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Spectrophotometric Determination of some Pharmaceutical Preparations using suitable Organic Reagent

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

وَاللَّهُ أَخْرَجَكُم مِّن بُطُونِ أُمَّهَاتِكُمْ لَا تَعْلَمُونَ شَيْئًا وَجَعَلَ لَكُمُ السَّمْعَ وَالْأَبْصَارَ وَالْأَفْئِدَةَ لَعَلَّكُمْ تَشْكُرُونَ ﴿٧٨﴾

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Supervisor Certification

Signature

I certify thesis entitle

Spectrophotometric Determination of some Pharmaceutical preparations using suitable Organic Reagent

Was prepared under my supervision at the University of Babylon / College of Science as a partial requirement for the degree of Master of Chemistry Science

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Dedication

- To every candle burns to enlighten the way to others.

My Teachers

- To my brothers, my wife, and my children.

Razan, Khalid

- To all my friends…

- I dedicate, with humility, my Thesis.

Mohammed Khalid

Acknowledgments

First of all, my great thanks and appreciation to Allah for granting me health and strength to facilitate my studies. My thanks, appreciation, and gratitude to my great supervisor **Prof. Dr. Abass Noor Alshirifi**, for the assistance, support, supervision, and encouragement. I would like to thank the Head and staff of the Department of Chemistry, College of Science, University of Babylon, for providing the necessary facilities, and for their assistance in this work. I want to thank my friends who have helped me to reach this stage. Finally, I want to thank everyone who helped me.

Summary:

This work includes the development of a simple rapid and sensitive spectrophotometric method for the determination of the Ceftriaxone (CF) and dapsone drugs, the method is based on the coupling of diazotized Ceftriaxone and dapsone drugs with 4,5-diphenylimidazole(4,5-DPI) reagent.

- Diazotized Ceftriaxone is coupled with the 4,5-diphenylimidazole reagent in a sodium hydroxide (NaOH) solution to form a relatively stable, water-soluble, and

purple-colored azo compound, which exhibits maximum absorption at wavelength λ_{max} 550nm, the suggested method obeys Beer's law in concentrations range (2-90) $\mu g.mL^{-1}$ with correlation coefficient R^2 (0.9976), Sandell's sensitivity S (0.22) $\mu g.cm^2$, molar absorption coefficient (3536.3) $L.mol^{-1}.cm^{-1}$, the limit of detection LOD (0.06) $\mu g.mL^{-1}$ and limit of quantitation LOQ (0.19) $\mu g.mL^{-1}$. The relative standard deviation RSD% (0.089).

- The results show that the proposed method is suitable for the simultaneous determination of some pharmaceutical preparations which contain Ceftriaxone drug, this method is compared with a standard method that exists in the British Pharmacopeia and found that the experimental value of (the T_{test} and F_{test}) is smaller than its table value, whereas there is no significant difference between the two methods.

- The diazotized dapsone drug as well as is determined by coupling with 4,5-DPI reagent in alkaline media, which has a maximum absorption at a wavelength of λ_{max} 501nm. Found that the method obeys Beer's law in concentrations range (2-18) $\mu g. mL^{-1}$ with correlation coefficient R^2(0.9942), Sandell's sensitivity S (0.154) $\mu g.cm^2$, molar absorption coefficient (34080) $L.mol^{-1}.cm^{-1}$, the limit of detection LOD (0.29) $\mu g. mL^{-1}$ and limit of quantitation LOQ (0.96) $\mu g.mL^{-1}$. The relative standard deviation RSD% is (0.88).

- The results show that the proposed method is suitable for the simultaneous determination of some pharmaceutical preparations, which contain dapsone drug, this method is compared with a standard method that exists in the US pharmacopeia and found that the experimental value of the (T_{test} and F_{test}) is smaller than its table value, whereas there is no significant difference between the two methods.

List of Contents

Shorten	Full name
ε	Molar absorptivity

2,5-DMP	2,5-dimethyl pyrrole
4,5-DPI	4,5-diphenylimidazole
4-TBP	4-Tertiary Butyl phenol
CF	Ceftriaxone
Conc.	Concentration
Da	Dalton
HPLC	High-performance liquid chromatography
LC	liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
p-CA	p-Chloranitic acid
PDAB	p-dimethyl amino benzaldehyde
ppm	Part per million
R^2	Correlation coefficient
RP-HPLC	Reversed-phase High-performance liquid chromatography
SDA	4,4-sulfonyl dianiline
pH	Potential of hydrogen
Rec%	Recovery percent
E%	Relative error percent
RSD%	Relative deviation percent

S	Sandell’s sensitivity Standard deviation
SD	Standard deviation
T	Temperature
UV–Vis	Ultraviolet-Visible Spectroscopy
R	Universal gas constant
λ_{max}	The wavelength of maximum absorbance

Chapter One

Introduction

1-General Introduction

1-1: Antibiotics

An antibiotic is a chemical compound that inhibits or abolishes the growth of microorganisms, such as bacteria, fungi, or certain parasites [1]. A parasite is a type of germ that needs to live on or in another living being (host) [2]. It does not

work against infections that are caused by viruses, for example, the common cold or flu. The term originally referred to any agent with biological activity against living organisms; however, "antibiotic" is now used to refer to substances with anti-bacterial, anti-fungal, or anti-parasitic activity. The first antibiotic compounds used in modern medicine were produced and isolated from living organisms, such as the penicillin class produced by fungi in the genus Penicillium or streptomycin from bacteria in the genus Streptomyces [3]. With advances in organic chemistry, many antibiotics are now also obtained by chemical syntheses, such as drugs. Many antibiotics are relatively small molecules, with a molecular weight less of than000 Dalton (Da=1.6×10^{-24} gram)[4].

1-2: Types of antibiotics [5-7]

There are various antibiotics available and they come in various different brand names. Antibiotics are usually grouped together based on how they work. Each type of antibiotic only works against certain types of bacteria or parasites. This is why different antibiotics are used to treat different types of infection. The main types of antibiotics include:

Penicillin’s -for example, phenoxymethylpenicillin, flucloxacillin and amoxicillin.

-Cephalosporin's - for example, cefaclor, cefadroxil , Ceftriaxone and cefalexin.

-Tetracycline's - for example, tetracycline, doxycycline and lymecycline.

-Aminoglycosides - for example, gentamicin and tobramycin.

-Macrolides - for example, erythromycin, azithromycin and clarithromycin.

-Clindamycin.

-Sulfonamides and trimethoprim - for example, co-trimoxazole, and dapsone.

-Metronidazole and tinidazole.

-Quinolones - for example, ciprofloxacin, levofloxacin and norfloxacin.

-Nitrofurantoin - used for urinary infections .

1-3: Ceftriaxone(CF)

Ceftriaxone(CF)(6R,7R)-3-[(acetyl-oxy)(methyl]-7-[[(2Z)-(2-amino-4-thiazolyl)(methoxyimino)-acetyl]amino]-8-oxo-5- thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid[8]. Cephalosporin antibiotic is a third-generation. It has broad spectrum activity against Gram negative and Gram positive bacteria[9]. Third-generation cephalosporins are broad-spectrum antimicrobial agents useful in a variety of clinical situations ,there is No cephalosporin is appropriate for all infectious disease problems[10]. Cefotaxime and ceftizoxime have the best gram-positive coverage of the third-generation agents. Ceftazidime and cefoperazone are the only third-generation drugs that provide antipseudomonal coverage CF is often used (in combination with macrolide and/or amino glycoside antibiotics) for the treatment of community-acquired pneumonia . It is also used as a routine prophylactic antibiotic for the patients undergoing orthopedic surgery [11]. The Figure (1-1) shows the structural formula of Ceftriaxone.

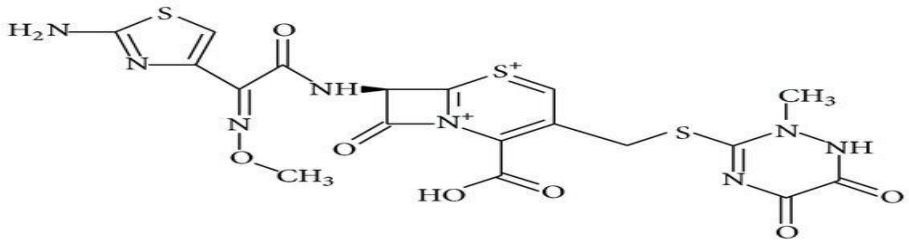

Figure (1-1): structure of ceftriaxone

Ceftriaxone is used for the treatment of a number of bacterial infections, such as middle ear infections, meningitis pneumonia, endocarditis, bone and joint infections, skin infections, and urinary tract infections [12].

Adverse effects of ceftriaxone used are changes in white blood cell counts, local reactions at site of administration, rash, and diarrhea [13].

1-4: Dapsone

Dapsone is an antibiotic commonly used in combination with rifampicin and clofazimine for the treatment of leprosy [14], also known as 4,4-sulfonyl dianiline (SDA) [15]. The Figure (1-2) shows the structural formula of dapsone.

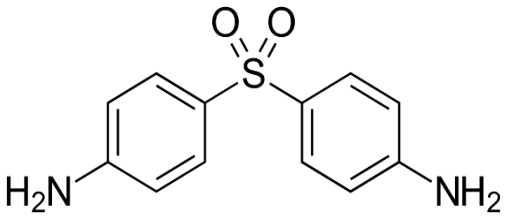

Figure (1-2): structure of dapsone

It is an antibiotic belonging to the sulfonamides family, a competitive inhibitor of para-amino benzoic acid, this drug works to reduce the production of folic acid necessary for the growth of the bacteria, which leads to the death of the bacteria [16]. dapsone still has a key role in the treatment of bullous dermatitis [17]. After being absorbed by the gastrointestinal tract, dapsone is widely distributed among body tissues and subsequently is selectively retained in the skin, the muscles, liver, and kidneys are partially subjected to acetylation (the introduction of an acetyl molecule into the compound) or join the liver and are finally excreted in the urine as metabolites [18]. Figure (1-3) shows the acetylation of dapsone.

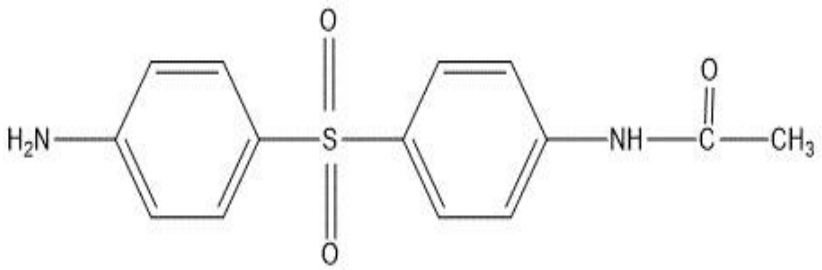

Figure (1- 3): Acetylation of dapsone

Anemia is one of the most important side effects of using dapsone, as a result of hemolysis, and this effect appears more in people who suffer from a deficiency in one of the enzymes called glucose-6-phosphate dehydrogenase [19].

1-5: Organic reagents and their importance in spectroscopy

In chemistry, of reagent or analytical reagent is a substance or compound added to a system to cause a chemical reaction[20], and its can be used to determine the analyte or absence of specific chemical substance [21]. For many years, organic reagents play an important role in the chemical and physiochemical methods of many analytical analysis. They are employed for qualitative or quantitative determination of chemical elements and compounds (both organic and inorganic) , as well as for separations, concentration, masking and other auxiliary operations that precede or accompany the analysis [22]. The reagent for the determination of metals should, first of all, contain a proper functional analytical group that enables its interactions with the determined element and subsequent observation of the respective analytical signal [23]. Currently, several tens of such functional analytical groups are known and documented [24].

The determination of organic compounds requires that the reagent molecule contained specific groups able to react with functional groups of determined compounds (hydroxyl , carbonyl , halogen , sulfur , nitrogen containing) with the formation of intensely colored or luminescent products[25]. Reactions used for the determination of functional groups differ considerably from those used for inorganic ions [26]. New problems that the chemical methods of analysis encounter with have stimulated wider studies of organic reagent. Novel directions have appeared, that are

associated with the modification and immobilization of the conventional organic reagents, as well as with the use of non-aqueous, water-organic and organized media [27]. At present, organic reagents are widely used not only in spectrophotometry,but also in atomic absorption, atomic emission spectrometry, inverse voltammetry and in liquid and gas chromatography. And also used in many other methods of determinations [28-30].

The 4,5-diphenylimidazole (DPI) is considered as an organic reagent, ionize in the basic medium, losing hydrogen ion, to be a nucleophile reagent. Figure (1-4) shows ionization of 4,5-DPI in alkaline media.

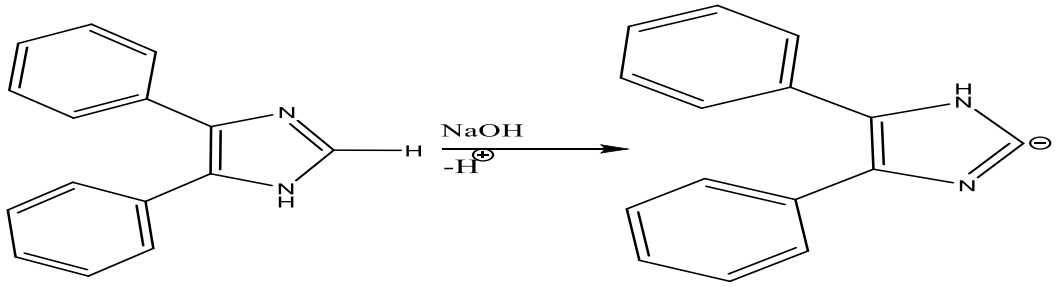

Figure (1-4): Ionization of 4,5-DPI reagent in alkaline media.

1-6: Diazotization

Diazotization refers to the chemical process of converting a primary aromatic amine into the corresponding diazonium salt of the amine [31]. In 1858, the German industrial chemist Peter Griess was the first to report such a reaction. He went on to discover many more diazonium salt reactions [32]. The reaction of an aromatic amine with nitrous acid in the presence of another acid results in the formation of these diazonium salt [33].The intermediates resulting from the diazotization of

primary aliphatic amines are unstable, they are rapidly converted into corresponding alcohols, and to different compounds according to the type of aliphatic amine used[34]. Nitrous acid is produced by the reaction between sodium nitrite and the other mineral acid (acid derived from one or more inorganic compounds) [35].

1-7: Mechanism of reaction

To understand the mechanism of the Diazotization reaction, it is necessary to understand the steps that the reaction goes through to reach the product [36]. Nitrous acid does not have a direct reaction with the amino group. Because it is an extremely unstable acid, the reaction is carried out with sodium nitrite, which decomposes to form nitrous acid under acidic conditions [37].

A-step1: Nitrosonium ion composition

Nitrosonium ion composition as shown in Figure (1-5).

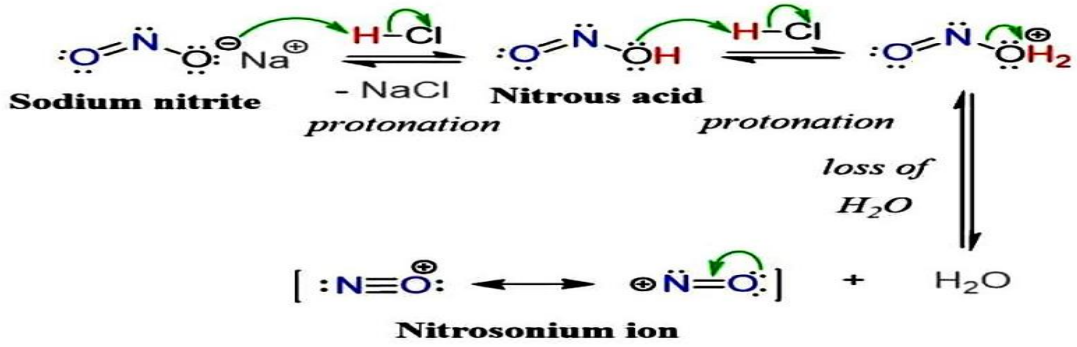

Figure (1-5): Nitrosonium ion composition

B-step 2: Formation of N-Nitrosamine

N- Nitrosamines are formed when nitrosonium ions react with aromatic amines. [38]. When a nitrosonium ion combines with an aromatic amine, its positive charge is transferred to the aromatic amine's nitrogen, which only provides a few electrons to the nitrosonium ion. As a result of the reaction between aromatic amine and nitrosonium ion, a nitrogen-nitrogen bond is formed. When deprotonation occurs, N-nitrosamines are formed [39]. As shown in Figure (1-6).

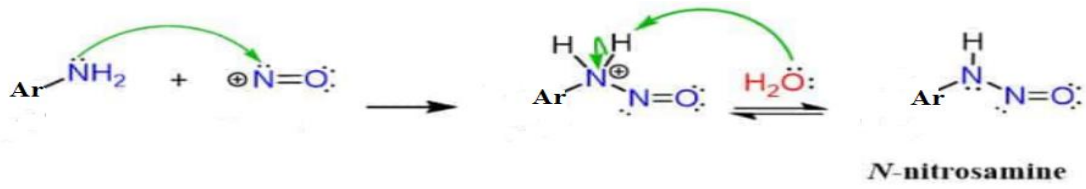

Figure (1-6): Formation of N-Nitrosamine

In low concentrations, the diazonium ion forms and immediately reacts with the amine present in the reaction mixture, shifting the equilibrium of this first step forward. The diazotization reaction begins with a nucleophilic attack of the amine on the diazonium ion, followed by deprotonation. Following a proton transfer, an N-

nitrosamine is formed, which is eventually converted into a diazonium salt via H_2O loss [40]. As shown in Figure (1-7).

C-step3: Formation of Diazonium Salt

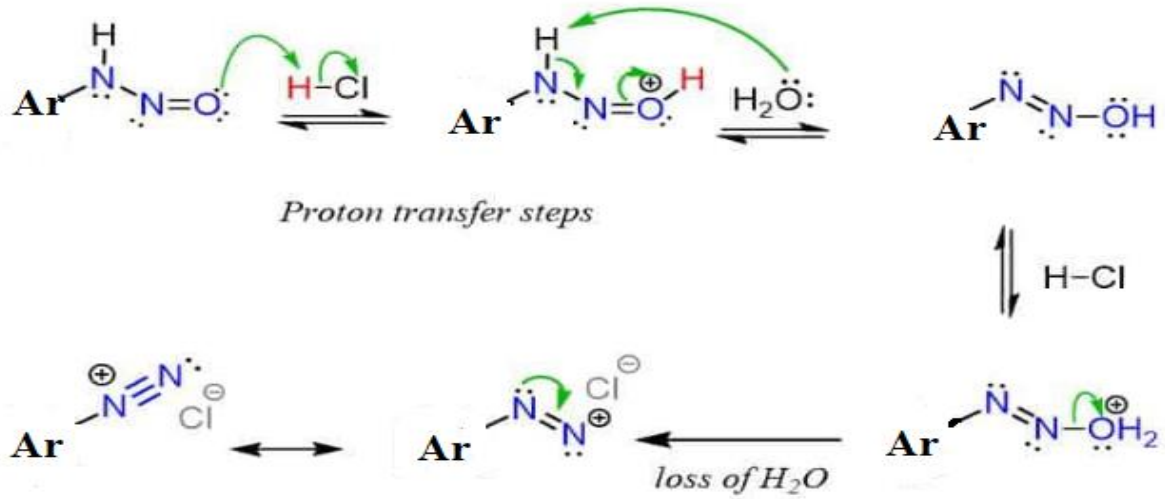

Figure (1-7) Formation of Diazonium Salt.

Aryl diazonium salts represent an important tool in organic chemistry since they're stable enough to be used as intermediates for preparing substituted benzene rings[41].

1-8: Importance of diazonium Salts

Diazonium compounds are standard reagents used in organic compound synthesis, especially aryl derivatives. Diazonium salts are sensitive to light and break down under Ultraviolet or violet light nearby [42]. The azo pigments are important in various industries such as paints, rubber, and plastics. They are preferred to the light fastness and their excellent coloring pigments. Many of the azo-pigments are nontoxic and only a few of them are carcinogenic and mutagenic. Even, many classes of the azo dyes are known that are produced by the reactions of diazonium salts, such

as substantive dyes, reactive dyes, and metal complex dyes. In the cellulose-based textiles, such as cotton, substantive dyes are used [43]. These dyes are bound to the textile by the non-electrostatic forces. Some of the azo compounds such as the methyl orange are being used as the acid-base indicators. Most of the proteins are cationic, so the dyeing of wool and leather corresponds to the ion exchange reaction. The anionic dyes adhere to the articles by the electrostatic forces. Quaternary ammonium centers are present in the cationic azo dyes. Azo pigments are also produced from the diazonium salts and their chemical structure is similar to the azo dyes [44].In the synthesis of organic compounds, diazonium compounds are standard reagents, especially the aryl derivatives. Substituted aromatic compounds cannot be obtained by direct substitution in the benzene and for these compounds, the replacement of diazo compounds is used in the diazonium salts. For introducing the fluoride, bromide, chloride, iodide, hydroxyl and -CN groups to the aromatic ring, diazonium salts are used as intermediates [45].

1-9: Diazonium salt coupling

Diazonium coupling reaction occurs when Aryl Diazonium salts react with compounds that contained an electron- donating groups such as (OH^-, NH_2^-) to form brightly colored azo compounds. This reaction is commonly used in the production of azo dyes [46].

The positive charge in diazonium ions is delocalized over the two nitrogen atoms, according to a resonance description. Nucleophiles cannot bond to the inner nitrogen, but bonding (or coupling) of negative nucleophiles to the terminal nitrogen results in neutral azo compounds [47]. As shown in Figure (1-8).

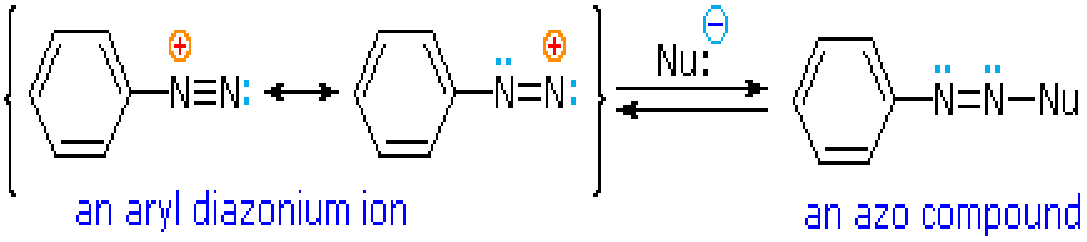

Figure (1-8): Formation of azo compound.

Because diazonium salts are weak electrophiles, the presence of the withdrawing and electrophilic groups affects their activity. The driving groups reduce the activity of the diazonium salts, while the withdrawing groups increase their activity by increasing the speed of compensation and an increasing the selectivity of the compensation site for nucleophiles (the coupling factor). The diazonium coupling reaction is useful for comparing the active or electronically active sites of nucleophile molecules [48].

1-10: Spectroscopy for chemical analysis

Spectroscopy can be defined as the study of the absorption and emission of light and other radiation by matter, depending on the wavelength of the radiation [49]. Spectroscopy methods are classified according to the type of radiation used, the interaction between the energy and the material, the material type, and the applications for which the technique is used [50]. The most common types of spectroscopy used for chemical analysis are atomic spectroscopy, ultraviolet and visible spectroscopy, infrared spectroscopy, Raman spectroscopy, and nuclear magnetic resonance [51].

A variety of analytical methods can be used to analyze chemicals quantitatively and qualitatively, but one major area of analysis is spectroscopy. Spectroscopy is the

study of the interaction between electromagnetic radiation and matter, which results in electronic excitations, molecular vibrations, or nuclear spin orientations [52].

1-11: Ultraviolet and Visible (UV-Visible) Spectroscopy

Ultraviolet (UV) and visible (Vis) spectroscopy examines compounds using the electromagnetic radiation spectrum range from 190 nm to 700nm.

Many atoms or compounds can emit or absorb visible light, and it is this absorption or reflectance that gives the apparent color of the chemicals being analyzed [53-55].

Absorption of visible and UV radiation is associated with electron excitation from a low energy ground state to a high energy excited state, and the energy can be absorbed by both non-bonding n-electrons and -electrons within a molecular orbital [56-58]. Wavelengths of light all have a particular energy associated with them, and it is only light with the right amount of energy that causes transitions from one level to another for absorption [59]. For larger gaps between energy levels, more energy is required for promotion to the higher energy level, so there will be higher frequency and shorter wavelength absorbed [60].

UV and visible spectroscopy can be used to measure sample concentration of sample by applying the principles of the Beer-Lambert Law, which states that absorbance is proportional to the concentration of the substance in solution and the path length [61,62]. UV and visible spectroscopy can be used to detect the presence of free electrons and double bonds within a molecule [63]. A UV-Vis spectrometer can be used as a detector for high-performance liquid chromatography in addition to being an analytical technique that can be used alone [64].

1-12: Methods for estimating ceftriaxone

1-12-1: spectrophotometric methods

Ceftriaxone is estimated by several spectrophotometric methods as shown in Table (1-1).

Table (1-1): The most important spectrophotometric methods used for the determination of Ceftriaxone in pharmaceuticals.

NO	Method Summary	Wavelength of maximum absorbance nm(λ)	Linear range of concentration $\mu g.ml^{-1}$	Reagent Concentration	References
1	The reaction of ceftriaxone with 2,5-DMP to form the red color of the azo dye	520	3.0 -50	$2x10^{-3}M$	[65]
2	coupling of ceftriaxone with 4-TBP reagent to form a red azo dye.	500	10-30	$2.5x10^{-3}M$	[65]
3	The reaction of ceftriaxone with P-dimethyl amino benzaldehyde (PDAB).	490.6	5-25	0.05(w/v)	[66]
	Hydrolysis of the drug using 0.5M of				

4	NaOH at 100 ºC , then react the sulfide ion that formed with 4-chloro-7-nitrobenzeno-2-oxa-1,3-diazole to form yellow colour.	390	20-80	$3.0x10^{-3}$M	[67]
5	Hydrolysis of β-Lactam ring of the ceftriaxone drug using NaOH ,then react with iodate to liberate iodine in acidic medium	556	0.2-7.0	0.1M of KI	[68]
6	Charge- transfer; between ceftriaxone drug (electron donor) and p-CA as Π-acceptor to form violet chromogen	520	20-240	0.05(w/v)	[69]
7	Complex formation with metal-chromium (VI) as a reagent.	520	0.2-28	0.01(w/v)	[70]

8	Diazotization of Ceftriaxone in acidic medium, then coupling with 3-amino Phenol to result orange-red colour.	500	20-160	3 x 10^{-3}M	[71]
9	Diazotization of Ceftriaxone, then coupling with acidified P-dimethyl amino Benzaldehyde	400-430	25-60	0.3%	[72]
10	Derivatization of ceftriaxone with 1,2-naphthaqinone-4-Sulfonic acid sodium in alkaline medium to produce of orange colour	480	25-175	0.5%	[73]
11	Diazotization of Ceftriaxone, then coupling with 4,5-DPI reagent in alkaline media to produce of purple color	550	2-90	0.001M	This work

1-12-2: Chromatography Methods

The Ceftriaxone is determined by several chromatographic methods illustrated in Table (1-2).

Table (1-2): The most important chromatographic ways used for the determination of ceftriaxone in pharmaceutical preparations.

No.	Type of Chromatographic	Characteristics of the method used	Linear range of concentration $\mu g.mL^{-1}$	Application	Reference
1	HPLC	Mobile phase phosphate buffer: water(40:4.8:55.2%v/v/v) and UV detection	1.0 -120	pharmaceutical	[74]
2	RP-HPLC	Mobile phase acetonitrile : water (70:15:20v/v/v) and UV-Vis detection	2.5-25	pharmaceutical	[75]

3	HPLC	Mobile phase: methanol: mono potassium phosphate (16:84, v/v); and UV-Vis detection	1-70	Standard and pharmaceutical form	[76]
4	HPLC	Mobile phase: ammonium phosphate buffer and methanol (90:10% v/v) and UV-Vis detection	10-50	pharmaceutical	[77]
5	HPLC	Mobile phase: triethylammonium acetate and acetonitrile (60:40 v/ v) and UV-Vis detection.	0.2-54	pharmaceutical	[78]

6	HPLC	Mobile phase: potassium dihydrogen with phosphoric acid) with triethylamine – methanol (70:30, v/v) and UV-Vis detection	0.5-250	Biological sample	[79]
7	HPLC	Mobile phase: potassium dihydrogen phosphate with phosphoric acid) with triethylamine – methanol (70:30, v/v) and UV-Vis detection	0.24-250	Human urine	[80]

8	HPLC	Mobile phase: acetonitrile and phosphate buffer(8:92 v/v)	0.1–50	Human plasma	[81]
9	HPLC	Mobile phase: dibasicpotassium phosphate and cetyltrimethyl ammonium bromide with acetonitrile (73:27 v:v)	0.005–120	plasma	[82]
10	HPLC	Mobile phase: tetraheptylammonium bromide, acetonitrile, pH 7 buffer, pH 5 buffer and water. (50% v/v) and water (50% v/v).18	1.153-6.92	stainless steel surface of pharmaceutical manufacturing equipments	[83]

1-12-3: Electrochemical Methods

Ceftriaxone was determined by several electrochemical methods are shown in Table (1-3)

Table (1-3): Several electrochemical methods are used for the determination of ceftriaxone.

No.	Method summary	Linear range of concentration $\mu g.ml^{-1}$	Applications	References
1	Oxidation of ceftriaxone on a carbon –nanotube-modified glass carbon electrode in the phosphate buffer solution PH=7.40	$1x10^{-4}$ - $1x10^{-3}$	Human serum albumin	[84]
2	Determination of Ceftriaxone through electrochemical sensor developed based on caffeic acid$C_9H_8O_4$ and chitosan-containing polyurethane (CAC-PU) films modified platinum electrode.	0.1-1.5	urine samples	[85]
3	Oxidation of Ceftriaxone on A copper hexacyanoferrate $C_6Cu_4FeN_6$ nanostructure was prepared on the	2-72	pharmaceutical samples	[86]

	surface of a disposable pencil graphite electrode			
4	Determination of Ceftriaxone by a glass carbon electrode was developed with platinum nanoparticle decorated multiwalled carbon nanotube	0.01– 10.0	pharmaceutical and clinical preparations.	[87]

1-12-4: Fluorescence Methods

Ceftriaxone is determined by several fluorescence methods are shown in Table (1-4).

Table (1-4): The most important fluorescence methods for the determination of ceftriaxone.

No.	Summary of method	Excitation and Emission wavelength Respectively	Linear range of concentration µg.mL^{-1}	Applications	References
1	conversion of ceftriaxone into a fluorescent product by reacting with ortho-phthalaldehyde (OPA) in the presence of sulfite	324nm-386 nm	0.4-20	Formations and human plasma	[88]

2	Sensitize of ceftriaxone by l-cysteine (Cys) coated CdS quantum dots (QDs) ,which proposed as sensitizers for the Ceftriaxone determination.	360nm-420nm	0.1-20	meat sample	[89]
3	Reaction of ceftriaxone with polyethyleneimine in aqueous solutions,then formation noncovalent molecular complex . The product hydrolysis when heated, Then the fluorescence spectrum of the resulting compound was taken	290nm-350nm	$3x10^{-6}$-$1x10^{-4}$	pharmaceutical	[90]

4	Determination of Ceftriaxone by reaction with L-cysteine capped ZnS (L-Cys-ZnS)	310nm-411nm	6 -20	pharmaceutical	[91]

1-13: Determination of dapsone

Dapsone is estimated by various spectroscopic methods are shown in the Table (1-5)

1-13-1: spectrophotometric methods

Table (1-5): The most important spectrophotometric methods used for the determination of dapsone in pharmaceuticals.

NO	Method Summary	The wavelength of maximum absorption nm(λ)	Linear range of concentration $\mu g.ml^{-1}$	Reagent Concentration	Reference

1	The method is based on the reaction of dapsone with sodium 1,2-naphthoquinone-4-sulfonic in pH 6.98 buffer solution to form a salmon pink compound.	525	0.4 - 10	0.2%	[92]
2	reaction of diazotized dapsone with phloroglucinol($C_6H_6O_3$) to form yellow colour.	435	0.4-10	0.5%	[93]
3	Determination of dapsone depending on charge transfer, complex formation with O-chloranil ($C_6Cl_4O_2$) in aqueous media forming coloured complex	394	0.125-1.25	5×10^{-3}M	[94]
4	Spectrophotometric Determination of dapsone in pure form by using methanol	260	3-18	30% methanol alcohol	[95]

	alcohol and water as solvent.				
5	coupling of hydrolyzed carbofuran($C_{12}H_{15}NO_3$) with diazotized dapsone in alkaline medium to give orange red colored product .	480	0.1- 4	$2x10^{-3}M$	[96]
6	The reaction of 4-nitro phenol$C_6H_5NO_3$ with dapsone in presence of acid medium and heating, forming yellow complex	460	3-50	0.1%	[97]
7	Diazotization of dapsone then coupling reaction with iminodibenzyl (IDB) $C_{14}H_{13}N$ in alcohol medium to form violet colour.	570	0.1–2.5	0.1%	[98]
	Diazotization of dapsone then				

8	coupling reaction with N-bromosuccinimide (NBS) $C_4H_4BrNO_2$ to form green colour.	610	0.5-5.0	0.05%	[98]
9	Diazotization of dapsone then coupling reaction with thymol.	460	0.3-4	0.02	[99]
10	Diazotization of dapsone then coupling reaction with Benzoyl acetone (BAC) $C_{10}H_{10}O_2$	437	0.9-15	$2x10^{-3}M$	[100]
11	Diazotization of dapsone then coupling with 4,5-DPI reagent.	502	2-18	$1x10^{-3}M$	This work

1-13-2: Chromatography Methods

Dapsone is determined by several chromatographic methods are illustrated in Table (1-6).

Table (1-6): The most important chromatographic ways for the determination of dapsone in pharmaceuticals.

NO	Type of chromatography	Characteristic of the method used	Linear range of concentration µg / mL	Application	reference
1	HPLC	Mobile phase: water and acetonitrile in the ratio of 50:50% (V:V) and UV-VIS detection	0.5-20	pharmaceutical	[101]
2	HPLC	The mobile phase: aqueous acetate buffer, and acetonitrile 27/63% (v/v) and UV-VIS detection.	0.5-5	pharmaceutical	[102]

3	RP- HPLC	The mobile phase :formic acid and ethanol (90:10, v/v) and UV detection.	0.2-50	pharmaceutical	[103]
4	HPLC	The mobile phase: Acetonitrile and acetic acid (25:75 v/v) diode-array detector.	5-25	crude rice brain oil.	[104]
5	RP-HPLC	The mobile phase: methanol, H_2O, glacial acetic acid and tri ethyl amine (20: 80: 0.06: 0.1)	0.050-62.5	pharmaceutical	[105]

6	RP-LC	The mobile phase: acetonitrile ammonium acetate with acetic acid (75:25, v/v) using UV detector.	1-30	pharmaceutical	[106]
7	HPLC	The mobile phase : methanol-water (65:35, v/v) and UV detection	5-65	pharmaceutical	[107]
8	RP-HPLC	The mobile phase : ammonium acetate and methanol (60:40 v/v) and UV detection	2-12	pharmaceutical	[108]

1-13-3: Electrochemical Methods

Dapsone is determined by several electrochemical methods are shown in Table (1-7)

Table (1-7): Several electrochemical methods were used for the determination of dapsone.

NO	Method summary	Linear range of concentration .	Applications	References
1	Using an electrochemical sensor and polymer Iron oxide nanoparticles (Fe_3O_4) as magnetic material was first deposited and followed by the electropolymerization of aniline monomer with and without a template molecule at the surface of the platinum electrode (Pt).	1×10^{-7} -1×10^{-5} M	pharmaceutical samples	[109]

2	Synthesis of the Co metal-organic framework molecularly imprinted polymer nanoparticles, which can be used as a highly selective and sensitive method for the determination of dapsone .	0.5 - 170 µM	pharmaceutical	[110]
3	Electrochemical oxidation of dapsone ,then adsorption it at a stationary glassy carbon electrode .	3.2 -29.4 mM	Pharmaceutical and urine	[111]

1-13-4: Fluorescence Methods

Dapsone is determined by several fluorescence methods are shown in Table (1-8).

Table (1-8) The most important fluorescence method used for the determination of dapsone.

No	Summary of method	Excitation and Emission wavelength Respectively	The linear range of concentration	Applications	References
	Formation complex from dapsone with β-Cyclodextrin in presence of linear alcohol , Then the fluorescence spectrum of the resulting compound was taken.	296nm,428nm	3.4 -1.5×10^3 ng. ml^{-1}	Pharmaceuti cal and human plasma	[112]

1-14: Objective of the research

1- Development of simple, rapid, and sensitive spectrophotometric methods used for the determination of some pharmaceutical compounds in their pure form and in some of their pharmaceutical preparations. These drugs are:

A- Ceftriaxone (CF).

B- Dapsone.

2- The study of the optimal conditions required to conduct the interaction of each drug with this reagent, such as the type and concentration of the reagent, the type of acid, the volume of sodium nitrite, the volume of sulfamic acid, the volume of the reagent, and the time required to complete the nitrification process, the time required to remove excess nitrite, and the time required for product stability.

3- Investigating the effect of additives (excipients) on the spectrophotometric method used to increase sensitivity, which is dependent on the shape and type of pharmaceutical product manufacturer that results from the presence of additives in pharmaceutical preparations on the studied drug compound.

4- Quantitative estimation of the pharmaceutical compounds under study in their pure form as well as in some types of pharmaceutical preparations containing them that are available on the local market.

5- Comparing the results of the methods used to estimate the studied compounds to the standard method of estimation recommended by the British or American Pharmacopoeia.

6- The possibility of using this method to determine the extent of these pharmaceutical compounds within their pharmaceutical preparations in their various forms.

Chapter Two

The Experimental Part

2-1: Experimental Instruments

1- Double-beam UV and visible light spectrophotometer, made by Shimadzu, 1800 model, Japan.

2- Japanese WB 710 model optima water bath device.

3- Sartorius Germany's analytical balance apparatus, model BP 3015.

2-2: Chemicals and reagent

All chemicals and reagents are used in this study without any additional and further purification. The supplier firm and the level of purity of the used chemicals and reagents are displayed in Table (2-1).

Table (2-1): chemicals and reagent

NO.	Chemical name	Molecular formula	company	purity%	Molar mass (g/mole)
1	Ceftriaxone	$C_{18}H_{18}N_8O_7S_3$	SDI-Iraq	Pure	554.58
2	Dapsone	$C_{12}H_{12}N_2O_2S$	Merck	98.00	248.302
3	4,5-DPI	$C_{12}H_{12}N_2O_2S$	Merck	98.00	220.27
4	Ethanol	C_2H_6O	Merck	absolute	46
5	acetone	C_3H_6O	SIGMA	98.00	58
6	Hydrochloric acid	HCl	SIGMA	Analar	36.50
7	Sodium nitrite	$NaNO_2$	LOBAChmie	98.00	69.00
8	Sodium hydroxide	NaOH	SIGMA	98.00	40
9	Sulfamic acid	H_3NSO_3	SIGMA	98.00	97.10

2-3: Excipients.

The types of additives and suppliers are displayed in Table (2-2).

Table (2-2): the excipient used with dapsone

No	Excipients
1	Lactose monohydrate
2	Magnesium stearate
3	Maize starch
4	Sodium lauryl sulphate
5	Colloidal anhydrous silica

2-4: Pharmaceutical Preparations

The pharmaceutical preparations are given in Table (2-3) along with their names, contents, and manufacturers.

Table (2-3) Pharmaceutical preparations of ceftriaxone and dapsone.

NO	pharmaceutical	Contains	company	origin
1	Ceftriaxone injection	500 mg	BRAWN	India
2	Ceftriaxone injection	1g	L.D.P	Spain
3	Ceftriaxone injection	1g	SANAVETA	Germany
4	Dapsone tablets	100mg	DOMINA	Syria
5	Dapsone tablets	100mg	GlaxoSmithKline	Britain

2-5: preparation of solutions

2-5-1: General solutions

A-Sodium hydroxide solution(1.0M):

This solution is prepared by dissolving (4.000g) of sodium hydroxide granules in (100mL) of distilled water in the volumetric flask.

B-Hydrochloric acid(1.0M):

This solution is prepared by using (8.800mL) of the concentrated solution and then diluted with distilled water to(100mL) in a volumetric flask.

C-sodium nitrite(0.5M):

This solution is prepared by dissolving (6.900g) of sodium nitrite granules in (100mL) of distilled water in a volumetric flask.

D-sulfamic acid(0.2M):

This solution is prepared by dissolving (1.942 g) sulfamic acid granules in (100mL) of distilled water in a volumetric flask.

2-5-2: preliminary solutions for spectrophotometric studies

2-5-2-1-A: Ceftriaxone drug solution (CF) 500 μg / mL

This solution is prepared by dissolving 0.01g of Ceftriaxone (CF) in (5mL) of absolute ethanol and complete to (20mL) by distilled water in a volumetric flask. The solutions of a drug concentration are prepared by further dilution. This solution is prepared daily.

2-5-2-1-B: Dapsone drug solution 100 µg / mL

This solution is daily prepared by dissolving 0.01 g of dapsone in (5mL) of acetone and complete to (100mL) by distilled water in a volumetric flask. The solution of a drug concentration of 25 µg / mL is prepared by further dilution.

2-5-2-2: 4,5-diphenylimidazole (4,5-DPI) reagent solution 0.001 mole / L

This solution is prepared by dissolving 0.022 g of 4,5-DPI in (5mL) absolute ethanol and complete to (100mL) by distilled water in a volumetric flask.

2-5-3: pharmaceutical preparation solutions

2-5-3-A: pharmaceutical preparation solution of ceftriaxone (CF) injection

This solution is prepared by weighing 0.01 g of powder, then dissolved in 10 mL of ethanol, and complete to 20 mL of the volumetric flask with distilled water, this weight is equivalent to 500µg / mL of ceftriaxone.

2-5-3-B: Pharmaceutical preparation solution of dapsone Tablets

Weighed and carefully crushed 10 dapsone tablets, then weighed 0.016 g of DOMINA company (Syria) and 0.012 g of GlaxoSmithKline company (Britain) of dapsone powder, equating to 0.01g of dapsone, which is dissolved in 5 mL of acetone and completed to 100 mL of distillate water in a volumetric flask to prepare 100 µg / mL of this solutions. Low concentrations are prepared by further dilution.

2-6: Development of Spectrophotometric method for determination of Ceftriaxone in pharmaceutical preparations.

2-6-1: Absorbance spectra study of ceftriaxone (CF)

The aim of the spectrophotometric investigation is to determine the maximum absorbance wavelength λ_{max} of the colored product formed from the reaction of ceftriaxone with the analytical reagent 4,5-diphenylimidazole (4,5-DPI).

In a volumetric flask of 10 mL, combine 1 mL of 500 μg/mL ceftriaxone solution with 0.5 mL of 1 M HCl solution and 0.75 mL of 0.5 M $NaNO_2$, then cool by ice from 0-5°C for 10 minutes before adding 0.75 mL of 0.001 M 4,5-DPI solution and 1.25 mL of 1.0 M NaOH solution, respectively. The final azo solution formed was left for 5 min until the azo was stable, and the absorbance of the stabilized solution was measured spectrophotometrically at λ_{max} 550 nm using 1.0 cm cells against the blank solution, which was prepared in the same manner as above but without the addition of ceftriaxone.

2-6-2: Optimum conditions

The optimal conditions and their effect on the color development and absorbance of the azo formed by the reaction of ceftriaxone with 4,5-DPI have been studied.

2-6-2-1: Effect of reagent concentration (4,5-DPI)

A concentration of (1×10^{-3} M) of 4,5-DPI and various volumes of 0.25, 0.50, 0.75, 1.00, 1.25, and 2.00 mL are used to study the optimal volume of the reagent 4,5-DPI in order to determine which volume of the reagent produces the maximum absorbance of the azo compound.

2-6-2-2: Effect of base

The best volume of sodium hydroxide (NaOH) at a concentration of (1.0 M) is studied on the formation of Ceftriaxone azo compound using various volumes of NaOH (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50) mL in order to reach the best volume of sodium hydroxide that gives the highest absorbance of the formed azo compound.

2-6-2-3: Effect of acid

The effect of the concentration of hydrochloric acid, on the absorbance of the colored azo produced is studied also, by using different volumes of (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50) mL of 0.10 M then observing the best volume that gave the highest absorbance of the azo dye that formed.

2-6-2-4: Effect of sodium nitrite

One of the solutions influencing the amount of the drug's diazotization solution, which in turn interacts with the reagent, is sodium nitrite (0.50 M). To determine the best volume for this solution, volumes of (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50) mL are used to obtain the highest absorbance intensity of the azo formed.

2-6-2-5: Effect of sulfamic acid.

Different volumes of sulfamic acid (0.25, 0.50, 0.75, 1.00, 1.25, and 1.50 mL) are used to determine the best amount to be used in order to break down the excess sodium nitrite and obtain the highest absorption intensity of the formed azo. The best amount of sulfamic acid with a concentration of (0.20 M) is studied based on the intensity of absorbance of the formed product.

2-6-2-6: Effect of temperature

Under the same conditions, a water bath is utilized to study the impact of various temperatures ranging from (0-50) ^{o}C on the absorbance of the azo produced, at 5 min of heating time used.

2-6-2-7: Effect of the time required to complete the formation of the diazonium salt.

The effect of time required to complete the diazonium salt production process was studied depending on the amount of azo that was absorbed at various times, from (1, 5, 10, 15, 20, 25, 30) minutes at 0-5 ^{o}C.

2-6-2-8: Effect of time necessary to remove excess nitrous acid.

The effect of the time required to remove excess nitrous acid by sulfamic acid is tested at times (1-5) minutes to achieve the maximum absorption intensity of the produced azo.

2-6-2-9: Effect of time on an azo-dye stability

In order to determine the ideal amount of time period to achieve a stable reaction product under optimal conditions, the stability of the reaction product was tested by measuring the intensity of absorbance of the resultant azo-colored solution after intervals of (5, 15, 25, 35, 45, 55, and 60) min.

2-6-3: Recommended procedure.

In a series of 10 mL volumetric flasks, amounts of ceftriaxone solution are added to give a final ceftriaxone concentration within a range of 2–90 μg/mL, followed by 0.5 mL of 1.00 M hydrochloric acid solution and 0.75 mL of 0.5M sodium nitrite solution to form a diazonium salt. All flasks are cooled in an ice bath at 0-5 °C for approximately 10 min. Finally, follow a cold solution containing 0.75 mL of (0.001

M) 4,5-DPI in 1.25 mL of (1.0 M) sodium hydroxide solution is added and left aside until a purple azo dye color appeared as a result of coupling between diazotized ceftriaxone and 4,5-DPI, and the 10 mL flask is complete with distilled water. Left aside for 5 minutes at 0 °C, then followed by adding (0.75 mL) of 0.2M of sulfamic acid, the absorbance of each solution was measured at a maximum wavelength of 550 nm using a 1.0 cm cell against a blank solution prepared using the same method but without the addition of the drug.

2-6-3-1: Molar absorptivity and Sandell's sensitivity

In order to determine how strongly a chemical species absorbs light at a specific wavelength, it is necessary to use a term called molar absorptivity.

According to Beer's Law

$$A=\varepsilon bc \qquad (2\text{-}1)$$

Where (A) is absorbance, (ε) is a constant called molar absorptivity, (b) is the light path, and (c) is concentration.

Molar absorptivity(ε) is defined as a measure of the chemical ability to absorb light at a specified wavelength, it depends on the chemical species; actual absorbance depends on chemical concentration and the path length.

Sandell's sensitivity is the lowest concentration of the analyte in ppm($\mu g/cm^3$), which give an absorbance of 0.001 in 1 cm path length.

This is valid only if the system conforms to Beer’s law indefinitely at low concentrations, this is true for all reactions [113].

$$\text{Molar absorptivity} = \text{Slope} \times \text{Molar mass} \times 10^3 \qquad (2\text{-}2)$$

$$\text{Sandell's sensitivity} = \frac{\text{Molar mass}}{\text{Molar absorptivity}} \qquad (2\text{-}3)$$

2-6-3-2: Limit of detection LOD and limit of quantification LOQ

The limit of detection (LOD) is the smallest amount or concentration of analyte in the test sample that can be distinguished from zero with certainty. LOQ is the lowest analyte concentration that can be quantitatively identified with a defined level of accuracy and precision. The main distinction between LOD and LOQ is that LOD is the smallest concentration of an analyte in a test sample that we can determine with acceptable repeatability and accuracy, whereas LOQ is the smallest concentration of an analyte in a test sample that we can easily distinguish from zero. The equations below are used to derive the LOD and LOQ in these procedures, which are determined by measuring the absorbance of ten blank solutions at the calibration curve's optimal condition [114].

$$\text{LOD} = \frac{3\text{SD}}{\text{slope}} \qquad (2\text{-}4)$$

$$\text{LOQ} = \frac{10\ \text{SD}}{\text{slope}} \qquad (2\text{-}5)$$

$$\text{SD} = \sqrt{\frac{\sum (Xi - \bar{X})^2}{n-1}} \qquad (2\text{-}6)$$

Where, SD is the standard deviation for ten readings of the used blank at the maximum wavelength of the method.

2-6-4 : Accuracy and precision

Accuracy is defined as the closeness of the calculated value to the true or accepted value. The precision of an analytical method reflects the similarity of results obtained from a series of measurements obtained from many samples of the same homogeneous sample under optimal conditions[115].

2-6-4-1: Relative Error E%

The relative error is calculated to determine how close or far the value is from the true value.

Relative errors were calculated in this method by using the equation (2-7).

$$\%E = \frac{X - X^{o}}{X^{o}} \times 100\% \quad (2\text{-}7)$$

X = measured value, X^{o}= true value.

2-6-4-2: Recovery Percent(Rec %) :

$$Rec\% = 100 \pm E\% \quad (2\text{-}8).$$

2-6-4-3 : Relative standard deviation percent (RSD %)

Relative standard deviation is also called percentage relative standard deviation formula , is the derivation measurement that tells us how the different numbers in a particular data set are scattered around the mean .

The RSD was calculated by the equation below .

$$\text{RSD}\% = \left(\frac{\text{SD}}{\bar{\text{X}}}\right) \times 100 \qquad (2\text{-}9)$$

$$\bar{\text{X}} = \frac{\sum \text{Xi}}{\text{n}} \qquad (2\text{-}10)$$

$$\text{SD} = \sqrt{\frac{\sum(\text{xi} - \bar{\text{x}})^2}{\text{n}-1}} \qquad (2\text{-}11)$$

Where xi is the absorbance

$\bar{x}$ is the mean of xi

(n) number of the measurement

The accuracy and precision are investigated using three concentrations of Ceftriaxone (1.0,1.2,1.4) mL, which corresponded at concentrations of (50,60,70) μg mL^{-1}. The final colored solution and completed colored azo dye product were prepared according to the method's procedure under optimal conditions. The preparation of the solution is repeated three times for each concentration, the absorbance is for each solution measured at the maximum wavelength against the blank using the method. The equations above are used to achieve accuracy and precision.

2-6-5: Continuous variation and Mole ratio method method

By using the continuous variation method (job's method) and the mole ratio method the reaction stoichiometric mechanism was obtained.

2-6-5-1: continuous variation method (Job's method)

The continuous variation method, or Job's method, is chosen for the estimation of the stoichiometry of the product formed from the reaction of diazotized ceftriaxone drug with 4,5-DPI as a reagent. In a series of 10 mL volumetric flask reactions, increasing volumes of the drug (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9) mL of diazotized ceftriaxone at a concentration of 1.0×10^{-4} M are reacting with the same concentration of 4,5-DPI as a reagent in decreasing volumes of it (0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3,0.2 and 0.1) mL each time. Each time, the absorbance is measured at 550 nm and plotted against the mole fraction of the drug.

2-6-5-2: Mole ratio method

The mole ratio method is used for the estimation of the stoichiometric ratio of the product formed from the reaction of diazotized ceftriaxone drug with 4,5-DPI as a reagent. A series of reactions is provided by reacting a fixed (1.0 mL) volume of (1.0×10^{-4}M) diazotized ceftriaxone drug concentration with the same concentration of 4,5-DPI as a reagent at different volumes (0.25, 0.5,0.75,1.0, 1.25, 1.5 and 2.0) mL. The absorbance of the final solution is measured at 550 nm maximum and plotted against the mole ratio[116].

2-6-6: Mechanism of reaction

The mechanism of the reaction between ceftriaxone and 4,5-DPI is suggested by the estimation of the stoichiometric ratio of the product formed by the continuous variation method and mole ratio method.

2-6-7: Analytical applications

In order to ascertain the selectivity of the method and benefit from the possibility of applying it to some pharmaceutical preparations containing ceftriaxone, solutions from ceftriaxone injection were prepared, as shown in paragraph 2-5-3-A.

2-6-8: F- test and T-test

T-test is a statistical calculation that measures the difference in means between two tester groups, to the known truth of the analytical process by comparisons of data obtained with standard methods, is used in hypothesis testing to check whether the variance of two samples is equal or not. t-test is necessary to know the truth of the analytical process by comparisons of data obtained.

A- Two methods

B- Two persons

C- The same person, but under different method conditions.

t-test can be calculated according to the equation (2-12).

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_{1-2}} \sqrt{\frac{N_1 N_2}{N_1 + N_2}} \qquad (2-12)$$

Where, $S_{1\text{-}2}$: the standard deviation of the data to two methods.

$$s_{1-2} = \sqrt{\frac{\sum(xi_1 - \bar{x}_1)^2 + \sum(xi_2 - \bar{x}_2)^2}{N_1 + N_2 - 2}} \qquad (2-13)$$

xi_1 and xi_2 are values of measurements of method one and method two respectively. $\bar{x}_1\ and\ \bar{x}_2$ are means of measurements of method one and method two. F-test is used to compare two methods to know the difference in the precisions, calculate by the equation below.

$$F = \frac{s_1^2}{s_2^2} \qquad (2-14)$$

Where, S_1 and S_2 are the standard deviations of the data to two methods on secession. In this method T-test and F-test were calculated by using equations above [117,118].

2-7: Spectrophotometric technique developed for determining dapsone in pharmaceutical preparations.

2-7-1: Study of the dapsone absorbance spectrum

The aim of the spectrophotometric investigation is to determine the maximum absorbance wavelength λ_{max} of the red-pink color product formed from the reaction of dapsone with the analytical reagent 4,5-DPI.

In a volumetric flask of 10 mL, combine 1 mL of 25 μg/mL dapsone solution with 1.0 mL of 1.0 M HCl solution and 1.0 mL of 0.5 M $NaNO_2$, then cool by ice from 0-5°C for 10 minutes before adding 2.0 mL of 1x 10^{-3}M of 4,5-DPI solution was mixed with 1.25 mL of 1.0 M NaOH solution, then followed by adding (1.25 mL) of 0.2M of sulfamic acid. The final azo solution formed is left for 5 min until the azo was stable, and the absorbance of the stabilized solution is measured spectrophotometrically at 501 nm using 1.0 cm cells against the blank solution, which is prepared in the same manner as above but without the addition of dapsone.

2-7-2:Optimum conditions

The ideal conditions and their impact on the color development and absorbance of the azo produced by the reaction of dapsone with 4,5-DPI.

2-7-2-1: Effect of reagent concentration

The effect of reagent concentration on absorbance was investigated by placing various volumes of reagent solution (0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 2.50) mL with a concentration of 1 x 10^{-3}M in a series of 10 mL volumetric flasks under other optimal conditions, then measuring the absorbance at 501 nm and recording the absorbance with the highest value.

2-7-2-2: Effect of the base

The synthesis of the azo compound of dapsone is explored using(0.25, 0.50, 0.75, 1.00, 1.50, and 2.00) mL of (1.0M) sodium hydroxide NaOH to determine the volume of sodium hydroxide that provides the maximum absorbance of the azo compound.

2-7-2-3: Effect of acid

The effect of hydrochloric acid concentration is also investigated by preparing various volumes of (0.1 M) acid solution of (0.25, 0.50, 0.75,1,1.25, and 1.75) mL and then determining the best volume to produce the azo-colored with the highest absorbance.

2-7-2-4 :Effect of sodium nitrite

In addition, different volumes of (0.5M) sodium nitrite solution (0.25, 0.50, 0.75, 1.00, 2.25, and 1.50) mL were taken and the absorbance for each volume is recorded.

2-7-2-5 : Effect of sulfamic acid

Different volumes of sulfamic acid (0.25, 0.5, 0.75, 1.00, 1.25, and 1.50) mL are used to determine the best amount to use to break down the excess sodium nitrite and obtain the highest absorbance intensity of the formed azo. Based on the intensity of absorbance of the formed product, the best amount of sulfamic acid with a concentration of 0.2 M is studied.

2-7-2-6: Effect of the time required to dismantle the excess of nitrous acid Formed

To get the maximal absorption intensity of the produced azo, the time needed to eliminate an excess of nitrous acid by sulfamic acid is studied at various times (1–5 min).

2-7-2-7: Effect of temperature

Different temperatures are used (25, 30, 35, 40, 45, 50, 55, and 60) ^{o}C to find out the effect of temperature on the absorbance values of the azo-dye compound, water bath is used and heated at (5 minutes).

2-7-2-8: Effect of time on the stability of azo-dye compound

The reaction product's stability was investigated by measuring the absorbance of the resulting colored solution after (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50) minutes. The best time to complete the reaction was known under the best conditions.

2-7-3: Recommended procedure of Dapsone

In a series of 10 mL volumetric flasks, amounts of dapsone solution are added to give a final dapsone concentration within a range of 2–18 µg/mL, followed by 1.0 mL of 1.0 M hydrochloric acid solution and 1.0 mL of 0.5 mole/L sodium nitrite solution to form a diazonium salt. All flasks are cooled in an ice bath at 0–5 °C for approximately 10 min. Finally, a cold solution containing 2.0 mL of (0.001M) of 4,5-DPI in 1.25 mL of 1.0 M sodium hydroxide solution is added and left aside until a red azo dye color appeared as a result of coupling between diazotized dapsone and 4,5-DPI, then followed by adding 1.25 mL of 0.2M of sulfamic acid, After 5 minutes

at 0°C, 10 mL flask was filled with distilled water, the absorbance of each solution is measured at a maximum wavelength of 501 nm using a 1.0 cm cell against a blank solution prepared using the same method but without the addition of the drug.

2-7-3-1: Molar absorptivity and Sandell's sensitivity

The molar absorptivity and Sandell's sensitivity were calculated using (2-2) and (2-3) equations.

2-7-3-2: Limit of detection LOD and limit of quantification LOQ

These calculations are also calculated using equations (2-4) and (2-5) mentioned previously in this chapter.

2-7-4: Accuracy and precision

Accuracy and precision are investigated by taking three volumes of dapsone solution (0.8, 1 and 1.2 mL), equivalent to (4, 8, and 12) ppm in the final volume, and after adding all the additions and completing the volume to 10 mL to prepare the compound produced by the reaction of dapsone with 4,5-DPI, absorbance is measured three times for each concentration, and accuracy and precision are assessed using equations (2-7), (2-8) and (2-9).

2-7-5: Mole ratio method and continuous variation method(job's method)

By using the molar ratio method and continuous variation method (Job' s method), the reaction mechanism is obtained.

2-7-5-1: Mole ratio method

The mole ratio method is used for the estimation of the stoichiometric ratio of the product formed from the reaction of diazotized dapsone drug with 4,5-DPI reagent. This experiment is completed by taking a series of volumetric flasks of 5 mL capacity and placing 1 mL of dapsone solution with a molar concentration of (0.001 M). Then, in the ice for 10 minutes, 1.0 mL of (1.0 M) hydrochloric acid and 1 mL of 0.5 M sodium nitrite solution are added to each of them. Then these solutions are

added to another series of solutions consisting of (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8) mL of the reagent solution with a concentration of 1×10^{-3} M in an alkaline medium 1.25 mL each of them at a concentration 1.0 M, The absorbance of the solutions is measured at λ_{max}501 nm to find the ratio of the reagent to the drug.

2-7-5-2 : continuous variation (job's method)

The continuous changes method is also used to find the ratio of the reagent to the drug for the product of the reaction of the dapsone drug with the 4,5-DPI reagent. This experiment is carried out by taking two series of volumetric flasks, The first series has a capacity of 10 mL, in which different volumes of the drug are placed (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4, 4.5) mL, and 1.0 mL of hydrochloric acid of (1.0 M) concentration is added to it, as well as 1mL of sodium nitrite at a concentration of 0.5M in ice at a temperature of 0-5ºC . As for the other series of volumetric flasks, they were with a capacity of 10mL , in which different volumes of the reagent are placed (4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5) mL, thus completing the final volume of the drug and the reagent equal to 10 mL , 1.25 mL of sodium hydroxide was added to it, then the solutions are mixed and left for a ten minutes to complete the reaction, Then 1 mL of sulfamic acid was added to it and the volume is completed to (10 mL) with distilled water and the absorbance of the resulting solutions is measured, by using 1cm cell at λ_{max}501nm.

2-7-6: Effect of the Excipients

The effect of some additives (excipients) that are present with pharmaceutical preparations is studied, where quantities of these substances are individually added to (4μg/mL) of dapsone at an increase of (10) folds more than the concentration of dapsone, i.e. 400 μg/mL, to a series of volumetric flasks of (10 mL) capacity, following the same steps as indicated in the standard calibration curve. The effect of additives is considered acceptable if the error rate does not exceed (+2%) compared

to the measurements in the absence of interferences, and through the values of (Error%) and (Recovery%), noticed that there is no effect of the presence of such additives on the spectral method used to estimate dapsone using the reagent 4,5-DPI.

2-7-7:Analytical applications

The solutions are prepared in the manner described in paragraph (2-5-3-B) in order to determine the method's selectivity so that it could be applied to some pharmaceutical preparations containing dapsone.

2-7-8 : Mechanism of reaction

The mechanism of the stoichiometric reaction between dapsone and 4,5-DPI is suggested by the estimation of the stoichiometric ratio of the product formed by the continuous variation method and mole ratio method.

Chapter Three

The Results and Discussions

3-1: Development of spectrophotometric method for the determination of ceftriaxone in pharmaceutical preparations

3-1-1: Absorbance spectral study of ceftriaxone

A purple azo dye is prepared under optimal conditions by combining diazotized ceftriaxone with 4,5-DPI in the alkaline medium as a reagent. This colored azo exhibited an absorbance maximum at λ_{max} 550 nm against reagent blank as shown in Figure (3-1).

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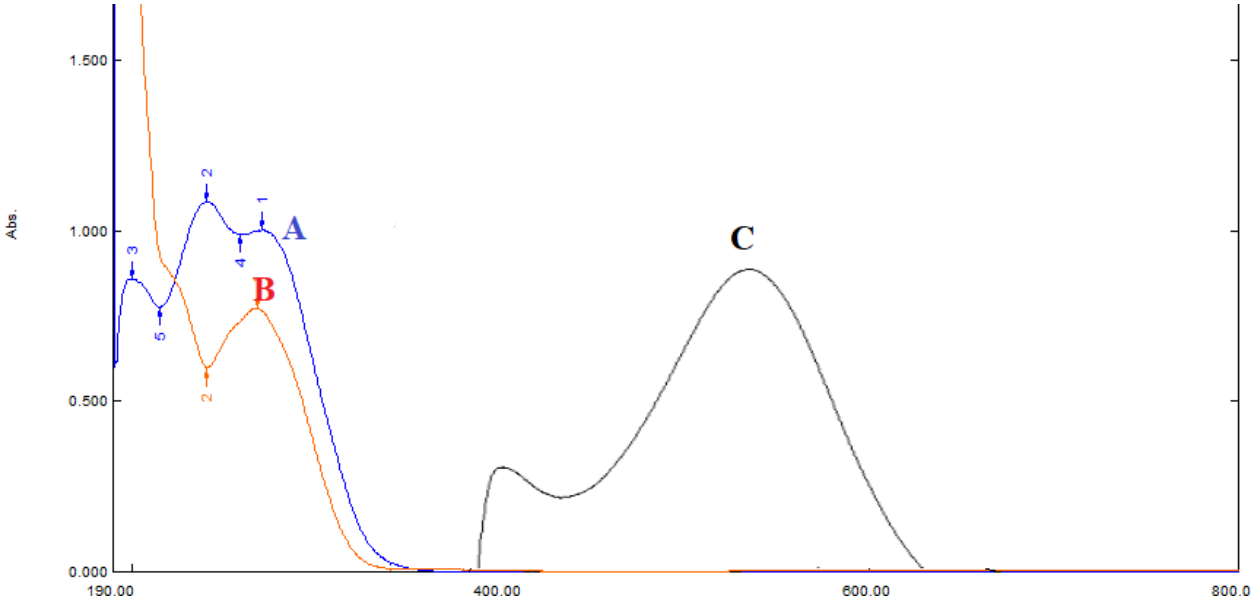


Figure (3-1): A-absorbance of Ceftriaxone drug λ_{max} (220 nm).

B-absorbance of (4,5-DPI) λ_{max}(250nm).

C-absorbance of a conjugated complex of (drug + reagent) λ_{max}550nm .

3-1-2: Optimum conditions

3-1-2-1: Effect of reagent concentration

The effect of various amounts of the 4,5-DPI reagent (1 $x10^{-3}$M) on the purple azo product's absorbance is studied as shown in paragraphs 2-6-2-1. The data is listed in Table (3-1) and Figure (3-2).

Table (3-1): absorbance of purple azo at different volumes of 4,5-DPI reagent.

NO	Volume of reagent(mL)	Absorbance
1	0.25	0.050
2	0.50	0.071
3	0.75	0.093
4	1.00	0.074
5	1.25	0.067
6	1.50	0.060

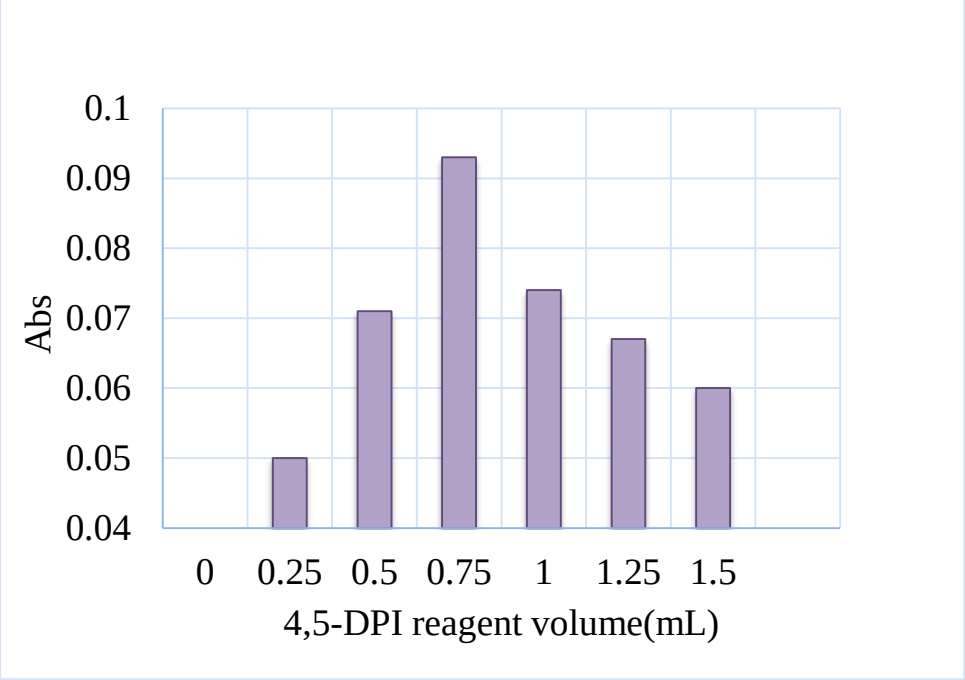


Figure (3-2): Absorbance of purple azo product at different volumes of 4,5-DPI.

From the previous results, it was found that the significant volume of reagent 4,5-DPI was 0.75ml. This volume gave the highest absorbance for ceftriaxone azo product with 4,5-DPI reagent.

3-1-2-2: Effect of base

The effect of various volumes of 1.0 M NaOH on the absorbance of the purple color of the ceftriaxone diazotization product mentioned in paragraph 2-6-2-2 is studied, and the results obtained are shown in Table (3-2) and Figure (3-3).

Table (3-2): Absorbance of purple azo at different volumes of NaOH

NO	Volume of NaOH (mL)	Absorbance
1	0.25	0.047
2	0.50	0.049
3	0.75	0.051
4	1.00	0.064
5	1.25	0.081
6	1.50	0.047

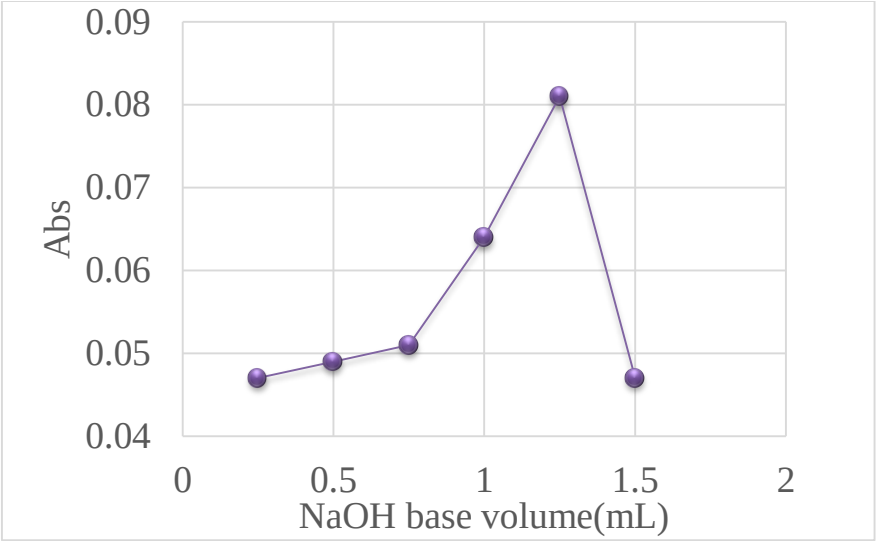


Figure (3-3): Absorbance of purple azo product at various volumes of NaOH

From the result, a significant volume of sodium hydroxide solution is found at 1.25 mL to give a higher absorbance of the product ceftriaxone with 4,5-DPI reagent, exceeding the base volume, causing a rapid decrease in the absorbance value of the azo dye. This may be due to the partial dissociation of the azo dye.

3-1-2-3: Effect of acid

As mentioned in paragraphs 2-6-2-3, the effect of volumes of (1.0M) HCl on the absorbance of purple azo products is studied. Table (3-3) and Figure (3-4) show the obtained results.

Table (3-3): Absorbance of purple azo at different volumes of HCl acid

NO	Volume of HCl(mL)	Absorbance
1	0.25	0.009
2	0.50	0.126
3	0.75	0.063
4	1.00	0.042
5	1.25	0.025

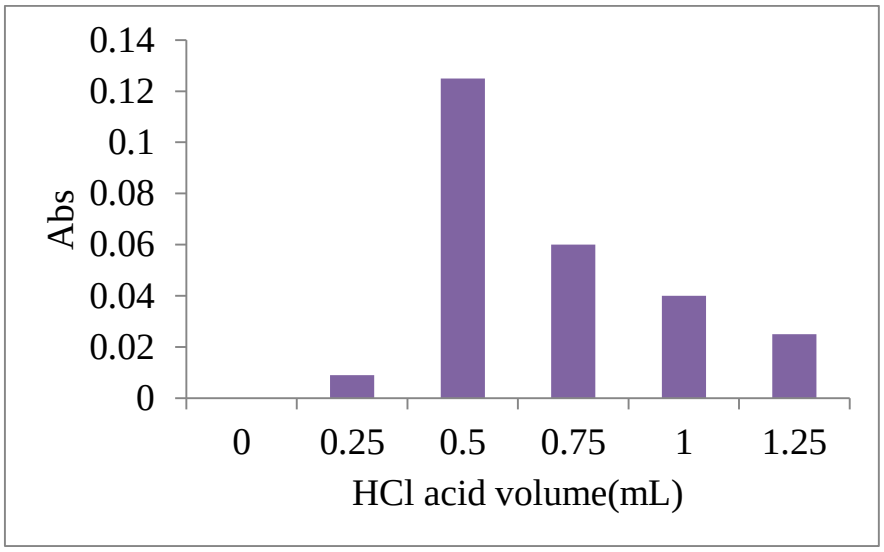


Figure (3-4): Absorbance of purple azo product at different volume of HCl.

According to the results, the optimal volume of (1.0M) HCl acidic solution is 0.5mL. Above this volume, the absorbance of the azo compound decreases rapidly, which may be attributed to the dissociation of the diazotized ceftriaxone. In this study, 0.5 mL of a (1.0M) HCl solution is used because it provided the highest absorbance value for the purple azo product.

3-1-2-4: Effect of Sodium Nitrite

As mentioned in paragraphs 2-6-2-4, the effect of different volumes of (0.5 M) sodium nitrite $NaNO_2$ on the absorbance of a purple azo product is studied. The obtained results are shown in Table (3-4) and Figure (3-5).

Table (3-4): Absorbance of purple azo at different volumes of $NaNO_2$.

NO	Volume of $NaNO_2$(mL)	Absorbance
1	0.25	0.068
2	0.50	0.107
3	0.75	0.143
4	1.00	0.105
5	1.25	0.064
6	1.75	0.042

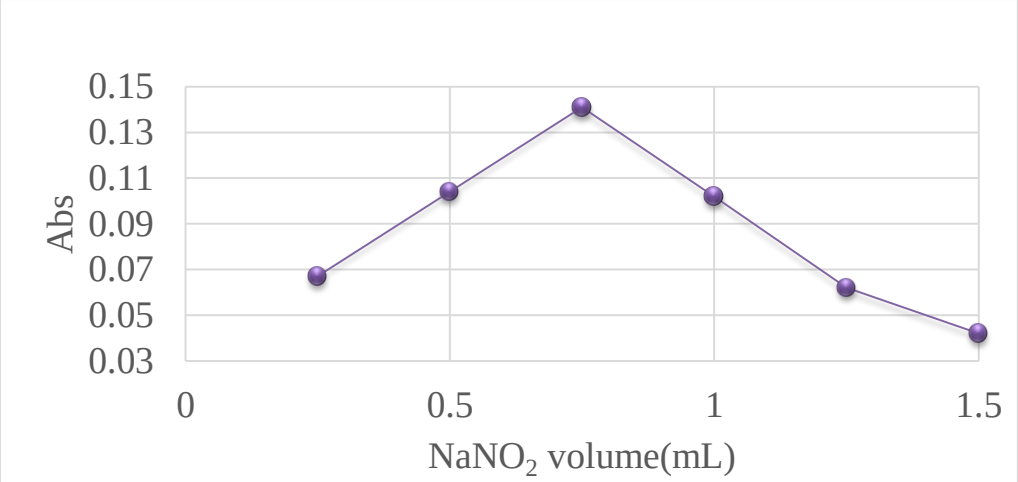


Figure (3-5): Absorbance of purple azo product at various volumes of $NaNO_2$

The results showed that the optimal volume of 0.5 M $NaNO_2$ was 0.75 mL. This volume of $NaNO_2$ is the significant due to give a highest absorbance of the purple azo product. Above 0.75 mL, its absorbance decreased due to the formation of a large excess of HNO_2, which affected the quantity of diazotized ceftriaxone.

3-1-2-5: Effect of sulfamic acid

As mentioned in paragraph 2-6-2-5, the effect of 0.2 M sulfamic acid NH_2SO_3H volumes on the absorbance of purple azo dye product is studied. The obtained results are shown in Table (3-5) and Figure (3-6).

Table (3-5): **Absorbance of purple azo at different volumes of sulfamic acid**.

NO	Volume of NH_2SO_3H(mL)	Absorbance
1	0.25	0.085
2	0.50	0.102
3	0.75	0.123
4	1.00	0.093
5	1.25	0.085
6	1.50	0.083

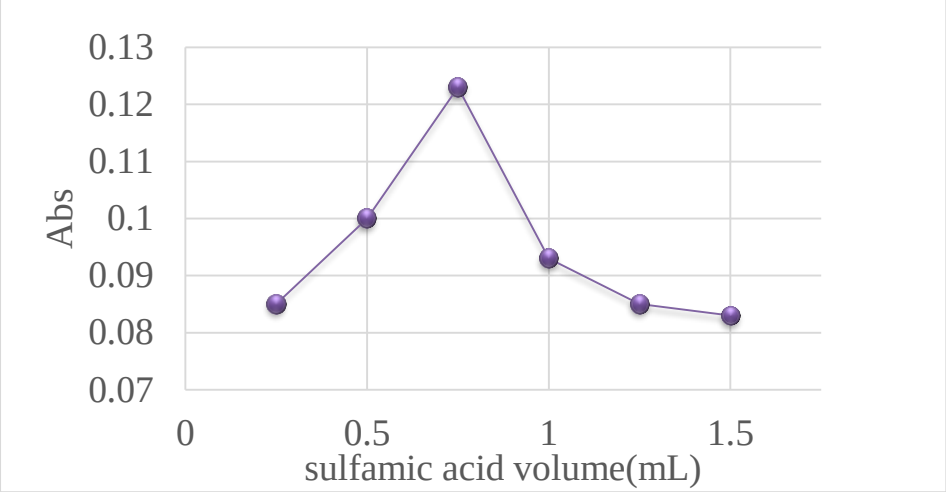


Figure (3-6): Absorbance of purple azo product at various volumes of sulfamic acid.

The results revealed that 0.75 mL of 0.2 M sulfamic acid (NH_2SO_3H) solution provided the highest absorbance value, which may have contributed to the removal of all excess HNO_2 formed in the diazotized ceftriaxone solution. The absorbance values decreased above 0.75 mL. It may be cauesed to a decrease in the amount of diazonium salt formed.

3-1-2-6: Effect of temperature

Temperature affects the stability of the compound, according to the summarized procedure mentioned in paragraphs 2-6-2-6, and this is known by measuring the absorbance at different temperatures. Table (3-6) and Figure (3-7) show the result.

Table (3-6): Absorbance of azo compound at different temperatures.

NO	Temperature(ºC)	Absorbance
1	0	0.168
2	10	0.162
3	20	0.156
4	30	0.144
5	40	0.128
6	50	0.098

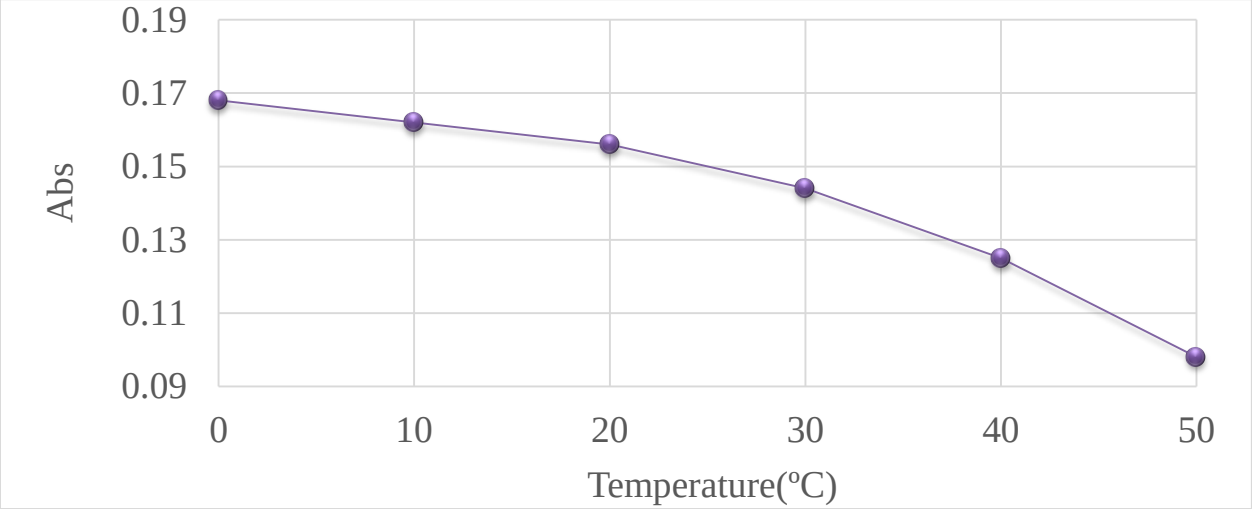


Figure (3-7): Absorbance of azo product at different temperatures.

Observed from the previous Table (3-6) and Figure (3-7) that the absorbance of the azo product decreases with increasing temperature and that the highest absorbance is at a temperature (0-20ºC) approximately, then decreases.

3-1-2-7: Effect of the time required to complete the formation of the diazonium salt.

The effect of the time required to complete the formation of the diazonium salt on the absorbance of a pinkish-red product is examined using the same procedure

described in paragraphs 2-6-2-7. The results are shown in Table (3-7) and Figure (3-8).

Table (3-7): Absorbance of purple azo at different times required to complete the formation of diazonium salt.

NO	Time(minutes)	Absorbance
1	1	0.560
2	5	0.780
3	10	0.942
4	15	0.607
5	20	0.481
6	25	0.418

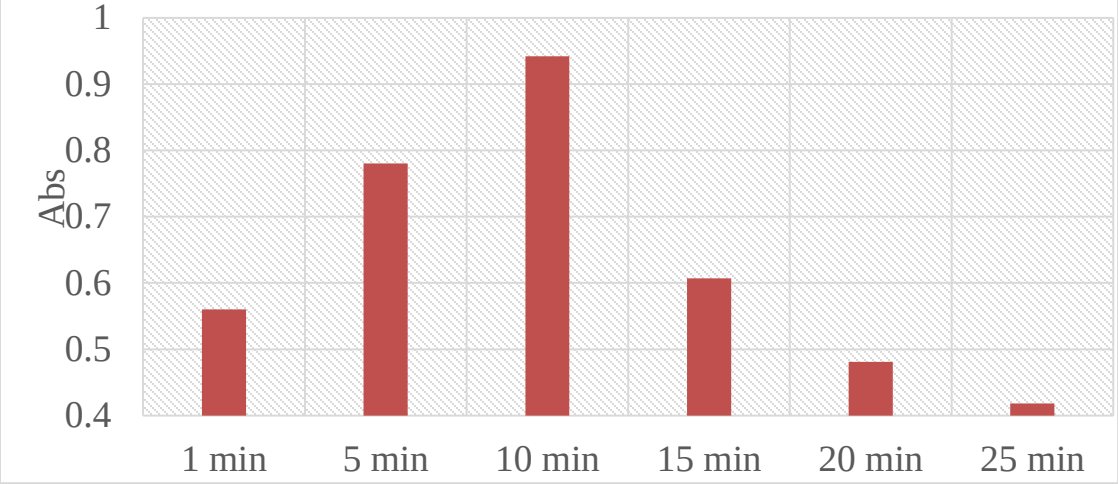


Figure (3-8): The absorbance of pinkish-red of diazonium salt at a different times.

The results from Table (3-7) and Figure (3-8) show that the optimum time required to complete the formation of the diazonium salt is 10 minutes at 0-5ºC. If it takes longer than 10 min. there will be a decrease in absorbance due to partially dissociation of a diazonium salt.

3-1-2-8: Effect of the removing time of nitrous acid

The effect of removal of an excess of nitrous acid solution remaining after diazotized ceftriaxone at various time intervals in the presence of 1.0 mL of (0.2 M)

sulfamic acid solution has been studied. Following the procedure outlined in paragraphs 2-6-2-8, the results are shown in Table (3-8) and Figure (3-9).

Table (3-8): The absorbance of purple azo at different times to breakdown of HNO_2.

NO	Time(minutes)	Absorbance
1	1	0.127
2	2	0.126
3	3	0.125
4	4	0.125
5	5	0.124

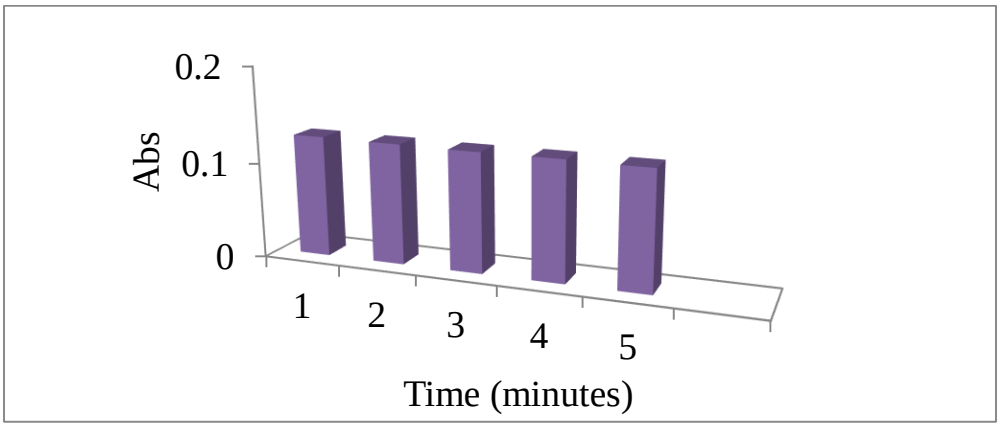


Figure (3-9) The absorbance of purple of AZO dye at different times to remove excess nitrous acid.

The results showed that one minute is the optimum time required to completely removed an excess of nitrous acid. Above this time, the absorbance of the purple azo product begins. This is confirmed in subsequent experiments.

3-1-2-9: Effect of stability time

The stability of the AZO dye product compound with time has been studied, as mentioned in paragraphs 2-6-2-9 of the previous chapter. Table (3-9) and Figure (3-10) shows the stability of the AZO product compound with time.

Table (3-9): Absorbance of the purple solution of azo dye at different times to determine the stability of the complex.

NO	Temperature(minutes)	Absorbance
1	5	0.144
2	10	0.162
3	15	0.161
4	20	0.157
5	25	0.151
6	30	0.147
7	35	0.144
8	40	0.140
9	45	0.139
10	50	0.131
11	55	0.129
12	60	0.124

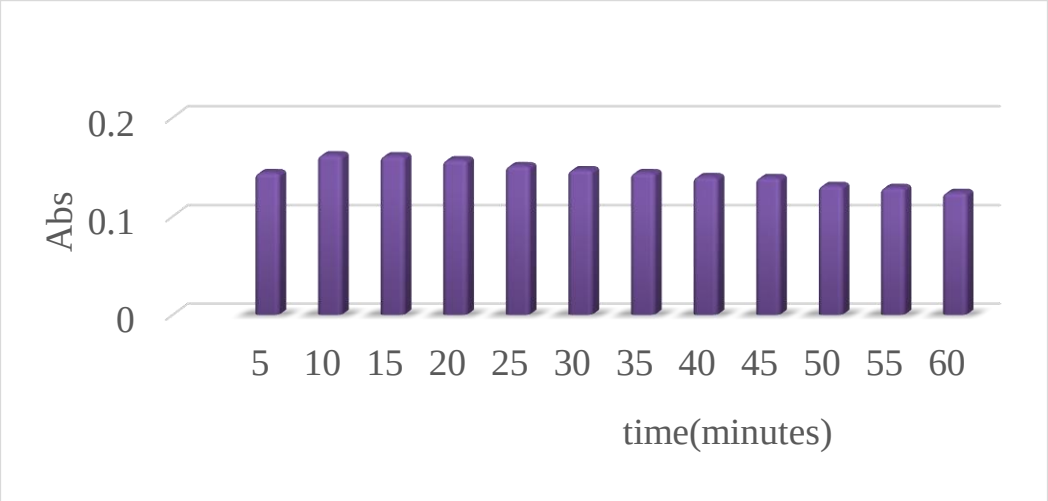


Figure (3-10): The absorbance of AZO dye product at different times to determine the stability.

It was noted from the previous Table and Figure that the absorbance slightly decreases after 10 minutes, due to the partial dissolution of the resulting compound

and that it remains stable for about an hour, therefore, the first ten minutes are chosen to measure the absorbance because it gave the highest intensity of absorbance, and this time was proven in subsequent experiments.

3-1-3: Calibration curve

After determining the optimum conditions, the calibration curve is constructed by using different concentrations of diazotized ceftriaxone and measuring the absorbance of each concentration (2,3,5,10,20,30,40,50,60,70,80, and 90) µg/mL of ceftriaxone solution, which is formed by reacting the drug with 4,5-DPI reagent as mentioned in paragraph 2-6-3. The results and calibration curve are shown in Figure (3-11).

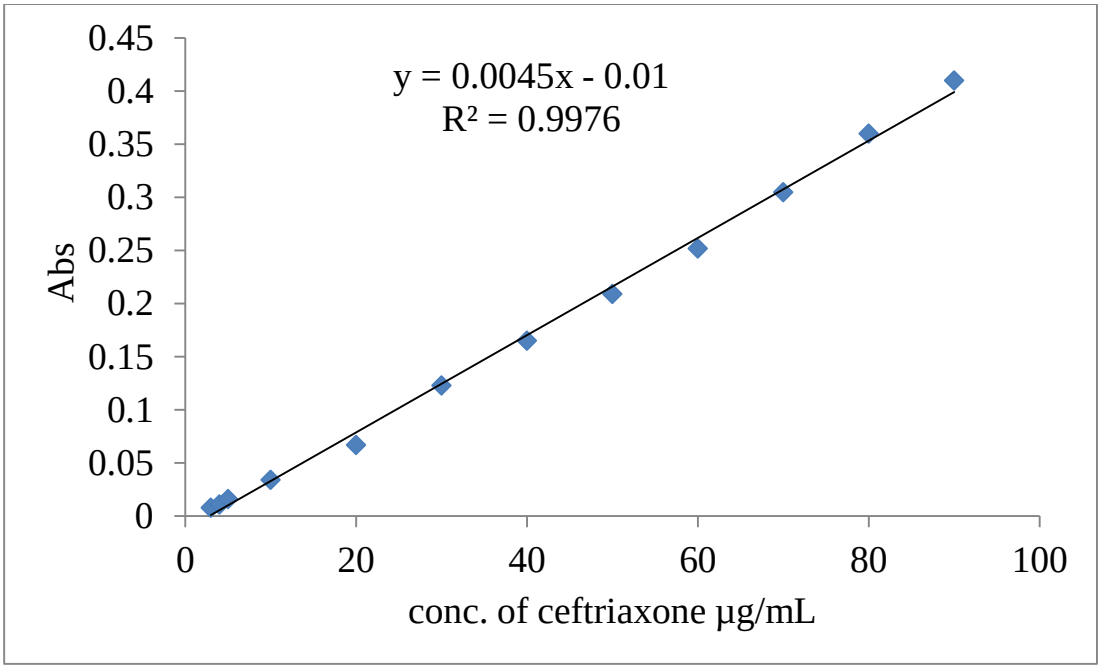


Figure (3-11): Calibration curve for the determination of ceftriaxone with 4,5-DPI reagent.

3-1-3-1: Sandell's sensitivity and molar absorptivity.

Sandell's sensitivity and molar absorptivity are calculated using equations (2-2) and (2-3) in this method, and other information obtained from the calibration curve are shown in Table (3-10).

3-1-3-2: Limit of detection LOD and limit of quantification LOQ.

The detection limit in this method LOD, and limit of quantifications LOQ are calculated using equations (2-4) and (2-5), and other information obtained from the calibration curve is shown in Table (3-10).

Table (3-10): Analytical values of statistical treatments of the calibration curve of the ceftriaxone determination procedure.

parameter	value
Equation of regression	Y=0.0045x-0.01
Slope	0.0045
Correlation coefficient	0.9976
Linear range (µg/mL)	2.0-90
ε (L. moL^{-1}. cm^{-1})	3536.3
L.O.D (µg.mL^{-1})	0.06
L.O.Q µg.mL^{-1}	0.19
S (µg.cm^{-2})	0.22
R.S.D%	0.089

3-1-4: Accuracy and precision

The accuracy and precision of this method are calculated using three parameters, Relative Error E%, Recovery percentage (Rec%), and Relative standard deviation percent (RSD%), using the equations (2-7), (2-8), and (2-9). The results from reading the absorbance of the purple azo product ceftriaxone with 4,5-DPI at three drug concentrations and calculating the concentrations from the calibration curve are shown in Tables (3-11) and (3-12).

Table (3-11): Concentration results were obtained using the method.

NO	Ceftriaxone Taken(µg/mL)	Absorbance	Ceftriaxone found (µg/mL)	RSD%
1	50	0.209	48.67	0.058
		0.208	48.45	
		0.208	48.45	
2	60	0.252	58.22	0.15
		0.249	57.56	
		0.250	57.78	
3	70	0.304	69.78	0.058
		0.303	69.56	
		0.303	69.56	

The method by results above is used to calculate the relative error E%, recovery percentage (Rec%), and relative standard deviation percent (RSD%), and it is

discovered that there is a good agreement of RSD%, Rec%, and E% with the conventional method.

Table (3-12): parameters values of accuracy and precision

NO	Ceftriaxone in µg/mL		E%	Recovery%
	taken	found		
1	50	48.52	-2.9	97.1
2	60	57.86	-3.5	96.5
3	70	69.63	-0.52	99.48

3-1-5: Continuous method (Job's method) and mole ratio method.

Using the job's continues variation method and the mole ratio method. Following the procedure outlined in paragraph 2-6-5-1. Figures (3-12) and (3-13) show the stoichiometric product formed from the reaction of diazotized ceftriaxone with 4,5-DPI using continuous variation (job's method) and mole ratio methods. The results show that the azo dye is formed in a 1:1 ratio (drug: reagent).

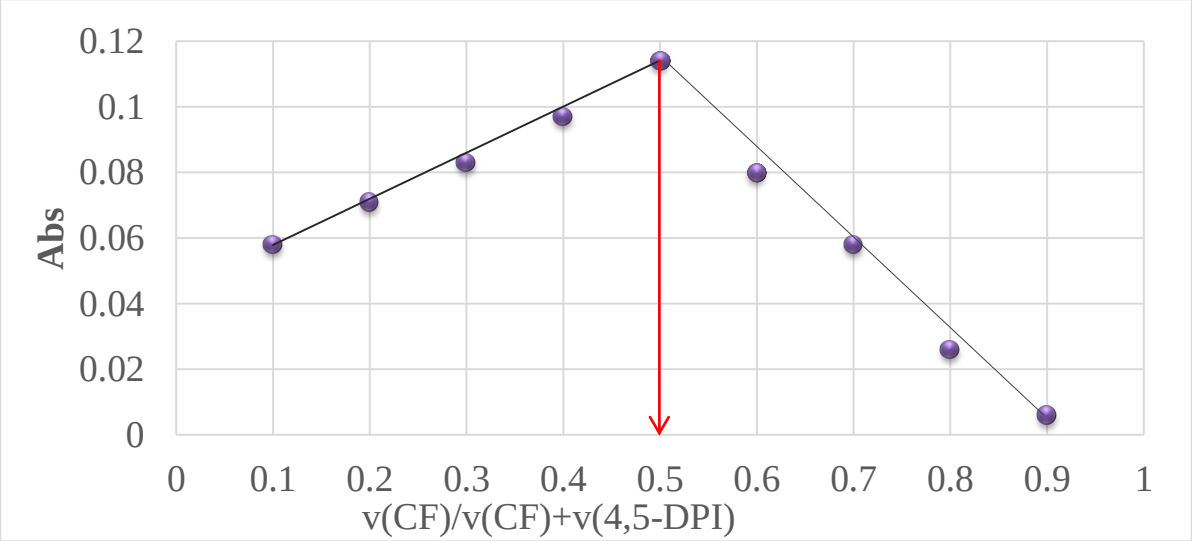


Figure (3-12): Job's method for the coupling between ceftriaxone with 4,5-DPI

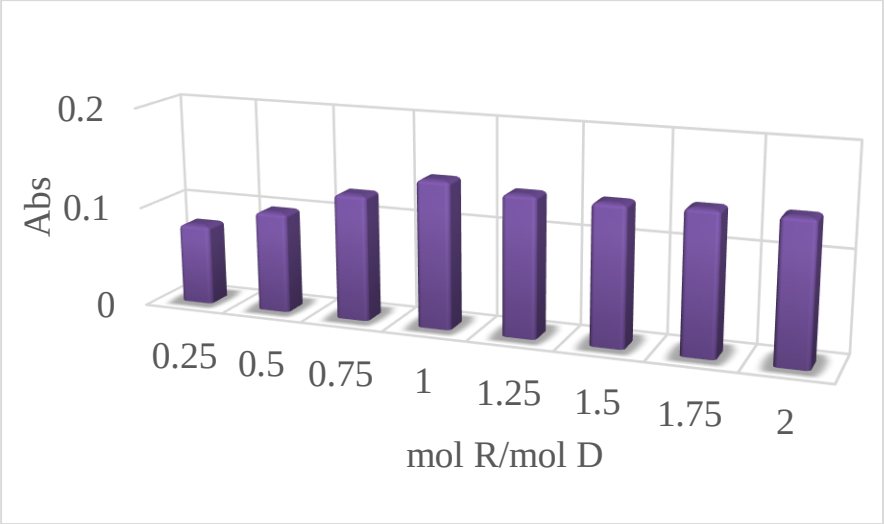
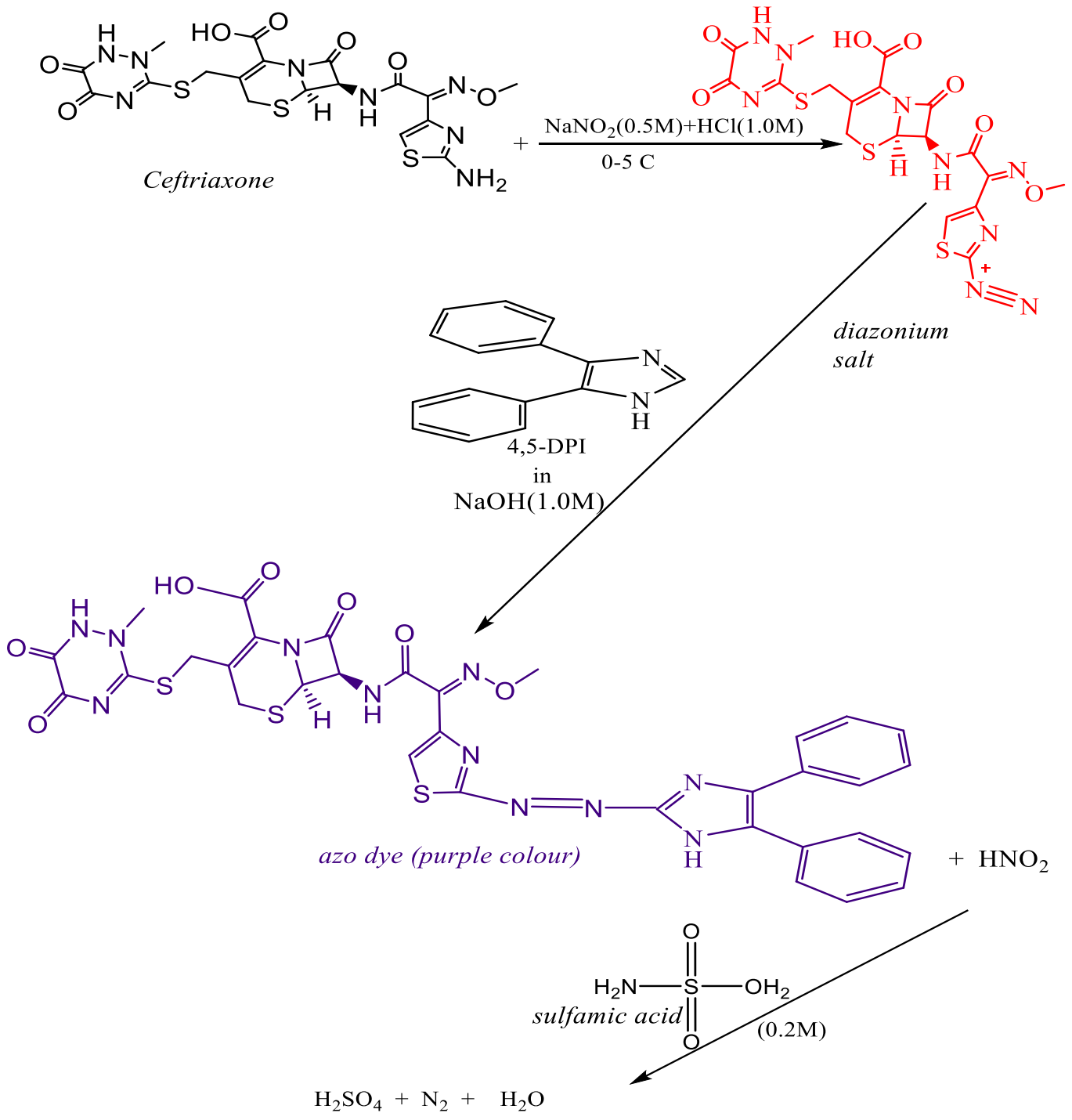


Figure (3-13): Mole ratio for the reaction between ceftriaxone with 4,5-DP

3-1-6: Mechanism of reaction



Scheme (3-1): Mechanism proposed for the reaction between ceftriaxone and 4,5-DPI.

3-1-7: Analytical applications

According to the procedure described in paragraph 2-6-8, the proposed method is successfully applied for the quantitative determination of Ceftriaxone in its pharmaceuticals injection. The obtained data are compared statistically to those obtained from the reported and official method to get T-test and F-test [119]. The results in Table (3-13) indicate that this method has good precision and accuracy.

Table (3-13): Applications of the determination of ceftriaxone in pharmaceutical preparations.

Applications in pharmaceutical	Con. Taken (µg /mL)	Con. Found (µg /mL)	E %	Recovery %	R.S.D %	T-test	F-test
Ceftriaxone Injection (500 mg) (India)	50	48.1	-3.8	96.2	1.68	0.46	0.198
	60	57.6	-4.0	96.0	4.05		
	70	67.5	-3.5	96. 5	1.68		
Ceftriaxone Injection (1g) (L.D.P) Spain	50	47.9	-4.2	95.8	1.98		
	60	58.32	-2.8	97.2	2.15		
	70	69.5	-0.72	99.28	2.25		
Ceftriaxone Injection(1g) SANAVITA Germany	50	48.4	-3.2	96.8	0.55		
	60	60.24	0.4	100. 4	0.57		
	70	69.1	-1.2	98.8	1.25		

3-2: Development of spectrophotometric Method for determination of dapsone in pharmaceuticals

3-2-1: Absorbance spectra study of dapsone

A red azo dye is created under optimal conditions by combining diazotized dapsone with 4,5-DPI in a basic medium as a reagent. As shown in Figure (3-17), this colored azo had an absorbance maximum at λ_{max} 501 nm when compared to the reagent blank.

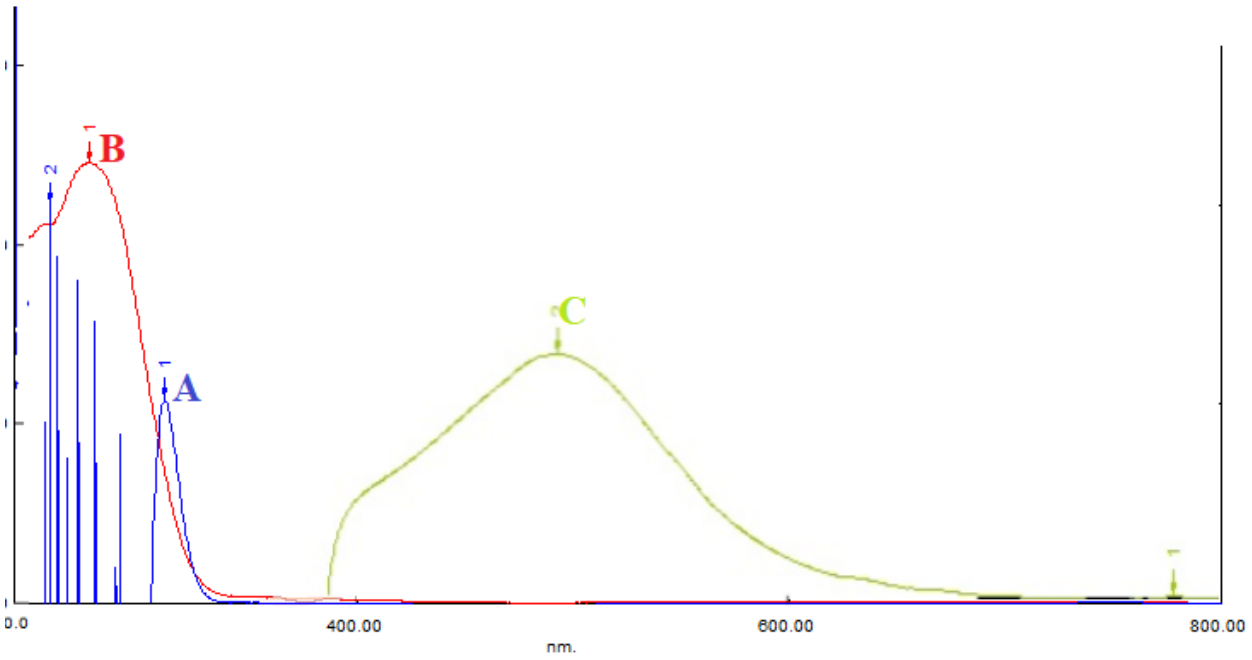


Figure (3-14): A-absorbance of dapsone drug λ_{max} (250 nm). B-absorbance of (4,5-DPI) λ_{max}(220nm) . C-absorbance of an azo dye of (drug + reagent) λ_{max}501nm.

3-2-2 Optimum conditions

3-2-2-1 Effect of concentration of 4,5-DPI reagent

As shown in paragraph 2-7-2-1, the effect of different amounts of the 4,5-DPI reagent (0.001M) on the absorbance of the red azo product is studied. Table (3-14) and Figure (3-15) show the data.

Table (3-14) absorbance of Red azo at different volumes of 4,5-DPI reagent.

NO	Volume of reagent(mL)	Absorbance
1	0.25	0.06
2	0.50	0.073
3	0.75	0.081
4	1.00	0.090
5	1.50	0.110
6	2.00	0.129
7	2.50	0.100
8	2.75	0.053

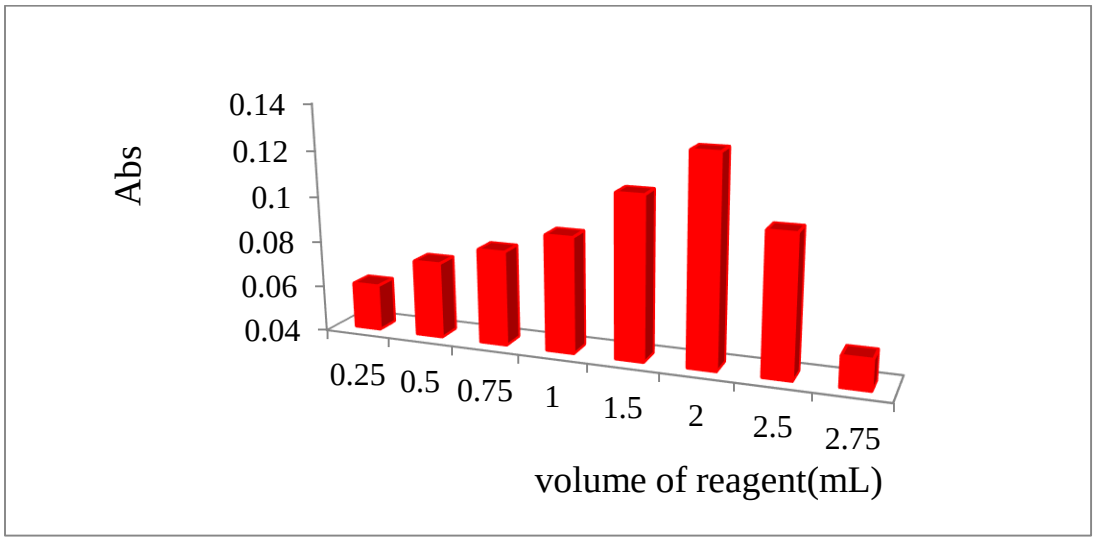


Figure (3-15): Absorbance of red azo product at different volumes of 4,5-DPI reagent.

From the previous results, it is found that the best volume of reagent 4,5-DPI is 2.0 mL. This volume gave the highest absorbance for dapsone azo products with 4,5-DPI reagent.

3-2-2-2: Effect of base

As mentioned in paragraph 2-7-2-2, the effect of different volumes of (1.0M) NaOH on the absorbance of the red azo product used in the spectrophotometric determination of dapsone was studied. The obtained results are shown in Table (3-15) and Figure (3-16).

Table (3-15): Absorbance of red azo at different volume of NaOH

NO	Volume of base(mL)	Absorbance
1	0.25	0.00
2	0.50	0.00
3	0.75	0.08
4	1.00	0.133
5	1.25	0.183
6	1.50	0.120
7	2.00	0.110

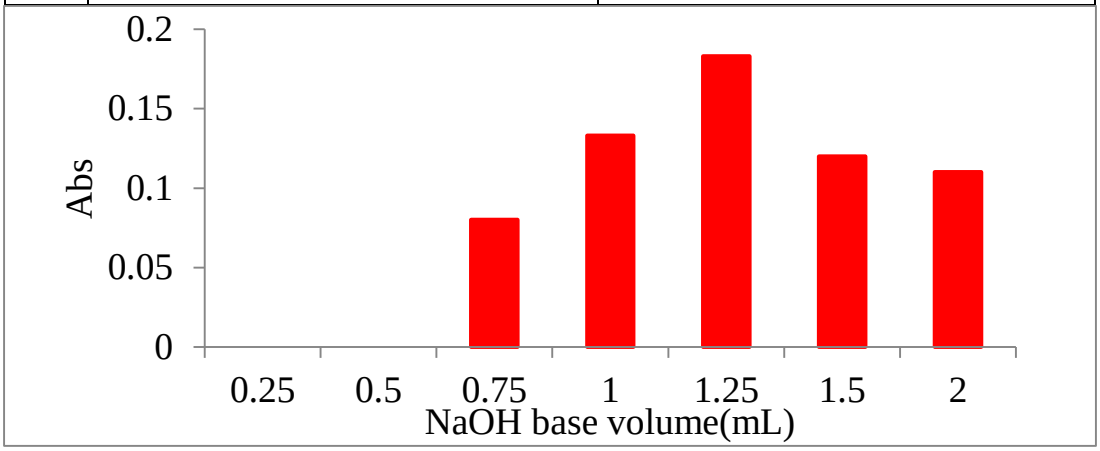


Figure (3-16): Absorbance of red azo product at various volumes of base.

From the previous results, it is found that the best volume of NaOH base is 1.25 mL. This volume gave the highest absorbance for dapsone azo products with 4,5-DPI reagent.

3-2-2-3: Effect of acid

The effect of volumes of (1.0 M) HCl acid on the absorbance of red azo products is studied, as mentioned in paragraphs 2-7-2-3. The results obtained are shown in Table (3-16) and Figure (3-17).

Table (3-16): Absorbance of red azo at different volume of HCl.

NO	Volume of acid(mL)	Absorbance
1	0.25	0.022
2	0.50	0.056
3	0.75	0.074
4	1.00	0.139
5	1.25	0.073
6	1.50	0.043
7	1.75	0.031

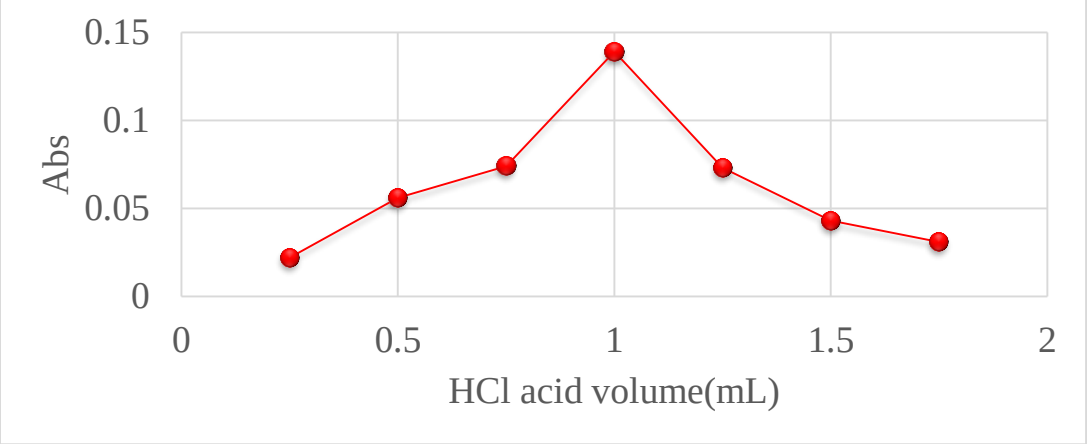


Figure (3-17): Absorbance of red azo product at various volumes of HCl acid.

According to the observations, the optimum volume of (1M) HCl acidic solution is 1.0 mL. As a result, 1.0 mL of HCl solution is used in this study because it gave the highest absorbance value of the red azo product.

3-2-2-4: Effect of Sodium nitrite

As mentioned in paragraphs 2-7-2-4, the effect of different volumes of (0.5M) sodium nitrite, $NaNO_2$ on the absorbance of the red azo product used is studied. The obtained results are shown in Table (3-17) and Figure (3-18).

Table (3-17): Absorbance of red azo at different volumes of $NaNO_2$

NO	Volume of $NaNO_2$(mL)	Absorbance
1	0.25	0.102
2	0.50	0.222
3	0.75	0.267
4	1.00	0.357
5	1.25	0.233
6	1.50	0.167
7	1.75	0.147

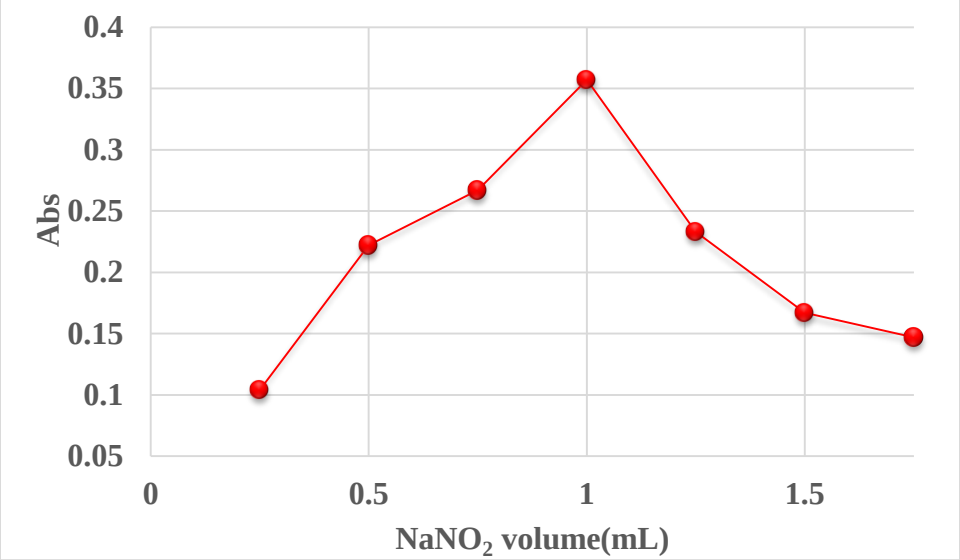


Figure (3-18): Absorbance of red azo product at various volumes of $NaNO_2$

According to the results, the best volume of (0.5 M) $NaNO_2$ is 1.0 mL, this volume of $NaNO_2$ is the best volume that will give a higher absorbance of the red

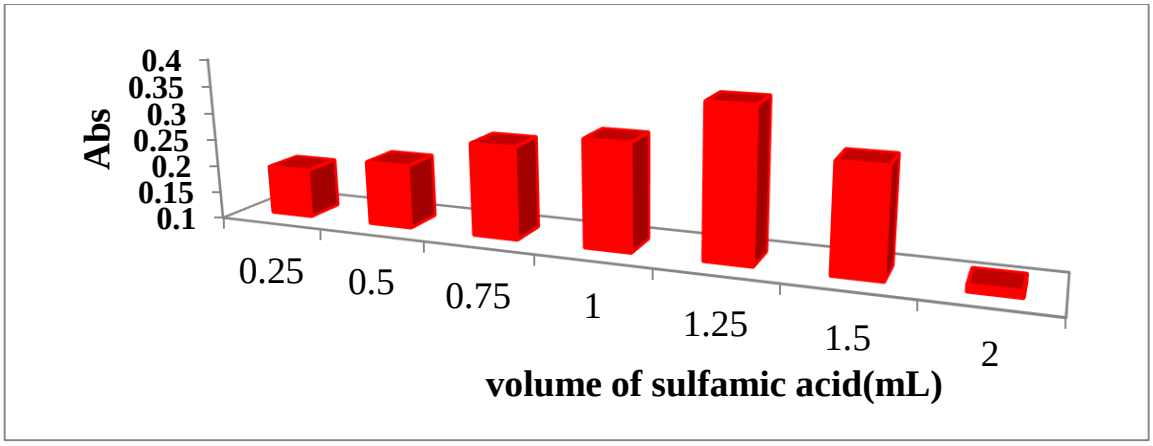
azo product. Excess sodium nitrite resulted in the formation of the nitrous acid HNO_2, which affected the amount of diazotized dapsone.

3-2-2-5: Effect of sulfamic acid

As mentioned in paragraphs 2-5-2-5, the effect of (0.20M) sulfamic acid volumes on the absorbance of red azo dye product used in the Spectrophotometric determination of dapsone. The obtained results are shown in Table (3-18) and Figure (3-19).

Table (3-18): Absorbance of red azo at different volumes of sulfamic acid.

NO	Volume of NH_2SO_3H(mL)	Absorbance
1	0.25	0.190
2	0.50	0.217
3	0.75	0.269
4	1.00	0.294
5	1.25	0.373
6	1.50	0.292
7	2.00	0.111



Figure(3-19): Absorbance of red azo product at various volumes of NH_2SO_3H.

According to the results, the highest absorbance value is 1.25 mL of (0.20M) sulfamic acid solution, which may have contributed to the removal of all excess

HNO_2 formed in diazotized dapsone solution. Absorbance values decrease above 1.25 mL. This concentration of sulfamic acid results in the greatest absorbance of the red azo product dapsone with 4,5-DPI.

3-2-2-6: Effect of the time required to remove the excess of nitrous acid.

The effect of removing an excess of nitrous acid solution remaining after diazotized dapsone at various time intervals in the presence of 1.0 mL of (0.20 M) sulfamic acid solution has been studied. Following the procedure outlined in paragraphs 2-5-2-6. The results are shown in Table (3-19) and Figure (3-20).

Table (3-19): The absorbance of red azo at different times to breakdown of nitrous acid

NO	Time (minute)	Absorbance
1	1	0.197
2	2	0.196
3	3	0.195
4	4	0.195
5	5	0.195

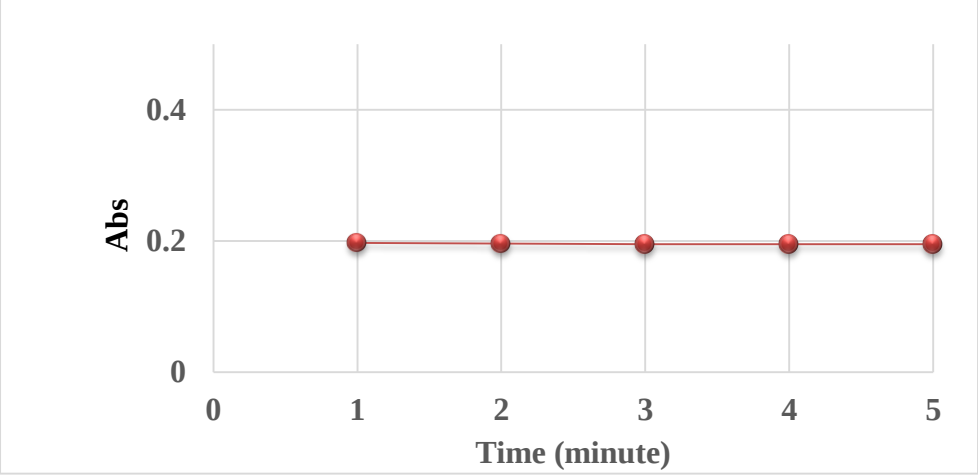


Figure (3-20): The absorbance of red of azo dye at different times to remove excess nitrous acid.

The results showed that one minute is the optimum time required to completely remove excess nitrous acid. The absorbance of the red azo product dapsone with 4,5-DPI decreases above this time, which is confirmed in subsequent experiments.

3-2-2-7: Effect of temperature

Temperature did not affect the stability of the compound formed by the reaction of dapsone with 4,5-DPI, as described in the summarized procedure in paragraph 2-7-2-7, and this is discovered by measuring absorption at various temperatures. The outcome is shown in Table (3-20) and Figure (3-21).

Table (3-20): Absorbance of azo compound at different temperatures.

NO	Temperature(°C)	Absorbance
1	0.0	0.204
2	10	0.205
3	20	0.205
4	30	0.204
5	40	0.204
6	50	0.204
7	60	0.203

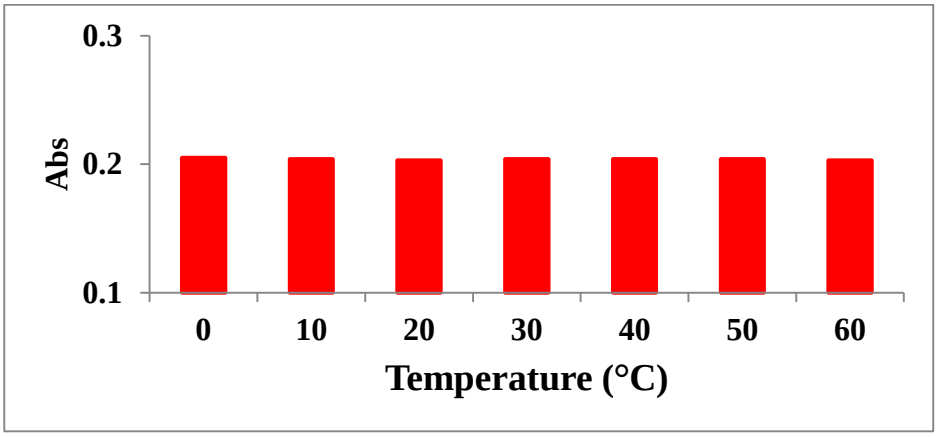


Figure (3-21): Absorbance of an azo dye of dapsone at different temperatures.

It was observed from Table (3-20) and Figure (3-24) that the compound produced from the coupling of dapsone with 4,5-DPI is stable at all temperatures.

3-2-2-8: Effect of stability time

The effect of stability time on the absorbance of a red azo product of dapsone is studied using the procedure described in paragraphs 2-7-2-8. The results are shown in Table (3-21) and Figure (3-22).

Table (3-21): Absorbance of red azo at different of stability time.

NO	Time (minute)	Absorbance
1	5	0.176
2	10	0.195
3	15	0.196
4	20	0.196
5	25	0.196
6	30	0.195
7	35	0.194
8	40	0.195
9	45	0.193
10	50	0.195

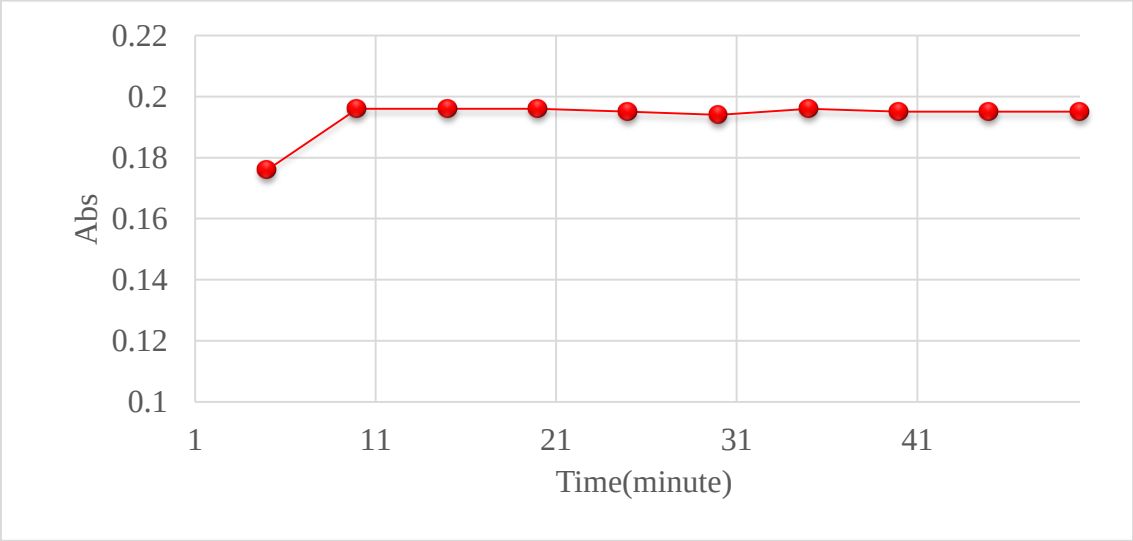


Figure (3-22): Colored product absorbance at various stability times.

Because the results show that the azo formed is stable for ten minutes because it gives the highest absorbance values at that time, ten minutes is chosen as an ideal time period in subsequent experiments.

3-2-3: Calibration curve

After determining the optimum conditions, the calibration curve is constructed by measuring the absorbance of each concentration (2,4,6,8,10,12, 14,16, and 18) µg/mL of azo dye solution, which is formed by reacting diazotized dapsone drug with 4,5-DPI reagent as mentioned in paragraphs Figure (3-23) depicts the results and calibration curve.

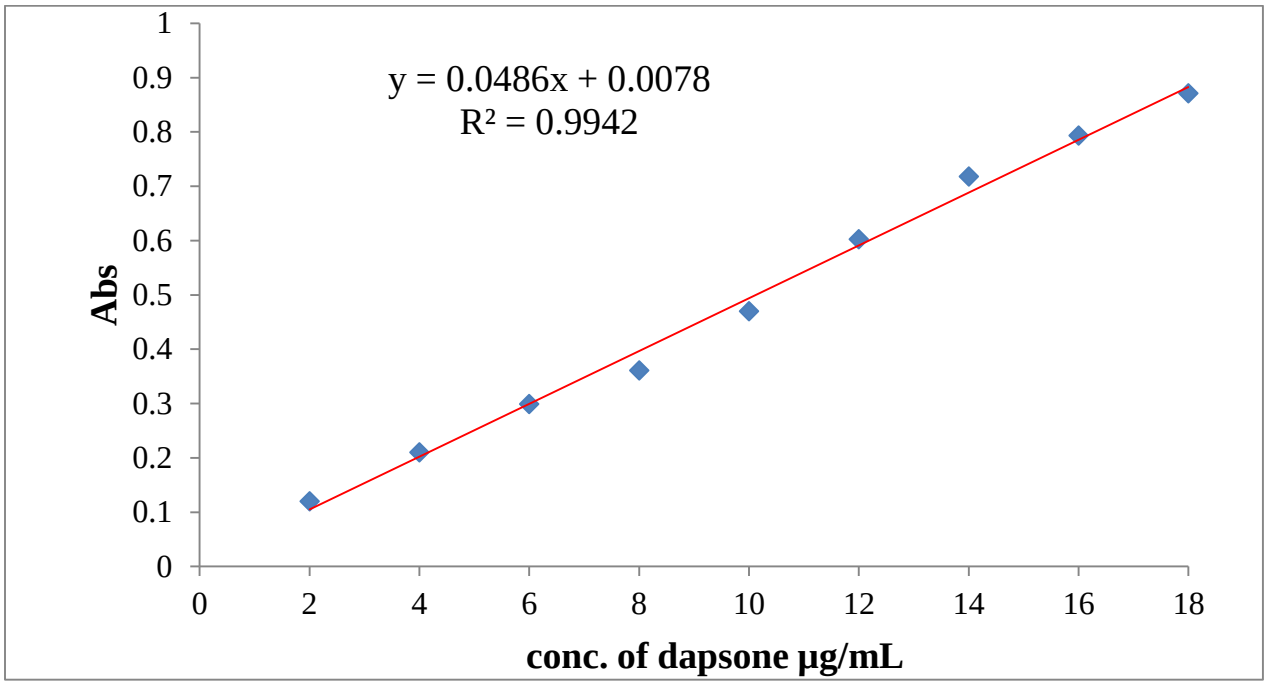


Figure (3-23): Calibration curve for the determination of dapsone with 4,5- DPI reagent.

3-2-3-1: molar absorptivity and Sandell's sensitivity

Sandell's sensitivity and molar absorptivity were calculated using equations (2-2) and (2-3). And Table (3-25) shows the other information obtained from the Calibration Curve.

3-2-3-2: Limit of detection LOD and limit of quantification LOQ

The detection limit in this method, LOD, and limit of quantifications LOQ were calculated using equations (2-4) and (2-5), And Table (3-22) shows the other information obtained from the Calibration Curve.

Table (3-22): Analytical values of statistical treatments of the calibration curve of the procedure developed to determine dapsone.

parameter	value
Equation of regression	Y=0.0486X+0.0078
Slope	0.0486
Correlation coefficient	0.9942
Linear range (µg/mL)	2-18
ε (L. moL^{-1}. cm^{-1})	34080
L.O.D (µg.mL^{-1})	0.29
L.O.Q (µg.mL^{-1})	0.96
S (µg.cm^{-2})	0.154
R.S.D%	0.88

3-2-4: Accuracy and precision

The accuracy and precision of the proposed method are calculated by using three individual concentrations of diazotized dapsone drug coupled with 4,5-DPI reagent and measuring three times the absorption at 501 nm. The parameters Relative Error (E%), Recovery Percentage (Rec%), and Relative Standard Deviation (RSD%) are investigated. The results are shown in Tables (3-23) and (3-24).

Table (3-23): Result and concentrations were found from the method.

NO	dapsone taken(µg/mL)	Absorbance	dapsone found (µg/mL)	R.S.D%
1	10	0.461	9.33	1.15
		0.472	9.55	
		0.475	9.61	
2	12	0.596	12.10	1.17
		0.605	12.28	
		0.610	12.39	
3	14	0.715	14.5	0.32
		0.711	14.4	
		0.711	14.4	

Table (3-24): values of parameters of accuracy and precision.

NO	dapsone in µg/mL		E%	Rec%
	taken	found		
1	10	9.5	-5	95
2	12	12.25	2	102
3	14	14.43	3	103

3-2- 5: Mole ratio method and continuous variation

Using the molar ratio method and the continuous variation method (jobs). According to the process mentioned in paragraphs 2-7-5. Figures (3-24) and (3-25) show the stoichiometric product formed from the reaction of diazotized dapsone with 4,5-DPI reagent. According to the results, the azo is formed in a 1:2 ratio (R2:1D).

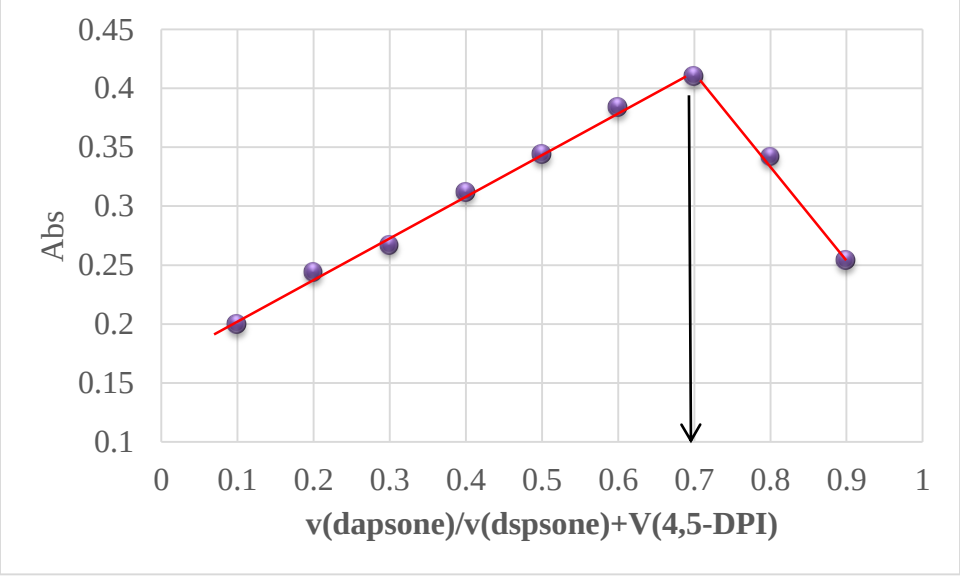


Figure (3-24): Job's method for the reaction between dapsone and 4,5-DPI.

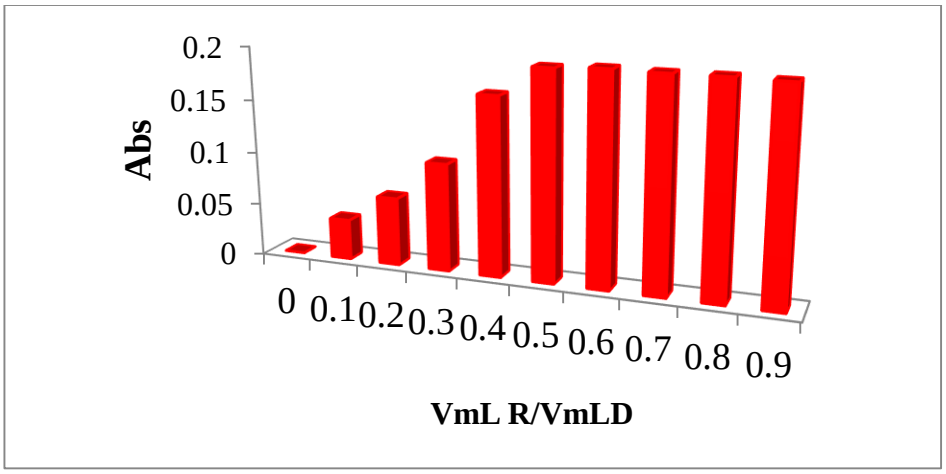
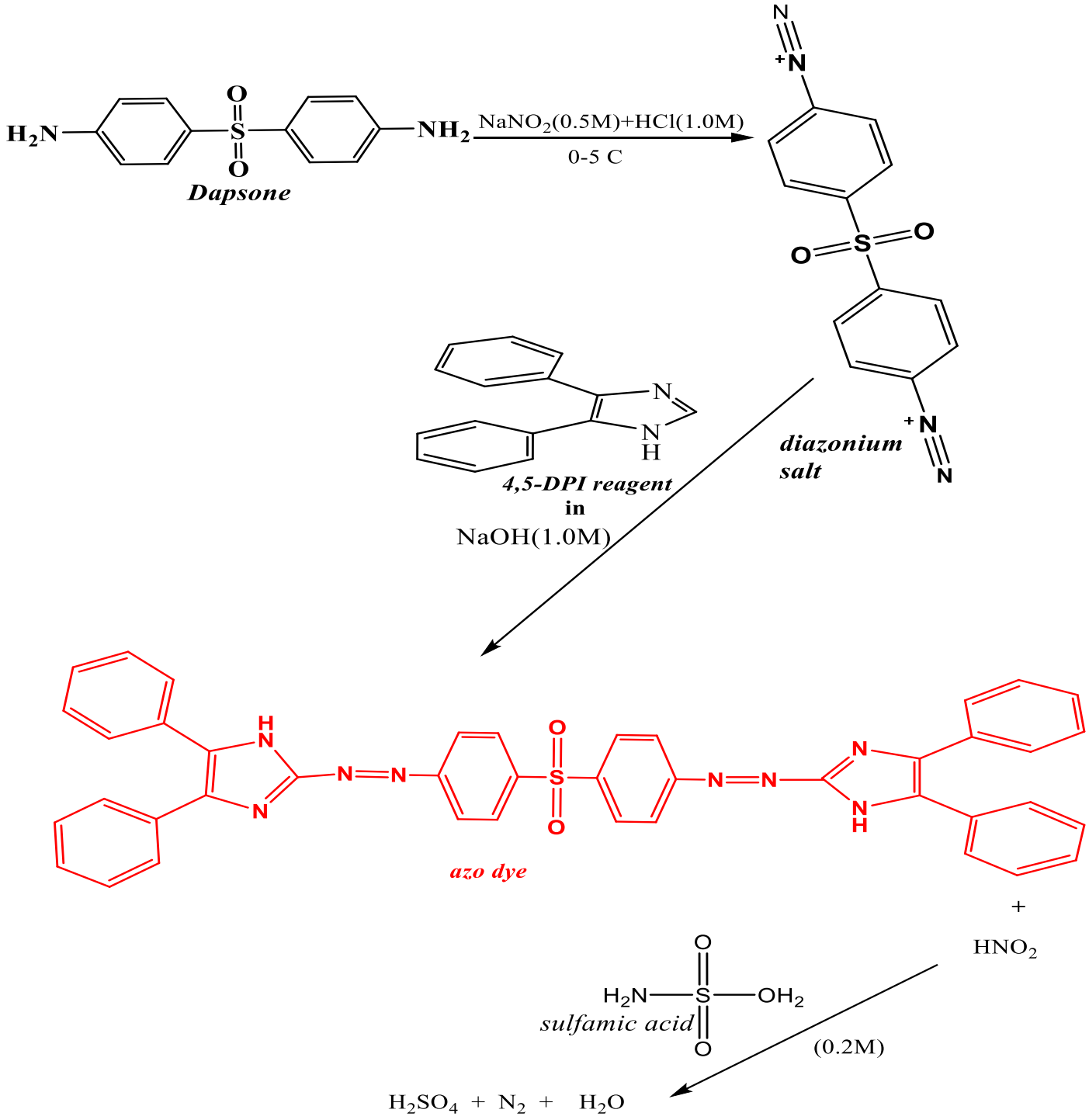


Figure (3-25): Mole ratio for the coupling between dapsone and 4,5-DPI.

3-2-6: Mechanism of reaction



Scheme (3-2): Mechanism proposed for the reaction between dapsone and 4,5-DPI.

3-2-7: Effect of Excipients

Studying the effect of adding the excipients (lactose monohydrate, magnesium stearate, maize starch, sodium lauryl sulfate, and colloidal anhydrous silica) present with dapsone does not affect absorbency because when crushing, grinding, and filtering the grains with acetone, the excipients do not dissolve in acetone, only dapsone does.

3-2-8: Analytical applications

The proposed method is successfully applied for the quantitative determination of dapsone in its tablet pharmaceutical preparations. Table (3-18) shows that the assay table solutions are prepared with good precision and accuracy, the absorbance of the amount taken from the reducing solution was measured, and the amount of dapsone is estimated, as shown in graphs 2-7-8. The relative error E% and recovery rate R% were also computed. The obtained data are compared statistically to those obtained from the reported and official method to get T-test and F-test [120]. The results are shown in Table (3-25).

Table (3-25): Applications of the method for determination of dapsone in pharmaceutical preparations.

Applications in pharmaceutical	concentration taken (µg / mL)	concentration found (µg / mL)	E%	Recovery (%)	R.S.D (%)	T-test	F-test
Dapsone Tablets GlaxoSmithKline Pharmaceutical Limited / Britain	4	3.96	-1	99.0	0.52	0.46	0.19
	8	7.8	-2.5	97.5	0.47		
	12	11.91	-0.75	99.25	0.49		
Dapsone Tablets DOMINA pharmaceuticals / Syria	4	3.81	-4.75	95.25	2.5		
	8	7.62	-4.75	95.25	2.71		
	12	11.92	-0.67	99.33	1.92		

3-2-9: Future prospects

1-Synchronous spectrophotometric method for simultaneous determination of the previously studied drugs and other amino drugs dithizied with the previous reagent studied.

2-Development of a new spectrofluorometric method for the determination of previously studied drugs.

3-Applied this method for the determination of the studied drugs in real samples.

References:

1-Kyung, K. H. (2012). Antimicrobial properties of allium species. Current opinion in biotechnology, *23*(2), 142-147.

2- Moya, A., Peretó, J., Gil, R., & Latorre, A. (2008). Learning how to live together: genomic insights into prokaryote–animal symbioses. Nature Reviews Genetics, 9(3), 218-229.

3-Chandra, N., and Kumar, S. (2017). Antibiotics producing soil microorganisms. In Antibiotics and antibiotics resistance genes in soils (pp. 1-18). Springer, Cham.

4-O'Shea, R., and Moser, H. E. (2008). Physicochemical properties of antibacterial compounds: implications for drug discovery. Journal of medicinal chemistry, 51(10), 2871-2878.

5-Berdy, J. (2005). Bioactive microbial metabolites. The Journal of antibiotics, 58(1), 1-26.

6- Mohr, K. I. (2016). History of antibiotics research. How to Overcome the Antibiotic Crisis, 237-272.

7- Parisien, A., Allain, B., Zhang, J., Mandeville, R., & Lan, C. Q. (2008). Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. Journal of applied microbiology, 104(1), 1-13.

8-Rind, F. M. A., Laghari, M. G. H., Memon, A. H., Khuhawar, M. Y., & Maheshwari, M. L. (2008). Spectrophotometric determination of ceftriaxone using 4-dimethylaminobenzaldehyde. Pakistan Journal of Analytical & Environmental Chemistry, *9*(1), 7.

9- Klein, N. C., and Cunha, B. A. (1995). Third-generation cephalosporins. The medical clinics of North America, *79*(4), 705-719.

10- Hafeez, S., Izhar, M., Ahmed, A., Zafar, A., & Naeem, M. (2000). In-vitro antimicrobial activity of Cefpirome: a new fourth-generation cephalosporin against clinically significant bacteria. JPMA, *50*(250).

11- Rubinstein, E., Isturiz, R., Standiford, H. C., Smith, L. G., Oliphant, T. H., Cammarata, S., and Remington, J. (2003). Worldwide assessment of linezolid's

clinical safety and tolerability: comparator-controlled phase III studies. Antimicrobial agents and chemotherapy, 47(6), 1824-1831.

12- Ji, B., Perani, E. G., Petinom, C., N'Deli, L., & Grosset, J. H. (1994). Clinical trial of ofloxacin alone and in combination with dapsone plus clofazimine for treatment of lepromatous leprosy. Antimicrobial agents and chemotherapy, *38*(4), 662-667.

13- Pal, R. R., Patil, P. S., Salunkhe, M. M., Maldar, N. N., & Wadgaonkar, P. P. (2005). Synthesis and characterization of aromatic polyamides containing an s-triazine ring with thiophenoxy linkages. Polymer international, 54(3), 569-575.

14- Capasso, C., & Supuran, C. T. (2014). Sulfa and trimethoprim-like drugs–antimetabolites acting as carbonic anhydrase, dihydropteroate synthase and dihydrofolate reductase inhibitors. Journal of enzyme inhibition and medicinal chemistry, 29(3), 379-387.

15- HALL, R. P., LAWLEY, T. J., SMITH, H. R., and KATZ, S. I. (1982). Bullous eruption of systemic lupus erythematosus: dramatic response to dapsone therapy. Annals of Internal Medicine, 97(2), 165-170.

16-Tripathi, K. D. (2013). Essentials of medical pharmacology. JP Medical Ltd.

17- Pamba, A., Richardson, N. D., Carter, N., Duparc, S., Premji, Z., Tiono, A. B., and, Luzzatto, L. (2012). Clinical spectrum and severity of hemolytic anemia in glucose 6-phosphate dehydrogenase–deficient children receiving dapsone. Blood, The Journal of the American Society of Hematology, 120(20), 4123-4133.

18- Peters, K., and Richards, F. M. (1977). Chemical cross-linking: reagents and problems in studies of membrane structure. Annual review of biochemistry, *46*(1), 523-551.

19- Kroes, R., Galli, C., Munro, I., Schilter, B., Tran, L. A., Walker, R., & Würtzen, G. (2000). Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing. Food and chemical toxicology, 38(2-3), 255-312.

20- Kot, A., and Namiesńik, J. (2000). The role of speciation in analytical chemistry. TrAC Trends in Analytical Chemistry, 19(2-3), 69-79.

21-Stalikas, C. D. (2002). Micelle-mediated extraction as a tool for separation and preconcentration in metal analysis. TrAC Trends in Analytical Chemistry, 21(5), 343-355.

22- Granlund, M., & Malmi, T. (2002). Moderate impact of ERPS on management accounting: a lag or permanent outcome. Management accounting research, 13(3), 299-321.

23- Yoon, N. M., Pak, C. S., Brown Herbert, C., Krishnamurthy, S., & Stocky, T. P. (1973). Selective reductions. XIX. Rapid reaction of carboxylic acids with borane-tetrahydrofuran. Remarkably convenient procedure for the selective conversion of carboxylic acids to the corresponding alcohols in the presence of other functional groups. The Journal of Organic Chemistry, 38(16), 2786-2792.

24- Haddad, P. R., Doble, P. and Macka, M. (1999). Developments in sample preparation and separation techniques for the determination of inorganic ions by ion chromatography and capillary electrophoresis. Journal of Chromatography A, *856*(1-2), 145-177.

25- O'Connor, D., Hou, D., Ok, Y. S., Song, Y., Sarmah, A. K., Li, X., and Tack, F. M. (2018). Sustainable in situ remediation of recalcitrant organic pollutants in groundwater with controlled release materials: A review. Journal of Controlled Release, *283*, 200-213.

26-Bersier, P. M., Howell, J., and Bruntlett, C. (1994). Tutorial review. Advanced electroanalytical techniques versus atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry in environmental analysis. Analyst, 119(2), 219-232.

27-Pettine, M., McDonald, T. J., Sohn, M., Anquandah, G. A., Zboril, R., and Sharma, V. K. (2015). A critical review of selenium analysis in natural water samples. Trends in Environmental Analytical Chemistry, 5, 1-7.

28-Ali, I., and Jain, C. K. (2004). Advances in arsenic speciation techniques. International Journal of Environmental Analytical Chemistry, 84(12), 947-964.

29-Zarei, A., Hajipour, A. R., Khazdooz, L., Mirjalili, B. F., and Chermahini, A. N. (2009). Rapid and efficient diazotization and diazo coupling reactions on silica sulfuric acid under solvent-free conditions. Dyes and Pigments, 81(3), 240-244.

30-Cliffe, W. H. (1959). The life and times of Peter Griess. Journal of the Society of Dyers and Colourists, 75(6), 278-285.

31-Doyle, M. P.,and Bryker, W. J. (1979). Alkyl nitrite-metal halide deamination reactions. 6. Direct synthesis of arenediazonium tetrafluoroborate salts from aromatic amines, tert-butyl nitrite, and boron trifluoride etherate in anhydrous media. The Journal of Organic Chemistry, 44(9), 1572-1574.

32- Yates, E., & Yates, A. (2016). Johann Peter Griess FRS (1829–88): Victorian brewer and synthetic dye chemist. Notes and Records: the Royal Society Journal of the History of Science, 70(1), 65-81.

33- Wang, B., and Laskin, A. (2014). Reactions between water-soluble organic acids and nitrates in atmospheric aerosols: Recycling of nitric acid and formation of organic salts. Journal of Geophysical Research: Atmospheres, 119(6), 3335-3351.

34- O'Donnell, V. B., Eiserich, J. P., Chumley, P. H., Jablonsky, M. J., Krishna, N. R., Kirk, M., ... & Freeman, B. A. (1999). Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid, nitrogen dioxide, and nitronium ion. Chemical research in toxicology, 12(1), 83-92.

35- Zolfigol, M. A. (2001). Silica sulfuric acid/NaNO2 as a novel heterogeneous system for production of thionitrites and disulfides under mild conditions. Tetrahedron, 57(46), 9509-9511.

36- Deadman, B. J., Collins, S. G., and Maguire, A. R. (2015). Taming hazardous chemistry in flow: the continuous processing of diazo and diazonium compounds. Chemistry–A European Journal, 21(6), 2298-2308.

37-O'Donnell, V. B., Eiserich, J. P., Chumley, P. H., Jablonsky, M. J., Krishna, N. R., Kirk, M., ...and Freeman, B. A. (1999). Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid,

nitrogen dioxide, and nitronium ion. Chemical research in toxicology, 12(1), 83-92.

38- Ridd, J. H. (1961). Nitrosation, diazotisation, and deamination. Quarterly Reviews, Chemical Society, 15(4), 418-441.

39-Peters, A. T., and Freeman, H. S. (Eds.). (1996). Physico-Chemical principles of color chemistry. Blackie Academic & Professional.

40- Zolton, L. (1963). Diazotization of Aliphatic and Aromatic Amines. The University of Arizona.

41-Broggi, J., Terme, T., and Vanelle, P. (2014). Organic electron donors as powerful single-electron reducing agents in organic synthesis. Angewandte Chemie International Edition, 53(2), 384-413.

42-Gordon, P. F., and Gregory, P. (2022). Organic chemistry in colour. In Organic Chemistry in Colour. De Gruyter.

43- Christie, R., and Abel, A. (2021). Disazo (Bishydrazone) pigments based on acetoacetanilides. Physical Sciences Reviews.

44- Aljamali, N. M. (2019). Review on (azo, formazane, sulfazane)-compounds. International Journal of Innovations in Scientific Engineering, 10, 19-45.

45-Sun, C. L., and Shi, Z. J. (2014). Transition-metal-free coupling reactions. Chemical reviews, 114(18), 9219-9280.

46- Kumar, D. (2016). Further Exploration on Reactivity of Diazonium Salts with Selective Nucleophiles and Enolates (Doctoral dissertation, IISER-M).

47-Huisgen,R.1,5,-Electrocyclizations—An Important Principle of Heterocyclic Chemistry. Angewandte Chemie International Edition in English, 19(12), 947-973.

48- Chen, J., Xie, X., Liu, J., Yu, Z., and Su, W. (2022). Revisiting aromatic diazotization and aryl diazonium salts in continuous flow: highlighted research during 2001–2021. Reaction Chemistry & Engineering, 7(6), 1247-1275.

49- Engel, M., Steiner, M., Lombardo, A., Ferrari, A. C., Löhneysen, H. V., Avouris, P., and Krupke, R. (2012). Light–matter interaction in a microcavity-controlled graphene transistor. Nature communications, *3*(1), 1-6.

50-Hahn, D. W., and Omenetto, N. (2012). Laser-induced breakdown spectroscopy (LIBS), part II: review of instrumental and methodological approaches to material analysis and applications to different fields. Applied spectroscopy, 66(4), 347-419.

51- Liu, X., Pang, H., Liu, X., Li, Q., Zhang, N., Mao, L., and Wang, X. (2021). Orderly porous covalent organic frameworks-based materials: superior adsorbents for pollutants removal from aqueous solutions. The innovation, 2(1), 100076.

52- Simonescu ,C.M.(2012). Application of FTIR spectroscopy in environmental studies. Advanced aspects of spectroscopy, 29(1), 77-86.

53- Kowalska, E., Mahaney, O. O. P., Abe, R., and Ohtani, B. (2010). Visible-light-induced photocatalysis through surface plasmon excitation of gold on titania surfaces. Physical Chemistry Chemical Physics, 12(10), 2344-2355.

54-Morris, R. (2015). Spectrophotometry. Current Protocols Essential Laboratory Techniques, 11(1), 2-1.

55- Kuznetsov, V. N., and Serpone, N. (2006). Visible light absorption by various titanium dioxide specimens. The Journal of Physical Chemistry B, 110(50), 25203-25209.

56- Akash, M. S. H., and Rehman, K. (2020). Ultraviolet-visible (UV-VIS) spectroscopy. In Essentials of Pharmaceutical Analysis (pp. 29-56). Springer, Singapore.

57- Pratiwi, R. A., and Nandiyanto, A. B. D. (2021). How to read and interpret UV-VIS spectrophotometric results in determining the structure of chemical compounds. Indonesian Journal of Educational Research and Technology, 2(1), 1-20.

58- Karuppaiah, S., and Krishnan, M. (2022). UV-Visible Spectroscopy for Colorimetric Applications. Colorimetry, 35.

59- Mirkovic, T., Ostroumov, E. E., Anna, J. M., Van Grondelle, R., and Scholes, G. D. (2017). Light absorption and energy transfer in the antenna complexes of photosynthetic organisms. Chemical reviews, 117(2), 249-293.

60- Sarina, S., Waclawik, E. R., & Zhu, H. (2013). Photocatalysis on supported gold and silver nanoparticles under ultraviolet and visible light irradiation. Green Chemistry, 15(7), 1814-1833.

61- Behera, S., Ghanty, S., Ahmad, F., Santra, S., and Banerjee, S. (2012). UV-visible spectrophotometric method development and validation of assay of paracetamol tablet formulation. J Anal Bioanal Techniques, 3(6), 151-7.

62- Akash, M. S. H., and Rehman, K. (2020). Ultraviolet-visible (UV-VIS) spectroscopy. In Essentials of Pharmaceutical Analysis (pp. 29-56). Springer, Singapore.

63- Karthikeyan, S., Gupta, V. K., Boopathy, R., Titus, A., & Sekaran, G. (2012). A new approach for the degradation of high concentration of aromatic amine by heterocatalytic Fenton oxidation: kinetic and spectroscopic studies. Journal of Molecular Liquids, 173, 153-163.

64- Hardwick, C. C., Herivel, T. R., Hernandez, S. C., Ruane, P. H., and Goodrich, R. P. (2004). Separation, Identification and Quantification of Riboflavin and its Photoproducts in Blood Products using High-performance Liquid Chromatography with Fluorescence Detection: A Method to Support Pathogen Reduction Technology. Photochemistry and Photobiology, 80(3), 609-615.

65- Ahmed, A. W., and Al-Ameri, S. A. (2020). Spectrophotometric Method for the Estimation of Ceftriaxone in Pure Form and Pharmaceuticals. Ibn AL-Haitham Journal For Pure and Applied Science, *33*(2), 33-42.

66- Jambulingam, M., Thangathurai, S. A., Kamalakannan, D., Punitha, S., Rincy, R., Santhi, S., Subarla, S. J. (2015). A Simple spectrophotometric estimation of ceftriaxone sodium in bulk and sterile formulation. PharmaTutor, 3(9), 48-52.

67- Rageh, A. H., El-Shaboury, S. R., Saleh, G. A.,and Mohamed, F. A. (2010). Spectophotometric method for determination of certain cephalosporins using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl). Natural Science, 2(08), 828.

68-Pasha, C., and Narayana, B. (2008). A simple method for the spectrophotometric determination of cephalosporins in pharmaceuticals using variamine blue. Eclética Química, 33, 41-46.

69-Saleh, G. A., Askal, H. F., Darwish, I. A., and El-Shorbagi, A. N. A. (2003). Spectroscopic analytical study for the charge-transfer complexation of certain cephalosporins with chloranilic acid. Analytical Sciences, 19(2), 281-287.

70- Amin, A. S., and Ragab, G. H. (2004). Spectrophotometric determination of certain cephalosporins in pure form and in pharmaceutical formulations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 60(12), 2831-2835.

71-Roopa, K. P., and Jayanna, B. K. (2016). Spectrophotometric determination of some cephalosporins in bulk and in pharmaceutical formulations. Analytical Chemistry Letters, 6(2), 143-152.

72-Adegoke, O. A., and Quadri, M. O. (2016). Novel spectrophotometric determinations of some cephalosporins following azo dye formation with p-dimethyl amino benzaldehyde. Arabian Journal of Chemistry, 9, S1272-S1282.

73-Kumar, C. A., Gurupadayya, B. M., Sloka, S. N., Chandan, R. S., and Thejaswini, J. C. (2011). Colorimetric determination of cefadroxil and ceftriazone in pharmaceutical dosage forms. Tropical Journal of Pharmaceutical Research, 10(1).

74-Rao, K. S., Rao, B., Patro, S., and Patnaik, A. (2012). Development and validation of new analytical method for ceftriaxone sodiumin bulk and pharmaceutical dosage forms. International Journal of Research and Reviews in Pharmacy and Applied Science, 2(3), 556-565.

75-Sultana, N., Arayne, M. S., and Shahzad, W. (2010). Simultaneous determination of ceftriaxone sodium and statin drugs in pharmaceutical formulations and human serum by RP-HPLC. Journal of the Chilean Chemical Society, 55(2), 193-198.

76- El-Adl, S. M., El-Shanawany, A. A., Abdel-Aziz, L. M., and Hassan, A. F. (2014). HPLC determination of three cephalosporins (cefepime, cefotriaxone and

cefotaxime) in their bulk and dosage forms. Asian Journal of Pharmaceutical Analysis, 4(3), 91-97.

77- Kale, R. S., Jain, H. K., Ghode, P. D., Mhaske, G. S., Puri, M. V., Raut, M. D., and Patil, H. S. (2011). An rphplc method for simultaneous estimation of Ceftriaxone sodium and sulbactam sodium in parenteral dosage form. International Journal of Pharmacy and Pharmaceutical Sciences, 3(supplement 5), 406-409.

78- Hiremath, B., and Mruthyunjayaswamy, B. H. M. (2009). Development and validation of a high-performance liquid chromatographic determination of ceftriaxone sodium and its application to drug quality control. Analytical Letters, 42(14), 2180-2191.

79- Shah, J., Rasul Jan, M., Shah, S., and Naeem Khan, M. (2013). Development and validation of HPLC method for simultaneous determination of ceftriaxone and cefaclor in commercial formulations and biological samples. Journal of the Mexican Chemical Society, 57(4), 314-320.

80-Sun, H., Wang, H., and Ge, X. (2012). Simultaneous determination of the combined drugs of ceftriaxone sodium, metronidazole, and levofloxacin in human urine by high-performance liquid chromatography. Journal of Clinical Laboratory Analysis, 26(6), 486-492.

81- Briscoe, S. E., McWhinney, B. C., Lipman, J., Roberts, J. A., and Ungerer, J. P. (2012). A method for determining the free (unbound) concentration of ten beta-lactam antibiotics in human plasma using high performance liquid chromatography with ultraviolet detection. Journal of Chromatography B, 907, 178-184.

82- Al-Rawithi, S., Hussein, R., Raines, D. A., AlShowaier, I., and Kurdi, W. (2000). Sensitive assay for the determination of cefazolin or ceftriaxone in plasma utilizing LC. Journal of pharmaceutical and biomedical analysis, 22(2), 281-286.

83- Akl, M. A., Ahmed, M. A., and Ramadan, A. (2011). Validation of an HPLC-UV method for the determination of ceftriaxone sodium residues on stainless steel surface of pharmaceutical manufacturing equipments. Journal of pharmaceutical and biomedical analysis, 55(2), 247-252.

84- Majdi, S., Jabbari, A., Heli, H., Yadegari, H., Moosavi-Movahedi, A. A., and Haghgoo, S. (2009). Electrochemical oxidation and determination of ceftriaxone on a glassy carbon and carbon-nanotube-modified glassy carbon electrodes. Journal of Solid State Electrochemistry, 13(3), 407-416.

85- Hassinea, C. B. A., Güngör, Ö. Z. N. U. R., Burc, M., Özcan, İ., Köytepe, S. Ü. L. E. Y. M. A. N., and Duran, S. T. (2022). Electrochemical determination of ceftriaxone using polyurethane-modified electrode containing caffeic acid and chitosan. Polymer-Plastics Technology and Materials.

86- Majidi, M. R., Asadpour-Zeynali, K., and Hafezi, B. (2011). Electrocatalytic oxidation and determination of ceftriaxone sodium antibiotic in pharmaceutical samples on a copper hexacyanoferrate nanostructure. Analytical Methods, 3(3), 646-652.

87- Shahrokhian, S., Hosseini-Nassab, N., and Kamalzadeh, Z. (2014). Fabrication of an electrochemical sensor based on the electrodeposition of Pt nanoparticles on multiwalled carbon nanotubes film for voltammetric determination of ceftriaxone in the presence of lidocaine, assisted by factorial-based response-surface methodology. Journal of Solid State Electrochemistry, 18(1), 77-88.

88-Shah, J., Jan, M. R., and Shah, S. (2013). Development and validation of a spectrofluorimetric method for the quantification of ceftriaxone in pharmaceutical formulations and plasma. Luminescence, 28(4), 516-522.

89-Sadeghi, S., and Olieaei, S. (2019). Capped cadmium sulfide quantum dots with a new ionic liquid as a fluorescent probe for sensitive detection of florfenicol in meat samples. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 223, 117349.

90-Karpov, V. M., Spektor, D. V., and Beklemishev, M. K. (2016). Determination of ceftriaxone by the fluorescence quenching of quantum dots using binding with polyethyleneimine. Journal of Analytical Chemistry, 71(5), 519-526.

91-Qu, J., Zhu, Z., Bai, X., and Qu, J. (2013). Determination of ceftriaxone sodium by fluorescence probes of L-Cysteine capped ZnS quantum dots. Nanoscience and Nanotechnology Letters, 5(10), 1051-1057.

92-Wang, H. Y., Xu, L. X., Xiao, Y., and Han, J. (2004). Spectrophotometric determination of dapsone in pharmaceutical products using sodium 1, 2-naphthoquinone-4-sulfonic as the chromogenic reagent. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 60(12), 2933-2939.

93-Daood, L. T. (2008). Spectrophotometric Determination of Dapsone Using Phloroglucinol Azo Coupling Reagent. Rafidain journal of science, 19(3 E).

94-Al-Enizzi, M. S., SHEEJ AHMAD, O. A., and Al-Sabha, T. A. N. (2020). Spectrophotometric Determination of Dapsone Using Charge Transfer Complex Formation Reaction. Egyptian Journal of Chemistry, 63(8), 3167-3177.

95-Nangare, G., Tegeli, V., Ingle, S., Matole, V., Birajdar, A., & Adlinge, S. (2021). UV Spectrophotometric Analysis and Validation of Dapsone in Semisolid Dosage Form. Research Journal of Pharmacy and Technology, 14(9), 5007-5009.

96-Nagaraja, P., and Bhaskara, B. L. (2006). Sensitive spectrophotometric assessment of dapsone using carbofuran as a new chromogenic reagent in formulations and environmental samples. Eclética Química, 31, 43-48.

97-Alhamid, S. M. A. (2015). Colorimetric and Kinetic method for determination of Dapsone in bulk and pharmaceutical preparations. Indian Journal of Research in Pharmacy and Biotechnology, *3*(1), 15.

98- Nagaraja, P., Yathirajan, H. S., Sunitha, K. R., and Vasantha, R. A. (2002). Novel methods for the rapid spectrophotometric determination of dapsone. Analytical letters, 35(9), 1531-1540.

99- Altahir, B. M., Mahdi, A. S., Al-hraishawi, T. J., and Taylor, K. E. (2021). Mutual Derivatization in the Determination of Dapsone and Thymol Using Cloud Point Extraction Followed by Spectrophotometric Detection. Analytical and Bioanalytical Chemistry Research, 8(2), 177-186.

100- Omran, A. A. (2005). Individual and simultaneous spectrophotometric determination of dapsone and metoclopramide HCl in pharmaceutical dosage forms and synthetic binary mixtures. Chemical and pharmaceutical bulletin, 53(11), 1498-1501.

101-A Sarsam, L. (2013). Spectrophotometric and high performance liquid chromatographic methods for the determination of dapsone in a pharmaceutical preparation. Rafidain Journal of Science, 24(1), 128-145.

102- Machado, S., Fernandes, S. R., Chaves, L. L., Lima, S. A., Silva, E. M., Barreiros, L., ... & Segundo, M. A. (2018). Chromatographic method for the simultaneous quantification of dapsone and clofazimine in nanoformulations. Journal of separation science, 41(17), 3382-3388.

103- Beheshti-Maal, L., Navidpour, L., and Afshar, M. (2016). An ecofriendly and stability-indicating RP-HPLC method for determination of dapsone: Application to pharmaceutical analysis. Chiang Mai J Sci, 43(3), 420-629.

104- Colomé, L. M., Freitas, G. M., de Medeiros Bastiani, J., Pereira, T. C. B., Bajerski, L., Bender, E. A., and Haas, S. E. (2017). Validation of analytical method by HPLC for determination of dapsone in polymeric nanocapsules based on crude rice brain oil. Journal of Applied Pharmaceutical Science, 7(7), 230-233.

105- LIU, G. F., ZHEN, L. M., and HUANG, L. J. (2010). RP-HPLC simultaneous determination of dapsone, caffeine and metoprolol in rabbit. Chinese Journal of Pharmaceutical Analysis, 30(12), 2263-2266.

106- Benjamin, T., & Ramachandran, D. (2015). Development and validation of RP-LC method for Dapsone in pharmaceutical formulations. *World J Pharm Res*, *20*, 1090-1099.

107- Salama, N. N. E. D. A., El Ries, M. A., Toubar, S., Hamide, M. A., and Walash, M. I. (2012). Validated TLC and HPLC stability-indicating methods for the quantitative determination of dapsone. JPC–Journal of Planar Chromatography–Modern TLC, 25(1), 65-71.

108- Khedekar, G., and Mirgane, S. (2017). Development and validation of stability indicating HPLC assay method for Clofazimine capsules. 5, 112-120.

109- Essousi, H., and Barhoumi, H. (2018). Electroanalytical application of molecular imprinted polyaniline matrix for dapsone determination in real pharmaceutical samples. Journal of Electroanalytical Chemistry, 818, 131-139.

110-Malekzadeh, M., Mohadesi, A., Karimi, M. A., and Ranjbar, M. (2021). Development of a New Sensitive Electrochemical Sensor for Dapsone Detection

Using a Cobalt Metal-Organic Framework/Molecularly Imprinted Polymer Nanostructures. Analytical and Bioanalytical Electrochemistry, 13(2), 226-238.

111- Manisankar, P., Sarpudeen, A.,and Viswanathan, S. (2001). Electroanalysis of dapsone, an anti-leprotic drug. Journal of pharmaceutical and biomedical analysis, 26(5-6), 873-881.

112- Ma, L., Tang, B., and Chu, C. (2002). Spectrofluorimetric study of the β-cyclodextrin–dapsone–linear alcohol supramolecular system and determination of dapsone. Analytica Chimica Acta, 469(2), 273-283.

113-Janghel, E. K., Rai, J. K., Rai, M. K., and Gupta, V. K. (2007). A new sensitive spectrophotometric determination of cypermethrin insecticide in environmental and biological samples. Journal of the Brazilian Chemical Society, 18, 590-594.

114- Harvey, D., Modern analytical chemistry. Vol. 1. 2000: McGraw-Hill New York.

115- Williams, P. B., Barnes, J. H., Szeinbach, S. L., and Sullivan, T. J. (2000). Analytic precision and accuracy of commercial immunoassays for specific IgE: establishing a standard. Journal of allergy and clinical immunology, 105(6), 1221-1230.

116- Vives, M., Gargallo, R., and Tauler, R. (2000). Multivariate extension of the continuous variation and mole-ratio methods for the study of the interaction of intercalators with polynucleotides. Analytica chimica acta, 424(1), 105-114.

117- Kruschke, J. K. (2013). Bayesian estimation supersedes the t test. Journal of Experimental Psychology: General, 142(2), 573.

118- Ludden, T. M., Beal, S. L., and Sheiner, L. B. (1994). Comparison of the Akaike Information Criterion, the Schwarz criterion and the F test as guides to model selection. Journal of pharmacokinetics and biopharmaceutics, 22(5), 431-445.

119-Shrestha, B., Bhuyan, N. R., and Sinha, B. N. (2013). Simultaneous determination of Ceftriaxone and Tazobactam in injectables by UHPLC method. Pharmaceutical Methods, 4(2), 46-51.

120- Wang, H. Y., Xu, L. X., Xiao, Y., and Han, J. (2004). Spectrophotometric determination of dapsone in pharmaceutical products using sodium 1, 2-naphthoquinone-4-sulfonic as the chromogenic reagent. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 60(12), 2933-2939.

الخلاصة:

يتضمن هذا العمل تطوير طريقة طيفية بسيطة سريعة وحساسة لتقدير ادوية السيفترياكزون CF والدابسون , وتعتمد الطريقة على ارتباط السيفترياكزون والدابسون مع كاشف 4,5-diphenylimidazole (4,5-DPI).

يرتبط السيفترياكزون المأزوت بكاشف 4,5-DPI في محلول هيدروكسيد الصوديوم لتشكيل مركب ازو بنفسجي مستقر نسبيا وقابل للذوبان في الماء , والذي يظهر امتصاص عند الطول الموجي λ_{max} عند الطول الموجي 550nm , الطريقة المقترحة تطيع قانون بير بالتراكيز من (2-90) مايكروغرام / مل , مع معامل ارتباط R^2 0.9976, وحساسية ساندل S 0.22 مايكروغرام .سنتمتر مربع, معامل الامتصاص المولي (3536.3) لتر.مول$^{-1}$.سنتمتر$^{-1}$, حد الكشف L.O.D 0.06 مايكروغرام / مل , وقيمة L.O.Q 0.19 مايكروغرام /مل , والانحراف المعياري R.S.D% 0.089.

وتم تطبيق الطريقة المقترحة بنجاح لتقدير بعض المستحضرات الصيدلانية التي تحتوي على السيفترياكزون ,وتمت مقارنة هذه الطريقة مع طريقة قياسية موجوده في دستور الادوية البريطاني ووجد ان القيمة التقريبية F-test, T-test اصغر من القيم المجدولة , ولايوجد فرق معنوي بين الطريقتين .

تم كذلك تقدير دواء الدابسون المازوت عن طريق الارتباط مع كاشف 4,5-DPI في الوسط القاعدي والتي لديها اقصى امتصاص عند طول موجيλ_{max} 501nm , وجد ان الطريقة تطيع قانون لامبرت بير في التراكيز من (2-18) مايكروغرام / مل , مع معامل ارتباط R^2 0.9942, وحساسية ساندل S 0.154 مايكروغرام .سنتمتر مربع, معامل الامتصاص المولي (34080) لتر.مول$^{-1}$.سنتمتر$^{-1}$, حد الكشف L.O.D 0.29 مايكروغرام / مل , وقيمة L.O.Q 0.96 مايكروغرام /مل , والانحراف المعياري R.S.D% 0.88.

وتم تطبيق الطريقة المقترحة بنجاح لتقدير بعض المستحضرات الصيدلانية التي تحتوي على الدابسون,وتمت مقارنة هذه الطريقة مع طريقة قياسية موجوده في دستور الادوية الامريكي ووجد ان القيمة التقريبية T-test F-test, اصغر من القيم المجدولة , ولايوجد فرق معنوي بين الطريقتين.

جمـهورية العراق
وزارة التعليم العالــي والبحث العلـمي
جـامعـة بابل/ كلية العلـوم
قسـم الكـيمـيـاء

التقـديـر الطـيفـي لبعض المستحضرات الصيدلانية باستخدام كواشف عضوية ملائمة

مقدمـة الـى مجلـس كلـية الـعلـوم – جامـعـة بـابـل
كجزء من متطلبات نـيـل درجة الماجستير في علـوم الكـيميـاء

مـن قـبـل
مـحـمـد خـالـد قـحطـان جـنديـل

إشـراف
الاسـتـاذ الـدكتـور عبـاس نـور الشـريفـي

2023 م 1444 هـ