Development of an Analytical Method for Determination of Dexchlorpheniramine Maleate Level in Tablet Preparations by UV Detector High-Performance Liquid Chromatography

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ARTICLE INFO

Article history
Received:
October 10, 2024

Revised:
November 29, 2024

Accepted:
December 31, 2024

DOI:
https://doi.org/10.54384/eruditio.v5i1.203

ABSTRACT / ABSTRAK

Dexchlorpheniramine maleate, an antihistamine for allergy treatment, is traditionally tested in tablet form using the UV spectrophotometric method referenced in United States Pharmacopeia (USP), NF 43. However, this method struggles to separate the active compound from other tablet components, such as dyes, necessitating an extraction process. Extraction has several drawbacks, including high waste production, extensive use of costly pure solvents, prolonged preparation times, and reliance on hazardous volatile solvents. This study aims to develop a safer, more efficient, and effective method using high-performance liquid chromatography (HPLC), modified from the USP, NF 43 standard. The experimental approach involves method development and validation, focusing on selectivity, precision, accuracy, linearity, and robustness. The selectivity test demonstrated a resolution value 19.6 between dexchlorpheniramine and maleic acid peaks. The specificity test confirmed identical retention times and spectra between standard and sample peaks. Precision testing yielded a relative standard deviation (RSD) below 0.37%. Accuracy tests at 80%, 100%, and 120% levels achieved an average recovery of 99% within the 97%–103% acceptance range. Linearity testing resulted in a correlation coefficient (r) 1.00 and Vx0 of 0.1. ANOVA test results on Robustness testing revealed no significant differences with column modification but identified sensitivity to pH and mobile phase composition changes. In conclusion, the developed HPLC method meets validation parameters, providing a reliable alternative for analyzing dexchlorpheniramine maleate tablets, with attention to pH and mobile phase suitability.

Deksklorfeniramin maleat adalah senyawa antihistamin yang digunakan dalam pengobatan alergi. Penetapan kadar deksklorfeniramin maleat tablet dilakukan sesuai acuan United States Pharmacopeia (USP), NF 43 dengan metoda spektrofotometri UV. Kelemahan metode ini yaitu tidak dapat memisahkan zat aktif yang akan diuji dari komponen lain yang ada dalam tablet seperti pewarna, sehingga perlu dilakukan proses ekstraksi sebelum dilakukan penetapan kadar. Namun metode ekstraksi mempunyai kelemahan, antara lain produksi cairan limbah yang besar, kebutuhan pelarut murni dalam jumlah besar dan mahal, waktu ekstraksi yang lebih lama, serta penggunaan pelarut berbahaya yang mudah menguap. Penelitian ini bertujuan untuk mengembangkan metode analisis penetapan kadar tablet deksklorfeniramin maleat yang lebih implementatif, efeisien, efektif dan aman dengan metode kromatografi cair kinerja tinggi (KCKT), yang merupakan modifikasi dari cara kerja penetapan kadar baku pembanding dekskloefeniramin maleat sesuai USP, NF 43. Metode penelitian yang digunakan adalah metode eksperimental, yaitu dengan melakukan

pengembangan dan validasi metode mencakup uji selektivitas/spesifitas, presisi, akurasi, linearitas, dan robustness. Uji selektivitas antara puncak deksklorfeniramin dan asam maleat menghasilkan nilai resolusi 19,6. Pada uji spesifitas, baku deksklorfeniramin maleat dan sampel yang diuji menghasilkan puncak pada waktu retensi dan spektrum yang identik. Uji presisi memberikan simpangan baku relatif (RSD) kurang dari 0,37%. Uji akurasi pada kadar 80%, 100% dan 120% menghasilkan rata-rata perolehan kembali 99% dengan rentang keberterimaan 97% – 103%. Uji linearitas memberikan nilai koefisien korelasi r = 1,00 dan Vx0 = 0,1. Hasil uji ANOVA pada uji ketangguhan metode menunjukkan hasil yang tidak berbeda signifikan pada modifikasi kolom, tetapi berbeda signifikan pada modifikasi pH dan komposisi fase gerak. Secara umum dapat disimpulkan bahwa metode uji yang dikembangkan memenuhi parameter validasi, tetapi dalam penerapannya harus memperhatikan kesesuaian pH dan perbandingan fase gerak yang digunakan.

Keywords: dexchlorpheniramine maleate, HPLC, method development, validation
Kata Kunci: Deksklorfeniramin maleat, KCKT, pengembangan metode, validasi

1. Introduction

Accurate test results are essential to ensuring the quality and safety of drugs and food circulating in the community. Laboratories must continuously develop analytical methods that are time-efficient, resource-saving, and aligned with the latest technological advancements (Pratiwi et al., 2021). Furthermore, validating test methods is crucial for ensuring reliable laboratory outcomes and instilling confidence in the results (Barnett et al., 2023).

Dexchlorpheniramine maleate is a first-generation antihistamine used to treat allergies. This compound exhibits sedative side effects due to its ability to penetrate the blood-brain barrier more effectively than second-generation antihistamines. Studies have shown that high concentrations of dexchlorpheniramine can induce genotoxicity in human lymphocytes (Chaves et al., 2022). Therefore, monitoring the quality and safety of dexchlorpheniramine maleate is critical for public health.

Dexchlorpheniramine maleate is available in dosage forms such as tablets and syrups. Determining its levels in tablets has been traditionally performed using the UV spectrophotometric method as per the United States Pharmacopeia (USP), NF 43 (United States Pharmacopeial Convention, 2022). However, this method struggles to separate the active substance from other tablet components, such as dyes, which may interfere with the results. To address this, an extraction process is often employed (Mustarichie Resmi, 2014). Extraction, however, has significant drawbacks, including large waste production, high solvent costs, extended preparation times, and hazardous volatile solvents (Hewage et al., 2022; Mandal et al., 2015).

Given these limitations, developing an efficient and safe analytical method is imperative. High-performance liquid chromatography (HPLC) offers a robust alternative for separating, identifying, and quantifying active substances (Sabir et al., 2016). Unlike UV spectrophotometry, the HPLC method eliminates the need for extraction, reducing costs and enhancing accuracy (Singh et al., 2021; Smolinska et al., 2022).

One prior study employed HPLC to analyze dexchlorpheniramine maleate levels in syrups (Le et al., 2019). This study highlights the need to extend similar methods to tablet preparations. Validation of such methods, even when adapted from standard practices, ensures accuracy and reliability in a broader range of applications (ISO/IEC 17025:2017).

This study aims to develop an HPLC method to effectively determine dexchlorpheniramine maleate levels in tablets. This will provide laboratories with a practical tool for ensuring product

quality and public safety. Ultimately, this will enhance regulatory oversight and address drug and food safety standards violations.

2. Methodology

The research method employed is experimental, focusing on developing and validating a method for determining the levels of dexchlorpheniramine maleate in tablet preparations using high-performance liquid chromatography (HPLC). Validation parameters include selectivity/specificity, precision, accuracy, linearity, and robustness (Kementerian Kesehatan Republik Indonesia, 2020; Barnett et al., 2023). The resulting data were statistically analyzed and compared against the acceptance criteria for each validation parameter (Riyanto, 2014; Belouafa et al., 2017).

The tablets were homogenously crushed and dissolved in the mobile phase, followed by analysis using an HPLC system (Shimadzu) equipped with a column containing Octadecylsilane X Bridge (Waters) measuring 25 mm in length, 4.6 mm in inner diameter and 5 µm particle size. The detector was a UV-PDA, set to a wavelength of 225 nm. The analysis was conducted in isocratic mode with a mobile phase consisting of phosphate-buffered saline (pH 3.0 ± 0.1) and acetonitrile in a 70:30 ratio, a 1.0 mL/min flow rate, and an injection volume of 20 µL.

The validation parameters assessed include:

2.1. Selectivity/specificity

Selectivity was determined by examining the resolution between dexchlorpheniramine and maleic acid peaks, comparing standard and sample spectra, and calculating the peak purity index.

2.2. Precision

Performed ten replicate tests and calculated the % RSD value.

2.3. Accuracy

The standard addition method was used at concentrations of 80%, 100%, and 120%, with three replicates for each concentration.

2.4. Linearity

Linearity was assessed using a series of concentrations (60%, 80%, 100%, 120%, and 140%), and the correlation coefficient (r) and Vx0 values were calculated from the chromatogram area and theoretical content.

2.5. Robustness

The robustness test evaluated the impact of variations in testing parameters on the levels of dexchlorpheniramine maleate. The tested variations included:

2.5.1. Variation of pH

Comparing results using phosphate-buffered saline with pH values of 2.5, 3.0, and 3.5.

2.5.2. Variation of mobile phase composition

Comparing results using mobile phase ratios of 65:35, 70:30, and 75:25 (phosphate-buffered saline: acetonitrile).

2.5.3. Variation of column brands

Comparing results using X Bridge (Waters), Luna (Phenomenex), and Zorbax (Agilent) columns.

3. Result and Discussion

In this validation process, a thorough evaluation has been carried out based on the test results of the validation parameters: Selectivity/Specificity, Precision/Repeatability, Accuracy, Linearity and Robustness.

3.1. Selectivity/Specificity

In chromatographic techniques, selectivity is demonstrated by a clear separation between the analyte and other components in the sample. This requirement is met when the resolution of the analyte from other components exceeds 2.0 (ICH, 2022). According to the analysis results, the resolution between the maleic acid and dexchlorpheniramine peaks was 19.6, as shown in Figure 1.

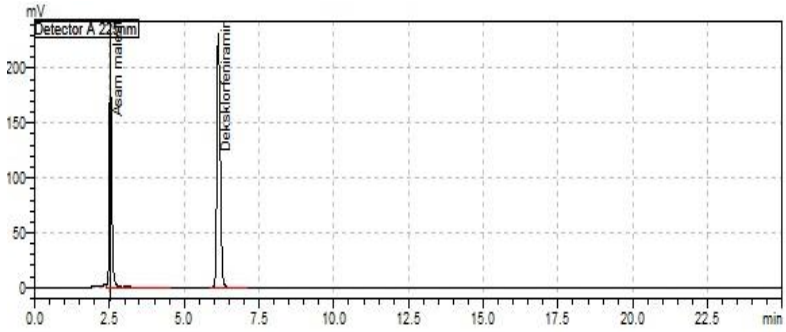

Figure 1. Chromatogram of Dexchlorpheniramine maleate

Specificity refers to the ability of an analytical method to assess an analyte in the presence of other components within the sample matrix. In HPLC, specificity can be evaluated by the peak purity index of the analyte chromatogram. Specificity is further determined by comparing the retention times of the sample and comparator standards, as well as ensuring the sample spectrum matches that of the standard. The chromatogram of dexchlorpheniramine maleate in the test solution showed an average retention time of 6.11 minutes. For the standard solution, the retention time averaged 6.14 minutes, with a single peak and a peak purity index of 1.00000 (Figure 2). The sample spectrum was identical to the standard spectrum (Figure 3).

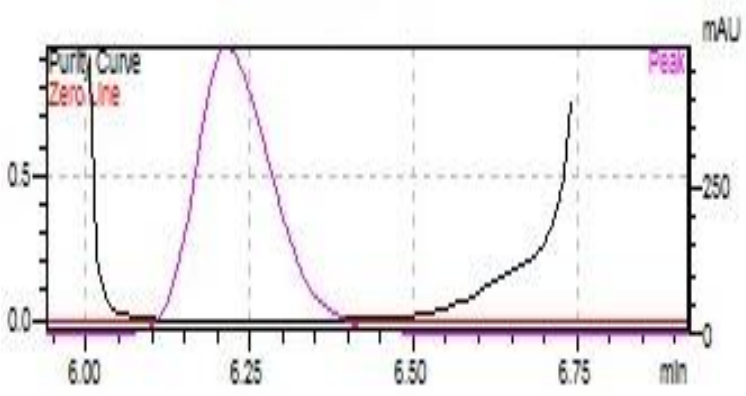

Figure 2. Peak purity of Dexchlorpheniramine

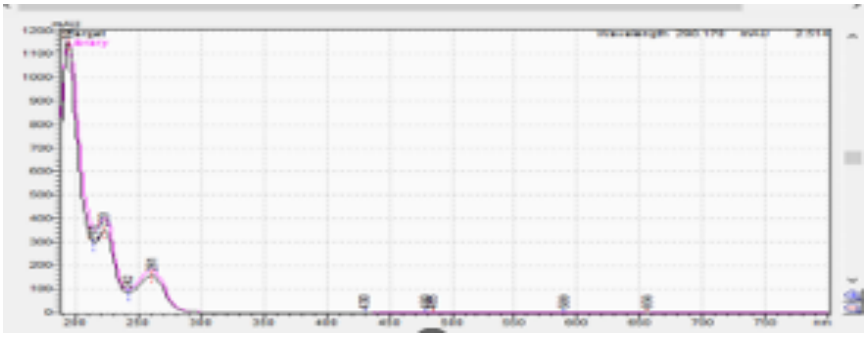

Figure 3. Overlay of sample and standard spectra of Dexchlorpheniramine

3.2. Precision/Repeatability

Precision indicates whether an analytical method produces consistent results upon repetition. It can be evaluated by analyzing individual results relative to the average value when the procedure is repeated. A method meets precision criteria if the relative standard deviation (RSD) or coefficient of variation (CV) is ≤2% (Kementerian Kesehatan Republik Indonesia, 2020). As shown in Table 1, the method produced an RSD of 0.37%.

Table 1. Results of Precision Test RSD Calculation

Replication	Content (mg)	Content (%)
1	2,028	101,40
2	2,034	101,68
3	2,040	102,01
4	2,045	102,23
5	2,028	101,38
6	2,026	101,28
7	2,049	102,43
8	2,036	101,79
9	2,035	101,74
10	2,037	101,85
Avarage	2,04	101,78
SD	0,01	0,37
RSD	0,37	0,37

3.3. Accuracy

Accuracy refers to the closeness between the measured and accepted reference values. This is assessed by determining the analyte recovery rate using a spiked sample. The standard addition method, which involves adding a known standard solution to the sample matrix, was used to evaluate accuracy. Results showed an average recovery of 99% (Table 2), within the acceptable range of 97%–103% (Association Of Official Analytical Collaboration (AOAC) International, 2016).

Table 2. % Recoveries Calculation Results

No.	Concentration (%)	Recoveries (%)	Recoveries (%)
1	80	99	
	80	100	100
	80	99	
2	100	100	
	100	99	99
	100	97	
3	120	99	
	120	98	99
	120	99	
	Average		**99**

3.4. Linearity

Linearity is determined by measuring different concentration ranges and calculating the slope, intercept, and correlation coefficient. The test results showed Vx0 = 0.1 and r = 1.0000, indicating a linear relationship for dexchlorpheniramine maleate concentrations between 31.761 µg/mL and 70.141 µg/mL (Yuwono et al., 2005).

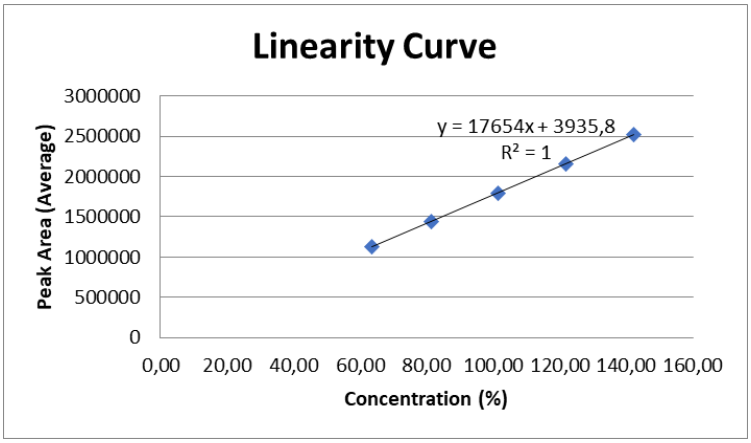

Figure 4. Linearity Curve

3.5. Robustness

Robustness assesses the ability of an analytical method to remain unaffected by minor but deliberate variations in parameters, ensuring reliability during routine use (Yuwono & Indrayanto, 2005). This study tested variations in mobile phase composition, pH, and column brand. Variations in pH and mobile phase composition caused significant differences, while column brand changes did not produce significant effects. These findings highlight the importance of maintaining consistent pH and mobile phase conditions.

4. Conclusion

The results of this validation study demonstrate that the analytical method meets the validation requirements of Indonesia Pharmacopeia and AOAC for specificity/selectivity, precision, accuracy,

and linearity. Consequently, this HPLC method is suitable for determining dexchlorpheniramine maleate levels in tablet preparations.

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