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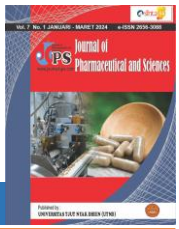

Validation of an HPLC Analytical Method for the Determination of Paracetamol in Generic and Branded Tablet Dosage Forms

Validasi Metode Analisis KCKT untuk Penetapan Kadar Parasetamol dalam Sediaan Tablet Generik dan Bermerek

M. Deddy Harfiansyah [a*], Gratia Apulina Cindylawsa Purba [b], Fitria [c], Ummi Khairani Rambe [a] and Ageng Azhar Amar [b]

[a] *Department of Pharmacy, Faculty of Health Sciences, Universitas Haji Sumatera Utara, North Sumatra, Indonesia.*
[b] *Department of Pharmacy, Faculty of Pharmacy, Institut Kesehatan Deli Husada Deli Tua, North Sumatra, Indonesia.*
[c] *Department of Pharmacy, Faculty of Health Sciences, Universitas Ubudiyah Indonesia, Aceh, Indonesia.*

Corresponding Authors: mdeddyh@gmail.com

Abstract

Background: Paracetamol is one of the most widely used analgesic and antipyretic drugs, and quality control of its dosage forms is crucial to ensure therapeutic effectiveness. **Objective:** This study aimed to validate a simple, rapid, and reliable High-Performance Liquid Chromatography (HPLC) method for the determination of paracetamol content in generic and branded tablet formulations. **Methods:** The chromatographic analysis was performed on a C18 column using a mobile phase of distilled water and methanol (3:1, v/v) with a flow rate of 1.0 mL.min^{-1}, injection volume of 20 µL, and detection wavelength at 243 nm. Validation parameters including system suitability, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were evaluated according to pharmacopeial guidelines. **Results:** The method demonstrated excellent linearity over the range of 8–12 µg.mL^{-1} (r = 0.9998), with LOD and LOQ values of 0.13 µg.mL^{-1} and 0.40 µg.mL^{-1}, respectively. Accuracy values were 99.00% for generic and 98.86% for branded tablets, while precision showed %RSD of 0.53% and 0.68%. The paracetamol content was 101.47% and 100.20% for generic and branded formulations, respectively, meeting pharmacopeial standards (90–110%). **Conclusion:** The validated HPLC method proved to be accurate, precise, and sensitive for the quantitative determination of paracetamol in tablet dosage forms. This method is suitable for routine quality control and can be extended for stability or pharmacokinetic studies.

Keywords: HPLC, Paracetamol, Method Validation, Generic Tablets, Branded Tablets.

Abstrak

Latar Belakang: Parasetamol merupakan obat analgesik dan antipiretik yang paling banyak digunakan, sehingga pengendalian mutu sediaannya penting untuk menjamin efektivitas terapi. **Tujuan:** Penelitian ini bertujuan untuk memvalidasi metode Kromatografi Cair Kinerja Tinggi (KCKT) yang sederhana, cepat, dan andal untuk penetapan kadar parasetamol dalam sediaan tablet generik dan bermerek. **Metode:** Analisis dilakukan menggunakan kolom C18 dengan fase gerak campuran air dan metanol (3:1, v/v), laju alir 1,0 mL.menit^{-1}, volume injeksi 20 µL, dan deteksi pada panjang gelombang 243 nm. Parameter validasi yang diuji meliputi kesesuaian sistem, linearitas, akurasi, presisi, batas deteksi (LOD), dan batas kuantifikasi (LOQ) sesuai pedoman farmakope. **Hasil:** Metode menunjukkan linearitas sangat baik pada rentang 8–12 µg.mL^{-1} (r = 0,9998) dengan nilai LOD 0,13 µg.mL^{-1} dan LOQ 0,40 µg.mL^{-1}. Nilai akurasi sebesar 99,00% untuk tablet generik dan 98,86% untuk tablet bermerek, sedangkan presisi menunjukkan %RSD 0,53% dan 0,68%. Kadar parasetamol yang diperoleh adalah 101,47% dan 100,20% untuk tablet generik dan bermerek, sesuai dengan standar farmakope (90–110%). **Kesimpulan:** Metode KCKT yang divalidasi terbukti akurat, presisi, dan

sensitif untuk penetapan kadar parasetamol dalam sediaan tablet. Metode ini layak digunakan untuk pengujian rutin mutu obat dan dapat dikembangkan untuk uji stabilitas maupun studi farmakokinetik.

Kata Kunci: KCKT, Parasetamol, Validasi Metode, Tablet Generik, Tablet Bermerek.

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Introduction

Paracetamol, also known as acetaminophen or N-acetyl-para-aminophenol (APAP), is an analgesic (pain reliever) and antipyretic (fever reducer) that is widely used across the world. It belongs to the non-opioid class of drugs and is generally prescribed for mild to moderate pain, such as headaches, muscle aches, or mild postoperative pain, as well as for reducing fever. Unlike many nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol does not exhibit strong anti-inflammatory properties, making it less effective as an anti-inflammatory agent [1–3]. Chemically, the structure of paracetamol consists of a benzene ring core substituted with a hydroxyl group and an amide group (acetamide) bonded through a nitrogen atom in a para (1,4) orientation, as illustrated in Figure 1 [2].

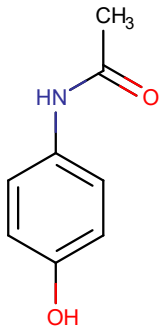

Figure 1. Chemical Structure of Paracetamol

Paracetamol in tablet form is one of the commonly available pharmaceutical preparations, produced both as generic medicines and branded products. A generic drug contains the same active ingredient as the branded (originator) product once its patent has expired. Pharmacologically, generic medicines must meet bioequivalence requirements with respect to the reference product, meaning they must demonstrate comparable release profiles of the active substance and equivalent pharmacokinetic parameters within the limits set by regulatory authorities. As a result, their therapeutic efficacy and safety are expected to be equivalent. The main advantage of generic medicines lies in their significantly lower production costs and market prices, thereby improving patient access to therapy [4,5]. Branded medicines, on the other hand, are products originally developed and patented by innovator pharmaceutical companies. During the period of patent protection and or market exclusivity, only the originator company is authorized to manufacture and distribute the product-resulting in higher prices due to research and development (R&D) expenses, marketing, and exclusivity rights. Once the patent expires, other manufacturers may produce generic versions provided they comply with regulatory standards [6,7].

The use of generic drugs is globally recognized as an effective strategy to enhance access to medicines and reduce healthcare costs [8,9]. However, challenges remain, including limited technical understanding of generic drugs, doubts regarding their quality and efficacy, and a tendency among both the public and

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healthcare professionals to prefer branded medications [9,10]. Recent studies indicate that despite the acknowledged economic benefits of generic drugs, concerns persist regarding their effectiveness and safety, with acceptance heavily influenced by healthcare providers' recommendations [9,10]. Therefore, tiered education, transparent public outreach, and strengthened quality regulations are essential to support the acceptance of generics within healthcare systems [8,11]. Quality regulations encompass various aspects, one of which is the determination of drug content, aimed at ensuring that the active ingredient in generic medicines aligns with pharmacopeial standards, thereby guaranteeing safety, efficacy, and bioequivalence with reference drugs [12]. In practice, content determination is typically conducted using analytical methods with high accuracy and precision, such as High Performance Liquid Chromatography (HPLC), which can reliably detect and quantify active ingredients in accordance with validated analytical method requirements [13,14].

Experimental Section

Materials and Apparatus

The materials employed in this study included analytical-grade paracetamol standard was obtained from Anqiu Lu'an Pharmaceutical Co., Ltd. HPLC-grade methanol, supplied by J.T. Baker, and distilled water (aqua bidest) from PT. Ikapharmindo Putramas. Paracetamol tablet samples, including both generic (A) and branded (B) formulations, were purchased from local pharmacies in Medan, Indonesia. The instruments employed in this study included a High-Performance Liquid Chromatography (HPLC) system Alliance e2695 separation module (Waters) equipped with a UV/Visible detector (Waters 2489) and a µBondapak™ C18 column (3.9 × 300 mm, 10 µm, 125 Å), an ultrasonic bath (ELMA, Type D-78224), and a digital balance (Sartorius). Additional laboratory equipment comprised 0.45 µm Phenex NY filters and standard glassware (Pyrex®).

Liquid Chromatography Conditions

The HPLC analysis was carried out using an Alliance e2695 separation module (Waters) equipped with a UV/Visible detector (Waters 2489) and a µBondapak™ C18 column (3.9 × 300 mm, 10 µm, 125 Å). The mobile phase consisted of distilled water and methanol mixed in the ratio of 3:1 (v/v) [15] and was delivered under isocratic conditions at a flow rate of 1.0 mL.min^{-1}. The injection volume was 20 µL, the column temperature was maintained at 25 °C, and the detection wavelength was set at 243 nm. The total chromatographic run time was 5.5 min. Prior to use, the mobile phase and samples were filtered through a 0.45 µm membrane filter and degassed by sonication for 15 min. A daily calibration curve was prepared using 5 standard of paracetamol in the concentration range of 8–12 µg.mL^{-1}.

Preparation of Standard Solution

The stock standard solution of paracetamol was prepared at a concentration of 500 µg.mL^{-1} by accurately weighing 50 mg of paracetamol and dissolving it in the mobile phase using a 100 mL volumetric flask. Working standard solutions of paracetamol (8–12 µg.mL^{-1}) were subsequently prepared by appropriate dilutions of the stock solution with the mobile phase. All solutions were filtered through a 0.45 µm membrane filter, with the initial portion of the filtrate discarded, and the remaining clear solutions were collected and used for analysis.

Validation of Paracetamol HPLC Assay

System Specificity

System specificity of the HPLC method for paracetamol was evaluated by performing six replicate injections of the working standard solution at 10 µg/mL. The suitability of the system was determined based on the percent relative standard deviation (%RSD) of both peak areas and retention times. The method was considered acceptable when the %RSD values did not exceed ≤ 2%, indicating adequate precision and consistency of the chromatographic system [15].

Linearity

Linearity is the ability of an analytical method to obtain test results that are directly proportional to the concentration of the analyte within a given range. Linearity of the process for paracetamol was assessed by

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three replicate injections of five different concentrations (8, 9, 10, 11, and 12 µg.mL^{-1}) prepared from the stock solution. The mean peak areas were plotted against the corresponding concentrations, and the linearity was evaluated from the calibration curve by calculating the slope, intercept, and correlation coefficient (r). In general, a correlation coefficient (r) ≤ 1 is considered indicative of an acceptable fit of the experimental data to the regression line [16].

Accuracy

The accuracy of an analytical method reflects the closeness between the expected concentration and the concentration obtained experimentally. Accuracy of the technique for paracetamol was determined by successive analysis (n = 3) at three concentration levels corresponding to 80%, 100%, and 120% of the target concentration. The percentage recovery was calculated using the formula [% Recovery = (Measured concentration/Theoretical concentration) × 100] to assess the method's recovery and validity. The acceptance criterion required mean recovery values within the range of 90–110% [16,17].

Precision

Precision of an analytical method is defined as the degree of agreement among replicate measurements when the procedure is applied repeatedly under specified conditions. In this study, precision was evaluated by performing six replicate analyses of paracetamol at a target concentration. The reproducibility of the method was expressed as %RSD, with a value ≤ 2% considered acceptable to demonstrate adequate precision. [16,18].

Limit of Detection and Limit of Quantification

The limit of detection (LOD) is defined as the lowest concentration of analyte in a sample that can be detected but not necessarily quantified under the described experimental conditions. The limit of quantification (LOQ) is the lowest concentration of analyte that can be measured with acceptable accuracy and precision. Both parameters were estimated using the equations LOD = 3.3 × SD/S and LOQ = 10 × SD/S, where SD represents the standard deviation of the response (peak area), and S denotes the slope of the calibration curve [16,18].

Sample Analysis of Paracetamol Tablets

The content of paracetamol in marketed tablet formulations, including generic (A) and branded (B) products, was determined by analyzing 20 tablets from each type. The tablets were weighed to obtain the mean weight, then finely powdered. A portion of the powder equivalent to 50 mg of paracetamol was accurately transferred into a 100 mL volumetric flask containing 50 mL of the mobile phase, sonicated for 15 min, and diluted to volume with the same solvent. From this solution, 1 mL was withdrawn and transferred into a 50 mL volumetric flask, then diluted to the mark. The resulting solution was filtered through a 0.45 µm membrane filter, discarding the first 10 mL of the filtrate, and the clear solution was collected for analysis. An aliquot of 20 µL of the prepared sample was injected into the HPLC system under the optimized chromatographic conditions described above. The peak areas were monitored at 243 nm, and the paracetamol concentrations in the tablets were calculated using a multilevel calibration curve based on linear regression analysis performed under identical conditions.

Results and Discussion

System Suitability

System suitability of the HPLC method for paracetamol was evaluated by conducting six replicate injections of the working standard solution at a concentration of 10 µg.mL^{-1} (Figure 2). The method was considered acceptable when %RSD ≤ 2% for both peak area and retention time, as summarized in Table 1 [19,20].

Linearity, LOD, LOQ

The resulting linearity value of 0.9998 was obtained between the concentration and the corresponding peak area of paracetamol in the range of 8-12 µ g.mL-1 (Table 2). The regression Equation describing the calibration curve of the drug concentration (X) versus the peak area (Y) with the calculated paracetamol

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Equation obtained y = 64714x + 5434.4, with the calibration curve that can be seen in Figure 3. LOD and LOQ are computed using the regression line Equation derived from the calibration curve. The LOD value obtained was 0.13 μg.mL^{-1} while the LOQ value for this method was 0.40 μg.mL^{-1}.

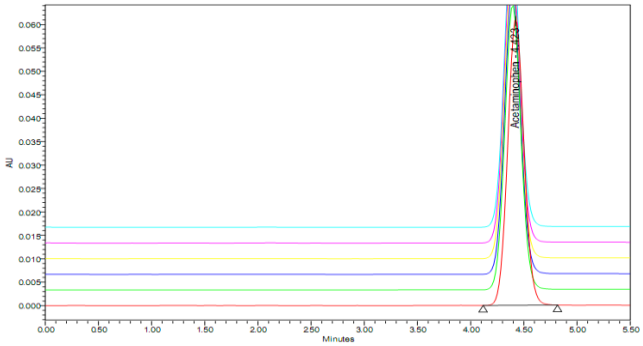

Figure 2. System Suitability Chromatogram of the Paracetamol Working Standard Solution

Table 1. System Suitability Test of the Paracetamol Working Standard Solution

Working Standard Solution Paracetamol (Replicate)	Retention Time (min)	Area (AU)
1	4.423	654331
2	4.394	645956
3	4.387	646996
4	4.388	646600
5	4.386	647444
6	4.391	647224
Mean		648092
%RSD		0.48

Table 2. Paracetamol Calibration Curve Data

Concentration (μg.mL^{-1})	Area (AU)
8	525902
9	584509
10	652024
11	717376
12	783036

Accuracy and Precision

Accuracy is a parameter in which the test is conducted at three appropriate concentration levels, and the return value is calculated, which can be called the percent recovery. The accuracy value is shown in Table 3, namely the average percent recovery across three specific ranges with three repetitions. In this case, the particular ranges used are 80, 100, and 120%. The resulting accuracy value shows that the method used meets the method validation requirements, namely the accuracy value requirement of 90-110% [16,17]. Precision is a parameter that measures how close the analysis results are across several repetitions, in this case, six repetitions. Precision shows that the method used yields close results even after several replications. The precision parameter is described by the resulting %RSD value, for the results obtained in Table 3 meet the validation requirements of %RSD ≤ 2% [16,18].

Analysis of Generic and Branded Tablet Dosage Forms

The determination of paracetamol content in generic (A) and branded (B) tablet formulations, with chromatograms generated in Figure 4, and the levels are presented in Table 4. The results indicate compliance with pharmacopeial standards, as the measured drug levels were within the acceptable range of 90–110%, in

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accordance with the specifications outlined in the United States Pharmacopeia (USP 38) and the National Formulary (NF 33) [21].

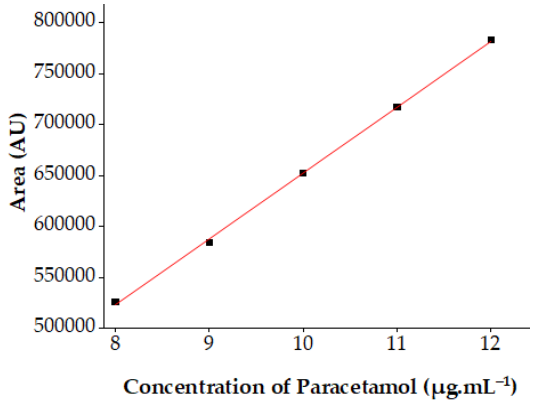

Figure 3. Calibration Curve of Paracetamol Constructed in the Mobile Phase Across the Concentration Range of 8–12 µg.mL/1.

Table 3. Accuracy and Precision Results for Paracetamol in Generic (A) and Branded (B) Tablet Formulations

Parameter	Paracetamol Tablet	
	Generic (A)	Branded (B)
Accuracy (%)	99.00	98.86
Precision (%)	0.53	0.68

Table 4. Analysis of Paracetamol in Generic (A) and Branded (B) Tablet Formulations

Paracetamol Tablet	Level (%)	Requirement (%)
Generic (A)	101.47	90–110
Branded (B)	100.20	90–110

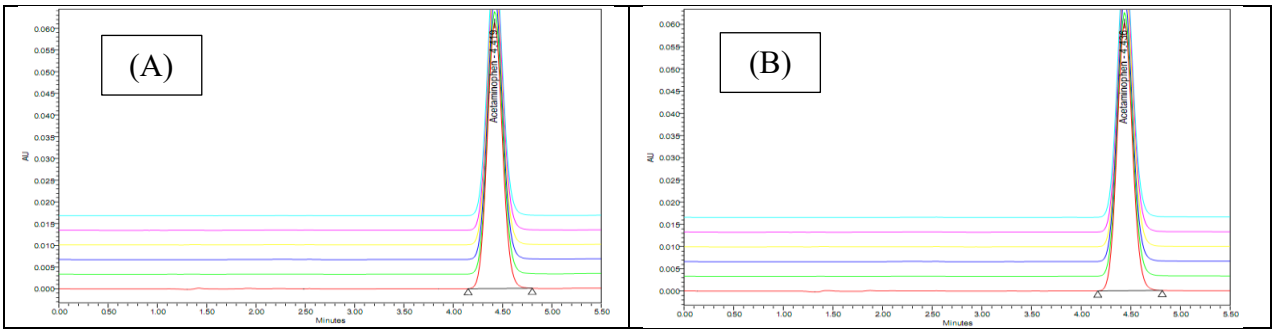

Figure 4. Chromatogram of paracetamol in Generic (A) and Branded (B) Tablet Formulations

Conclusions

A validated HPLC method was established for the determination of paracetamol, offering a simple, rapid, and sensitive approach. The short chromatographic runtime enabled efficient analysis, while statistical evaluation confirmed the method's reliability for determining paracetamol content in both generic and branded tablet formulations, with no interference from excipients. In addition, the technique shows potential for broader applications, including stability assessments (e.g., degradation kinetics) and the determination of paracetamol in biological matrices (e.g., plasma).

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