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Original scientific paper

A validated and discriminatory *in vitro* release test for evaluation of marketed Clotrimazole cream formulations

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Abstract

In vitro surrogate tests are broadly used for evaluation of the critical release characteristics of semi-solid dosage forms. In general, these tests are limited to assess the effect of crucial manufacturing process related steps on the physicochemical characteristics and overall drug product performance. *In vitro* release tests (IVRT) do not directly anticipate *in vivo* performance, but may detect *in vitro* changes that may correlate with *in vivo* performance. The objective of this work was to develop a suitable *in vitro* release test for evaluation of the similarity between two marketed Clotrimazole 1% Cream formulations. A systematic approach was used to address some essential qualification parameters and validation concepts described in EMA`s draft guideline on quality and equivalence of topical products. The procedure included suitable evaluation of the receptor medium, membrane qualification, followed by evaluation of method precision and method robustness. For evaluation of data, the comparison of Clotrimazole release profile met the relevant acceptance criteria for the 90% Confidence Interval for the ratio of means of the pairwise comparisons falling inside the limits of 90–111%. The linearity of the IVRT method as function of the drug concentration in the formulation was evaluated with 50% and 200% API formulations. In addition, the discriminatory power of the method was confirmed with formulation with altered viscosity as critical quality attribute. After validation of the method, the Clotrimazole 1% cream formulations were compared and their similarity was assessed. The approach was found to be useful and comprehensive in performing validation activities.

Key words: IVRT, Clotrimazole cream formulation, method validation, discriminatory power

Introduction

In vitro surrogate tests are broadly used for evaluation of the critical release characteristics of semi-solid dosage forms. They are recognized by EMA and FDA as useful in establishing a correlation between critical physical and chemical parameters of topical products such as solubility, particle size and rheological properties and their impact on the product performance. In general, these tests are limited to justify small changes in the manufacturing process of the semi-solid product or for post-approval changes. However, in cases where two formulations have the same qualitative and quantitative composition the differences are in the manufacturing process and in the scale up, so, they often serve to evaluate similarity between formulations (Lionberger, 2008).

Therapeutically, Clotrimazole belongs to the class of imidazole antifungal drugs with broad spectrum of uses such as topical treatment of tinea pedis (athlete`s foot),

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tinea cruris and tinea corporis caused by isolates of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis* and *Candida Albicans.* It`s mechanism of antifungal action consists of inhibition of a crucial step in the biosynthesis of ergosterol, a component of the fungal cytoplasmic membrane, which causes a cascade of reactions which lead to inhibition of the fungal growth. Its potential in other medical treatments has been also assessed such as promising antineoplastic properties when combined with various metals etc. (Crowley and Gallagher, 2014). It is available in multiple pharmaceutical dosage forms such as topical crema, gel, lotions, powder, lozenges, topical solutions and vaginal inserts/tablets.

When being used for fungal skin infections it is usually available as cream. Science data shows that when applied locally on the skin, Clotrimazole does not target the systematic circulation, it acts to a large extent locally on the skin in the process of destroying the causative fungi (Bolla et al., 2019), so as permeation trough skin is not relevant to efficacy, for evaluation of similarity of generic drug products with same composition, a nonclinical approach, such as IVRT can be considered as a biowaiver for claiming extended pharmaceutical equivalence of the products (EMA`s Draft guideline on quality and equivalence of topical products, 2018).

The objective of this study was to develop a suitable *in vitro* release test for evaluation of the similarity between two marketed Clotrimazole 1% Cream formulations: Mycotreat® manufactured by Bionika pharmaceuticals and Canesten manufactured by Bayer® using the novel approaches of method development and validation of *in vitro* release tests described in EMA`s draft guideline on quality and equivalence of topical products. The procedure was developed and validated at Alkaloid AD R&D Laboratories.

Material and methods

Chemicals

Clotrimazole working standard was used, supplied by Bionika Pharmaceuticals, North Macedonia. For preparation of the mobile phase for HPLC analysis, dipotassium hydrogen phosphate (Merck, Germany) and acetonitrile isocratic grade (Supelco, Germany) were used. The column for HPLC analysis was supplied by Merck, Germany. For the IVRT test, for preparation of the receptor medium solution, Phosphate Buffer Saline (PBS) pH 7.4 supplied by VWR, USA, and Ethanol absolute obtained from Merck, Germany were used. The membranes used in the development of the IVRT study were Regenerated Cellulose filter membranes with pore size of 0.2 microns and diameter of 25 mm supplied by Sartoirius, Germany, Nuclepore track-etch membrane with pore size of 0.05 microns and dimeter of 25 mm supplied by Whatman, USA.

Drug formulations

Clotrimazole 1% cream formulations – Mycotreat® and Canesten®, used in the method development and evaluation, were obtained from Bionika Pharmaceuticals, North Macedonia.

VDC system

The VDC system used in the study is an automated Hanson Vision Microette Vertical Diffusion Cell Apparatus with 6 cells. The VDC system has Type A diffusion cells as described in detail in USP chapter <1724>. 12 mL cells were used whose accurate volume is stated on every cell. In the cells the receptor medium is continuously stirred with the aid of metallic helix and magnetic stirrer located at the bottom of the cells. For maintaining a constant temperature of the receptor medium a circulating water bath is supplied with the apparatus. For the dosage supply, dosage wafers were used, which supply a dose of about 600 mg. Validation of the procedure for application of the cream was performed in order to ensure that with every experiment, in every cell, a suitable amount of drug - a dose of about 600 mg was applied.

HPLC method for evaluation of analytical results

The HPLC method for evaluation of the analytical results was fully validated by Bionika.Pharmaceuticals. The chromatographic conditions are as follows: Stationary phase: Purospher® STAR RP18e (5μm), 250 x 4.6 mm (i.d.); Column temperature: 25 ºC; Mobile phase: Acetonitrile:buffer = 75:25 (*v/v*); Buffer: 4.35 mg/mL of dibasic potassium phosphate; Flow rate: 1.5 mL/min; UV Detection: 254 nm; Injection volume: 25 μL; Run time: approximately 9 minutes.

IVRT Parameters

For evaluation of the Clotrimazole semi-solid formulations a set of parameters regarding the IVRT apparatus were applied. The parameters are presented in Table 1.

The set of parametars used were chosen based on general recommendations for performing IVRT test (Klein et al., 2018). The receptor medium solution was selected after performing a study to confirm sink conditions are maintained troughout the experiments. The temperature used during the experiment was 32 °C, as the product is intended for application to the skin surface. The stirring speed was set to 400 rpm as it was established that the medium is sufficiently stirred with this agitation speed. The duration of the test was set to 6 h as in accordance with the recommendations of EMA's draft guideline on quality and equivalence of topical products. The sampling schedule was set to collecting samples every hour in order to cover the overall release profile of Clotrimazole from the drug product. Twelve samples of every formulation were analyzed obtained from two separate experiments of 6 samples applied.

Table 1. IVRT tested parametars

Release Medium	10% PBS pH 7.4 : EtOH = 50:50
Franz Cells	12 mL volume (ca.1.8 cm² diffusion area)
Membranes	1.Nucleopore Track Etch, 0.05 µm 2. Regenerated Cellulose , 0.2 µm
Stirring speed	400 rpm
Temperature	32.0 °C ± 1 °C
Duration	6 hours
Time points	1h;2h;3h;4h;5h;6h

Evaluation of results

The active substance release from matrix systems such as creams, is trough diffusion mechanism. The mathematical model which fits best the release kinetics of the active substance from this kind of a matrix system is explained with the Higuchi model (Higuchi, 1961). According to this model, the release amount of active substance per unit area is linearly proportional to the square root of time. This assumption is based on a pseudo-steady state approach of drug release from thin ointment films containing finely dispersed drug into a perfect sink. This means that an infinite dose needs to be ensured during the experiment to obtain optimum efficiency of the method. To apply this linear model, Higuchi assumptions believe that the percentage of active substance release from the cream base should be less than 30% of the total amount of active substance applied. This mathematical model was applied for evaluation of the analytical results. The Higuchi plot is created for each cell as the individual amount of Clotrimazole released per unit area is plotted versus the square root of time, which yields a straight line. The slope of the release curve of Clotrimazole obtained is the rate of release. The average of 12 slopes for the tested formulation represents the drug release rate of Clotrimazole from the dosage form.

For evaluation of the results, the average cumulative amount of Clotrimazole released per unit area (µg/cm²) after 6 hours and the slope of the release curve representing the release rate of Clotrimazole from 12 samples of formulation analyzed were used. Statistical approach used for comparison of the obtained results for these two parameters was trough calculation of the 90% Confidence Interval for the ratio of means of the pairwise comparisons. The obtained upper and lower limit of the interval should fall within the limits 90-111% as proposed by EMA's draft guideline on quality and equivalence of topical products.

Results and discussion

FDA and EMA have encouraged the regulatory background for performing *in vitro* release tests as an essential part for development and evaluation of semi-solid formulations. The guidelines provide basic approaches that would lead to suitable development of a reliable and effective IVRT method. Although there is no uniform standardized protocol for development and validation of *in vitro* release test for topicals, several different frameworks have recently been published (Rath and Kanfer, 2020; Tiffner et al., 2018) that are found to be useful and comprehensive in performing validation activities. The procedure applied for development and validation of the IVRT method for Clotrimazole in this study, included suitable evaluation of the receptor medium based on sink conditions criteria, membrane qualification, followed by evaluation of method precision, linearity, discriminatory power and method robustness. The method was developed and validated on Mycotreat® formulation. Later on, the IVRT method was used for evaluation of similarity between Mycotreat® formulation and Canesten® formulation.

Receptor medium qualification

The choice of the receptor medium is one of the crucial steps in the development of a suitable IVRT method. The primary factor in establishing a proper receptor medium is to ensure appropriate solubility of the active substance in the medium and satisfactory sink conditions. In this context, the receiving medium should provide adequate amount of dissolved active substance in reasonable time period in order to ensure suitable evaluation of the release rate of the active component. Another factor that is preferable to take into consideration when choosing a receptor medium, is a medium which mimics the physiological conditions of the skin (Thakker and Chern, 2003). To simulate the physiological conditions, phosphate buffer aqueous solution (PBS) with pH 7.4 was chosen. As it is already well known that Clotrimazole has poor solubility in aqueous solutions, a different portion of ethanol as additive in the release medium (30%, 40% and 50%) was added in order to meet criteria for sink conditions. The solubility of the active component in the aforementioned media was determined through calculation of the concentration of saturation of solutions of Clotrimazole tested in triplicate for every medium, using

Table 2. Sink conditions for Clotrimazole obtained in 10% Phosphate Buffer Solution (PBS); 10% PBS : EtOH = 70:30 (*v/v*), 10% PBS : EtOH = 60:40 (*v/v*); 10% PBS : EtOH = 50:50 (*v/v*)

Release Medium	Saturation Concentration (mg/mL)
PBS	BLQ
PBS/EtOH=70/30	0.08 mg/mL
PBS/EtOH=60/40	0.74 mg/mL
PBS/EtOH=50/50	3.02 mg/mL

the calibration curve for determination of the linearity of the method. The results for solubility in the predefined release media are presented in Table 2. It was concluded that, Clotrimazole has very poor solubility in PBS solution with controlled pH of 7.4. This is rather expected, as it is known that Clotrimazole is slightly soluble in aqueous solutions. The addition of co-solvent ethanol increases the solubility of Clotrimazole.

To apply the linear Higuchi model, the percentage of active substance released from the cream base should be less than 30% of the total amount of active substance applied. In theory the highest possible concentration (assumption 30% release) of Clotrimazole in the receptor medium amounts 0.15 mg/mL when a dose of about 600 mg cream is applied in a 12 mL volume of VDC. Sink conditions are ensured if the determined solubility is at least 3 to 10 times higher than the concentration obtained at the end of the IVRT experiment. This calculated concentration theoretically is 1.5 mg/mL. As presented in Table 2, it can be concluded that satisfactory solubility is obtained in PBS: ethanol = 50:50 (*v/v*), approximatively 3 mg/mL, so this medium was chosen as the most suitable receptor medium for the IVRT evaluation, as it complies with the sink conditions acceptance criteria.

Membrane selection

The membrane used in IVRT methods serves as an inert barrier which separates the formulation and the medium during the course of the experiment. In order to maintain the assumptions of the Higuchi model, the membrane should offer no resistance to diffusion of the active component, but at the same time it should prevent other components from the formulation diffuse in the receptor medium. It should be chemically compatible with the receptor medium and the formulation in terms of not binding the active component, additionally it is desirable that the membrane should provide optimum release rate of the active substance that would result in capability to distinguish different formulations.

Artificial or synthetic membranes are commonly used for these purposes as they offer good reproducibility for product quality control purposes. It is also desirable during IVRT method development to evaluate different membrane chemistries. For these purposes two different types, hydrophilic and polycarbonate type of membrane were tested:

- Nucleopore track etched with pores of 0.05 µm – a polymer-based membrane that has been shown to be high-flux membrane in drug diffusion studies (Fern Ng et al., 2012);
- Regenerated Cellulose with pores of 0.2 µm - hydrophilic type of membrane, commonly used as synthetic membrane for release studies for drug control purposes.

As primary criteria, the membrane's ability to bind the active substance was evaluated. The membranes were incubated with Clotrimazole solution for 24 h at 32 °C. Samples after 24 h were analyzed to assess any decrease of Clotrimazole in solution. The results of the recoveries are presented in Table 3.

Table 3. Recovery of Standard incubated with Nucleopore Track Etch. 0.05 µm and RC 0.2 µm

	Recovery (%)	
24h Incubation	Regenereted Celluulose 0.2um	Nucleopore Track Etch. 0.05um
Sample 1	99.73	100.06
Sample 2	100.2	100.22
Sample 3	99.69	99.97
Average	99.87	100.08
RSD	0.28	0.12

Table 4. IVRT of Mycotreat® on Nucleopore Track Etch. 0.05 µm and Regenerated Cellulose 0.2 µm

	Cumulative Transport of API after 6 hours (µg/cm²) (Average of 12 samples tested)	Slope of the release curve (µg/cm²/h) (Average of 12 samples tested)	Relative standard deviation of Cumulative transport of API	Relative standard deviation of slopes
Nucleopore Track Etch. 0.05µm	531.76	284.56	9.62	11.08
Regenerated Cellulose. 0.2µm	447.88	269.49	7.02	6.99

Both membranes showed satisfactory recovery after 24 h incubation with standard solution of Clotrimazole, showing no binding to the active substance. Since none of the membranes showed a significant advantage in terms of recovery and the critical parametars, were both further submitted for IVRT analysis, in order to gain a better insight of the release characteristics of the drug product formulation. The IVRT conditions were applied for both membranes. For each membrane the average amount of Clotrimazole released per unit area and the slope of the release rate from 12 samples of formulation were evaluated. The summarized results of the obtained results from both membranes are given in Table 4.

For each membrane the Higuchi plot is created. The release profile of Clotrimazole from the formulation performed with both membranes is shown in Figure 1.

Both membranes showed no remarkable difference in the amount of drug released, Nucleopore Track etch 0.05 µm showed a dose depletion of ~15.9% of Clotrimazole, whereas Regenerated Cellulose 0.2 µm showed a dose depletion of ~13.4% of Clotrimazole, after 6 hours. For both membranes evaluated, a linear region of release was obtained. Accordingly, the drug release profiles with both membranes exhibited linearity with respect to the square root of time (Higuchi model) for the active compound, with determination coefficient > 0.9%. Furthermore, relative standard deviation of the cumulative transport (in µg/cm^2) and the slope of the release curve were evaluated, in order to determine the variability of the obtained results with the set of parameters used. Preferable limit for this parameter was RSD < 10 %. When comparing the two different membranes, in this term, the Nucleopore Track Etch. 0.05 µm had a higher RSD% of the slope (~11%). As the RSD% was lower with Regenerated Cellulose 0.2 µm (6.99%), due to less variability in the results, this membrane was chosen for further development.

Method precision

In order to assess inter-run and intra-run precision and reproducibility of the developed IVRT method, the IVRT analysis was performed by two different analysts on two different days. The Relative standard deviation among slopes and among the cumulative transport was evaluated. The results of the cumulative amount of Clotrimazole released per unit area at the end of the test and the slope representing the release rate of the active substance are given in Table 5. The acceptance criteria for a suitable reproducibility of the method were RSD < 10% for both the cumulative amount and the slope of the release curve. The Highuchi plot for both IVRT experiments performed by Analyst I and Analyst II is given in Figure 2.

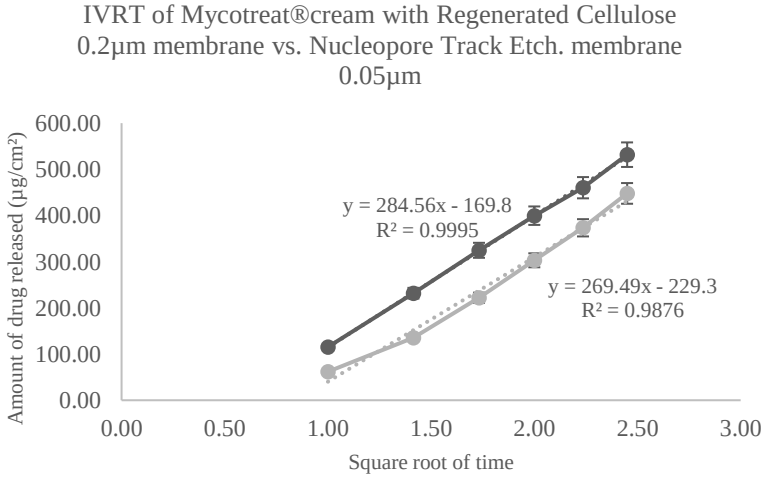

Fig. 1. Cumulative amount of Clotrimazole released from Mycotreat® formulation in IVRT experiment performed with Regenerated Cellulose and Nucleopore Track Etch. membrane.

Table 5. IVRT of Mycotreat® on Regenerated Cellulose 0.2 µm – Method Precision

	Cumulative Transport of API after 6 hours (µg/cm²) (Average of 12 samples tested)	Slope of the release curve (µg/cm²/h) (Average of 12 samples tested)	Relative standard deviation of Cumulative transport of API	Relative standard deviation of slopes
Analyst I	344.61	211.76	3.51	4.62
Analyst II	328.19	204.39	5.08	4.83

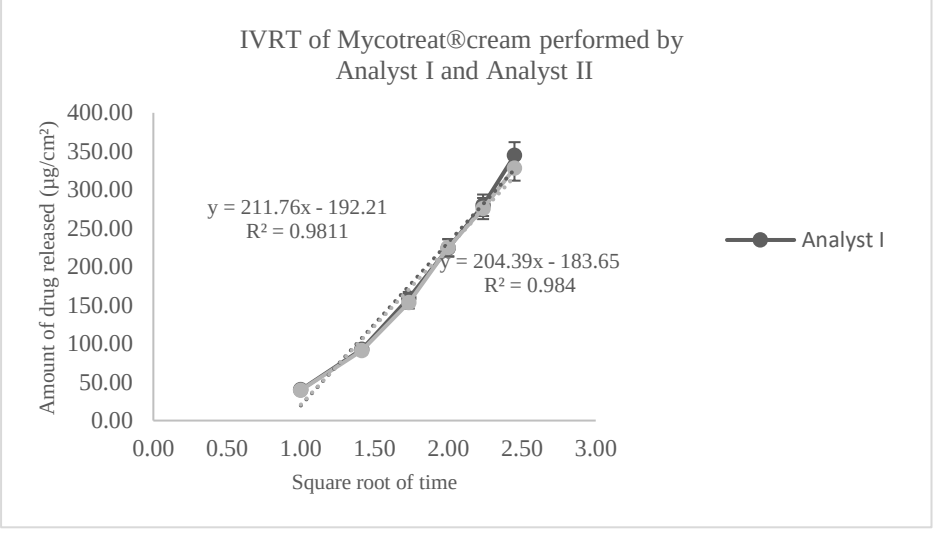

Fig. 2. Cumulative amount of Clotrimazole released from Mycotreat® formulation in IVRT experiment performed by Analyst I and Analyst II

Both experiments showed satisfactory linear trend of the results. For additional evaluation of the similarity of the experiments, the 90% Confidence Interval between the two experiments was evaluated. The obtained results are presented in Table 6.

As the upper and lower limit of the 90% Confidence limit for the ratio of means of the cumulative amount and of the slopes for both experiments falls between 90-111%, the method was found to be precise and furthermore reproducible with low variation of the obtained samples.

Discrimination sensitivity, specificity and selectivity

IVRT methods should have a suitable capability to distinguish different formulations. In this term, the parameters sensitivity, specificity and selectivity of the method play an important role. For evaluation of these parameters, modulation in the formulation regarding the dose amount was made, and the release rate as a function of the drug concentration was observed. Formulations with 50%, 100% and 200% API concentration were examined. The average amount of Clotrimazole released per unit area from 12 samples of each formulation and the slope of the release curve representing the release rate of Clotrimazole from the formulations were evaluated. The summarized results from 12 samples are given in Table 7, and the Highuchi plot for each formulation is presented in Figure 3.

Table 6. 90% Confidence Interval for evaluation of Method Precision

Formulation	90% Confidence Interval for cumulative transport		90% Confidence Interval for slopes		Significant difference
	Lower limit	Upper limit	Lower limit	Upper limit	
Analyst I vs. Analyst II	95%	96%	96%	98%	NO

Table 7. IVRT of 50%, 100% and 200% API Formulation

	Cumulative Transport of API after 6 hours (µg/cm²) (Average of 12 samples tested)	Slope of the release curve (µg/cm²/h) (Average of 12 samples tested)	Relative standard deviation of Cumulative transport of API	Relative standard deviation of slopes
50% API Formulation	197.45	119.14	9.72	9.98
100% API Formulation	344.61	211.76	3.51	4.62
200% API Formulation	805.28	488.07	7.92	7.53

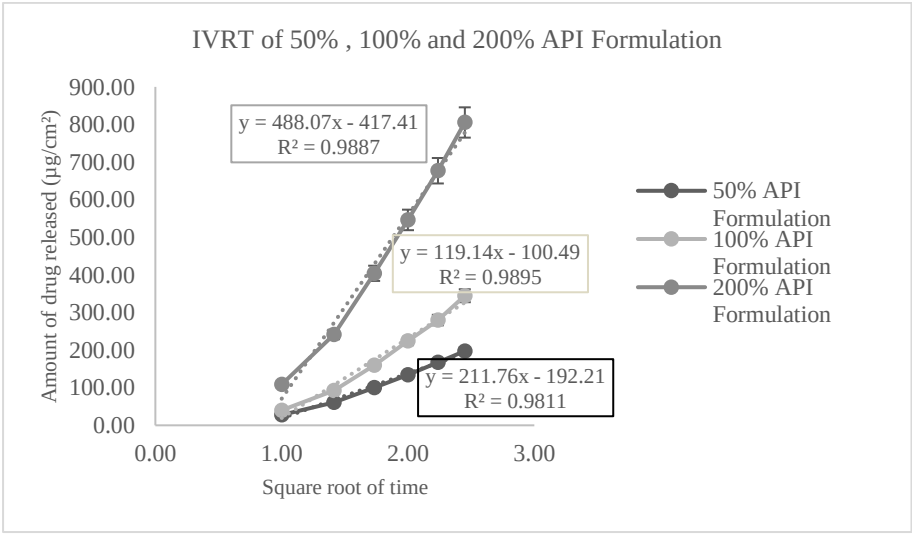

Fig. 3. Cumulative amount of Clotrimazole released from 50%, 100% and 200% API Formulation.

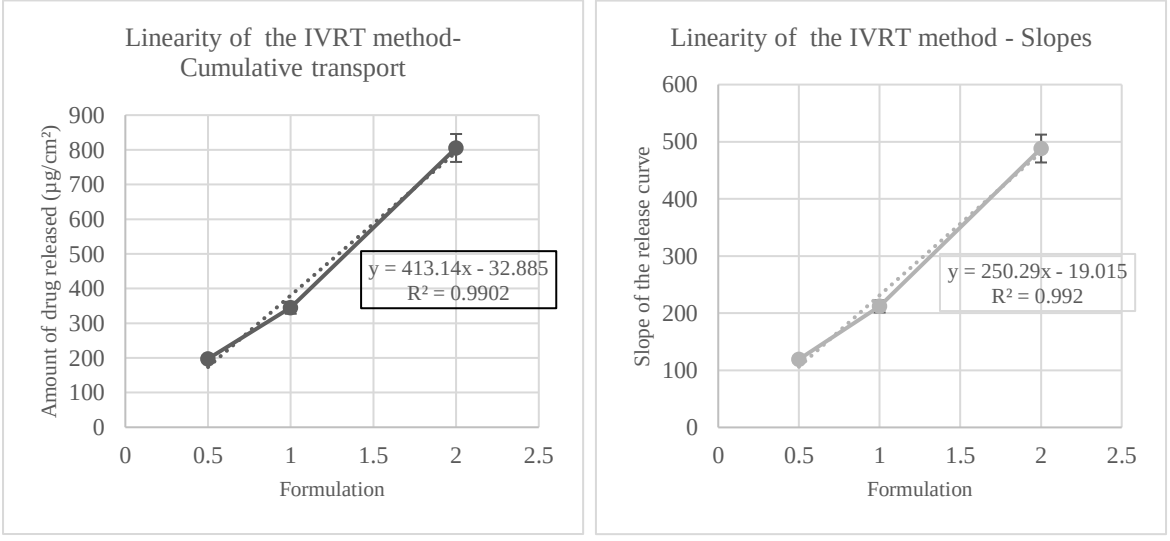

Fig. 4. Linearity of the IVRT method evaluated trough the cumulative amount and the slope of the release curve.

Table 8. 90% Confidence Interval for evaluation of IVRT Method Selectivity

Formulation	90% Confidence Interval for cumulative transport		90% Confidence Interval for slopes		Significant difference
	Lower limit	Upper limit	Lower limit	Upper limit	
Clotrimazole 0.5% Cream vs. Clotrimazole 1% Cream	57%	58%	56%	57%	YES
Clotrimazole 2% Cream vs. Clotrimazole 1% Cream	231%	237%	228%	234%	YES

The graphical representation shows that the IVRT method consistently identifies higher or lower rates of release as a function of the active substance concentration and accurately monitors these altered drug concentrations of the formulation, which confirms that the method has suitable sensitivity and specificity. The linearity of the method trough the function of the released amount of Clotrimazole and the slope of the release curve is presented in Figure 4. The method was found to be sensitive and linear, as the amount of active substance released was proportional to the concentration of active substance in the formulation.

In order to evaluate the method selectivity, a comparison was made between the three formulations in terms of the cumulative transport and the slope of the release curve of Clotrimazole, representing the release rate. The results of the 90% Confidence Interval are presented in a Table 8.

The 90% confidence interval falls out of the specified limits 90%-111%, showing that there is a significant difference between the formulations, which confirmed that the IVRT method is selective.

Discrimination: formulation changes

In terms of evaluating the discriminative power of the method, furthermore, as amended in EMA's draft guideline on quality and equivalence of topical products, formulation change in the amount of excipient composition was made, that would result in changes of the critical quality attributes of the formulation such as the rheological properties. The composition changes resulted in a formulation with much higher viscosity than that of the proposed formulation. The release profile of Clotrimazole form this discriminatory formulation was studied by preforming two separate experiments. This release profile of Clotrimazole was compared to Mycotreat® formulation with evaluation of the 90% confidence interval. The summarized results are given in Table 9 and the Highuchi plot for the Discriminatory formulation vs.Mycotreat® formulation is presented in Figure 5.

Table 9. IVRT of Discriminatory vs. Mycotreat® Formulation

	Cumulative Transport of API after 6 hours (μg/cm²) (Average of 12 samples tested)	Slope of the release curve (μg/cm²/h) (Average of 12 samples tested)	Relative standard deviation of Cumulative transport of API	Relative standard deviation of slopes
Discriminatory Formulation	254.83	135.46	6.36	7.25
Mycotreat® Formulation	344.61	211.76	3.51	4.62

Table 10. 90% Confidence Interval for evaluation of IVRT Method-Discriminative power

Formulation	90% Confidence Interval for cumulative transport		90% Confidence Interval for slopes		Significant difference
	Lower limit	Upper limit	Lower limit	Upper limit	
Discriminatory vs. Mycotreat®	73%	75%	63%	65%	YES

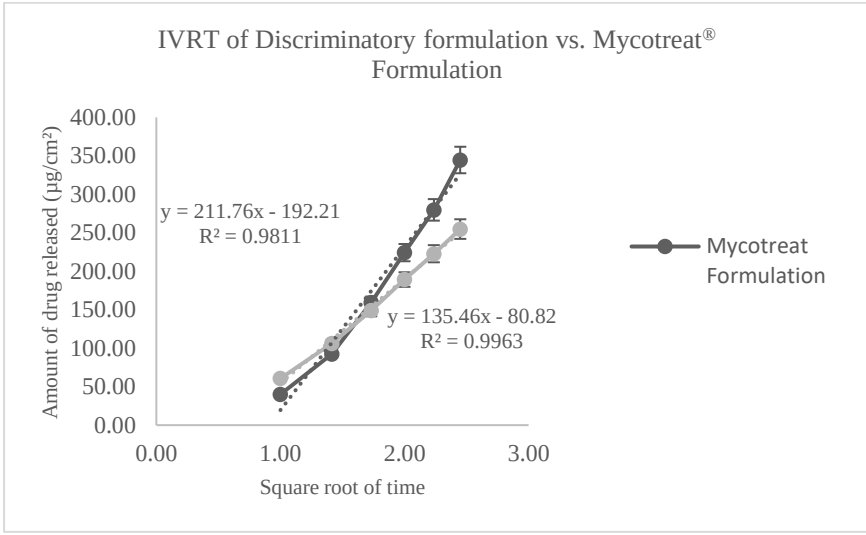

Fig. 5. Cumulative amount of Clotrimazole released from Discriminatory Formulation vs. Mycotreat® cream Formulation.

Evaluation of the 90% confidence interval (Table 10) shows that there is a significant difference between the two formulations and the IVRT method is efficient in discriminating between formulations with changed critical quality characteristics.

Method robustness

In order to assess method robustness, small deliberate changes in the method were made in terms of variation of the temperature of the cells (± 1 °C) and the rpm (± 50 rpm). The changes in the release rate were observed relative to the nominal method. The IVRT method was considered to be robust to the changes in method parameters, if the resulting mean release rate for the corresponding IVRT run did not deviate by more than 15% from the mean release rate for the nominal IVRT run during the precision and reproducibility investigations. The results are presented in Table 11.

The 90% confidence interval was evaluated as well, presented in Table 12.

The method was found to be robust and small changes in the parameters did not affect the method performance.

Table 11. IVRT – Method Robustnes

	Cumulative transport (µg/cm²)	Slopes (µg/cm²/h)
Nominal method	344.61	211.76
Altered temperature (31 °C)	334.28	210.38
Altered temperature (33 °C)	362.39	225.36
Altered rpm (350 rpm)	352.04	218.92
Altered rpm (450 rpm)	355.91	221.74

Table 12. 90% Confidence Interval for evaluation of IVRT Method – Robustness

Formulation	90% Confidence Interval for cumulative transport		90% Confidence Interval for slopes		Significant difference
	Lower limit	Upper limit	Lower limit	Upper limit	
31 °C vs. 32 °C	97%	98%	99%	100%	NO
33 °C vs. 32 °C	104%	106%	105%	108%	NO
350 rpm vs. 400 rpm	101%	103%	103%	104%	NO
450 rpm vs. 400 rpm	103%	104%	104%	106%	NO

Table 13. 90% Confidence Interval for evaluation of similarity between Clotrimazole creams: Mycotreat® vs. Canesten®

Formulation	90% Confidence Interval for cumulative transport		90% Confidence Interval for slopes		Significant difference
	Lower limit	Upper limit	Lower limit	Upper limit	
Mycotreat® batch no.1 vs. Canesten®	97%	99%	100%	103%	NO
Mycotreat® batch no.2 vs. Canesten®	101%	103%	108%	110%	NO
Mycotreat® batch no.3 vs. Canesten®	104%	106%	108%	111%	NO

Comparison of the drug product formulations

After a suitable method was developed and validated, the Clotrimazole formulations were compared and their similarity was evaluated. Three different batches of Mycotreat® formulation were compared with Canesten® formulation. Mycotreat® formulations showed a cumulative amount released of 321.16 μg/cm^2, 334.53 μg/cm^2, 344.61 μg/cm^2 of Clotrimazole after 6 hours respectively, with slopes of the release curve 195.81 μg/cm^2/h, 213.49 μg/cm^2/h, and 211.76 μg/cm^2/h. The Canesten® formulation showed a cumulative amount released of 328.70 μg/cm^2 of Clotrimazole after 6 hours and slope of the release curve 193.85 μg/cm^2/h. The 90% confidence interval was calculated for these formulations and evaluation of the results was performed (Table 13).

The calculated upper and lower limits for the pairwise comparisons were inside the CI limits of 90-111% for both creams. All three batches of the Mycotreat® formulation were found to be similar with Canesten® formulation. As both formulations have same qualitative and quantitative excipients, and penetration of Clotrimazole trough skin is not relevant to efficacy, the *in vitro* release test can be used as a nonclinical approach of demonstration of similarity of a generic drug product with a reference drug product, based on the concept of extended pharmaceutical equivalence for topicals (EMA`s Draft guideline on quality and equivalence of topical products, 2018).

Conclusion

A suitable IVRT method was developed and validated in order to meet the requirements for evaluation of the release profile of the active substance in marketed Clotrimazole cream formulations. The procedure was performed according to the framework of EMA's draft guideline on quality and equivalence of topical products. The approach used, offers an effective and easy-to-use evaluation of the critical validation parameters of an IVRT method. After successful validation, the IVRT method was applied to assess the similarity of the formulations. Both Clotrimazole 1% cream formulations were found to be similar. The developed and validated IVRT procedure can be potentially applied as a valuable tool for ensuring the quality of on-going production and batch to batch performance, as well as evaluation of other Clotrimazole cream formulations.

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Резиме

Валидиран и дискриминаторен *in vitro* тест за евалуација на Клотримазол крем формулации присутни на пазарот

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Клучни зборови: IVRT, клотримазол крем, валидација на метод, дискриминаторна моќ

In vitro сурогат тестовите широко се користат за евалуација на критичните карактеристики што влијаат врз перформансот на полуцврсти дозирани форми. Генерално, употребата на овие тестови е ограничена и служи за да се процени ефектот на клучните чекори на производниот процес врз физичко-хемиските карактеристики на формулацијата. *In vitro* тестовите за евалуација на профилот на ослободена активна компонента (IVRT) немаат способност да дадат предвидување за својствата на производот во *in vivo* услови, но може да детектираат промени во *in vitro* услови што корелираат со *in vivo* карактеристиките на производот. Целта на оваа студија беше да се развие соодветен IVRT метод наменет за евалуација на сличноста меѓу две клотримазол 1% крем формулации, присутни на пазарот. Валидацијата беше извршена согласно насоките на регулаторниот водич на EMA за евалуација на производи за надворешна употреба. Постапката опфати квалификација на медиумот за ослободување на активната компонента и мембраната, евалуација на прецизноста и робусноста на методот. За евалуација на резултатите од профилот на ослободена активна компонента беше користен статистички пристап преку пресметка на 90% интервал на доверба, чиишто горен и долен лимит треба да биде во рамките на границите од 90 - 111%. Линеарноста на IVRT методот беше евалуирана со 50% и 200% формулации на активна компонента. Дополнително,

дискриминаторната моќ беше потврдена со формулација со изменета вискозност како критичен атрибут за квалитетот. По развојот и валидацијата на IVRT методот, двата производи на клотримазол 1% крем беа споредени и беше потврдена нивната сличност. Предложениот систематски пристап се покажа корисен и сеопфатен за валидација на IVRT метод.