IVRT Method Development and Validation of Dapsone Gel, 7.5% Using the Phoenix RDS Diffusion Cell System

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Application Note H-AN-012

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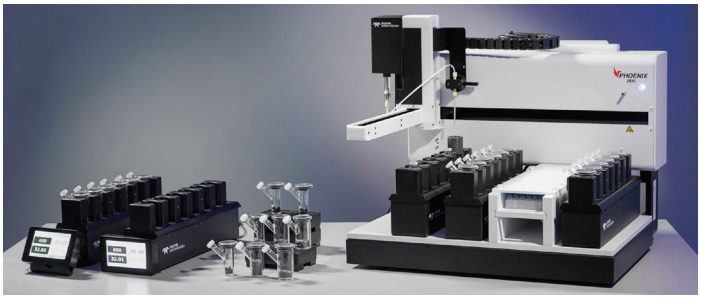
Introduction

In vitro release rate testing (IVRT) can reflect numerous or combined effects of several physical and chemical parameters, including solubility, particle size of the active pharmaceutical ingredient (API), and rheological properties of the dosage form. IVRT has been recommended by the United States Food and Drug Administration (US FDA) as an important test to assess pharmaceutical equivalence or inequivalence between two products, such as reference listed drugs versus proposed generics, or between products with pre- and post-approval product changes.[1]

In October of 2022, the US FDA published "In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs Guidance for Industry."[2] The utility of IVRT was previously extended in 2012 when the US FDA published a draft guidance for assessing the bioequivalence (BE) of acyclovir topical ointment, which was the first such publication recommending IVRT for use as a waiver of BE studies for a locally acting topical product.[3] This particular guidance provided useful and promising information for the future application of IVRT as a method to assess the sameness of topical formulations intended for local action. Subsequently, the US FDA has published several draft guidances for using in vitro methods for biowaivers for topical products.[3, 4, 5] In addition, a 2018 draft guideline published by the European Medicines Agency also makes provision to use IVRT for the approval of generic products.[6]

However, a comprehensive validation of the IVRT method is imperative to ensure that the resulting method has the requisite attributes of sensitivity, precision, selectivity, and reproducibility necessary to detect differences relating to qualitative (Q1) and quantitative (Q2) properties and the microstructure and arrangement of matter (Q3) between products.

In light of the facts described above, an IVRT method was developed to assess Dapsone Gel (7.5%). One marketed product containing 7.5% Dapsone and three additional Dapsone gels specifically manufactured to contain 3.75%, 7.5%, and 11.25% Dapsone were studied. A positive control was included to ensure that the method had the necessary capability to confirm sameness, and negative controls ensured that the method had the requisite discriminatory power to detect significant differences.



The Phoenix RDS diffusion apparatus

Materials and Methods

Chemicals and Formulations

Dapsone Certified Reference Standard was purchased from Sigma Aldrich. High-performance liquid chromatography (HPLC) grade acetonitrile and ethanol (95%) were purchased from Cole-Parmer. Dapsone Gel, 7.5%, lot number AC64371 and expiry dated March 2024, was manufactured by Taro Pharmaceuticals and is an approved, commercially available product. This gel was purchased from a local pharmacy. Topical products containing Dapsone were specially manufactured in a laboratory for use as test products and were identified as Dapsone Gel A (7.5%), Gel B (3.25%), and Gel C (11.25%). These three gels were manufactured by a well-educated 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.

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

An in-house qualified HPLC system (Shimazu Scientific, model LC-2010) was used. It contained a photo diode array (PDA) detector and was recalibrated in February of 2023. An ACE® Equivalence™ C18 chromatographic column (4.6 x 250 mm, 5 µ) was used for the entire study.

Membrane Screening

Membrane screening was carried out using various synthetic membranes such as nylon, polyethersulfone (PES), and polyvinylidene difluoride (PVDF). On the basis of the study data, the PVDF membrane was selected to perform the test. The binding of Dapsone was investigated on PVDF membranes. Each membrane was immersed in 10 mL of the test solution containing 2.0 µg/mL of Dapsone in the receptor medium of ethanol and water (60/40 v/v) at 32 ± 1 °C for six hours. The results of membrane screening are presented below in Table 1.

Membrane	Concentration of solution before membrane soaking Dapsone mg/ml	Concentration of solution after membrane soaking Dapsone mg/ml	% Recovery
Nylon	1.9965	1.9739	98.9
PES	1.9965	1.9719	98.8
PVDF	1.9965	1.9859	99.5
Cellulose Acetate	1.9965	1.9613	98.2

Table 1. *Membrane soaking into Dapsone solution of about 2.0 mg/mL.*

After receiving the data shown above, all four membranes were tested per the defined test method for the full IVRT study. The data from these tests is presented in Table 2 below.

	Dapsone release (µg/cm2)			
Time (Hrs)	Nylon	PES	PVDF	Cellulose Acetate
0.5	152.68	245.46	242.35	136.26
1	270.62	409.35	421.75	287.36
2	449.64	641.17	647.48	371.07
3	573.58	804.98	825.40	441.31
4	680.79	944.37	987.96	600.73
6	857.13	1179.06	1262.99	786.06
Average Slope	52.34	69.08	72.84	111.53
R 2	0.9991	0.9970	0.9971	0.9767
%RSD-released amount	7.05	5.85	7.61	7.85

Table 2. *Data for membrane screening, with 30% ethyl alcohol as receptor solution.*

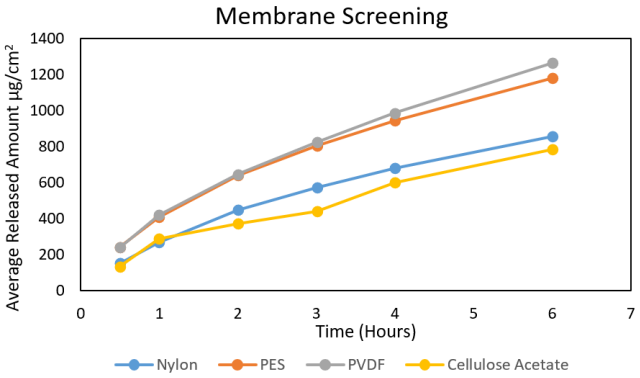


Figure 1. *Graphical presentation of membrane screening data.*

Dapsone Solubility and Receptor Solution Selection

Various receptor solutions were examined for suitability for use with the IVRT method. Various concentrations of isopropyl alcohol (IPA), ethyl alcohol, and dimethyl sulfoxide (DMSO) with water were individually evaluated.

The solubility of Dapsone in receptor fluid was investigated. The Certified Reference Standard of 20 mg was dissolved in 100 mL of receptor solution, which resulted in a 0.2 mg/mL concentration. There were no undissolved particles remaining in the flask after the solution was diluted to the 100 mL mark in a class A volumetric flask. The stock solution containing 0.2 mg/mL was far below the level of the expected released amount of Dapsone in receptor solution at the end of the IVRT study. The diluted standard solution of preparation 1 was evaluated against the second preparation of diluted standard solution and resulted in recovery of 99.8%.

Time (Hrs)	PVDF cumulative released Dapsone (µg/cm²)					
	10% IPA	10% DMSO	10% Ethanol	30% IPA	30% DMSO	30% Ethanol
0.5	105.63	115.43	73.16	214.30	145.50	242.35
1	183.54	196.32	124.20	379.59	225.80	421.75
2	303.85	309.86	196.13	615.06	348.30	647.48
3	389.82	396.31	257.37	764.06	425.50	825.40
4	465.16	469.87	311.30	961.03	501.90	987.96
6	593.53	594.11	400.35	1234.30	685.50	1262.99
Slope (Average)	36.19	35.42	24.24	75.05	94.75	72.84
R-Square (Average)	0.9997	0.9999	0.9988	0.9948	0.9885	0.9971
Slope (%RSD)	6.44	1.83	2.52	4.84	5.25	7.61

Table 3. *Solvent screening data using the PVDF membrane.*

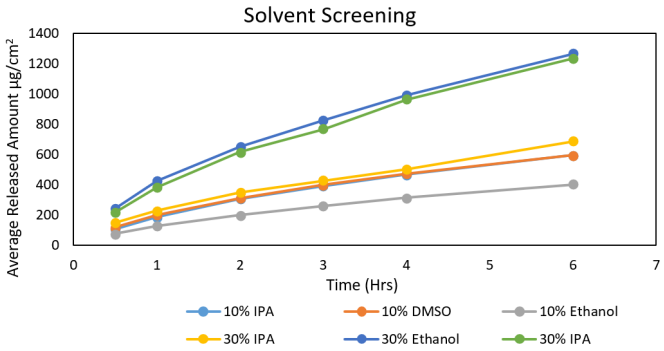


Figure 2. *Solvent screening profiles.*

Vertical Diffusion Cell and Assembly

In vitro release studies were performed using six vertical diffusion cells (each with a 1.0 cm^2 diffusional surface area) mounted on a Teledyne Hanson Phoenix RDS 12-station diffusion apparatus equipped with individual stirrer motors. The cells were heated with air; that is, they were heated on a dry heat block, which eliminates conventional water baths and improves temperature control and stability. The diffusion cells and apparatus were assembled with donor and receptor chambers separated by the selected synthetic membrane.

Apparatus Qualification

Environmental conditions such as the amount of suitable working area and workbench levelness, exposure to direct sunlight and direct cooling vents, capacity of each vertical diffusion cell (VDC), diameter of the VDC orifice, temperature of the receptor medium, stirring speed, mass of the magnetic stirrers, and dispensed sample volume were assessed.

Method development of the IVRT method

Successful IVRT is contingent on reliable drug transport from the test material through a membrane and into the receiving medium. Therefore, in identifying the optimal experimental parameters, the focus is on the active pharmaceutical ingredient's physiochemical properties and selecting the proper membrane, receiving medium, and sampling schedule.

The IVRT system was developed by assessing and optimizing membrane inertness, solubility of Dapsone in receptor fluid, linearity, sensitivity, specificity, selectivity, and precision parameters. Formulations of gels containing 50% (3.25% Dapsone gel), 100% (7.5% Dapsone gel), and 150% (11.25% Dapsone gel) were specially manufactured for the purposes of testing the sensitivity, selectivity, and specificity of the IVRT method. Generic Dapsone Gel, 7.5% was compared to in-house Dapsone Gel, 7.5%. Understanding and defining the release of an API from semisolid formulations is a critical aspect of product development. The objective of this project was to develop an IVRT method to assess the release and diffusion of Dapsone from different strengths of gel formulations intended for local action and to link the differences in release rates to formulation factors.

In Vitro Release Test of Gel Containing Dapsone

The studies were conducted in accordance with the US FDA's Scale-Up and Post Approval Changes Semisolids (SUPAC-SS) Working Group guidance. Dapsone Gel, 7.5% (~400 mg) was applied to PVDF membranes mounted on each cell. The donor compartments were then covered with glass discs to prevent evaporation of vehicle and ensure the integrity of the formulations throughout the respective study periods. The receptor chamber was filled with 10.0 mL of ethanol/water solution (60/40 v/v) and maintained at 32 °C. The receptor solutions were continuously stirred at 400 rpm using an individual magnetic stirrer in each VDC.

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

Calculation of Release Rates

Based on the periodical concentrations of Dapsone that were measured using RP-HPLC with PDA detection, the release rates were calculated using the Higuchi model, which assumes perfect sink conditions, as depicted in the equation below. Dilution of the receptor medium due to replacement of the sampled amount was taken into account, and the concentrations of Dapsone in the receptor medium at different sampling times were calculated using the equation

$$Q_n = C_n \frac{V_c}{A_c} + \frac{V_s}{A_c} \sum_{i=1}^{n} C_{i-1} \quad ,$$

where Q_n is the amount of drug released per unit area at each time (n) (µg/cm^2); C_n is concentration of drug in receptor medium at different sampling times (n) (µg/ cm^3); V_c is volume of cell (cm^3); A_c is area of the orifice of cell (cm^2); and V_s is volume of the sample (cm^3).

The release rate corresponds to the slope of the regression line of the plot of Q_n versus the square root of time. Q_n is affected by sample volume, VDC volume, and by the diameter of the orifice of the VDC. Consequently, the dimensions of these parameters were verified during apparatus qualification.

Statistical Analysis

The statistical approach that was used to perform the sameness test is described in USP General Chapter <1724>.[7] The release rates of the generic formulation ("marketed") and each of the Dapsone gel test formulations ("in-house") were used to calculate test/reference (T/R) ratios. A total of 36 T/R ratios were calculated. A list of the 36 T/R ratios was sorted from the lowest to the highest number. The 90% confidence interval (CI) was subsequently determined from the list of T/R ratios, whereby the 8th and the 29th ratio was set as the lower and upper limit, respectively. The predetermined criterion for equivalence is that the range of the 90% CI should be within 75%–133.33%.

Comparative IVRT of Dapsone Gel

The validated IVRT method for the analysis of topical Dapsone gel was applied to commercially available gel, as well as to a formulated 7.5% gel. These creams were assessed in accordance with the SUPAC-SS guidance published by the US FDA.[1] One gel product containing 7.5% Dapsone that is commercially available in the USA and Canada was included in this investigation. To test if the IVRT method had the necessary ability to determine both sameness and difference, a generic formulation was compared to itself as the positive control, and three gels specifically manufactured to contain 7.5%, 3.25%, and 11.25% Dapsone (Gels A, B, and C, respectively) were also included in these studies. The gels containing 3.25% and 11.25% Dapsone were used in the partial validation and served as negative controls.

Partial Validation of the IVRT Method

In accordance with the SUPAC-SS guidance, the resulting release rates for each VDC were calculated using linear regression.[1] If the release of Dapsone from its formulation follows the Higuchi equation, the amount released per unit area should be linear with respect to the square root of time. An R^2 value greater than 0.95 was considered acceptable to demonstrate linearity. The mean release rate and the variance components (inter-run and intra-run) were used to calculate the coefficient of variation (CV). The resultant slopes from six runs (n = 6) were used to calculate intra-run variability, and the mean release rate from each run (n = 6) was used to calculate inter-run variability. A CV of less than 15% indicated acceptable precision and reproducibility.[8] Inter-run and inter-run variability were within mean release rates of 28.331977.3 and 2012.2 mg/cm^2/min$^{1/2}$, respectively. Consequently, the intra-run and inter-run CVs were 1.92% and 1.35%, respectively. The CV values were less than 15%; therefore, acceptable precision and reproducibility of the method was confirmed, as illustrated in Figure 3.

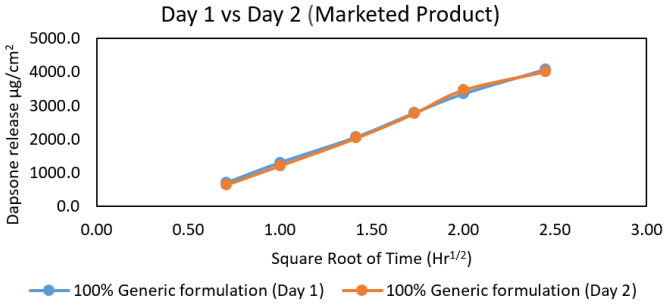


Figure 3. *Release profile for IVRT of marketed generic product: Dapsone Gel, 7.5%.*

Parameter	Acceptance Criteria	Results	Decision
Intra run variability	Day 1: Intra-run CV for (n = 6): < 15%	1.92%	Complies
	Day 2: Intra-run CV for (n = 6): < 15%	1.35%	Complies
Inter run variability	Inter-run CV for both runs (n = 12): < 15%	1.82%	Complies
Product sameness test (Positive control)	90% CI: 75%–133%	100.03% - 103.80%	Complies

Table 4. *Repeatability and sameness of generic Dapsone Gel, 7.5%.*

Sensitivity and selectivity were evaluated by determining the effect of changing the concentration of Dapsone in the gel formulations on Dapsone release rates. The method was considered sensitive, as the mean Dapsone release rate from the 3.25% Dapsone test gel was lower than that of the 7.5% Dapsone test gel, and the mean release rate from the 7.5% Dapsone test gel was lower than that of the 11.25% Dapsone test gel. The mean release rates (n = 3) from the runs with 3.25%, 7.5%, and 11.25% Dapsone gels increased with increasing Dapsone concentration: 2144.3, < 3538.4, and < 4679.5 µg/cm^2/min$^{1/2}$, respectively (Table 5).

Sr. No.	Dapsone Concentration (%)	Mean Slope (µg/cm^2/hour$^{1/2}$)
1	50	2144.3
2	100	3583.4
3	150	4679.5

Table 5. *Data for sensitivity and selectivity.*

Specificity was determined by evaluating whether the change in the Dapsone release rate was directly proportional to the three levels of Dapsone concentrations of the test gels. The IVRT method successfully distinguished between the three different products, as depicted by Figure 4. A linear relationship between Dapsone concentration and release rate was evident from the results with an R^2 value of 0.9939 (Figure 4).

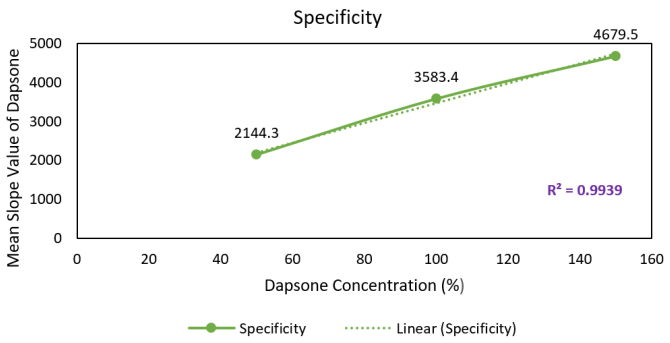


Figure 4. *Release rates of in-house Dapsone gels for 50%, 100%, and 150% label claim.*

Comparative IVRT of Two Topical Gel Products:

The results obtained when generic Dapsone Gel, 7.5% was compared to itself to provide a positive control (Figure 1). The 90% CI, which was determined to be 100.03%–103.80%, clearly indicates that the method was able to determine sameness (Table 4). Comparison of the release rates of Dapsone Gel A and generic gel clearly indicated inequivalence (Figure 5, Table 6). Although both products contained 7.5% Dapsone, formulation differences relating to the types of excipients and amounts as well as Q3 factors likely contributed to the results.

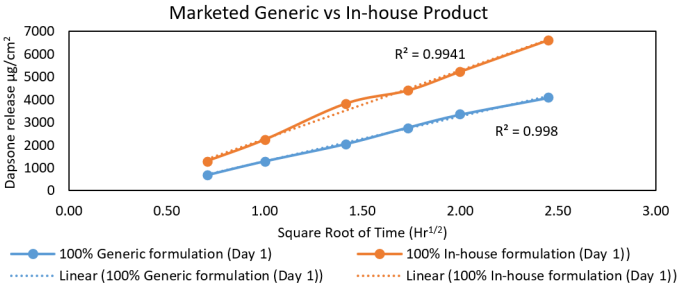


Figure 5. *IVRT data of marketed generics and in-house product at 100% label claim.*

Parameter	Acceptance Criteria	Results	Decision
Intra run variability	Day-1: Intra-run CV for (n = 6): < 15%	1.91%	Complies
	Day-2: Intra-run CV for (n = 6): < 15%	1.43%	Complies
Inter run variability	Inter-run CV for both runs (n = 12): < 15%	4.18%	Complies
Comparative study as per SUPAC-SS Guidance	90% CI: 75%–133%	147.98% - 154.51%	Fail

Table 6. *Repeatability and sameness of in-house Dapsone Gel, 7.5%.*

The specially prepared gels containing 7.5% Dapsone were found to be inequivalent to the marketed product of Dapsone Gel, 7.5%, thereby providing further evidence that the method had the necessary discriminatory power to determine the differences between products. The only difference in this case can be specifically related to differences in Q2 and, furthermore, also served as appropriate negative controls.

Comparative IVRT Between Two Laboratories

Marketed generic Dapsone Gel, 7.5% was tested at two laboratories: Laboratory 1 in New York, USA and Laboratory 2 at the VES College of Pharmacy at Mumbai, India. Comparative results obtained at both laboratories are presented in Table 7 below.

Square Root of Time (Hrs)	Average Release µg/cm²	
	Laboratory 1	Laboratory 2
0.71	695	759
1.00	1297	1378
1.41	2058	2188
1.73	2781	2727
2.00	3351	3189
2.45	4087	3903
Slope Average	1977	1801
%RSD of slope	1.70	3.70
Average R²	0.9971	0.9967
%RSD of R²	0.20	0.17

Table 7. *IVRT data for marketed generics of Dapsone Gel, 7.5% between two laboratories.*

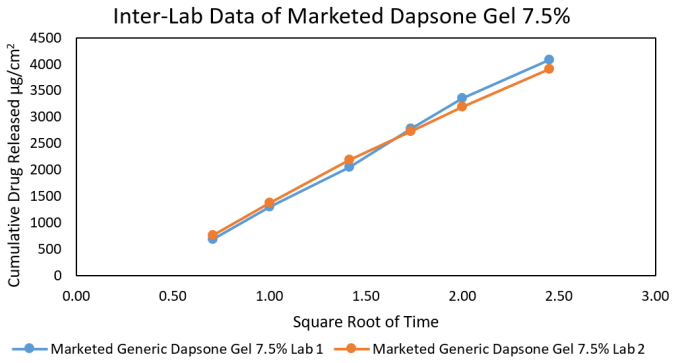


Figure 6. *IVRT data for marketed generics of Dapsone Gel, 7.5% between two laboratories.*

Conclusions

A comprehensive characterization of the operational parameters of an IVRT method was performed, and an IVRT method for Dapsone Gel, 7.5% was developed and partially validated. The IVRT method for the analysis of topical Dapsone Gel, 7.5% was applied to commercially available generic Dapsone gel. These gels were assessed in accordance with the SUPAC-SS guidance published by the US FDA.

The approved and the commercially available products were found to be in vitro inequivalent. This difference in the release of Dapsone between the two gels is explained by the polymer difference in both of the formulations. Positive controls (Generic vs Generic) show the good reproducibility of the method, and negative controls (Gels B and C) provided necessary evidence to confirm the discriminatory ability of the IVRT method. In addition, the resulting data indicated the potential of the IVRT method to identify differences in formulation and/or process variables (Q1/Q2/Q3). Furthermore, the comparative data between the different strengths of specially manufactured Dapsone Gel, 7.5% demonstrated that the IVRT method was able to show differences between these products which, irrefutably, are due to differences in Q2 only. The results indicate that the IVRT method was very precise and reproducible, thereby confirming its suitability to discriminate differences in release rates of Dapsone from topical gel formulations and its value as a useful tool in formulation development of topical products. Inter-laboratory data also conforms the robustness of both the IVRT and HPLC methods and their easy transferability between testing sites.

The study results obtained as mentioned above also prove that Teledyne Hanson's Phoenix RDS system is capable of producing excellent results meeting regulatory requirements for a biowaiver study using the IVRT approach.

References:

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