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Utility of in vitro release testing (IVRT) to assess 'sameness' of 1% clotrimazole creams for use as a biowaiver

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Abstract

The October 2022 draft United States Food and Drug Administration (FDA) guidance presents an option of in vitro release test (IVRT) studies as a biowaiver for topical drug products submitted in abbreviated new drug applications (ANDAs). However, the product-specific guidance (PSG) for 1% clotrimazole (CLZ) topical cream does not provide an in vitro option for biowaiver and requires a clinical endpoint study to demonstrate bioequivalence (BE). Therefore, the main objective was to use IVRT to investigate pharmaceutical equivalence of several 1% CLZ topical creams from two countries — South Africa (SA) and Canada. This investigation aims at demonstrating the utility of IVRT to determine 'sameness' and/or differences between topical creams containing 1% CLZ and the potential of IVRT for supporting biowaivers, thereby obviating the necessity to conduct clinical endpoint studies in patients. A validated IVRT method was applied to conduct comparative IVRT runs on five generic products marketed in SA and one Canadian generic, which were compared against a relevant comparator product from their country of origin in accordance with the FDA's acceptance criteria of 75–133.33%. All five SA-marketed generic creams showed pharmaceutical inequivalence to the SA comparator product indicating Q1/Q2/Q3 differences. Despite containing the same excipients as both comparator products, the Canadian generic showed substantially lower release rate compared to the comparator products which could be attributed to Q2/Q3 differences. The IVRT method displayed the requisite ability to assess the various 1% CLZ creams and confirmed the potential of the IVRT method to support a biowaiver for 1% CLZ topical creams.

Keywords In vitro release testing (IVRT), Sameness, Pharmaceutical equivalence, Biowaivers, Topical clotrimazole creams

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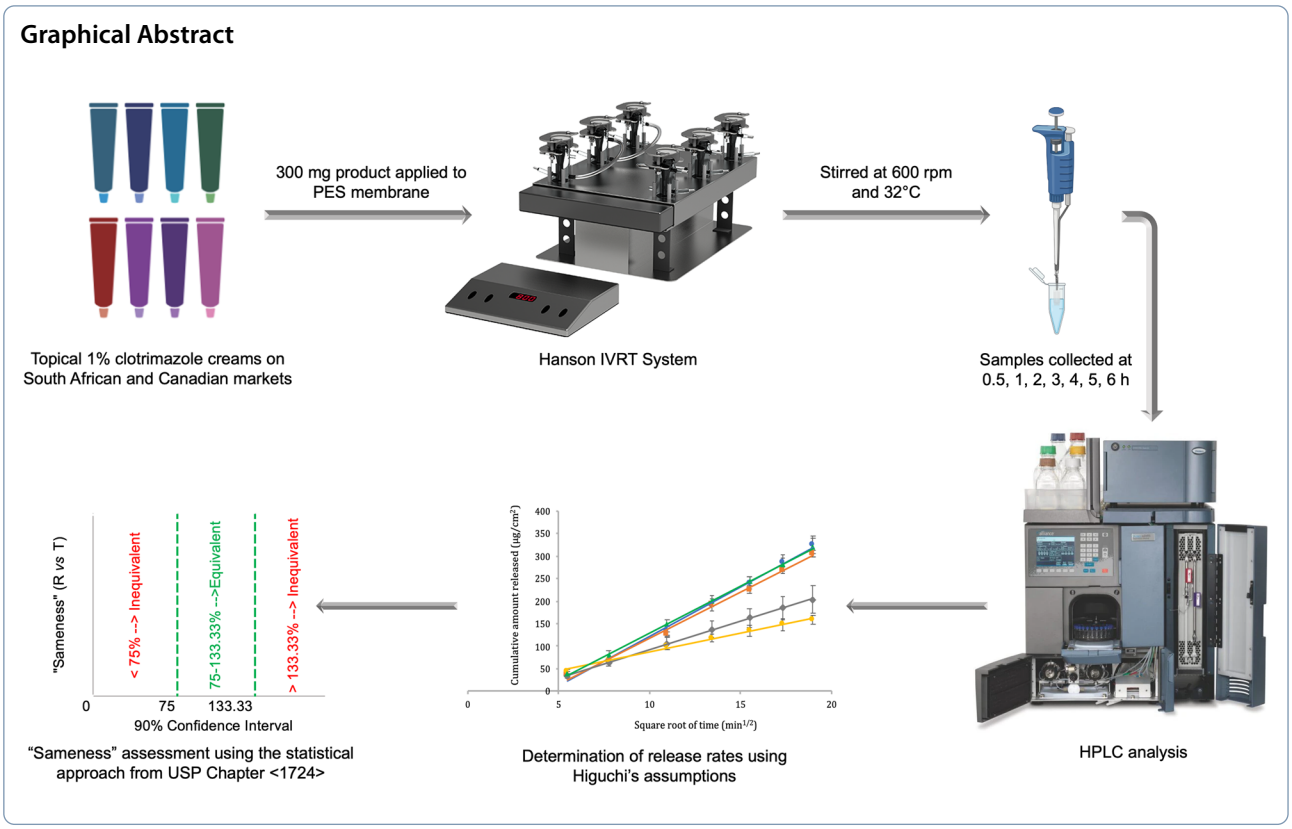

Introduction

Topical product performance is assessed by measuring the rate and extent of drug release from the dosage form, as only once the drug is liberated from the dosage form can it diffuse into the skin, which is the target site (Shah et al. 1999). Measuring drug release rates from topical dermatological products can be achieved by in vitro release test (IVRT) using vertical diffusion cells (VDC) containing an appropriate receptor medium and a corresponding acceptable synthetic inert membrane (Yacobi et al. 2014; United States Pharmacopeial Convention 2018; Shah and Williams 2014). The capability of IVRT to detect differences in release rates of formulations due to changes in product composition, manufacturing process, and production site has rendered it an important quality control (QC) tool. Therefore, the application of IVRT to assess product 'sameness' of topical semisolid products after post-approval change has been recommended by the United States Food and Drug Administration (FDA) in the scale-up and post-approval changes-semisolid (SUPAC-SS) document (US Food and Drug Administration - Centre for Drug Evaluation and Research 1997). Moreover, IVRT is outlined as a compendial method for testing semisolid product performance in United States Pharmacopeia (USP) chapter < 1724 > (United States Pharmacopeial Convention 2018). While IVRT is well established as a crucial QC tool, it has shown promise as a potential surrogate measure for biowaiver justification.

The major challenge encountered by proposed generic topical products reaching the market is that regulatory authorities require comparative clinical studies to demonstrate BE to a relevant comparator product, which is lengthy and expensive (Shah et al. 2014). Therefore, generic competition is low for topical drug products, negatively impacting patients as there is limited access to affordable products. Considering this challenge, the FDA, and European Medicines Agency (EMA) in the past decade, have released several product-specific guidance (PSG) documents in which IVRT has been recommended for biowaiver justification (US Food and Drug Administration - Office of Generic Drugs 2012, 2014, 2018a, 2018b, 2017a, 2017b). This affordable and efficient approach for BE assessment is applicable to generic (test) products that have the same qualitative (Q1) and quantitative (Q2) compositional attributes as the comparator. Notably, despite Q1 and Q2 similarity, generic product performance can differ significantly due to microstructural arrangement of matter (Q3), which can be a consequence of different manufacturing processes. Therefore, it is imperative that the IVRT method is selective and discriminatory to evaluate Q3 attributes on product performance.

In addition to PSG documents, the FDA has implemented several initiatives, such as the Drug Competition Action Plan and Generic Drug User Fee Act (GDUFA) programme to promote the entry of generic topical products on the market, thereby increasing generic competition (US Food and Drug Administration - Centre for Drug Evaluation and Research 2022). The regulatory frameworks for the FDA and EMA differ slightly in terms of 'sameness' acceptance criteria, with the SUPAC-SS 90% confidence interval limits being 75–133.33% (US Food and Drug Administration - Centre for Drug Evaluation and Research 1997), while the EMA has a much narrower interval of 90–110% (European Medicines Agency 2018).

IVRT can provide valuable information to support the demonstration of BE, and several comparative studies of marketed topical formulations have been performed using it. However, without validation of the IVRT method, the results produced may be inaccurate and unreliable to deduce meaningful conclusions. Hence, the FDA recommends validation (US Food and Drug Administration - Centre for Drug Evaluation and Research 2022) to ensure that the method and equipment possess the requisite discriminatory power to accurately determine 'sameness' between topical products. Despite the numerous published IVRT methods that have been developed and applied for comparative assessment of topical products, there has been limited literature on the use of appropriately validated methods until recently (Tiffner et al. 2018; Tiffner et al. 2021; Rath and Kanfer 2020; Mudyahoto et al. 2020; Purazi et al. 2020). Recently, however, regulatory authorities such as the US FDA and the EMA have published guidelines with specific recommendations for validation of IVRT methods (US Food and Drug Administration - Centre for Drug Evaluation and Research 2022; European Medicines Agency 2018).

Although PSGs for several topical products recommend IVRT to support biowaivers, the PSG for topical 1% clotrimazole (CLZ) cream does not provide such an in vitro option, necessitating the conduct of a clinical endpoint study to demonstrate bioequivalence (BE) (US Food and Drug Administration - Office of Generic Drugs 2010). Therefore, the objective of this research was to demonstrate the utility of IVRT to assess 'sameness' of some topical 1% CLZ creams from South Africa (SA) and Canada (CA) and thereby support a biowaiver based on the evaluation of qualitative (Q1) and quantitative (Q2) properties and structural arrangement of matter (Q3).

Materials

Chemicals and drug formulations

All the creams used in these comparative studies contained 1% CLZ. Two comparator products were used — Canesten® (Bayer, Gauteng, SA) assigned R_1 and Canesten® (Bayer Inc., Ontario, CA, USA) assigned R_2. These two comparator products were compared to 8 test products — 5 South African-marketed generic creams (SA_1, SA_2, SA_3, SA_4, and SA_5), one Canadian-marketed generic cream (CA_1), and two extemporaneously prepared creams (T_1 and T_2).

The extemporaneously compounded creams were prepared using different excipients (Table 1). Cream T_1 was prepared using CLZ powder, propylene glycol, and placebo cream which had been generously donated by Aspen Pharmacare, SA. This placebo cream comprised of cetostearyl alcohol, paraffin (white soft and liquid), sorbitan monostearate, polysorbate 60, citric acid, disodium phosphate, propylene glycol, benzyl alcohol, and water. Cream T_2 was compounded using cetyl palmitate (Emprove® Essential, Merck KGaA, Darmstadt, Germany), cetostearyl alcohol (Sigma Aldrich®, Merck Life Science, Modderfontein, South Africa), octyldodecanol (Xi'an Lynsey Biotech Co. Ltd., China), benzyl alcohol,

Table 1 Overview of inactive ingredients (excipients) for the 1% CLZ creams (R_1, R_2, CA_1, T_1, and T_2)

SA Canesten® (R_1)	CA Canesten® (R_2)	CA generic CA_1	Cream T_1	Cream T_2
Cetostearyl alcohol	Cetostearyl alcohol	Cetostearyl alcohol	Cetostearyl alcohol	Cetostearyl alcohol
Cetyl esters wax	Cetyl esters wax	Cetyl esters wax	Paraffin white soft	Cetyl palmitate
Sorbitan monostearate	Sorbitan monostearate	Sorbitan monostearate	Paraffin liquid	Sorbitan monostearate
Polysorbate 60	Polysorbate 60	Polysorbate 60	Sorbitan monostearate	Polysorbate 60
Octyldodecanol	Octyldodecanol	2-Octyldodecanol	Polysorbate 60	Octyldodecanol
Benzyl alcohol	Benzyl alcohol	Benzyl alcohol	Citric acid	Benzyl alcohol
Purified water	Purified water	Purified water	Disodium phosphate	Purified water
			Benzyl alcohol	
			Propylene glycol	
			Purified water	

sorbitan monostearate, polysorbate 60, and CLZ powder which were donated by Aspen Pharmacare, SA.

IVRT system

A Hanson manual diffusion test system (Hanson Research Corporation, Chatsworth, USA) consisting of six closed-top diffusion cells (volume: 7.9 mL, orifice diameter: 15 mm) mounted onto a stand (Variomag®, CA, USA), containing six stirrer drive units, controlled by a stirring unit (Telemodul 40S, H + P Labortechnik, Munich, Germany), and coupled to a circulating water bath (Polyscience®, IL, USA).

High-performance liquid chromatography (HPLC) system

A chromatographic system consisting of a Waters Alliance® (e2695) high-performance liquid chromatography (HPLC) machine coupled to a PDA detector (2998) (Waters® Corporation, MA, USA) and equipped with Waters Empower® 3 software was used for the sample analysis.

Samples and standards were weighed using a Mettler® model AE analytical balance and Mettler® MX5 Toledo microbalance (Mettler® Inc., Zurich, Switzerland) respectively. Pipetman™ pipette sizes P100 and P1000 (Gilson International, Villiers Le Bel, France) were used to transfer sample and standard solutions. Illustrative chromatograms have been provided as supplementary material (Supplementary Figs. S1–S4).

Methods

IVRT system qualification and method validation

Critical operational parameters of the VDC system were investigated to assess their compliance with proposed predefined acceptance criteria published in Pharmacopeial Forum in 2009 (Ueda et al. 2009). These parameters included cell capacity and internal diameter, temperature maintenance, stirring speed, and sample volume dispensed as well as environmental parameters to assess if the working area and positioning of the VDC system were optimal. A performance verification test (PVT) of the IVRT system as described in the Pharmacopeial Forum was also carried out (Ueda et al. 2009).

An IVRT method was developed to measure CLZ release rates from cream products containing 1% CLZ. The optimized parameters are summarized in Table 2. Subsequently, a comprehensive validation including positive and negative controls was conducted to assess the precision, reproducibility, reliability, robustness, sensitivity, specificity, selectivity, and discriminatory power of the IVRT method (Wellington 2023). Moreover, the additional parameter of supplemental activity was assessed according to the latest FDA draft guidance (US Food and Drug Administration - Centre for Drug Evaluation and Research 2022).

Release rates

The developed and validated IVRT method, as described by Wellington et al. (Wellington 2023), was used for the 'sameness' assessment of marketed 1% CLZ creams. The comparative studies were divided into two sets: (1) South African-marketed 1% CLZ generic creams vs. SA comparator product — Canesten® SA and (2) Canadian-marketed 1% CLZ generic cream vs. CA comparator product — Canesten® CA and extemporaneously prepared 1% CLZ creams vs. Canesten® SA. Six comparative IVRT runs were performed for the first set and five for the second set. Additionally, two IVRT runs were performed as a positive control comparing the comparator product against itself to determine if the method could confirm 'sameness' between equivalent products. Two different batches (Batch A and B) of SA Canesten® (R_1) cream were used to confirm 'sameness'. These comparative IVRT tests were performed in congruence with the FDA's SUPAC-SS guidance (US Food and Drug Administration - Centre for Drug Evaluation and Research 1997), which suggests that all products being tested should be

Table 2 Optimized IVRT parameters (Wellington 2023)

VDC system	6-cell diffusion manual apparatus (Hanson)
Cell orifice	15 mm
Cell capacity (volume)	7.9 mL
Temperature	32 ± 1 °C
Receptor medium	Phosphate buffer and ethanol (50:50% v/v)
Synthetic membranes	PES (0.45 µm, 25 mm, pre-soaked in receptor medium)
Dose	300 mg
Stirring speed	600 ± 60 rpm
Sampling volume	200 µL
Sampling interval	0.5, 1, 2, 3, 4, 5, and 6 h

on each of the VDCs in the runs to ensure unbiased comparison of the products in case of any system variability between the runs. The arrangement of the creams on the VDCs in each run was selected systematically by alternating the pattern for each successive run. This ensured that the release rates from each cream were measured on all six VDCs at the end of the comparative studies, with the product dosing scheme shown in Table 3.

The cumulative amount of CLZ released per unit area as a function of the time was plotted, generating six release rates per cream at the end of each set of IVRT runs shown in Table 3 where 12 release rates were generated for Canesten® SA vs. itself, 36 release rates for the SA-marketed 1% CLZ generic creams vs. Canesten® SA, and 30 release rates for CA generic and extemporaneously prepared creams vs. their relevant comparator products. In order to calculate the cumulative amount of CLZ released, Eq. 1 was used, where Q_n refers to the cumulative amount released (μg/cm^2), C_n was the measured concentration in the samples at time n (μg/cm^3), A_c was the area of the VDC orifice (cm^2), V_s was the volume of the VDC (cm^3), and V_s was the volume of the sample (cm^3).

$$Q_n = C_n \frac{Vc}{Ac} + \frac{Vs}{Ac} \sum_{i=1}^{n} C_{i-1} \quad (1)$$

Equation 1 Calculation of release rate (Q_n) for the different CLZ creams.

Statistical analyses — 'sameness' assessment

The equivalence of the test and comparator products was determined using the Wilcoxon rank-sum/Mann–Whitney statistical test described in USP < 1724 > (United States Pharmacopeial Convention 2018), which was appropriate as these data followed a nonnormal distribution (Conover 1980). This nonparametric method requires the calculation of the 90% CI using the 36 T/R ratios. These ratios are ordered from smallest to largest, and the 8th and 29th values correspond to the lower and upper limits of the CI, respectively. The predefined equivalence/'sameness' acceptance criterion, according to the FDA SUPAC-SS guidance, was that the computed 90% CI limits fall within the range of 75–133.33% (US Food and Drug Administration - Centre for Drug Evaluation and Research 1997). Equivalence was evaluated using the pairwise comparison of drug release rates for the comparator products with that for each of the eight CLZ test products, as well as for itself, to compute the 90% CI according to the approach outlined in USP chapter < 1724 > (United States Pharmacopeial Convention 2018) and the relevant FDA guidances (US Food and Drug Administration - Centre for Drug Evaluation and Research. FDA-SUPAC-SS 1997; US Food and Drug Administration - Centre for Drug Evaluation and Research 2022).

Table 3 VDC product dosing scheme

	VDC 1	VDC 2	VDC 3	VDC 4	VDC 5	VDC 6
Canesten® SA vs. itself (batch A — R_{1A} and batch B — R_{1B})						
Run 1	R_{1A}	R_{1B}	R_{1A}	R_{1B}	R_{1A}	R_{1B}
Run 2	R_{1B}	R_{1A}	R_{1B}	R_{1A}	R_{1B}	R_{1A}
SA-marketed 1% CLZ generic creams (SA_n) vs. Canesten® SA (R_1)						
Run 1	R_1	SA_1	SA_2	SA_3	SA_4	SA_5
Run 2	SA_1	SA_2	SA_3	SA_4	SA_5	R_1
Run 3	SA_2	SA_3	SA_4	SA_5	R_1	SA_1
Run 4	SA_3	SA_4	SA_5	R_1	SA_1	SA_2
Run 5	SA_4	SA_5	R_1	SA_1	SA_2	SA_3
Run 6	SA_5	R_1	SA_1	SA_2	SA_3	SA_4
1% CLZ creams (CA generic CA_1 and extemporaneously prepared creams T_1 and T_2) vs. relevant comparator products — Canesten® SA and CA (R_1 and R_2)						
Run 1	R_1	R_2	CA_1	T_1	T_2	R_1
Run 2	R_2	CA_1	T_1	T_2	R_1	R_2
Run 3	CA_1	T_1	T_2	R_1	R_2	CA_1
Run 4	T_1	T_2	R_1	R_2	CA_1	T_1
Run 5	T_2	R_1	R_2	CA_1	T_1	T_2

Results

IVRT system qualification and method validation

Apparatus qualification, PVT, and method validation results are summarized under supplementary material (Supplementary Tables S1–S3).

Release rates

South African comparator product versus itself as a positive control

The SA comparator product, Canesten®, was compared with itself as a positive control. Creams from two different batches A and B were compared against each other (Fig. 1).

South African marketed generic creams versus comparator product, Canesten® SA

The release rate profiles were linear for all the products, with R^2 values ranging between 0.988 and 0.998. The mean release rates ± SD of the five generic products were 8.17 ± 0.31 µg/cm^2/min$^{1/2}$ (SA_1), 8.46 ± 0.69 µg/cm^2/min$^{1/2}$ (SA_2), 8.36 ± 0.74 µg/cm^2/min$^{1/2}$ (SA_3), 11.60 ± 1.17 µg/cm^2/min$^{1/2}$ (SA_4), and 7.49 ± 0.25 µg/cm^2/min$^{1/2}$ (SA_5), which were all significantly lower than the comparator product (R_1), 21.25 ± 1.06 µg/cm^2/min$^{1/2}$. These differences in the mean release rates are depicted in Fig. 2.

1% CLZ creams from South Africa, Canada, and extemporaneously compounded creams

There was a linear relationship between the cumulative amount of CLZ released and $\sqrt{t}$ for all products, with R^2 values ranging from 0.992 to 1.000 (Fig. 3). The mean release rates ± SD were 21.82 ± 1.01 µg/cm^2/min$^{1/2}$ for R_1, 20.38 ± 2.22 µg/cm^2/min$^{1/2}$ for R_2, 12.63 ± 1.02 µg/cm^2/min$^{1/2}$ for CA_1, 8.48 ± 0.51 µg/cm^2/min$^{1/2}$ for T_1 and 21.08 ± 1.03 µg/cm^2/min$^{1/2}$ for T_2.

Comparative 'sameness' assessment

South African-marketed generic creams versus comparator product, Canesten® SA

In order to determine the ability of the IVRT to accurately distinguish between the release rates of the comparator product and the five generic products, a nonparametric statistical test was used to assess the equivalence for each pairwise comparison. The computed lower and upper limits of the 90% CI for all five generic creams fell outside the 75–133.33% range, as shown in Table 4.

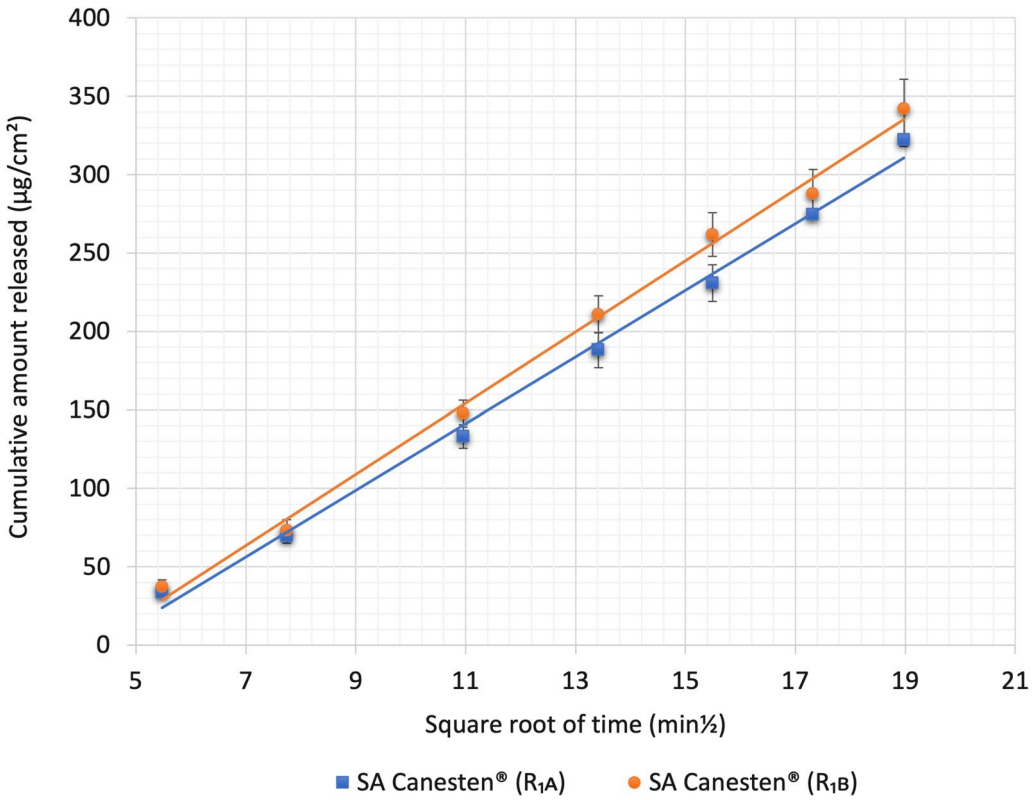

Fig. 1 Release rate profiles comparing comparator product, Canesten.® vs. itself (R_{1a} and R_{1b}) ($n = 6$)

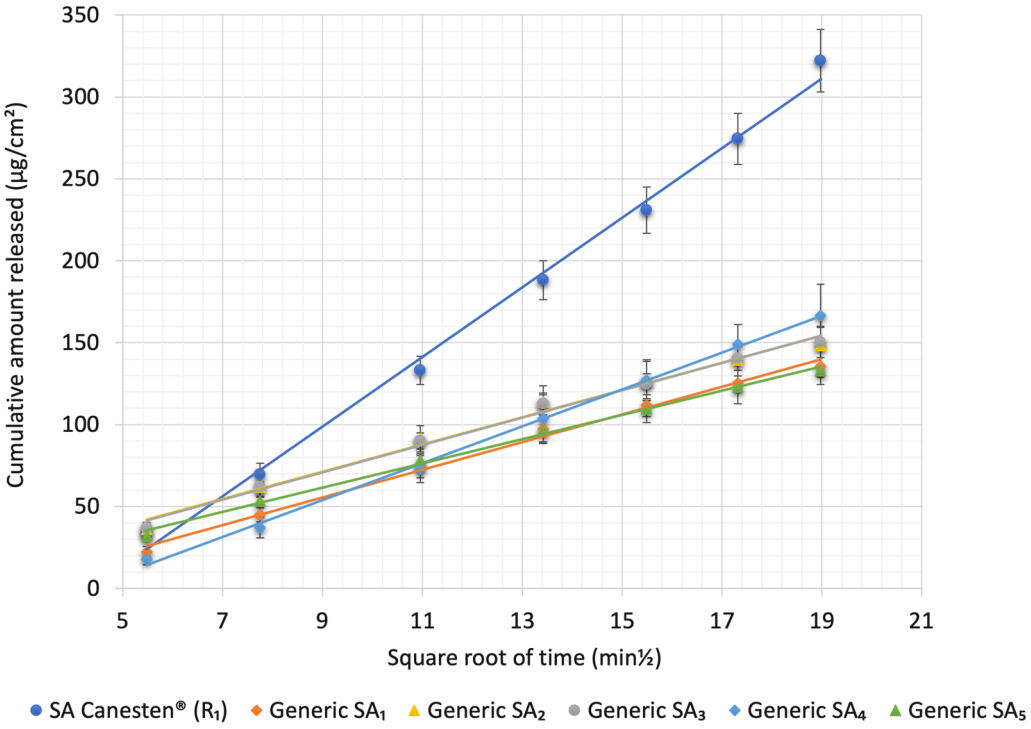

Fig. 2 Release rate profiles from SA-marketed 1% CLZ generic creams (SA_n) and Canesten.® 1% CLZ (R_1) ($n = 6$)

1% CLZ creams from South Africa, Canada, and extemporaneously compounded creams

Table 5 depicts a summary of the calculated 90% CIs for the pairwise comparisons of 1% CLZ creams from Canada and extemporaneously compounded creams vs. the respective comparator product (R_1 or R_2).

Discussion

IVRT system qualification and method validation

All assessment parameters, except one (cell capacity), met the requirements demonstrating that the VDC system and environment were acceptable to perform requisite IVRT studies. The IVRT method complied with all the requirements (Wellington 2023). Although the cell capacity did not conform to the manufacturer's specifications, it was still considered suitable for the IVRT functional parameters because the range of variation between the six VDCs was 0.04 mL, indicating low intercell variability and acceptable precision.

Release rates

South African comparator product versus itself as a positive control

When the SA comparator product, Canesten®, was compared with itself as a positive control, the release rate profiles of the comparator product against itself indicated similar release rates, confirming equivalence.

South African marketed generic creams versus comparator product, Canesten® SA

The release rate profiles for all the SA marketed 1% CLZ products were significantly lower than the comparator product. Interestingly, visual clustering of the release rate profiles from all test products was evident, reflecting the relative compositional similarity of the generic products (Tiffner et al. 2021).

1% CLZ creams from South Africa, Canada, and extemporaneously compounded creams

Notably, the CA generic product (CA_1) and one of the compounded test creams (T_1) had significantly lower release rates as compared to the two comparator products (R_1 and R_2), and cream T_2, which was compounded using the same ingredients as the comparator products. This illustrates that compositional differences in the cream formulations can significantly impact the drug release rates, as R_1, R_2 and T_2, which had the same excipients qualitatively and quantitatively (Q1/Q2 equivalent), demonstrated almost identical release rates. In contrast, T_1, which had several different excipients, had the slowest release rate, possibly due to the presence of hydrocarbon bases (i.e. paraffin) and propylene glycol (Tiffner et al. 2021). Interestingly, the generic cream (CA_1), which had the same excipients (Q1) as the comparator products (R_1

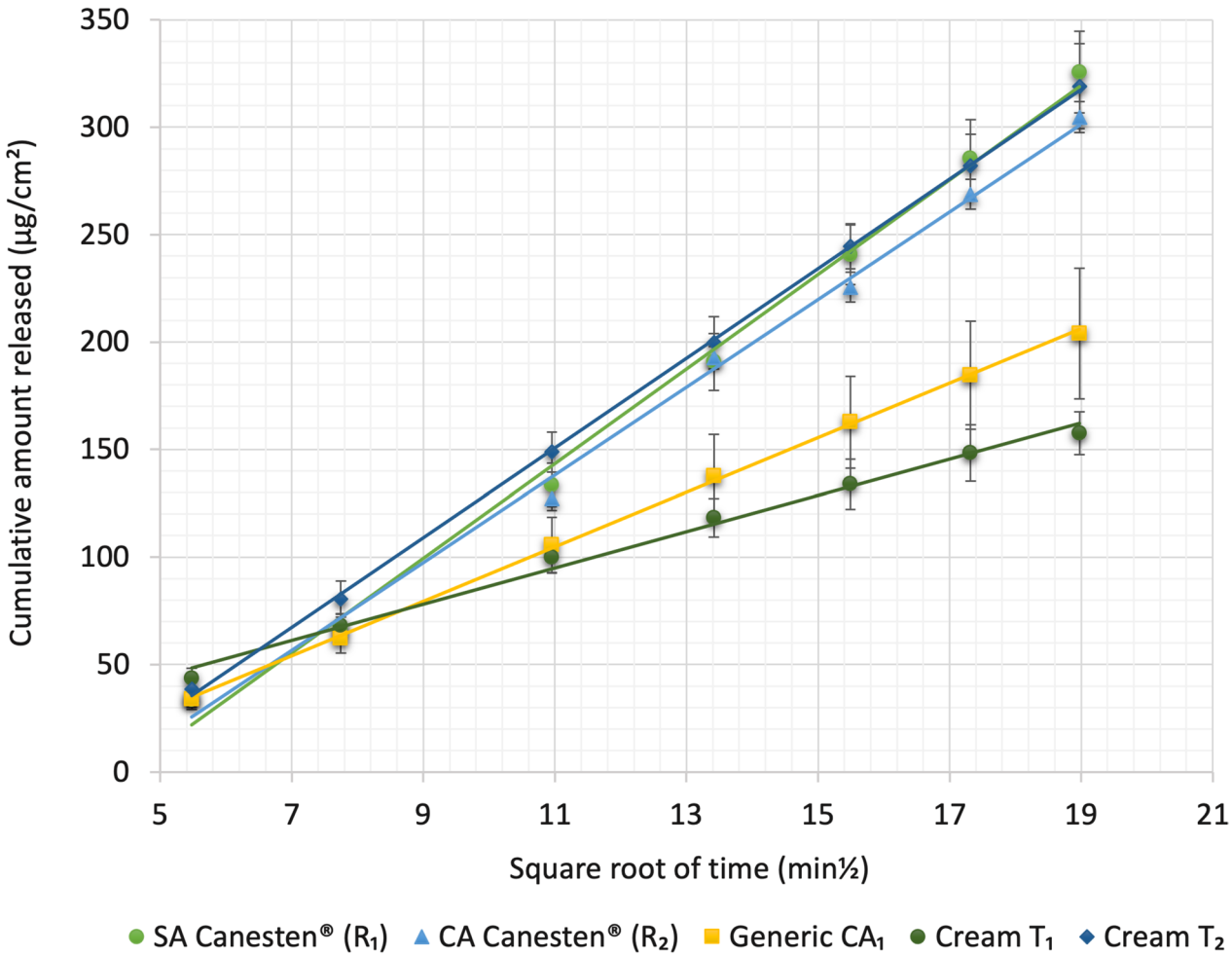

Fig. 3 Release rate profiles from SA and CA Canesten.® 1% CLZ (R_1 and R_2), CA-marketed 1% CLZ generic cream (CA_1), and extemporaneously compounded creams T_1 and T_2 ($n=6$)

Table 4 Results from the pairwise comparison of test vs. comparator products and the predefined acceptance criteria for 'sameness'

Pairwise comparison	'Sameness' acceptance criteria	Results	'Sameness' pass/fail
Generic SA_1 vs. SA Canesten® (R_1)	90% CI for the marketed generic 1% CLZ creams should lie within the limits of 75–133.33% according to the FDA's SUPAC-SS guidance	LL: 36.87% UL: 40.18%	Fail
Generic SA_2 vs. SA Canesten® (R_1)		LL: 36.26% UL: 43.15%	Fail
Generic SA_3 vs. SA Canesten® (R_1)		LL: 51.61% UL: 59.38%	Fail
Generic SA_4 vs. SA Canesten® (R_1)		LL: 37.52% UL: 43.08%	Fail
Generic SA_5 vs. SA Canesten® (R_1)		LL: 33.94% UL: 36.33%	Fail
SA Canesten® (R_1) vs. itself (positive control)		LL: 84.03% UL: 102.86%	Pass

LL lower limit, *UL* upper limit

Table 5 Results from the pairwise comparison of test vs. comparator products and the predefined acceptance criteria for 'sameness'

Pairwise comparison	'Sameness' acceptance criteria	Results	'Sameness' pass/fail
SA Canesten® (R_1) vs. CA Canesten® (R_2)	90% CI for the marketed generic 1% CLZ creams should lie within the limits of 75–133.33% according to the FDA's SUPAC-SS guidance	LL: 85.78% UL: 101.99%	Pass
Generic CA_1 vs. CA Canesten® (R_2)		LL: 56.54% UL: 70.18%	Fail
Cream T_1 vs. SA Canesten® (R_1)		LL: 36.28% UL: 40.25%	Fail
Cream T_2 vs. SA Canesten® (R_1)		LL: 92.30% UL: 102.40%	Pass

LL lower limit, *UL* upper limit

and R_2), had a substantially lower release rate than the comparator products, which could be due to differences in the quantities (Q2) of excipients used (Tiffner et al. 2021) and/ or differences in Q3.

As seen from the results, the vehicle composition and microstructure of the semisolid formulation can significantly impact in vitro drug release rates and potentially permeation into the skin. This is because different excipients may alter the physicochemical properties and thermodynamic activity of the formulation in addition to changes in attributes of the structural arrangement of matter (Q3) characteristics (Raghavan et al. 2019; PharmTech 2016).

Comparative 'sameness' assessment

South African-marketed generic creams versus comparator product, Canesten® SA

The 90% CI for all five generic creams fell outside the 75–133.33% range, which indicated that these products were statistically inequivalent or different to the comparator product, Canesten® 1% cream, thus showing that the IVRT method could identify differences should they exist. The mean release rates from two different batches of Canesten® 1% cream sourced from Bayer South Africa were used to calculate the lower and upper limits of the 90% CI, which fell within the equivalence range, thus passing the statistical equivalence test as shown in Table 4. This displays the ability of the method to accurately identify 'sameness' between these products.

Inequivalence between the marketed generic 1% CLZ topical creams and the comparator product was possibly due to differences in qualitative (Q1) and quantitative (Q2) properties and arrangement of matter (Q3) between the products. These data indicate that differences in release rates are highly likely to have a negative outcome on bioavailability (BA) and consequently result in bioinequivalence (BIE) (Tiffner et al. 2021).

1% CLZ creams from South Africa, Canada, and extemporaneously compounded creams

SA Canesten® (R_1), CA Canesten® (R_2), and cream T_2 consisted of the same formulation ingredients and unsurprisingly passed the 'sameness' test, where the 90% CI limits fell within the acceptable range of 75–133.33%. However, the generic cream (CA_1), when compared to the Canadian comparator product, failed the equivalence test, despite being qualitatively identical to the comparator product. Cream T_1, which was compositionally different to the SA comparator product, was found to be inequivalent as a result of considerable differences in the type and amounts of inactive ingredients used.

Regulatory approval requirements "Generally, topical 1% CLZ products are marketed as over-the-counter (OTC) products based on its long history and use relating to safety and efficacy, hence in vivo studies are generally not required for marketing approval as substantiated in an FDA monograph entitled "Subpart C-Topical Antifungal Drug Products" (21 CFR 333.201-333.280) (United States of America, Federal Register -Code of Federal Regulations 2023; United States of America, Federal Register -Code of Federal Regulations 2001) where it is mentioned that 1% topical CLZ products do not require clinical data for marketing authorization since 1989. Furthermore, unlike new drug products which require placebo-controlled clinical trials in patients to establish effectiveness on a non-inferiority basis, comparative in vivo studies to demonstrate BE for topical generic drug products are regarded as inefficient, risky, very expensive and also considered to be the least accurate, sensitive, and reproducible of the general approaches for measuring BA or demonstrating BE for such products (United States of America, Federal Register - Code of Federal Regulations 2003).

Our results indicate that several of the marketed generic 1% CLZ products did not meet the

'sameness' requirements when compared to their respective reference products. Since all the studied generic products were previously approved for marketing, the results could conjure up the possibility of over-discrimination. However, such a notion can be readily refuted since our data confirmed 'sameness' when the reference product was compared against itself and when reference products from the same manufacturer and containing the same excipients (Q1, Q2) from two countries were compared against each other. In addition, the inclusion of positive and negative controls provided compelling evidence of the discriminatory power of the method, thereby indicating that over-discrimination is highly unlikely."

Conclusions

IVRT is a well-established QC tool widely accepted globally by regulatory authorities, including EMA, US FDA, World Health Organization (WHO) (World Health Organization (WHO) 2017), and South African Health Products Regulatory Authority (SAHPRA) (South African Health Products Regulatory Authority (SAHPRA) 2019) to detect similarities and differences should they exist among topical semisolid products. Furthermore, IVRT has been proposed as an acceptable method for the justification of biowaivers in numerous FDA product-specific guidelines (US Food and Drug Administration 2023). However, failure to comply with appropriate validation requirements can result in erroneous approvals which may have significant implications on the clinical performance of generic products. Our data, generated using an appropriately validated IVRT method, clearly indicate that the investigated marketed generic 1% CLZ creams were not pharmaceutically equivalent to their respective comparator products. The validated IVRT method showed the potential to accurately measure the release from 1% CLZ creams and demonstrated appropriate discriminatory power to identify 'sameness' and/ or differences, if any. Furthermore, it displayed the requisite ability to discriminate between the various marketed 1% CLZ creams, including some products with relatively insignificant formulation differences and others with substantially dissimilar attributes relating to Q1/Q2/Q3. Hence, IVRT is a valuable tool which can be used to predict possible differences in clinical performance as a result of formulation differences which result in differences in release rates. Based on our results, it is evident that an appropriately validated IVRT method is an extremely valuable tool and reinforces the potential for its use as a biowaiver for topical products for local action. The foregoing promotes the possibility to establish IVRT as an acceptable option to assess BE of such products, thereby obviating the need for cost-, labour-, and time-intensive clinical studies and facilitating faster entry of affordable generic products into the market.

Abbreviations

ANDA	Abbreviated new drug applications
BA	Bioavailability
BE	Bioequivalence
BIE	Bioinequivalence
CA	Canada
CLZ	Clotrimazole
CI	Confidence interval
EMA	European Medicines Agency
FDA	United States Food and Drug Administration
GDUFA	Generic Drug User Fee Act
HPLC	High-performance liquid chromatography
IVRT	In vitro release test
PVT	Performance verification test
PSG	Product-specific guidance
QC	Quality control
SUPAC-SS	Scale-up and post-approval changes-semisolids
SA	South Africa
SAHPRA	South African Health Products Regulatory Authority
USP	United States Pharmacopeia
VDC	Vertical diffusion cells
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41120-023-00087-4.

Additional file 1: Fig. S1. Chromatogram depicting a 1 µg/mL CLZ peak, **Fig. S2.** Chromatogram depicting a 5 µg/mL CLZ peak, **Fig. S3.** Chromatogram of the negative control (i.e., receptor medium) showing the absence of interfering peaks around CLZ retention time of 4.3 min, **Fig. S4.** Chromatogram depicting CLZ peak in positive control (i.e., receptor medium spiked with 50 µg/mL CLZ).

Additional file 2: Table S1. Summary of apparatus qualification results, **Table S2.** Summary of performance verification test (PVT) results, **Table S3.** Summary of IVRT method validation results.

Authors' contributions

HW, contributed to the methodology, software analysis, validation, formal analysis, investigation, data curation, writing—original draft preparation, and writing—review and editing. SR, contributed to the methodology, software, validation, data visualization, supervision, writing—original draft preparation, and writing—review and editing. SA, helped secure and manage resources for the project. IK, contributed to conceptualization, funding acquisition, supervision, writing—original draft preparation, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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