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Research Article

Development and Validation of New RP-HPLC Method for the Quantitative Estimation of Guaifenesin in Tablet Dosage Form

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Abstract

A novel very rapid, sensitive, reverse phase High Performance Liquid Chromatography (RP-HPLC) technique was developed for the quantitative estimation of Guaifenesin in bulk and tablet dosage form. The developed HPLC has several advantages over reported HPLC methods with respect to speed, solvent consumption, resolution and cost of analysis. It was resolved by using a mobile phase of Phosphate buffer: Acetonitrile in the ratio 60:40 v/v at a flow rate of 1 mL/min. using UV-Visible detector at the wavelength of 232 nm for quantification. Efficient separation was achieved for guaifenesin on Cosmosil C18 column (100 × 2.1 mm, 5 µm). The retention time of guaifenesin was 2.783 min. The calibration graphs were linear and the method showed excellent recovery for tablet dosage form. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness.

Keywords: Guaifenesin; HPLC; New method development; validation

Introduction

Guaifenesin is chemically known as is (+)-3-(2-methoxyphenoxy)-propane-1, 2-diol, is a widely used expectorant and treatment of coughing. [1] The structure of Guaifenesin shown in figure1. [2] Useful for the symptomatic relief of respiratory conditions. 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. Its solid oral dosage form is available as extended release tablets for oral administration Guaifenesin is used to relive chest congestion. Guaifenesin may help control symptoms but does not treat the cause of symptoms or speed recovery.

Several HPLC methods were reported for simultaneous determination of Guaifenesin and other expectorants in pharmaceutical preparations [3-8]. The LC-MS method was also reported for quantification of guaifenesin in human plasma [9]. The UV method have been reported for guaifenesin. [10] and only two HPLC methods were related for individual estimation of guaifenesin. [11,12] The reported HPLC methods were more time consuming, complex mobile phase mixtures, use high flow rate of analysis, lack of sensitivity and peak symmetry. It is, therefore, felt necessary to develop a new rapid method for the determination of Guaifenesin by HPLC method. Hence a reproducible RP HPLC method was developed for the quantitative determination of Guaifenesin tablets by using Cosmosil C_{18} column (100 × 2.1 mm, 5 µm) HPLC column. The proposed method was validated as per the guidelines suggested by ICH.

Experimental details

Materials and Reagents

Guaifenesin Working Standard was procured from Aurabindo laboratories, Hyderabad, India. Commercially available guaifenesin purchased from local pharmacy. Acetonitrile HPLC Grade and Ortho phosphoric acid AR grade were obtained from Merck chemicals, Mumbai. Water was prepared by using Millipore Milli Q Plus water purification system.

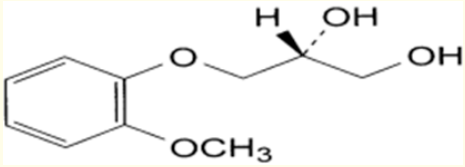

Figure 1: Chemical structure of guaifenesin.

Chromatographic conditions

Chromatography separation was performed on LC Solution HPLC with UV detector. The output signal was monitored and processed using Chrom-work station HPLC V4.0 software. The chromatographic column used Cosmosil C_{18} (100 × 2.1 mm, 5 µm). The mobile phase of phosphate buffer: acetonitrile in the ratio 40:60 v/v at a flow rate of 1.0 ml/min. The detection was monitored at the Wavelength of 232 nm. The injection volume was 20.0 µL and the chromatographic runtime of 8 min was used.

Preparation of solutions

Preparation of Phosphate buffer

Weighed 7.0 grams of Potassium di hydrogen phosphate into a 1000 mL beaker, dissolve and diluted to 1000 mL with mille pore water. Adjusted the pH to 4.0 with ortho phosphoric acid.

Preparation of mobile phase

Mixed a mixture of above buffer 400 mL (40%) and 600 mL of acetonitrile (60%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of the Guaifenesin Standard & Sample Solution

Standard Solution Preparation

Accurately transferred sample equivalent to 10 mg of guaifenesin working standard into a 10 mL volumetric flask and take 1 mL of diluents added 9 ml of mobile phase then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further pipette out 1 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluents. Mix well and filter through 0.45 µm filter.

Sample Solution Preparation

Accurately transferred the sample equivalent to 10 mg of guaifenesin into a 10 mL volumetric flask. About 1 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluent. Mixed well and filtered through 0.45 µm filter. Further pipette 1 ml of the above stock solution into a 10 ml volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45 µm filter.

Method validation

Precision: The precision of the method was evaluated by carrying out five independent assays of test sample against a qualified reference standard and the %RSD of assay was calculated (%RSD should not be more than 2%).

Intermediate Precision/Ruggedness

Intra-day precision

The precision of the assay method was evaluated by carrying out five independent assays of Guaifenesin (50,100, 150% i.e. 5.0, 7.5, 10.0 µg/ml.) test samples against qualified reference standard. The percentage of RSD of five assay values was calculated.

Intermediate precision (inter-day)

Different analyst from the same laboratory and by using different column of same brand evaluated the intermediate precision of the method. This was performed by assaying the five samples of Guaifenesin against qualified reference standard. The percentage of RSD of five assay values was calculated. The %RSD for the area of five replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

Accuracy

Recovery of the assay method for Guaifenesin was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyte concentration. Each solution was injected thrice (n = 3) into HPLC system and the average peak area was calculated from which Percentage recoveries were calculated. (% Recovery should be between 98.0 to 102.0%).

Linearity: Test solutions were prepared from stock solution at 5 concentration levels (20, 30, 40, 50, and 60µg/ml). The peak area vs concentration data treated by least square linear regression analysis (Correlation coefficient should be not less than 0.999).

Limit of Detection (LOD) Limit of Quantification (LOQ): LOD and LOQ for the were determined at signal to noise ratios of 3:1 and 10:1, respectively by injecting series of dilute solutions with known concentrations.

Robustness: To prove the reliability of the analytical method during normal usage, some small but deliberate changes were made in the analytical method (e.g., flow rate, column temperature, and mobile phase composition). Changes in the chromatographic parameters (i.e., theoretical plates and the tailing factor) were evaluated for the studies.

Results

Method development: Different chromatographic conditions were experimented to achieve better efficiency of the chromatographic system. Parameters such as mobile phase composition, wavelength of detection, column, column temperature, pH of mobile phase, and diluents were optimized. Several proportions of buffer, and solvents (water, methanol and acetonitrile) were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, tailing, theoretical plates, and run time were the major tasks while developing the method. Buffers like sodium dihydrogen orthophosphate, dipotassiumhydrogenorthophosphate, and disodium hydrogen orthophosphate did not yield desired results. Use of ion pair reagents also did not yield the expected peak. At 40:60 (buffer:solvent) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 40:60 (buffer:solvent) in an isocratic mobile phase flow rate. The typical chromatogram obtained for Guaifenesin from final HPLC conditions are depicted in Figure 2.

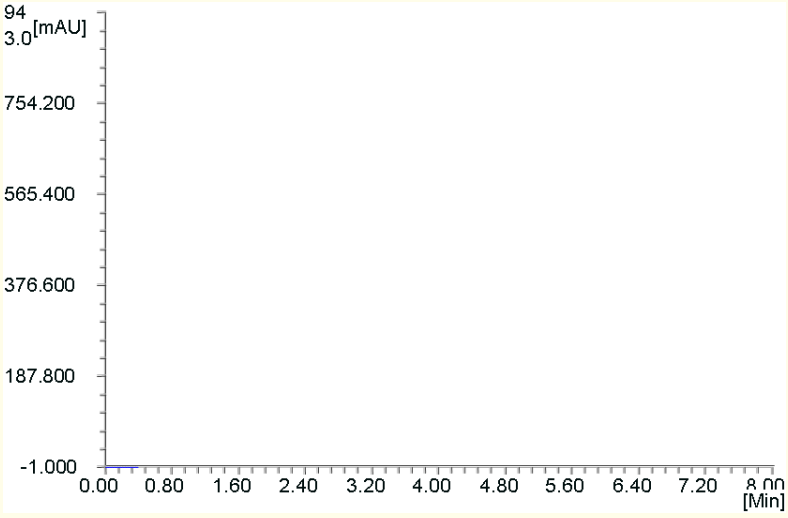

Figure 2: Typical chromatogram of Guaifenesin by proposed method.

Method validation: Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ , robustness and sensitivity as follows.

System suitability: The system suitability results for the proposed HPLC method are Tailing factor Obtained from the standard injection is 1.28. Theoretical Plates Obtained from the standard injection is 11105.3. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

Precision: The %R.S.D. of guaifenesin assay during the method precision was found to be 1.73%, indicating good precision of the method. The results we summarized in table no1.

Injection	Area
Injection-1	70131.8
Injection-2	70650.7
Injection-3	71284.2
Injection-4	72547.3
Injection-5	72653.8
Injection-6	73241.6
Average	71751.57
Standard Deviation	1242.691
%RSD	1.73%

Table 1: Results of precision:

Limits of detection (LOD) and quantification (LOQ):

Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for Guaifenesin were 0.01 and 0.05 µg/ml, respectively. Since the LOQ and LOD values of Guaifenesin are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

Accuracy: Percentage recovery of Guaifenesin samples ranged from 98.8% to 101.4% and the mean recovery is 99.96%, showing the good accuracy of the method. The result was shown in Table 2.

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	75587.167	5.0	5.0	99.95%	99.96%
100%	161756.43	7.5	7.5	99.98%	
150%	208555.8	10.0	10.2	99.95%	

Table 2: Results of Accuracy

Linearity: The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 50-90 µg/ml for three times, and the correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

Robustness: In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is 10-50 µg/ml, (n = 3). It can be concluded that the variation in flow rate and the variation in 10% Organic composition do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ± 10% and change in the Mobile phase ± 10%. The results are summarized in table 3.

Chromatographic changes	USP Plate Count	USP Tailing
Flow rate (ml/min)		
0.8	10478.5	1.29
1.0*	10297.33	1.35
1.2	9898.94	1.36
Change in organic composition in the mobile phase		
10% less	9350.33	1.28
40:60(Buffer: acetonitrile)*	10297.33	1.35
10% more	10044.49	1.28
UV wavelength (nm)		
230	8680.99	1.9
232*	12965.45	1.29
234	8273.24	1.26

* optimized parameters.

Table 3: Results of Robustness.

Application of the developed method to commercial Guaifenesin tablets

When the developed method was used to analyze a commercial brand of guaifenesin tablet formulation, the mean recovery of five replicates was 99.96% with %R.S.D. of 1.73. The %recovery value indicates non-interference from the excipients present in the dosage form.

Discussion

Method development and optimization

The main aim of the developed method was to achieve separation and quantification of Guaifenesin using an isocratic mobile phase with HPLC system. Developing a HPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of Guaifenesin was adequate at 232 nm. The initial trial was conducted using HPLC and chromatographic separation was obtained on C_{18} column (100 × 2.1 mm, particle size 5 µm). The mobile phase was optimized in the ratio of Phosphate buffer: Acetonitrile in the ratio 40:60% v/v at a flow rate of 1 ml/min. While developing the HPLC method, basic chromatographic conditions such as the column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the HPLC column, its stability at the lower pH was taken into consideration to preserve the long life of the column. Most commercial C_{18} columns are not stable at lower pH on the longer run, thus shortening their life span. Column Cosmosil C_{18} (100 × 2.1 mm, 5 µm). Was found to be more suitable and stable at this pH. The peak was sharp and acceptable. The flow rate also is scaled down from 2.0 to 1.0 mL/min. When these operating conditions were applied to the developed method, a satisfactory peak was achieved for Guaifenesin, which eluted at around 2.783 min giving a total run time of 8 min.

Conclusion

The new, isocratic RP-HPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 2.783 min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of guaifenesin in tablet formulations.

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