent studies.

Table 4.2 Study of the quantity of oxidizing agent

X ml of $K_2Cr_2O_7$ (1×10^{-2} M)	Absorbance	
	Methyldopa	Levodopa
0.10	0.211	0.235
0.30	0.265	0.282
0.50	0.248	0.256
0.75	0.237	0.245
1.00	0.224	0.231
1.25	0.207	0.202

4.7 Study on the Effect of Types of Acids

This study was conducted to select the appropriate acid by adding 1 ml of different acids at a concentration of 0.1 M in order to achieve the highest possible sensitivity. Figure 4.6 illustrates that phosphoric acid providing the highest absorption for both Methyldopa and levodopa. Therefore, it was adopted for further studies.

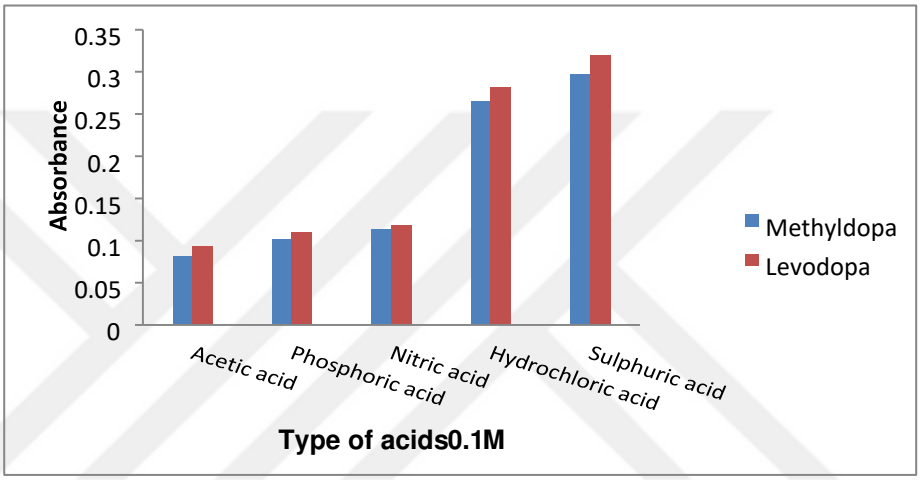


Figure 4.6 Graphical representation of effect of different types of acids on absorption spectrum

4.8 Study the Effect of Acid Volumes

In this study, increasing volumes of phosphoric acid at a concentration of 0.1 M were investigated, as shown in Table 4.3. It was found that 0.75 ml the optimal volume of acid for Methyldopa and 1.0 ml for levodopa. Hence. These quantities were selected for further studies.

Table 4.3 Study the effect of volume of acid

Volume ml of H_3PO_4 (0.1M)	Absorbance	
	Methyldopa	**Levodopa**
0.25	0.193	0.287
0.50	0.241	0.311
0.75	0.273	0.343
1.00	0.297	0.320
1.25	0.276	0.257
1.50	0.231	0.224

4.9 Study the Effect of Temperature

The impact of different temperatures on the absorption of the formed complex was investigated, as shown in Figure 4.7 and Figure 4.8. It indicates that the optimal temperature of 60 °C for both methyldopa and levodopa in oxidation and coupling reactions. Which give maximum absorption at specified wavelength with highest stability of up to two hours of formed complexes for methyldopa and approximately 20 minutes of stable product for levodopa.

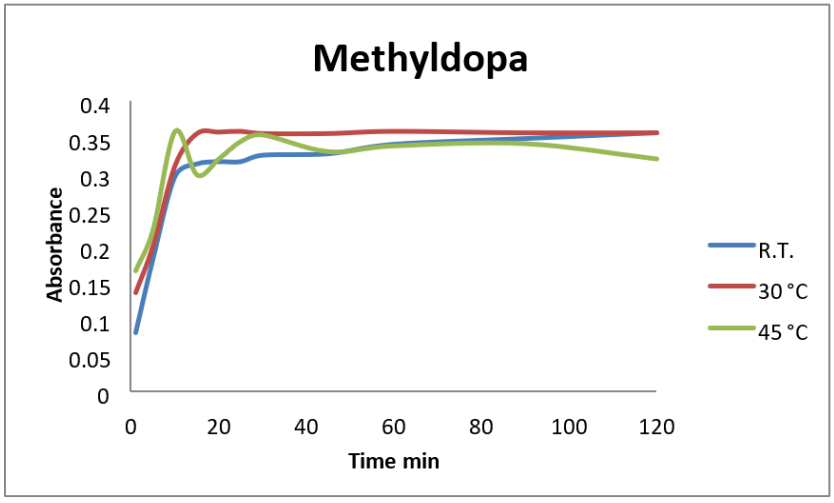


Figure 4.7 Effect of temperature on complex formation for Methyldopa

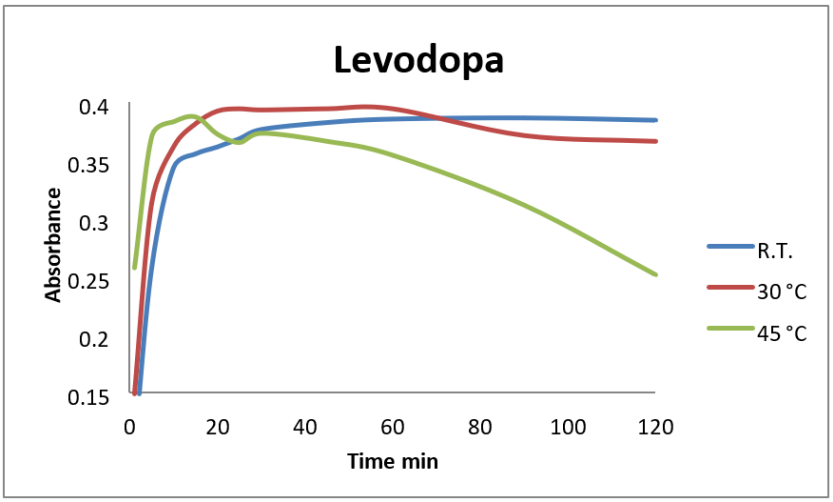


Figure 4.8 Effect of temperature on complex formation for Levodopa

4.10 Study the Effect of Addition Order of Reagents

The impact of different addition order of reagents on the absorption values of the formed complex was investigated by adding the components in different orders. The results shown in Table 4.4 demonstrate that the sequence adopted in previous studies was the best one, which yield the highest absorption. Therefore, this sequence was fixed and used in subsequent studies.

Table 4.4 Study the effect of addition order of reagents

Order Number	Reaction component	Absorbance	
		Methyldopa	Levodopa
I.	D+R+O+A	0.357	0.391
II.	D+O+R+A	0.321	0.361
III.	D+A+R+O	0.313	0.344

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

However, the established optimal conditions for methyldopa and the levodopa from above studies was further summarized in the Table 4.5 given below:

Table 4.5 Summary of optimal conditions

Experimental conditions	Methyldopa	Levodopa
$_{Max}(nm)\lambda$	404	417
2-amino-5-(ρ-aminophenyl)-1,3,4-thiadiazol 1×10^{-3}M (ml)	1.0	0.75
$K_2Cr_2O_7$ 1×10^{-2}M (ml)	0.3	0.3
H_3PO_4 0.1 M (ml)	1.0	0.75
Temperature (°C)	30	30
Development Time (min.)	15	20
Stability period (min.)	≤105	40

4.11 The Final Absorption Spectrum

After establishing the optimal conditions for the formed complexes of methyldopa and levodopa, the absorption spectra of the complexes were plotted against the reference solution for each compound. The complexes exhibited the highest absorption at 404 and 417 nanometers for methyldopa and levodopa, respectively, as shown in Figure 4.9 and Figure 4.10 below.

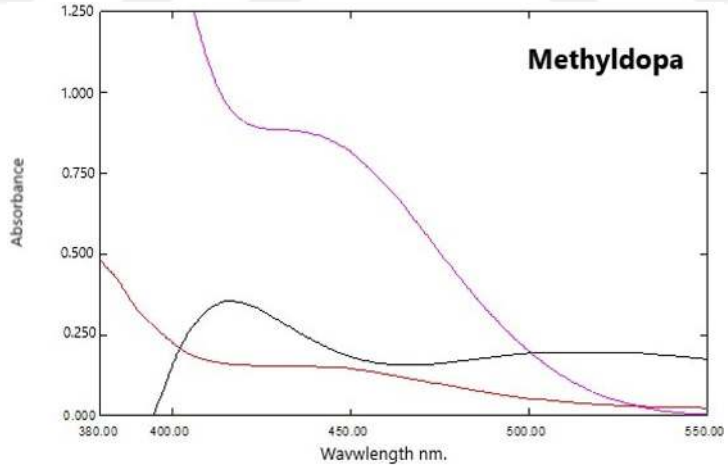


Figure 4.9 UV-VIS spectra of complexes formed of methyldopa

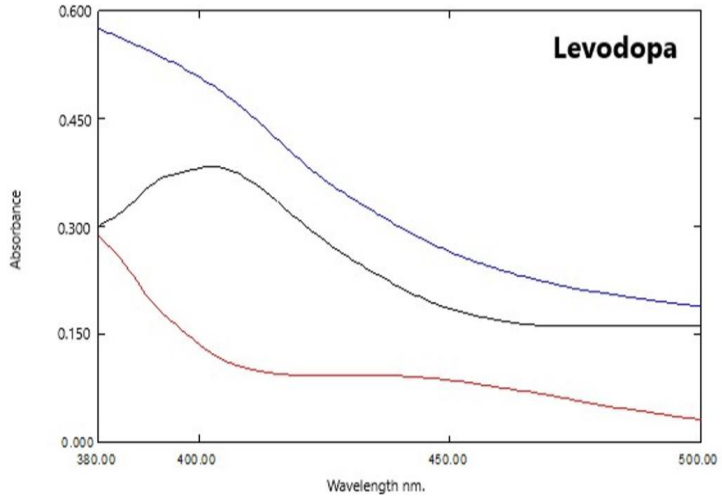


Figure 4.10 UV-VIS spectra of complexes formed of levodopa

A: Complex solution of the drug compound versus distilled water.
B: Complex solution of the drug compound versus the reference solution.
C: Reference solution versus distilled water.

4.12 Establishment of Standard Curve

The standard curve was plotted by applying the optimal conditions listed in Table 4.7, to determine both Methyldopa and levodopa in the aqueous solution within the range of the different concentrations from (2.5-40) and (7.5-60) µg/ml, respectively. The resulting standard curve was shown in Figure 4.8 below.

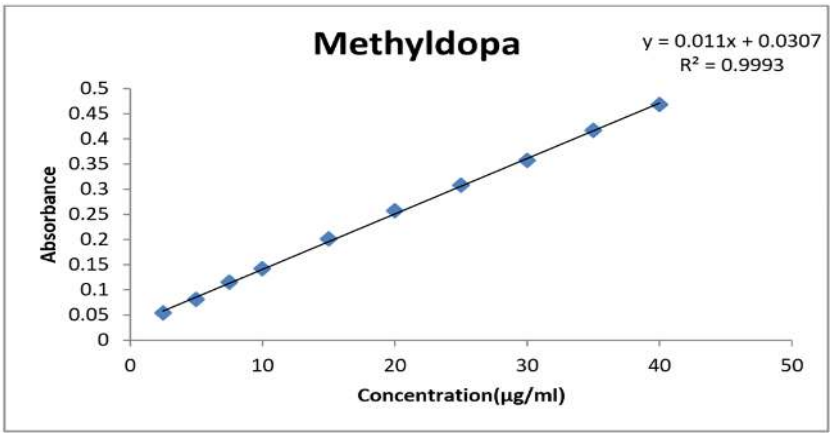


Figure 4.11 Standard curve of methyldopa complexes

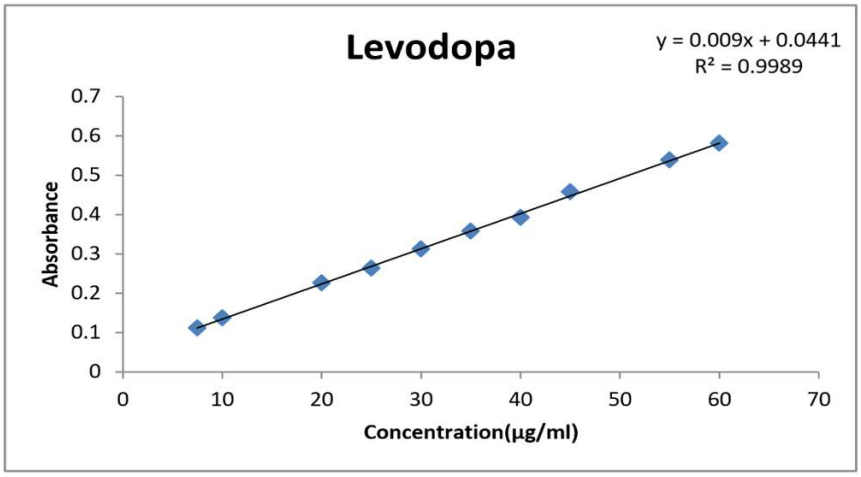


Figure 4.12 Standard curve of levodopa complexes

4.13 The Accuracy and Compatibility of the Method

To assess the accuracy and compatibility of the method, five-time samples were taken for three different concentrations of methyldopa. The results in Table 4.6 indicate that the developed method is good in compatibility, sensitive and accurate.

Table 4.6 Accuracy and compatibility data of developed method

Compound	Amount added(µg.ml^{-1})		Recovery* (%)	Average Recovery (%)	RSD*(%)
	Taken	Found			
Methyldopa	5	4.905	98.11	99.663	1.87
	20	19.91	99.54		1.23
	35	35.469	101.34		0.971
Levodopa	10	100.12	100.12	100.06	1.03
	30	29.379	97.93		1.52
	50	51.065	102.13		1.19
* Average of five determinations					

4.14 Study the Nature of the Resulting Product

To study the nature of the complex formed from the reaction of each of the methyldopa and the levodopa with the prepared oxadiazole reagent, the methods of Job and molar ratios were applied.

4.14.1 The continuous variation method (JOB's Method)

To determine the compositional molar ratios, the Job's method was applied to diluted solutions. Figure 4.13 and Figure 4.14 illustrates the obtained results for both drugs, indicating a 2:1 ratio between the drug compound and the 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole reagent.

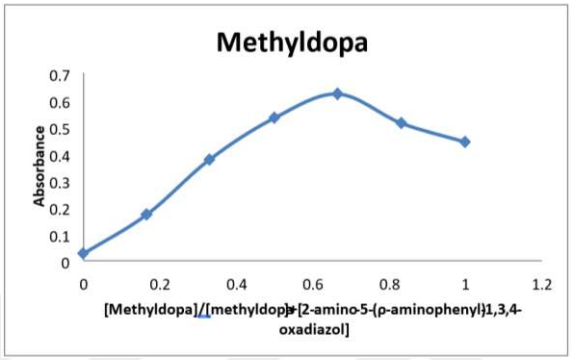


Figure 4.13 Graphical representation results of jobs method for methyldopa

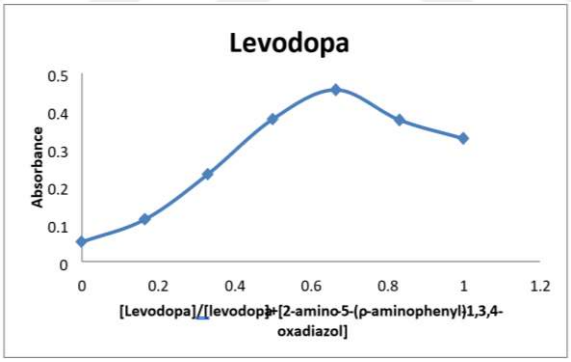


Figure 4.14 Graphical representation results of jobs method for levodopa

4.14.2 The molar ratio method

To validate the results obtained from the Job's method, the molar ratio method was applied. In this method, varying volumes of the drug compound were added to a fixed volume of the reagent. The results obtained from this method, as shown in Figure 4.15 and Figure 4.16, confirm the accuracy of the ratios obtained from the Job's method.

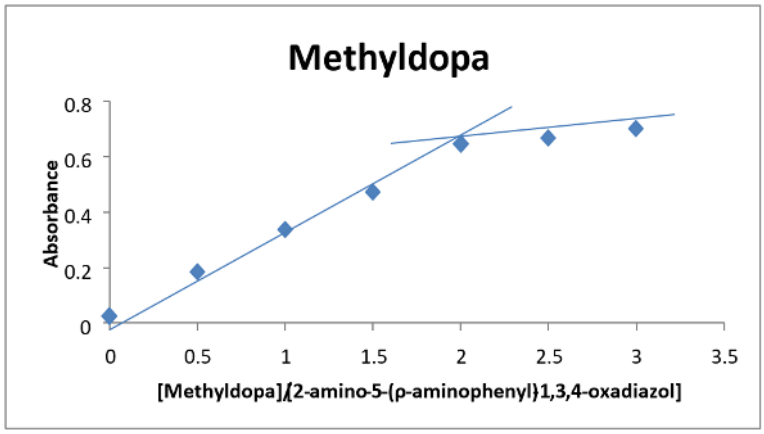


Figure 4.15 Graphical representation of results of Molar ratio method for methyldopa

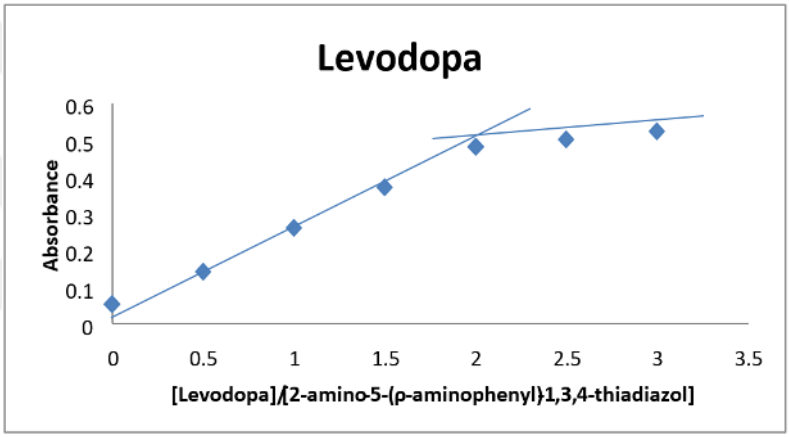


Figure 4.16 Graphical representation of results of Molar ratio method for levodopa

4.15 Calculation of the Stability Constants of the Formed Complexes

The stability constants of the formed complexes at a 2:1 ratio for both methyldopa and levodopa with the 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole reagent were calculated. Table 4.7 showed that the stability constants of the formed complexes are very high.

Table 4.7 The stability constants of the two complexes formed

Compound	Conc. (mol. l^{-1})	Absorbance		α	Average K_{st} ($l^2.mol^{-2}$)
		As	Am		
Methyldopa	1×10^{-5}	0.115	0.182	0.368	21.87×10^{11}
	1×10^{-4}	0.4387	0.441	0.877	
	1×10^{-3}	0.461	0.542	0.850	
Levodopa	1×10^{-5}	0.103	0.175	0.4114	20.37×10^{11}
	1×10^{-4}	0.361	0.423	0.1466	
	1×10^{-3}	0.425	0.501	0.1517	

4.16 Application of the Developed Method on the Pharmaceutical Synthesis

The developed method was applied using the standard curve of pure methyldopa. The concentration of methyldopa in the tablets was determined, and the results obtained are shown in Table 4.8.

Table 4.8 Determination of methyldopa in pharmaceutical Synthesis

Pharmaceutical Preparation	Certified Value (mg)	Amount Present(µg/ml)		Drug content found*mg	Recovery (%)*	Average Recovery (%)
		Taken	Found			
Tablets Bristol Laboratories ltd	250	5	4.92	245.8	98.32	99.58
		10	9.876	246.9	98.76	
		20	20.164	252.05	100.82	
		35	35.413	252.95	101.18	
Tablets S.D.I.-IRAQ		5	5.04	252.225	100.89	100.225
		10	10.128	253.2	101.28	
		20	19.814	247.675	99.07	
		35	36.029	257.35	102.94	
*Average of five determinations						

According to the Table 9, it can say that the developed method to determine methyldopa in the pharmaceutical Synthesis was high in accuracy and compatibility level.

4.17 Evaluate the Results of the Proposed Method with the Standard Addition Method

To demonstrate the efficiency of the developed method, the standard addition method was applied to determine the compound of methyldopa. The results obtained, as shown in Figure 4.11 (A: S.D.I.-IRAQ, B: Bristol Laboratories ltd) and presented in Table 4.9, indicate that the obtained results were well matched with the proposed method. Which indicate this method have potential to be selected.

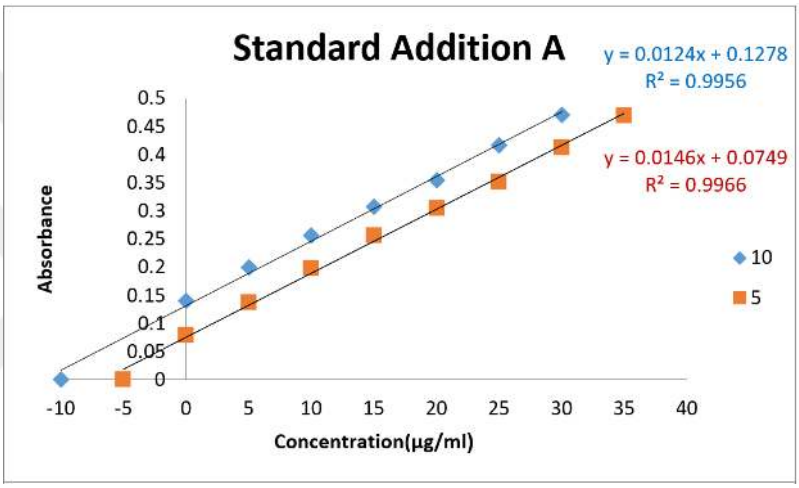


Figure 4.17 Standard addition A curve for the determination of methyldopa in pharmaceutical tablets

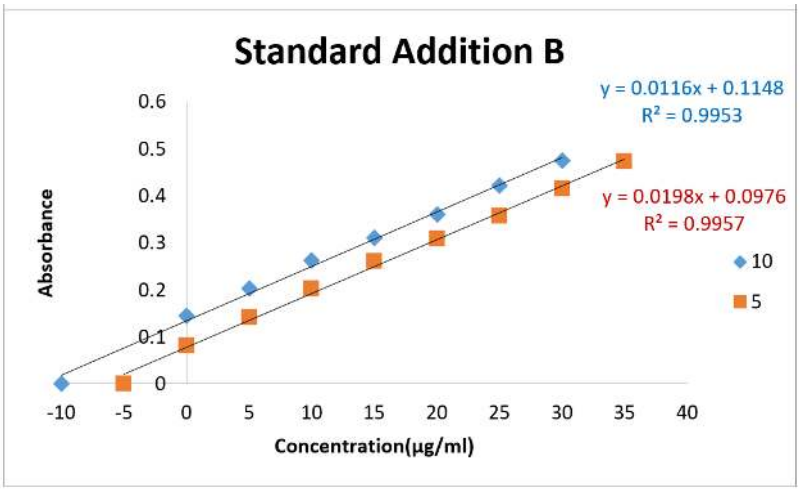


Figure 4.18 Standard addition B curve for the determination of methyldopa in pharmaceutical tablets

Table 4.9 Determination of Methyldopa in both methods (Standard addition and proposed)

Pharmaceutical Preparation	Certified Value(mg)	Amount Present (µg/ml)	Drug content found (mg)		Recovery (%) of standard Addition procedure
			Present method*	Standard Addition procedure	
Tablets Bristol Laboratories ltd	250	5	245.8	246.45	98.58
		10	246.9	247.4	98.96
Tablets S.D.I.-IRAQ		5	252.225	256.5	102.60
		10	253.2	257.65	103.06

4.18 Results of Biological Study

The compound 2-amino-5-(para-aminophenyl)-4,3,1-oxadiazole exhibited effectiveness against both Gram-positive and Gram-negative bacteria, such as Escherichia coli (E. coli) and Staphylococcus aureus. The inhibitory activity of the compound was found to be greater than that of known pharmaceutical compounds, as indicated in the table below:

Table 4.10 The inhibitory efficacy of the prepared reagent on the growth of both Gram-negative and Gram-positive bacteria (inhibition zone diameter measured in mm) using DMSO as a solvent

Comp No.	Staphylococcus aureus		E. Coli	
	10 (mg/ml)	1 (mg/ml)	10 (mg/ml)	1 (mg/ml)
2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole	32	26	24	22
Amoxicillin 10 mg/disk	25		-	
Ciprofloxacin mg/disk .5	-		15	

5. CONCLUSIONS AND RECOMMENDATION

A novel organic compound containing an active amine group was synthesized and employed for the estimation of pharmaceutical compounds through the diazotization reaction. The method was utilized for the quantitative determination of the pharmaceutical compounds Methyldopa and Levodopa. The developed method was proven highly sensitive and exhibiting high precision and accuracy. Additionally, a spectrophotometric method was developed for the determination quantities of both Methyldopa and Levodopa in µg using the detector 2-amino-5-(para-aminophenyl)-1,3,4-oxadiazole at wavelengths of 404 and 417 nanometers, respectively. The proposed method relied accurately on the oxidative coupling reaction. The developed method was successfully applied to estimate Methyldopa in the form of pharmaceutical tablets.

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APPENDICES

APPENDIX 1. Statistical

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1- Yield

$$\text{Yield} = \frac{\text{O}}{\text{T}} \times 100$$

As:

O = Actual Weight

T = Theoretical Weight

2- Recovery

$$\text{Recovery} = \frac{\text{Xi}}{\text{u}} \times 100$$

As:

Xi = the analytical result for the concentrations

u = the actual result for the concentrations

3- Standard deviation

$$S = \sqrt{\frac{\Sigma(Xi-\bar{X})^2}{N-1}}$$

As:

$\overline{\text{X}}$ = Calculation Medium

Xi = The results appear on the screen

N = The number of the results appear on the screen

4- Relative Standard deviation

$$\text{RSD}\% = \frac{\text{S}}{\overline{\text{X}}} \times 100$$

As:

S = Standard deviation

$\overline{X}$ = Calculation Medium

5- Relative Error

$$RE = \frac{F-T}{T} \times 100$$

As:

F = The measured value of concentrations

T = The Actual value of concentrations

6- The detection limit and quantification limit

It can be calculated for the identical solution by measuring absorption in ten replicates of the identical solution. The following relationships were applied:

$$LOD = \frac{3\ \sigma\ B}{S}$$

$$LOQ = \frac{10\ \sigma\ B}{S}$$

σ_B = The standard deviation of the identical solution.

S = Slop

7- Molecular absorptivity

ε = Slope × M. wt. × 1000

Slope = Slop

M.Wt = The molecular weight of the pharmaceutical compound

8- Sandel Sensitivity

$$S = \frac{M.Wt}{\varepsilon}$$

As:

M.Wt = The molecular weight of the pharmaceutical compound

ε = Molecular absorptivity

9- Dissociation degree and stability constant for the formed complex

$$\alpha = \frac{Am - As}{Am}$$

α = Dissociation degree

The stability constant for the formed complex with a ratio of 1:2 was calculated using the equation below:

$$K_{st} = \frac{1-\alpha}{4\,\alpha^3 C^2}$$

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