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Pengembangan dan Validasi Metode HPLC sebagai Metode Kunci dalam Analisis Olopatadine HCl

Development and Method Validation of HPLC Method as an Key Method in the Analysis of Olopatadine HCl

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Abstract

Olopatadine hydrochloride (HCl) is a widely used second-generation antihistamine, and its accurate quantification is critical to ensure product quality and therapeutic efficacy. Reliable analytical methods are therefore essential for regulatory compliance and routine quality control. This study aimed to develop and validate a simple and robust high-performance liquid chromatography (HPLC) method for the determination of olopatadine HCl in pharmaceutical samples. The method was validated in accordance with USP <1225>, AOAC, and ICH Q2(R1) guidelines, with evaluation of key parameters including linearity, accuracy, precision, selectivity, sensitivity, and system suitability. The validation outcomes confirmed that the method provided strong linearity across the tested range, acceptable recovery values, excellent repeatability, and sufficient sensitivity for low-level detection. Overall, the developed HPLC method proved to be accurate, precise, selective, and reliable. Its simplicity and cost-effectiveness make it particularly suitable for routine quality control of olopatadine HCl in research and pharmaceutical industry laboratories.

Keywords**:** ***:*** *Olopatadine HCl; HPLC; Method validation; Accuracy; Precision.*

1. INTRODUCTION

Olopatadine hydrochloride is a second-generation antihistamine that exerts its therapeutic effects through dual mechanisms: selective antagonism of histamine H_1 receptors and stabilization of mast cells. This pharmacological profile enables olopatadine to effectively relieve symptoms associated with allergic conjunctivitis and allergic rhinitis, including itching, redness, sneezing, nasal congestion, and watery eyes [1][2]. Therefore, Olopatadine HCl is considered as first-line therapy in treating moderate to severe allergic conjunctivitis [3]. It is formulated in various dosage forms such as ophthalmic drops and nasal sprays, and its widespread use underscores the importance of precise and reliable quantification in pharmaceutical products.

As a critical active pharmaceutical ingredient (API), olopatadine HCl must be present in precise concentrations within a formulation to ensure consistent therapeutic outcomes. Underdosing may lead to suboptimal symptom control, while overdosing may increase the risk of adverse effects [4]. Therefore, the development of validated analytical methods to quantify olopatadine in pharmaceutical products is essential for quality control and regulatory compliance.

High-performance liquid chromatography (HPLC) is widely regarded as the method of choice for the quantification of APIs due to its high accuracy, reproducibility, and ability to resolve compounds in complex matrices [5][6]. Unlike simpler techniques such as UV-Vis spectrophotometry, HPLC provides superior specificity, enabling the separation and quantification of the analyte even in the presence of excipients, degradation products, or other interfering substances [7][8]. For this reason, HPLC is routinely recommended in official pharmacopoeias and international guidelines for the analysis of drug substances and drug products as well as natural products [9][10].

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Analytical method validation is a mandatory requirement in pharmaceutical analysis to ensure the reliability of results obtained from methods like HPLC [11]. International guidelines, including ICH Q1(R1)and the updated ICH Q1(R2), require the evaluation of parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ), along with recommended validation strategies and acceptance criteria [12][13]. Similarly, USP <1225> provides harmonized definitions and regulatory expectations for these parameters, supporting global compliance and consistent quality assurance in pharmaceutical testing [14].

In this study, a HPLC method was developed and validated for the quantification of olopatadine HCl in solution. The method was evaluated for its suitability in routine quality control by assessing key validation parameters, and recovery testing was performed using the standard addition method. The results are expected to provide a reliable analytical foundation for use in pharmaceutical quality assurance settings.

2. METHODS

Equipment and Materials

Instrumentation

HPLC analysis was performed using a Shimadzu LC-20AT system (Shimadzu, Kyoto, Japan) equipped with a UV-Vis detector SPD-20A and a reversed-phase C18 column (250 mm × 4.6 mm, 5 µm; Waters Symmetry, PT. LabMart Indonesia, Jakarta, Indonesia). The mobile phase consisted of methanol (AR grade; PT. SMARTLAB INDONESIA, Jakarta, Indonesia) and distilled water in a ratio of 70:30 (v/v). Additional laboratory equipment included micropipettes (10–100 µL and 100–1000 µL, Baoshishan, China) with disposable tips, syringe filters (0.22 µm, HPLC grade; Microlab Scientific ,PT. Diontek Prima Analisa, Indonesia), an analytical balance (±0.1 mg accuracy; OHAUS, Japan), a vortex mixer (Scientific Industries, USA), an ultrasonicator (Bransonic 1510E-MT, Branson Ultrasonics Corp., USA), and standard laboratory glassware.

Materials

Olopatadine hydrochloride reference standard (≥99% purity) was obtained from INFALABS BPOM (Jakarta, Indonesia). Methanol (AR grade) and other analytical grade reagents were purchased from PT. SMARTLAB INDONESIA (Jakarta, Indonesia). A commercially available olopatadine HCl ophthalmic solution (eye drops) was purchased from a local pharmacy in Jakarta, Indonesia, and used as the test sample.

Procedural

The analytical procedure was designed to develop and validate a high-performance liquid chromatography (HPLC) method for the quantification of olopatadine HCl. The analysis was performed on a Shimadzu LC-20AT system equipped with a UV-Vis detector SPD-20A, using a reversed-phase C18 column The mobile phase consisted of methanol and distilled water in a ratio of 70:30 (v/v), delivered at a flow rate of 0.35 mL/min. Detection was carried out at 299 nm with an injection volume of 10 µL. These chromatographic conditions were selected after preliminary optimization to achieve sharp peak shapes, consistent retention times, and baseline stability.

A stock solution of olopatadine HCl was prepared at 2000 ppm in methanol and stored at 4°C protected from light. From this stock, calibration standards at 20, 30, 40, 50, 60, 70, and 80 ppm were freshly prepared by serial dilution prior to analysis. The test sample consisted of a commercially available olopatadine HCl ophthalmic solution purchased from a local pharmacy. Before analysis, the eye drop sample was homogenized by vortex mixing for 2 minutes and filtered through a 0.45 µm syringe filter to remove particulates.

For validation, accuracy and precision were assessed using the standard addition method at three concentration levels (low, medium, and high), corresponding to 30, 45, and 60 ppm. Each level was prepared in triplicate and analyzed across three consecutive days to evaluate both intraday and interday variability. Linearity was assessed from the calibration curve in the range of 20–80 ppm, while sensitivity was expressed as the limit of detection (LOD) and limit of quantification (LOQ), calculated from the standard deviation of the response and the slope of the regression line. Selectivity and specificity were verified through spiking experiments and chromatographic resolution analysis.

All experimental procedures were conducted under standard laboratory safety protocols. The validation parameters were evaluated in accordance with USP <1225>, AOAC, and ICH Q2(R1) guidelines.

3. RESULT AND DISCUSSIONS

1) Sampel Preparation

To support the method's development and validation, a series of standard and sample solutions were prepared, a stock solution of olopatadine HCl at 2000 ppm was prepared using absolute methanol as the solvent. This stock was then serially diluted to produce working standard solutions at concentrations of 20, 30, 40, 50, 60, 70, and 80 ppm. These standards were used to construct a calibration curve that served as the

In parallel, a 50 ppm sample solution was prepared to simulate the target concentration in pharmaceutical dosage forms. To assess the method's accuracy, a standard addition protocol was implemented. This involved preparing three additional spiked samples by adding 0.2 mL, 0.35 mL, and 0.5 mL of the 2000 ppm stock standard solution to 0.2 mL of the 50 ppm sample solution. Each mixture was then diluted to a final volume of 10 mL with methanol, resulting low, medium, and high concentration, respectively. These samples were analyzed in triplicate to ensure precision and reproducibility across the concentration range.

All solutions were filtered through a 0.45 µm membrane filter prior to injection into the HPLC system to eliminate any particulates that could potentially interfere with the chromatographic separation or detection. The systematic preparation of standards and spiked samples provided a robust framework for evaluating the performance of the HPLC method in terms of accuracy, precision, linearity, and specificity

2) Optimization of HPLC Analytical Conditions

In order to obtain accurate and reproducible quantification of olopatadine HCl, the chromatographic conditions of the HPLC method were carefully optimized. The method was carried out using a reversed-phase C18 column (150 mm × 4.6 mm, 5 µm particle size), which is widely used for the separation of moderately polar pharmaceutical compounds. Methanol and water in a ratio of 70:30 (v/v) were selected as the mobile phase. This composition was found to provide good elution strength, sufficient peak sharpness, and adequate resolution under isocratic conditions.

The flow rate was set at 0.35 mL/min, a value chosen to balance optimal separation with reasonable analysis time and column backpressure. Detection was performed at a wavelength of 299 nm, corresponding to the maximum UV absorbance of olopatadine HCl. This wavelength ensures sensitivity to even low concentrations while maintaining selectivity against possible interfering components in the matrix. An injection volume of 10 µL was used for all standard and sample runs to maintain consistency and reproducibility.

Under these conditions, olopatadine HCl was consistently eluted at a retention time of approximately 7.8 minutes. The chromatographic peaks obtained were sharp, symmetrical, and well-resolved, indicating efficient interaction between the analyte and the stationary phase. The baseline remained stable throughout the analysis, and no ghost peaks or overlapping signals were observed in the region of interest. These results confirmed the suitability of the selected conditions for the routine analysis of olopatadine HCl and ensured the reliability of the subsequent validation steps. The representative chromatogram of olopatadine HCl obtained under these optimized conditions is presented in Figure 1.

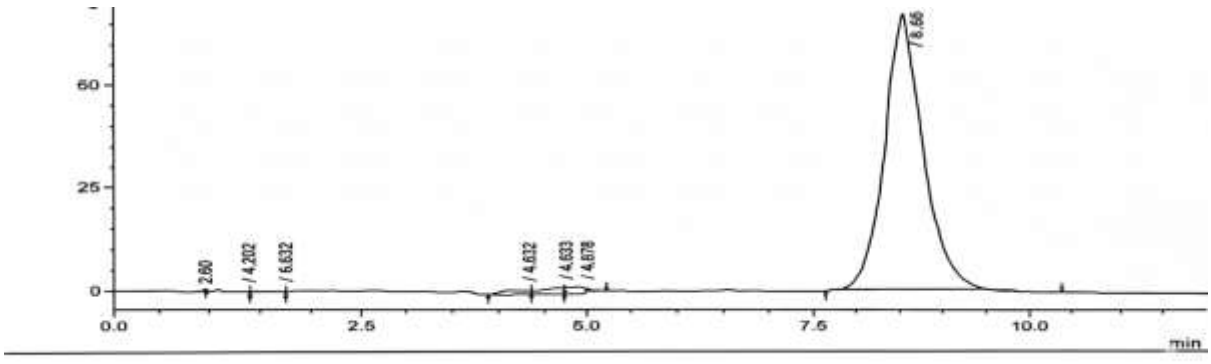

Figure 1. Olopatadine HCl Chromatogram

3) Selectivity and Specificity

Selectivity and specificity are critical parameters in validating an analytical method, particularly for pharmaceutical compounds such as olopatadine HCl. Selectivity refers to the method's ability to distinguish the analyte from other substances present in the sample matrix, while specificity is the method's capability to accurately identify and quantify the analyte of interest without interference from degradation products, impurities, solvents, or excipients.

In this study, selectivity was assessed through a spiking approach by comparing the chromatograms of standard olopatadine HCl, unspiked sample, and spiked sample. The chromatographic results showed a distinct, sharp, and well-resolved peak for olopatadine HCl at its characteristic retention time, with no co-eluting peaks observed in the vicinity. The spiked sample did not exhibit any additional or interfering peaks at the analyte's retention time, confirming the absence of matrix interference and suggesting high specificity.

Specificity testing was also performed using the spiking method, considering that the detector employed was UV. Under these conditions, specificity was evaluated by ensuring that no peak splitting occurred at the analyte's retention time, thereby strengthening the evidence that the reference standard and the analyte in the sample are indeed the same compound.

To further evaluate the resolution between olopatadine HCl and the nearest neighboring peak, resolution (Rs) was calculated. The obtained resolution value was 3.1180, which exceeds the minimum acceptable threshold of 2.0 as recommended by chromatographic validation guidelines. This result indicates that the analyte was well-separated from any adjacent components, further supporting the method's selectivity [15].

No interfering signals were detected from the diluent (methanol), mobile phase, or other background components, and no degradation peaks were observed in the chromatograms. This reinforces the method's specificity and confirms that the detected peak corresponds exclusively to olopatadine HCl. Overall, the chromatographic method demonstrated excellent selectivity and specificity, making it suitable for the reliable quantification of olopatadine HCl in pharmaceutical preparations or stability testing environments.

4) System Suitability Test (SST)

System Suitability Test (SST) is an essential part of analytical method validation, ensuring that the chromatographic system is functioning properly and consistently before proceeding with sample analysis. It evaluates key parameters such as retention time consistency, peak area reproducibility, column efficiency, peak symmetry, and resolution.

In this study, SST was performed using seven replicate injections (n = 7) of a 50 ppm standard solution of olopatadine HCl. The average peak area was 1,333,313, with a standard deviation (SD) of 2,685.81, resulting in a coefficient of variation (%CV) of 0.201%. This %CV falls well below the USP <621> threshold of 2.0%, and even satisfies the more stringent criterion of <0.73% for highly reproducible methods, indicating excellent injection repeatability and system precision [15].

The retention time (RT) for the olopatadine HCl peak was consistently observed at approximately 7.8 minutes, with no significant shift across the seven injections. The peak shape was sharp and symmetrical, as reflected by an asymmetry factor (As) value of less than 2, which is within the acceptable range recommended by USP for reversed-phase HPLC methods.

Furthermore, the system demonstrated satisfactory column efficiency, with a theoretical plate number (N) greater than 8,533, indicating high column performance and minimal band broadening. Additionally, a resolution (Rs) value greater than 2.0 was achieved between adjacent peaks, confirming that the chromatographic conditions provided adequate separation without co-elution or interference.

Taken together, these results confirm that the HPLC system used in this study met all suitability criteria. The method is therefore considered robust, reproducible, and suitable for the quantitative determination of olopatadine HCl in pharmaceutical samples.

5) Linearity and Range

Linearity evaluation was performed to assess the ability of the HPLC method to produce results directly proportional to the concentration of olopatadine HCl within a defined range. Seven standard solutions with concentrations of 20, 30, 40, 50, 60, 70, and 80 ppm were prepared and analyzed under the optimized chromatographic conditions. The resulting chromatographic peak areas were plotted against their respective concentrations to construct the calibration curve.

The calibration data revealed a strong linear relationship between analyte concentration and detector response. Regression analysis yielded a coefficient of correlation (r) of 0.998, indicating excellent linearity across the working range. The linear regression equation was determined to be y = 25262x + 91851, which was suitable for use in quantitative analysis and prediction of unknown sample concentrations [16]. The range measurement produces a regression equation y=25560x + 60393 and shows an r value of 0.99. This indicates that the method used is accurate and reliable for measurements within the specified range.

The selected concentration range also met the requirements for method validation, covering the lower and upper expected limits of olopatadine HCl in pharmaceutical formulations. In addition, the consistent and reproducible peak areas obtained for each concentration level further supported the reliability of the method over the specified range. Based on these findings, the method was deemed linear and appropriately validated for use within the 20–80 ppm range.

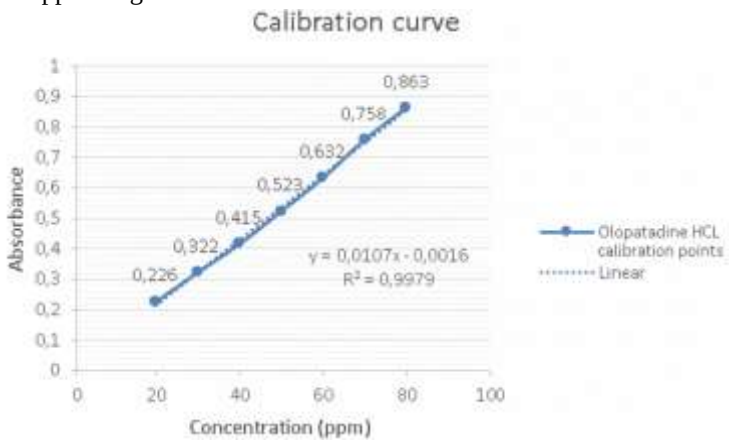

Figure 2. Calibration curve of olopatadine HCl showing the linear relationship between concentration (20–80 ppm) and absorbance

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

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are crucial parameters for evaluating the sensitivity of an analytical method. The LOD represents the lowest concentration of an analyte that can be detected but not necessarily quantified under stated conditions, while the LOQ indicates the minimum level at which the analyte can be quantitatively determined with acceptable accuracy and precision.

In this study, the LOD and LOQ for olopatadine HCl were calculated using the standard deviation of the response and the slope of the calibration curve, following the ICH Q2(R1) and USP <1225> guidelines. Based on the calculations from the HPLC calibration data, the LOD was determined to be 0.0485 ppm, while the LOQ was 0.1617 ppm. These values indicate that the developed HPLC method is highly sensitive, capable of detecting and quantifying olopatadine HCl at sub-ppm levels. Such sensitivity is particularly beneficial for pharmaceutical applications where trace-level detection is often required, including impurity profiling and stability studies. The low LOD and LOQ also affirm the method's robustness for routine quality control analysis in drug formulation and development.

7) Accuracy and Precision

The accuracy and precision of the HPLC method were evaluated using a standard addition (spiking) approach at three concentration levels (low, medium and high) achieved by adding 0.2 mL, 0.35 mL, and 0.5 mL, respectively, of a 2000 ppm olopatadine HCl stock solution to a sample. Each concentration level was prepared in triplicate and analyzed over three consecutive days to assess both intraday (within-day) and interday (between-day) performance.

Accuracy was determined by calculating the percent recovery of the added analyte relative to its theoretical concentration. The overall average recovery across all concentration levels was 105.63%, which falls within the AOAC (2019) acceptable range of 95%–110%, demonstrating that the method delivers accurate quantification of olopatadine HCl across the tested range.[17].

Precision was assessed by calculating the coefficient of variation (%CV) for each spike level across the three days. The pooled average %CV was 0.52%, indicating excellent repeatability and supporting the method's reliability for routine application. Collectively, these results confirm that the developed HPLC method is both accurate and precise, making it suitable for the quantitative analysis of olopatadine HCl in pharmaceutical preparations.

8) Overall Evaluation and Method Suitability

The overall evaluation of the HPLC method for the determination of olopatadine HCl demonstrates that the analytical procedure fulfills the essential validation parameters in accordance with USP <1225>, ICH Q2(R1), and AOAC guidelines. The method was designed to support accurate and reproducible quantification of olopatadine HCl in bulk or finished pharmaceutical formulations, aligning with Category I procedures as outlined in the USP Validation of Compendial Procedures, which includes assays for active pharmaceutical ingredients (APIs).

The method exhibited excellent linearity across the working range of 20–80 ppm, with a r value consistently exceeding 0.998, indicating that the response was directly proportional to the analyte concentration. The accuracy of the method, assessed through percent recovery using the standard addition method, with a total average recovery of 105.63%, falling within the AOAC-acceptable range of 95%–110% for analytes in this concentration range.

Precision, evaluated through both intraday and interday assessments, yielded consistently low %CV values with an overall average of 0.515%, indicating excellent repeatability and intermediate precision. The method's limit of detection (LOD) was calculated to be 0.0485 ppm, and the limit of quantification (LOQ) was 0.1617 ppm, demonstrating the method's high sensitivity in detecting and quantifying olopatadine HCl at low concentrations.

System Suitability Testing (SST) confirmed the method's operational robustness, with results meeting all critical parameters: %CV of peak area was 0.201%, theoretical plate number exceeded 8,533, asymmetry factor was <2, and resolution between adjacent peaks was >2. The determination of olopatadine HCl content from five replicate injections (n=5) of the sample yielded an average concentration of 0.095 ± 0.001%. This finding highlights that the method is simple, economical, and reliable, making it suitable for routine quality control in both research laboratories and the pharmaceutical industry.

Altogether, the method satisfies the validation criteria for specificity, linearity, accuracy, precision, LOD, LOQ, and system suitability. Thus, it can be concluded that the developed HPLC method is both scientifically sound and practically applicable for routine quality control testing of olopatadine HCl in pharmaceutical analysis. It offers a validated, selective, and robust approach to ensure the identity and quantitative determination of the API in compliance with regulatory expectations.

4. CONCLUSION

The validated HPLC method for the quantification of olopatadine HCl demonstrated excellent performance in terms of linearity, accuracy, precision, selectivity, sensitivity, and system suitability. The method showed a high correlation coefficient ($R^2 > 0.99$), recovery within acceptable limits, and low variability (%CV < 1%). The LOD

(0.05 ppm) and LOQ (0.16 ppm) indicate adequate sensitivity for pharmaceutical analysis. System suitability parameters, including retention time consistency and peak resolution, confirmed the robustness of the chromatographic conditions. Overall, the method is validated and suitable for routine quality control of olopatadine HCl in pharmaceutical formulations, offering a reliable tool for ensuring product quality and compliance with regulatory standards.

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