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Ministry of Higher Education and Scientific Research
University of Kerbala
College of Education for Pure Sciences
chemistry department

RP-HPLC and spectrophotometric (charge transfer) determination of mebeverine -HCl in their pure state and pharmaceutical preparation

A Thesis submitted to
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In conformity with the requirements for the Degree of Master
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Certificate

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I certify that the linguistic evaluator of this thesis (RP-HPLC and spectrophotometer (charge transfer) determination of mebeverine -HCl in their pure state and pharmaceutical preparation) was carried out by me and it is linguistically sound

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We certify that, we read this thesis (RP-HPLC and spectrophotometer (charge transpher) determination of mebeverine –HCl in their pure state and pharmaceutical preparation) and as examining committee examined the student (Zainab Abas Abdal-Zahra) in its content , and that in our opinion it is adequate( ) with standing as a thesis for degree of master in chemistry sciences.

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Chapter one

introduction

Chapter two

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Abstract

The first part of the thesis was the spectral method, which involved the formation of three ion-association complexes for the determination of mebeverine hydrochloride drug in pure and pharmaceuticals preparation.

The first complex between Mebeverine and phenol red(λmax=396nm), The second complex between mebeverine and picric acid (λmax=382nm),The third complex between mebeverine and Iodine(λmax=360nm), Beers law is obeyed over the range(2 – 25 ,1-30 ,1.2-28)ppm for complex I,II,III respectively with molar absorptivity of (7.57×10^4, 3.41×10^4 , 9.08×10^3) L/ Mol.Cm for complex I,II,III respectively a sandal sensitivity of (0.00615, 0.013, 0.05) µg. Cm $^{-2}$ for complex I,II,III respectively, and the relative standard deviation RSD (1.39, 0.63, 2.05)% for complex I,II,III respectively, and correlation coefficient of (R^2 = 0.9941, 0.9904, 0.9979) for complex I,II,III respectively, The limit of detection (LOD)(0.21, 0.77, 0.72) µg/ml for complex I,II,III respectively and limit of quantitation (LOQ)(0.71,2.57,2.50) µg/ml for complex I,II,III respectively .The method was applied successfully for the determination of Mebeverine. HCl in bulk and pharmaceutical preparations.

The Second part was High perfect liquid chromatography (HPLC)method for simultaneous quantitative estimation of Mebeverine hydrochloride in pure and pharmaceuticals preparation, the optimized method uses a reverse phase column ODS2-C18 (150mm×4.6mm particle), a mobile phase of acetonitrile: 0.02 M potassium dihydrogen phosphate (70: 30: v/v) pH 3.6, flow rate of 1.0 ml/min and a detection wavelength of 240 nm using a UV detector. The developed method resulted in Mebeverine eluting at 1.03 min, linearity in the range 1-100µg/ml, relative standard deviations of 3.2%. A simple, accurate, precise, linear and rapid RP-HPLC method was developed for simultaneous quantitative estimation of Mebeverine hydrochloride in pure in various pharmaceutical industries

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List of symbols and abbreviation

Po	Incident radiant
P	Transmitted radiant
UV	Ultra-vault
IR	Infrared radiation
C	speed of light
W	Frequency
ʎ	Wave length
VIS	Visible
DR	Dissociative recombination
LMCT	ligand to metal charge transfer
CT	charge transfer
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
PLOT	porous-layer open tubular columns
GLC	Gas liquid Chromatography
LLC	Liquid- liquid Chromatography
LC	Liquid Chromatography
RP-HPLC	Reversed phase- High performance liquid chromatography
SEC	Size exclusion chromatography
RPC	Reversed phase Chromatography
MBV	Mebeverine hydrochloride
Kd	distribution constants
DCM	Dichloromethane
D.W	Distilled water
λmax	Wave length maximum
R.S.D	Relative standard deviation
LOD	limit of detection
LOQ	limit of quantification
SD	standard deviation
K	slope of the calibration graph
S.D. I	The state company for Drugs industry and medical appliance/Iraq
K^{-}	Capacity factor
As	symmetry coefficient

(1-1) Light Absorption Process & Beer-Lambert law

The mathematician Lambert laid down the bases for photometry hypothesis in the eighteenth century. The photometry hypothesis depends on reducing the energy of the electromagnetic radiation which leads to attenuate beam absorption obeying Beer law [(1).] Actually, the molecular structure of each sample characterized by ability to absorb light at the time of exposing to the incident radiation, and the absorbed light can be translated into absorption spectrum graph as shown in figurer (1.1). [(1)]

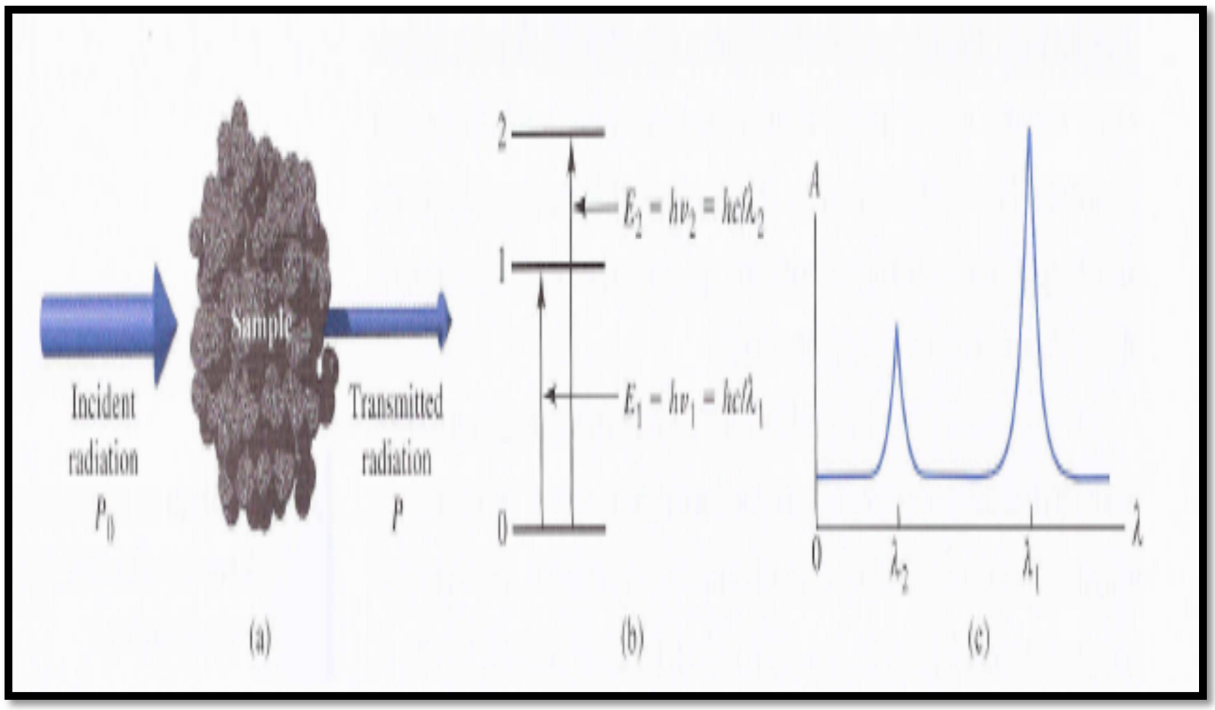


Figure (1.1): Illustrating the steps for the absorption methods: (A) refers to the time of incident radiant power (Po), which can be absorbed by the analytic. (B) Transmitted beam of the lower radiant power (P) (b) refers to the counting of the energy that comes from the incident beam. (C) The graph spectrum explains the absorption spectrum. Beer law is a quantitative law deals with the concentration of the absorbed molecules and path length over which absorption occurs [(2).] For example, analytic solution of known concentration, if the

light pass through longer medium, the light will increase its absorption time and increase its attenuation, as shown in figurer (1.2) [3].

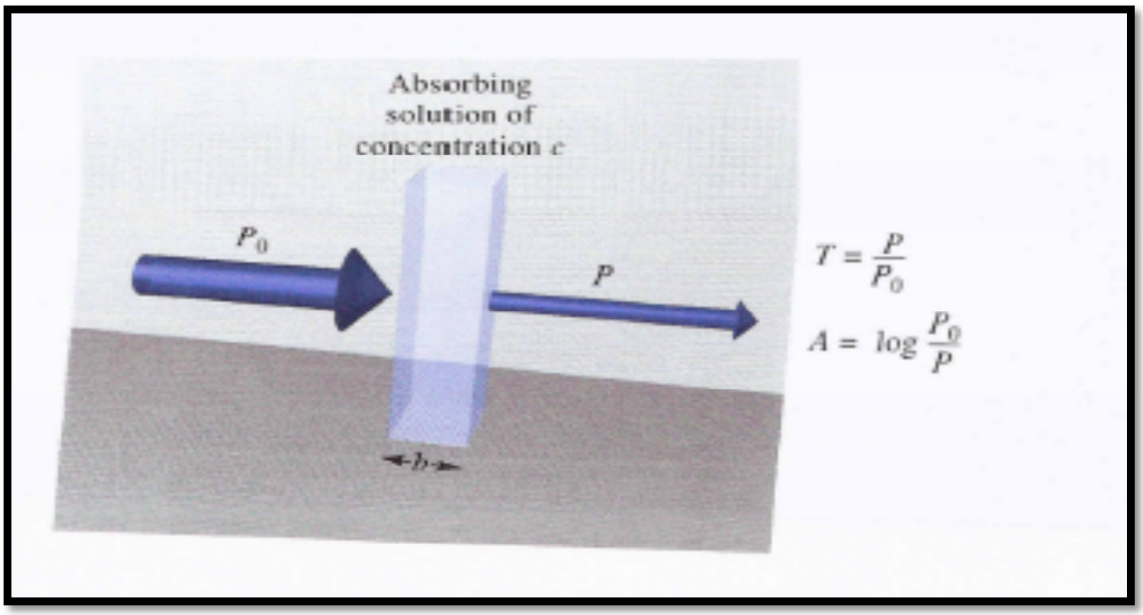


Figure (1-2) Shows attenuation steps of a radiation beam by absorbing solution, the wider arrow refers to the high radiant power than is transmitted by solution. (b) Refers to the path length of the absorbing solution (c) refers to the concentration of the absorbing solution

(1-2) Beer's law deviation

Deviation refers to the nonlinear regression of the Beer's law plot. Such cases could be referring to the high concentrations of the analytic, which through using different chemicals (chemical deviation) or different instruments (instrumental deviation), see figure (1-3) .[4]

However, to understand the exact definition of instrumental deviation, we have to know the normal range working is 15 to 80 percentages in parallel to the absorbance values range 0.10-0.82. So that it is recommended

for the researchers to prepare their standards within that range, and in case of unknown sample, the sample has to be diluted into different concentrations to see which concentration from the standard values can fit the unknown sample. Hence, it is not possible for the instrument to be extremely high or if very low transmitted values. (4)

Chemical deviation refers to the interferences that occur when we use either high or low analytic concentration, which leads to shift the chemical equilibrium in such a way the affects its absorbance. For this reason, the chemists have to dilute the unknown samples in the range would be expected as in the instrumental deviation (4).

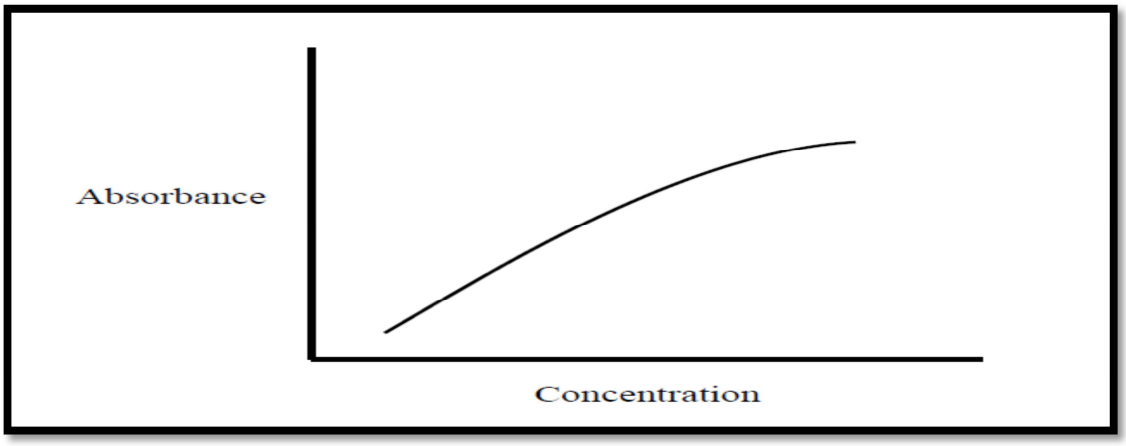


Figure (1-3): represents the nonlinear regression plot of Beer's law, which often manifested at the high concentration of the analytic (4).

(1-3) The electromagnetic spectrum

Wave length refers to the distance two adjacent peaks in the time-frozen electromagnetic wave, and is measured in meters, centimeters and nanometers like 10^{-10} see figure (1-4). Interestingly, light can give rise to a variety of transitions and different kinds of spectroscopy. The UV light is part of the electromagnetic spectrum, which is characterized by wavelength range from high-energy UV 190 nm to 800 nm low energy red. Infrared IR radiation for example can induce excitation but not emission of the molecules. For this reason, IR radiation. In addition, visible wavelength occurs within 400 to 700 nm. However, to understand the nature of the electromagnetic spectrum, we need to learn some photometric terms; υ refers to the frequency, which means the cycle of wave numbers that pass through frozen point per unit of time. Frequency υ is always measured in cycles per second or in Hertz (Hz). Frequency and wavelength can be summarized in the following equation:

$$\lambda = c/\nu = \frac{2\pi c}{\omega}$$

C= light speed

w=2πv= radiant per second= υ

White light has to react by colored substance to allow a characteristic portion of the spectrum to be absorbed. The remaining light or the part of

light does not absorb and exhibit a harmony color to the wavelength. For example, absorb light between 420-430 nm leave a yellow substance [5]

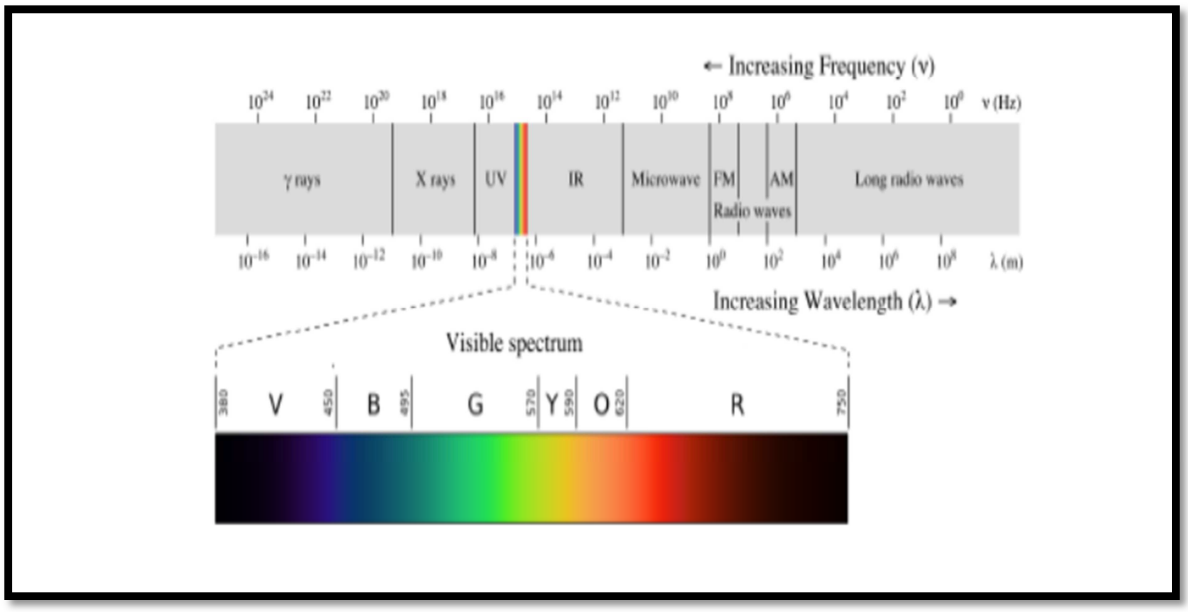


Figure (1-4): represents the electromagnetic wavelength.

(1-4) Ultra Violet Visible absorption spectroscopy

UV-VIS is instrumental method that used to measure the interaction between molecules and electromagnetic radiation, which arranges between 190-800 nm, and is which divided into ultraviolet 190-400 nm and visible 400-800 nm [6- 7].

The interaction between molecules and electromagnetic radiation leads to generate energy, which is absorbed through analytic species to stimulate their valance electrons from the ground state to the excitation state. Then the excitation state generates light which can be measured as an indicator of the

frequency or wavelength [6]. Absorption species include organic and inorganic ions and molecules as well as inorganic anions [8]. However, the absorption of UV or visible radiation resembles three kinds of electron transmission: first π, σ, and *n* electrons transition, charge transfer electrons transition and d and f electrons transition [9]. Although UV-VIS counts the most instrumental useful method, it has limited application in the field of the quantitative analysis because of the maximum and minimum numbers of absorptions are relatively few comparing to the Beer law method, which offers a valuable, easy and simple analytical method in the quantitative analysis field [7, 8].

(1-5) General applications of UV-Visible:

UV-Visible can apply for a variety of species including inorganic metal, organic compounds and biochemical species, which are able to absorb UV and visible radiation and thus flexible to direct quantitative measurement. Furthermore, many of the non-visible molecules can also be determined by using ultra violet or visible radiation after converting these molecules into absorbed derivatives by adding some chemical compounds. Statistically, it has been estimated that more than 90% of the analyses in the chemical laboratories have done or determined by using UV/Visible spectroscopy. Some of these applications are widely known such as estimated medication, drug, and pesticides in the sub micro organ level, vitamins and steroids [10].

In addition, visible spectrophotometric analytical method has been developed to measure and analyze colored metal complex and colored compounds [11].

(1-6) Ionic bonding complexes

Is one of the ion pair extractive and quantitative analytical method which is used by many pharmaceutical companies [12-14]. The method depends on the Dissociative Recombination (DR) which is the process when neutral and charged fragments are formed. In case of diatomic molecules, the reaction is represented by the following equation:

$$AB^+ + e^- \longrightarrow AB^* \longrightarrow A(n) + B(n'),$$

Actually, the neutral fragments can be detected in different excited phases. Most of the experimental studies of DR and all neutral are measurable and the total cross section for DR is detected. In addition, in ion pair formation opposite charge fragments are formed as an ion pair [15].

$$AB^+ + e^- \longrightarrow AB^* \longrightarrow A^+ + B^-.$$

Transition metal complexes with the octahedral geometry, which is the most important type of the electronic transmission that is called d-d transition in which an electron moves from the lower level t_2g to the upper level. Interestingly the transitions give the color to the complex as soon as it happens in both visible and ultraviolet part of the spectrum. However, the values for the molar extinction coefficient are low because they obey LaPorte forbidden transition law.

The value of the molar extinction coefficient ε comes from 0.5 to 20 $Lmol^{-1}$ cm^{-1} when the electron is transmitted in the phase of d-d transitions. In the other cases when the absorption bands occur within UV or visible light or in the other words occur between the range 1000-55000 $Lmol^{-1}$ cm^{-1},

the absorption happens due to charge transfer bands which is characterized by so much intense, which could be translated to the deep color and to the respectful transition metal complex. As comparing to the normal crystal field transition, charge transfer transitions are of much higher energy, but they show the representative color in case of falling transition in the visible part of the spectrum [16].

(1-7) Charge transfer transitions

Charge transfer refers to the movement of electrons from one point to another in the complex to generate energy. Electron movement may also occur from the ligand to the metal, which is called ligand to metal charge transfer LMCT. In addition, electron transition can flow from the central metal to the ligand. In such cases, the flow names metal to ligand charge transfer [17].

Charge transfer CT exactions are the term that are generally used to describe the electron flow events in conventional molecular crystals. Actually, in the field of electron excitation, we see many terminologies used to describe the nature of exactions such as and CT exactions which are both used conventionally to describe the exactions in the molecular solid field, which recently highly adapted by the conjugated polymer community. However, several possible mechanisms explain the mechanism of CT exciting formation in the conjugated materials. Photo generation for example works through a single excited molecule, and then the excited electron may move to a neighboring polymer chain leaving a hole on the original chain and leaving an electron that bind together coulomb interaction. The electron-hole pair establishes the spatially indirect exciting by the other hand it is

believed that positive and negative species on the adjacent chains form positive and negative poltroons, which lead to another explanation like geminate interchange bound polar on pairs. In addition to the photo generation mechanism, we have excited-state dimer (excimer) or a ground-state dimer (aggregation). Briefly, excimer is stable because of using resonance contributions from exactions and CT exactions. Hence, the possibilities of formation such as species are depending on the proximity of the neighboring molecules and their relative orientation [18].

(1-8) Types of charge transfer spectra

There are two types of CT: ligand to metal charge transferred LMCT and metal to ligand charge transfer MLCT. LMCT the electron has to transfer from the low-lying molecular orbital to the naturally metal orbital. In the case of MLCT the electron is transferred from the molecular orbital that are naturally metal to the empty π^* orbitals of the ligand which are of higher energy comparing to the metal orbitals (show figure 1-5) for better understanding the comparative relationship [19].

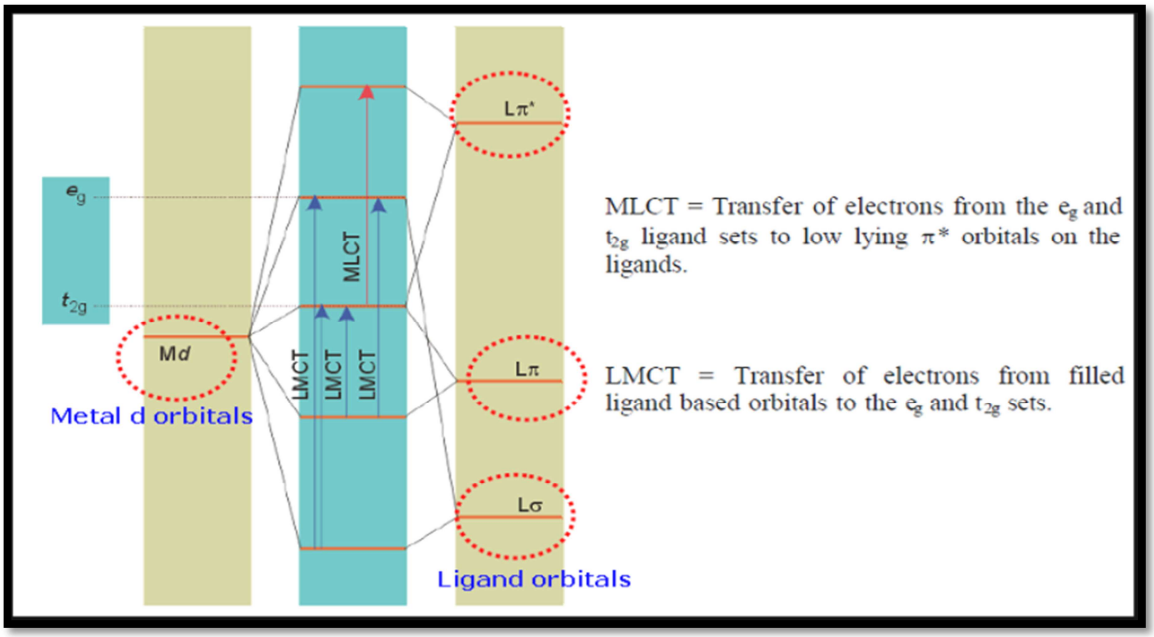


Figure (1-5) shows the comparative of the charge transfer in organic species

(1-9) Types of chromatography:

Depending on the mobile phase, there are two main types of chromatography: gas chromatography and solid chromatography. Depending on the absorbance substance, Gas chromatography can be classified into two categories called: Gas-Solid and Gas-Liquid. Similarly, Liquid chromatography is classified into two kinds Liquid-Liquid chromatography and Liquid-solid chromatography see figure (1-6).

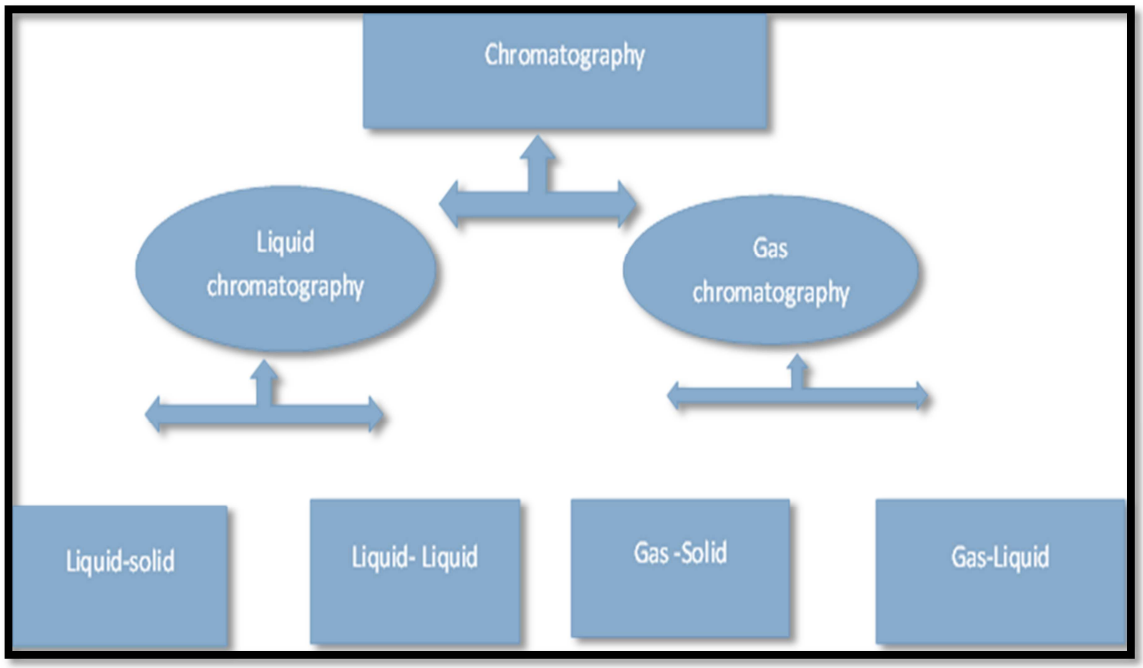


Figure (1-6) summarizes chromatography classification

(1-10) High Performance Liquid Chromatography HPLC:

HPLC is the most popular analytical advance technique that uses column liquid chromatography. The principle of HPLC technique is separation, identification and quantification of each molecule in a complex or in a mixture. The HPLC works through many complicated steps start by using solvents flow through column under the effect of high pressure 400 atmospheres, which allow the sample to be separated into different constituents with different affinities [20- 22].

(1-10-1) Gas chromatography

Mikhail has discovered gas chromatography GC in 1900s, and it is known since that time as analytical technique used to separate complex compound constitutes. Although there are variety types of GC in the analytical chemistry field, Gas-Liquid chromatography is the most common type used to separate organic compounds. Recently with the updating systems, engineering has combined GC and mass spectrometry to determine molecules. However, a typical GC machine contains from an injection port, a column, equipment to control gas flow, maintaining temperature equipment including heaters and ovens, integrator chart recorder and a detector [23]

Organic solution sample has to be injected into the sample port to vaporize under high gas pressure, then the vaporized sample happened by inert gas mostly either helium or nitrogen. The inert gas goes through a glass column filled with silica and coated with a liquid. Then the less soluble liquid soluble compounds will increase the reader is comparison to the high liquid soluble compounds. The column is counting packed when the glass or metal column tubing is filled with small spherical inert supports. The liquid phase absorbs in a thin layer on to the surface of beads. In a capillary column, the tubing walls are coated the stationary phase or absorbent layer that able to support the liquid phase. Hence, Gas liquid chromatography has limited application in the chemical analytical lab because of the sever peak tailing and the semi-permanent retention of polar compounds in the column [23].

(1-10-1-A) Gas-solid chromatograph

This type of the instrument depends on the adsorb of gaseous substances on solid phases. Distribution of coli cines is usually larger than gas-liquid chromatography. For this reason, gas-solid chromatography is ideal for the separation of compounds, which are not determined by gas-liquid columns like components of air, hydrogen sulfate, carbon dioxide and carbon monoxide. The other advantage of gas-solid chromatography is using packed and open tubular columns, which are beneficial for thin layer of the adsorbent. Such columns are called porous-layer open columns or we can call them PLOT columns [24].

(1-10-1-B) Gas – Liquid chromatography (GLC)

GLC is the most useful technique in analytical chemistry field. GLC characterizes by a film-coated station on the solid and mobile support phase in an inert gas like Nitrogen N_2, which call gas carrier, over the liquid film surface in a controlled manner. However, GLC works after vaporizing the sample under conditions of high gas pressure and high temperature. Then the vaporized sample fractioning between a mobile gaseous phase and a liquid stationary phase in a column [25].

(1-10-2) liquid chromatography

Liquid chromatography works in principles similar to gas chromatography, but liquid chromatography uses of liquid mobile phase instead of gaseous phase. The mobile phase is inert in a solid station that packed with Silica gel SIO2.xH2O, Alumina AL2O3.xH2O or cellulose in glass columns. The reader reduces if silica and alumina absorb water, but the

reduction reverse by increasing the heat to 200-400 C. In general, Silica is acidic, so that it easily absorbs basic solution, while alumina is basic and strongly absorbs acidic solutes. Furthermore, there many other stationary phases like magnesia MgO.xH2O which is good to use for the separation of unsaturated and organic compounds, and dextran a polymer of glucose which is a good choice to use to separate unsaturated organic compounds as show figure (1-7) [26].

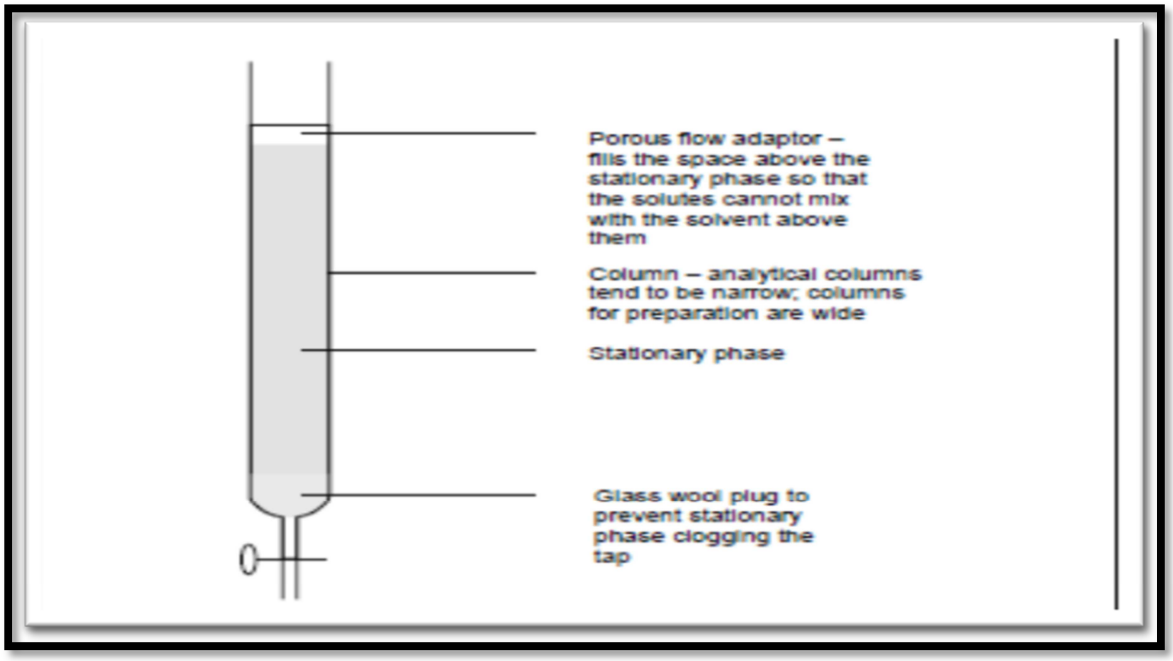


Figure (1-7) represents liquid chromatography Colum

(1-10-2-A) Liquid -solid chromatography

Also known adsorption chromatography see figure 1-8, it is one of the oldest analytical technique that uses mobilize liquid or gaseous station, which is adsorbed on the solid phase. Then the balance between the mobile and the stationary phases use to separate the compounds into different constitutes [27].

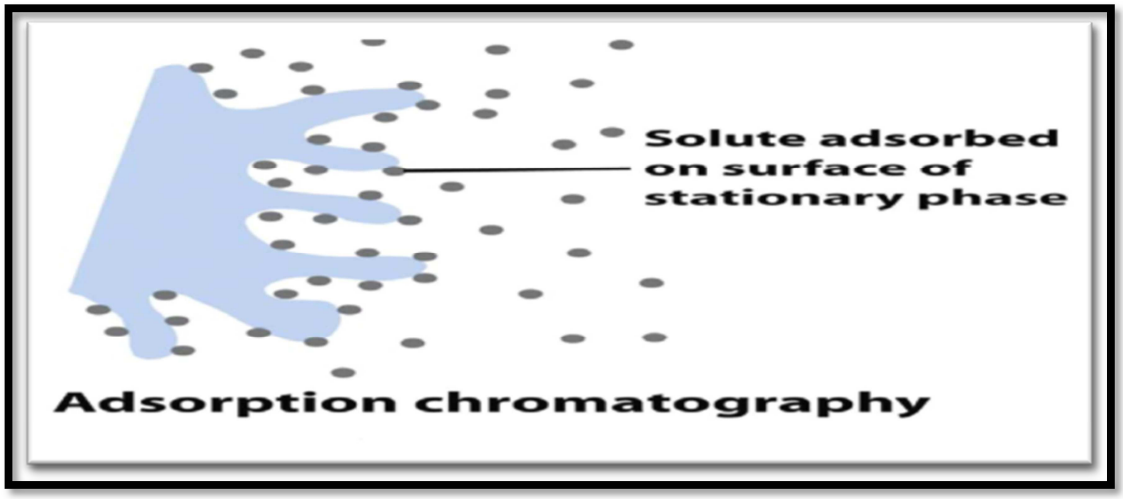


Figure (1-8) shows the principle of adsorption chromatography

(1-10-2-B) Liquid – Liquid Chromatography (LLC):

LLC is useful chromatography analytical technique, which has been used successfully for the separation and measurement of acids and protein compounds. LLC uses liquid and stationary phases under high performance pressure. Small molecules with particles are binding to the liquid surface to generate a thin liquid properties film. However, there are a variety of bounding agents, which are available. For example, a non-polar molecule has properties to bind to the solid and mobile phase, and this kind of method named reverse-phase liquid chromatography. The petition coefficient counts the molecular nature of mobile and stationary phase. However, such case the number of the stationary phase will be very few, while there is a large number of liquids and combination of the liquid solutions used for mobile station. This kind of method called isocratic show as figure (1-9) The disadvantage of using LLC is using the proper elution. The usual problem of using elution is the encountering with liquid chromatography of samples that

have both weak and strong retained solvents. This need heating and temperature similar to the programming that used in gas chromatography. In a process needs gradient elution, the concentration of retained solutes in the mobile phase is increased by constantly changing the compounds, and then the polarity of the mobile phase through the separation [28].

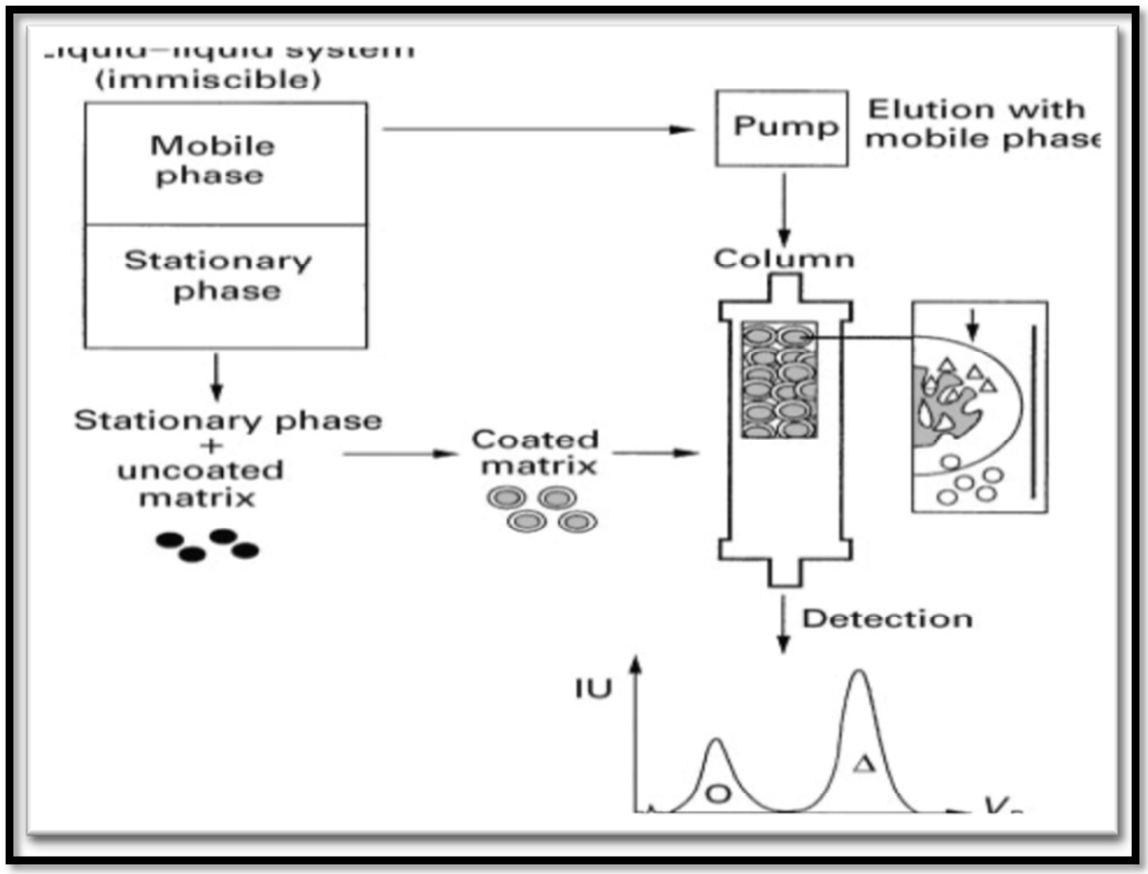
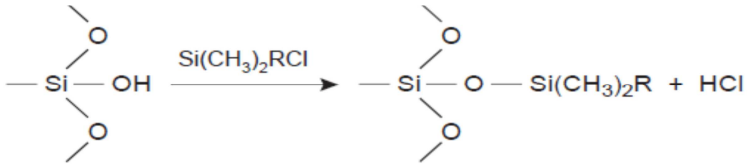


Figure (1-9) represents the diagram of Liquid-Liquid chromatography. IU=international units, VR=retention volume

(1-11) Stationary phases

In liquid-liquid chromatography, the stationary phase is liquid film coated on materials composed from 3-10 µg porous silica particles. However, the stationary phase may be partially soluble in the solid mobile phase that causes it to flow (bleed) from the column over time, and eventually cause losing of the stationary phase. However, to prevent the bleeding lost, the stationary phase covalently bound to the silica particles. For example, bounded stationary phases are created by the silica particles with an organ chloral silane of the chemical form Si(CH3)2RCl, where R is an alkyl or substituted alkyl group.



However, to prevent unwanted reaction which may happen between solutes and any kind of hydroxyl group like SiOH groups, the silica can be capped by reacting it with $Si(CH_3)_3CL$; this kind of columns are designed to be end-capped. The properties of the stationary phase are diagnosed by the chemical structure of the organ silane alkyl group. If R is a polar functional group, then the stationary phase will be pollard too. There different types of polar stationary phases such as Cyano C_2H_4CN, diol $C_3H_6OCH_2CHOHCH_2OH$, or amino $C_3H_6NH_2$ functional group. Unlike polarity of the stationary phase, the mobile phase is non-polar or moderate polar solvent. However, mixing polar and non-polar stationary phase results of new state that called normal phase chromatography. Unlike normal phase chromatography, the phase

chromatography, which is the most common encountered type of HPLC, composed of nonpolar stationary phase and mobile polar phase. However, the most popular type of nonpolar stationary phases adds an organ Chloro silane for which the R group is an n-Octal C8 or n-Octal C18 hydrocarbon chain. The most interesting common example of reverse phase separation uses a buffered aqueous solution as a polar mobile phase. Hence, the mobile phase has to have PH less than 7.5 because the silica substrate is subject to dissolve in basic solutions [29].

(1-12) Mobile phase

The molecular nature of the sample components and polarity of the stationary phase are the two important factors that determine the power of the solvent. Table (1-1) illustrates some widely used solvents in order of their elution power, and the series often call eluotropic series. However, practically getting better separation is by using least polar solvent and using mixture of solvents to achieve better separation conditions.

the other hand, the chemist has to make sure the purity of the polar nature like no more water or acids, alcohol in chloroform, aromatic in saturated hydrocarbons, which may impair the reader and the resolution. Furthermore, some types of solvent combinations are unstable which impaired the resolution too. For example, basic alumina generally uses to polymerize acetone [30].

Table (1-1) represents some selective examples of an eluotropic

Solvent	Uv cut of nm	RI 25C^{O}	Viscosity cp 25C^{o}	Solvent polarity (p^{-} partition - based)	Solvent polarity (ε^{o} adsorption-based)
n-hexane	190	1.372	0.30	0.1	0.01
Cyclohexane	200	1.423	0.90	-0.2	0.04
Carbon tetrachloride	265	1405.457	0.90	1.6	0.18
Toluene	285	1.494	0.55	2.4	0.29
Benzene	280	1.498	0.60	2.7	0.32
Methylene chloride	233	1.421	0.41	3.1	0.42
n-propanol	240	1.385	1.9	4.0	0.82
Tetrahydrofuran	212	1.405	0.46	4.0	0.57
Ethyl acetate	256	1.370	0.43	4.4	0.58
Iso-propanol	205	1.384	1.9	3.9	0.82
Chloroform	245	1.443	0.53	4.1	0.40
Acetone	330	1.356	0.3	5.1	0.56
Ethanol	210	1.359	1.08	4.3	0.88
Acetonitrile	190	1.341	0.34	5.8	0.65
Methanol	205	1.326	0.54	5.1	0.95
Water		1.333	0.89	10.2	

(1-13) Types of separation modes in HPLC

(1-13-1) Normal phase chromatography:

Generally, the components contain different constitutes which have different rates of polarity. So the mixture will elute at different degree depending on the polarity. At the time of using column in the separation, which is more polar method than using mobile phase, the analytical experiment name normal phase method. However, the stationary phase is a polarize phase in normal phase chromatography, so that the polar solutes separate by this method leads to generate more adherences to the stationary adsorbent phase.

When the solvent or gradients of solvents solution flows through the column, less polar compounds will elute in a degree higher than the polar ones. Then the components can be collected separately, assuming adequate separation achieved, due to increase polarity. Hence, we can use column phase method in Liquid-Liquid chromatography, and in Solid-Liquid chromatography, which both mobile and stationary phases are liquid, the solid absorbent column has to coat by a solid absorbent combined with polar liquid [31].

(1-13-2) Reversed phase:

Chromatography is analytical physical separation method uses to separate complex compounds into its components through run the sample between two phases; one of the two phases is stationary station whilst the second is for moving into definite direction. However, in HPLC, the stationary phase will be either solid, porous, liquid in which the surface

coated with micro particle beads of an inert solid, which usually made from silica. The mobile station is a liquid, which flows through inert packed beads of the stationary phase in the column under high performance pressure show as figure (1-10) that represents the typical components of HPLC instrument.

The most common applicable type of HPLC is reversed phase high performance liquid chromatography of (RP-HPLC). RP-HPLC composed of from two stations; the first one is non-polar stationary phase, which is mostly made from silica micro particles that coated with C18 chain. The second one is polar mobile phase. The mobile phase has to fill with solvent, which can be isocratic elution or it can run through gradient elution. Generally, in RP-HPLC, gradient elution is starting from polar composition to less polarity solutions.

Actually, the separation principle of RP-HPLC instrument is to run the analyte between two phases: the stationary phase and mobile phase. Injected analyte samples through the analytical column leads to transport the sample along the column at different gradients depends on the sample solubility. Therefore, separation occurs through differences in the distribution constants (Kd) of the individual analyte in a sample mixture [(32).]

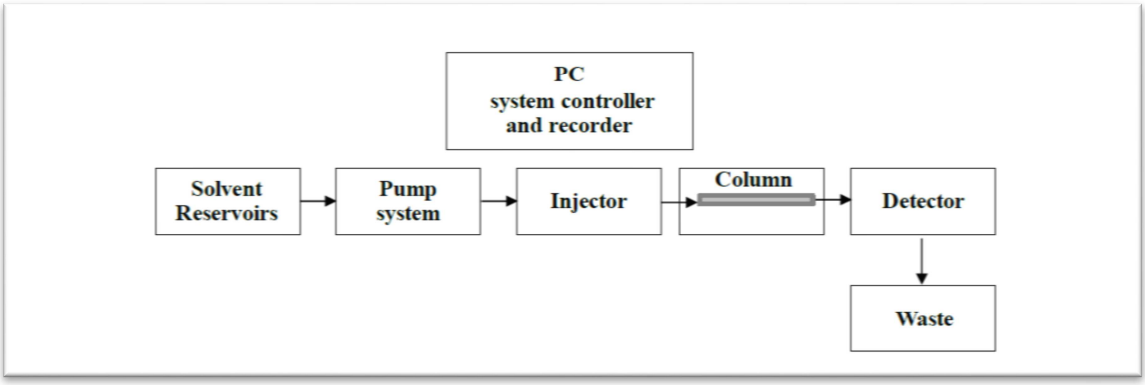


Figure (1-10) schematic figure represents the typical components of HPLC.

(1-13-2-1) Mechanism of reversed phase of HPLC:

The reason for using RP-HPLC is the situation of using high polarity in the mobile phase comparing to the low polarity in the stationary phase. So that, the method is named (Reverse Phase) because it refers to the opposite straightforward phase, where the polar phase used in conjunction with a lower mobile phase. However, typical reverse phase is hydrophobic that has affinity to bind to the surface of a silica support particles see figure (1-11). More over the most applicable and usable stationary phases are shown in figure (1-12).

In neutral analyte, the mobile phase consists of water, which is polar compound, and an organic solvent that to fluctuate the retention of analyte by reducing the polarity of the mobile phase.

However, increasing water content lead to get red or squeeze the hydrophobic non-polar analyte out of the mobile phase, repeat on to the non-polar stationary phase where they will wait for more time until partitioning out into the mobile phase again. However, for every on-off cycle is called Theoretical Plate. In some exception cases like ionizable analyte, we need to add additives like buffers or ion pairing reagents to the mobile phase to manage retention and experiment reproducibility. Therefore, we need to keep in our mind when we work with ionizable analyte, the hydrophobicity and the retention properties will be affected depending on the ionize or non-ionize state of the analyte see figure (1-13) [33].

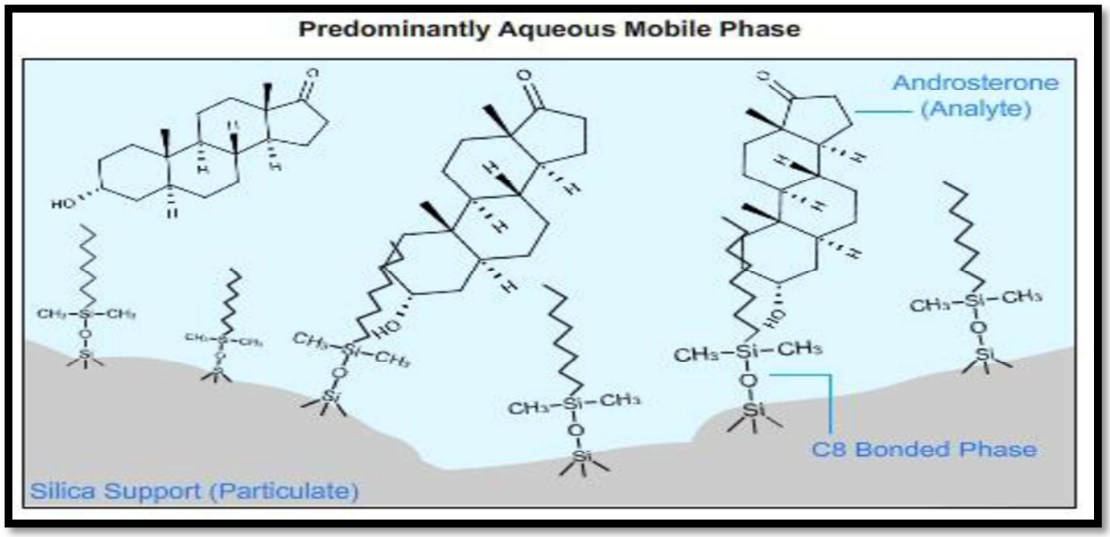


Figure (1-11): illustrates of the reverse phase of HPLC

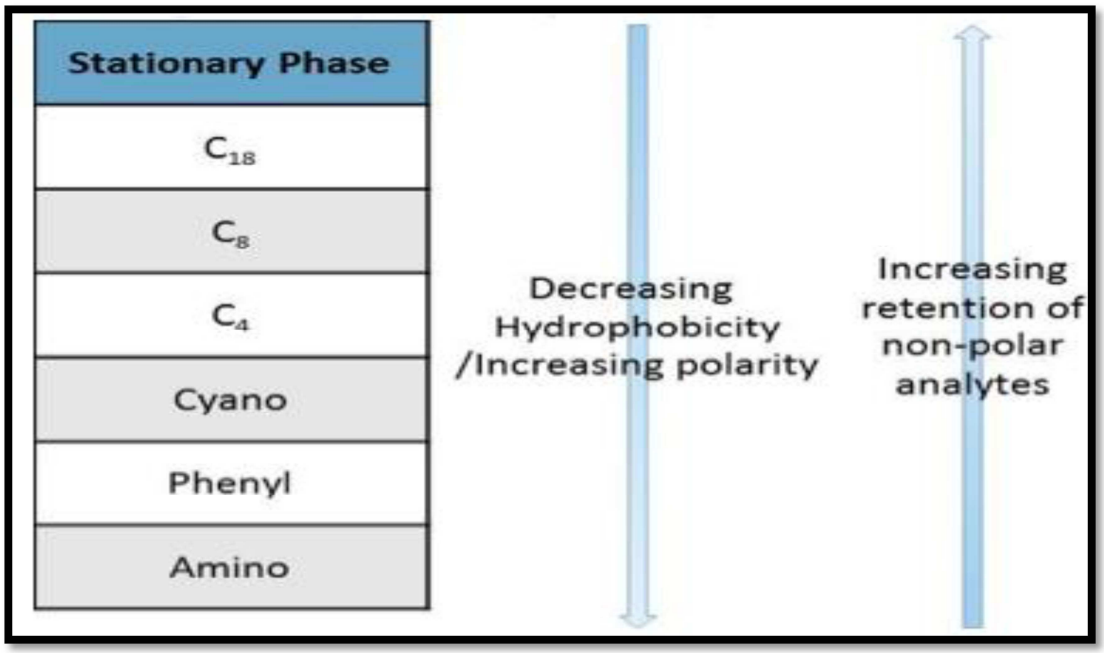


Figure (1-12): shows the common type of the RP-HPLC

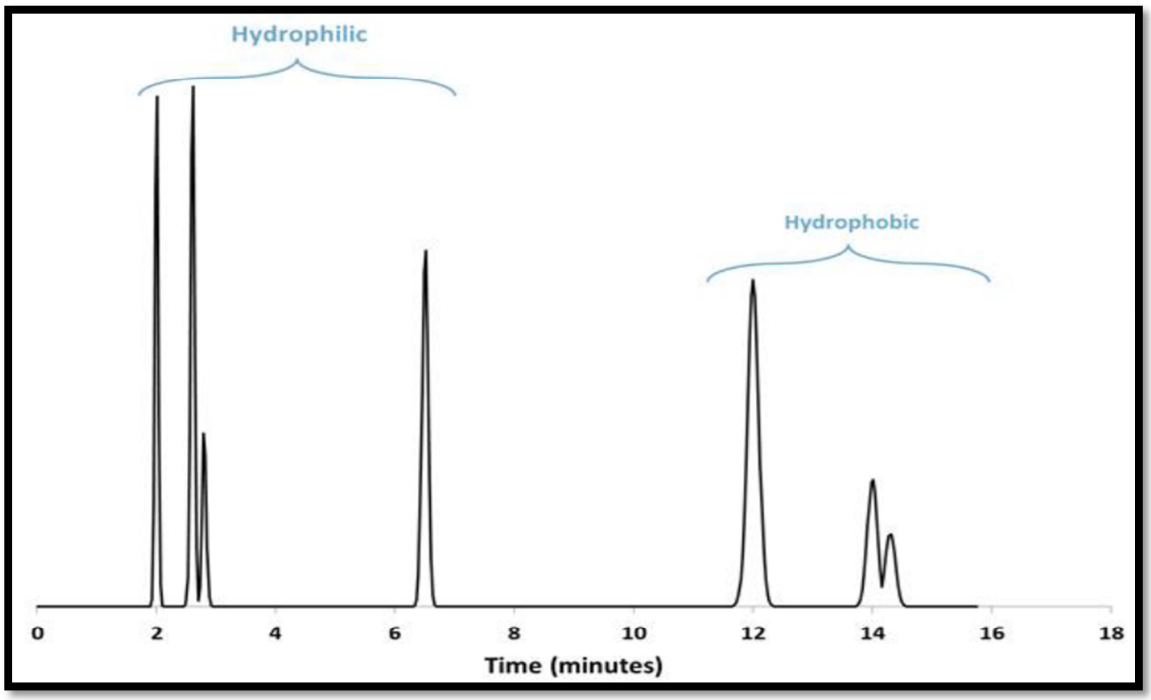


Figure (1-13): representing the elution order of the hydrophilic and hydrophobic

(1-13-3) Ion exchange:

Similar to partition chromatography, ion exchange chromatography composed from coated solid station. The coated area like a risen and it has ions which either anions or cations that bounded covalently to the risen coat. While the opposite ions site is electrostatically bound to the surface. When the liquid mobile phase is eluted through risen coat, the charged ions will release while other ions are bonded preferentially see figure (1-14) as an example of ion exchange chromatography [34].

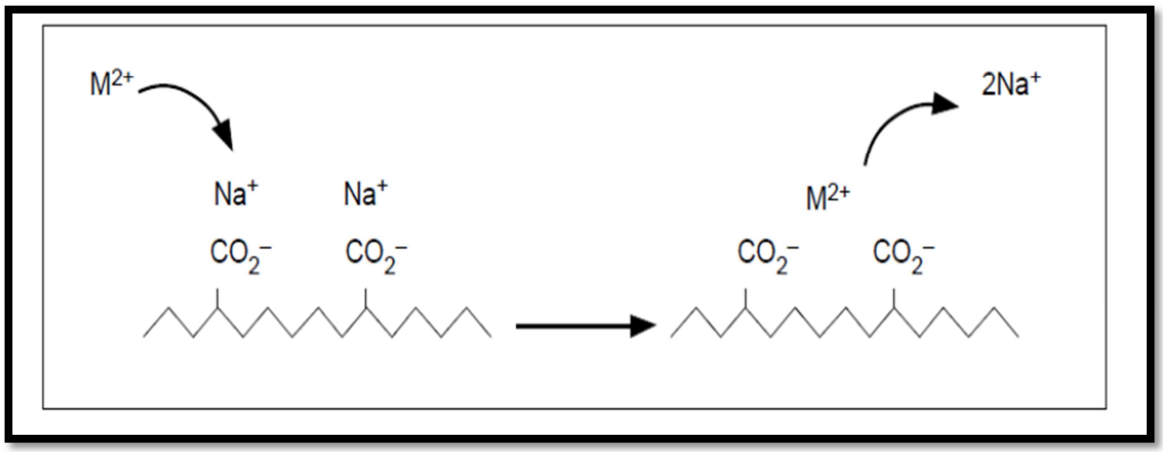


Figure (1-14) domestic water is an example of ion exchange chromatography

(1-13-4) Size exclusion:

In addition to the above chromatography types, size exclusion chromatography SEC or Gel filtration chromatography is one of the useful, applicable separation methods. SEC separates the components depending on the particles size. Moreover, SEC is useful method to draw the tertiary structure and quaternary structure of protein and amino acids as well as the molecular structure of polysaccharides [35].

(1-13-5) Affinity chromatography:

Affinity chromatography is an updated type of chromatography, and unlike the other types of chromatography, affinity chromatography is limited to low pressure operations. For sure, the growing pharmaceutical populations, generating recombinant molecules and purified complex biological compounds are asking for major updating in the stationary phases of the high-performance pressure for affinity chromatography. Affinity

chromatography depends on the affinity of the molecules for specific analyte. For example, the affinity ligand on the stationary phase differed from peptide to protein to oligopeptide to monoclonal antibody. In some experiment, the chemist can tag the desired molecules with an affinity tag to simplify the separation. Furthermore, the affinity chromatography uses in the synthesis of recombinant proteins when the protein is engineered then the desired structure labelled with affinity tag such as polyhistidine. In the stationary phase amino acids and nickel chelate use to remove out the required molecule by chelating with polyhistidine [36].

(1-14) Instruments of HPLC:

(1-14-1) Pump

The pump part in the HPLC must be strong and solid enough to generate high performance pressure up to 350 or even 500 bars. It is always looking for the high flow accuracy and precision, which can be set up or chosen on demand. The range of the flow occurs between 0.1 ml/min to 5 ml/min, which can be established at any value in the step of 0.1 ml/min. However, it must be independently between, flow rate and pressure rate, even if the changes occur through a separation, which is always the case of gradient elution. In addition, the flow rate must be pulseless especially at the time of using electrochemical or conductively detectors or refractive index [37].

(1-14-2) Column

In typical and classical column chromatography, the movement of the mobile phase does not depend on the external pressure instead; it depends on the gravity forces only. Analyses can prolong for several hours and the separation efficiency is low. To get efficient and precise separation, we have to use stationary phase that coated with particles ≤10um of uniform size that homogenously filled in the column. Interestingly, these kinds of coated particles create a large resistance to mobile phase movement and then high pressure has to apply (tens or MPa). Hence, the method that uses high performance pressure called High Performance Liquid Chromatography HPLC. Separation by using LC technique can be affected by chosen either mobile or stationary phases. For more information about HPLC components, it is easier and cheaper to choose changing the composition of mobile phase, change the solvent type, or change the pH and ion of the mobile phase than deciding to change the chromatographer column. [38]

According to the ancient analytical chemical rule (similar is dissolved in similar), the mobile phase has to dissolve the analyte and should not interfere with the detection. So that we can work at the fixed composition of the mobile phase or it is called isocratic elution, or we can choose to change the composition of the analyte, which called gradient elution. Therefore, choosing of the stationary and mobile phase depends on the chemical composition of the analyte. When the stationary phase characterizes with the absorbance coated with silica gel and the separation depends on the adsorbent mechanism, then the method is called adsorption chromatography. Because of the polarity of the silica gel, we choose a non-polar substance like hexane to work as mobile phase and we choose polar solvent like water,

alcohols with concentration less than 1% to increase its polarity. So that the adsorption chromatography is a good choice for separation, isomers compound like phenols. However, the most common type of chromatography that uses non-polar chemically bound phases is reverse phase chromatography or refers to it as RP-HPLC. The principle of the RP is quite sophisticated; briefly, it is depending on combine dissolution and adsorption. The phases packed with the inert support materials which are either non-polar compounds like Octyl, Octadic, or polar chemical compounds like phenyl, cyan propyl, amino propyl. For separation of non-polar analyte, it is better to choose aqueous solutions like methanol, acetonitrile, or some other available solvents. [38]

During gradient elution, it is better to pick gradient of organic solvents. If the substance is ionizing, then we need to destroy their dissociation by changing the PH (PH adjustment) through using buffers or trifluoroacetic acid). It is widely recommended for the chemist to add ion-pairing substances, which could be organic acid or organic basic that form neutral ion pairs with ionic analyte and work on the analysis on non-polar stationary phases ion-pair chromatography. [38]

the other hand, ionic structures can be separated by Ion Exchange Chromatography (IEC) separation method as well. In IEC ionizing functional groups like -SO3 that characterize strong cation exchanger, -COO that characterize weak cation exchanger, $-N^{+} = (CH_3)_4$ that known strong anion exchanger, and –NH3 mostly know week anion exchanger are use on the stationary phase. [38]

Ions in the analyte solution are exchanged with H^+ or OH^- ions in the principle of ion exchanger. Then using buffers on the mobile phase to apply gradients of highly acidic compounds or concentrated salt are applied. It is interesting to know that IEC is only applicable to ionize compounds strong acid or strong base and their concentrated salts. To apply IEC on the weak acids and weak base, we need to increase the dissociation of these compounds by changing the PH. Amino acids are example for the weak electrolytes, and more often they are applicable to analyze by using IEC. The analyses are more often carrying out in acidic medium with gradient elution, where amino acids are created in the cations from [38].

(1-14-3) Detector

Detectors are generally depending on the elective response for the solution like UV absorbance or Fluorescence absorbance. In the other hand, detectors depend on the bulk properties of the mobile phase that can be modified by the solute such as refractive index see Table (1-2) [39].

Table (1-2) showing the characteristics of different types of HPLC detector

Detector	Sensitivity g cm^{-3}	Linear range	Characteristics
Uv -visible absorbance			Good sensitivity, most widely used, selective for unsaturated groups and structures. Not Can be used with gradient elution
Filter -photometer	5×10^{-10}	10^4	
spectrophotometer	5×10^{-10}	10^5	
Diode- array spectrometer	$>5\times10^{-10}$	10^5	
Fluorescence	10^{-12}	10^4	Excellent sensitivity, selective, including fluorescent derivatives. Not flow or temperature sensitive
Refractive index	5×10^{-7}	10^4	Almost universal, but only moderate sensitivity. Very temperature sensitive (control to $\pm0.001^oC$). Cannot be used with gradient elution
Electrochemical			Flow and moderately temperature sensitive. Cannot be used with gradient elution. Detects only ionic solutes. Excellent sensitivity, selective but problems with electrode contamination
Conductimetric	10^{-8}	10^4	
Amperometry	10^{-12}	10^5	

(1-14-3-1) Spectroscopic detectors:

Spectroscopic is one of the most popular types of HPLC detectors. A spectroscopic detector includes visible light, UV and fluorescence absorption. Spectroscopic range from simple or old design in which choosing the suitable absorption depends on the choosing appropriate filter to the more complicated or modified design which has special flow cells. However, using UV/V detector leads to plot the results of the absorbance as a function of elution time as chromatograph figure (1-16).

The chromatograph figure can utilize in three-dimension which represents elution time and the absorbance as a function of wavelength when we use diode array spectrometer, which record the entire spectrum. The flow cell of the modern HPLC detectors has a volume of 1-10 µl and a path length of 0.2-1 cm. The disadvantage of using detectors with the flow cells is that the mobile phase should not strongly absorb see figure 1-16 which represents schematic diagram of the flow cell detectors. Chosen the right absorbance wavelength is critical for detection, so that UV/V cannot be used for different mobile phases. However, detectors provide limit detection of as little as 100 pg^{-1} ng of injected analyte. While fluorescence detectors provide more choices because of fewer solutes are able to absorb fluorescent. The chromatograph figure represents a plot of the fluorescence intensity as a function of time, and the detection limits as little as 1-10 pg of injected analyte [40].

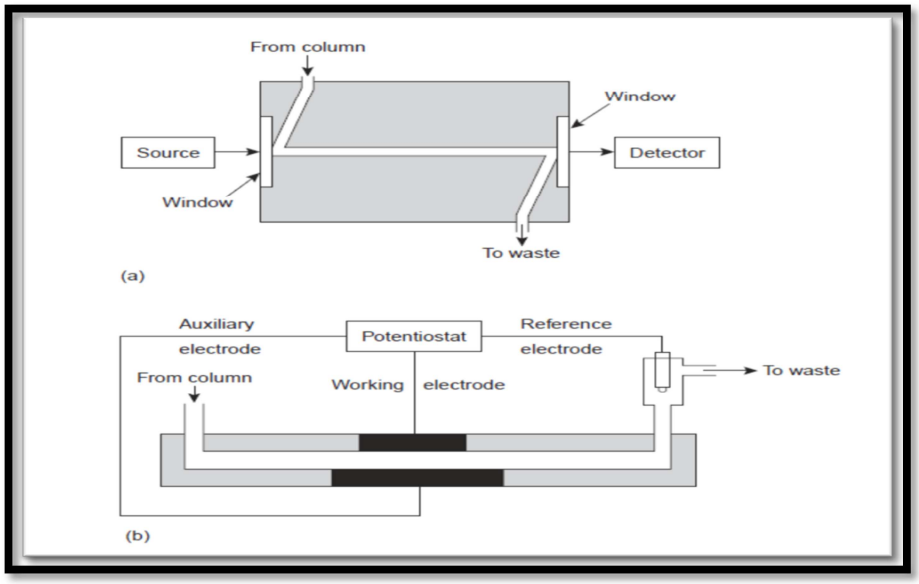


Figure (1-15) showing the flow cell detectors for HPLC using a: UV/V is absorption spectroscopy (b) amperometry

(1-15) Mebeverine hydrochloride MBV

MBV is a white crystalline structure, having molecular formula $C_{25}H_{35}NO_5$.HCL with molecular weight 466g/mol see figure 1-18 for more details. MBV is water and ethanol 96% soluble structure, while it is insoluble in dietheyether. According to the IUPAC, the nomenclature of MBV is 3, 4-Dimethoxy benzoic acid [ethyl (2-(4-methoxy 4-phenyl)-1-methyl ethyl) amino]-butyl ester Medically, MBV is a relaxant anti-muscarinic substance that belongs to a group of drugs called mucosotropic. So that, MBV.HCL uses antispasmodic especially for colonic spasm and for treatment irritable bowel syndrome [41].

MBV.HCL works directly on the gastrointestinal muscles at the cellular level to cause relaxation. In addition, MBV.HCL is calcium channel replenishment, therefore it can be used as dual action drug that normalize the

small intestine motility [42]. Interestingly, more than 75% of the MBV.HCL binds to the plasma protein with very short half time of 2.5 hours. In addition, MBV.HCL is rapidly absorbed after oral administration with peak concentration occurring after 1-3 hours.

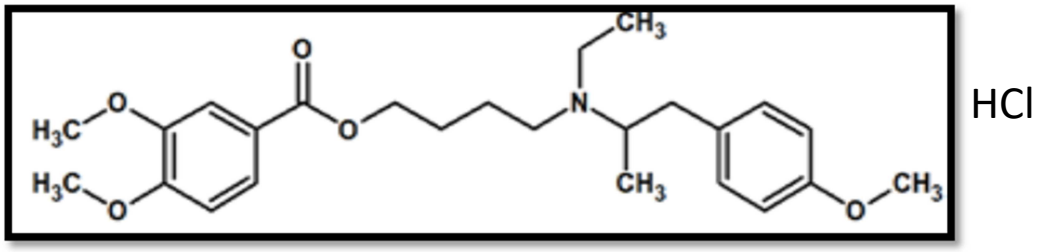


Figure (1-16) represents the chemical structure of Mebeverine hydrochloride structure.

However, the recommended dose of MBV.HCL of 135mg, which appears to have effective pharmaceutical action to relief spasmodic symptoms or irritable bowel syndrome. More often uses of MBV.HCL medication may lead to high plasma concentration, which lead to systemic side effects like tachycardia and hypotension. [43]

(1-16) Literature review

Literature review reveals that MBV hydrochloride can be detected in biological fluids such as blood, plasma and urine, and in pharmaceutical formulations. The techniques used in the determination includes thin layer chromatography, potentiometric, ultraviolet and visible spectrometry. Hence the present study suggests a modified updating analytical chemical method to determine MBV.HCL in its pharmaceutical dosage

The following Tables summarize the most common analytical methods that used to determine MBV.HCL in its pharmaceutical dosage.

name metho d	Wavelength (nm) And LOD, LOQ(ppm)	Linearity (ppm) and RSD%	Correlation coefficient, Molar absorptivity (mol/L. Cm)	Method	Ref
Uv spectrophotometric method development and validation for the simultaneous quantitative estimation of mebeverine hydrochloride and chlordiazepoxide in capsules	260 321,973 …….	10-40 0.499	0.996 ……..	The optimized method uses adiluent100%Triethylammonium phosphate buffer (pH 3.0) for the measurement of assay of MBV. HCL and Chlordiazepoxide in Capsules, which are analyzed at a detection wavelength of 260nm.	44
Method development of mebeverine hydrochloride by using UV spectrophotometric method.	246 0.4369, 1.31724	12.5-200 1.046	0.998 ……	Dissolved in DI water/ethanol (96%)	45

Development and validation of spectrophotometric Method for the simultaneous determination of mebeverine hydrochloride and chlordiazepoxide in bulk and in dosage form	234.8 …….	5-81 0.128, 0.388	0.9997 …….	Dissolving in Methanol	46
Simultaneous quantitative estimation of mebeverine hydrochloride and chlordiazepoxide in capsules using spectrophotometry	263 0.528,1.6	7.5-22.5 0.589	0.993 …….	Triethylammonium/Water phosphate buffer (pH=4)	4 7

Spectrophotometric determination of mebeverine hydrochloride	625 ……	2.0-40 0.64	0.9999 9.32×10^{3}	Both A and B are the case of ion formation (association complex structure) between the MBV and fast green FCF (FGFFCF, λmax 625nm) or bromothymol blue (BTB, λmax 405 nm). While method C works through the formation of a complex compound between the drug and cobalt thiocyanate (CTC, λmax 625 nm).	48
	405 …..	2-25 1.15	0.9998 1.67×10^{4}		
	625 ……	100-600 1.45	0.9999 6.06×10^{2}		

Spectrophotometric determination of benzylamine HCl, levamisole HCl and mebeverine HCl through ion-pair complex formation with methyl orange	422 ……	4-14 ……	0.9998 ……..	The compound is prepared by interaction with methyl orange substance in buffer aqueous solution at acidic environment (PH 3.6). Then the yellow ion pair structure is extracted by adding dichloromethane.	49
An application of eosin Y for the selective spectrophotometric and spectrofluorometric determination of mebeverine hydrochloride	551 ……	1-12 ……	0.998 …….	The quench effect of the target drug is measured by spectrofluorometric using eosin Y filter at 540 nm emission and 390 nm excitation. Then the absorbance of the creative structure is measured at 551 nm.	50

Colorimetric determination of mebeverine hydrochloride in Tablets by charge transfer complexation.	292 ……	0.5-3 ……	0.9986 $10^5 \times 1.42$	The reaction of MBV hydrochloride with iodine will generate yellow charge transfer complexation in chloroform.	51
	416 …….	5.0-15 …….		The principle of the method depends on the interaction of MBV hydrochloride with tetracyanoethylene 0.1% solution in acetonitrile.	
	840 …….	5.0-25 ……..		The methods work on the chemical reaction between MBV hydrochloride with 0.2% 7, 7, 8, 8-tetracyanoqulnodimethane.	

New approach for determine of mebeverine by quenched Fluorescence of analytically interested species using continuous flow injection laser diode fluorimeter anal user.	405	0.05-10 1	0.9629 …….	The method works through quenching the effect of fluorescent by MBV HCL in aqueous medium after apply fluorescein sodium salt.	52
Simultaneous determination of mebeverine hydrochloride and sulpiride using first derivative of ratio Spectro and chemometric method	263.7 234.9 0.72	4-40 1.238	0.9998 ……..	Different concertation of 0.1M HCl	53

Colorimetric determination of some kinds of drug that contain methoxy group in their chemical structure like Methyl-2-Benzothiazolinone Hydrochloride MBTH	670 …….	200-1400 0.511	0.9999 0.293×10^3	The method works through oxidation of the methoxy to o-quinone groups of the target drug. Then o-quinone is coupling with MBTH to generate the colored substrates.	54
Spectrophotometric micro determination of nefopam, mebeverine and phenylpropanolamine hydrochloride in pharmaceutical formulations using alizarins	553 0.22	0.5-28 0.80	0.9989 5.9×10^3	The analytical method works through the reaction between the cited drug MBV HCL with alizarin (I), alizarin red S (II), alizarin yellow G (III) and quinalizarin (IV) to generate ion pairing complex structure that can be measured at the optimum wavelength.	55
	483 0.45	0.5-28 0.90	0.9969 8.6×10^3		
	391 0.5	0.5-30 1.20	0.9869 19.3×10^3		
	586 0.44	0.5-25 1.10	0.9990 5.6×10^3		

Name of method	Flow rate Ml/min	t_R min	Detector (nm)	R.S.D%	Linearity, LOD	Mobil phase	Ref.
HPLC fluorescent detector method can be applied to determine of sulpiride and MBV HCL in human biological fluid like plasma.	1	5	365	o.756	10-100 0.85,2.57	Acetonitrile and 0.01M dihydrogen phosphate pH=4	56
Validation of ultra-performance high liquid chromatography on the assay of MBV HCL.	1	1.306	242	0.23	50-400	Mixture buffer acetonitrile in the ration 60:44 with phosphoric acid pH 5.2	57
Development and validation of gradient HPLC-DAD analytical assay method to determine the concentration of ternary mixture that composed from amebicide and analgesic drugs.	1	3-4	260	1.11	1-20 0.3	0.05M phosphate buffer with 1% (v/v) of triethyl amine pH=3, methanol	58

Developed a novel analytical method for the coupling mixture of MBV HCL and Chlordiazepoxide in pharmaceutical formula that uses in stress studies.	1	3.4	262	0.348	27-216	The method required 40:60 v/v ration of methanol and triethyl amine buffer pH=7	59
Simultaneous quantification method for determine alprazolam and MBVHCL in pharmaceutical dosage form by using Liquid Chromatography.	1	3.5	225	0.016	0.2-40	Methanol: buffer (o. o2M KH_2PO_4) (70:30)	60
A quantitative analytical developed method called RP-HPLC to determine MBV HCL and Chlordiazepoxide in capsules formula.	1	5.8	240	1.45	250-750 91.67	Triethylammonium phosphate buffer (pH=2.5): acetonitrile 60:40 v/v	61

An updating and modified method of the RP-HPLC was developed to estimate the stability of MBVHCL in the present of its degradation products.	1.2	13.24	219		52-78 0.32	0,1%diethyl amine in methanol,20mM ammonium bicarbonate pH4.6 adjusted with trifluoracetic acid 0.1% diethyl amine in isopropyl alcohol (55:15:30 v/v/v)	62
	1.2	11.17	219		56-84 0.30		
Quantitative analyses method to measure MBV HCL concentration in dosage form by using HPLC.	1	4.76	263		0.5-10 5	0.05M ammonium acetate buffer and acetonitrile 45% v/v	63
Analytical method was developed to validate MBV HCL in pharmaceutical bulk formula by using RP-HPLC.	0.8	4.334	271	1	1-5 0.105	Ration of triethanolamine to acetonitrile pH=3. The ration =40:60 v/v	64

Use HPLC to determine two fixed known doses of combination of Chlordiazepoxide Hydrochloride and MBV HCL; and the second combination between Carvedilol and Hydrochlorothiazide in their Tablets.	1	6	220		0.25200	We need to use 0.5 M of acetonitrile disodium hydrogen phosphate (50:50, V/V) pH=4	65
Determination the stability of Sulpiride and MBV HCL are done by using HPLC and HPTLC methods.	1		221	o.910	5-60	The method needs a fixed ration of acetonitrile and water (70:30 V/V) pH=7	66

Simultaneous quantitative analysis of Chlordiazepoxide and MBV HCL in their degradation products or in the impurities form was done by using HPLC.	1	6.9	260	0.99	10-200 0.67	The needs to use a fixed ratio between three substances acetonitrile: 0.1 M Potassium dihydrogen: trimethylamine (35:65:0.2, V/V) pH=4.5	67
HPLC method was used to validate MBV HCL and Chlordiazepoxide in capsule formula.	1	3.4	262	0.0262	27-21 2.2	mobile phase consisting of methanol: TEA buffer =7 (40: 60) (v/v) in an isocratic mode	68

(1-17) Aim of this study

This study include two method for estimation of mebeverine –HCl

1. **Charge transfer complex formation method**

-Development three spectrophotometric method using charge transfer method for estimation

of mebeverine –HCl

--Validated of three methods

-Application of three methods on three pharmaceutical preparation as tablets

-Comparison of three method with standard method.

2. **Chromatographic method (Rp-HPLC)**

- Development a new chromatographic method by using C18 column and Uv-detector .
- - Choosing a new mobile phase
- - decreasing the analysis time
- -validated the method
- -comparison of the precision and accuracy of the method with standard method
- - Application of the proposed method to estimation of the meveberine –HCl in pure and pharmaceutical preparation .

(2-1) Apparatus:

1.Double beam UV- Visible-spectrophotometer -1800, shimadzu, (Japan)equipped with quartz cell (1 cm).

2.pH meter (Hanna Instrument, Italy).

3.Sensitive Balance (DANFER.Germany).

4.High Performance Liquid Chromatography HPLC, UFLC-Shimadzu, CBM 20A, (Japan), equipped with ODS2-C18 (150mm×4.6mm particle) analytical column, UV-visible detector.

5.100µl glass micro-syringe(Japan)

6. Ultrasonic (Ultrasonic cleaning machine, model: CLEAN50.

7.FT-IR spectrophotometer (Broker, Enro).

8. Potentiometric Apparatus Phywe, Cobra3Chem-Unit (Germany)SI Analytics Titranic universal

(2-2) Drugs and chemical materials:

Table (2-1): The drugs and chemical materials used in this study.

Name	Chemical structure	M.wt g/mol	Manufactured by companies	Purity%
mebeverine Hydrochloride	$C_{25}H_{35}NO_5 \cdot HCl$	466	S.D.I-Iraq	99.8
Phenol red	$C_{19}H_{14}O_5S$	354.38	HIMEDA	99.8
Potassium dihydrogen phosphate	KH_2PO_4	136.09	HIMEDIA	99.8
Sodium hydroxide	NaOH	40	HIMEDIA	99.8
Chloroform	$CHCl_3$	119.38	HIMEDIA	99.8
Picric acid	$C_6H_3N_3O_7$	229.1	HIMEDIA	99.8

Di chloro methane	**CH_2Cl_2**	**84.93**	**HIMEDIA**	**99.8**
Iodine	**I_2**	**253.809**	**HIMEDIA**	**99.8**
Hydrochloric acid	**HCl**	**36.5**	**HIMEDIA**	**35%**
Water for HPLC	**H_2O**	**18.015**	**Chem-lab NV**	**99.999**
Acetonitrile	**CH_3CN**	**58.08**	**Chem-Iab NV**	**99.8**
Phosphoric acid	**H_3PO_4**	**97.994**	**HIMEDI**	**99.8**
Glucose	**$C_6H_{12}O_6$**	**180.156**	**HIMEDI**	**99.7**
Lactose	**$C_{12}H_{22}O_{11}$**	**342.3**	**HIMEDI**	**99.7**
Starch	**$C_6H_{10}O_5$**	**Change**	**HIMEDI**	**99.7**
Magnesium stearate	**$Mg(C_{18}H_{35}O_2)_2$**	**591.27**	**HIMEDI**	**99.7**
Carbon tetrachloride	**CCL_4**	**153.82**	**HIMEDI**	**99.8**
Dichloreethane	**$C_2H_4Cl_2$**	**98.95**	**HIMEDI**	**99.8**
Ether	**CH_4O_{10}**	**176**	**HIMEDI**	**99.8**
Benzene	**C_6H_6**	**78.11**	**HIMEDI**	**99.8**

(2-3) Preparation of Standard stock solution

Method one: A stock solution of Mebeverine(MBV) 100ppm was prepared by dissolving (0.01 gm) from the drug and transferring into 100 ml volumetric flask and complete the volume in Distilled water.

Method two and method three: A stock solution of Mebeverine(MBV) **100 ppm** was prepared by dissolving (0.01 gm) from the drug and transferring it into 100 ml volumetric flask and complete the volume in Dichloromethane DCM.

(2-4) preparation of sample solution

Ten Tablets of each pharmaceutical preparation mebeverine hydrochloride (Duspatalin 135mg, EVACOL 135 mg, MEVA 135mg) were weighted and finely powdered. An accurately weight portions of powder equivalent to 0.01gm of MBV was transferred to a 100 ml volumetric flask and was dissolved and completed to the mark with distilled water to obtain 100 ppm and stirred for 10 min followed by filtration through to the 0.5µ Filter paper and the filtrated placed at 100mL volumetric flask and complete to the mark with the same solvent (water in method one, DCM in method two and method three).

(2-5) preparation of phenol red

Phenol red ($3.203x10^{-3}$ M) prepared by dissolving (0.056 gm) of phenol red in 25 ml ethanol and made up to 50 ml volumetric flask with distilled water.

(2-6) preparation of buffer solution (pH=2.3)

A buffer has a pH=2.3 was prepared by dissolving (1.287 gm of NaOH) in distilled water and complete to 250 ml with distilled water (D.W), and (3.4206 gm KH_2PO4) was dissolving in (D. W) and makes up to 250ml Volumetric flask with the same solvent.

(2-7) Preparation of picric acid

A (2.145×10^{-4}M) of picric acid was prepared by dissolving (0.00245gm) of picric acid is small amount of DCM and transferring to 50 ml volumetric flask and complete to the mark with same solvent.

(2-8) preparation of iodine

A (3.9×10^{-3}M) of Iodine was prepared by dissolving of (0.04949gm)) I_2 in DCM and transfer to 50 ml volumetric flask and complete to the mark with same solvent.

.

(2-9) Preparation of Standard stock solution for HPLC

A stock solution of 100ppm Mebeverine(MBV) was prepared by dissolving weighting (0.01gm) from the drug in the mobile phase and then to be transferred into 100 ml volumetric flask which is to be and complete with the same solvent

(2-10) Solution of pharmaceutical preparation for HPLC

Ten Tablets of each pharmaceutical preparation mebeverine hydrochloride (Duspatalin 135mg, EVACOL 135 mg, MEVA 135mg) were weighted and finely powdered. An accurately weight portions of powder equivalent to 0.01 gm of MBV was transferred to a 100 ml volumetric flask and was dissolved and completed to the mark with mobile phase to obtain 100 ppm and stirred for 10 min followed by filtration through the 5µ Filter paper and the filtrated placed at 100mL volumetric flask and completed to the mark with the mobile phase.

Chapter three

(3-1) Wavelength selection of Mebeverine hydrochloride

The standard solution of 8ppm bebeerine hydrochloride that dissolved in distilled water were scanned verses the reagent blank by UV-Visible spectrophotometer at the range (200-400) nm. Form the overlain UV spectra, suitable wavelength considered for monitoring the drug were 263nm and 227 nm on basis of higher response. Figure (3-1)

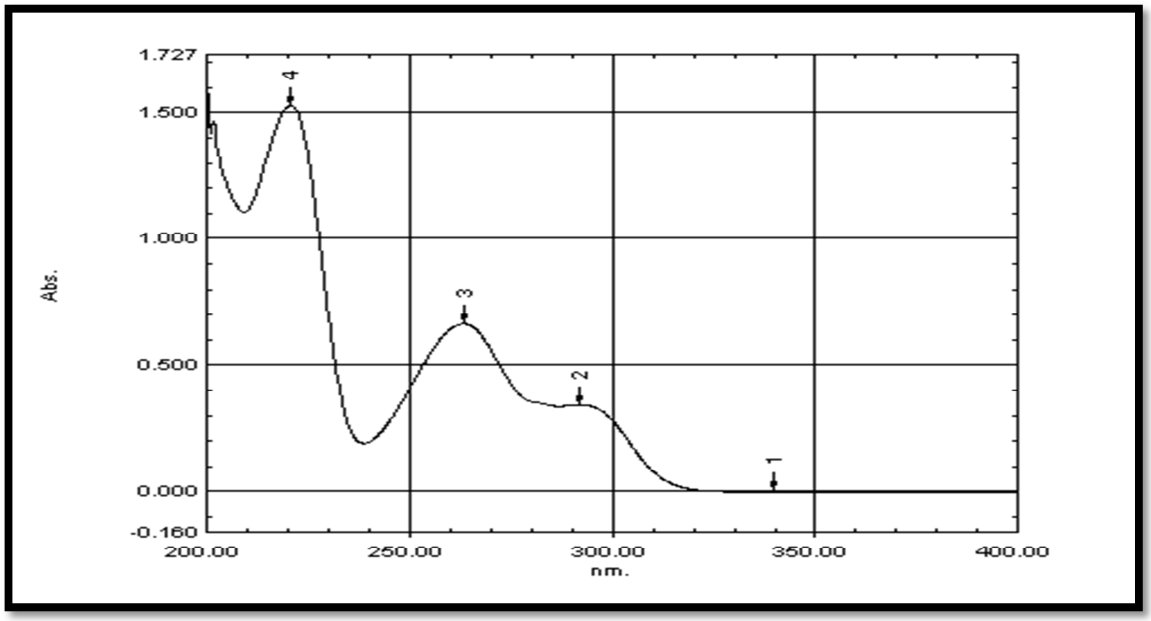


Figure (3-1) The spectrum of 8 ppm of Mebeverine HCl

(3-2) Study of FT-IR spectra [69]

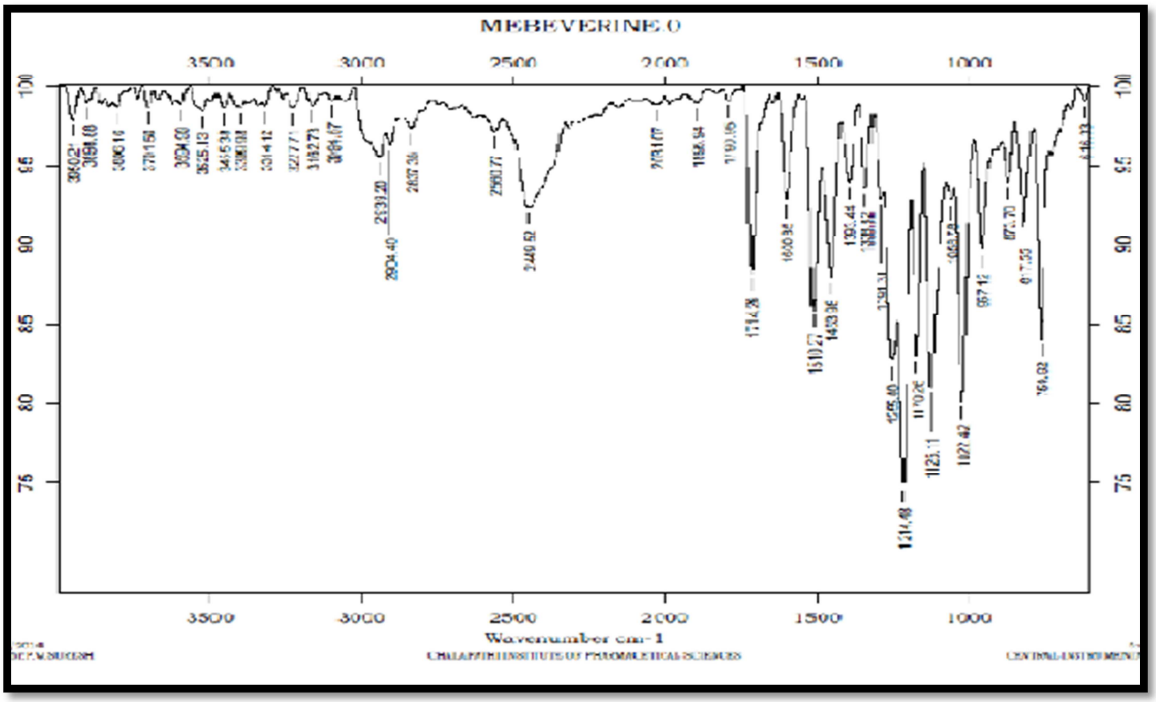


Figure (3-2) FT-IR for Standard mebeverine (sigma-Aldrich)

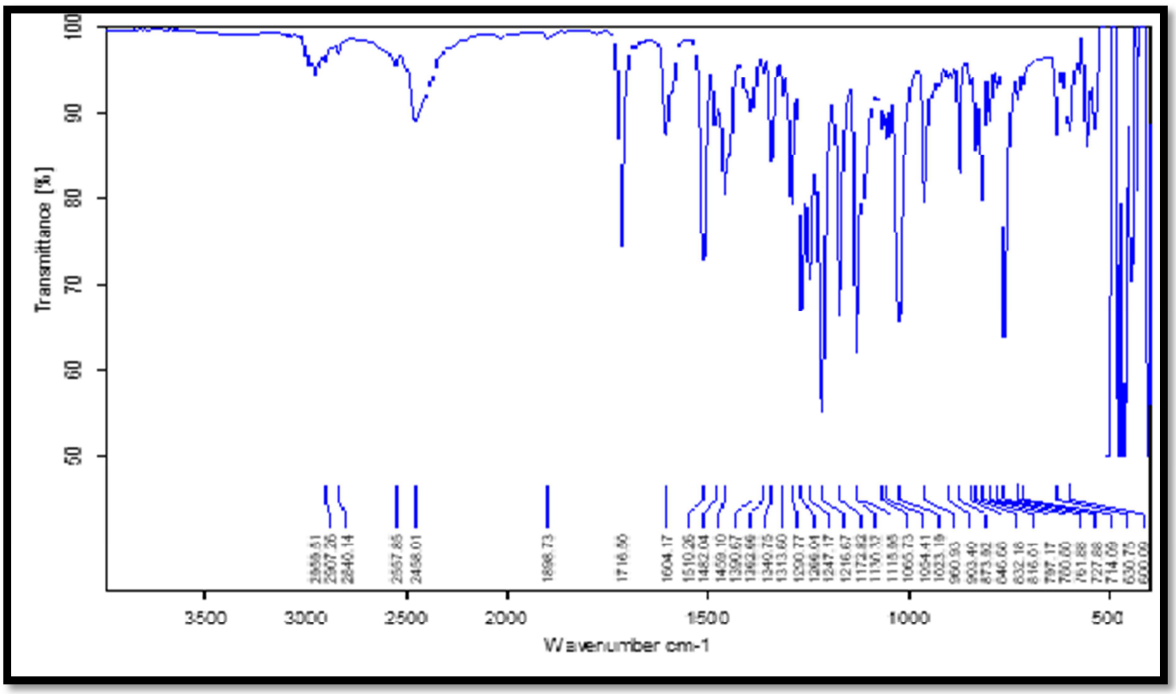


Figure (3-3) FT-IR spectrum for working mebeverine HCl. (using Double beam UV-Visible-spectrophotometer -1800, shimadzu, (Japan)equipped with quartz cell (1 cm))

Part one
Spectrophotometric method

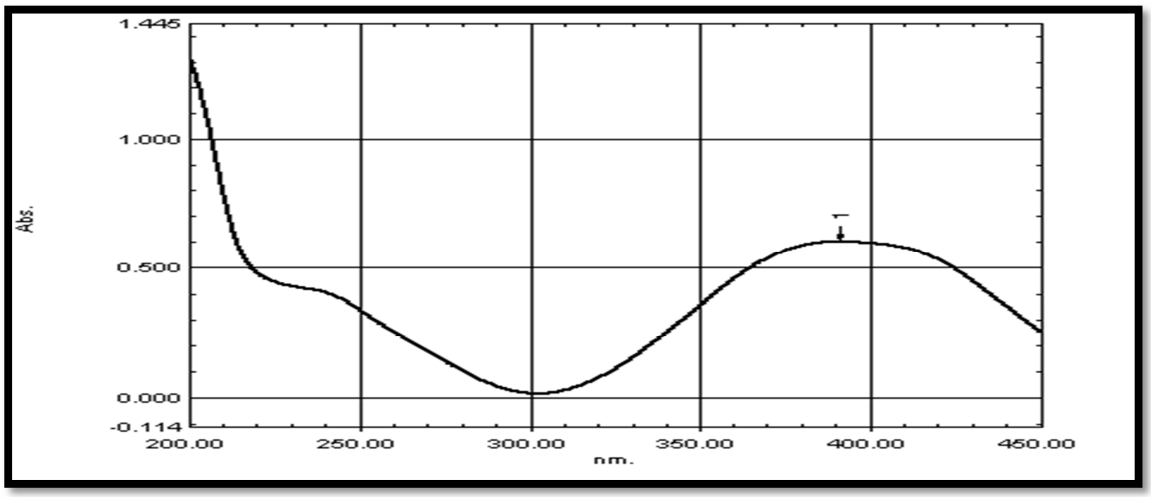
(3-3- A) Method (I) mebeverine and phenol red complex

(3-3-A-1) Recommended procedure:

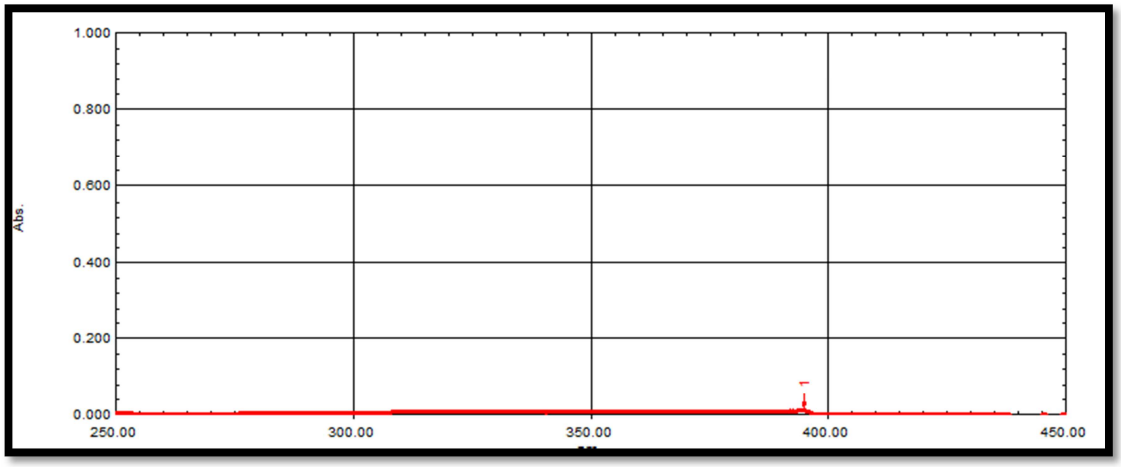
Transfer a suitable concentration (0.2 - 2.5 ml) 100ppm standard solution of MBV into 125 ml separating funnel containing 8 ml of buffer solution (pH = 2.3) and 5 ml of $3.203x10^{-3}$ M of phenol red. The above solution, shake well for 2 minutes, then 10ml of Chloroform was added and the contents shaken for 2 min. The two aqueous phases are allowed to separate. The organic layer was placed in the 10mL volumetric flask and dilute the solution to the mark with chloroform. The separated Chloroform layer was standing for 5 minutes and measured at 396nm against a reagent blank prepared at the same way expect absence of the drug. To obtain the best possible results for the developed method various parameter that effect of absorbance of complex was studied.

(3-3-A-2) Absorption spectrum

Spectrum of colored compound of a concentration of (10 ppm) MBV with phenol was scanned by UV-Visible spectrophotometer in the range (200-400) nm. A typical spectrum of the colored compound was measured versus the reagent blank which has negligible absorbance at λ_{max} 396 nm Figure (3-5)



A



B

Figure (3-4) A: Absorption spectra of the colored compound (10 PPm) 0f MBV with phenol red (3.2×10^{-3}M).

B: Absorption spectra of blank(chloroform)

(3-3-A-3) Optimization of the experimental Conditions

Various parameters that effect the absorption intensity of the complex formed were studied and the reaction conditions were optimized.

(3-3-A-3-1) Effect of phenol red volume:

Various Volume of the phenol red $3.20\text{x}10^{-3}$ M solution was added to fix amount of MBV and was found that 5 mL of $3.203\text{x}10^{-3}$ M of phenol red gave the full intensity and gave a minimum blank value of the absorbance and this is considered to be optimum for the concentration (2 ppm) of the drug as in Figure (3-4)

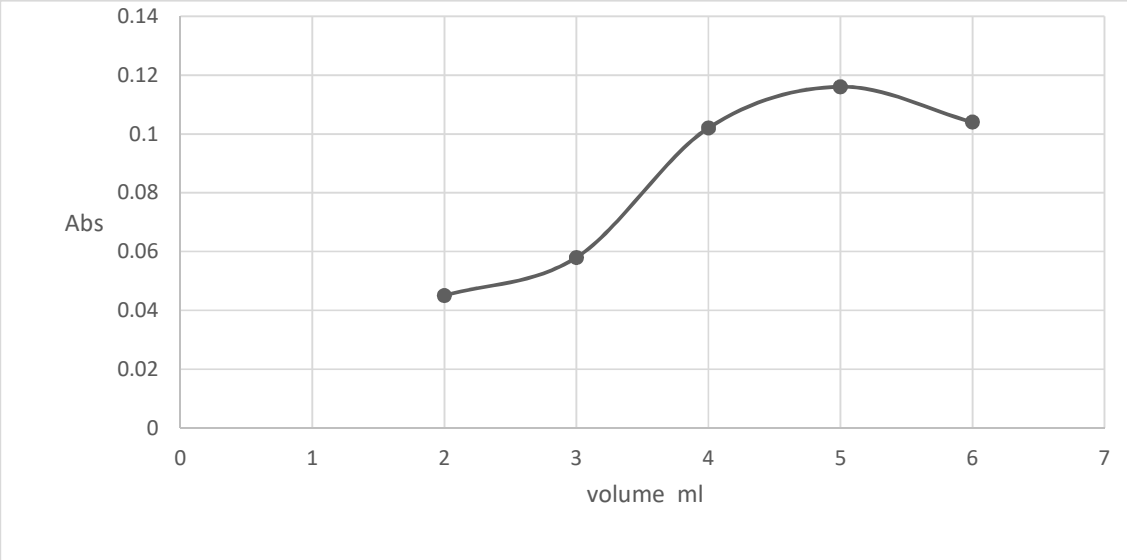


Figure (3-5) Effect of different volume of (1ppm) phenol red on absorption of the complex between MBV and phenol red, at 25C^{o} and 5min

(3-3-A-3-2) Effect of Time:

The color intensity reached a maximum absorption after Mebeverine has been reacted with phenol red at 20min. Therefore, 20 min development time was chosen for further experiment. The results obtained are shown in Figure (3-3)

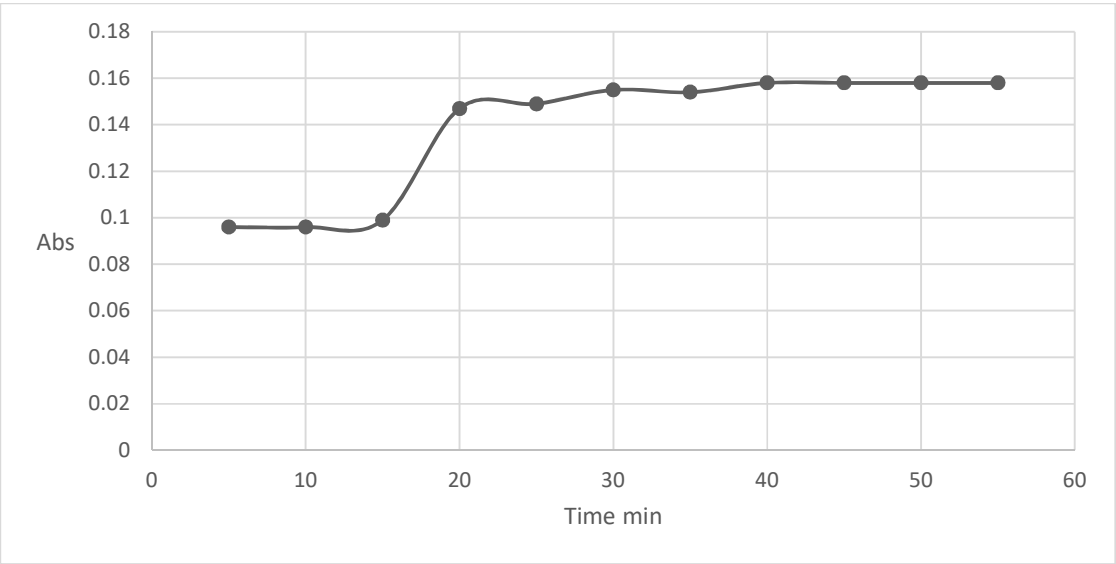


Figure (3-6) Effect of Time of absorption intensity of MBV(20 ppm).

(3-3-A-3-3) Effect of Temperature

The effect of temperature on the absorbance of the formed compound was studied and found that 50 C^o gave the highest absorption at λ max 396 nm, therefore it is recommended that the colored compound should be carried out at 50 C^0 as in figure (3-6)

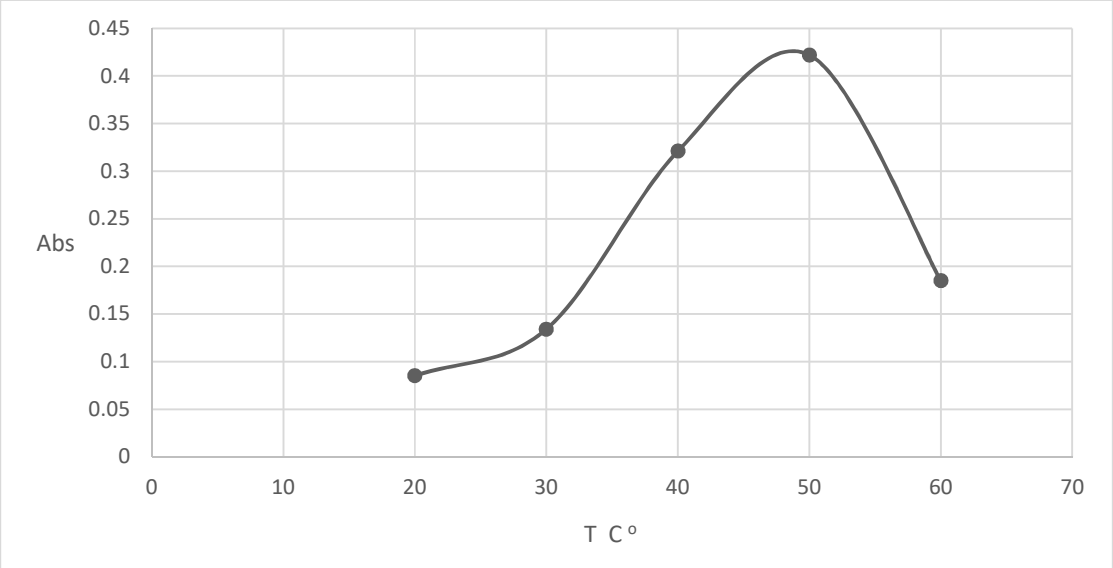


Figure (3-7) effect of temperature on the absorbance in MBV (10ppm),10 min.

(3-3-A-3-4) Choice of organic solvent:

A difference of organic solvents as chloroform, carbon tetrachloride, dichloromethane, dichloromethane and ether were tested to choose the suitable solvent to give a high absorbance. Chloroform was preferred to other solvents for its selectivity and gave highest absorbance. It was found that one extracted is adequate to obtain high recovery of the complex and short time to reach the equilibrium between the two phases.at MBV (20 ppm) ,20 min and 25C^o.

Table (3-1) Effect of Solvents on Absorption

Solvent	Abs
Chloroform	0.176
Carbon tetrachloride	0.059
Dichloromethane	0.063
Dichloreethane	0.034
Ether	0.021

(3-3-A-4) Calibration curve

A linear Calibration curve was obtained when employing the condition described above for the determination of MBV which shows that Beer's law obeyed over the concentration range (2-25) ppm with a correlation coefficient of (0.9956). The molar absorptivity [70] of the formed compound was found to be 1.87 $x10^4$ L. mol^{-1}. cm^{-1} and a sandal sensitivity of 0.0615 µg.$cm^{-2.}$ The result obtained shown in figure (3-8).

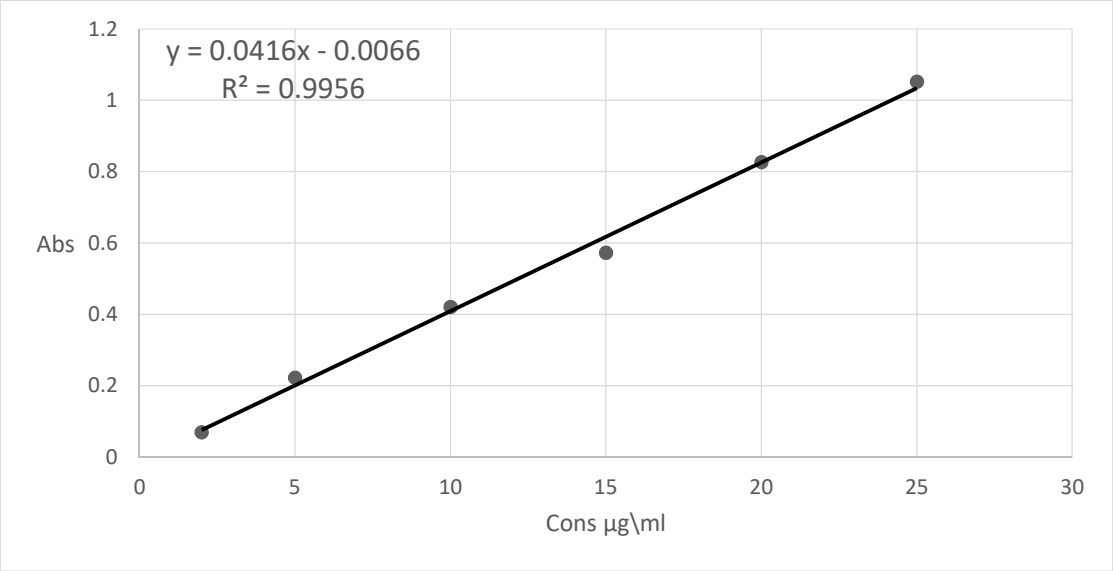


Figure (3-8) Calibration curve for the determination of MBV using phenol red.

(3-3-A-5) Precision and Accuracy of the proposed method

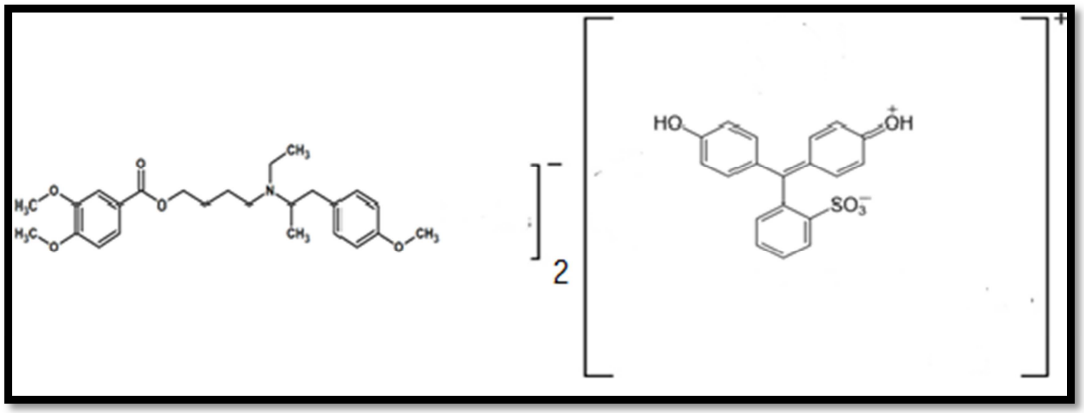
The result shows that proposed method was accurate according to the values of the recovery with the range of (96.74-99.39) when the three levels of concentration were measured also the low values of R.S. D% [71] indicated that the method was highly precise (0.479-1.986) %. Table (3-2)

Table (3-2) Precision and Accuracy of the proposed method

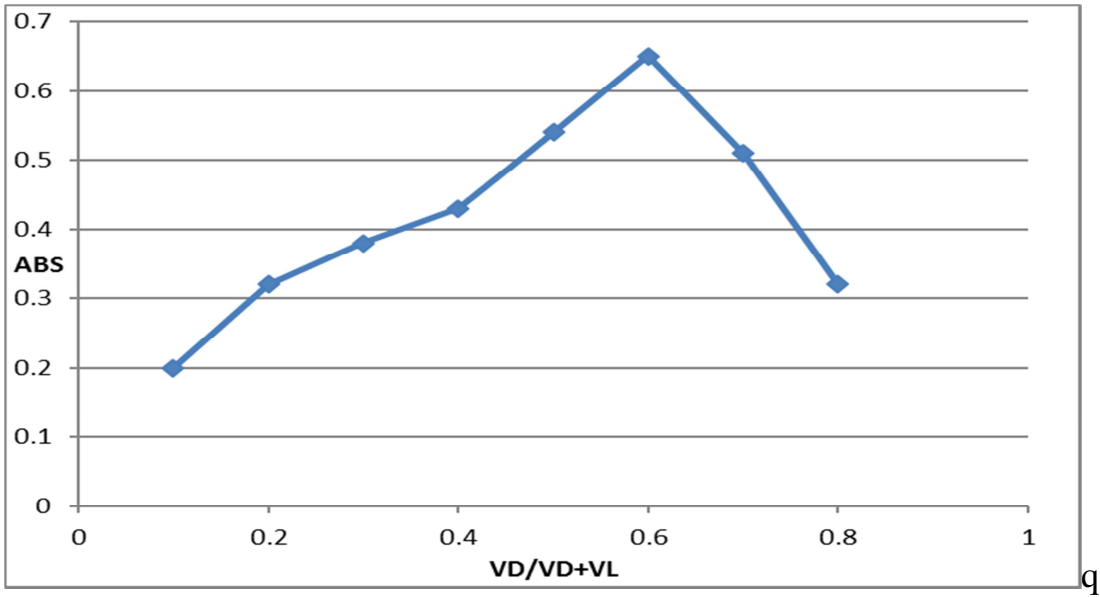
N	Concentration In ppm	Found ppm	R.S.D%	Recovery (%)
1	**8**	**7.70**	**0.48**	**96.75**
2	**16**	**15.63**	**0.61**	**97.66**
3	**20**	**19.52**	**1.99**	**97.59**

(3-3-A-6) Nature of the dye product [72-74]:

The stoichiometry of the reaction between Mebeverine and phenol red (3.2×10^{-3}M) dye was investigated using the mole ratio and Jobs Method. The Results obtained and Figure (3-8) A and B shows that a (2:1) product is formed between the drug and the reagent; therefore, the formation of the dye probably occurs as follows.

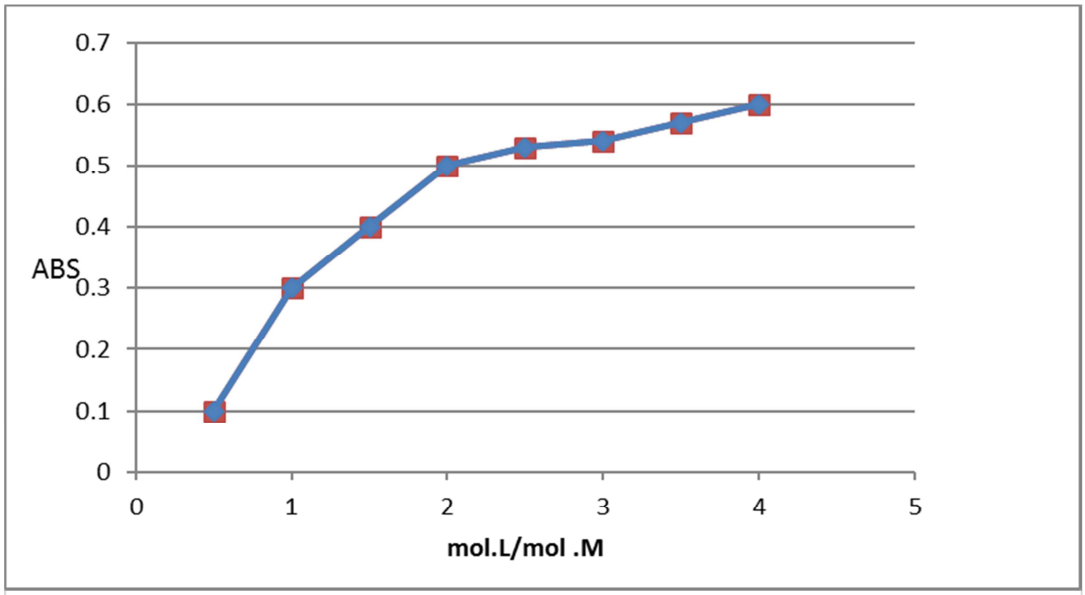


Scheme 3-1. Suggested of MBV- Ph. Red ion-pair formation



q

A



B

Figure (3-9) A mole fraction of drug and B represented the mole ratio

(3-3-A-7) Stability of the ion-pair complexes:

The stability of the formed complex between the MBV and phenol red was evaluated. Although the constant observance readings were obtained at short time 20 min and 50 C^0. The formed complex was stable for at least 2h without any change in intensity of observance or in λ_{max} The conditional constant was calculated according to literature [75], and found to $1.315x10^7$, and this supported the stability of the formed complex.

(3-3-A-8) Interference studies:

The results showed that no interferences were found in the presence of 40 ppm for each excipient (Glucose, Lactose, Starch, Sucrose, Magnesium Stearate, Talk, Aacia) with these concentration different concentrations of the drug up to (2,10,20) ppm in the determination of MBV

Table (3-3) shows the effect represented excipient as men recovery.

Concentration of MBV. ppm	Found ppm	Mean Recovery (%)
2	**1.95**	**97.59**
10	**9.61**	**96.10**
20	**19.86**	**99.30**

(3-3-A-9) Detection and quantification limits:

The detection limit (LOD) and the limit of quantification, LOQ was define as LOD= 3SD/k and LOQ = 10SD / k where SD is the standard deviation of replicate determination value under the same condition in the absence of the drug and k is the slop of the Calibration curve [76].

According to these two parameters the LOD and LOQ were found to be 0.213 ppm and 0.713 ppm respectfully.

(3-1-A-10) Analytical Applications:

The result obtained from the application of the proposed method **for** the analysis of MBV in its pure form and in its pharmaceutical preparations indicates that the proposed method was accurate and precise. The low value of relative standard deviation (R.S. D%) indicated good reproducibility and precision. The

mean of percent recoveries obtained were in the range of (97.18-99.55) indicating good accuracy of the proposed method.

Table (3-4). The obtained results from the application of the proposed method.

Company	Claimed	Found	R.S.D%	Recovery (%)
Duspatalin	**135mg**	**131.2**	**1.32**	**97.18**
EVACOL	**135mg**	**133.5**	**1.49**	**98.88**
MEVA	**135mg**	**134.4**	**1.38**	**99.55**

Table (3-5) statistical parameters of the proposed method (phenol read)

Parameters	Value
Maximum absorption	396 nm
Beers law is range	2 – 25 ppm
Molar absorptivity	(7.57×10^4L/ Mol. Cm)
sandal sensitivity	(0.00615 µg. Cm^{-2}),
RSD %	(1.3974%)
mean recovery	(96-99 %)
correlation coefficient	($R^2 = 0.9956$)
LOD	0.21 ppm
LOQ	0.71 ppm

Table (3-6) F-Test and T-Test for comparison of Accuracy and precision between proposed method and standard method

Pharmaceutical preparation containing MBV	Proposed method		Standard method	
	Recovery%	SD^2	Recovery%	SD^2
Duspatalin	**97.18**	**2.99**	**98.27**	**2.00**
EVACOL	**98.88**	**3.95**	**98.51**	**2.64**
MEVA	**99.55**	**3.43**	**98.85**	**1.73**
	x^-=98.53	**Ɛ=10.37**	**x^-=98.54**	**Ɛ=6.37**

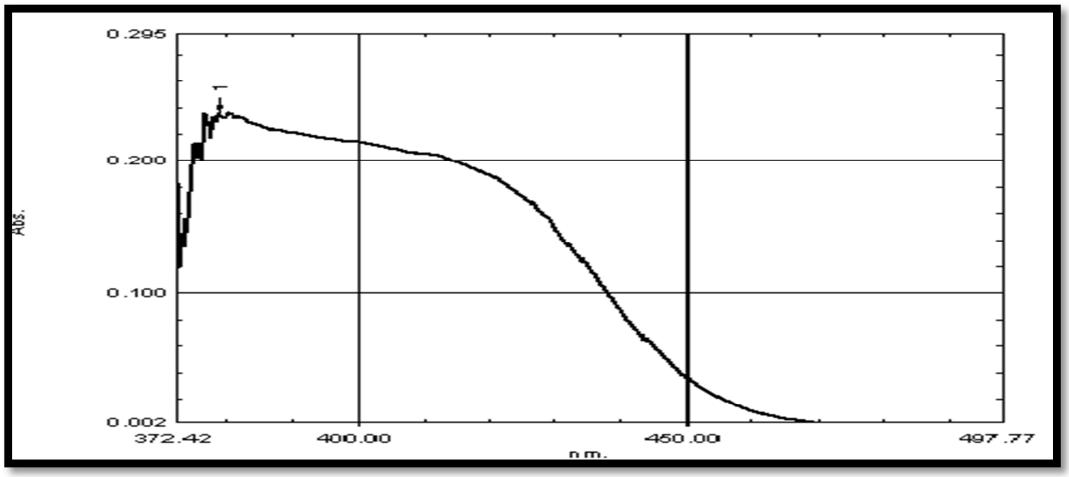
(3-4-B) Method (II) mebeverine and picric acid

(3-4-B-1) Recommended procedure:

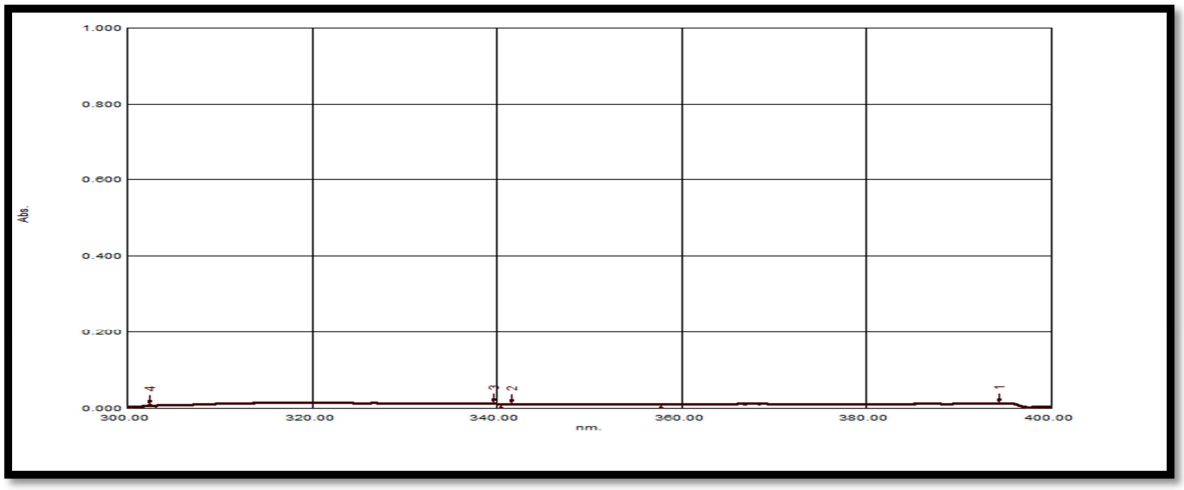
Transfer 0.1-3ml of 100 ppm standard solution MBV into volumetric flask(50ml). Add 2ml picric acid solution to each flask, mix and dilute to volume with dichloromethane. Keep the solution in the dark for 30 minutes. Measure the absorbance at 382 nm against simultaneously prepared blank.

(3-4-B-2) Absorption spectrum

A colored compound was scanned in the range 200-400 nm by using U.V spectrophotometer in the range 200-400 nm the scanning of the solution of MBV was carried out. As examined the complex show the maximum absorbance at 382nm was selected as the specific wave length for the determination of the drag Shown in figure (3-10)



A



B

Figure (3-10) A: Absorption spectra of the colored compound (1 ppm) 0f MBV with 2 ml of picric acid 25C^{o} and 30 min.

B: Absorption spectra of the Blank(Dichloromethane).

(3-4-B-3) Optimization of the experimental Conditions

Various parameters that effect the absorption intensity of the complex formed were studied and the reaction conditions were optimized.

(3-4-B-3-1) Effect of picric acid Volume:

Different volumes were taken from the picric acid ($1.745x10^{-2)}$ M in the range (1-6 ml). The volume 2ml gave the best absorption intensity at λmax 382nm, while 5ml gave very high absorption Show in figure (3-8)

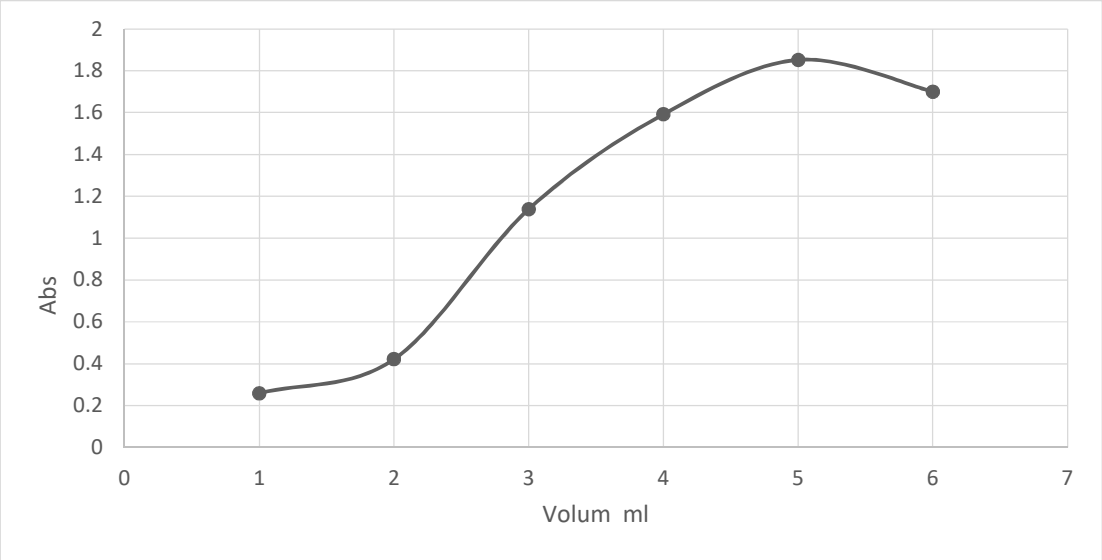


Figure (3-11): Effect of different volume of ($1.745x10^{-2}$) M picric acid on absorption of the complex between MBV(10PPM) and picric acid.

(3-4-B-3-2) Effect of reaction time:

The color intensity reached a maximum absorption after MBV has been reacted with picric acid at 30 min. Therefore 30 min development time was chosen for further use. The results obtained are shown in figure (3-9)

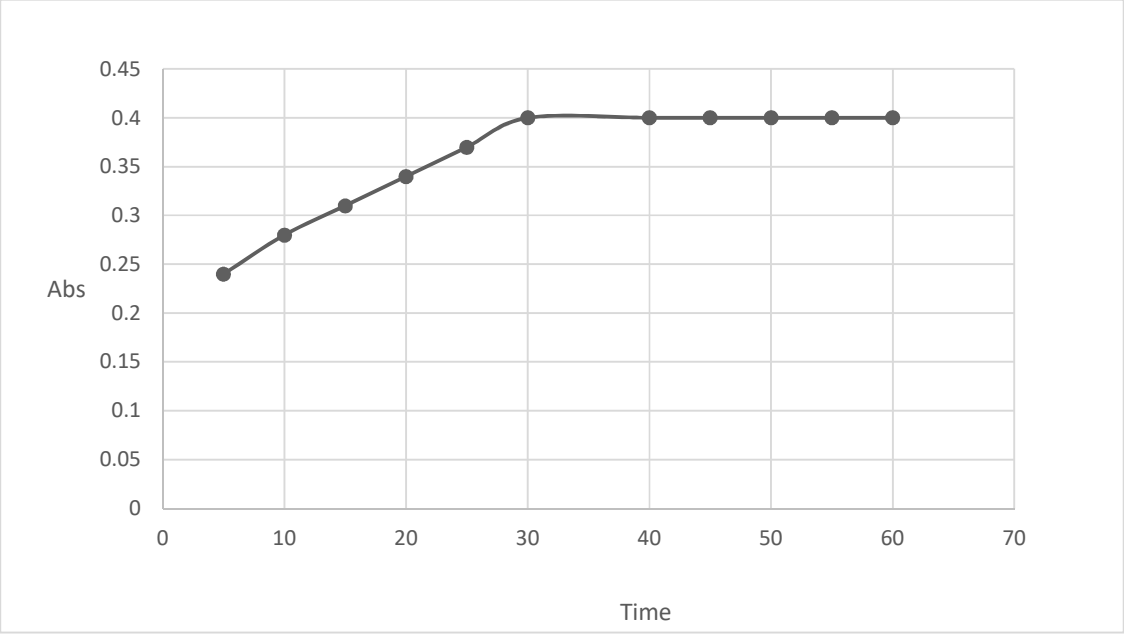


Figure (3-12): Effect of time on the absorption of MBV(10PPM).

(3-4-B-3-3) Effect of Temperature:

The effect of temperature on the complex stability was not studied due to the fact the solvent used is (DCM) and the boiling point of the solvent is less than 40 c°.

(3-4-B-3-4) Choice of Organic Solvent:

A different organic solvent as Dichloreethane, dichloromethane, chloroform, acetone and ether were tested to choose the suitable solvent to give a high absorbance and low absorption of a blank. Dichloromethane was preferred to other solvents for its selectivity and highest absorbance.

Table (3-7) Effect of Solvents on Absorption

Solvent	**Abs**
Dichloreethane	0.022
Dichloromethane	0.382
Chloroform	0.188
Benzene	0.248

(3-4-B-4) Calibration curve:

Under the optimum conditions, a linear Calibration curve for the estimation of MBV was obtained over the concentration range of (1-30 ppm). The linear regression equation is Y=0.0206X+0.1786 with molar absorptivity [70] of (3.417×10^4 Mol L^{-1} cm^{-1}, a sandal sensitivity of 0.013 μg.cm^{-2} ,and correlation coefficient of 0.9904 as show in figure(3-12)

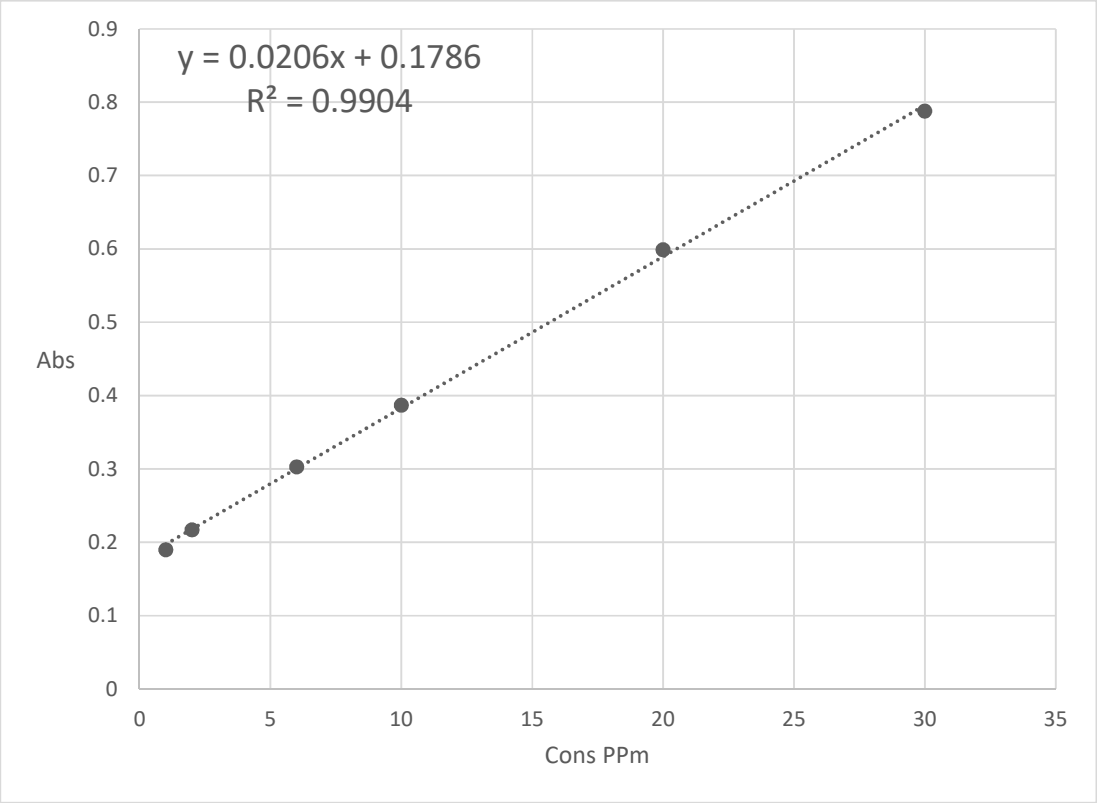


Figure (3-13) Calibration curve for the determination of MBV.

(3-4-B-5) Precision and Accuracy of the proposed method

The result shows the proposed method was accurate according to the values of the recovery with a range of (94.85--99.31 %) when the three levels of concentration were measured also the low values of R.S.D % [71] indicated that the method was highly precise (0.4076-2.949) % Table (3-7)

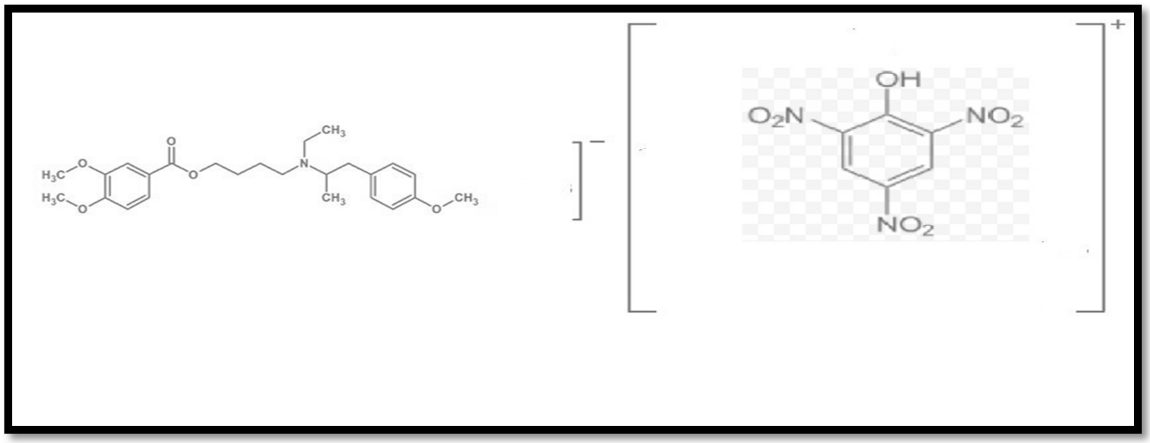
Table (3-8) Precision and Accuracy of the proposed method

N	Concentration of MBV In ppm	Found	R.S.D%	Recovery (%)
1	2	1.96	2.95	98.23
2	10	9.93	2.00	99.31
3	30	28.45	0.40	94.85

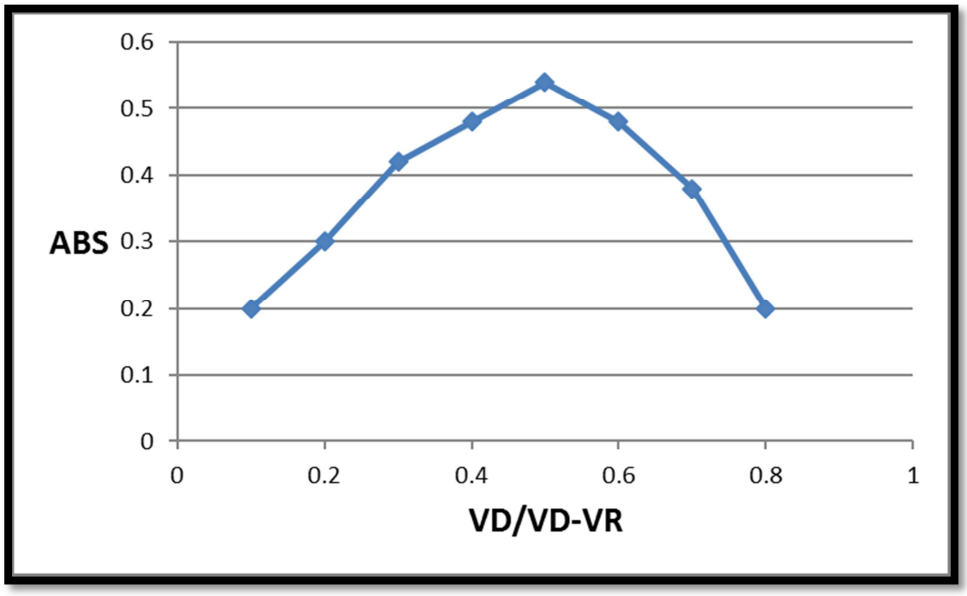
(3-4-B-6) Nature of the dye product [72,73,74]:

The stoichiometry of the reaction between Mebeverine and picric acid (2.145×10^{-4}M) was investigated, using mole ratio and jobs method and the results obtained show that the reaction of the drug with ligand follow the percent 1:1 drugs / reagent as in figure (3-14) A, and B.

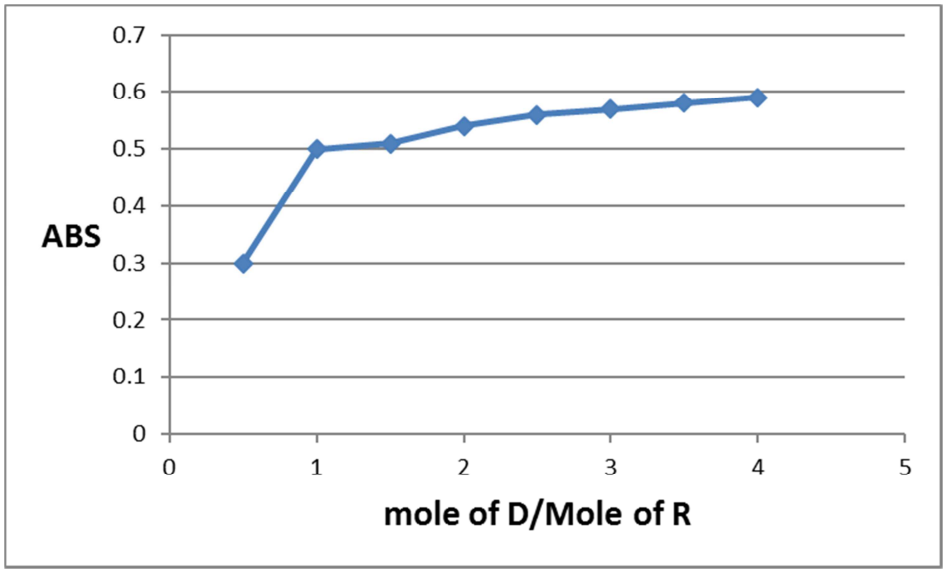
And this supported that the reaction was follow as the path in scheme.



Scheme 3-2. Suggested mechanism of MBV- formation



A



B

Figure (3-15) A mole fraction of drug and B represented the mole ratio

(3-4-B-7) Stability of the ion-pair complexes:

The stability of the formed complex between the MBV and picric acid was evaluated. Although the constant observance readings were obtained at short time 30 min and 20 c°. The formed complex was stable for at least 2h without any change in absorbance. The conditional constant was calculated according to literature [75] and found to 3.949×10^{3} , and this supported the stability of the forming complex.

(3-4-B-8) Interference studies:

The results showed that no interferences were found in the presence of 20 ppm for each excipient (Glucose, lactose, starch, magnesium, stearate, talk, Aacia) with these concentration different concentrations of the drug up to (2,10,20) ppm in the determination of MBV.

Table (3-9) shows the effect of occupant interfering materials that present in pharmaceutical preparations of the drug

Concentration of MBV. ppm	Found ppm	Mean Recovery (%)
2	**1.96**	**98.80**
10	**9.79**	**97.90**
30	**28.4**	**94.66**

(3-4-B-9) Detection and quantification limits:

The detection limit (LOD) and the limit of quantification (LOQ) was definitely as LOD=3 SD/K and LOQ=10 SD/K where SD is the standard deviation of five replicate determination value under the same condition in the absence of the drug and K is the slop of the Calibration curve [76].

According to these two parameters the LOD and LOQ were found to be (0.77) (2.57) respectfully.

(3-4-B-10) Analytical Applications:

The results obtained from the application of the proposed method for the analysis of MBV in its pure from and its pharmaceutical preparations indicate that the proposed method was accurate and precise. The low value of relative standard deviation (R.S. D%) indicated good reproducibility and precision. The mean of percent recoveries obtained were in the range of (97.18-99.55) indicating good accuracy of the proposed method.

Table (3-10). The obtained results from the application of the proposed method.

Company	Claimed	Found	R.S.D%	Recovery (%)
Duspatalin	**135mg**	**131.10**	**1.31**	**97.11**
EVACO	**135mg**	**133.10**	**1.49**	**98.85**
LMEVA	**135mg**	**133.98**	**1.34**	**99.24**

Table (3-11) statistical parameters of the proposed method

Parameters	**Value**
Maximum absorption	382 nm
Beers law is range	1 – 30 ppm
Molar absorptivity	(3.417×10^4L/ Mol. Cm)
sandal sensitivity	(0.013 µg. Cm^{-2}),
RSD %	(0.64%)
mean recovery	(90-98 %)
correlation coefficient	(R^2 = 0.9904
LOD	0.77 ppm
LOQ	2.57 ppm

Table (3-12) F-Test and T-Test for comparison of Accuracy and precision between proposed method and standard method

Pharmaceutical preparation containing MBV	**Proposed method**		**Standard method**	
	Recovery%	SD^2	Recovery%	SD^2
Duspatalin	97.11	2.94	98.27	2.00
EVACOL	98.85	3.93	98.51	2.64
MEVA	99.24	3.22	98.85	1.73
	$\bar{x}$=98.40	Ɛ=10.09	$\bar{x}$=98.54	Ɛ=6.37

(3-4-C) Method (III) mebeverine and iodine

(3-4-C-1) Recommended procedure:

Transfer 0.3-7 ml of 100 ppm of standard MBV into a series of 10 ml volumetric flask then 1.5 ml of standard solution of (3.9×10^{-3} M) Iodine was added, mixed and diluted to volume with dichloromethane. Keep the solution in the dark for 30 minutes. Measure the absorbance at 360 nm against simultaneously prepared blank.

(3-4-C-2) Absorption spectrum

The scanning of the colored complex was carried out, by using U.V spectrophotometer in the range 200-400 nm. It examined the complex which

shows the maximum absorbance at 360nm with was selected as the specifically wave length for determination of the drag. Shown in figure (3-15)

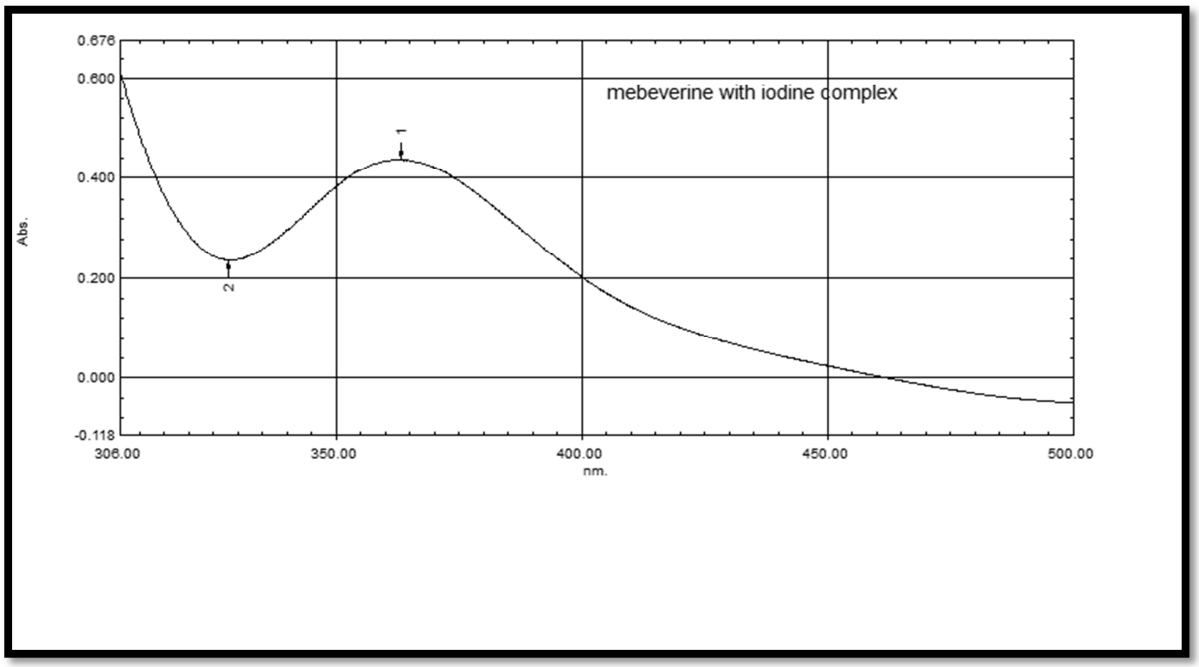


Figure (3-16) Absorption spectra of the colored compound (20 PPM) 0f MBV with Iodine (3.9×10^{-3}M).

(3-2-C-3) Optimization of the experimental Conditions

Various parameters that effect on the absorption intensity of the complex formed were studied and the reaction conditions were optimized

(3-2-C-3-1) Effect of Iodine Volume:

Different volumes were taken from the ligand in the range (1 - 3 ml). The volume1.5ml of $2.9\text{x}10^{-3}$ M gave the best absorption intensity at λ max 360nm, while 3,3.5,4 gave very high absorption: Shown in figure (3-13)

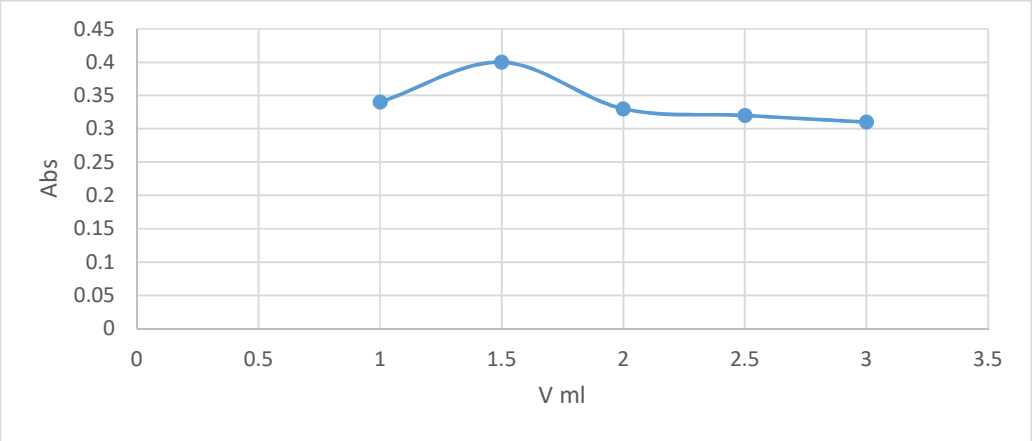


Figure (3-17) Effect of different volume of 3.9x10 $^{-3}$ M Iodine on absorption of the complex between MBV(20μg/ml) and Iodine.

(3-4-C-3-2) Effect of reaction time:

The color intensity of the complex reached a maximum absorption after MBV had been reacted with Iodine at 30 min. Therefore 30 min development time was chosen for further we. The results obtained are shown in figure (3-16)

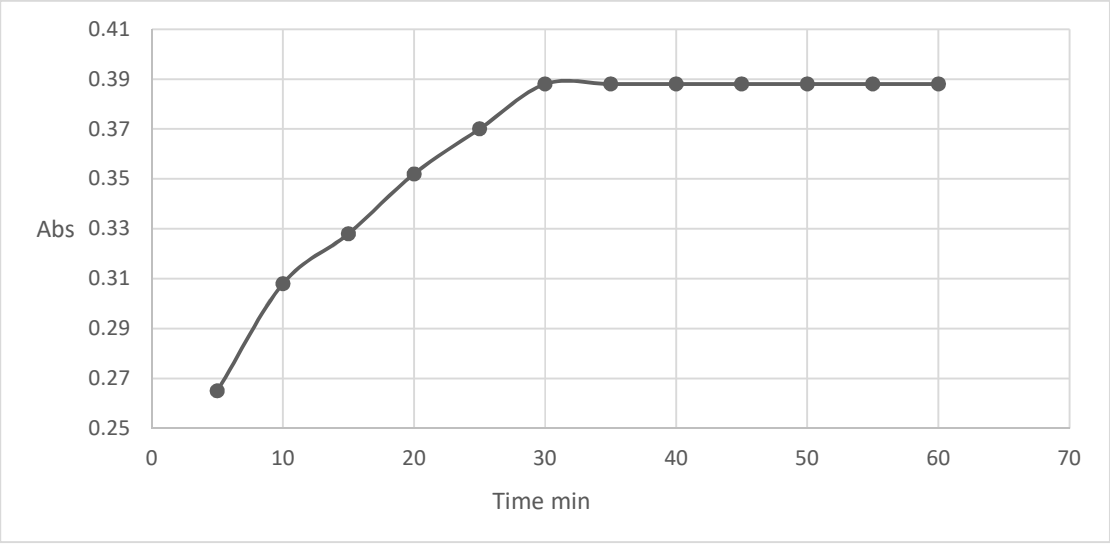


Figure (3-18): Effect of Time on the absorption of MBV(20μg/ml).

(3-4-C-3-3) Effect of temperature:

The effect of temperature on the complex stability was not studied due to the fact the solvent used is DCM and the boiling point of the solvent is less than 40c°

(3-4-C-3-4) Choice of organic solvent:

A different organic solvent as Dichloreethane, dichloromethane, chloroform and acetone were tested to choose the suitable solvent to give high absorbance. Dichloromethane was preferred to other solvents for its selectivity and giving the highest absorbance

Table (3-17) Effect of Solvents on Absorption

Solvent	Abs
Dichloroethane	0.299
Dichloromethane	0.410
Chloroform	0.101
Acetone	0.107

(3-4-C-4) Calibration curve:

Under the optimum conditions, a linear Calibration curve for the determination of MBV was obtained over the range of (1.2-28 ppm). the linear regression equation Y=0.0195X+0.0025 with molar absorptivity of (9.08×10^3mol L^{-1} cm^{-1}) [70]and correlation coefficient of 0.9979.and sandal sensitive is 0.05 μg.cm^{-2} the linear Calibration curve is shown in figure (3-16)

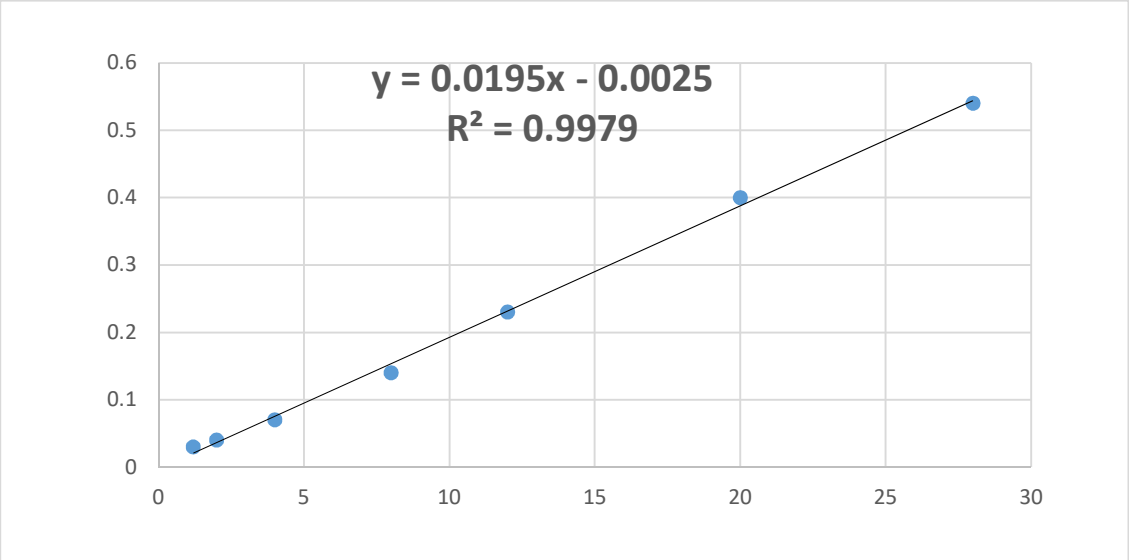


Figure (3-19) Calibration curve for the determination of MBV

(3-4-C-5) Precision and Accuracy of the proposed method

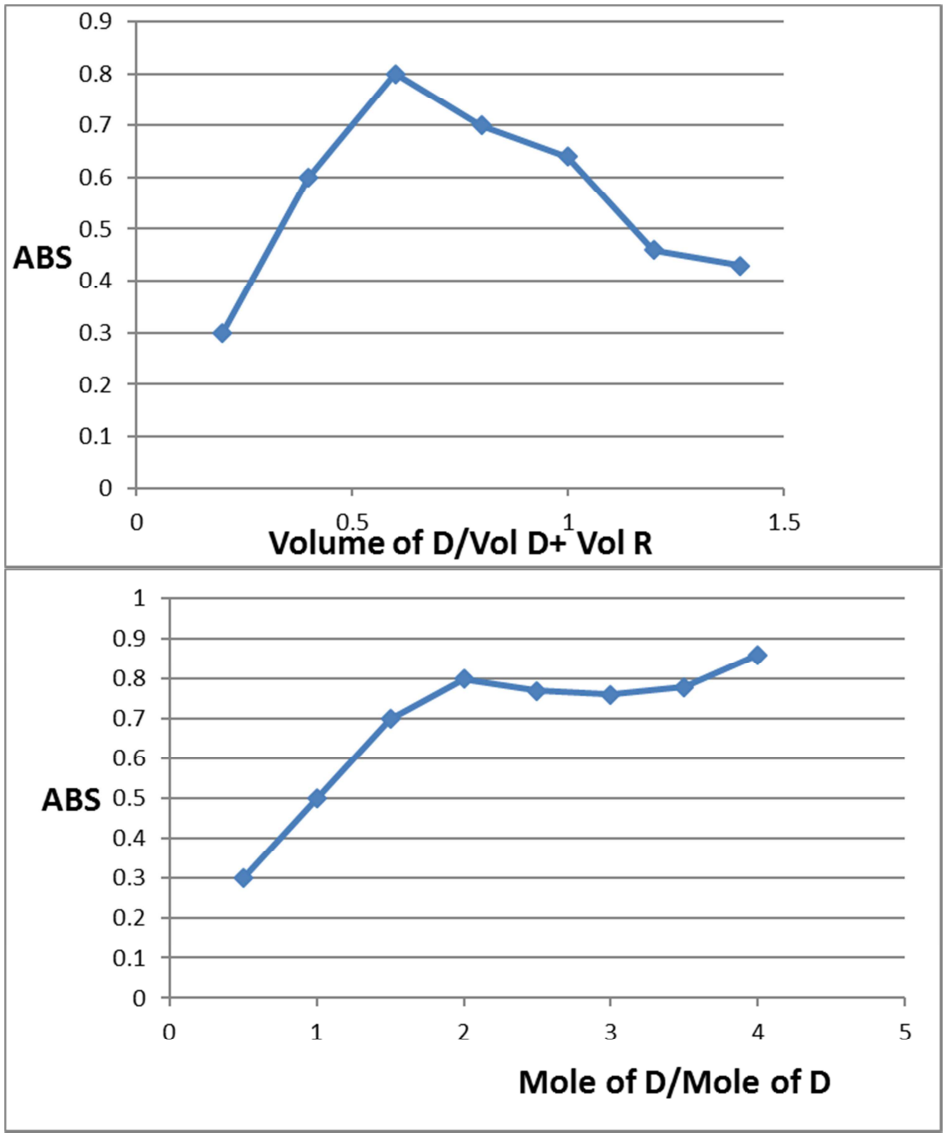
The result shows the proposed method was accurate to according the values of the recovery with range of (91.25 – 103.20) when the three levels of concentration were measured also the low values of R.S.D % [71] indicted that the method was highly precise (2.23 – 2.28) %.

Table. (3-14) Precision and Accuracy of the proposed method

N	Concentration in ppm	Found	R.S.D%	Recovery%
1	8	7.30	2.23	91.25
2	20	20.64	1.66	103.20
3	28	27.82	2.28	99.35

(3-4-C-6) Nature of the dye product [72-74]:

The stoichiometry of the reaction between Mebeverine and Iodine (3.9×10^{-3}M) was investigated using the mole ratio and Jobs method. The results obtained in figure (3-16) show 2: 1 drugs to reagent product was formed



A B

Figure (3-20) A mole fraction of drug and B represented the mole ratio

(3-4-C-7) Stability of the ion-pair complexes

The stability of the formed complex between the MBV and Iodine was evaluated. Although the constant absorbance readings were obtained at short time 30 min and 25 c°. The formed complex was stable for at least 2h without and change in intensity of absorbance or in λ max. The conditional constant was calculated according to literature [75], and found to 75.55×10^5 , and this supported the stability of the formed complex

(3-4-C-8) Interference studies:

The results showed that no interferences where found in the presence of 20 ppm for each excipient (Glucose, lactose, starch, magnesium, stearate, talk, Aacia) with these concentrations different concentration of the drug up to (2,4,28) ppm in the determination of MBV.

Table (3-15) shows the effect of excipient interfering materials that present in pharmaceutical preparations of the drug

Concentration of MBV.ppm	Found ppm	Mean Recovery%
2	**1.85**	**92.50**
4	**3.94**	**98.50**
28	**27.69**	**98.89**

(3-4-C-9) Detection and quantification limits:

The detection limit (LOD) and the limit of quantification (LOQ) was defined as LOD=3 SD/K and LOQ=10 SD/K where SD is the standard deviation of replicate determination value under the same condition in the absence of the drug and K is the slop of the Calibration curve [76].According to these two parameters the LOD and LOQ were found to be (0.72) (2.50) respectfully.

(3-4-C-10) Analytical application:

The results obtained from the application of proposed method for the analysis of MBV in its pure form and its pharmaceutical preparations indicate that the proposed method was accurate and precise

Table (3-16) application of the proposed method

Company	Claimed	Found	R.S.D%	Recovery (%)
Duspatalin	**135mg**	**132.20**	**1.33**	**97.92**
EVACOL	**135mg**	**133.51**	**1.49**	**98.88**
MEVA	**135mg**	**132.17**	**1.38**	**99.55**

Table (3-17) statistical of parameters of the proposed method

Parameters	**Value**
Maximum absorption	**360 nm**
Beers law is range	**1.2 – 28 ppm**
Molar absorptivity	**$9.08.\times 10^{3}$L/ Mol. Cm**
sandal sensitivity	**0.05 µg. Cm^{-2}**
RSD %	**2.05%**
mean recovery	**91-103** %
correlation coefficient	**$R^{2} = 0.9979$**
LOD	**0.72 ppm**
LOQ	**2.50 ppm**

Table (3-18) F-Test and T-Test for comparison of Accuracy and precision between proposed method and standard method

Pharmaceutical preparation containing MBV	**Proposed method**		**Standard method**	
	Recovery%	SD^2	Recovery%	SD^2
Duspatalin	97.92	1.75	98.27	2.00
EVACOL	98.88	1.98	98.51	2.64
MEVA	99.55	1.82	98.85	1.73
	x^{-}=98.78	Ɛ=5.55	x^{-}=98.54	Ɛ=6.37

Part two
HPLC Method

(3-5) The operating conditions

Table (3-19) show the operating conditions

Mobile phase	acetonitrile: 0.02 M potassium dihydrogen phosphate (70: 30: v/v) PH 3.6
Wavelength	263nm
Run time	2.5 min
pH of mobile phase	3.5
Flow rate	1 ml /min
Injection volume	20µl
Temperature	Ambient ,25C^{o}
Mode of operation	Isocratic elution

(3-6) Preliminary Investigation

Note that when injecting 20 µl of the standard mebeverine solution using a mobile phase acetonitrile: 0.02 M potassium dihydrogen phosphate (70: 30: v/v) PH 3.5 at a wavelength of 263 and a flow rate of 1 ml/ min. The chromatogram showed a peak at minute Retention 1.09 min as shown in Figure (3-21)

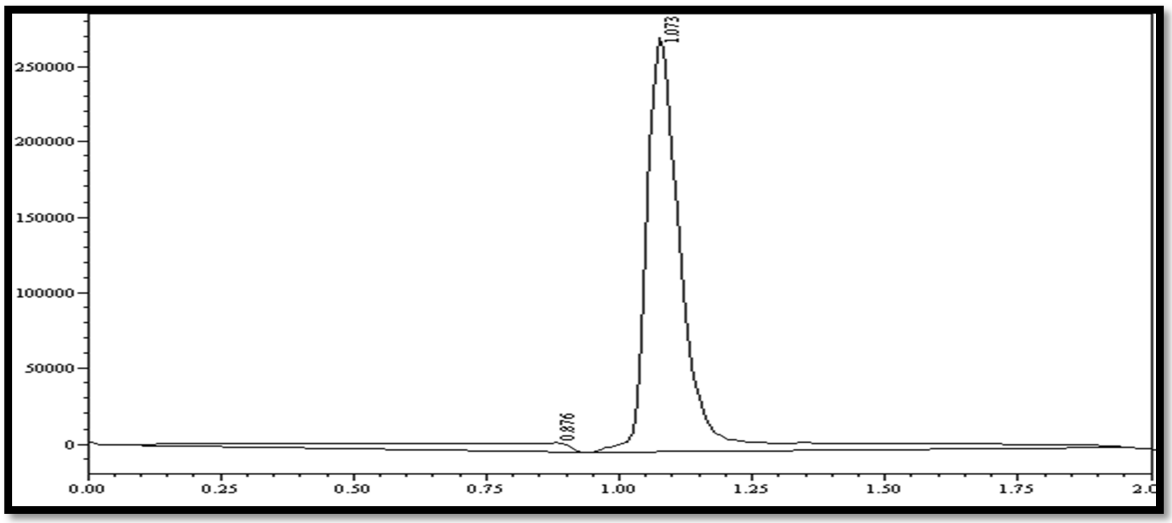


Figure (3-21) chromatograph response of 25ppm of MBV.

(3-7) Optimization of the experimental conditions

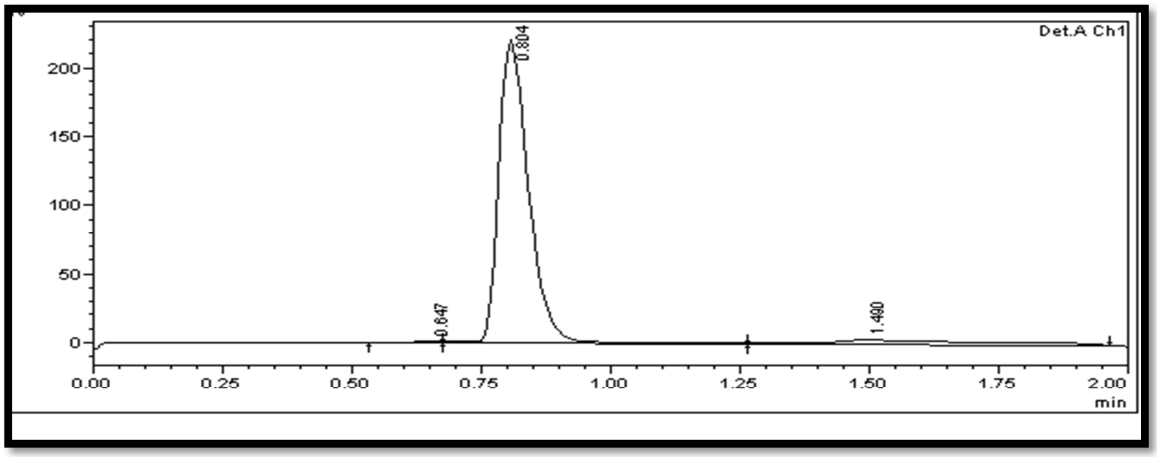
Subsequent experiments were performed using 20 µl of solution with a concentration of 25 ppm of the pure mebeverine and the retention time of the solutions at a wavelength λmax=263nm.

(3-7-1) Effect of the Flow rate of Mobile phase

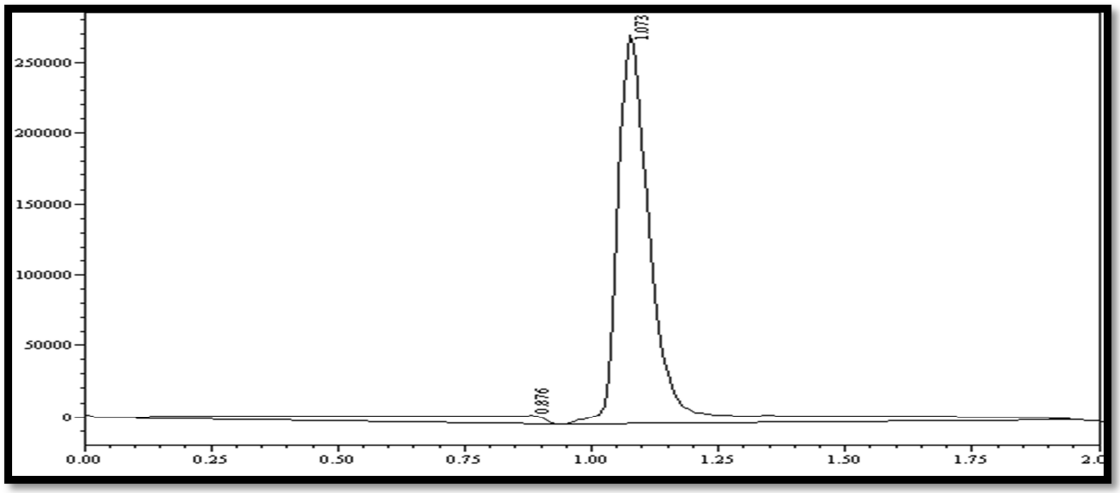
To find the best flow rate of the mobile, several experiments were performed by injecting 20 µl of mebeverine standard solution onto column C18-ODS using Acetonitrile: potassium dihydrogen phosphate (70: 30: v/v) PH 3.5 a mobile phase. Table (203-) and Figure (3-18) shows that 1 ml/min of the mobile phase the best flow rate based on the calculation of the capacitance factor [77] (K^-) and the symmetry coefficient [78](As)

Table(3-20)show capacitance factor (K^-) and the symmetry coefficient (As)
Of the Standard MBV using difrent flow rate.

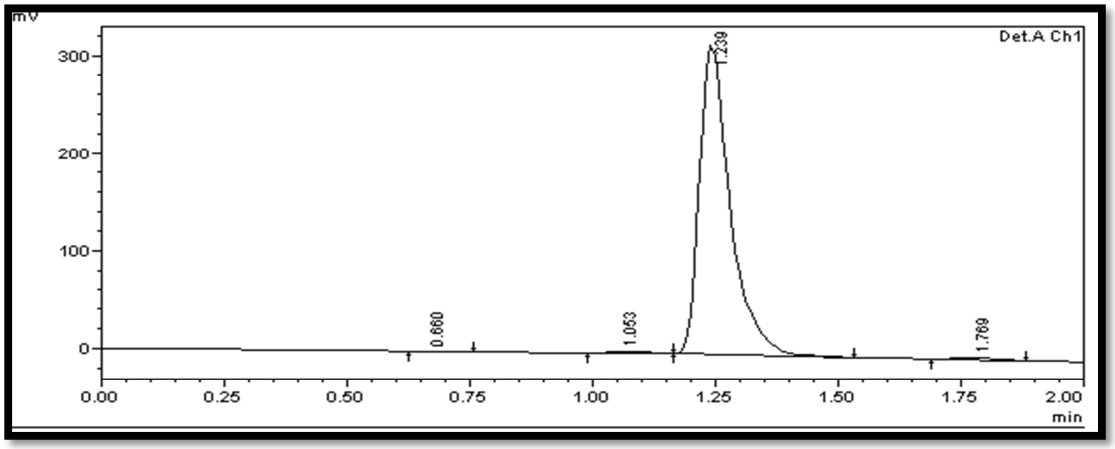
N	Flow rate ($ml.min^{-1}$)	Capacity factor(k^-)	Symmetrical factor (A_s)	Retention time(t_R)(min)
A	0.5	0.89	0.075	0.8
B	1	1.44	0.1	1.03
C	1.5	4.7725	0.125	2.309



A



B



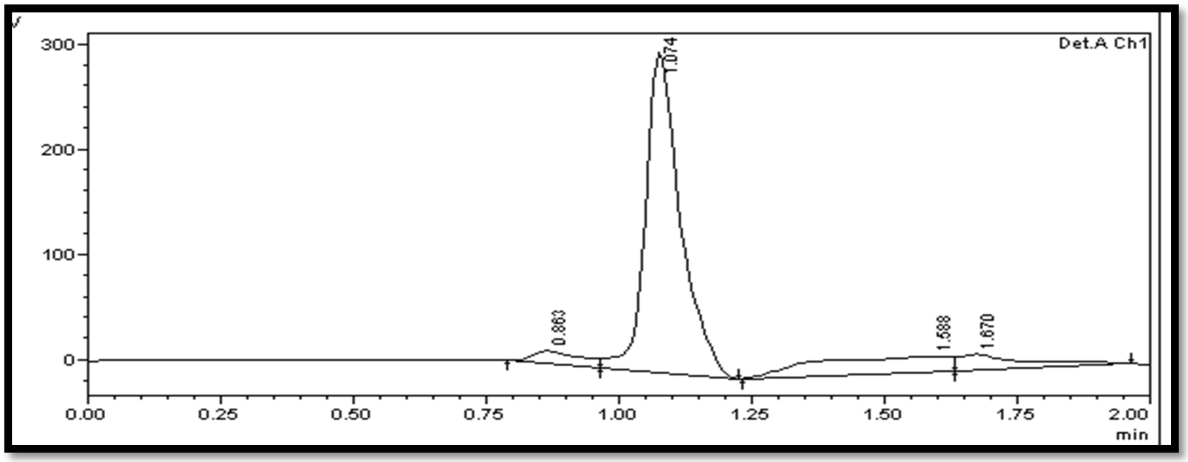
C Figure(3-22) Effect of the Flow rate of Mobile phase.

(3-7-2) Percentage of organic modifier

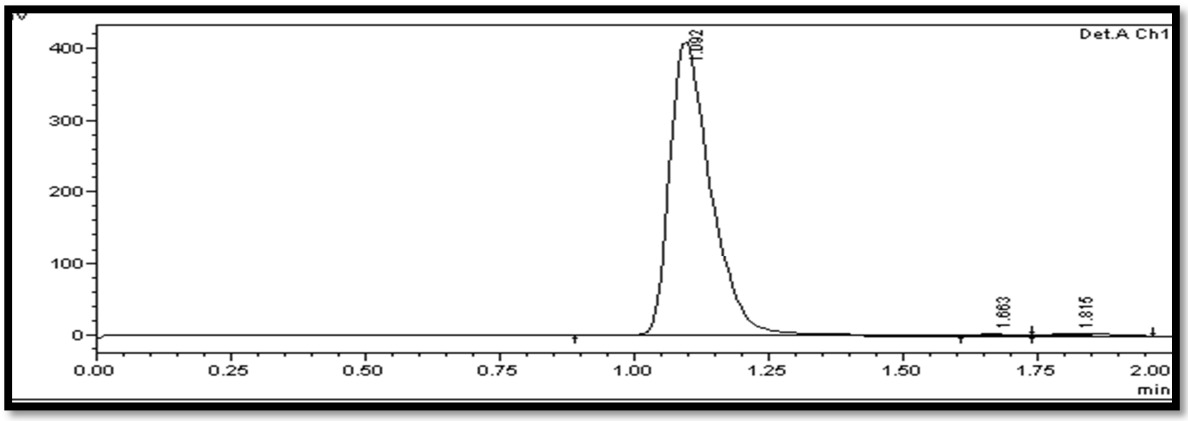
The effect of mobile phase component ratios on the retention time was studied using the best conditions obtained from previous experiments. It is clear from this study that the best ratio of the mobile phase components is 30%buffer and 70%acetonitrill, depending on the values of the K^- and the as parameters as shown in Table (3-21) and Figure (3-19)
Table (3-4) show capacitance factor (K^-) and the symmetry coefficient (As)
Of the Standard MBV using different Percentage of organic modifier.

Table (3-21) The effect of percentage of organic modifier on the parameter separation.

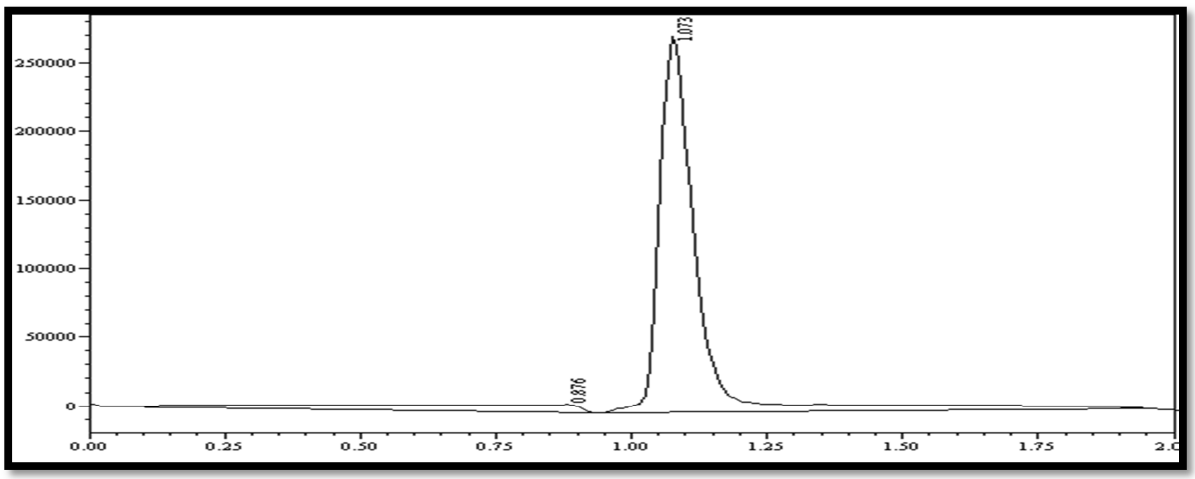
N	Mobile Phase	Ratio	Capacity factor (k^-)	Symmetrical factor (A_s)	Retention time (T_R) (min)
A	ACN: Buffer	90:10	1.74	0.1	1.07
B		80:20	1.58	0.125	1,09
C		70:30	1.44	0.1	1.03
D		60;40	1.55	0.1	1.08
E		50;50	4.505	0.12	1.202


Det.A Ch1
1.074
0.863
1.588
1.670
300
200
100
0
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
min

A


Det.A Ch1
1.092
1.663
1.815
400
300
200
100
0
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
min

B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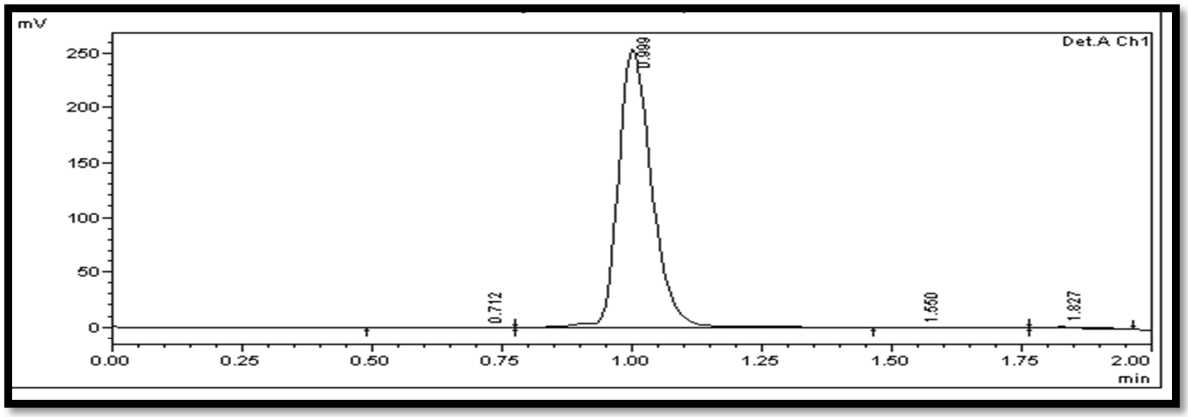
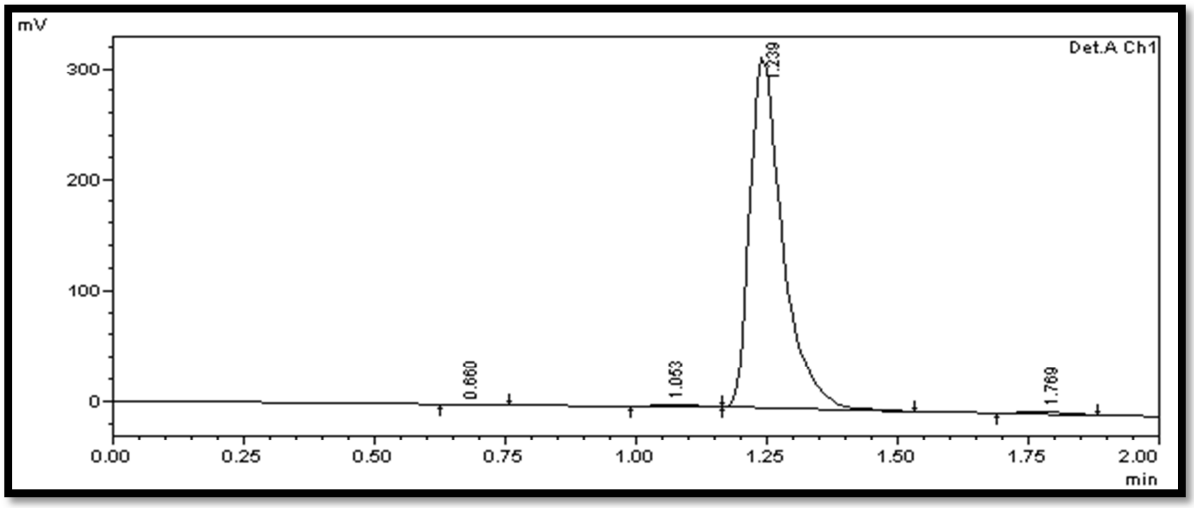
1.25
1.50
1.75

C



D



E

Figure(3-23) Effect of Ratio of the Mobile Phase contains

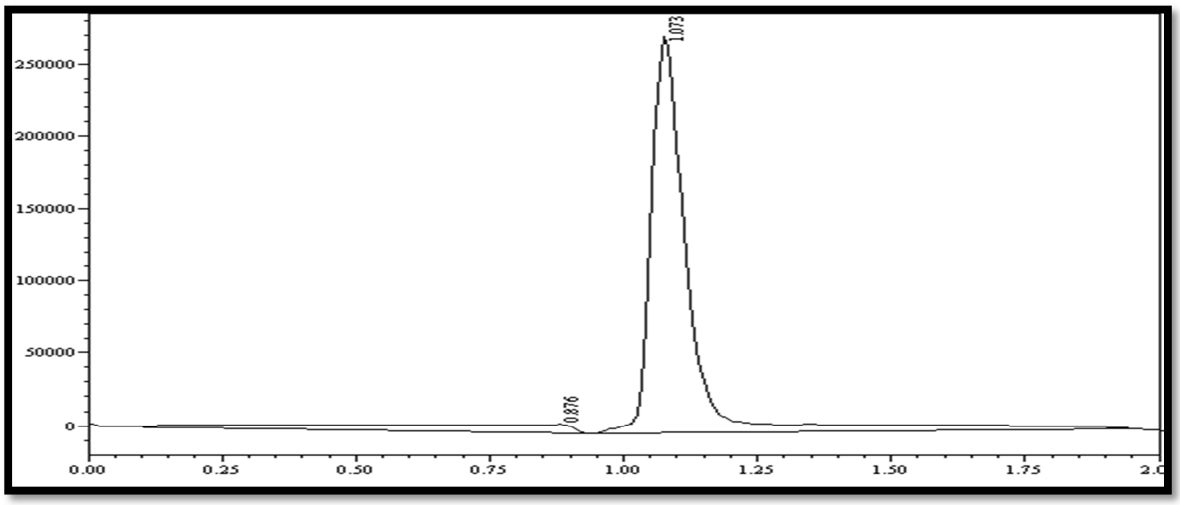
(3-7-3) effect of pH

To find the best pH of the mobile phase, several experiments were performed by injecting 20 microliters of the standard Mebeverine solution and using 30%buffer and 70%acetonitryle as a mobile phase. Table (3-22) and Figure (3-20) show that pH 3.5 of the mobile phase is the best pH depending on the calculation of the capacitance factor (K-) and the asymmetry coefficient (As)

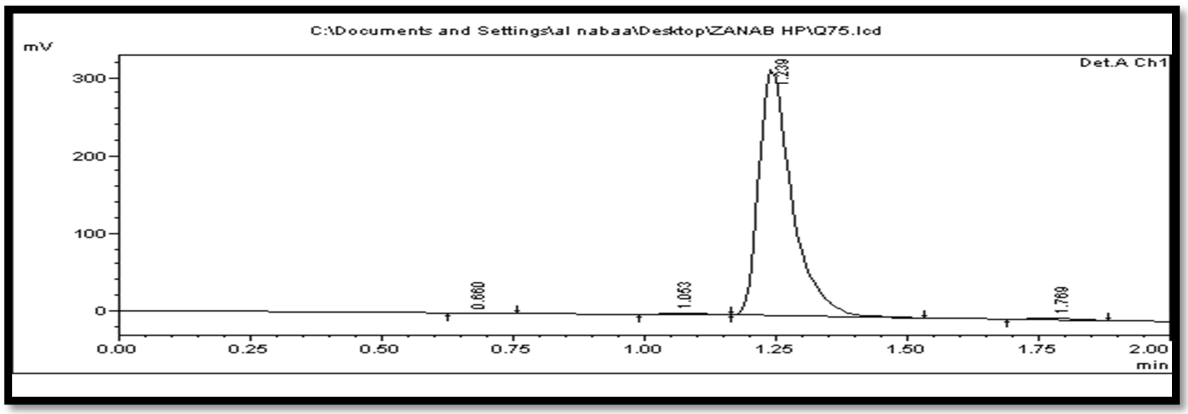
Table (3-22) show capacitance factor (K^-) and the symmetry coefficient (As)
Of the Standard MBV using different pH of mobile phase

N	pH	**Capacity factor (k^-)**	**Symmetrical factor (A_s)**	**Retention time (t_R(min)**
A	3.5	1.44	0.1	1.03
B	4	1.84	0.075	1.2
C	4.5	2.08	0.056	1.3
D	5	2.31	0.1	1.4

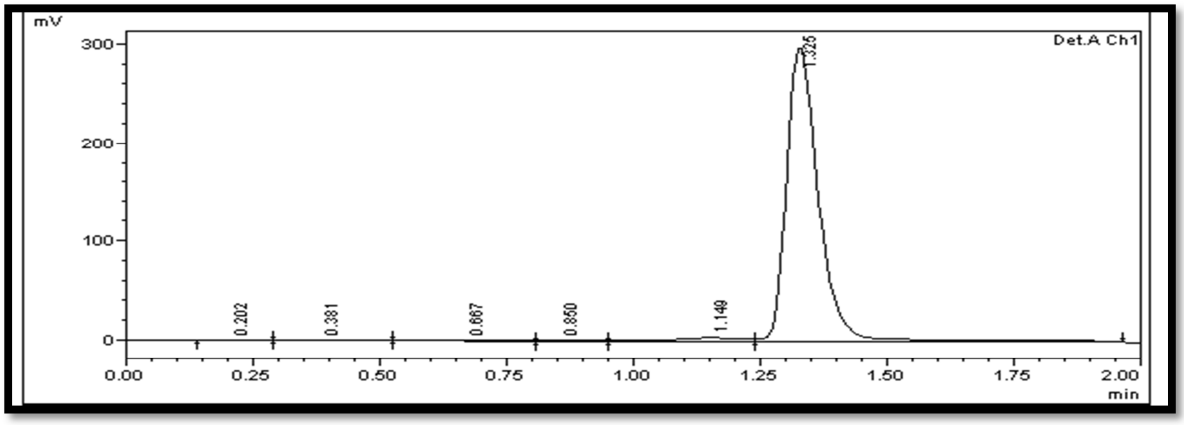
.



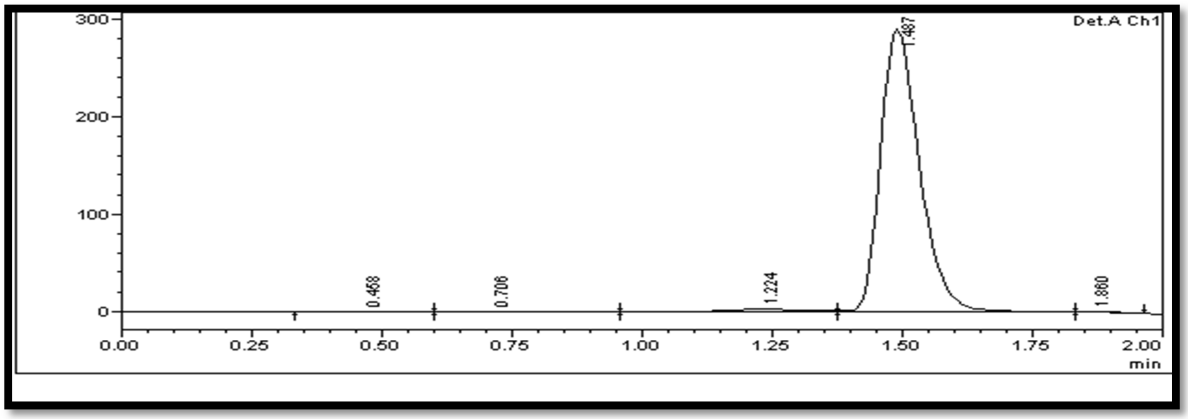
A



B



C



D

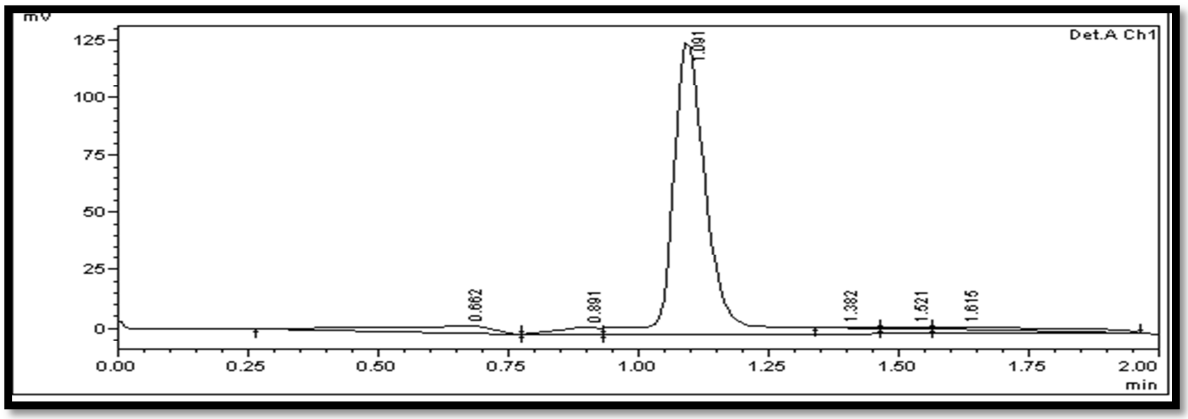
Figure (3-24) effect of pH

(3-7-4)Effect of volume injection

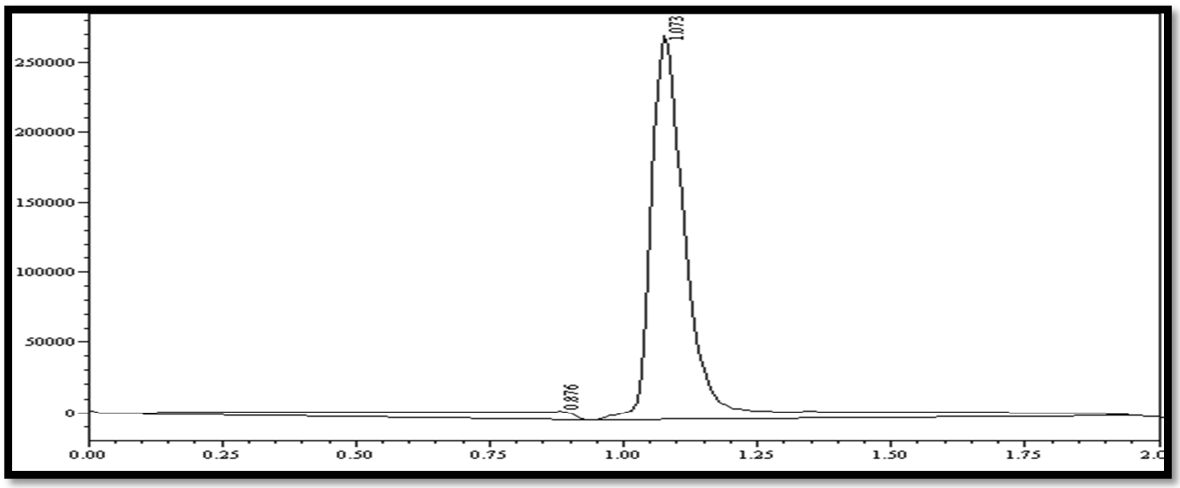
To obtain the best volume of injection from the mobile phase, several experiments were performed by injecting different volumes of the standard mebeverine solution using 100µl loop and 30% of the buffer and 70% of acetonitrile pH 3.5 as a mobile phase. Table (3-23) and Figure (3-21) show that the mobile phase is the best size 20µl based on the calculation of the capacitance factor (K-) and the asymmetric factor (As).

Table (3-23) show capacitance factor (K^-) and the symmetry coefficient (As) Of the Standard MBV using different volum injeaction .

N	Volume injection	**Capacity factor (k^-)**	**Symmetrical factor (A_s)**	**Retention time (t_R) (min)**
A	10	1.58	0.075	1.09
B	20	1.44	0.1	1.03
C	30	1.825	0.06	1.130



A



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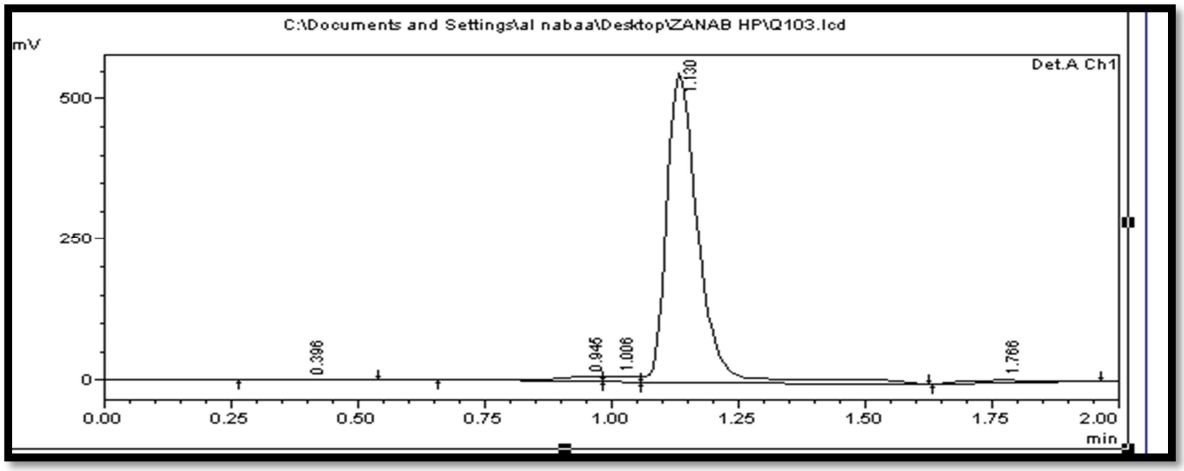


C

Figure (3-25) Effect of volume injection

(3-7-5) Calibration curve

A series of standard solutions was mebeverine within the range of 1-100ppm and 20 µl of each standard solution was applied to the HPLC column. Operating conditions were used. The peak area was calculated at a 263 nm wavelength and the standard linear curve between the area of the summit and the concentration Figure(3-22) shows the results obtained. The linear limits fall within the range of 1-100 ppm. The deviation follows with a highest concentrations

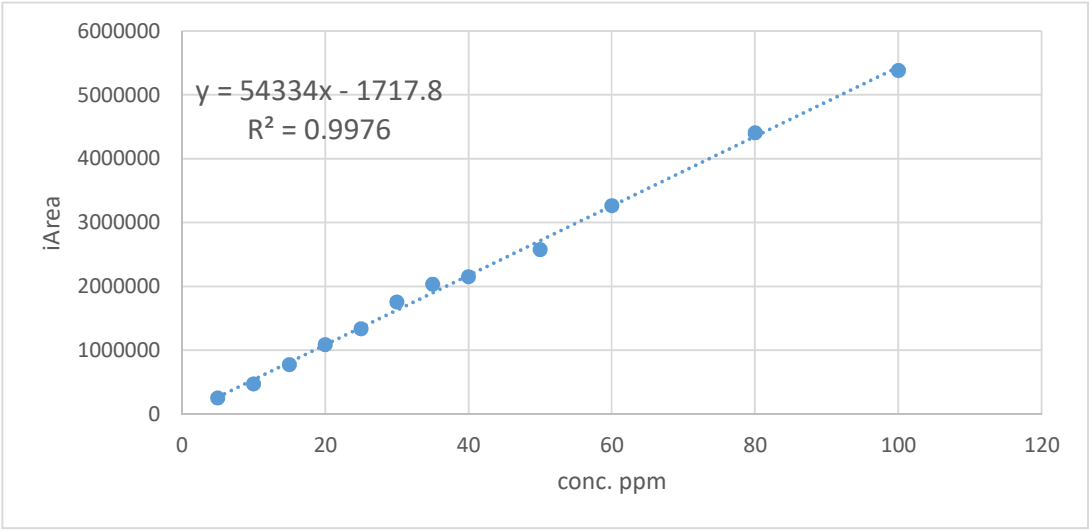


Figure (3-26) Calibration curve of estimation of mebeverine HCl.

(3-7-6) Statistical Treatment of the Analytical Results

Table (3-25) shows the statistical values obtained

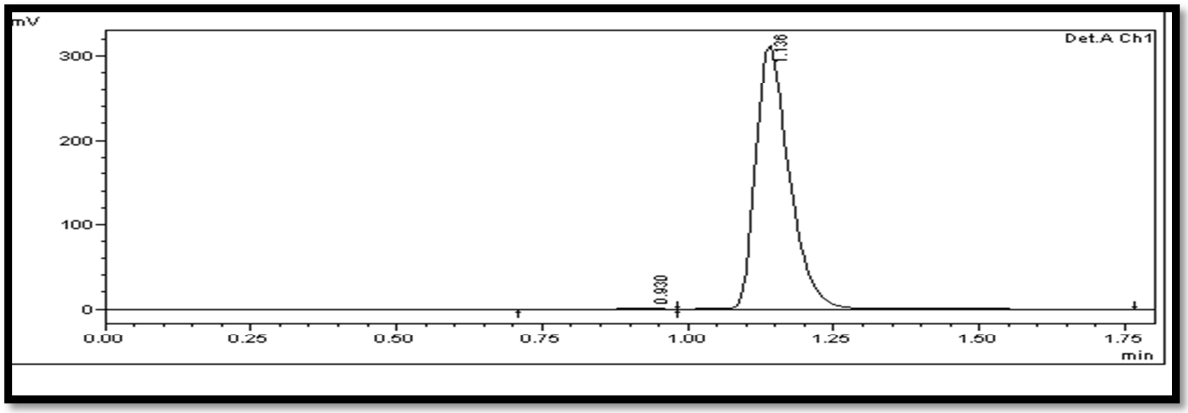
Parameters	Value
Correlation coefficient (R^2)	0.9976
Slope(b)	54334
Intercept (a)	-1717.8
Calculated F-test	0.0006
Calculated T-test	2.06

(3-8) Precision and Accuracy the proposed Method

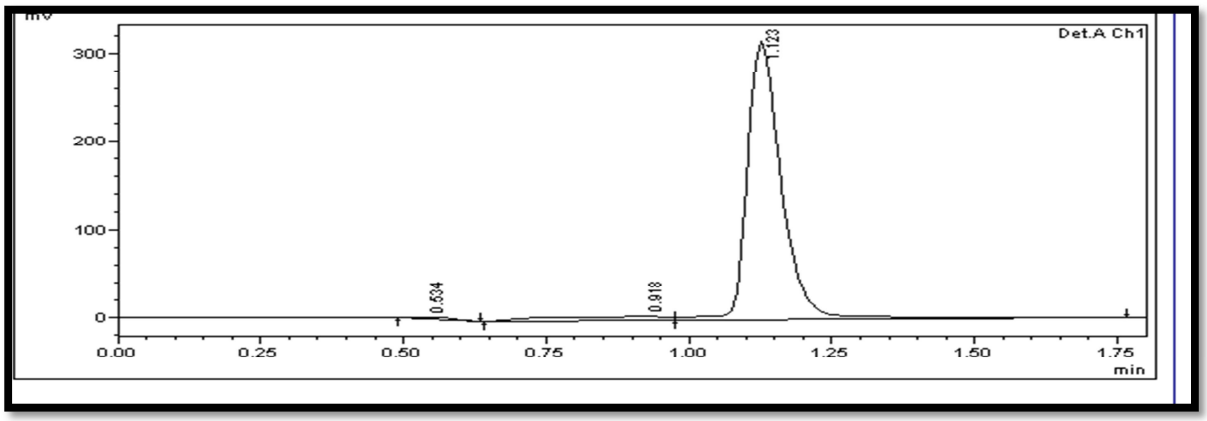
The relative standard deviation [79-81] (RSD) compatibility method was calculated and the accuracy of the percentage error and regression method was measured by measuring three different concentrations of 25,50 and 100 ppm and each three measurements ,the mean of the data was estimation, according to the standard working method under the standard curve and summarizing the results in Table (3-26).

Table (3-26) System precision and recovery [82,83] results of Mebeverine HCl.

N	Conc. of Mebeverine µg.ml^{-1}		Recovery%	R.S.D%
	Present	Found		
1	25	24.42	97.69	3.03
2	50	45.51	91.02	3.75
3	100	93.09	93.09	3.37



A1



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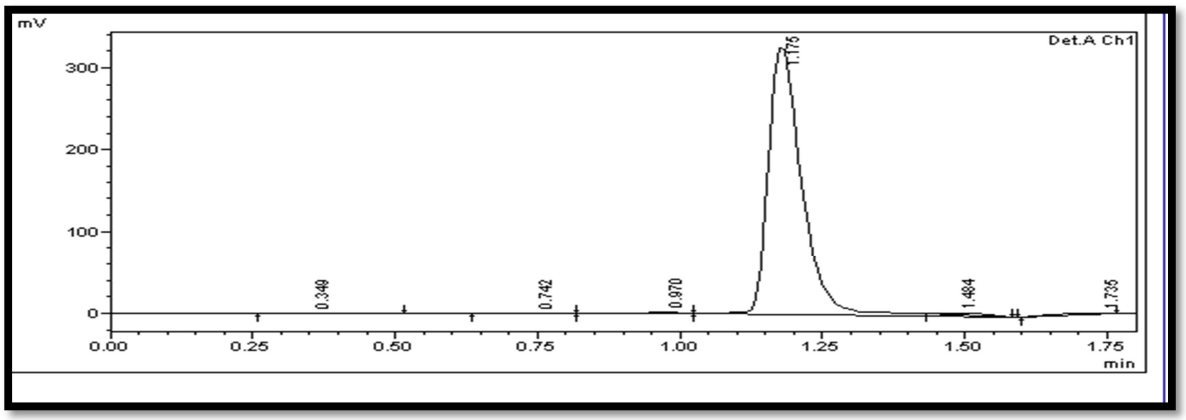
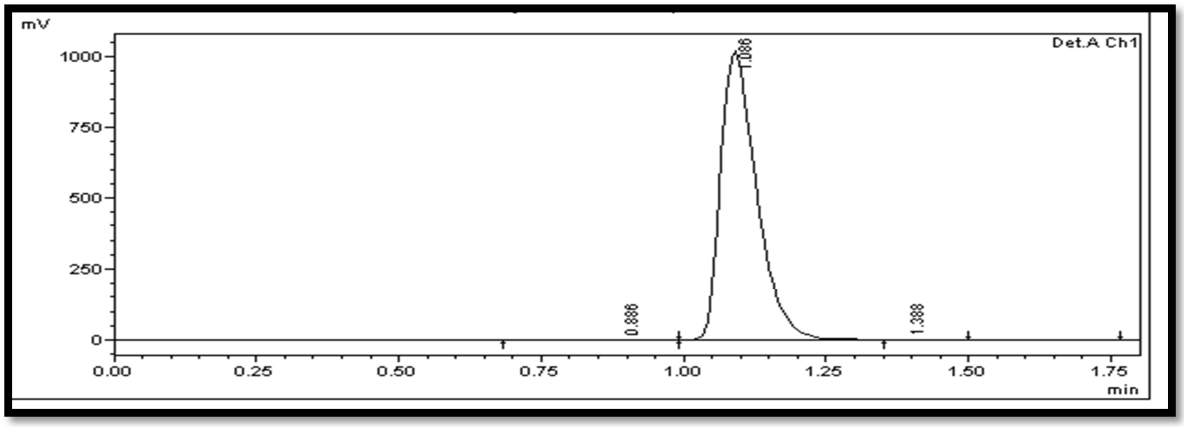
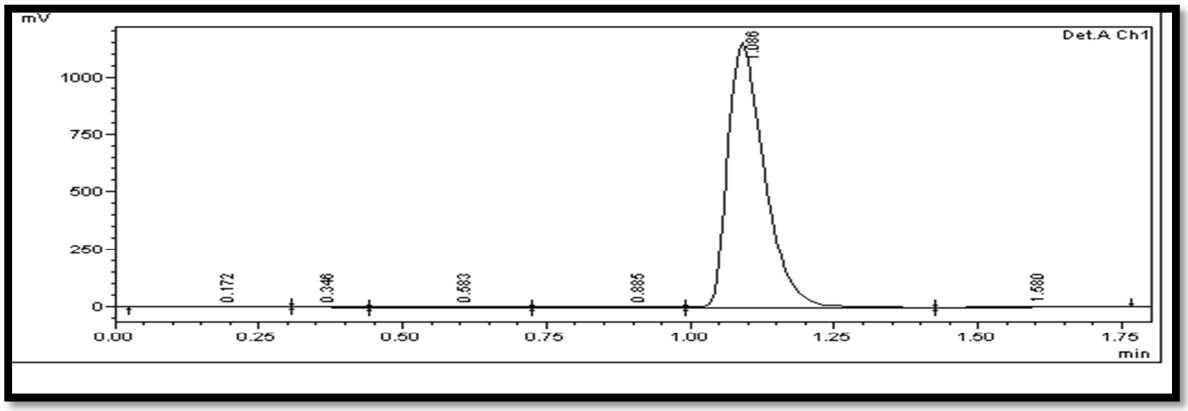


A

Figure (3-27) Represents the repeatability of the results to a concentration of 25 µg. ml^{-1} of the standard mebeverine solution.



B1



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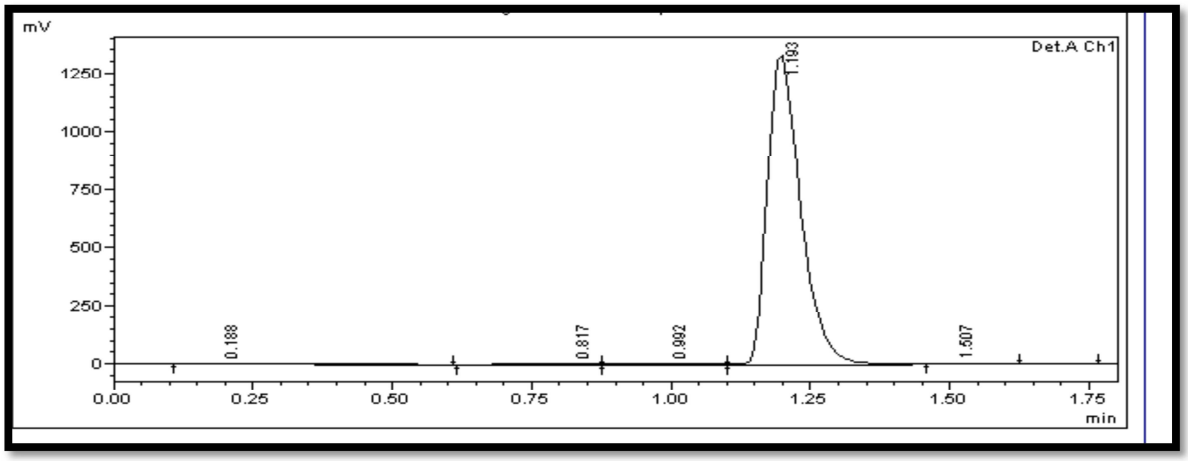


B3

Figure (3-28) Represents the repeatability of the results to a concentration of 100 µg. ml^{-1} of the standard mebeverine solution.

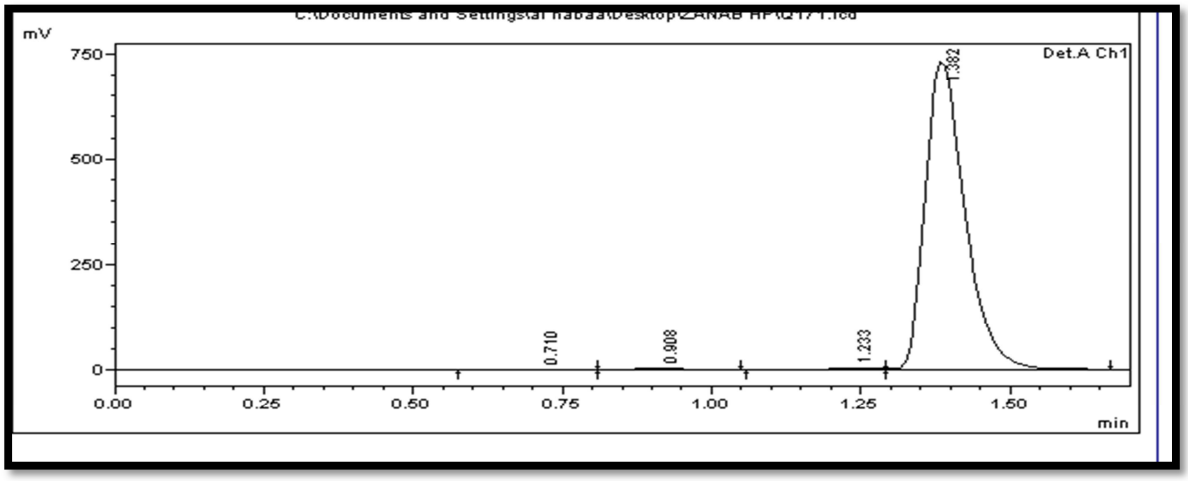
(3-9) Application of the Proposed method in pharmaceutical preparations.

The result obtained from the application of the proposed method **for** the analysis of MBV in its pure form and in its pharmaceutical preparations indicate that the proposed method was accurate and precise. The low value of relative standard deviation (R.S. D%) indicated good reproducibility and precision. The mean of percent recoveries obtained were in the range of (100.69-105.83) indicating good accuracy of the proposed method.

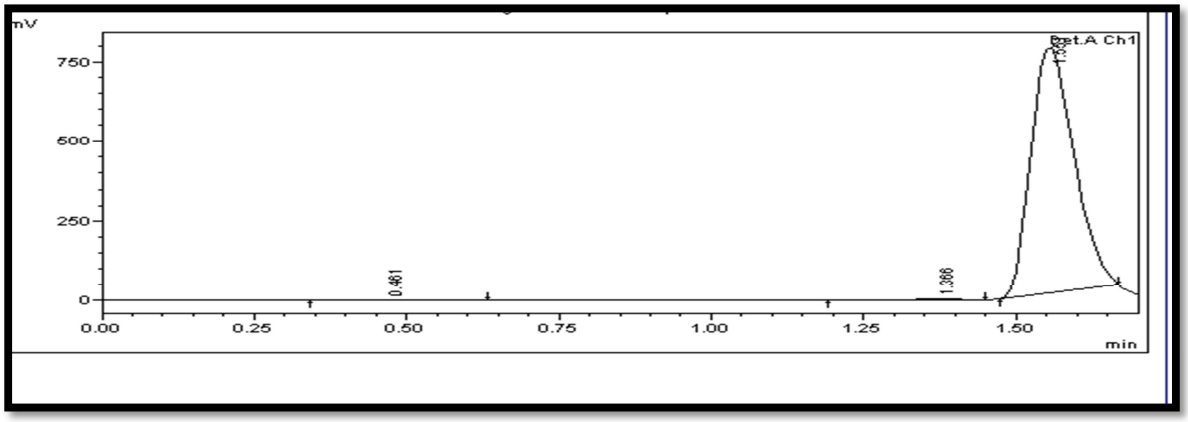
Table (3-27). The obtained results from the application of the proposed method.

Pharmaceutical Preparations	**Conc. of MBV μg.ml^{-1}**		**Recovery %**	**R.S.D%**
	Present	**Found**		
EVACOL	135	135.87	100.61	0.55
Duspatalin	135	136.53	101.13	1.72
MEVA	135	135.93	100.68	0.63

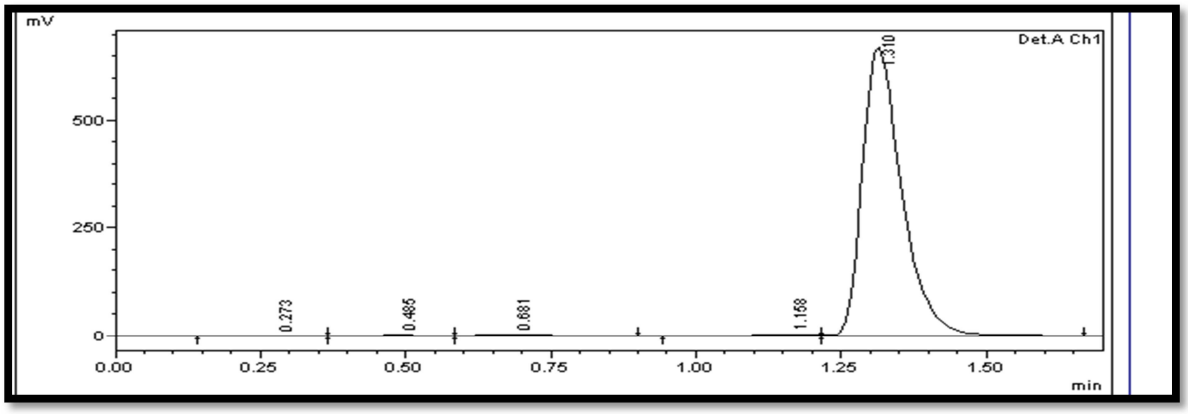
(each concentration was measured of three replete and the mean of the data was estimation.)



A1

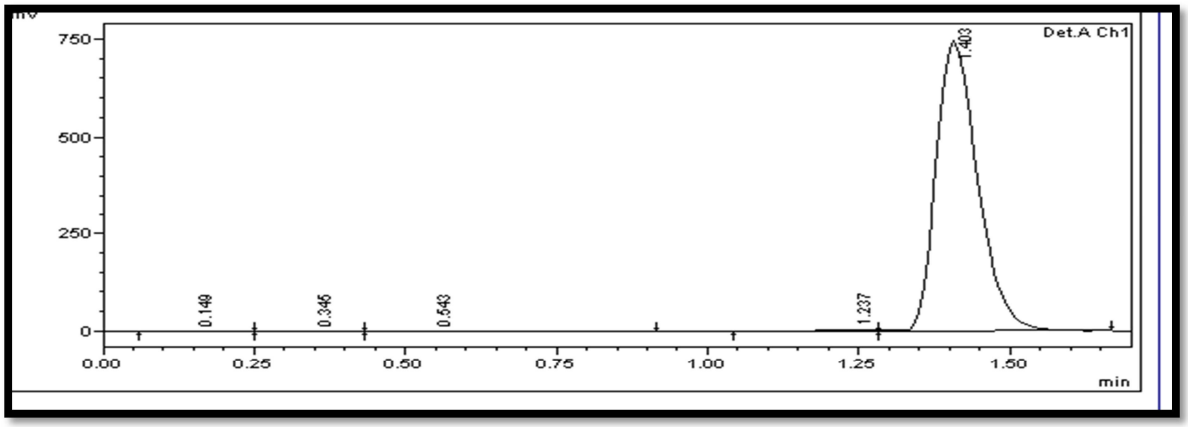


A2

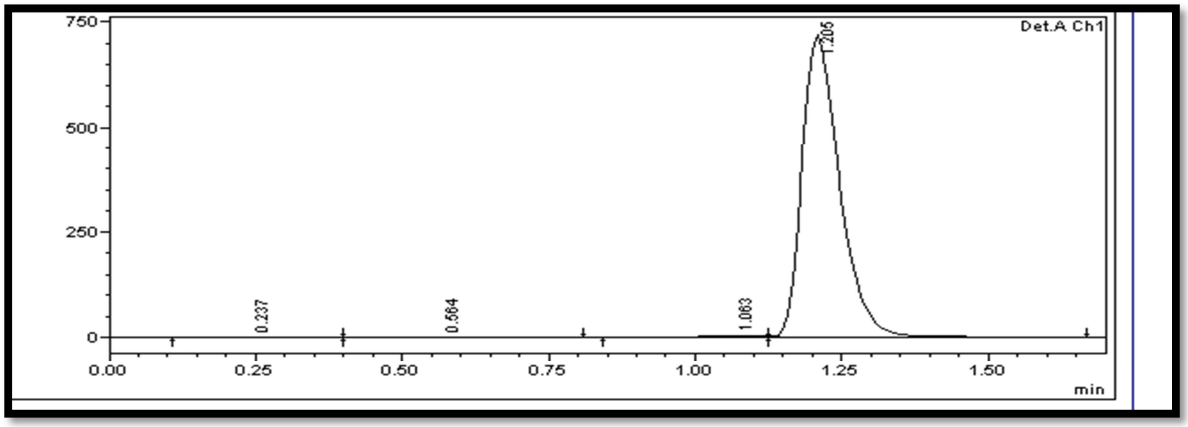


A3

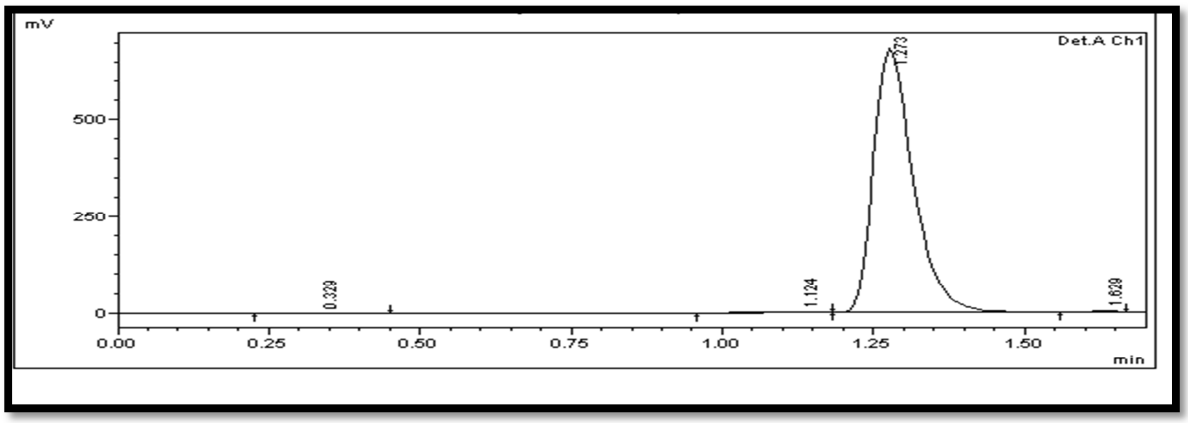
Figure (3-29) Represents the repeatability of the results to a concentration of 60 µg. ml^{-1} of the standard mebeverine (COLES-135mg) solution.



B1

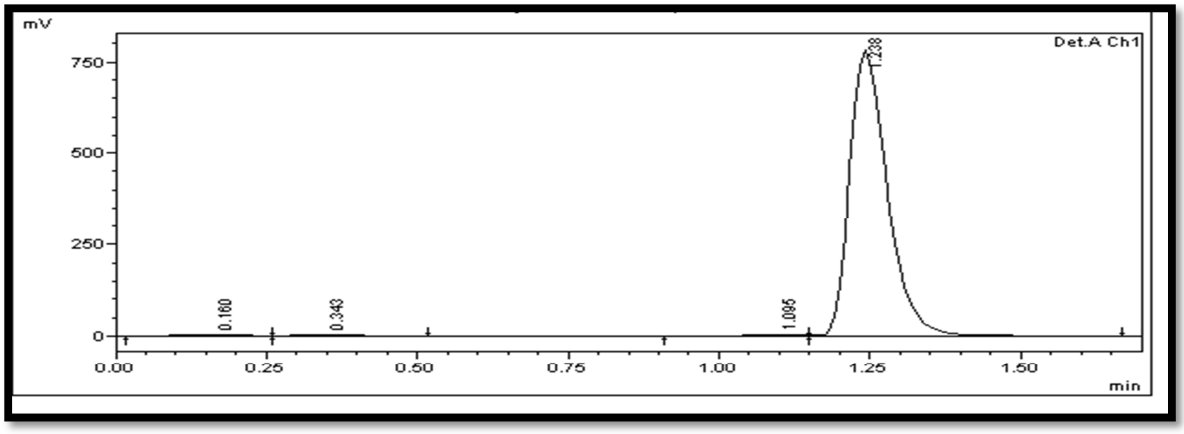


B2

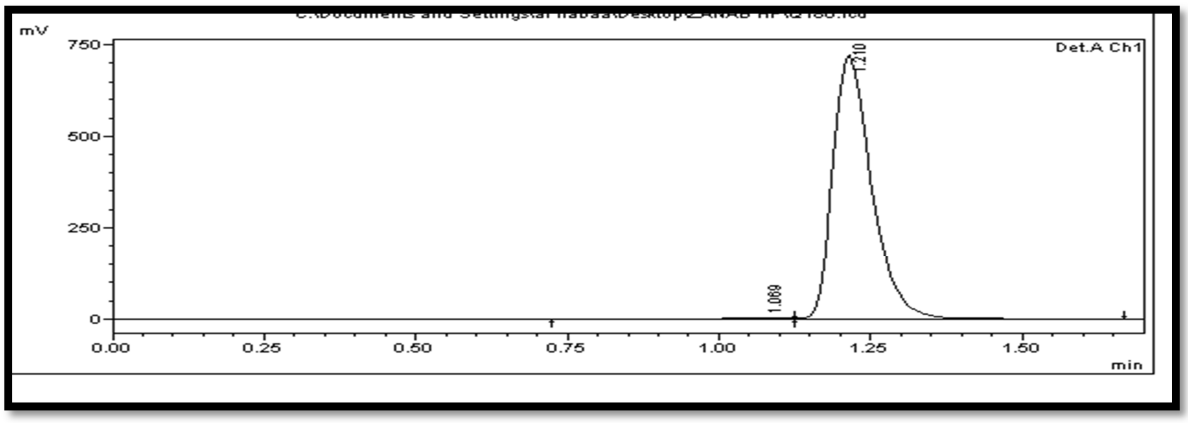


B3

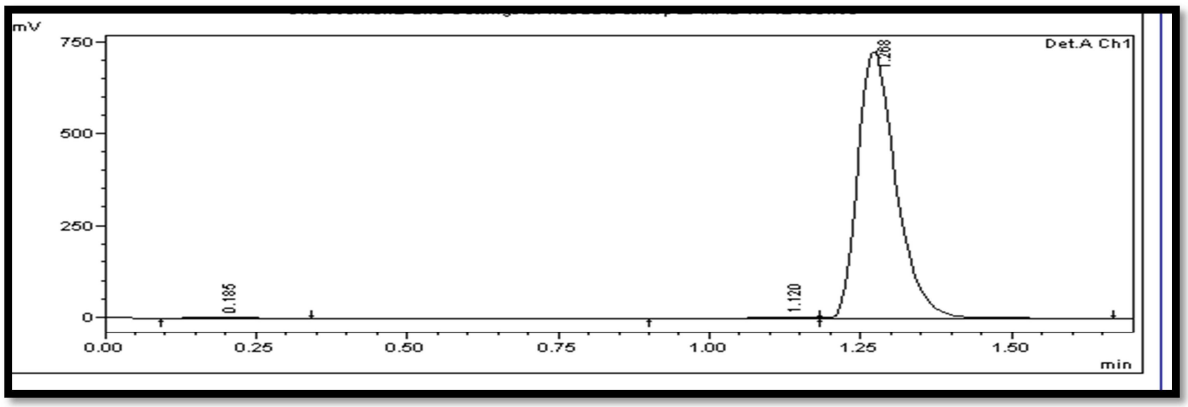
Figure (3-30) Represents the repeatability of the results to a concentration of 100 μg. ml^{-1} of the standard mebeverine (DUSPATALIN(Abbott)-135mg) solution.



C1



C2



C3

Figure (3-31) Represents the repeatability of the results to a concentration of 100 µg. ml^{-1} of the standard mebeverine MEVA-135mg solution.

Table (3-28)F-Test and T-Test for comparison of Accuracy and precision between proposed method and standard method

Pharmaceutical preparation containing MBV	**Proposed method**		**Standard method**	
	Recovery%	SD^2	Recovery%	SD^2
Duspatalin	101.13	2.35	98.27	2.00
EVACOL	100.61	0.74	98.51	2.64
MEVA	100.68	0.86	98.85	1.73
	$\bar{x}$=100.80	Ɛ=3.95	$\bar{x}$=98.54	Ɛ=6.37

(4-1) Recommendations

1. Possibility to apply Rp-HPLC for estimation of mebeverine HCl in various pharmaceuticals preparation and biological samples.
2. Possibility to apply Rp-HPLC for determination of related compounds of mebeverine HCl.
3. Possibility to apply ion pair method for determination of some drug that contain amino group.
4. Possibility to apply ion pair method for estimation of mebeverine HCl by flow-injection method.

(4-2) conclusion

1-The spectrophotometer method accuracy, sensitive, The proposed method has some advantage like fast determination ion of the drug in its pure and in pharmaceutical preparation. The wide ranges of the linearity of the method gave a good application for the pharmaceutical preparation, The absence of the effect of the interference in the analysis made the method useful for routine analysis and quality control assay of the drug in raw material and in Tablets.

The HPLC method is fast, simple, and accurate stability-indicating HPLC method has been developed for the simultaneous determination of MBV, the method allows identification, quantitative analysis, and purity assessment to be performed simultaneously. The proposed method appears to be suitable for quality control laboratories, where economy and time-saving are essential.

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Appendix

Analytical and statistical laws used in research

1-A=Ɛbc

Where A absorbance, Ɛ molar an absorbance, b sample pathlength, c concentration

2- S= M/Ɛ

Where S Sandal sensitive, M Molecular weight, Ɛ molar a absorbance.

3-- S.D =

Where SD is the Standard deviation, Xi is the experimental value, X^- is theoretical value, n number of value.

4- R.S.D% = (S.D /) *100

Where R.S.D % is the relative Standard deviation, SD is the Standard deviation, X^- is theoretical value

5-As= A/B

Where As is the peak asymmetry factor, B peak Width after the peak center at 10% peak height, A peak width at baseline before the perk center.

6- ќ=(tR-to)/to

Where ќ is capacity factor, tR is the retention time, to is the void time.

8-F test = SD12/SD2 2

Where SD1 Standard deviation for method one ,SD2 Standard deviation for method two.

9-T test=lrl √(n-2)/√(1-r^2)

Where r is the correlation coefficient, n Number of concentrations studied in the calibration curve

Republic of Iraq
Ministry of Higher Education
& Scientific Research
University of Kerbala / College of Science

CERTIFICATE

This is to certify that

Abdulbari Mahdi and Zainab Abas

has presented a scientific paper entitled

Spectrophotometric Determination of mebeverine hydrochloride in pharmaceutical preparation viaIonAssociation Reaction

The 6th Conference of College of Science - University of Kerbala
Renewable Energy and its Applications
which has held on 21 - 22 Feb. 2018

Prof. Dr. Amir Al Qaraawi
Dean of College of Science

-

الخلاصة

تضمنت الرسالة جزئين الجزء الاول الطريقة الطيفية حيث شملت على تكوين ثلاث معقدات ذات ترابط ايوني لتقدير عقار المبفرين هايدروكلورايد بصورة نقية وفي المستحضرات الصيدلانية .المعقد الاول(I) بين المبفرين والفينول الأحمر ($\lambda max = 396nm$) ، والمعقد الثاني (II) بين المبفرين وحامض البكرك ($\lambda max = 382nm$) ، المعقد الثلاث (III) بين المبفرين واليود ($\lambda max = 360nm$) .وكانت حدود قانون بير ضمن مدى التراكيز (2 - 25، 1-30 ، 1.2-28) ميكروغرام / مل لمركب الأول والثاني والثالث على التوالي والامتصاصية المولالية من $10^4 \times 7.57$،$10^4 \times$ 3.41 ، 10^3 9.08) لتر/مول.سم للمعقد I، II، III على التوالي ،حساسية ساندال 0.00615 ، 0.013 ، 0.05 ميكروغرام. سم$^{-2}$ للمعقد I و II و III على التوالي ، ونسبة الانحراف المعياري النسبي (3.3974 ، 0.63 ، 2.05)٪ للمعقدة I، II، III على التوالي ، ومعامل الارتباط (0.9941 ، 0.9904 ، 0.9979) للمعقد III,II,Iعلى التوالي،,بلغ حد الكشف النوعي (LOD) (0.21 ، 0.77 ، 0.72) ميكروغرام/مل للمعقد I، II، III على التوالي وحد الكشف الكمي) (0.71،2.57،2.50) ميكروغرام / ملل للمعقد I، II، III على التوالي. الطريقة كانت سهلة وسريعة وحساسة ودقيقة وتم تطبيقها بنجاح من أجل تقدير المبفرين هايدروكلورايد. بصورة نقية وفي المستحضرات الصيدلانية.

اما الجزء الثاني كان طريقة الكروماتوغرافيا العمود السائلة عالية الاداء (HPLC) للتقدير الكمي للمبفرين بصورتة النقية والمستحضرات الصيدلانية .استخدم في هذة الطريقة الطور العكوس والعمود المستخدم نوع ODS2-C18 (جسيم 4.6mm150 × mm) ، الطور المتحرك يتكون من : 0.02 M الاسيتونايتريل و فوسفات هيدروجين البوتاسيوم3.6 PH (30:70 : v / v) ، معدل سرعة الجريان 1.0 مل / دقيقة والطول الموجي يبلغ 240 نانومتر باستخدام كاشف للأشعة فوق البنفسجية. زمن الاحتباس للمبفرين 1.03 دقيقة ،كانت الحدود الخطية للتراكيز في المدى 1-100 مايكروغرام/مل ، والانحراف المعياري النسبي 3.2 ٪. .وكانت الطريقة RP-HPLC بسيطة ودقيقة وخطية وسريعة للتقدير الكمي المبفرين في صورتة النقية وفي مختلف الصناعات الدوائية

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الاهداء .....

بدانا باكثر من يد وقاسينا اكثر من هم وعانينا الكثير من الصعوبات وها نحن اليوم والحمد للة نطوي سهر الليالي وتعب الايام وخلاصة مشوارنا بين دفتي هذا العمل المتواضع ...

الى منارة العلم وسيد الخلق اجمعين الى رسولنا الكريم.....الى محمد صلى اللة علية والة وسلم

الى من علمونا حروف من ذهب وكلمات من درر سيرة العلم والنجاح ....الى اساتذتي الكرام

الى الغائبة الحاضرة شوقا ووفاء الى روحها الطاهرة والتي لم تفارقنا ابدا...عمتي رحمها اللة

الى من سعى وشقى لانعم بالراحة والهناء الذي لم يبخل بشي من اجل دفعي في طريق النجاح الذي علمني ان ارتقي سلم الحياة بحكمة وصبر....الى والدي العزيز

الى الينبوع الذي لايمل العطاء الى من حاكت سعادتي بخيوط منسوجة من قلبها..... الى والدتي العزيزة.

الى اروع من جسد الحب بكل معانية فكان السند والعطاء قدم لي الكثير في صور من صبر ..وامل..ومحبة ..لن اقول شكرا بل ساعيش الشكر معك دوما ...الى زوجي

الى تلك الانسانة التي يعجز لساني عن شكرها واول من مدت يد العون لي واخذت على عاتقها مسئولية كبيرة..... الى خالتي ام احمد

الى من حبهم يجري في عروقي ويلهج بذكراهم فؤادي..... الى اخواني واخواتي .

الى العينين التي استمد منها القوة والاستمرار اعذب مافي عمري.....الى ولدي

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(وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ ۚ عَلَيْهِ
تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ)

صدق الله العلي العظيم

(سورة هود الأية 88)

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية التربية للعلوم الصرفة
قسم الكيمياء

استخدام تقنية كروموتغرافية العمود السائلة عالية الاداء-الطور العكوس والتقنية الطيفية - معقدات انتقال الشحنة في تقدير عقار المبيفرين هيدروكلورايد في الحالة النقية وفي المستحضرات الصيدلانية

رسالة مقدمة الى
كلية التربية للعلوم الصرفة – جامعة كربلاء
وهي جزء من متطلبات نيل درجة الماجستير
من قبل

زينب عباس عبد الزهرة البيضاني
بكالوريوس تربية كيمياء - جامعة كربلاء -2015
بأشراف
الاستاذ الدكتور
عبد الباري مهدي ماهود

1440 **2018**

الشكر والتقدير

الحمد لله رب العالمين والصلاة والسلام على اشرف خلق الله نبينا وحبيبنا محمد (ص) والﻫ الطيبين الطاهرين .

يسعدني وانا انهي رسالتي هذه بفضل الله وتوفيقﻫ ان اتقدم بجزيل الشكر واوفر الامتنان إلى اساتذي المشرف الاستاذ الدكتور عبد الباري مهدي ماهود الحلفي لاقتراحﻫ موضوع البحث ورعايتة العلمية ولجهودة المبذولة طيلة مدة انجاز البحث ، فلﻫ مني كل العرفان والامتنان وجزاه الله عني خير الجزاء راجية لﻫ السعادة والموفقية والعمر المديد ودوام الرقي العلمي .

ويطيب لي ان اقدم شكري وتقديري إلى عمادة كلية الصيدلة لتوفيرهم كافة مستلزمات البحث كما اتقدم بخالص شكري وتقديري الى عمادة كلية التربية للعلوم الصرفة ورئيس قسم الكيمياء أ.د حميدة عيدان سلمان واساتذة القسم كافة لما ابدوة من مساعدة في توفير متطلبات انجاز هذه الرسالة وتوجيهاتهم السديدة خلال فترة الدراسة واتقدم بخالص شكري وتقديري الى استاذي أ.د منير عبد العالي الدعمي لدعمة العلمي المتواصل وتشجيعة .

ومن الوفاء ان اشكر الاخوات (فاطمة وسرى ونهلة وحنان وزهراء وايناس) وطلبة الدراسات العليا جميعهم متمنيا لهم الموفقية والنجاح

واخيرا تحية وشكر من اعماق قلبي إلى زوجي و اهلي وافراد اسرتي لاهتمامهم وتشجيعهم المتواصل لي اسأل الله العلي القدير ان يوفق الجميع لما فيﻫ صلاح الاسلام والمسلمين .

زينب عباس