High performance liquid chromatographic method for the
determination of guaifenesin in pharmaceutical syrups and in
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 guaifenesin in pure from pharmaceutical formulations and industrial effluent. Chromatography was carried out on supelco L7 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 for guaifenesin 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 guaifenesinsyrups and industrial effluent samples.

Keywords: HPLC, Guaifenesin, Pharmaceutical preparations, Industrial effluent

Introduction:
Guaifenesin is chemically known as 1, 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 or speed recovery. Guaifenesin is in a class of medications calledexpectorants. It works by thinning the mucus and clear the airways. The usual does is 100 to 200 mg every 2 to 4 hours[3-5]

Analytical procedures for the determination of guaifenesin include titrimetry[1], various spectrophotometric[6-13], HPLC[14-20], micellarelectrokinetic chromatography[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 guaifenesin in different types of syrups and environmental water samples.

Materials and Methods:

Apparatus

Chromatographic system consisted of a Shimadzu HPLC model LC-20AT with UV detector model SPD-20A and C8 Supelco column (25 cm × 4.6 mm), 5μm particle size. HPLC conditions are given in Table [1].

<table>
<thead>
<tr>
<th>Table(1) : HPLC conditions</th>
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</thead>
<tbody>
<tr>
<td>Column</td>
</tr>
<tr>
<td>Wavelength</td>
</tr>
<tr>
<td>Mobile phase</td>
</tr>
<tr>
<td>Retention time</td>
</tr>
<tr>
<td>Flow rate</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>Injection volume</td>
</tr>
</tbody>
</table>

Reagents

All chemicals used were of analytical or pharmaceutical grade and HPLC grade methanol and acetonitrile were 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 determining guaifenesin

A series of standard solutions 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 (Exidil 30mg/5ml, Pulmocodain 100mg/5ml, Tussilet 50mg/5ml and Bronquium 30mg/5ml) were selected for analysis. The content of 5 bottles of each type were mixed well in a 1L dried beaker. Aliquots equivalent to 300 mg of guaifenesin were transferred into 1L volumetric flasks and diluted with mobile phase to the volume. 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 until analyzed which shows negative results, then the samples were spiked with the concentrations ranging from 0.2-0.6 mg.ml⁻¹ of guaifenesin and then 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 of guaifenesin in pure from its pharmaceutical formulations and industrial waste water samples using the most commonly employed RP L₇ column 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. Guaifenesin was determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r=0.999) over the concentration range 0.08-0.8mg/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 guaifenesin peak. The standard deviation for the 6 injections for each concentration was calculated. The standard deviation at concentration 0 was calculated and this 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: \( \text{LOD} = (3.3 \sigma/s) \) and \( \text{LOQ} = (10 \sigma/s) \) where \( \sigma \) 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.

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.

**Method precision**

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

<table>
<thead>
<tr>
<th>Guaifenesin concentration mg/ml</th>
<th>% Assay Mean(n=6)</th>
<th>%RSD of Assay (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mg/ml</td>
<td>101.6</td>
<td>1.02</td>
</tr>
<tr>
<td>0.3 mg/ml</td>
<td>101.4</td>
<td>1.15</td>
</tr>
<tr>
<td>0.6 mg/ml</td>
<td>99.6</td>
<td>0.86</td>
</tr>
<tr>
<td>Mean</td>
<td>100.8661.01</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Guaifenesin Amount added Mg</th>
<th>Amount found mg</th>
<th>%Recovery n =5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0.201</td>
<td>100.5</td>
</tr>
<tr>
<td>0.40</td>
<td>0.398</td>
<td>99.5</td>
</tr>
<tr>
<td>0.60</td>
<td>0.602</td>
<td>100.33</td>
</tr>
<tr>
<td>Mean=</td>
<td>100.11 ± 0.39</td>
<td></td>
</tr>
</tbody>
</table>

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 label 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

<table>
<thead>
<tr>
<th>Pharmaceutical formulations</th>
<th>Proposed method found*</th>
<th>Label amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exidil syrup(NDI)</td>
<td>6.04 mg/ml</td>
<td>6 mg/ml</td>
</tr>
<tr>
<td>Pulmocodin syrup(NDI)</td>
<td>19.92 mg/ml</td>
<td>20 mg/ml</td>
</tr>
<tr>
<td>Tussilet syrup(NDI)</td>
<td>10.06 mg/ml</td>
<td>10 mg/ml</td>
</tr>
<tr>
<td>Bronquium(Ferrer)</td>
<td>6.0 mg/ml</td>
<td>6.0mg/ml</td>
</tr>
</tbody>
</table>

*Mean of five determinations
Table(6) : Determination of guaifenesin in industrial wastewater samples

<table>
<thead>
<tr>
<th>Wastewater samples</th>
<th>Added mg/ml</th>
<th>Found* mg/ml</th>
<th>Recovery % (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial wastewater</td>
<td>0.2</td>
<td>0.201</td>
<td>100.5</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.399</td>
<td>99.75</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.607</td>
<td>101.16</td>
</tr>
</tbody>
</table>

* 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 guaifenesin in pharmaceutical formulations (syrups) and wastewater samples. The method presented in this study was selective enough using a conventional RP L7 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.

Acknowledgments
The first author (Nief R. Ahmed) wishes to express gratitude to his former company [the state company of drug industries and medical appliance (NDI) (Nineveh – Iraq.) for providing gift sample of guaifenesin standard materials and pharmaceutical preparations (syrups) and for permission and facilities to carry out the research work.

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تقدير الكوافنسين بطريقة كروماتوغرافيا السائل ذات الأداء العالي في مستحضرات الشراب وفي المياه الصناعية المطروحة

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**الخلاصة:**
تم اختبار طريقة كروماتوغرافيا السائل ذات الأداء العالي حيث تميزت بطريقة بالبساطة والدقة والسرعة والضبط العالي لتقدير الكوافنسين في حالتهم النقية وفي بعض مستحضراته الصيدلانية وفي المياه الصناعية المطروحة حيث تم الفصل باستخدام كولوم نوع (L7) و استخدام مزيج الميثانول الماء واستيروتريلكوبست ناقل نسبة (80:10:10) حجم حجم حجم حجم حجم حجم حجم. وبسرعة جريان 1 مل/دقيقة واستخدام مكشاف الإشعة فوق البنفسجية عند الطول الموجي 254 نانومتر وفي درجة حرارة المحيط حيث كان زمن الاحتباس 2.4 دقيقة، وامكنت تقدير الكميات التي تتراوح بين 0.8-0.08 ملغ/مليjl. 0 و 6 و 18 ملغ/مليjl. على التوالي واختبر مصداقية الطريقة بقياس استقامة الخط البياني والضبط والدقة واستخدمت الطريقة بنجاح لتقدير الكوافنسين في مستحضرات الشراب وفي المياه الصناعية المطروحة.