Introduction and Literature Review

1.1 General Introduction

Guaifenesin is used to control cough and is sometimes combined with dextromethorphan, an antitussive, such as in Mucinex DM or Robitussin DM.It increases the analgesic effect of paracetamol (acetaminophen) and aspirin, increases the sedative effects of alcohol, tranquilisers, sleep-pills and total anesthetics. It increases the effects of medication that decrease muscle tone. This medication is not usually used for ongoing coughs from smoking, asthma, other long-term breathing problems (e.g., emphysema), or for coughs with a lot of mucus, unless directed by your doctor (Aluri and Stavchansky , 1993).

Similar medicines derived from the guaiac tree were in use as a generic remedy by American indigenous peoples when explorers reached North America in the 16th century. The Spanish encountered guaiacum wood "when they conquered Santo Domingo; it was soon brought back to Europe, where it acquired an immense reputation in the sixteenth century as a cure for syphilis and certain other diseases.. The 1955 edition of the *Textbook of Pharmacognosy* states: "Guaiacum has a local stimulant action which is sometimes useful in sore throat. The resin is used in chronic gout and rheumatism, whilst the wood is an ingredient in the compound concentrated solution of sarsaparilla, which was formerly much used as an alternative in syphilis.

Guaifenesin was first approved by the Food and Drug Administration (FDA) in 1952. Although previously deemed "Generally Regarded as Safe" in its original approval, the drug received a New Drug Application for the extended-release version, which won approval on July 12, 2002. Because of this, Guaifenesin is sold as pills or syrups under many brand names (Bennett *et. al.,* 2004) .

1.2 Introduction for HPLC techniques :-

Chromatography comprises of closely related group of separation methods initiated by David day and Mikhail. Tsucelf in between 1897-1906 which were now developed into very important potential techniques for the preparation of highly pure compounds in pharmaceutical chemistry and for quality control .The method use for separating a mixture of components into individual components through equilibrium distribution between two phases . (Anjaneyulu *et. al.,* 2006).

High performance liquid chromatography(HPLC)**3-7** is a process, which separates mixture containing two or more components under high pressure. in this the stationary phase is packed in a column one end of which is attached to a source of pressurized liquid mobile phase.High performance liquid chromatography is the fastest growing analytical technique for the analysis of drugs. Its simplicity, high specificity and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids.

HPLC is also known as high pressure liquid chromatography It is essential form of column chromatography in which the stationary phase is consist of small particles (3-50µm) pickings contained in a column with a small pore(2-5mm) one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three form of high performance liquid chromatography most often used are ion-exchange, partition and adsorption (Anjaneyulu *et. al.,* 2006).

HPLC provides a specific, sensitive and precise method for analysis of different complicated samples ,there is speed of analysis,the analysis by HPLC is specific, accurate and precise and It offers advantage over gas chromatography in analysis of many polar substances, metabolic products and thermo labile as well non-volatile substances (Srivastava, 1991).

Analytical method development and validation are key elements of any pharmaceutical development program. HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products.

HPLC method development and validation play important role in the discovery, development and manufacture of agro chemicals , pharmaceutical products and every day many chromatographers face the need to develop HPLC separation ,whereas individual approaches mayexhibit considerable diversity, method development often follows the series of steps summarized in Figure 1 (Syder, *et. al*., 1997).

Information concerning the sample, for example, molecular mass, structure functionality, pKa values, UV spectra and solubility of the compound . The sample solubility should be identified whether it is organic solvent soluble or water soluble, as this helps to select the best mobile phase and column to be used in HPLC method development (Nguyen, *et. al*., 2010).

Selectivity can be manipulated by combination of different factors like solvent composition, type of stationary phase, mobile phase, buffers and pH. Changing solvents and stationary phases are the most comfortable approaches to achieve the separation. The proper range of pH is an important tool for separation of ionizable compounds.

Acidic compounds are retained at low pH while basic compounds are more retained at higher pH. The neutral compounds remain unaffected. The pH range 4-8 is not generally employed because slight change in pH in this range would result in a dramatic shift in retention time (Nguyen, *et. al.*, 2010).

Information on sample

↓

Defining separation goals.

↓

Choose of detector.

↓

Choose LC method; preliminary runs; estimate , Best separation conditions.

↓

Optimize separation conditions

↓

Requirements for separation procedures

↓ ↓

a. Recovery of purified material b.Quantitative method

c. Qualitative method

↓

Validated method for laboratories, released to routine

Figure (1.1) : Flow chart for Method development steps

1.2.1 Stationary Phases for RPLC

The introduction of chemically modified stationary phases has had a remarkable impact in the field of liquid chromatography. Successful development and improvement in the technology of manufacturing reproducible bonded layers has revolutionized many chromatographic techniques. Porous silica stationary phases have been modified with ligands of various chemistry and size.

The composition and the structure of the bonded organic layer is varied by changing the size of the modifier, specific surface area of the adsorbent, and the bonding density. Chemical bonding of organic ligands with high bonding density on the inner surface of silica pores alters the adsorbent geometry.

The effect of surface modification on adsorbent geometric parameters (surface area, pore volume, pore size) has been investigated on several different silica gels . It was shown that a decrease in mean pore diameter and in pore volume are associated with the molecular volume of bonded ligands and bonding density. Similar effects were also observed by other researchers clearly (kazakevich , 2007).

surface modification has a significant impact on the adsorbent geometry of reversed-phase columns, which will also influence the separation mechanism itself .Silica-based packing materials dominate in applications for RP separations in the pharmaceutical industry. Hydrophobic surface of these packings typically are made by covalent bonding of organosilanes on the silica surface.

This modification involves the reaction of monofunctional alkyldimethylchlorosilanes with the surface silanol groups. Octadecylsilane was the first commercially available silica-based bonded phase and is still the most commonly utilized.

Also, alkyl-type ligands of different number of carbon atoms (C1,C4, C8, C12) are often used as well as phases with phenyl functionality; also,polar end-capped, polar embedded phases have been introduced .Polar embedded phases provide an additional avenue for potential modification of the chromatographic selectivity, and some of these phases offer an enhancement of retention of polar analytes . These phases can be used with aqueous mobile phases, even 100% aqueous, without loss of analyte retention that sometimes could be observed for more hydrophobic phases.

Screening several different types of stationary phases during method development for a particular separation is often useful because different columns usually have different selectivity for components in a sample.

Most silica-based reversed-phase packing materials have a relatively narrow applicable pH range. Below pH 2, the linkage of the bonded phase to the silica substrate is prone to hydrolytic cleavage. Above pH 7, the silica substrate is prone to dissolution, particularly in aqueous-rich mobile phases (kazakevich , 2007).

In addition, basic compounds may exhibit peak asymmetry above pH 3 due to secondary interactions between the ionized form of the solute and accessible residual silanols. Some new developments in column chemistry have been adopted to address the issues of limited pH working range and reduction of surface density of silanols (kazakevich , 2007).

1.2.2 Mobile Phases for RPLC

Mobile phases commonly used in reversed-phase HPLC are hydro-organic mixtures. The most common reversed-phase organic modifiers include methanol and acetonitrile and/or combinations of these two modifiers.

Other mobile-phase modifiers such as tetrahydrofuran, IPA, and DMSO have been also used for minor selectivity adjustment; however, they are not common due to their high backpressure limitations and/or high background UV absorbance.

The concentration of organic modifier in the eluent is the predominant factor that governs the retention of analytes in RPLC. Highly purified solvents (HPLC grade) are recommended in order to minimize contamination of the stationary phase with impurities of the solvents and reduction of the background absorbance if they contain impurities that have UV chromophores >190nm.

Considerations for choice of mobile-phase solvents include compatibility between solvents, solubility of the sample in the eluent, polarity, light transmission, viscosity, stability, and pH. The mobile-phase solvents should be miscible and should not trigger precipitation when they are mixed together. For example, dichloromethane and water are immiscible at most compositions and should not be used as mobile phase components. Similarly, high concentrations of phosphate buffer should not be used with high levels of acetonitrile because the phosphate will eventually precipitate out, resulting in damage in the pump head and blockage of the column frit.The sample should also be soluble in the mobile phase to avoid precipitation in the column (kazakevich , 2007).

Table (1.1) , Solvent UV Cutoff for the Most Typical Solvents Used in HPLC.

Solvent	**UV Cutoff**
Acetonitrile	190
Isopropyl alcohol	205
Methanol	205
Ethanol	205
Uninhibited THF	215
Ethyl acetate	256
DMSO	268

Transmission is an important parameter when using UV detection; see table for UV cutoffs of common reversed-phase organic modifiers.

Solvents with high UV cutoffs such as acetone (UV cutoff 330 nm) and ethyl acetate (UV cutoff 256 nm) cannot be used for analyses at low wavelengths such as 210nm. Acetonitrile has a very low UV cutoff (<190nm) and is one of the contributing factors toward its common use as a solvent for reversed-phase separations. Methanol, ethanol, and isopropanol have a UV cutoff of <205nm, and at higher organic concentrations the mobile phase transmits less light. It is generally recommended to work at wavelengths >210nm with these solvents. Also the viscosity of the mobile phase plays an important role in the back pressure generated in the HPLC column (pressure drop). The viscosity is not a linear function and is dependent upon the type and concentration of the organic solvent as well as the operation temperature Also, highly viscous solvents such as methanol andisopropanol can lead to reduced diffusion rates, resulting in peak broadening as well as creating excessively high backpressures in the column. Solvents such as tetrahydrofuran(THF) and other ethers are prone to oxidation to form peroxides. These peroxides can react with the solute or with other mobile-phase components, causing the appearance of spurious peaks.

(kazakevich , 2007).

1.2.3 pH of The Mobile-Phase

Most pharmaceutical compounds contain ionizable functionalities such as amino, pyridinal, or carboxylic groups. Mobile-phase pH and composition are among the main parameters used to control HPLC retention of most pharmaceutical compounds and to optimize separations. The introduction of new packings that are stable over a wider pH range up to pH 12 allows for a broader applicability of mobile-phase pH as a retention/selectivity adjustment parameter . The pH of the mobile phase has a strong influence on the retention of protolytic solutes and should be controlled in reversed phase HPLC. Buffers are recommended to control the pH stability of the mobile phase. Note that the common volatile buffers trifluoroacetate (p*Ka* 0.5), acetate (p*Ka* 4.8), and formate (p*Ka* 3.8) can be used for mass-spectrometric detection; however, they have significant background absorption, depending on their concentration at wavelengths below 220nm.This usually leads to descending baselines when running gradient separations (since the aqueous portion of the mobile phase is being diluted with the organic and there is a consequent decrease in the background absorbance). It is generally recommended to add the same concentration of acid modifier or salt buffer that is in the aqueous phase to the organic phase to suppress the descending baseline effect (*Note*: Check the solubility of buffering agents in organic phase). However, even though this leads to a flatter baseline, it still reduces the detection sensitivity because the mobile phase is absorbing at the wavelength of interest (<220nm).

The pH specified in analytical methods for pharmaceutical analysis should be that of the aqueous solvent. Note that the addition of organic modifier to aqueous buffer generally results in a shift in mobile-phase pH. The p*Ka* of the solute is also subject to variation and is dependent on the type and concentration of the organic modifier in the eluent (kazakevich , 2007).

1.3 METHOD VALIDATION

The method validation process is to confirm that the method is suited for its intended purpose. Although the requirements of validation have been clearly documented by regulatory authorities [ICH, USP, and FDA], the approach to validation is varied and open to interpretation.

1.3.1 Types of analytical procedures to be validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests.
- Quantitative tests for impurities' content.
- Limit tests for the control of impurities.
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated.

[ICH Q2(R1) Geneva, 2005].

1.3.2 Typical validation characteristics which should be considered are listed below

1. Accuracy
2. Precision
3. Repeatability
4. Intermediate Precision
5. Specificity

6. Detection Limit
7. Quantitation Limit
8. Linearity
9. Range

1.3.2.1 Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.This is sometimes termed trueness.

1.3.2.2 Precision

The precision of an analytical procedure expresses the closeness of agreement(degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

1.3.2.3 Reproducibility

Repeatability expresses the precision under the same operating conditions over Short interval of time. Repeatability is also termed intra-assay precision .

1.3.2.4 Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

1.3.2.5 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants,matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification: to ensure the identity of an analyte.

Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency):

to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

1.3.2.6 Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

1.3.2.7 Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

1.3.2.8 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

1.3.2.9 Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

1.3.2.10 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage （ICH Q2(R1) Geneva, 2005).

1.4 Chemistry of guaifenesin

Guaifenesin (GN), (+)-3-(2-)methoxyphenoxy-propane-1,2-diol, . Its empirical formula is C_{10} H_{14} O_4 ,which corresponds to a molecular weight of 198.21 , it is a white or slightly gray crystalline substance with a slightly bitter aromatic taste , it soluble in water ,alcohol ,chloroform , and in propylene glycol , it sparingly soluble in glycerin.

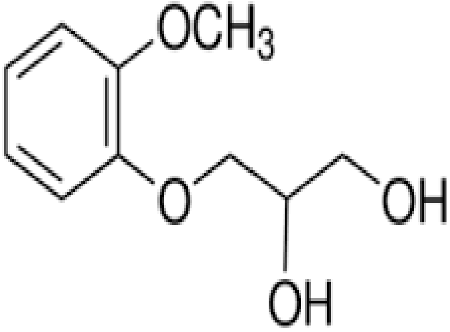
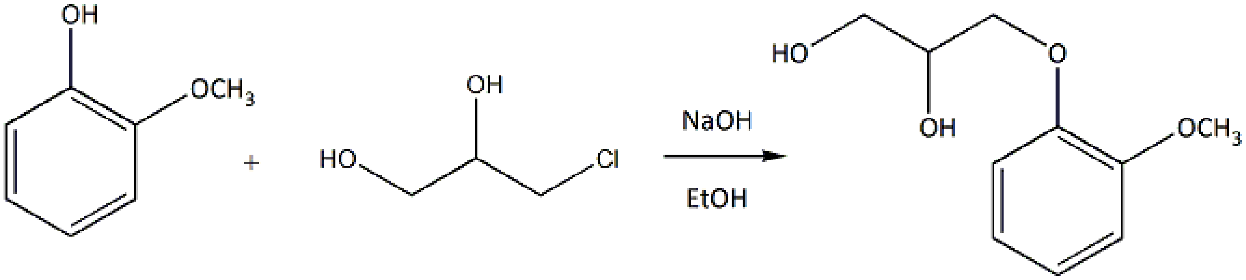
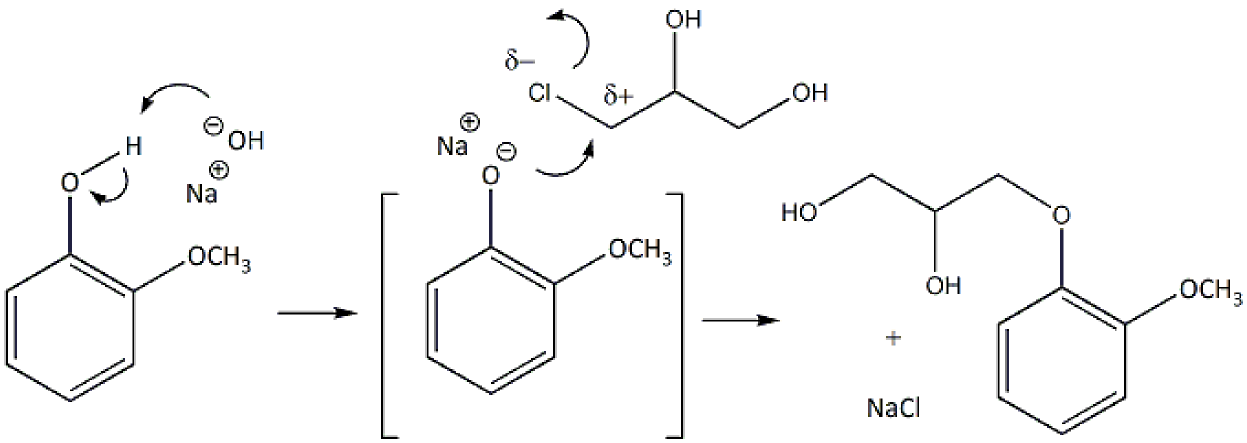


Figure. 2.1 : Structure of guaifenesin

guaifenesin is prepared through the Williamson ether synthesis which involves an SN_2 mechanism between the sodium phenoxide salt dervied from guaiacol (2-methoxyphenol) and 3-chloro-1,2-propanediol (Aluri, and Stavchansky, 1993).



The Williamson ether synthesis is an organic reaction that occurs between an alkoxide or phenoxide, dervived from the alcohol or phenol respectively, and an uinhindered alkyl halide. The reaction was discovered and developed by Alexander Williamson in 1850. In this reaction, the alkoxide or phenoxide is generally generated *in situ* and the alkyl halide is subsequently added to the reaction mixture. The reaction occurs through an SN_2 mechanism and the alkoxide/phenoxide serves as the nucleophile and the alkyl halide the electrophilic substrate. In this experiment, the Williamson ether synthesis is used to prepare guaifenesin. 2-Methoxyphenol (as referred to as guaiacol) is reacted with sodium hydroxide (NaOH) to generate the phenoxide anion. This nucleophilic anion reacts with the primary alkyl chloride carbon of 3-chloro-1,2-propanediol to give the target product. The mechanism of the reaction is provided below (Bennett, *et. al*.,2004).



Guaifenesin (glyceryl guaiacolate) is an expectorant drug sold over the counter and usually taken orally to assist the bringing up of phlegm from the airways in acute respiratory tract infections .the medical use for guaifenesin to control cough and sometimes combined with dextromethorphan ,an antitussive , such as in Mucinex DM .

Guaifenesin mechanism of action is thought to act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It has been said to aid in the flow of respiratory tract secretions , allowing ciliary movement to carry the loosened secretions upward the pharynx .thus ,it may increase the efficiency of the cough reflex and facilitate removal of the secretions .it has muscle relaxant and anticonvulsant properties and may be acting as an NMDA receptor antagonist (Bennett, et. al., 2004) .

1.5 Objective :-

Base on the important and advantage of HPLC method in Pharmaceutical industry and for the benefit of guaifenesin in used to treat cough , congestion and other breathing illnesses this work aimed to estimate all validation parameters (precision, accuracy , selectivity , linearity , limit of detection, limit of quantitation ,and range) of HPLC method for guaifenesin and established a simple and precise HPLC method with fewest cost to determination of guaifenesin raw material .

2.1 Materials

Acetonitrile, for preparative HPLC , S.D fine –chem limited , Mumbai, india .
Guaifenesin , BP, Stellar Chemical Laboratories Pvt . Ltd , Mumbai, india .
Orthophosphoric acid abt. 85 % LR . S.D fine –chem limited , Mumbai, india .
Triethylamine AR , S.D fine –chem limited , Mumbai, india .

2.2 Methods

2.3.1 IR identification of guaifenesin

The FT-IR Spectrophotometer, model No. FT/IR -4100, Jasco was used to determine the identity of guaifenesin before used . the IR spectrum is shown in figure (2.1) .

2.3.2 UV identification and weave length selection

A 50.0 mg of guaifenesin working standard was weighed and transferred accurately into a 100 ml volumetric flask by 60 mlof diluents, then was sonicated for 10 minu -tes , allowed to cool at room temperature and the volume was make up to the mark by diluent , 5 ml of the solution was diluted to the 100 ml volumetric flask by diluent. UV spectrum was recorded in 200 – 400 nm range by used UV Spectrophotometer , UV-1800, Shimadzu . Results are shown in figure (2.2) .

2.3.3 preparation of buffer :-

3 ml of triethylamine was added to 900 ml of filtered de-ionizaed water in 1000 mlvolumetric flask , the pH was adjusted to 6.5 with orthophosphoric acid and completed the volume to the mark with filtered de-ionizaed water .

2.3.4 preparation of the mobile phase :-

A mixture of acetonitrile and buffer in ratio of (25 : 75) respectively .

2.3.5 preparation of the diluent :-

A mixture of acetonitrile and filtered de-ionizaed water in ratio of (10:30) respectively.

2.3.6 test solution preparation :-

A 50.0 mg of guaifenesin working standard was weighed by (Balance , Sartorius , AG , Germany) and transferred accurately into a 100 ml volumetric flask by 60 ml of diluents, then was sonicated for 10 minutes , allowed to cool at room temperature and the volume was make up to the mark by diluent , 10 ml of the solution was diluted to the 100 ml volumetric flask by diluent .

2.3.7 Chromatographic conditions :-

Column: Octadecylsiyl silica gel (ODS 3) .
  Length : 250 mm .
  Internal diameter : 4.6 mm .
  Particle size : 5 µm .
Flow rate : 1 ml / min
Injection volume : 10 µl
Detector : UV 225

High performance liquid chromatography , SYKAM, Germany was used , the Instrument consists of the following components :

S1121 solvet delivery system , S3200 UV-VIS detector , SRI model 333 peak simple chromatography data system and Software (Peak 378 rar).

2.3.8 System suitability testing :-

A 50.0 mg of guaifenesin working standard was weighed and transferred accurately into a 100 ml volumetric flask by 60 ml of diluents, then was sonicated for 10 minutes , allowed to cool at room temperature and the volume was make up to the mark by diluent , 10 ml of the solution was diluted to the 100 mℓ volumetric flask by diluent to obtain concentration equal to 50 µg /mℓ.

2.3.9 Determination of Specificity :-

solution of mixture of acetonitrile and filtered de-ionizaed water in ratio of (10:30) respectively was prepared , one injection was carried out to determine the Specificity , The results are shown in figure (2.4).

2.3.10 Determination of linearity

Serial solutions , 30 , 40 , 50 , 60 , 70 µg / mℓ were prepared from stock solution had concentration equal 100 µg / mℓ .

Three injections from each concentration were carried out .The calibration curve was drawn using the average response from each concentration against the Target Concentration Solution equal 100 % (50 µg / mℓ) .

2.3.11 Determination of limit of detection

The result of linearity were used to calculation the detection limit by method of standard deviation of response and the slope in which the detection limit is expressed by the following relation .

$$LOD = \frac{3.3\ \sigma}{s}$$

where :-

σ : the standard deviation of response factor.

s : the slope of the calibration curve .

2.3.12 Determination of limit of quantitation

The results of linearity were used to find out the quantitation limit using the method of standard deviation of response and the slope in which it expressed by the following relation

$$LOQ = \frac{10\ \sigma}{s}$$

where :-

σ : the standard deviation of response factor.

s : the slope of the calibration curve .

2.3.13 Determination of precision

2.3.13.1 Repeatability precision

100 % solution of guaifenesin was prepared , six injections were carried out to determine the repeatability precision of the results, The results are shown in table (2.3).

2.3.13.2 intermediate precision

100 % solution of guaifenesin was prepared by different analysts (X , Y and Z), on a different system and in different day . Three injections of the solution were carried by each analyst and the results are shown in tables (2.4) , (2,5) and (2.6) .

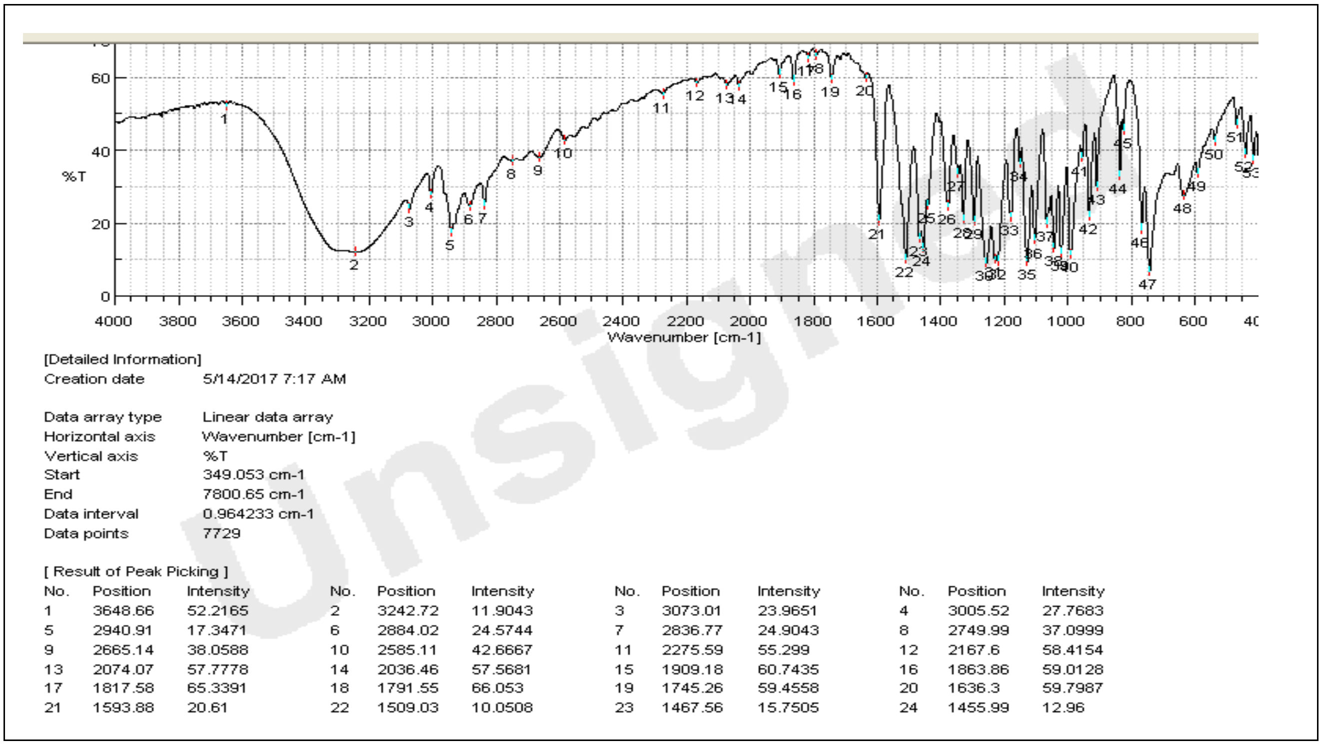
2.3.14 Determination of accuracy

Serial solutions 80 % , 100 % and 120 % of guaifenesin were prepared from stock solution had concentration 1mg / mℓ .

Three injections from each solution were carried out separately and starting with the lowest concentration . Three injections of each solutions were carried out and the results are shown in tables (2.7) .

3.1 Result and Discussion

The IR spectrum of guaifenesin in figure (2.1) shows that , the compound has two ether groups , appear at 1250 cm^{-1} . Primary and secondary Alcohol (hydroxyl group) appeared in range of 3000 cm^{-1} - 3600 cm^{-1}.aromatic hydrocarbon group appear at 1433 cm^{-1} and 1600 cm^{-1}. alkane group appear at 2955 cm^{-1} .



[Detailed Information]

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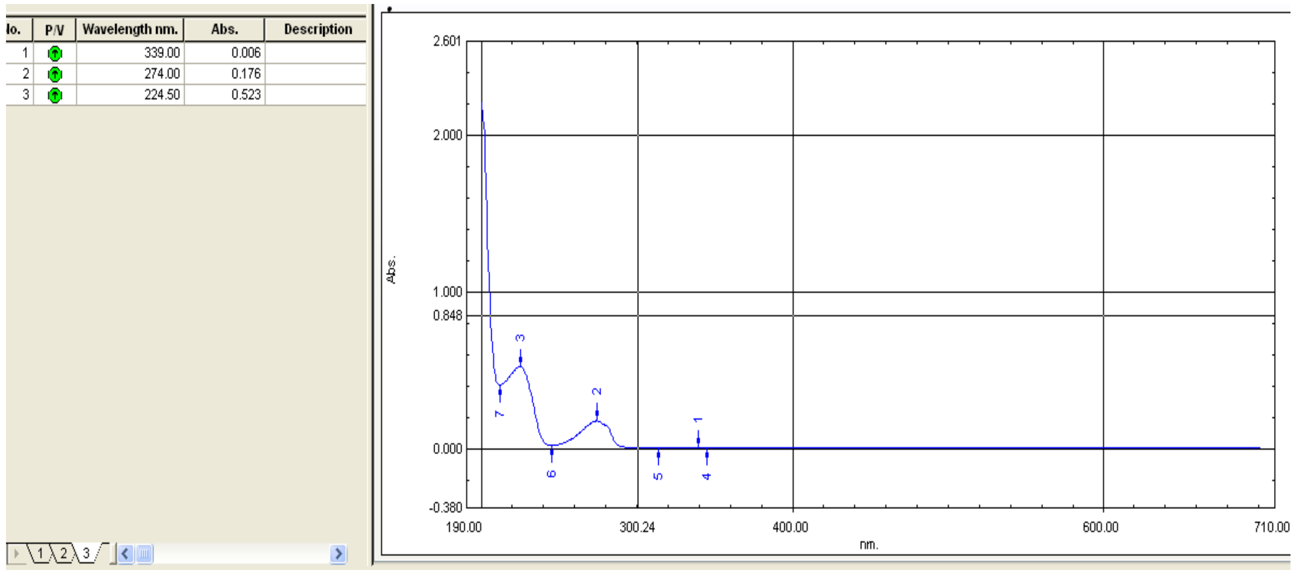
[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3648.66	52.2165	2	3242.72	11.9043	3	3073.01	23.9651	4	3005.52	27.7683
5	2940.91	17.3471	6	2884.02	24.5744	7	2836.77	24.9043	8	2749.99	37.0999
9	2665.14	38.0588	10	2585.11	42.6667	11	2275.59	55.299	12	2167.6	58.4154
13	2074.07	57.7778	14	2036.46	57.5681	15	1909.18	60.7435	16	1863.86	59.0128
17	1817.58	65.3391	18	1791.55	66.053	19	1745.26	59.4558	20	1636.3	59.7987
21	1593.88	20.61	22	1509.03	10.0508	23	1467.56	15.7505	24	1455.99	12.96

Figure (3.1) IR spectrum for guaifenesin .

The results of UV identification and weave length selection are shown in in figure (3.2) . to choice the mobile phase compostion and weavelength of the detector which show that guaifenesin has a maximum absorbance at 276 nm and 225 nm using water , 100 % methanol and in 20 % methanol (diluent) .



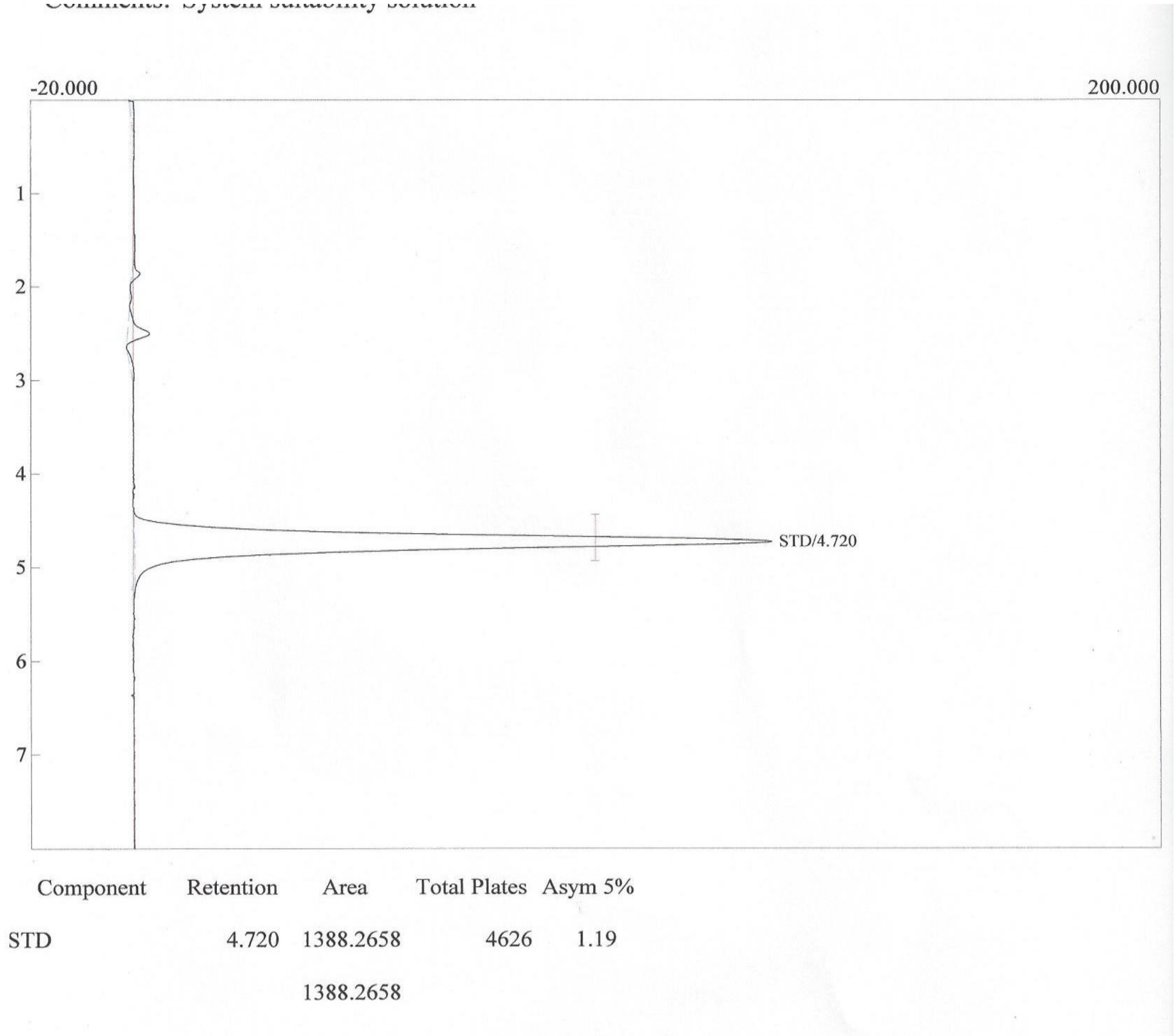
lo.	P/V	Wavelength nm.	Abs.	Description
1	↑	339.00	0.006	
2	↑	274.00	0.176	
3	↑	224.50	0.523	

figure (3.2) UV spectrum of guaifenesin .

The system suitability parameters of the HPLC method for determination of guaifenesin are shown in table (2.1) , the number of theoretical plates (N) was 4254 , asymmetry 5 % was 1.19 and RSD % for six injections was 0.4 which are within the acceptance values of N ≥ 2000 , asymmetry 5 % between 0.8 – 2 and RSD % for six injections must be ≤ 2 [shabbier , 2003].

Table (3.1) System suitability test results

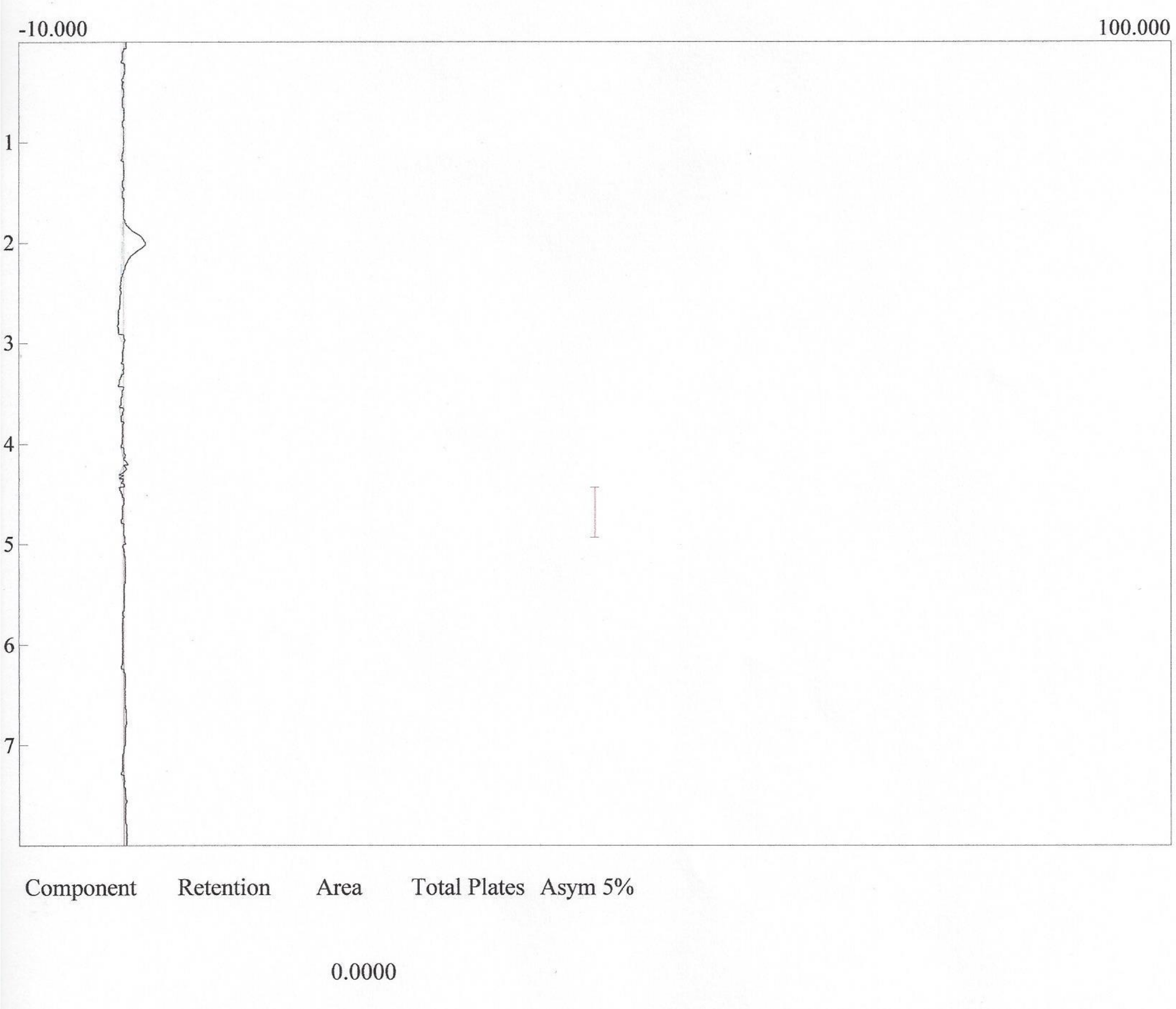
No.of injections	Retention time	Peak area	Tailing factor	Total plates	RSD %
6	4.8	1379.3397	1.19	4254	0.4


Comments: System suitability solution

Component	Retention	Area	Total Plates	Asym 5%
STD	4.720	1388.2658	4626	1.19
		1388.2658		

Figure (3.3) Chromatogram for System suitability test

A reverse phase HPLC method for guaifenesin determination using buffer of 3 mℓ of triethylamine was added to 900 mℓ of filtered de-ionizaed water in 1000 mL volumetric flask , the pH was adjusted to 6.5 with orthophosphoric acid and completed the volume to the mark with filtered de-ionizaed water and a mixture acetonitrile with buffer in ratio of (25 : 75) respectively as a mobile phase , at a flow rate 1 mℓ / min using ODS 3 column at room temperature and uv / vis detector at 225 nm to give the best resolution and best peak shape .



Component	Retention	Area	Total Plates	Asym 5%
		0.0000		

Figure (3.4) Chromatogram for Specificity

The method validation requirements were applied after the system suitability was checked to determine it was suitable for use or not according to ICH guideline , than validation parameters (linearity , accuracy , precision , limit of detection and limit of quantization) were evaluated .

For linearity measurement , the calibration curve appendix [3] was carried using solutions with a concentration 30 , 40 , 50 , 60 and 70 µg / mℓ .

TABLE (3.2) . Linearity Results :-

Solution Number	Target Concentration Solution (%)	Final Concentration of solution (µg / mL)	Peak Area	Response factor	Average for Peak Areas
1	**60**	**30**	**830.9827**	**27.699**	**832.7812**
			832.1984	**27.739**	
			835.1624	**27.838**	
2	**80**	**40**	**1104.9686**	**27.624**	**1103.7143**
			1104.5935	**27.614**	
			1101.5808	**27.539**	
3	**100**	**50**	**1369.5866**	**27.391**	**1372.5747**
			1372.4164	**27.448**	
			1375.5687	**27.511**	
4	**120**	**60**	**1646.7300**	**27.445**	**1653.0087**
			1654.6352	**27.577**	
			1657.6610	**27.627**	
5	**140**	**70**	**1932.4688**	**27.606**	**1918.9816**
			1914.7827	**27.354**	
			1909.6934	**27.281**	
				SD=0.151	

*** Average for Peak Areas of the Target Concentration Solution 100 % (STD) = 1378.0185**

The calibration curve was constructed using five standard solutions of different concentration . Result are shown in table (2.2) . the data of the calibration curve were treated statistically , and the linear regression equation was found to be Y = 13.752 X with r^2 = 0.99997 ; where y represents the peak area of analyte And x represents analyte concentration .

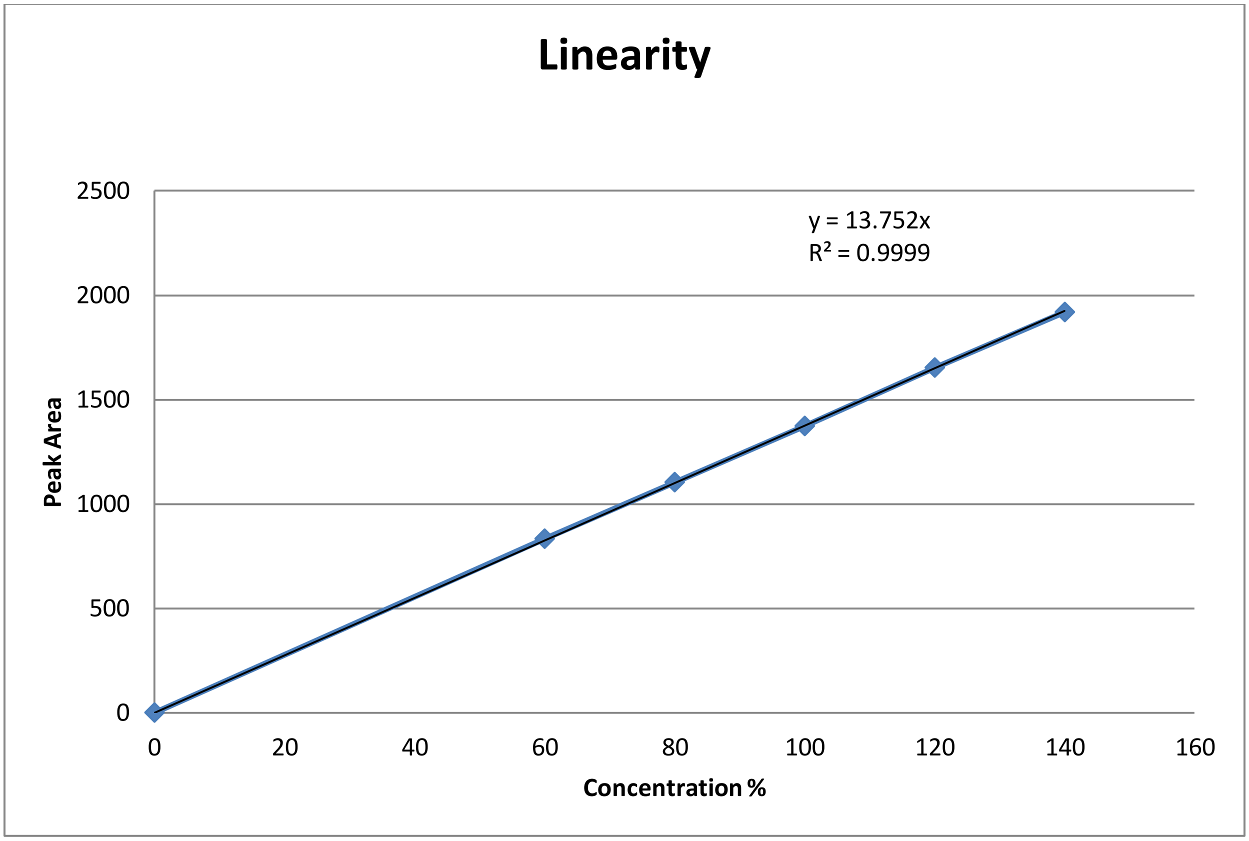


Figure (3.5) The calibration curve for Linearity determination

The result are satisfactory , because there is significant correlation between response factor and concentration of drug within the concentration range . Acceptance criteria for coefficient of correlation should be not less than 0.999 [Shrivastava et al , 2011] .

For precision measurements , repeatability of the method was achieved using 100% solution of guaifenesin . The results obtained shown in table (2.3) , show that the method is repeatable and precise as the RSD % of the results is less than 2 as recommended by ICH guideline .

Table (3.3) : Results of repeatability precision

Injection Number	Peak Area
1	**1384.1258**
2	**1370.5570**
3	**1372.8488**
4	**1386.9400**
5	**1377.1472**
6	**1384.4196**
RSD % = 0.5	

Intermediate precision of the method was carried out by different analysts X, Y and Z , are shown in tables 2.3 , 2.4 and 2.5 respectively , indicating the precision of the method , the calculated percent of relative standard deviation of the results were , 0.13 , 0.06 and 0.42 respectively which are less than 1 of the three analysts .

Table (3.4) : Results of intermediate precision carried by analyst X

Injection Number	Peak Area
1	1368.1350
2	1368.6764
3	1375.0902
X^- of STD= 1371.4135 RSD % = 0.13	

Table (3.5) : Results of intermediate precision carried by analyst Y

Injection Number	Peak Area
1	1360.9702
2	1380.2489
3	1375.6098
X^- of STD= 1372.1362 RSD % = 0.06	

Table (3.6) : Results of intermediate precision carried by analyst Z

Injection Number	Peak Area
1	**1383.8010**
2	**1381.4579**
3	**1379.5268**
X^{-} of STD= 1363.5518 **RSD % = 0.42**	

These results for inter and intra precision of the results prove that the precision requirement of the HPLC method is fulfilled , even though the method used by different analysts on different instrument , the results will be certainly precise and acceptable .

Table (3.7): Result of Accuracy

Injection number	Peak area	Theoretical content	Actual content	Recovery	RSD
1	1104.9686	80 %	80.18 %	99.77	0.17
2	1104.5935		80.15 %	99.81	
3	1101.5808		79.93 %	100.08	
1	1369.5866	100 %	99.38 %	100.62	0.22
2	1372.4164		99.59 %	100.41	
3	1375.5687		99.82 %	100.18	
1	1646.7300	120 %	119.49 %	100.42	0.34
2	1654.6352		120.07 %	99.94	
3	1657.6610		120.29 %	99.75	

*** Average for Peak Areas of the Target Concentration Solution 100 % (STD) = 1378.0185**

The accuracy of the method was tested by using standard solutions of 80 % , 100 % and 120 % of guaifenesin and the recoveries were calculated . good recoveries obtained (99.77% - 100.62 %) with a mean of 100.10 % . The results obtained are shown in table (2.6) the actual values were very close to the true values showing that the method is accurate .

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the method based on standard deviation of the response and the slope (ICH , 1996) ,using the expressions :-

$$LOD = \frac{3.3\ \sigma}{s}$$

And

$$LOQ = \frac{10\ \sigma}{s}$$

where :-

σ : the standard deviation of response factor.

s : the slope of the calibration curve .

$$LOD = \frac{3.3 \text{ x } 0.151}{0.0367} = 0.036\ \mu g / m\ell$$

And

$$LOQ = \frac{10 \text{ x } 0.151}{0.0367} = 10.98\ \mu g / m\ell$$

the values that obtained for LOD and LOQ were *0.036* µg / mℓ and 10.98 µg / mℓ , respectuively . these values indicate that the method is sensitive and good to determination the low dosage .

Table (3.8) : Result of the validation parameters

Validation parameter	**Acceptance criteria**	**Results**
Specificity	**Complete separation**	No interference impurities
Linearity **Slope of regression**	**$R^2 \geq 0.99$**	0.999 13.75
precision **repeatability** **intermediate precision**	**Precision ≤ 1.0 %** **RSD % ≤ 2.0 %** **RSD % ≤ 2.0 %**	0.4 0.5 0.1, 0.06 and 0.4 for analyst X, Y and Z .
Accuracy	**Mean recovery for three concentration (98 – 102 %) .**	100.10 %
Limit of detection	**-**	*0.036* µg / mℓ
Limit of quantitation	**-**	*10.98* µg / mℓ
Range	**-**	*30 – 70* µg / mℓ

This HPLC method for guaifenesin determination is valid at all validation characteristics (selectivity , precision , accuracy , linearity , range , Limit of detection (LOD) and limit of quantitation (LOQ).) ; values are within the acceptable values as shown in table (2.7) , and thus it is valid and suitable for its purpose .

The work proves that the described HPLC procedure for guaifenesin is valid and suitable for use in this analytical conditions . if the conditions of the analysis is changed , the method should be revalidated to ensure that the change doesn’t affect on the system suitability of the method for analysis .

3.2 Conclusions and recommendations

The following Conclusions and recommendations from the results of this study :-

1. The method was found to be simple , precise , accurate and selective for determination of guaifenesin .
2. The mobile phase is simple to prepare , it consist of mixture from acetonitrile and buffer in ratio of (25 : 75) respectively .
3. The preparation steps of buffer and sample for analysis are simple and rapid .
4. The method is valid for estimation of drug and convenient for use in routine analysis for guaifenesin in pharmaceutical dosage forms .
5. These obtaining results could be compared with another validation results from other laboratories to check the reproducibility of procedure .
6. Shorter retention time could be obtain , if the ratio of the component of mobile phase varied , this lead to minimum analysis time . this modified procedure should be validated to see if this change affect on the validation requirements of the method .

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