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Analytical Method Development and Validation of Guaifenesin by RP-HPLC Method

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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. It was resolved by using a mobile phase Potassium di-hydrogen phosphate: methanol in the ratio (60:40 v/v) at a flow rate of 0.8mL/min. using UV - Visible detector at the wavelength of 228 nm for quantification. Efficient separation was achieved for Guaifenesin on used Waters Acquity HSS T-3 C_{18} (100 × 2.1 mm, 5µm). The retention time of Guaifenesin was 2.433min. 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.

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1. INTRODUCTION:
"Guaifenesin, is a widely used expectorant, 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 tables for oral administration".[1]

"Guaifenesin 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 toward the pharynx Thus it may increase the efficiency of the cough reflex and facilitate removal of the secretions Guaifenesin has muscle

Structure of drug

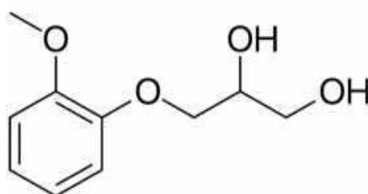

Figure 1: Structure of Guaifenesin

2. EXPERIMENTAL DETAILS:

2.1 Materials and Reagents: Guaifenesin Working Standard was procured from Teena laboratories, Hyderabad, India. Commercially available Guaifenesin purchased from local pharmacy. Methanol 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.

2.2 Chromatographic conditions: Chromatography separation was performed on LC Solution HPLC with UV detector. The output signal was monitored and processed using Chrome- work station HPLC V4.0 software. The chromatographic column used Inertsil ODS C18 5µm (4.6*250 mm) mobile section magnitude relation was Phosphate buffer (0.05 M) pH 4.6: ACN (55:45% v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm at a flow rate of 0.8 mL/min. The detection was monitored at the Wavelength of at 255 nm. The injection volume was 20.0 µL and the chromatographic runtime of 5 min was used.

2.3 Preparation of solutions

2.3.1 Preparation of Phosphate buffer: Weigh 7.0 grams of Potassium di hydrogen phosphate into a 1000 ml beaker, dissolve and diluted to 1000 ml with milli pore water. Adjusted the pH to 4.0 with ortho phosphoric acid.

2.3.2 Preparation of mobile phase: Mix a mixture of above buffer 600 mL (60%) and 400 mL of methanol (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

2.4 Preparation of the Guaifenesin Standard and sample Solution:

2.4.1 Standard Solution Preparation. Accurately transferred 10mg of Guaifenesin working standard into a 10 mL volumetric flask and about 7 mL of Diluent added then sonicated to dissolve it completely and the volume was made up to the mark with the same solvent (Stock solution). Further pipette 5 ml of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluents. Mix well and filter through 0.45µm filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter.

2.4.2 Sample Solution Preparation: Accurately transferred the sample equivalent to 10 mg of Guaifenesin into a 10 mL volumetric flask. About 7 mL of diluent added and sonicated to dissolve it completely and the volume is made up to the mark with diluents. Mixed well and filtered through 0.45µm filter. Further pipette 5 ml of the above stock solution into a 50mL volumetric flask and diluted up to the mark with diluents. Mix well and filter through 0.45µm filter. Further pipette 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter.

2.5 Method validation

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

2.5.2 Intermediate Precision/Ruggedness:

2.5.2.1 Intra-day precision: The precision of the assay method was evaluated by carrying out six independent assays Guaifenesin (50,100, 150% i.e. 5.0, 10.0, 15.0µg/Mr.) test samples against qualified reference standard. The percentage of RSD of six assay values was calculated.

2.5.2.2 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 six samples of Guaifenesin against qualified reference standard. The percentage of RSD of six assay values was calculated. The % RSD for the area of six replicate injections was found to be within the specified limits (% RSD should not be more than 2%).

2.5.3 Accuracy: Recovery of the assay method for Guaifenesin was established by three determinations of test sample using tablets at 50%, 100% and 150% of analyse 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%).

2.5.4 Linearity: Test solutions were prepared from stock solution at 5 concentration levels (10, 20, 30, 40 and 50 µm/mol). The peak area vs concentration data treated by least square linear regression analysis. (Correlation coefficient should be not less than 0.999.)

2.5.5 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

2.5.6 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.

3. RESULTS:

3.1 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, Phosphate buffer 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. Potassium di-hydrogen phosphate: ACN in the ratio (55:45% v/v) ratio of the mobile phase, a perfect peak was eluted. Thus the mobile phase ratio was fixed at 55:45 (Potassium di-hydrogen phosphate: ACN) in an isocratic mobile phase flow rate. The typical chromatogram obtained for from final HPLC conditions are depicted in Figure2.

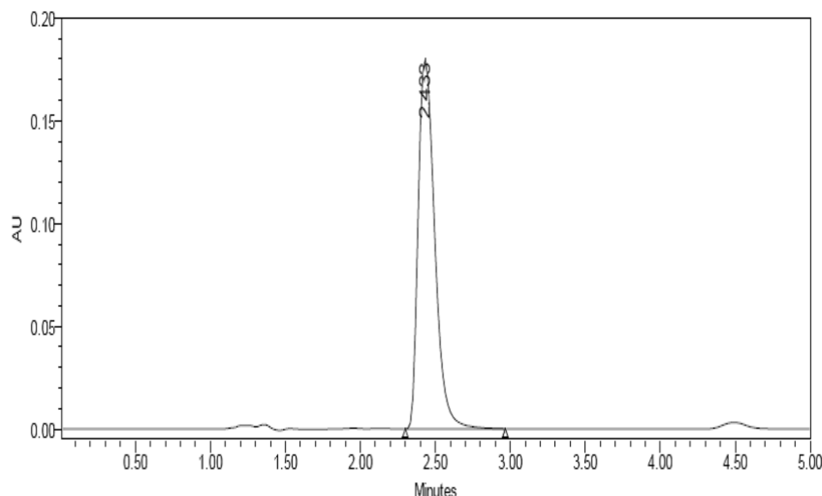

Figure 2: typical chromatogram of by proposed method

3.2 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.

3.2.1 System suitability: The system suitability results for the proposed HPLC method are Tailing Factor Obtained from the standard injection is 1.4. Theoretical Plates Obtained from the standard injection is 4184.7. The results proved that the optimized HPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

3.2.2 Precision: The % R.S.D. of Guaifenesin assay during the method precision was found to be 0.45%, indicating good precision of the method. The results are summarized in table 1.

Table 1- Results of precision

Injection	Area
Injection-1	696977
Injection-2	696531
Injection-3	696214
Injection-4	695535
Injection-5	696708
Average	696393
Standard deviation	8624.7
%RSD	1.1%

3.2.3 Limits of detection (LOD) and quantification (LOQ): LOD and LOQ for Guaifenesin were 0.024and 0.07µg/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.

3.2.4 Accuracy: Percentage recovery of Guaifenesin samples ranged from 99.69 % to 101.2% and the

mean recovery is 100.5%, showing the good accuracy of the method. The result is shown in Table 3.

Table 3 - Results of Accuracy

%Concentration (at specification Level)	Mean Peak Area (n=3)	Amount Added (mg)	Amount Found (mg)	Average % Recovery	Mean Recovery
50%	688287	5.68	5.78	100.9%	
100%	1378200	10.0	9.97	99.7%	
150%	2065480	14.05	13.93	98.2%	100.7%

3.2.5 Linearity: The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e. 10 - 50µg/ml for three times, and the correlation coefficient obtained was 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte.

3.2.6 Robustness: In all the deliberately varied chromatographic conditions in the concentration range for the evaluation of robustness is10 -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 4.

Table- 4- Results of Robustness

Chromatographic changes	*USP Plate Count*	*USP Tailing*
Flow rate(ml/min)		
0.6	4187.6	1.5
0.8*	4184.7	1.3
1.0	4084.7	1.4
Change in organic composition in the mobile phase		
10% less	4194.5	1.5
60:40(Buffer: methanol)*	3156.0	1.3
10% more	3097.0	1.4
UV wavelength(nm)		
226	4205.0	1.5
228*	3648.3	1.3
230	3354.2	1.4

3.2.7 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 triplicates was 99.69 % with % R.S.D. of 0.52. The % recovery value indicates non-interference from the excipients present in the dosage form

4. 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 255 nm.The initial trial was conducted using HPLC and chromatographic separation was obtained on Inertsil ODS C18 5µm (4.6*250mm),The mobile phase was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), flow rate of 0.8ml/min. While developing the HPLC method, basic chromatographic conditions such as the used Inertsil ODS C18 5µm (4.6*250mm) 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 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 2.433 min giving a total run time of 5 min.

5. 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 2.433 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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