RESEARCH PAPER

Development of QbD-Based HPLC Method for Guaiphenesin

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ABSTRACT

Background and Objective: A systematic and structured Quality by Design (QbD) strategy was employed for the development, optimization, and validation of a rapid, precise, and reliable chromatographic method for the quantitative determination of Guaiphenesin in pharmaceutical dosage forms. The primary objective of this study was to establish a robust analytical method by identifying and optimizing critical method parameters that significantly influence chromatographic performance.

Methods: A Central Composite Design (CCD) was utilized to evaluate the combined effects of key experimental variables, including mobile phase composition and flow rate, on critical quality attributes such as retention time, peak symmetry, resolution, and theoretical plate count. Statistical modelling and response surface methodology facilitated the establishment of optimized chromatographic conditions and an analytical design space.

Results: Under the optimized conditions, efficient separation of Guaiphenesin was achieved with sharp, symmetrical peaks and acceptable analysis time. The developed method demonstrated excellent linearity across the concentration range of 10–50 µg/mL, with a high correlation coefficient. Validation studies confirmed satisfactory precision, accuracy, repeatability, specificity, robustness, and system suitability, in compliance with the requirements of ICH Q2(R1) guidelines. Recovery experiments further substantiated the accuracy and reliability of the proposed method.

Conclusion: The application of QbD principles enabled enhanced method understanding, improved control of analytical variability, and increased confidence in method performance. The validated method is simple, economical, reproducible, and suitable for routine quality control, stability testing, and batch release analysis of Guaiphenesin in pharmaceutical formulations. This study highlights the significance of a QbD-driven analytical development approach in achieving consistent, high-quality, and regulatory-compliant analytical outcomes.

Keywords: Guaiphenesin, Identification, QbD, Standardization, Validation.

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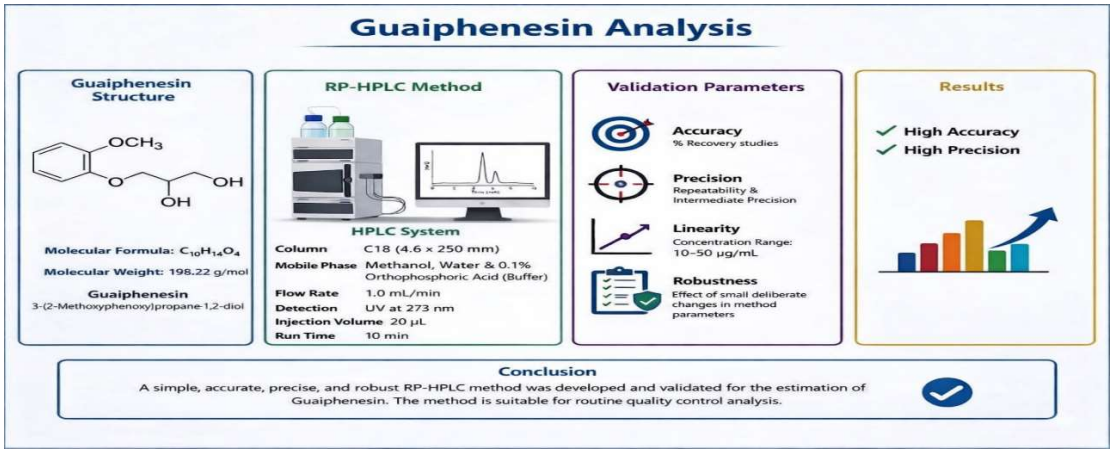
Graphical Abstract:



Introduction

The phrase "Quality by Design" (QbD) was coined as an overall approach that encompasses a broader scientific understanding critical process and product properties, constructing the controls and the test from the scientific limits of comprehension within the evolutionary process and taking advantage of the learned experiences over the life-course of the product to function in a world of relentless improvement. QbD is a pharmaceutical development procedure for design of formulation and manufacturing procedures so as to guarantee the prescribed quality of products. Guidelines and mathematical models are used to ensure the development and implementation of the acquired knowledge on the topic independently and in an integrated fashion. ICH guidelines Q8 for pharmaceutical development, Q9 for quality risk management and Q10 for quality systems are the basis of QbD.[1]

Guaiphenesin as a natural medication has been used since the 1500s. The Native Americans used guaiac tree (Guaiacum officinale) extracts for treating various diseases. The US Food and Drug Administration (FDA) first approved the medication in 1952. The drug was included in the Final Monograph for "Cold, Cough, Allergy, Bronchodilator, and Anti-asthmatic Drug Products for Over-the-Counter Human Use", 21 CFR 341, in 1989. Inclusion in the monograph implied that Guaiphenesin is a safe and effective medication for relieving the symptoms of acute upper respiratory tract infections (URTIs) and also permitted it for the use in stable chronic bronchitis.[2]

Guaiphenesin, one of the key ingredients often included in these combinations, acts as an expectorant. It works by loosening and thinning mucus in the airways, making it easier to cough out. This helps relieve chest congestion associated with the common cold, flu, acute bronchitis, and other breathing disorders that involve excessive mucus production.3

- Mechanism of action of Guaiphenesin:

  Guaiphenesin is an expectorant, meaning it helps the body clear mucus from the airways. Its action is primarily focused on the respiratory tract, where it improves the efficiency of coughing and mucus removal. After oral intake, Guaiphenesin stimulates the cells lining the respiratory tract to produce more fluid. This added fluid mixes with the thick mucus already present, making it less sticky and easier to move. The mucus becomes thinner and less adhesive, which helps the tiny hair-like structures (cilia) in the airways move it upward toward the throat.

  With thinner mucus, coughing becomes more productive, allowing the body to expel the mucus more easily. As mucus is cleared, chest congestion decreases, and breathing becomes more comfortable. Other additional physiological action by which Guaiphenesin act is by vagal stimulation inducing broncho dilation and gastric secretion stimulation. [4](Refer Figure 1).

- Pharmacokinetics:

  Guaiphenesin is rapidly absorbed from the gastrointestinal tract following oral administration. The onset of action usually occurs within a short time, and peak plasma concentrations are generally reached within about 15–30 minutes. After

absorption, the drug is distributed widely throughout body fluids. It undergoes metabolism mainly in the liver, where it is converted into inactive metabolites such as β-(2-methoxyphenoxy) lactic acid. The elimination half-life of Guaiphenesin is relatively short, approximately one hour. The metabolites are primarily excreted through the kidneys in the urine. Because of its short half-life, the drug may require repeated dosing to maintain its therapeutic effect.[5,6]

- Toxicity and Adverse Effects:
  Guaiphenesin is generally considered safe and well tolerated when used in recommended doses. However, some individuals may experience mild adverse effects. The most common side effects include nausea, vomiting, dizziness, headache, and gastrointestinal discomfort. These effects are usually mild and transient. In rare cases, allergic reactions such as skin rash may occur. Overdose of Guaiphenesin is uncommon but may lead to symptoms such as severe nausea, vomiting, and stomach upset. Very high doses taken for prolonged periods have occasionally been associated with the formation of kidney stones. Overall, the toxicity profile of Guaiphenesin is low compared with many other respiratory medications.[7]

- Therapeutic Uses:
  Guaiphenesin is widely used for the symptomatic relief of productive cough associated with respiratory tract infections such as the common cold, acute bronchitis, and other upper respiratory conditions. By loosening and thinning mucus, it helps patients cough up phlegm more effectively and clear the airways. It is also used in some cases of chronic respiratory diseases where mucus accumulation is present. The drug is frequently included in combination cough and cold preparations with agents such as Dextromethorphan, Pseudoephedrine, or Paracetamol to provide multiple symptomatic benefits.[8]

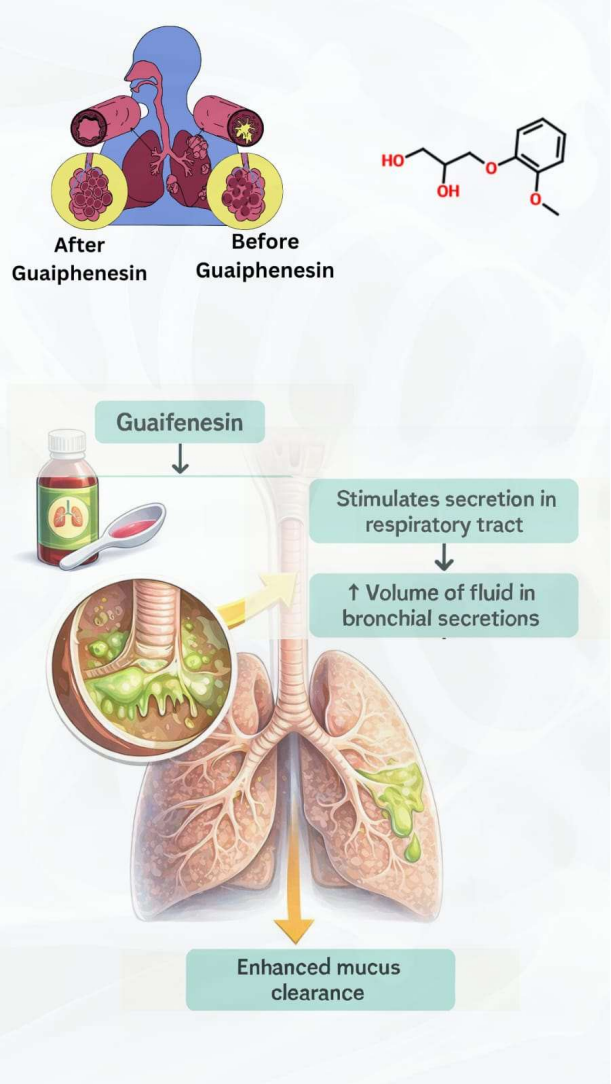


Figure 1: Mechanism of Action

Objectives

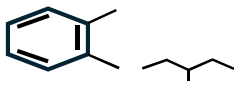
- To develop a robust and stability-indicating HPLC method for the

quantitative determination of Guaiphenesin.

- To separate and quantify Guaiphenesin in the presence of its degradation products and related impurities.[9]
- To evaluate the purity of bulk drug substance and assess the stability of its tablet dosage form.
- To validate the developed method in accordance with ICH guidelines and international regulatory requirements.[10]
- To establish a method that is simple, accurate, precise, reproducible, and reliable.
- To reduce analysis time while maintaining high analytical performance.
- To ensure the method is suitable for routine quality control analysis of Guaiphenesin in pharmaceutical formulations.[11]

Methods:

1) **Chemicals :**
   Guaiphenesin, Distilled water, Ortho phosphoric acid and Methanol
2) **Structure:**



3-(-2methoxyphenoxy) propane-1,2-diol

Figure 2: Chemical Structure of Guaiphenesin

3) **Apparatus:**
   The apparatus used for experimentation was UV visible spectrophotometer by Analytical Technology 2080, sample cell used was quartz and type was Double Beam Spectroscopy by using UV-visible Analyst as software which had pace line – z. The other apparatus used was High Performance Liquid Chromatography Agilent model 1100 column used was [column 18 (4.6 ×250 mm)

4) **Central composite design:** Central composite design is the most widely used response surface designed experiment.[12] Central composite designs are a factorial or fractional factorial design that includes centre points and augmented with a set of axial points (also known as star points) that enable you to estimate curvature.[13] You can use a central composite design for: Easily estimate first- and second-order terms. Make a curvature model for a response variable by adding centre and axial points to a previously run factorial design. Central composite designs are useful for sequential experiments because you can generally include axial and centre points to augment previous factorial experiments. In experimental design, the central composite design or CCD is a useful and powerful tool for modelling and optimizing processes with a curvilinear or "second-order" relationship between the inputs and outputs. As part of response surface methodology (RSM), a CCD sequentially adds points to a minimum factorial experiment so researchers have a way of determining the curvature of a response surface with significantly fewer experimental runs than a full three-level factorial design.[14] The power and capability of this design have made it a widely used method in many industries from

chemical engineering to pharmaceutical manufacturing.

The general structure of a CCD is created based on a standard factorial design and augmented with two essential sets of points: central and axial points. Factorial points determine the high and low level of each factor and form the vertices of the design space. The repeated centre points, placed at the centre of all factor ranges, play a critical role in the estimation of experimental error and determining whether there is curvature in the response. The axial points, placed outside the range of the factorial points, are the necessary additions that enable the estimation of coefficients of quadratic models, which are the causes of curvature. By adding these five various levels for every factor ($-\alpha$, -1, 0, +1, $+\alpha$), a CCD can build a second-order polynomial model that effectively simulates the behaviour of the process. One of the advantages of the CCD is that it can be utilized in sequential experimentation.[15] A researcher could begin with a simpler, two-level factorial design to have an understanding of which factors have the strongest impact. If the results actually do indicate a high degree of curvature, they can subsequently enrich the existing experimental data by adding the axial and centre points to complete the full central composite design. It is an extremely effective technique with substantial time and cost savings over a full re-run of a new big experiment. It is an adaptable, step-by-step procedure which facilitates a more informed and sensitive optimization strategy.[16]

The effectiveness and flexibility of central composite designs have rendered them commonplace in process optimization throughout scientific and industrial disciplines. In chemical engineering, CCDs are applied to establish the reaction optimums of temperature, pressure, and catalyst concentration to yield optimal yield and minimal waste. Food scientists apply CCDs to modify recipes and manufacturing conditions, optimizing variables for taste, texture, and shelf life. In the same way, in the pharmaceutical sector, CCDs are essential in the synthesis of new drugs by adjusting ingredient levels in a bid to achieve desired properties and effectiveness. By enabling researchers to model and comprehend complex relationships with very little experimental effort, the central composite design continues to be a stalwart of modern process optimization.

5) **Preparation of Mobile Phase**- The mobile phase was created by combining 75 ml of methanol with 25 ml of water that contained 0.1% concentrated orthophosphoric acid, achieving a 75:25 %v/v ratio. Subsequently, the mobile phase was vacuum filtered to eliminate impurities. Following this, it was sonicated for a duration of 10 minutes to ensure thorough mixing and the removal of any trapped air bubbles.
6) **Preparation of Stock Solution** - A 1000 µg/mL standard solution of Guaiphenesin was made by dissolving 10 mg of the substance in 10 mL of methanol. To create samples with different concentrations (10, 20, 30, 40, and 50 µg/mL), varying amounts of this standard solution were diluted to 10 mL with the mobile phase.
7) **Ethical Statement -** This study involves analytical method development and validation and does not involve human or animal subjects.

Therefore, ethical approval was not required.

8) **Statistical Analysis** - Statistical analysis was performed using Design-Expert software (version 13) and Microsoft Excel. Calibration curves were constructed using linear regression analysis. Method validation parameters such as accuracy, precision, and robustness were evaluated, and results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was applied where appropriate, and a p-value of less than 0.05 was considered statistically significant.

Results

I) **Optimization**

Experimental design for optimizing chromatographic conditions

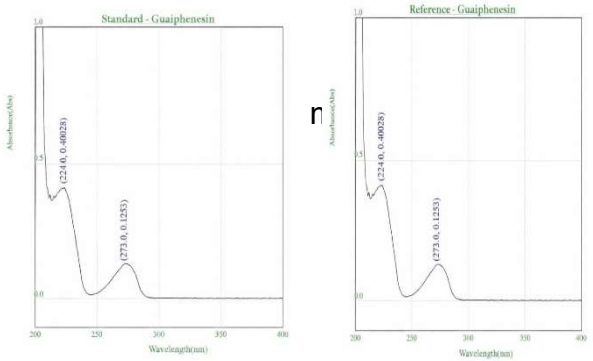
The optimization of the methanol and water as mobile phase led to significant enhancements in chromatographic performance, which included improved peak symmetry, shorter retention times, and greater column efficiency, as indicated by elevated theoretical plate counts. Together, these results illustrate the efficacy of the optimized conditions.

Optimized Chromatographic Conditions

Among the investigated conditions, the methanol–water ratio of 75:25 (v/v) at a flow rate of 0.7 mL/min exhibited the most desirable chromatographic performance. This composition provided optimal retention time (~4.4 min), maximum peak area, high theoretical plate count, and excellent peak symmetry. The sharp and symmetrical peak obtained at this ratio indicates superior resolution and sensitivity. Therefore, the 75:25 mobile phase composition was identified as the optimized condition and selected for further experimental analysis, as it ensures reliable, efficient, and reproducible chromatographic performance with buffer 0.1% orthophosphoric acid, flow rate 0.7 ml/min. Analytical column: C18 column Agilent (4.6 * 250mm), UV detection: 273 nm, run time 10 min.[17](Refer Table 1)

Table 1: Factor considered for the study by software used.

RUN	Standard (mcg)	Factor 1 Methanol	Factor 2 water	Flow rate ml/min	RT retention time	P A peak area	TP Theoretical plates	T F Tailing factor
1	30	90	10	0.7	3.945	113.895	3368	1.28
2	30	80	20	0.7	4.191	297.287	5660	0.76
3	30	70	30	0.7	4.642	296.366	5385	0.78
4	30	60	40	0.7	5.497	294.343	4944	0.93
5	30	50	50	0.7	7.478	295.800	4702	0.58
6	30	50	50	0.7	7.423	316.251	7241	0.72
7	30	50	50	0.9	5.903	245.91	6951	0.70



						3		
8	30	75	25	0.7	4.438	358.9800	6138	0.66
9	30	75	25	0.7	4.414	344.641	5837	0.71

II) **Method validation**

A comprehensive validation process, as proposed by the International Conference for Harmonization (ICH[7]) was adopted for the evaluation of the proposed HPLC method, which included the testing of the performance characteristics of the proposed HPLC method. The validation process involved testing the repeatability, specificity, precision, accuracy, and robustness of the proposed HPLC method.[18]

The UV spectrophotometric method was used to analyze Guaiphenesin standard and reference samples in the wavelength range of 200–400 nm. Both samples exhibited characteristic absorption maxima at 224 nm and 273 nm. The overlay spectra showed excellent agreement, confirming the spectral similarity between standard and reference. This validates the method for qualitative identification of Guaiphenesin and confirms the identity of the reference sample. (Refer Figure 3).

Figure 3: UV Spectroscopy of Standard and Reference Guaiphenesin sample.

III) **Linearity and Range**

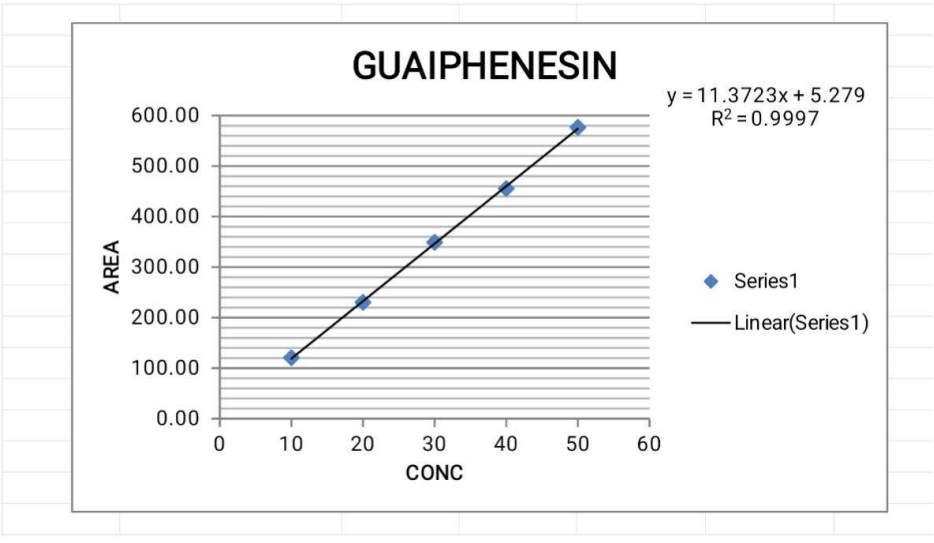
To quantify Guaiphenesin, a five-point calibration curve was constructed using independent dilutions of a stock solution, spanning concentrations from 10-50µg/ml. The regression line established a strong linear relationship within this range, evidenced by a high correlation coefficient, minimal y-intercept, and well defined slope.[19,20] (Refer Table 2) (Refer Figure 4).

Figure 4: Guaiphenesin Calibration Curve

Table 2- Result of linearity and range study linearity and range study

Concentration	Area of peak	SD	%RSD
10	120.70	2.13	1.77
20	230.30	0.97	0.42
30	349.01	0.15	0.04
40	455.53	0.30	0.07
50	576.70	1.28	0.22
Average SD		0.97	

Linearity parameter



A. Equation: y= 11.37x + 5.279
B. Slope: 11.37
C. Intercept: 5.279
D. Regression: 0.999

IV) **Repeatability**

Repeatability of measurements describes how consistent the results are when the same measurement is performed multiple times on the same subject under unchanged conditions. In this process, the same instrument or method is used, measurements are taken by the same observer (if applicable), and all readings are recorded within a short time interval so that the true value does not change. Therefore, the observed differences in repeated measurements arise only from errors in the measurement method and not from variation in the subject.[21](Refer Table 3).

Table 3: Results of repeatability

Sr. No	Concentration	Area 1	Area 2	Mean	SD	%RSD
1	30	352.155	351.979	352.07	0.12	0.04
2	30	352.120	351.964	352.04	0.11	0.03
3	30	352.184	352.028	352.10	0.11	0.03
4	30	351.992	352.184	352.08	0.14	0.04
5	30	352.063	351.907	351.98	0.11	0.03
6	30	352.148	351.996	352.07	0.11	0.03

V) **Specificity**

The specificity of the proposed method for Guaiphenesin was confirmed by injecting blank solutions and working standard samples into the chromatographic system to evaluate any possible co-elution at the drug's retention time. No interfering peaks were observed at the retention time (4.426 ± 0.017) mins demonstrating that the method is highly specific for Guaiphenesin. In addition, the influence of commonly used excipients and formulation additives present in the dosage form was examined. Under optimized chromatographic conditions, the selected mobile phase achieved efficient and clear separation of the drug from other components. Guaiphenesin produced a sharp, symmetrical, and well-resolved peak, and detection at 273 nm was selected as it provided optimum sensitivity for its accurate quantification.[22]

VI) **Precision**

Precision refers to the degree of proximity among data values obtained from multiple measurements conducted under identical analytical conditions. Repeatability was evaluated by performing at least six determinations at the full test concentration of 100%. Both the standard deviation and the relative standard deviation were documented to represent precision.[23]

The injection repeatability was assessed through the analysis of three different concentrations of pure components (10, 30, and 50 µg mL-1 for Guaiphenesin), conducted in duplicate on the same day. The experiment was subsequently repeated the following day using the same concentrations to evaluate the intermediate precision. The results were favorable, yielding an acceptable RSD% from the peak area.[24,25, 26]

(Refer Table 4).

Table 4- Result of Validation Paramet	**PRECISION**
	Result of Intraday Precision

Result of Precision	**Sr.no**	**Concentration**	**Area 1**	**Area 2**	**Mean**	**SD**	**%RSD**
	1	10	120.999	120.274	120.636	0.51	0.425
	2	30	351.805	351.724	351.764	0.06	0.02
	3	50	579.905	579.405	579.655	0.35	0.061
	Result of Interday Precision						
	Sr.no	**Concentration**	**Area 1**	**Area 2**	**Mean**	**SD**	**%RSD**
	1	10	119.329	119.279	119.304	0.04	0.03
	2	30	351.758	351.849	351.803	0.06	0.02
	3	50	581.261	579.16	580.210	1.49	0.26

RUGGEDDNESS

Result of Ruggedness	**Conc**	**Area**	**(C)**	**(M)**	**CM**	**Amt Found**	**LC**	**% Amt Found**
	40	456.80	5.279	11.37	451.5160	39.71117	0.9927792	99.28
	40	457.18	5.279	11.37	451.9010	39.74503	0.9936258	99.36
	Mean	456.99				39.73		99.32
	SD	0.272				0.024		0.060
	%RSD	0.060				0.060		0.060

RECOVERY

Result of Recovery	**80% Accuracy**	**µg/ml**	**Amt Found**	**Amt Recovery**	**% Recovery**
		10	17.88	7.88	98.54
		10	17.93	7.93	99.18
				Mean	98.86
	100% Accuracy	**µg/ml**	**Amt Found**	**Amt Recovery**	**% Recovery**
		10	20.000352	10.0003518	100.00
		10	20.026913	10.02691293	100.27
				Mean	100.14
	120% Accuracy	**µg/ml**	**Amt Found**	**Amt Recovery**	**% Recovery**
		10	22.067458	12.06745822	100.56
		10	22.049516	12.04951627	100.41
				Mean	100.49

ROBUSTNESS

	Mobile	**Concentration**	**Area**	**Mean**	**SD**	**%RSD**

Result of Robustness	**Phase 72.1+ 27.9**	30	350.328	350.6	0.38	0.11
		30	350.862			
	Mobile Phase 74.1 + 25.9	**Concentration**	**Area**	**Mean**	**SD**	**%RSD**
		30	354.471	354.32	0.21	0.06
		30	354.171			
	Wavelength 272 nm	**Concentration**	**Area**	**Mean**	**SD**	**%RSD**
		30	337.308	338.2	1.24	0.37
		30	339.068			
	Wavelength 274 nm	**Concentration**	**Area**	**Mean**	**SD**	**%RSD**
		30	388.388	389.08	0.98	0.25
		30	389.78			

VII) **Ruggedness**

The ruggedness of an analytical method refers to the extent to which test results can be reproduced when analyzing the same samples across various conditions, including different laboratories, analysts, instruments, and batches of reagents or over different days.

According to the given methodology the dilution was administered twice. Given that the relative standard deviation (RSD) is below 2.0%, this HPLC method can be regarded to exhibit ruggedness for the quantitative analysis of the specified compounds under the outlined experimental conditions.[27] (Refer Table 4).

VIII) **Recovery**

The proposed methodology was validated by conducting recover studies using the standard addition technique to ensure the accuracy of the results. Previously analysed Guaiphenesin samples (10µg/ml) were spiked were further supplemented with Guaiphensin standards at 80%, 100%, 120%. The mixtures were then analysed using the proposed method.[28] We then calculated the standard deviation of the % recovery and mentioned in the table.

The % recovery of Guaiphenesin was found to be 99.8%. (Refer Table 4).

IX) **Robustness**

The robustness of the chromatographic method was investigated by introducing small, deliberate changes in key analytical conditions. Parameters such as melting point, mobile phase composition and detection wavelength were varied individually to determine their effect on retention time, peak response, recovery, and system performance indicators like theoretical plates. The results showed no significant changes in chromatographic behaviour or repeatability, confirming that the method is stable and reliable for accurate quantification of the drug solution 30 µg/ml under minor operational variations.[29] (Refer Table 4).

Limit of Detection and Limit of Quantification

Calculation of LOD = 3.3* Avg SD/ Slope

Calculation of LOQ = 10 * Avg SD/ Slope

LOD & LOQ:

LOD: 0.28075311µg

LOQ: 0.850767µg

Label Claim / Analysis of Marketed Formulation

The marketed tablet formulation was analysed to evaluate compliance with the stated label claim. The assay results showed that the amount of drug present in the formulation was 599.92 mg against the labelled claim of 600 mg per tablet. The percentage assay was found to be 99.98%, which is in close agreement with the declared label claim. These results indicate that the marketed formulation meets the required specifications and confirms the accuracy of the analytical method. No interference from tablet excipients was observed during analysis, demonstrating the suitability and reliability of the method for routine quality control of the formulation.[30]

Result of Label Claim

Amount Found	599.92 mg
Label Claim	600 mg
% Assay	99.98

Discussion

The analytical method developed for the estimation of Guaiphenesin was found to be simple, rapid, and reliable for routine quality control analysis. The method exhibited excellent linearity over the concentration range of 10-50 µg/mL, with a correlation coefficient (r^2) close to 1, indicating a strong linear relationship between concentration and response.

The accuracy of the method was confirmed by recovery studies, where the percentage recovery was found to be within acceptable limits (98–102%), suggesting that the method is free from interference by excipients present in the formulation. Precision studies, including intra-day and inter-day variations, showed %RSD values less than 2%, indicating a high degree of reproducibility and reliability.

The specificity of the method was demonstrated by the absence of interference from other components in the pharmaceutical formulation, confirming that the method selectively measures the analyte of interest. The limit of detection (LOD) and limit of quantification (LOQ) were found to be sufficiently low, indicating the sensitivity of the method for detecting even small quantities of the drug. Robustness studies revealed that small deliberate variations in analytical parameters, such as changes in wavelength or mobile phase composition, did not significantly affect the results, confirming the robustness of the method.

All validation parameters were found to be within acceptable limits as per guidelines of the International Council for Harmonisation. Therefore, the developed method can be considered accurate, precise, sensitive, and robust, making it suitable for routine analysis of Guaiphenesin in pharmaceutical dosage forms.

Conclusion

Guaiphenesin is an effective expectorant that can be used as a pharmaceutical aspect to relieve productive cough through the loosening of mucus secretions to facilitate airway clearance. This drug has shown to be effective in terms of safety as well as efficacy, making it a commonly used drug in respiratory therapy. The solvent used are easily available, nature friendly and are cheaper. This method has been successfully validated to obtain accurate results, which can be utilised for further analysis in research studies.

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testing procedures and granting access to advanced analytical instruments, especially the HPLC apparatus, which was essential for accurate analysis and NCRD'S Sterling Institute of Pharmacy for providing the infrastructure, laboratory facilities, and academic guidance needed to carry out this work efficiently.

Abbreviations

QbD: Quality by Design; CCD: Central Composite Design; FDA: Food and Drug Administration; URTIs: Upper respiratory tract infections; RSM: Response surface methodology; ANOVA: Analysis of Variance; HPLC: High Performance Liquid Chromatography; UV: Ultraviolet; Conc: Concentration; RSD: Relative standard deviation; SD: Standard deviation; LOD: Limit of Detection; LOQ: Limit of Quantification; RT: Retention time; PA: Peak area; TP: Theoretical plates; TF: Tailing factor.

Conflict of Interest

The authors have no conflict of interest.

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The authors declare that there was no financial support or sponsorship for this study.

Author Contributions

All authors contributed to the research work. Dr Deepak Pokharkar and co-authors performed the experimental work, data collection, and analysis. The manuscript was prepared by Dr Deepak Pokharkar with inputs from all co-authors. Dr. Deepak Pokharkar supervised the study and reviewed the manuscript. All authors read and approved the final manuscript.

Summary

Method development for the quantitative determination of Guaiphenesin was carried out using a high-performance liquid chromatography (HPLC) technique to achieve accurate, precise, and reliable analysis in pharmaceutical formulations. Guaiphenesin is widely used as an expectorant in cough and cold preparations; therefore, a robust analytical method is essential for quality control and regulatory compliance. The chromatographic separation was performed using a reverse-phase C18 column, which provided adequate retention and resolution of the analyte. Various mobile phase compositions were investigated to obtain optimal peak shape and retention time. A mixture of organic solvent and aqueous buffer was optimized to achieve good chromatographic performance. The flow rate of the mobile phase and column temperature were adjusted to enhance separation efficiency and reduce analysis time. Detection was carried out using a UV detector at an appropriate wavelength corresponding to the maximum absorbance of Guaiphenesin. Sample preparation involved dissolving accurately weighed quantities of the drug or pharmaceutical formulation in a suitable solvent followed by filtration to remove particulate matter before injection into the HPLC system.The developed method was optimized by evaluating several chromatographic parameters, including mobile phase composition, flow rate, injection volume, and detection wavelength. The optimized conditions provided a sharp and symmetrical peak for Guaiphenesin with minimal interference from excipients present in the formulation. After optimization, the analytical method was validated according to the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. Validation parameters included linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), and robustness. The results demonstrated that the developed method is reliable and suitable for routine analysis of Guaiphenesin in pharmaceutical

dosage forms. Overall, the validated method provides a simple, rapid, and cost-effective analytical approach for the determination of Guaiphenesin in quality control laboratories.

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