**RESEARCH ARTICLE**

# Application of Quality by Design in RP-HPLC for Robust Impurity Profiling and Stability Assessment of Doxylamine

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**ABSTRACT**

The primary purpose of this research was to create and validate a doxylamine detection method using reverse phase high performance liquid chromatography (RP-HPLC) for use in pharmaceutical formulations and bulk materials. Quality by design (QbD) served as the inspiration for this approach. The method was developed by running a C18 column at 1-mL/min through a mobile phase of 60 parts methanol to 40 parts 0.1% OPA water (pH 2.8). The detection was done with a UV detector set to 259 nm. All requirements for system applicability were satisfied by the suggested approach, including an acceptable asymmetry factor and an adequate number of theoretical plates. A correlation coefficient (R2) of 0.9990 was used to confirm linearity over a concentration of 10–50 g/mL. The approach showed good accuracy by a mean %recovery ranging from 99.59–101.45% and a %RSD between 0.11 and 0.81. Precision tests conducted both intraday and across days showed that the medication content stayed within allowable bounds. The approach was found to be robust, with only minor variations in flow rate, mobile phase composition, and detecting wavelength significantly affecting the accuracy and specificity. A 0.71 g/mL LoQ and a 0.23 g/mL LoD were determined to be analytical limits of detection and quantification, respectively. This study substantiates the development of a reliable, long-lasting, and cost-effective QbD-based RP-HPLC method suitable for the comprehensive analysis of doxylamine in tablet formulations and bulk dosages.

**Keywords:** Drug analysis, LoQ, LoD, Doxylamine, RP-HPLC, QbD, Pharmaceutical Formulations.

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## INTRODUCTION

A first-generation antihistamine with several medicinal uses, including as a sleep aid and in over-the-counter cold and allergy medicines, is doxylamine. It is essential for many physiological processes and is important for the central nervous system. There are several ways to take doxylamine, including pills and liquid preparations. It is essential to guarantee the purity and quality of doxylamine in medicinal goods due to its extensive use.[1]

Active pharmaceutical ingredients (APIs) are safeguarded for their safety and effectiveness through impurity profiling, a crucial component of pharmaceutical quality control. Doxylamine (Figure 1), one of the APIs, is important in many therapeutic applications. But, impurities, whether they develop during the synthesis process or as a result of storage, can materially affect the efficacy and security of these formulations. Due to its great sensitivity and specificity, great high-performance liquid chromatography (HPLC) has been a pillar in this analytical landscape. The QbD strategy has become popular in recent years as a methodical way for improving analytical processes, notably for creating stability-indicating techniques necessary for long-term quality assurance of pharmaceutical goods.[2]

Development and validation of a stability-indicating RP-HPLC method for doxylamine impurity profiling is the main goal of this study. To achieve dependability and robustness, this development will use AQbD approach. Secondary goals include determining the method's applicability to pharmaceutical and bulk dose forms of doxylamine, analyzing its accuracy, precision, and robustness, and determining if it is appropriate for routine quality control analysis.

The creation, verification, and implementation of a Doxylamine-specific RP-HPLC technique are all included in the scope of this work. Both its bulk form and its medicinal dosage forms, such as tablets and liquid formulations, will be covered by this method. The importance of this discovery lies in its potential to significantly improve quality control procedures for drugs based on doxylamine. The project intends to establish a new benchmark for analytical robustness by using an AQbD technique, which will assist pharmaceutical

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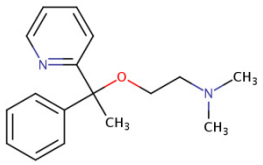

**Figure 1:** Doxylamine chemical structure

makers and customers by enhancing the quality and safety of their products.

### Analytical Methods for Impurity Profiling of Doxylamine

The impurity profile of doxylamine, a first-generation antihistamine widely utilized in medical applications, has been done before utilizing various types of analysis. GC-MS has gained popularity due to its high sensitivity and specificity; nonetheless, it can only detect volatile pollutants. Although it has a wider range of applications, including those involving non-volatile contaminants, LC-MS has the disadvantage of having high operating costs. Another widely utilized technique is traditional HPLC, which is praised for being affordable and widely accessible but may necessitate substantial sample preparation. While CE is quick and requires little sample preparation, it lacks the sensitivity of other techniques. Due to the requirement for extremely pure samples, nuclear magnetic resonance (NMR) is typically not employed for routine analysis but offers precise structural information on doxylamine and its impurities. However, rapid and simple, fourier-transform infrared spectroscopy (FTIR) is mostly used for qualitative investigation. The choice typically depends on the specific analytical needs, such as the type of contaminants, the equipment that is available, and the necessity for sensitivity and specificity. Each of these methods has a unique set of benefits and drawbacks.[3]

### RP-HPLC Methods for Doxylamine Impurity Profiling Include

RP-HPLC has become a popular analytical method for doxylamine impurity profiling over time. The mobile phase in early techniques consisted of a combination of acetonitrile and water and C18 columns with isocratic elution. Although these techniques worked, they lacked the sensitivity required for detecting traces of contaminants. Gradient elution techniques were later developed, greatly improving resolution and sensitivity and enabling the identification of contaminants at the ppm level. To increase the method's specificity, some investigations have additionally used UV detection at various wavelengths. The addition of ion-pairing agents in the mobile phase, which significantly enhanced the separation of doxylamine from its impurities, was another significant development. To ensure robustness and dependability, RP-HPLC techniques for doxylamine have more recently been investigated with the incorporation of QbD concepts. These QbD-optimized techniques have demonstrated promise in speeding up execution and enhancing method resilience, making them appropriate for high-throughput quality control settings. Despite these developments, difficulties with the identification of polar and volatile impurities still exist, motivating current research to improve RP-HPLC techniques for thorough impurity profiling of doxylamine.[4]

### QbD in Analytical Techniques

Innovating analytical approaches, QbD shifts the emphasis from merely meeting quality requirements to incorporating quality into the process from the outset. QbD is a methodical framework for method development used in analytical techniques like RP-HPLC, which begins with a thorough knowledge of the method's intended use and the identification of critical quality attributes (CQAs). When assessing elements that could affect these CQAs, tools for risk evaluation like failure mode and effects analysis (FMEA) are frequently used. Using this information as a foundation, a Design of Experiments (DoE) is created to investigate the effects of these variables and determine the ideal settings for robustness, sensitivity, and specificity. The end result is a design environment where operating parameters ensure reliable performance. Then, control measures are implemented to keep an eye on these variables and guarantee a constant level of quality. The QbD methodology improves method resilience while also speeding up the validation procedure because the method is created to operate at its best inside the defined design area. As a result, analytical methods are developed that are both regulatory requirements and industry needs-aligned, as well as robust, dependable, efficient, and cost-effective.[5-8]

**Table 1:** DoE with central composite design

| *Std* | *Run* | *Factor 1: A:Flow rate (mL/min)* | *Factor 2: B:Methanol (%)* | *Response 1: RT (min)* | *Response 2: Peak Area* |
|---|---|---|---|---|---|
| 8 | 1 | 1 | 65 | 6.1 | 3758.04 |
| 1 | 2 | 0.9 | 55 | 11.12 | 4272.7 |
| 3 | 3 | 1.1 | 55 | 9.2 | 3229.32 |
| 5 | 4 | 1 | 60 | 7.8 | 3742.55 |
| 6 | 5 | 1.1 | 60 | 7.1 | 3341.53 |
| 4 | 6 | 0.9 | 60 | 8.7 | 4278.26 |
| 7 | 7 | 0.9 | 65 | 6.9 | 4285.84 |
| 2 | 8 | 1 | 55 | 10.2 | 3742.55 |
| 9 | 9 | 1.1 | 65 | 5.6 | 3337.77 |

### Research Gaps on Analytical Methods of Doxylamine Currently Available

While there has been substantial study on Doxylamine impurity profiling and the use of RP-HPLC in pharmaceutical analysis, there is a glaring hole in the literature about the incorporation of QbD strategy in this particular situation. QbD framework for method optimization is rarely included into most existing methods, which typically either focus on the analytical technique itself or the substance under analysis, in this case doxylamine. This creates a gap in our knowledge of how a systematic design strategy could improve the stability and dependability of RP-HPLC techniques developed for doxylamine. There is also a dearth of studies examining the application of such QbD-optimized techniques to different doxylamine dose forms, including both its bulk and medicinal dosage forms such tablets and liquid formulations. This study gap highlights an unmet need for thorough investigations that not only apply QbD principles to method creation but also evaluate their applicability across various Doxylamine forms, hence assisting in the development of more effective and trustworthy quality control procedures.[9]

## METHOD AND MATERIALS

Chemicals and Reagents: Sparrow Pharma in Mumbai, India, was used to obtain high-purity doxylamine. Acetonitrile, methanol, and water were purchased from Merk in India as HPLC quality solvents.

### Procedures for Preparing Standard Samples and Testing of Doxylamine using HPLC

A stock solution (STOCK-1) of 1000 g/mL of doxylamine is made by dissolving 10 mg in 10 mL of methanol. Various concentrations are then created by diluting this stock solution with a mobile phase (M.P). A second stock solution (STOCK-II) is prepared using tablet powder to achieve a 1000 g/mL concentration. The ideal wavelength for testing is identified as 275 nm through UV scans. The HPLC system is calibrated using the CHEMSTATION program, and mobile phase consisting of a 60:40 ratio of methanol to 0.1% OPA water (pH 2.8) is used. Flow rate is set at 1.0 mL per minute, and a detecting wavelength of 259 nm is chosen. Finally, the effectiveness of the method is confirmed by comparing the outcomes with known concentrations. QbD Optimization: QbD strategy was used. Using DoE, critical parameters such were discovered and optimized.[10]

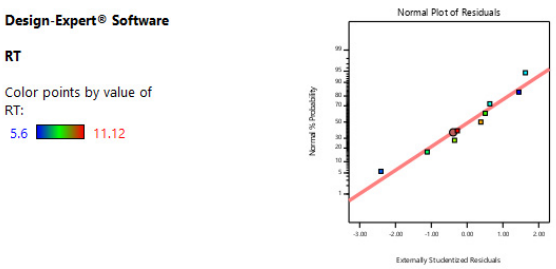


**Figure 2:** Normal probability plot of the residuals

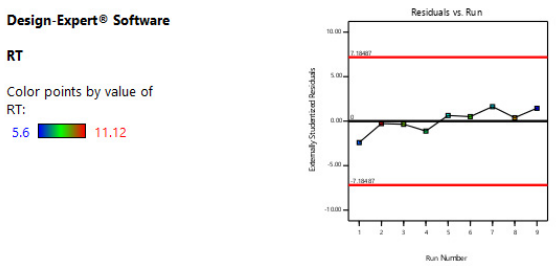


**Figure 3:** Residuals versus run

### Validation and Robustness Testing of the RP-HPLC Method

Linearity is confirmed through a calibration curve using 10-50 g/mLconcentrations. The method's accuracy is verified through recovery investigations at 80, 100, and 120% concentrations and by assessing both intraday and interday precision. LoD and LoQ are determined as per ICH guidelines. Robustness is evaluated by altering variables like column temperature, detection wavelength, and mobile phase ratio. Additionally, the method undergoes rigorous stress testing, including hydrolytic, oxidative, thermodynamic, and photolytic stress, to profile impurities and assess Doxylamine stability, thereby confirming the method's suitability for impurity profiling.[11-15]

## RESULTS

### Development and Improvement of Methods

Doxylamine chromatographic analysis using HPLC
Devices and chromatographic situations

- AGILET (1100) HPLC System
- Application: CHEMSTATION
- Column: ID 4.6 x length 100 mm
- Packing Particle Size: 2.5 m
- C18 (AGILENT) is the stationary phase.
- Methanol: 0.1% OPA Water (pH 2.8) in a 60:40 ratio makes up mobile phase.
- 259 nm for detection wavelength
- 1.0 ml/min flow rate
- Ambient temperature
- 20 l sample size

### QbD Methodology

To explore the effects of two parameters, "flow rate" (Factor 1) and "Methanol%" (Factor 2), on two responses, "RT" (Response 1) and "Peak area" (Response 2), a DOE study was conducted. The design is shown in Table 1. The quadratic model is recommended with an Adjusted (R2) of 0.9988 and a Predicted (R2) of 0.9945. Upon analysis of the outcomes subsequent to the implementation of the model, it is evident that the data points exhibit a general linear trend. Notably, the highlighted run displays a greater deviation from its projected value compared to other runs. However, it is important to note that this deviation remains within the established red control limits, as illustrated in Figures 2 and 3, where the red control limits are visually depicted. Based on the response surface contour plot and 3D response surface plot presented in

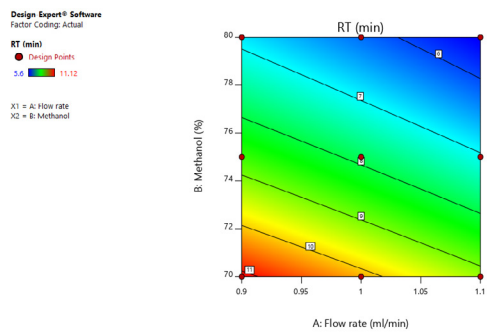


**Figure 4:** Response surface contour plot

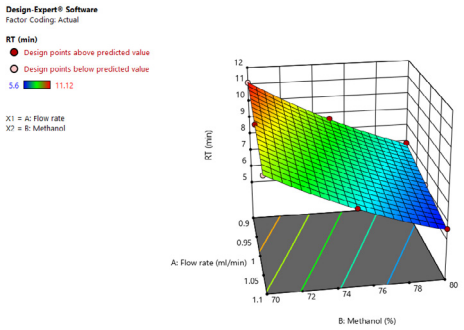


**Figure 5:** 3D response surface plot

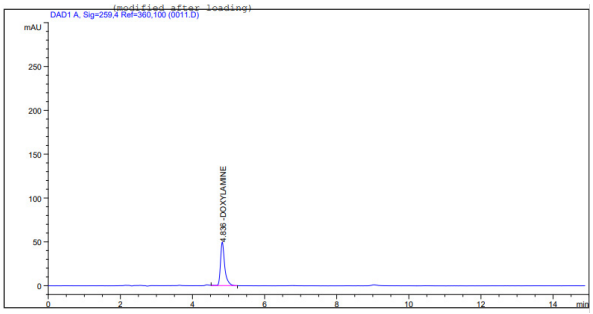


**Figure 6:** Doxylamine standard chromatogram

**Table 1:** Linearity results of the given compound

| *Sr. No.* | *Conc (µg/mL)* | *Avg. Area* |
|---|---|---|
| 1. | 10.00 | 86.38 |
| 2. | 20.00 | 183.56 |
| 3. | 30.00 | 265.36 |
| 4. | 40.00 | 354.52 |
| 5. | 50.00 | 446.27 |

$R^2$ (Correlation coefficient): 0.999
Regression equation: y = 8.907x - 9.643

**Table 2:** Summary of recovery data in HPLC

| *Level of Recovery (µg/mL)* | *Mean %Recovery* | *Standard deviation (SD)* | *%RSD* |
|---|---|---|---|
| 80 | 101.45 | 0.82 | 0.81 |
| 100 | 100.75 | 0.50 | 0.50 |
| 120 | 99.59 | 0.10 | 0.11 |

All three levels display high Mean %Recovery values, which reflect great precision, and low %RSD values, which signal good accuracy.

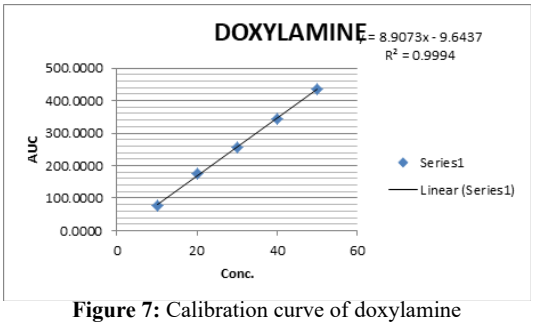


**Figure 7:** Calibration curve of doxylamine

**Table 3:** Summary of intraday data in HPLC

| *Concentration (conc)* | *Mean area* | *Mean amt found* | *% Amt found* | *Standard deviation (SD)* | *%RSD* |
|---|---|---|---|---|---|
| 20 | 169.25 | 20.08 | 100.42 | 2.17 | 1.28 |
| 30 | 256.12 | 29.84 | 99.46 | 1.18 | 0.46 |
| 40 | 343.81 | 39.68 | 99.21 | 3.68 | 1.07 |

**Table 4:** Summary of interday data in HPLC

| *Concentration (conc)* | *Mean area* | *Mean amt found* | *%Amt found* | *Standard deviation (SD)* | *%RSD* |
|---|---|---|---|---|---|
| 20 | 170.20 | 20.19 | 100.96 | 1.03 | 0.61 |
| 30 | 255.05 | 29.72 | 99.06 | 0.13 | 0.05 |
| 40 | 348.21 | 40.18 | 100.44 | 0.00 | 0.00 |

**Table 5:** LoD and LoQ

| *Sr. No* | *Parameter* | *Measured value* |
|---|---|---|
| 1. | LoD | 0.2371169 |
| 2. | LoQ | 0.718536 |

**Table 6:** Robustness study

| *Parameter modification* | *Level ± SD* | *%RSD* |
|---|---|---|
| Flow rate ± 0.1 mL/min | 0.9 | 0.30 |
| | 1.1 | 1.22 |
| Wavelength ± 1 nm | 258 | 0.18 |
| | 260 | 0.00 |
| Mobile phase ± 1 mL | 61:39 | 0.24 |

Figures 4 and 5, respectively, it can be observed that the replication of center points within the experimental zone yields a highly accurate power of prediction in the vicinity of its central region. According to the study, changing the flow rate and methanol concentration can greatly impact RT. To regulate and optimize processes more precisely, use the quadratic model. DoE with CCE is shown in Table 1.

The study comprehensively analyzes the factors affecting HPLC for doxylamine, particularly focusing on the interplay between flow rate and methanol percentage in the mobile phase. It reveals that these variables significantly interact to influence key parameters like retention time (RT) and peak area. For example, altering flow rate from 1 to 0.9 mL/min and adjusting the methanol percentage from 65 to 55% led to RT and peak area changes. The method's sensitivity to environmental changes necessitates careful calibration for accurate quantitative analysis. The study found that the

**Table 7:** Results of stability indicating impurity profiling for doxylamine

| *Sr. No.* | *Time* | *Degradation* | *Area of Standard* | *Area of Degraded Sample* | *Degraded upto%* | *Actual %Degradation* |
|---|---|---|---|---|---|---|
| 1 | After 1 hour | Acid degradation | 436.6277 | 405.9502 | 92.97 | 7.03 |
| 2 | | Basic degradation | 436.6277 | 388.5477 | 88.99 | 11.01 |
| 3 | | $H_2O_2$ degradation | 436.6277 | 411.044 | 94.14 | 5.86 |
| 4 | | Neutral | 436.8821 | 420.8055 | 96.32 | 3.68 |
| 5 | After 2 hours | Acid degradation | 436.6277 | 394.6168 | 90.38 | 9.62 |
| 6 | | Basic degradation | 436.6277 | 394.6796 | 90.39 | 9.61 |
| 7 | | $H_2O_2$ degradation | 436.6277 | 400.4638 | 91.72 | 8.28 |
| 8 | | Neutral | 436.6277 | 425.3667 | 97.42 | 2.58 |

RT could range from 5.6 to 11.12 minutes and the peak area from 3229.32 to 4285.84, depending on the settings. Optimal outcomes were achieved at a flow rate of 0.9 mL/min with 65% methanol, emphasizing the need for method optimization that considers individual variables and their interactions for reliable analytical performance. Even though the highlighted run in Figure 2 deviates more from its projected value than any other run, there is no reason to be alarmed because it is still within the red control boundaries. As Figure 3 indicated, we obtain a high predictive power in the middle of your experimental zone. The ideal outcomes are depicted in Figures 4 and 5.

The reduced quadratic model's ANOVA (Analysis of Variance) results shed more light on how the variables "Flow rate" and "Methanol" relate to the outcome ("RT"). The whole model has a p-value of 0.0001 and an F-value of 1223.46, which both indicate high significance. Significant factors influencing the reaction include "Flow rate" (A) and "Methanol" (B), with F-values of 680.30 and 4160.66, respectively, and p-values of 0.0001. The interaction term (AB), which has an F-value of 16.88 and a p-value of 0.0147, is likewise significant. It is significant that the quadratic component for methanol (B2) has an F-value of 35.98 and a p-value of 0.0039.The ideal parameters for the RP-HPLC method were established after applying QbD approach. Ultimate mobile phase composition was 60:40 Methanol: 0.1% OPA Water (pH 2.8). Figure 6 illustrates how these circumstances led to outstanding peak symmetry and resolution with a retention period for doxylamine of about 4.8 minutes.

**Method Validation**

*Linearity*

Table 1 and Figure 7 show that the procedure demonstrated excellent linearity over the 10 to 100 g/mL concentration range for doxylamine. A strong linear relationship between concentration and peak area was discovered to exist, as indicated by the correlation coefficient ($R^2$), which was found 0.999.

*Accuracy*

Summary of recovery data in HPLC as shown in Table 2

*Precision*

Precision was assessed on an intraday and an interday basis. Relative standard deviation for intraday precision indicated high precision was less than 2%, and for interday precision, it was less than 2.0% (Table 3 to Table 5).

*Robustness*

Robustness was evaluated by making minor adjustments to the method's flow rate and mobile phase composition. Since the peak area and retention duration didn't alter significantly, the approach was deemed to be reliable (Table 6).

*Stability indication and impurity profiling*

The results of successfully using the devised method to identify and measure contaminants in both bulk and pharmaceutical dosage forms of doxylamine are shown in Table 7. The approach was determined to be stable because it could successfully separate doxylamine from its degradation products even when subjected to stress conditions, including heat, light, and oxidation. This demonstrates that the technique is appropriate for doxylamine long-term stability tests.

The degree of degradation varies with time and condition. While $H_2O_2$ and neutral circumstances led to less deterioration, acid and basic environments did. With the exception of the neutral condition, where degradation slightly increased after 2 hours, the sample often experienced more degradation after 1 hour compared to 2 hours.

## CONCLUSION

The study successfully developed and validated a robust method for impurity profiling and stability assessment of the pharmaceutical chemical doxylamine using RP-HPLC. Utilizing DoE, the method was optimized, considering key variables like flow rate and methanol proportion in mobile phase, and demonstrated excellent accuracy, and precision linearity. Method proved to be robust and stability-indicating, effectively separating doxylamine from its degradation products under various stress conditions such as acidic, basic, and oxidative environments. This makes it suitable for long-term stability studies in pharmaceutical formulations. Additionally, the method enabled identifying unknown degradation products and quantifying known contaminants, offering valuable insights into doxylamine stability. Overall, the research enhances quality control for doxylamine-based medications, providing a systematic approach that pharmaceutical companies and regulatory bodies can utilize to ensure product quality and patient safety.

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