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Comparative Assessment of 1% Luliconazole Cream Release Profile Through Franz Diffusion Cells – A Way Towards Qualification and Validation of Critical In Vitro Parameters

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ABSTRACT

Introduction: The in vitro release test (IVRT) is an established method used to characterize the rate of active pharmaceutical ingredient (API) release and assess the sameness in product quality attributes. This study aims to present a systematic approach for validating critical IVRT parameters, alongside high-performance liquid chromatography (HPLC) method validation and qualification of the IVRT procedure for estimating luliconazole (LCZ) from semisolid formulations. **Methods:** The comparative release profile of LCZ from its cream formulations was evaluated using vertical Franz diffusion cells. Samples collected during the in vitro studies were analyzed using a HPLC system equipped with an ultraviolet detector. **Results:** The drug release demonstrated linearity, with a coefficient of determination (R^2) ≥ 0.90, indicating a strong correlation between the amount of drug released and the square root of time (√t) through the LCZ semisolid matrices. Statistical analysis confirmed equivalence between reference formulations when IVRT was performed on 2 separate days; however, non-equivalence was observed between the reference and test formulations, as the 90% confidence interval exceeded the acceptable range of 75–133.33%, according to SUPAC-SS guidelines. **Conclusion:** These results confirm that the developed IVRT method is sensitive, selective, and specific for evaluating the product sameness of LCZ formulations.

KEYWORDS: In vitro release testing, dissolution, Franz diffusion cell, apparatus qualification, luliconazole

INTRODUCTION

Luliconazole (LCZ), an imidazole antifungal agent, has demonstrated potent activity against a variety of fungi. Fungal infections are generally classified into two categories: superficial and invasive. Superficial fungal infections are often associated with poor quality of life and neglect of treatment and affect approximately 25% of the world's population. Invasive fungal infections, which typically occur in patients who are critically ill or immune-compromised, are a significant cause of hospitalization.

The R-enantiomer of LCZ exhibits strong antifungal activity by inhibiting the enzyme lanosterol demethylase, thereby disrupting the synthesis of ergosterol. This inhibition results in decreased levels of ergosterol and an accumulation of lanosterol (*1*). LCZ cream is approved for topical use in the treatment of interdigital tinea pedis, tinea cruris, and tinea corporis, caused by *Trichophyton rubrum* and *Epidermophyton floccosum*, in patients aged 18 years and older (*2*).

Draft guidance published by the United States Food and Drug Administration (FDA) on LCZ states that the test product and reference standard should exhibit equivalent LCZ release rates as demonstrated through an acceptable IVRT bioequivalence study (*3*). This study should compare

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at least one batch of the test product with one batch of the reference standard using a properly validated IVRT method (*3*).

The physical and structural properties of a semisolid topical formulation can significantly influence the release rate of the active pharmaceutical ingredient (API). Characterizing the release behavior of an API is essential throughout the drug development process. The IVRT method serves as a critical tool for determining the release rate and diffusion behavior of an API from topical formulations. For semisolid dosage forms, it is imperative to evaluate drug release characteristics using IVRT techniques.

The IVRT method offers several advantages, including its application in the quality control of drug formulations, prediction of in vivo performance, evaluation and confirmation of formulation design intent, and assessment of formulation quality and product equivalence following post-approval changes (*4*). IVRT is also a valuable tool for optimizing formulations during the early stages of development, serving as a cost-effective means of generating predictive insights into a drug product's in vivo behavior. In each IVRT experiment, certain validated parameters—such as temperature, sample application technique, membrane preparation, stirring efficiency, Franz diffusion cell (FDC) dimensions, and sampling intervals—are maintained consistently to ensure the robustness and reproducibility of the study. In contrast, variables such as the type of synthetic membrane and the choice of receptor fluid can significantly influence the drug release characteristics of the dosage form.

An extensive literature review revealed a previously reported comparative IVRT study of LCZ, which primarily focused on validation of the IVRT and HPLC methods; however, it lacked comprehensive methodology and relevant data for conducting an in-depth comparative release study (*5*). Another study focused on the application of mathematical models, but similarly did not provide adequate comparative release data (*6*). Although additional literature was identified for LCZ HPLC analysis, no well-qualified and validated IVRT and HPLC method has been reported (*7*).

This study aims to develop and validate an IVRT method for LCZ cream with high sensitivity, specificity, selectivity, and reproducibility. This study also outlines a procedure for determining product equivalence or non-equivalence using the test/reference (T/R) ratio calculation. Moreover, this study presents a comprehensive evaluation of IVRT parameters, resulting in a simple and reliable method that can be applied to the characterization of other topical dosage forms as well.

METHODS

Chemicals and Reagents

LCZ (working standard) was obtained from Clearsynth Labs Ltd. (Mumbai, India). Brij O20 and HPLC-grade methanol were sourced from Sigma Aldrich Chemical Pvt. Ltd. (Bengaluru, India). Ammonium bicarbonate was acquired from Fluka, Honeywell (Mumbai, India), and phosphate buffer saline (PBS) was procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India).

Drug Products

Lulifin cream (LCZ 1% w/w, batch no. SXB0257C, Sun Pharmaceutical Industries Ltd., Gurugram, India) was employed as the reference formulation, while LCZ cream (LCZ, 1% w/w, batch no. F51/PRS/175) served as the test formulation. Additionally, to evaluate IVRT selectivity, specificity, and sensitivity, two other test formulations were included: LCZ 0.5% cream (LCZ, 0.5% w/w, batch no. F88/ASR/001) and LCZ 1.5% cream (LCZ, 1.5% w/w, batch no. F88/ASR/003).

High-Performance Liquid Chromatography (HPLC) Method Validation

The reverse phase (RP)-HPLC method validation was performed over a concentration range of 0.200–200.244 µg/mL at a detection wavelength of 295 nm using a Zorbax SB CN column (150 × 4.6 mm, 5 µm) from Agilent (Mumbai, India). A gradient elution technique was employed, where mobile phase A consisted of buffer 1 and methanol (40:60, v/v), and mobile phase B contained buffer 1 and methanol (10:90, v/v). Buffer 1 was prepared as 20 ± 1 mM ammonium bicarbonate. The flow rate was maintained at 1.000 mL/min, following the gradient program outlined in Table 1.

Table 1. HPLC Time Program for Luliconazole Estimation.

Time (min)	Mobile Phase	Flow (%)
0.01	A	100
	B	0.0
3.00	A	100
	B	0.0
3.01	A	0.0
	B	100
5.01	Stop	-

HPLC: high performance liquid chromatography; mobile phase A: Buffer 1: Methanol; 40:60, v/v; mobile phase B: Buffer 1: Methanol; 10:90, v/v; and buffer 1 was 20 ± 1 mM ammonium bicarbonate.

The injection volume was set to 10 µL, and the column oven temperature was maintained at 45 °C. A stock solution of LCZ was prepared in methanol at a

concentration of 1 mg/mL, which was used to construct the calibration curve and prepare quality control (QC) samples. This stock solution was further diluted with the mobile phase A to obtain the following calibration standards: 0.200, 0.501, 4.005, 20.024, 40.049, 80.098, 160.196, and 200.244 µg/mL.

During each analytical run for IVRT samples, eight calibration standards and one blank were injected. The calibration curve was generated based on these standards. Additionally, three QC samples at low, medium, and high concentrations (0.586, 79.220, and 158.440 µg/mL, respectively) were included in each IVRT run to ensure analytical accuracy and reliability.

Linearity and Range

Method linearity was evaluated by analyzing an eight-point standard calibration curve. The curve demonstrated excellent linearity over the concentration range of 0.200 µg/mL (limit of quantitation, LOQ) to 200.244 µg/mL (upper limit of quantitation, ULOQ), with a regression equation of $y = 1.0019x + 0.1183$ and a R^2 of 0.9998. This calibration curve was then used to back-calculate the concentrations of LCZ in unknown samples.

Selectivity and Specificity

The synthetic membrane Ultipor N66 was immersed in the receptor medium for 6 hours. Simultaneously, 300 µL of placebo was mixed with 20 mL of receptor solution, vortexed, and allowed to stand at room temperature for the same duration to simulate the experimental conditions. This procedure was performed in triplicate. After processing the selectivity samples, the peak area response at the analyte's retention time was evaluated.

Precision and Accuracy

In this study, both intra-batch (within-batch) and inter-batch (between-batch) precision and accuracy were evaluated. Intra-batch assessments involved six replicates of QC samples at three concentration levels: 0.562 µg/mL (low), 61.069 µg/mL (medium), and 156.588 µg/mL (high), all prepared in receptor solution and analyzed on the same day. For inter-batch evaluation, 18 replicates at each QC level were analyzed across three precision and accuracy runs conducted over 2 consecutive validation days.

In Vitro Release Test (IVRT) Method

The IVRT system was qualified by evaluating all critical parameters of the FDC, including receptor chamber capacity, cell diameter, membrane surface area, receptor solution temperature, stirring speed, dispensing volume, and environmental conditions (*8*). These parameters were measured using standard techniques for assessing length, weight, and temperature. The results are summarized in Table 2.

The IVRT experiment was carried out using an FDC system (PermeGear, PA, USA) with a receptor chamber volume of 20 mL. The experimental setup included the donor and receptor chambers, clamp, magnetic stirrer, and synthetic membrane, all properly assembled. A magnetic stir bar was placed in the receptor chamber, which was filled with receptor medium composed of 0.5% Brij O20 (w/v) in a mixture of 10× PBS and water (10:90, v/v).

The membrane was carefully placed over the receptor chamber to ensure full contact with the junction between the donor and receptor chambers. The donor chamber was aligned on top of the membrane, and a clamp was used to secure the assembly. The underside of the membrane was checked for air bubbles, which were eliminated by gently tilting the FDC assembly, if needed.

The entire setup was mounted in the cell holder, and the water jacket was connected to a recirculating system using flexible tubing. A heating circulator bath was activated to maintain the membrane temperature at 32 ± 1 °C. The magnetic stirrer was operated at a consistent speed of 560 ± 20 rpm throughout the experiment. The membrane was allowed to equilibrate for at least 30 minutes, with its surface temperature monitored using a calibrated infrared thermometer.

Table 2. Results of Apparatus Qualification Test

Parameter	Acceptance Criteria	Result	Acceptable
Franz diffusion cell capacity (mL)	20 ± 1.0	20 ± 0.16	YES
Orifice diameter (mm)	15 ± 0.75	15 ± 0.2	YES
Temperature of receptor solution (°C)	32 ± 1	32 ± 0.5	YES
Temperature on membrane surface (°C)	32 ± 1	32 ± 0.6	YES
Speed of magnetic stirrer (rpm)	600 ± 60	565 ± 5	YES
Dispensed sampling volume (µL)	300 ± 9	302 ± 5	YES

Values are presented as mean ± SD (n = 6).

Before application of the test formulation, pre-dose samples (300 µL) were collected from the center of the receptor chamber in each FDC and stored in sample vials. After each sampling, the receptor chamber was refilled with fresh receptor solution to maintain consistent volume and conditions.

Quantification of IVRT samples was carried out with a Shimadzu HPLC system coupled to a UV detector, along with Analyst 1.6.3 software for data analysis.

Laboratory Qualification

Laboratory qualification was conducted by evaluating the release rates of LCZ reference formulations using the developed and validated IVRT and HPLC-UV methods. Release rates from two reference formulations were measured over 2 separate days using six FDCs per day (n = 6). Reproducibility, along with intra- and inter-run variability, was calculated as the percent coefficient of variation (%CV), which was required to remain below 15%.

The intra-run %CV for the first and second IVRT runs was 5.06% and 4.09%, respectively, while the inter-run %CV (n = 12 FDCs) was 3.89%. Product equivalence was evaluated using the 90% CI method in accordance with SUPAC-SS guidelines (*9*). Individual test-to-reference (T/R) ratios were expressed as percentages, with Day 1 considered the reference and Day 2 the test run. The 90% CI was calculated from the ordered T/R ratios, with the 8^{th} and 29^{th} ranked ratios representing the lower and upper confidence limits, respectively (*9*). The resulting 90% CI ranged from 100.94–112.51%, which falls within the acceptable equivalence range of 75–133.33%, indicating successful qualification and reproducibility of the IVRT system.

Receptor Solution Selection

Various receptor solutions and synthetic membranes were evaluated in this study to optimize the drug release rate. The receptor solutions tested included different ratios of methanol-water and isopropyl alcohol-water mixtures. Cumulative drug release percentages were measured using hydro-alcoholic solutions containing 5–50% isopropyl alcohol or methanol in water. Notably, even with as little as 5% organic content, the cumulative drug release exceeded 30%, indicating a deviation from Higuchi theory (*10*). Additionally, these hydro-alcoholic receptor solutions showed high inter-cell variability (n = 6), with release rates exceeding 15% and a coefficient of determination (R^2) below 0.90 across the FDCs.

Subsequently, PBS was considered as a receptor solution. However, due to the lipophilic nature of LCZ, with a Log P value of 4.07, inadequate solubility and inconsistent release results were observed in PBS alone. To address this issue, various concentrations (0.1%, 0.25%, 0.5%, and 1.0%) of a hydrophilic-lipophilic balance (HLB) surfactant, Brij O20, were added to the PBS receptor solution to enhance drug solubility and maintain sink conditions (*11–13*).

Surfactant concentrations above 0.5% were effective in maintaining sink conditions throughout the experiment; however, a concentration of 1.0% Brij O20 resulted in excessive bubble formation in the receptor solution (*14*). Therefore, the optimal concentration was determined to be 0.5% Brij O20 in 10-mM PBS. This composition maintained sink conditions, provided consistent results, minimized variability between cells, and yielded an R^2 value close to 1. The solubility of LCZ in the selected receptor solution was further confirmed by dissolving 1 mg of LCZ in 1 mL of the solution.

Synthetic Membrane Selection

A suitable membrane should be selected to ensure consistent drug release, providing inertness and minimal resistance to diffusion from the dosage form. In this study, three different synthetic membranes were evaluated: Supor 200, Ultipor Nylon 6,6 [N66], and Tuffryn HT200, procured from Pall Life Sciences (Mumbai, India). All membranes had a pore size of 0.2 µm and a diameter of 25 mm. Temperature monitoring of the synthetic membranes was performed using an infrared thermometer (Metravi MT4, West Bengal, India).

To assess drug binding to the membranes, each was immersed in a known concentration of LCZ prepared in the receptor solution for over 6 hours. Following the incubation period, the peak area responses of the LCZ solutions (after membrane immersion) were measured and compared with the peak area response of a control stock solution. This comparison allowed for the evaluation of drug loss due to membrane binding.

Drug Application and Sample Collection

Approximately 300 µL of the formulation was evenly applied to the synthetic membrane via the donor chamber of the FDC. After application, the donor chamber was occluded with parafilm to prevent evaporation. According to regulatory guidance, a minimum of six sampling time points is required to establish linearity (*8*). In this study, the sampling time points were set as: pre-dose, 0.5, 1, 2, 3, 4, 5, and 6 hours.

The maximum duration of the IVRT was limited to 6 hours, which is sufficient to distinguish the release rates between different strengths of LCZ. At each designated

time point, approximately 300 µL of receptor solution was withdrawn and transferred into HPLC vials for analysis. The receptor chamber was immediately replenished with pre-warmed receptor solution to maintain volume consistency and sink conditions.

Estimation and Comparison of Release Rates

Concentration of collected samples was estimated through HPLC-UV analysis. For calculating the amount of drug released at each time point (µg/cm^2), the cumulative concentration (µg) obtained at each sampling time point was multiplied by the FDC volume (20 mL) and by the volume of sample removed at each time point, which was then divided by the effective surface area of membrane (i.e., surface area of orifice = 1.77 cm^2). The cumulative amount removed in the previous sampling was calculated by adding the volume of sample removed (mL) from the FDC at each sampling time.

For calculation of release rate, the slope of a straight line (which denotes release rate) was obtained by plotting the cumulative amount of drug release per unit area (µg/cm^2) versus time ($h^{1/2}$). Mass balance was evaluated by dividing the cumulative amount of drug released (µg) by the concentration of the applied formulation.

Comparison of the in vitro release rate was conducted following the SUPAC-SS guidelines. Six individual release rate slopes were obtained for both the test and reference formulations. From these slopes, 36 individual T/R ratios were calculated and expressed as percentages (i.e., T/R ratio × 100). These T/R ratios were then ordered from lowest to highest. The 8th and 29th values in the ordered list were used to define the lower and upper limits, respectively, of the 90% CI for the calculated T/R ratios. According to the guidelines, the 90% CI must fall within the acceptance range of 75–133.33% (*9*).

All statistical analyses were performed using Microsoft Excel 2021.

RESULTS

HPLC Method Validation

Selectivity and Specificity

The results showed no significant interference at the analyte's retention time in any of the blank selectivity samples, confirming that the method is specific for detecting LCZ in its cream formulation.

Precision and Accuracy

Method precision reflects the reproducibility of results, and accuracy indicates how close the measured values are to the true value. Precision is typically expressed as the percentage coefficient of variation (%CV), and accuracy is reported as the percentage deviation from the nominal concentration at each level. The percentage accuracies ranged from 91.00–97.01% for intra-batch and from 95.89–98.16% for inter-batch. The mean %CV for intra-batch precision ranged between 0.14–1.28%, and inter-batch precision ranged from 1.03–1.20%.

IVRT Method Validation

Solubility of Drug in Receptor Solution

The receptor medium must maintain sink conditions, meaning it should be able to dissolve at least three times the amount of drug present in the dosage form. In this study, 300 µL of a formulation containing 1% w/w LCZ was applied, so the receptor medium needed to dissolve at least 450 µg/mL of LCZ. Experimental results showed that the solubility of LCZ in the chosen receptor medium was 464.398 µg/mL, confirming that sink conditions were properly maintained. Additionally, using this receptor medium produced reproducible drug release profiles and consistent R^2 values across all trials.

Selection of Synthetic Membrane

Among the membranes tested, Supor 200 and Tuffryn HT200 showed significant LCZ binding at 3.81% and 2.68%, respectively. In contrast, the Ultipor N66 membrane demonstrated minimal drug binding of 1.58%, resulting in a higher recovery rate of 98.42%. Due to its lower drug retention and cost-effectiveness, Ultipor N66 was selected as the most suitable membrane for conducting IVRT experiments.

Sensitivity, Specificity, and Recovery

The developed IVRT method demonstrated sensitivity by effectively distinguishing among the three concentrations, with average release rates increasing proportionally with LCZ strength: 30.3829, 63.5267, and 103.1695 µg/cm^2/$h^{1/2}$ for the 0.5%, 1.0%, and 1.5% formulations, respectively (Fig. 1).

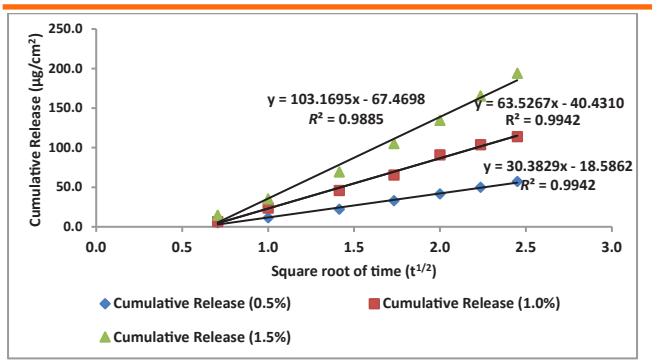

Figure 1. Cumulative release of different strengths of luliconazole formulations (0.5%, 1.0%, and 1.5%), showing sensitivity of the method.

Specificity was assessed through linear regression analysis, using release rate as the dependent variable and LCZ concentration as the independent variable. The analysis showed a strong linear correlation, with an R^2 value of 0.9918 (Fig. 2).

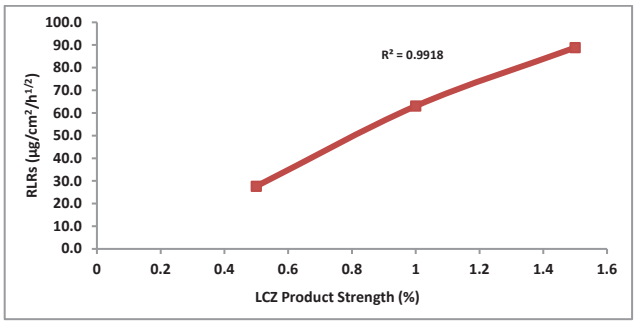

Figure 2. R^2 between different strengths of luliconazole (LCZ) formulations (0.5%, 1.0%, and 1.5%), showing specificity of the method (n = 6). RLR: release rate.

To evaluate selectivity, pairwise comparisons were conducted between the 1.0% LCZ cream and both the 0.5% and 1.5% formulations (Tables 3 and 4). The method's ability to detect performance differences fell outside the acceptance range of 75–133.33%, indicating non-equivalence between the products.

Recovery studies were performed over three separate IVRT runs, each utilizing six FDCs with the reference formulation applied. The recovery values obtained were 6.62%, 6.48%, and 6.13%, respectively. Because all recovery values remained below 30.00% and the LCZ release rates exhibited consistent linearity over time, the extent of drug depletion was considered acceptable.

Comparison of Release Rates

Release rates were calculated for both products; the R^2 exceeded 0.90, indicating consistent drug release over the 6-h period. The intra-day variation in release rate, expressed as the %CV between cells, was below 15%, demonstrating minimal variability and confirming the reproducibility of the method. Collectively, these results support that the developed IVRT method conforms to the principles of the Higuchi release model (*10*).

Case 1: Reference Versus Test Formulation

The 90% CI was calculated based on the release data of the reference and test formulations. As shown in Table 5, the 90% CI bounds (8th and 29th ranked values) are 123.95% and 151.45%, respectively. This indicates that the 90% CI falls outside the acceptable limits of 75–133.33%, as specified by the SUPAC-SS guidance (*9*). Therefore, the reference and test formulations are considered non-equivalent.

Table 3. Calculated T/R Ratios for Release Rates (Slope) of Luliconazole (LCZ) 1% (Reference [R]) Versus LCZ 0.5% (Test [T]).

Test Release Rate, µg/cm²/h$^{1/2}$	Reference Release Rate, µg/cm²/h$^{1/2}$					
-	**63.5267**	**67.5495**	**60.8094**	**63.2525**	**62.8280**	**60.3677**
30.3829	0.4783	0.4498	0.4996	0.4803	0.4836	0.5033
23.8143	0.3749	0.3525	0.3916	0.3765	0.3790	0.3945
27.8709	0.4387	0.4126	0.4583	0.4406	0.4436	0.4617
25.6603	0.4039 (8th)[a]	0.3799	0.4220	0.4057	0.4084	0.4251
29.2862	0.4610	0.4336	0.4816	0.4630	0.4661	0.4851
28.8480	0.4541	0.4271	0.4744	0.4561	0.4592	0.4779 (29th)[a]

Bold values are mean release rates (slope) over time obtained from six Franz diffusion cells.
[a]Rank order is given in parentheses for the lower (8th) and upper (29th) bounds of the 90% CI.

Table 4. Calculated T/R Ratios for Release Rates (Slope) of Luliconazole (LCZ) 1% (Reference [R]) Versus LCZ 1.5% (Test [T]).

Test Release Rate, µg/cm²/h$^{1/2}$	Reference Release Rate, µg/cm²/h$^{1/2}$					
-	**63.5267**	**67.5495**	**60.8094**	**63.2525**	**62.8280**	**60.3677**
97.1062	1.5286	1.4376	1.5969	1.5352	1.5456	1.6086
70.4472	1.1089	1.0429	1.1585	1.1137	1.1213	1.1670
78.9356	1.2426 (8th)[a]	1.1686	1.2981	1.2479	1.2564	1.3076
103.1695	1.6240	1.5273	1.6966	1.6311	1.6421	1.7090
86.1924	1.3568	1.2760	1.4174	1.3627	1.3719	1.4278
97.1584	1.5294	1.4383	1.5978 (29th)[a]	1.5360	1.5464	1.6094

Bold values are mean release rates (slope) over time obtained from six Franz diffusion cells.
[a]Rank order is given in parentheses for the lower (8th) and upper (29th) bounds of the 90% CI.

Case 2: Reference Versus Reference Formulation

Conversely, the 90% CI was calculated using release data from the reference formulation obtained on 2 different days. As shown in Table 6, the 90% CI bounds (8th and 29th ranked values) are 91.58% and 112.14%, respectively. This indicates that the 90% CI falls within the acceptable limits of 75–133.33%, in accordance with the SUPAC-SS guidance (*9*).

Thus, when comparing inter-day data of the reference formulation, the method is discriminatory between reference vs. test formulations, as well as consistent between reference vs. reference formulations.

DISCUSSION

To ensure the reproducibility and reliability of an IVRT method, comprehensive validation is essential prior to its application in product evaluation. During the qualification of the IVRT apparatus, all critical parameters of the FDC system were rigorously assessed, including receptor chamber volume, cell diameter, membrane surface temperature, temperature control, stirring speed, and sampling volume. Each parameter was tested in triplicate, with all results falling within predefined acceptable limits. Laboratory qualification further confirmed system compliance, as intra-run %CV values for two IVRT runs remained below 15%, and the 90% CI for release rate comparisons across 2 days fell within the established acceptance range, confirming reproducibility of the method.

Quantification of LCZ in IVRT samples was performed using a validated HPLC method. Key validation parameters such as sensitivity, specificity, and selectivity were also evaluated, demonstrating the method's capability to effectively differentiate formulations based on drug concentration.

The IVRT method showed suitability through consistent drug release profiles throughout the study, indicated by an R^2 exceeding 0.99. Additionally, the coefficient of variation among diffusion cells (intra-cell variability) remained below 15%, confirming excellent reproducibility. After 6 hours, cumulative drug release from each FDC was below 30% of the applied dose, confirming maintenance of sink conditions during the experiment.

Comparison of release rates revealed a significant difference between the test and reference formulations, with the test formulation exhibiting approximately 35%

Table 5. Calculated T/R Ratios for Release Rates (Slope) of Lulifin 1% (Reference [R]) Versus Luliconazole 1% (Test [T])

Test Release Rate, µg/cm²/h$^{1/2}$	Reference Release Rate, µg/cm²/h$^{1/2}$					
-	**40.3060**	**46.5885**	**41.4833**	**51.6100**	**48.7019**	**49.3251**
63.5267	1.5761	1.3636	1.5314	1.2309	1.3044	1.2879
67.5495	1.6759	1.4499	1.6284	1.3088	1.3870	1.3695
60.8094	1.5087	1.3052	1.4659	1.1782	1.2486	1.2328
63.2525	1.5693	1.3577	1.5248	1.2256	1.2988	1.2824
62.8280	1.5588	1.3486	1.5145 (29th)[a]	1.2174	1.2901	1.2738
60.3677	1.4977	1.2958	1.4552	1.1697	1.2395 (8th)[a]	1.2239

Bold values are mean release rates (slope) over time obtained from six Franz diffusion cells.
[a]Rank order for is given in parentheses lower (8th) and upper (29th) bounds of the 90% CI.

Table 6. Calculated T/R Ratios for Release Rates (Slope) of Lulifin 1% (Reference [R]) Versus Lulifin 1% (Test [T]) performed on 2 different days.

Test Release Rate, µg/cm²/h$^{1/2}$	Reference Release Rate, µg/cm²/h$^{1/2}$					
-	**40.3060**	**46.5885**	**41.4833**	**51.6100**	**48.7019**	**49.3251**
42.6671	1.0586	0.9158 (8th)[a]	1.0285	0.8267	0.8761	0.8650
50.1927	1.2453	1.0774	1.2099	0.9725	1.0306	1.0176
49.1491	1.2194	1.0550	1.1848	0.9523	1.0092	0.9964
46.5193	1.1542	0.9985	1.1214 (29th)[a]	0.9014	0.9552	0.9431
51.5718	1.2795	1.1070	1.2432	0.9993	1.0589	1.0455
43.2849	1.0739	0.9291	1.0434	0.8387	0.8888	0.8775

Bold values are mean release rates (slope) over time obtained from six different Franz diffusion cells.
[a]Rank order is given in parentheses for the lower (8th) and upper (29th) bounds of the 90% CI.

higher release. Conversely, comparison of release data from the reference formulation collected on 2 separate days indicated equivalence, thereby confirming the method's ability to demonstrate the correct release profile, which is influenced by formulation excipients. Collectively, these findings support that the validated IVRT method is robust and appropriate for routine quality control testing.

CONCLUSION

The primary objective of this study was to develop a sensitive, specific, and reproducible IVRT method for quantifying the release of LCZ from topical cream formulations. Statistical comparison of release rates between test and reference products, using the T/R ratio approach, showed that the results fell outside the 90% CI, indicating nonequivalence. However, release data for the reference formulation obtained on different days demonstrated equivalence within the 90% CI limit. The validated IVRT and HPLC methods developed in this study are suitable for routine release testing of LCZ cream formulations and can be extended to evaluate release profiles of other LCZ-based topical products.

DISCLOSURES

The authors received no financial support for this work and have no conflicting interests

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