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Thermodynamic, Kinetic And Spectrophotometric Studies Of Hydroxychloroquine And Lopinavir

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ.}

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We certify that thesis(**Thermodynamic, kinetic and spectrophorometric studies of hydroxychloroquine and lopinavir)** was papered under our supervision in the chemistry department-college of Education for pure sciences-University of kerbala, in partial fulfillment of the requirements for the master degree in chemistry sciences by the student (**Maryam Ali Abd Ulsamad**) .

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Dedication

To Those Who have supported me throughout my life and cared for me more than themselves to those who spent sleepless nights for my comfort to the most precious thing in my life to the light of my eyes, my dear parents

My mother and My father

Maryam

2022

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ABSTRACT

This study tried to find a quick, easy and inexpensive spectral way to detect two kinds of drugs (HCQ and LPV) in pharmacological formula through the reaction of reagent (2,4-Dinitrophenylhydrazin) (2,4-DNPHz) with the oxidizing agent (potassium periodate) (KIO_4) in presence of drug in alkaline medium.

The results revealed a high absorption for drugs (HCQ and LPV) at the wavelengths (332nm and 322nm) respectively, while the highest absorption of the reagent (2,4-DNPHz) at wavelengths (359nm). Moreover the highest absorptions of the azo dye product were at (620nm) and (482nm) respectively.

This study was conducted to find out that the optimized conditions were experimented to estimate two drugs. It was shown that the optimized concentration reagent of drug (HCQ) and (LPV) was equal to (0.0075M), and (0.005M) respectively. While the optimal volumes of the reagent were (1ml) and (1.25ml) respectively. And the optimized volume of oxidizing agent was (0.75 ml) for each drugs. while the optimized volume of the base solution were (0.5ml) and (1.5ml) of drugs (HCQ and LPV) respectively.

Added to that the effect of temperature and time were conducted, a higher absorbance was obtained when temperature was (25ºC) for HCQ and (35ºC) for LPV ,while at the best time it was obtained at (10min) for each drug for the stability of the azo dye product.

The sequence of addition for each drug, reagent (2,4-DNPHz), oxidizing agent (KIO_4) and the base solution(NaOH) also studied .The best sequences added to drugs (HCQ) and (LPV) were (2,4-DNPHz + KIO_4 + drug +NaOH).

The calibration curve of the two drugs was constructed after establishing the optimal conditions, The calibration curves were constructed and subsequently the Beer's law was obeyed within the concentration ranges of drugs (HCQ and LPV) which were equal (2.5 – 22.5$\mu g.ml^{-1}$). The molar absorptivity (Ɛ) values were (1048.28) $L.mol^{-1}.cm^{-1}$, and (3143.75)$L.mol^{-1}.cm^{-1}$ respectively, and the Sandell's sensitivity values were t (0.3205) and (0.20002) $\mu g.cm^2$ .The Detection

limit (D.L) value were (0.243) and (0.933) μg.ml^{-1}.The %R.S.D value were (%1.44) , and (%1.12).the %Erel value were (%-0.012), and (%-0.2525) with a correlation coefficient (0.9978) and (0.9967) respectively.

The stoichiometry of the reaction between two drugs with the (2,4-DNPHz) reagent was by using the continuous variations and mole ratio methods respectively equal to (1:1) for each drug and the stability constant (Ksta) was ($7.52x10^6$) L.mol^{-1} for (HCQ) , while the stability constant (Ksta) of (LPV) was ($2.818x10^6$) L.mol^{-1} .

Interferences were studied. The results reveal no effect on the determination of the two drugs.This technique has been used to successfully identify the drug (HCQ) in pharmaceutical formulation. And also to determine of (LPV) in serum.

Enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG), among other thermodynamic characteristics, were also estimated. It was found that the reaction between the two drugs and (2,4-DNPHz) reagent was endothermic, Spontaneous, and more regular.

The activation energy of the reaction between the two drugs and (2,4-DNPHz) reagent was also calculated and its value was (143.6) and (203.8) J/mol respectively.

Finally, kinetics of the reaction were studied, It was found that the reaction was pseudo second order of the two drugs.

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Abbreviation and Symbols	The Meaning
HCQ	Hydroxychloroquine
LPV	Lopinavir
A	Frequency factor
Abs	Absorbance
2,4-DNPHz	2,4-Dinitrophenyl hydrazine
Ea	Activation energy
ΔG	Change of free energy
ΔH	Change of enthalpy
h	Rate of reaction
J	Joule
K	Kelvin
k	Rate constant
keq	Equilibrium constant
kins	Instability constant
ksts	Stability constant
L.O.D	Limit of detection
L.O.Q	Limit of quantification
qe	The concentration at equilibrium

qt	The concentration at any time
R	Universal gas constant
%R.S.D	Relative standard deviation
S	Sandell sensitivity
S.D	Standard deviation
ΔS	Change of entropy
T	Temperature
t	Time
λmax	Maximum wavelength
ε	Molar absorption coefficient

Chapter One Introduction

1.Introduction

1.1.The Drugs:

The drugs are natural or highly selective chemicals used in pharmaceutical form aimed at modifying or exploring the patient's conditions. Pharmaceuticals contain one or more drugs along with other substances that are organic acids, bases or salts, and are in various forms including vitamins, hormones, proteins and mineral salts that are used when the organism lacks them, or the drugs are in the form of vaccines used to improve the body's immunity towards a particular disease or in the form of antibiotics used against infection and to treat the deficiencies of a particular function in the body of the organism[1,2].

The process of drug detection and development is divided into two important parts: the main first part : the discovery of drugs involving the isolation of the active ingredient, its purification, and standard. The second main part, the evolution of the drug, begins with a solitary compound, at which stage various studies are used aimed at supporting its support as a new drug[3].

A pharmaceutical compound is a drug specific to human or veterinary uses in treating, alleviating, preventing or diagnosing the disease. Use of ineffective, dangerous, or subpar medications results in health damage and financial waste. A weak medicine supply chain and unfavorable weather conditions make the issue worse (including storage and transport).These causes lead to a deteriorate in the quality of the drug, loss of effectiveness and may turn into harmful decomposition products[4].

1.1.1.The Drugs analysis:

In general, the drug analysis is a practical application so as to determine the drug (once or combination) in the form of a large dose or pharmaceutical. Chemical, physical, and occasionally microbiological analysis is used to test pharmaceutical items[5]. Drug analysis is a crucial method for monitoring drug quality. [1]. Nearly all medications are dangerous or even lethal when taken in large doses[2]. Determining the drug's action and conducting pharmacokinetic and pharmacodynamic research for pharmaceutical formulations is crucial[6]. Drug analysis is a crucial step in the process of creating new drugs. All active ingredients, impurities, excipients, stability of the active ingredients (degradation intermediates or end products), and additional factors including content homogeneity, solubility, and dissolving rate are all determined[7].

Because of the development of pharmaceutical preparations, it has attracted researchers, as this development is revolutionizing human health[8], that these drugs do not achieve their goal unless they are free of impurities, as they are given to the patient in an appropriate amount [9]. In order for medicines to serve their intended function, a variety of chemical techniques and practical tools must be produced on a regular basis[10]. The contribution of chemistry, pharmacology, microbiology, and biochemistry has established a standard in the field of drug discovery, where new medications are being developed through collaboration between biologists and chemists rather than solely through the creative process of chemists[11].

Numerous analytical techniques, including high performance liquid chromatography, have been documented in the literature for the therapeutic monitoring of pharmaceuticals in commercial dosage form and biological fluids (HPLC)[12], spectral method, LC method,

electrochemical , and electrophoretic method . The primary issues with adopting such techniques are the need for either time-consuming extraction operations or the requirement for derivatization[13,14].

The interaction of ultraviolet light and some infrared light with the sample causes the quantitative analysis of optical spectrometry, which has an impact on many fields of research and technology. Due to technological advancements over the past few decades, spectrometers have undergone numerous alterations, adding numerous additional functions for various types of materials and optical qualities[15].

1.1.2. The Drug stability:

The capacity of a pharmaceutical product to maintain its efficacy, qualities, and properties over the course of its shelf life in a particular container is known as stability[16]. For commercial pharmaceutical goods, a suggested shelf life (expiration date) is three to five years. The drug's concentration shouldn't drop below 95% of what it was when it was first manufactured at this point[17].

In the production of drugs, the pharmacist is concerned with five different types of stability:

1- Chemically: The product keeps its potency and chemical integrity (including photochemically).

2- Physical:During storage or handling, the pharmaceutical product's conformance (color, appearance, dissolution, etc.) does not alter.

3-Microbiologically,sterilized goods must continue to be sterile.

4-Therapeutic:The prescribed dose scheme has no effect on the therapeutic effect, which remains constant.

5- Toxicologically,there has been no discernible rise in the expected toxicity effect.

The medicinal, microbiological, and hazardous types of stability are determined by the drug's chemical and physical properties. Knowing the drug's chemical stability is crucial for selecting the best storage environment to protect it from environmental factors like light, temperature, humidity, etc. and to prepare for drug interactions with excipients or other substances[18,19].

Many medications are derivatives of carboxylic acid or contain functional groups based on this moiety, such as esters, amides, lactones, lactams, and imides. These processes, in turn, lead to drug degradation[17]. As a result, the medicine may degrade as a result of several chemical processes. These processes include dehydration, oxidation, polymerization, racemization, isomerization, hydrolysis, and photochemical reactions[16].

1.1.3.Photochemical degradation:

These are the reactions that result from the absorption of visible or ultraviolet light. where the excited reactive molecule absorbs photons of light(energy). A result of photodecomposition is then produced by the stimulating molecule. Numerous photochemical processes could fail to take the reactive molecule into account. Radiation does not reach the reactive molecule directly; instead, it travels through an intermediary that absorbs the accident's radiation and then transfers its energy to it. An example of this kind of device is a light sensor[20].

Most therapeutic substances are white-looking, meaning they may be absorbed into the UV area based on their chemical composition[21].

Many industrial processes also include photochemistry changes. In photography, for example, some silver salts in a film absorb light when the image is taken. The absorbed light chemically changes these salts. When the film acidifies, the changing salts emit dark images on the negative. research into photochemistry these days has involved the development of technical uses of solar energy. Some photochemists are seeking ways to mimic the process of photosynthesis with artificially created atoms. These chemists hope to convert sunlight into electricity in a more efficient way than is possible now. Other chemists are studying ways to use sunlight to produce fuels, such as hydrogen gas and methanol. Some of these methods include the fragmentation of water atoms with solar energy[22].

1.2. Some Basic Concepts:

The science that means (Interaction) the electromagnetic beam with the material is called spectroscopy. The absorption or emission of the electromagnetic beam by the material leads to the effect and then energy transitions between specific levels, Quantification of the intensity of electromagnetic radiation at one or more wavelengths using a measurement-designed sensor called spectroscopy[23]. The absorption spectrum is formed when there is an effect between the fallen electromagnetic beam and the material, which represents the amount of radiation absorbed at a certain wavelength, Depending on this wavelength, this method can be used in qualitative and quantitative analysis of absorbent matter[24].

1.3. Analytical methods:

In the research and development of medications and pharmaceuticals, analytical method development and validation methods are essential. Drug synthesis is aided by analytical procedures, which are also used to assess possible drug candidates, support formulation studies, monitor the stability of bulk drugs and pharmaceuticals, and test the finished products before they are released. The success of medication and formulation development is significantly influenced by the caliber of the analytical data[25].

One of the most important methods used in the analysis of drugs and their components[26]:

1-chromatographic technology.

2-method of electrochemical analysis.

3-spectroscopy methods.

1.4. Spectrometric Method of Analysis:

The Bear-Lambert Act, which forms the foundation of spectrum analysis, governs the relationship between the absorbed beam and the concentration of absorbent particles in the solution, In order for spectral estimation to occur, the material must be converted into colored varieties, if not colored, that absorb radiation in the visible area by adding chemical reagents that interact selectively with the components to be estimated, consisting of colorful variants, the intensity of which is determined by the amount of those ingredients in the solution [26,27].

These rays can be absorbed by organic or inorganic compounds after specific conditions are met.

1.4.1.UV-Visible Spectrometry:

Although absorption in the UV-Vis region is limited for qualitative analysis, it is considered one of the most important means for quantitative analysis because of its sensitivity and selectivity, good accuracy, In addition to the ease and speed of analytical performance when using spectral devices. As a simple and inexpensive method, it is widely used in various analyses,[28,29].

Spectral measurements are also frequently used in the qualitative control of substances, compound diagnosis, as well as in clinical, environmental and other analyses[30,31].

The UV area extends from about 10 nm to 350 nm, but the area most commonly used in the analysis is 200 nm to 350 nm, which is called nearby ultraviolet, The visible area forms a small part of the electromagnetic spectrum and includes wavelengths stretching from a nearby ultraviolet area of 350 nm to about 770 nm, and most of the energy absorbed at wavelength in the visible area appears colored so you can see with the naked eye[32,33].

1.4.2. Applications of UV-Visible in general:

Biochemical species, inorganic metals, organic compounds, and other species that can absorb UV and visible light can all be affected by ultraviolet radiation. These species can then be used to quantify measurements. After transforming many invisible molecules into absorbed derivatives by adding various chemical compounds, it is also feasible to identify numerous invisible molecules using UV or visible radiation. According to statistics, more than 90% of analysis in chemistry labs have been carried out or identified using UV/visible spectroscopy.

These applications, include estimated medications, and insecticides at the sub-microorgan level, vitamins, and stimulants, are well known[34].

In addition, the analytical spectroscopy method was used in estimating and measuring colored metals and colored compounds[35].

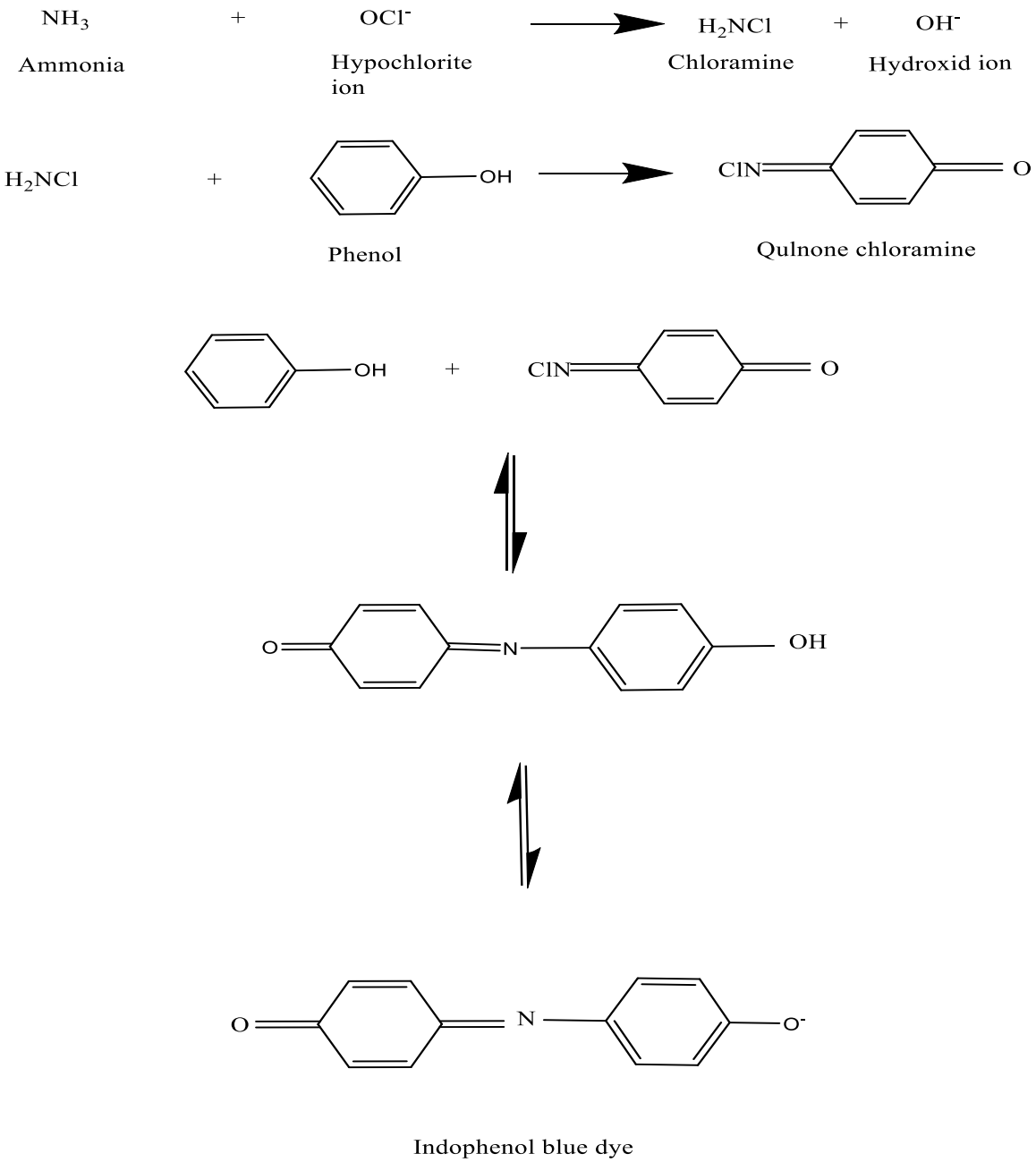
1.5.Oxidative coupling reaction:

Oxidative reaction consists of the association of two or more organic substances with the presence of an oxidized agent suitable and in appropriate interaction conditions. When the oxidation of these materials occurs, intermediate compounds are formed that interact with each other to form a color compound that can be spectrally measured, and can be utilized for the purposes of quantifying several compounds[36].

Studies have shown that there are many factors that affect the interaction of oxidative association, the most important of which are:

- Temperature: The temperature activates some unstimated reactions, where The temperature used in this can reach 100°C while most These interactions are at 25°C and this usually makes the analysis simpler without need to control temperature[37].
- Interaction: Oxidative reaction can occur in acid, alkaline or moderate reactions depending on the conditions of interaction and the nature of the reactive materials and the resulting colored materials.
- Contributing factors: It was found that some compounds and ions can act as helping factors such as sulfuric acid, chromium ion and manganese ion (ll) in many oxidative reactions[38].

The first oxidative pairing reaction was observed in 1859 by the scientist Berthelot[39]. It is referred to as the indophenol reaction created the indophenol dye, which consists of The maximum absorbance was produced at 630 nm by the interaction between phenol and hypochlorite when ammonia was present[40]. Show in scheme (1-1)



Scheme (1-1) indophenol reaction mechanism.

1.6. Azo Coupling reaction:

A diazonium compound and another aromatic compound undergo a chemical process known as an azo coupling, which results in an azo compound[41]. due to the extended conjugated systems, aromatic azo compounds frequently have vivid colors. numerous are used as dyes (azo dye)[42]. so organic substances with the functional group RN=NR′, in which R and R′ are typically aryl, are known as azo dyes[43].

Diazotization is the process of turning primary aromatic amines into their diazonium salt. diazonium salts are significant synthetic intermediates that can be converted into azo dyes through coupling reactions and functional groups into other molecules through electrophilic substitution processes[44].

Due to their common use as pH indicators in chemistry studies, vehicles with aromatic fragments attached to a double bond N = N. are often cited. examples include methyl yellow, methyl orange, methyl red, congo red, and alizarine yellow, they are easy to made and essential for industry. in a two-step procedure, azo dyes are made, the creation of an aromatic diazonium ion from an aniline derivative being the first. the diazonium salt is combined with an aromatic component in the following step. A variety of hues like yellow, red, orange, brown, and blue can be found in azo dyes[45].

In a previous study, the indirect determination method of dopamine and paracetamol was used by electrochemical impedance spectroscopy using the azo coupling reaction with oxidized 2,4-dinitrophenylhydrazine (2,4-DNPH)[46]. in another study, the voltametric determination of amoxicillin was used in a dosage form using the reaction of azo coupling with sulfanilamide[47]. and The Azo coupling reaction was also used for

indirect spectroscopy estimation of furosemide using resorcinol as a detector by the researchers (Ali I. Abdullah and Sumayha M. Abass)[48].

1.7.Chemical reagents:

They are compounds with high molecular weights, which are poorly soluble in water because they have covalent bonds, as they are characterized by possessing effective groups to give colored complexes in most cases that qualify them to interact with many chemical elements, and these compounds have multiple advantages that made their use spread widely and in different fields such as medicine, Technology and science have proven this by giving results of great importance[49].

In the field of analytical chemistry, it is possible to exploit the chromaticity of organic reagents (azo reagents and their complex compounds including) with compounds in their aqueous solutions and the clear difference in the colors of those complex compounds from the colors of the reagents and elements used with them, which gave the opportunity to be used in spectroscopic analysis[50].

Reagents are used to detect or identify another substance by chemical or microscopic means, especially analysis. The most important types of reagents are sediments, solvents, oxidants, and color reagents[51]. It is also considered " a material or compound that is given to a system in order to cause a chemical reaction or to test whether a reaction occurs. Reagents are substances or combinations, typically made up of inorganic or tiny organic molecules, that are utilized in organic chemistry to change the organic substrate. Examples of organic reagents include Collins Detector, Fenton Detector, and Gregnard Detector. There are also analytical reagents and they are used to confirm the presence of another substance[52].

1.7.1. 2,4-Dinitrophenyl hydrazine reagent:

2,4-Dinitrophenylhydrazine (2,4-DNPHz) is a hydrazine substitute. It is a structure containing hydrazine, which is the main active functional group. 2.4-DNPHz is an important reagent in analytical chemistry that is often used first in determining (qualitative analysis) of carbonyl groups (aldehyde compounds and ketone)[53]. It is used in the quantitative spectral estimate of different compounds through different types of reactions[54], where the detector was used to identify drugs such as (propranolol hydrochloride and isoprobramide as iodide)[55].

For a figure (1-1) shows the synthetic formula of the 2,4-DNPHz detector, and The table (1-1) shows general characteristics of the detector[56,57]:

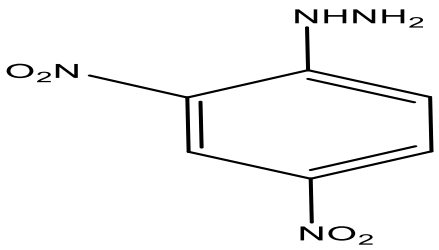
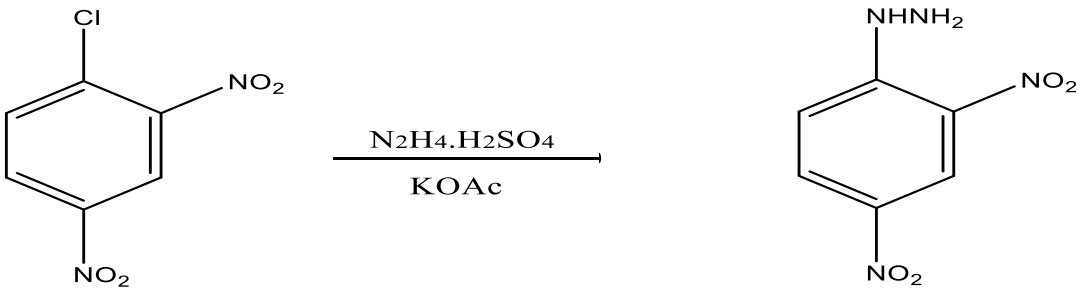


Figure (1-1): Synthetic formula for 2,4-DNPHz.

One of the methods of preparation of the reagent compound (2,4-DNPHz)



Scheme(1-2) The preparation of 2,4-DNPHz reagent.

Table (1-1): General characteristics for 2,4-DNPHz.

Systematic name (IUPAC)	**2,4-dinitrophenylhydrazine**
Additional name	**2,4-DNPHz,2,4-DNP,Brady's**
Molecular formula	**$C_6H_6N_4O_4$**
Molecular weight ($g.mol^{-1}$)	**198.14**
Melting point (ºC)	**198 to 202**
Solubility	**Slightly soluble in water, soluble in concentrated H_2SO_4**
Storage	**Stored wet and for no more than 5 years**
Stability	**The product is stable**
Appearance	**orange powder**
Uses	**Laboratory reagent**

1.8. Anti-viral drugs:

A class of medication called antiviral medications is used to treat viral infections[58]. While most antivirals target particular viruses, broad-spectrum antivirals work against a variety of viruses[59]. Antiviral medications differ from other antibiotics in that they suppress the growth of their target pathogen rather than eradicating it. Antimicrobials, a broader category that also includes antibiotics (also known as antibacterial), antifungal, and antiphrastic medications, or antiviral medications based on monoclonal antibodies, are one class of which includes antiviral medications[60].

It was initially intended to cure and prevent malaria, but it is now also used to treat rheumatoid arthritis, certain lupus erythematosus symptoms, juvenile idiopathic arthritis, pediatric arthritis, and other autoimmune illnesses. To prevent and treat coronavirus illness 2019 (COVID 19), hydroxychloroquine is being explored; however, all clinical trials completed in 2020 revealed that it is useless and may have risky side effects[61].

Many antiviral medications are prodrugs ; in order for them to work, they need to be phosphorylated by viral or cellular enzymes[62]. Antiviral medications prevent active replication, thus once the medication is stopped, viral growth continues. [63]. and the most crucial characteristics of antiviral medications, able to infiltrate virus-infected cells, obstruct the control or production of viral nucleic acids, Some medications prevent viruses from adhering to cells, Some medications boost the immune system of the body, Patients with strong immune systems respond to antiviral medications well, and a strong immune system cooperates with the treatment to stop or inhibit viral activity[64].

Latent or nonreplicating viruses are not eradicated by modern antiviral medications[65]. A strong host immune response is still necessary for recovering from a viral infection[66]. Obtaining inhibitory conc. at the site of infection within the infected cells is necessary for clinical efficacy[67]. Since viral infections have caused millions of fatalities globally throughout the history of human civilization, there is an urgent need for the development of dynamic antiviral medications[68].

One of the most important ways to find antivirals is to discover computer-based drugs and this nelfinavir approach is an example discovered, in the 1990s to treat HIV infection[69]. Despite modern tools

and strict quality control procedures, only a few antiviral drugs are approved for human use and therefore for several reasons including side effects or antivirals resistance drugs ,with increased awareness about viruses their infection mechanism and the rapid development of new antiviral strategies and techniques will further develop new antiviral drugs [(70)]. The current situation around the world indicates that microbial threats continue to emerge rapidly, especially because of unprecedented climate change and globalization[(71)] .

1.8.1. Hydroxychloroquine :

In combination therapy for RA, hydroxychloroquine (HCQ), a good anti-rheumatism infectious disease drug (DMARD), is more frequently used than chloroquine[(72)],The IUPAC name of HCQ(C18H26ClN3O) is (RS)- 2-[4-(7-Chloro-4- quinolylamino) pentyl (ethyl)amino]-ethanol[(73)], In 1946, the hydroxyl group was added to the structure of the antimalarial medication chloroquine (CQ), which allowed for the production of HCQ. As a result of the discovery of HCQ, which is two to three times less hazardous than CQ[(74)]. HCQ figure. (1-2) is 4-aminoquinolin where it possesses the activities described above . These drugs also increase immunity , symptoms resulting with HCQ Compared to CQ, treatment is uncommon; an acute hepatic lesion is also related[(75)].

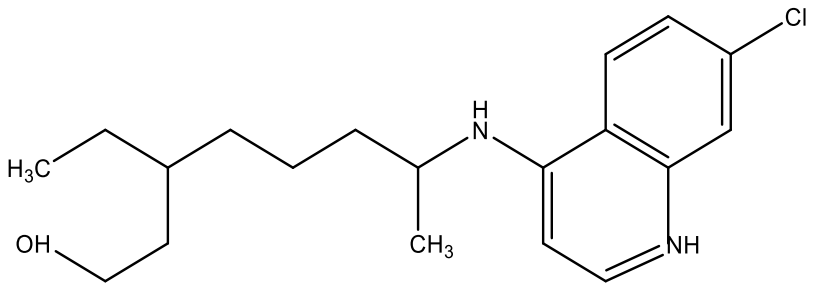


Figure (1-2): Chemical structures of HCQ.

German researchers in 1946, Alexander Sari and Henry Hammer, First, HCQ is created by adding a hydroxyl function to the structure of chloroquine in order to lessen toxicity when treating malaria[76]. Tablet versions of HCQ are offered on the market. A daily dose of 100 mg to 1.2 grams of HCQ is taken, and it is promptly absorbed within two to four hours. About 74% of the total is absorbed, leaving 13%. The blood concentration of HCQ increases early after ingestion but drops quickly due to fast organ diffusion[77], Hydroxy chloroquine (HCQ)is amino-4-quinolin derived from quinolone Quinine nucleus, consisting of two aromatic nuclei, Malaria and autoimmune illnesses are two conditions where it is often utilized[78].

Azithromycin is connected with the COVID-19 virus because it has features that are comparable to those of Chloroquine while being less harmful. Whereas research on animals have revealed that HCQ is roughly 40% less hazardous than chloroquine. HCQ is nearly complete, rapidly absorbed by the digestive system, and soluble in water (estimated to be about 70 percent)[79]. The most important thing that distinguishes HCQ is the rapid absorption of the digestive system and its elimination through the kidneys. When entering the digestive system, it passes easily in the blood and quickly reaches the maximum level of plasma[80]. HCQ has advantages, however at 6.5 mg/kg of body weight, it becomes hazardous[81]. Side effects of a rapidly increasing overdose include breathing issues, blood circulatory issues, and arrhythmia, as well as insufficient[82].

Additionally, laboratory studies have demonstrated that HCQ testing of human blood, serum, and urine can reveal details about the clinical state of infected people[83]. In addition, it has In tissues, HCQ is widely dispersed. They adhere to the tenacity of ruptured cells, primarily red

cells that have invaded. It is abundantly present on tissues that contain melanin as well as the kidneys, lungs, liver, and spleen[84].

Long-term chloroquinemia and sluggish elimination are the effects of this reflectable binding. Additionally, negative effects are brought on by the drug's central nervous system passage[85,86].

And In addition, hydroxychloroquine is characterized by its metabolic liver before eliminating the kidneys and slow urine secretion[87]. The rest is metabolized with metabolism using cytochrome partially[88].

Numerous investigations have demonstrated a connection between the clinical response and the level of the medication hydroxychloroquine in the entire blood[89]. Inadequate analytical methods for HCQ analysis in blood samples lead to more patients and fatalities[90]. It is crucial to assess whether HCQ is present in biological medium. A crucial first step in accurately identifying this medicine is to select the best analytical technique. For screening, identifying, and quantifying HCQ, analysts can employ a very wide variety of analytical techniques[91].

1.8.2. Lopinavir:

Is used in conjunction with other anti-retrovirals to treat HIV-1 infection and is regarded as an antiviral protein enzyme inhibitor. Due to LPV's poor oral bioavailability and significant biotransformation, it is only sold and administered in conjunction with ritonavir. This combination was first marketed by Abbott under the brand name Kaletra in 2000[92].

Lopinavir is a dicarboxylic acid diamide that is amphetamine is substituted on nitrogen by a (2,6-dimethylphenoxy)acetyl group and on

the carbon alpha- to nitrogen by a (1S,3S)-1-hydroxy-3-{[(2S)-3-methyl-2-(2-oxotetrahydropyrimidin-1-yl)butanoyl]amino}-4-phenylbutyl group[93].

Lopinavir has the molecular formula $C_{37}H_{48}N_4O_5$, molecular weight 628.80 g mol^{-1}, and the chemical structure[94] in figure (1-3).

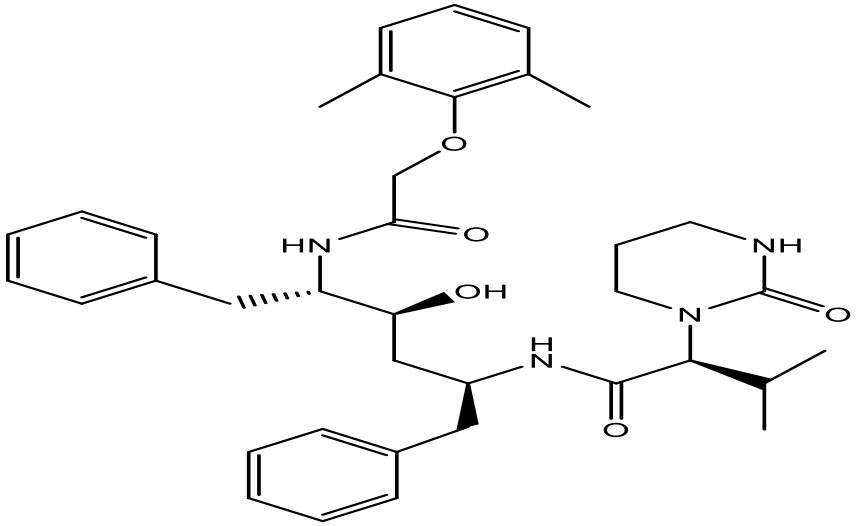


Figure (1-3): The chemical structure of LPV.

Currently used highly active antiretroviral therapy (HAART) regimens contain an important component known as the therapeutic class of HIV protease inhibitors (PI)[95].

A PI derived from ritonavir and structurally linked to lopinavir (RTV)[96]. Additionally, the dosage of the two medications is always 33 mg RTV and 133 mg LPV (Kaletra). This substance has an antiretroviral effect because of LPV, whereas RTV functions as a booster because it inhibits the cytochrome P450 CYP3A isoenzymes that render LPV inactive[97]. The extensive binding of LPV and RTV to plasma proteins, primarily a1-acid glycoprotein (99%), and their attraction for drug-transporting proteins, particularly P-glycoprotein, restrict their dispersion in the body (ABCB1 or MDR1)[98]. Because of this, the amounts of LPV

and RTV in brain, semen, and saliva are much lower than their combined concentrations in blood (unbound plus protein-bound)[99]. at these sanctuary areas, and might not be adequate for antiviral activity [100]. Due to the risk of viral recovery from these reservoirs if antiretroviral medication is stopped, sanctuary sites continue to be a significant barrier to eliminating HIV from the body[101].

Developed by Abbott Laboratories, Kaletra has been used to treat HIV infection. Kaletra consists of two protein enzyme inhibitors lupinavir and retunavir. An accurate and quick analytical method is necessary to produce reliable data in order to support clinical development and track drug exposure. Numerous analytical techniques for lopinavir and ritonavir have been documented in the literature[102.103].

Lopinavir may occasionally result in a clinically evident, acute liver injury as well as brief, generally asymptomatic rises in blood aminotransferase levels[104]. Highly active antiretroviral therapy with lopinavir may cause the underlying chronic hepatitis B or C to worsen in patients with HBV or HCV coinfection[105].

Experience with acute lopinavir overdose in isolation is few because lopinavir is only offered in conjunction with ritonavir. The risk of an overdose seems to be greater in pediatric patients[106]. Following an approximately 10-fold overdose of Kaletra oral solution, one case report described a fatal cardiogenic shock in a 2.1kg child. Other recorded infant overdose reactions include full AV block, cardiomyopathy, lactic acidosis, and acute renal failure[107], which increases the likelihood of overdose. Overdose from lopinavir The oral Kaletra solution contains 42 percent (v/v) ethanol, which significantly raises the risk of overdose in children and infants. It is also extremely concentrated has no antidote[108].

The majority of an overdose's treatment should focus on supportive measures and close monitoring of the patient's clinical condition and vital signs[109]. If clinically necessary, activated charcoal or stomach lavage should be used to remove any unabsorbed medication. Due to lopinavir's high protein binding, dialysis is unlikely to be beneficial, however it might assist remove ethanol and propylene glycol from the bloodstream in the event of a Kaletra oral solution overdose[110].

1.9. The Literature review:

There are many methods by different techniques for the determination of the (HCQ and LPV) drugs. Table (1-2) and (1-3) shows these technique.

Table(1-2): Different Techniques used for Determination of HCQ.

Analytical Technique	Outline of the Method	Liner Rang (mol L^{-1})	DL (mol L^{-1})	R.S. D%	Ref.
Liquid chromatography–tandem mass spectrometry (LC-MS/MS)	HCQ and its metabolites were examined in human blood using liquid chromatography tandem mass spectrometry (LC-MS/MS) in 2014 by Ying Qu et al.	3×10^{-8} to 5.95×10^{-6}	1.48×10^{-8}	7.6	111
Fluorescence chromatographic detection technique (HPLC-FLD)	A clinical study has shown that HCQ is detected and metabolized in the blood using fluorescent chromatography detection (HPLC-FLD).	1.48×10^{-7} to 1.19×10^{-9}	1.48×10^{-7}	4.3	112
(LC-MS/MS)	Using high performance liquid chromatography, hydroxy-chloroquine and its three metabolites have also been examined simultaneously.	7.44×10^{-8} to 5.95×10^{-6}	5.95×10^{-8}	------	113
Differential pulse voltammetry. (DPV)	Lara Maria Utilizing cyclic voltammetry and differential pulse voltammetry, P.M. Arguelho et al. investigated the electrochemical reactivity of HCQ using the glassy carbon electrode.	2×10^{-5} to 5×10^{-4}	3.4×10^{-5}	0.46	114

square wave voltammetry (SWV)	.. Square wave voltammetry was used by Patrcia Batista Deroco et al. in 2014 to calculate the HCQ by a boron-doped diamond electrode	0.1×10^{-6} to 1.9×10^{-6}	6×10^{-8}	4.5	115
Liquid-phase micro extraction (LPME)	Hydroxychloroquine was estimated using LPME in urine samples.	2.97×10^{-8} to 2.97×10^{-6}	2.97×10^{-8}	-----	116

Table(1-3): Different Techniques used for Determination of LPV.

Analytical Technique	**Outline of the Method**	**Liner Rang**	**DL**	**R.S.D %**	**Ref.**
Reverse Phase High-Performance Liquid Chromatograph (RP-HPLC)	developed a high-performance liquid chromatography technique for the reverse stage that validates the detection of ultraviolet radiation for the study of lupinavir in mice plasma.	250 to 4000 $ng.ml^{-1}$	-------	4.73	117
Liquid chromatography–tandem mass spectrometry (LC-MS/MS)	A quick and accurate liquid chromatography-mass spectrometric (LCMS-MS) technique for lopinavir and ritonavir detection in human plasma	50 to 20000 $ng.ml^{-1}$	-------	4.3	118
high-performance liquid chromatography and ultraviole (HPLC-UV)	Using high-performance liquid chromatography (HPLC) and titania-based column with UV detection	5 to 35 $\mu g.mL^{-1}$	0.844 $\mu g.mL^{-1}$	-----	119
(RP-HPLC)	Development and Validation of RP-HPLC Method for Determination of Ritonavir and Lopinavir .	50 to 150 $\mu g.mL^{-1}$	0.013 $\mu g.mL^{-1}$	0.35	120

HPLC	In another study, lopinavir (LPV) was developed in soft gelatin capsules and fully validated using an HPLC approach that indicates stability.	40 to 360 $\mu g.ml^{-1}$	200 $\mu g.mL^{-1}$	0.7	121

1.10. The aims of study:

1-The objective of work is to develop new spectrophotometric methods for the determination of some anti- viral drugs such as Hydroxychloroquine and lopinavir in pharmaceutical formulations by using 2,4-DNPHz organic reagents, which are accurate, precise and sensitive.

2-Study the analytical parameters for each method such as the reagent of reaction (volume of reagent, pH, temperature, time of stability, order of addition, stoichiometry and etc.

3-The methods will be validated with respect to accuracy, precision, linearity, selectivity and sensitivity.

4-Study the kinetic for determination method by calculating the rate and order of reaction.

5-Calculate the thermodynamics parameters such as enthalpy, Gibbs energy and entropy and also activation energy.

Chapter Two
Experiments
Part

2.1 Chemicals

The current table includes the chemical materials that are used in the current study:

Table (2-1): Chemicals used in the Research

No.	Chemicals	Molecular Formula	Molecular weight (g /mol)	Purity %	Company
1	Hydroxychloroquine (HCQ)	$C_{18}H_{26}ClN_3O$	335.87	99%	China-Nanjing Duly Biotech
2	Lopinavir (LPV)	$C_{37}H_{48}N_4O_5$	628.8	99.15%	China-Nanjing Duly Biotech
3	Brady`s reagents (2,4-Dinitrophenylhydrazine)	$C_6H_6N_4O_4$	198.14	98%	UK
4	Potassium periodate	KIO_4	230	99.5%	BDH
5	Potassium iodate	KIO_3	214	99%	BDH
6	Sodium periodate	$NaIO_4$	213.89	99%	BDH
7	Sodium hydroxide	NaOH	39.99	99.9%	India
8	Sulfuric acid	H_2SO_4	98.08	98.0 %	GCC
9	Ethanol	C_2H_6O	46.07	99.5%	UAE
10	Starch	$(C_6H_{10}O_5)n$	------	Pure powder	BDH
11	Magnesium stearate	$Mg(C_{18}H_{35}O_2)_2$	591.27	90%	England
12	Di basic calcium phosphate	$CaHPO_4$	136.06	90%	UAE
13	Poly ethylene glycol	$C_{2n}H_{4n+2}O_{n+1}$	------	Pure powder	England

2.2 Apparatus

There are various instruments required in the study. The details are explained in the table (2-2).

Table (2-2): Apparatus used in the Research.

No.	Apparatus	Origin	Company	The Place
1	UV–Visible single beam Spectrophotometer	Germany	EMSLAB	University of kerbala\ College of Education for Pure Sciences
2	1800 PC UV-Visible spectrophotometer,Shimadzu, Japan(Double beam).	Japan	Shimadzu	University of kerbala\ College of Education for Pure Sciences
3	Electrical Sensitive Balance	Germany	Lap. BL210,Sartorius median	University of kerbala\ College of Education for Pure Sciences
4	pH – Meter	Germany	WTW-720 ionlap	University of kerbala\ College of Education for Pure Sciences
5	Centrifuge.	Germany	Univerasal 320R, Hettich	University of kerbala\ College of Education for Pure Sciences
6	Shaking water bath	Korea	VISON	University of kerbala\ College of Education for Pure Sciences
7	Hot plate with magnetic stirrer	India	Ijlassco, India	University of kerbala\ College of Education for Pure Sciences

2.3 Preparation of Standard Solutions

2.3.1 Solution of drugs (HCQ and LPV)

The stock solution of (HCQ and LPV) was prepared by dissolving the precisely measured 0.1g of the pure medication in 10 mL of ethanol, and then adding the appropriate volume of the ethanol to a 100 mL volumetric flask. and they were prepared by dissolving 10 mL of the stock solution into 100 mL of ethanol in a volumetric flask.

2.3.2 Solution of 2,4-Dinitrophenylhydrazine (Brady`s reagent)

It was prepared by dissolving 0.01 g of 2,4-DNPHz in 2 mL of concentrated sulfuric acid, and then completing this volume to the mark

in a 100 mL volumetric flask with deionized water. The resulting solution had a concentration of (5×10^{-4}M) and keep away from light.

2.3.3 Solution of sodium Periodate ($NaIO_4$)

By dissolving 0.014g of $NaIO_4$ in the necessary volume of deionized water, and then transferring the solution to a 100 ml volumetric flask and topping it off with deionized water, the oxidizing agent solution ($NaIO_4$) is created at a concentration of (6.5×10^{-4}M).

2.3.4 Solution of Potassium iodate (KIO_3)

The oxidized agent solution(KIO_3) is prepared at a concentration of (6.5×10^{-4}M) by dissolving 0.014g of KIO_3 in an appropriate volume of deionized water and then transferred to a 100 ml volumetric flask and complements the size to the mark with deionized water And heat at 40 ˚C with stirring by using the shaking water bath.

2.3.5 Solution of Potassium Periodate (KIO_4)

First The oxidized agent solution(KIO_4) is prepared at a concentration of (6×10^{-4}M) by dissolving 0.014g of KIO_4 in an appropriate volume of deionized water and then transferred to a 100 ml volumetric flask and complements the size to the mark with deionized water And heat at 40 ˚C by using hot plate with magnetic stirrer .

Second The oxidized agent solution(KIO_4) is prepared at a concentration of (2.6×10^{-3} M) by dissolving 0.056g of KIO_4 in an appropriate volume of deionized water and then transferred to a 100 ml volumetric flask and complements the volume to the mark with deionized water, And heat at 40 ˚C with stirring by using the shaking water bath.

2.3.6 Solution of Sodium Hydroxide (NaOH)

First The alkaline solution sodium hydroxide (NaOH) is prepared with concentration (1M) by dissolving 4g of sodium hydroxide in an appropriate volume of deionized water and then transferred to a 100 ml volumetric flask and complements the size to the mark with deionized water.

Second The base solution sodium hydroxide (NaOH) is prepared with concentration (1.25M) by dissolving 5g of sodium hydroxide in an appropriate volume of deionized water and then transferred to a 100 ml volumetric flask and complements the size to the mark with deionized water.

2.4 Optimization of the Experimental Conditions

2.4.1 Effect of the type of oxidized factor

The study was conducted by adding 1 ml of 2,4-dinitro phenyl hydrazine solution(5×10^{-4} M) to 1 ml of different types of oxidizing agents (6×10^{-4}M) (such as: sodium periodate , potassium periodate, and potassium iodate) and 1ml of drug than 0.5 ml of sodium hydroxide solution(1M). Than calculate the highest absorbance against the blank solution of λmax for each drug.

2.4.2 Effect of 2,4-dinitrophenylhydrazine Concentration

Various weight of reagent (0.001 to 0.03) g were dissolve in 2 ml of Concentrated sulphuric acid (H_2SO_4) and this volume has been completed to a mark in 100 mL with deionized water.by added 1 mL of reagent with 1 mL of potassium periodate solution (6×10^{-4}M),1 mL of drugs (100µg.ml^{-1}) solution was added, and 0.5 ml of sodium hydroxide(1M) for each HCQ and LPV respectively in In a series of volumetric flask with a volume of 10ml, Then mix the solution well. finally the volume was completed to the mark with deionized water. The absorbance of color

product was recorded at the λmax for each HCQ and LPV to detect the best absorbance ,against a reagent blank preparation in same way without drugs.

2.4.3 Effect of Concentrated sulphuric acid (H_2SO_4) Volume

Prepare the reagent (5×10^{-4}M) using various volumes of concentrated sulphuric acid (0.15 to 0.45) mL , 1 ml of prepared reagent has been added for each (HCQ and LPV) were taken to prepare of various blank solutions of this volume .Then the volumes were taken and mixed with 1mL of potassium periodate solution , 1 mL of drugs solution , and 0.5 ml of sodium hydroxide .Then the highest absorbance was recorded.

2.4.4 Effect of 2,4-Dinitrophenylhydrazine Volume

Various volumes of reagent (0.25 to 2.5) mL (5×10^{-4}M), for each (HCQ and LPV) were taken to prepare of various blank solutions of this volume .Then the volumes were taken and mixed with 1 mL potassium periodate solution, 1 mL of drugs solution, and 0.5 ml of sodium hydroxide solution .Then the highest absorbance was recorded.

2.4.5 Effect of potassium periodate (KIO_4) Concentration

Various weight of KIO_4 (0.007 to 0.0563)g were dissolve in 10 mL of deionized water put in volumetric flask . take 1 ml of this solution and the optimum volume and concentration of reagents solution , 1ml of drugs and o.5 ml of sodium hydroxide solution with a blank solution were added , then the highest absorbance was recorded for the solution.

2.4.6 Effect of potassium periodate (KIO_4) volume

Various volumes of KIO_4 (0.25 to 2.5) mL were added in 10 ml volumetric flask and the limitation volume of the reagent , drugs and sodium hydroxide and the volume were completed to 10ml to the mark

,the blank solution was prepare of this volume .The highest absorbance of the best volume was recorded.

2.4.7 Effect of sodium hydroxide (NaOH) Concentration

Various weight of NaOH (0.1 to 0.5)g were dissolve in 10 mL of deionized water put in volumetric flask. Take the optimum volume and concentration for both reagent and oxidant factor solution and 1ml of drugs than 0.5 ml of this solution, with a blank solution were added , then the highest absorbance was recorded for the solution.

2.4.8 Effect of sodium hydroxide (NaOH) volume

after detecting the best concentration of sodium hydroxide solution for each drugs. Various volumes of NaOH (0.05 to 2) mL were added in 10 ml volumetric flask and the limitation volume of the reagent , potassium periodate and drugs, and the volume were completed to 10ml to the mark ,the blank solution was prepare of this volume .The highest absorbance of each drug volume was recorded.

2.4.9 Effect drugs (HCQ and LPV) Volume

After the detection of the best concentration and volume from the reagent, the oxidized factor and the base Various volumes of drugs solution (0.25-2.5) mL (100µg.ml^{-1}) were taken and reacted with the best volume and concentration of the reagent, the oxidized factor and the base (each drugs with special volume and concentration) in 10 mL conical flask ,The blank was prepared in the same way without drugs Then the highest absorbance was recorded.

2.4.10 Effect of Reaction Time

The effect of time was studied in order to signify the stabilities of the colored azo dye product at the studied optimum conditions . The highest solution absorbance was noted at various times (0 to 120) min.

2.4.11 Effect of Temperature

Using a water bath, the effect of temperature on azo dye solution absorbance was examined at various temperatures (5,10,15,20,25,30,35,40°C). The azo dye solution's absorbance was measured.

2.4.12 Effect of the Order of Addition

The influence of addition on the absorbance of solution was studied depending upon the highest absorbance during the adding of the drugs, reagent , oxidant factor, and sodium hydroxide.

2.5 Construction of Calibration Curve

Calibration curve was constructed by preparing standard solutions of drugs (HCQ and LPV) in various concentration (2.5-22.5µg.ml^{-1}), tike obtain a optimum volume of reagent, oxidant factor then taking different volumes from drugs solutions and base solution in the volumetric flask (10ml) in optimum time and temperature . The absorbance of solution was recorded at maximum wavelength of each drugs against the blank solution which was prepared in the same way without drugs.

2.6 Precision and Accuracy

To be sure , the precision and accuracy of the analytic method of drugs (HCQ and LPV) were determined according to the relative stander deviation %R.S.D and %Erel for five solution in the same concentration in the optimum condition . The absorbance of solution was recorded at

wavelength for each drugs against the blank solution which was prepared in the same way without drugs.

2.7 Applications

2.7.1 Pharmaceutical solutions for (HCQ)

The standard solution of drug from pharmacological material (4.5µg.ml^{-1}) was prepared by taking One tablet (0.3381 g/tablet , 0.272 g/tablet and 0.664 g/tablet) to be weighted and finely-grinded, and dissolved in10 mL ethanol and filtered. added 1 mL of (2,4-DNPHz) was obtain by adding and follow by 0.75 ml oxidized agent solution than 1 ml of drug solution and 0.5 ml of base solution in volumetric flask 10 mL and complete by deionized water to mark. the reaction which was completed to the optimum condition. The solution result was measurement at maximum wavelength against reagent blank which was treated similar.

2.7.2 Applications for (LPV)

Due to the lack of pharmaceutical preparations for lopinavir, five serum samples were collected from healthy people (males and females) by drawing 5 ml of venous blood through a medical syringe used only once and placing the blood in Gel tubes with tight lids, after 30 min these tubes were placed in Centrifuge for 5 min at 3000 rpm, after which serum was withdrawn by micropipette. 1 ml of serum was added to different concentrations of drug (2.5-12.5 µg.ml^{-1}) . with the optimum volume and concentration of each of the reagent, oxidizing agent and base solution, then the absorbance of the solution is measured.

2.8 Effect of Interferences

To be sure the method of permeability in the studies of pharmacological application ,was conducted to obtain the effect of foreign chemical materials found in pharmacological formula.

2.9 Continuous Variations (Job's) Method

To determine the stoichiometric ratio of the reactant substance, the Job's method of continuous variations was used[122]. The Master equal molar solution of HCQ and 2,4-DNPHz (7.5×10^{-3} M) and the different Master equal molar solution of LPV and 2,4-DNPHz (1.5×10^{-3} M) were produced for LPV. The Series of 10 mL portions of the master solution of drugs and 2,4-DNPHz were made up comprising different complementary proportions

(0.1:0.9-0.9:0.1, inclusive) in a volumetric flask of ten milliliters. The solution was further manipulated as described under the general recommended procedures. Than record the absorption to each drug in maximum wavelength.

2.10 Mole-Ratio Method

Volumetric flask (10 mL) ,was taken the constant volume of drugs , 1mL of drugs with a different volume of the reagent (0.25-2 mL) of master equal malar (7.5×10^{-4}M) solution of HCQ and 2,4-DNPHz were prepare. while LPV the concentration of drug and reagent were (5×10^{-4}M). The absorbance of solution was recorded at max wavelength of each drugs against blank solution prepared in the same way without drugs .

2.11 Calculation of Dissociation Degree and Stability of Product[123]

Depending on the results obtained from the determination of the ratio of drugs to reagent (2,4-DNPHz) and in the methods of job's and mole

ratios the degree of disintegration and stability of each drugs were calculated according to the following equations:

$$\alpha = \frac{Am - As}{Am} \quad \text{.....(2-1)}$$

Am: The absorption value of the product at the greatest absorption.

As: The absorption value of the product at the point of equivalence.

The disintegration equation can be written as follows:

$$ABn \rightleftharpoons A + nB$$

C	**0**	**0**
C(1-α)	**αC**	**nαC**

C: Total concentration of output expressed in unit (mol . L^{-1}) can be written resulting non-stability constant (K_{ins}) as follows:

$$K_{ins} = \frac{(\alpha C)(n\,\alpha\,C)^{n}}{C(1-\alpha)} \quad \text{.....(2- 2)}$$

n: the number of mole reagent.

As for stability constant (K_{sta}),it is expressed in relation to the following:

$$K_{sta} = \frac{1}{K_{ins}} \quad \text{.....(2-3)}$$

2.12.Thermodynamic parameters for the reaction of 2,4-DNPHz reagent with drugs

It is possible to calculate the thermodynamic parameters (free energy change [ΔG], entropy [ΔS], and enthalpy [ΔH]) by examining the impact of temperature[124].

(ΔG) With the use of the following equation, values can be calculated:

$\Delta G=-RT \ln k$ ……..(2-4)

Where:

ΔG: free energy change ($J.mol^{-1}$)

R: universal gas constant (8.314 $J.mol^{-1}.K^{-1}$)

keq : equilibrium constant

(keq) the following equation can be used to compute it at each temperature:

$$k_{eq}=\frac{Qe\ m}{Ce\ V} \text{ ……..(2- 5)}$$

(ΔH) determined using the Vant-Hoff-Arrhenius equation and the link between ln Keq and 1/T:

$\ln keq = (-\Delta H/RT) + constant$ ……..(2-6)

(ΔS) values are calculated from this equation:

$\Delta G = \Delta H - T\Delta S$ ……..(2-7)

2.13.Kinetic parameters for the reaction of 2,4-DNPHz reagent with drugs

The kinetic parameters (order and rate of reaction) can be determined by studying the effect of time.

Two kinetic models first order and second pseudo-order models—are used to fit experimental data:

1- First order equation[125]:

This equation is described by Lagergren as:

$\ln q_t = \ln q_e + k_1 t$ – – – –(2-8)

2- Pseudo second order equation[126]:

It is possible to express the pseudo-second order kinetics as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{qe}t \quad \text{– – – –} \quad \text{– – – –} \quad \text{(2-9)}$$

Where:

qt: the concentration at any time.

qe : the concentration at equilibrium.

k : rate constant.

t : time.

The following equation is used to compute the rate law for a second-order reaction:

$$\mathbf{h = k_2 [q_e]^2} \quad \text{– – –} \quad \text{– – – –} \quad \text{(2-10)}$$

2.14. The activation energy for the reaction of 2,4-DNPHz reagent with drugs

activation energy can be calculated using the arrhenius equation[127] (2-11) and then by drawing the relationship between 1/T and ln k according to the following equation:

$$\mathbf{Ln\ k = Ln\ A - \frac{Ea}{RT}} \quad \text{– – –} \quad \text{– – – –} \quad \text{(2-11)}$$

Where:

Ea : the energy of activation.

A : Frequency factor.

K : rate constant.

R : standard gas constant

Chapter Three
Results And Discussion

3.Results and Discussion

3.1 Absorption Spectra

The comparison of absorption spectrum of hydroxychloroquine with ethanol was reported where it have a maximum absorption peak (λmax) at 332nm for hydroxychloroquine. After performing the reaction between HCQ and 2,4-DNPHz, the product's absorption spectra was recorded in comparison to the reagent blank (Figure 3-1). It was found the product was azo dye colored exhibiting (λmax) at 620 nm while 2,4-DNPHz (λmax) at 359 nm. The λmax of azo dye was blue shifted,eliminating any potential interference. Therefor, the measurements were carried out at 620nm.

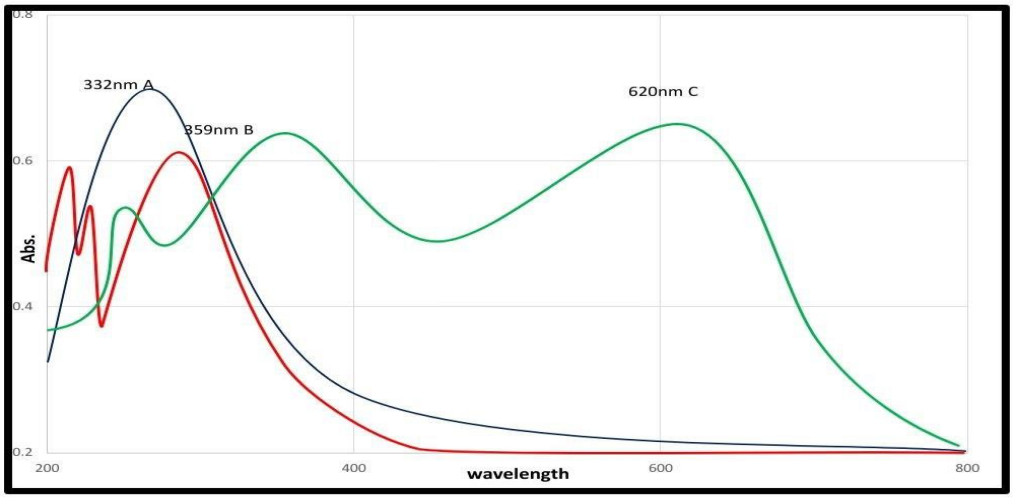


Figure (3-1) Absorption spectra of **(A)** HCQ (100 μg.ml^{-1}). **(B)** 2,4-DNPHz (0.005 M) **(C)** azo dye product of HCQ with 2,4-DNPHz.

Against ethanol, Lopinavire (LPV) was observed. demonstrates a maximum absorption peak (λmax) at 322nm, according to research on the drug lopinavire. After performing the reaction between LPV and 2,4-DNPHz, the product's absorption spectra was recorded in comparison to the reagent blank (Figure 3-2). The product was discovered to be colored, exhibiting (λmax) at 482nm, while the 2,4-DNPHz (λmax) was 359 nm.

The λmax of azo dye was yellowish green shifted,eliminating any potential interference. Therefor, the measurements were carried out at 482nm.

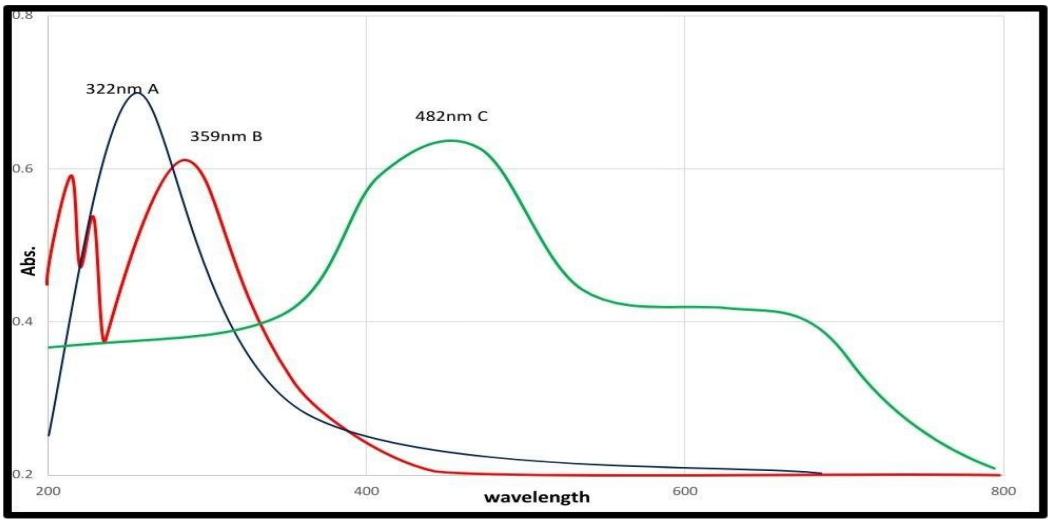


Figure (3-2) Absorption spectra of **(A)** LPV (100 μg.ml^{-1}). **(B)** 2,4-DNPHz (0.005 M) **(C)** azo dye product of LPV with 2,4-DNPHz.

3.2 Optimization of the Reaction Condition

3.2.1 .Effect of the type of oxidized factor

The study was carried out by adding 1 mL of 2,4-dinitro phenyl hydrazine solution (5× 10^{-4} M) to of various oxidizing agents (6 ×10^{-4} M) than added 1ml of drug solution and 0.5mLof sodium hydroxide solution.The results showed that potassium periodate solution gives a higher intensity for colored azo dye and highest absorption, than other oxidizing agents used as shown in table (3-1). so this oxidizing agent was chosen in subsequent experiments.

Table (3-1): Effect of oxidizing agent type on the reaction

Type of oxidizing factor	Absorption of blue azo dye (620nm)	Absorption of yellowish green azo dye(482nm)
2,4-DNPHz + KIO_4+drug+NaOH	0.422	0.513
2,4-DNPHz + KIO_3+drug+NaOH	0.312	0.169
2,4-DNPHz + $NaIO_4$+drug+NaOH	0.305	0.416

3.2.2 Effect of (2,4-DNPHz) Concentration

The analysis of (2,4-DNPHz) concentrations showed that the reagent (2,4-DNPHz) was necessary for the reaction.The maximum absorption obtained when concentration was (0.0075M) of the reagent for the hydroxychloroquine, while the maximum absorption of Lopinavir was obtained when concentration was (0.005M), as shown in Figure (3-3).

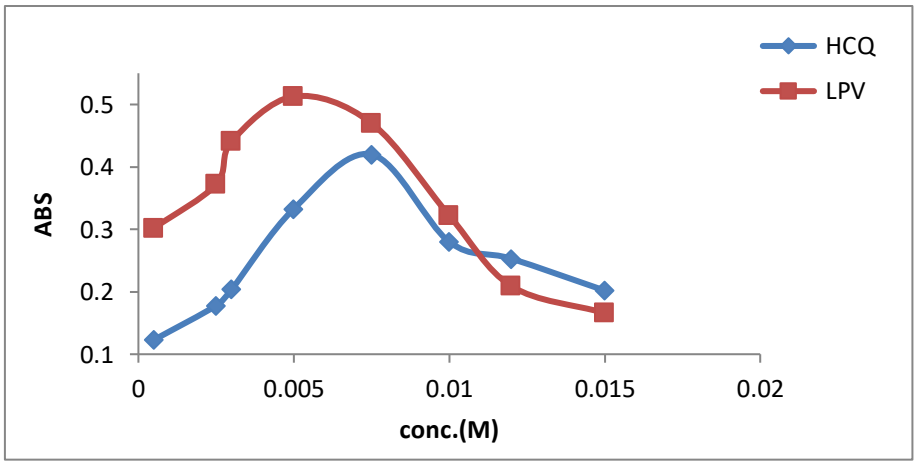


Figure (3- 3) Effect of 2,4-DNPHz concentration on the reaction with HCQ and LPV.

3.2.3 Effect of (H_2SO_4) volume

By using various volumes of concentrated H_2SO_4 after the optimal 2,4-DNPHz concentration, the influence of concentrated sulfuric acid

volume on the intensity of the ensuing absorption was seen in the current investigation. with a concentration of (2,4-DNPHz) was (0.0075M) for drug (HCQ) and (0.005M) for drug (LPV). For the drugs HCQ and LPV, the highest absorption intensity was found at volumes of (H_2SO_4) of (0.3 and 0.25)mL, respectively, while the larger volume of (H_2SO_4) with (0.45mL) had low impact on the absorption values, as shown in(figure 3-4).

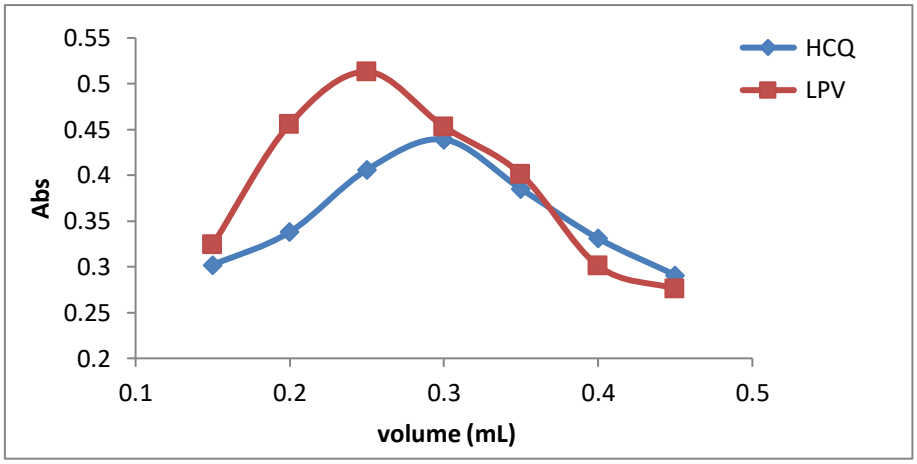


Figure (3- 4) Effect of volume (H_2SO_4) on absorption of HCQ and LPV with 2.4-DNPHz.

3.2.4 Effect of Reagent (2,4-DNPHz) Volume

In the current investigation, the influence of reagent volume was noted on the intensity of absorption caused by ingesting various amounts of 2,4-DNPHz after the best concentration of 2.4-DNPHz and the best volume of H_2SO_4, as demonstrated in with a volume of (2,4-DNPHz) were (1mL and 1.25mL) for drugs (HCQ and LPV) respectively (figure 3- 5). the greater volume of (2,4-DNPHz) (2.5mL) had low impact on the absorption values.

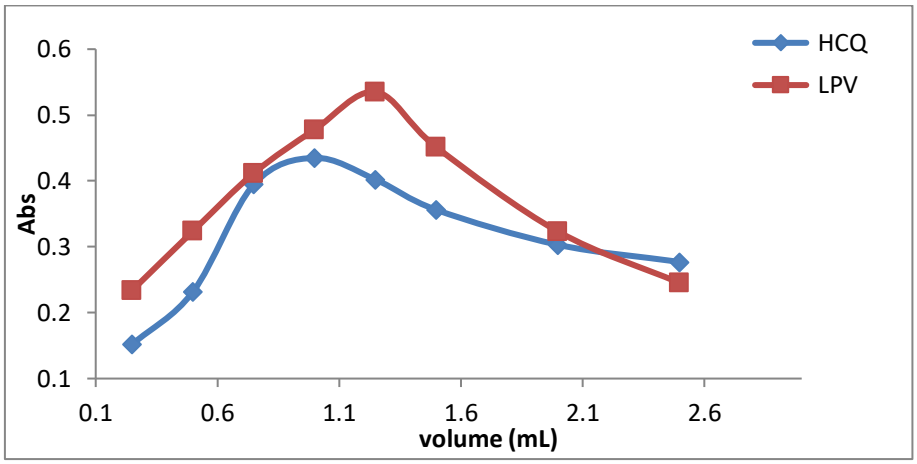
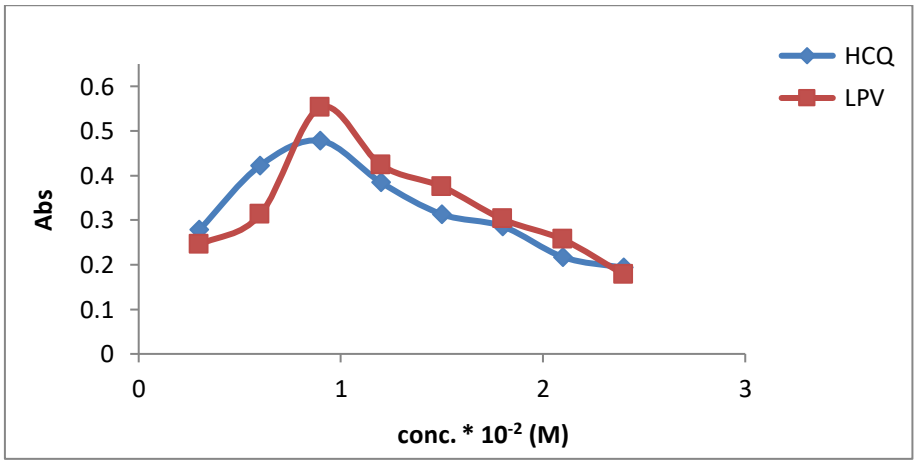


Figure (3- 5) Effect of volume (2,4-DNPHz) on absorption of HCQ and LPV with 2.4-DNPHz.

3.2.5 Effect of potassium periodate concentration (KIO_4)

Following the discovery of the optimal 2,4-DNPHz concentration and volume, the study examined the impact of oxidizing agent concentration by employing various KIO_4 concentrations and evaluating the absorption intensity for each solution using the wavelength of each drug. The greatest amount of absorption intensity was found. the optimal KIO_4 concentration for both drugs was (0.009 M). and the results are indicated that the higher concentration of the oxidizing agent (0.024M) had low impact on the absorption values (figure 3-6).



Figure(3- 6) Effect of concentration (KIO_4) on absorption of HCQ and LPV with 2.4-DNPHz.

3.2.6 Effect of oxidizing factor volume (KIO_4)

Following the discovery of the optimal KIO_4 concentration, the study examined the impact of KIO_4 quantity using various volumes of oxidizing factor by evaluating the intensity of absorption of each mixture of solution using the wavelength of each drug. It was found that the absorption intensity was maximum at the optimal volume of KIO_4 was (0.75 mL) of hydroxychloroquine and (1 mL) of lopinavir, while (2.5 mL) of oxidizing agent had low effect on the absorption values, as demonstrated in (figure3-7).

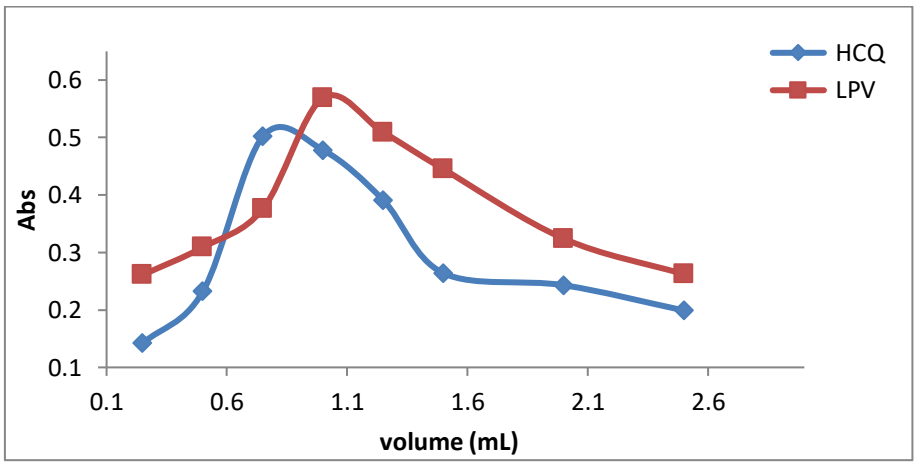


Figure (3- 7) Effect of volume (KIO_4) on absorption of HCQ and LPV with 2.4-DNPHz.

3.2.7 Effect of sodium hydroxide concentration (NaOH)

After knowing the best conditions in previous experiences were study effect of base solution concentration on the reaction . The study was investigated using different concentrations of sodium hydroxide, as shown in Figure (3- 8), by determining each mixture's absorption intensity using the wavelengths of each drug. It was found the best concentration of NaOH was (0.625M) of hydroxychloroquine and (1M) of Lopinavir, and the higher concentration of sodium hydroxide (1.25M) had low effect on the absorption values.

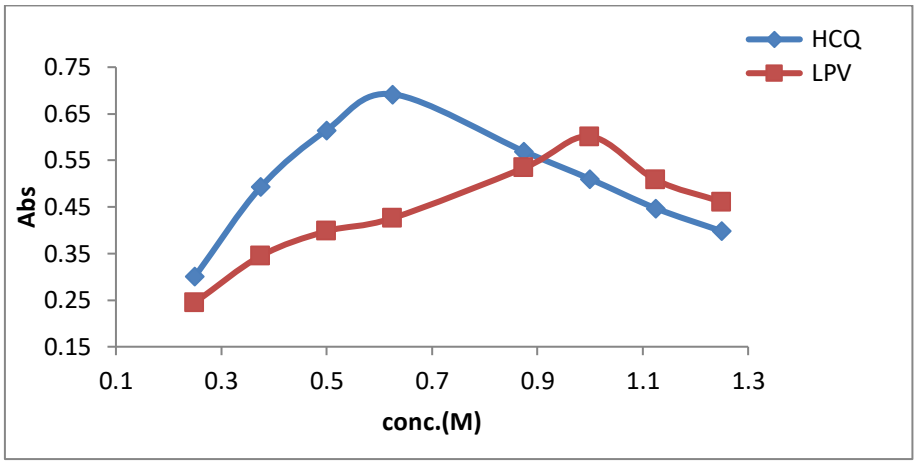


Figure (3- 8) Effect concentration of (NaOH) on absorption of HCQ and LPV with 2.4-DNPHz.

3.2.8 Effect of sodium hydroxide Volume (NaOH)

Additionally, the impact of the drugs' sodium hydroxide amount (HCQ and LPV) on the azo dye product's absorbance was investigated. by using different amounts of base solution (NaOH). It was found The highest absorption intensity is depicted in (figure 3-9).The best volume of NaOH was (0.5 mL) for hydroxychloroquine and (1.5 mL) for Lopinavir . and the higher volume of base solution (2mL) had low effect on the absorption values.

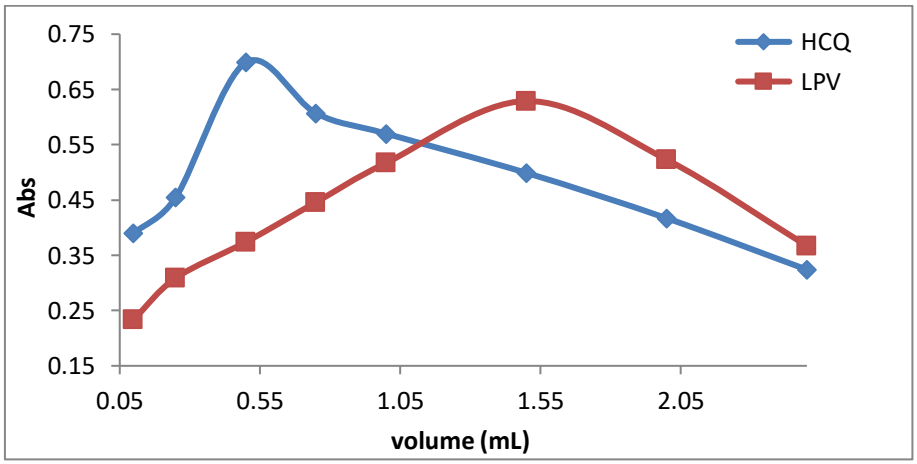


Figure (3- 9) Effect volume of (NaOH) on absorption of HCQ and LPV with 2.4-DNPHz.

3.2.9 Effect of Volume drugs

Following the discovery of the optimal volume and concentration of 2,4-DNPHz, the oxidized factor, and the base, the study examined the effects of amount for drugs(HCQ and LPV), evaluating the absorption intensity of each mixture of azo dye solution using the wavelength of each drug. The greatest amount of absorption was found, at the optimal volume of HCQ was (1.25mL) and (1mL) of LPV, as well as the larger volume of drugs at (2.5mL), had low effect on the absorption values, as indicated in (figure3-10).

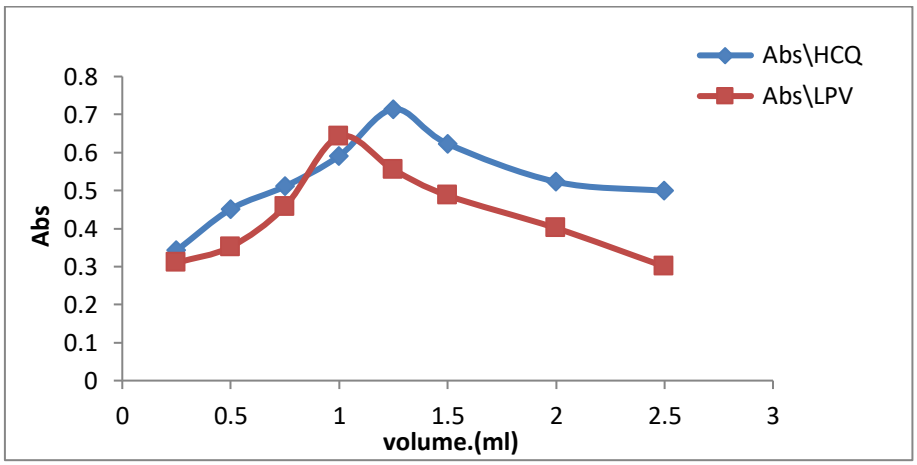


Figure (3- 10) Effect volume of (HCQ and LPV) on the reaction with 2,4-DNPHz.

3.2.10 Effect of Reaction Time

ln order to investigate how the time effects on the stability of a mixture of 2,4-DNPHz and KIO_4 with drugs in a base medium. The reaction's absorbance was assessed at a different time as shown in (figure 3-11). The azo dye product's absorbance was measured after standing for various lengths of time under other ideal conditions. The mixture's best reaction times was identified based on the high absorbance at 10 minutes for each drugs.

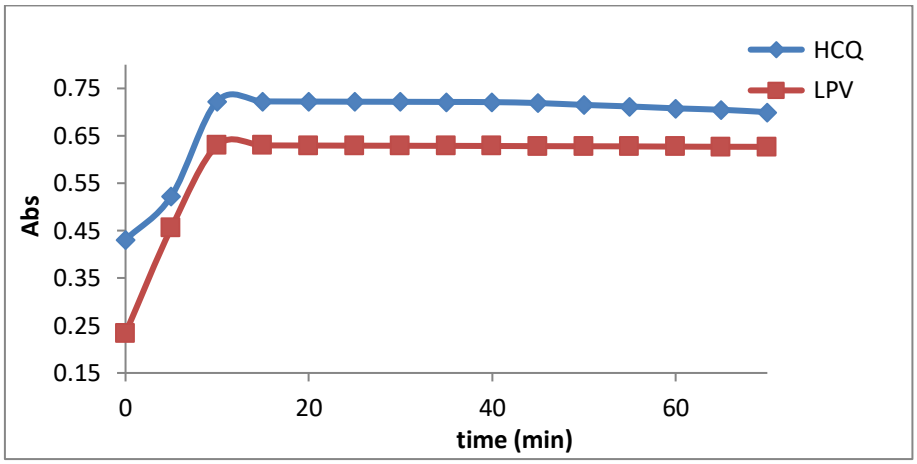


Figure (3- 11) Effect of time for reaction HCQ and LPV with 2,4-DNPHz.

3.2.11 Effect of Temperature

The effect of temperature on the stability of 2,4-DNPHz and drugs reaction was detected in (figure 3-12) which reveals the best temperature from the reaction was (25°C) for HCQ and (35°C) for LPV where they offered the highest absorption.

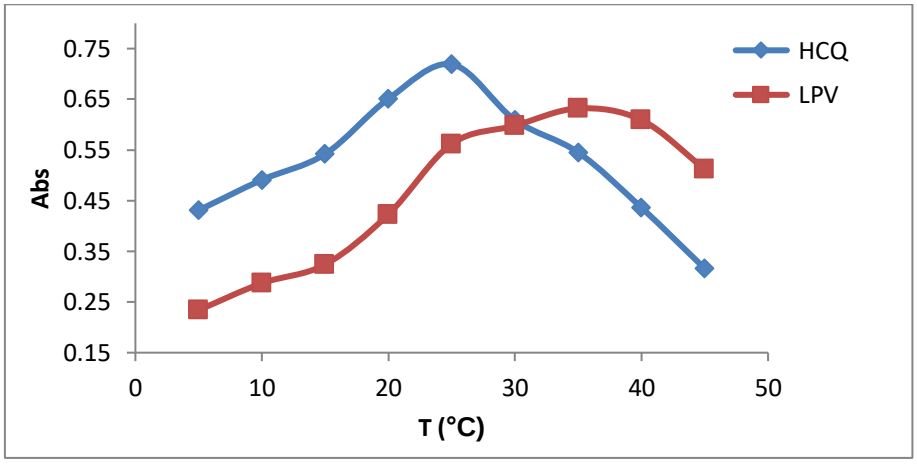


Figure (3- 12) Effect of Temperature on the reaction of (HCQ and LPV) with 2,4-DNPHz.

3.2.12 Effect of Addition Order

The impact of the addition material's sequence on the reaction's absorption was examined based on four arrangements to ensure the best outcomes in tables (3- 2 and 3-3), to observe the next common material

that provided a larger absorption due to the occurrence of a chemical reaction.

Table(3-2) : Order of addition drug (HCQ).

NO.	Addition	Abs
1	2,4-DNPHZ + KIO_4+ Drug + NaOH	0.725
2	Drug + NaOH + 2,4-DNPHZ + KIO_4	0.287
3	2,4-DNPHZ + Drug + KIO_4 + NaOH	0.415
4	Drug+2,4-DNPHZ + KIO_4 + NaOH	0.502

Table (3-3) : Order of addition drug (LPV).

NO.	Addition	Abs
1	2,4-DNPHZ + KIO_4+Drug + NaOH	0.634
2	Drug + NaOH + 2,4-DNPHZ + KIO_4	0.509
3	2,4-DNPHZ + Drug + KIO_4 + NaOH	0.239
4	Drug+2,4-DNPHZ + KIO_4 + NaOH	0.326

3.3 Construction Calibration Curve

3.3.1 Calibration Curve for (HCQ) with 2,4-DNPHz

The standard calibration curve for the determination of HCQ is shown in Figure (3-13). It comes out that under ideal circumstances at the maximum wavelength of (620) nm and the correlation coefficient (R=0.9978), it obeys Beer's Law between the range of (2.5 to 22.5)$g.ml^{-1}$. As a result, the molar absorptive (Ɛ) value was (1048.28) $L.mol^{-1}.cm^{-1}$.

The detection limit was (0.243)µg.mL^{-1}, the quantitation limit was (0.738) µg.mL^{-1}, %Erel was (%-0.012) and the value of %R.S.D was (%1.44). The coefficient of the specific absorption (a) of the following relationship, which was (0.3204) g.mL^{-1}, was used to compute the sandall's sensitivity. This analytical approach is preferred for (HCQ) measurement at low concentrations due to its sensitivity. As shown in table (3-4).

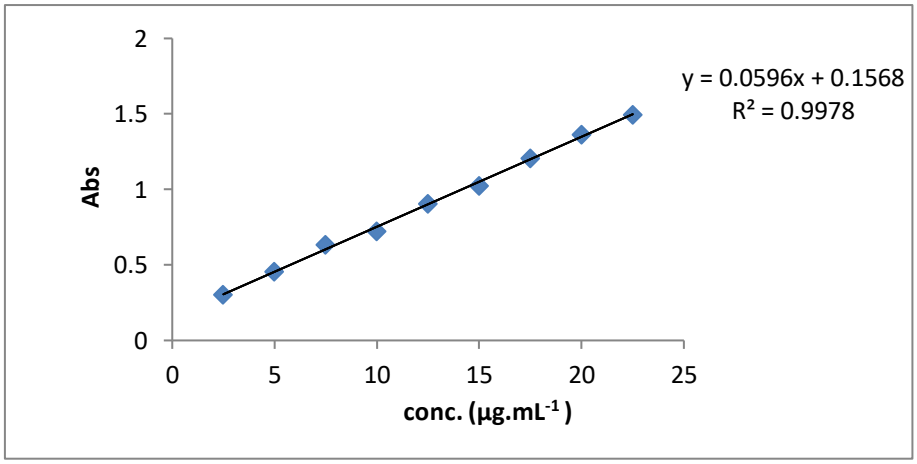


Figure (3- 13) Calibration Curve (HCQ) with 2,4-DNPHz

3.3.2 Calibration Curve for (LPV) with 2,4-DNPHz

Figure (3- 14) shows the standard calibration curve for the estimation of LPV. It turns out that it obeys the Beer's Law between the range of (2.5 to 22.5) µg.ml^{-1} under the best conditions at the maximum wavelength of (482) nm and the correlation coefficient(R=0.9967). therefore, the value of the molar absorptive (Ɛ) equals to (3143.75) L.mol^{-1} .cm $^{-1}$. The detection limit was (0.933)µg.mL^{-1}, the quantitation limit was (2.82) µg.mL^{-1}, %Erel was (%-0.2525) and the value of %R.S.D was (%1.12). The sandall's sensitivity was calculated by calculating the coefficient of the specific absorption (a) of the following relationship which was (0.20002)µg. mL^{-1} . sensitivity make this analytical method

preferred for (LPV) determination at low concentrations. As shown in table (3-4).

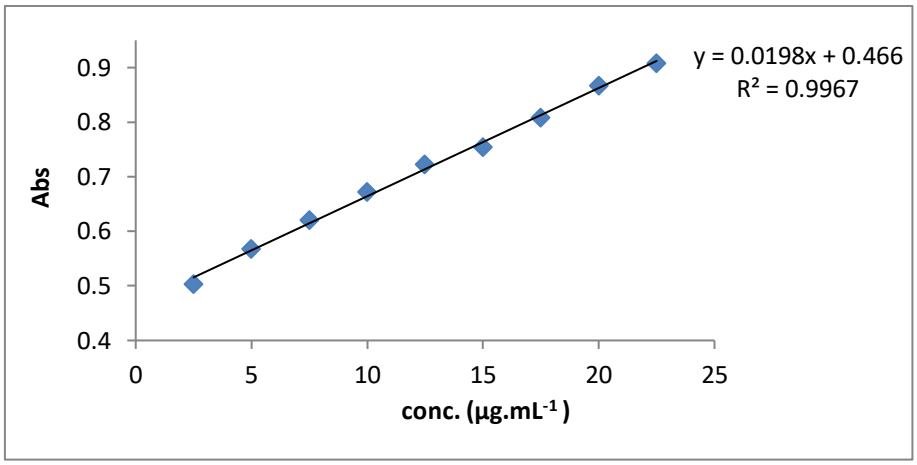


Figure (3- 14) Calibration Curve (LPV) with 2,4-DNPHz

Table(3-4): Analytic parameter for (HCQ) and (LPV) determination.

Parameter value	Value\HCQ	Value\LPV
Beer's law limit (µg.ml^{-1})	(2.5-22.5)	(2.5-22.5)
Molar absorptivity (L.mol^{-1} .cm^{-1})	1048.28	3143.75
Sandell's sensitivity[128] (µg.cm^{-2})	0.3204	0.20002
Detection limit[129] (µg.ml^{-1})	0.243	0.933
Quantitation limit[130] (µg.ml^{-1})	0.738	2.82
Determination coefficient(R^2)	0.9978	0.9967
Slope (b)	0.0596	0.0198
Intercept (a)	0.1568	0.466
%Erel	%-0.012	%-0.2525
%R.S.D	%1.44	%1.12

3.4 Estimation the Composition of the Product

3.4.1 Hydroxychloroquine

This study used the stoichometric methods (continuous variation method) (Job's method) and the mole ratio method to evaluate the amount of HCQ with(2,4-DNPHz) in the alkaline medium to the formation of the azo dye product under the optimal condition at a maximum wavelength (620 nm) as in figure (3- 14) and (3-15) whene the ratio of drug to the reagent was (1:1).

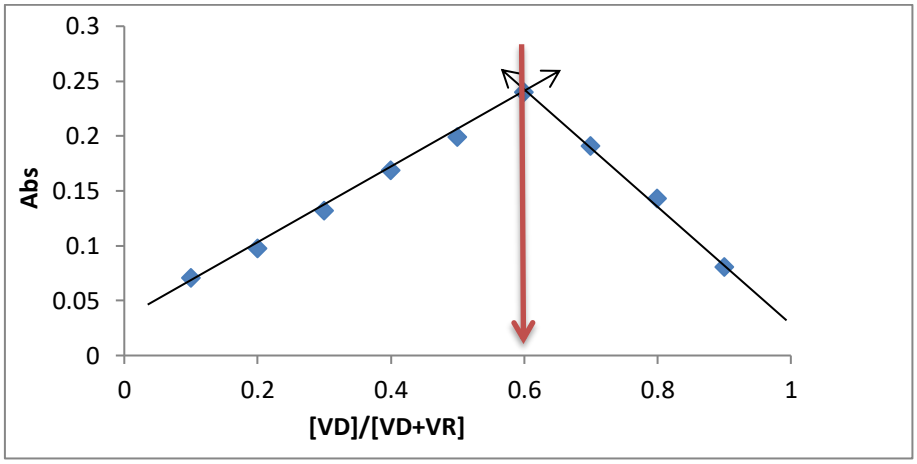


Figure (3- 15) the continuous variation (Job's method) for HCQ with 2,4-DNPHz.

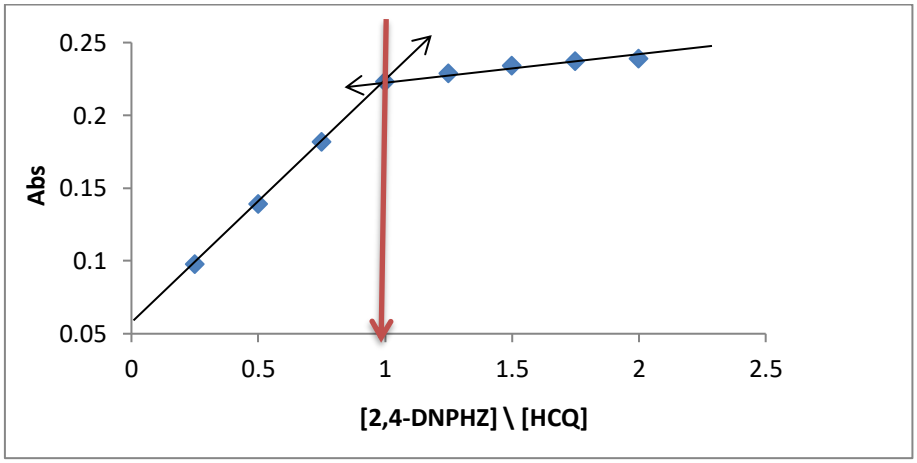
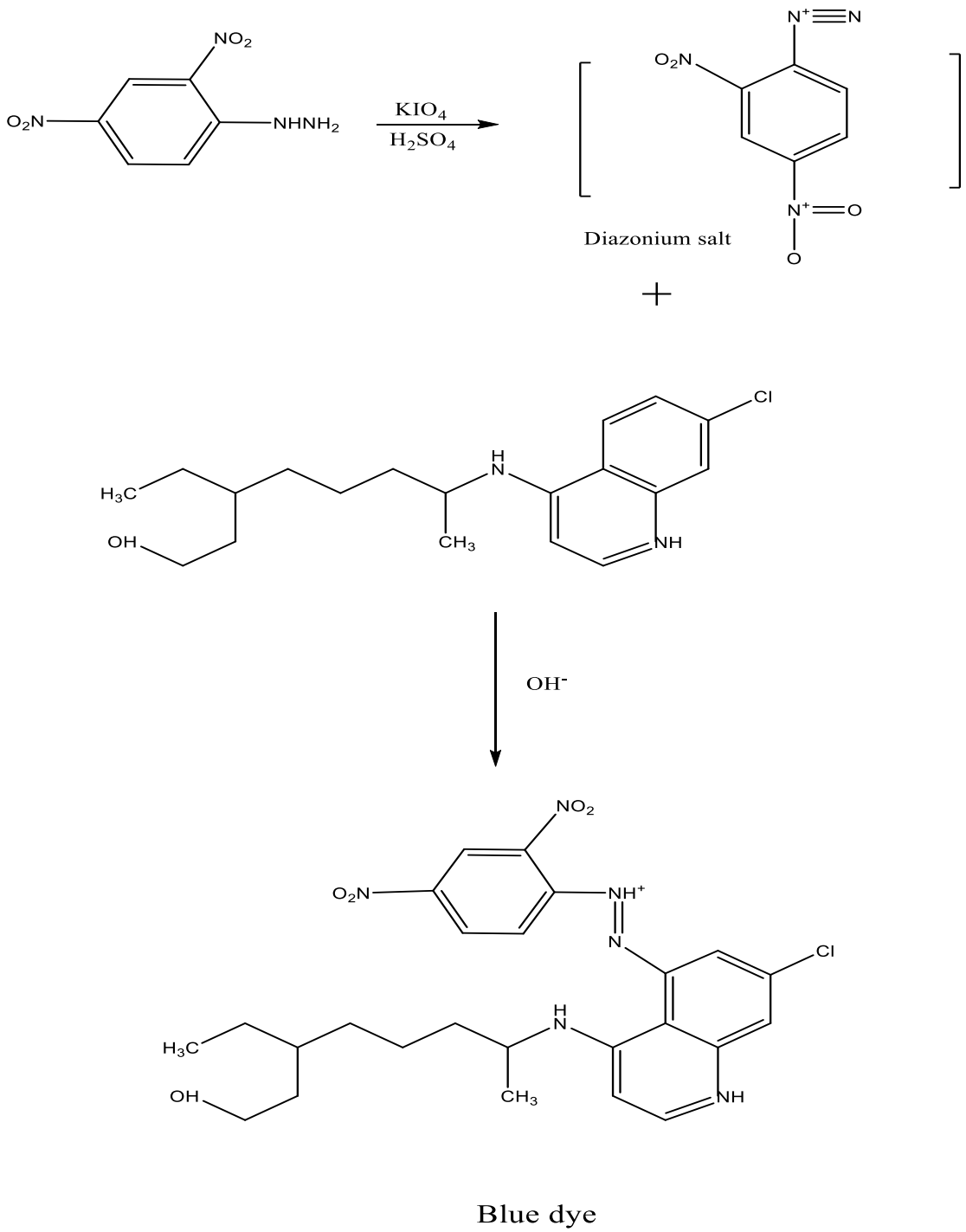


Figure (3- 16) mole ratio for (HCQ) with 2,4-DNPHz.

3.4.2 Suggest reaction of 2,4-DNPHz in presence of KIO_4 with HCQ



Scheme (3-1) Suggested structure for azo dye product of HCQ with 2,4-DNPHz**.**

3.4.3 Lopinavir

The stoichiometric ratio for the formation of the reaction between the reagent (2,4-DNPHz) and (LPV) under the ideal conditions at a wavelength (482nm) was evaluated using the Job's method (continuous variation) and the mole ratio, and the ratio of drug to reagent was (1:1) as noted in figure(3-17) and (3-18).

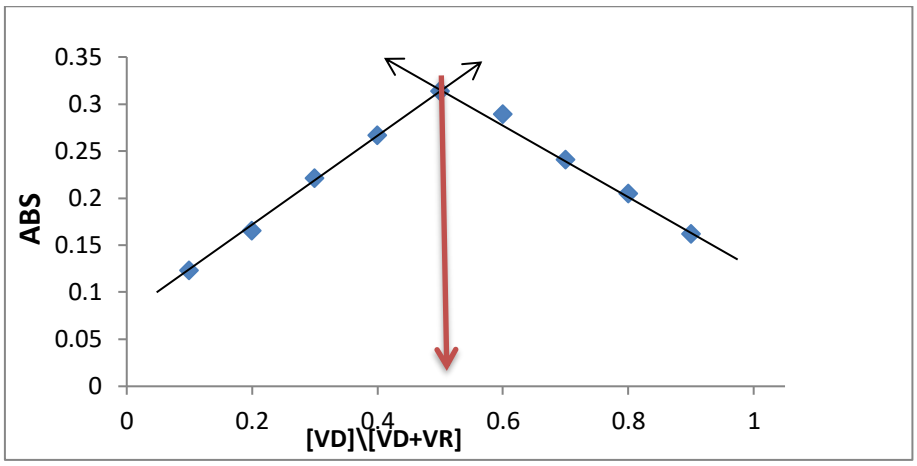


Figure (3- 17) the continuous variation (Job's method) for (LPV) with 2,4-DNPHz.

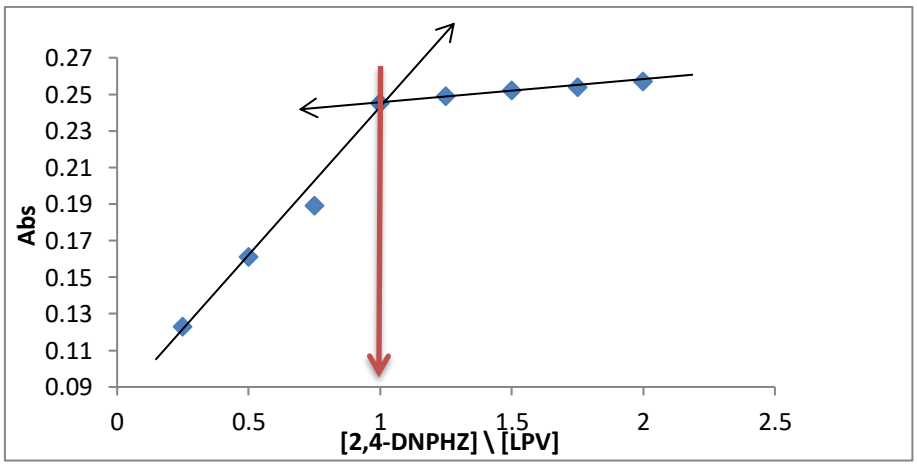
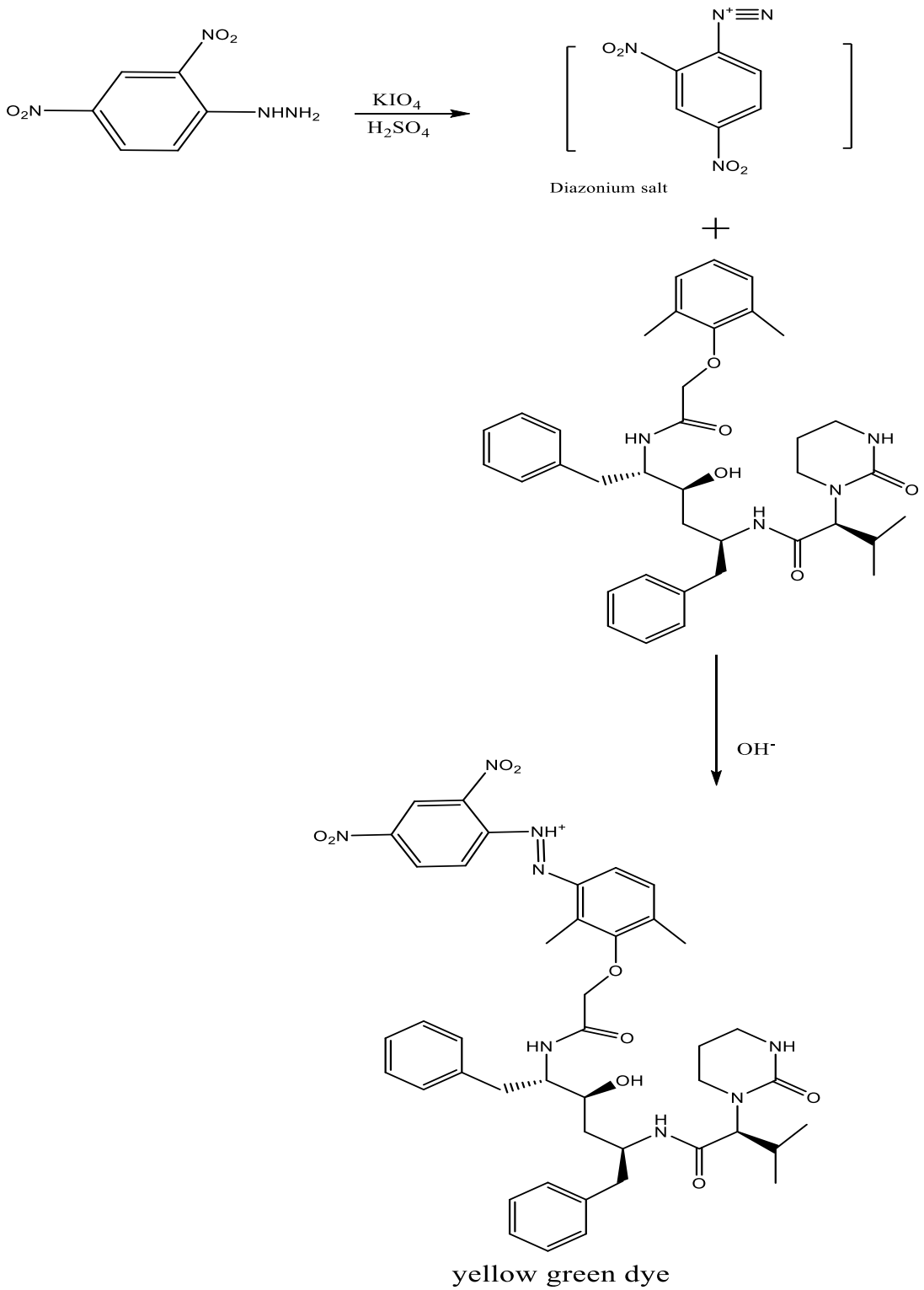


Figure (3- 18) mole ratio for (LPV) with 2,4-DNPHz.

3.4.4 Suggest reaction of 2,4-DNPHz in presence of KIO_4 with LPV



yellow green dye

Scheme (3-2) Suggested structure for azo dye product of LPV with 2,4-DNPHz.

3.5 Calculation of Dissociation Degree and Stability of Product

To obtain the stability of the formed product and the possibility of studying by spectral methods ,the degree of the disintegration and constant stability was calculated by the equations (2-1) (2-2) and (2-3) . It was shown after the application of these equations that the degree of the disintegration was (0.066), the value of the stability constant was ($7.52x10^6$)L. mol^{-1} this for hydroxylchloroquinen, while the degree of disintegration and the stable stability of the lopinavir were (0.046) and ($2.818x10^6$) L. mol^{-1}.Therefore, the high value of the stability constant indicates that the high stability of the azo dye product.

3.6 Accuracy and Precision[123]

First, we calculated using five replicates at the concentration from each of the two drugs mixtures (5 $g.ml^{-1}$) to verify the accuracy and precision of the proposed method (HCQ and LPV) . Tables (3-5) and (3-6) for each drugs (HCQ and LPV with 2,4-DNPHz reagent) show the calculation of %R.S.D and the its values were equal to (1.288%) and (0.248%) .Those drugs determined by this method are highly precise.

Table(3-5): The value of (%R.S.D) for the studied method to determining drug (HCQ) with 2,4-DNPHz.

X	$X-X^-$	$(X-X^-)^2$
0.450	-0.008	6.4×10^{-5}
0.466	0.008	6.4×10^{-5}
0.460	0.002	4×10^{-6}
0.459	0.001	1×10^{-6}
0.456	-0.002	4×10^{-6}
$\Sigma X=2.291$		$\Sigma(X-X^-)^2=1.4\times10^{-4}$

$$\mathbf{X^{-}=\frac{\Sigma X}{n}=\frac{2.291}{5}=0.458}$$

$$\mathbf{S.D=\sqrt{\frac{\Sigma(X-X^{-})^2}{n-1}}=\sqrt{\frac{1.4\times10^{-4}}{5-1}}=5.9\times10^{-3}}$$

$$\mathbf{\%R.S.D=\frac{S.D}{X^{-}}\times100=\frac{5.9\times10^{-3}}{0.458}\times100=1.288}$$

Table(3-6): The value of (%R.S.D) for the studied method to determine drug (LPV) with 2,4-DNPHz.

X	$X-X^{-}$	$(X-X^{-})^2$
0.568	0	0
0.566	-0.002	4×10^{-6}
0.572	0.004	1.6×10^{-5}
0.570	0.002	4×10^{-6}
0.564	0.004	1.6×10^{-4}
ΣX=2.84		$\Sigma(X-X^{-})^2=4\times10^{-5}$

$$\mathbf{X^{-}=\frac{\Sigma X}{n}=\frac{2.84}{5}=0.568}$$

$$\mathbf{S.D=\sqrt{\frac{\Sigma(X-X^{-})^2}{n-1}}=\sqrt{\frac{4\times10^{-5}}{5-1}}=3.162\times10^{-3}}$$

$$\mathbf{\%R.S.D=\frac{S.D}{X^{-}}\times100=\frac{3.162\times10^{-3}}{0.568}\times100=\%0.557}$$

Table (3-7): Relative errors and recovery as parameters expressing accuracy of methods to determining (HCQ and LPV) with 2,4-DNPHz

Values	HCQ	LPV
wavelength(nm)	620	482
Conc. (µg.ml-1)	5	5
$\bar{X}$	0.456	0.568
%R.S.D	1.288%	0.557%
%Erel	0.334%	0.0303%
D.L(µg.ml-1)	0.326	0.527
%Recovery	100.33%	97.08%

3.7 Effect of interferences:

The purpose of this study is to demonstrate how certain substances included in pharmaceuticals formulation can affect how well they are absorbed. The realest no effect of the absorption value by material interference because this material that not content of carbonyl groups and (2,4-DNPHz) specific to compounds containing carbonyl group and aromatic compounds Table (3-8 and 3-9)

Table(3-8): Effect of interferences on the determination of HCQ

interferences	Abs.
Magnesium stearate	**0**
Starch	**0.002**
Polyethylene glycol	**0**
Dibasic calcium phosphate	**0**
Polysorbate	**0.001**
Hydroxypropyl methylcellulose	**0.006**

Table(3-9): Effect of interferences on the determination of LPV.

interferences	Abs.
Glycerin	**0**
Oleic acid	**-0.001**
Polyoxyl 35 castor oil	**0**
Propylene glycol	**0**
Sorbitol	**0**

3.8.Applications

3.8.1 Determination of (HCQ) in Pharmacological Formulation:

The determination of HCQ (the tablet) in the pharmacological formulation by spectrophotometric method with 2,4-DNPHz . as shown in paragraph (2.7.1), the results in table (3-10) indicate that the method was satisfactery .

Table(3-10): Analytical applications (HCQ) by 2,4-DNPHz.

Sample	Conc. mg/mL		%Error	%Recovery	%R.S.D
	Present	Found			
Quenil tablets (France)	20	19.98	0.1	100.01	0.381%
Quinoric tablets (British)	20	20.18	-0.9	99.1	0.455%
Dolquine tablets (Spain)	20	20.406	-1.6	98.4	0.663%

3.8.2 Analysis of recovery of (LPV) from serum samples

Serum samples were prepared to analyze the recovery of the lopinavir in these studied methods, where serum samples were contain different amount respectively of pure drug as in table (3-11). High accuracy and recovery that have been obtained, indicating that the studied method of restoring lupinavir can be successfully applied in serum samples.

Table (3-11): The recovery of lopinavir in serum

Amount added (µg/ml)	Amount found (µg/ml)	Recovery (%)	%Erel	%R.S.D
2.5	2	100.2	0.2	1.392%
5	4.9	100.02	0.02	0.844%
7.5	7.67	99.98	-0.02	0.564%
10	10.11	99.99	-0.01	0.638%

3.9 Thermodynamic parameters for determination of HCQ and LPV with 2,4-DNPHz.

The thermodynamic variables (free energy change,ΔG, enthalpy change,ΔH, and entropy change,ΔS) were calculated by studying the effect of temperature. Enthalpy (ΔH) was calculated by drawing Vant – Hoff Arrhenius equation for HCQ and LPV , as shown in figure (3-19) and (3-20).

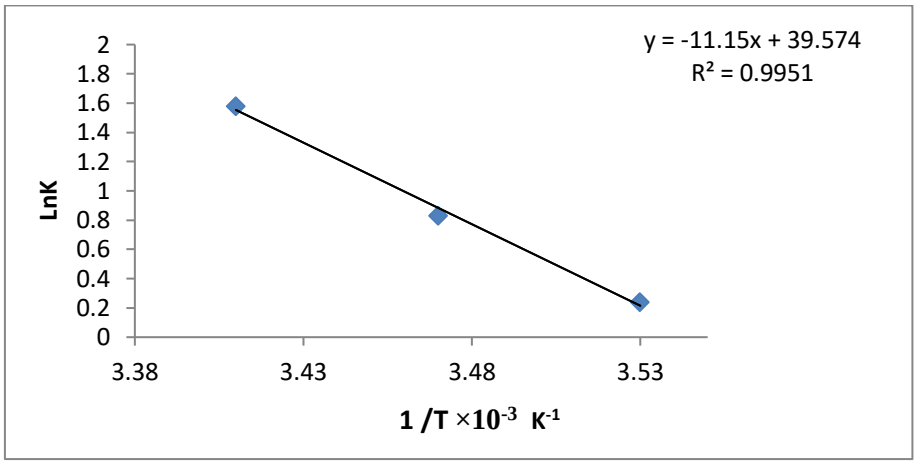
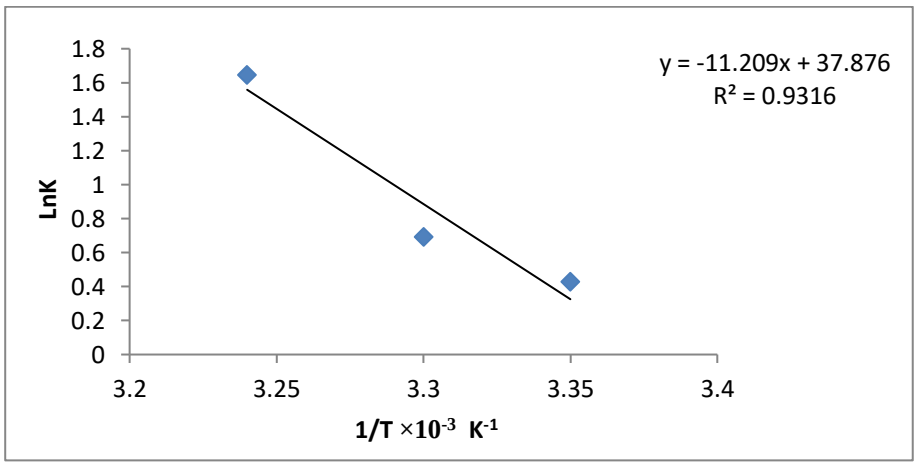


Figure (3-19) the relationship between ln Keq and 1/T for HCQ with 2,4-DNPHz.



Figure(3-20) the relationship between ln Keq and 1/T for LPV with 2,4-DNPHz.

The results of thermodynamic parameters are shown in table(3-12), the positive values of (ΔH) indicate that the process of HCQ and LPV is a endothermic process, This indicates that increased temperature reduces absorption of (HCQ and LPV). Negative values of (ΔG) denote a spontaneous process, and negative values of (ΔS) denote a regular system. [131] .

Table (3-12): Thermodynamic parameters for the reaction 2,4-DNPHz with (HCQ and LPV)

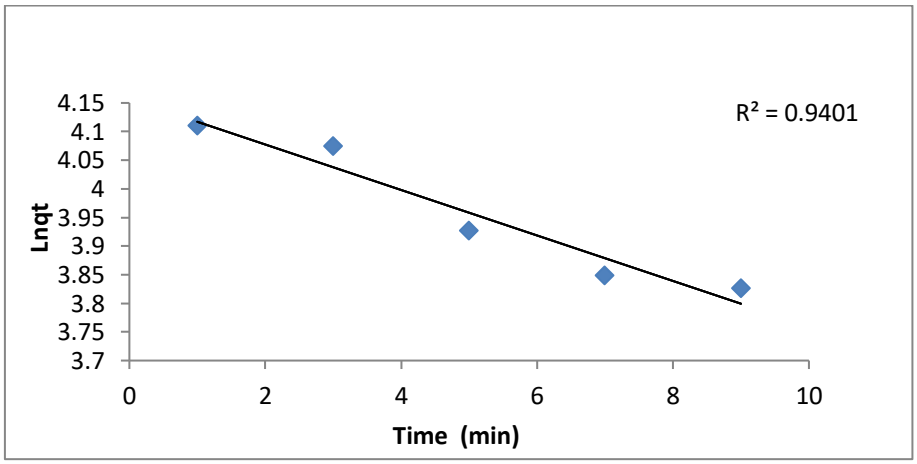
	T (K)	ΔG (kJ/mol.K)	ΔH (kJ/mol.K)	ΔS (J/mol)
HCQ	288	-0.56939	0.0927	-0.5694
	293	-1.98738		-1.9874
	298	-3.84888		-3.8489
LPV	298	-1.03147	0.0932	-3.91
	303	-1.68851		-6.08
	308	-4.0787		-14

3.10 Kinetic parameters of drugs (HCQ and LPV) with 2,4-DNPHz

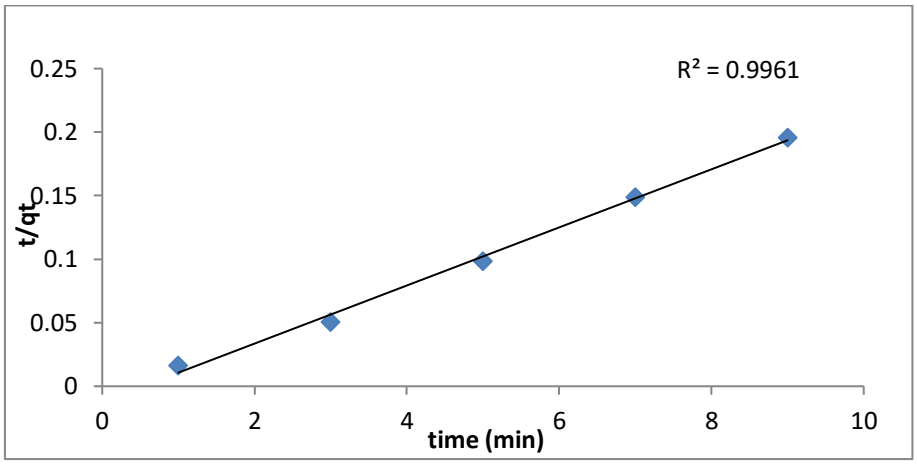
By examining the effects of time, to identify the kinetic parameters (order and rate of reaction), we used two distinct models to calculate the order of reaction: first order (eq.2-8) and pseudo second order (eq.2-9) kinetics, as shown in figure (3-21),(3-22),(3-23) and (3-24).

Table(3-13): Application of first and pseudo-second order kinetic model on the reaction data

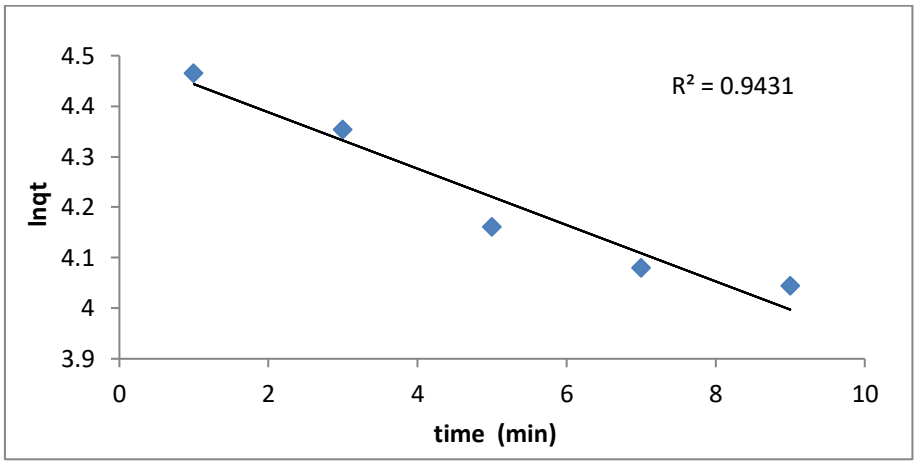
Time (min)	HCQ			LPV		
	qt (mg/g)	lnqt	t/qt	qt (mg/g)	lnqt	t/qt
1	61.04	4.115	0.0164	93.9	4.54	0.0106
3	58.8	4.075	0.0509	90.8	4.509	0.033
5	50.9	3.928	0.0984	73.2	4.293	0.068
7	46.9	3.849	0.1491	68.1	4.222	0.1026
9	45.8	3.827	0.1959	67.6	4.214	0.1329
10	qe (mg/g) = 30			qe (mg/g) = 20.7		



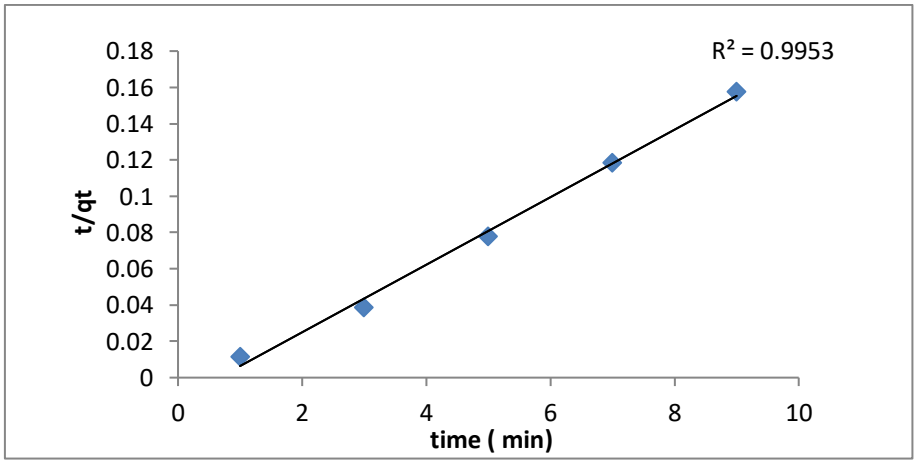
Figure(3-21) First kinetic models of HCQ with 2,4-DNPHz.



Figure(3-22) pseudo Second kinetic models of HCQ with 2,4-DNPHz.



Figure(3-23) First kinetic models of LPV with 2,4-DNPHz.



Figure(3-24) pseudo Second kinetic models of LPV with 2,4-DNPHz.

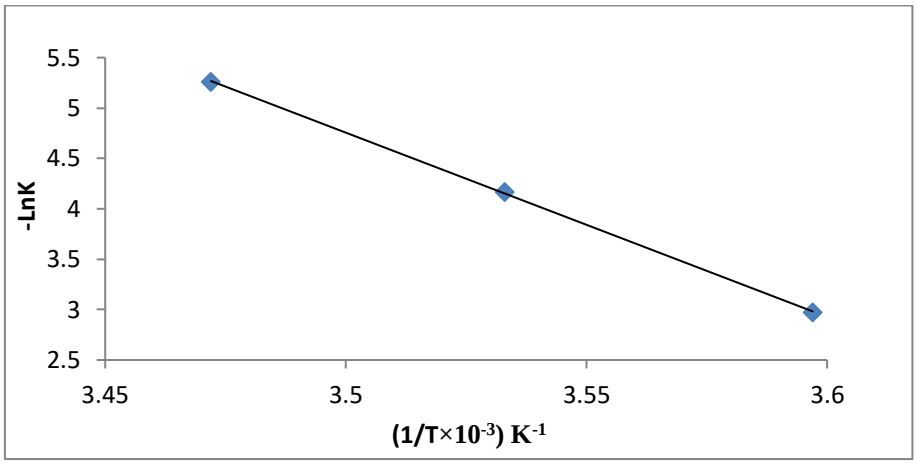
The results of the kinetic parameters are shown in table (3-13), According to the first-order equation, the correlation coefficient (R^2) was known, and the deviation of the values from the straight line was noted, which indicates the non-compliance of this process to the first order. Therefore, the pseudo second-order equation was applied to get the straight line, and thus we were able to extract the values of the rate constant of the second order(K_2)and the values of(R^2), The rate of reaction was calculated using equation (2-10).

Table(3-11): kinetic parameters for the reaction 2,4-DNPHz with (HCQ and LPV)

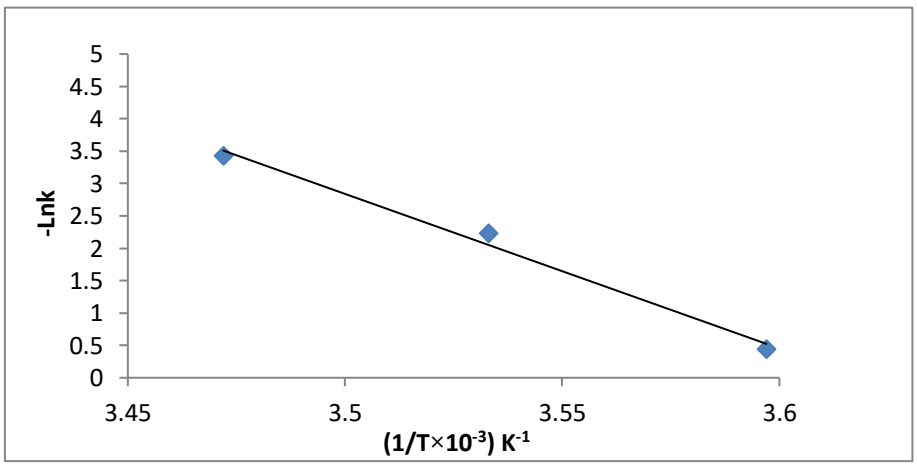
drugs	first kinetic model		Pseudo-Second kinetic model		
	k (min^{-1})	R^2	k ($g.mg^{-1}.min^{-1}$)	h ($mg.g^{-1}.min^{-1}$)	R^2
HCQ	0.0397	0.9401	0.0229	20.61	0.9961
LPV	0.0558	0.9431	0.0186	7.97	0.9953

3.11 The activation energy for reaction of the (HCQ and LPV) with 2,4-DNPHz

The activation energy have been estimating by examining the impact of temperature and the rate constant and from drawing the equation (2-11) as shown in figure (3-27) and (3-28) for both HCQ and LPV, the results showed that the activation energy is equal to (143.6J/mol) for HCQ and (203.7J/mol) for LPV. Low values of activation energy indicate an increase in the rate of occurrence of the reaction at a certain temperature[132].



Figure(3-25) the relationship between ln K and 1/T for HCQ with 2,4-DNPHz.



Figure(3-26) the relationship between ln K and 1/T for LPV with 2,4-DNPHz.

Conclusions And Recommendations

Conclusions:

1-The analytical method was quick, easy and inexpensive for the determination of drugs (HCQ and LPV) spectrophotometrically also it has the high accuracy and precision.

2-The best oxidizing agent was KIO_4 which gives high absorbance for the drug (HCQ and LPV) with 2,4-DNPHz reagent in base medium.

3-The method obeys Bear's lambert law in linear of range (2.5-22.5µg.mL^{-1}) with high sensitivity. and good limit of detection and limit of quantification.

4-It was a selective method for determining of (HCQ and LPV) with 2,4-DNPHz in presence of different interference

5- This method has stable product (azo dye) after a period of time which is to min for two anti-viral drugs.

6- The stoichiometric for the two anti-viral drugs (azo dye) is 1:1.

7-This method was thermodynamically spontaneous, endothermic and was more regular for two anti-viral drug (HCQ and LPV).

8-The reaction of 2,4-DNPHz with two anti-viral drug in presence of KIO_4 and NaOH is the pseudo second order kinetically.

Recommendation:

1- The determination of anti-viral drugs (HCQ and LPV) by different analytical technique such as flow injection and cyclic voltammetry.

2- The determination of different drug by the reaction with 2,4-DNPHz to form shift base or azo dye products.

3-Estimating of anti-viral drugs (HCQ and LPV) by other orange reagent, folien reagent and others.

4-Using this method for studying the concentration of (HCQ and LPV) drugs in a number of Pharmaceuticals formulation and body fluid.

5-Studying the biological activity of compound can prepared by azo dye.

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الخلاصة

تضمنت هذه الدراسة ايجاد طريقة طيفية سهلة و سريعة وغير مكلفة لتقدير (هيدروكسي كلوروكوين ولوبينافير) بصورة نقية وفي المركبات الصيدلانية من خلال تفاعل الكاشف (٢، ٤- ثنائي نايترو فينيل هيدرازين) بوجود العامل المؤكسد بيرايودات البوتاسيوم مع الدواء في الوسط القاعدي .

وكشفت النتائج عن امتصاص عال للأدوية (HCQ وLPV) عند الأطوال الموجية (332nm و 322nm) على التوالي ، في حين أن أعلى امتصاص للكاشف -2,4-DNPHz) عند الأطوال الموجية (359nm). علاوة على ذلك ، كانت أعلى نسبة امتصاص لمنتج صبغة الآزو عند (620nm) و(482nm) على التوالي

أجريت هذه الدراسة لمعرفة أن الظروف المثلى تم تجربتها لتقدير عقارين. وقد تبين أن كاشف التركيز الأمثل للدواء (HCQ) و (LPV) كان يساوي (0.0075M) ، و (0.005M) على التوالي. في حين أن الكميات المثلى للكاشف كانت(1mL) و (1.25mL) على التوالي. وكان الحجم الأمثل للعامل المؤكسد (0.75mL) لكل دواء. في حين أن الحجم الأمثل للمحلول الأساسي كان (0.5mL) و (1.5mL) من الأدوية (HCQ و LPV) على التوالي.

إضافة إلى ذلك تم إجراء تأثير درجة الحرارة والوقت ، تم الحصول على امتصاص أعلى عندما كانت درجة الحرارة (25°C) ل HCQ و (35°C) ل LPV ، بينما في أفضل وقت تم الحصول عليها في (10 min) لكل دواء لاستقرار منتج صبغة الآزو.

كما تمت دراسة تسلسل الإضافة لكل دواء ، كاشف (2,4-DNPHz) ، عامل مؤكسد (KIO_4) والمحلول الأساسي (NaOH) . أفضل التسلسلات المضافة إلى الأدوية (HCQ) و (LPV) كانت ($NaOH + Drug + 2,4\text{-}DNPHz + KIO_4$).

بعد تثبيت الظروف المثلى تم بناء منحني المعايرة للدواءين وقد وجد انها مطاوعة لقانون بيرلامبرت ضمن مدى التركيز $\mu g.ml^{-1}$ (2.5-22.5) و الامتصاصية المولارية (1048.28) و (3143.75) $L.mol^{-1}.cm^{-1}$ على التوالي. وحساسية ساندل تساوي (0.3205) و (0.20002) $\mu g.cm^{-1}$ وحدة الكشف كانت (0.243) و (0.933) $\mu g.ml^{-1}$ ، وقيم R.S.D% كانت (1.44%) و (1.12%) مع معامل الارتباط (0.9978) و (0.9967) تباعا.

تم اقتراح التركيب الفراغي المتكون من تفاعل الدواءين مع الكاشف باستعمال طريقتي النسب المولية و التغيرات المستمرة على التوالي فكانت تساوي (1:1) وثابت الاستقرارية (7.52×10^6) و (2.818×10^6) $L.mol^{-1}$ على التوالي .

تم دراسة المتداخلات والتي لم تؤثر على تقدير الدواءين. كما تم تطبيق هذه الطريقة بنجاح على عدد من المركبات الصيدلانية بالنسبة لدواء (HCQ) وايضا تم تطبيقها بنجاح على عدد من عينات المصل بالنسبة لدواء (LPV).

الدوال الثرموديناميكية مثل الانثالبي ΔH ، الانتروبي ΔS و طاقة كبس الحرة ΔG تم حسابها ايضا. ووجد ان التفاعل بين الدواءين و الكاشف كان تفاعل تلقائي ، ماص للحرارة و اكثر انتظاما.

كما تم حساب طاقة تنشيط التفاعل بين العقارين والكاشف (2,4-DNPHz) وكانت قيمته (143.6) و (203.7) J/mol على التوالي

واخيرا تم دراسة حركية التفاعل حيث وجد ان التفاعل من المرتبة الثانية الكاذبة لكلا الدواءين.

جامعة كربلاء

كلية التربية للعلوم الصرفة

قسم الكيمياء

دراسة ثرموديناميكية وحركية للقياس الطيفي لهيدروكسي كلوروكين ولوبينافير

رسالة مقدمة الى

مجلس كلية التربية للعلوم الصرفة – جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء

تقدمت بها

مريم علي عبد الصمد

بإشراف

أ.د. حميدة عيدان سلمان

الاشراف الثاني:

أ.د. مثنى صالح مشكور

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