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Release Rate Determination of Eucrisa (Crisaborole 2%) Topical Ointment Using an IVRT Method Performed on the RDS Phoenix Automated Diffusion Station

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keywords: Phoenix RDS, diffusion, Eucrisa, IVRT study

The Phoenix RDS diffusion apparatus

Introduction

Eucrisa® is a brand-named Reference Listed Drug (RLD) product owned by Anacor Pharmaceuticals Inc., a part of Pfizer. The ointment contains 2% Crisaborole; it is useful to treat minor to mild eczema (atopic dermatitis). The patent on this product may expire on December 14, 2026.

Teledyne Hanson has developed and validated an in vitro release testing (IVRT) method for Eucrisa using the Phoenix RDS, an automated diffusion tester. A validated IVRT method can be used to support a demonstration of bioequivalence (BE) of generic drug product to the RLD. The use of the IVRT method is recommended by the United States Food and Drug Administration (US FDA) to assess the drug product sameness in the SUPAC-SS guidance [1, 2]. The IVRT is also established as a compendial procedure in the United States Pharmacopeia (USP) under the General Chapter <1724>. In this chapter, the test procedure, apparatus, and statistical methods to prove the product's similarity or sameness are described [3]. For certain types of products, the FDA's regulations generally require that the generic products be qualitatively (Q1) and quantitatively (Q2) the same as for the RLD [4]. The FDA also provides the recommendations for physicochemical and structural (collectively, Q3) characterizations that can be used to identify the dosage form of a proposed generic (test) topical product and to describe properties of the drug product that may be critical to its performance to support a demonstration of bioequivalence (BE) when comparing the Q3 attributes of two topical products [5].

Recently, a huge interest has been generated for the use of the IVRT method as an additional measure to prove product similarity in an Abbreviated New Drug Application (ANDA). The US FDA has published a product-specific draft guidance for Crisaborole 2% [6]. The European Medical Agency (EMA) has also published a guidance document for use of an IVRT procedure to access product quality and support for the equivalence of topical products [7,8,9].

Chemicals and Formulations

Crisaborole Certified Reference Standard was purchased from Sigma Aldrich. High-performance liquid chromatography (HPLC) grade acetonitrile and ethanol (95%) were purchased from Cole Parmer. RLD of Eucrisa (Crisaborole) 2% was purchased from a local pharmacy, lot number SDAF; expiry date: March 2024. The product was distributed by Pfizer. Topical products containing 50%, 100%, and 150% of the labeled amount of Crisaborole (2%)

were specially manufactured in a laboratory for use as test products and were identified as Crisaborole (2%). These products were manufactured by a well-trained scientist under the supervision of an experienced professor of Pharmacy in a laboratory at the Swami Vivekananda Education Society (VES) School of Pharmacy, Mumbai, India. Part of the analysis of this research work has been conducted in the same facility.

Formulation details and comparison

Sr No	Name of Excipients	Eucrisa	In-house
1	Crisaborole	√	√
2	Propylene Glycol	√	√
3	Butylated Hydroxytoluene	√	√
4	Mono- and Di- Glycerides	√	√
5	Paraffin	√	√
6	White Petrolatum	√	√
7	Edetate Calcium Disodium	√	√

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

The Crisaborole concentration in the IVRT samples were determined by an in-house qualified HPLC system (Shimadzu Scientific, model LC-2010). It contained a photo diode array (PDA) detector and was recalibrated in August of 2023. A Kromasil C18 chromatographic column (4.6 x 150 mm, 5 µ) was used for the entire study. The mobile phase consisting of 0.05% THF in water:acetonitrile in the ratio of 55:45 pumped at a flow rate of 1.0 mL/min following a 10 µL sample injection. The column temperature was maintained at 35 °C while the autosampler was kept at 15°C throughout the chromatographic runs. The eluate was monitored at a wavelength 250 nm.

Method development of the IVRT method

The IVRT method was developed at Teledyne Hanson's research laboratory. The method was validated by assessing membrane inertness, the solubility of Crisaborole in the receptor medium, linearity, precision, reproducibility, sensitivity, specificity, selectivity, and other parameters. A detailed method development and validation data are stored per the internal policy and procedures of Teledyne Hanson Research.

In Vitro Release Test of Crisaborole

The study was completed per the guidance provided in the US FDA's Scale-Up and Post Approval Changes Semisolids (SUPAC-SS). The receptor compartment of Vertical Diffusion Cells (VDC) was filled with 10 mL of 0.5% acetic acid in water:tetrahydrofuran:ethanol solution (55:15:30 V/V) and was maintained at 32 ±1°C; nylon membranes were mounted on each cell. After approximately 30 minutes, the cells were equilibrated, and about 400 mg of Crisaborole, 2% was applied to the membrane. To prevent evaporation and to maintain product integrity, the donor compartments were covered using glass discs. The receptor solution was kept continuously mixed throughout the test period using magnetic stirrers set at 400 rpm.

Diffusion Parameters

Cell Size	Small, 10 mL volume
Mixer Size	30 mm
Cell Cap	11.3 mm orifice × 4 mm
Temperature	32.0 ± 1 °C
Stirring Speed	400 rpm
Membrane	Nylon, 0.45 µ
Sampling Time Points	0, 0.5, 1, 2, 3, 4, and 6 hours
Sample Volume	400 µL
Replacement Volume	400 µL
Average Diffusional Surface Area	1.0 cm2

Calculation of Release Rates

The release rate was calculated using the Higuchi model, which assumes perfect conditions for the test. Obvious dilutions of the receptor media due to replacement was considered, and the concentration at each time point was determined using a RP-HPLC with PDA detector. The concentration of Crisaborole in the receptor medium at different sampling times and the cumulative amount of drug released were calculated using an in-house validated Microsoft Excel spreadsheet.

The release rate corresponds to the slope of the regression line for the plot of the amount of drug released (µg/cm^2) versus the square root of time (√t) and is affected by sample volume, cell volume, and the cell orifice diameter. Consequently, these parameters were verified during the process of apparatus qualification.

Statistical Analysis

As mentioned in the USP General Chapter <1724>, the statistical approach was used to calculate the release rates of the RLD product formulation ("marketed"), and each of the Crisaborole test formulations ("in-house") were used to calculate the Test/Reference (T/R) ratios. Six diffusion cells were used to test both products, hence, a total of 36 T/R ratios were obtained and placed in numerical order from lowest to highest. As required, the 90% confidence interval (CI) was determined from the listed T/R ratios, in which the 8th and the 29th ratio are the lower and upper limit, respectively. When the 90% CI is within the range of 75%–133.33%, the products are considered to be equivalent. The IVRT studies were conducted in accordance with the FDA's SUPAC-SS guidance [1]. The test products, i.e., in-house Crisaborole (2%), were compared against the reference product, Eucrisa (Crisaborole) 2%, as shown in Figure 1.

The samples were placed randomly on Vertical Diffusion Cells as test (T) and reference (R) products in accordance with the SUPAC-SS guidance [1]. The individual cumulative amounts of drug released from R and T were plotted versus the square root of time. Because of common testing artifacts such as air bubbles, membrane defects, and yield measurements that are not normally distributed, a nonparametric statistical technique is used to evaluate the test results. Since a few outliers are expected to occur during IVRT (e.g., due to air bubble formation), a nonpara-

metric method that tends to be resistant to the presence of such outliers was used. As suggested in the USP general Chapter 1724, the Mann-Whitney U test was used to calculate the 90% confidence interval (CI) for the ratio of slopes between Reference and Test products.

Comparative IVRT of Two Products

The results obtained when RLD of Crisaborole Ointment 2% known as Eucrisa was compared against an in-house Crisaborole (2%), as shown in Table 1. Comparison of the release rates between two products indicates inequivalence between them (Figure 1). At the end of the study, both products display a similar amount released; the differences in initial time point data are responsible for mismatch of the two products profiles. Although both products contained 2% Crisaborole, the difference in formulation in terms of the excipient types and quantities, as well as Q3 factors, may have impacted the study results.

Crisaborole RLD Product Data of Cumulative Amount of Drug (µg/cm2)							
√T (Hrs)	Cell-01	Cell-02	Cell-03	Cell-04	Cell-05	Cell-06	Mean
0.71	139	145	142	136	148	139	141.5
1.00	183.6	192.8	204.7	197.4	183.9	196.6	193.2
1.41	276.7	283.3	302.6	288.1	276	294.2	286.8
1.73	354.2	333.1	355.2	352.1	331.6	370.4	349.4
2.00	397.5	397.4	418.4	400.2	394.9	433.3	407
2.45	491.9	489.9	514.6	495.8	491.2	523.1	501.1
SLOPE	206.7	198.7	212.7	205.6	200.2	224.8	208.1
% RSD				4.6			
Regression	0.9973	0.9978	0.9987	0.9994	0.9953	0.9989	0.9994
Crisaborole 2% In-house Product Data of Cumulative Amount of Drug (µg/cm2)							
√T (Hrs)	Cell-01	Cell-02	Cell-03	Cell-04	Cell-05	Cell-06	Mean
0.71	217	215	214	215	233	214	218
1.00	291.7	290.6	289.6	275.6	306.3	286.6	290.1
1.41	365	363.9	362.8	354.3	378.2	358.7	363.8
1.73	402.8	400.6	400.5	400.7	413.5	397.2	402.6
2.00	443.6	443.3	441.2	430.4	453.6	438.8	441.8
2.45	504.4	503.1	497.9	478.7	512.7	496.4	498.9
SLOPE	160.2	160.6	158.6	151.9	155.7	158.2	157.5
% RSD				2.07			
Regression	0.9851	0.9845	0.9824	0.9795	0.9841	0.9857	0.9843

Table 1. Eucrisa RLD vs In-house product.

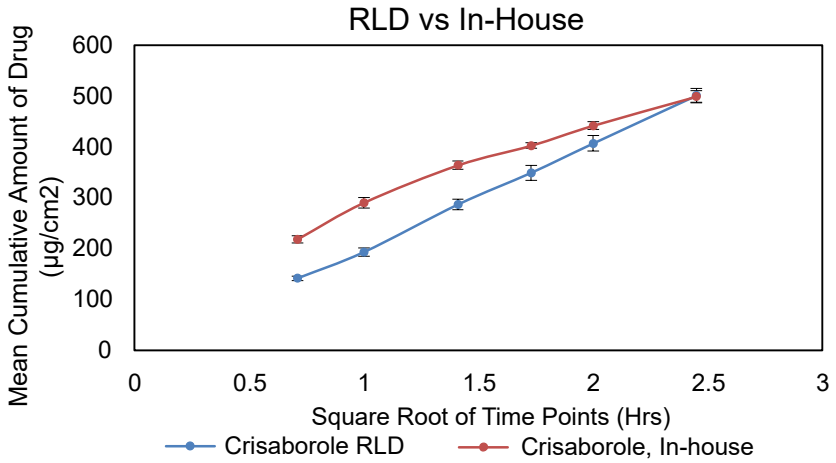

Figure 1. Eucrisa RLD vs In-house product.

Comparative IVRT of RLD in two different labs

The IVRT results of RLD, Eucrisa Crisaborole Ointment 2% obtained at two different labs were compared against each other and shown Table 2. Comparison of the release rates between the two lab's data indicates that if the test method is followed as indicated, it will produce same results. The graphical representation of the release rate is shown in Figure 2.

Conclusions

In vitro release tests (IVRT) of an in-house manufactured product and approved RLD products of Crisaborole were conducted according to recommendations of the US FDA Draft Guidance for Crisaborole, as well as the SUPAC-SS Guidance for non-sterile semisolid dosage forms and the USP General Chapter <1724>. IVRT study results of an in-house Crisaborole (2%) and RLD did not meet the acceptance criteria of 75%-133.33%. The comparison between the generics indicated that these were not equivalent in terms of release rate per the analytical test method used. This inequivalence may be because the generic formulations are not Q1/Q2 with RLD; possibly, the main reason is that a different grade of polymer was used in their formulation. However, the test results obtained at two different labs of RLD samples matched each other, and the specificity of in-house formulated products with 50%, 100% and 150% of labeled amount of 2% Crisaborole met the acceptance criteria for method specificity and sensitivity criteria.

It can be concluded that the IVRT method is very useful to accurately discriminate release rates, which could reflect the difference or similarity in product performance. Furthermore, the results indicate that the developed IVRT method and the tools used have an incredible ability to detect changes in a formulation. The results obtained in the study provide the evidence that Phoenix RDS equipment and the validated test method have the capability to accurately determine the release rate of Crisaborole from topical products. The combination of the two reliably provides compelling data that may be used in biowaiver applications.

RLD Analysis at Hanson Lab								
√T	Cell-01	Cell-02	Cell-03	Cell-04	Cell-05	Cell-06	Mean	%RSD
0.71	110.3	105.5	114.2	109.7	108.7	101.7	108.4	3.6
1.00	172.2	165.8	158.5	169.9	169.9	156.6	165.5	3.6
1.41	262.1	257.5	270.1	260.9	254.9	236.4	257.0	4.0
1.73	322.4	333.8	320.3	327.2	326.0	293.4	320.5	4.0
2.00	385.3	367.3	370.2	377.6	364.6	337.4	367.1	4.1
2.45	465.5	471.2	482.3	475.3	460.8	429.8	464.2	3.6
Slope	205.5	209.0	210.9	209.5	201.1	186.5	203.7	4.1
RLD Analysis at VES College								
√T	Cell-01	Cell-02	Cell-03	Cell-04	Cell-05	Cell-06	Mean	%RSD
0.71	139.0	145.0	142.0	136.0	148.0	139.0	141.5	2.8
1.00	183.6	192.8	204.7	197.4	183.9	196.6	193.2	3.9
1.41	276.7	283.3	302.6	288.1	276.0	294.2	286.8	3.3
1.73	354.2	333.1	355.2	352.1	331.6	370.4	349.4	3.9
2.00	397.5	397.4	418.4	400.2	394.9	433.3	407.0	3.5
2.45	491.9	489.9	514.6	495.8	491.2	523.1	501.1	2.6
Slope	206.7	198.7	212.7	205.6	200.2	224.8	208.1	4.2

Table 2. IVRT data of Eucrisa RLD tested at two different labs.

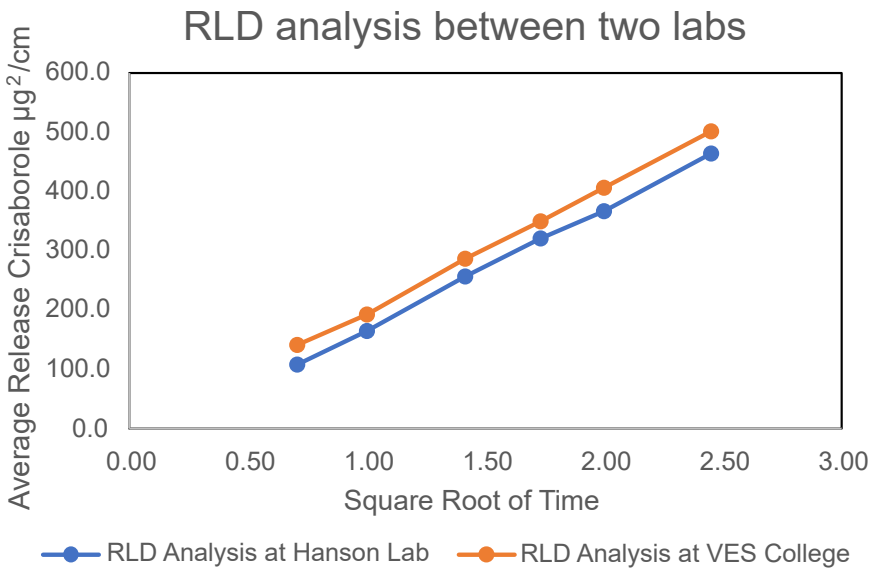

Figure 2. IVRT data of Eucrisa RLD tested at two different labs.

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