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A Novel and an Alternative In Vitro Release Test for Hydrophobic Diflorasone Diacetate Ointment with Dialysis Method by using Reciprocating Cylinder, USP Apparatus 3

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Abstract

A novel and reproducible in vitro release test method was developed for the performance assessment of an ointment formulation containing an anti-inflammatory drug substance called as Diflorasone Diacetate which is insoluble in water and its performance comparison with a reference drug product. In vitro release test was performed by using Reciprocating Cylinder known as USP Apparatus 3, alternatively to Franz Diffusion Cell and Flow Through Cell. Experiments were designed to evaluate the effect of the composition of receptor media containing phosphate buffer pH 5.8: Ethanol under the conditions at 32ºC±0.5, 20 dpm for 24 h. A dialysis membrane was evaluated: 12-14 KD MWCO, which is inert and practical. Experimental results indicated that the release amount of drug substance increased when ethanol ratio was higher. The requirement for the similarity between the generic dermatological preparation, which is corticosteroid in potent group with ATC code D07AC10 (AI Drug)* and Diflorasone Diacetate Ointment USP, 0.05% (RLD)** based on USP <1724> monograph for in vitro release performance has been provided.

Keywords: In vitro; Release; Performance tests; Semisolid; USP apparatus 3; Ointment release; Diflorasone diacetate; Franz diffusion cell; Flow through cell; dialysis

Introduction

A dosage form according to the USP pharmacopoeia is a pharmaceutical preparation consisting of drug substance (s) and / or excipient (s) to facilitate the dosage, administration and administration of drug product or placebo ingredient to the patient. [1]. Topically applied drug products are examined in two general categories. The first of these categories are those applied to provide local effect and the other is applied to achieve systemic effects after being absorbed into the bloodstream through the skin [2]. One of the advantages of administering drugs to the patient through the skin is that it can provide controlled and continuous application of the drug. In addition, drugs with short biological half-lives are ensured in continuous application. [3].

The most important similarity parameter for any drug product is its efficacy, as demonstrated in controlled clinical studies. When such trials are evaluated considering time and costs, they cannot be used as a routine quality control method and performance measurement. For this reason, in vitro testing is often used to ensure that product quality and performance is maintained over time and whenever there is a change. Various physical and chemical tests performed for semi-solid products and their components provide reasonable evidence of consistent performance in the past [4].

For pharmaceutical products, semisolid dosage form quality control tests include identification, assay, impurities, viscosity, particle size, and testing. An in vitro release test is used to monitor the release and diffusion of drug products from semisolid dosage form [5]. An in vitro release rate reflects the combined effect of several physical and chemical parameters, including the solubility of the active ingredient, particle size, and rheological properties of the dosage form. The in vitro release rate is a useful test to evaluate product identity between products before and after exchange [4]. In vitro methods allow the use of synthetic membranes and animal or human skin (biological membrane), and contribute toward the reduction, refinement, and replacement of in vivo testing.

In vitro release comparisons are based on recommendation texts described in the United States Food and Drug Administration (FDA) Draft Guidelines for acyclovir ointment [6] and the SUPAC-SS Guidelines for non-sterile semi-solid dosage forms [4]. Plots of release rates (slopes) of cumulative amounts of acyclovir released versus square root of time for various generic formulations compared to the reference product in the Draft Guide for acyclovir ointment were found to range from 75.00% to 133.33% with a 90% confidence interval. These experiments show that generic acyclovir cream formulations exhibit release rates comparable to the innovative product and can be considered pharmaceutically equivalent [5].

Vertical Diffusion Cell Apparatus and USP Apparatus 4 (Flow Through Cell) are recommended in USP <1724> monograph for in vitro release test for semisolid drug products to carry out the performance tests. Also for semi-solid dosage forms in same monograph, it is stated that they are acceptable as extended release preparations [7]. Therefore, USP Apparatus 3 (Reciprocating Cylinder) device recommended for in vitro performance tests of extended release drugs can be used by placing the sample in a dialysis bag membrane.

A thick layer of semi-solid product is placed in contact with a medium in a reservoir. Diffusion takes place across an inert, highly permeable support membrane. The membrane needs to keep the product and the receptor environment separate and different. Membranes should offer the least possible diffusion resistance. In addition, the membranes should not be speed controllers [7]. Diflorasone Diacetate is a topically applied drug in the ointment dosage form on the market. It is used in the treatment of psoriasis and eczema as an indication. Diflorasone Diacetate is in a class of drugs called corticosteroids. It works by activating ingredients in the skin to reduce swelling, redness, and itching [8].

Diflorasone Diacetate can be absorbed through the skin and entered the systemic circulation. After absorption through the skin, topical corticosteroids are used in pharmacokinetic routes like systemically administered corticosteroids. Corticosteroids bind to different degrees of plasma proteins. They are primarily metabolized in the liver and then excreted by the kidneys. Some of the topical corticosteroids and their metabolites are also excreted in bile [9]. In this study, performance similarity of AI Drug and RLD was evaluated by using Franz Diffusion Cell and Flow Though Cell (USP Apparatus 4) recommended in USP <1724> monograph; alternatively, by using Recipricating Cylinder Apparatus (USP Apparatus 3).

Materials and Methods

Materials

a. Diflorasone Diacetate RS, 99.5% purity was purchased from USP (Maryland, USA)

b. Acetonitrile (JTB, USA; Catalog Number:104873)

c. Ethanol (Fisher, Hampton, USA; Catalog Number: E/0600DF/F21)

d. Sodium Hydroxide (Sigma, Missouri, USA; Catalog Number:06203)

e. Potassium dihydrogen phosphate (Merck, Darmstadt, Germany; Catalog Number:104873)

f. Dialysis membrane 12-14 KD MWCO was purchased

g. Glass Microfiber Filters Diameter 25 mm, GF/D (Whatman)

h. Cellulose Acetate Membranes, 0.45 µm (Whatman)

Equipments

a. Franz Diffusion Cell Apparatus (Hanson)

b. Flow Through Cell Apparatus, USP Apparatus 4 (Sotax)

c. Reciprocating Cylinder Dissolution Apparatus, USP Apparatus 3

d. Waters High Performance Liquid Chromatography equipped with an ultroviolet/visible dedector Waters 2489; a reversed phase column: Waters Symmetry C18 150 mm x 4.6 mm x 3.5 µm; a data recorder and processor system: Empower Software.

Composition of the formulations

AI Drug was developed by Abdi İbrahim R&D Center, Turkey. RLD was used as reference product was obtained from Taro Pharmaceuticals Inc, Canada. The composition of the formulation contain; Diflorasone Diacetate (Drug Substance), Propylene Glycol (softener), Glycerol (emulsifier), Vaseline (ointment base).

Methods

Preparation of samples

Dialysis membranes in appropriate length were cutter and saturated with receptor media. One side of the membrane was clamped with a dialysis clamp and filled with the ointment sample. Then, the other side of the membrane was clamped with a dialysis clamp, depicted in Figure 1. Each dialysis sample was prepared by carrying out this procedure and placed into the each vessel.

Release determination

The release profiles of AI Drug and RLD were measured at 32ºC ± 0.5 °C with using different ratio of receptor media containing phosphate buffer pH 5.8 and ethanol for 24 h, 20dpm (sampling time: 1h, 3h, 6h, 9h, 12h, 18h ve 24h) by Reciprocating Cylinder Dissolution Apparatus (USP Apparatus 3). Volume of receptor media 250 ml per each vessel. The receptor compartment was not renewed with the solution; therefore, cumulative calculation was used for the determination of the results obtained from HPLC method.

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HPLC method

The mobile phase was a mixture of acetonitrile and water (50:50, v:v). Mixed well and degassed before application. Experimental conditions were as follows: Injection volume 50 µl ; the mobile phase performed isocritically pumped at a flow rate 1.0 ml/min; column temperature 25°C; sample temperature 5°C; the detection wavelength 254 nm. Method validation has been successfully completed by applying specificity, linearity and range, precision, accuracy and recovery, robustness parameters to In Vitro Release Test method in Diflorasone Diacetate 0.05% Ointment.

According to validation studies results: In Vitro Release Test method is specific for Diflorasone Diacetate and robust in view of changes to flow rate, wavelength, column temperature. The linearity, accuracy and recovery of the method in the range of 0.0004 mg/ml-0.0060 mg/ml has been proved.

METHOD DEVELOPMENT

It is necessary that drug substance have a sufficient solubility in the media throughout the study without an effect on sink conditions while choosing a compatible receptor media for in vitro release test. Especially for the drug substances which has lower solubility characteristics in water, the aqueous buffer solutions or hydro-alcoholic medias can be used as a receptor solvent with an appropriate justification [10]. Any receptor media for release performance of the ointments containing Diflorasone Diacetate has not indicated by FDA or any pharmaceutical guideline. Therefore, the points below were considered to choose of the receptor media:

A. Determination of Diflorasone Diacetate solubility in the solutions

B. Determination of the hydrophobic/hydrophilic characteristics of the excipients

C. Determination of appropriate pH value, considering the indications of the drug and its target area

While selecting of the receptor media it was considered that oil of skin, sweat glands and fatty acid breakdowns by Staphylococcus brings about a slightly acidic pH value for human skin. Therefore, phosphate buffer pH 5.8 was chosen as a main component of the receptor media [11]. Diflorasone Diacetate is a white to pale yellow crystalline powder which is soluble in methanol and in acetone, sparingly soluble in ethyl acetate, slightly soluble in toluene, very slightly soluble in ether and insoluble in water [12].

Besides, the excipients in the formulation were evaluated. Propylene Glycol is miscible with water and with ethanol (96%) [13]. Glycerol is miscible with water and with ethanol (96%), slightly soluble in acetone practically insoluble in fatty oils and in essential oils [14]. Vaseline is insoluble in water, soluble in dichloromethane, chloroform, benzene, diethyl ether, carbon disulfide and turpentine [15]. In result, addition of an alcoholic structure to the receptor media was decided. Ethanol was selected because of high amount solubility of Diflorasone Diacetate in alcoholic structures, high amount vaseline in the ointment formulation which has an insoluble characteristic in water and the other excipients which are miscible with ethanol.

Receptor Media and Apparatus Selection

Vertical Diffusion Cell Apparatus and USP Apparatus 4 (Flow Through Cell) are recommended in USP <1724> monograph for in vitro release test for semisolid drug products to carry out the performance tests. Therefore, both were used to evaluate the release of the formulations in this study. In addition to that, USP Apparatus 3 (Reciprocating Cylinder) was alternatively used to determine the release performance.

Phosphate buffer pH 5.8

Diflorasone Diacetate raw material and reference standard were insoluble in phosphate buffer pH 5.8 based on its insolubility in water. In result, phosphate buffer pH 5.8 100 % was not selected as a receptor media.

Phosphate buffer pH 5.8: ethanol (80:20, v/v) and phosphate buffer pH 5.8: ethanol (50:50, v/v)

Firstly, both Franz Diffusion Cell and Flow Through Apparatus were used to perform in vitro release test for AI Drug and RLD with phosphate buffer pH 5.8: Ethanol (80:20, v/v) and phosphate buffer pH 5.8: Ethanol (50:50, v/v) as receptor medias. The studies were carried out by 0.45 µm cellulose acetate synthetic membranes and 25 mm microfiber filters for Franz Diffusion Cell and Flow Through Apparatus, respectively. There was no significant data for each study because of inability of release from the membrane surface, given in Table 1.

Alternatively, in vitro release studies were performed by using Reciprocating Cylinder, USP Apparatus 3, on AI Drug and RLD with phosphate buffer pH 5.8: Ethanol (80:20, v/v) as a receptor media under the conditions at 32ºC±0.5, 20 dpm for 24 h. The release was achieved with dialysis membrane 12-14 KD MWCO. Main calculated cumulative release of Diflorasone Diacetate (%) reached 11.2 % and 11.8 % at 24 hours respectively. When the dissolved amounts are considered for each period, both ointment formulation showed similar relationship between calculated cumulative release versus time. Results of phosphate buffer pH 5.8: ethanol (80:20, v/v) study were given in table 2 & figure 2.

Furthermore, in vitro release studies were performed by using Reciprocating Cylinder, USP Apparatus 3, on AI Drug and RLD with phosphate buffer pH 5.8: Ethanol (50:50, v/v) as a receptor media under the conditions at 32ºC±0.5, 20 dpm for 24 h. The release was achived with dialysis membrane 12-14 KD MWCO. The results of phosphate buffer pH 5.8: ethanol (50:50, v/v) study was given in table 3, Table 4 & Figure 3. The results

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proved that main cumulative release of Diflorasone Diacetate (%) was higher when the receptor media phosphate buffer pH 5.8: ethanol (50:50, v/v) was selected. Due to increase in ethanol ratio of the receptor media, Diflorasone Diacetate cumulative release (%) reached 23.6 % and 24.4 % at 24 hours for AI Drug and RLD, respectively. Hence, phosphate buffer pH 5.8: ethanol (50:50, v/v) was finalized as receptor media for in vitro release test study.

Table 1: Summary of in vitro release studies for in phosphate buffer pH 5.8: ethanol (80:20, v/v) and buffer pH 5.8: ethanol (50:50, v/v) for AI Drug and RLD by using Franz Diffusion Cell and Flow Through Apparatus.

Apparatus	Receptor Media	Conditions	Result
Franz Diffusion Cell	Phosphate Buffer pH 5.8: Ethanol (80:20, v/v)	600 rpm, 32ºC ± 0.5, 0.45 µm cellulose acetate synthetic membrane filters	A significant release profile was not obtained.
	Phosphate Buffer pH 5.8: Ethanol (50:50, v/v)	600 rpm, 32ºC ± 0.5, 0.45 µm cellulose acetate synthetic membrane filters	A significant release profile was not obtained.
Flow Through Cell	Phosphate Buffer pH 5.8: Ethanol (80:20, v/v)	200 ml volume, 10 ml/min, 32ºC ± 0.5, 25 mm microfiber filters	A significant release profile was not obtained.
	Phosphate Buffer pH 5.8: Ethanol (50:50, v/v)	200 ml volume, 10 ml/min, 32ºC ± 0.5, 25 mm microfiber filters	A significant release profile was not obtained.

Table 2: Release of Diflorasone Diacetate in phosphate buffer pH 5.8: ethanol (80:20, v/v) for AI Drug and RLD by using Reciprocating Cylinder, USP Apparatus 3.

Time (h)	AI Drug (6261806P1) (%)	RLD (J768529434) (%)
0	0	0
1	3.3	3.6
3	4.6	4.7
6	5.9	5.0
9	7.1	5.9
12	8.2	6.3
18	10.6	8.2
24	11.2	11.8

Table 3: Release of Diflorasone Diacetate in phosphate buffer pH 5.8: ethanol (50:50, v/v) for AI Drug and RLD by using Reciprocating Cylinder, USP Apparatus 3.

Time (h)	AI Drug (6261806P1) (%)	RLD (J768529434) (%)
0	0	0
1	2.6	7.8
3	7.5	10.4
6	13.7	12.9
9	17.3	14.3
12	19.7	15.1
18	22.4	18.6
24	23.6	24.4

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Table 4: Comperation of the release performance between AI Drug and RLD in two medias which have different ethanol ratio, phosphate buffer pH 5.8: ethanol (80:20, v/v) and phosphate buffer pH 5.8: ethanol (50:50, v/v) by using Reciprocating Cylinder, USP Apparatus 3.

	Phosphate Buffer pH 5.8: Ethanol (80:20, v: v)		Phosphate Buffer pH 5.8: ethanol (50:50, v: v)	
Time (hour)	AI Drug (6261806P1) (%)	RLD (J768529434) (%)	AI Drug (6261806P1) (%)	RLD (J768529434) (%)
0	0	0	0	0
1	3.3	3.6	2.6	7.8
3	4.6	4.7	7.5	10.4
6	5.9	5	13.7	12.9
9	7.1	5.9	17.3	14.3
12	8.2	6.3	19.7	15.1
18	10.6	8.2	22.4	18.6
24	11.2	11.8	23.6	24.4

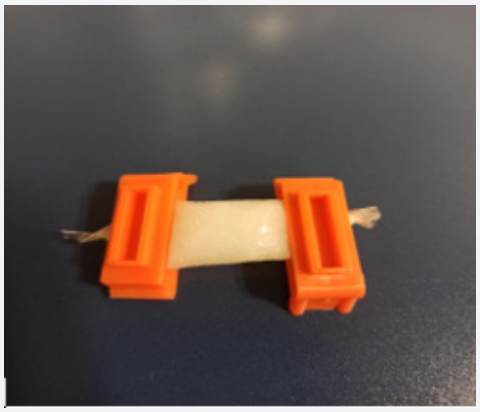

Figure 1: A dialysis tubing filled with the ointment sample.

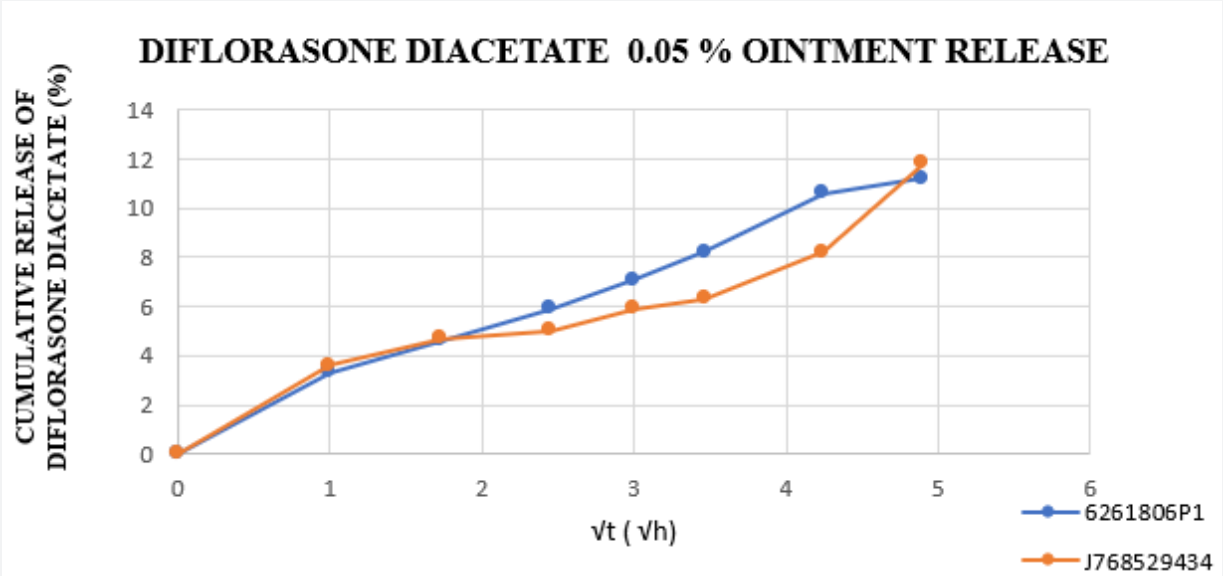

Figure 2: Cumulative release (%) vs √t (hours) graph for AI drug and RLD in in phosphate buffer pH 5.8: ethanol (80:20, v/v) by using Reciprocating Cylinder, USP apparatus 3.

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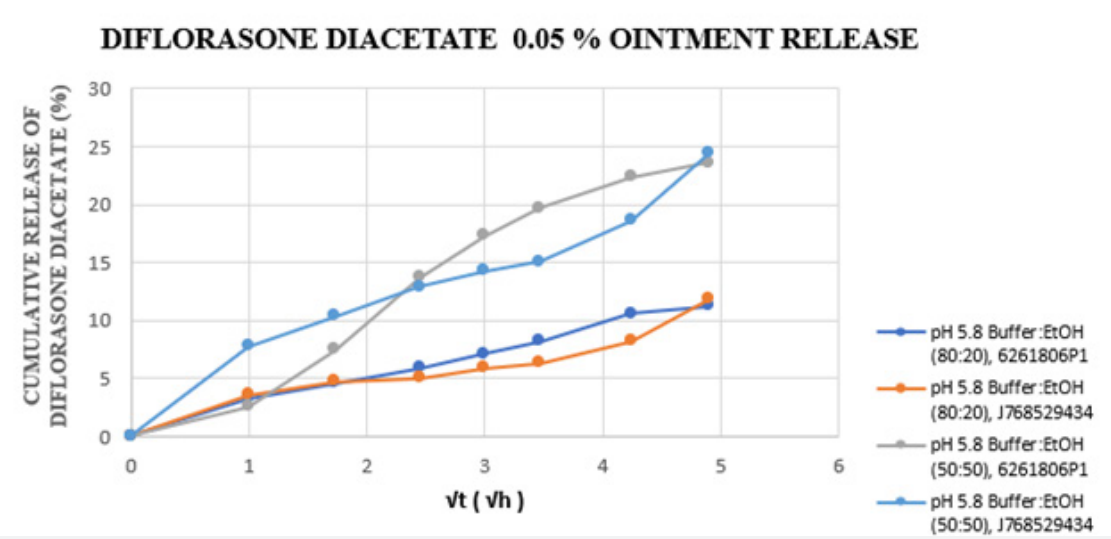

Figure 3: Cumulative release (%) vs square root of time √t (hours) graph for AI drug and RLD in two medias: phosphate buffer pH 5.8: ethanol (80:20, v/v) and phosphate buffer pH 5.8: ethanol (50:50, v/v) by using Reciprocating Cylinder, USP apparatus 3.

Experimental results showed that USP Apparatus 3 can be used to evaluate the performance of the drug products as an alternative method of Franz Diffusion Cell and Flow Through Cell. Besides, the release amount of drug substance increased when ethanol ratio was higher. Therefore, Reciprocating Cylinder USP Apparatus 3 was selected and phosphate buffer pH 5.8: Ethanol (50:50) was chosen as receptor media for in vitro release test.

Results and Discussion

Table 5: The cumulative Diflorasone Diacetate release amount for AI Drug with batch number: 6261806P1 as a function of time in the selected media, which is phosphate buffer pH 5.8: ethanol (50:50, v/v).

Diflorasone Diacetate Release %							
Sample Number	**1st hour (%)**	**3rd hour (%)**	**6th hour (%)**	**9th hour (%)**	**12th hour (%)**	**18th hour (%)**	**24th hour (%)**
1	2.5	6.3	11.5	15.1	18.9	22.2	24.7
2	2.8	6.8	12.3	16.2	19.6	22.9	24.5
3	2.3	7.7	14.5	17.7	19.8	22.4	22.9
4	2.5	7.7	14.4	17.6	19.4	22.1	22.6
5	2.7	8.4	15.0	18.8	20.2	22.5	23.6
6	2.9	7.8	14.6	18.1	20.2	22.2	23.2
Average	2.6	7.5	13.7	17.3	19.7	22.4	23.6
SD	0.2	0.8	1.4	1.4	0.5	0.3	0.9
RSD %	8.5	10.2	10.5	7.9	2.5	1.3	3.6
Min.	2.3	6.3	11.5	15.1	18.9	22.1	22.6
Max.	2.9	8.4	15	18.8	20.2	22.9	24.7

These studies were carried out based on USP <1724> monograph to monitor in vitro release performance of different ointment formulations, which were AI Drug (Batch Number: 6261806P1 & 6261806P2, manufactured by Abdi İbrahim İlaç San. Ve Tic.A.Ş) and RLD (Batch Number: J768529434, manufactured by Taro Pharmaceuticals Inc.) and prove the similarity in the release rate of the drug substance for phosphate buffer pH 5.8: Ethanol (50:50, v/v) receptor media under the conditions at 32ºC±0.5, 20 dpm for 24h.

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Each ointment formulation was tested for the release of the drug through a dialysis tubing membrane and showed an ability to release Diflorasone Diacetate active substance from the membrane surface. Tables 5 & 6 show the results of the cumulative release amount for the different formulations as a function of time. Table 7 show mean cumulative release amount by time for three ointment formulations. Cumulative release amount vs square root of time graph for each formulation is depicted in figure 4. As a result, it can be stated that each formulation gives almost parallel released amount after 6h period by reaching 13.7 %, 13.9 % and 12.9 % respectively.

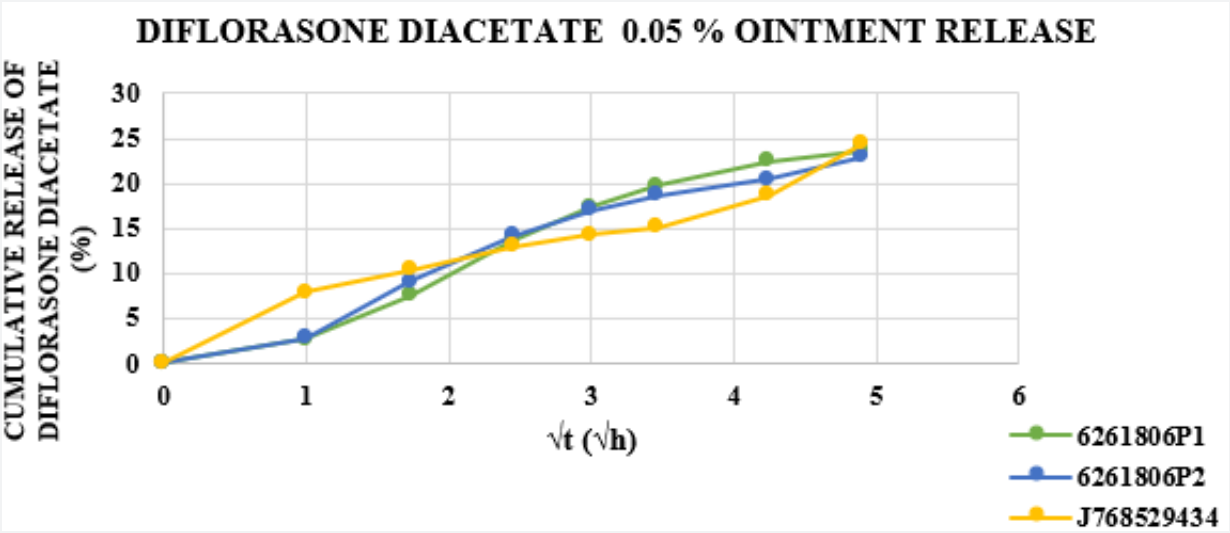

Figure 4: Cumulative Release (%) vs square root of time √t (hours) graph for AI Drug with batch numbers: 6261806P1 & 6261806P2 and RLD with batch number: J768529434, in the selected media: phosphate buffer pH 5.8: ethanol (50:50, v/v).

Table 6: The cumulative Diflorasone Diacetate release amount for AI Drug with batch number: 6261806P2 as a function of time in the selected media, which is phosphate buffer pH 5.8: ethanol (50:50, v/v)

Diflorasone Diacetate Release %							
Sample Number	**1st hour (%)**	**3rd hour (%)**	**6th hour (%)**	**9th hour (%)**	**12th hour (%)**	**18th hour (%)**	**24th hour (%)**
1	2.6	9.1	14.1	17	18.5	20.0	21.5
2	3.0	9.4	13.4	15.5	16.5	17.4	21.6
3	2.3	8.0	12.9	15.9	17.9	20.0	22
4	2.4	7.7	13.6	17.7	19.5	22.7	25.5
5	2.8	9.8	14.4	16.5	18.3	19.4	21.3
6	2.8	9.5	14.7	16.6	18.0	18.3	21.1
Average	2.7	8.9	13.9	16.5	18.1	19.6	22.2
SD	0.3	0.9	0.7	0.8	1.0	1.8	1.7
RSD %	10.1	9.7	4.9	4.7	5.4	9.3	7.5
Min.	2.3	7.7	12.9	15.5	16.5	17.4	21.1
Max.	3.0	9.8	14.7	17.7	19.5	22.7	25.5

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Table 7: The cumulative Diflorasone Diacetate release amount for RLD with batch number: J768529434 as a function of time in the selected media, which is phosphate buffer pH 5.8: ethanol (50:50, v/v).

Diflorasone Diacetate Release %							
Sample Number	**1st hour (%)**	**3rd hour (%)**	**6th hour (%)**	**9th hour (%)**	**12th hour (%)**	**18th hour (%)**	**24th hour (%)**
1	9.2	11.9	14.3	16.1	16.6	18.9	24.4
2	8.6	11.1	13.7	15.3	15.8	19.0	23.2
3	6.7	8.4	10.6	11.4	12.3	19.8	25.7
4	8.4	11.6	14.3	15.8	16.5	18.4	25.4
5	6.9	9.7	12.3	13.7	14.5	17.5	23.1
6	6.8	9.6	12.4	13.4	15.1	17.7	24.5
Average	7.8	10.4	12.9	14.3	15.1	18.6	24.4
SD	1.1	1.4	1.4	1.8	1.6	0.9	1.1
RSD %	14.1	13.1	11.2	12.6	10.6	4.7	4.4
Min.	6.7	8.4	10.6	11.4	12.3	17.5	23.1
Max.	9.2	11.9	14.3	16.1	16.6	19.8	25.7

To acquire a quantitative approach on the performance of the generic drug products, the amount of drug released (%) at each sampling time (t_1, t_2, etc.) is determined for each vessel and the cumulative amount released plotted versus square root of time. The momentary rate of the release for the drug substance, which is dm/dt, becomes proportional to $1/\sqrt{t}$, which mirrors the slowing of drug release in the course of time. The slope of the cumulative release versus square root of time graph gives the rate of drug release [7]. The average value of 6 slopes from the graphs for each formulation is a measurement of the drug release rate from the test products and reference product.

The Mann-Whitney U test is used to determine the 90% confidence interval for the ratio of the slopes between the test and the reference products. The T/R slope ratios are calculated for each test-to-reference slope, which test batch is T and reference batch is R. The calculations are given with a table where the values for the slopes for test product and reference product are listed down the left side and across the top of the table, respectively. Then, The T/R slope ratios are calculated by multiplying 100. After the T/R ratios have been determined, the results are ordered from the lowest one to the highest one. The 8th and 29th T/R ratios must be within the range of 75.0 %–133.33% [7].

Table 8: The T/R ratios for AI Drug (Batch Number: 6261806P1) / RLD (Batch number: J768529434) listed from the lowest to the highest.

T/R ratios			
Number	**AI Drug (Batch Number: 6261806P1) / RLD(Batch Number: J768529434)**	**Number**	**AI Drug (Batch Number: 6261806P1) / RLD (Batch Number: J768529434)**
1	113.79	**19**	125.75
2	115.71	**20**	125.78
3	115.93	**21**	126.18
4	117.35	**22**	126.27
5	118.72	**23**	126.70
6	119.74	**24**	127.03
7	119.90	**25**	127.88
8	120.55	**26**	128.12
9	120.73	**27**	128.40
10	120.96	**28**	128.65
11	121.93	**29**	129.27
12	122.16	**30**	129.69
13	122.44	**31**	130.15
14	122.84	**32**	130.23
15	123.66	**33**	132.33
16	124.92	**34**	132.88
17	124.94	**35**	133.23
18	125.16	**36**	133.78

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Cumulative release (%) versus square root of time graphs (√t) plotted and their slopes for each vessel are given in figures 5-7. The ratio of the slopes between test batch and reference batch is listed in tables 7 & 8 and the summary is shown in Table 9. As a result, the performance test for AI Drug (batch numbers: 6261806P1 & 6261806P2) over RLD (batch number: J768529434) was accomplished. The slopes obtaining from the graphs for each vessel was calculated for each formulation. The T/R ratios was then determined. The 8th and 29th T/R ratios are identified. The ratios were between the range of 75.0 %–133.33% for both comparative studies.

Table 9: The T/R ratios for AI Drug (Batch Number: 6261806P2) / RLD (Batch number: J768529434) listed from the lowest to the highest.

T/R ratios			
Sıra No	**AI Drug (Batch Number: 6261806P2) / RLD (Batch Number: J768529434)**	**Sıra No**	**AI Drug (Batch Number: 6261806P2) / RLD (Batch Number: J768529434**
1	96.03	**19**	109.25
2	98.45	**20**	109.85
3	100.20	**21**	110.92
4	101.05	**22**	111.67
5	101.19	**23**	112.02
6	102.72	**24**	112.14
7	103.68	**25**	112.54
8	103.74	**26**	114.77
9	104.25	**27**	115.20
10	105.43	**28**	115.68
11	106.13	**29**	117.48
12	106.28	**30**	117.97
13	106.31	**31**	123.02
14	106.48	**32**	128.35
15	106.57	**33**	129.63
16	108.77	**34**	132.81
17	108.80	**35**	135.95
18	109.09	**36**	136.51

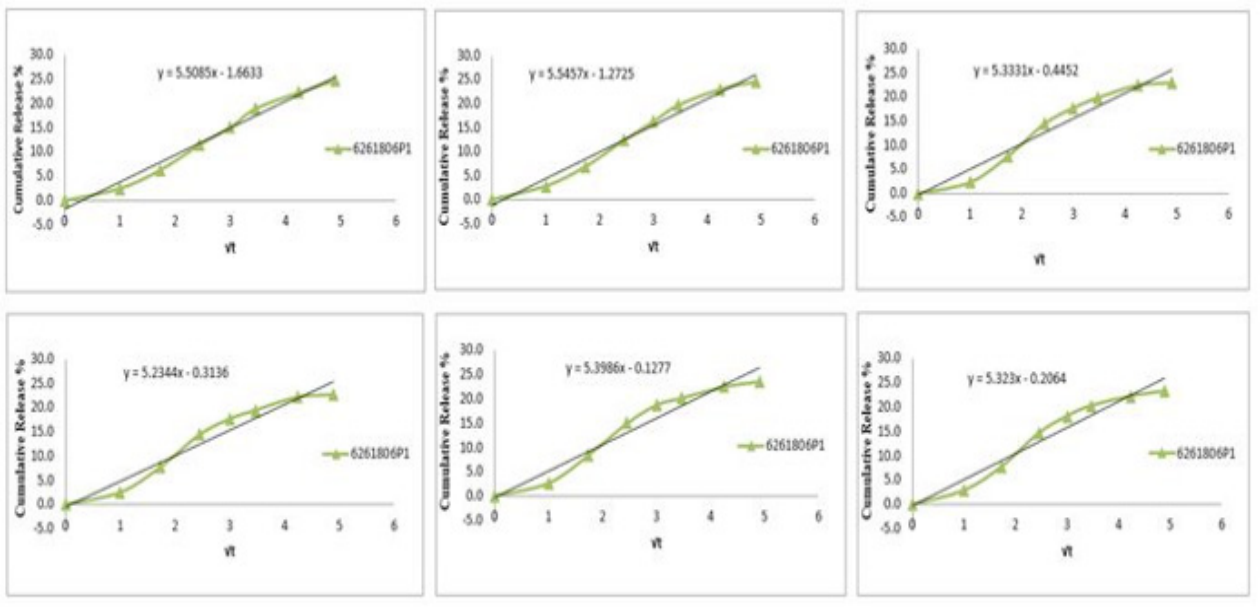

Figure 5: Cumulative Release (%) vs square root of time √t (hours) graph resulting from each vessel for AI Drug with batch numbers: 6261806P1 in the selected media: phosphate buffer pH 5.8: ethanol (50:50, v/v).

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Table 10: The 8th and 29th T/R ratios for AI Drug (Batch Number: 6261806P1 & 6261806P2) and RLD (Batch number: J768529434).

Mann-Whitney U Test Results (90% Confidence Interval)			
Test	**Reference**	**8th T/R ratio (%)**	**29th T/R ratio (%)**
AI Drug (Batch Number: 6261806P1)	RLD(Batch Number: J768529434)	120.55	129.27
AI Drug (Batch Number: 6261806P2)	RLD (Batch Number: J768529434)	103.74	117.48

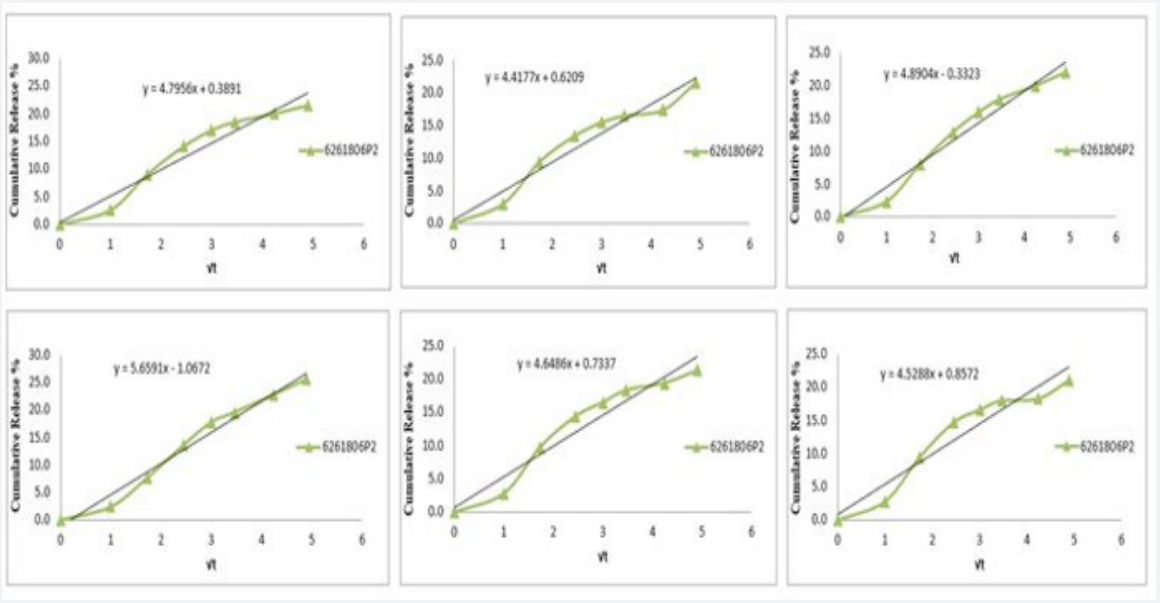

Figure 6: Cumulative Release (%) vs square root of time √t (hours) graph resulting from each vessel for AI Drug with batch numbers: 6261806P2 in the selected media: phosphate buffer pH 5.8: ethanol (50:50, v/v).

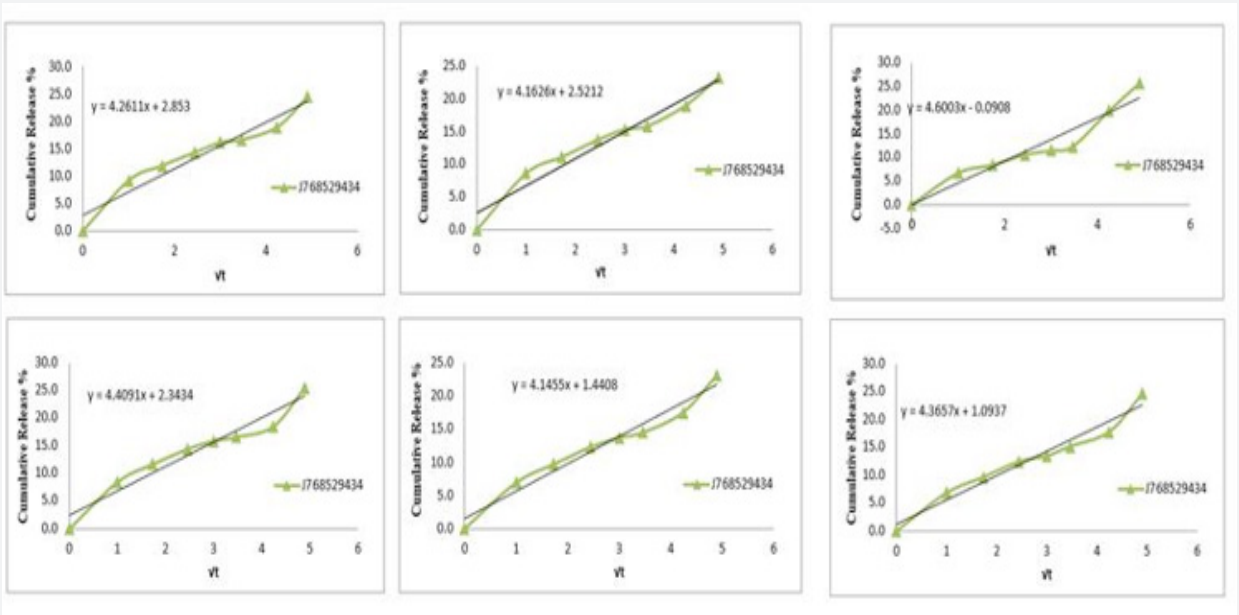

Figure 7: Cumulative release (%) vs square root of time √t (hours) graph resulting from each vessel for RLD with batch number: 768529434 in the selected media: phosphate buffer pH 5.8: ethanol (50:50, v/v).

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Conclusion

A novel and alternative in vitro release test method was developed for the ointment formulations, which have a hydrophobic characteristic impacting ability of release for drug substance from the product in a negative way, included Diflorasone Diacetate drug substance which is insoluble in water. The performance of the generic formulations against the reference drug was evaluated and similarity between the formulations based on USP <1724> was provided.

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