ASSESSMENT OF SIMULTANEOUS METHOD DEVELOPMENT AND VALIDATION OF FUSIDIC ACID AND LULICONAZOLE VIA HPLC AND ITS PERCENTAGE RECOVERY FROM MARKETED PREPARATION

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Abstract:
The combination of the luliconazole antifungal drug amd fusidic acid antibiotic drug combination is very rare but in pharmaceutical research it will be more helpful to reduce the dermal infection on the skin and patient will receive the simpler regimen at lower cost and take the benefit of this combinational therapy .But for the combination purity of the sample required and it is easily confirmed from the simultaneous method by using high performance liquid chromatography techniques (HPLC). In the present research work a HPLC method was developed and validated for assay of Fusidic acid and Luliconazole and its percentage recovery study in Topical pharmaceutical formulation. The chromatographic separation was achieved on reverse phase C18 , 5µm (125 X 4.6 mm) column at ambient temperature of 25^0C, at a flow rate 1 mL/min using Empower software of Waters HPLC . Different mobile phases were used on trial and error basis for separation of two drugs. The final mobile phase selected for analysis comprised of mixture of Methanol and water in the ratio of 85:15% (v/v/v). Both the drugs showed maximum absorbance at 246 nm which was selected as the wavelength of detection throughout the experimental work. Validation of developed method was carried out according to ICH guidelines. HPLC method was successfully developed for separation of fusidic acid and luliconazole with good resolution and after assessment of various parameters indicated low % RSD within an acceptable limit of < 2.0%. The developed HPLC method for estimation of FA and BD is rapid, reliable, precise, and reproducible.

Keywords: Fusidic acid, Luliconazole , HPLC, Method Validation etc.

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1. INTRODUCTION

Fusidic acid chemically is, 2-[(1S,2S,5R,6S,7S,10S,11S,13S,14Z,15R,17R)-13-(acetyloxy)-5,17-dihydroxy-2,6,10,11-tetramethyltetracyclo [8.7.0.0^{2,7}.0^{11,15}] heptadecan-14-ylidene]-6-methylhept-5-enoic acid. [1] Therapeutic class of Fusidic acid is an antibiotic & it is used for treatment of bacterial infection. It interferes with the bacterial protein synthesis which is require for multiplication of bacteria. It may not able to kill the bacteria but reduces its capacity to multiply further. As bacterial growth is restricted it eventually destroyed by the natural immune system of body. Fusidic acid is included in pharmaceutical preparation for the treatment of bacterial infection including one occurs in eczema.

Luliconazole is chemically known as (2E)-2-[(4R)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene]-2-imidazol-1-ylacetonitrile . Luliconazole is in a class of antifungal medications called azoles. It works by slowing the growth of fungi that cause infection. The combination of both these drugs is useful for the treatment of fungal infection; a skin disease. [2] It stops the growth of and reduces the itching, redness, swelling and crusting of the skin sores.Although, various analytical techniques have beendeveloped for estimation of Fusidic acid and Luliconazole individually or with other components in bulk drug and pharmaceutical dosage forms, the efficient and cost-effective analytical method has not yet been determined for estimation of these drugs. [5]

According to the AOAC International and analytical chemistry, all quantitative analytical techniques should, in general, satisfy a set of minimal performance requirements. [5] Currently, obtaining the required approvals requires the submission of analytical method validation data as a mandatory regulatory requirement. Particular guidelines have been released by the USFDA and ICH for carrying out analytical method validations.. [6-7]

One of the categories of analytical procedures to be validated is "quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product," per ICH guideline Q2 (R1). [7] Any analytical procedure that is validated must demonstrate that the analytical technique used is suitable for the intended application and that it is dependable, accurate, precise, sensitive, and specific enough to yield repeatable results.

This work developed and validated an HPLC method for the simultaneous analysis of Luliconazole and Fusidic acid in bulk drugs and combined topical pharmaceutical formulations

2. MATERIALS & METHODS

Chemicals and Reagents

Fusidic acid and Luliconazole were Purchased from the Sigma Aldrich and were used as working standards.

Marketed preparation Fusiwell cream and Lulizole creampurchased from the medical store. They were used without further purification and certified to contain 99.62 % and 99.80 % (w/w) fusidic acid and luliconzole respectively on dry weight basis. All analytical grade chemicals and reagents were purchased from Merck.

Instrumentation

Waters HPLC system with software Empower Ver. PDA detector, reverse phase C_{18}, 5µm (125 X 4.6 mm) analytical column, , Mettler Tolledo precision balance, pH meter (LAB INDIA) and Ultrasonic Cleaner along with Grade A certified glassware (Borosil) were used for the study.

Liquid Chromatography

The chromatographic separation was achieved using reverse phase C_{18}, 5µm (125 X 4.6 mm) analytical column at isocratic mode. The mobile phase consists of a mixture of Methanol, and HPLC grade water in the ratio of 85:15 (v/v/v) and degas by sonication. The flow rate and column temperature were maintained as 1 mL/min and 25ºC respectively throughout the analysis. The injection volume was maintained as 10µL.[8]

Standard and Sample preparation

Luliconazole standard stock solution

Standard stock solution of Luliconazole was prepared by transferring an accurately weighed 20 mg of luliconazole in 20mL volumetric flask. 20mL of methanol was added and the resulting solution was sonicated to dissolve the drug. The volume was made with methanol to prepare 1000µg/mL.[9]

Fusidic acid standard stock solution

Standard stock solution was prepared by transferring an accurately weighed 20 mg of FA in 20mL volumetric flask. 20mL of methanol was added and resulting solution was sonicated to dissolve the drug. The volume was made with methanol to prepare 1000µg/mL.[10]

Standard mixture preparation

Standard mixture was prepared by transferring 2mL of the standard stock solution of

Luliconazole and 1mL Standard Stock solution of Fusidic acid to 10mL volumetric flask. The volume was made with methanol to prepare 20µg/mL & 10µg/mL respectively.

Preparation of laboratory batch of cream formulation

Weigh 1 gram of cream , Dissolve it in 50 mL of methanol and sonicate it for 10 minutes. Heat at 60–65℃ until the base was dissolved and cool it at room temperature. Filter the extract through Whatman filters paper no. 42 and make up the volume up to 25 mL with methanol. Final stock solution containing Luliconazole (20 µg/mL) + Fusidic acid (10 µg/mL). From the above solution, 3, 4, 5, 6, and 7 mL was pipette out and transferred to 10 mL volumetric flask, and volume was made up to mark with methanol to give a solution containing luliconazole and fusidic acid for the concentration (3,4,5,6 and 7 ml)[11]

Method development

Mixture of working standard solution containing 10 µg/mL of FA and 20 µg/mL of Luliconazole were used for separation of two drugs and for development of method.

Optimization of HPLC method

Optimization of mobile phase

The mobile phases using combinations of various solvents such as Methanol: water (85: 15) . All solvents were filtered and sonicated for degassing and mixed in suitable combinations.

Optimization of flow rate

Optimization of flow rate was carried out by trying 1, 2, 2.5 mL/min of flow rates. The column was conditioned with methanol and allowed to saturate with mobile phase. Separation of both the drugs was recorded with different flow rate

Analysis of Laboratory batches

For mixed standard solution

Aliquots of 1 mL from working solution of Luliconazole (20 µg/mL) and 5 mL from the working solution of Fusidic acid (10 µg/mL) were taken into a common volumetric flask and diluted up to 10 mL with mobile phase to make final concentration .

For cream formulation

The experiment was carried out on six samples from a single batch of cream formulation. Solutions were injected in HPLC system and % RSD was determined.

Method validation

Linearity and Range

Linearity of the method for Luliconazole and Fusidic acid were evaluated by calibration equation and determination coefficient. According to calibration curves the method was found linear within the concentration range of 5-30 µg/mL. The linearity was studied over the increasing drug concentration and plotting the graph of peak area vs. concentration in µg. Standard solution of Luliconazole and Fusidic acid were prepared as described previously. Working standards of mixed standard stock solution were prepared at levels from 40% to 140%. Solutions were injected in the system and range was established from linearity study.

System Precision

The system precision of an analytical method is the degree of repeatability of the results in a series of experiments run during a single session operator with identical reagents and equipment**.** Solutions were injected and % RSD for retention times, standard areas, average tailing factor and number of theoretical plates were determined.

Method Precision

The method precision of an analytical procedure expresses the closeness of agreement from the multiple sampling of same homogeneous sample under prescribed conditions. The experiment was carried out using six assays from a single batch. Standard preparation in replicate (6 injections) was injected and % RSD for six assays of Luliconazole and Fusidic acid were determined.

Specificity

Placebo solutions (prepared similarly as the sample solution) and sample solution were analysed as per the method and the peak purity of Luliconazole and Fusidic acid peaks were checked

Limits of detection (LOD) and quantitation (LOQ) [Sensitivity]

The limits of detection and quantitation were defined as 3 times and 10 times the signal-to-noise ratio and were calculated using a mixed standard solution at a suitably low concentration level.

Accuracy

To ensure the accuracy of method, recovery studies were performed by standard addition method at 80 %, 100 % and 120 % concentration levels. Known amount of placebo was taken and spiked with known amount of Luliconazole and

Fusidic acid at three different levels, each in triplicate. The solutions were prepared and analysed by the proposed method. Percentage drug recovery for both the drugs was then determined.

Ruggedness

Ruggedness of the method was verified by analyzing six samples of a single batch of creams by two different analysts using similar operational and environmental conditions.

Robustness

Robustness of the method was checked by the system suitability parameters by deliberately varying the instrumental conditions such as flow rate (± 10 %), Methanol content in Mobile phase (± 2 % absolute), column oven temperature (± 5°C), and wavelength of detection (± 5 nm).

3. OBSERVATIONS AND RESULTS

Development of method and optimization of mobile phase

The mobile phase consisting of Methanol and Water in varying proportions and change in pH was tried. Finally, the ratio of 85:15 selected because it was found to give good separation for the peaks of Luliconazole (Rt- 1.25 minutes) and Fusidic acid (Rt- 0.25 to1 minutes). In addition, UV spectra of individual drugs were recorded at the wavelength range from 200 to 400 nm, and the response for optimization was compared. The choice of wavelength 295nm and 215 nm for luliconazole and fusidic acid were considered satisfactory, permitting the detection of both drugs with adequate sensitivity..

Several trials have been taken for accurate and precise method development. After using different solvents, column temperature, flow rates and good peak shape was obtained in reverse phase C18 ODS (250×4.6 mm,5µm) column with isocratic mobile phase Methanol: Water (85: 15). The standard solution of Luliconazole and fusidic acid in mobile phase were screened over 200 to 400 nm using photodiode array detector. On the basis of peak absorption maxima and peak purity index, 246 nm was decided as a detection wavelength which provided the maximum chromatographic compatibility to the method.

Chromatographic system

Column : 4.6-mm × 150 -cm, L1 (C18)
Wavelength: 295 nm luniconazole and 215 nm Fusidic acid and common wavelength 246 nm
Flow Rate : 1.00ml / min
Inject volume : 20 µl
Temperature : 25^0C
Run Time : 5.0 minutes

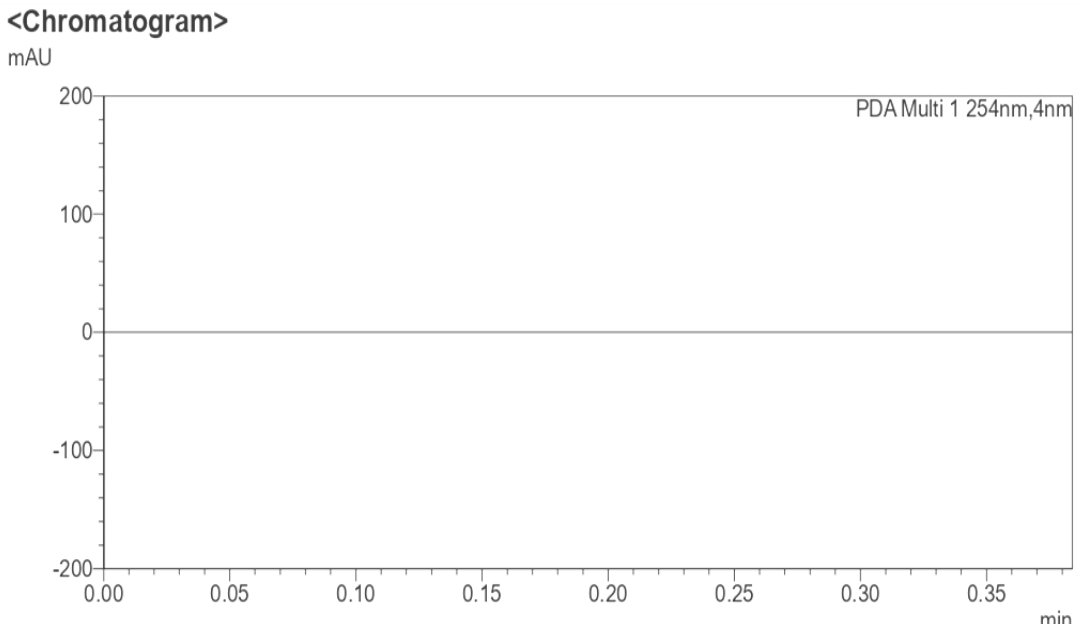


Fig1: HPLC graph of Blank (only solvent)

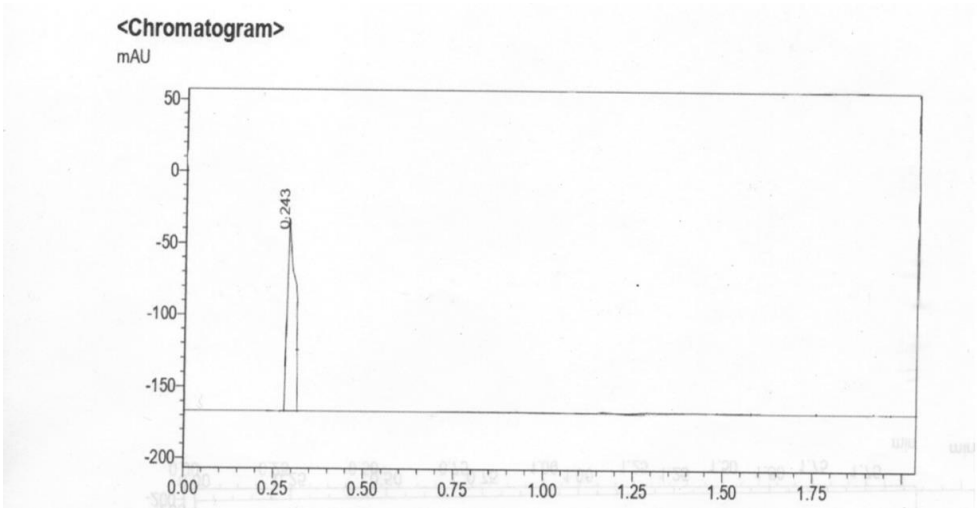


Fig2: HPLC graph of the fusidic acid

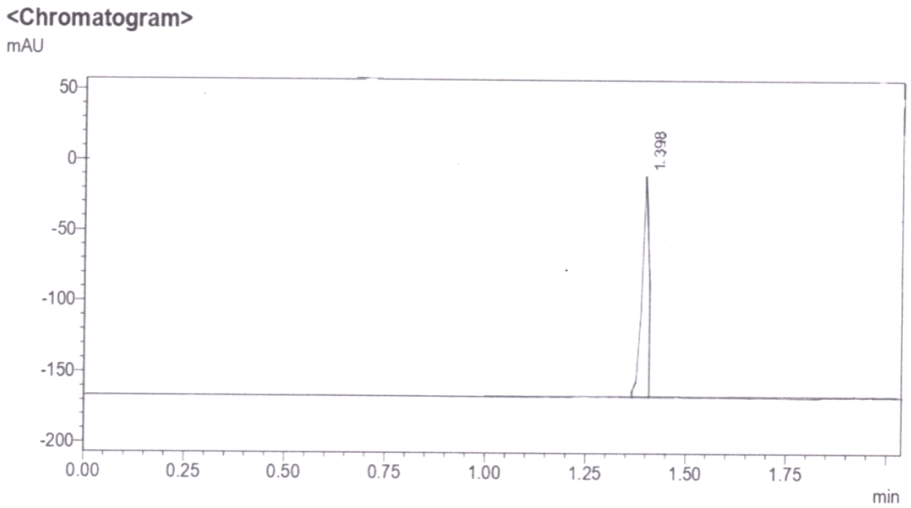


Fig3: HPLC graph of the combination of Luliconazole

Method validation

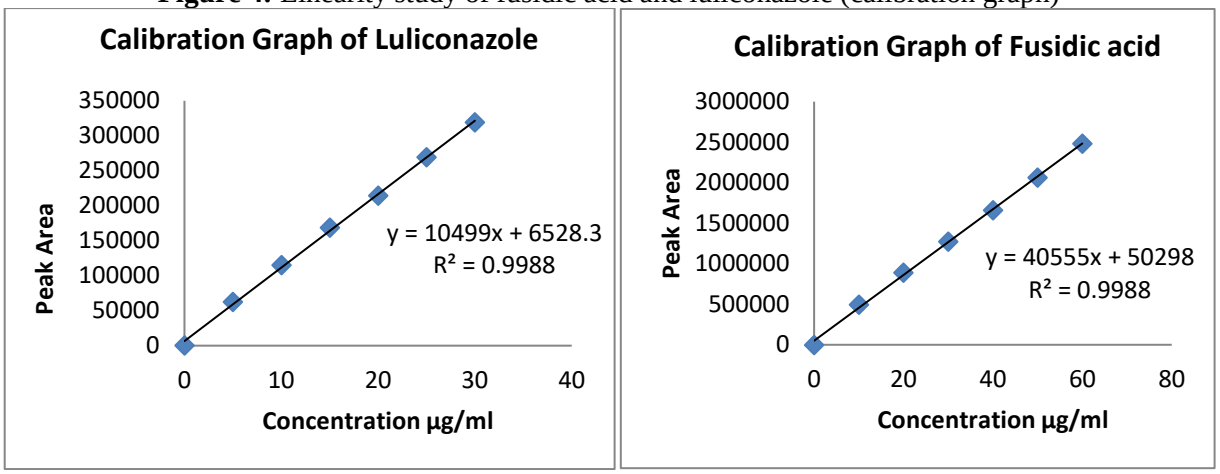
Assessment of linearity and range

Specificity involves quantitative detection of an analyte in the presence of those components that may be expected to be part of the sample matrix. The specificity of the developed method were established by spiking of luliconazole and fusidic acid in hypothetical placebo (i.e. might be expected to be present) . The linearity for Luliconazole and Fusidic acid were determined in the range of 5 µg/mL to 30 µg/mL and 10 µg/mL - 60 µg/mL. A graph was plotted with concentration on X axis and mean areas on Y axis and correlation coefficient was determined. Result shows that, with increasing concentration of both the drugs, peak area goes on increasing proportionately indicating the linear relationship. Similarly, the regression coefficient (r2) value was > 0.998. The linear range of detectability obeyed Beer's Law and it was well within higher and lower linear concentration of drugs. Observations of linearity studies of Luliconazole and Fusidic acid were highlighted in table1and fig1-4

Table 1 : Results of Linearity study

Luliconazole Conc (µg/mL)	**Luliconazole Mean Peak Area**	**Fusidic acid Conc (µg/mL)**	**Fusidic acid Mean Peak Area**
5	62366	10	494372
10	114941	20	891641
15	168367	30	1275468
20	214357	40	1660798
25	269040	50	2064219
30	319040	60	2482219

Figure 4: Linearity study of fusidic acid and luliconazole (calibration graph)



System Suitability Studies

Evaluation of system suitability was done by analyzing six replicate of Luliconazole and Fusidic acid in a mixture at a concentration of 5 µg/mL of Luliconazole, and 10 µg/mL of Fusidic acid. The column efficiency, peak asymmetry, and resolution were calculated for each replicate. As a result from the system suitability data it was found that the efficiency of the column was good for analysis and through the resolution it was indicated that the component was easily separated from the column.

Table 2: System suitability data

Drugs	Parameters	Mean ± SD (n=6)	% RSD
Luliconazole	Retention Time	2.2 ± 0.0112	0.509
	Theoretical Plate	24932.52 ± 75.19	0.301
	Tailing Factor	0.934 ± 0.0067	0.717
Resolution		2.026 ± 0.0125	0.616
Fusidic acid	Retention Time	1.5 ± 0.0307	0.204
	Theoretical Plate	27764.51 ± 153.19	0.551
	Tailing Factor	0.8106 ± 0.0081	0.999
Resolution		4.166 ± 0.0265	0.636

Method Precision

Six replicate injections of standard solution were given into the HPLC system. Data shown in table 5 indicate an acceptable level of precision for the analytical system. The inter day and intraday analysis reveals that method was précised for both the drug Luliconazole and Fusidic acid and not exceeded the value ±2. And the % RSD of each drug in both inter day and intraday not exceeded the 1 percent this value shows that the very low count of impurity and good efficiency of the column and also the develop method was precisely performed. The average % RSD of the Luliconazole and fusidic acid in interday was 0.60 and 0.546 and intraday was 0.495 and 0.319**.**

Table 3: Intraday data for Luliconazole and Fusidic acid

Drugs	Concentration (µg/mL)	Mean Peak Area ± SD (n=3)	% RSD.
Luliconazole	5	65246 ± 434.7836	0.666
	10	103557 ± 514.0205	0.496
	15	162823 ± 527.3503	0.323
Average RSD %			**0.495**
Fusidic acid	10	1192264 ± 3338.237	0.279
	20	1476219 ± 4747.698	0.321
	30	1751743 ± 6255.65	0.357
Average RSD %			**0.319**

Table 4: Interday data for luliconazole and Fusidic acid

Drugs	Concentration (µg/mL)	Mean Peak Area ± S.D. (n=)	% R.S.D.
Luliconazole	5	115179 ± 486.4195	0.422
	10	148315 ± 1051.752	0.709
	15	184807 ± 1241.625	0.671
Average RSD %			**0.60**
Fusidic acid	10	1192231 ± 5475.735	0.459

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD of the luniconazole and Fusidic acid was 4.89 and 12.09 while the LOQ of the luniconazole and Fusidic acid was 0.647 and 0.221, this was shows that the lowest concentration of an analyte in a sample that can be consistently detected with a stated probability.

Table 5: Limit of Detection (LOD) and Limit of Quantification (LOQ)

Parameters	**Fusidic acid**	**Luniconazole**
LOD (µg/mL) (n=5)	12.09	0.647
LOQ (µg/mL) (n=5)	4.89	0.221

Accuracy (Recovery studies)

For accuracy study data determinations over three concentrations at 80%, 100%, and 120% of expected sample concentration covering the specified range was determined & expressed as recovery values. The accuracy of the drug luliconazole and fusidic acid were analysed and the result were not shows any significance change at the concentration 80%,100% and 120 % of the dilution concentration luliconazole 5 µg/ml and 10 µg/ml of fusidic acid. The percentage recovery were not exceeded 100 percent it was equal and less than 100 percent.

Table 6 : Accuracy data for luliconazole and Fusidic acid

Drugs	Level (%)	Amount of sample (µg/mL)	Amount of std. spiked (µg/mL)	Total Amount (µg/mL)	Mean Peak Area ± S.D. (n=3)	Amount of sample found (µg/mL)	Mean %Recovery ± S.D. (n=3)
Luliconazole	0	5	0	5	97850± 1406.587	2.980	99.36 ± 0.015
	80	5	2.4	7.4	164396± 4567.657	5.431	100.46 ± 0.202
	100	5	3	9	193706 ± 1114.785	5.990	99.84 ± 0.015
	120	5	3.6	8.6	214169 ± 643.021	6.583	99.79 ± 0.066
Fusidic acid	0	10	0	10	542184± 32704.174	119.564	99.68 ± 0.075
	80	10	6	16	1303901± 59708.003	215.605	99.86 ± 0.020
	100	10	9	19	1572134 ± 13460.513	240.022	100.35 ± 0.277
	120	10	12	22	1434372 ± 6863.9153	263.568	99.89 ± 0.058

Robustness

The robustness of the method was determined to check the reliability of analysis concerning deliberate variation in method parameters. The typical variations are given below: Variation in mobile phase composition by ± 2 nm volume of solvent, Variation in flow rate by ± 0.2 units, the robustness parameters for the method. The robustness of luliconazole and fusidic acid was determined by taking the the two parameter moile phse and flow rate . The peak area and percentage RSD of both the drug not exceeded the ± 2 and it shows the method was robust for both the drug luliconazole and fusidic acid.

Table 7: Robustness data for Luliconazole

Parameters	**Level**	**Mean Peak Area ± SD (n=3)**	**%RSD**	**Rt ± SD (n=3)**	**%RSD**
Mobile Phase (85: 15 v/v)	80:8 v/v	67759 ± 406.006	0.599	1.60 ± 0.013	0.812
	90:12 v/v	68659 ± 626.172	0.091	1.75 ± 0.021	1.2
Flow rate (1.0 mL/min)	0.5mL/min	69059 ± 779.487	1.128	1.5 ± 0.010	0.60
	1.2 mL/min	69287 ± 514.304	0.742	1.96 ± 0.019	0.969

Table 8: Robustness data for Fusidic acid

Parameters	Level	Mean Peak Area ± SD (n=3)	% RSD.	Rt ± SD (n=3)	%RSD
Mobile Phase (85: 15v/v)	80:8 v/v	148265 ± 2611.25	1.76	0.98 ± 0.019	1.9
	90:12 v/v	147912 ± 2266.005	1.56	1.02 ± 0.017	1.6
Flow rate (1.0 mL/min)	0.5mL/min	148169 ± 2874.236	1.93	1.15 ± 0.009	0.782
	1.2 mL/min	147917 ± 2730.084	1.84	1.20 ± 0.018	1.5

For mixed standard solution

Mixed standard solution of Luliconazole and Fusidic acid were prepared with mobile phase in such a way that the final concentration of Luliconazole and Fusidic acid were in the range of 5–30 µg/mL, and 10-60 µg/mL respectively. The peak area was recorded for all the peaks as shown in below result tables for linearity of Luliconazole and Fusidic acid. The plots of peak area versus the respective concentration were found to be linear with regression coefficient (R2 =0.9998) for Luliconazole, and (R2=0.9981) for Fusidic acid.

Six replicate injections of the standard preparation 5 µg/mL and 10 µg/mL of Luliconazole and Fusidic acid were injected into HPLC system. The mean, SD and %RSD for peaks Luliconazole and Fusidic acid was calculated .The % RSD for peak area and retention time was <2.0 the same was describe under table .

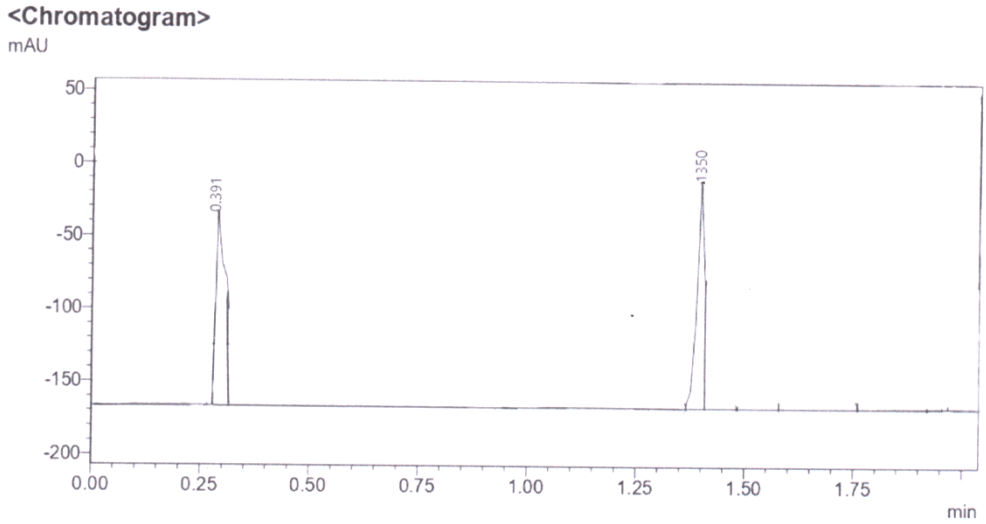


Fig 5 : HPLC graph of the combination of Luliconazle and fusidic acid

Table 9 : Mixed concentration HPLC of the Luliconazole and Fusidic acid

Sr.	FusidicAcid(FA)				Luliconazole			
No.	Peak area	Retention Time	Theoretical plates	Tailing Factor	Peak area	Retention Time	Theoretical plates	Tailing Factor
1	1192265	0.9	6458.3	1.29	65246	1.5	8632.56	1.13
2	1193242	1.02	6978.36	1.237	67143	1.9	7901.5	1.196
3	1189265	1.3	7970.44	1.355	68215	1.8	7916.22	1.104
4	1493663	1.1	7512.61	1.364	66649	2	7927.49	1.214
5	1294647	0.98	6695.51	1.355	67556	1.6	8412.52	1.102
6	1393269	1.06	8597.12	1.354	65211	2.1	8356.8	1.209
Mean	1292725.17	1.06	7368.72	1.32583	66670	1.81666667	8191.18	1.15917
SD	127422.071	0.13623509	----	----	1229.03605	0.21147629	----	----
% RSD	0.09856857	0.128523671	----	----	0.018434619	0.116408968	----	----
SEM	52030.24523	0.055628865	----	----	501.852203	0.0863521	----	----

MARKETED PREPARATION STUDY

The validated HPLC method was applied to the simultaneous determination of Luliconazole and Fusidic acid, in marketed pharmaceutical dosage form, i.e., cream (Fusiwal (containing Fusidic Acid and Lunizole containing Luniconazole) . The contains Weigh 1 gram of cream, dissolve it in 50 mL of methanol and sonicate it for 10 minutes. Then heat at 60–65°C until the base was dissolved and cool it at room temperature. Filter the extract through Whatman filters paper no. 42 and make up the volume up to 25 mL with

methanol. Final stock solution containing Fusidic acid (10 µg/ mL) and luniconazole (5µg/mL). From the above solution, 5 mL was pipette out and transferred to 10 mL volumetric flask and volume was made up to mark with methanol to give a solution containing Fusidic acid (10 µg/ mL) and luniconazole (5µg/mL).

After performing the simultaneous for both the drug cream formulation named lunizole containing luniconazole 1% in 15gm of preparation and Fusiwal containing 1 % w/w in 5gm of preparation shows the percentage recovery 99 % which was close to the 100% . On comparing with the assay of the formulation it shows the ranges from 98 -100 percent. Overall the percentage recovery from the dilution of the standard drug to the formulation shows equivalent percentage recovery which was not less than 98 percent. From that it was concluded that the method which was developed for the luniconazole and fusidic acid was validated.

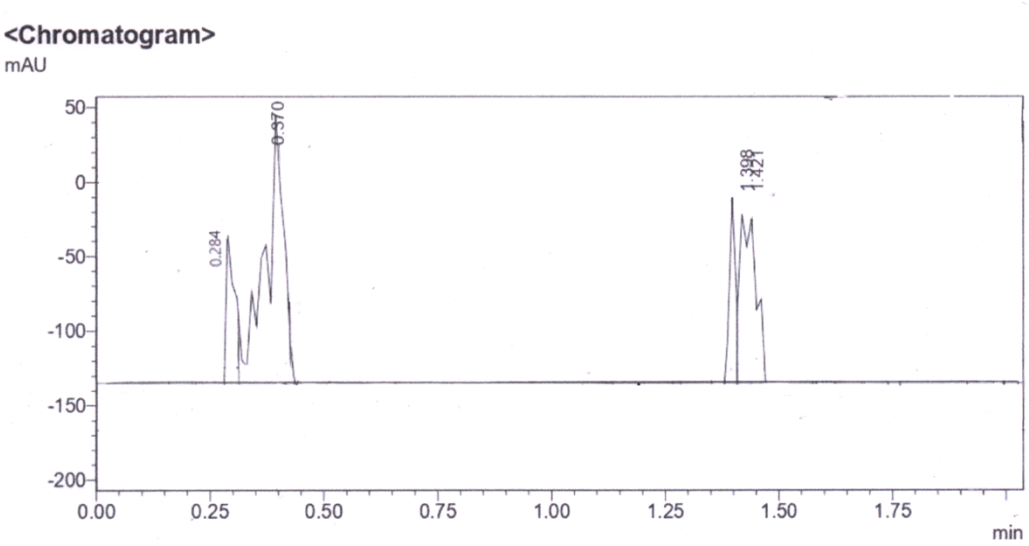


Fig 6: HPLC graph of the combination of Luliconazle and fusidic acid marketed preapartion (Lunizole and fusiwell)

Table 10 : Analysis of the marketed preparation of Fusidic acid and Luniconazole

SampleNo.	FusidicAcid(FA) (Fusiwal)		Luliconazole (Lunizole)	
	PeakArea	Assay	PeakArea	Assay
1	1145419	98.80%	66862	99.28%
2	1253916	99.98%	68231	98.71%
3	1135354	99.90%	68449	100.60%
Mean	----	99.56%	----	99.53%
SD	----	0.006593937	----	0.00969484
%RSD	----	15098.71872	----	10266.286
SEM	----	0.003807	----	0.005597

Table 11: Analysis of marketed formulation

Marketed Formulation	Amount taken (µg/mL)	Amount Obtained Mean ± SD. (µg/mL)	% Amount Obtained Mean ± S.D.(n=5)	
Luniconazole (Lunizole)	5	4.95 ± 0.030	CP	99.86 ± 1.031
Fusidic acid (Fusiwal)	15	14.90 ± 0.042	MP	99.06 ± 0.642

4. Discussion And Overall Conclusion

Any drug molecule's identification, quantification, and characterization in combination dosage forms and organic fluids can be aided by evaluation. The stages of formulation assessment and drug development require the use of analytical techniques. The information gathered about impurities (related to medication safety), bioavailability (which includes important drug traits like crystal kind, uniformity of drug, and release of drug), stability (which illustrates the degradation product), and the impact of manufacturing parameters are all made possible by the analytical methods. Efficiency data, which

may be directly related to the need for an identified dose, are also generated by these methods. These data sets are necessary to confirm that the drug product is produced in a consistent and repeatable manner.

Chromatographic techniques are significant and have become more popular due to their affordability, ability to shorten analysis times, increased viability, and decreased environmental waste output. Additionally, the analyst will be able to carry out the analysis in a safer manner by utilising chromatographic techniques. Furthermore, a lot of biotechnology and pharmaceutical companies use high-performance liquid chromatography (HPLC) as an analytical tool these days for drug discovery, development, and manufacturing cycles.HPLC is the preferred technique for determining a new chemical entity's peak purity, tracking reaction changes during synthetic or scale-up processes, assessing novel formulations, and performing quality control and assurance on the finished pharmaceutical products.

Development and validation of analytical method is required for measurement of different parameters and setting performance limits. It is an ongoing, connected process. Laboratory research is used to validate analytical methods to ensure that their execution characteristics satisfy the needs of the intended scientific application. Any new or modified procedure must be validated to ensure that it can produce results that can be relied upon, even when used by different administrators in completely different or similar laboratories with similar equipment.A programme known as "method validation" guarantees that the processing system will provide a high degree of confirmation in order to satisfy its predicated acceptance basis.

Current study was based on the development and validation of HPLC method for estimation of Fusidic acid and Luliconazole in pharmaceutical dosage form. For experimental work, Waters HPLC instrumentation system was used with less manual activities and more automation which has helped to reduce the errors. Each compound travels different distances up on the column depending on the solvent. According to these specifications, the suitable selected solvent in this study was methanol because both the drugs show significant solubility in methanol. However, the combination of different solvents was used for better resolution and separation.

5. REFERENCES

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