High performance liquid chromatographic method for the determination ofguaifenesin in pharmaceutical syrups andin environmental samples

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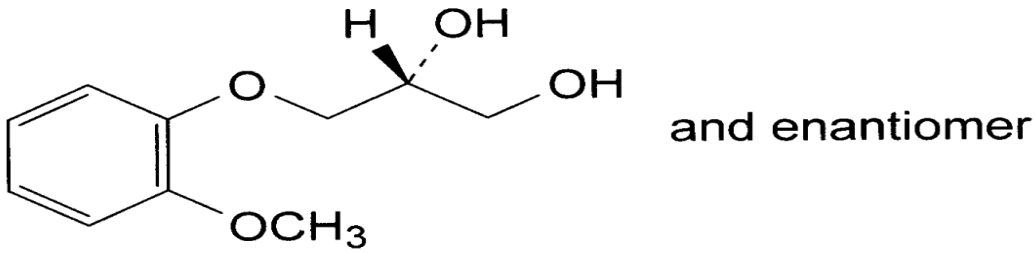
Abstract:

A simple, precise, rapid, and accurate reversed – phase high performance liquid chromatographic method has been developed for the determination of guaifenesinin pure from pharmaceutical formulations.andindustrial effluent. Chromatography was carried out on supelco L_7 reversed- phase column (25cm × 4.6mm), 5 microns, using a mixture of methanol –acetonitrile-water: (80: 10 :10 v/v/v) as a mobile phase at a flow rate of 1.0 ml.min^{-1}. Detection was performed at 254nm at ambient temperature. The retention time forguaifenesin was found 2.4 minutes. The calibration curve was linear (r= 0.9998) over a concentration range from 0.08 to 0.8mg/ml. Limit of detection (LOD) and limit of quantification (LOQ) were found 6µg/ml and 18µg/ml respectively. The method was validated for its linearity, precision and accuracy .The proposed method was successfully applied for the determination of guaifenesininsyrups and industrial effluent samples.

Keywords: HPLC, Guaifenesin,Pharmaceutical preparations, Industrial effluent

Introduction:

Guaifenesinis chemically known as1, 2- propanediol3-(2-methoxyphenoxy) (FIG.1)[1] is an expectorant and widely used in the treatment of coughing[2],guaifenesin may help control symptoms but does not treat the cause of symptoms orspeed recovery. Guaifenesin is in a class of medications calledexpectorants. It works by thinning the mucus and clear theairways .The usual does is 100 to200 mg every 2 to 4 hours[3-5]



Molecular formula: $C_{10}H_{14}O_4$ =198.2
Fig(1):Chemical structure ofguaifenesin.

Analytical procedures for the determination of guaifenesin include titrimetry [1],various spectrophotometric[6-13], HPLC[14-20], micellarelectrokineticchromatography[21,22]Voltammetric assay[23], Capillary gas chromatography[24,25] and ion pair high performance liquid chromatography[26] methods are also

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reported in the literature for the estimation of guaifenesin.High performance liquid chromatography (HPLC) can be used for determination of drugs and for purposes of control throughout the entire manufacturing process of drugs, as well as quality control of the finished product .It has the advantages of being sensitive, selective, rapid, accurate and reproducible. The present paper reports the development of a new high performance liquid chromatography (HPLC) method for determination of guaifenesinin different type of syrups and environmental water samples.

Materials and Methods:

Apparatus

Chromatographic system consisted of an shimadzu HPLC model LC-20AT with UV detector model SPD-20A and C_8supelco column (25cm ×4.6mm),5µm particle size HPLC condition are given in Table [1]

Table(1) : HPLC conditions

Column	SupelcoL_7(25cm×4.6mm),5 µm
Wavelength	254-nm
Mobile phase	Methanol-acetonitrile –H_{2O}
Retention time	2.4min
Flow rate	1.0ml/min
Temperature	Ambient
Injection volume	10 µL

Reagents

All chemicals used were of analytical or pharmaceutical grade and HPLC grade methanol andacetonitrilewere used throughout .
A standard stock solution of guaifenesin (1 mg/ml) was prepared in mobile phase .Working standard solutions in a range of (0.08-0.8 mg/ml) were prepared by dilution from this stock solution.

HPLC method for determiningguaifenesin

A series of standard solution containing 0.08-0.8 mg/ml of guaifenesin and the sample solution of pharmaceutical preparation were applied respectively. 10µl aliquot of each solution was injected into the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of guaifenesin.The concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from the concentration and peak area data.

Procedures for pharmaceutical preparations (syrups):

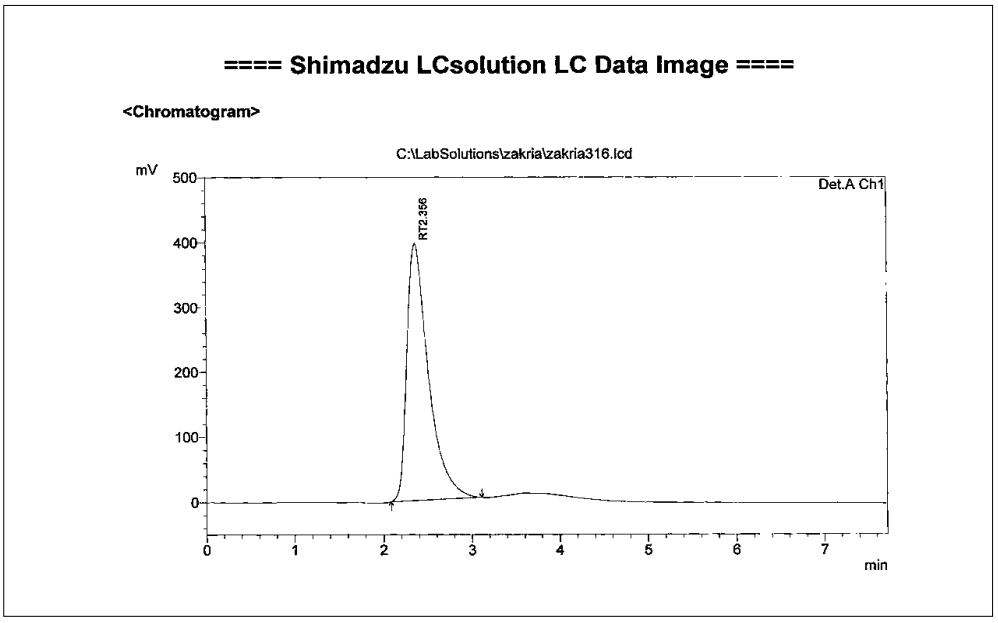
Four different marketed guaifenesin syrup formulations (Exidil30mg/5ml,Pulmocodain 100mg/5ml,Tussilet 50mg/5ml andBronquium30mg/5ml)were selected for analysis.The content of 5 bottles of each typeswere mixed well in 1L dried beaker. Aliquots equivalent to 300 mg of guaifenesin were transferred into 1L volumetric flasks and diluted with mobile phase to the volume.and the amount of guaifenesin was determined by comparing the peak area of the assay preparation with the standard preparation at the same concentration .

Procedure for industrial waste water

To demonstrate the practical applicability of the proposed method, industrial waste water samples from the state company for drug industries and medical appliances, Mosul-Iraq, were collected in polyethylene container cleaned with nitric acid ,and filtered through Whatman No.41 filter paper. Filtered samples were stored at 4 c^0 until analyzedwhich shows negative results, then the samples werespiked with the concentrations ranging from 0.2-0.6 $mg.ml^{-1}$ ofguaifenesin andThen determined the concentration of guaifenesin as described under HPLC method for determining guaifenesin. Calculate the percentage recovery using a calibration graph previously prepared

Results and Discussion:

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and pharmaceutical products. The aim of this study was to develop a rapid HPLC method for the determination ofguaifenesin in pure from ,its pharmaceutical formulations andindustrial waste water samplesusing the most commonly employed RP L_7column with UV detection. The detection wavelength of 254nm was chosen in order to achieve a good sensitivity for quantitative determination of guaifenesin in syrups and wastewater.The mobile phase consisting of methanol: acetonitrile :water (80:10:10) offered a good separation at ambient temperature under these conditions using a flow rate of 1.0ml/min and retention time of 2.4 min as shown in the chromatogram, Fig[2] .



Fig(2): Typical chromatogram (guaifenesin 0. 12mg/ml).

Under the described experimental conditions,the analyte peak were well defined and free from tailing . Guaifenesinwas determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r=0.999) over the concentration range0.08-0.8mg/ml. Using regression analysis, the linear equation Y=2E+06x+16983 was obtained where Y is the mean peak area and X is the concentration in mg/ml fig 3.

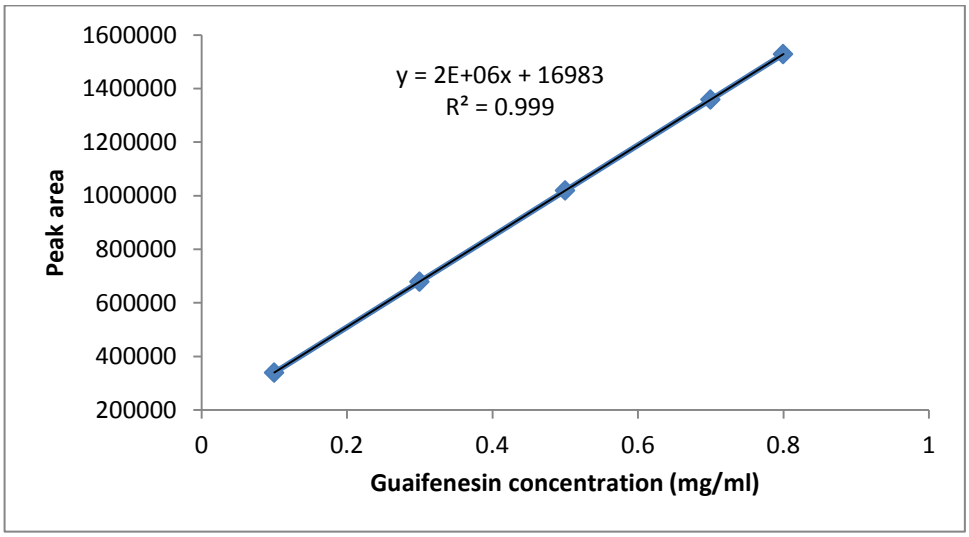


Fig (3)Calibration curve for guaifenesin

Determination of limit of detection and limit of quantitation (sensitivity) .A series of dilute solutions were prepared in the range of 0.1%,0.5% and 1% of the assay concentration (0.3µg/ml) using the standard solutions .10µl of each of the above solutions were injected in 6 times and the areas were calculated due to guaifenesinpeak. The standard deviation for the 6 injections for each concentration was calculated. The standard deviation at concentration 0 was calculated and this The results indication that the method was sensitive enough to detect a concentration of 6 µg/ml and able to quantify at a concentration of above 18 µg/ml.

value was used for the calculation of the limit of detection and limit of quantitation. The limits of detection (LOD) and quantification (LOQ)were calculated using the following formulae : LOD= (3.3σ/s) and LOQ=(10 σ/s) where σ is the standard deviation of the response and s is the slope of the regression line .[27]. Limit of detection (LOD) and limit of quantification (LOQ) were found 6µg/ml and 18µg/ml respectively .

Method precision

The precision of the method was established by carrying out the analysis

ofguaifenesin (n=6) using the proposed method .The low value of standard deviation showed that the method was precise. The results obtained were presented in Table[2].

Table (2) :Method precision

Guaifenesin concentration mg/ml	% Assay Mean(n=6)	%RSD of Assay (n=6)
0.1	101.6	1.02
0.3	101.4	1.15
0.6	99.6	0.86
Mean =	100.8661.01	

Method accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were found to be satisfactorily high, mean recoveries being 100.263±0.388 (n=5) as shown in Table[3]

Table(3) : Method accuracy

Guaifenesin Amount added Mg	Amount found mg	%Recovery n =5
0.20	0.201	100.5
0.40	0.398	99.5
0.60	0.602	100.33
Mean=	100.11 ± 0.39	

Analytical application

The proposed method was successfully applied to the assay of guaifenesin in pharmaceutical syrups and wastewater samples. No interfering peaks were found in the chromatogram, indicating that the excipients did not interfere with the estimation of the drug by the proposed HPLC method. The results obtained are presented in Table [5] ,[6] which reveals that there is close agreement between the results obtained by the proposed method and the lable claim for the determination of guaifenesin in pharmaceutical formulations and good agreement between results and known values indicated the successfully applicability of the proposed method for determination of guaifenesin in environmental samples.

Table (5) Determination of guaifenesin formulations

Pharmaceutical formulations	**Proposed method found***	**Label amount**
Exidil syrup(NDI)	6.04mg/ml	6 mg/ml
Pulmocodin syrup(NDI)	19.92 mg/ml	20 mg/ml
Tussilet syrup(NDI)	10.06 mg/ml	10 mg/ml
Bronquium(Ferrer)	6.0 mg/ml	6.0mg/ml

*Mean of five determinations

Table(6) : Determination of guaifenesinin industrial wastewater samples

Wastewater samples	Added mg/ml	Found* mg/ml	Recovery %(n=10)
Industrial wastewater	0.2	0.201	100.5
	0.4	0.399	99.75
	0.6	0.607	101.16

* mean value of ten determinations.

Conclusion: In this study, a simple, fast, efficient and reliable HPLC method was developed and validated for the determination of guaifenesinin pharmaceutical formulations (syrups) and wastewater samples .The method presented in this study was selective enough using a conventional RP L_7 analytical column and applicable to pharmaceutical preparation after simple extraction with mobile phase. Thus the developed method is recommended for control throughout the entire manufacturing process of drugs as well as quality control of the finished product in view of its high recovery, precision and accuracy.

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تقدير الكوافنسينبطريقة كروماتوغرافيا السائل ذات الاداء العالي في مستحضرات الشراب وفي المياه الصناعية المطروحة

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الخلاصه:
تم اختبار طريقة كروماتوغرافيا السائل ذات الأداء العالي حيثتميزت الطريقة بالبساطة والدقة والسرعة والضبط العالي لتقدير الكوافنسين فيحالته النقية وفي بعض مستحضراته الصيدلانيةوفي المياه الصناعية المطروحة.حيث تم الفصل باستخدام كولوم نوع(L_7)و استخدام مزيج الميثانول الماء واسيتونتريلكوسط ناقل نسبة (10:10:80) حجم\حجم\حجم. وبسرعة جريان 1 مل/دقيقة واستخدام مكشاف الاشعة فوق البنفسجية عند الطول الموجي 254 نانوميتر وفي درجة حرارة المحيط حيث كان زمن الاحتباس 2.4 دقيقه، وامكن تقدير الكميات التي تتراوح بين 0.8-0.08ملغرام\مل وبحدي كشف وكمي هما 6 و 18 مابكروغرام\مل على التوالي واختبر مصداقية الطريقة بقياس استقامة الخط البياني والضبط والدقة واستخدمت الطريقة بنجاح لتقدير الكوافنسين في مستحضرات الشراب وفي المياه الصناعية المطروحة.