การศึกษาความเท่าเทียมทางเภสัชกรรมของยาเมโทโพรลอลทาร์เทรตชนิดเม็ดที่จำหน่ายในจังหวัดอุบลราชธานี โดยวิธี RP-HPLC ที่ผ่านการตรวจสอบความถูกต้อง
Pharmaceutical Equivalence Study of Marketed Metoprolol Tartrate Tablets in Ubon Ratchathani via a Validated RP-HPLC Method

นิพนธ์ต้นฉบับ

Original Article

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บทคัดย่อ

วัตถุประสงค์: เพื่อเปรียบเทียบพารามิเตอร์ของความเท่าเทียมทางเภสัชกรรมของยาสามัญเมโทโพรลอลทาร์เทรตชนิดเม็ดขนาด 100 มิลลิกรัมที่ผลิตในประเทศ 2 ตำรับที่วางจำหน่ายใน จ.อุบลราชธานี เทียบกับผลิตภัณฑ์อ้างอิง **วิธีการศึกษา:** ประเมินตัวแปรต่าง ๆ ได้แก่ ความคลาดเคลื่อนของน้ำหนักเม็ดยา ระยะเวลาการแตกตัว ความสม่ำเสมอของปริมาณตัวยาสำคัญ และการละลายในหลอดทดลองในกรดไฮโดรคลอริก 0.1 N ตามมาตรฐานของสหรัฐอเมริกา (USP 39/NF 34) วิธีโครมาโทกราฟีของเหลวสมรรถนะสูงแบบเฟสกลับ (RP-HPLC) ที่พัฒนาขึ้นและผ่านการตรวจสอบความถูกต้องเพื่อการวิเคราะห์ปริมาณเมโทโพรลอลทาร์เทรตโดยเครื่องตรวจวัดแสงยูวีที่ความยาวคลื่น 274 นาโนเมตร แยกสารบนคอลัมน์รีเวอร์สเฟส C18 โดยใช้เฟสเคลื่อนที่แบบไอโซเครติกที่มีเมทานอล กรด 1-เฮปเทนซัลโฟนิก 0.01 โมลาร์ และกรดอะซิติกเข้มข้นอัตราส่วน 51.5:48.0:0.5 ตามลำดับ **ผลการศึกษา:** วิธีวิเคราะห์มีความเป็นเส้นตรงในช่วงความเข้มข้น 60 – 300 ไมโครกรัมต่อมิลลิลิตร (R^2 = 0.9999) ความแม่นยำภายในวันและระหว่างวัน (%RSD) เท่ากับ 1.94% และ 4.15% ตามลำดับ ความถูกต้อง (%การกลับคืน) อยู่ในช่วง 100.00 – 101.63% พบว่ายาสามัญที่ผลิตในประเทศทั้งสองตำรับและผลิตภัณฑ์อ้างอิงผ่านเกณฑ์มาตรฐาน USP ได้แก่ ความคลาดเคลื่อนของน้ำหนักเม็ดยาไม่เกิน 15% ระยะเวลาการแตกตัวไม่เกิน 30 นาที และปริมาณตัวยาสำคัญอยู่ในช่วง 91.50% ถึง 93.51% ของปริมาณที่ระบุบนฉลาก ผลการละลายต่างกันแต่ไม่มีนัยสำคัญทางสถิติ (P-value > 0.05) โดยมีค่าปัจจัยความแตกต่าง (f_1) เท่ากับ 1.04 และ 1.78 และค่าปัจจัยความเหมือน (f_2) เท่ากับ 71.72 และ 62.12 ตามลำดับ **สรุป:** ผลิตภัณฑ์ยาสามัญเมโทโพรลอลทาร์เทรตชนิดเม็ดขนาด 100 มิลลิกรัมที่ผลิตในประเทศทั้งสองตำรับมีความเท่าเทียมทางเภสัชกรรมกับผลิตภัณฑ์อ้างอิง

Keywords: ความเท่าเทียมทางเภสัชกรรม; โครมาโทกราฟีของเหลวสมรรถนะสูงแบบเฟสกลับ; การละลายในหลอดทดลอง; ยาเมโทโพรลอลทาร์เทรต; อุบลราชธานี

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Abstract

Objective: To compare parameters of pharmaceutical equivalence of two locally manufactured metoprolol tartrate 100 mg tablet formulations available in Ubon Ratchathani, Thailand compared with the reference product. **Methods:** The parameters evaluated included weight variation, disintegration time, uniformity of dosage units, and *in vitro* dissolution in 0.1 N hydrochloric acid, following the United States Pharmacopeia (USP 39/NF 34) standards. The reverse-phase high-performance liquid chromatography (RP-HPLC) method with ultraviolet detection at 274 nm was developed and validated for metoprolol tartrate quantification. Chromatographic separation was performed on a C18 reversed-phase column with an isocratic mobile phase of methanol, 0.01 M 1-heptanesulfonic acid, and glacial acetic acid (51.5:48.0:0.5). The method showed linearity from 60–300 µg/mL (R^2 = 0.9999). The intra- and inter-day precisions (%RSD) were 1.94% and 4.15%, respectively, with accuracies (%recovery) of 100.00 – 101.63%. The system suitability parameters, including the tailing factor (1.1) and theoretical plates, met the acceptance criteria. **Results:** Both generic formulations and the reference product met the following USP specifications: weight variation with acceptance values < 15%, disintegration < 30 minutes, and uniformity of dosage units with acceptance values within limits. The individual assay results ranged from 91.50% to 93.51% of the labeled amount. The dissolution profiles did not significantly differ from those of the reference product (P-value > 0.05), with difference factors (f_1) of 1.04 and 1.78 and similarity factors (f_2) of 71.72 and 62.12, respectively. **Conclusion:** The two locally manufactured metoprolol tartrate 100 mg tablet formulations available in Ubon Ratchathani had pharmaceutical equivalence with the reference product for the specific batches evaluated.

Keywords: pharmaceutical equivalence; reverse-phase high-performance liquid chromatography; *in vitro* dissolution; metoprolol tartrate; Ubon Ratchathani

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Introduction

Cardiovascular diseases (CVDs) remain the leading cause of death globally, accounting for approximately 17.9 million deaths annually, and are responsible for 32% of all global mortalities.[1] In Asia, the burden of CVDs is projected to rise dramatically, with estimates indicating a 91.2% increase in crude cardiovascular mortality between 2025 and 2050, amounting to nearly 24.1 million deaths across the region.[2] In Thailand, hypertension and ischemic heart

disease are among the most prevalent chronic conditions, placing increasing demands on healthcare systems and necessitating long-term access to safe, effective, and affordable medications.[3]

Metoprolol tartrate, a selective β_1-adrenergic receptor blocker, is commonly prescribed for the treatment of hypertension, angina pectoris, arrhythmias, and heart failure.[4] It is included in Thailand's National List of Essential Medicines (NLEM) and the World Health Organization (WHO) Model List of Essential Medicines, indicating its clinical importance and widespread use.[5] To increase access and reduce treatment costs, generic formulations of metoprolol are routinely substituted for innovator products in the public and private sectors. However, despite their regulatory approval, concerns remain among healthcare professionals and patients regarding the quality, efficacy, and therapeutic equivalence of generic drugs.[6]

Recent studies in Thailand and Southeast Asian countries have documented quality variability in cardiovascular medications, particularly among generic formulations. A comprehensive survey of Thai community pharmacists revealed that 68% expressed concerns about generic drug quality, with cardiovascular medications being among the most frequently questioned therapeutic categories.[7] Additionally, post-marketing surveillance studies in the ASEAN region have identified substandard cardiovascular drugs, with failure rates ranging from 5 – 15% for basic quality parameters.[8] These concerns are particularly relevant for beta-blockers, where consistent drug release and bioavailability are critical for maintaining therapeutic efficacy and preventing cardiovascular events.

Generic medicines are expected to demonstrate pharmaceutical equivalence and bioequivalence to the reference product to ensure therapeutic equivalence. Pharmaceutical equivalence refers to products that contain the same active ingredient in the same dosage form, route of administration, and strength and meet similar quality standards.[9] For Biopharmaceutics Classification System (BCS) Class I drugs, such as metoprolol tartrate, *in vitro* dissolution testing plays a critical role in predicting *in vivo* bioavailability, especially when performed under discriminatory conditions.[10]

In low- and middle-income countries (LMICs), including Thailand, there is increasing reliance on generics due to economic constraints. However, studies have revealed variability in the pharmaceutical quality of generic drugs, particularly cardiovascular medications, in these settings.[11,12] A study conducted in sub-Saharan Africa (the SEVEN study) reported that up to 15% of cardiac drugs failed basic quality tests, reinforcing the need for regular post-marketing surveillance.[13] In Thailand, limited research has been conducted to evaluate the *in vitro* quality of antihypertensive generics, especially at the regional level.[14] As a result, prescribers may lack confidence in locally manufactured generics potentially leading to unnecessary expenditures on more expensive innovator brands.

Recent literature further emphasizes the importance of pharmaceutical equivalence testing. Luo et al reported that generic cardiovascular medicines demonstrate safety and efficacy comparable to brand-name counterparts.[15] Methodological advances, such as the framework for bioequivalence assessment proposed by Insolia et al and the multivariate equivalence test described by Wang et al, also highlights evolving approaches to evaluate equivalence beyond traditional dissolution testing.[16,17] Despite these advances, no recent study has specifically examined metoprolol tartrate tablets in Thailand, underscoring the novelty of this work.

Given this background, evaluating the pharmaceutical equivalence of generic metoprolol formulations is essential to support the rational use of generics, ensure consistent clinical efficacy, and promote trust in public health systems. This study was therefore designed to assess and compare the pharmaceutical equivalence of two generic formulations of 100 mg metoprolol tartrate tablets collected from community pharmacies in Ubon Ratchathani, Thailand, against the reference product. The evaluation was based on key parameters defined in the United States Pharmacopeia (USP) 39/NF34 General Chapter ⟨905⟩ Uniformity of Dosage Units, ⟨701⟩ Disintegration, and specific monograph requirements including weight variation, disintegration, uniformity of dosage units, and dissolution behavior in 0.1 N HCl.[18] The findings are expected to inform local quality assurance efforts and support evidence-based decisions in generic substitution.

Methods

1. Chemicals and reagents

The USP metoprolol tartrate reference standard (99.8% purity) and propranolol hydrochloride were purchased from Sigma—Aldrich (USA). HPLC-grade methanol (Fisher Scientific Co., Japan), glacial acetic acid (Fisher Scientific Co., Japan), analytical-grade sodium 1-heptanesulfonic acid monohydrate (Fluka Co., Switzerland) and hydrochloric acid (Fisher Scientific Co., Japan) were used in this study. Pyrex-grade glassware, disposable 0.45 µm nylon syringe filters (Fisher Scientific, UK), and Milli-Q ultrapure (Type 1) water (Millipore, Bedford, MA, USA) were used throughout the study.

2. Sample information

Two generic formulations of 100 mg of metoprolol tartrate tablets (Test A and Test B) were selected from the available generic products in community pharmacies in Ubon Ratchathani, Thailand. Test A was a white, round, uncoated tablet, while Test B was a pale pink, film-coated tablet. Both generics were marketed at a substantially lower price (approximately 30 – 40% of the reference product cost). Information from product leaflets indicated that Test A contained lactose monohydrate and maize starch, while Test B contained microcrystalline cellulose and croscarmellose sodium as excipients.

At the time of sample collection, four generic formulations were available on the local market. The two selected formulations represented the most commonly dispensed generic products on the basis of pharmacy survey data. The innovator product is referred to as the “reference product” throughout the manuscript. All the samples were within their shelf life and stored under the recommended conditions prior to analysis.

3. Instrumental analysis

RP-HPLC analysis was carried out via a high-performance liquid chromatography system: Shimadzu LC-10 Series (Shimadzu, Japan) equipped with an isocratic LC-10A pump, a 100 µL injection loop, an SIL-10A autosampler, a degasser DGU-14A, an auto injector SIL-10AD, a UV—Vis spectrometric detector SPD-10A, and a system controller SCL-10A. The UV detector was set at 274 nm. Chromatographic separation was achieved on a reversed-phase Nova–Pak® C18 column (5 µm, 3.9 × 150 mm I.D., Waters, USA) maintained at ambient temperature. The isocratic mobile phase consisted of methanol, 0.01 M 1-heptanesulfonic acid, and glacial acetic acid at a ratio of 51.5:48.0:0.5 and was delivered at a flow rate of 1.0 mL/min. The injection volume was 20 µL.

The system suitability parameters, including the tailing factor (< 2.0), theoretical plates (> 2000), and baseline resolution, were evaluated before each analytical run. The system was considered suitable when all the parameters met the acceptance criteria. Method validation parameters, including linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ), were assessed following the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines.[19] The chromatographic conditions were optimized from the original USP method to achieve superior peak symmetry and resolution using available instrumentation. The modified mobile phase composition improved the analytical performance while maintaining equivalence to the pharmacopeial requirements, as confirmed through comprehensive method validation.

4. Sample preparation

4A. Standard solution preparation

Stock solutions of metoprolol tartrate were prepared by accurately weighing the USP reference standard and dissolving it in a methanol:0.1 N HCl (1:1) mixture to achieve a concentration of 1000 µg/mL. Working standard solutions were prepared by appropriate dilution of the stock solution with the mobile phase to obtain concentrations suitable for calibration curve construction and system suitability assessment. Propranolol hydrochloride internal standard solutions were prepared at 400 µg/mL in the same solvent system.[20]

4B. Sample solution preparation

For assay determination and content uniformity testing, individual tablets were accurately weighed and powdered separately via a mortar and pestle. An amount of powder equivalent to one tablet (approximately 100 mg metoprolol tartrate) was transferred to a 100 mL volumetric flask. The powder was dissolved in 70 mL of a methanol:0.1 N HCl

(1:1) mixture and sonicated for 30 minutes at room temperature. The solution was then heated in a water bath at 60°C for 10 minutes to ensure complete dissolution, as validated to not cause drug degradation on the basis of preliminary stability studies. After cooling to room temperature, the volume was adjusted to 100 mL with the same solvent mixture. The mixture was subsequently centrifuged at 2000 rpm for 10 minutes at room temperature to remove undissolved excipients. The supernatant was filtered through a 0.45 µm nylon membrane filter and diluted with the mobile phase to achieve a target concentration of approximately 200 µg/mL for RP-HPLC analysis. Each sample was processed individually and analyzed separately, with six replicates analyzed per formulation to provide adequate statistical power for equivalence assessment.[21]

5. Method validation

The assay method was validated following the ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision (repeatability and intermediate precision), limit of detection/limit of quantification (LOD/LOQ), and robustness. The system suitability parameters, including the tailing factor, theoretical plates, and peak resolution, were evaluated and met the acceptance criteria for each analytical run. Specificity was confirmed by demonstrating no interfering peaks in blank or placebo injections at the metoprolol retention time. Placebo tablets were prepared using the same excipients identified from pharmaceutical databases without the active pharmaceutical ingredient. Linearity was evaluated over the target range (60 – 300 µg/mL) at seven concentrations, with a correlation coefficient requirement of $\geq$ 0.999. Accuracy was assessed via recovery studies via standard addition methodology at three concentrations (80%, 100%, and 120% of the nominal concentration), with three replicates each. The recovery percentage was calculated as (found amount/added amount) × 100. Precision was determined from intra-day and inter-day replicate analyses. Intra-day precision was evaluated via six replicate analyses of the same sample on a single day. Inter-day precision was assessed by analyzing samples on three consecutive days with different analysts. The acceptance criterion was an RSD $\leq$ 5% according to the ICH Q2(R1) guidelines for drug substance assays. LODs and LOQs were estimated via signal—to—noise ratio criteria (S/N = 3 for the LOD and S/N = 10 for the LOQ). LOQ verification was performed through precision and accuracy testing at the determined concentration level to confirm analytical reliability. Robustness was tested by deliberate small changes in critical parameters, including flow rate (±0.1 mL/min), mobile phase composition (±2%), and temperature (ambient ±2°C), ensuring that retention time variations remained < 0.1 minutes and that assay variations remained < 2%.[22]

6. Determination of pharmaceutical quality

6A. Weight variation

Twenty tablets from each product were individually weighed using an analytical balance (AT 400, Mettler–Toledo Ltd., Switzerland). Weight variation was assessed according to USP 39 ⟨905⟩ Uniformity of Dosage Units via acceptance value (AV) calculation methodology. The AV was calculated via the following formula: $AV = |M - \bar{X}| + ks$, where M is the reference value (100%), $\bar{X}$ is the sample mean, k is the acceptability constant (2.4 for n = 10, 2.0 for n = 30), and s is the sample standard deviation. The test passes if AV $\leq$ 15.0, providing a more comprehensive statistical evaluation than traditional individual weight deviation criteria do.[23]

6B. Disintegration time

A tablet disintegration tester (ZT70, ERWEKA, USA) was used to record the disintegration time (minutes). A total of six tablets of each brand were randomly selected and individually placed into the tubes of the basket-rack assembly, with one tablet per tube. The immersion medium consisted of 600 mL of distilled water and was maintained at a controlled temperature of 37 $\pm$ 0.5 °C throughout the experiment. The basket rack was operated at a frequency of 29 – 32 cycles per minute, with a stroke height not less than 5.3 cm, in accordance with the specifications outlined in the United States Pharmacopeia (USP 39/NF 34) <701> Disintegration.[24] USP specifies 800–900 mL; however, 600 mL was used due to instrument capacity and validated in preliminary experiments). The disintegration test was carried out over a 1800-second period. At the conclusion of the test, the basket rack was raised, and the tablets were visually inspected for disintegration. If one or two tablets failed to disintegrate completely, an additional 12 tablets were subjected to the same test conditions, as stipulated by the compendial requirements.

6C. Uniformity of dosage units

The uniformity of the dosage units was determined according to USP 39 ⟨905⟩ by the content uniformity method. The drug contents of ten tablets were individually analyzed via the validated RP-HPLC method. Each tablet was processed as described in Section 2.4 and analyzed in triplicate. The acceptance value was calculated for content uniformity via the same statistical approach as weight variation, with the acceptance criterion of AV $\leq$ 15.0 for the first ten tablets tested. This methodology considers both individual tablet deviations and overall population statistics, providing superior discrimination of manufacturing consistency compared with simple percentage ranges.[23]

7. In vitro dissolution study

The dissolution medium used was 0.1 N hydrochloric acid to provide a discriminatory environment for detecting potential differences between formulations, as recommended for immediate-release solid dosage forms of BCS Class I drugs.[10] The *in vitro* dissolution profiles were determined via USP Apparatus I (basket method) (VK 7000, Vankel Industries, Inc., USA) according to USP 39/NF 34 ⟨711⟩ dissolution specifications. Testing was conducted at 100 rpm (±4 rpm tolerance) in 900 mL of 0.1 N hydrochloric acid (pH 1.2) maintained at 37 ± 0.5°C. Twelve tablets of each formulation were tested individually to meet regulatory requirements for dissolution profile comparison.[25] Aliquots (5 mL) were withdrawn at 5, 10, 15, 20, 30, and 40 min, with equal volumes of fresh medium maintained at the same temperature. RP-HPLC analysis was selected over UV spectrophotometry to ensure specificity and eliminate potential interference from excipients that might absorb at similar wavelengths. The dissolution samples were analyzed using the same RP-HPLC conditions as those described for the assay method. Propranolol HCl (20 µL of 400 µg/mL solution) was added as an internal standard to each dissolution sample to improve analytical precision and account for any injection volume variations during the extended dissolution study. Standard solutions for dissolution analysis were prepared at concentrations corresponding to the expected dissolution levels, ensuring analytical accuracy across the dissolution profile. The percentage of drug dissolved was calculated via the following formula:

% Dissolved = (Sample concentration × Volume × Dilution factor × 100)/Theoretical tablet content[25]

8. Comparison of in vitro dissolution profiles

Dissolution profiles were compared using model-independent approaches as outlined in FDA guidance for industry.[26] The difference factor (f_1) and similarity factor (f_2) were calculated according to regulatory requirements, with f_1 values $\leq$ 15 and f_2 values between 50 and 100 indicating similarity. The calculations utilized at least 12 individual data points from each formulation, with dissolution testing continuing until at least 85% drug release was achieved. Statistical significance was evaluated via repeated-measures ANOVA with Tukey's post hoc test.[26] The f_1 and f_2 values of the test products were calculated using the following equations (Eqs. (1) and (2)):

$$f_1 = \left[\frac{\sum_{t=1}^{n} R_t - T_t}{\sum_{t=1}^{n} R_t}\right] \times 100 \quad (1)$$

$$f_2 = 50 \times log\left[\left(1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right)^{-0.5}\right] \times 100 \quad (2)$$

where n is the number of sample time points and R_t and T_t are the percent cumulative release of drug from the reference product and the test product (at sample time point t), respectively.

9. Statistical analysis

The data are expressed as the means ± standard deviations (SDs). Statistical analysis was performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA).[27] One-way ANOVA followed by Tukey's post hoc test was employed to assess significant differences among formulations for individual parameters, with P-value < 0.05 considered significant. For dissolution profile comparisons, repeated-measures ANOVA was used to evaluate differences across multiple time points, with twelve replicates (n = 12) for each formulation at each sampling interval.

Results

1. Method validation

The RP-HPLC method developed for metoprolol tartrate quantification demonstrated acceptable performance characteristics according to the ICH Q2(R1) guidelines. The retention time for metoprolol tartrate was approximately 8.5 minutes under the described chromatographic conditions.

The system suitability parameters met the acceptance criteria: the tailing factor was 1.1 (acceptance criterion < 2.0), the number of theoretical plates exceeded 3000 (acceptance criterion > 2000), and baseline resolution was achieved between the metoprolol and potential excipient peaks (Table 1). The calibration curve was linear over the concentration range of 60 – 300 µg/mL, with a correlation coefficient (R^2) of 0.9999. The linear regression equation was y = 12,450x + 125.6, where y represents the peak area and x represents the concentration in µg/mL. The intra-day and inter-day precisions, expressed as percentages of the relative standard deviation (%RSD), were 1.94% and 4.15%, respectively. While the inter-day precision approaches the upper acceptance limit, it remains within the ICH Q2(R1) specification of ≤ 5% RSD for drug substance assays, confirming method acceptability for pharmaceutical analysis.[19]

Table 1 Method validation summary.

Parameter	Results	Acceptance criteria	Status
Linearity range	60 – 300 µg/mL	50 – 150% of target	Pass
Correlation coefficient (R^2)	0.9999	≥ 0.999	Pass
Intra-day precision (%RSD)	1.94%	≤ 5%	Pass
Inter-day precision (%RSD)	4.15%	≤ 5%	Pass
Accuracy – 80% level	100.00 ± 1.2%	98.0 – 102.0%	Pass
Accuracy – 100% level	101.22 ± 0.9%	98.0 – 102.0%	Pass
Accuracy – 120% level	101.63 ± 1.5%	98.0 – 102.0%	Pass
LOD	0.6	S/N ≥ 3	Pass
LOQ	2.0	S/N ≥ 10	Pass
Tailing factor	1.1	≤ 2.0	Pass
Theoretical plates	3250	≥ 2000	Pass

The accuracy assessment using standard addition methodology revealed recoveries ranging from 100.00% to 101.63% across the tested concentration levels (80%, 100%, and 120% of the nominal concentration), with RSD values below 2% for all levels, indicating excellent method accuracy and precision.[25] The LOD and LOQ determinations based on single-to-noise criteria yielded values of 0.6 µg/mL and 2.0 µg/mL, respectively. LOQ verification through precision and accuracy testing at 2.0 µg/mL demonstrated an RSD of 4.8% and recovery of 99.2%, confirming analytical reliability at the quantification limit. Robustness testing revealed that small deliberate changes in the flow rate (± 0.1 mL/min), mobile phase composition (± 2%), and temperature (± 2°C) resulted in retention time variations < 0.05 minutes and assay variations < 1.5%, well within acceptable limits, confirming the robustness of the method for routine analysis.[28]

2. Weight variation

Weight variation testing was performed on twenty tablets from each formulation according to the USP 39 <905> methodology (Table 2).[23] The mean weights were 315.1 ± 3.4 mg for reference product, 343.1 ± 3.9 mg for Test A, and 356.6 ± 3.2 mg for Test B. Individual tablet weights showed percentage deviations from the mean weight within ± 5% for all formulations, with no individual tablet exceeding ± 10% deviation from the formulation mean. The calculated acceptance values (AVs) were 2.8, 3.1, and 2.4 for reference product, Test A, and Test B, respectively, which are all well below the acceptance criterion of 15.0. These results demonstrate excellent manufacturing consistency and compliance with USP weight variation specifications across all the tested formulations.[29]

Table 2 Pharmaceutical quality control parameters of the reference and test products of metoprolol tartrate tablets.

Tablet brand codes	Tablet weight (mg)	AV for weight variation	Disintegration time (second)	Content uniformity (% content)	AV for content uniformity
Reference product	315.1 ± 3.4	2.8	965 ± 199	91.97 ± 0.24	8.1
Test A	343.1 ± 3.9	3.1	431 ± 112	91.50 ± 0.16	8.7
Test B	356.6 ± 3.2	2.4	278 ± 101	93.51 ± 0.27	6.8

Note: AV = Acceptance Value, calculated according to USP <905> Uniformity of Dosage Units (criterion: AV ≤ 15.0).

3. Disintegration time

The disintegration test results revealed a mean time of 965 ± 199 seconds for reference product, 431 ± 112 seconds for Test A, and 278 ± 101 seconds for Test B. Statistical analysis revealed significant differences between formulations (P-value < 0.05), with both test products demonstrating faster disintegration than the reference product. However, all formulations completely disintegrated well within the USP specification of ≤1800 seconds (30 minutes), confirming compliance with the pharmacopeial requirements.[21] The faster disintegration observed in the test products may be attributed to the different disintegrant types or concentrations used in the formulations, such as superdisintegrants such as croscarmellose sodium or sodium starch glycolate, which are commonly employed to increase tablet breakdown in aqueous media.[30]

4. Uniformity of dosage units

Individual tablet assays for uniformity were performed via the validated RP-HPLC method on ten tablets from each formulation. The mean assay values were 91.97 ± 0.24% for reference product, 91.50 ± 0.16% for Test A, and 93.51 ± 0.27% for Test B (expressed as a percentage of labeled content). The relative deviation (%RSD) values were 0.26%, 0.17%, and 0.29%, respectively, which are all well below the 6% criterion typically applied for content uniformity assessment. The acceptance values calculated via the USP <905> methodology were 8.1, 8.7, and 6.8 for reference product, Test A, and Test B, respectively, all of which met the acceptance criteria of AV ≤ 15.0. These results demonstrate excellent dose uniformity across individual tablets within each formulation, indicating consistent manufacturing processes and adequate content control. The assay results, ranging from 91.50% to 93.51%, fall within the acceptable pharmaceutical range of 90 - 110% of the labeled content. While these values are at the lower end of the specification range, they reflect actual pharmaceutical content rather than manufacturing deficiency, as evidenced by the rapid dissolution profiles that demonstrate complete drug availability despite the slightly lower assay values.[31]

5. In vitro dissolution study and profile comparison

As shown in Figure 1, the dissolution profiles of all three formulations demonstrated rapid drug release, with mean dissolution percentages at 30 minutes of 89.4 ± 2.1% for reference product, 91.2 ± 1.8% for Test A, and 88.7 ± 2.4% for Test B. All formulations achieved >85% dissolution within 30 minutes, exceeding the USP requirement of > 75% at 30 minutes. Statistical analysis using repeated-measures ANOVA revealed no significant differences between the dissolution profiles of the test products and the reference products ($F_{2,33}$ = 1.24, P-value = 0.302). Individual time point comparisons via Tukey's post hoc test confirmed no significant differences at any sampling interval (P-value > 0.05 for all comparisons). The dissolution profile similarity assessment yielded f_1 values of 1.04 and 1.78 for Test A and Test B, respectively (acceptance criterion $f_1 \leq 15$), whereas the f_2 values were 71.72 and 62.12, respectively (acceptance criterion f_2 = 50 - 100). These values clearly demonstrate the similarity between the test formulations and reference product dissolution profiles, confirming pharmaceutical equivalence on the basis of regulatory criteria.[32]

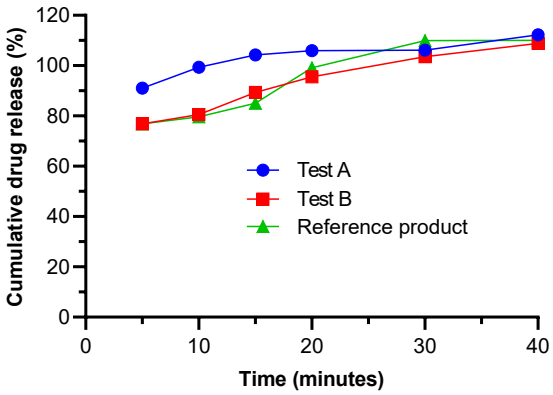

Figure 1 Dissolution profiles of metoprolol tartrate from two test products (A and B) compared with the reference product (mean ± SD, error bars shown) (n = 12).

Discussions and Conclusion

The pharmaceutical equivalence evaluation of the two generic metoprolol tartrate formulations demonstrated comprehensive compliance with the USP 39/NF 34 standards across all tested parameters.[18] The results support the potential interchangeability of these generic formulations with the reference product for the specific batches evaluated, providing important evidence for clinical decision-making regarding generic substitution. The validated RP-HPLC method proved suitable for pharmaceutical equivalence assessment, meeting all the ICH Q2(R1) validation requirements. While the inter-day precision of 4.15% RSD approaches the upper acceptance limit of 5%, this value remains within the established guidelines for drug substance assays and provides adequate analytical precision for regulatory purposes.[16] The method's specificity, accuracy, and robustness characteristics confirm its reliability for routine quality assessment of metoprolol tartrate formulations.

According to USP 39 ⟨905⟩ Uniformity of Dosage Units, uniformity can be demonstrated through either content uniformity or weight variation methods. This study applied both approaches comprehensively, with weight variation assessment showing excellent consistency (AV values of 2.4 - 3.1) and content uniformity testing confirming dose consistency across individual tablets (AV values of 6.8 - 8.7). The statistical approach using acceptance value calculations

provides superior discrimination compared with traditional percentage-based criteria, offering a more reliable assessment of manufacturing quality.[33]

The observed differences in disintegration times, while statistically significant, remained within acceptable pharmaceutical limits and did not negatively impact dissolution performance. Faster disintegration in the test formulations likely reflects formulation optimization through the use of effective disintegrants, which is acceptable provided that drug release characteristics remain equivalent. This finding demonstrates that formulation modifications can improve certain performance parameters while maintaining therapeutic equivalence. The content uniformity results, with individual tablet assay values ranging from 91.50% to 93.51% of the labeled content, demonstrated consistent manufacturing practices across all the evaluated products. The excellent uniformity (RSD < 0.3%) indicates that precise dosing control is essential for maintaining therapeutic efficacy and minimizing the risk of subtherapeutic dosing or adverse effects. While these assay values are at the lower end of the 90 – 110% specification range, they remain fully compliant and consistent with the dissolution data showing complete drug availability.[34]

The dissolution study results provide strong evidence of pharmaceutical equivalence. All formulations demonstrated rapid and complete drug release profiles, with no statistically significant differences (P-value > 0.05). The f_1 and f_2 values clearly meet regulatory acceptance criteria, confirming profile similarity. For metoprolol tartrate, a BCS Class I drug with high solubility and permeability, these dissolution characteristics strongly predict *in vivo* bioequivalence, making *in vitro* dissolution testing a reliable surrogate for clinical performance assessment.[10]

From a public health perspective, these findings support the adoption of high-quality generic formulations as cost-effective alternatives to branded medications. In Thailand where cardiovascular diseases represent a leading cause of morbidity and mortality, the availability of pharmaceutically equivalent generics is critical for improving medication access and adherence, particularly in rural or economically constrained regions. The demonstrated equivalence of locally manufactured generics can help build confidence among healthcare providers and patients, potentially improving treatment compliance and outcomes.

However, this study acknowledges several limitations that should be considered when interpreting the results. First, dissolution testing was conducted in a single medium (0.1 N HCl), which simulates gastric conditions but may not fully represent gastrointestinal variability. While this approach is appropriate for BCS Class I drugs and provides discriminatory conditions, regulatory guidelines recommend additional testing in pH 4.5 and pH 6.8 buffers to better evaluate pH sensitivity and intestinal dissolution characteristics.[35,36] Second, the study employed a convenience sampling approach limited to two generic formulations available in a specific geographic region (Ubon Ratchathani), which may limit generalizability to other generic products or market areas. The findings apply specifically to the tested batches and may not represent consistent quality across different manufacturing lots or facilities.

Third, other quality control tests such as friability and hardness were not performed due to resource constraints. These tests are important indicators of mechanical strength and may influence disintegration and dissolution behavior. Their omission represents a limitation of the current study; therefore, future research should incorporate friability and hardness testing to provide a more comprehensive quality assessment. Fourth, while dissolution similarity is strongly predictive of bioequivalence for BCS Class I drugs, *in vivo* bioequivalence studies remain the definitive approach for establishing therapeutic equivalence. Clinical correlation studies could provide additional confidence in the interchangeability of these formulations.

Finally, *in vivo* bioequivalence studies were not performed; although dissolution similarity is strongly predictive for BCS Class I drugs, clinical correlation remains the gold standard. The limitations of the analytical methodology should also be acknowledged. While the inter-day precision of 4.15% RSD meets the ICH acceptance criteria, it represents the upper limit of acceptable variation. This precision level, while suitable for pharmaceutical equivalence assessment, approaches the threshold where analytical variability could impact discrimination between similar formulations.[19]

Future studies might benefit from additional method optimization or alternative analytical approaches to achieve enhanced precision. Despite these limitations, a comprehensive evaluation demonstrated that well-

manufactured generic formulations could meet stringent pharmaceutical quality standards when appropriate manufacturing practices and quality control measures are implemented. The validated analytical methodology provides a reliable framework for ongoing quality assessment of cardiovascular medications in similar healthcare settings.

In conclusion, the developed and validated RP-HPLC method proved suitable for the qualitative and quantitative analysis of metoprolol tartrate in tablet formulations, meeting all ICH validation requirements for pharmaceutical analysis. The method demonstrated acceptable precision, accuracy, and robustness for routine quantity assessment applications. The pharmaceutical equivalent evaluation confirmed that both locally manufactured generic metoprolol tartrate formulations available at the Ubon Ratchathani community pharmacies comprehensively met the USP 39/NF 34 quality standards. All tested parameters, including weight variation, disintegration time, uniformity of dosage units, and dissolution profiles, complied with pharmacopeial specifications. The dissolution profile similarity demonstrated through f_1 and f_2 factor analysis confirmed the pharmaceutical equivalence with the reference product based on established regulatory criteria. The validated analytical methodology offers a reliable approach for ongoing post-marketing surveillance and quality assessment of generic cardiovascular medications. While friability and hardness testing were not performed, future studies should address these quality parameters for more comprehensive assessment. In addition, future studies should incorporate dissolution testing across multiple pH media and include broader sampling of available generic products to strengthen regulatory confidence and provide more comprehensive quality assessments across different manufacturing sources and batch variations. These findings have important implications for public health policy, supporting the broader adoption of quality-assured generic medications in resource-limited settings while emphasizing the importance of continued quality surveillance and analytical method validation for ensuring consistent pharmaceutical standards.

Authors contributions

All the authors contributed significantly to the study design, data collection, analysis, manuscript preparation, and critical review. Each author approved the final version for publication and accepts responsibility for the work's integrity, meeting ICMJE authorship criteria.

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Ethical approvals

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Data availability

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The authors confirm that no AI tools were used for manuscript writing, editing, or image manipulation.

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