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DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMINO DRUG COMPOUNDS IN PHARMACEUTICAL PREPARATIO NS BY MOLECULAR ABSORPTION. UV-VIS SPECTROPHOTOMETER

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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June 2023

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

DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMINO DRUG COMPOUNDS IN THEIR PHARMACEUTICAL PREPARATIONS BY MOLECULAR ABSORPTION. UV-VIS SPECTROPHOTOMETER

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Master of Science in Chemistry

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For the determination of drug and its quantity in pharmaceutics, there was a eed of development of new, highly sensitive, and effecient approach. İn this study, spectrophotometric method was developed for sodium diclofenic and thiamin hydrochloride. Both water soluble drugs give maximum absorbance at 600 nm and 441 nm of wavelength. İt was found that, developed method is highly sensitive and obtained final product is stable for long time to get enough measurment to determine its presence and quantity precisely. İt was found that, we can detect sodium diclofenic in the range between 0.8-16 µg/ml and thiamin hydrochloride 2.4-60 µg/ml accurately, as the developed method obey the Beer's Law. The molar absorption values, the sandell sensitivity index of 2.8756 $Lmol^{-1}$ cm^{-1} and 0.0110 $\mu g.cm^{-2}$ for sodium diclofenic and 8.526103 $lmol^{-1}cm^{-1}$ and 0.0390 µg/ml for thiamin hydrochloride was measured. The developed method was successfully applied for the determination of diclofenac sodium and thiamin hydrochloride in pharmaceutical preparations (pharmaceutical tablets and capsules) with reproducibility ranged of 98.4-99.9 %.

2022, 67 pages

Keywords: Spectrometric, Amino group, Sodium diclofenic, Thiamin hydrochloride

ÖZET

FARMASÖTİK HAZIRLIKLARINDAKİ (AMİNO İLAÇ BİLEŞİKLERİNİN) MOLEKÜLER SORSİYON İLE BELİRLENMESİ İÇİN SPEKTROFOTOMETRİK YÖNTEMLERİN GELİŞTİRİLMESİ. UV-VIS SPEKTROFOTOMETRE

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Eczacılıkta ilaç ve miktarının belirlenmesi için yeni, duyarlılığı yüksek ve etkin bir yaklaşımın geliştirilmesine ihtiyaç duyulmuştur. Bu çalışmada sodyum diklofenik ve tiamin hidroklorür için spektrofotometrik yöntem geliştirilmiştir. Her iki suda çözünür ilaç da 600 nm ve 441 nm dalga boyunda maksimum absorbans verir. Geliştirilen yöntemin oldukça hassas olduğu ve elde edilen nihai ürünün varlığını ve miktarını tam olarak belirlemeye yetecek kadar ölçüm elde etmek için uzun süre stabil kaldığı görülmüştür. Geliştirilen yöntem Beer Yasasına uyduğu için 0,8-16 µg/ml aralığındaki sodyum diklofenik ve 2,4-60 µg/ml aralığındaki tiamin hidroklorürü doğru olarak tespit edebildiğimiz saptanmıştır. Molar absorpsiyon değerleri, sodyum diklofenik için 2,8756 $Lmol^{-1}$ cm^{-1} ve 0,0110 $\mu g.cm^{-2}$ ve tiamin hidroklorür için 8,526103 $lmol^{-1}cm^{-1}$ ve 0,0390 µg/ml sandell duyarlılık indeksi ölçüldü. Geliştirilen yöntem, diklofenak sodyum ve tiamin hidroklorürün farmasötik müstahzarlarda (farmasötik tabletler ve kapsüller) tayini için 98.4-99.9 % tekrarlanabilirlik ile başarıyla uygulandı.

2022, 67 sayfa

Anahtar Kelimeler: Spektrometrik, Amino grup, Sodyum diklofenik, Tiamin hidroklorür

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Hashem Khalaf Thamer THAMER
Çankırı-2023

CONTENTS

LIST OF SYMBOLS

µg	Microgram
g	Gram
mg	Milligram
mL	Milliliter
nm	Nanometre

LIST OF ABBREVIATIONS

$\overline{X}$	Reading rate
B	Blank VS weter
BW	Solution from distilled water
CD-ROM	Compact disc
D L	Detection limit
DW	Distilled water
HPLC	Reverse phase columb
O	The top
RE	Relative error
RSD	Retieval calculation and relative standard deviation
S	Sample VS blank
SB	Absorbing the from vs famous solution
SBS	Absorbance of the Sample VS placebo
SDI	Atrodemark of the Samra pharmaceuti cacs factory of Iraq
T	The real value
TLC	Layer chromatography

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1. INTRODUCTION

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) that is used to relieve pain and inflammation. It works by blocking the production of certain chemicals in the body that cause pain and inflammation (Small, 1989). It is available in various forms, including tablets, capsules, injections, and topical gels. Diclofenac is commonly used to treat a variety of conditions, such as arthritis, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, menstrual cramps, and acute pain. It is also used to relieve pain and inflammation after surgery or injury (Menase 1978). Like other NSAIDs, diclofenac can cause side effects, such as stomach upset, nausea, vomiting, diarrhea, headache, dizziness, and skin rash. It may also increase the risk of serious cardiovascular events, such as heart attack and stroke, especially when used for long periods of time or in high doses (Gökçimen *et al.* 2001).

On the other hand, Vitamin B1 is known as thiamin or thiamine, a water-soluble vitamin that plays a key role in energy metabolism. It helps in to convert carbohydrates into glucose for fulfilment of required energy need of body (Martel *et al*. 2021, Fattal-Valevski 2011. It is also involved in the metabolism of fats and proteins. Thiamin is found naturally in many foods, such as whole grains, meat, fish, beans, and nuts. It is also added to many fortified foods, such as bread, cereal, and pasta. Thiamin deficiency is rare in developed countries, but it can occur in people who consume a diet lacking in thiamin-rich foods or in people with conditions that affect thiamin absorption, such as chronic alcoholism (Gibson *et al*. 2016, Rapala-Kozik 2011). Symptoms of thiamin deficiency can include fatigue, muscle weakness, loss of appetite, and nerve damage, which can lead to conditions such as beriberi and Wernicke-Korsakoff syndrome. Treatment for thiamin deficiency typically involves supplementation with thiamin hydrochloride, which is available in various forms, including tablets, capsules, injections, and oral solutions.

1.1 Objectives of Study

The goal of our study was to develop a novel, sensitive, and direct or quick responce spectrophotometric approach for identifying a class of significant and frequently used locally made medications, including diclofenac sodium and thiamine hydrochloride.

- Selection and optimization of appropriate organic and inorganic reagents for coupling reaction to produce detectable colored product.

- The possibility of applying the developed methods in analyzing the pharmaceutical preparations.

- Study the affect of chemical and physical variables and their interaction to established the best optimized conditions for developed approach to get highly sensitivity and accurate results.

2. LITERATURE REVIEW

2.1 Sodium Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) (Small, 1989) with chemical formula of Sodium (o-{(2,6-dichlorophenyl)amino}phenyl)acetate CO_2Na (Eisele et al. 2013), that was first introduced in 1973 by the pharmaceutical company Ciba-Geigy (now Novartis). Over the years, diclofenac has become one of the most widely used NSAIDs in the world, with indications for the treatment of a wide range of con ditions, including osteoarthritis, ankylosing spondylitis, and menstrual pain (Rodriguez et al. 2008, Mahood and Hamezh 2009).

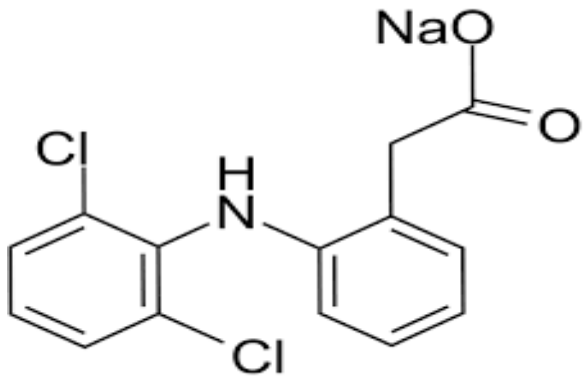

Figure 1.1 Structural formula of Sodium diclofenac (Eisele et al. 2013).

Diclofenic was initially developed as a eplacement for aspirin, which was the most commonly used NSAID at the time. As the goal of creation of a drug was to synthesis an alternative with a better safety and fewer side effects. It was initially approved for use in the United Kingdom and other European countries for the treatment of rheumatoid arthritis and other inflammatory conditions (Small, 1989, Sallmann 1986). Moreover, It is available in various forms, including tablets, capsules, injections, and topical gels. In addition, sodium diclofenic drug has several trade names, including: voltaren, olfen, Cataflam, Diclogesic, Diclomax, Rofenac, Tablflex, Votex (Eisele *et al.* 2013). In recent years, diclofenac has been the subject of controversy due to its potential

side effects, particularly its cardiovascular risks (Gökçimen *et al.* 2001). In 2013, the European Medicines Agency (EMA) conducted a review of the safety of diclofenac and recommended that it should not be used in people with certain pre-existing cardiovascular conditions, such as heart failure and coronary artery disease. The EMA also recommended that diclofenac should be used at the lowest effective dose for the shortest possible duration. Despite these concerns, diclofenac continues to be widely used for the treatment of pain and inflammation (Gen 2010). It is considered an effective and affordable option for many patients, but its use should always be carefully considered and monitored by a healthcare Professional (Gökçimen *et al.* 2001).

2.1.1 Physical Properties Of Sodium Diclofenic

Sodium diclofenac is a nonsteroidal anti-inflammatory drug (NSAID), a benzeneacetic acid derivative with molecular weight of 318.1 g/mol. It is a odorless, white or slightly yellowish crystalline powder (Adeyeye *et al*.1990) with a density of approximately 1.5 g/cm^3, having melting point of around 280-290°C. In addition, diclofenac is barely soluble in water, highly soluble in methanol, ethanol (96%) and aceton (Eisele *et al.* 2013). The drug is produced in the form of tablets, injections, and capsules.

2.1.2 Pharmacokinetic And Pharmacodynamic Study Of Sodium Diclofenic

Pharmacokinetics refers to the way a drug is absorbed, distributed, metabolized, and eliminated by the body. However, pharmacodynamics refers to the effects of the drug on the body and how it produces those effects. Pharmacokinetic study of diclofenac has shown that it is rapidly absorbed after oral administration, with peak blood concentrations reached within 1 to 2 hours (Lotsch *et al*. 2000, Ray *et al.* 2010, Narayanan *et al.* 2019, Ji *et al*. 2002). The bioavailability of diclofenac is about 50% due to extensive first-pass metabolism in the liver. However, food may delay the absorption of diclofenac, but does not significantly affect the extent of absorption. İn addition, diclofenac is extensively bound to plasma proteins, mainly albumin. It is metabolized in the liver by several pathways, including hydroxylation, demethylation, and glucuronidation. The major metabolite of diclofenac, 4'-hydroxydiclofenac, has

similar anti-inflammatory activity to diclofenac itself. Both diclofenac and its metabolites are eliminated in the urine and feces, with a half-life of approximately 2 hours (Lotsch *et al*. 2000, Ray *et al.* 2010, Narayanan *et al.* 2019, Ji *et al*. 2002). The pharmacodynamic study of diclofenac has shown that it works by inhibiting the production of prostaglandins, which are responsible for inflammation, pain, and fever (Gen 2010). Diclofenac is a non-selective inhibitor of cyclooxygenase (COX), which is the enzyme responsible for the production of prostaglandins (Tonussi and Ferreira 1994). By inhibiting COX, diclofenac reduces the production of prostaglandins, thereby reducing pain and inflammation. Diclofenac also has other pharmacological effects, such as inhibiting platelet aggregation and decreasing leukocyte migration. These effects may contribute to its anti-inflammatory and analgesic properties (Gen 2010). The pharmacokinetic and pharmacodynamic properties of diclofenac are important to understand in order to determine appropriate dosages, potential side effects, and drug interactions (Gökçimen *et al.* 2001).

2.1.3 Mechanisum Of Action Of Sodium Diclofenic

Diclofenac works by inhibiting the activity of the cyclooxygenase (COX) enzymes, which are responsible for the production of prostaglandins. Prostaglandins are hormone-like substances that are involved in pain, inflammation, and fever. By reducing the production of prostaglandins, diclofenac can help to relieve pain, reduce inflammation, and lower fever (Menase 1978, Paez-Hurtado *et al.* 2023). There are two main isoforms of COX: COX-1 and COX-2. COX-1 is expressed in most tissues and is involved in the normal physiological functions of various organs, such as the stomach, kidney, and platelets. COX-2, on the other hand, is induced during inflammation and is responsible for the production of prostaglandins that cause pain and inflammation. Moreove, diclofenac is a non-selective inhibitor of both COX-1 and COX-2 (Tonussi and Ferreira 1994, Alfero and Davis 2020, Menase 1978). T his means that it can inhibit the production of prostaglandins in various tissues, including those involved in the normal physiological functions of the body. This can lead to side effects, such as stomach upset, bleeding, and kidney damage. However, diclofenac is more selective for COX-2 at lower doses, which may explain its anti-inflammatory and analgesic effects. Overall, the mechanism of action of diclofenac involves inhibiting the production of prostaglandins

through the inhibition of the COX enzymes, leading to a reduction in pain, inflammation, and fever (Tonussi and Ferreira 1994, Alfero and Davis 2020).

2.1.4 Synthesis Of Sodium Diclofenic

Sodium diclofenac is a salt of diclofenac, which is a benzeneacetic acid derivative. It can be synthesized by reacting diclofenac acid with sodium hydroxide (NaOH). Diclofenac acid can be synthesized by reacting 2-acetylphenylacetic acid with phenylacetic acid in the presence of a catalyst such as zinc chloride (Altman *et al.* 2015). The resulting diclofenac acid can be purified by recrystallization from a suitable solvent. A solution of diclofenac acid in a suitable solvent such as methanol is treated with a stoichiometric amount of sodium hydroxide. The mixture is heated and stirred until complete dissolution of the starting material is achieved. The resulting solution is then allowed to cool, and the product sodium diclofenac salt is obtained by filtration, washing, and drying (Altman *et al.* 2015). The reaction can be represented as follows:

$$\text{Diclofenac acid} + \text{NaOH} \rightarrow \text{Sodium diclofenac} + H_2O$$

According to Aymen Labedi, synthesis of sodium diclofenac can be done by the reaction between 2-chlorobenzoic acid and 2,6- dichloroaniline an aromatic nucleophilic substitution reaction, also known as ipso-subsitution of addition-elimination reacton. During reaction $LiAlH_4$ an inorganic compound was used as reducing agent in organic syntheis (LABIDI 2021). The reduction of carboxylic acid, amides and esters. It converted the carboxylic acid into primary alcohol (1.2) as shown in Figure 2.2. Thionyl chloride SOCl2 is an inorganic compound, used as a chlorinating reagent to convert hydroxyl group into chlorine 2- and [(2,6-dichlorophenyl)-amino]-benzylchloride intermidiate formed (1.3). Which, react with sodium cynanide through a nuclephilic subsitution reaction leading to 2-[(2,6-dicholorophenyl)-amino]benzyl cyanide (1.4), leading to the Alkaline Hydrolysis and formation of the final product (1.5) as shown in Figure 2.2.

An other study reported the mechanisum of synthesis of diclofenic by heating 2-chlorobenzoic acid and KOH dissolved in 1-pentanol at 100°C under stirring for 1 h. After cooling, added the 2,6-dichloroaniline and copper powder and the mixture was refluxed for 120 h. A solution of Na_2CO_3 was added to the mixture then cooling and extraction of organic layer was done with pentanol. A brown solid ws obtained after precipitation of organic phases with drop wise addition of concentrated HCl, and precipitates were washed filter and dryed (LABIDI 2021).

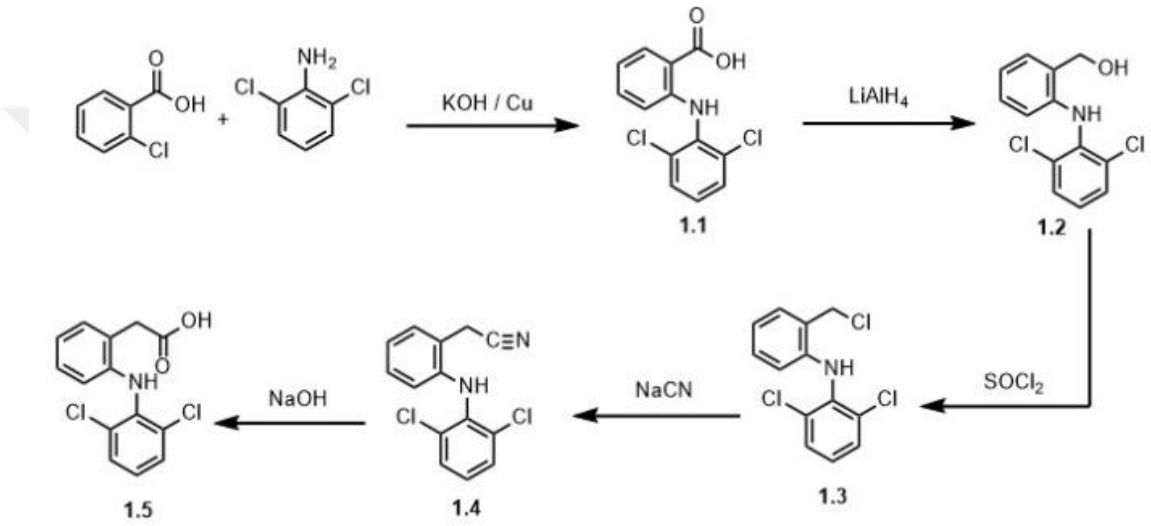

Figure 2.2 Synthesis method of sodium diclofenac (LABIDI 2021).

The obtained compound 1 was taken into THF with dropwise addition to a suspension of $LiAlH_4$ and cooled at 5°C–15°C. The final mixture was refluxed for 15 h. After that, 20 ml of water and NaOH was added and coold it. The suspension was filtered, washed with THF and added ethyl ether to get final product 2. İn addition, the obtained solution of compound 2 was added into diethyl ether and anhydrous pyridine dropwise into a solution of $SOCl_2$ with anhydrous pentane and cooled and pored on ice (Petroni *et al*. 2011).

The obtained mixture was extracted with 30 ml of 2N HCl and washed with water and 2N NaOH solution. The organic phase was filtered to get product 3 and dried over K_2CO_3. After that, Potassium cyanide was added to a solution im capped V-shaped vial. The mixture containing platinum wool heated at 1000°C. 25 µl of 1 M NaOH and 1.5 M

KCN was added (Petroni *et al*. 2011). The solvent was evaporated under nitrogen flow, and the resulting sodium cyanide solution was used without purification. Over it, 2.8. 1-[11C]-2-{2-[(2,6-Dichlorophenyl) amino]phenyl]} acetonitrile added to the vial containing $Na_{11}CN$. The vial containing crude 11C-labeled compound 4 was added with 10N NaOH and 30% H_2O_2, and the mixture was heated at 135°C for 10 min under stirring (Petroni *et al*. 2011). And final form of diclofenic product was obtained as shown in Fiure 2.3.

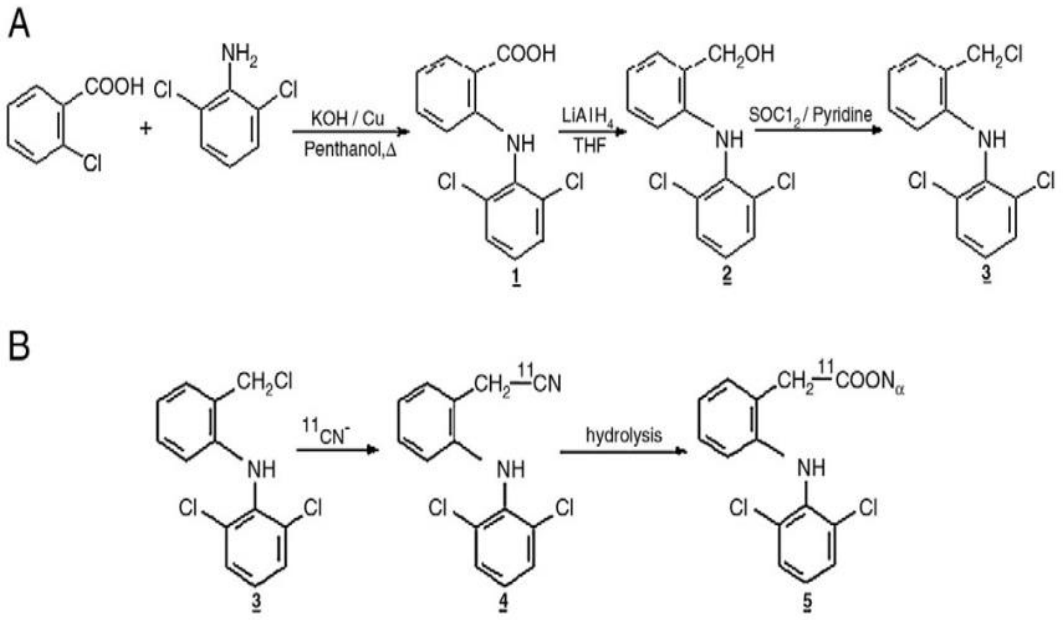

Figure 2.3. Synthesis of [^{11}C] diclofenac and its precursors (Petroni *et al*. 2011).

2.2 Thiamine Hydrochloride

Thiamin hydrochloride with a chemical formula of 3-[(4-amino-2-methylpyrimidin-5-yl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride, also known as thiamine or vitamin B1, a water-soluble vitamin that is essential for the proper functioning of the body (Eisele et al. 2013). It plays a crucial role in energy metabolism, nerve function, and the metabolism of carbohydrates. Thiamin hydrochloride is derived from thiamin and is commonly used as a dietary supplement to treat or prevent thiamin deficiency (Martel *et al*. 2021). The discovery of vitamin B1, is credited to the Polish

biochemist Casimir Funk in 1912. Who was studying beriberi, a disease that was prevalent in Asia and caused by a deficiency of an unknown nutrient. Funk hypothesized that the disease was caused by a lack of a specific nutrient, which he called a "vitamine". Funk and his team began to search for this vitamine and identified a substance in rice polishings that prevented and cured beriberi in chickens (Martel *et al*. 2021, Fattal-Valevski 2011, Gibson *et al.* 2016, Rapala-Kozik 2011). They isolated this substance and called it "thiamin." The chemical structure of thiamin was later elucidated by Robert Williams and his team in 1936 as shown in Figure 2.4.

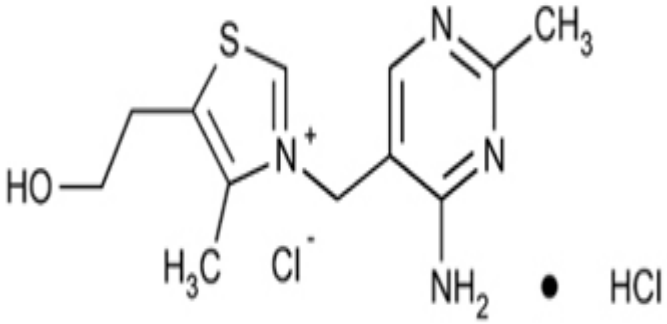

Figure 2.4 Structural formula of thiamine hydrochloride (Voelker *et al*. 2021).

Thiamin hydrochloride was first synthesized in 1926 by the German chemist E. L. Sommer, and its chemical structure was confirmed by Williams and his team in 1936. Since then, thiamin hydrochloride has been widely used as a dietary supplement and food additive to prevent and treat thiamin deficiency. Thiamin deficiency was a significant health problem in the early 20th century, particularly in countries where polished rice was a staple food (Gibson *et al.* 2016, Rapala-Kozik 2011). The fortification of foods with thiamin hydrochloride, along with improvements in food processing and distribution, has led to a dramatic reduction in thiamin deficiency worldwide. Thiamin hydrochloride can be found in a variety of foods, including whole grains, meat, fish, legumes, and fortified cereals. However, some individuals may require higher doses of thiamin due to certain medical conditions or lifestyle factors, such as alcoholism or malabsorption syndromes.

2.2.1 Properties And Mechanisum Of Action Of Thiamin Hydrochloride

vitamin B1 hydrochloride or thiamin hydrochloride is a is a white or off-white crystalline powder with molecular formula of 337.27 g/mol, soluble in water and slightly soluble in alcohol. Vitamin B1 is sensitive to heat, light, and acidic conditions, therefore, it should be protected from light and stored in a cool, dry place. It has been available in different brand names in market such as Surbex, Theravite, vicon - c, Z - bec, Nwrvovit and Beblx. However, the mechanisum of action of vitamin B1 in the body start by its conversion to its active form of thiamin pyrophosphate (TPP) (Gibson *et al.* 2016). Which is a essential cofactor for several enzymes involved in energy metabolism, including pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. There is an enzyme pyruvate dehydrogenase, that converts pyruvate, a product of glucose metabolism, into acetyl-CoA a precursor for the production of ATP and main source of energy in the body. Alpha-ketoglutarate dehydrogenase is an enzyme that is involved in the metabolism of amino acids and also produces ATP. Thiamine also helps in the treatment of type 2 diabetes and peripheral neuritis. Thiamine is taken either in the form of pills, or injection is taken into the muscle (Rodriguez et al. 2008). Moreover, vitamin B1 and TPP play a critical role in nerve function and the production of neurotransmitters (Martel *et al*. 2021). TPP is a cofactor for the enzyme transketolase, which is involved in the production of neurotransmitters such as acetylcholine and gamma-aminobutyric acid (GABA). İn addition, thiamin hydrochloride deficiency can lead to a range of neurological and cardiovascular disorders, including beriberi, Wernicke-Korsakoff syndrome, and heart failure. These conditions are caused by a dysfunction in energy metabolism and nerve function, which can be corrected by supplementing with thiamin hydrochloride (Novoa *et al.* 2023).

2.2.2 Pharmacokinetic And Pharmacodynamic Study Of Vitamin B1

Absorbtion, distribution, metabolizum of drug and its elimination from body is refered as pharmacokinetics of the drug. However, the effect of a drug on the body, including its mechanisum of action and therapeutic effects is called pharmacodynamics of drug. İn case of thiamin hydrochloride, Thiamin hydrochloride, exists in nature both in free

(thiamin) and esterified form, is rapidly absorbed in the small intestine by passive diffusion, and the absorption is not affected by food (*Smithline et al. 2011*). Moreover, it distributed throughout the body, with high concentrations found in the liver, kidneys, heart, and brain. İn addition, it is metabolized in the liver and other tissues to its active form, thiamin pyrophosphate (TPP), which is involved in several enzymatic reactions in the body. Hence, excreted mainly in the urine, with a half-life of approximately 1-1.5 hours (Hes *et al.* 2021, Koski *et al*. 2005, Novoa *et al*. 2023, Thakur *et al.* 2023). However, the pharmacodynamic study of vitamin B1 showed that, it is converted to TPP, which is an essential cofactor for several enzymes involved in energy metabolism, including pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase. Thiamin also plays a critical role in nerve function and the production of neurotransmitters. Overall, thiamin hydrochloride is a safe and effective dietary supplement that plays a critical role in energy metabolism, nerve function, and other physiological processes in the body (Smithline *et al*. 2011).

2.2.3 Synthesis of Thiamin hydrochloride

Vitamin B1 hydrochloride, can be synthesized in the laboratory using several methods. One of the most common methods is the thiazole synthesis, which involves the condensation of 2-methyl-4-amino-5-hydroxymethylpyrimidine with thioacetamide to form 2-(2-methyl-4-amino-5-pyrimidylmethyl)-5-(2-hydroxyethyl) thiazole. This compound is then hydrolyzed to form thiamin. In addition, Williams and j.k cline in 1936, first time synthesized thiamin by the rute dipicted in Figure 2.5. During the synthesis of thiamin, 5-(2-hydroxyethyl)-4-methylthiazole was subjected to the quaternization reaction with 4-amino-5-ethoxymethyl-2-methylpyrimidine. To get the expected product this method is still successfully used with some published study about modification in thiazole ring (Tylicki *et al*. 2018).

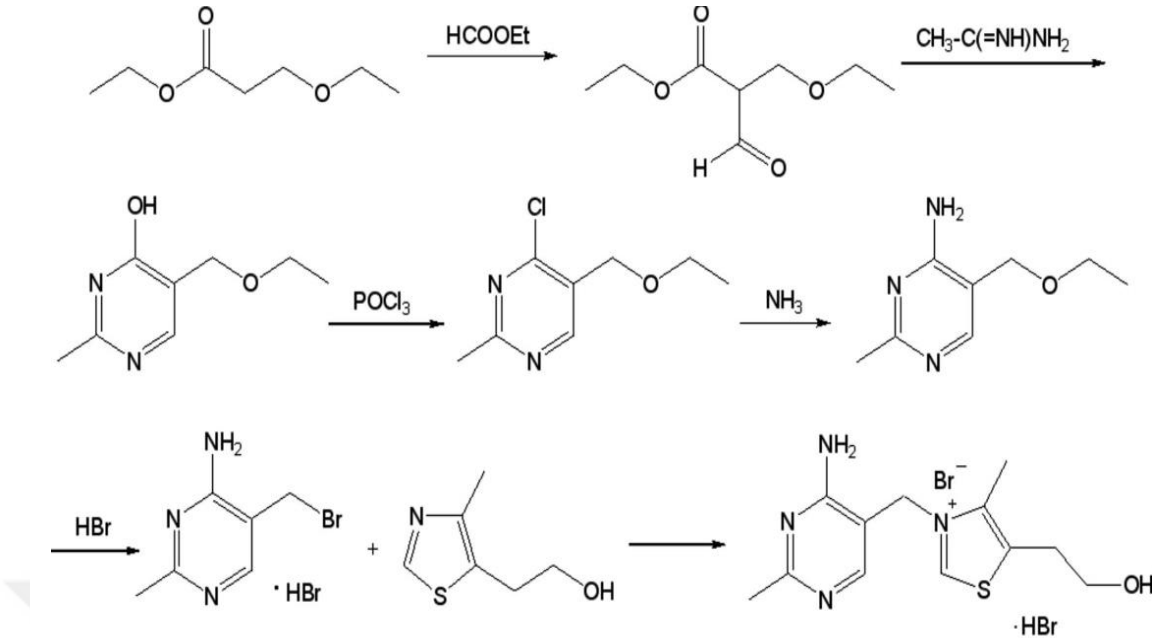

Figure 2.6 Williams synthsis method of thiamin hydrochloride (Tylicki *et al*. 2018).

Another method for the synthesis of thiamin hydrochloride involves the condensation of 4-methyl-5-(2-hydroxyethyl) thiazole with 2-methyl-4-amino-5-pyrimidine methiodide to form 2-(2-methyl-4-amino-5-pyrimidylmethyl)-5-(2-hydroxyethyl) thiazole. This compound is then reacted with hydrochloric acid to form thiamin hydrochloride (Contant *et al*.1990).

2.3 Oxidative Coupling Reactions

Oxidative coupling reactions have been widely exploited in the determination of important compounds in the fields of agriculture, food, environment, and clinical and pharmaceutical analyses by applying various analytical techniques such as spectroscopic (Sastry and Krishna 1996), fluorescent (Hušek *et al.*1990) and chromatography (Pietrogrande *et al.* 1985). The most important factors affecting oxidative duplication reactions were studied, as it was noted that the presence of certain ions such as the chromium ion, binary iron or manganese, and others, behave as catalysts in many oxidative duplication reactions. Moreover, the temperature plays very important role in activating some of these uncatalyzed reactions till at 100 °C to obtain a

color product with high intensity (Borsook1935). The influence of the nature of the reaction medium has also been investigated. It has been found that oxidative coupling reactions can take place in neutral, acidic and basic media, depending on the quality of reactants, reaction conditions and the stability of colored products in different reaction media.

Moreover, study about the mechanism of oxidative doubling reactions of aromatic compounds, it was found that some of these reactions depend on the formation of free radicals entering the reaction in the presence of cofactors (Mottola and Hanna 1978), and the other part of the reactions depend on the formation of intermediate compounds that have the ability to pair with other compounds (Amiani and Jagannodham 1990). The unsubstituted amines and phenols are among the most important reagents used in the oxidative coupling reactions for their interaction with the compounds to be determined. However, when these reagents are substituted on the parasite, it is difficult to obtain the reaction with the substance except for the halogenated substituted phenols. That these compounds participate in this type of reaction because of the ability of the halogen atom to move from one parasite to another site in the other ring when suitable oxidation conditions are available, then the compound is more susceptible to the duplication process (Soloway and Santoro1955). Berthelot (Searle1984) was considered the first to study the reactions of oxidative coupling in 1859 AD. The reaction was named after him. Sometimes it is called the indophenol reaction. This reaction involves the reaction of ammonium ions with a phenol reagent under suitable oxidation conditions to form the blue dye indophenol whose absorbance is measured at the two-maximum wavelength of 630 and 720 nm (Önal 2011).

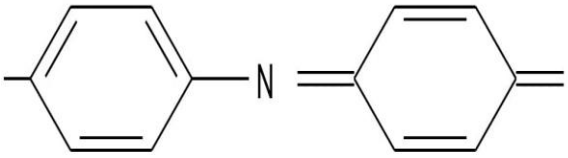

Figure 2.7 Structural formula of Indophenol

Morover, there is an evaluation of the sulfonamides, involved in oxidative coupling reaction with phenothiazine in the presence of sodium metaperiodate, an oxidizing agent. The absorbance of the resulting green dye was then measured at a wavelength of 612 nm. This approach is successfully used in certain pharmaceutical formulations, since the relative error value was less than 1.5 % with the relative standard deviation (S.D.) of less than 1.8 % (Azeez *et al.* 2006). After being combined with the reagent prominazine hydrochloride in the presence of hypochlorite as an oxidizing substance, chloramphenicol (Chloramphenicol) in its pure form and pharmaceutical preparations were reduced with zinc powder in an acidic medium. The resultant compound had the highest absorption intensity at the wavelength of 600 nm, having a sandel 0.0172 g/cm^2, and relative standard deviation 1.83 % (Sinan and Al-Abachi 2010). The Önal and his research team in 2011 created a spectrophotometric method by combining iodine with 4-chloro-7-nitrobenzofuranone. This method allowed them to easily determine carvedilol's dosage using a spectrophotometer as long as they added 7-60 µg/ml into the medium. The average slice concentration range, RSD value, linear concentration range and detection limit of carvedilol were 7-60 µg/ml, 0.98, 480 nm and 0.055 µg/ml, respectively. Additionally, their method returned more than 99.75% of the original carvedilol when tested multiple times. Benzocaine, lidocaine, and procaine hydrochloride have also been determined spectroscopically in their pure state and in some pharmaceutical preparations. This approach depends on the interaction of these chemicals with p-benzoquinone in an acidic solution to generate a colored charge transfer complex with a 525 nm absorbance. This method works well with Beer's law. The molar absorption factors of the resulting complexes were between 5-70 $\mu g.ml^{-1}$ for benzocaine, 5-60 $\mu g.ml^{-1}$ for lidocaine and 5-90 $\mu g.ml^{-1}$ for Procaine Hydrochloride (Amin and El-Didamony 2003).

Morover, it was reported that, salbutamol sulfate in medicinal formulations was also determined by using spectrophotometry. The reaction of salbutamol sulfate with a nitrified ortho-nitroaniline reagent in an alkaline medium was the base of the approach. Where it gives a stable, and water soluble yellow-orange product give maximum absorbance at 448 nm of wavelength. The procedure follows Beer's Law in the range of 50 to 1000 µg with a final volume of 25 ml, or 40 ppm. Similarly, study reported about

chloramphenicol determination by the reagent promethazine hydrochloride reaction with concentrated hydrochloric acid in the presence of hypochlorite ions at 600 °C. After reducing the nitro group of chloramphenicol to an amino group in the presence of zinc powder in pharmaceutical preparations, spectrometric analysis was studied (Hadi 2008). In addition, a very simple and sensitive method for determining the number of apricots was developed. Oxidative coupling reactions were performed in the presence of potassium ferrocyanide, aniline reagent and water. The resulting purple compound was studied at 530 nm and found that it obey's Beer's Law in the limit of 16-0.4 $\mu g.ml^{-1}$. The recovery rate was 100.62 % and the resulting molar absorbance of 2.2525 x 10 ml. Spectroscopic determination of sulfacetamide using trifluoperazine hydrochloride by oxidative coupling reaction in the presence of oxidant quaternary cerium. The relative ratio between 0.5-2.33 % and the relative standard deviation of 4.56 % with molar absorbance value of 0.7×10 liter.mol·cm, and sandal significance of 0.0303 $\mu g.cm^2$ was calculated. Moreover, study about paracetamol identification by oxidative coupling reaction of (1-naphthyl) ethylenediamine in acidic solution with sodium periodate as oxidant was reported. According to Sandel, the MILLA and molar absorbance of 7.0 x 10 liter.mol·cm, 0.021 μg and 2 maked it significant with S.D of less than 2.65 % (Sabeh and Abdul Aleem 2007).

2.3.1 Spectroscopic Study Of Sodium Diclofenic

The team of researchers led by Rodriguez used sodium diclofenic combined with potassium permanganate to create a spectroscopic method for measuring sodium diclofenic in urine samples and medical preparations. This method uses alkaline medium with an interaction of NaOH that has a linear range between 10-100 μg/ml, with a detection limit of 5 μg/ml. İn another study, sodium diclofenic reacts with iron chloride to form iron (II) through a process that takes only a short time. The procedure involves sodium diclofenic interacting with potassium and ferric hexacyanide in order to create a blue compound. A method for determining sodium diclofenic concentration in pharmaceuticals needed quick and easily. The correlation value for this method was 0.9980. It has a concentration range of 1-6 μg/ml, and molar absorptivity of 2.0581 x 10

liters.mol.cm, and 0.2 % of relative standard deviation with 97.83 to 104.9 regression from the mean values (Mahood 2009).

The researcher (Choksi et al. 2013) and his team were successful in developing a spectroscopic approach for concurrently analyzing sodium diclofenic and thiocolchicoside. The derivative may be measured spectrophotometrically to determine sodium diclofenic at 249 nm with a minor contribution from thiocolchicoside. Likewise, sodium diclofenic did not affect thiocolchicoside measured at 246 nm. For sodium diclofenic and thiochalkoxide, the technique exhibited linearity at concentrations of 4-63 and 2-10 g.mL- respectively, with a relative recall of 99.55 % sodium diclofenic and 99.32 % THIO, with relative standard deviations overlapping less than 2 % of the data. A straightforward spectrophotometric technique was created employing the area under the curve approach at a wavelength of 250–260 nm for concurrent and extensive measurements of sodium diclofenic. The relative standard deviation was likewise smaller than 2 % (Patel and Hariyani2013). For the simultaneous measurement of diclofenac sodium and paracetamol in the majority of dosage forms and tablets, an innovative and repeatable approach has been put forth. Each of the two medications was estimated using the wavelength-based absorption approach. The approach had a percentage inaccuracy of less than 2.0 % and a standard deviation of less than 1.0 % (Sharma et al. 2010). The researcher (Nirmal and Shinde 2013) also estimated sodium diclofenic and Tolperisone HCI in the form of tablets. The approach yielded a linear concentration range of sodium diclofenic 5-50 g. ml and Tolperisone 5-60 g. ml.

For the measurement of sodium diclofenic and eperisone HCl in tables and capsules, a spectrophotometric approach has been established. The linear range of 2-20 and 3-30 g/mL, for sodium diclofenic and Epperson were measured through this method. Correlations with 0.9990 at 281 nm and 0.996 at 255 nm in methanol yield a 99.8% and 101.2% accuracy rate, respectively. The detection limit for this method is (0.452) with a detection range between (0.149) µg/mL for DCF and Pearson (Harde *et al.* 2013). Moreover, according to another study, 60:20:20 ratio of ionized water, acetonitrile and methanol used in a mobile phase for HPLC. This mixture was then combined with 1 ml of flow at a 283 nm wavelength. The two-minute separation process took place on a 150

x 4.6.5 μm.cm column that was fitted onto a C8 cartridge from Supelco. At standard deviation of 1.2 %, the methodology's linear range extended from 0.25 to 4.0 μg/ml. The approach was used to accurately measure the quantity of diclofenac sodium in various pharmacological formulations (Atto 2012). By using a C18 column with 4.6 x 25 mm of dimensions and ethanol as a "mobile phase," the researchers (Ahmed and Lottfi, 2011) presented a specific approach for sodium diclofenic in both its undoped condition and in its pharmaceutical formulation. Use a 1 ml of flow rate. At a wavelength of 283 nm, measurements are made, and it become possible to estimate the quantity between 5- 80 μg/ml. The relative standard deviation of the approach was discovered to be less than 1% (Ahmed and Lottfi 2011). In addition, a technique devised for determining sodium diclofenic levels in rats serum, was accomplished after extracting 1 molar of orthophosphoric acid solution and a mixed solution consisting of hexane and isopropyl alcohol, with a correlation coefficient of 0.9999 (Hamad and Yahya 2013). In 2008, Kasperek successfully created an HPLC method for analyzing papaverine and dichlorofluoromethane in tablets using a Zorbax SB C18 column and a mobile phase composed of methanol and water in a 60:40 ratios. A Flow rate of 1 milliliter was used with a wavelength of 278 nanometers. (Kasperek 2008).

A method has been proposed to estimate sodium diclofenic using fluorescence injection (FIA) coupled analysis of luminol fluorescence suppression with hydrogen peroxide in the presence of Court ions (Co2 catalyst), 0.32% relative to the benchmark (Almahod et al., 2010). A sample solution of 5-50 g/ml of diclofenac sodium was injected into DCNP containing 0.01% (w/w) methanol at 451 nm in order to test the presence of diclofenac sodium in pharmaceutical formulations (FIA) using the flow-through injection method. The color of charge transfer complex creation at nm wavelengths was measured and detected with relative standard deviation of less than 1.6 % (Kamath et al. 1994). Diclofenac sodium was determined by forming MBTH-supplied compounds in 2-10 x 3 M sulfuric acid media (García et al. 1998).

Using the FIA chemiluminescent detector (CL), a sensitive technique for determining sodium diclofenic in pharmaceutical formulations has been established, With a concentration range of 0.040-5.0 g. The approach demonstrated linearity, standard

deviation and a concentration of diclofenac sodium 0.020 μg/ml, 0.040 μg/ml 2.0% (Song *et al.* 2015). An electrical method for determining sodium diclofenic was created by using a glassy carbon electrode paired with cyclically changing voltammetry. Tyrosine, an amino acid, was used as a calibration standard that matched the linear range of 10 to 140 μg of phosphate. A method using urine samples and diclofenac was developed. It determined the amount of the drug in each sample by calculating the mean, standard deviation 3.28 grain limit of detection. The method was accurate to 99.5 % with a grain recall of 99.5 %. (Chethana *et al.* 2012). Sodium diclofenic discs were examined by a technique called capillary electrophoresis. Applying this method to other discs to determine using voltammetry with multi-walled carbon nanotubes and a glassy carbon electrode. A dehexadocyl hydrogen phosphate film covers the glassy carbon electrode, and 0.83 volts were applied to power the amperometric detector. The kV range of XRF was used to find the sodium diclofenic in the urine. The detection limit was 2.5 to 10 μg/ml, and injection time of 10 seconds. 0.8% variation in electromigration rate and 4.7 % variation in peak current were also checked by this method (Jin and Zhang 2000).

2.3.2 Spectrometric Study Of Thiamine Hydrochloride

A spectroscopic method for determining thiamin in vitamin form and as a pharmaceutical has been established. This is accomplished by reacting vitamin B1 and chromates CrO_4 in an acid-based redox reaction. Then, 1,5-diphenylcarbazide was exposed to the unprocessed chromates. After this, the colored material turns into a stable pink-purple compound that is soluble in water. Maximum absorption at a specific wavelength of 543 nm indicates the greatest amount of thiamin present in the original material. The molar absorptivity was 1.5 x 10 liters.mol and Sandel's sensitivity index of 0.0228 $\mu g.cm^2$ and the relative standard deviation ranged from 0.31 – 0.75 (Shekho 2013). Utilizing solid-state absorbance at 278 and 320 nm with a Sephadex ion exchanger, a spectrophotometric technique for the detection of thiamine in the presence of vitamins B12, B6, and B2 showed that vitamin B1 selective for 25-CMC at pH 4.8. The molar absorbance varies from 5.63×10^5 to 2.79×10^7 L. The ability to detect 30-80 μg/ml allows for molar centimeters to work with smaller volumes of samples. For

example, using 1000 ml of sample would require a MCO of 10-1000 (Ortega-Barrales et al., 1998).

Researcher "Hassan" also calculated the thiamine content in the aqueous medium. The process includes nitrating sulfanilic acid, conjugating it with thiamine, and then removing the excess sodium hydroxide to create a soluble dye with a coffee-red color that can be seen at 490 nm in the spectrum with 7.74. mol.cm and 0.045 g.cm of molar absorbance and Sandel significance (Hassan 2005). Thiamine is measured using spectrophotometric method, involves in adding the vitamin to a water solution of ferric nitrate to produce iron. Which in turn reacts with ferric hexacyanohexacyanoide to form a blue dye that absorbs maximum wavelength at 747 nm. Its relative standard deviation is -0.617 and its average slope is 95.34 to 104.4 %. The detection limit for Sandel is 0.106 mg/ml, and the mean of all measurements was 95.34 to 104.4 %. The technology has been used with great success in pharmaceutical formulations (Husssin and Da‘amy 2010). Moreover, HPLC based method has been used to establish a procedure for determining thiamine in a combination with pyridoxine. To separate these compounds, the researchers used a Nucleosil 100-5 C18 column with a "mobile phase" of 0.1 M aluminum carbonate, water and methanol. The ratio of water to other components was 80:15:5 (v/v), as stated by (Dinç *et al.* 2000). This method determines the amount of vitamin B6 contained in mixtures with pyridoxine and cyanocobalamin. It can also determine the contents of supplements containing B1 and B12. This is done by using an electrochemical detector and UV light on a 4.6 x 25 cm (flexible) HPLC column. The vitamins have a retention time of 2, 2.7, or 2 minutes and are highly sensitive with accuracy of 99.6 to 102.7 %. (Marszałł *et al.* 2005).

The concentration of thiamine in syrup combinations including riboflavin, niacinamide, and pyridoxine was measured by HPLC using a C18 column with dimensions of (300 x 3.9 mm) and a solution of 1% methanol and 80% acetic acid. Mobile phase composition with a flow rate of 1 ml/min on average was measured at 280 nm (Yantih *et al.* 2011). The development of a method that can determine a mixture of riboflavin and thiamine by reversed-phase HPLC with a C18 column at 35°C was achieved. The method involves using ammonium acetate as the mobile phase at a pH of 6.7 and methanol in a

ratio of 28 v/v when performing the analysis. The 72 % reliability for separating riboflavin from thiamine had an RSD of 2.66 % and 2.21 %. Additionally, the accuracy of measuring riboflavin, thiamine and their percentage averages were 95.52 %, 90.08 % and RSD of 2.66 % and 2.21 %. (Sánchez-Machado *et al.* 2004). The backflow and normal injection approach were used to offer a method for determining thiamine. To detect metoclopramide, an alkaline medium replaced thiamine in the technique. The minimum sample size is 0.839 mg/ml and it takes 80-90 injections per hour for either a normal or reverse approach. (Al Abachi and Hadi 2012). Thiamine was estimated by injecting some reagents into the FIA-CL continuous flow system. Luminol and potassium periodate. The chemiluminescence method for thiamin determination involves injecting the vitamin into a closed loop system. The accuracy of this method is high, and it can bde used to detect thiamine in in-vivo samples and pharmaceuticals. Moreover, luminol and KIO4 interact to generate the CL signal, which decreases with increasing KIO4 concentrations. This method can be completed in under a minute, has little variation and excellent stability (Wasielczuk *et al.* 2004). The process first oxidizes thiamine to potassium ferric cyanide and then to chromium. Detection limit is 5-10 µg/ml (Halvatzis *et al.* 1990).

3. MATERIALS AND METHODS

3.1 Instrument And Equipments

Table 3.1 Instruments or equipments used in this study

Instruments	**Company Name**
UV-Vis Spectrometer	Shimadzu UV-1800
Sensitive scale	Sartorius 2100
Double beam and silica cells (1cm)	Clifton
PH meter	Jenway 3310

3.2 Chemicals And Reagents Used

All the chemical and reagent used in this study was of analytical grade.

Table 3.2 List of chemicals and reagents used in this study

Chemical name	Chemical formula
Diclofenac Sodium	$C_{14}H_{10}Cl_2NNaO_2$
2,4-dinitrophenylhydrazine	$C_6H_6N_4O_2$
potassium periodate	KIO_4
Sodium Hydroxide	NaOH
Thiamine hydrochloride	$C_{12}H_{17}ClN_4OS,HCl$
p- Amino phenol.HCl	C_6H_7NO,HCl

3.3 - Establishment Of Calibration Curve Of Sodium Diclofenic And Thiamin Hydrochloride

For the establishment of the standard curve of both sodium diclofenic and thiamin hydrochloride drugs, standard solution was formed by disolving 16µg and 30 µg respectively. Maximum absorbance of solution of both drug was taken by diluting (16, 14, 12, 10, 8,7,6,5,4,3,2,1 µg) of diclofenic sodium and (60, 50,40, 35,30,25,20,15,10, 5 µg) of thiamin hydrochloride drugs at 600 nm and 441nm respectively.

3.4 Spectrometric Study Of Sodium Diclofenic And Thiamin Hydrochloride With Coupling Reagents

For spectrometric determination of pharmaceutics, they were treated with coupling reagents and the final formed product was studied under spectrometer. 4.2^{-1} x $10^{\wedge 3}$ M of dihydronitrazine was taken in series of 25 ml of graduated bottels. Solution of potassium periodate 1 x 10^2 M as oxidizing agent was added over dinitrohidrazine solution (1 x 10^3 M) with the addition of 1ml of diclofenac solution. final solution was mixed well and 2.5 ml of NaOH (1M) was added and incubated for 5 minutes with continuous stirring. Final mixture was studied under UV-VIS spectrometer.

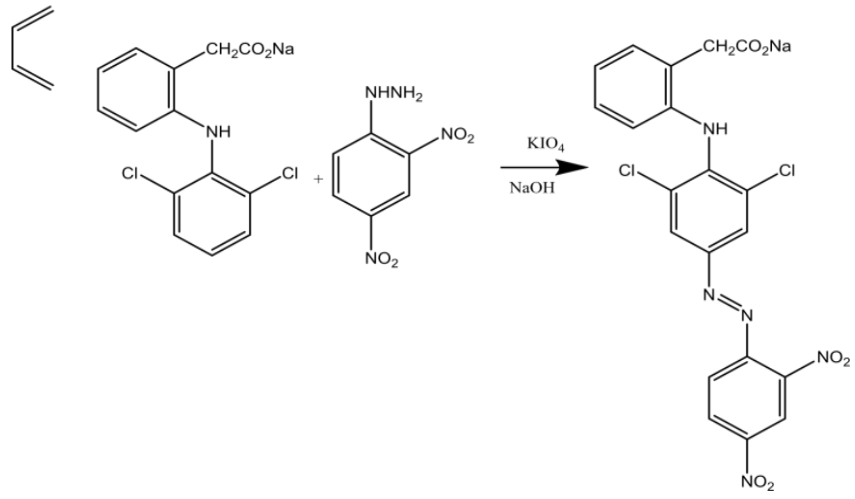

Figure 3.1 The reaction between sodium diclofenac and coupling reagents (Reference).

Similarly, 1.5 ml of p-aminophenol (1×10^{-2} M) was taken in series of 25 ml of graduated bottels, a thiamin solution was added in increasing amounts of (0-2.5 ml) from stock solution. After that, 1 ml of potassium periodate as an oxidizing agent was added and allowed to mix well on magnetic stir. 1 ml of NaOH (1M) was added into the mixture and incubate for 5 minutes with continuous stirring. Spectrometric analysis of final solutions was measured to find out the maximum absorbance of final product.

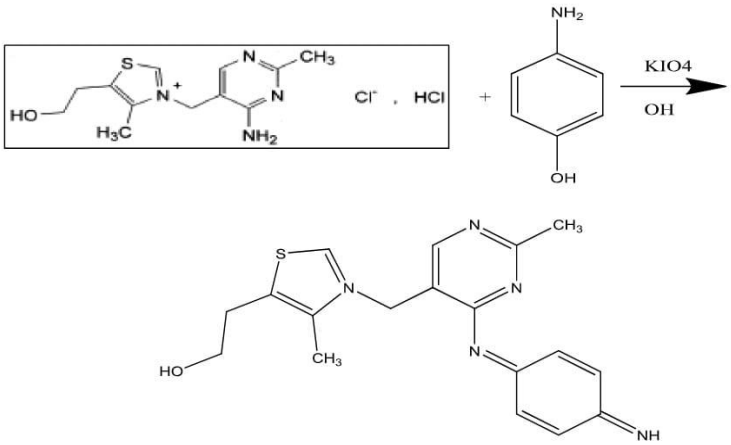

Figure 3.2 The reaction between thiamin hydrochloride and Coupling reagents reagent (Reference).

3.5 Spectrometric Analysis According To Molar Ratio Method And Contineous Changes Method (Job's Method)

Determination of pharmaceutics, two different approaches the molar ratio and continuous changes method were applied by measuring the formation of final product (Drug-reagent conjugate) of reaction spectrometrically. According to Job's method, first of all, 0.5 to 4.5 ml of sodium diclofenac drug was taken in 25 ml of measuring beaker. After that, 5 ml of the reagent solution with additives were added 1:1 ratio of drug and reagent solution into it.

According to molar ratio approach for sodium diclofenac pharmaceutics, 1:1 molar ratio of sodium diclofenac and coupling reagent was used in a series of 25 ml of volumetric flask. 1 ml of drug solution was placed in each flask, while the volume of the reagent solution was taken in the range of 0.5-4.5 ml, the remaning addition was done in optimal size and fill it up to the mark for dilution. The final solution was studied under UV-VIS spectrometer after 10 minutes of incubation.

3.6 Detrmination Of Drug Concentration In Pharmaceutics Using Direct Method

To determine the quantitiy of sodium diclofenac in pharmaceutical tablets, 50 mg of tablet were grinded in fine powder, and solution of different concentration of (2, 4, 8 µg) was prepare in different volume of (0.5, 1, 2 ml) were taken in 25 ml of volumetric flask. Coupling reagent and oxidizing agent was added into drug solution in already optimized quantities.

Similarly, direct method was lsoapplied on thiamin hydrochloride containing capsule Beblex of 5mg. The capsule of Beblex was dissolved into distal water in different volume of (0.5, 0.2, and 3 ml) to make the final concentration of solutions (6, 24, and 36) µg. the amount of coupling reagnt and oxidizing agent was added according to already established method and spectrometric anlysis was studied.

3.7 Determination Of Effectivness and Accuracy Of Developed Method By Standard Addition Method

To determine the effectiveness and accuracy of the proposed approach, the quantity of sodium diclofenac and thiamin hydrochloride in pharmaceutical formulations was evaluated by applying conventional additive method. In accordance with the established approach, two set of solution with pre set volume of (0.5 to 1 ml) was taken in 25 ml of volumetric flask to make the drug concentration 100 $\mu g \cdot ml^{-1}$. Of sodium diclofenac and 500 $\mu g \cdot ml^{-1}$ of thiamin hydrochloride. Afterthat, spectrometric analysis was studied of standard solution at different volume of (0.2, 0.5, 0.8, and 1 ml) of 100 $\mu g \cdot ml^{-1}$ sodium

diclofenic solution and (1.5, 1.0, 0.5, 0.3 mL) of 500 $\mu g \cdot ml^{-1}$ of thiamin hydrochloride solution respectively.

3.8 Priliminary Test Of Establishing Method For Determination Of Drugs

For establishment of the method for the determination of pharmaceuticlas, 2 ml solution of 100 μg of sodium diclofenac and 300 μg of thiamin hydrochloride was taken into 25 ml of volumetric flask. 1 ml of 1 x 10^2 molar potassium periodate solution was added into drugs solution and mixed well. After that 2 ml 4,2-dinitrophenylhydrazine to a concentration of 1 x 10^3 M was added into previous mixed solution for sodium diclofenac and 2 ml of p-aminophenol (1 x 10-2 M) solution for thiamin hydrochloride with addition of 1M NaOH solution. The mixture solution was mixed well and incubate for 10 minutes at room temperature till a colored product was observed. The spectrometric analysis of final product was analysed.

3.9 Optimization Of Parameter For Proposed Method For Pharmaceuticals Determination

For establishment of method, optimization study was done by doing multiple experiment to find out the suitable quantity of reagents for proposed approach. There for effect of different parameters such as pH, Volume, Temperature and ordered of addition of reagents were studied.

3.9.1 Effect Of Oxidizing Agent

For studying th effect of oxidizing agent, 2 ml of 1x10^2 M diclofenac and 2 ml of thiamin solution containing contained 300 $\mu g.ml^{-1}$ was taken into 25 ml of volumetric flask. 1 ml of different oxidizing agents of 1×10^{-2} M of KIO_4, KIO_3, $K_2Cr_2O_7$, $K_3[Fe(CN)_6]$ and 3×10^{-2}M of KIO_4, KIO_3, $K_2Cr_2O_7$, $Na_2[Fe(CN)_6NO]$ were study by reacting them with diclofenic and thiamin hydrochloride drug respectively. After mixing both

drugs and oxidizing agent, 3 ml of (1 M) and 1 ml of (0.1 M) NaOH solution was added and mixed well. After 10 minuts of incubation, spectrometric analysis was done.

3.9.2 Selection Of Best Coupling Factor

For the establishment of method for pharmaceutical determination, coupling reagent that give maximum sensitivity to reaction, selection of best coupling reagent was very important. Therefore, 2ml solution of sodium diclofenac and 1 ml of 1 x 10^3 M different coupling reagent such as 2,4-dinitrophenylhydrazine, N,N-dimethyl-phenylenediamine.2HCl, 4-aminoantiypirin, p-phenylendiamine was taken into volumetric flask. Similarly, 2 ml of thiamine hydrochloride and p-Aminophenol.HCl, 8-hydroxyquinoline, Resorcinol as coupling reagent was taken separately in 25 ml of volumetric flask. with the addition of potassium periodate and NaOH the solution was put into 10 minute of incubation and maximum absorbance was measured between 200 nm to 800 nm of wavelength.

3.9.3 Effect Of Time, PH and Temperature On Oxididation Reaction

For establishment of optimum condition for developing method, pH, Temperature and time of the oxidation reaction was kept in consideration. İn case of optimizing condition for both pharmaceuticals, Time was considere between 5-20 minute, pH concentration by 1.5-3.5 ml by volüme of 1M NaOH solution and temperature of 20, 25, 30 and 40 ^{o}C with time interval of 5 minutes for 1 hour. Spectrometric analysis was done by taking absorption spectra for each reading at 600 nm for sodium diclofenac and 441 for thiamin hydrochloride.

3.9.4 Effect of The Amount Of Oxidizing Agent, Type of Solvent And Order Of Addition of Reagents

For optimization of parameters for establishing method, effect of volume of oxidizing agent, type of solvent and order of addition of reagent was studied. For measurement of

effect of potassium periodate ($10^{-2}\times1$M) was studied in the range of 0.2 to 3 ml of solution of oxidizing agent for sodium diclofenac. While, 0.4 to 5 ml of potassium periodate was utilized in the study to find out the best volume of oxidizing agent for optimization of reaction.

Moreover, to determine the effect of solvent on chemical reaction, 25 ml of water, ethanol, methanol and aceton was taken in graduated cylancder and used for sodium diclofenac drug-based reaction. While, 10 ml of water, ethanol and methanol were used for thiamin hydrochloride to find out the difference in absorption spectra of final colored product.

In addition, order of addition of the reagent for coupling reaction also play important role, therefore in this study order of addition of reagent was studierd. Different type of sequances such as (D + R + O +B), (D + O + B + R), (D + B + R + O), (D + O + R + B), (D + O + B + R) and (R + O + B + D) were studied for establishment of the method for sodium diclofenic. Similarly, different sequance of reagents (D + R + B + O), (D + R + O + B), (D + B + R + O) and (D + O + R + B) were tested for thiamin hydrochloride. Maximum absorbance was measured using spectrometer.

3.9.5 Effect of Amount of Reagent

İt was importent to know the effect of final concentration of coupling reagent in the reaction mixture. Therefore, 0.5, 4, 3, 2, 1.5, 1.0 ml of dinitrophenylhydrazine 1×10^{-2} M solution was treated with sodium diclofanic in the presesence of potassium periodate as an oxidizing agent. The final solution was studied under spectrometer to find ut the maximum absorption value.

However, to find out the optimum value of coupling reagent for thiamin hydrochloride, different amount in volume of p-aminophenol 0.5, 1.0, 1.5, 2, 3 ml was trated against thaimin hydrochloride and postasium periodate solution. maximum absorbance of the final soluion was measured using spectrometer.

3.9.6 Effect Of Time On Stability Of The Generated Output

The absorbance value of diclofenac solution was monitored at a concentration of 4, 8 and 12 μg.ml^{-1} was measured at 600 nm of wavelength for an hour at each 5 minute of interval of time. Similarly, stability of final product of thiamin hydrochloride was also measured at 441 nm of wavelength. Different concentration of 24, 36 and 48 μg.ml^{-1} thaimin hydrochloride solution was studied against time. Each reading was measured at 5 minutes of interval for one hour.

3.10 Statistical Analysis And Evaluation Of Outcomes Of Suggested Method

The following two tests, t -test and f-test were used to assess the precision and validity of the analytical application of the suggested approach in comparison to standard procedures.

4. RESULTS AND DISCUSSION

4.1 Establishment Of Calibration Curve Of Sodium Diclofenic And Thiamin Hydrochloride

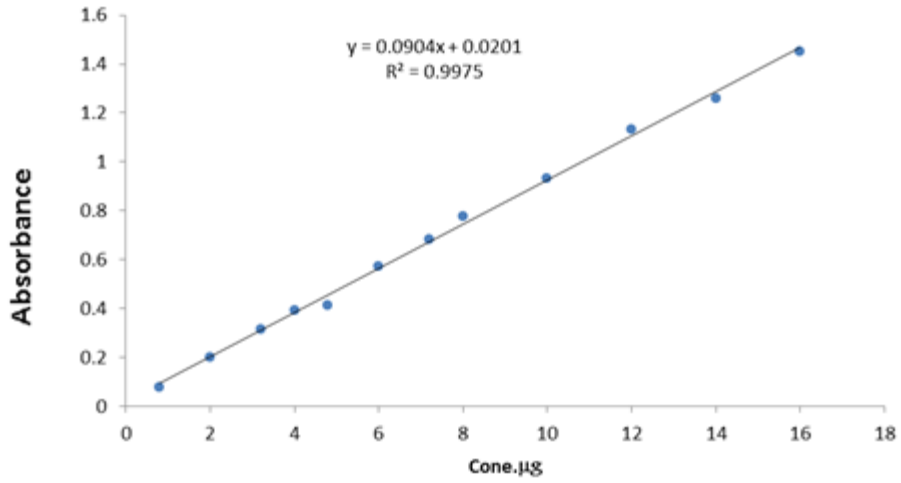

Figure 4.1 Standard curve for sodium diclofenac

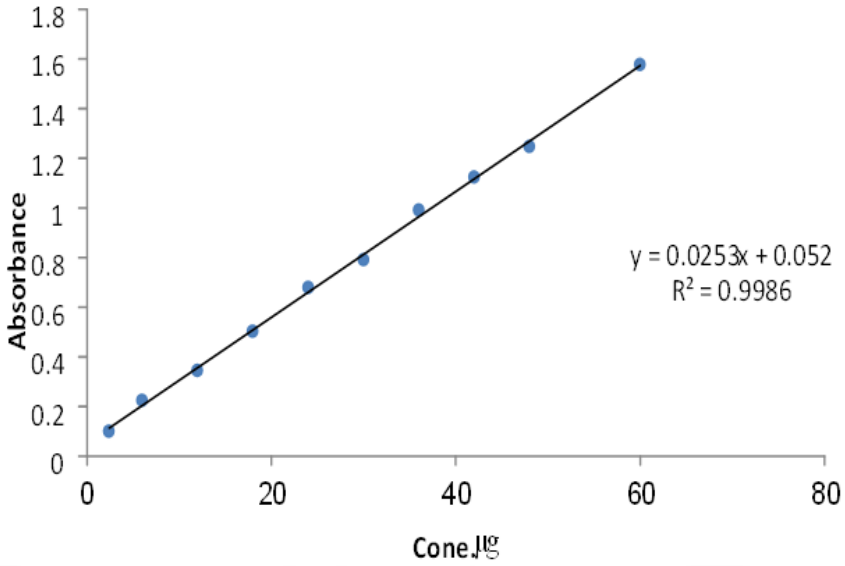

Figure 4.2 Standard curve for thiamine hydrochloride

Calibration curve of sodium diclofenac and thiamin hydrochloride shown in Figure 4.1 and Figure 4.2 showed the linearity in concentration verses absorption with R2 value of 0.997 and 0.998 respectively.

4.2 Spectrometric Study Of Sodium Diclofenic And Thiamin Hydrochloride With Coupling Reagents

The spectrometric study of final product formed by the reaction of sodium diclofenac and coupling reagent was measured at 600 nm of wavelength. The maximum absorbance of series of reactions final prduct having different concentration values were measured as shown in Figure 4.3.

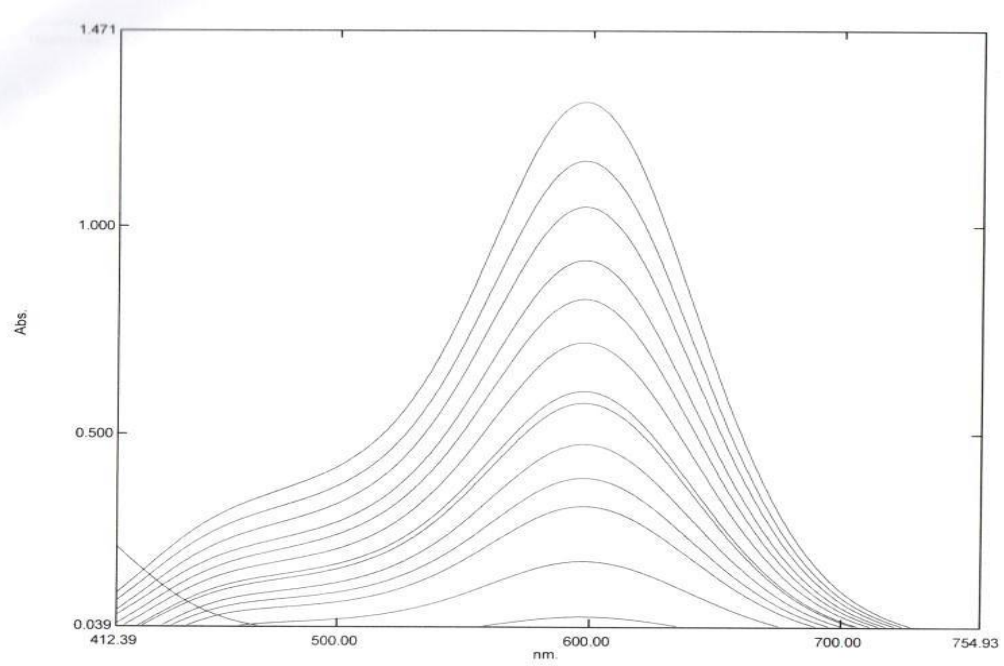

Figure 4.3 Absorption spectrum of final product of reaction between 0.8 to 16 µg. ml^{-1} diclofenac and coupling reagent in the presence of oxidizing agent.

It was found that the concentration of sodium diclofenic can be calculated in the range between 0.8-16 µg. ml^{-1} as it follows the Beer's law. Moreover, as compare to the data of standard curve, it sowed a linear relationship with correlation coefficient of 0.9975.

Similarly, spectrometric analysis of final product of thiamin hydrochloride and coupling reagents was studied at 441nm to determine the concentration of drug. It was found that, thiamin hydrochloride in the range of 4.2-60 µg.ml^{-1} was obeying the Beer's law as shown in Figure 4.4. The Molar Absorbance, Sandel signigficance and correlation coefficient calculated as 8.5286103L mol^{-1} cm^{-1}, 0.0395 $g.cm^2$ and 0.9986 respectively, indicating the linearity of the standard curve.

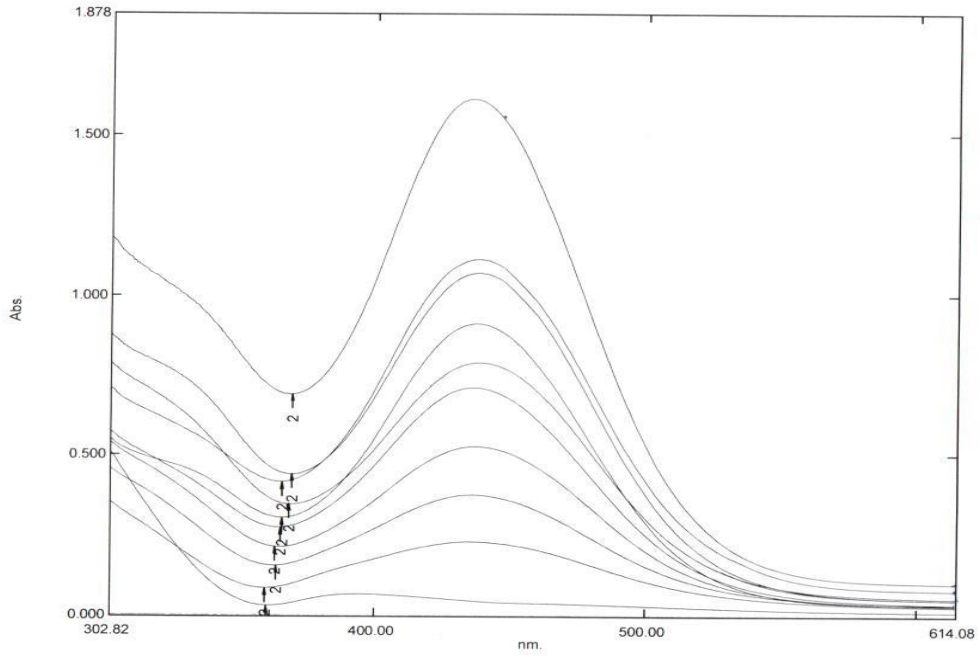

Figure 4.4 Absorption spectrum of final product of reaction between 4.2-60 μg. ml^{-1} thiamin hydrochloride and coupling reagent in the presence of oxidizing agent.

4.3 Spectrometric Analysis According To Molar Ratio Method And Contineous Changes Method (Job's Method)

Detection of coloured product formed after reaction of sodium diclofenac and coupling reagent were observed using spectrometer. However, sticiometric determination was done by following job's method as well as mole ratio method.

Jobs Method:

According to method proposed by Jobs, the nature of the product was measured by reacting reactents at same mole ratios. The concentration of the solution vary but molarity of both solution remaon constant. In binding activity between dinitrohidrazine and sodium diclofenic were done at same ratio and final product was observed at 600 nm. Figure 4.5 showd the spectrum of conjugates observed at different concentration values.

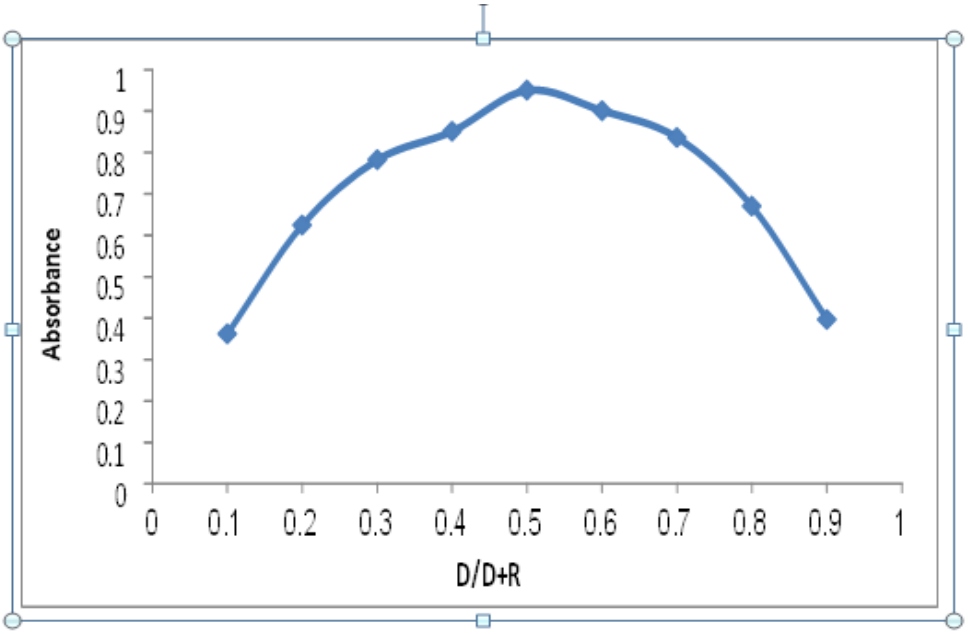

Figure 4.5 Spectrometric analysis by Continuous changes method (Job Method)

Molar Ratio Method

To find out the concentrtion of drug bounded to reagent molecule and produce final conjugated product were also tested using mole ratio approach. As an alternative method to job's method, the value of different concentration of sodium diclofenic to dinitrohidrazine was observed at 600 nm as shown in Figure 4.6. The data obtaind from mole ratio analysis approach, shows a straight line in start of reaction but after achiving maximum range of binding saturation is observable.

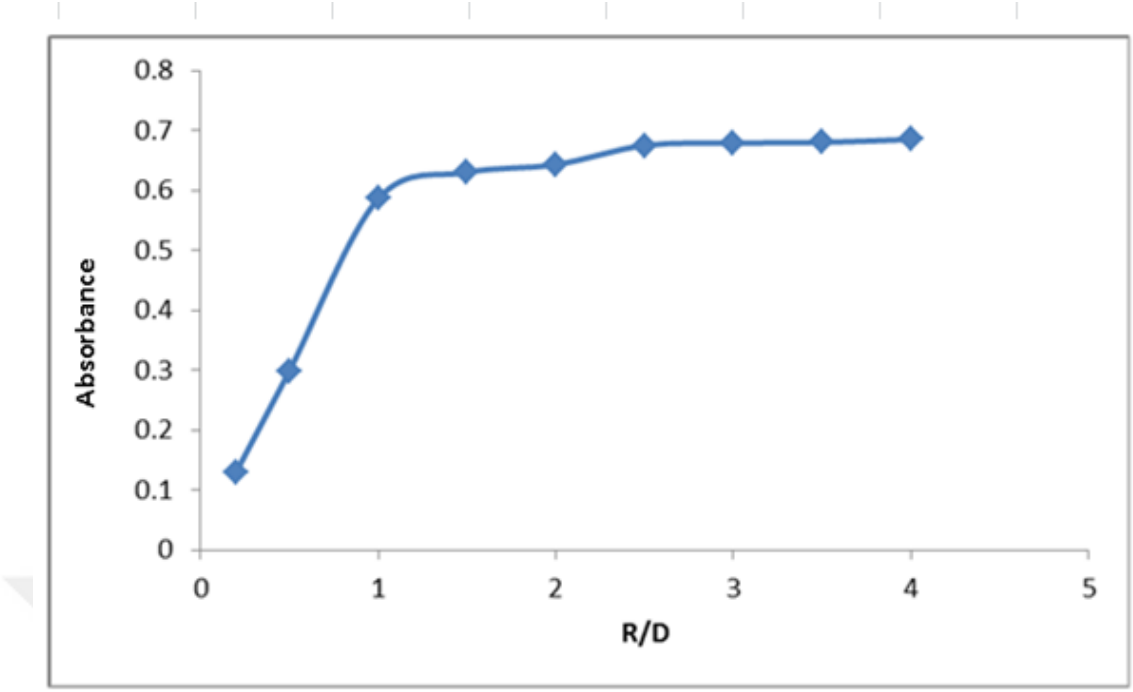

Figure 4.6 Spectrometric analysis of sodium diclofenac reactents by molar ratio method

Similary, for thaimin hydrochloride, spectrometric detection of conjugate of thiamin hydrochloride and p-aminophenol were studied. For stoichiometric determination of formed product were done by following two different analytical method mentiond below:

Jobs Method:

According to Jobs method, stoichiometric binding event of p-aminophenol and thiamin hydrochloride were constant. The binding of both molecule with each other was calculated using spectrometric analysis of product at 441 nm as shown in Figure 4.7. Thiamine was used in a 1:1 ratio with the reagent p-aminophenol, which show that maximum conjugation was observed at 0.5 µg of the drug.

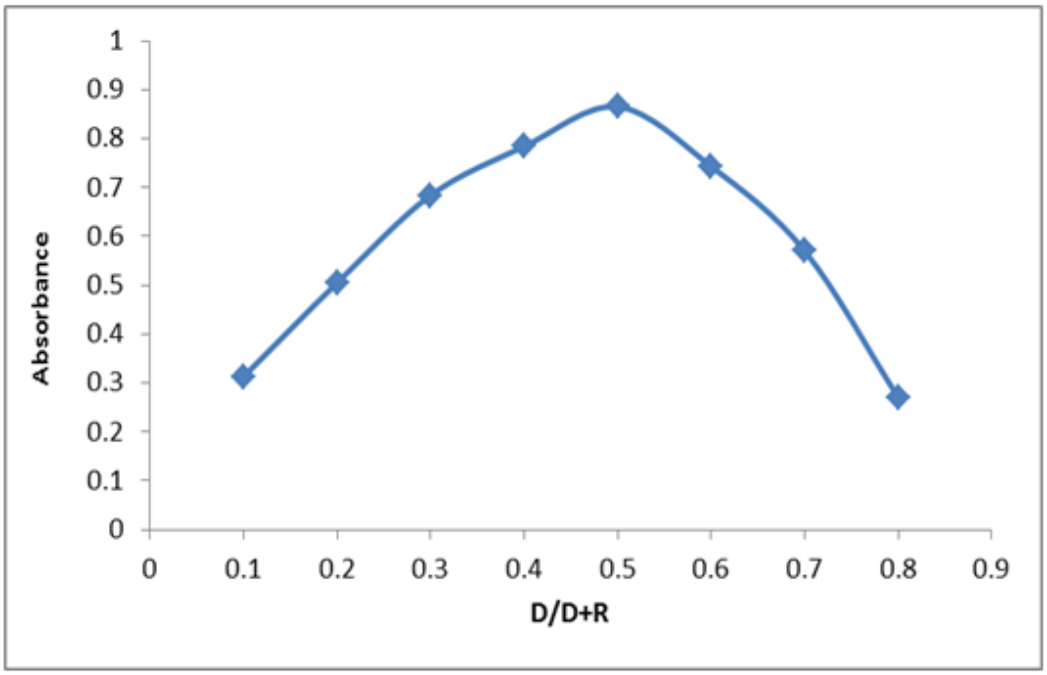

Figure 4.7 Spectrometric analysis of thiamine hydrochloride by continuous changes method (Joepe's method)

Molar Ratio Method:

The Molar Ratio based stictiometry is alternative method to jobe's method in measurement of reactant in analytical based analysis. In which concentration of p-aminophenol was kept constant while thiamine hydrochloride was varied. Here we determined the stictiometric binding of both reactant by ploting a graph of UV-VIS spectra at 441 nm as shown in Figure 4.8. It was found that increase in volume of thiamin hydrochloride, conjugation of the reagent also increases but after saturation of the reagent the absorbance value started to give a straight line.

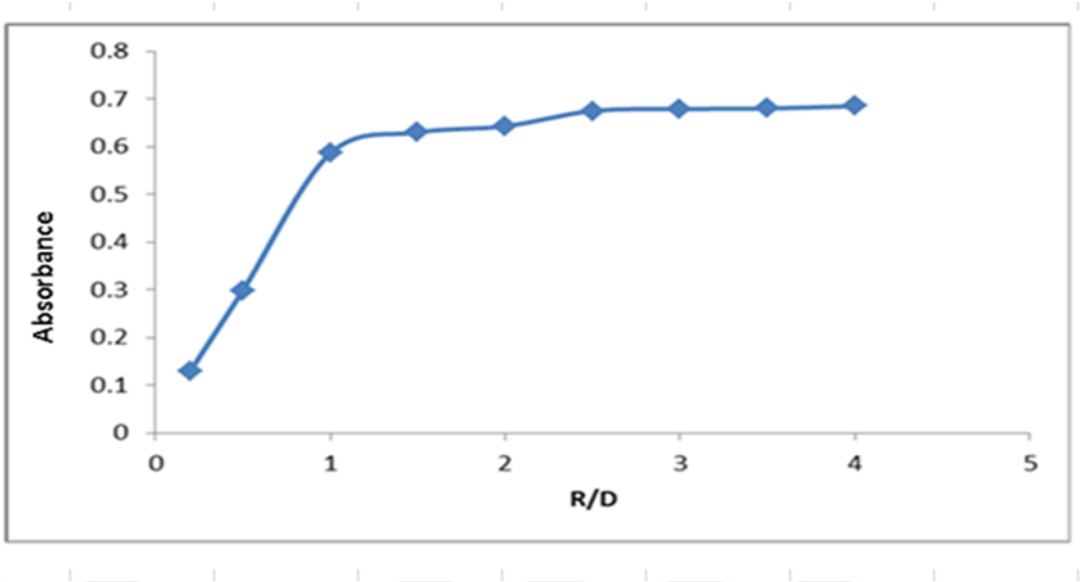

Figure 4.8 Spectrometric analysis of thiamine hydrochloride by continuous changes detrmination of drug concentration in pharmaceutics using direct method

For the determination of diclofenac using direct method, spectrometric analysis was done at 600 nm of wavelength and each experiment was repeated 6 time and averge of all measurement was taken. The maximum recovery rate determined by direct method is given in Table 4.1 shoed the affectivness of the established method.

Table 4.1 Estimation of diclofenac in a tablet (50 mg) by the direct method

Amount of Diclofenac taken (µg/ml)	Diclofenac Measured (µg/ml)	RE.%	Recovery. %	Average Recovery .%	RSD. %
2	1.99	-0. 5	99.5	100.2	1.076
4	4.16	4	104		0.562
8	7.78	-2.7	97.3		0.218

Similarly for thiamin hydrochloride determination from 50 mg Beplex capsule, average of six different reading was measured by using spectrometr at maximum absorbance of

441 nm as shown in Table 4.2. It was sfound that, esblished direct method also give maximum recovery of 99.4 % from tablet.

Table 4.2 Determination of thiamine in Beblex capsule (5 mg) by the direct method

Amount of Beblex Tablets taken (µg/ml)	Thiamine.HCl Measured (µg/ml)	RE.%	Recovery .%	Average Recovery .%	RSD .%
6	5.94	-1	99	99.4	0.18
24	23.86	-0.58	99.42		2.57
36	36.02	0.05	99.9		1.17

4.5-Determination Of Effectivness And Accuracy Of Developed Method By Standard Addition Method.

For detrermining the efficiency and accuracy of developed method, average of six observation was studied at 600 nm of wavelength as shown in Table 4.3. maximum recovery of drug was observed using standard addition method.

Table 4.3 Method of standard addition for recovery of sodium diclofenac

Type of Drug	Diclofenac Present (µg/ml)	Diclofenac Measured (µg/ml)	Recovery (%)
Tablets (voltarin) 50mg S.D.I Iraq	0.8	0.800	100.0
	2	2.08	104.0

Moreover, a standard curve of both solution at different concentration and volume were drawn as shoen in Figure 4.9. it was found that, standard addition technique has good agreement and maximum tolerances, which indicating that the obtained results are reasonable and free of glitches.

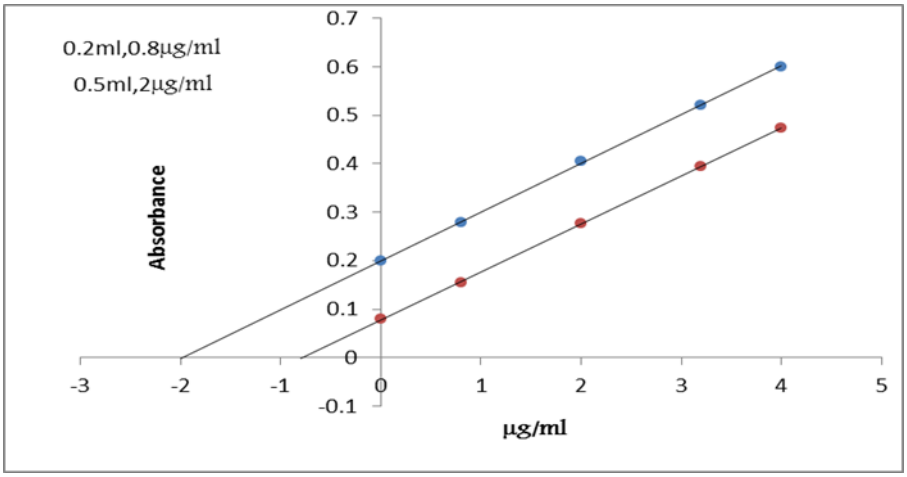

Figure 4.9 Graphical representation of curve of standard additives for the determination of diclofenac in 50 mg tablets

Similar to sodium diclofenac, thiamin hydrochloride was also measured by using standard addition method. Maximum absorbance of six measurement was observed at 441 nm of wavelength and average was taken as seen in Table 4.4. Moreover, a standard curve of both solutions was drawn as shown in Figure 4.10. It was found tht masximum recovery of 99 % was achived at 6 µg/ml of concentration. A straight line was observed which showed the accuracy and authenticity of the standard addition method.

Table 4.4 Standard additive method for recovery of thiamin hydrochloride

Type of Drug	Thiamine.HCl Present µg/ml	Thiamine.HCl Measured µg/ml	Recovery. (%)
Tablets Beblx Thiamine.HCl 5mg S.D.I Iraq	6	5.94	99.01
	12	12.01	0.0108

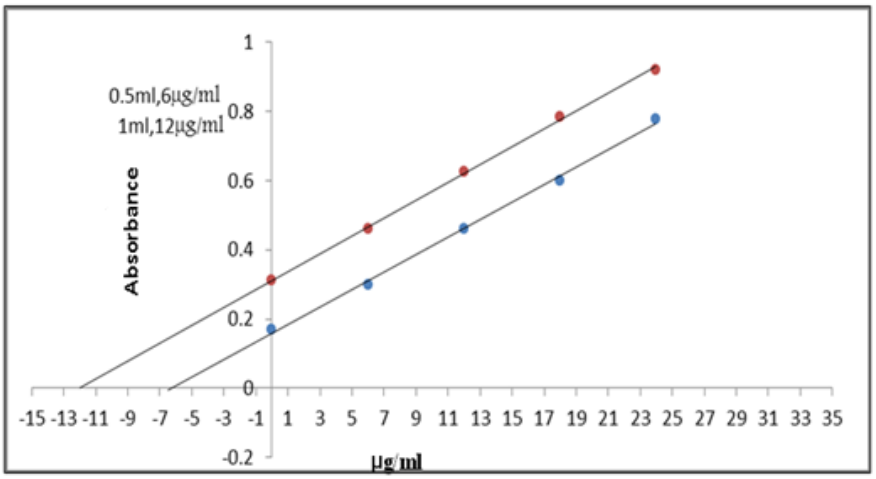

Figure 4.10 Graphical representation of curve of standard supplements for the determination of thiamine in a 5 mg capsule

4.4 Comparison Study Of Developed Method With Already Developed Approaches

The comparison study of the finding of multiple parameters of developed method with already established method was shown in Table 4.5 for sodium diclofenac and in Table 4.6 for thiamin hydrochloride.

Table 4.5 Comparison of other spectroscopic approaches to our developed method for sodium diclofenac

Analytical Parameters	Literature Method	Literature Method	Present method
Reagent	Phenanthroline	Methelen blue	2,4-dinitro phenylhydrizine
Medium	Acide	Base	Alkaline
Colour	Red	Blue	Blue
λ_{max} (nm)	510	---------	600
Temperature (C)°	-----	-------	25
Solvent	-----	-------	Water
ε(L.mol^{-1}. cm^{-1})	1.15×10^{2}		2.8756×10^{4}
Beer's law range µg/mL	10-32	0.8-6.4	0.8-16
Sandel Index µg.cm^{-2}	2.76	---------	0.0110
D.L µg/mL	-----	0.37	0.088
Average recovery %	99.2	-------	100.8
RSD,%	----	-------	2.39

Table 4.6 Comparison of other spectroscopic approaches to our developed method for thiamin hydrochloride

Analytical Parameters	Literature Method	Literature Method	Present method
Reagent	Silphanilic acid	Sodium phenylhydrazine - 4-Salphonate	p-Amino phenol
Medium	Alkaline	Alkaline	Alkaline
Colour	Red	Orange	Orange
λ_{max} (nm)	491	491	441
Temperature	-----	-----	15
Solvent	-----	------	Water
$\varepsilon(L.mol^{-1}.cm^{-1})$	10^3x7.74	-----	8.528×10^3
Beer's lawµg/mL	------	1-16	2.4-60
Sandel Index	0.045	-----	0.0390
D.L µg/mL	----	-----	1.007
Average recovery %	------	%9987-11186	99.6
RSD,%	-----	0.35	3.21

Table 4.6 shows that the proposed method performs well compared to the specified one.

4.5 Priliminary Test Of Establishing Method For Determination

The final solution obtaind after completion of coupling reaction in the presence of oxidizing agent give blue colored product. The final product of sodium diclofenic and thiamin hydrochloride give maximum absorbance at 600 nm and 441 nm respectively.

4.6 Optimization Of Parameter For Proposed Method For Pharmaceuticals Detrmination

For optimization study of established method, different parameter were taken under consideration. According to the obtained findings of different parameters, suitable results were considered for establishment of the method.

4.6.1 Effect Of Oxidizing Agent

The effect of reaction of different oxidizing agent with sodium diclofenac and thaimin hydrochloride was studied under spectrometer. It was found that, among different

oxidizing agent KIO_4, KIO_3, $K_2Cr_2O_7$, $K_3[Fe(CN)_6]$ final product of sodium diclofenic give maximum absorbance at 600, 385, 353 and 435 respectively. Similarly, coupling reaction in the presence of KIO_4, KIO_3, $K_2Cr_2O_7$, $Na_2[Fe(CN)_6NO]$ oxidizing agent for thiamin hydrochloride gave maximum absorbance at 91, 99, 227, 70 nm respectively by spectrometer as shown in Table 4.7 and Table 4.8 respectivelly. From the finding, potassium periodate was selected for further study.

Table 4.7 Effect of different type of oxidizing agent for sodium diclofenac

Oxidizing Agent (1×10^{-2} M)	Absorbance	λmax (nm)	Ɛ.L.mol-1. cm-1
KIO_4	0.625	600	2.480×10^4
KIO_3	0.305	385	1.2113×10^4
$K_2Cr_2O_7$	0.231	353	9.166×10^3
$K_3[Fe(CN)_6]$	0.282	435	1.1191×10^3

Table 4.8 The effect of the type of oxidizing agent for thiamine hydrochloride

Oxidizing Agent (3×10^{-2}M)	$\Delta\lambda$ (nm)	Absorbance		Ɛ.L.mol^{-1}.cm^{-1}
		S	B	
KIO_4	91	0.626	0.118	8.816×10^3
KIO3	99	0.474	0.616	6.657×10^3
$Na_2[Fe(CN)_6NO]$	227	0.634	0.523	8.904×10^3
K_2CrO_4	77	0.660	0.457	9.269×10^3

4.6.2 Selection Of Best Coupling Agent

It was found that, among 2,4-dinitrophenylhydrazine, N,N-dimethyl-phenylenediamine.2HCl, 4-aminoantiypirin, p-phenylendiamine, 2-4DNH was the best coupling agent because it give maximum absorption at 600 nm compare to control solutions about 2.5 times maximum as seen in Table 4.9. However, in case of thiamin hydrochloride, as seen in Table 4.10, among p-Aminophenol.HCl, 8-hydroxyquinoline, Resorcino, it wass found that p-aminophenol as a coupling agent give maximum absorbance 441 nm of wavelength.

Table 4.9 Selection of the best coupling agent for sodium diclofenac

Reagent 1×10^{-3} M	Variable	Absorbance	λ max (nm)	$\Delta\lambda$ (nm)	Ɛ.L.mol^{-1}. cm-1
2,4-dinitrophenylhydrazine	S	0.620	600	219	2.468×10^{4}
	B	0.011	381		
N,N-dimethyl-p phenylenediamine.2HCl	S	0.350	565	203	1.392×10^{4}
	B	0.055	362		
4-aminoantiypirin	S	0.092	400	91	3.651×10^{3}
	B	0.001	310		
p-phenylendiamine	S	0.195	399	16	7.738×10^{3}
	B	0.121	383		

Δ λ max = color contrast = λ max (S) - λ max (B) S = sample and blank

Table 4.10 Selection of the best coupling agent for thiamin hydrochloride

Reagent1×10^{-2}M	Variable	Absorbance	λ max (nm)	λ (nm)	Ɛ.L.mol-1. Cm-1
p-Amino phenol.HCl	S	0.658	441	95	9.143×10^{3}
	B	0.260	350		
8 hydroxyquinoline	S	0.348	710	11	4.8887×10^{3}
	B	0.259	700		
Resorcinol	S	0.301	384	42	4.227×10^{3}
	B	0.261	342		

4.6.3 Effect Of Time, pH And Temperature On Oxididation Coupling Reaction

The effect of time on coupling reaction was studied between 3-20 minuts for both drugs. İt was found, duration of 5 minute for sodium diclofenic and 11 minutes for thiamin hydrochloride give maximum absorbance and considerable for further study as shown in Table 4.11 for sodium diclofenic and 4.12 for thiamin hydrochloride.

Table 4.11 Effect of time on oxidation coupling reaction for sodium diclofenac

Time/min.	3	5	10	15	20
Absorbance	0.787	0.763	0.622	0.420	0.385

Table 4.12 Effect of time on oxidation coupling reaction for thiamin hydrochloride

Time Minutes	3	5	10	15	20
Absorbance	0.377	0.558	0.632	0.621	0.618

Moreover, the effect of change in pH was studied by adding different concentraion of NaOH into the reaction mixture. It was found that, 2.5 ml of 1 M NaOH for sodium diclofenic and 1 ml for thiamin hydrochloride give best results which was utilized in

further study as shown in Table 4.13 for sodium diclofenic and Table 4.14 for thiamin hydrochloride.

Table 4.13 The affect of volume of NaOH on coupling reaction for sodium diclofenic

1M NaOH (ml)	Absorbance	pH
1.5	0.417	12.0
1.8	0.564	12.23
2	0.621	12.36
2.2	0.730	12.45
2.5	0.766	12.57
2.8	0.781	12.72
3	0.787	12.83
3.5	0.791	12.99

Table 4.14 The affect of volume of NaOH on coupling reaction for thiamin hydrochloride

(0.1) M NaOH (ml)		0.2	0.5	0.7	1	1.5	2	2.5
Absorbance	S.B	0.456	0.618	0.623	0.627	0.632	0.651	0.665
	B.W	0.034	0.083	0.107	0.121	0.151	0.260	0.313
	pH	8.23	10.71	10.98	11.11	11.76	11.78	11.80

In addition, the effect of temperature from 15, 25, 35 and 45 was measured for one hour of 5 minutes of interval. It was found that, reaction give best result at 25 ^{o}C, while with increase in temperature the value of absorbtion decrease with passage of time. Hence ideal temperature for established methd was considere as 25 ^{o}C which was applied in further study as shown in Table 4.15 for sodium diclofanic.

Table 4.15 Effect of temperature over time on absorbance spectra of coupling reaction of sodium diclofenic

Temperature (C^{o})	Absorbance/min of 8µg / ml of diclofenac								
	5	10	15	20	25	30	40	50	60
15	0.413	0.425	0.435	0.432	0.432	0.431	0.430	0.428	0.425
25	0.768	.0745	0.735	0.731	0.729	0.725	0.716	0.715	0.715
35	0.682	0.680	0.678	0.673	0.671	0.686	0.666	0.657	0.653
45	0.544	0.536	0.525	0.514	0.510	0.503	0.495	0.480	0.473

However, in case of thiamin hydrochloride 15 ^{o}C was found to be optimum temperature at which reaction mixture give maximum absorbance as shown in Table 4.16.

Table 4.16 Effect of temperature over time on absorbance spectra of coupling reaction of thiamine hydrochloride

Temperature (C^{o})	Absorbance/ minute								
	5	10	15	20	25	30	40	50	60
R.T (15)	0.568	0.625	0.622	0.618	0.611	0.603	0.537	0.527	0.515
20	0.599	0.560	0.551	0.509	0.485	0.450	0.437	0.398	0.384
25	0.551	0.501	0.450	0.428	0.422	0.414	0.393	0.375	0.370
30	0.454	0.385	0.361	0.338	0.321	0.318	0.305	0.296	0.283
40	0.380	0.360	0.300	0.280	0.265	0.243	0.221	0.200	0.181

4.6.4 Effect Of The Amount Of Oxidizing Agent, Type Of Solvent And Order Of Addition Of Reagents

While determining the effect of amount of oxidizing agent, it was found that, maximum absorbance was obtained at 600 nm in the range of 0.8 to 1.5 ml of oxidizing agent. Hence 1 ml was decided to be utilized in further study as seen in Table 4.17.

Table 4.17 The effect of volume of oxidizing agent's on coupling reaction for sodium diclofenac

Volume in (ml) of Potassium Periodate $10^{-2}\times1M$	Absorbanse
0.2	0.581
0.5	0.668
0.8	0.720
1	0.763
1.5	0.775
2	0.777
2.5	0.781
3	0.785

The amount of potassium periodate determined by reactionof different amount of addition of oxidizing agent are shown in Table 4.18. Which showed that, addition volume of potassium periodate at the range of 0.5-1 ml gives maximum absorbance at 441 nm of wavelength. Therefore, we consider 0.1 ml of oxidizing agent volume for our further study.

Table 4.18 The effect of volume of oxidizing agent's on coupling reaction for thiamin hydrochloride

Amount (ml) Potassium periodate $^{2-}10\times3M$	Absorbance of 24 μg /ml	
	S.B	B.W
0.5	0.834	0.344
0.7	0.766	0.300
1	0.623	0.111
1.5	0.420	0.101
2	0.385	0.070
2.5	0.362	0.056
3	0.328	0.043
4	0.301	0.034

Maximum absorbance of the resuting solvent was observed at 600 nm for sodium diclofenac and 441 nm for thiamin hydrochloride. It was found that, as shown in table 4.19 and 4.20, that water as a solvent was consider the best media for solution formation. Therefor, water was used in whole study for each experiment.

Table 4.19 The effect of solvents on coupling reaction for sodium diclofenac

Solvent	λ_{max} (nm)	Absorbance
Water	600	0.767
Ethanol	602	0.766
Acetone	602	0.761
Methanol	601	0.763

Table 4.20 The effect of solvents on coupling reaction for thiamine hydrochloride

Solvent	λ_{max} (nm)	Absorbance
Water	441	0.626
Ethanol	434	1.093
Methanol	436	1.484

To find out the best sequence of addition of reagent in the cemical ractio, maximum absorbance of final product was measured after addition of reagent in different addition order. It was fount that, as shown in table 4.21 the best sequence of addition of reagents (D + O + R + B) give maximum absorbance at 600 nm for sodium diclofenic. While Table 4.22 showed the order of addition of reagent that give maximum absorbance of 0.767 was for (D + R + B + O) sequance. Therefore these sequance were considered for development of optimized condition for method.

Table 4.21 Effect of the sequence of additions of reagents for developed method for sodium diclofenac

No	Order of additions	Absorbance of 8 µg / ml of diclofenac
1	D + R + O +B	0.698
2	D + O + B + R	0.241
3	D + B + R + O	0.266
4	D + O + R + B	0.769
5	D + O + B + R	0.223
6	R + O + B + D	0.234

Where, Diclofenac sodium (D), Potassium periodate (O), 2,4-dinitrophenylhydrazine (R) and NaOH (B) as a base were used.

Table 4.22 Effect of the sequence of additions of reagents for developed method for thiamine hydrochloride

No	Order of additions	Absorbance
1	D + R + B + O	0.633
2	D + R + O + B	0.275
3	D + B + R + O	0.512
4	D + O + R + B	0.115

Here, Thiamine hydrochloride (D), Para-Aminophenol (R), Potassium periodate (O), Alkaline Sodium Hydroxide (B).

4.6.5 Effect Of Volume Of Coupling Reagent

Reaction of couplin agent dinitrophenylhydrazine with sodium diclofanic in the presence of oxidizing agent was studied at 600 nm of wavelength. Among different volume of 0.5, 4, 3, 2, 1.5, 1.0 ml of dinitrophenylhydrazine 1×10^{-2} M solution was treated with sodium diclofenic solution. İt was found that as shown in Table 4.23, 2ml of 1×10^{-2} M solution of dinitrophenylhydrazine give maximum absorbance at 600 nm of wavelength. Hence this volume was considered suitable for further study.

Table 4.23 Effect of volume of coupling reagent on developed method for sodium diclofenac

Amount (ml)of Reagant 1×10^{-2} M	Absorbance of µg /ml diclofenac					
0.5	2	4	6	8	12	16
	0.159	0.285	0.382	0.461	0.466	0.553
1	0.244	0.363	0.492	0.567	0.670	0.683
1.5	0.249	0.421	0.510	0.606	0.710	0.827
2	0.255	0.561	0.591	0.770	1.014	1.310
2.5	0.201	0.358	0.517	0.725	0.890	1.252
3	0.197	0.343	0.496	0.626	0.850	1.003

Similarly, reaction of thaimin hydrochloride with 1×10^{-2}M p-aminophenol as a coupling agent was measured at 441 nm of wavelength. It was found that among 0.5, 1.0, 1.5, 2, 3 ml volume of p-aminophenol 1.5 ml of coupling reagent give maximum absorbance value as shown in Table 4.24. Hence, 1.5 ml was considerd more suitable volume for further study.

Table 4.24 Effect of volume of coupling reagent on developed method for thiamine hydrochloride

Amount(ml)of p-aminophenol 1×10^{-2}M		Absorbance of µg/mL of thiamine. HCl				
	B.W	6	12	18	24	36
0.5	0.035	0.075	0.218	0.323	0.392	0.524
1	0.045	0.116	0.279	0.335	0.579	0.730
1.5	0.074	0.175	0.297	0.469	0.627	0.818
2	0.113	0.224	0.373	0.483	0.630	0.831
2.5	0.234	0.120	0.206	0.276	0.427	0.557

4.6.6 Effect Of Time On Stability Of The Generated Output

The stability of the final product of sodium diclofenac reaction with reagents at different concentration was measured at 600 nm of wavelength at different time interval. It was found that, formation of final product was finished in first 5 minutes as shown in Table 4.25. Morover, it was also observed that, the final product formed after completion of reaction was stable atleast for one hour.

Table 4.25 The stability of the generated output of developed method for sodium diclofenac

Time (Minute)	Absorbance of diclofenac (μg/ml)		
	4	8	12
0	0.437	0.776	1.014
5	0.456	0.761	1.013
10	0.452	0.759	1.015
15	0.449	0.766	1.013
20	0.451	0.765	1.011
25	0.454	0.764	1.012
30	0.452	0.766	1.01
35	0.458	0.764	1.011
40	0.454	0.763	1.012
45	0.451	0.760	1.009
50	0.447	0.756	1.006
55	0.442	0.751	1.005
60	0.440	0.748	1.002

Similar results were obtained for thiamin hydrochloride solution. When different concentration of thiamin hydrochloride was treated with coupling reagent and readings were measured at 441 nm of wavelength for 5 minutes of interval till one hour. It was found that, the reaction completes within 10 minutes as shown in Table 4.26. However,

it was observed that, the final reaction product was stable for 30 minutes, that is sufficient time for measurement of final product.

Table 4.26 The stability of the generated output of developed method for thiamine hydrochloride

Time (min)	Absorbance of Thiamine hydrochloride (µg / ml)		
	24	36	48
0	0.358	0.697	0.989
5	0.564	0.749	1.008
10	0.628	0.844	1.213
15	0.624	0.839	1.283
20	0.621	0.828	1.276
25	0.609	0.810	1.263
30	0.574	0.775	1.232
35	0.557	0.744	1.213
40	0.535	0.734	1.198
50	0.498	0.700	1.172
60	0.484	0.689	1.146

4.7 Final Absorption Spectrum

Through the results of previous experiments, the optimal conditions for the determination of diclofenac were summarized as show in Table 4.27.

Table 4.27 Summary of optimum conditions for determination of Diclofenac sodium

Experimental Conditions	
λ max	600
Amount (mL) of 1×10^{-2} M potasium periodate	1.0mL
Amount (mL)of1×10^{-2}M2,4-dinitrophenyl Hydrazine	2mL
Buffer Solution (B F) (NaOH)	2.5 ML
Oxidation time	5min.
Solvent	Water
Temperature (C^o)	25 C^o

By evaluating the absorption spectra of the resultant solution under ideal working enviornment for the detection of sodium diclofenac, the wavelength of maximum absorption was determined. Figure 4.11 showed the maximum absorbtion of diclofenac pharmaceutics, pure drug at 600 nm of wavelength.

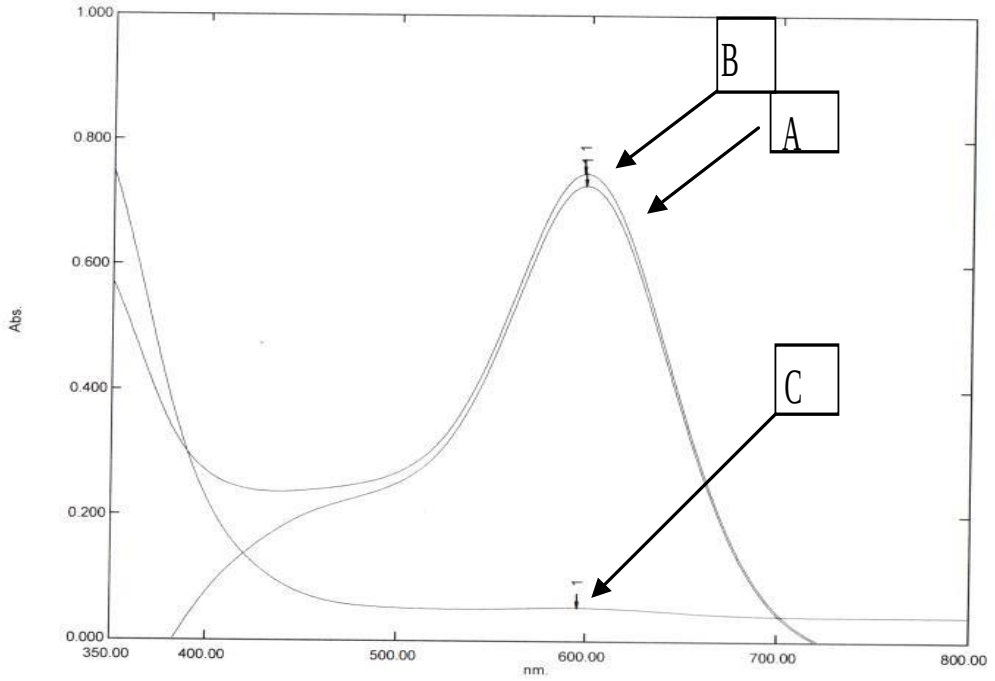

Figure 4.11 Absorption spectrum of diclofenac

Here, A: 8 μg solution mL of pure diclofenac solution versus the mock solution. B: Diclofenac solution versus water. C: Mock solution versus distilled water. Similarly, conditions optimized for thiamin hydrochloride by the data obtained from findings of earlier research were summarized, as shown in Table 4.28.

Table 4.28 Summary of optimum conditions for determination of thiamine hydrochloride

Experimental Conditions	
λ max	441
Amount (mL) of 1×10^{-2} M p-Amino phenol hydrochloride	1.5mL
Amount (mL) of 3×10^{-2} M potassium periodate	1.0 mL
Amount (mL) of NaOH 0.1M	1 mL
Oxidation time	11Min.
Solvent	Water
Temperature (C^o)	15 C^o

By evaluating the absorption spectra of the resultant solution under ideal working condition for the detection of thiamine hydrochloride, the maximum absorbance waswas observed at 441 nm of wavelength as shown in Figure 4.12.

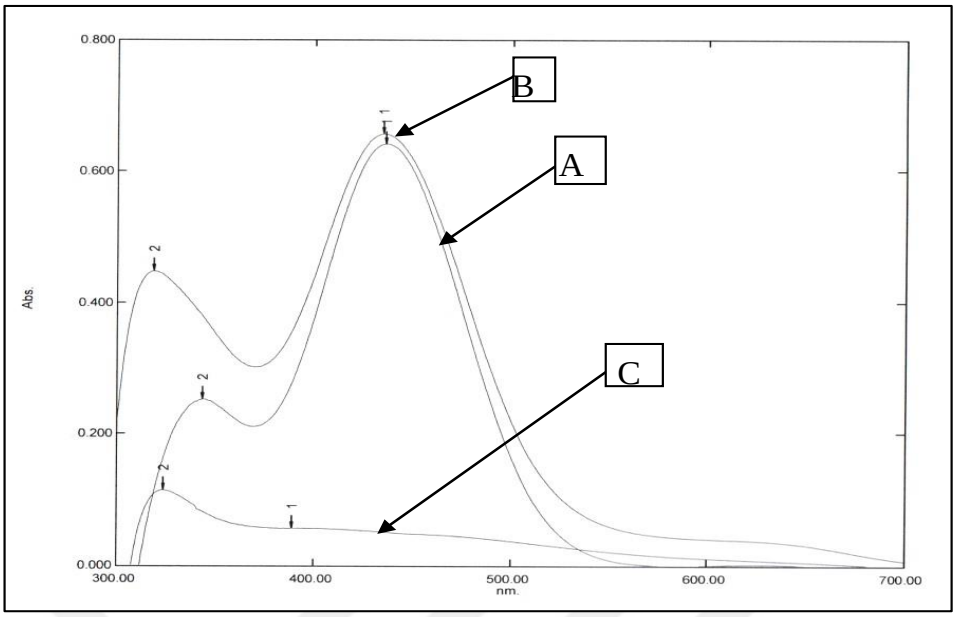

Figure 4.12 Absorption spectrum of thiamine hydrochloride.

Here, A: 24 µg.ml^{-1} solution of pure thiamine versus the mock solution, B: Thiamine solution versus water, and C: Mock solution vs. distilled water.

4.8 Detection Limit, Precision And Compatibility Of The Established Method

Detection limit of the method showed the sensitivity of the developed approach that at how much least concentration it can detect the presence of active molecule. Therefore, detection limit of sodium diclofanic was tested by proposed method. It was found that, 0.8 µg.ml^{-1} of drug can be determined by proposed method at the wavelength of 600 nm by spectrometer as shown in Table 4.29.

Table 4.29 Limit of detection of sodium diclofenac by developed method

Concentration µg/mL	X	S	D.L µg/mL
0.8	0.07031	0.00258	0.08806

Moreover, the precision of method and accuracy of developed approached was measured for the determination of sodium diclofenac. For this purpose, 2, 4 and 8 $\mu g.ml^{-1}$ of sodium diclofenic was tested. İt was found that, 100.4 % of recovery of drug was obtaind with 2.97 of standard deviation as shown in Table 4.30. from the obtained results, it can be say that proposed method is highly precise, accurate and sensitive.

Table 4.30 Accuracy and compatibility of the developed method for sodium diclofenac

Amount of Diclofenac (µg/ml)	RE.%	Recovery.%	Average recovery.%	RSD. %
2	-0. 036	99.9	100.4	2.97
4	4.00	104.0		0.788
8	-2.64	97.3		0.422

Similar to sodium diclofenac pharmaceutics, thaimin hydrochloride was also determined with its limit of detection value as shon in Table 4.31. 2.4 $\mu g.ml^{-1}$ of thiamin hydrochloride can be detected easily by the proposed method at 441 nmof wavelength.

Table 4.31 Limit of detection of thiamine hydrochloride by developed method

Concentration µg/mL	$\overline{X}$	S	D.L µg/mL
2.4	0.1043	0.0146	1.001

Howver, the average of six measurements for each of the three distinct concentrations (12, 30, and 42 $\mu g.ml^{-1}$) was used to calculate the accuracy of the proposed method for thiamine hydrochloride determination. Hence, it was found that, 99.6 % of drug can be recovered by appling the already established approach with a standard deviation of 3.211 as shown in Table 4.32.

Table 4.32 Accuracy and compatibility of the developed method for thiamine hydrochloride

Amount of thiamine Taken µg/mL	RE.%	Recovery.%	Average recovery.%	RSD.%
12	-1.5	98.4	99.6	3.211
30	0.4	100.4		0.935
42	0.2	100.2		0.864

4.9 Statistical Analysis And Evaluation Of Outcomes Of Suggested Method

Statistical analysis of data obtains for both sodium diclofanic and thiamin hydrochloride was analysed by applying t-test and F-test. It was found that, at a 95% of confidence level with four degrees of freedom, the experimental value of [+1.43] was determined to be smaller than the tabular t-value of 2.776. Which indicates that the two methods do not differ in terms of reliability of the approach. Moreover, the goal of the test was to ascertain whether the finding of suggested technique differ significantly that that of conventional approach. Since there are no degrees of freedom and the experimental F value of 1.33 is smaller than the F value of 6.26 in the table at the 95 % of confidence level, it is found by using the statistical equation in the appendix that there is no statistically significant difference between both techniques. Hence, the data obtained form the approach showed compare to standard deviation that, the suggested oxidative coupling approach has high potential in the application of manufacturing of pharmaceuticals. Similarly, data obtaind for thaimin hydrochloride was statistically analysed. It was found that, the tabulated statistic of 776.2 is larger than 95 % of confidence level with four of degrees of freedom. Which indicate the developed approaches are relaiable. However, this test was designed to examine if the results of the suggested technique and the findings of the standard method differ significantly or not. Although, the experimental F-value of 1.18 determined to be less than the tabulated F-value of 9.28 at the 95 % of confidence level and three degrees of freedom using the

statistical approach. This means that the suggested oxidative conjugation approach has a high potential as an application for the validityof the established method.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

İn conclusion, spectrometric method developed for detrmination of sodium diclofenic rely on the direct use of 4,2-dinitrophenylhydrazine coupling reagent in the presence of oxidizing agent in an alkaline media. Which makes developed approach for the measurment of diclofenic easy, senstive and reliable with maximum absorption at 600 nm of wavelength. İt was also found that, the sensitivity of devloped method was in the range of 0.8-16 μg of sodium diclofenic drug which folllow the Beer's Law. Moreover, this method was successfully applied for the determination of diclofenac in pharmaceutical preparations (tablets), with reproducibility between 98.4 % and 99.9 % and Sandel's significance of 0.0110 g. However, the relative standard deviation does not exceed to 2.97 %, with the detection limit of 0.0881 μg. ml^{-1}. Similar to sodium diclofenic, the method developed for thiamin hydrochloride was found to be highly sensitive, fast and easy. According to the developed method thiamine hydrochloride was treated with p-aminophenol as a coupling aagent in the presence of oxidizing agent potassium periodate in basic media at pH of 11.11. After incubation yelowish to orange colored product was formed which was studied by using spectrophotometer at 441 nm of wavelength. İt was found that, the devloped method also follow the Beer's Law in the range of 2.4-60 $g.ml^{-1}$ and have a correlation value of 0.9986. However, the obtained standard deviation of the measurements was about 3.21 % or less. Additionally, 1.001 micrograms per milliliter is the detection limit with a significance of 0.0390 μg. ml^{-1}. Hence, this technique has potential to be used to determine and recover the thiamine hydrochloride in pharmaceutical preparations such as capsules. It works by taking a sample and calculating the standard deviation, along with the detection limit. These two numbers need to be less than 3.21% and 1.001 μg. ml^{-1}, respectively.

5.2 Recommendations

- Possibility of determination of sodium diclofenac and thiamine hydrochloride using coupling reagents in presence of oxidizing agent in basic media and their application in pharmaceutical preparations.

- Implementation of the proposed reactions in the methods developed by the flow injection technique and chromatography.

- The possibility of applying the developed method fort he determination of drug in blood and urine of the patient.

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