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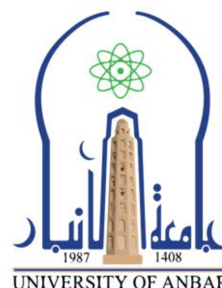

Developing Spectrophotometric Method for Some Amino Drugs determination and Study of Their Effect on ALP Activity

A thesis
Submitted to the College of Science at
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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

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صدق الله العظيم

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Declaration

I dedicate this work to:

My dear mom and dad.

My husband (Mohammed)

My daughters (Dyan, Aleen, Sela)

My dear brothers and sisters

My dear friend (Taghreed Sabah).

As a small tribute to their infinite love and sacrifice for me. For standing by my side and supporting me throughout my studies.

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Nagham

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ABSTRACT

The present work is divided into three chapters.

Chapter one: Includes a brief review of the drugs under study [Phenylephrine (PHE), Bromohexine (BRH) and Tenoxicam (TNX), and Ranitidine (RAN)]and their analytical methods for determination described in chapter one. Then a brief review on the principle of ion pair reaction, cloud point extractions (CPE), dispersive liquid-liquid micro-extraction (DLLME) and direct extraction. Finally, the chapter ended with the aim of the present work.

Chapter two: Describes the experimental part for the development of a new analytical method for the determination of the above studied drugs. This includes the chemicals and instruments used throughout the work as well as a description of the general procedure for the preparation of the standard stock solutions, a procedure for the optimization of conditions and general procedures for the determination of the studied drugs.

Chapter three: Was divided into four parts.

Part I: This part includes the development of a spectroscopy method for the determination of phenylephrine and Bromohexine based on ion-pair reactions with alizarine yellow reagent. in acidic media. Tenoxicam and Ranitidine were reacted with 2-hydroxy propyl beta-cyclodextrin(2-HPβCD) in suitable media. The color of the produced was yellow with maximum wavelengths 430, 480,385 and 330 nm from phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively. The concentration ranges that obey Beer's law by direct extraction were 1 to 20 µg/mL for phenylephrine, bromohexine and 1 to 45 µg/mL for tenoxicam and ranitidine. Correlation coefficients (R^2) 0.997,0.997, 0.998, and 0.999 from phenylephrine, Bromohexine, Tenoxicam, and

Ranitidine, respectively. Molar absorptivity 14459.9,16504.0,2200.8,6072.88 L/mol.cm and a detection limit 0.34, 0.0814, 0.60, 0.17 µg/mL for phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively.

Part II: Cloud point extraction (CPE) was employed for the trace determination of the produced from the studied drugs followed by measuring the absorbance of the yellow color with λ_{max} of 430, 480,385, and 330 nm for phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively. The concentration ranges that obey Beer's law were (1 – 35) µg/mL for phenylephrine, bromohexine, and tenoxicam and (1-20) µg/mL for ranitidine drug. Correlation coefficients (R^2) 0.999,0.995,0.996 and 0.996 from phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively. Molar absorptivity 4073.2,13202.88,1572.0,1572.0 L/mol.cm, and limit of the detection (LOD) 0.065, 0.141,0.164, and 0.17 µg/mL respectively.

Part III: DLLME was used to determine the trace of ion pair from the studied drugs, by use optimal conditions, followed by measurement of absorption of colors at λ_{max} 430, 480,385, and 330 nm for phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively. The concentration ranges that obey Beer's law were (1 – 13) µg/mL for phenylephrine, ranitidine and (1-23), (1-21) µg/mL for bromohexine, and tenoxicam, respectively. correlation coefficients (R^2) 0.996,0.998,0.996, and 0.998 for phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively. Molar absorptivity 12423.3, 23930.2, 2515.2, and 25303.2 $L.mol^{-1}.cm^{-1}$ from phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively. The limit of detection (LOD) 0.094, 0.055, 0.079, and 0.04 µg/mL from phenylephrine, Bromohexine, Tenoxicam, and Ranitidine, respectively.

part IV: The activity of the alkaline phosphatase (ALP) enzyme was also studied on one of the studied compounds, bromohexine hydrochloride, and the azo compound formed from the same drug. The ability of the enzyme ALP to inhibit the drug and the azo compound was evaluated significantly, indicating the importance of the drug in the enzymatic activity.

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List of Abbreviations

Abbrev	Full name
BRH	Bromohexine HCl
ALP	Alkaline Phosphatase
CPE	Cloud point extraction
CMC	Critical micelle concentration
DLLME	Dispersive liquid liquid micro extraction
FT-IR	Fourier Transform Infra-Red
HPLC	High Performance Liquid Chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
pH	Potential of hydrogen
PEH	Phenylephrine HCl
RAN-HCl	Ranitidine HCl
SDS	Sodium Dodecyl Sulfate
TNX	Tenoxicam
Triton X-114	Polyethylene glycol tert-octyl phenyl ether
Triton X-100	Polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether.
Tween	Polyoxymethylene Sorbitan Mono Oleate
R1	Diethanolamine, buffer (pH10.2), Magnesium chloride 0.625mmol/L
R2	P-Nitro phenyl phosphate 50mmol/L

List of schemes

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CHAPTER ONE

INTRODUCTION

CHAPTER ONE

1. INTRODUCTION

1.1 General Introduction

Medicines are necessary for human health, for the drug to function appropriately; it should be free of impurities and given appropriate quantities. In many stages of drug development, transportation and storage, impurities may increase and develop in pharmaceutical preparations, which makes the drug risky, therefore, drugs need to be distinguished and quantified (1).

Pharmaceutical research assumed that microbiology, chemistry, biochemistry, and pharmacology were critical in developing pharmaceutical drugs. However, new pharmaceuticals are no longer created just by chemists' imaginations but rather by a collaboration between chemists and biologists. The creation of pharmaceutical products showed the therapeutic benefits to control, check out or cure diseases led to the advancement of the procedure of drug industries such compounds called active pharmaceutical drug ingredients and their analysis getting initial information on safety and therapeutic efficacy is required before identifying potential pharmaceutical candidates for further investigation. Therefore, to produce drugs that serve the need, different chemicals and instrumental procedures were developed regularly to evaluate drugs (2–6). The analytical separation techniques applied in the study of pharmaceutical preparations are Chromatography (7,8), titrimetric (9), spectroscopy (10–12), electrophoresis (13), electrochemical and other basic drug analysis methods (14).

1.2 Phenylephrine Hydrochloride (PEH)

Phenylephrine hydrochloride, known as [(R)-1-3-(hydroxyl phenyl)-2-(methylamino) ethanol hydrochloride, C9H13O2N.HCl. The structure of phenylephrine HCl is depicted in Figure 1-1 (15).

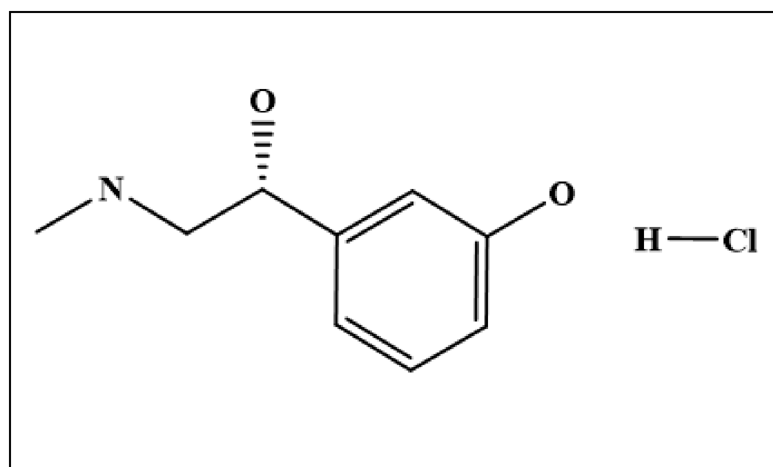


Figure 1-1: Phenylephrine HCl structure

The drug PEH is found in vasopressor medicines as eyewashes, nasal decongestant, and syrup (16,17). The Table 1-1, shown commonly properties of phenylephrine HCl.

Table 1-1: Common properties of PEH (18)

Chemical nomenclature	[(R)-1-3-(hydroxyl phenyl)-2-(methyl amino) ethanol] hydrochloride
Traditional nomenclature	Neo-Synephrine, Neofrin
Appearance	white crystalline powder
Molecular formula	$C_9H_{13}O_2N.HCl$
Molecular mass	167.205gm.mol^{-1}
Solubility	Soluble in 0.1N HCl, soluble in water.
Storage	Protected from light
Melting point	143 ºC

Many analytical techniques were used to estimate PEH in medicinal formulations, as shown in Table 1-2.

Table 1-2: Other techniques used for the determination of PEH

Methods	The Reagent	Linearity µg/ml	Limited of detection µg/mL	Recovery%	Ref.
Conductometric titration	bismuth (III) tetraiodide	8.0-50	2.5	100.113	(17)
Thin-layer chromatography	Dimetindene maleate	1.00 -10.00	0.30	98.70 ± 1.494	(19)
voltammetry	Iron nanoparticle (INPs)	100–800	0.76	101.1 ± 0.3	(20)
Flow injection	-2,4 dinitrophenylhydrazine (DNPH)	2-50	1.044	/	(21)
UV-Vis Spectrophotometry	————	5 -30	0.200	99.90	(22)
UV-Vis Spectrophotometry	————	10-70	0.0557	100.27-100.31	(23)
UV-Vis Spectrophotometry	————	10-100	0.892	101.20	(24)
Derivative spectrophotometric	————	2-50	0.0638	101.1	(25)
HPLC	4-aminophenol	3-7	0.06	101.14	(26)

1.3 Bromohexine Hydrochloride (BRH)

Bromohexine HCl, known as 2,4-dibromo-6- [[cyclohexyl (methyl) amino] methyl] aniline; hydrochloride, $C_{14}H_{20}Br_2N_2.HCl$. The structure of Bromohexine HCl is depicted in Figure 1-2 (27).

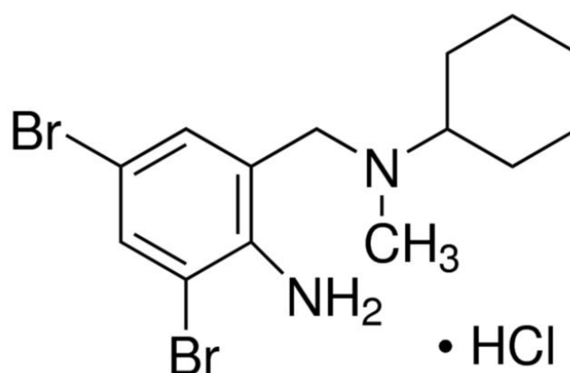


Figure 1-2: Bromohexine HCl Structure

BRH is a benzyl amine-derived cardiac depressant generated from the plant Adhatoda vasica. Bromhexine HCl is an expectorant that decreases the viscosity of the material, making coughing and expulsion easier (28). The mechanisms of action are based on sputum decomposition and dark coughing; Respiratory production helps in the formation of thinner, less thick phlegm. Assisting vasomotor secretion generates a vasomotor secretory effect (29). The properties of BRH are shown in Table 1-3.

Table 1-3: Common properties of BRH (30,31)**.**

Chemical nomenclature	2,4-dibromo-6-[[cyclohexyl(methyl) amino] methyl] aniline; hydrochloride.
Traditional nomenclature	Bromohexane, Bisolvon, Solvodin.
Appearance	white crystalline powder
Molecular formula	$C_{14}H_{20}Br_2N_2.HCl$
Molecular mass	412.59gm.mol^{-1}
Solubility	Chloroform and methylene chloride are both soluble in them.
Storage	Mucolytic
Melting point	84.70℃

Several analytical techniques were used to estimate BRH in medicinal formulations, as shown in the Table 1-4.

Table 1-4: Other techniques used for the determination of BRH.

Method	Reagent	Linearity	Limited of detection	Recovery%	Ref.
Potentiometric Flow Injection	conventional and coated wire electrodes	3.16×10^{-5} - 1×10^{-2}M	/	98.2-99.8	(32)
Thin-layer chromatography	———	4-40 µg/ml	0.521 µg/ml	98.67	(33)
Spectrophotometric Quantitative	paradimethylaminobenzaldehyde (PDEAB)	2-20 µg/ml	0.2011 µg/ml	99.63	(34)
UV-Vis Spectrophotometry	———	1-14 µg/ml	/	100.083	(35)
UV-Vis Spectrophotometry	triphenylmethane dyes	2.5–25 2.5–25 2.0–25 µg/ml	412.00 414.00 415.00 µg/ml	100.47 99.84 99.57	(36)
HPLC	———	10-70 µg/ml	0.13 µg/ml	103.2	(37)
HPLC	Ambroxol HCl	0.391-100 µg/ml	0.195 µg/ml	97.88-100.68	(38)

1.4 Tenoxicam (TNX)

Tenoxicam is an example of the oxicam class (39,40), as it is derived from enolic acid, known as 4-hydroxy-2-methyl-1,1-dioxo-N-pyridin-2-ylthieno[2,3-e] thiazine-3-carboxamide, $C_{13}H_{11}N_3O_4S_2$. The structure of Tenoxicam is depicted in Figure 1-3 (41).

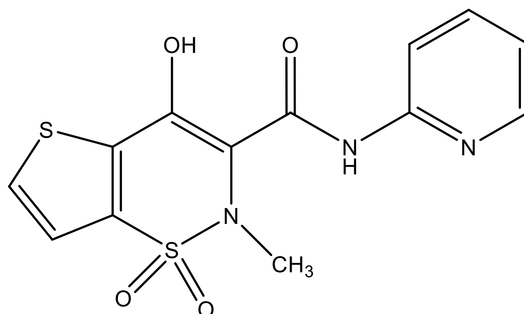


Figure 1-3: Tenoxicam structure

The maximum plasma concentration of TNX is 1.7-3.6 µg/mL (42), so it has long half-life of 60-75 hours, which is one of the essential advantages of this drug so that it can be given as one dose per day (43),and limiting first-pass metabolic, keep drug delivery, and have a regular and lengthy level of plasma (44). Tenoxicam relates to the family of non-steroidal anti-inflammatory medicines (45). It is used to reduce inflammation and pain, reduce the enzyme cyclo-oxygenase and inhibit the formation of prostaglandins (46). Prostaglandins are sensitive to pain receptors, so tenoxicam is commonly used to treat rheumatic diseases and joints, bones and muscles diseases (47). The properties of TNX as depicted in Table 1-5.

Table 1-5: Common properties of TNX (48)

Chemical nomenclature	4-hydroxy-2-methyl-1,1-dioxo-N-pyridin-2-ylthieno[2,3-e] thiazine-3-carboxamide
Traditional nomenclature	Mobiflex, Telecoil, Tenoctil
Appearance	Yellow powder
Molecular formula	$C_{13}H_{11}N_3O_4S_2$
Molecular mass	337.376 gm.mol^{-1}
Solubility	Insoluble in water (49) .soluble in 0.1N NaOH
Storage	The effect of heat and sunlight on temperature of 25C°
Melting point	209 – 213 °C

The analytical techniques were used in the estimation of TNX in medical formulations can be shown in the following Table 1-6.

Table 1-6: Other techniques used for the determination of TNX

Method	Reagent	Linearity	LOD	Recovery%	Ref.
HPLC	———	5-2000 ng/ml	5 ng/ml	98.99 ± 4.3	(50)
UV-Vis spectrophotometer	———	4-24 mg/mL	/	/	(51)
Spectrophotometric and Potentiometric	Potassium iodate N-bromo-succinimide	0.05 - 0.6 µg/mL 0.33 - 3.37 µg/mL	/	100.66 99.8	(52)
Fluorescence technique	———	4-30 µg/mL	1.31 µg/mL	100.04	(53)
UV-Vis Spectrophotometry	diluents 0.1N NaOH	2-12 µg/mL	2.67 µg/mL	98-99	(54)

1.5 Ranitidine HCl (RAN-HCl)

Ranitidine HCl, known as N, N – Dim ethyl -5- [2(1-methyl amino - 2 - nitro vinyl amino) ethyl thio methyl]. Empirical Formula $C_{13}H_{22}N_4O_3S.HCl$ (55) .The Ran-HCl is depicted in Figure 1-4.

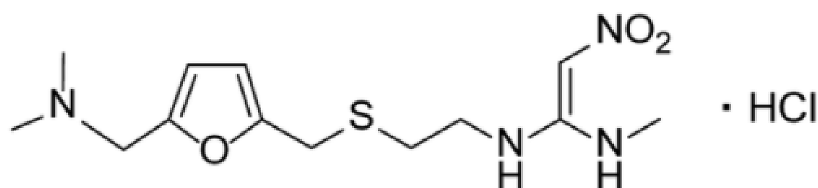


Figure 1-4: Ranitidine HCl Structure

White ranitidine or light-yellow crystalline particles, characterized by being easily soluble in water and methanol (56). RAN-HCl has the

advantage of being a short-term treatment of hypersecretion disorders (57), including peptic ulcer disease (58). The drugs are used as H2-receptor antagonists (59). Competitive inhibition reduces gastric acid secretion. For example, ranitidine reduces stomach acid, which secretes several stimuli such as caffeine, insulin, food, Penta gastrin, and betazole (60). In addition, RAN-HCl increases gastric flora, such as bacteria that reduce nitrates (61).The properties of Ranitidine HCl as shown in Table 1-7.

Table 1-7: Common properties of RAN-HCl (62)

Chemical nomenclature	N, N – Dim ethyl -5- [2(1-methyl amino - 2 - nitro vinyl amino) ethyl thio methyl].
Traditional nomenclature	Antagonin, Zantac, Melfax.
Appearance	White to off white powder.
Molecular formula	$C_{13}H_{22}N_4O_3S.HCl$
Molecular mass	350.9 $gm.mol^{-1}$
Solubility	Soluble in water, acetic acid and methanol.
Storage	The effect of light
Melting point	133 – 134 ºC

The analytical techniques were used in the estimation of RAN-HCl in medical formulations can be shown in the following Table 1-8.

Table 1-8: Other methods used for the estimation of RAN-HCl

Methods	Reagent	Linearity	Limited of detection μg/ mL	Recovery %	Ref.
HPLC	———	0.05 - 60 μg/mL	0.016	96.29-97.58	(63)
Spectrophotometric method	Ninhydrin	8.98×10^{3} - 9.90×10^{4} μg/L	0.0997	/	(64)
Spectrophotometric method	p-Dimethylaminobenzaldehyde	50.00 - 350.00 μg/mL	0.00346	/	(57)
Potentiometric	———	2×10^{-5}-1×10^{-2} M	/	100.91	(65)
Flow injection	Cerium (IV) and Ferroin	2.5 x 10^{-4} - 1.25 x 10^{-3} mol $L^{-1.}$	1.6 x 10-6	96.6 - 103	(66)
First Order Derivative Spectroscopy Method	———	7.5-37.5 μg/mL	0.66	100.50	(67)

1.6 Ion-Pair Reaction

Ion pairs are chemical compounds, electrically neutral structures. Coulomb forces are used to hold two ions of opposite charges together without formation of a covalent bond (68).

The scientist Saul Winstein proposed the ion-pair interaction. An ion-pair is described as a reaction between an anion and a cation and are surrounded by the solvent's molecules. Two ions with different charges are connected to form the ion-pair by using less polar solvents; there are no solvent particles between the ions in the ion pair that has been formed (69).

The purpose of an ion pair reaction is to create a strong bond bet ween two charged molecules so that the proportion of molecules produced by ion pair interactions in solution is as high as possible (70).

In the pharmaceutical field, ion pairing refers to the binding of a charged drug molecule to an opposite ion. The ion pair method is used

to investigate electrolyte solutions using inorganic equilibrium ions (71).

An ion pair is created by a large cation and a small anion. The solvation with electrophilic molecules is caused by the high negative charge of alcohols and chloroform. Solvation with nucleophilic molecules is caused by a modest cation relation and a large anion. The structure of the ions involved in the equilibrium of the ion pair has three types of ion pairs. (a) inorganic ion pairs (both ions are inorganic), (b) ion pairs formed between an organic molecule in ionized form and an inorganic ion and (c) organic ion pairs (both ions are organic substances in ionized form) (72,73).

1.6.1 Basic requirements for ion-pair complex

One of the main conditions for ion-pair formation success is that both the drug and the reagent can dissolve in the same solvent and the resultant product has less solubility in aqueous solvents; that is quantitatively extractable in organic solvents. In addition, because the unreacted reagent (dye) molecules are insoluble in the organic phase, one of the primary conditions for the formation of the complex is their inability to be extracted in it (74,75).

1.6.2 Advantages of the ion-pair complex

This technique increases the sensitivity of medications that are poorly absorb in the UV spectrum. It was also established that the complex formation included no excipients; hence it was widely utilized in most analysis methods. This approach is also considered very specific. Because it is a complex between a particular medicine and a reagent that does not form unless a specific pH is given (75).

1.7 2-Hydroxypropyl-β-cyclodextrin (HPβCD)

Beta Cyclodextrin is cyclic oligosaccharides containing seven sub glucose units joined to α-1,4 glycosidic bonds. β-CD is a cone-shaped molecule including the hydrophilic outer surface of the bore and hydrophobic in the central bore (76,77).The 2-HPβCD is depicted in Figure 1-5 and Table 1-9.

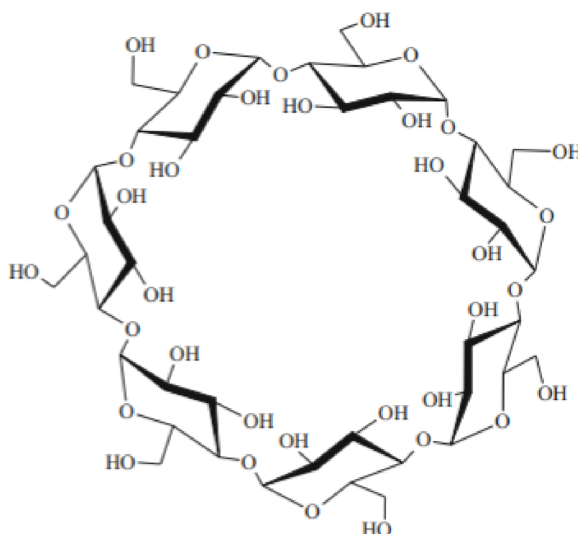


Figure 1-5: 2-Hydroxypropyl-β-cyclodextrin (78)

Table 1-9: Common properties of 2-hydroxypropyl-β-cyclodextrin (79)

Chemical names	Cycloheptaamylose
Glucose monomers	7
SYNONYMS	Beta-cyclodextrin, ßCD, BCD, ß-Schardinger dextrin, cyclodextrin B.
Chemical formula	$(C_6H_{10}O_5)_7$
Molecular weight	1135.00
Assay	Not less than 98.0% of $(C_6H_{10}O_5)_7$on an anhydrous basis
Water Solubility(g/mL;25 ºC)	1.85
Melting range(ºC)	255-265
Molecules of water in cavity	11

The medication dosage form is hampered by the medicine's poor aqueous solubility, severely limiting their therapeutical application. In addition, the slow dissolution of solid-state drug formulation and side influences of some medicines result from poor aqueous solubility. Various methods were used to increase the solubility of pharmaceutical preparations and advance their therapeutic efficiencies, such as organic solvents, emulsions, liposomes, micelles, chemical modification, and complexation of the drugs with an appropriate organic or inorganic complexing agent(80–86).

The effect of HPβCD on drug solubility depends on the drug's chemical and physical properties, such as molecular mass, lipophilicity, and chemical composition. In addition, HPβCD has the advantage of being is palatable, less toxic, non-accumulative, chemically stable, and easily separated. So, its formed complex (BCD-Drug) components dissolved in water due to sliding the drug partially or completely inside the cavity and linking it with hydrogen bonds expected to the presence of hydroxyl groups on the external surface. The reaction of beta-cyclodextrin with the medication under investigation is primarily affected by numerous fundamental factors such as the drug's polarity, hydrophobicity, size, and shape (85).

1.8 Extraction techniques

Extraction is separating combined substances by dissolving all components in suitable solvents. The results are distributed in two phases, the origin phase (rich insolvent) and the extraction phase (rich in solute); the phases are immiscible (87). The basic extraction procedure is indicated in Figure 1-6.

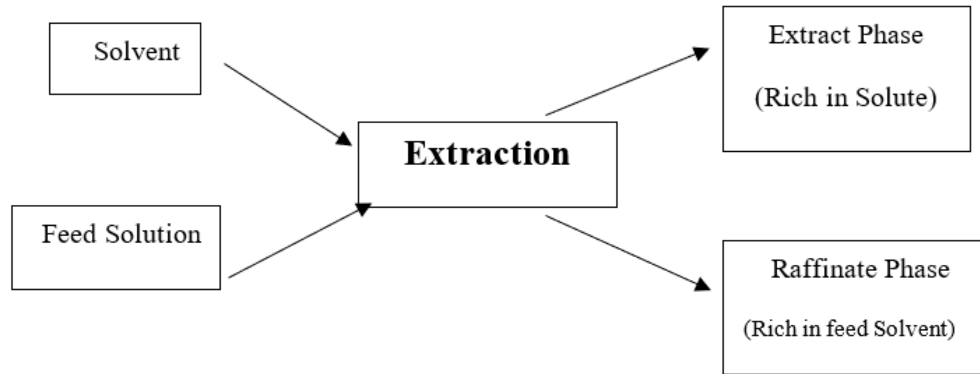


Figure 1-6: The basic extraction procedure (87)

Extraction techniques are divided into solid phase extraction, liquid-liquid extraction, solid-liquid extraction, and supercritical extraction. advanced extraction techniques include microwave extraction, ultrasonic extraction, and pressurized fluid extraction (88).

The principal determinants about how a solute is distributed between two phases (89) :

- Polarity
- Hydrogen bonding
- Degree of ionization
- Electrostatic interaction

1.8.1 Liquid – Liquid Extraction

Solvent Extraction is the process of separating the components of a liquid combination by contacting it with an insoluble liquid solvent that preferentially dissolves one or more parts (90).

Liquid-liquid extraction depended on their solubilities in two different immiscible, one of the aqueous solvents (polar) and another non-aqueous solvent (non-polar).

The expression solvent extraction refers to the fact that the substance you need to isolate is soluble in one phase but insoluble in the other, indicating that it is evenly distributed between the two phases (91).

1.8.2 Dispersive liquid–liquid microextraction

DLLME is a liquid-liquid micro-extraction method that uses a ternary solvent system for dispersion and extraction. (Aqueous and organic phases are immiscible) (92).

DLLME is a liquid-liquid mini-extraction method for Liquid-Liquid Extraction (LLE) using a microliter-sized amount of extraction solvent (93). It's a new and efficient preconcentration method (94,95).

One of the most critical steps in any analytical approach is the extraction and pre-concentration of the sample; there are many methods like liquid-liquid extraction (LLE) and solid phase extraction (SPE) (96,97).

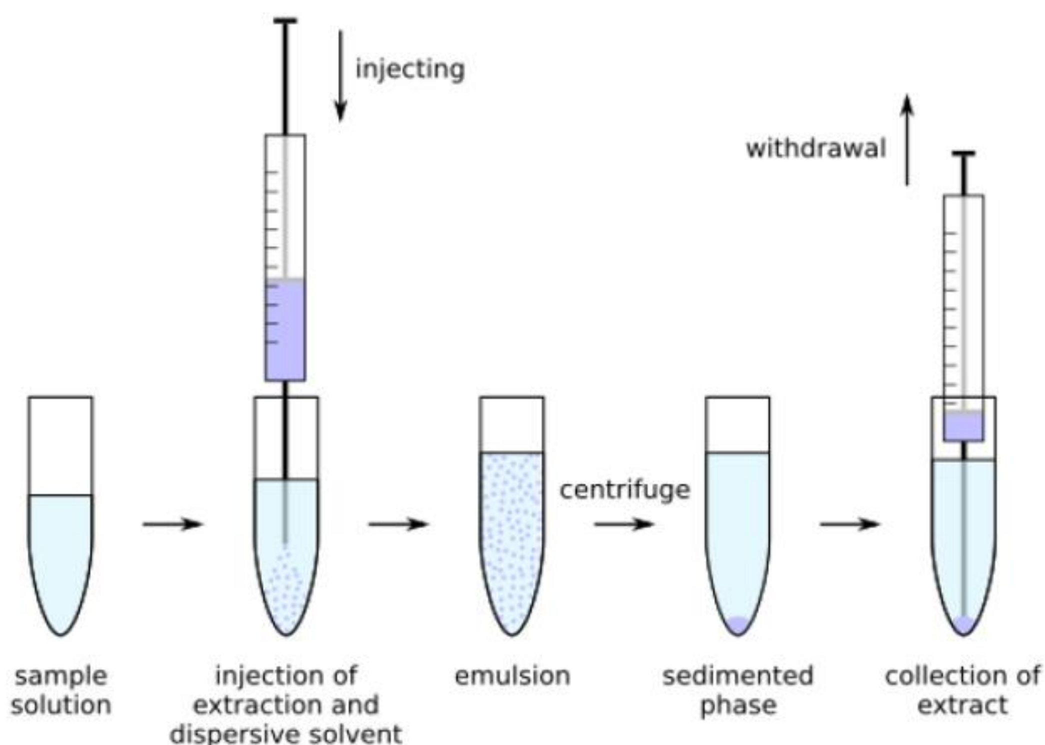
Liquid-liquid Extraction (LLE) takes a long time and usually involves using significant amounts of organic solvent, which are expensive, time-consuming, and damaging to the environment and human health. More recently, modern, minimized and cost-effective methods have been used, such as the DLLME method by Rezaee and colleagues, which was first discovered in 2006 (93,98,99).

The organic solvents used in the extraction are considered harmful to human health, so the DLLME method is used because it is a method that uses solvents in minimal volume. Hence, the advantage of the DLLME technique is fast, simple, cheap, time-saving, low-cost, high recovery and environmentally friendly (100–103).

1.8.2.1 Dispersive liquid-liquid microextraction principle

The sample (aqueous solution) is rapidly injected with a mix of extraction and dispersion solvents (104–106). The result is a cloudy solution, and the cloudy solution is separated into two phases by fracturing the emulsion solution used for the centrifuge system, allowing simple recovery of the determinate sample (107,108).

The principles of dispersive liquid–liquid microextraction as shown in Scheme1-1.



Scheme1-1: Principles of dispersive liquid liquid extraction (DLLME) (109)

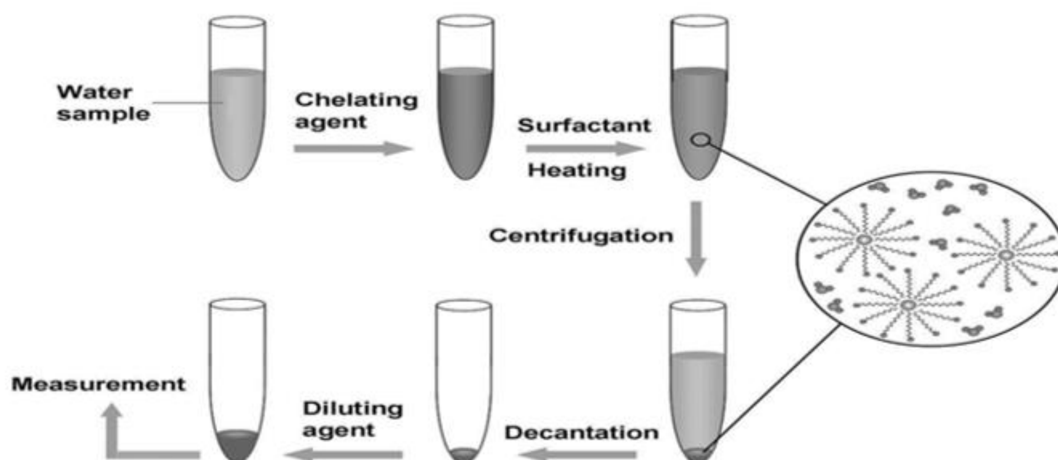
1.8.3 Cloud point extraction (CPE)

In1976A.D Watanabe and colleagues used CPE to extract metal ions using non-ionic surfactants (TritonX-100) as a replacement solvent for the extraction of metal ion (110–113).

In analytical and environmental chemistry, separation and pre concentration methods are considered of extreme importance; as a result, CPE is commonly utilized surfactant instead of organic solvents. Especially for analytical chemists (114).

CPE is a type Liquid-Liquid Extraction. It is a safe, simple, and environmentally friendly method that does not need organic solvents or costly sample preparation devices (115).

The essential CPE is dependent on the diffusion of hydrophobic analyte between a surfactant and aqueous phase. The solution is heated to a critical temperature for a non-ionic surfactant; the cloud point temperature is reached. The hydrophobic group can interact with micelles, allowing them to be extracted and concentrated in tiny amounts in the dissolved surfactant–rich phase (116) as shown in Scheme1-2.



Scheme1-2: Principles of cloud point extraction(CPE) (117)

The surfactant employed in the CPE method are determined mainly by the best conditions for extracting the required material;

however, in the hydrophilic phase (polar), water is preferred as opposed to any surfactant appropriate in the hydrophobic phase (118).

In CPE, there are two phases: a surfactant-rich phase (low volume) of analysis confined by organized micelles and a bulk aqueous phase or surfactant-poor phase with a small surfactant molecule at critical micelle concentration (CMC) (119). The shape of the surfactant molecule is shown in the following Figure 1-7.

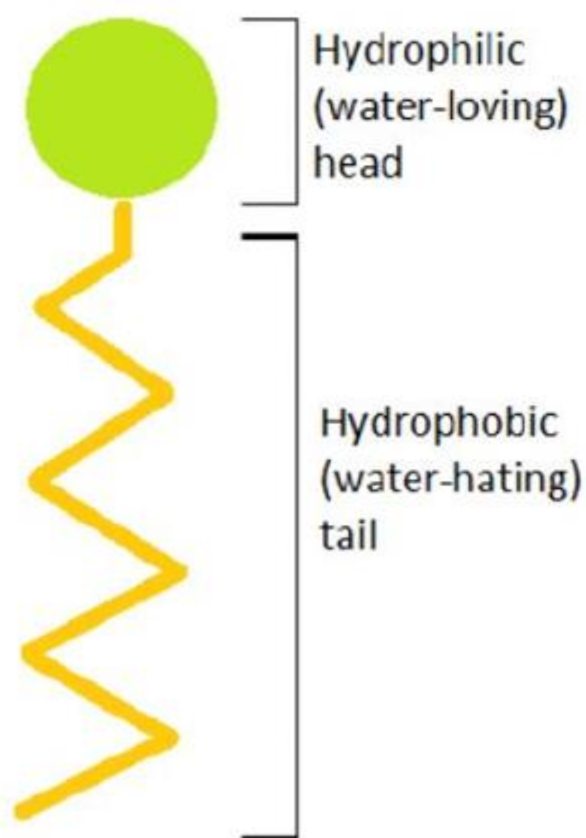


Figure 1-7: Shape of surfactant molecule (120)

1.9 Surfactants

Surfactant is an organic compound with an amphiphilic surfactant molecule (dual affinity) (120). The term surfactant (short for surface-active-agent), has a polar group that combine to form a hydrophilic mass (hydrophilic head), and it has a non-polar group bound together

in a hydrophobic chain (hydrophobic tail) (121). These surfactant molecules are known as micelles and are able to dissolve in both polar and non-polar solvents (122). On this basis, the polar groups provide the possibility of dissolution in polar solvents, such as water, while non polar groups for solubility in non-polar solvents such as chloroform (123,124).

When surfactant molecules are present in an aqueous solution, the hydrophilic head connects with water, while the hydrophobic tail avoids contact with the water (125,126), as shown in the Figure 1-8.

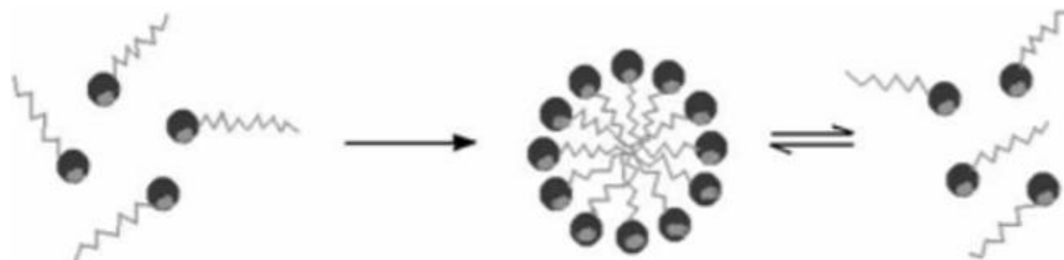


Figure 1-8: Surfactant-micelles (120).

The cloud point temperature (CPT) separates into two immiscible phases, both of which have the surfactant but at various concentrations (127).

1.9.1 Classification of surfactants

Surfactants have a different nature and charge. It's divided into (128–130): Cationic surfactant has a positive charge, for example, the Quaternary ammonium halides ($R4N^+ X^-$), anionic surfactant bears a negative charge, as, sulfate ($ROSO_3^- M^+$), nonionic surfactant does not have any positive or negative charges, for example polyoxymethylene ($R-OCH_2CH_2O-$) and Zwitterionic molecule has both positive and

negative charges, such as, (Sulfobetaine RN $(CH_3)_2CH_2CH_2SO_3^-$ (131–134). Figure 1-9, shown different surfactant structures and types.

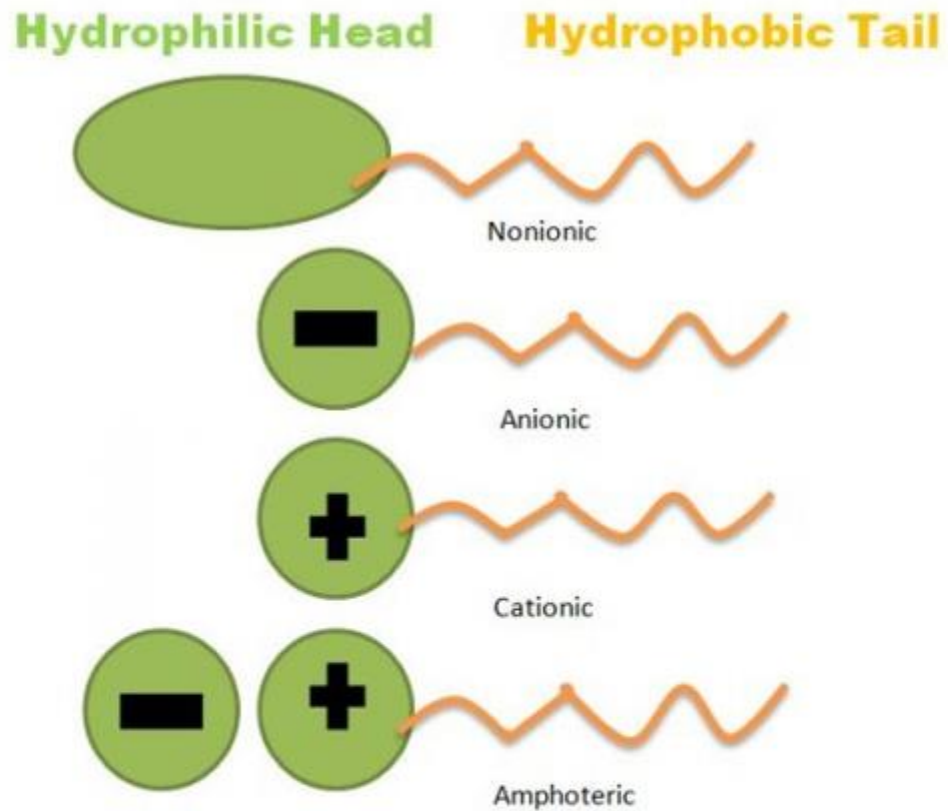


Figure 1-9: Types of surfactants (135)

1.10 Micelle formation

Surfactants appear properties phase phenomena of separation upon heating a certain threshold temperature (136,137). When the temperature is towered above in an aqueous solution, it will lead to the formation of micelles. The hydrophilic parts are oriented toward the water, while the hydrophobic parts that are long in the hydrocarbon chain are protected from water molecules to be inside the tail structure (138,139). CMC is the critical micelle concentration of surface-active molecules at which micelles create spontaneously, with all surfactant additions converting the solution to micelles. As a result, it is a feature

of surfactants. The surface tension of the liquid changes fast as the concentration of the surfactant solution increases. However, the surface tension remains relatively constant or decreases with a low slope as the solution reaches the CMC (140,141), as shown in Figure 1-10.

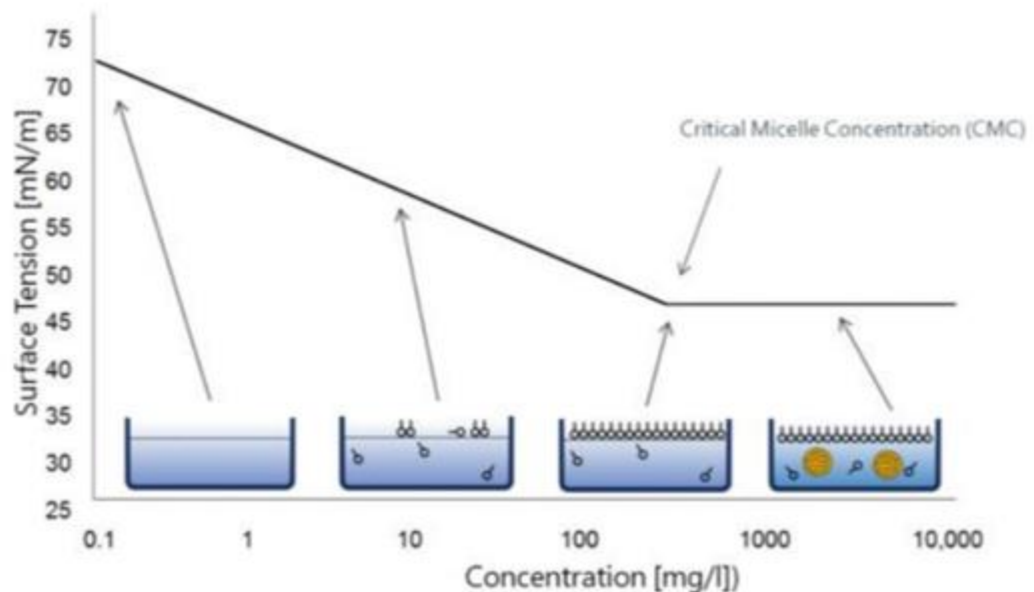


Figure 1-10: Surface tension of a surfactant solution with increasing concentration (141)

The insoluble part away from water is most suitable for concentration and separation to form a micelle (142).

The form and size of the micelle affects many conditions, like pH, ionic strength, temperature, and surfactant concentration; therefore, the micellar aggregates can be different shapes in solution, which is spherical, cylindrical, viscous isotropic, middle phase and neat phase, as shown in Figure 1-11 (143–145).

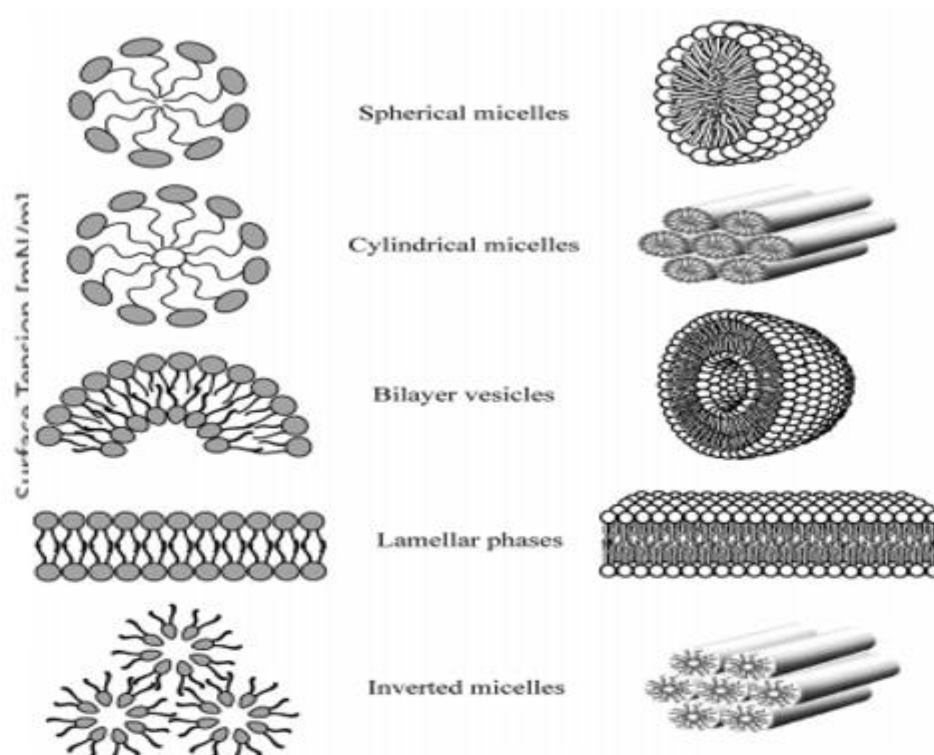


Figure 1-11: Shapes of Micellar formation (145)

The solution will be cloudy, and this will lead to the separation. The methods in surfactant solutions are usually referred to as cloudiness phenomenon (146–149). The phase isolation causes the dehydration of the hydrophilic surfactant group upon increasing the temperature. Dehydration stimulates micellar growth while also lowering intermicellar repulsive forces, resulting in phase separation. We must know the clouding method for a significant advance has been made in applying this phenomenon to a range of separation and extraction process. Micelle formation used to be a multistep technique. It also explains the increasing variety of the physical properties in the concentration range close to the CMC. There is a distribution of monomers and premicellar at concentrations below the CMC (150).

1.11 Biochemistry

Biochemistry studies the chemical principles that allow biological molecules to carry out processes within living cells (151,152).

Biochemistry has an essential and fundamental role in medicine in metabolic pathways, mechanism and storage conditions for different biomolecules or cellular communications (153,154).

1.11.1 Enzymes

Protein catalysts for chemical reactions in biological systems are known as enzymes as it speeds up the rate of chemical reactions inside living cells. In contrast, enzymes remain unchanged, as they act as an oxidizing agent (155). In 1878, German scientist Wilhelm Kohn used the term "enzyme" to describe the ability of yeast to create alcohol from carbohydrates (156,157).

Simple enzymes are made up of only protein molecules not coupled to any other proteins. It could also take the form of a holo-enzyme, which consists of both protein and non-protein components. There are two parts of holoenzymes; the apoenzyme is the protein component of these holoenzymes. The cofactor is the non-protein component of the holoenzyme (158).

1.11.2 Enzyme's inhibition

Inhibitors are substances that lower the activity of an enzyme-catalyzed process. They influence the catalytic characteristics of the active site either directly or indirectly. Inhibitors might be foreign or natural components of the cell. Those in the latter category can play a significant role in cell metabolism regulation. In addition, many poisons and pharmacologically active compounds (illegal and prescription and over-the-counter medications) work by inhibiting enzyme-catalyzed

activities (159–162). The enzyme inhibitor consists of two kinds: non-reversible and reversible, as in Figure 1-12.

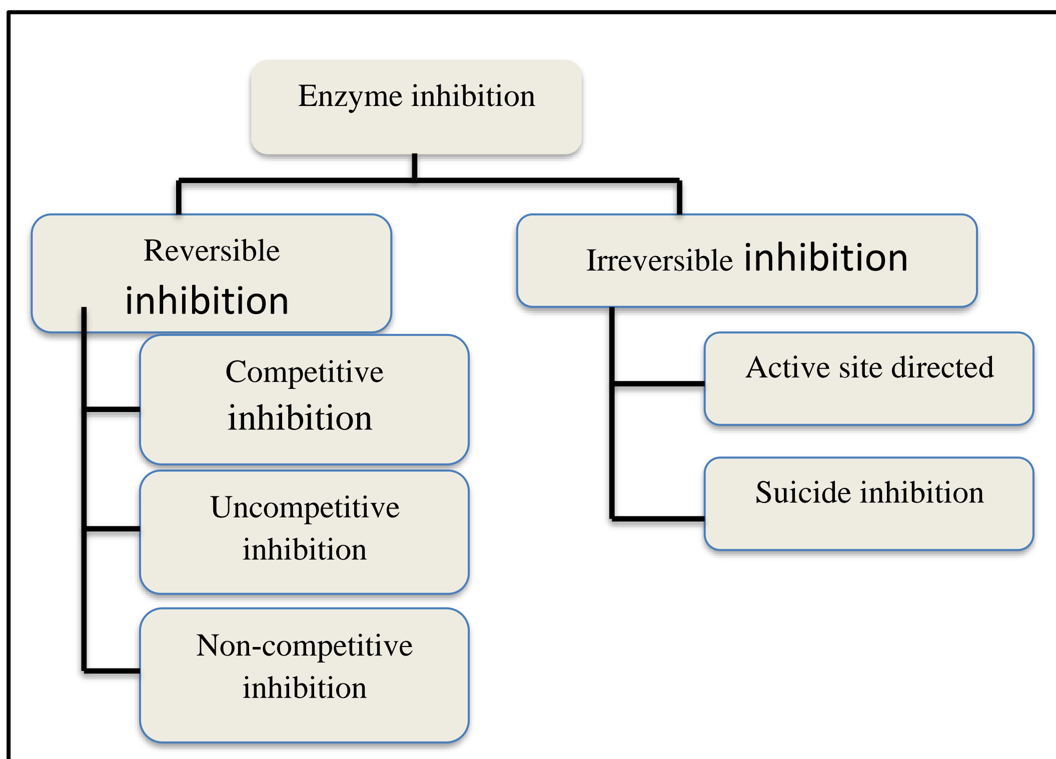


Figure 1-12: Type of enzyme inhibitors (163)

Enzymes' activity can be inhibited in several methods (164):-

1- Competitive inhibitors - a chemical that obstructs an enzyme's active site, causing a substrate to compete for the enzyme's attention with the inhibitor.

2- Non-competitive inhibitors attach to an enzyme in a different position than the active site, reducing its effectiveness

3 -Uncompetitive inhibitors bind to the enzyme and substrate after being connected. As a result, the products leave the active site less efficiently, slowing down the reaction.

4- Irreversible inhibitors bind to an enzyme and keep it inactive for the rest of its life.

1.11.3 Alkaline phosphatase enzyme (EC 3.1.3.1)

Alkaline phosphatase is a type of glycoprotein. It's orthophosphoric-monoester phosphohydrolase (EC 3.1.3.1), a monomer containing five cysteine residues, two zinc atoms, and one magnesium atom, all of which are required for the catalytic function of hydrolyzing monophosphate esters at an alkaline pH. The ideal pH levels for these enzymes are 10, (165)

Alkaline phosphatase is an enzyme located in the liver, bone, and placenta. Alkaline phosphate is released into the bloodstream during injuries, moderate bone growth, and pregnancy. On the other hand, it has a lot of overlapping and discontinuous variations. Only two or three types are recognized in any blood sample. It's assumed to form in the liver, where the skeletal system accounts for up to half of all activity (166–168)

ALP activity is a significant biochemical diagnostic of liver disease in the blood. Increases in ALP activity in serum and other body fluids, on the other hand, may represent physiological or pathological alterations such as hepatic dysfunction (169).

The enzyme level in the blood depends on a variety facet, including ages, gender, and blood type. Inflammatory reactions in patients with chronic kidney disease are influenced by alkaline phosphatase (ALP), and erythropoiesis is connected to factor-resistant anemia (170,171).

Hepatitis C is an example of a disease that does not influence alkaline phosphatase levels. The presence of high levels of this enzyme does not affect liver damage (172).

Lowered ALP levels are less usual than elevated levels. They may occur in conditions and diseases such as hypophosphatasia, postmenopausal women receiving estrogen therapy for osteoporosis, men who have recently undergone heart surgery, malnutrition, magnesium deficiency, hypothyroidism, and severe anemia, among others (173).

1.12 The aim of work

1- Describe analytical methods for determining medicines in their pure state and in pharmaceuticals that are simple, economical, and environmentally friendly.

2- To generate the best ideal experimental conditions for evaluating medicines utilizing a spectrophotometric approach incorporating the product reaction employing alizarin yellow reagent or HPβCD, as well as determining the best experimental conditions, the concentration of reagent, the concentration of acid, the temperature, and the concentration of medication are all parameters that affect the reaction.

3- Using three methods; direct extraction, DLLME, and CPE as an analytical method for estimating the proposed drugs and studying the optimal conditions for this method in terms of the concentration of the drug and the reagent, the volume of the drug and the reagent, in addition to the volume of the extracted and dispersed solvent.

4- Studying the effect of bromohexine hydrochloride in its pure and azo form on the activity of the ALP enzyme.

CHAPTER TWO

EXPREMENTAL PART

CHAPTER TWO

2-EXPREMENTAL PART

2.1 Apparatuses

The significant instruments utilized in this study are as follows:

- **UV-Visible spectrophotometer**

The spectral measurements have been recorded with a T80 UV-Vis double beam spectrophotometer instruments, and used quartz cell size 1.0cm.

- **Infrared spectra**

Spectrophotometer from Shimadzu with a range of 600-4000 cm^{-1}.

- **A Centrifuge**

Kaida Professional Co. is used. Model TDL5M Series is used to separate urine and serum. It is German made.

- **pH meter**

The pH meter type ino Lab 7110, WTW from Germany. To determination and control the acidity of the solution.

- **Sensitive Digital Balance**

All weighing was done on a Sartorius TE215 S scientific balance with a sensitive balance of ± 0.0001gm, Gottingen, Germany.

- **Vortex Mixer**

It is used type IKA®VORTEX GENIUS 3. To mix and stir the liquid samples under study in test tubes.

- **Hotplate magnetic stirrer**

It’s used type IKA®RH basic 2, made in Germany. It’s used for mixing and heating sample solution.

2.2 Chemicals and reagents

Each chemical indicated in the table was used in the study, showing the compounds, formula, molecular weight and purity of each compound Table 2-1.

Table 2-1: The chemical compounds, chemical formula, molecular weight, purity and companies

Compounds	Chemical formula	M.WT g/mol	Purity (%)	Company name
Phenylephrine HCl	$C_9H_{13}O_2N.HCl$	203.66	99.7	Sigma–Aldrich
Bromohexine HCl	$C_{14}H_{20}Br_2N_2.HCl$	412.59	99.3	SDI
Tenoxicam	$C_{13}H_{11}N_3O_4S_2$	337.376	99.7	SDI
Ranitidine HCl	$C_{13}H_{22}N_4O_3S.HCl$	350.9	99.7	SDI
Hydrochloric acid	HCl	36.46	pure	Scharla
Alizarin yellow	$C_{13}H_8N_3NaO_5$	287.23	99.5	sigma-Aldrich
2-hydroxypropyl-ß-cyclodextrin	$C_{63}H_{112}O_{42}$	1541.5	99.7	Siga Ultra UK
Ethanol	C_2H_5OH	46.07	98.0	BDH
Chloroform	$CHCl_3$	119.37	98.0	BDH

carbon tetrachloride	CCl_4	153.81	97.0	BDH
8-Hydroxy quinoline	C_9H_7NO	145.16	98.0	BDH
D-Glucose	$C_6H_{12}O_6$	180.16	99.5	BDH
Lactose	$C_{12}H_{22}O_{11}.H_2O$	360.32	99.0	BDH
Starch	$(C_6H_{10}O_5)_n$	$(162)_n$	99.0	BDH
Maltose	$C_{12}H_{22}O_{11}$	342.30	99.5	BDH
Fructose	$C_6H_{12}O_6$	180.16	98.0	BDH
Sodium hydroxide	NaOH	39.99	99.9	GCC
TritonX-114	$C_{14}H_{22}O(C_2H_4O)_{7-8}$	536.00	99.6	Sigma Aldrich UK
TritonX-100	$C_8H_{17}C_6H_4(OC_2H_4)_n$	625.00	99.6	Sigma Aldrich UK
Tween 80	$C_{32}H_{60}O_{10}$	604.822	99.0	Siga Ultra UK
Tween 20	$C_{18}H_{34}O_6(C_2H_4O)_n$	522.6692	99.6	Siga Ultra UK
SDS	$NaC_{12}H_{25}O_4$	288.38	99.0	GCC

CTAB	$C_{19}H_{42}BrN$	364.45	99.0	Siga Ultra UK
Glacial acetic acid	CH_3COOH	60.05	99.5	BDH (England)
Acetonitrile	C_2H_3N	41.05	99.8	BDH England
Acetone	$(CH_3)_2CO$	58.08	99.5	BDH

2.3 Preparation of Stock solution and reagent solution

The glassware cleaned with double- distilled water and dried at 50°C for 30 minutes before use in batch tests to ensure the reproducibility of data and the average result. As a result, every drug ion employed was of the utmost purity, and the majority of the solutions were prepared with distilled water.

2.3.1 Preparation of Stock solution of a PEH and BRH

500 µg/mL stock solution of PEH or BRH was prepared by dissolving 0.05gm from the PEH or BRH in 10mL from 0.1N HCl for PEH, 10 mL from D.W for BRH and diluting it, to the mark in a 100mL the volumetric flask. The working standard solution was prepared fresh by diluting the stock standard solution to a require concentration.

2.3.2 Preparation of Stock solution of a Tenoxicam

TNX 500 µg/mL stock solution was prepared by dissolving 0.05gm from the TNX in 10 mL of 0.1 N NaOH and completed 100mL

distilled water to the mark on the volumetric flask. The working standard solution was prepared fresh by diluting the stock standard solution to a require concentration.

2.3.3 Preparation of Stock solution of a Ranitidine. HCl

RAN-HCl 500 µg/mL stock solution was made by dissolving 0.05gm from the RAN in distilled water and diluting them to the mark in volumetric flask. The working standard solution was prepared fresh by diluting the stock standard solution to a require concentration.

2.3.4 Preparation of stock solution of an Alizarin yellow and 2-HPβCD reagents under investigation

Alizarin yellow or HPβCD reagent 500 µg/mL stock solution was prepared by dissolving 0.05gm from the Alizarin yellow or 2-HPβCD reagent in distilled water and completed to the mark in 100mLvolumetric flask. The working standard solution was made fresh by diluting the stock standard solution to a require concentration.

2.4 Preparation of solution of the pharmaceutical formulation

As shown in Table 2-3, the medicinal formulations used were obtained from well-known commercial sources. Table 2-2.

Table 2-2: The pharmaceutical formulation.

Drugs	Type of pharmaceutical formulation	Declared Compositions	Company
Phenylephrine HCl	Phenylephrine/ cooper	Sterile eye drops 10% w/v	European Union
Phenylephrine HCl	Dolo-cold	Tablets (10mg)	Micro Labs Limited India
Bromohexine HCl	Solvodin	Syrup of Bromohexine HCl (4mg)	SDI
Bromohexine HCl	Biosolvon	Syrup of Bromohexine HCl (4mg)	Egypt
Tenoxicam	Tilcotil	Tablets (20mg)	France
Tenoxicam	Tenoctil	Tablets BP (20mg)	Syria
Ranitidine HCl	BARKADIN	Syrup of Ranitidine (75mg/5mL)	Syria
Ranitidine HCl	HISTAC ^{R}150	Tablets (150mg)	India

2.4.1 Preparation solution of Phenylephrine HCl drops samples

Diluting (0.5mL) of PEH drops /cooper and completing with 0.1N HCl in a 100mL volumetric flask yielded 500 µg/mL solution of PEH drops samples. The solutions were prepared fresh by diluting the solution suitably.

2.4.2 Preparation solution of Phenylephrine HCl tablets samples

Five tablets containing PEH have been carefully powdered and accurately weighed. A quantity of is powder equal to 50 mg of PEH was dissolved in 0.1N HCl. The solution is filtered before dilution in a volumetric flask to 100 mL.

2.4.3 Preparation solution of Bromohexine HCl syrup samples

To prepare a 500 µg/mL BRH solution in Solvodin and Bisolvon, dilute 1.5 mL of Solvodin and 1.4 mL of Bisolvon in distilled water in a 50 mL volumetric flask. The solution was prepared fresh by diluting the solution suitably.

2.4.4 Preparation solution of Tenoxicam tablets samples

Tilcotil and Tenoctil tablets containing TNX were powdered and weighed accurately. A quantity of is powder equal to 50mg of Tenoxicam was dissolved in distilled water. The solution was filtered before diluting by D.W in the volumetric flask to 100mL, yielded 500 µg/ mL solution of TNX.

2.4.5 Preparation solution of Ranitidine HCl Syrup samples

To prepare a 500 µg/mL RAN solution in BARKADIN, diluted 0.166 mL of BARKADIN in distilled water in a 100 mL volumetric flask. The solution was made fresh and diluted to the original resolution appropriately.

2.4.6 Preparation solution of Ranitidine HCl tablets samples

HISTAC R150 tablets containing RAN were powdered and accurately weighed. A quantity of is powder equal to 50mg of RAN was dissolved in distilled water. The solution was filtered before

diluting D.W in a volumetric flask to 100mL, yielded 500 µg/mL solution of RAN.

2.5 Preparation of materials solutions

2.5.1 Hydrochloric acid solution 0.1N(HCl)

In a 100mL volumetric flask, 0.83mL of concentrated HCl was diluted with distilled water up to the mark to make hydrochloric acid 0.1N.

2.5.2 Preparation of buffer solutions

The buffer solution was made by blending the following solutions in the appropriate amounts. A pH meter was used to determine the exact pH of the solutions Table **2-3**.

Table 2-3: Preparation of buffer solutions (174)

Buffer solution	pH	Preparing a buffer solution
Phosphate buffer	1	50mL 0.1M KCl +134mlL 0.1M HCl
	2	50mL 0.1M KCl + 13mL 0.1M HCl
	3	100mL 0.1M KHPth + 44.6 mL 0.1M HCl
	4	100mL 0.1M KHPth + 0.2 mL 0.1M HCl
	5	100mL 0.1M KHPth + 45.2 mL 0.1M NaOH
	6	100mL 0.1M KH_2PO_4 + 11.2 mL 0.1M NaOH
	7	100mL 0.1M KH_2PO_4 + 58.2 mL 0.1M NaOH
	8	100mL 0.1M KH_2PO_4 + 93.4 mL 0.1M NaOH
Acetate buffer	4	41mL 0.58M CH_3COOH + 9mL 0.82M CH_3COONa
$Na_2HPO_4.12H_2O$ +NaOH	10	100mL 0.05M $Na_2HPO_4.12H_2O$ + 53.8mL 0.1M NaOH
KCl + NaOH	10	50mL 0.2M KCl + 12mL 0.2M NaOH
Na_2CO_3 + $NaHCO_3$	10	27.5ml 0.1M Na_2CO_3 + 22.5 ml 0.1M $NaHCO_3$
Borax +HCl	8	100mL 0.025 $Na_2B_4O_7$ + 41mL 0.1M HCl

2.5.3 Preparation of surfactants

Surfactants, including Triton X-114, Triton X-100, Tween 20, CTAB, and SDS was, prepared for working on using in the cloud point extraction.

2.5.3.1 Preparation of Triton X-114, Triton X-100, Tween 20

The surfactants (TX-114, TX-100, TW20) were prepared at a concentration of 10% v/v by taking a 10 mL volume of the surfactant

solution, pouring it into the volumetric flask, completing the volume for 100 mL D.W and allowing the sample to settle for one day before use.

2.5.3.2 Preparation of CTAB, SDS

The surfactants (CTAB, SDS) were prepared at a concentration of 10% wt./v by taking a 10gm volume of CTAB, pouring it into the volumetric flask, completing the volume for 100 mL D.W.

2.5.4 Preparation of interference solution

The interference solution is prepared by dissolving 0.1gm of each of the following components in D.W: starch, glucose, sucrose, maltose, lactose, and glycine, then transferring to a volumetric flask of 100mL and completing D.W to 100mL.

2.6 Spectrophotometric determination of PEH and BRH by alizarin yellow reagent using ion-pair reaction

2.6.1 General procedure of direct extraction method for PEH and BRH

2.6.1.1 Preliminary Studies

0.5 mL, 500µg/mL of standard amino drug solution (PEH or BRH) was mixed with 2 mL, 500µg/mL alizarin yellow reagent solution and 1mL of phosphate buffer (PH=1). The solution was transferred to a test tube containing stoppered. The volume was completed to 5mL with 0.1N Hydrochloric acid (HCl) for PEH, distill water for BRH. 5mL of chloroform solvent was added to the mixture and blended well for 1 minute utilizing a vortex. The drugs were reacted with alizarin yellow and formed ion-pair products and extracted by chloroform. Then, the chloroform layer was separated. The absorbance of the colored solution was scanned on a spectrophotometer in the range of 200-800nm against blank to calculate the λ_{max}.

2.6.1.2 Optimization of parameters

The influence of various factors on the intensity absorbance for extracting and forming an ion-pair substance by coupling medications (PEH and BRH) with alizarin yellow reagent was investigated. The optimum conditions are essential in improving the limit of detection and sensitivity of the method by changing one parameter and keeping the other parameter constant.

2.6.1.3 Effect of pH

The effect of pH on the formation of ion-pair complex was studied for both PEH and BRH in direct extraction method. 2 mL of alizarin yellow reagent was added to 0.5mL(500µg/mL) of (PEH or BRH) drug, following added 1 mL buffer with series values pH values from 1 to 8. The solution was transferred to a test tube containing stoppered. The volume was completed to 5mL with 0.1N Hydrochloric acid (HCl) for PEH, distilled water for BRH. 5mL of chloroform solvent was added to the mixture and blended well for 1 minute utilizing a vortex. The solution is separated into two phases. The required method involves measuring the absorbance at a maximum wavelength for each product to get pest pH for the reaction.

2.6.1.4 Effect of buffer type

The effect of the buffer solutions on the formation of the ion-pair complex was examined in direct extraction method, while the other factors are constant. Various types of buffer solutions (phosphate, citrate, and acetate solution) were prepared at a concentration of 0.1M in a 100mL volumetric flask. After mixing 0.5mL of the (PEH or BRH) drug with 2 mL of alizarin yellow reagent, 1mL optimal pH from types of buffers was added to a 15mL test tube containing stoppered. The absorbance was measured at the maximum wavelength of each product

to obtain the good buffer for the reaction.

2.6.1.5 Effect of buffer volume

The effect of the volume of buffer solutions on the formation of the ion-pair product was examined in direct extraction method, while the other factors are constant. Quantity of 10mL solution contains 2 mL of alizarin yellow reagent with 0.5 mL solution of standard (PEH or BRH) medicine. Various volumes between 0.2 and 2 mL of the selected buffer solution was added to15mL test tube containing stoppered. The absorption was measured at the maximum wavelength of each solution to obtain the optimum absorption.

2.6.1.6 Effect of volume of alizarin yellow solution

The effect of volumes of alizarin yellow solution on the formation of the ion-pair complex was examined in direct extraction method, while the other factors are constant. 10mL solution contains 0.5 mL of standard (PEH or BRH) medicine and the required volume of selected buffer solution. Various volumes of alizarin yellow reagent between 0.5and 3 mL was added to a 15 mL test tube containing stoppered. The absorption was measured at the maximum wavelength of each solution to obtain the optimum absorption.

2.6.1.7 Effect of organic solvent type

The effect of the type of organic solvent on the extraction of the ion-pair product was examined in direct extraction method, while the other factors are constant. In a test tube, the volume of selected of alizarin yellow reagent was mixed with 0.5 mL standard solution of (PEH or BRH) medicine and the required volume of selected buffer solution. Volumes of 5 mL of different solvents, including chloroform, hexane, benzene, and tetra chlorocarbon were added to the solution. The

absorptions were measured at the maximum wavelength of each solution to obtain the best solvent.

2.6.1.8 Effect of interference

Different substances could interfere with the ion-pair product of the drug including; lactose, sucrose, maltose, glucose, starch, glycine and fructose. It was examined the effect of previous interferences by preparing 0.1 % of each of them. A volume of 1 mL of each substance was added to 0.5 mL of 500 µg/mL of a drug under study (PEH or BRH), and added the selected volume of alizarin yellow reagent and the buffer solution. The absorption was taken at the maximum wavelength of each solution to obtain the optimum absorption.

2.6.1.9 Stoichiometric determination (PEH, BRH) of complex

a) Mole ratio method (175)

A series of solutions were prepared by mixing 1 mL of PEH (2×10^{-3} M) or BRH (1×10^{-3} M) with variable volumes between 0.2-2 mL of 500 µg/mL of alizarin yellow reagent. Other additives were then added as ideal conditions and diluted with distilled water in a 10 mL. The maximum absorption was measured at the wavelength of each solution. Then, the relationship between the reagent alizarin yellow to the drug was plotted against the absorbance, and the conjugation ratio was determined.

b) Continuous variation method (176)

The continuous variation approach (Job's method) was used to estimate the ratio of alizarin yellow reagent to medication (PEH, BRH). This method was required series volumes (0.1-0.9) mL from 500 µg/mL of alizarin yellow reagent and series volumes (0.9-0.1) mL from the drug (PEH, BRH), the total volume of each solution is 1 mL. Other

chemicals were added based on the optimal conditions, then completed with distilled water to 10mL. The absorbance was taken for each solution, which shows the values of the concentration ratios of the drug and reagent at the maximum wavelength of each complex at a concentration of (2 x 10^{-3} M) for PEH (1 x 10^{-3} M) for BRH. Then, the relationship between the reagent alizarin yellow to the drug was plotted against the absorbance, and the conjugation ratio was determined.

2.6.1.10 Calibration curve for PEH, BRH by direct extraction method

After selecting the best circumstances for the two medications under investigation, a series of concentrations of PEH and BRH (1-50µg/mL). The required volume of each concentration of drug was mixed with (2 or 1.5 mL) alizarin yellow reagent solution for (PEH or BRH) and 1.2mL of acetate buffer (PH = 4) for PEH, 1mL of phosphate buffer (pH = 5) for BRH. The solution was transferred to a test tube containing stoppered. The volume was completed to 5 mL (0.1N Hydrochloric acid for PEH and distill water for BRH). Chloroform solvent 5mL was added to the result and blended well for 1 minute utilizing a vortex the medicine: reagent ion-pair formation extracted in chloroform. Then, the chloroform layer was separated. The absorbance of the colored solution was scanned on a spectrophotometer in the range of 200-800 nm against blank.

2.6.1.11 Accuracy and precision

The accuracy was determined using the standard addition procedure, which took three concentrations (5, 10 and 15) µg/ mL of (PEH or BRH) from the pure drug and mixing it with alizarin yellow reagent, with paragraph 2.6.1.10 being the optimum conditions. The absorbance was measured for each solution using a spectrophotometer

and experiment was repeated three times for each concentration.

2.6.1.12 Application of the direct extraction method on pharmaceuticals formulation PEH, BRH drug

The pharmaceutical solutions for (PEH BRH) were prepared using the best conditions determined in the direct extraction method. PEH, BRH was evaluated at three different concentrations 5,10,15 µg/mL in (phenylephrine eye drops and Dolo-cold PEP) for PEH and (Solvodin, Bisolvon Elixir) for BRH. The absorbance of each pharmaceutical formulation (PEH or BRH) was taken against a specified blank at 430,480nm, respectively.

2.6.2 General procedure of dispersive liquid liquid microextraction (DLLME) for PEH, BRH

2.6.2.1 Preliminary study

In a 15 mL glass centrifuge tube, 0.5 mL (20 µg/ mL) of the drug (PEH or BRH) was blended with 1 mL of alizarin yellow reagent and 1 mL of phosphate buffer (PH = 1), then filled to 5 mL with 0.1N HCl and completed to 10 mL with distilled water. A cloudy solution was generated using a micro-syringe by rapidly infusing 300µL of chloroform as an extraction solvent and 700µL of ethanol as a dispersive solvent into the solution. The combination was centrifuged at 5000rpm for 5 minutes. The yellow ion-pair product was obtained using a micro-syringe then placed in a 1cm quartz microcell after the absorbance at the correct wavelength was measured against the blank.

2.6.2.2 Optimization parameters

The factors that influence the absorption density of colored DLLME were selected, including kind of solvent, kind of dispersion, the volume of the dispersion, volume of the solvent, time and speed of

the centrifugation method, and the extraction time.

2.6.2.3 Effect of type of extraction and disperser solvents

Different extraction solvents (300µL) such as chloroform, benzene, hexane, and carbon tetrachloride were investigated on the extraction of ion-pair product in the DLLME method. In a 15 mL glass centrifuge tube, 0.5 mL of the drug (PEH or BRH) was blended with 1 mL of alizarin yellow reagent and 1 mL of phosphate buffer (PH = 1), then filled to 5 mL with 0.1N HCl for PEH, distilled water for BRH, and completed to 10 mL with distilled water. The mixture was injected each time with a different extracting solvent of 300 µL and 700 µL of disperser solvent ethanol using micro-syringe. The mixture was centrifuged at 5000 rpm for 5 minutes. The product was separated using a micro-syringe, then placed in a 1cm quartz microcell after dissolved ethanol, and the absorbance was measured for PEH and BRH at 430 and 480 nm, respectively, against a blank.

The study was repeated under the same conditions using different disperse solvents such as ethanol, methanol, acetonitrile and acetone with the chosen extraction solvent.

2.6.2.4 Effect of extraction and dispersive solvent volume

1 mL of alizarin yellow reagent and 0.5 mL standard solution of (PEH or BRH) medicine was added to a glass tube containing 1mL of phosphate buffer solution pH = 1. The solution was completed to 5 mL with 0.1N HCl, then filled to 10 mL with distilled water. The different volumes (200-500) µL of extraction solvent chloroform and variable volumes (500-1500) µL of dispersive solvent ethanol were used in the current investigation. The solvents were injected into the mixture by micro-syringe. The mixture was centrifuged at 5000 rpm for 5 minutes. The yellow ion-pair complex was collected using a micro-syringe, then

placed in a 1cm quartz microcell after dissolved ethanol, and the absorbance was measured for PEH, BRH at 430,480 nm, respectively, against a blank. The amount of extracting solvent and dispersive that provided the best absorbance was used in subsequent studies.

2.6.2.5 Effect of pH

The effect of pH solution on the complex formation of ion-pair product reaction between the (PEH or BRH) drug and alizarin yellow in the DLLME method was tested. It was added 1 mL of different values from pH from between 1 – 8, to 1mL of alizarin yellow reagent and 0.5 mL standard solution of (PEH or BRH) medicine. The solution was filled to 5 mL with 0.1N HCl, then completed to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300µL of chloroform as an extraction solvent and 700µL of ethanol as a dispersive solvent into the solution. The mixture was centrifuged at 5000 rpm for 5 minutes. The yellow ion-pair complex was obtained using a micro-syringe, then placed in a 1cm quartz microcell. The absorbance was measured for PEH and BRH at 430 and 480 nm, respectively, against a blank.

2.6.2.6 Effect of buffer type

The type of buffer was investigated on the formation of ion-pair complex to (PEH or BRH) drug in the DLLME method. Various types of buffer solutions (phosphate, citrate and acetate solution) were prepared at a concentration of 0.1M in a 100 mL volumetric flask. After mixing 0.5mL of the (PEH or BRH) drug and 1mL of alizarin yellow reagent, 1mL optimum pH was added. The absorbance was measured at the maximum wavelength of each complex to obtain the optimum condition for reaction.

2.6.2.7 Effect of buffer volume

The effect of the volume of pH buffer solution was examined on the formation of ion-pair product to (PEH or BRH) drug in the DLLME method. To a solution containing 1 mL of alizarin yellow reagent with 0.5 mL solution of standard (PEH or BRH) medicine, various volumes between 0.2 and 2 mL of buffer solution (acetate buffer for PEH and phosphate buffer for BRH) were added in a 15 mL glass centrifuge tube. The solution was completed to 5mL with 0.1N HCl, then to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300µL of chloroform as an extraction solvent and 700µL of ethanol as a dispersive solvent into the solution. The mixture was centrifuged at 5000 rpm for 5 minutes. The yellow ion-pair complex was obtained using a micro-syringe, then placed in a 1cm quartz microcell. The absorption was measured at the maximum wavelength of each solution to obtain the optimum reaction condition.

2.6.2.8 Effect of volume of alizarin yellow solution

The effect of the volume of alizarin yellow reagent was investigated on the ion-pair drug product in the DLLME method. In a solution of 0.5 mL of standard (PEH or BRH) medicine and the required the volume of selected buffer solution. Various volumes of alizarin yellow reagent between 0.5 and 3 mL in a 15 mL glass centrifuge tube. The mixture was filled to 5mL with 0.1N HCl, then to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300µL of chloroform as an extraction solvent and 700 µL of ethanol as a dispersive solvent into the solution. The mixture was centrifuged at 5000rpm for 5 minutes. The yellow ion-pair product was obtained using a micro-syringe, then placed in a 1cm quartz microcell. The absorption was measured at the maximum wavelength

of each solution to obtain the optimum reaction condition.

2.6.2.9 Effect of centrifuge speed and time

The effect of centrifuge speed and time was tested on the formation of an ion-pair drug product in DLLME method. To a solution containing the volume of selected of alizarin yellow reagent with 0.5 mL solution of standard (PEH or BRH) medicine and the required the volume of selected buffer solution in a 15 mL glass centrifuge tube. The mixture was filled to 5mL with 0.1N HCl, then completed to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300µL of chloroform as an extraction solvent and 700µL of ethanol as a dispersive solvent into the solution. The centrifuge speeds between 1000 to 6000 rpm and intervals between 2.0 to 10.0 min were changed. The absorption of each solution was measured at their respective maximum. The duration and speed that produced the best absorption were used in subsequent studies.

2.6.2.10 Calibration curve for PEH and BRH by DLLME method

After selecting the optimal conditions for the drug under study (PEH, BRH), it was found that the optimal method of action for each drug is:

In a 15 mL glass centrifuge tube, add various concentration (0.5-20µg/mL) of medication (PEH or BRH) and (1.0 or1.5 mL) of alizarin yellow reagent for PEH and BRH, respectively, then add 0.8 mL of acetate buffer (PH = 4) for PEH, 1.2 mL of phosphate buffer (PH = 5) for BRH, then complete the volume to 5 mL with 0.1N HCl, then to 10 mL with distilled water. The volume of (400 or 300µL), chloroform as an extraction solvent and ethanol (700 or 900µL), respectively as dispersive solvent was rapidly injected into the solution using a micro

syringe to produce the formation of a cloudy solution. The mixture was centrifuged at (2000 or 4000 rpm) for (6 or 4 minutes) respectively. The color ion-pair product was obtained with a micro syringe, then placed in a 1cm quartz microcell, and the absorbance was taken for PEH, BRH at 430,480nm, respectively, against a blank.

2.6.2.11 Accuracy and Precision

The accuracy was determined using the standard addition procedure, which took three concentrations (3, 5 and 7) µg/mL for (PEH or BRH) from the pure drug and mixing it with alizarin yellow reagent, with paragraph 2.6.2.10 being the optimum conditions. The absorbance was measured for each solution using a spectrophotometer and experiment was repeated three times for each concentration.

2.6.2.12 Application of the DLLME method on pharmaceuticals formulation PEH, BRH drug

The pharmaceutical solutions for (PEH BRH) were prepared using the best conditions determined in the DLLME method. PEH, BRH was evaluated at three different concentrations 3,5,7 µg/mL in (phenylephrine eye drops and Dolo-cold PEP) for PEH and (Solvodin, Bisolvon Elixir) for BRH. The absorbance of each pharmaceutical solution (PEH BRH) was measured against a specified blank at 430,480 nm, respectively.

2.6.3 General procedure of cloud point extraction (CPE) for PEH, BRH

2.6.3.1 Preliminary study

A 0.5mL, 500µg/mL of standard drug solution of (PEH or BRH) was mixed with 2 mL, 500µg/mL of alizarin yellow reagent and 1 mL of phosphate buffer (pH = 1), then added 1 mL of (10%) TX-114 in a

15mL test tube containing stoppered, and completed the volume with distilled water to 10 mL and placed in a water bath at 50ºC for 20 minutes. The solution was centrifuged at 3000 for 4 minutes to separate the two phases. The cloud was split and dissolved in 2mL of methanol. The absorbance of the colored solution was scanned on a spectrophotometer in the range of 200-800 nm against a drug-free blank solution.

2.6.3.2 Optimization of CPE method

The impact of various factors on the intensity absorbance of the production of an ion-pair substance by coupling pharmaceuticals with alizarin yellow reagent was studied. By changing one parameter such as type of surfactant, the volume surfactant, effect of temperature and incubation time, and the effect of centrifuge speed and time of while keeping the other constant, the optimum circumstances are critical for improving the detection limit and sensitivity of the approach.

2.6.3.3 Effect of type of surfactant

The effect of type surfactant on CPE method was investigated. A solution was prepared from 0.5mL (500µg/mL) of PEH, BRH, 2mL alizarin yellow reagent, 1mL(pH=1) phosphate buffer solution for produce ion-pair product. The surfactants TX-114, TX-100, TW20, CTAB, and SDS at a concentration of 10% were prepared. A 1mL of each surfactant was added to the mixture and completed the volume with distilled water to 10 mL and placed in a water bath at 50 ºC for 20 minutes. The mixture was separated into two phases with a centrifuge at 3000rmp for 4 minutes. The cloud was split and dissolved in 2 mL of methanol. The absorbance was measured at the maximum wavelength.

2.6.3.4 Effect of surfactant volume

The effect of surfactant volume CPE method was studied. A solution was prepared from 0.5 mL (500µg/mL) of PEH or BRH, 2mL alizarin yellow reagent, 1mL (pH=1) phosphate buffer solution for produce ion-pair product. The optimal surfactant was added in different volumes between 0.2 and 3 mL. The complex was completed to 10 mL by distilled water and placed in a water bath at 50 ^{o}C for 20 minutes. The mixture was separated into two phases using a centrifuge at 3000rmp for 4 minutes. The cloud was split and dissolved in 2 mL of methanol. The absorbance was measured at the maximum wavelength.

2.6.3.5 Effect of temperature and incubation time

The effect of temperature and incubation time on CPE method was studied. Temperature and incubation time are the most critical steps in extracting cloud points. A solution was prepared from 0.5mL (500µg/mL) of PEH or BRH, 2 mL alizarin yellow reagent, 1mL phosphate buffer solution to produce ion-pair product. A 1mL of TX-114 was added to the mixture then completed to 10 mL by distilled water. The mixture was placed in a water bath at different temperatures between (30 and 80) ^{o}C at different incubation periods from 10 to 60 minutes. The mixture was separated into two phases using a centrifuge at 3000 rpm for 4 minutes. The cloud was split and dissolved in 2 mL of methanol. The absorbance was measured at the maximum wavelength.

2.6.3.6 The effect of centrifuge speed and time

One of the most critical steps in extracting cloud points is to study the influence of centrifugal speed and time when the others factor constant. The speed ranged from 1000 to 6000 rpm and the time ranged

from 1 to 6 minutes. The speed and period that gave the maximum absorption in the procedure were chosen at an appropriate wavelength.

2.6.3.7 The effect of best solvent

Using optimal conditions, the final product of a complex reaction (PEH or BRH) with alizarin yellow reagent was diluted with 2 mL of different solvents [ethanol, methanol, chloroform, and hexane]. Absorbance was measured at the maximum wavelength of each color product and recorded the highest absorbance for the best solvent.

2.6.3.8 Calibration curve for PEH, BRH by CPE method

After studying the optimal conditions for the drug under study (PEH or BRH), it was found that the optimal method of action for each drug is:

In a 15mL glass centrifuge tube, add various concentration (1-50μg/mL) of medication (PEH or BRH) was transferred to a 15mL glass centrifuge tube stoppered tube, and 1 mL of acetate buffer (pH = 5) for PEH, phosphate buffer (pH = 5) for BRH was added to it, then (1.0 or 0.8 mL) of alizarin yellow reagent was added. Then added (1.0 or 0.8 mL) of (10%) TX-114 and completed the volume with double distilled water to reach 10 mL and placed in a water bath at (60 or 50ºC) for 20 minutes. After using a centrifuge at (4000 or 5000rpm) for (5) minutes to separate the two phases. The cloud was split and dissolved in 2 mL of (methanol or ethanol). The absorbance of the colored solution was scanned on a spectrophotometer in the range of 200-800 nm against a drug-free blank solution.

2.6.3.9 Accuracy and precision

The accuracy was determined using the standard addition procedure, which took three concentrations (5, 10 and 15) µg/mL of

(PEH or BRH) from the pure drug and mixing it with alizarin yellow reagent, with paragraph 2.6.3.8 being the optimum conditions. The absorbance was measured for each solution using a spectrophotometer and experiment was repeated three times for each concentration.

2.6.3.10 Application of the CPE method on pharmaceuticals formulation PEH, BRH drug

The pharmaceutical solutions for (PEH and BRH) were prepared using the best conditions determined in the direct extraction method. PEH, BRH was evaluated at three different concentrations (5,10,15) µg/mL in (phenylephrine eye drops and Dolo-cold PEP) for PEH and (Solvodin, Bisolvon Elixir) for BRH. The absorbance of each pharmaceutical solution (PEH BRH) was measured against a specified blank at 430 and 480nm, respectively.

2.7 Spectrophotometric determination of TNX and RAN-HCl by the hydroxypropyl-β-cyclodextrin reagent

2.7.1 General procedure of direct extraction method for TNX and RAN-HCl

2.7.1.1 Preliminary Studies

A 1mL, 500µg/mL standard drug solution (TNX or RAN-HCl) was mixed with 1mL,500µg/mL HPβCD reagent solution and 1mL of phosphate buffer (PH=1). The solution was transferred to a test tube containing stoppered. The volume was completed to 10 mL distilled water. The solution was placed in water bath at 35 ^{o}C for 5 min. 5mL of Chloroform solvent was added to the mixture and blended well for 1 minute utilizing a vortex. The medicines were reacted with HPβCD reagent and formed complexes and extracted by chloroform solvent. Then, the chloroform layer was separated. The absorbance of the

colored solution was scanned on a spectrophotometer in the range of 200-800 nm against a reagent blank. The blank was prepared similarly method but without drug (TNX or RAN).

2.7.1.2 Optimization parameters for complication of TNX and RAN-HCl

The influence of various factors on the intensity absorbance for extracting and forming a product by reaction medication (TNX or RAN-HCl) with HPβCD reagent was investigated. The optimum conditions are essential in improving the limit of detection and sensitivity of the method by changing one parameter and keeping the other parameter constant.

2.7.1.3 Effect of pH

The effect of pH on the extraction of the complex was studied for both TNX and RAN-HCl drug in direct extraction method. A 1 mL of HPβCD reagent was added to 1mL(500µg/mL) of (TNX or RAN-HCl) drug, followed added 1ml of the pH with series values from 1 to 12. The volume was completed to 10mL distill water. The solution was placed in water bath at 35 ºC for 5min. 5 mL of Chloroform solvent was added to the mixture and blended well for 1 minute utilizing a vortex. The required method involves measuring the absorbance at a maximum wavelength for each complex to get optimum conditions for the reaction.

2.7.1.4 Effect of buffer type

The effect of the buffer solutions on the extraction of the complex was examined while the other factors are constant. Various type of buffer solutions pH=10 ($Na_2HPO_4.12H_2O$+NaOH, KCl+NaOH and carbonate-bicarbonate buffer solution) for TNX, PH = 8 (phosphate,

Borax+HCl buffer solution) for RAN were prepared at a concentration in a Table **2-3** .After mixing 1mL of the (TNX or RAN-HCl) drug with 1mL of 2-HPβCD reagent, 1mL optimal pH from types of buffers was added to a 15 ml test tube containing stoppered, the volume was completed to 10 mL with distilled water, then heating in water bath at 35 ^{o}C for 5 min, the others factor constant. The absorbance was measured at the maximum wavelength to each product in order to obtain optimum condition for reaction.

2.7.1.5 Effect of buffer volume

The effect of the volume of buffer solutions on the extraction of complex was examined in direct extraction method, while the other factors are constant. 10 mL solution contains 1 mL of HPβCD reagent with 1 mL solution of standard (TNX or RAN-HCl) medicine. Various volumes between 0.2 and 3 mL of the selected buffer solution (KCl+NaOH buffer) for TNX, phosphate buffer for RAN-HCl was added to15mL test tube containing stoppered. The volume was completed to 10 mL with distilled water. The solution was placed in water bath at 35 ^{o}C for 5min, the others factor constant. The absorption was measured at the maximum wavelength of each solution to obtain the optimum absorption.

2.7.1.6 Effect of volume of HPβCD reagent solution

The effect of volumes of HPβCD solution on the extraction on the formation of the complex was examined in direct extraction method, while the other factors are constant. A 10 mL solution contains 1 mL of standard (TNX or RAN-HCl) medicine and the required volume of selected buffer solution. Various volumes of the of 2-HPβCD reagent between 0.5 and 3 mL was added to a 15 mL test tube containing stoppered., the volume was completed to 10 mL distilled water. The

solution was placed in water bath at 35 ºC for 5min. The absorption was measured at the maximum wavelength of each solution to obtain the optimum reaction condition.

2.7.1.7 Effect of temperature

The influence of the temperature on the formation of the color product was studied in direct extraction method, while the other factors are constant. 10mL solution contains 1 mL of 2-HPβCD reagent with 1 mL of standard drug (TNX or RAN-HCl) medicine and the required volume of selected buffer solution. The volume was completed to 10mL with distilled water. The solution was placed in water bath at different temperature (25-50 ºC) for 5 min. The absorption is taken at the maximum wavelength of each solution to obtain the optimum reaction condition.

2.7.1.8 Effect of organic solvent type

The effect of the type of organic solvent on the extraction of the color product was examined in direct extraction method, while the other factors are constant. In a test tube, 10 mL solution contains the volume of selected of 2-HPβCD reagent was mixed with 1 mL solution of standard (TNX or RAN-HCl) medicine and the required volume of selected buffer solution. A series of 5 mL of different solvents, including chloroform, hexane, benzene, and tetra chlorocarbon were added to the solution. The absorptions were measured at the maximum wavelength of each solution to obtain the optimum reaction conditions.

2.7.1.9 Effect of interference

Different substances could interfere with the color product of the drug including; lactose, sucrose, maltose, glucose, starch, glycine, fructose. It was studied, the effect of previous interferences by

preparing 0.1 % of each of them. A volume of 1 mL of each substance was added to 1mL of 500µg/mL of a drug under study (TNX or RAN-HCl), and added the selected volume of 2-HPβCD reagent and the buffer solution. The absorption was taken at the maximum wavelength of each solution to obtain the optimum reaction condition.

2.7.1.10 Stoichiometric determination (TNX, RAN) of complex

A. Mole ratio method (175)

A series of solutions were prepared by mixing a fixed volume of 1 mL of TNX (3×10^{-3} M), RAN-HCl (3×10^{-3} M) with a variable volume between 0.2-2 mL of HPβCD reagent. Other additives were then added as ideal conditions and diluted with distilled water in a 10 mL. The maximum absorption was measured at the wavelength of each solution. Then the relationship between the reagent 2-HPβCD to the drug was plotted against the absorbance, and the conjugation ratio is determined.

B. Continuous variation method (176)

The continuous variation approach (Job's method) was used to estimate the ratio of 2-HPβCD reagent to medication (TNX or RAN-HCl), this method was required series volume (0.1-0.9) mL from HPβCD reagent and a series volume (0.9-0.1) mL from the drug (TNX or RAN-HCl). The total volume of each solution is 1 mL. Other chemicals were added based on the optimal conditions, then completed with distilled water to 10 mL. The absorbance was taken for each solution, which shows the values of the concentration ratios of the drug and reagent at the maximum wavelength of each product at a concentration of (3 x 10 -3 M) for TNX (3 x 10 -3 M) for RAN-HCl. Then, the relationship between the reagent 2-HPβCD to the drug, was

plotted against the absorbance, and the conjugation ratio was determined.

2.7.1.11 Calibration curve of TNX, RAN-HCl by direct extraction method

After selecting the best circumstances of the two medications under investigation, a series of concentrations of TNX and RAN-HCl (1-50µg/mL) were added in 15mL stoppered tube containing, (1.0 mL) 2-HPβCD reagent solution for (TNX or RAN-HCl), and 1.2mL of (KCl+NaOH) for TNX, 0.8 mL phosphate buffer for RAN. The volume was completed to 10mL distilled water. The solution was placed in water bath at (30 or 25 ºC) for 5min. Chloroform 5mL was added and blended for 1 minute utilizing a vortex. The medicine: reagent complex was extracted in chloroform. Then, the chloroform layer was separated. The absorbance of the colored solution was scanned on a spectrophotometer in the range of 200-800 nm against a reagent blank. The blank was prepared similarly method but without drug (TNX or RAN).

2.7.1.12 Accuracy and precision

The accuracy was determined using the standard addition procedure, which took three concentrations (5, 10 and 15) µg/mL of (TNX or RAN-HCl) from the pure drug and mixing it with 2-HPβCD reagent, with paragraph 2.7.1.11. being the optimum conditions. The absorbance was measured for each solution using a spectrophotometer and experiment was repeated three times for each concentration.

2.7.1.13 Application of the direct extraction method on pharmaceuticals formulation TNX and RAN-HCl drug

The pharmaceutical solutions for (TNX or RAN-HCl) were

prepared using the best conditions determined in the direct extraction method. TNX or RAN was evaluated at three different concentrations 5,10,15 µg/ mL in (Tilcotil and Tenoctil) for TNX and (Barkadin, HISTAC R150) for RAN. The absorbance of each pharmaceutical solution was taken against a specified blank.

2.7.2 General procedure of dispersive liquid liquid microextraction (DLLME) for TNX, RAN-HCl

2.7.2.1 Preliminary study

In a 15 mL glass centrifuge tube, 1 mL, 20 µg/ mL of the drug (TNX or RAN-HCl) was blended with 1 mL of 2-HPβCD reagent and the required volume of selected buffer solution for TNX or RAN-HCl, then was completed to 10 mL with distilled water, and then heating in water bath at 35 ^{o}C for 5 min. A cloudy solution was generated using a micro-syringe by rapidly infusing 300 µL of chloroform as an extraction solvent and 700 µL of ethanol as a dispersive solvent into the solution. The combination was centrifuged at 5000 rpm for 5 minutes. The product complex was obtained using a micro syringe, then placed in a 1cm quartz microcell after dissolved with ethanol, and the absorbance at the correct wavelength was measured against a blank.

2.7.2.2 Optimization parameters of TNX and RAN-HCl with DLLME method

The factors which influence the absorption density of colored DLLME were selected, including the kind of solvent, kind of the dispersion, volume of the dispersion and solvent, time and speed of the centrifugation method, and the extraction time.

2.7.2.3 Effect type of extraction and dispersive solvents

Different extraction solvents (300 µL) such as chloroform,

benzene, hexane, and carbon tetrachloride were investigated on the extraction of ion-pair color Type in the DLLME method. In a 15 mL glass centrifuge tube, 1 mL of the drug (TNX, RAN-HCl) was mixed with 1 mL of 2-HPβCD reagent and 1 mL of (KCl+NaOH) buffer (PH = 10) for TNX, phosphate buffer pH=8 for RAN-HCl, then completed to 10 mL with distilled water and then, heating in water bath at 35 ºC for 5 min. The mixture was injected each time with a different extracting solvent of 300 µL and 700 µL of disperser solvent ethanol using micro-syringe. The mixture was centrifuged at 5000 rpm for 5 minutes. The product was separated using a micro syringe, then placed in a 1cm quartz microcell after dissolved ethanol. The absorbance was taken for TNX or RAN-HCl, against a blank. The study was repeated under the same conditions using different disperse solvents such as ethanol, methanol, acetonitrile and acetone with the chosen extraction solvent.

2.7.2.4 Effect of extraction and disperser solvent volume

1 mL of 2-HPβCD reagent and 1 mL standard of solution (TNX or RAN-HCl) medicine was added to a glass tube containing the 1mL of (KCl+NaOH) buffer PH=10 for TNX, phosphate buffer pH=8 for RAN-HCl. The solution was filled to 10 mL with distilled water then, heating in water bath at 35ºC for 5min. The different volumes (200-500) µL of chloroform were used as a solvent, and variable volumes (500-1500) µL of ethanol for TXN, methanol for RAN-HCl, as a disperser were used in the current investigation. The solvents were injected into the mixture by micro-syringe. The mixture was centrifuged at 5000 rpm for 5 minutes. The complex was collected using a micro-syringe, then placed in a 1cm quartz microcell after dissolved ethanol or methanol, and the absorbance was measured for TNX, RAN-HCl, against a blank.

The amount of extracting solvent and dispersive that provided the best absorbance was used in subsequent studies.

2.7.2.5 Effect of buffer volume

The effect of the volume of buffer solution was examined on the complex extraction of (TNX or RAN-HCl) medicine in the DLLME method. To a solution containing 1 mL of 2-HPβCD reagent with 1mL standard solution of (TNX or RAN-HCl) medicine, various volumes between 0.2 and 2 mL of buffer solution (PH=10 for TNX, phosphate buffer pH=8 for RAN-HCl) were added in 15 mL glass centrifuge tube. The solution was completed to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300μL of chloroform as an extraction solvent and 700 μL of ethanol as a dispersive solvent into the solution. The mixture was centrifuged at 5000 rpm for 5 minutes. The product was collected using a micro-syringe, then placed in a 1cm quartz microcell. The absorption is taken at the maximum wavelength of each solution to obtain the optimum reaction condition.

2.7.2.6 Effect of volume of 2-HPβCD solution

The effect of the volume of 2-HPβCD reagent was investigated on the drug complex in the DLLME method. In a solution of 1 mL of standard (TNX or RAN-HCl) medicine and the 1mL of (KCl+NaOH) buffer PH=10 for TNX,1.2mL phosphate buffer pH=8 for RAN-HCl. Various volumes of a 0.5–3 mL HPβCD reagent in a 15 mL glass centrifuge tube. The mixture was completed to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300μL of chloroform as an extraction solvent and 700μL of ethanol as a dispersive solvent into the solution. The mixture was centrifuged at 5000 rpm for 5 minutes. The complex was collected

using a micro-syringe, then placed in a 1cm quartz microcell. The absorption was measured at the maximum wavelength of each solution to obtain the optimum reaction condition.

2.7.2.7 Effect of centrifuge rate and time

The effect of centrifuge speed and time was tested on the extraction of drug complex in DLLME method. To a solution containing 1 mL of 2-HPβCD reagent with 1 mL solution of standard (TNX or RAN-HCl) medicine and then add 1mL of (KCl+NaOH) buffer PH=10 for TNX,1.2 mL phosphate buffer pH=8 for RAN-HCl, in a 15 mL glass centrifuge tube. The mixture was filled to 10 mL with distilled water. Using a micro-syringe, a cloudy solution was generated by rapidly infusing 300 µL of chloroform as an extraction solvent and 700 µL of ethanol as a dispersive solvent into the solution. The centrifuge rates between 1000 to 6000 rpm and intervals between 2.0 to 10.0 min were changed. The absorption of each solution was measured at their respective maximum. The duration and speed that produced the best absorption were used in subsequent studies.

2.7.2.8 Calibration curve for TNX and RAN-HCl by DLLME method

After selecting the optimal conditions for the drug under study (TNX or RAN-HCl), it was found that the optimal method of action for each drug is:

In a 15 mL glass centrifuge tube, add 1 mL of medication (TNX or RAN-HCl) 20 µg/mL and (1.5 mL) of 2-HPβCD reagent 20 µg/mL, then add 1 mL of (KCl+NaOH) buffer PH=10 for TNX,1.2 mL phosphate buffer pH=8 for RAN-HCl, then filled to 10 mL with

distilled water, then heating in water bath at 35 ºC for 5min. The volume of (400 or 300 µL) chloroform as an extraction solvent and ethanol (700 or 800 µL) as dispersive solvent was rapidly injected into the solution using a micro syringe to produce the formation of a cloudy solution. The mixture was centrifuged at (5000 or 4000 rpm) for (4 or 6 minutes). The product complex was obtained with a micro syringe, put in a 1cm quartz microcell after dissolved ethanol, and the absorbance was measured for TNX or RAN-HCl, against a blank.

2.7.2.9 Accuracy and precision

The accuracy was determined using the standard addition procedure, which took three concentrations (3, 5 and 7) µg/mL of (TNX or RAN-HCl) from the pure drug and mixing it with 2-HPβCD, with paragraph 2.7.2.8. being the optimum conditions. The absorbance was measured for each solution using a spectrophotometer and experiment was repeated three times for each concentration.

2.7.2.10 Application of the DLLME method on pharmaceuticals formulation TNX and RAN-HCl

The pharmaceutical solutions for (TNX or RAN-HCl) were prepared using the best conditions determined in the DLLME extraction study. TNX or RAN-HCl was evaluated at three various concentrations 3,5,10 µg/mL in (Tilcotil and Tenoctil) for TNX and (Barkadin, HISTAC R150) for RAN-HCl. The absorbance of each pharmaceutical solution was taken against a specified blank.

2.7.3 General procedure of cloud point extraction (CPE) of TNX and RAN-HCl

2.7.3.1 Preliminary study

In a 15 mL test tube containing stoppered, 1 mL, 500 µg/mL of

standard drug solution of (TNX or RAN-HCl) was mixed with 1 mL, 500 μg/mL of HPβCD reagent, and 1 mL of (KCl+NaOH) buffer PH=10 for TNX, phosphate buffer pH = 8 for RAN-HCl, then added 1 mL of (10%) TX-114 and completed the volume with distilled water to 10 mL and placed in a water bath at 50 ºC for 20 minutes. The solution was centrifuged at 3000 for 4 minutes to separate the two phases. The cloud was split and dissolved in 2 mL of methanol. The absorbance was scanned on a spectrophotometer in the range of 200-800 nm against a drug-free blank solution.

2.7.3.2 Optimization parameters for complication of TNX and RAN-HCl of CPE method

The effect of various factors on the intensity absorbance of the production of complex by reaction pharmaceutical (TNX or RAN-HCl) with 2-HPβCD reagent was studied. By changing one parameter such as type of surfactant (TX-114, TX-100, TW20, CTAB, and SDS) and the volume surfactant while keeping the other constant, the optimum circumstances are critical for improving the limit of detection and sensitivity of the approach.

2.7.3.3 Effect of type surfactant

The effect of type surfactant on CPE method. A solution was prepared from 1mL (500 μg/mL) of TNX or RAN-HCl, 1 mL 2-HPβCD reagent, 1mL of (KCl+NaOH) buffer PH=10 for TNX, phosphate buffer pH=8 for RAN-HCl for produce the product. The surfactants TX-114, TX-100, TW20, CTAB, and SDS at a concentration of 10% were prepared. A 1mL of each surfactant was added to the mixture and completed the volume with distilled water to 10 mL and placed in a water bath at 50 ºC for 20 minutes. After separating the two phases with a centrifuge at 3000 rpm for 4 minutes. The cloud was split and

dissolved in 2 mL of methanol. The absorbance taken at maximum wavelength.

2.7.3.4 Effect of surfactant volume

The effect of surfactant volume on the CPE method was achieved. A solution was prepared from 1mL (500 µg/ml) of TNX, RAN-HCl, 1 mL HPβCD reagent,1 mL of (KCl+NaOH) buffer PH=10 for TNX, phosphate buffer pH = 8 for RAN-HCl for produce the product. The optimal surfactant was added in different volumes between 0.2-3 mL. The solution was completed to 10 mL by double distilled water and placed in a water bath at 50 ^{o}C for 20 minutes. The mixture was separated into two phases using a centrifuge at 3000 for 4 minutes. The cloud was split and dissolved in 2 mL of methanol. The absorbance was measured at maximum wavelength.

2.7.3.5 Effect of temperature and incubation time

The effect of temperature and incubation time on CPE method was investigated. Temperature and incubation time are the most critical steps in CPE. A solution was prepared from 1mL(500 µg/mL) of (TNX, RAN-HCl), 1 mL 2-HPβCD reagent, 1 mL of (KCl+NaOH) buffer PH=10 for TNX, phosphate buffer pH= 8 for RAN-HCl to produce complex. 1.4 mL of TX-114 for TNX, 1mL for RAN-HCl, was added to the mixture, then completed to 10 mL by distilled water. The mixture was placed in a water bath at different temperatures between (30- 80) ^{o}C for 20 minutes. The mixture was separated into two phases using a centrifuge at 3000 for 4 minutes. The cloud was split and dissolved in 2mL of methanol. The absorbance was measured at the maximum wavelength.

The study was repeated under the same conditions with incubation periods varying from 10 to 60 minutes after determining the optimum

temperature for the procedure (60 ºC for TNX, 50 ºC for RAN-HCl). After 4 minutes of centrifugation at 3000 rpm, the two phases were separated. The cloud was separate and dissolved in 2 mL of methanol. At the maximum wavelength, the absorbance was measured.

2.7.3.6 The effect of centrifuge speed and time

One of the most critical steps in CPE is to study the influence of centrifugal speed and time when the others factor constant. The speed ranged from 1000 to 6000 rpm and the time ranged from 1 to 6 minutes. The speed and period that gave the maximum absorption in the procedure were chosen at an appropriate wavelength.

2.7.3.7 Effect of solvents

Using optimal conditions, the final product of a complex reaction (TNX, RAN-HCl) with 2-HPβCD reagent was diluted with 2 mL of different solvents [ethanol, methanol, chloroform, and hexane]. Absorbance was measured at the maximum wavelength of each product and recorded the highest absorbance for the best solvent.

2.7.3.8 Calibration curve for TNX and RAN-HCl by CPE method

After selecting the optimal conditions for the drug under study (TNX or RAN-HCl), it was found that the optimal method of action for each drug is:

A series of the drug solution (1-50 µg/mL) of (TNX or RAN-HCl) was transferred to a 10 mL glass centrifuge tube stoppered tube, and 1 mL of (KCl+NaOH) buffer PH=10 for TNX, phosphate buffer pH=8 for RAN-HCl was added to it, then (1.0 or 0.8 mL) of HPβCD reagent (500 µg/mL) was added. Then added (1.4 or1.0 mL) of (10%) TX-114 and completed the volume with distilled water to 10 mL and placed in a water bath at (40 ºC) for 20 minutes. After using a centrifuge at (5000

or 4000 rpm) for (5 or 4) minutes to separate the two phases. The cloud was split and dissolved in 2 mL of methanol. The absorbance of the colored solution was scanned on a spectrophotometer in the range of 200-800 nm against a drug-free blank solution.

2.7.3.9 Accuracy and precision

The accuracy was determined using the standard addition procedure, which took three concentrations (5, 10 and 15) µg/mL of (TNX or RAN-HCl) from the pure drug and mixing it with 2-HPβCD reagent, with paragraph 2.7.3.8. being the optimum conditions. The absorbance was measured for each solution using a spectrophotometer and experiment was repeated three times for each concentration.

2.7.3.10 Application of the CPE method on pharmaceuticals formulation TNX and RAN-HCl

The pharmaceutical solutions for (TNX, RAN-HCl) were prepared using the best conditions determined in the cloud point extraction study. TNX, RAN-HCl was evaluated at three different concentrations 5,10,15 µg/ mL in (Telecoil and Tenoctil) for TNX and (Barkadin, HISTAC R150 for RAN-HCl. The absorbance of each pharmaceutical solution was measured against a specified blank.

2.8 Synthesis of azo compound from BRH

The dye used in this study was synthesized according to a published procedure. An 8- hydroxyquinoline (72.58 mg, 0.0005 mole), sodium hydroxide (3M, 0.5 mL) was mixed. The mixture was cooled to 0 ^{o}C. A solution of bromohexine. HCl (220 mg, 0.00053 mole), concentrated hydrochloric acid (12 M, 0.25 mL) and 0.5 mL distilled water were warmed and a solution of sodium nitrite (50 mg, 0.00053 mole) in 0.5 mL of distilled water at 0 ^{o}C was added and this prepared

solution was added to the above cold mixture with good stirring. A red solid was formed and reaction left up to 20 min in ice-bath. The crude product formed was filtered, washed with cold water, dried and purified by recrystallization from ethanol. The resulting compound was proven using UV and I.R spectra. The purity of synthesis compound was carried out by TLC.

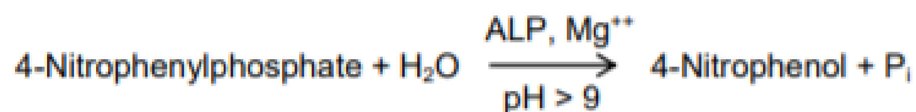
2.9 Study of biological effect for BRH pure and BRH in an azo compound

To study the biological effect of BRH drug under study in its pure form and its azo compound form, alkaline phosphatase ALP, were used. First, the stock solution of pure BRH medication was prepared of a concentration of 0. 1 M, A series of dilute solutions (10-100 µg/mL) were prepared, and their effect on the proposed enzymes was determined. The prepared 0.1 M of azo compound for BRH. A series of dilute solutions (10-100 µg/mL).

The serum was then separated using a centrifuge at 4000 rpm for 10 minutes after blood was extracted from a vein with a syringe and left at room temperature to clot. After getting the required serum, it was transferred to tubes, and the known concentrations of the drugs under investigate were added to study the influence of enzymes ALP on drugs.

2.10 Determination of enzymes activity

Alkaline phosphatase catalyzes the hydrolysis of 4-nitrophenyl phosphate to produce free 4-nitrophenol and inorganic phosphate, with the alkaline buffer serving as the phosphate-group acceptor. The rate of synthesis of 4-nitrophenol, related to the activity of ALP current in the sample under investigation, is monitored kinetically at 405 nm.



2.10.1 Reagent preparation

Use (4 mL R1 + 1 mL R2) as a work reagent. Based on the remaining caducity of both reagents, stable for five days at 20-25 ºC or 15-30 days at 2-8 ºC. Light is being obstructed.

2.10.2Samples

Free of hemolysis serum or heparinized plasma. Many anticoagulants like EDTA, oxalate and citrate inhibit the enzyme by complexing Mg^{+2} and should not be utilized. Alkaline phosphatase in serum or plasma is stable for seven days.

2.10.3 Procedure

1. Set the reaction temperature for the working reagent(R1+R2), samples and controls.
2. Transfer to the cuvette in the following manner:

Procedure	Working reagent	Sample (serum)	Inhibitor (drug)
Control	1000µL	20µL	
Test	980 µL	20 µL	20 µL

3. Incubate for one minute at 37 °C after mixing.
4. Record the change in (ΔA/min) during 3 minutes.

2.10.4 Calculation

Alkaline phosphatase activity was measured in U/L using the following equation

ALP Activity (U/L) = (ΔA/min) × 2750.

The inhibition Percent was estimated by measuring activity under

identical conditions with and without the inhibitor, using the equation:

$$\% \text{ Inhibition} = 1 - \frac{The\ activity\ in\ the\ absence\ of\ inhibitor}{The\ activity\ in\ the\ presence\ of\ inhibitor}\ X\ 100$$

CHAPTER THREE

RESULTS AND DISCUSSION

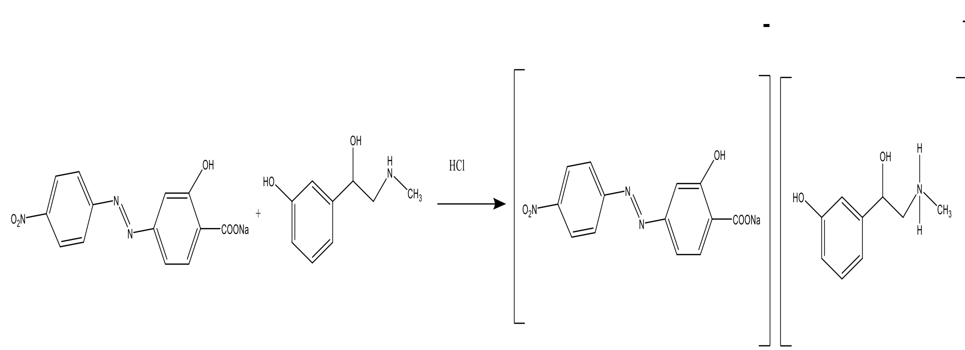
CHAPTER THREE

3-RESULTS AND DISCUSSION

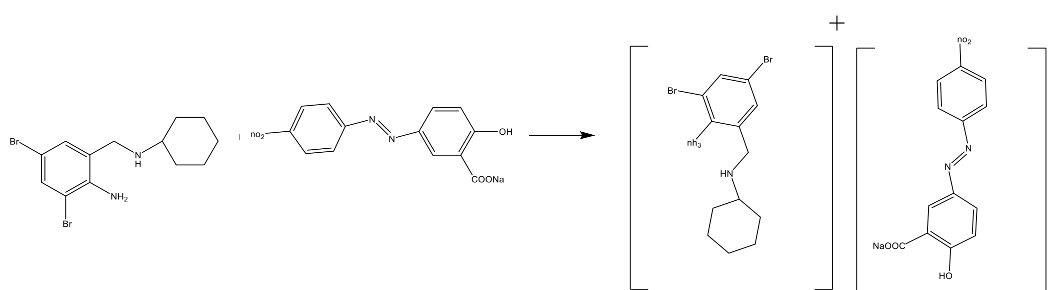
The developed techniques for evaluating Phenylephrine hydrochloride, Bromohexine hydrochloride, Tenoxicam, and Ranitidine-HCl use the methods of direct extraction, liquid-disperse, and cloud point extraction. Various reagents (alizarin yellow and hydroxypropyl-beta-cyclodextrin (2-HPβCD) also react with pharmaceuticals to form colored products that may be measured using spectrophotometry. The suggested methods, direct extraction, liquid-disperse extraction, and cloud point extraction are utilized to determine amino drug trace concentration (µg/mL).

3.1 Ion – Pair formation for PEH and BRH

The ion-pair extraction technique includes combining an aqueous solution of amines or quaternary ammonium compounds with a suitable reagent (dye) to generate an ion pair between positive (+), or negative (-) charged nitrogen compounds with a countercharge reagent. The resulting ion-pair product is characterized by its solubility in organic solvents such as chloroform. It is also characterized by the resulting compound being colored. The spectrophotometric technique can estimate that. The ionic pair products were formed by connecting amino drugs in their cationic state (PEH, BRH) with a suitable anionic reagent (alizarin yellow reagent). The following Schemes (3-1) and (3-2) can be used to express it:



Scheme 3-1: Ion-pair product for PEH: Alizarin yellow reagent



Scheme 3-2: Ion-pair product for BRH: Alizarin yellow reagent

3.2 Study of formation of ion-pair formation of PEH

The ion-pair is formed when the cation of phenylephrine HCl (PH^{+}) binds to the anion of the alizarin yellow reagent(A^{-}) to create an intense yellow-colored ion-pair product (A^{-} - PH^{+}). The absorbance of the yellow or orange product can be measured by spectrophotometer analysis in pH 4 at 430nm against blank; the result is shown in Figure 3-1.

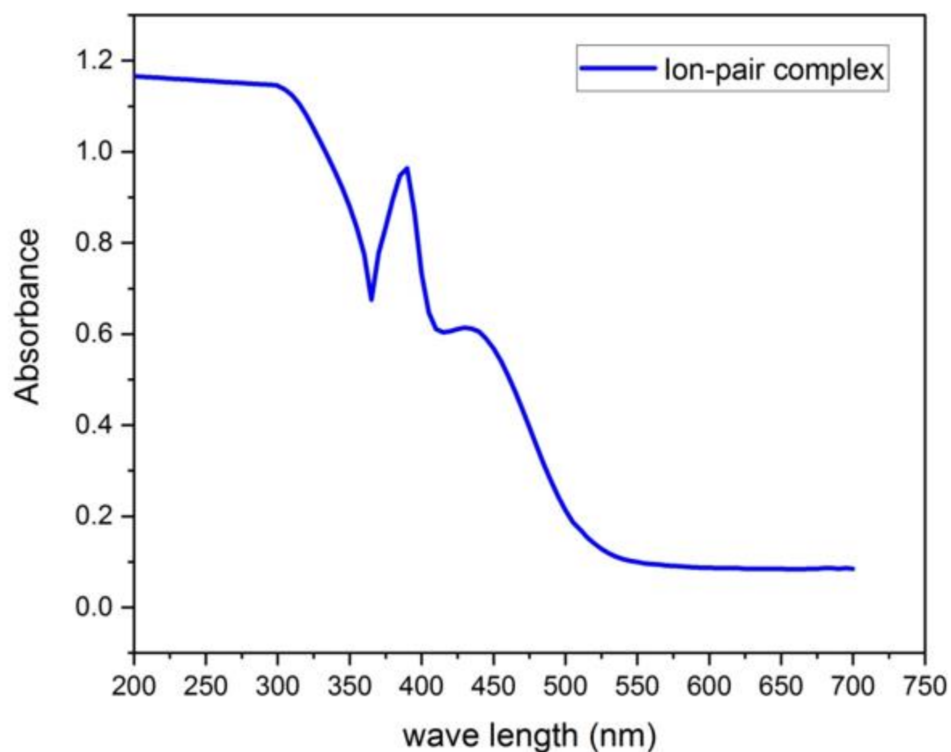


Figure 3-1:Absorption Spectrum of the ion-pair product of PEH

3.3 Study of formation of ion-pair product of BRH

A yellow ion-pair compound is created when BRH combines with alizarin yellow reagent. The absorbance of the yellow product can be estimated by spectrophotometer analysis in pH 5 at 480 nm against blank. The result is illustrated in Figure 3-2.

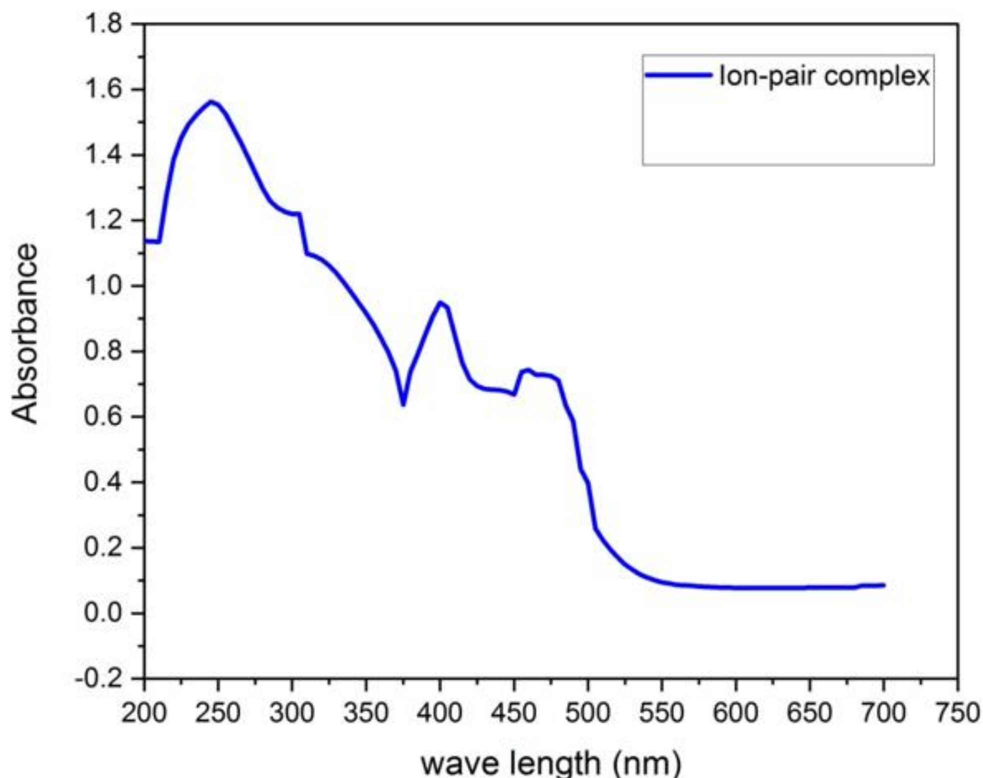


Figure 3-2:Absorption Spectrum of the ion-pair product of BRH

3.4 Direct Extraction method

The ion-pair product of two drugs, PEH and BRH was determined using direct extraction technique, and the absorbance was measured at 430 and 480 nm, respectively (maximum wavelength for the product).

3.4.1 Optimization of direct extraction

The direct extraction combined with the UV-Vis spectrophotometer was used to select the suitable conditions for the product of PEH drug and BRH drug with an alizarin yellow reagent at wavelength 430 and 480 nm, the most critical factor in this product formation process is the acidic function (pH), type and volume of buffer solution, reagent volume and type solvent. Therefore, it has been thoroughly investigated.

3.4.1.1 Effect of pH value

The pH range for the phosphate buffer was used between 1 to 8, and it was noticed that the best acidity function for the PEH product formation was at pH = 4, as for the BRH drug, it had an acidic function at pH=5, as shown in Figure 3-3.

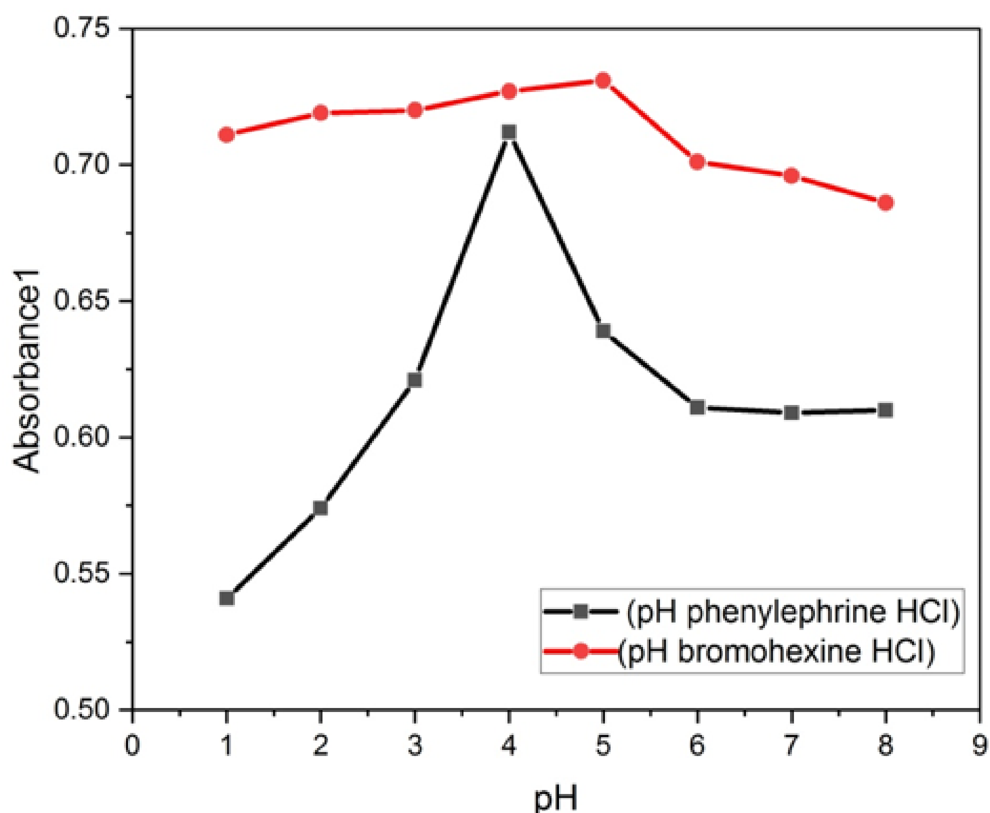


Figure 3-3:Effect of pH on the product formation

3.4.1.2 Effect of buffer type

Since the type of buffer possesses an effect on the formation of products, a variety of buffer solutions such as phosphate, acetate, and citrate buffer were used. It was noticed that the acetate buffer provided the highest absorption value for PEH and phosphate buffer for BRH, the results obtained are shown in Table 3-1.

Table 3-1: Effect type of buffer solution on absorbance of PEH

Type of buffer	Absorbance at 430nm PEH	Absorbance at 480nm BRH
Phosphate	0.620	0.732
Citrate	0.570	0.572
Acetate	0.640	0.601

3.4.1.3 Effect of buffer volume

The different volumes of acetate and phosphate were examined, it was noticed that the volume which recorded the highest absorption was 1.2 mL and 1 mL at 430 and 480 nm, respectively, as shown in Figure 3-4.

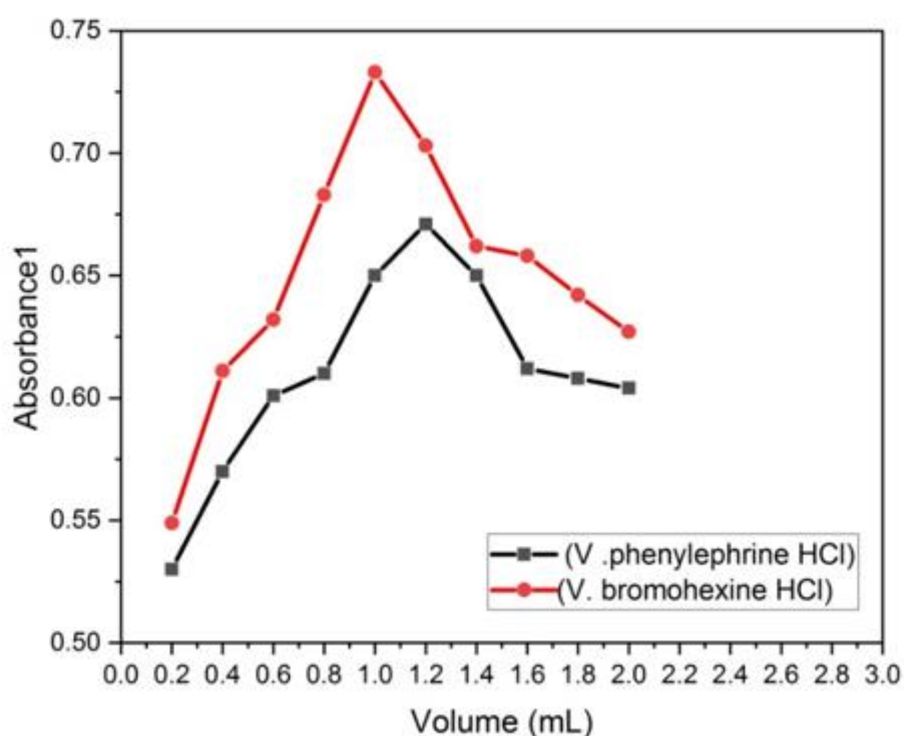


Figure 3-4:Effect of buffer volume

3.4.1.4 Effect of reagent volume

The best reagent volume was considered for forming products between PEH and an alizarin yellow reagent, as well as between BRH and an alizarin yellow reagent. It was determined that 2 mL and 1.5 mL of alizarin reagent were required for product formation, respectively, as shown in Figure 3-5.

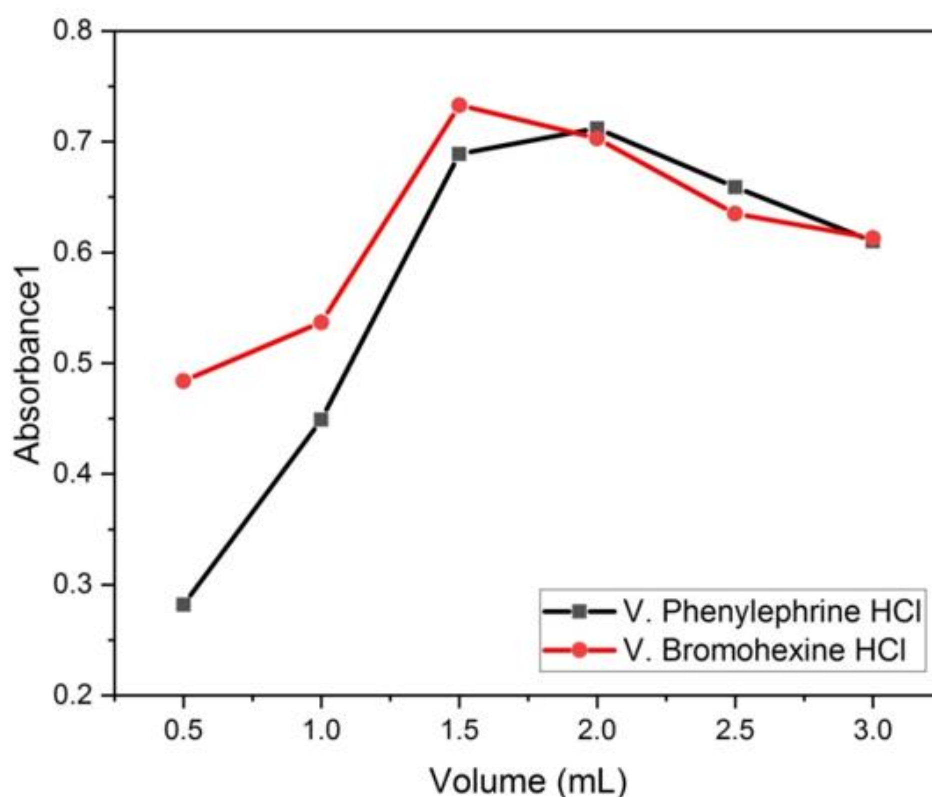


Figure 3-5:Effect of reagent volume

3.4.1.5 Effect of organic solvent type

The influence of several solvents (chloroform, tetra chlorocarbon, benzene and hexane) on the extraction of product composition of both medications was also investigated; Chloroform was shown to be the best solvent for both drugs absorption as shown in Table 3-2.

Table 3-2: Effect of solvent type

Type Solvent	Absorbance at 430nm PEH	Absorbance at 480nm BRH
Chloroform	0.721	0.730
CCL_4	0.448	/
Benzene	0.501	0.646
Hexane	/	/

3.4.1.6 Stoichiometric evaluation PEH and BRH of complex

The ratio of PEH to alizarin yellow reagent, as well as the ratio of BRH to the same reagent, were determined using two methods; continuous variation and molar ratio techniques.

I. Continuous variation method (Job´s method)

The ratio of each medication to alizarin yellow reagent was expressed using the continuous variance technique. The process was proved to have a 1:1 ratio (PEH: alizarin yellow reagent), (BRH: alizarin yellow reagent), as shown in Figure 3-6 and Figure 3-7.

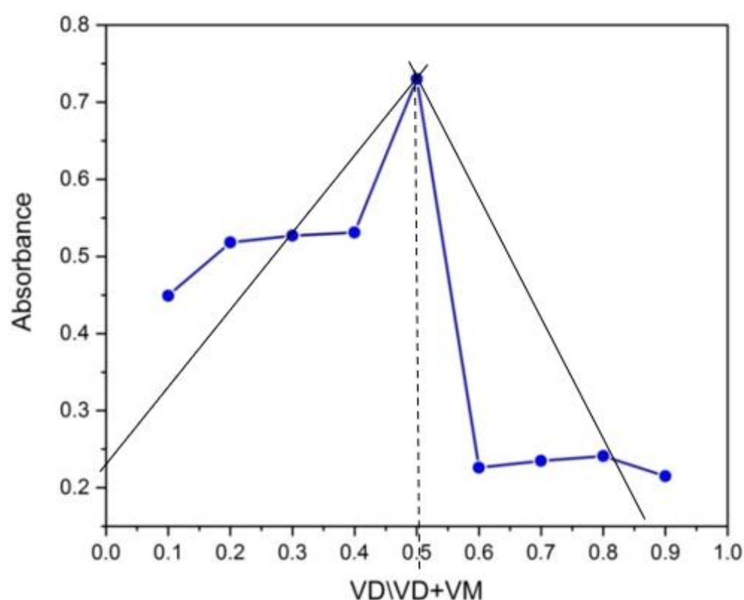


Figure 3-6: Continuous variation method of PEH

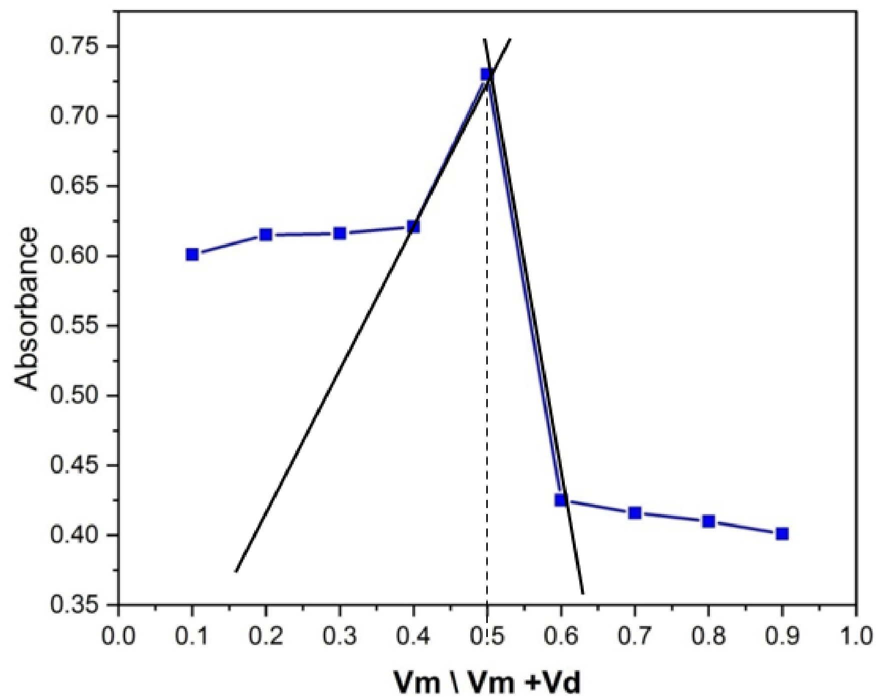


Figure 3-7:Continuous variation method of BRH

II. Mole- ratio method

The ratio of PEH and BRH with alizarin yellow reagent was determined using the molar ratio technique. To combine both medications and the reagent, a 1:1 ratio was observed, as shown in Figure 3-8 and Figure 3-9.

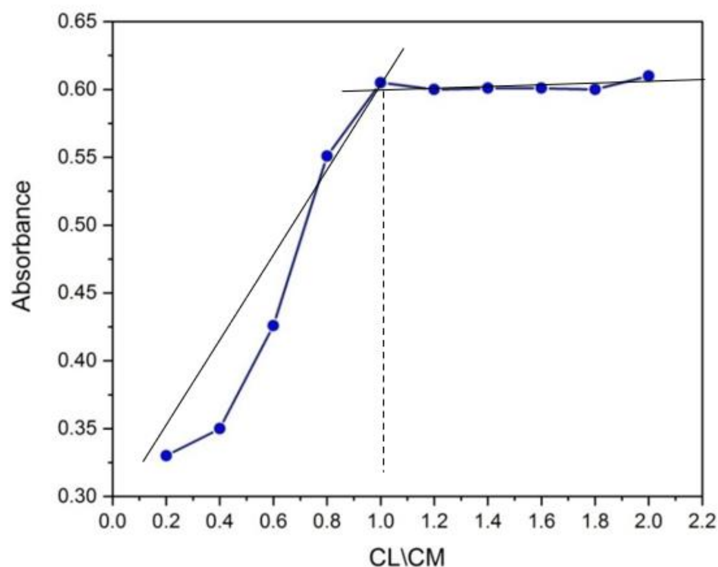


Figure 3-8:Mole-ratio method of PEH

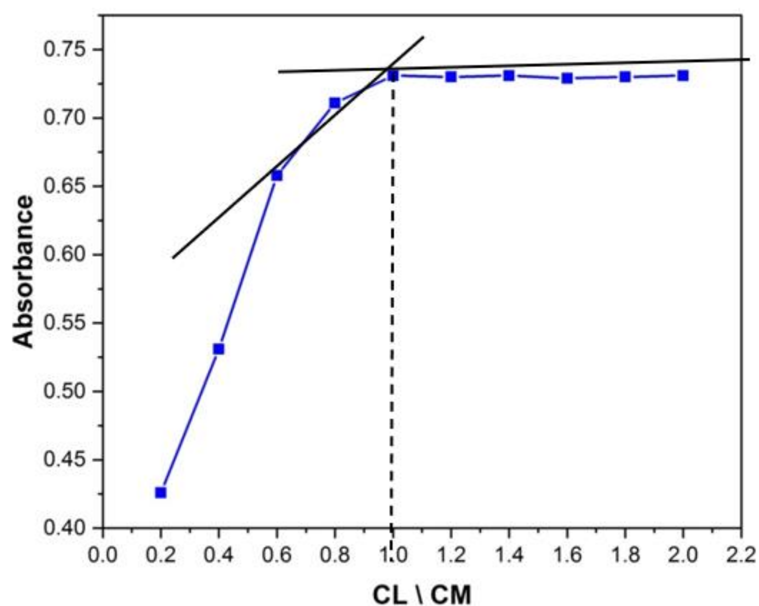


Figure 3-9: Mole-ratio method of BRH

3.4.1.7 Effect of stability

The influence of time on color stability for both complexes was investigated, within period ranged between 5 to 60 minutes. It was

detected that after 25 minutes is the time to stable the color for PEH and after 30 minutes for BRH. as shown in Figure 3-10.

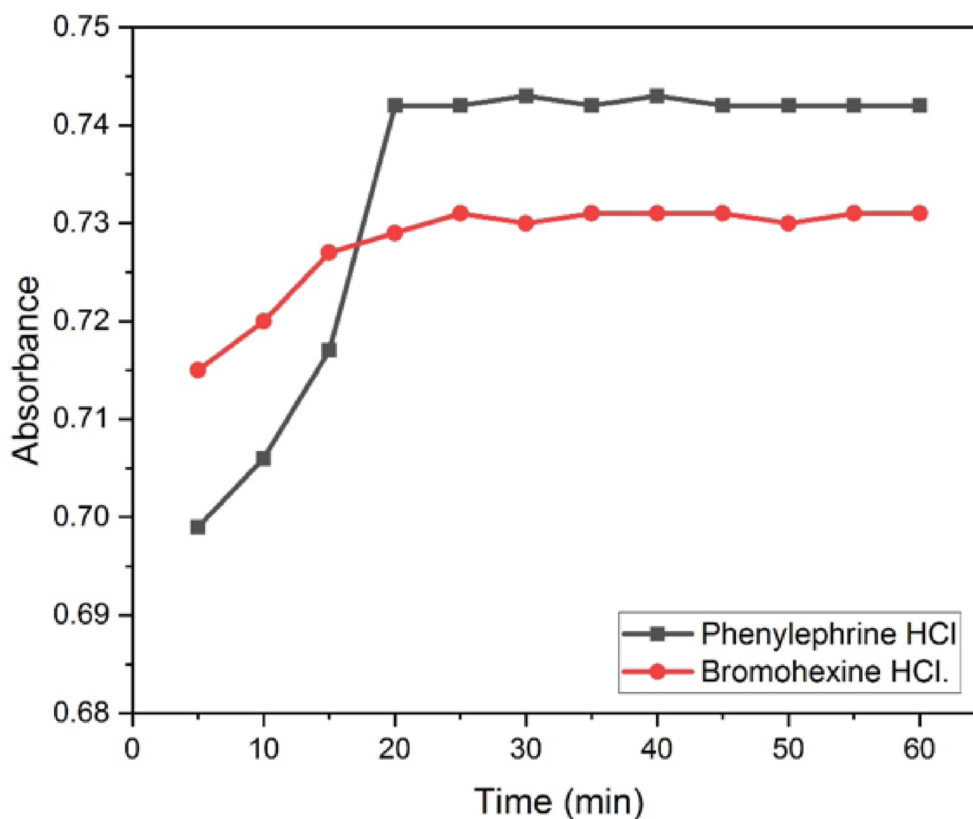


Figure 3-10: Effect of stability

3.4.1.8 Effect of interference

Interferences that may be added to pharmaceutical preparations such as (glucose, fructose, lactose, etc.) was investigated. It was found that they had no clear effect on the absorbance. The result obtained is shown in Table 3-3.

Table 3-3: Extraction recovery% with different interference compound

Compound	Recovery% BRH	Recovery% PEH
Maltose	99.4	99.32
Starch	97.8	100.41
Glucose	98.3	99.04
Lactose	96.6	98.5
Fructose	99.5	98.6
Glysine	96.6	99.73

3.4.2 Calibration curve and statistical treatments

After determining the optimal conditions for the extraction of both medicines' products (PEH and BRH), the calibration curve was created by plotting the absorbance of the two medications against their solution concentration. The concentration range of PEH was determined to be (1-20 µg/mL), as illustrated in Figure 3-11, using the regression equation $Y = 0.071X + 0.061$ and $R^2 = 0.997$. BRH concentrations ranged from 1 to 35 µg/mL, with the regression equation $Y = 0.040X + 0.295$ and $R^2 = 0.995$ from the linear calibration as shown in Figure 3-12.

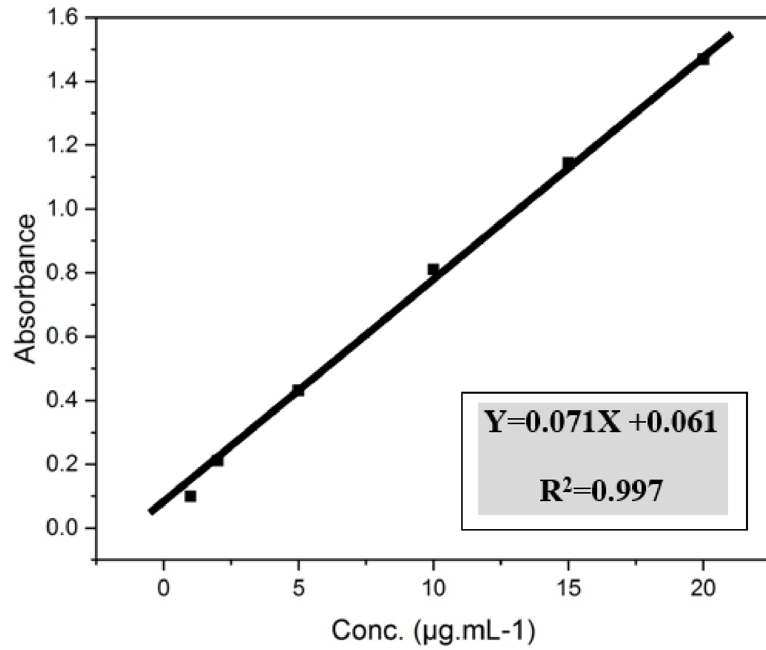


Figure 3-11: Calibration curve of PEH by direct extraction method.

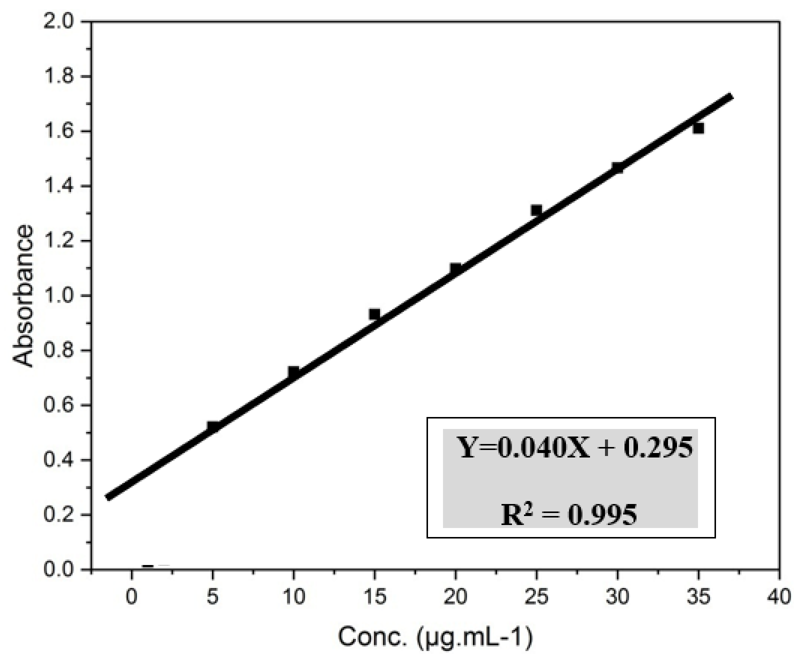


Figure 3-12: Calibration curve of BRH by direct extraction method

Table 3-4: Analytical parameter of direct extraction

Parameter	Direct extraction for PEH	Direct extraction for BRH
λ_{max} nm	430	480
Color	yellow	yellow
Regression equation	Y=0.071X+0.061	Y=0.040X + 0.295
Linearity range (µg/mL)	1-20	1-35
Correlation Coefficient (R^2)	0.997	0.995
Ɛ($L.mol^{-1}.cm^{-1}$)	14459.9	16504
Sandell' sensivity (µg . cm^{-2})	0.0141	0.025
Slope (b)	0.071	0. 040
Intercept(a)	0.061	0.295
Limit of detection (µg/mL)	0.34	0.0814
Limit of quantification (µg/mL)	1.12	0.244
C.L.for the slope($b \pm t_{sb}$) at 95%	0.071± 0.275	$0.040 \pm 8.6\times10^{-4}$
C.L. for the intercept ($a \pm t_{sa}$) at 95%	0.061±2.96	$0.295 \pm 9.5\times10^{-3}$
Standard error for regression line ($S_{y/x}$)	0.45	1.414×10^{-3}
C.L for Conc.5µg/ mL at 95%	5.09± 0.1241	$4.8 \pm 2.5\times10^{-3}$
C.L for Conc.10µg/mL at 95%	9.82± 0.099	$10.03 \pm 5\times10^{-3}$
C.L for Conc.15µg/mL at 95%	14.04±0.124	$14.5 \pm 2.5\times10^{-3}$

3.4.3 Accuracy and precision

The accuracy and precision were studied of drug (PEH or BRH). These experimentations are carried out with three different drug concentrations (5, 10, 15 µg/mL for PEH and BRH) for three replications before using the direct extraction approach at optimum conditions. It is clear from these findings that the process has good accuracy and precision since the recovery rate for PEH is (97.90%) and for BRH is (96.76%), depending on the exact procedure is applied in

clause (2.6.1.11). F-test, T-test was used (two-tailed) to calculate the accuracy and compared the practical value F-test with value in the critical statistical if the practical value less than the value in a table does not differ significantly between the suggested value method and standard method.

The results were signed between the standard HPLC method and suggested method with PEH; BRH found no significant difference between the two methods. The statistical analysis results shown in the Table 3-5 proved that the calculated T-values and F-values for PEH BRH determination in different pharmaceuticals were less than t-critical and F-critical at 95% confidence interval and (n-1) degrees of freedom. The new methods have higher accuracy and precision than the literature (26,38).

Table 3-5: Accuracy and pression of direct extraction procedure of pure PEH and BRH drug

drug	**Direct extraction method**							
	Con. µg/mL		Relative Error%	Recov. %	Average Recov.%	T-value	F-value	RSD%
PEH	5	5.09	-1.88	101.87	97.90	2.132	5.4	0.98
	10	9.82	1.8	98.2				0.41
	15	14.05	6.36	93.64				0.5
BRH	5	4.8	4	96	96.76	2.23	7.21	0.04
	10	9.7	3	97				0.03
	15	14.6	2.6	97.3				0.01
Critical value at 95% confidence limit, t = 2.7764, F= 19.								

3.4.4 Application the suggested method on pharmaceutical preparation of PEH and BRH

In the direct extraction method, it was observed that PEH in phenylephrine eye drops and Dolo-cold (tablets) are suitable for

evaluating PEH in pharmaceutical preparations, as well as Solvodin and Biosolvon in BRH. As demonstrated in the Table 3-6, the high accuracy and acceptable results were achieved.

Table 3-6: Application of the proposed direct extraction for the evaluation of PEH and BRH

PEH	**direct extraction**					
	Concentration of drug µg/mL		**Relative Error %**	**Reco. %**	**Avg. Recov %**	**RSD% (n=3)**
	Taken	**Found**				
Eye drops	5	5.2	-4	104	100	0.02
	10	10.2	-2	102		0.98
	15	14.1	6	94		0.015
Dolo-cold (tablet)	5	4.91	1.8	98.2	97.9	0.024
	10	9.35	6.5	93.5		0.021
	15	15.3	-2	102		0.04
BRH	**direct extraction**					
	Concentration of drug µg/mL		**Relative Error%**	**Recov. %**	**Average Recov %**	**RSD% (n=3)**
	Taken	**Found**				
Solvodin	5	4.6	8	92		0.022
	10	9.8	2	98.3		0.01
	15	14.4	4	96	95.4	0.03
Biosolvon	5	4.7	6	94	95.6	0.04
	10	9.6	4	96		0.01
	15	15.3	-2	102		0.04

3.5 Dispersive Liquid Liquid Extraction (DLLME) method

The DLLME method is used to evaluate, and extract the ion-pair complex for PEH and BRH, its spectra were examined at 430, 480 nm, respectively.

3.5.1 Optimization of DLLME

The DLLME technique combined with the UV-Vis spectrophotometer was utilized to select the suitable conditions for the extraction of PEH and BRH complex with an alizarin yellow reagent at the wavelengths 430 and 480 nm. The most critical factors in these complexes formation process is the acidic function (pH), type, volume of pH, reagent volume and type solvent. Therefore, it has been thoroughly investigated.

3.5.1.1 Effect of the extraction and dispersive solvents

The influence of chloroform, tetra chlorocarbon, hexane and benzene on the extraction was studied. Chloroform was proved to be the best extraction solvent for both drugs tested. The result obtained is shown in Table 3-7.

Table 3-7: Selection type of extraction solvent

Type of extraction solvent	Absorbance at 430nm PEH	Absorbanceat480nm BRH
Chloroform	0.860	0.641
CCl_4	0.801	0.611
Benzene	0.799	0.527
Hexane	/	/

The influence of dispersed solvent such as ethanol, methanol, acetone, and acetonitrile were studied. The result was showed that ethanol was the best dispersion solvent for PEH and BRH.As shown in Table 3-9.

Table 3-8: Selection type of dispersive solvent

Type of dispersive solvent	Absorbance at 430nm PEH	Absorbance at 480nm BRH
Ethanol	0.861	0.642
Methanol	0.815	0.639
Acetone	0.820	0.620
Acetonitrile	0.726	0.539

3.5.1.2 Effect of pH value

In the DLLME method, the pH range for the phosphate buffer was employed between (1-8). It was seen that the best acidity function for the PEH complex formation was at pH = 4 and pH = 5 for the BRH drug, as shown in Figure 3-13.

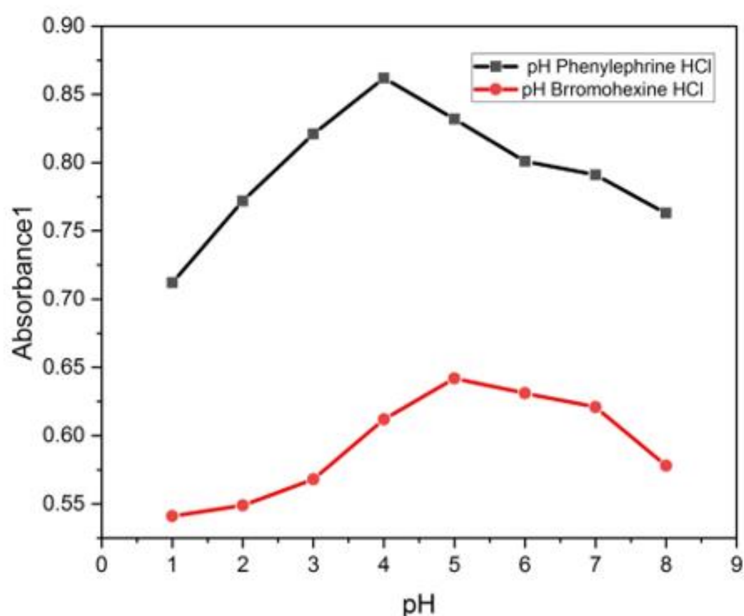


Figure 3-13: Effect of pH buffer

3.5.1.3 Effect of type of buffer

A variety of buffer solutions were utilized since the type of buffer affects the formation of complexes (phosphate, acetate and citrate). The acetate solution was found to have the maximum absorption value in the PEH medication. In the case of BRH, however, the phosphate

solution was shown to have the maximum absorption, as shown in Table 3-9.

Table 3-9: Effect of type of buffer

Buffer type	Absorbance at 430 nm PEH	Absorbance at 480 nm BRH
Phosphate buffer	0.861	0.641
Acetate buffer	0.966	0.582
Citrate buffer	0.811	0.561

3.5.1.4 Effect of buffer volume

The absorbance values for different amounts of the selected buffer solutions were examined. It was observed that when using the drug PEH, the volume of 0.8 mL of acetate solution was recorded the highest absorption value at 430 nm, but when using the drug BRH, the volume of 1.2 mL of phosphate solution was the appropriate volume, giving the highest value at 480 nm, as shown in Figure 3-14.

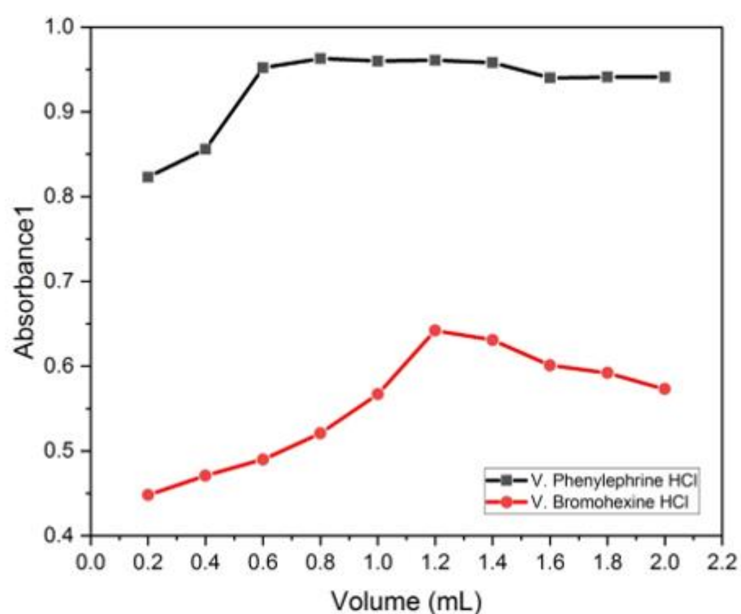


Figure 3-14: Effect of buffer volume

3.5.1.5 Effect of reagent volume

The extraction of the product in both drugs (PEH and BRH) requires a specific volume of alizarin reagent. It was found that the volume of 1 mL of alizarin gave the highest absorbance in the PEH drug, and the volume of 1.5 mL was the appropriate volume for the BRH drug, as shown in the Figure 3-15.

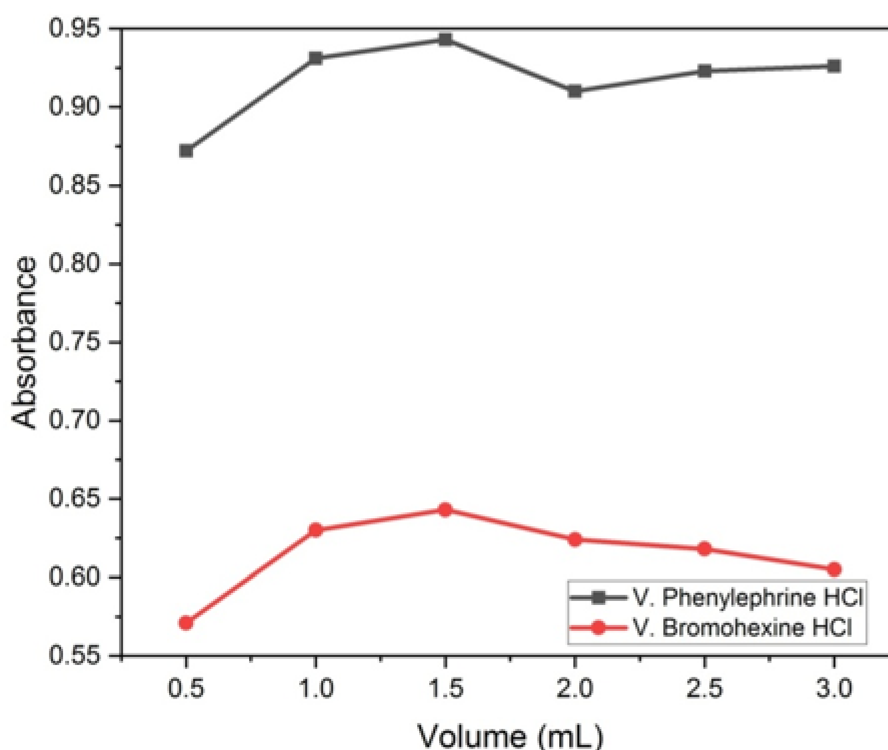


Figure 3-15: Effect of reagent volume

3.5.1.6 Effect of extraction and dispersion volume solvent

Different amounts of extraction and dispersal solvents have different effects on the extraction of product composition of both drugs used. Therefore, it was noticed that the optimal volume of PEH for extraction and dispersion is 400 µL for extraction and 700 µL for dispersion. In addition, the optimal volume of BRH is 300 µL for the extraction solvent and 900 µL for the dispersion solvent, as shown in Table 3-10 and Table 3-11.

Table 3-10: Effect of the extraction solvent volume

Extraction solvent volume (chloroform)µL	Dispersive solvent volume (Ethanol) µL	Absorbance at 430 nm PEH
200	700	0.899
300		0.913
400		0.940
500		0.928
Extraction solvent volume (chloroform) µL	**Dispersive solvent volume (Ethanol) µL**	**Absorbance at 480 nm BRH**
200	700	0.529
300		0.640
400		0.639
500		0.570

Table 3-11: Effect of the dispersive solvent volume

Extraction solvent volume (chloroform) µL	Dispersive solvent volume (Ethanol) µL	Absorbance at 430 nm PEH
400	500	0.901
	600	0.922
	700	0.941
	800	0.939
	900	0.910
	1000	0.899
	1100	0.904
	1200	0.872
	1300	0.870
	1400	0.864
	1500	0.863
Extraction solvent volume (chloroform) µL	**Dispersive solvent volume (Ethanol) µL**	**Absorbance at 480 nm BRH**
300	500	0.901
	600	0.922
	700	0.941
	800	0.939
	900	0.899
	1000	0.899
	1100	0.904
	1200	0.872
	1300	0.870
	1400	0.864
	1500	0.863

3.5.1.7 Effect of speed and time in the centrifuge

The effects of centrifuge speed and time are significant in the separation and extraction of drug products. The optimal extraction time and speed for PEH was 6 minutes and 2000 rpm, whereas for BRH was 4 minutes and 4000 rpm, as shown in Figure 3-16 and Figure 3-17.

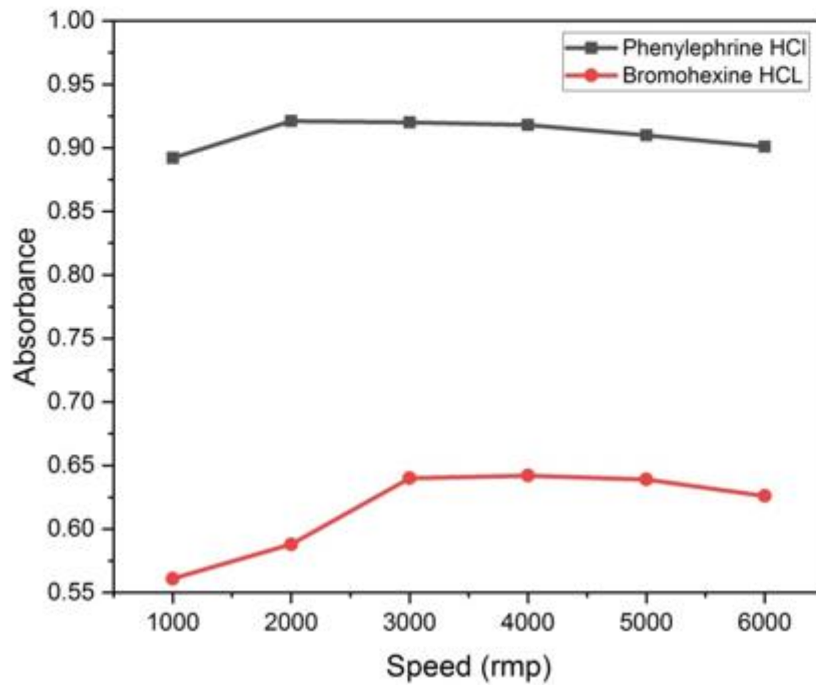


Figure 3-16: Effect of the centrifuge speed

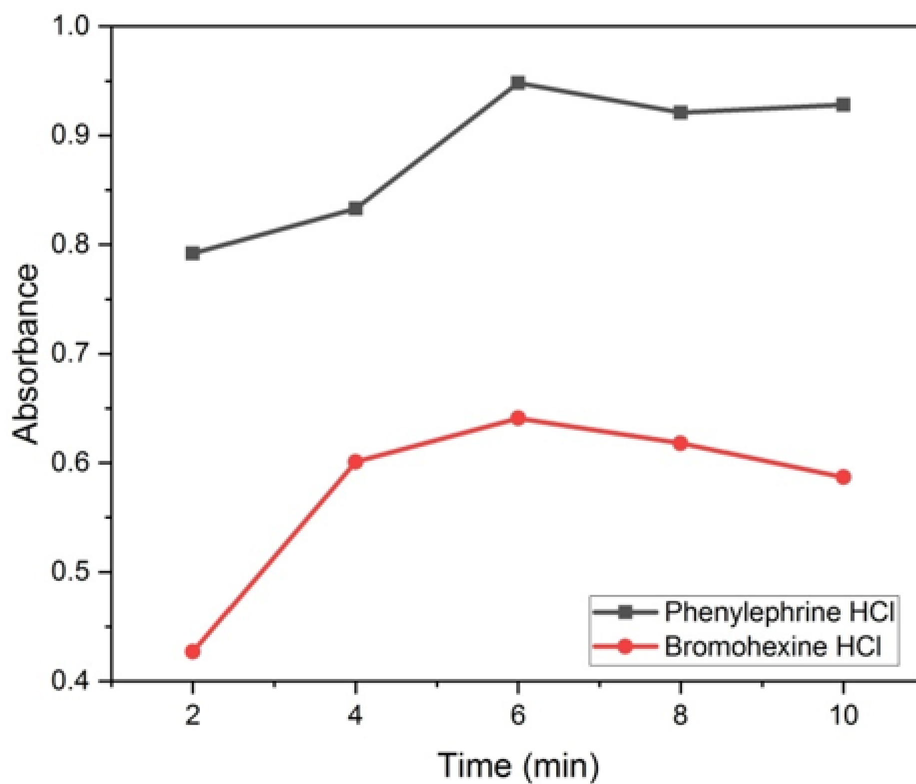


Figure 3-17: Effect of the centrifuge time

3.5.1.8 Effect of the stability

The influence of time on stability for both products was studied, within period ranged between 5-60 minutes. It was detected after 15min is the time to stable for PEH and after 20 min for BRH. As shown in Figure 3-18.

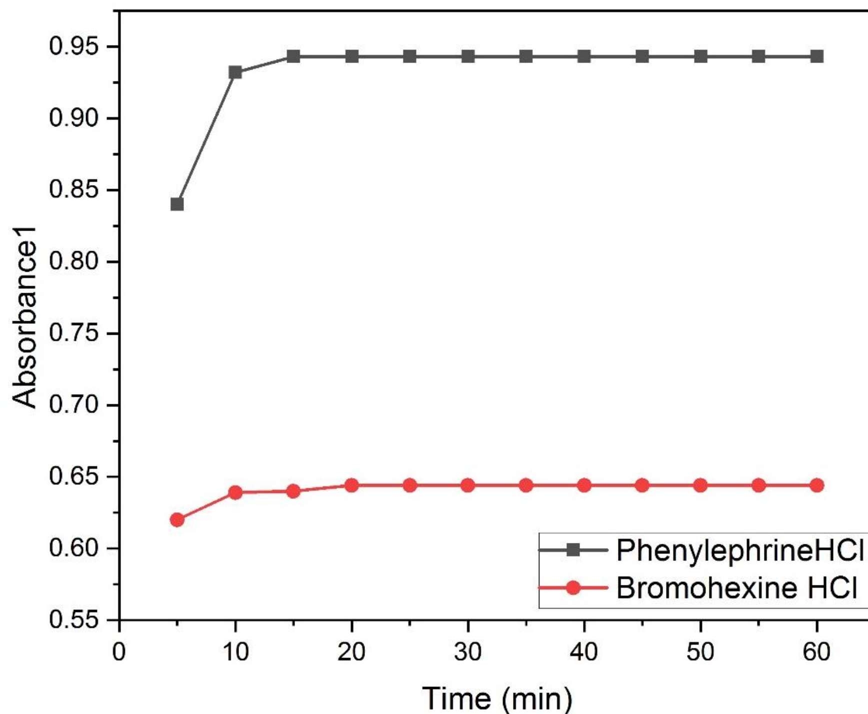


Figure 3-18 : Effect of stability

3.5.1.9 Effect of the interference

Interferences effect from several compounds such as glucose, fructose, lactose and other sugars that may be added to pharmaceutical preparations was investigated. They were not affected for the sugar interference on the PEH and BRH extraction procedures as shown in Table 3-12.

Table 3-12: Extraction recovery with different interference compound

Interference	Recovery% Absorbance at 430 nm PEH	Recovery% Absorbance at 480 nm BRH
Starch	96.8	98.1
Glucose	95.3	96.9
Maltose	96	98.4
Lactose	97.8	97.5
Glysine	97.7	98.0
Fructose	95	98.3

3.5.2 Calibration curve and statistical treatments

After determining the optimal conditions for the formation of both medicines' complexes (PEH, BHH), the calibration curve was created by plotting the absorbance of the two medications against their solution concentration. The concentration range of phenylephrine hydrochloride was determined to be (1-13 µg/mL), as illustrated in Figure 3-19, using the regression equation $Y = 0.061X - 0.032$ and $R^2 = 0.996$. Bromohexine hydrochloride concentrations ranged from (1 -23µg/mL), with the regression equation $Y = 0.058X-0.015$ and $R^2 = 0.998$ from the linear calibration as shown in Figure 3-20.

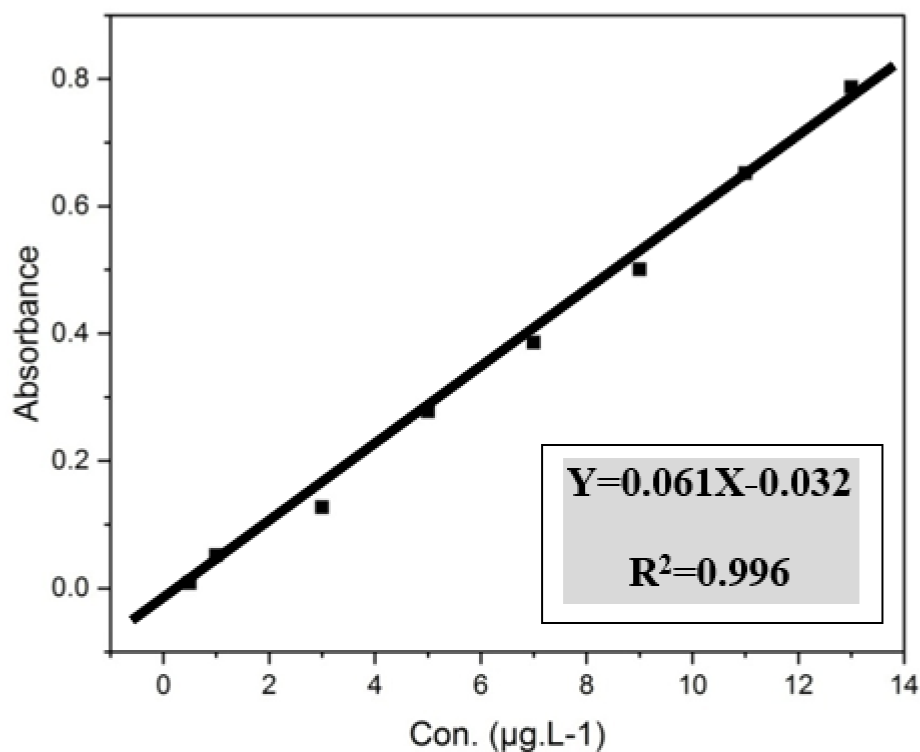


Figure 3-19: Calibration curve of PEH by DLLME method

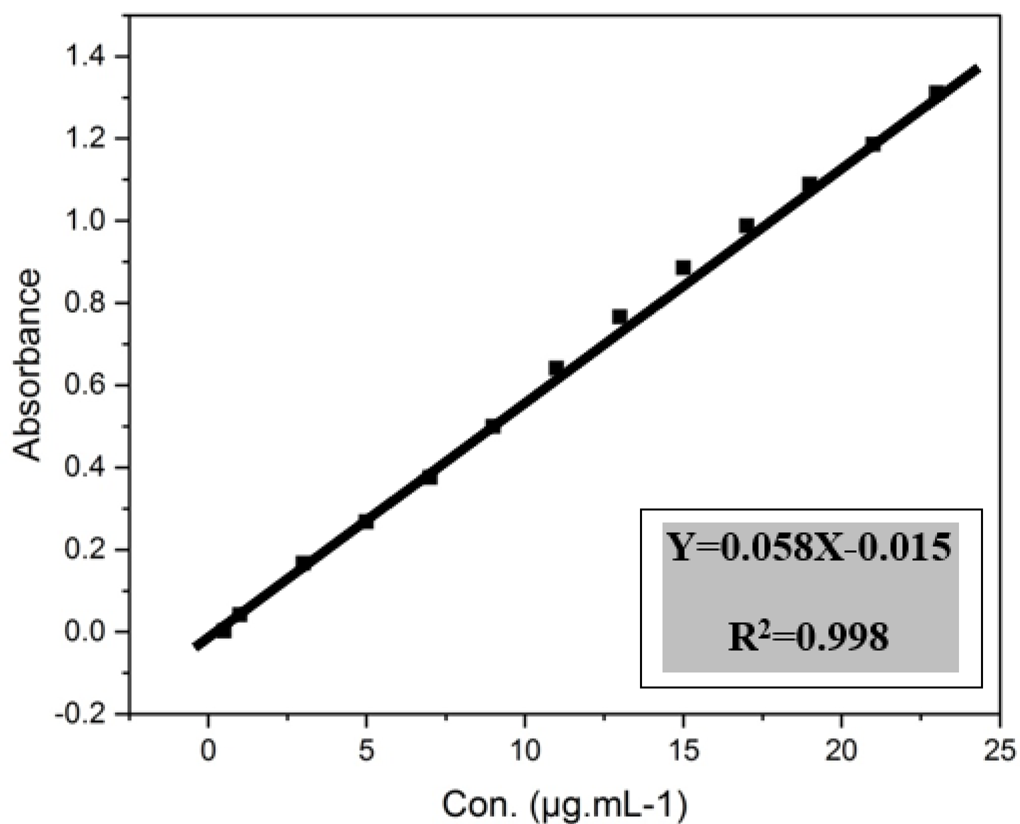


Figure 3-20: Calibration curve of BRH by DLLME method

Table 3-13: Analytical parameter of DLLME Method

Parameter	DLLME for PEH	DLLME for BRH
λ_{max} nm	430	480
Color	yellow	yellow
Regression equation	Y=0.061X-0.032	Y=0.058X-0.015
Linearity range (µg/mL)	1-13	1 – 23
Correlation Coefficient (R^2)	0.996	0.998
Ɛ($L.mol^{-1}.cm^{-1}$)	12423.3	23930.2
Sandell·ssensivity ($\mu g . cm^{-2}$)	0.0164	0.0172
Slope (b)	0.061	0.058
Intercept(a)	0.032	0.015
Limit of detection (µg/mL)	0.094	0.055
Limit of quantification (µg/mL)	0.31	0.183
C.L.for the slope($b \pm ts_b$) at 95%	0.061± 0.266	0.058 ± 0.2249
C.L.for the intercept ($a \pm ts_a$) at 95%	0.032±1.42	0.015 ± 1.1825
Standard error for regression line ($S_{y/x}$)	0.176	0.1479
C.L for Conc.3µg/mL at 95%	2.8± 0.0015	$3.14 \pm 2.48 \times 10^{-3}$
C.L for Conc.5µg/mL at 95%	5.12± 0.025	$4.85 \pm 2.48 \times 10^{-3}$
C.L for Conc.7µg/ mL at 95%	6.85±0.0025	$6.7 \pm 2.48 \times 10^{-3}$

3.5.3 Accuracy and precision

The accuracy and precision were studied of drug (PEH or BRH). These experimentations are carried out with three different drug concentrations (3, 5, 7 µg/mL for PEH and BRH) for three replications before using the DLLME approach at optimum conditions. It is clear from these findings that the process has good accuracy and precision since the recovery rate for PEH is (97.86%) and for BRH is (99.4%), depending on the exact procedure is applied in clause (2.6.2.12). F-test, T-test was used (two-tailed) to calculate the accuracy and compared the

practical value F-test with value in the critical statistical if the practical value less than the value in a table does not differ significantly between the suggested value method and standard method.

The results were signed between the standard HPLC method and suggested method with PEH; BRH found no significant difference between the two methods. The statistical analysis results shown in the Table 3-14 proved that the calculated T-values and F-values for PEH BRH determination in different pharmaceuticals were less than t-critical and F-critical at 95% confidence interval and (n-1) degrees of freedom. The new methods have higher accuracy and precision than the literature (26,38).

Table 3-14: Accuracy and pression of DLLME procedure of pure PEH, BRH drug

drug	DLLME method							
	Con. µg/mL		Relative Error%	Recov. %	Average Recov.%	T-value	F-value	RSD%
PEH	3	2.8	6.7	93.3	97.86	1.232	8.5	0.02
	5	5.12	-2.4	102.4				0.021
	7	6.85	2.14	97.9				0.015
BRH	3	3.1	-3.3	103.3	99.4	2.132	1.28	0.01
	5	4.91	1.8	98.2				0.02
	7	6.78	0.3	96.8				0.01
Critical value at 95% confidence limit, t = 2.7764, F= 19.								

3.5.4 Application the suggested method on pharmaceutical preparation for PEH and BRH

Using a DLLME method, it was discovered that PEH in the phenylephrine eye drops and Dolo-cold (tablets) are suitable for evaluating PEH in pharmaceutical preparations, as well as Solvodin and

Biosolvon in BRH. As demonstrated in the Table 3-15. High accuracy and acceptable results were achieved.

Table 3-15: Application of the proposed DLLME for the evaluation of PEH and BRH

PEH	DLLME					
	Conc. of drug µg/mL		Relative Error%	Recov. %	Avg. Recov %	RSD % (n=3)
	Taken	Found				
Eye drops	3	2.6	13.3	86.7	95.5	0.02
	5	5.2	-4	104		0.01
	7	6.7	4.3	95.7		0.04
Dolo-cold (tablet)	3	2.64	12	88	96.3	0.08
	5	5.1	-2	102		0.04
	7	6.92	1.1	98.8		0.01
BRH	**DLLME**					
	Conc. of drug µg/mL		**Relative Error%**	**Recov. %**	**Avg. Recov %**	**RSD % (n=3)**
	Taken	**Found**				
Solvodin	3	2.76	8	92	96	0.02
	5	5.1	-2	102		0.02
	7	6.6	5.7	94		0.02
Biosolvon	3	2.8	6.6	93.3	96.2	0.02
	5	4.7	6	94		0.02
	7	7.1	-1.4	101.4		0.014

3.6 Cloud point method

The cloud point extraction was utilized to extract and determine of both medications (PEH and BRH) using ion-pair product at 430 and 480 nm, respectively.

3.6.1 Optimization of cloud point extraction

The cloud point method combined with the UV-Vis spectrophotometer was used to select the optimal conditions to extracted and evaluated of PEH and BRH product with an alizarin yellow reagent at wavelength 430 and 480 nm, respectively. Critical factors are effect on the process of the product formation including acidic function (pH)**,** type and volume of pH, reagent volume and type solvent. Therefore, it was studied in detail.

3.6.1.1 Effect of pH value

The acidity function was studied using pH range of 1 - 8 for the phosphate buffer. The optimum pH function for creating both medicines (PEH and BRH) in the development of the ion-pair product was similarly discovered to be pH = 5, as shown in Figure 3-21.

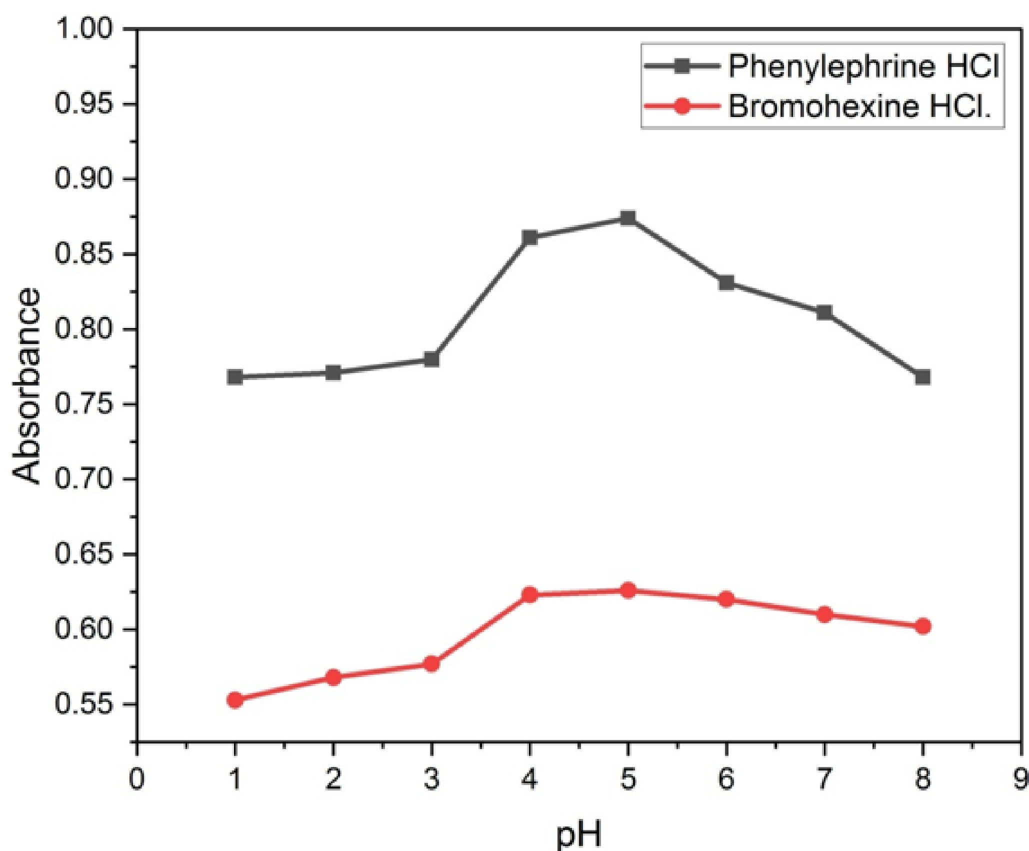


Figure 3-21: Effect of pH on the complex formation

3.6.1.2 Effect of buffer type

The phosphate, acetate, and citrate buffer solutions were examined, it was observed that the acetate buffer produce the highest absorption value for PEH. In contrast, the phosphate buffer made the best absorption value for BRH. As shown in the Table 3-16.

Table 3-16: Effect type of buffer solution absorbance

Type of buffer	Absorbance at 430 nm Phenylephrine HCl	Absorbance at 480 nm Bromohexine HCl
Phosphate	0.874	0.625
Citrate	0.823	0.504
Acetate	0.891	0.618

3.6.1.3 Effect of the buffer volume

The absorbance values of various quantities of the chosen buffer solutions were investigated. As a consequence, it was revealed that while using the drug PEH, 1.0 mL of acetate solution produced the maximum absorption value at 430 nm, and when using the drug BRH, 0.8 mL of phosphate solution produced the highest absorption value at 480 nm. See Figure 3-22.

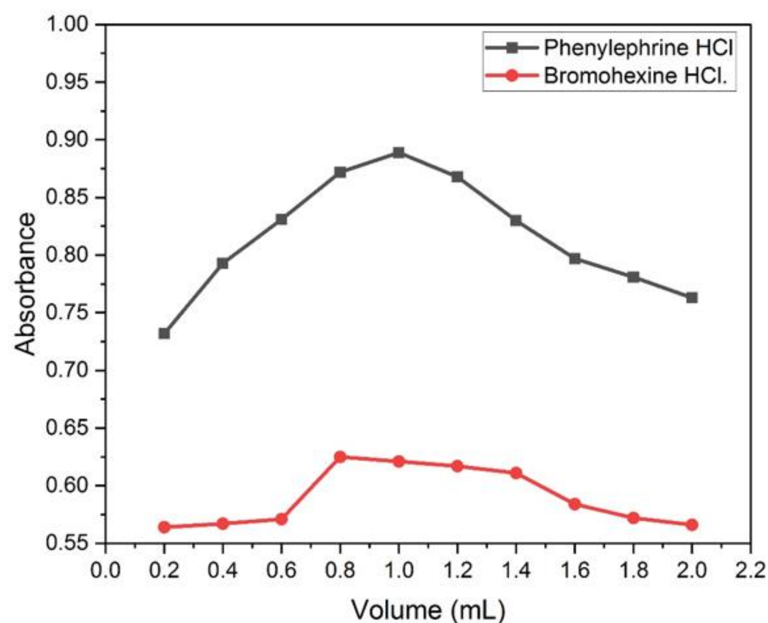


Figure 3-22: Effect of volume buffer

3.6.1.4 Effect of surfactant type

Triton X-114, Triton X-100, Tween20, CTAB, and SDS were among the surfactant solutions examined. It was founded that Triton X-114 give the maximum absorption value for both drugs (PEH and BRH) at 430 nm and at 480 nm, respectively. The results obtained are shown in Table 3-17.

Table 3-17: Effect of surfactant type

Type of surfactant	Absorbance at 430 nm PEH	Absorbance at 480 nm BRH
Triton X-114	0.871	0.626
Triton X-100	/	/
Tween 20	0.527	/
CTAB	/	/
SDS	/	/

3.6.1.5 Effect of surfactant volume

The different volumes of surfactant were examined; it was observed that 1.0 mL had the maximum absorption value at 430 nm when using the drug PEH. In comparison, 0.8 mL had the highest absorption value at 480 nm when using BRH. as shown in Figure 3-23.

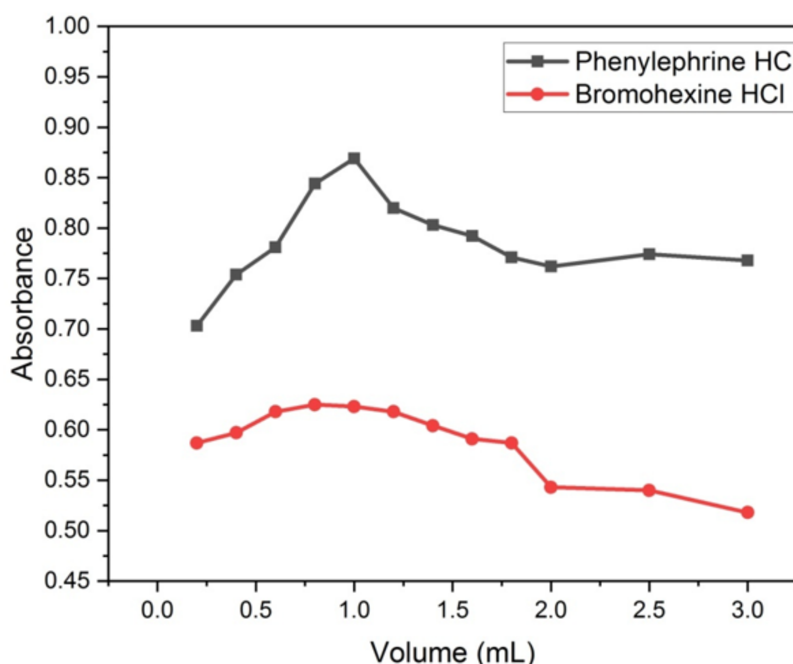


Figure 3-23: Effect of surfactant volume

3.6.1.6 Effect of temperature

Temperatures of 30 to 80 ºC were tested using a water bath to estimate (PEH, BRH), using a surfactant; it was discovered that 60 ºC had the maximum absorption value of PEH at 430 nm. The highest absorption value for BRH was found to be 50 ºC at 480 nm, as shown in Figure 3-24.

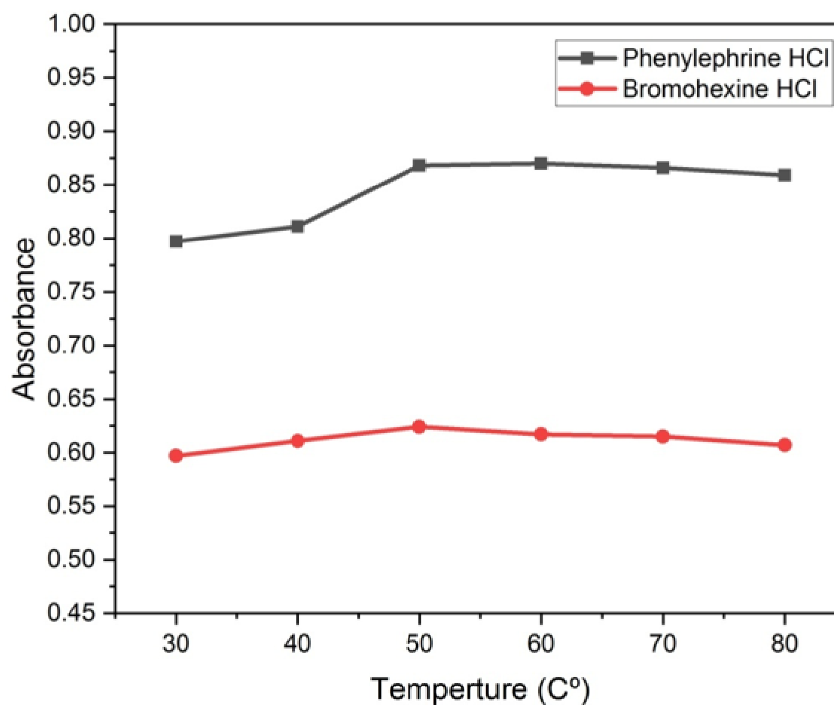


Figure 3-24: Effect of temperature

3.6.1.7 Effect of incubation time

The effect of time required for product extraction in CPE method was studied. The time ranged from 10 to 60 minutes, it was observed that 20 minutes is gave the highest absorption value for PEH. Whereas BRH, it was discovered that 40 minutes is the best time incubation to give the maximum absorption value at 480 nm. As shown in Table 3-18.

Table 3-18: Effect of incubation time (min)

Time (min)	Absorbance at 430 nm PEH	Absorbance at 480nm BRH
10	/	/
20	0.869	/
30	0.852	0.618
40	0.741	0.625
50	0.734	0.621
60	0.711	0.619

3.6.1.8 Effect of Centrifuge time

The role of time in centrifuge for product extraction is significant. Both PEH and BRH had the fastest extraction times of 5 minutes. as shown in Table 3-19.

Table 3-19: Effect of centrifuge time (min)

Time (min)	Absorbance at 430 nm PEH	Absorbance at 480 nm BRH
1	/	/
2	/	/
3	0.832	/
4	0.838	0.622
5	0.868	0.624
6	0.812	0.620

3.6.1.9 Effect of Centrifuge rate (rpm)

In the centrifuge, the effect of speed on the extraction of complexes is critical. The most excellent extraction speed for PEH was 4000 rmp, while the highest extraction speed for BRH was 5000 rpm. As shown in Table 3-20.

Table 3-20: Effect of centrifuge rate (rpm)

Centrifuge rate (rmp)	Absorbance at 430 nm PEH	Absorbance at 480 nm BRH
1000	/	/
2000	0.748	/
3000	0.783	0.611
4000	0.866	0.620
5000	0.831	0.624
6000	0.824	0.617

3.6.1.10 Effect of the best organic solvent type

The influence of numerous solvents (Methanol, Ethanol, Chloroform, and Hexane) on complex absorbance was tested. Methanol was demonstrated to be the optimum solvent for achieving the greatest

absorbance for PEH. While the best solvent for BRH was ethanol. As shown in Table 3-21.

Table 3-21: Select of best organic solvent

Solvent	Absorbance at 430 nm PEH	Absorbance at 480 nm BRH
Ethanol	0.692	0.624
Methanol	0.864	0.620
Chloroform	0.454	0.518
CCl4	0.441	0.501
Hexane	/	/

3.6.1.11 Effect of stability

The influence of time on stability for both product was studied, within period ranged between 5-60 minutes. It was detected after 20 minutes is the time to stable for PEH and BRH. As shown in Figure 3-25.

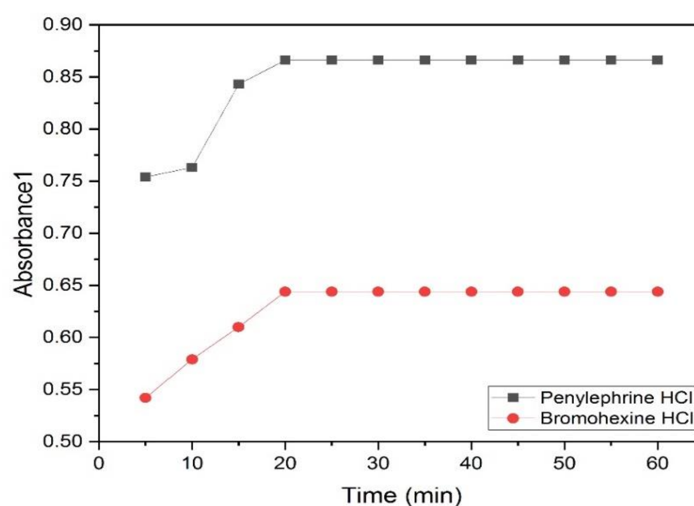


Figure 3-25: Effect of stability

3.6.1.12 Effect of interference

Table 3-22 shows that the interference that could be added to the pharmaceutical preparations, such as (glucose, fructose, lactose, etc.) it was observed that not effect of previous interference on the estimation of the drugs (PEH or BRH).

Table 3-22: Extraction recovery with different interference compound

Interference	Recovery% PEH	Recovery% BRH
Starch	97.3	99.04
Glucose	99.3	99.36
Maltose	98.6	100.3
Lactose	99.5	98.1
Glycine	99.2	96.8
Fructose	98.8	98.7

3.6.2 Calibration curve and statistical treatments

After determining the optimal conditions for the extraction of both medicines' complexes (PEH or BHH) method, the calibration curve was created by plotting the absorbance of the two medications against their solution concentration. The concentration range of PEH was determined to be (1-35 µg/mL), as illustrated in Figure 3-26, using the regression equation Y = 0.029X - 0.020 and R^2 = 0.999. BRH concentrations were ranged from (1 - 40µg/mL), with the regression equation Y = 0.032X - 0.032 and R^2 = 0.998 from the linear calibration as shown in Figure 3-27.

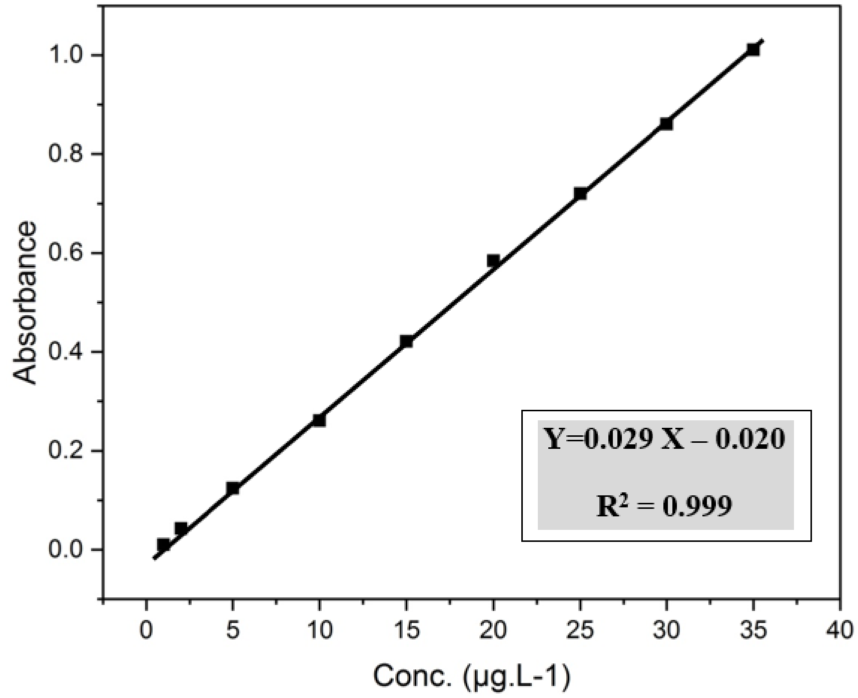


Figure 3-26: Calibration curve of PEH by CPE method

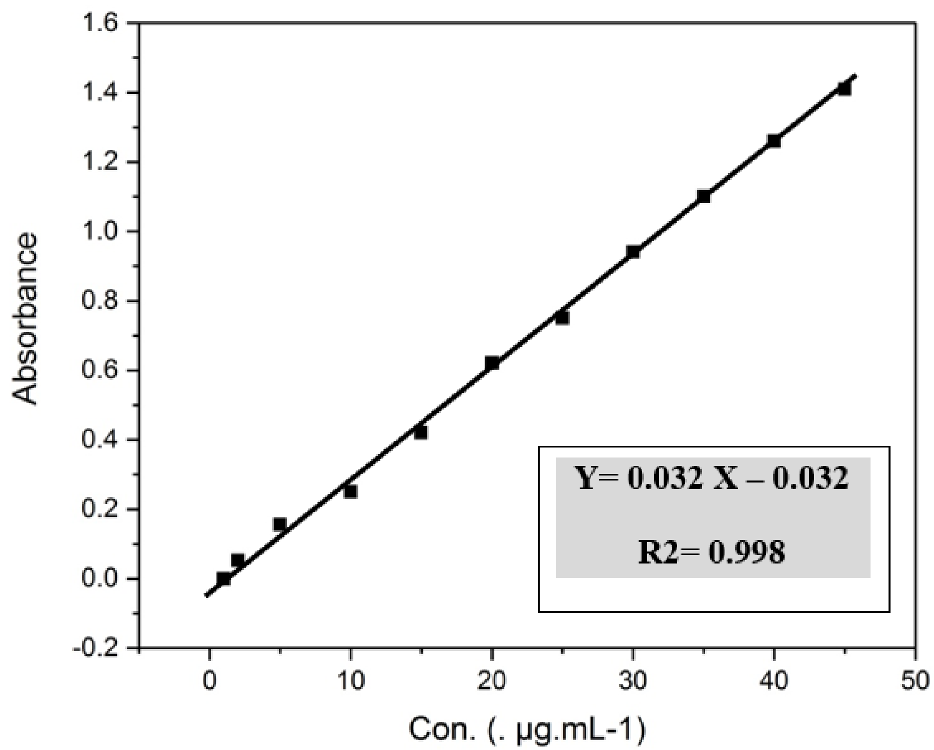


Figure 3-27: Calibration curve of BRH by CPE method

Table 3-23: Analytical parameter of cloud point extraction

Parameter	Cloud point for PEH	Cloud point for BRH
λ_{max} nm	430	480
Color	yellow	yellow
Regression equation	Y=0.029X-0.020	Y=0.032X-0.032
Linearity range (µg/mL)	1-35	1-40
Correlation Coefficient (R^2)	0.999	0.998
Ɛ($L.mol^{-1}.cm^{-1}$)	4073.2	13202.88
Sandell·ssensivity ($\mu g\ .\ cm^{-2}$)	0.05	3.121×10^{-3}
Slope (b)	0.029	0.032
Intercept(a)	0.020	0.032
Limit of detection (µg/mL)	1.065	0.141
Limit of quantification (µg/mL)	3.515	0.465
C.L.for the slope($b\pm ts_b$) at 95%	0.029±0.35	$0.032 \pm 6.5\times10^{-3}$
C.L.for the intercept ($a\pm ts_a$) at 95%	0.020±0.87	0.032 ± 0.487
Standard error for regression line ($S_{y/x}$)	0.132	0.2263
C.L for Conc.5µg/ mL at 95%	4.9±0.024	$4.8 \pm 2.5\times10^{-3}$

C.L for Conc.10µg/ mL at 95%	10.3±0.049	9.7 ± 5×10^{-3}
C.L for Conc.15µg/ mL at 95%	14.5±0.075	14.8 ±2.5×10^{-3}

3.6.3 Accuracy and precision

The accuracy and precision were studied of drug (PEH, BRH). These experimentations are carried out with three different drug concentrations (5, 10, 15 µg/mL for PEH and BRH) for three replications before using the CPE approach at optimum conditions. It is clear from these findings that the process has good accuracy and precision since the recovery rate for PEH is (99.23%) and for BRH is (98.5%), depending on the exact procedure is applied in clause (2.6.3.9). F-test, T-test was used (two-tailed) to calculate the accuracy and compared the practical value F-test with value in the critical statistical if the practical value less than the value in a table does not differ significantly between the suggested value method and standard method. The results were signed between the standard HPLC method and suggested method with PEH; BRH found no significant difference between the two methods. The statistical analysis results shown in the Table 3-24 proved that the calculated T-values and F-values for PEH BRH determination in different pharmaceuticals were less than t-critical and F-critical at 95% confidence interval and (n-1) degrees of freedom. The new methods have higher accuracy and precision than the literature (26,38).

Table 3-24: Accuracy and pression of CPE procedure for PEH, BRH drug

drug	DLLME method							
	Con. µg/mL		Relative Error%	Recov. %	Average Recov.%	T-value	F-value	RSD%
PEH	5	4.9	2	98	99.23	0.97	10.6	0.01
	10	10.3	-3	103				0.02
	15	14.5	3.3	96.7				0.01
BRH	5	5.1	-2	102	98.5	2.25	2.38	0.015
	10	9.5	5	95				0.02
	15	14.8	1.3	98.6				0.02
Critical value at 95% confidence limit, t = 2.7764, F= 19.								

3.6.4 Application the suggested method on pharmaceutical preparation for PEH and BRH

Using a cloud point method, it was discovered that PEH in the phenylephrine eye drops and Dolo-cold (tablets) are suitable for evaluating phenylephrine HCl in pharmaceutical preparations, as well as Solvodin and Biosolvon in BRH. As demonstrated in the Table 3-25, high accuracy and acceptable results were achieved.

Table 3-25: Application of the proposed cloud point for the evaluation of PEH and BRH

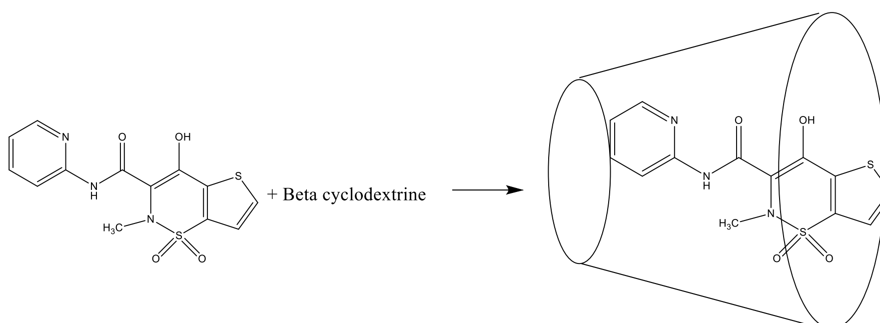
PEH	Cloud point					
	Conc. of drug µg/mL		Relative Error %	Recov. %	Average Recov %	RSD % (n=3)
	Taken	Found				
eye drops	5	4.6	8	92	96.2	0.07
	10	9.4	6	94		0.02
	15	15.4	-2.7	102.6		0.01
	5	5.2	-4	104	99.4	0.04
	10	9.7	3	97		0.03

Dolo-cold (tablet)	15	14.6	2.7	97.3		0.01

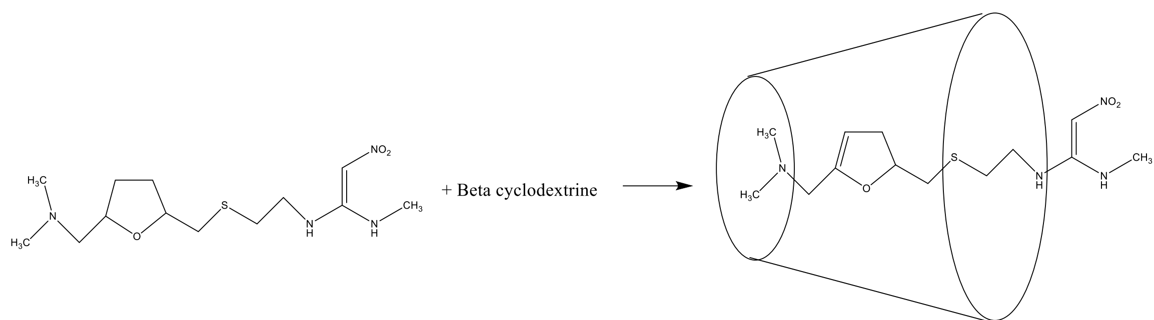
BRH	**Cloud point**					
	Conc. of drug µg/mL		**Relative Error %**	**Recov. %**	**Average Recov %**	**RSD % (n=3)**
	Taken	**Found**				
Solvodin	5	4.8	4	96	97.2	0.02
	10	9.7	3	97		0.021
	15	14.8	1.3	98.6		0.7
Biosolvon	5	4.7	6	94	97.8	0.02
	10	10.2	-2	102		0.02
	15	14.6	2.6	97.3		0.7

3.7 Spectrophotometric extraction of TNX and RAN-HCl using 2-HPβCD

The 2-hydroxypropyl-β-cyclodextrin reagent was used, a cyclic oligosaccharide consisting of six, seven or eight glycol pyranose units. The cyclodextrin is conical truncated with a lipophilic central cavity and a hydrophilic outer surface. The reagent can be product with the drug molecules represented by TNX, RAN-HCl, sliding partially or entirely inside the cavity. Thus, the formed product can have significantly improved physicochemical properties such as solubility, reactivity and stability. As in the following schemes:



Scheme 3-3: Structure of TNX: 2-HPβCD product



Scheme3-4: Structure of RAN- HCl: 2-HPβCD product

3.8 Study of formation of complex of TNX

Through the reaction of TNX with the hydroxypropyl-ß-cyclodextrin reagent, a complex was easily formed by entrapping the drug fully or partially into the reagent cavity. The absorbance of the complex can be measured by spectrophotometer analysis in pH 10 at 385nm against blank; the results were illustrated in Figure 3-28.

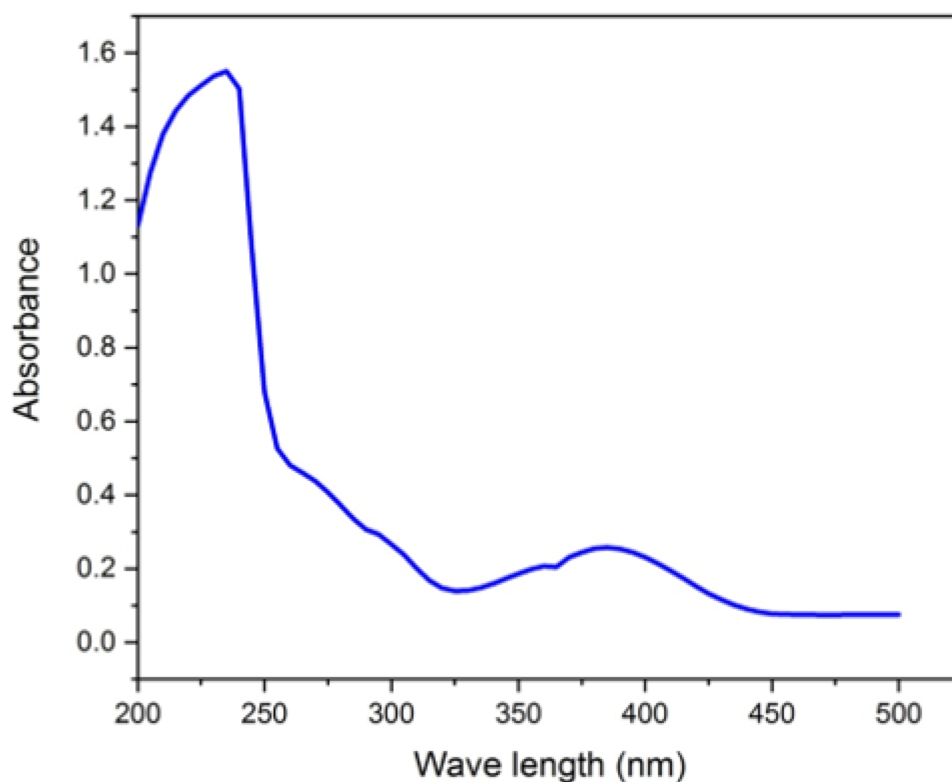


Figure 3-28: Absorption spectrum of formation product of TNX

3.9 Study of formation of complex of RAN-HCl

A complex was quickly produced by entrapping the drug partial or complete into2- hydroxypropyl-ß-cyclodextrin reagent cavity through the interaction of RAN-HCl with the hydroxypropyl-ß-cyclodextrin reagent. The complex's absorbance can be measured using a spectrophotometer in pH 8 at 330 nm against a blank, as shown in Figure 3-29.

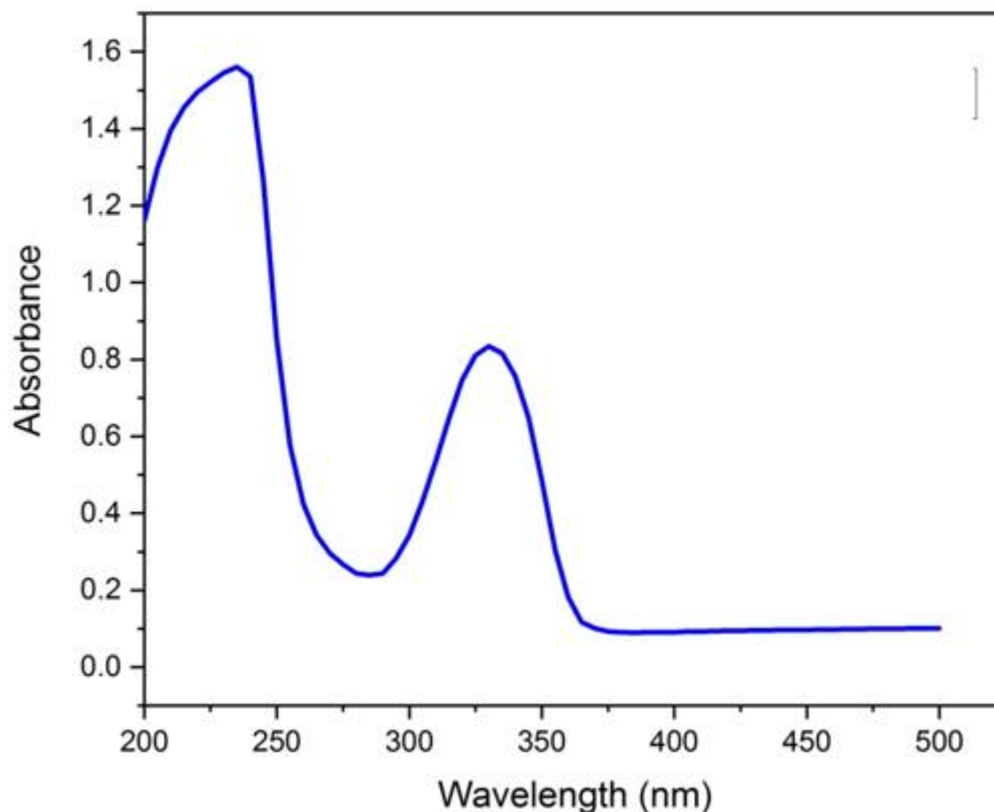


Figure 3-29: Absorption spectrum of formation product of RAN-HCl

3.10 Direct extraction method

The ion-pair product of two drugs, TNX and RAN-HCl, was determined using direct extraction technique, and the absorbance was measured at 385 and 330 nm, respectively.

3.10.1 Optimization of direct extraction

Testing parameters were used, including pH type and volume, reagent volume, and solvent type, to determine the optimal circumstances for the extraction of TNX and RAN-HCl drug with 2-hydroxypropyl-ß-cyclodextrin reagent at wavelength 385 and 330 nm. As a result, they were thoroughly explored and optimized.

3.10.1.1 Effect of pH value

The 2-hydroxypropyl-ß-cyclodextrin reagent was used to choose the appropriate conditions for the product of TNX or RAN-HCl drugs with a UV-Vis spectrophotometer at wavelengths of 385 and 330 nm. The pH range for solution buffer was 1-12. It was discovered that the best acidity function for the TNX product formation was at pH = 10, while the acidity function for the RAN-HCl medication was at pH = 8, as shown in Figure 3-30.

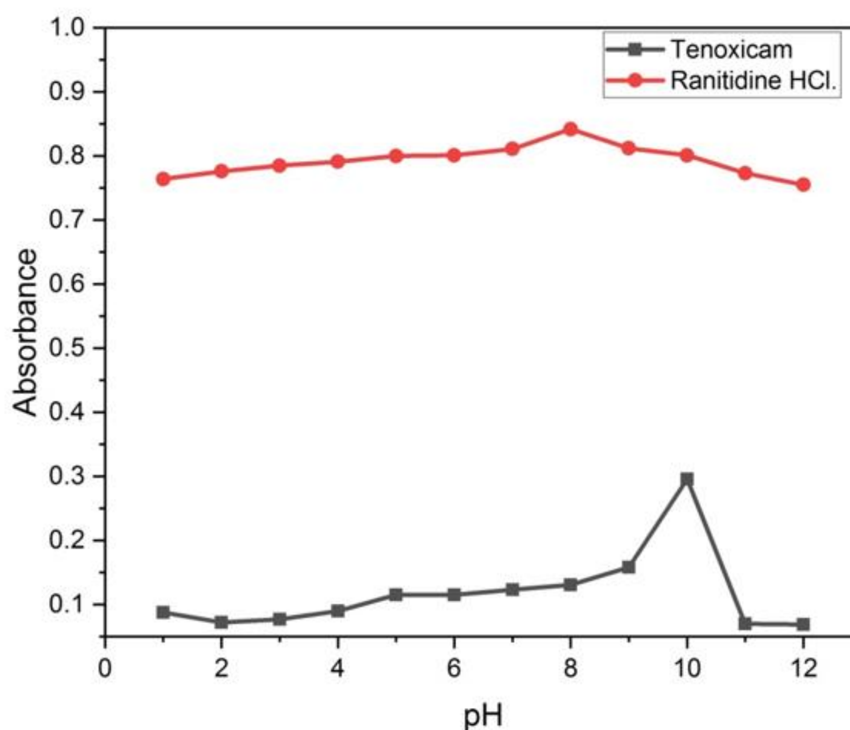


Figure 3-30: Effect of pH on the product formation

3.10.1.2 Effect of buffer type

Since buffer type impacts product formation, a variety of buffer solutions at pH 10 (phosphate buffer, (KCl+NaOH), and Na_2CO_3 + $NaHCO_3$) were examined in the production of the product (TNX–HPβCD). It was revealed that (KCl+ NaOH) at pH 10 is the best. At pH 8, the effect of buffer type (Phosphate Buffer, Borax + HCl) on forming

the (RAN-HCl - HPBCD) complex was investigated. Phosphate at pH = 8 was the best buffer, as shown in Table 3-26.

Table 3-26: Effect type of buffer solution absorbance of TNX, RAN-HCl

Type of buffer at PH=10	Absorbance at 385 nm TNX
$Na_2HPO_4.12H_2O$ + NaOH	0.257
Na_2CO_3 + $NaHCO_3$	0.086
KCl + NaOH	0.297
Type of buffer at PH=8	**Absorbance at 385 nm RAN- HCl**
Phosphate buffer	0.837
Borax ($Na_2B_4O_7.10H_2O$) + HCl	0.524

3.10.1.3 Effect of buffer volume

The different volumes of (KCl+NaOH) and phosphate buffer were examined, it was noted that the best volume recorded the highest absorption was 1.2mL and 0.8 mL at 385 and 330 nm, respectively, as shown Figure 3-31.

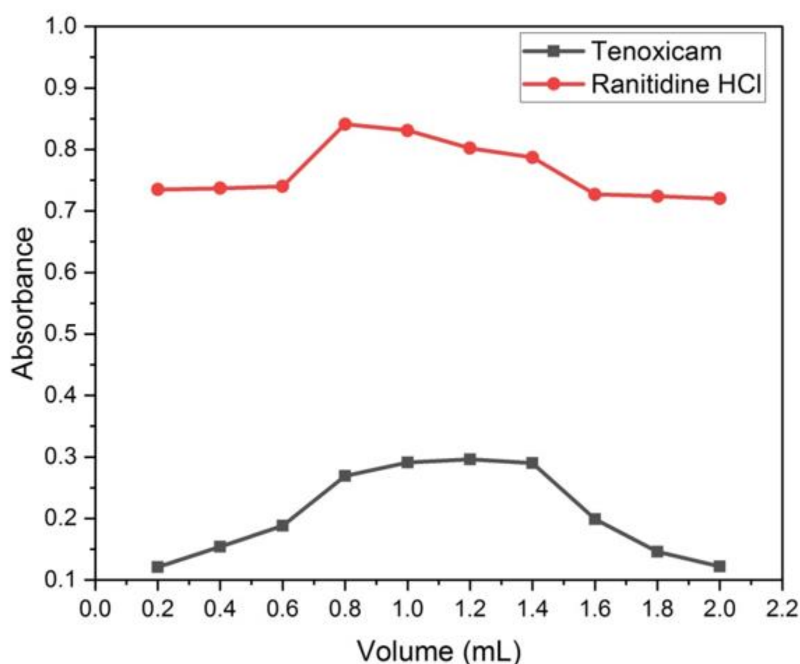


Figure 3-31: Effect of buffer volume

3.10.1.4 Effect of reagent volume

This volume was considered best for forming products between TNX and 2-hydroxypropyl-ß-cyclodextrin reagent, as well as between RAN- HCl and a 2-hydroxypropyl-ß-cyclodextrin reagent. It was determined that 1.0 mL of reagent were required for product formation, respectively, as shown in Figure 3-32.

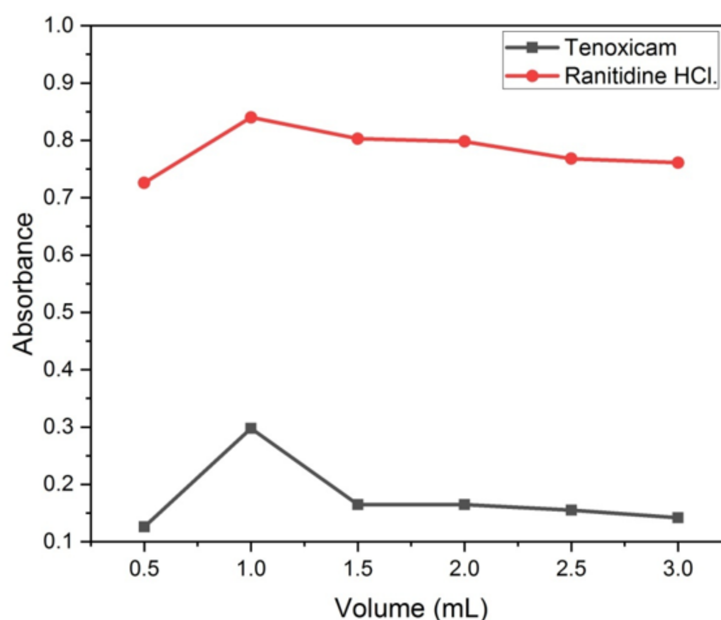


Figure 3-32: Effect of reagent volume

3.10.1.5 Effect of solvent type

The influence of several solvents (chloroform, tetra chlorocarbon, benzene, and hexane) on the extraction of complex composition of both medications was also investigated; chloroform was shown to be the best solvent for both drugs absorption. As shown in Table 3-27.

Table 3-27: Effect of solvent type

Type Solvent	Absorbance at 385nm TNX	Absorbance at 330 nm RAN- HCl
Chloroform	0.297	0.841
CCL_4	0.152	0.832
Benzene	0.081	0.616
Hexane	———	———

3.10.1.6 Effect of temperature

The temperature on extraction was studied using temperatures ranging from 25 to 50 ºC, with the results revealing that 30 ºC, is the best temperature for TNX absorption, and 25 ºC, is the optimal temperature for ranitidine HCl absorption, as shown in Figure 3-33.

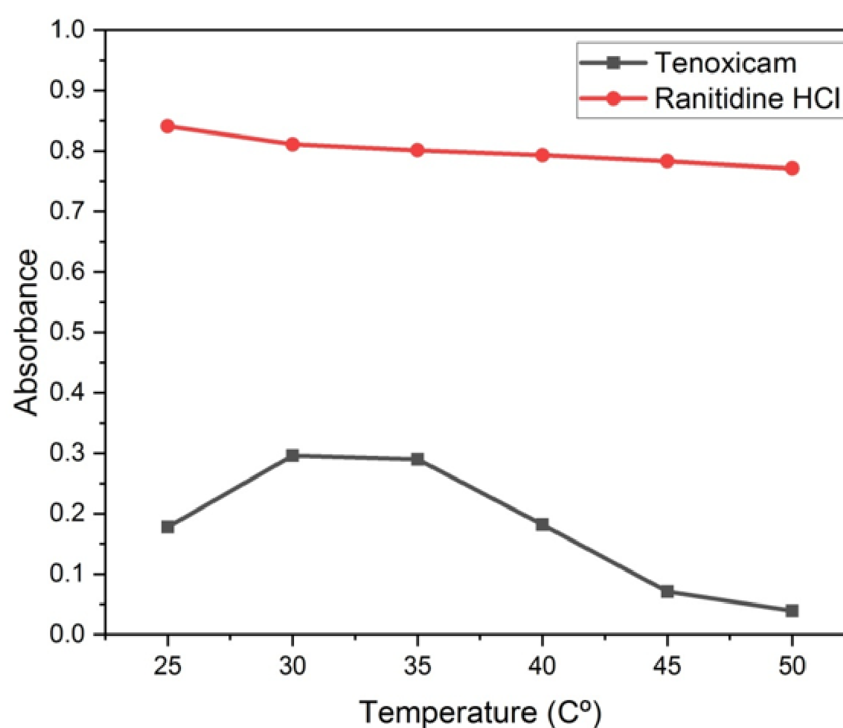


Figure 3-33: Effect of temperature

3.10.1.7 Stoichiometric evaluation of color complex

a. Continuous variation method (Jobs method).

The ratio of each medication to 2-hydroxypropyl-ß-cyclodextrin reagent was expressed using the continuous variance technique. The process was proved to have a 1:1 ratio (TNX: 2-hydroxypropyl-ß-cyclodextrin reagent), (RAN-HCl: 2-hydroxypropyl-ß-cyclodextrin reagent), as shown in Figure 3-34 and Figure 3-35.

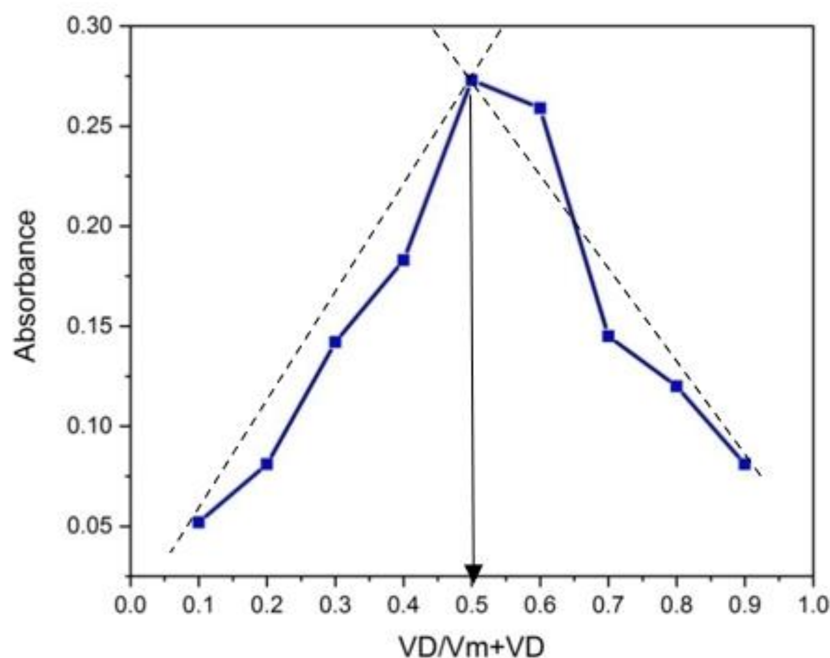


Figure 3-34: Continuous variation method of TNX

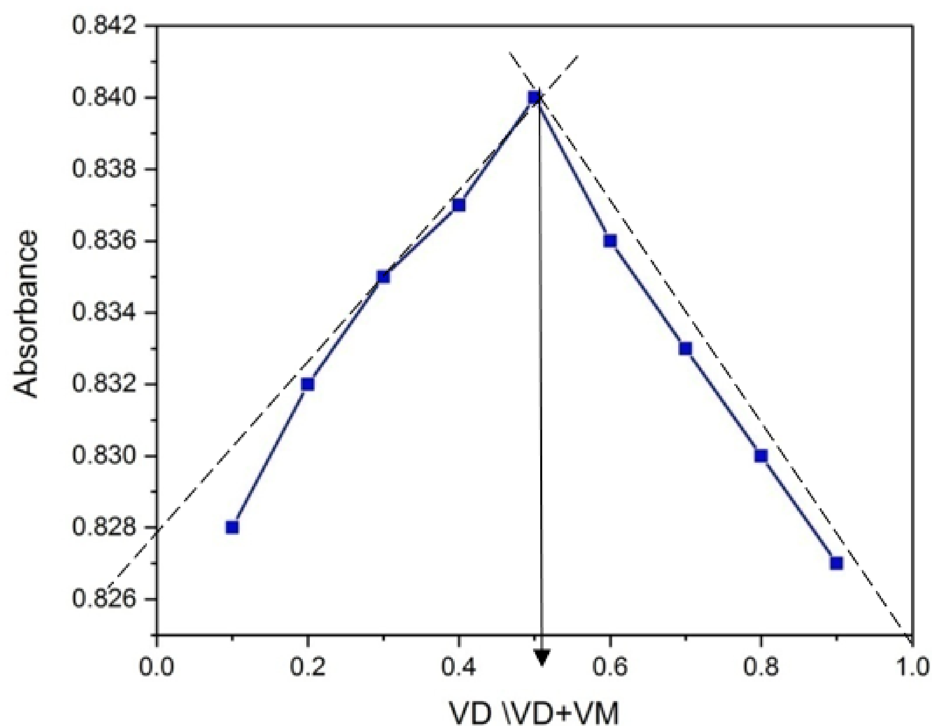


Figure 3-35: Continuous variation method of RAN-HCl

b. Mole- ratio method

The ratio of TNX and RAN-HCl with 2-hydroxypropyl-ß-cyclodextrin reagent was determined using the molar ratio technique. To combine both medications and the reagent, a 1:1 ratio was observed, as shown in Figure 3-36 and Figure 3-37.

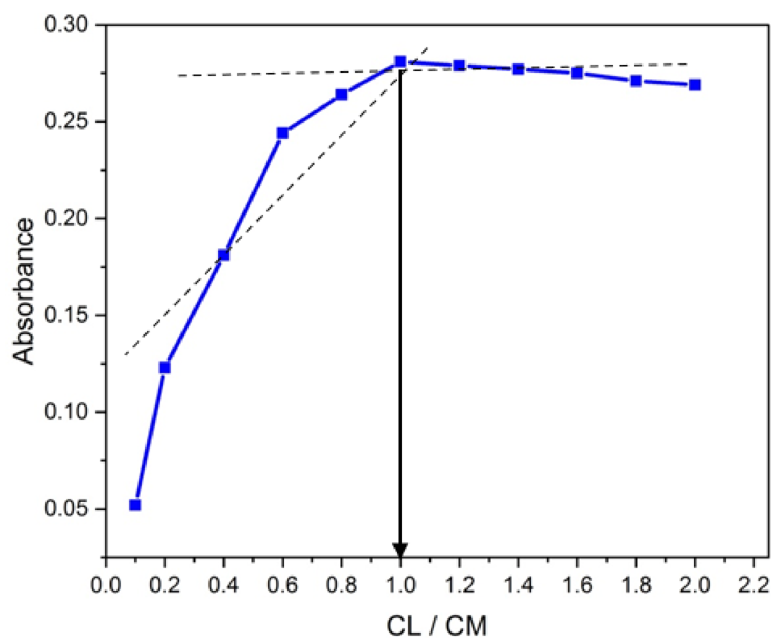


Figure 3-36: Mole-ratio method of TNX

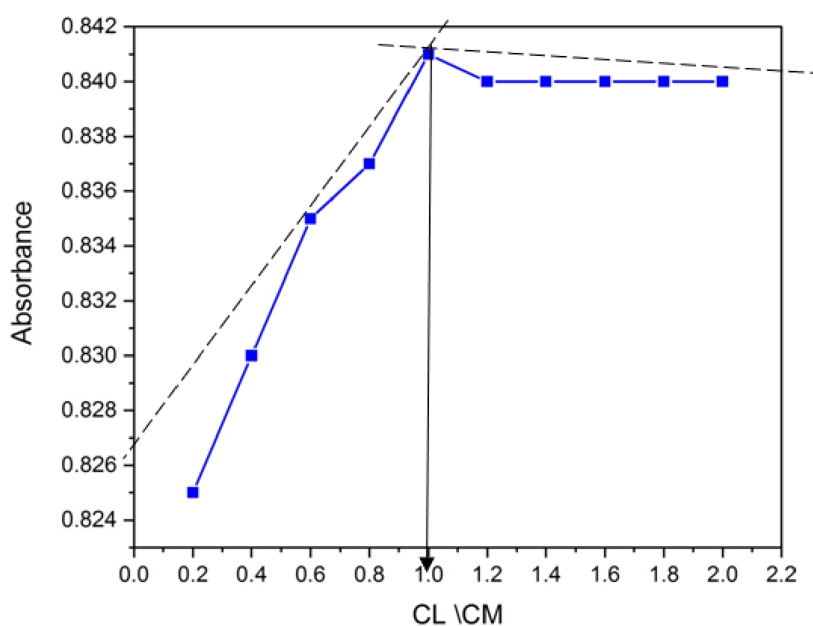


Figure 3-37: Mole-ratio method of RAN-HCl

3.10.1.8 Effect of stability

The influence of time on color stability for with products was investigated, within period ranged from 5 to 60 minutes. It was detected

after 15 minutes is the time to stable the color for TNX and after 20 minutes for ranitidine HCl. As shown in Figure 3-38.

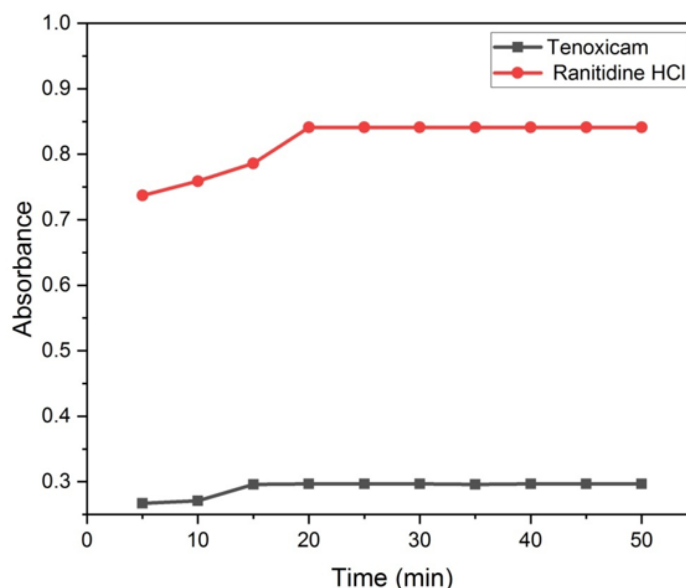


Figure 3-38: Effect of Color stability

3.10.1.9 Effect of interference

Interferences that may be added to pharmaceutical preparations such as (glucose, fructose, lactose, etc.) was investigated. It was found that they had no effect on the medications, as shown in Table 3-28.

Table 3-28: Extraction recovery% with different interference compound

Compound	Recovery% TNX	Recovery% RAN-HCl
Maltose	98.64	99.2
Starch	100.7	97.6
Glucose	97.3	98.8
Lactose	96.9	97.5
Fructose	95.6	97.6
Glysine	97.62	96.4

3.10.2 Calibration curve and statistical treatments

After determining the optimal conditions for the extraction of both medicines' complexes (TNX and RAN-HCl), the calibration curve was created by plotting the absorbance of the two medications against their solution concentration. The concentration range of TNX was determined to be (1-45 µg/mL), as illustrated in Figure 3-39, using the regression equation Y = 0.007X + 0.05 and R^2 = 0.998. RAN-HCl concentrations ranged from (1 -45µg/mL), with the regression equation Y = 0.018X - 0.016 and R^2 = 0.999 from the linear calibration as shown in Figure 3-40.

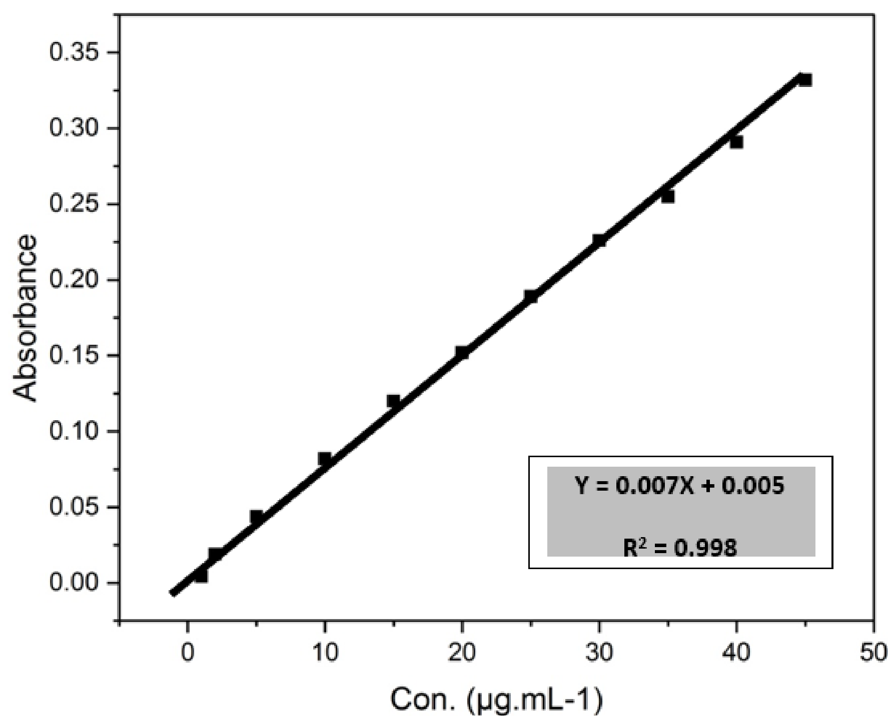


Figure 3-39: Calibration curve of TNX by direct extraction method

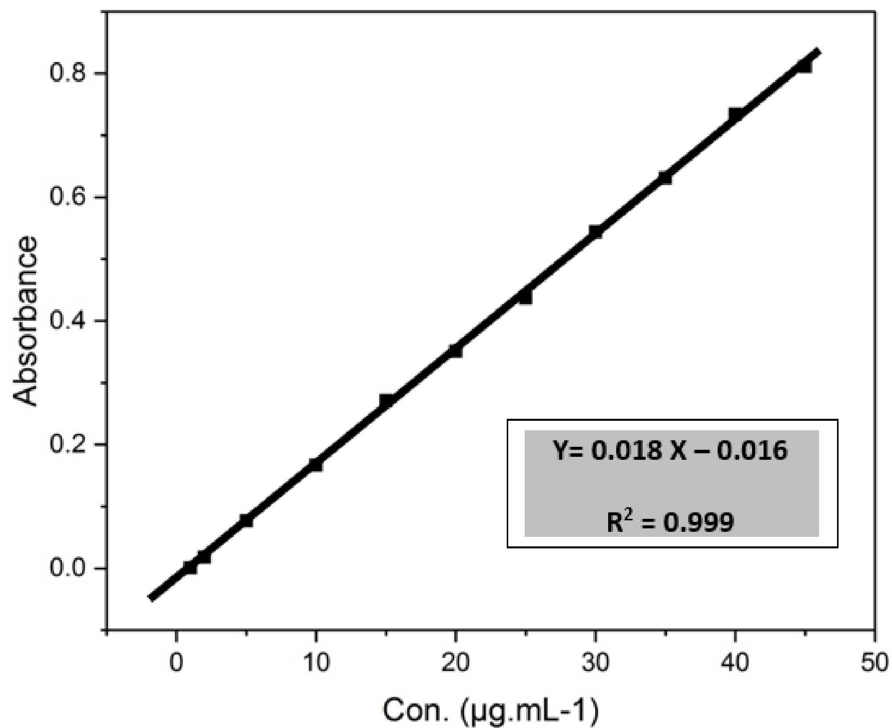


Figure 3-40: Calibration curve of RAN-HCl by direct extraction method

Table 3-29: Analytical parameter of direct extraction

Parameter	**Direct extraction for TNX**	**Direct extraction for RAN-HCl**
λ_{max} nm	385	330
Color	Light yellow	Light yellow
Regression equation	Y= 0.007X+0.005	Y= 0.018X - 0.016
Linearty range (µg/mL)	1-45	1-45
Correlation Coefficient (R^2)	0.998	0.999
Ɛ($L.mol^{-1}.cm^{-1}$)	2200.8	6072.77
Sandell'ssensivity (µg . cm^{-2})	0.143	0.0555
Slope (b)	0.005	0.018
Intercept(a)	0.007	0.016
Limit of detection (µg/mL)	0. 6	0.17
Limit of quantification (µg/mL)	1.98	0.55

C.L.for the slope($b \pm ts_b$) at 95%	$0.005 \pm 13\times10^{-4}$	$0.018 \pm 34.9\times10^{-4}$
C.L.for the intercept ($a \pm ts_a$) at 95%	0.007 ± 0.31	0.016 ± 0.802
Standard error for regression line ($S_{y/x}$)	0.0461	0.122
C.L for Conc.5µg/ mL at 95%	$5.3 \pm 2.5\times10^{-3}$	$5.2 \pm 4.96 \times 10^{-3}$
C.L for Conc.10µg/mL at 95%	$9.6 \pm 2.5\times10^{-3}$	$9.8 \pm 2.483 \times 10^{-3}$
C.L for Conc.15µg/mL at 95%	$14.8 \pm 5\times10^{-3}$	$14.7 \pm 2.483 \times 10^{-3}$

3.10.3 Accuracy and precision

The accuracy and precision were studied of drug (TNX or RAN-HCl). These experimentations are carried out with three different drug concentrations (5, 10, 15 µg/mL for TNX and RAN-HCl) for three replications before using the direct extraction approach at optimum conditions. It is clear from these findings that the process has good accuracy and precision since the recovery rate for TNX is (101%) and for RAN-HCl is (99.77%), depending on the exact procedure is applied in clause (2.7.1.12). F-test, T-test was used (two-tailed) to calculate the accuracy and compared the practical value F-test with value in the critical statistical if the practical value less than the value in a table does not differ significantly between the suggested value method and standard method.

The results were signed between the standard HPLC method and suggested method with TNX; RAN-HCl found no significant difference between the two methods. The statistical analysis results shown in the Table 3-30 proved that the calculated T-values and F-values for TNX and RAN-HCl determination in different pharmaceuticals were less than t-critical and F-critical at 95% confidence interval and (n-1)

degrees of freedom. The new methods have higher accuracy and precision than the literature (50,63).

Table 3-30: Accuracy and pression of direct extraction procedure for TNX, RAN-HCl drug.

drug	Direct extraction method							
	Con. μg/mL		Relative Error%	Recov. %	Average Recov.%	T-value	F-value	RSD%
TNX	5	5.2	-4	104	101	0.73	6.81	0.01
	10	10.1	-1	101				0.02
	15	14.7	2	98				0.01
RAN-HCl	5	5.11	-2.2	102.2	99.77	2.01	10.87	0.015
	10	9.78	2.2	97.8				0.02
	15	14.9	0.6	99.3				0.02
Critical value at 95% confidence limit, t = 2.7764, F= 19.								

3.10.4 Application the suggested method on pharmaceutical preparation for TNX and RAN-HCl

Using a direct extraction method, it was discovered that TNX in Tilcotil and Tenoctil are suitable for evaluating TNX in pharmaceutical preparations, as well as Barkadin and HISTAC R150 in RAN-HCl. As demonstrated in the Table 3-31, below, high accuracy and acceptable results were achieved.

Table 3-31: Application of the proposed direct extraction for the evaluation of TNX, RAN-HCl

TNX	direct extraction					
	Conc. of drug µg/mL		Relative Error%	Recov. %	Average Recov %	RSD % (n=3)
	Taken	Found				
Tilcotil	5	5.37	-7.4	107.4	100.6	0.018
	10	9.6	4	96		0.01
	15	14.8	1.3	98.6		0.03
Tenoctil	5	5.3	-6	106	100.7	0.01
	10	9.8	2	98		0.01
	15	14.7	2	98		0.02
RAN-HCl	**direct extraction**					
	Conc. of drug µg/mL		**Relative Error%**	**Recov. %**	**Average Recov %**	**RSD % (n=3)**
	Taken	**Found**				
Barkadin	5	4.6	8	92	95.6	0.04
	10	9.6	4	96		0.01
	15	14.8	1.3	98.7		6.8×10^{-3}
HISTAC R150	5	4.7	6	94	96	0.02
	10	9.6	4	96		0.01
	15	14.7	2	98		6.8×10^{-3}

3.11 Dispersive liquid liquid microextraction (DLLME) method

For TNX and RAN-HCl, the DLLME method was utilized to evaluate and extract the ion-pair formation, and its spectra were studied at 385 and 330 nm, respectively.

3.11.1 Optimization of DLLME

3.11.1.1 Effect of the extraction and dispersive solvents

The effect of chloroform, carbon tetrachloride, hexane and benzene on the extraction was investigated. Chloroform was proved to be the best extraction solvent for both drugs TNX and RAN-HCl tested. The result obtained is shown in Table 3-32.

Table 3-32: Selection type of extraction solvent

Type of extraction solvent	Absorbance at 385 nm TNX	Absorbance at 330nm RAN-HCl
Chloroform	0.145	0.701
carbon tetrachloride	0.130	0.681
Benzene	0.096	0.074
Hexane	———	———

The influence of dispersed solvents (ethanol, methanol, acetone, and acetonitrile) was studied. The result showed that ethanol was the best dispersion solvent for TNX and RAN-HCl.As shown in Table 3-33.

Table 3-33: Selection type of dispersive solvent

Type of dispersive solvent	Absorbance at 385nm TNX	Absorbance at 330nm RAN-HCl
Ethanol	0.146	0.700
Methanol	0.144	0.706
Acetone	0.072	0.465
Acetonitrile	0.121	0.145

3.11.1.2 Effect of type of buffer

A variety of buffer solutions were studied since the type of buffer affects the extraction complex in DLLME method. A type of buffer solutions at pH 10 (phosphate buffer, (KCl+NaOH), and Na_2CO_3 + $NaHCO_3$) were examined in the production of the complex (TNX: HP-ß-CD), and it was revealed that (KCl+ NaOH) at pH 10 is the best. At pH 8, the effect of buffer type (Phosphate Buffer, Borax + HCl) on the formation of the (RAN-HCl: HPßCD) complex was studied. Phosphate at pH = 8 was found to be the best buffer. As shown in Table 3-34.

Table 3-34: Effect type of buffer solution absorbance of TNX and RAN-HCl

Type of buffer at PH = 10	**Absorbance at 385 nm TNX**
$Na_2HPO_4.12H_2O$ + NaOH	0.140
Na_2CO_3 + $NaHCO_3$	0.074
KCl + NaOH	0.147
Type of buffer at PH = 8	**Absorbance at 385 nm RAN-HCl**
Phosphate buffer	0.712
Borax ($Na_2B_4O_7.10H_2O$) + HCl	0.285

3.11.1.3 Effect of buffer volume

The different volumes of (KCl+NaOH) and phosphate buffer were examined. It was noted that the volume that recorded the highest absorption was 1.0 mL and 1.2 mL at 385 and 330 nm, respectively. As shown in Figure 3-41.

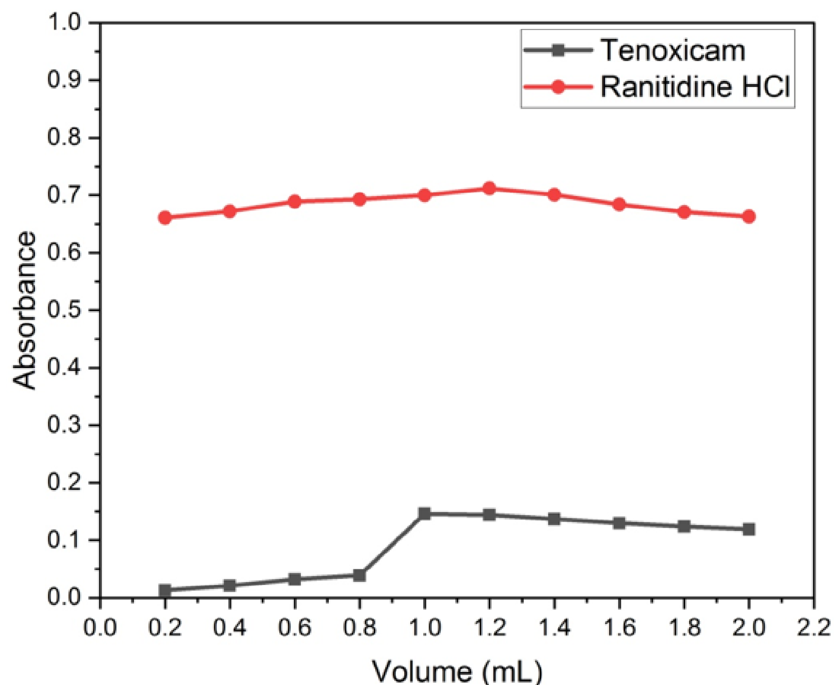


Figure 3-41: Effect of buffer volume

3.11.1.4 Effect of reagent volume

Both medications (TNX and RAN-HCl) require a particular volume of 2-HPßCD reagent to form the product. It was discovered that 1.5 mL generated the greatest absorbance in the TNX and RAN-HCl. As shown in Figure 3-42.

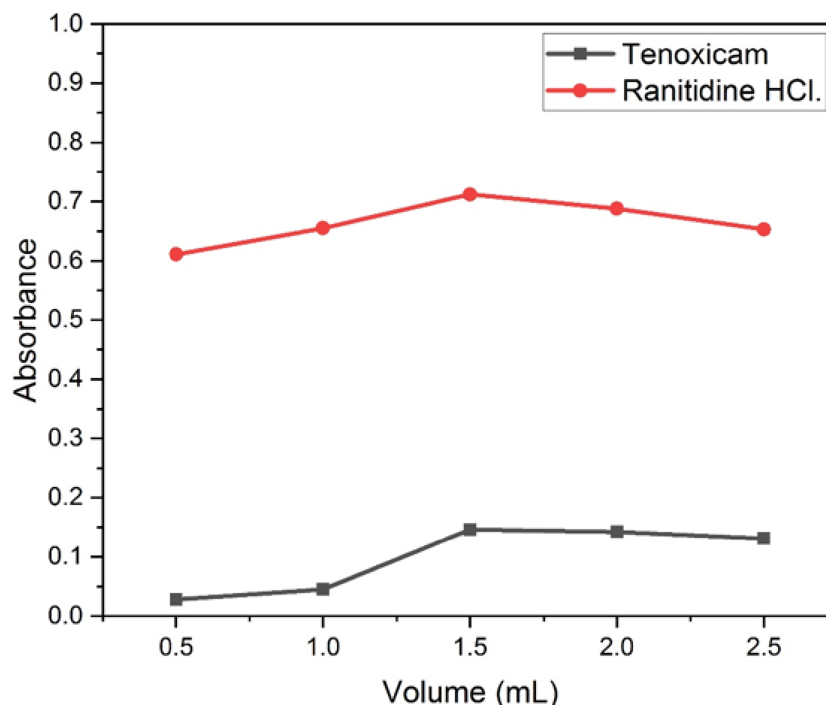


Figure 3-42: Effect of reagent volume

3.11.1.5 Effect of extraction and dispersion volume solvent

Different amounts of extraction and dispersal solvents have different effects on the extraction of product composition of both drugs used. Therefore, it was discovered that the best volume of TNX for extraction and dispersion is 400 µL for extraction and 700 µL for dispersion. According to the results, the optimal volume RAN-HCl is 300 µL for the extraction solvent and 800 µL for the dispersion solvent, The results got are shown in Table 3-35 and Table 3-36.

Table 3-35: Effect of the extraction solvent volume

Extraction solvent volume (chloroform) µL	Dispersive solvent volume (Ethanol) µL	Absorbance at 385 nm TNX
200	700	0.096
300		0.140
400		0.146
500		0.137
Extraction solvent volume (chloroform) µL	**Dispersive solvent volume (Methanol) µL**	**Absorbance at 330 nm RAN-HCl**
200	700	0.706
300		0.711
400		0.705
500		0.701

Table 3-36: Effect of the dispersive solvent volume

Extraction solvent volume (chloroform) µL	Dispersive solvent volume (Ethanol) µL	Absorbance at 385 nm TNX
400	500	0.132
	600	0.141
	700	0.147
	800	0.145
	900	0.139
	1000	0.118
	1100	0.104
	1200	0.087
	1300	0.081
	1400	0.078
	1500	0.070
Extraction solvent volume (chloroform) µL	**Dispersive solvent volume (Methanol) µL**	**Absorbance at 330 nm RAN-HCl**
	500	0.682
	600	0.701
	700	0.708

300	800	0.712
	900	0.710
	1000	0.708
	1100	0.703
	1200	0.692
	1300	0.685
	1400	0.677
	1500	0.674

3.11.1.6 Effect of rate and time in the centrifuge

The effects of centrifuge rate and time are significant in the separation and extraction of complexes. The optimal extraction speed for TNX was 4 minutes and 5000 rpm, whereas the best extraction speed for RAN-HCl was 6 minutes and 4000 rpm, as shown in Figure 3-43 and Figure 3-44.

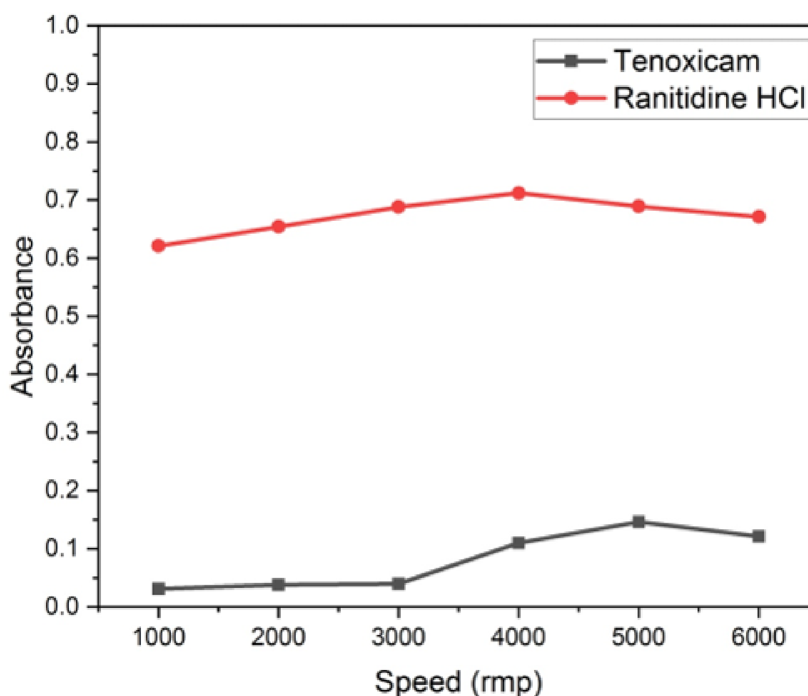


Figure 3-43: Effect of the centrifuge rate

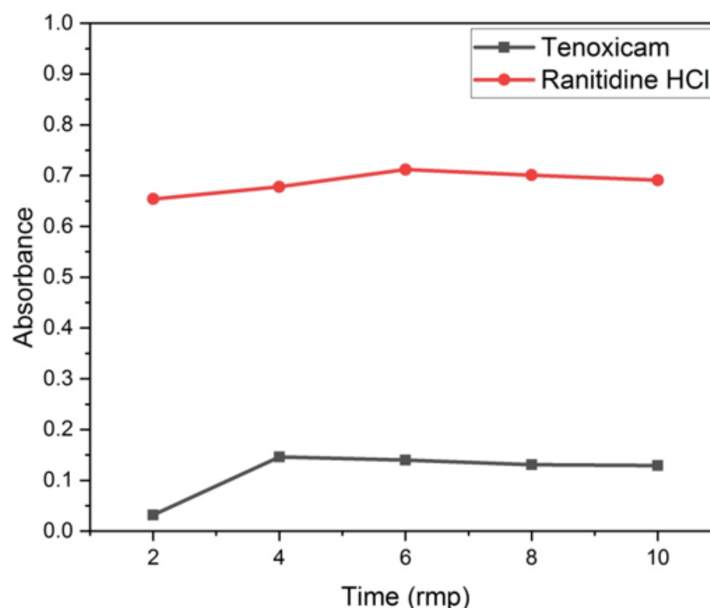


Figure 3-44: Effect of the centrifuge time

3.11.1.7 Effect of the stability

The influence of time on color stability for both products was investigated, within period ranged from 5 to 60 minutes. It was detected after 15 minutes is the time to stable the color for TNX and after 20 minutes for ranitidine HCl. As shown in Figure 3-45.

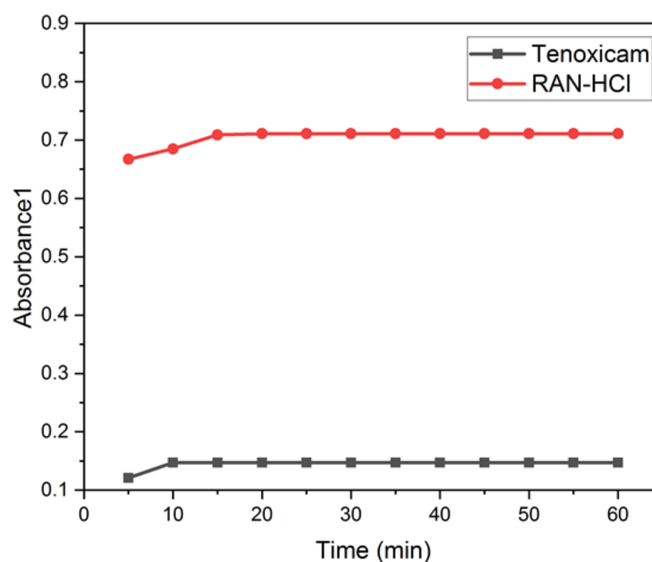


Figure 3-45: Effect of stability

3.11.1.8 Effect of interference

Carbohydrates added to pharmaceutical formulations such as glucose, fructose, lactose, and other sugars do not affect on the extraction of medicine (TNX and RAN-HCl). As shown in Table 3-37.

Table 3-37: Extraction recovery with different interference compound

Interference	Recovery% Absorbance at 385 nm TNX	Recovery% Absorbance at 330 nm RAN-HCl
Starch	95.1	99.3
Glucose	96.7	97.1
Maltose	94.4	98.6
Lactose	95.2	98.7
Glysine	98	96.4
Fructose	97.7	98.2

3.11.2 Calibration curve and statistical treatments

The calibration curve was created by graphing the absorbance intensity of the included reaction complex at 385 nm for TNX and 330nm for RAN-HCl versus the concentration of both medicines under optimization conditions. The resulting calibration curve was linear, the concentration range of TNX was determined to be (1-21 µg/mL), as illustrated in Figure 3-46, using the regression equation Y = 0.008X + 0.032 and R^2 = 0.996. RAN-HCl concentrations ranged from (1-13µg/mL), with the regression equation Y = 0.075X-0.054 and R^2 = 0.998 from the linear calibration as shown in Figure 3-47.

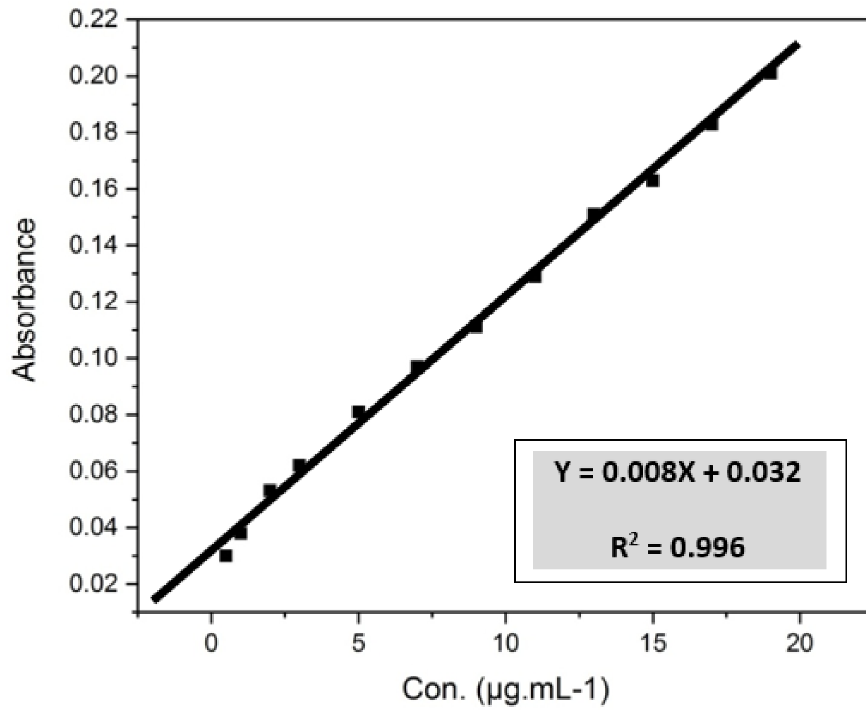


Figure 3-46: Calibration curve of TNX by DLLME method

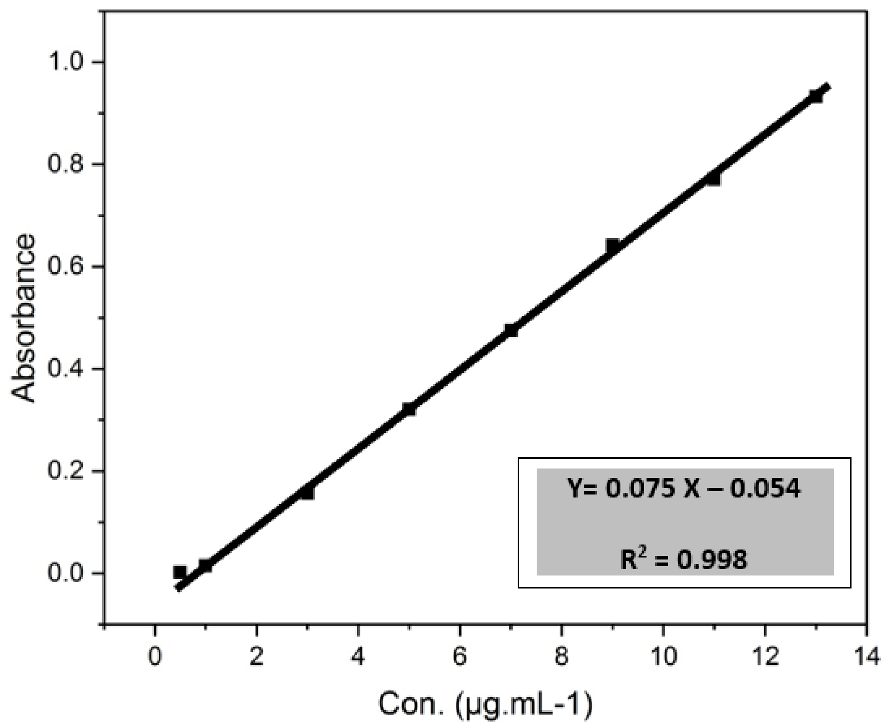


Figure 3-47: Calibration curve of RAN-HCl by DLLME method

Table 3-38: Analytical parameter of DLLME

Parameter	DLLME for TNX	DLLME for RAN-HCl
λ_{max} nm	385	330
Color	Light yellow	Light yellow
Regression equation	Y=0.008X+0.032	Y=0.075X -0.054
Linearity range (µg/mL)	1 – 21	1-13
Correlation Coefficient (R^2)	0.996	0.998
Ɛ($L.mol^{-1}.cm^{-1}$)	2515.2	25303.2
Sandell·ssensivity (µg . cm^{-2})	0.125	0.0133
Slope (b)	0.008	0.075
Intercept(a)	0.032	0.054
Limit of detection (µg/mL)	0.079	0.04
Limit of quantification (µg/mL)	0.254	0.132
C.L.for the slope($b\pm ts_b$) at 95%	$0.008 \pm 10.8\times10^{-4}$	$0.075 \pm 6 \times10^{-3}$
C.L.for the intercept ($a\pm ts_a$) at 95%	0.032 ± 0.25	0.054 ± 1.367
Standard error for regression line ($S_{y/x}$)	0.038	0.208
C.L for Conc.3µg/ mL at 95%	$3.2 \pm 2.5\times10^{-3}$	$2.8\pm2.48 \times10^{-3}$
C.L for Conc.5µg/ mL at 95%	$4.7 \pm 5\times10^{-3}$	$5.1\pm2.48 \times10^{-3}$
C.L for Conc.7µg/mL at 95%	$9.7 \pm 2.5\times10^{-3}$	$6.7\pm2.48 \times10^{-3}$

3.11.3Accuracy and precision

The accuracy and precision were studied of drug (TNX or RAN-HCl). These experimentations are carried out with three different drug concentrations (3, 5, 7 µg/mL for TNX and RAN-HCl) for three replications before using the DLLME approach at optimum conditions. It is clear from these findings that the process has good accuracy and precision since the recovery rate for TNX is (99.5%) and for RAN-HCl is (97.78%), depending on the exact procedure is applied in clause

(2.7.2.10). F-test, T-test was used (two-tailed) to calculate the accuracy and compared the practical value F-test with value in the critical statistical if the practical value less than the value in a table does not differ significantly between the suggested value method and standard method.

The results were signed between the standard HPLC method and suggested method with TNX; RAN-HCl found no significant difference between the two methods. The statistical analysis results shown in the Table 3-39 proved that the calculated T-values and F-values for TNX and RAN-HCl determination in different pharmaceuticals were less than t-critical and F-critical at 95% confidence interval and (n-1) degrees of freedom. The new methods have higher accuracy and precision than the literature (50,63).

Table 3-39: Accuracy and Pression of DLLME procedure for TNX, RAN-HCl drug

<table>
<tr><th rowspan="2">drug</th><th colspan="8">DLLME method</th></tr>
<tr><th colspan="2">Con. µg/mL</th><th>Relative Error%</th><th>Recov. %</th><th>Average Recov.%</th><th>T-value</th><th>F-value</th><th>RSD%</th></tr>
<tr><td rowspan="3">TNX</td><td>3</td><td>2.8</td><td>4</td><td>96</td><td rowspan="3">99.5</td><td rowspan="3">0.43</td><td rowspan="3">6.22</td><td>0.01</td></tr>
<tr><td>5</td><td>5.2</td><td>-2</td><td>102</td><td>0.02</td></tr>
<tr><td>7</td><td>7.1</td><td>0.6</td><td>100.6</td><td>0.01</td></tr>
<tr><td rowspan="3">RAN-HCl</td><td>3</td><td>2.7</td><td>6</td><td>94</td><td rowspan="3">97.78</td><td rowspan="3">0.33</td><td rowspan="3">9.02</td><td>0.015</td></tr>
<tr><td>5</td><td>4.8</td><td>2</td><td>98</td><td>0.02</td></tr>
<tr><td>7</td><td>7.2</td><td>1.3</td><td>101.3</td><td>0.02</td></tr>
<tr><td colspan="9">Critical value at 95% confidence limit, t = 2.7764, F= 19.</td></tr>
</table>

3.11.4 Application the suggested method on pharmaceutical preparation TNX and RAN-HCl

Using a DLLME method, it was discovered that TNX in Tilcotil and Tenoctil are suitable for evaluating TNX in pharmaceutical preparations, as well as Barkadinand HISTAC R150 in RAN-HCl. As demonstrated in the Table 3-40, high accuracy and acceptable results were achieved.

Table 3-40: Application of the proposed DLLME for the evaluation of TNX and RAN-HCl

TNX	DLLME					
	Conc. of drug µg/mL		Relative Error%	Recov. %	Average Recov %	RSD % (n=3)
	Taken	Found				
Tilcotil	3	2.7	10	90	93.3	0.01
	5	4.7	6	94		0.02
	7	6.7	4.3	96		0.01
Tenoctil	3	2.7	10	90	93.7	0.01
	5	4.7	6	94		0.02
	7	6.8	2.9	97		0.02
RAN-HCl	**DLLME**					
	Conc. of drug µg/mL		**Relative Error%**	**Recov. %**	**Average Recov %**	**RSD % (n=3)**
	Taken	**Found**				
Barkadin	3	2.7	10	90	96	0.04
	5	4.8	4	96		0.042
	7	7.2	-2.8	102		0.03
HISTAC R150	3	2.8	6.6	93.3	94.8	0.012
	5	4.7	6	94		0.02
	7	6.8	2.9	97		0.015

3.12 Cloud point method

Cloud point extraction was utilized to evaluate and extract both medications (TNX and RAN-HCl) at 380 and 330 nm, respectively.

3.12.1 Optimization of cloud point

3.12.1.1 Effect of buffer type

A variety of buffer solutions were studied since the type of buffer affects the extraction complex in CPE method. A type of buffer solutions at pH 10 (phosphate buffer, (KCl+NaOH), and in the production of the complex (TNX: 2-HPBCD), and it was revealed that (KCl+ NaOH) at pH 10 is the best. At pH 8, the effect of buffer type (Phosphate Buffer, Borax + HCl) on the forming of the (RAN-HCl: 2-HPBCD) complex was investigated. Phosphate at pH = 8 was found to be the best buffer. As shown in Table 3-41.

Table 3-41: Effect type of buffer solution absorbance of TNX and RAN-HCl

Type of buffer at PH = 10	Absorbance at 380 nm TNX
$Na_2HPO_4.12H_2O + NaOH$	0.179
$Na_2CO_3 + NaHCO_3$	0.126
KCl + NaOH	0.194
Type of buffer at PH = 8	**Absorbance at 330 nm RAN-HCl**
Phosphate buffer	0. 220
Borax ($Na_2B_4O_7.10H_2O$) + HCl	0.035

3.12.1.2 Effect of buffer volume

The different volumes of (KCl+NaOH) and phosphate buffer were tested, and the volume that recorded the maximum absorption for both medicines was 1.0 mL at 380 and 330 nm, respectively. As shown in Figure 3-48.

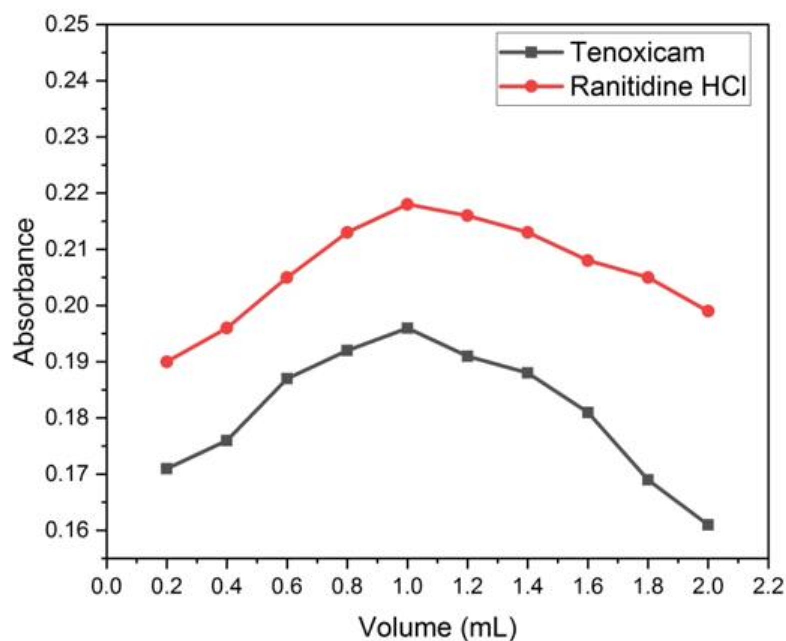


Figure 3-48 : Effect of volume buffer

3.12.1.3 Effect of surfactant type

Triton X-114, Triton X-100, Tween20, CTAB, and SDS were among the surfactant solutions examined. It was determined that Triton X-114 created the maximum absorption value for both drugs (TNX and RAN-HCl). As shown in Table 3-42.

Table 3-42: Effect of surfactant type

Type of surfactant	Absorbance at 380 nm TNX	Absorbance at 330 nm RAN-HCl
Triton X-114	0.196	0.217
Triton X-100	———	———
Tween 20	0.057	———
CTAB	———	———
SDS	———	———

3.12.1.4 Effect of surfactant volume

The different volumes of surfactant were examined, and it was observed that 1.4 mL had the maximum absorption value at 380 nm

when using the drug TNX. In comparison, 1.0 mL had the highest absorption value at 330 nm when using RAN-HCl. As shown in Figure 3-49.

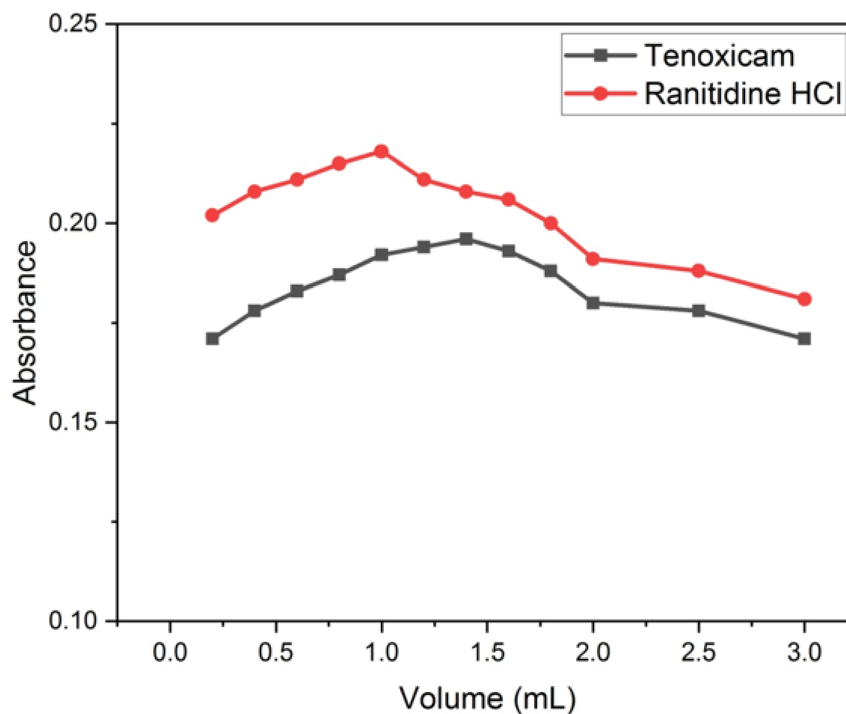


Figure 3-49: Effect of surfactant volume

3.12.1.5 Effect of Temperature in water bath

Using a water bath, temperatures ranging from 30 to 80 ^{0}C were investigated to determine TNX and RAN-HCl, using surfactant. It was found that 40 ^{0}C had the highest absorption value for TNX and RAN-HCl at 380 nm, 330 nm, respectively, as shown in Figure 3-50.

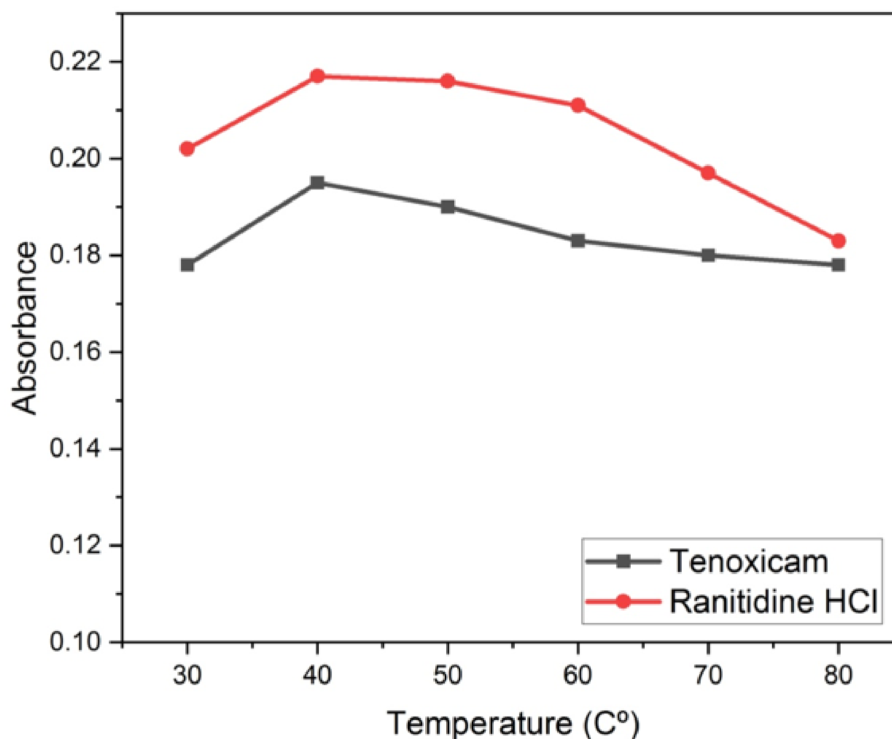


Figure 3-50: Effect of Temperature in water bath

3.12.1.6 Effect of incubation time

The effect of time required for product extraction in CPE method was studied. The time ranged from 10 to 60 minutes, it was observed that 20 minutes is gave the highest absorption value for TNX and RAN-HCl at 380 nm, 330 nm, respectively, as shown in Table 3-43.

Table 3-43: Effect of incubation time (min)

Time (min)	Absorbance at 380 nm TNX	Absorbance at 330nm RAN-HCl
10	———	0.209
20	0.196	0.218
30	0.191	0.214
40	0.188	0.211
50	0.185	0.208
60	0.183	0.205

3.12.1.7 Effect of Centrifuge time

In the centrifuge, time plays an essential role on the extraction of complex. The best extraction times were 5 minutes for TNX and 4 minutes for RAN- HCl, respectively. As shown in Table 3-44.

Table 3-44: Effect of centrifuge time (min)

Time (min)	Absorbance at 380 nm TNX	Absorbance at 330nm RAN-HCl
1	———	———
2	———	———
3	———	0.215
4	0.193	0.217
5	0.197	0.214
6	0.195	0.210

3.12.1.8 Effect of Centrifuge speed (rpm)

In the centrifuge, the effect of speed on the extraction of complexes is critical. The most excellent extraction speed for TNX was 5000 rpm, while the highest extraction speed for RAN-HCl was 4000 rpm.as shown in Table 3-45.

Table 3-45: Effect of centrifuge speed(rpm)

Centrifuge rate(rmp)	Absorbance at 380 nm TNX	Absorbance at 330nm RAN-HCl
1000	0.180	0.208
2000	0.183	0.211
3000	0.185	0.215
4000	0.190	0.217
5000	0.196	0.213
6000	0.192	0.210

3.12.1.9 Effect of the best of solvent

The influence of numerous solvents (Methanol, Ethanol, Chloroform, and Hexane) on absorbance of product was tested;

methanol was demonstrated to be the optimum solvent for achieving the greatest absorbance for TNX and RAN-HCl. As shown in Table 3-46.

Table 3-46: Select of best solvent

Solvent	Absorbance at 380 nm TNX	Absorbance at 330 nm RAN-HCl
Ethanol	0.191	0.214
Methanol	0.196	0.217
Chloroform	0.121	0.201
CCl4	0.173	0.185
Hexane	——	——

3.12.1.10 Effect of stability

The influence of time on color stability for with products was investigated, within period ranged from 5 to 60 minutes. It was detected after 15 minutes is the time to stable the color for TNX and RAN-HCl. As shown in Figure 3-51.

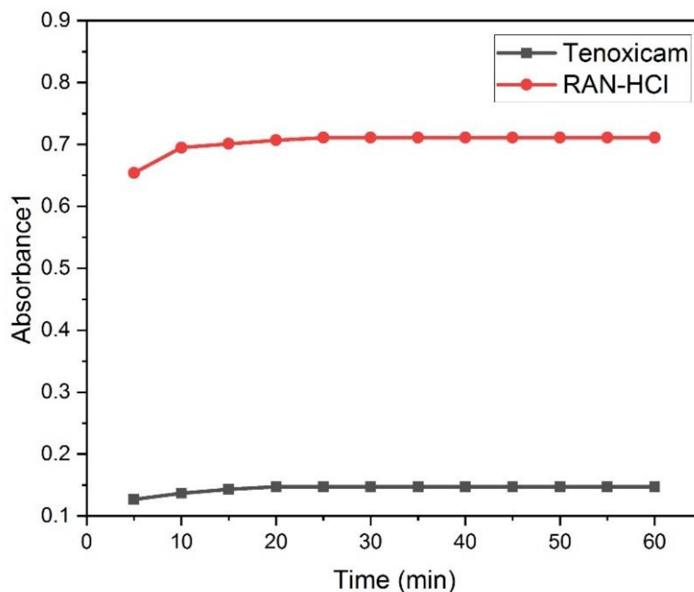


Figure 3-51: Effect of stability

3.12.1.11 Effect of interference

Table 9 shows that the interference that could be added to the pharmaceutical preparations, such as (glucose, fructose, lactose, etc.). It was observed that not effect of previous interference on the estimation of the drugs (TNX and RAN-HCl). As shown in Table 3-47.

Table 3-47: Extraction recovery with different interference compound

Interference	Recovery% TNX	Recovery% RAN-HCl
Starch	98.1	99
Glucose	99.2	97.3
Maltose	97.8	98.4
Lactose	98.3	97.7
Glycine	98.7	96.4
Fructose	96.6	98.1

3.12.2 Calibration curve and statistical treatments

After determining the optimal conditions for the formation of both medicines' complexes (TNX and RAN-HCl), the calibration curve was created by plotting the absorbance of the two medications against their solution concentration. The concentration range of TNX was determined to be (1-35 µg/mL), as illustrated in Figure 3-52, using the regression equation $Y = 0.005X + 0.015$ and $R^2 = 0.996$. RAN-HCl concentrations ranged from (1 -20µg/mL), with the regression equation $Y = 0.013X - 0.015$ and $R^2 = 0.999$ from the linear calibration as shown in Figure 3-53.

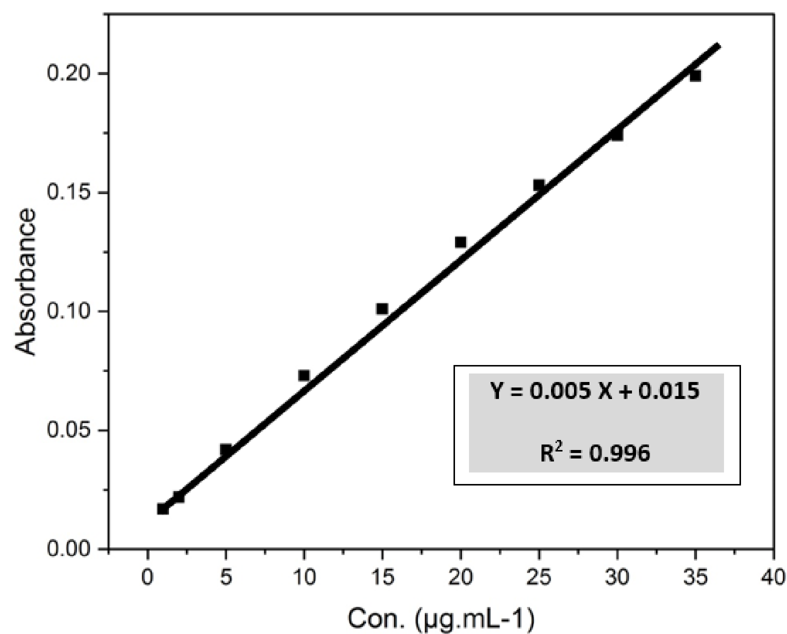


Figure 3-52: Calibration curve of TNX by CPE method

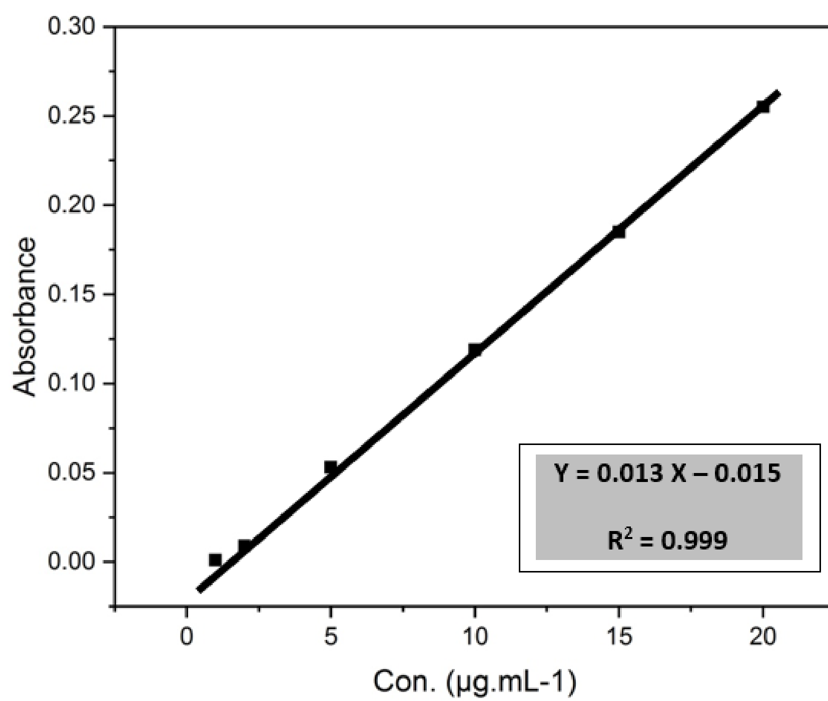


Figure 3-53: Calibration curve of RAN-HCl by CPE method

Table 3-48: Analytical parameter of cloud point

Parameter	Cloud point for TNX	Cloud point for RAN-HCl
λ_{max} nm	380	330
Color	Light yellow	Light yellow
Regression equation	Y=0.005X+0.015	Y=0.013X - 0.015
Linearity range (µg/mL)	1 – 35	1-20
Correlation Coefficient (R^2)	0.996	0.999
Ɛ($L.mol^{-1}.cm^{-1}$)	1572.0	4385.89
Sandell's sensivity (µg . cm^{-2})	0.2	0.077
Slope (b)	0.015	0.013
Intercept(a)	0.005	0.015
Limit of detection (µg/mL)	0.164	0.5
Limit of quantification (µg/mL)	0. 54	1.523
C.L.for the slope($b\pm ts_b$) at 95%	$0.015 \pm 15.1\times10^{-4}$	$0.013 \pm 26.4\times10^{-4}$
C.L.for the intercept ($a\pm ts_a$) at 95%	0.005 ± 0.344	0.015 ± 0.61
Standard error for regression line ($S_{y/x}$)	0.0521	0.092
C.L for Conc.5µg/ mL at 95%	$4.8 \pm 2.4\times10^{-3}$	$4.8 \pm 6.46\times10^{-3}$
C.L for Conc.10µg/mL at 95%	$10.1 \pm 2.51\times10^{-3}$	$10.1 \pm 5\times10^{-3}$
C.Lfor Conc.15µg/ mL at 95%	$14.7 \pm 2.5\times10^{-3}$	$14.8 \pm 2.5\times10^{-3}$

3.12.3Accuracy and precision

The accuracy and precision were studied of drug (PEH, BRH). These experimentations are carried out with three different drug concentrations (5, 10, 15 µg/mL for TNX and RAN-HCl) for three replications before using the CPE approach at optimum conditions. It is clear from these findings that the process has good accuracy and precision since the recovery rate for TNX is (100.1%) and for RAN-HCl is (100.2%), depending on the exact procedure is applied in clause

(2.7.3.10). F-test, T-test was used (two-tailed) to calculate the accuracy and compared the practical value F-test with value in the critical statistical if the practical value less than the value in a table does not differ significantly between the suggested value method and standard method. The results were signed between the standard HPLC method and suggested method with TNX; RAN-HCl found no significant difference between the two methods. The statistical analysis results shown in the Table 3-49 proved that the calculated T-values and F-values for TNX and RAN-HCl determination in different pharmaceuticals were less than t-critical and F-critical at 95% confidence interval and (n-1) degrees of freedom. The new methods have higher accuracy and precision than the literature (50,63).

Table 3-49: Accuracy and pression of CPE procedure for TNX, RAN-HCl drug

drug	CPE method							
	Con. µg/mL		Relative Error%	Recov. %	Average Recov.%	T-value	F-value	RSD%
TNX	5	5.2	-4	104	100.1	0.53	5.17	0.01
	10	9.76	2.4	97.6				0.02
	15	14.8	1.3	98.6				0.01
RAN-HCl	5	5.1	-2	102	100.2	2.54	8.94	0.015
	10	9.8	2	98				0.02
	15	15.1	-0.6	100.6				0.02
Critical value at 95% confidence limit, t = 2.7764, F= 19.								

3.12.4 Application the suggested method on pharmaceutical preparation for TNX and RAN-HCl

Using a cloud point method, it was discovered that TNX in Tilcotil and Tenoctil are suitable for evaluating TNX in pharmaceutical preparations, as well as Barkadin and HISTAC R150 in RAN-HCl. As

demonstrated in the Table 3-50, high accuracy and acceptable results were achieved.

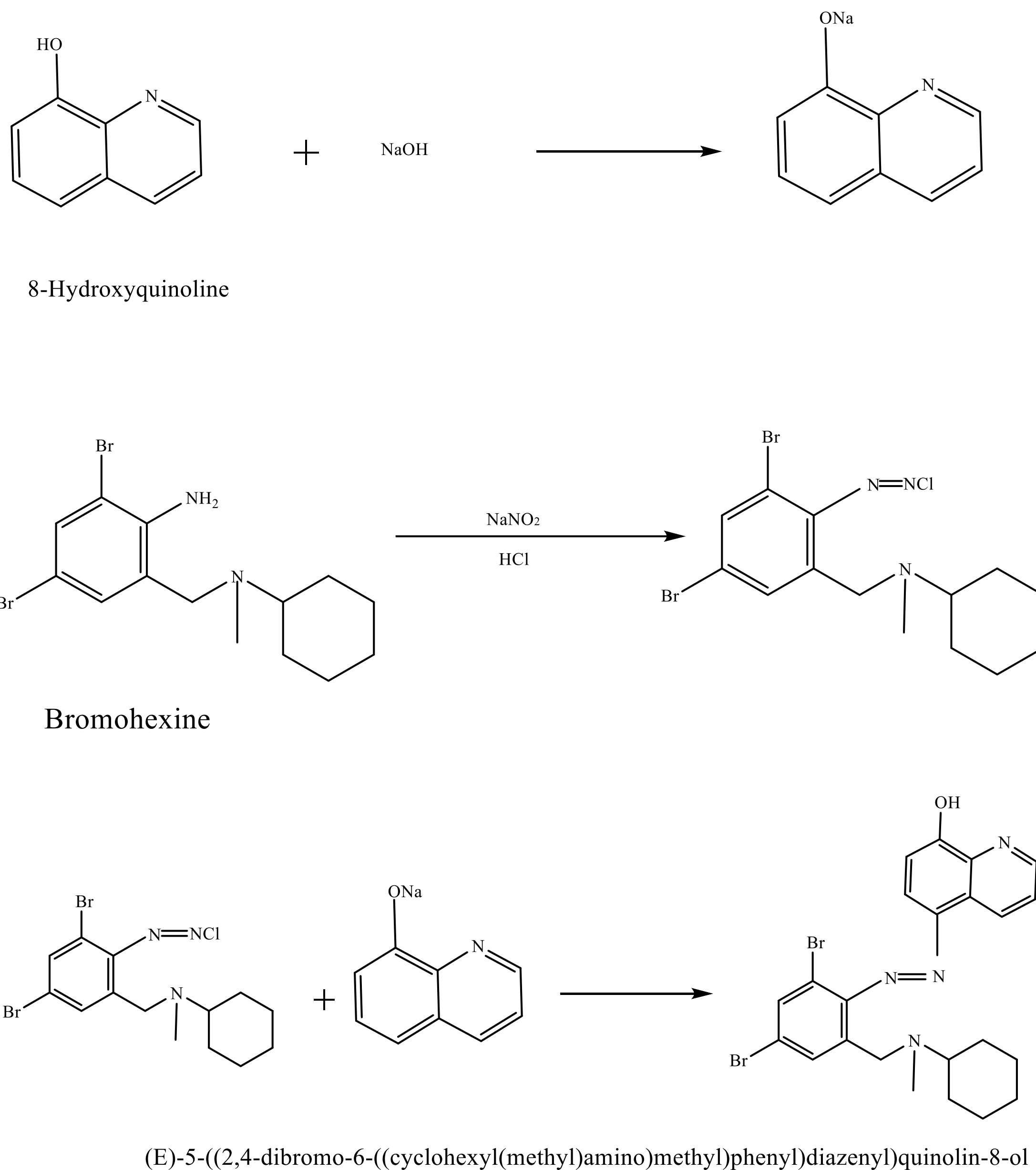
Table 3-50: Application of the proposed cloud point for the evaluation of TNX and RAN-HCl

TNX	Cloud point					
	Conc. of drug µg/mL		Relative Error%	Recov. %	Average Recov %	RSD % (n=3)
	Taken	Found				
Tilcotil	5	4.6	8	92	96.4	0.01
	10	9.6	4	96		0.01
	15	15.2	-1.3	101.3		0.02
Tenoctil	5	5.1	-2	102	98.6	0.01
	10	9.6	4	96		0.01
	15	14.7	2	98		0.02
RAN-HCl	**Cloud point**					
	Conc. of drug µg/mL		**Relative Error%**	**Recov. %**	**Average Recov %**	**RSD % (n=3)**
	Taken	**Found**				
Barkadin	5	4.5	10	90	94.2	0.07
	10	9.6	4	96		0.021
	15	14.5	3.3	96.6		6.9×10^{-3}
HISTAC ^{R}150	5	4.6	8	92	94.5	0.04
	10	9.5	5	95		0.011
	15	14.5	3.3	96.6		6.9×10^{-3}

3.13 Synthesis of azo-dye for BRH

The azo compound was synthesized by diazotizing of BRH at 0-5 ^{o}C and combining it with 8-hydroxy quinoline at the same temperature,

as shown in Scheme3-5, then, the azo was filtrated and washed in cold water, dried and purified by recrystallization with ethanol. Red crystals were produced.



Scheme 3-5: Synthesis of azo-dye for BRH

The compound has a melting point of 231.8 - 233.0 °C and the percentage 80.41%. The physical characteristics resulting from the synthesis of the azo compound as shown in Table 3-51.

Table 3-51: physical characteristics resulting from the preparation of the azo compound

Drug derivatives	Formula	Yield (%)	M.wt (g /mol)	Melting point (°C)
BRH-Azo	$C_{23}H_{24}ON_3Br_2$	80.41	518.034	231.8-233.0

The azo compound was also diagnosed by FT-IR, as shown in Figure 3-54.

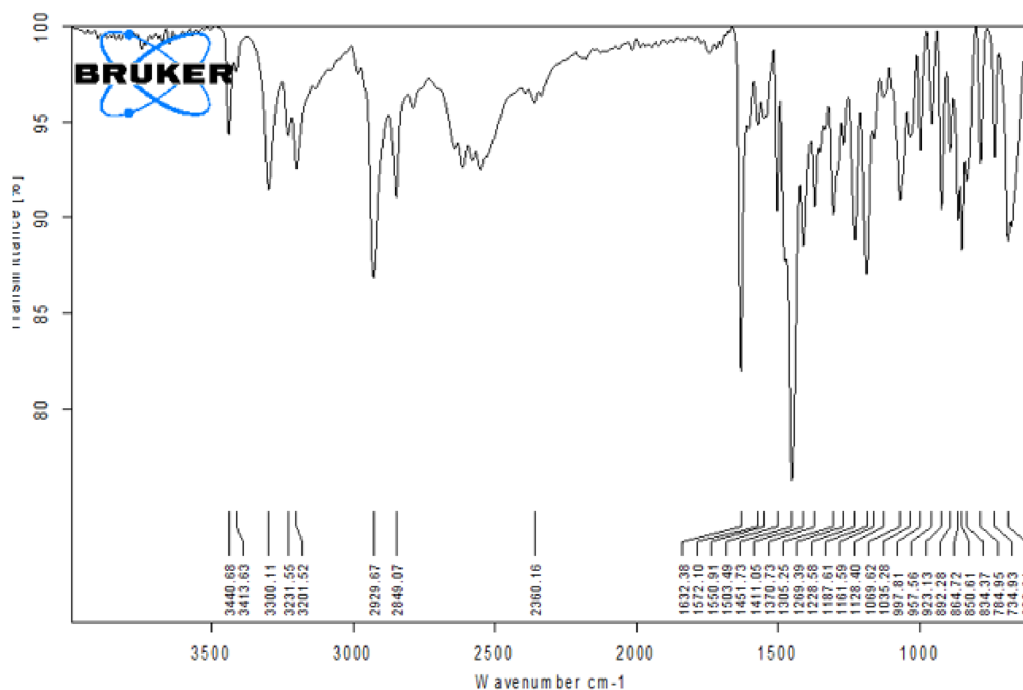


Figure 3-54: FT-IR spectrum of BRH-Azo

The I.R spectrum showed absorption at 3440 cm^{-1} (O-H stretch), 1411 cm^{-1} (N=N stretch), 2929-2849 cm^{-1} (Aliphatic C-H), 3001 cm^{-1} (Aromatic C-H), 1632 cm^{-1} (C=C aromatic). The presence of the stretching vibration of N=N at 1411Cm^{-1} is solid evidence for the reaction complete under the present condition. As indicated in the Table 3-52.

Table 3-52: Frequencies of functional group

Functional group	Frequencies (cm^{-1})
O-H	3440
N=N	1411
C- H Aliphatic	2929-2849
C- Br	834
C=C Aromatic	1632
C=O or C=C	1632

The UV-visible spectrum showed the main beam at 435 nm, which represents the greatest absorption of the azo compound of bromohexine HCl, which means the azo bridge group (N=N) for the charge transfer, as shown in the Figure 3-55.

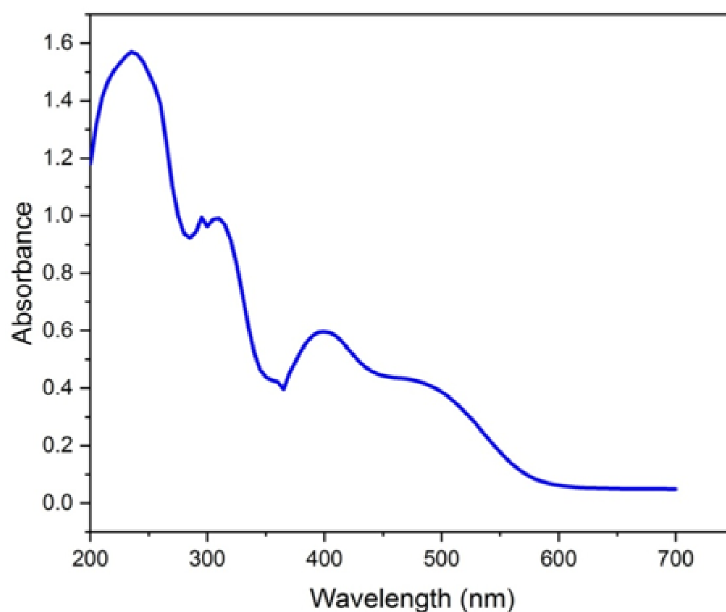


Figure 3-55:Uv-Vis for Azo compound for BRH

Thin-layer chromatography was also examined to determine the difference between the drug alone BRH and the synthesized azo compound, to know the purity of the azo compound of BRH, and it was

found that $R_F = 0.32$, which represents the distance of the derivative to the distance of the solvent, as shown in the Figure 3-56.

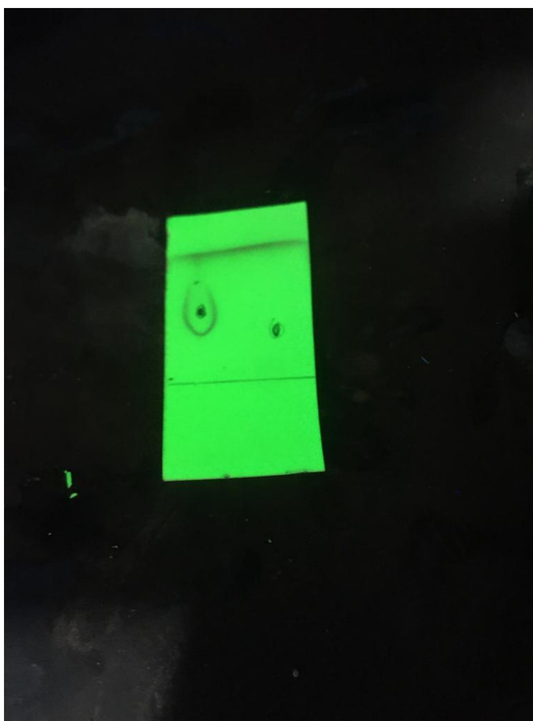


Figure 3-56: Thin layer chromatography for pure BRH and Azo: BRH

3.14 Study of biological activity for Bromohexine HCl (BRH) pure and BRH in an azo compound

The biological effect was studied in pure form and as an azo compound, and study the effect of two compounds BRH pure and BRH in an azo compound on the activity of alkaline phosphatase (ALP).

3.14.1 Estimation of the ALP activity

The alkaline phosphatase activity in a healthy person was estimated. The enzyme activity value was 280.31UL, within the standard men's range in kit at ALP (80-306UL). This value is considered the activity of the enzyme in the absence of the medication. Then the alkaline phosphatase activity was examined in the presence

pure BRH and azo: BRH compound. The results showed decreased in enzyme activity of ALP in the presence of difference concentrations 10-100 mmol/L of pure BRH and azo: BRH compound.

Furthermore, the effect of the compounds (pure BRH and azo: BRH) on the activity of the enzyme was studied, and the results found that the inhibition increased as the drug concentration increased and that the rate of inhibition of the azo: BRH compound was more inhibitory than the pure drug alone. As shown in Table 3-53 and Figure 3-57.

Table 3-53: Effect of different concentrations of the (pure BRH, Azo: BRH) on serum enzyme activity

Enzyme activity Concentration (UL)	Concentration of BRH or Azo: BRH (mmol/L)	Enzyme activity In pure BRH	Percentage of Inhibition (%)	Enzyme activity In Azo:BRH	Percentage of Inhibition (%)
280.31	10	260.03	7.23	248.40	14.24
	20	233.77	16.60	229.51	18.12
	30	214.13	23.61	198.01	29.36
	40	201.60	28.08	186.95	33.31
	50	180.90	35.46	149.17	46.78
	60	171.01	38.99	125.62	55.19
	70	161.36	42.44	119.71	57.29
	80	142.20	49.27	107.12	61.79
	90	138.71	50.52	80.13	71.41
	100	122.09	56.44	71.41	74.52

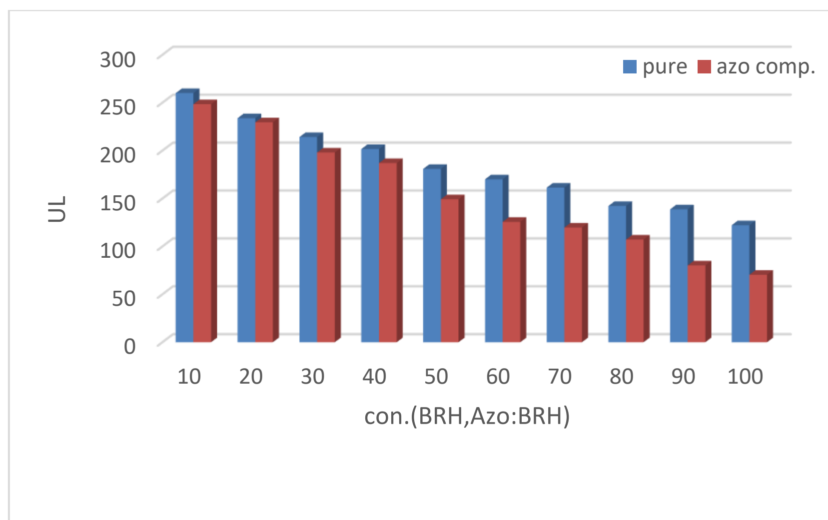


Figure 3-57: Effect of different concentrations of the (pure BRH, Azo: BRH) on serum enzyme activity

3.14.2 The inhibition in pure BRH drug

The kinetic parameters of ALP (maxi velocity (Vmax) and Michaelis constant (Km)) were determined by measuring enzyme activity in the presence of different concentrations of BRH drug as an inhibitor (10, 30, and 50 mmol/L) to difference concentrations of a substrate (P-Nitro phenyl phosphate) (0.2,0.4,0.6,0.8,1 mmol/L) under optimal conditions (pH 10 at 25°C) using the Lineweaver-Burk equation , and plotted 1/[S] vs 1/V in the presence and without BRH drug, as shown in Figure 3-58, Table 3-54.

$$\frac{1}{V} = \frac{Km}{Vmax} \cdot \frac{1}{S} + \frac{1}{Vmax}$$

Scheme 3-6: Lineweaver-Burk equation

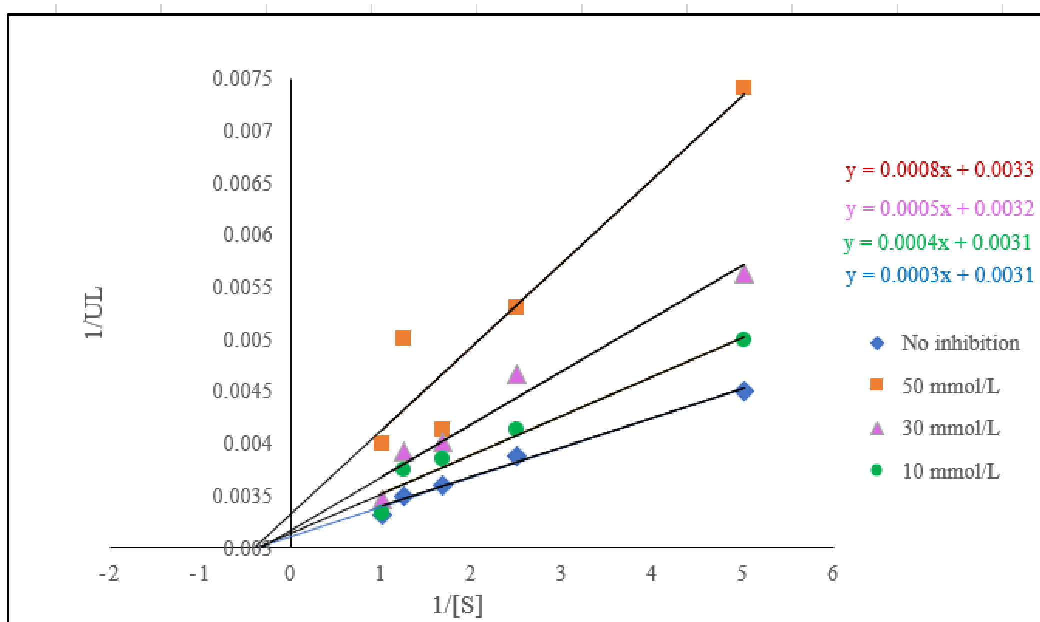


Figure 3-58: Effect of different concentrations of the (pure BRH) on serum enzyme activity

Table 3-54: The kinetic parameters of ALP inhibited by BRH

Parameters		Vmax	Km
Control		322.58	0.097
BRH conc.	10	322.58	0.129
	30	319.03	0.242
	50	312.5	1.563

According to the results, the pure BRH medication acts as a mixed inhibitor. When Vmax values converge or decrease while Km increases as drug concentrations increase (160), the inhibitor promotes free enzyme binding because it closely resembles competitive binding.

3.14.3 The inhibition in Azo: BRH compound

The kinetic parameters of ALP (maxi velocity (Vmax) and Michaelis constant (Km)) were determined by measuring enzyme activity in the presence of different concentrations of Azo: BRH

compound inhibitor (10, 30, and 50 mmol/L) to difference concentrations of a substrate (P-Nitro phenyl phosphate) (0.2,0.4,0.6,0.8,1 mmol/L) under optimal conditions (pH 10 at 25 ºC) using the Lineweaver-Burk equation, and plotted 1/[S] vs 1/V in the presence and without Azo: BRH compound, as shown in Figure 3-59 and Table 3-55.

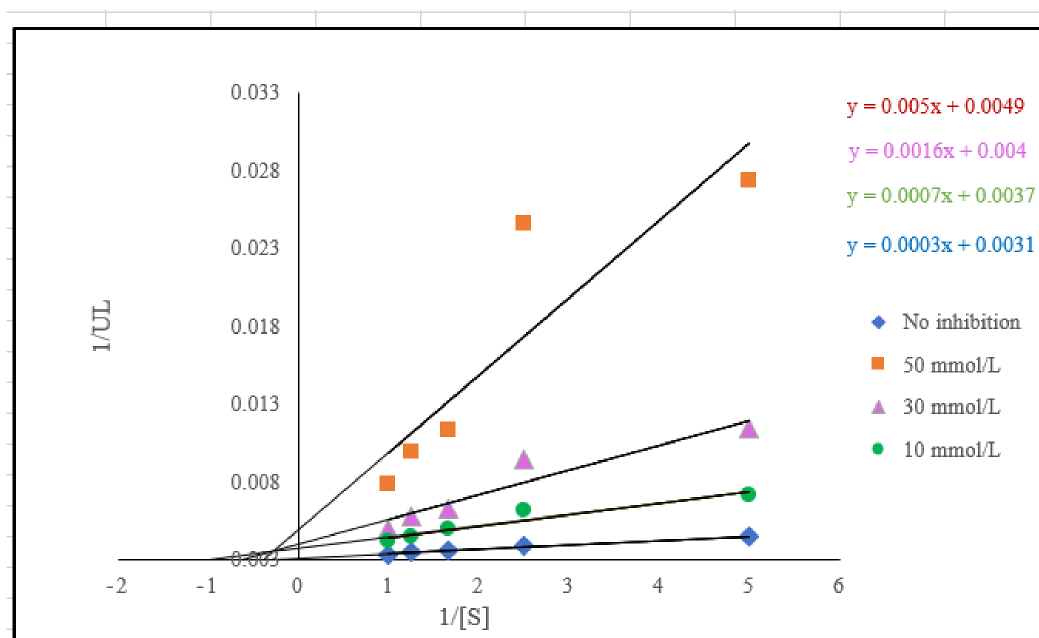


Figure 3-59: Effect of different concentrations of the (Azo: BRH) on serum enzyme activity.

Table 3-55: The kinetic parameters of ALP inhibited by Azo: BRH compound

Parameters		Vmax	Km
Control		322.58	0.097
Azo: BRH conc.	10	290.27	0.189
	30	281.0	0.40
	50	272.08	1.02

According to the results, the Azo: BRH compound acts as a mixed inhibitor. When Vmax values decrease while Km increases as drug concentrations increase (177), the inhibitor promotes free enzyme binding because it closely resembles competitive binding.

Conclusions

1-The two extraction methods, CPE and DLLME, were more accessible and accurate than the direct extraction method. In addition, these methods are considered environmentally friendly because they use trace solvents or surfactants instead of a large amount of solvents, reducing their danger to the environment and human health.

2-The interaction of drugs with one of the reagents, Alizarin yellow and HPβCD, colored results were obtained, which were able to be accurately estimated by spectrophotometry.

3-The optimal conditions obtained by CPE and DLLME methods were more efficient, accurate and precise than the direct extraction method.

4-The BRH drug was found to act as an inhibitor for ALP enzyme in serum. A specialist doctor must strictly supervise this medication, and liver faction should be checked when taken.

Recommendations and future studies

1. The reactions can evaluate other organic medicine compounds containing phenolic and amino groups

2. There is a need for thermodynamic studies which is importance to understand the solubilization behavior of drugs and product in surfactant micelles that used as mediator extraction.

3. Try to spectrophotometric determination and direct extraction, DLLME and CPE of these drugs by reaction with other reagents and formation new products to obtain more sensitivity and selectivity and comparison with official methods.

4. The possibility of using Phenylephrine hydrochloride, Bromohexine hydrochloride, Tenoxicam, and Ranitidine-HCl medicaments as an inexpensive chemical reagent in the extraction, preconcentration and determination of other drugs in different materials by using direct extraction, DLLME and CPE spectrophotometry.

5. The enzyme activity can study on all of the studied compounds, and the azo synthesized from the same drug.

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APPENDIX A

APPENDIX A

1- ε = slope × 1000 × Molecular weight.

ε = Molar Absorbance Coefficient

2- ε = A / Con. × I

A= Absorbance

Con.= Concentration.

I= Length optical path (1cm).

3- a = ε / M.wt × 1000

4- S = M.wt / ε

S= Sandall's sensitivity in ($\mu g/cm^2$).

M.wt = Molecular weight of the drug to be determined (gm/mol).

5- C.L = X ± $t_{(n-1)}$ S.D

C.L = Confidence Limit for the concentration (µg/mL).

X= Mean of measurement (concentration or absorbance).

S.D = Standard deviation.

n = The number of samples.

T = Test-t at (n-1) from the degree of freedom at C.L.

6- $S.D = \frac{\sqrt{(Xi-X)2}}{n-1}$

7- C.L = b ± t_{sb} , C. L = a ± t_{sa}

C.L = The confidence Limit for slope and intercept.

8- $S\,y/x = \frac{\sqrt{(yi - y)2}}{n-2}$

S y/x = Standard deviation of change (y) value.

9- LOD = 3.3 SD / slope

LOD= Limit of detection.

10- LOQ = 10 SD / slope

LOQ = Limit of quantitation.

11- RSD% = SD / X . 100

RSD = Relative standard deviation.

12- Relative error % = Found – Taken / Taken × 100

Found = Analytical result of the concentration.

Taken = The real result of the concentration.

13- F-test = SD^2_1 / SD^2_2

14- T-test = Xi-X / SD ×Root(N).

الخلاصة :-

الأطروحة مقسمة إلى ثلاثة فصول.

الفصل الأول: يتضمن استعراضًا موجزًا للأدوية قيد الدراسة: فينيليفرين ، البروموهكسين والتنوكسكام ، والرانتيدين وكذلك تم استعراض الطرق التحليل الخاصة بها في الفصل الأول. ثم مراجعة موجزة لمبدأ التفاعل الزوج الأيوني ، واستخلاص النقطة السحابية ، والاستخلاص المباشر ، والاستخلاص الدقيق للسائل السائل المشتت ،كذلك تم التطرق إلى الهدف من العمل.

الفصل الثاني: في هذا الفصل (الجزء التجريبي) هو تطوير طرق تحليلية لتحديد الأدوية المذكورة أعلاه. بالإضافة إلى الأجهزة والأدوات والمواد الكيميائية المستخدمة في العمل وكذلك وصف الإجراء العام لإعداد المحاليل وإجراءات المخزون القياسي لتحسين الشروط والإجراءات العامة لتحديد الأدوية قيد الدراسة.

الفصل الثالث: قسم إلى اربعة أجزاء.

الجزء الأول: يتضمن تطوير طرق طيفية لتقدير العقاقير باستخدام تفاعلات الازدواج للفنيليفرين والبروموهكسين مع الكاشف الاليزارين الاصفر بوجود وسط حامضي. اما عقار التنوكسكام والرانتدين فتم تفاعله مع كاشف الهيدروكسي بروبيل بيتا سايكلودكسترين في وسط مناسب. وكان لون الناتج هو الاصفر عند طول موجي اعظم 430 ,480 و385و330 نانوميتر للفنيليفرين والبروموهكسين والتنكسوكام و الرانتدين على التوالي. التراكيز التي اطاعت قانون بير بطريقة الاستخلاص المباشر فكانت من 1 الى 20 مايكرو غرام / مل للفنيليفرين والبروموهكسين و 1 الى 45 نانوميتر للتنكسوكام و الرانتدين. معامل الارتباط كان 0.997, 0.997 و0.998 و 0.999 للفينيليفرين ، البروموهكسين والتنوكسكام ، والرانتيدين على التوالي . الامتصاصية المولارية 14459.9 ,16504.0 و 2200.8 و6072.77 لتر/ مول سم وحد الكشف 0.34 , 0.0814 و0.6 و 0.17 مايكرو غرام / مل للفينيليفرين ، البروموهكسين والتنوكسكام ، والرانتيدين على التوالي.

الجزء الثاني : تم استخدام تقنية الاستخلاص بنقطة الغيمة لتقدير التراكيز النزرة المتكونة من الأدوية قيد الدراسة متبوعًا بقياس امتصاص الألوان عند λ بحد أقصى 430 ,480 و385و330 نانومتر للفينيليفرين ، البروموهكسين والتنوكسكام ، والرانتيدين ، على التوالي.

التراكيز التي تخضع لقانون بير كانت من 1 الى 35ميكروغرام / مل للفنيليفرين والبرومو هكسين والتنكسوكام و من 1 الى 20ميكروغرام / مل لعقار الرانتدين . معاملات الارتباط 0.999 ، 0.995 و 0.996 و 0.996 للفينيليفرين ، البرومو هكسين والتنوكسكام ، والرانتيدين على التوالي. الامتصاصية المولارية 4073.2 و13202.88و 1572.0 و 1572.0لتر مول $^{-1}$ سم $^{-1}$ ، وحد الكشف (1.065، 0.141 و 0.164 و 0.17 ميكروغرام / مل على التوالي.

الجزء الثالث: استخلاص دقيق للسائل السائل المشتت حيث تم استخدامه لتحديد الأدوية المدروسة ، باستخدام الظروف المثلى ، متبوعًا بقياس امتصاص الألوان عند اقصى طول موجي 430 ,480 و385و330 نانومتر للفينيليفرين ، البروموهكسين والتنوكسكام ، والرانتيدين على التوالي. نطاقات التركيز التي تخضع لقانون بير كانت من (1 - 13) ميكروغرام / مل للفنيلفرين والرانتدين و (1-23) و(1-21) ميكروغرام / مل للبروموهكسين والتنكسوكام على التوالي. معاملات الارتباط 0.996 ،0.998، 0.996 و 0.998 لكل من فينيليفرين ، البرومو هكسين والتنوكسكام ، والرانتيدين على التوالي . ا لامتصاصية المولارية 12423.3، 23930.2، 2515.2 ، 25303.2 لتر مول $^{-1}$ سم$^{-1}$ للفينيليفرين ، البرومو هكسين والتنوكسكام ، والرانتيدين على التوالي. وحدود الكشف 0.094و 0.055و 0.079و 0.04 ميكروغرام / مل للفينيليفرين ، البرومو هكسين والتنوكسكام ، والرانتيدين على التوالي.

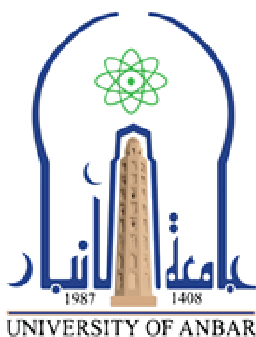
الجزء الرابع: تم دراسة نشاط انزيم الفوسفاتيز القلوي (ALP)على أحد المركبات المدروسة وهو عقار البرومو هكسين هيدروكلورايد، ومركب الأزو المتكون من نفس العقار. وتم تقييم قدرة الإنزيم ALP على تثبيط العقار ومركب الازوبشكل ملحوظ دلالة على اهمية العقار في النشاط الانزيمي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة الانبار

كلية العلوم-قسم الكيمياء



تطوير طرائق طيفية لتقدير بعض الادوية الامينية ودراسة تأثيرها على فعالية انزيم الفوسفاتيز القاعدي

أطروحة

مقدمة الى مجلس كلية العلوم – جامعة الانبار

وهي جزء من متطلبات الحصول على درجة الدكتوراه فلسفة في علوم الكيمياء

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