Research Article

Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Luliconazole and Salicylic Acid in Topical Formulation

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ABSTRACT

A specific, accurate, and precise stability-indicating RP-HPLC method was developed and validated for the simultaneous estimation of Luliconazole and Salicylic Acid in a topical formulation. This method ensures reliable quantification of both active ingredients, even in the presence of degradation products, following ICH guidelines for stability studies. RP-HPLC method was developed using Inertsil ODS 3V (250 x 4.6) mm, 5µ as stationary phase and mobile phase Buffer pH 4.5: Methanol (45:55 % v/v) used in isocratic mode. Sample was injected at 1.0 ml/min flow rate and detected at 240 nm wavelength. The method is validated according to ICH guidelines. The degradation was found in acidic, basic, oxidative, photo, and thermal condition.

Keywords: Luliconazole, Salicylic Acid, RP-HPLC, Forced degradation, Method Validation.

INTRODUCTION

FUNGAL INFECTION[1]

A fungal infection, or mycosis, is a skin disease caused by fungi. There are millions of fungal species, and they exist in various environments, including soil, plants, household surfaces, and even human skin.

There are four main types of fungal infections.[2]

1) Athlete's foot

2) Jock itch

3) Ringworm

4) Yeast infection

Luliconazole (LULI)[3]

It is known as (2*E*)-2-[(4*R*)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile (Shown in figure 1). It has a molecular formula of $C_{14}H_9Cl_2N_3S_2$ and molecular weight of 354.27 g/mol. It is a topical antifungal agent that belongs to a class of drugs called antifungal primarily used to treat fungal infections of the skin like ringworm, jock itch, and athlete's foot. It is official in Indian Pharmacopoeia, United Pharmacopoeia, British Pharmacopoeia, Japanese Pharmacopoeia, and European Pharmacopeia.

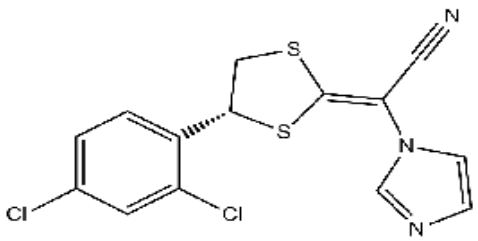

Figure 1: Chemical structure of LULI

Salicylic Acid (SALI) [4]

It is known as 2-hydroxybenzoic acid. (Shown in figure 2). It has a molecular formula of $C_7H_6O_3$ and a molecular weight of 138.12 g/mol. It is a topical medication that treats acne and other skin conditions. It's also known as a keratolytic agent. It is official in Indian Pharmacopoeia, United state Pharmacopoeia, British Pharmacopoeia, and Japanese Pharmacopeia.

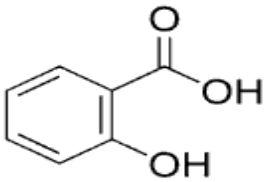

Figure 2: Chemical structure of Salicylic acid

The literature review reveals that few analytical methods were reported like RP-HPLC methods, UV Spectrophotometric, HPLC, chromatograph hy and response surface methodology in single or in Combination with other drug in bulk and pharmaceutical dosage form. However, there was only one UV Spectroscopy method reported for these drugs combination in pharmaceutical dosage forms and bulk drugs and hence present study aimed to develop a new Stability Indicating RP-HPLC Method Development and Validation for Simultaneous Estimation of Luliconazole and Salicylic Acid in a topical formulation e suitable for routine quality control analysis.

MATERIALS AND METHOD

Materials

Gift sample were obtained from Metro Chem PVT. LTD, Ahmedabad for Luliconazole and Salicylic acid respectively. HPLC grade water, dipotassium hydrogen phosphate, Potassium dihydrogen phosphate was purchased from

Merck Life Science Pvt. Ltd. Acetonitrile and Methanol were purchased from Rankem (HPLC grade) Pvt. Ltd. All other chemical used were of analytical reagent grade.

Instrumentation:

A High-performance liquid chromatography Shimadzu Model LC 2010 CHT Chromatography system equipped and UV detector used. Sample were injected at 100µL Volume. ODS 3V (250 × 4.6 mm, 5µ) column was utilized to develop an analytical method. Data acquisition and integration were performed using software.

Selection of UV wavelength

An acceptable response of two drugs was obtained at 240 nm and 225 nm.

Mobile phase selection and optimization.

Based on different trials, the mixture of Phosphate buffer (PH 4.5) and potassium dihydrogen ortho-phosphate at 6.8 gm/l. flow rate has proven to be better than the other mixtures of mobile phase in terms of peak shape, theoretical plates and asymmetry. HPLC chromatogram of Luliconazole and Salicylic acid at optimized condition was shown in Figure 3.

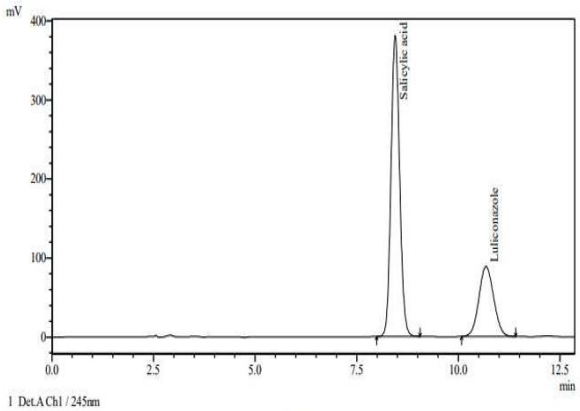

Figure 3: HPLC chromatogram of Luliconazole (30µg/mL, Rt-10.673 min) and Salicylic Acid (90µg/mL, Rt-8.441 min) using Phosphate buffer (pH 4.5) and methanol (45:55 % v/v)

Procedure:

- **Preparation of Mobile phase: (**0.05 M phosphate buffer) was prepared by using 6.8 g of potassium dihydrogen ortho-phosphate was dissolved in 1 Liter of water. pH 4.5 adjusted with diluted ortho phosphoric acid.
- **Preparation of standard stock solution:**
- **LULI stock solution (300 µg/ml):** Approximately 15 mg of luliconazole working standard was precisely weighed and transferred into a 50 mL volumetric flask. About 30 mL of methanol was added, and the mixture was sonicated to ensure complete dissolution. The solution was then diluted to volume with methanol to prepare the stock solution.
- **SALI stock solution (900 µg/ml):** Approximately 45 mg of salicylic acid working standard was precisely weighed and transferred into a 50 mL volumetric flask. About 30 mL of methanol was added, and the mixture was sonicated to ensure complete dissolution. The solution was then diluted to volume with methanol to prepare the stock solution.
- **LULI standard solution (30 µg/ml):** Pipette out 1 ml from stock solution into 10 ml volumetric flask and was diluted with methanol up to the mark. To prepare the working standard solution.
- **SALI standard solution (90 µg/ml):** Pipette out 1 ml from stock solution into 10 ml volumetric flask and was diluted with methanol up to the mark in the flask to prepare the working standard solution.
- **Mixed Standard Solution (30 µg/ml LULI + 90 µg/ml SALI):** Pipette out 1 ml of LULI stock solution and 1 ml of SALI stock solution into 10 ml volumetric flask. Volume was made up to the mark with methanol.

Forced degradation study [5-9]

A forced degradation study investigates the stability of a chemical or pharmaceutical product under stressful conditions (forced degradation research is also known as stress testing). “Stress” in this case means any physical or environmental conditions that a product will encounter that could cause a chemical change.

Stress conditions of acid (0.1 N HCl- 10 ml for 8 hours at room temperature), base (0.1 N NaOH - 10 ml for 8hours at room temperature), oxidation (0.3% hydrogen peroxide - 10ml for 8 hours at room temperature), photolytic stress (solution kept for in UV light for 1 day), heat (exposed at 50^0 C for 6 hours) were applied on sample stock solution containing 300 µg/ml Luliconazole and 900 µg/ml Salicylic acid. after completion of degradation, solutions were injected and monitored the chromatograms under optimized conditions and % degradation was calculated.

Method validation

Method validation is the process of confirming that an analytical procedure is suitable for its intended purpose and can produce reliable and consistent results.

Precision-Inter-day, intra-day precision and repeatability were assessed by injecting 6 independent sample solutions containing LULI (30 µg/mL) and SALI (90 µg/mL)

Specificity - Retention time was compared between the same concentration of standard and sample solution. Chromatograms were shown in figure-9.

Linearity and range - The linear correlation was obtained between peak area and concentration of 15–45 µg/mL for LULI and 45–125 µg/mL for SALI.

Accuracy: LULI (30 µg/mL) and SALI (90 µg/mL) solution were taken in three different flasks and 50%, 100% and 150% of the respective standard solution was spiked in it to carry out a triplicate recovery study and % recovery was calculated.

LOD and LOQ - LOD and LOQ for LULI (30 µg/mL) and SALI (90 µg/mL) were calculated by using the formula LOD = 3.3*SD/S and LOQ = 10*SD/S, Where SD: the standard deviation of the intercept, S: the slope of the calibration curve.

Robustness - It was studied by variations in method parameters like changes in flow rate, Wavelength and pH of the mobile phase

Assay of synthetic mixture:

An assay of a synthetic mixture involves determining the amount or concentration of specific components within the mixture. This process typically uses analytical techniques like chromatography or spectroscopy to separate and quantify the desired analytes. The goal is to determine the accuracy and precision of the method, often by comparing results to known standards.

RESULTS

Result of Forced degradation studies in synthetic mixture:

Luliconazole and Salicylic acid were undergoing degradation in synthetic mixture to different extents under different stress conditions. The result of Forced degradation studies is shown in Table 1.

Result of method Validation

Specificity- The difference between the retention time of the test and standard was found to be ±0.012min for LULI and ±0.010min for SALI.

Intraday, Interday Precision- Results were reported as %RSD. The results found are shown in Table 3.

Linearity and range: Linear correlation were obtained between peak area and concentration of SITA and GLIM in the concentration range of 50-150µg/mL and 1-3µg/mL respectively. Linearity graph is shown in figure 4 and 5.

LOD and LOQ- LOD and LOQ were found 1.68 µg/ml, 2.25 µg/ml and 5.09 µg/ml, 6.83 µg/ml for LULI and SALI respectively.

Accuracy study and recovery- % Recoveries were found in a range of 98.7-101.7% for LULI and 98.5-101.7% for SALI shown in table 4.

Table 1: % Degradation of Luliconazole

Condition	Sample		API	
	Standard area: 2302268		**Standard area:** 2360783	
	Area	**% Degradation**	**Area**	**%Degradation**
Acid	2124189	**7.7**	2124272	**10.0**
Base	2075253	**9.9**	2066290	**12.5**
Peroxide	2222292	**3.5**	2225217	**5.7**
Thermal	1889618	**17.9**	1880931	**20.3**
Photo	2124683	**7.7**	2178085	**7.7**

Table 2: % Degradation of Salicylic acid

Condition	Sample		API	
	Standard area: 5614309		**Standard area:** 5545347	
	Area	**% Degradation**	**Area**	**% Degradation**
Acid	5444437	3.0	5438089	1.9
Base	5444437	5.9	5278064	4.8
Peroxide	5262496	6.3	5076783	8.4
Thermal	5082826	9.5	5090982	8.2
Photo	5241496	6.6	5147731	7.2

Table 3: Result of Intraday, Interday Precision

Luliconazole (Intraday)			**Salicylic acid (Intraday)**		
Conc.	**Area Mean ± SD(n=6)**	**%RSD**	**Conc.**	**Area Mean ± SD(n=6)**	**%RSD**
30 µg/mL	2280810.667 ± 30594.45	0.43	90 µg/mL	5596190.6 ± 73982.17	0.28
Luliconazole (Interday)			**Salicylic acid (Interday)**		
Conc.	**Area Mean ± SD(n=3)**	**%RSD**	**Conc.**	**Area Mean ± SD(n=3)**	**%RSD**
15	1106607 ± 4225.83	0.38	45	2855303.3 ± 8315.360	0.29
30	2454481 ± 41248.47	1.68	90	5698134 ± 21013.33	0.37
45	3442933 ± 26292.36	0.76	125	7904097 ± 53319.40	0.37

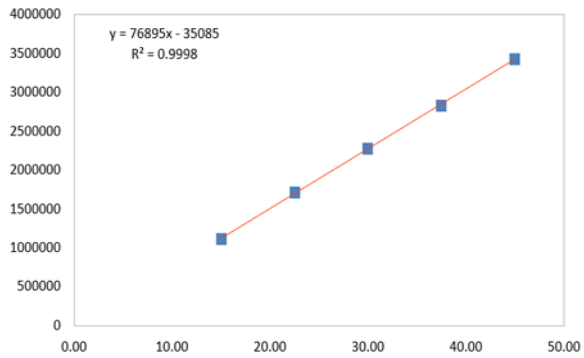

Figure 4: Linearity curve of LULI

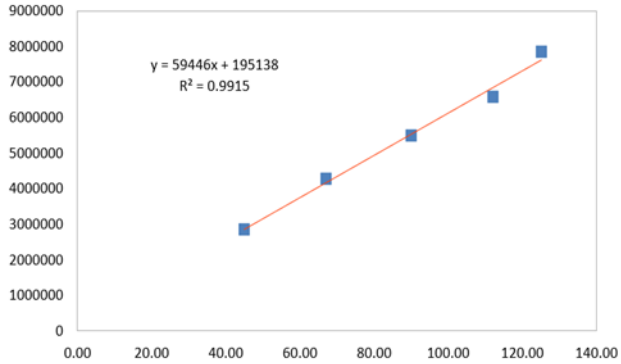

Figure 5: Linearity curve of SALI

Table 4: Result of recovery data of LULI and SALI

	Luliconazole		Salicylic acid	
Sr No	**Amount of added (mg)**	**% Mean recovery**	**Amount of added (mg)**	**% Mean recovery**
1	0.150	98.7	0.450	100.8
2	0.300	101.5	0.900	101.7
3	0.450	100.7	1.350	98.5

Table 5: Result of Robustness

	%RSD					
Drug	**Area at Temp. (-5°C)**	**Area at Temp. (+5°C)**	**Area at Flow rate (-10%)**	**Area at Flow rate (+10%)**	**Area at Organic phase (-2%)**	**Area at Organic phase (+2%)**
LULI	2263931	2291311	2517971	2070481	2246972	2301900
SALI	5668210	5592939	6167280	5106751	5680156	5529828

Robustness- The robustness of the method was studied by deliberate variation in method parameters like changes in flow rate, mobile phase ratio and pH of the mobile phase. %RSD was calculated. The mean %RSD was found to be less than 2. The results found are shown in Table 5.

Assay of marketed formulation: The result of % assay was found 100.7 ± 0.245 with RSD of 0.25 for LULI. For SALI it was found about 101.3 ± 0.133 and RSD was 0.13.

CONCLUSION

A specific, selective, sensitive and simple forced degradation RP-HPLC method was developed which is suitable for the determination of LULI and SALI in the presence of its degradation products in drug formulation. As per ICH guidelines, this method is robust, sensitive, accurate, selective and precise. The developed method is less time-consuming as well as cost-effective. It can be routinely applied for simultaneous estimation of LULI and SALI.

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