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# A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF GUAIFENESIN IN BULK DOSAGE FORM

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**Abstract:**

*A simple and reproducible method was developed for Guaifenesin by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Guaifenesinwas separated on Greece C18 (4.6ID x 250mm, Particle size: 5 micron), using ortho phosphoric acid buffer with pH of 3.0 at the UV detection of 225 nm. Isocratic elution of methanol and water was used as a mobile phase with various ratios and flow rates, eventually 80:20 v/v methanol and water was being set with the flow rate of 0.8mL/min. The statistical validation parameters such as linearity, accuracy, precision, LOD, LOQ, Robustness, system suitability were checked, further the limit of detection and limit of quantification of Guaifenesin concentrations were found to be 57.98µg/mL and 175.71µg/mL .*

***Keywords:*** *Guaifenesin, Methanol, Development, RP-HPLC, Validation.*

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## INTRODUCTION:

Guaiphenesin is an expectorant which clears chest congestion by loosening and reducing the viscosity of phlegm, increasing the volume of phlegm and making coughs more productive [1]. Guaifenesin has musclerelaxant and anticonvulsant properties and may be acting as an NMDA receptor antagonist [2]. Its solid oral dosage form is available as extended release tablets for oral administration.Guaifenesin is readily absorbed from the GI tract and is rapidly metabolized and excreted in the urine. Guaifenesin has a plasma half-life of one hour. The major urinary metabolite is β-(2-methoxyphenoxy) lactic acid [3,4]. Use in pregnancy Guaiphenesin has been taken by a large number of pregnant women and women of child bearing age without any proven increase in the frequency of malformations or other direct or indirect harmful effects on the foetus having been observed.[5,6]. It is not known whether guaiphenesin is excreted in breast milk or whether it has a harmful effect on the breastfeeding infant [3]. Therefore it is not recommended for breastfeeding mothers unless the potential benefits to the patient are weighed against the possible risk to the infant [7,8]. In literature several analytical techniques like colorimetric, spectroflurimetric methods have been reported on assay of Guaifenesin in combination with other drugs. In the present study, novel method was developed with methanol as solvent in HPLC; it is a simple method to study, detect and separate the Guaifenesin from mixture of com-pounds and can be adopted for regular quality assessment in pharmaceutical industry and scientific laboratories.

## MATERIALS AND METHOD:

All reagents used were of analytical-reagent grade. Water purification systems, reverse osmosis and ultrapure water (Nanopure Human Corporation, Korea), sonicator (Digital citizen ultra sonic cleaner) for degassing of HPLC grade Methanol and ortho phosphoric acid 88% (S.D. FineChem Limited, Mumbai, India) and pure Guaifenesin drug.

The RP-HPLC system composed of HPLC Binary Gradient System of HPLC 3000 series instant pilot software: HPLC Workstation and certified for pharmaceutical QA/QC. It constitutes Detector: UV-3000-M (Single Wavelength) Pump: P-3000-M Reciprocating (40MPa) Column: Greece C18 (4.6ID x 250mm, Particle size: 5 micron). It is most flexible configuration for the maximum in gradient and low flow rate accuracy and precision, high-speed, multi-wavelength and full spectral UV-visible detection for peak purity analysis and spectral confirmation. The chromatographic and integrated data were recorded in computer system. Two solvents were used, solvent-A containing methanol filtered through 0.22µm filter paper and solvent-B containing ultrapure water filtered through 0.22µm Borosil (1 liter), 0.45micron cellulose membrane filter [pH adjusted to 3.0 with 88% ortho phosphoric acid], de-gassed with sonication. Various flow rates and solvent compositions were provided at room temperature [28°C] to develop a method. The detection was performed at 225nm using UV detector 3000-M (Single Wavelength). Solution 10mg/mL of pure Guaifenesin was pre-pared in the mobile phase. The solution was adequately diluted and it was taken to develop a method by various solvent ratios and flow rates.

**Validation of the Method**

Validation of the optimized HPLC method was carried out with the following parameters.

**Linearity**

Guaifenesin standard stock solution of 10mg/mL was used for preparation of subsequent aliquots; aliquots of 20, 40, 60, 80, 100µg/mL concentrations were prepared. All measurements were repeated three times for each concentration. The calibration curves of the area under curve versus concentration were recorded.

**Accuracy**

Guaifenesin standard stock solution of 10mg/mL was used to prepare 20, 60, 100µg/mL concentrations and injected for the accuracy studies. The area under curve obtained was checked and analyzed for the recovery percentage.

**Precision**

The precision of method was checked and verified by repeatability .Repeatability was checked by injecting 20,69,100µg/ml concentration of Guaifenesin for 3 times on the same day was injected and analyzed at different time intervals on the same day.

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The LOD and LOQ of Guaifenesin were separately determined on the basis of signal (S) and noise (N) ratio, LOD and LOQ concentrations ofGuaifenesin.

**Robustness of the Method**

To determine the robustness of the developed method, minute changes were made in the flow rate, percentage of Methanol and the pH of the mobile phase and is studied for the deviations from optimized method.

**System Suitability Parameters**

To perform the system suitability tests the standard solution was freshly prepared and injected under the condition of optimized method to study the following parameters.

## RESULTS:

**Table 1: Parameters of Experiment**

| Concentration of solvent | Flow Rate (ml/min) | Retention Time (min) | Area Under Curve | % Recovery |
|---|---|---|---|---|
| 20 | 0.8 | 3.578 | 519641 | 59.48718 |
| 40 | 0.8 | 3.576 | 1357747 | 79.74359 |
| 60 | 0.8 | 3.578 | 2296980 | 86.495727 |
| 80 | 0.8 | 3.581 | 3140325 | 89.871795 |
| 100 | 0.8 | 3.585 | 3981717 | 91.897436 |

**Linearity**

The method gave a linear response to Guaifenesin drug within the concentration range of 20-100µg/mL with R2= 0.9996 as shown in figure 2.

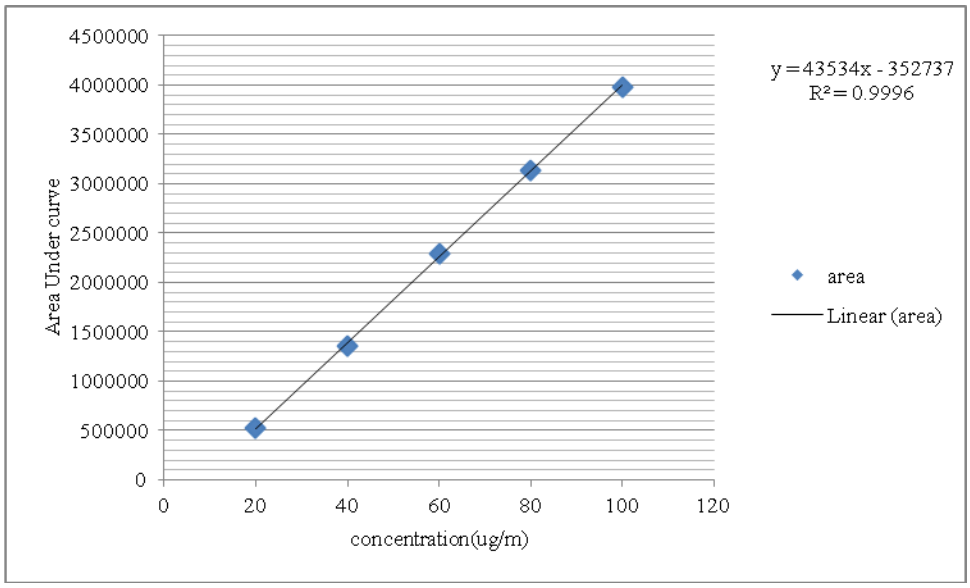


**Fig 2: Linearity Curve**

**Accuracy**

The Guaifenesin was used for various concentrations and standard deviation is calculated for same as shown in table 2.

**Table 2: Accuracy studies of Guaifenesin.**

| **Accuracy of Guaifenesin.** | | | |
|---|---|---|---|
| **Guaifenesinconcμg/mL** | | | |
| | **Conc-1** | **Conc-2** | **Conc-5** |
| | 20 | 60 | 100 |
| Replicate-1 | 519641 | 2296980 | 3981717 |
| Replicate-2 | 522855 | 2301097 | 4062112 |
| Replicate-3 | 517982 | 2308149 | 4019887 |
| Average | 520159.3333 | 2302075.333 | 4021238.667 |
| S.D | 2477.505668 | 5648.406176 | 40214.54038 |

*The value of Standard Deviation is calculated for accuracy studies.*

**Precision**

**Repeatability**

The repeatability, The RSD values were below 2%, indicating a good precision. The t-test value for inter-day precision was less than 0.1%, indicating the significant precision.

**Table 3: Precision study of Guaifenesin**

| **Precision of Guaifenesin.** | | | |
|---|---|---|---|
| **Guaifenesinconcμg/mL** | | | |
| | **Conc-1** | **Conc-2** | **Conc-5** |
| | 20 | 60 | 100 |
| Replicate-1 | 519641 | 2296980 | 3981717 |
| Replicate-2 | 522855 | 2301097 | 4062112 |
| Replicate-3 | 517982 | 2308149 | 4019887 |
| Average | 520159.3333 | 2302075.333 | 4021238.667 |
| S.D | 2477.505668 | 5648.406176 | 40214.54038 |
| %RSD | 0.476297455 | 0.245361483 | 1.000053559 |

Relative Standard Deviation = Less than 2 %

**LOD and LOQ:**

The LOD and LOQ concentrations of Guaifenesinwere found to be 57.98μg/mL and 175.71μg/mL

**Robustness of the Method**

**Table 4: Robustness Studies**

| Sr. No | Concentration(µg/ml) | Area(mAU) |
|---|---|---|
| 1 | 20 | 494549 |
| 2 | 40 | 1366487 |
| 3 | 60 | 2275262 |
| 4 | 80 | 3123424 |
| 5 | 100 | 3971699 |

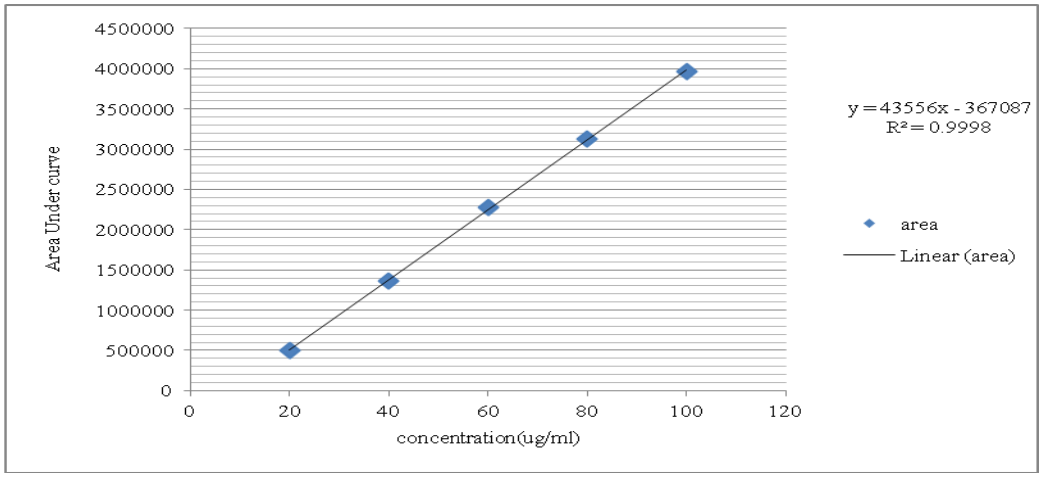


**Fig 3: Robustness Study Using Linearity Curve**

The regression coefficient of the curve is 0.9998

**System Suitability Studies**

The system suitability parameters such as retention time, theoretical plate number, Asymmetry factor were associated with confined values as shown in the table 5.

**Table 5: System Suitability Studies.**

| Concentration | Retention time | Theoretical plate no. | Asymmetry factor |
|---|---|---|---|
| 20 | 3.578 | 6689 | 1.18 |
| 40 | 3.576 | 7077 | 1.19 |
| 60 | 3.578 | 7283 | 1.19 |
| 80 | 3.581 | 6890 | 1.18 |
| 100 | 3.585 | 6813 | 1.18 |

*Number of theoretical plates is more than 2000 and Asymmetry factor should be less than 2.*

**Chromatographs of Guaifenesin**

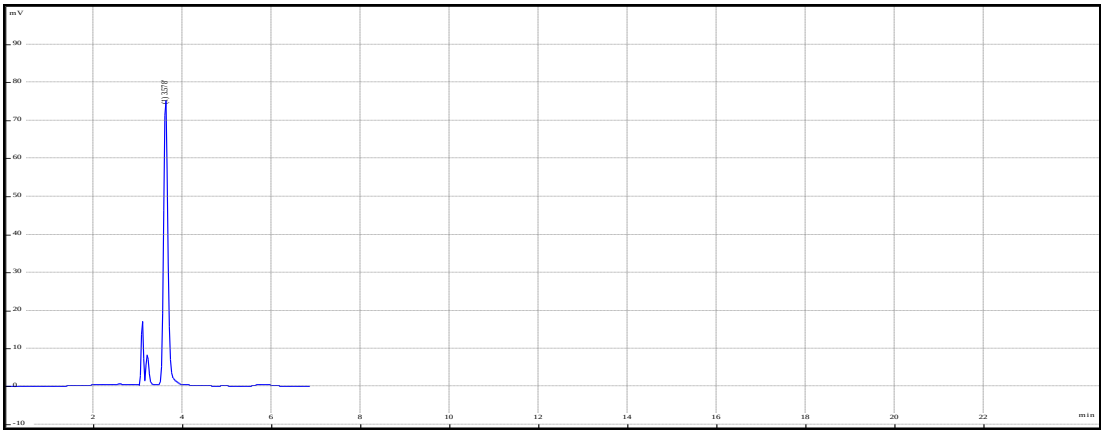


**Fig 4: chromatogram of 20µg/mL**

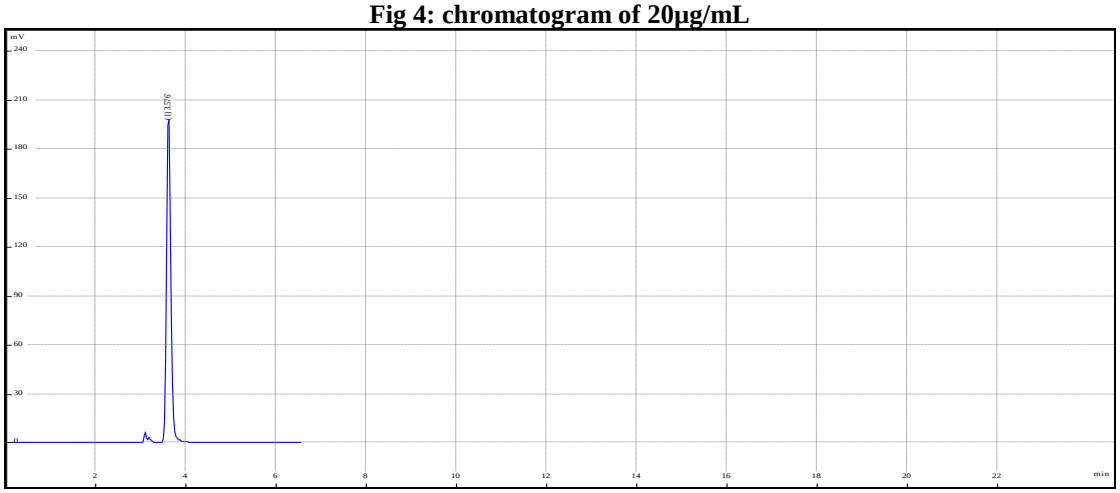


**Fig 5: chromatogram of 40µg/mL**

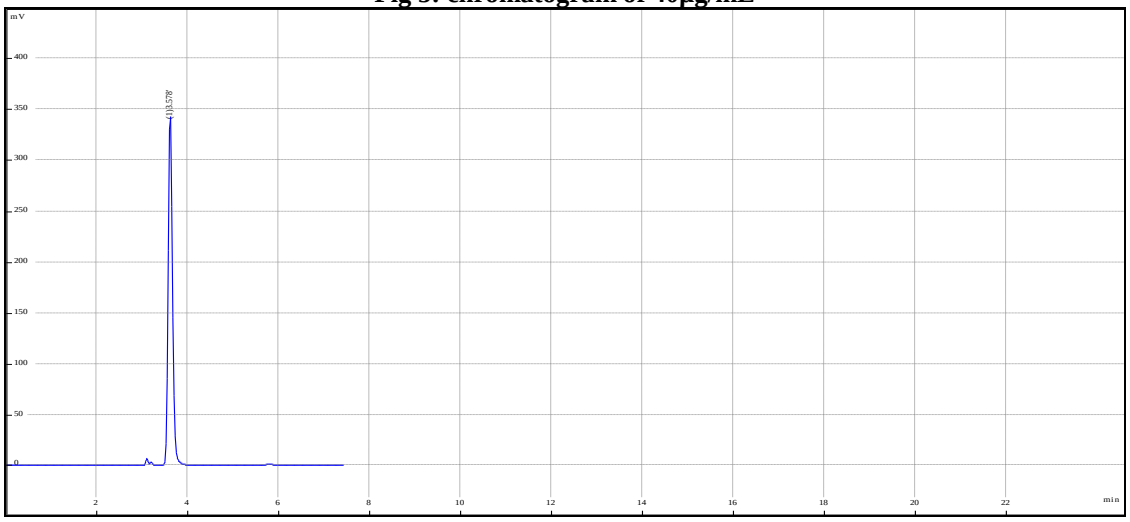


**Fig 6: chromatogram of 60µg/mL**

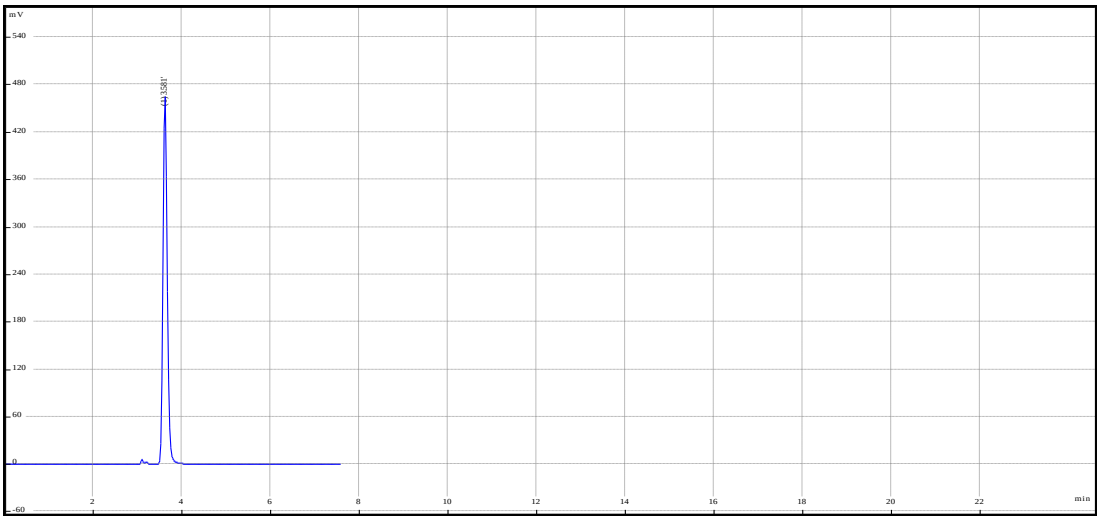


**Fig 7: chromatogram of 80µg/mL**

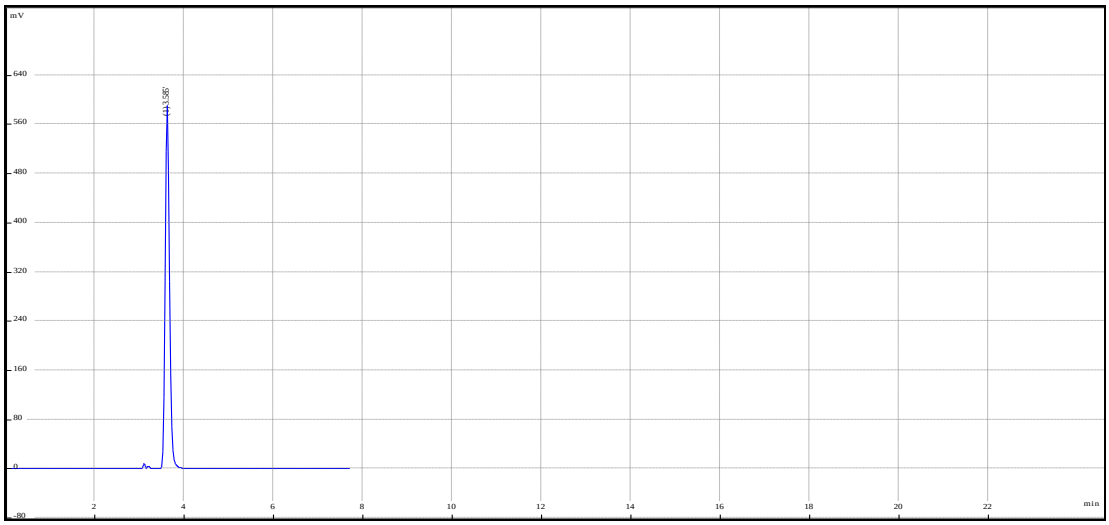


**Fig 8: chromatogram of 100µg/mL**

## DISCUSSION:

The developed and validated method of Guaifenesin was aimed to establish chromatographic conditions, capable of qualitative and quantitative de-termination of Guaifenesin in pharmaceutical preparations. Guaifenesin was completely separated on C18 column by RP-HPLC using the isocratic elution of Methanol and water as mobile phase. When the Methanol percentage was increased starting from 50% by a decrement of every 5%, sharp peaks were observed. As a result of higher concentrations of Methanol in mobile phase and increase in the percentage of Methanol the peak was sharp pointed and well separated. The unusual peaks could be the result of improper dissolution of Guaifenesin in lower concentration of Methanol, therefore the chromatographic column was not able to resolve the Guaifenesin properly and even there was decrease in the recovery percentage ofGuaifenesin. As Methanol concentration gradually decreases the peak broadening, fronting and tailing were remarkably reduced. It is evident that the flow rate of mobile phase in chromatography plays an important role in resolving theGuaifenesin, as the flow rate in-creases from 0.75 mL/min to 0.9 mL/min the retention time also decreased with fluctuation in Guaifenesin recovery (Table 1), eventually proper resolution was achieved at flow rate of 0.8mL/min and retention time of 3.5 minutes.

## CONCLUSION:

The optimized reverse phase HPLC method forGuaifenesin is linear, accurate, precise, robust, simple, rapid and selective. It can be adopted apparently for routine quality control analysis of raw materials, formulations and testing. The analysis time can be reduced due to less retention time and also cost effective mobile phase is useful for analysis.

## REFERENCES:

1.Aluri JB, Stavchansky S. Determination of Guaifenesin in human plasma by liquid chromatography in the presence of pseudoephedrine. J Pharm Biomed Anal, 1993; 11 (9): 803–8.

2.Smith SM, Schroeder K, Fahey T. Over-the-counter medications for acute cough in children and adults in ambulatory settings. Cochrane Database System Rev.2008 Jan 23:(1) .

3.Bennett S, Hoffman N, Monga M "Ephedrine- and guaifenesin-induced nephrolithiasis". J Altern Complement Med 2004; 10 (6): 967–9.

4.Check JH, Adelson HG, Wu C-H "Improvement of cervical factor with guaifenesin". Fertility and Sterility; 1982 May; 37(5):707-8.

5.Weschler, Toni (2002). Taking Charge of Your Fertility (Revised Ed.).

6.Keshavarz M, Showrasky A, Emamghoreishi M. "Anticonvulsant Effect of Guaifenesin against Pentylene Tetrazole-Induced seizure in Mice" Iran J Med Sci 2013;38 (2): 116–21.

52.

7.Tranquilli WJ, Thurmon JC, Grimm KA. (2007). Lumb and Jones' Veterinary Anesthesia and Analgesia. Blackwell Publishing. Chapter: Centrally Acting Muscle Relaxants.

8.Valverde A. "Balanced anesthesia and constant-rate infusions in horses". Vet Clin North Am Equine Pract; (Apr 2013) 29 (1): 89–122.