Assessment of Fusidic acid (FA) and Beclomethasone Dipropionate (BD) In Semi-solid Dosage Form Using Validated High-Performance Liquid Chromatography Method

Sunil Shewale[1]*, Vaishali Undale[1], Atul Deshmukh[2], Vikas Wawale[3], Sachin Gundecha[1], Vrushali Bhalchim[1]

[1] Dr. D.Y. Patil Institute of Pharmaceutical Sciences & Research, University of Pune. Maharashtra (India)-411018.

[2] Vasantidevi Patil Institute of Pharmacy, Shivaji University, Kolhapur, Maharashtra (India)-416114.

[3] Cloudbyz India Pvt. Ltd., Bengaluru, Karnataka (India)-560102.

Email: ms.shewale85@gamil.com[1]

DOI: 10.47750/pnr.2022.13.S01.154

Abstract

Objective: To develop a validated analytical method for simultaneous estimation of Fusidic acid and Beclomethasone dipropionate in pharmaceutical combined dosage form by HPLC. **Method:** A HPLC method was developed and validated for assay of Fusidic acid (FA) and Beclomethasone dipropionate (BD) in Topical pharmaceutical formulation. The chromatographic separation was achieved on Kromasil $C_{18,}$ 5µm (125 X 4.6 mm) column at ambient temperature of 25^0C, at a flow rate 2.5 mL/min using LC-2010 CHT Liquid Chromatography, Shimadzu HPLC system. 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, Ortho-Phosphoric acid and Acetonitrile in the ratio of 10:45:45% (v/v/v). Both the drugs showed maximum absorbance at 220 nm which was selected as the wavelength of detection throughout the experimental work. Validation of developed method was carried out according to ICH guidelines. **Result:** HPLC method was successfully developed for separation of FA and BD with good resolution. Method validation after assessment of various parameters indicated low % RSD within an acceptable limit of < 2.0%. **Conclusion:** The developed HPLC method for estimation of FA and BD is rapid, reliable, precise, and reproducible.

Keywords: Fusidic acid, Beclomethasone dipropionate, HPLC, Method Validation etc.

1. INTRODUCTION

FA 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. [2]

BD is chemically known as 2-[(1R,2S,10S,11S,13S,14R,15S,17S)-1-chloro-17-hydroxy-2,13,15-trimethyl-5-oxo-14-(propanoyloxy)tetracyclo[8.7.0.0^{2,7}.0^{11,15}] heptadeca-3,6-dien-14-yl]-2-oxoethyl propionate. [3] The BD is synthetic glucocorticoid used as an anti-inflammatory agent in topical preparation or in aerosol form for the treatment of asthma. [4] The combination of both these drugs is useful for the treatment of atopic dermatitis; a skin disease. It stops the growth of bacteria and reduces the itching, redness, swelling and crusting of the skin sores. [5] Although, various analytical techniques have been developed for estimation of FA and BD 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. [2,6-10]

In general, from the analytical chemistry point of view and as per the AOAC International; any analytical quantitative methods should meet the certain minimal performance criteria. [11] Now a days, submission of analytical method validation data is mandatory regulatory requirements for getting the necessary approvals. ICH and USFDA have issued specific guidance for performing analytical method validations. [12-13]

According to the guideline Q2 (R1) of ICH, "quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product" is one of the types of analytical procedures to be validated. [12] The validation of any analytical procedure implies that the applied analytical technique, is reliable, accurate, precise, sensitive and specific enough to give reproducible results, proving its suitability to the intended application.

In this study, an HPLC method was developed and validated for simultaneous analysis of FA & BD in bulk drugs and combined topical pharmaceutical formulation.

2. MATERIALS & METHODS

2.1 Chemicals and Reagents

FA and BD were obtained as a gift samples from Ciron Drugs and Pharmaceutical Pvt. Ltd., India and were used as working standards. They were used without further purification and certified to contain 99.62 % and 99.80 % (w/w) of FA and BD respectively on dry weight basis. All analytical grade chemicals and reagents were purchased from S. D. Fine Chemical Laboratories, Mumbai, India.

2.2 Instrumentation

LC-2010 CHT Liquid Chromatography Shimadzu HPLC system, UV-Vis Shimadzu detector, Kromasil $C_{18,}$ 5µm (125 X 4.6 mm) analytical column, LC solution software for data processing and evaluation, Mettler Tolledo precision balance, pH meter (LAB INDIA) and FASTCLEAN Ultrasonic Cleaner along with Grade A certified glassware (Borosil) were used for the study.

2.3 Liquid Chromatography

The chromatographic separation was achieved using Kromasil $C_{18,}$ 5µm (125 X 4.6 mm) analytical column at isocratic mode. The mobile phase consists of a mixture of Methanol, Ortho-Phosphoric acid and Acetonitrile in the ratio of 10:45:45 (v/v/v) and degas by sonication. The flow rate and column temperature were maintained as 2.5 mL/min and 25ºC respectively throughout the analysis. The injection volume was maintained as 10µL.

2.4 Standard and Sample preparation

2.4.1 Preparation of 0.05M solution of Orthophosphoric acid

0.05M solution of orthophosphoric acid was prepared by dissolving 4.9gm of orthophosphoric acid in 1000mL of distilled water.

2.4.2 Beclomethasone Dipropionate standard stock solution

Standard stock solution of BD was prepared by transferring an accurately weighed 25 mg of BD in 200mL volumetric flask. 150mL of methanol was added and the resulting solution was sonicated to dissolve the drug. The volume was made with methanol to prepare 125µg/mL.

2.4.3 Fusidic acid standard stock solution

Standard stock solution was prepared by transferring an accurately weighed 200 mg of FA in 50mL volumetric flask. 30mL of methanol was added and resulting solution was sonicated to dissolve the drug. The volume was made with methanol to prepare 4000µg/mL.

2.4.4 Standard mixture preparation

Standard mixture was prepared by transferring 2mL of the standard stock solution of BD and 5mL Standard Stock solution of FA to 100mL volumetric flask. The volume was made with methanol to prepare 2.5µg/mL & 200µg/mL respectively.

2.4.5 Preparation of laboratory batch of cream formulation

Laboratory batch of cream formulation was prepared by transferring an accurately weighed cream equivalent to 40 mg of FA and 0.5 mg of BD in 200mL volumetric flask, 150mL methanol was added and heated until the cream melt and shaken vigorously for 15 min. The solution was cooled below 10°C, the volume was made with methanol and the solution was filtered through glass microfiber filter paper (whatman GF/C) to prepare 200µg/mL and 2.5µg/mL respectively.

2.5 Method development

Mixture of working standard solution containing 200 µg/mL of FA and 2.5µg/mL of BD was used for separation of two drugs and for development of method.

2.6 Determination of analytical wavelength

FA and BD each 10µg/mL working standard solutions were prepared and these solutions were scanned by using UV-spectrophotometer in the range of 200 - 400 nm. The overlain spectra of both the drugs were observed for isobestic point for determination of analytical wavelength.

2.7 Optimization of HPLC method

2.7.1 Optimization of mobile phase

The mobile phases using combinations of various solvents such as Acetonitrile, Disodium hydrogen phosphate, Glacial acetic acid, Methanol, Orthophosphoric acid and water were prepared and each combination was tried to check the separation of both the drugs. All solvents were filtered and sonicated for degassing and mixed in suitable combinations.

2.7.2 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. Other chromatographic conditions such as Kromasil $C_{18,}$ 5µm (125 X 4.6) mm analytical column, an ambient temperature of 25^0C, analytical wavelength was kept same for different flow rates. Separation of both the drugs was recorded with different flow rate.

2.8 Solution Stability

Stability of analytical solution is the ability of the analyte to remain stable during course of time and under the temperature mentioned. Standard mix solution and laboratory batch formulation solution were prepared as described previously. Mixed standard solution and cream formulation solution were injected at different time intervals and cumulative % RSD was determined.

2.9 Analysis of Laboratory batches

2.9.1 For mixed standard solution

Mixed standard solution of 200 µg/mL and 2.5 µg/mL of FA and BD respectively was injected in HPLC system. The % RSD for retention times, standard areas, average tailing factor and number of theoretical plates were determined.

2.9.2 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.

2.10 Method validation

2.10.1 Linearity and Range

The linearity was studied over the increasing drug concentration and plotting the graph of peak area vs. concentration in µg. Standard solution of FA and BD 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.

2.10.2 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.

2.10.2 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 FA and BD were determined.

2.10.3 Specificity

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

2.10.4 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.

2.10.5 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 FA and BD 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.

2.10.6 Ruggedness

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

2.10.7 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), Acetonitrile content in Mobile phase (± 2 % absolute), column oven temperature (± 5°C), and wavelength of detection (± 5 nm).

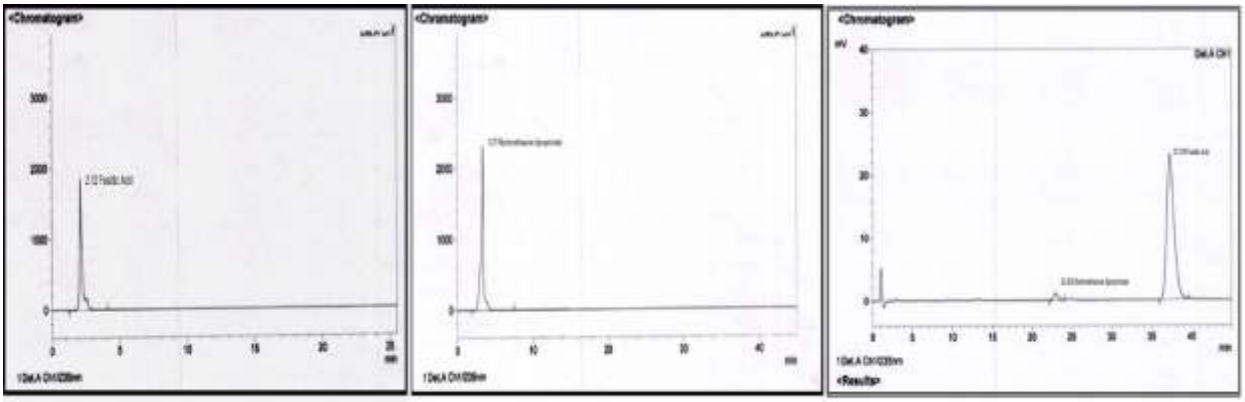
3. OBSERVATIONS AND RESULTS

3.1 Development of method and optimization of mobile phase

Fusidic acid contains a single carboxylic acid functional group with a pKa of 5.3. Therefore, the retention time of FA is affected by the pH of mobile phase. In order to ensure that the molecule remains completely protonated in solution a suitable mobile phase should have a pH of 2 units below the pKa of the acidic group. For this reason, a mobile phase acidified with phosphoric acid was chosen. BD, on the other hand, is a neutral compound and its retention on the analytical column was not affected by pH. The solution mixture of standard FA and BD was injected in system and chromatograph was developed in different solvent systems. First solvent system of Actonitile:0.01M Disodium hydrogen orthophosphate (70:30 % v/v) adjusted to pH 6 with Glacial acetic acid and second solvent system of Methanol: Water (70:30% v/v), at flow rate - 1,2,2.5 mL/min shows only one

peak with no separation of two drugs. Mobile phase combination Methanol, Ortho-Phosphoric acid and Acetonitrile in the ratio of 10:45:45% v/v/v & Flow rate 2.5 mL/min was able to separate peaks of FA and BD properly and peaks were observed distinctly in the chromatogram. This satisfactory result has given confirmation for use of HPLC method for further analysis. The method development for separation of FA & BD using optimized mobile phase is illustrated in figure 1.

Figure 1 HPLC method development for separation of FA & BD

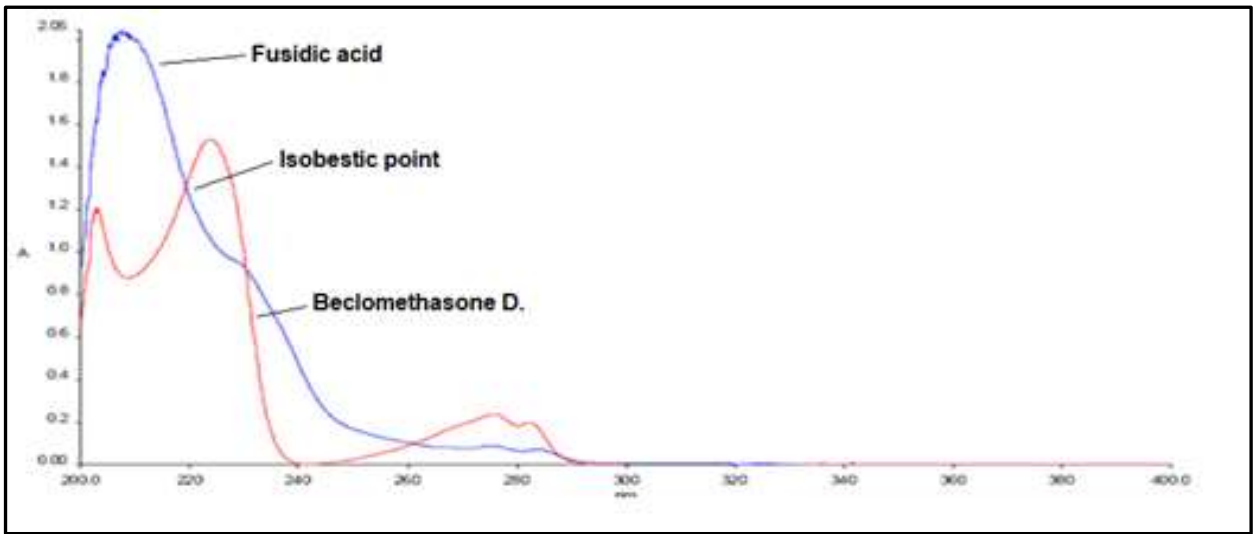


1a) Actonitile: Disodium hydrogen orthophosphate (70:30% v/v) 1b) Methanol: Water (70:30% v/v) 1c) Methanol: Ortho-Phosphoric acid: Acetonitrile (10:45:45% v/v/v)

3.2 Determination of absorption maximum & isosbestic point

Spectra of both the drugs were examined and λ max of Fusidic acid was observed at 207 nm and that of Beclomethasone Dipropionate at 225 nm. Overlain of both the absorbance spectrum indicated that both the drugs showed significant absorbance at 220 nm. Hence 220 nm was selected as analytical wavelength (λ max). (Figure 2)

Figure 2: Overlain absorption spectrum of FA and BD



3.3 Solution Stability

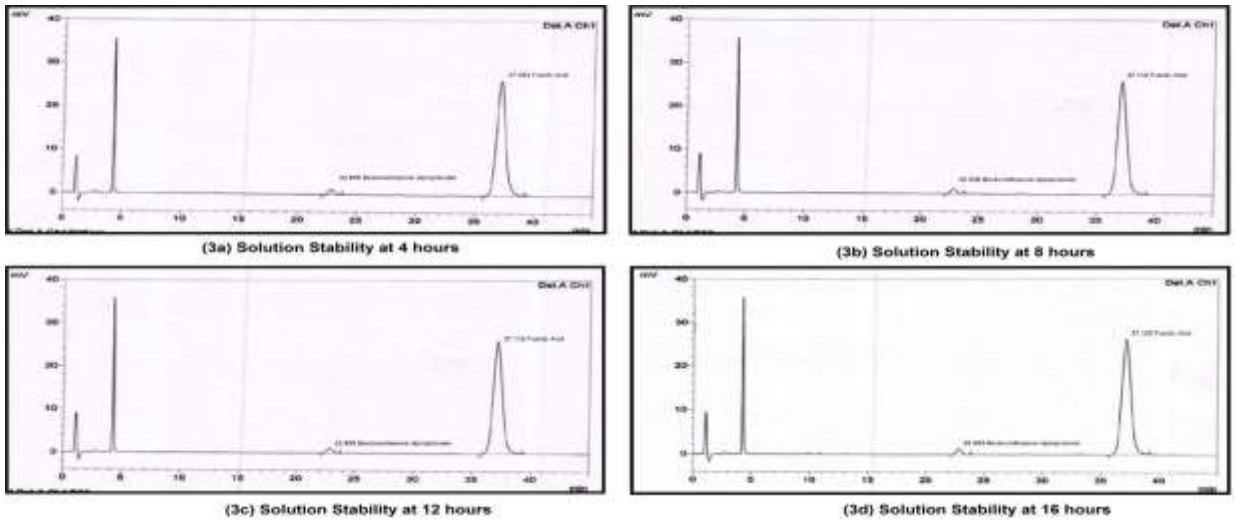
Stability of known area of Beclomethasone Dipropionate and Fusidic acid in analytical solution was verified by analyzing the sample initially and at 4 hr, 8 hr, 12 hr and 16 hr by storing at 25°C. The cumulative % RSD for the peak areas of

Beclomethasone Dipropionate and Fusidic acid were within the acceptance criteria for stability of sample solution. It was thus, concluded that sample solution was stable up to 16 hours at 25°C. The results of solution stability of FA and BD are mentioned in table 1 & figure 3.

Table 1: Results of solution stability of FA & BD at different time point

Time in Hours	Peak area of FA	Peak area of BD
0	1385975	38492
4	1387744	38450
8	1390237	38516
12	1396506	39052
16	1403148	39405
Mean	1392722	38783
SD	7062.19	426.06
%RSD	0.50	1.10
SEM	3158.30	190.54

Figure 3 FA & BD solution stability at different time point



(3a) Solution Stability at 4 hours

(3b) Solution Stability at 8 hours

(3c) Solution Stability at 12 hours

(3d) Solution Stability at 16 hours

3.4 Analysis of laboratory batches

3.4.1 For mixed standard solution

Six replicate injections of the standard preparation were injected into HPLC system. The mean, SD and %RSD for peaks of Beclomethasone Dipropionate and Fusidic acid was calculated. The % RSD for peak area and retention time was < 2.0 the same is describe under table 2.

Table 2: Results of mixed standard solution

Sr. No.	Fusidic Acid (FA)				Beclomethasone Dipropionate (BD)			
	Peak area	Retention Time	Theoretical plates	Tailing Factor	Peak area	Retention Time	Theoretical plates	Tailing Factor
1	1474483	37.282	8498.598	1.290	41184	22.967	8532.766	1.230
2	1474235	37.219	7968.859	1.337	40936	22.925	8001.675	1.296
3	1476980	37.203	7990.741	1.355	41101	22.917	8116.348	1.304
4	1473845	37.148	8012.809	1.364	41115	22.907	8027.391	1.314
5	1475358	37.081	8645.809	1.355	41039	22.838	8592.122	1.302
6	1479848	37.070	8797.717	1.354	41184	22.836	8749.196	1.309
Mean	1475791.50	37.167	8319.070	1.340	41093.17	22.900	8336.580	1.290
SD	2279.45	0.08	----	----	94.53	0.05	----	----
% RSD	0.15	0.22	----	----	0.23	0.23	----	----
SEM	930.39	0.03	----	----	38.58	0.02	----	----

3.4.2 For cream formulation

Three samples of a single batch were analysed as per test method. The Assay of three samples shows percentage RSD < 2.0. The data for analysis of laboratory batches of formulation is included in table 3.

Table 3: Results for analysis of laboratory batches of formulation

Sample No.	Fusidic Acid (FA)		Beclomethasone Dipropionate (BD)	
	Peak Area	Assay	Peak Area	Assay
1	1349419.0	97.88 %	36862.0	99.28 %
2	1353916.0	95.98 %	38231.0	98.71 %
3	1385344.0	98.90 %	38449.0	100.60 %
Mean	----	97.59 %	----	99.56 %
SD	----	1.48	----	1.01
% RSD	----	1.52	----	1.02
SEM	----	0.86	----	0.58

3.4 Method validation

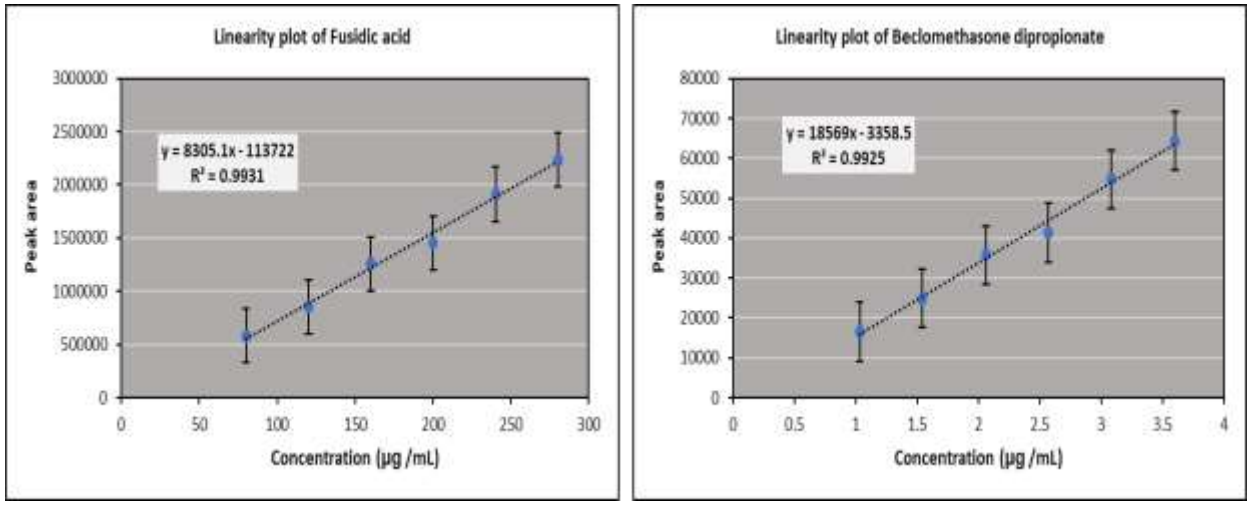
3.4.1 Assessment of linearity and range

The linearity for Beclomethasone Dipropionate and Fusidic acid were determined in the range of 1.03 µg/mL to 3.60 µg/mL for Beclomethasone dipropionate (about 40% - 140% of the test concentration as Beclomethasone dipropionate) and 80.08 µg/mL to 280.28 µg/mL for Fusidic acid (about 40% - 140% of the test concentration as Fusidic acid). 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 (r^2) value was > 0.99. 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 FA & BD are highlighted in table 4 & figure 4. Range inferred from the data of linearity found is 1.03µg/mL to 3.60µg/mL for BD and 80.08µg/mL to 280.28µg/mL for the estimation of FA.

Table 4: Results of Linearity study

Added Level In %	Fusidic Acid (FA)		Beclomethasone Dipropionate (BD)	
	Concentration in µg/mL	Peak Area	Concentration in µg/mL	Peak Area
40	80.08	581641.5	1.03	16553.0
60	120.12	855770.0	1.54	24898.0
80	160.16	1256251.5	2.06	35741.5
100	200.20	1454945.5	2.57	41349.0
120	240.24	1911008.5	3.08	54758.5
140	280.28	2236507.0	3.60	64286.5
Slope	8305.1		18569	
y-intercept	-113722		-3358.5	
Correlation coefficient	0.9931		0.9925	
Slope error	346.95		806.96	

Figure 4: Linearity study of FA & BD



3.4.2 System 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.

Table 5: Results of system precision

Sr. No.	Fusidic Acid (FA)				Beclomethasone Dipropionate (BD)			
	Peak area	Retention Time	Theoretical plates	Tailing Factor	Peak area	Retention Time	Theoretical plates	Tailing Factor
1	1383881	36.21	8394.543	1.247	38459.00	21.75	8345.658	1.31
2	1396265	36.27	8174.714	1.429	38405.00	21.78	8318.628	1.285
3	1379580	36.29	7984.726	1.361	38415.00	21.80	8217.226	1.315
4	1385930	36.45	8331.878	1.353	38443.00	21.77	8051.323	1.322
5	1374589	36.11	8564.833	1.371	38419.00	21.72	8487.545	1.309
6	1388936	36.52	8679.762	1.349	38444.00	21.79	8613.69	1.298
Mean	1384863.5	36.31	8355.076	1.352	38430.83	21.77	8339.012	1.307
SD	7512.16	0.15	----	----	20.85	0.03	----	----
% RSD	0.54	0.42	----	----	0.05	0.14	----	----
SEM	3066.19	0.06	----	----	8.51	0.01	----	----

3.4.3 Method Precision

Six samples of a single batch of cream were prepared and analysed as per the proposed method. Data is shown in table 6. The % RSD values indicate that the method has an acceptable level of precision.

Table 6: Results for method precision

Sample No.	**Fusidic Acid (FA)**		**Beclomethasone Dipropionate (BD)**	
	Peak Area	**Assay**	**Peak Area**	**Assay**
1	1385344.0	99.59 %	38449.0	100.68 %

2	1355312.0	97.29 %	39339.0	102.85 %
3	1349419.0	99.78 %	36862.0	99.28 %
4	1351806.0	97.02 %	38829.0	101.50 %
5	1353916.0	95.98 %	38231.0	98.71 %
6	1353601.0	99.57 %	37636.0	100.84 %
Mean	----	98.21 %	----	100.64 %
SD	----	1.64	----	1.50
% RSD	----	1.67	----	1.49
SEM	----	0.67	----	0.61

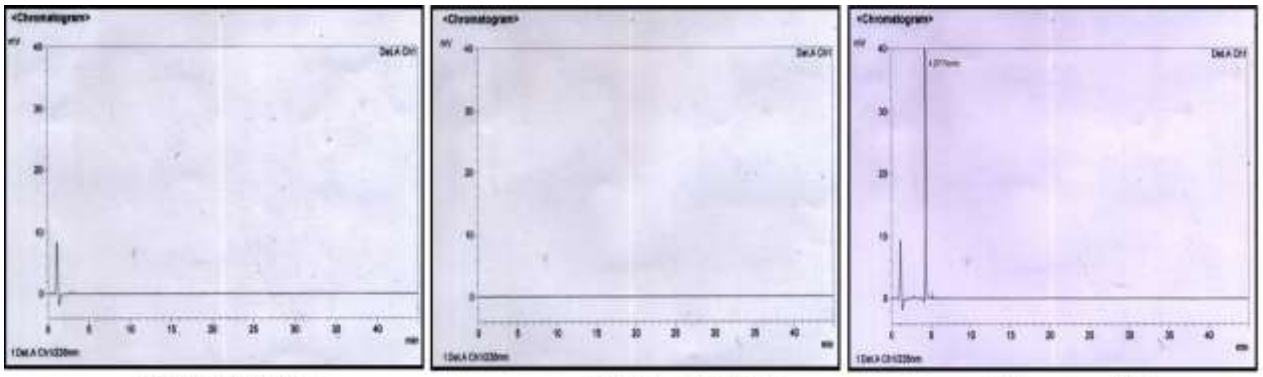
3.4.4 Limits of detection (LOD) and quantitation (LOQ) [Sensitivity]

The detection limit for FA was found to be 29.84 µg/mL and for BD it was 0.08 µg/mL. These can be correlates to linearity study of both the drugs where lower concentration of drugs detected was 80.08 and 1.03 µg/mL respectively. Similarly, the limit of quantitation was 87.52 µg/mL and 3.40 µg/mL approximately, three times that of detection limit.

3.4.5 Assessment of specificity

There was no interference from the diluent (Methanol), mobile phase and placebo at the retention time of analyte peak. There was no interference at the retention time of BD and FA peak. The method is specific for the estimation of BD and FA in cream formulation (figure 5)

Figure 5 Specificity of HPLC method



(5a) Chromatogram of diluent (5b) Chromatogram of mobile phase (5c) Chromatogram of placebo

3.4.6 Accuracy (Recovery studies)

The percentage purity obtained after assay of drugs in cream formulation was 101.60 for FA and 101.30 for BD and the % RSD was < 2.0. (table 7) As per British Pharmacopoeia, both the drugs in formulation contains not less than 97.5 % and not more than 101.0 % w/w and not less than 97.0 % and not more than 102.0 % w/w of the stated amount of FA and BD respectively. It has been evident in this assay. Similarly, the relative standard deviation was also within acceptable limit.

Table 7: Results of Recovery studies

Level	Fusidic Acid (FA)					Beclomethasone Dipropionate (BD)				
	Initial amount (ppm)	Excess drug added (ppm)	Drug recovered (ppm)	% drug Recovery	% RSD/ SEM	Initial amount (ppm)	Excess drug added (ppm)	Drug recovered (ppm)	% dug Recovery	% RSD/ SEM
Level I (80%)	6.43	5.14	5.04	98.088	1.62/ 0.93	2.57	2.06	2.09	101.702	0.35/ 0.20
	6.43	5.14	5.19	100.936		2.57	2.06	2.08	101.054	
	6.43	5.14	5.18	100.834		2.57	2.06	2.08	101.131	
Level II (100%)	6.43	6.43	6.44	100.245	0.90/ 0.52	2.57	2.57	2.60	101.277	0.18/ 0.10
	6.43	6.43	6.38	99.332		2.57	2.57	2.60	101.130	
	6.43	6.43	6.50	101.138		2.57	2.57	2.59	100.916	
Level III (120%)	6.43	7.71	7.85	101.862	0.28/ 0.16	2.57	3.08	3.11	100.763	0.75/ 0.43
	6.43	7.71	7.81	101.303		2.57	3.08	3.11	100.842	
	6.43	7.71	7.84	101.646		2.57	3.08	3.07	99.505	

3.4.7 Ruggedness

In the ruggedness study, the drugs BD and FA were estimated with HPLC I and HPLC II by proposed analytical method. The assay for FA by HPLC I and HPLC II were not showing significant change. % RSD values were less than 2 and as per acceptance criteria. (table 8)

Table 8: Results of Ruggedness

Sample No.	% Assay			
	Fusidic Acid (FA)		Beclomethasone Dipropionate (BD)	
	Method Precision (HPLC-I)	Ruggedness (HPLC-II)	Method Precision (HPLC-I)	Ruggedness (HPLC-II)
1	99.59	97.85	100.68	97.72
2	97.29	98.76	102.85	99.25
3	99.78	95.50	99.28	100.88
4	97.02	98.46	101.50	96.33
5	95.98	96.46	98.71	99.33
6	99.57	96.45	100.84	99.63
Mean	98.21	97.25	100.64	98.86
SD	1.64	1.30	1.50	1.60
% RSD	1.67	1.33	1.49	1.61
SEM	0.67	0.53	0.61	0.65

3.4.8 Robustness

Sample solutions were analyzed under each condition and assay of FA, and BD was calculated. The mean, standard deviation and % RSD for each set of data are shown in table 9 Robustness of method is indicated by the % RSD and overall % RSD values between the data of control and data of at each variable condition.

Table 9: Results of Robustness

Sample No.	% Assay							
	Robustness (with changed conditions)							
	Fusidic Acid (FA)				Beclomethasone Dipropionate (BD)			
	Flow rate	MP	Temperature	Wavelength	Flow rate	MP	Temperature	Wavelength
1	98.85	98.69	96.20	95.75	101.53	100.29	100.91	97.84
2	99.24	97.33	99.43	97.14	100.31	97.54	101.45	98.73
3	99.72	96.19	98.46	99.37	101.86	98.41	97.44	100.94
4	98.18	99.25	95.12	94.89	98.84	101.07	97.35	97.68
5	97.07	98.88	97.67	98.62	97.78	100.62	101.11	101.09
6	99.66	98.51	95.92	98.50	100.67	98.98	100.28	101.19
Mean	98.79	98.14	97.13	97.38	100.17	99.49	99.76	99.58
SD	1.02	1.16	1.66	1.77	1.58	1.39	1.86	1.68
% RSD	1.03	1.18	1.71	1.82	1.58	1.40	1.87	1.69
SEM	0.42	0.47	0.68	0.72	0.64	0.57	0.76	0.69

4. DISCUSSION AND OVERALL CONCLUSION

Evaluation of any drug molecule is helpful for its identification, determination and characterization in combined dosage forms and organic fluids. The analytical techniques are necessary during drug development and formulation assessment stage. The analytical methods are useful in generating efficiency data (which might be directly connected with the need of an identified dose), gathering information related to impurity (related to safety of the medication), bioavailability (consists of key drug traits like crystal kind, uniformity of drug and release of drug), stability (that shows the degradation product), and effect of manufacturing parameters. [14] These data sets are required to verify that the production of drug product is steady and reproducible.

Chromatographic methods are important and have gained prominence because they are economic, reduces analysis time, more viable and reduce the quantities of waste to the environment. Moreover, analyst will also be able to perform the analysis using chromatographic techniques in safer way. Furthermore, many pharmaceutical & biotechnology organizations now a days, utilizes the high-performance liquid chromatography (HPLC) analytical tool at all stages of drug discovery, development, and production cycle. [15] HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes in synthetic or scale up procedures, evaluating new formulations and carrying out quality control / assurance of the final drug products. [16]

Measurement of various parameters and establishing the performance limits is depends on development and validation of analytical method. It is continuous and interrelated process. Validation of an analytical method is established through laboratory research, that the execution attributes of the procedure meet the requirements for the proposed scientific application. Validation is required for any new or altered procedure to verify that it is fit for giving predictable and dependable outcomes, once used by various administrators by usage of comparable instrumentation inside the similar or absolutely distinct laboratories. [17] Method validation is a reported program that offers with that the processing system will give a high level of affirmation to meet its predicated acceptance basis. [18]

Current study was based on the development and validation of HPLC method for estimation of Fusidic acid and Beclomethasone dipropionate in pharmaceutical dosage form. For experimental work, Shimadzu 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. The validation of HPTLC method indicated that the method is simple, precise, robust, sensitive, and reproducible for estimation of FA and BD drugs in combination.

5. FUNDING SOURCE

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

6. CONFLICT OF INTEREST

The study was carried out as a part of academic research work. There is no conflict of interest.

7. ACKNOWLEDGMENT

All individuals listed as authors have contributed substantially to write this review. The authors are thankful to Ciron Drugs and Pharmaceutical Pvt. Ltd., India for providing drug samples of FA & BD.

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