Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

RP-HPLC Method Development and Validation for Simultaneous Estimation of Clobetasol Propionate and Fusidic Acid in Cream

Deepali P. Mohapatra[1*], Alisha Patel[1], Tapas K. Mohapatra[2]

[1]*Department of Pharmaceutical Quality Assurance at ROFEL Shri G. M. Bilakhia College of Pharmacy, Vapi-396191, Gujarat, India*
[2]*Department of Pharmaceutics at Gayatri College of Pharmacy, Sambalpur-768200, Odisha, India*

ARTICLE INFO

Article history:
Received: 08 May, 2021
Revised: 18 June, 2021
Accepted: 30 June, 2021
Published: 30 July, 2021

Keywords:
Clobetasol propionate,
Cream dosage form
Fusidic acid, RP-HPLC,
Simultaneous estimation.

DOI:
10.25004/IJPSDR.2021.130406

ABSTRACT

A simple, rapid, precise, and accurate high-performance liquid chromatography method was developed for simultaneous estimation of Clobetasol propionate and Fusidic acid in cream. The separation was obtained using a mobile phase consisting of Acetonitrile and Water in the ratio of 90:10 and adjusting pH 5.0 with Glacial acetic acid (10%) using Shim-pack solar C18 (250 × 4.6 mm, 5 µm) column. The flow rate of 1.0 mL/min and UV detection at 240 nm was employed. The retention time for Clobetasol propionate and Fusidic acid was 4.787 min and 6.006 min respectively. Linearity for Clobetasol propionate and Fusidic acid was found to be in the range of 3-7 µg/mL and 120-280 µg/mL, respectively. The method was validated as per the ICH guidelines and the results were within the acceptance criteria for precision, linearity, specificity, and robustness.

Introduction

Clobetasol propionate (21-Chloro-9-fluoro-11β-hydroxy-16βmethyl-3, 20-dioxopregna-1, 4-dien-17-yl propanoate) is a derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity. Glucocorticoids inhibit phospholipase A_2, which decreases the formation of arachidonic acid derivatives, they inhibit HF-Kappa B and other inflammatory transcription factors such as prostaglandins and leukotrienes, molecular weight 467.0 g/mol and melting point is 196°C. [Fig1 (a)].[1] Fusidic acid [ent-(17Z)-16α- (Acetyloxy)-3β, 11β-dihydroxy-4β, 8, 14- trimethyl-18-nor-5β, 10α-cholesta-17(20), 24-dien21-oic acid hemihydrate] is an bacteriostatic activity. It works by interfering with bacterial protein synthesis, preventing the translocation of the elongation factor G from the ribosome and inhibiting chloramphenicol acetyltransferase enzymes with molecular weight 516.71 g/mol a melting point is 192–193°C—[Fig 1(b)]. Methylparaben [methyl 4-hydroxybenzoate] is a preservative that prevents the decomposition of food products and pharmaceutical formulation by preventing the growth of fungi or bacteria. [Fig 1 (c)].

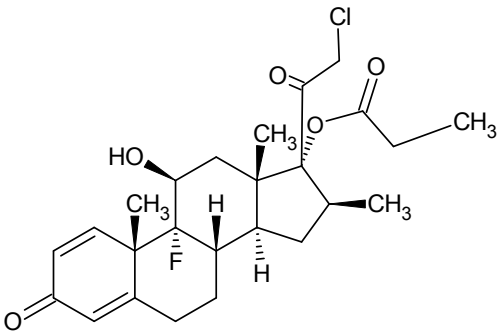

Fig.1 (a): Structure of Clobetasol Propionate

*Corresponding Author: Deepali P. Mohapatra

Address: F-203, Sairuchi C-1 appt, Kanchanjunga Society, opp Jagan Park, Kabarstan Road, Chala, Vapi-396191, Gujarat, India

Email ✉: mohapatrad43@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

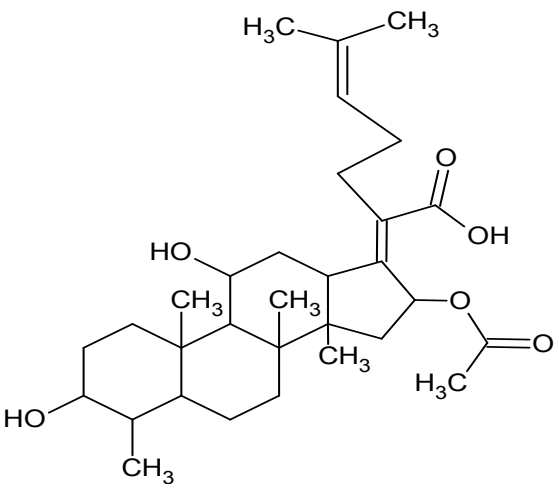

Fig.1 (b): Structure of Fusidic acid

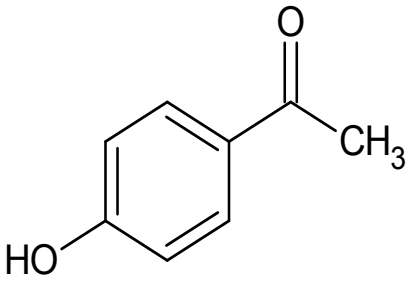

Fig.1(C): Structure of Methylparaben

Clobetasol propionate (CP) and Fusidic acid (FA) drugs were combined in a single dosage form (cream) in the brand name of Lozivate®-F for the treatment of various skin diseases like eczema, psoriasis, dermatitis, etc., also reduces redness, swelling by acting against infection-causing bacterias. Lozivate®-F cream contains (0.05%w/w Clobetasol propionate, 2 %w/w Fusidic acid and 0.1%w/w Methylparaben as preservative). CDSCO approves the combination on 17/07/2015.

Several analytical methods like UV,[4,5] HPLC,[6-9] and HPTLC[7] are reported alone and in combination with other drugs to determine Clobetasol propionate and Fusidic acid in the literature for its assay. However, there is a reported RP-HPLC[8] method reported for simulation estimation of Clobetasol propionate and Fusidic acid in combination by using Acetonitrile: water (80:20 %v/v) and adjusted with pH 5.0 with glacial acetic acid (10%), and the reported Rt were Clobetasol Propionate: 5.55 minutes, Fusidic acid: 7.48 minutes have been reported, during this method use of 20%, Water HPLC grade may increase the cost of analysis. The present study proposes a new RP-HPLC method using a mixture of ACN: Water (90:10 % v/v) as a mobile phase for simultaneous estimation of Clobetasol Propionate, Fusidic acid, and Methylparaben and validation of developed methods as per ICH guidelines,[10] Criteria employed for assessing the suitability of the proposed method were cost-effectiveness and speed of analysis.

Material and Methods

Instrumentation

The HPLC system used was gradient HPLC Shimadzu LC-2010 CHT, series equipped with a 10 µL sample loop and UV detector. The output signal was monitored and integrated using software LC solution version 1.25. Shim-pack solar C18 (250 × 4.6 mm, 5µm) column was used for the separation.

Materials

The drug sample of Clobetasol propionate and Fusidic acid was obtained from Avik Pharmaceutical Ltd, Vapi and Aroma Remedies, Daman, respectively. The creams LOZIVATE®-F which are marketed and manufactured by Canixa Life Science Pvt, Uttarakhand. It was procured from the market. Label claims for Clobetasol propionate and Fusidic acid were 0.05% w/w and 2.0% w/w per cream. Acetonitrile HPLC Grade (Rankem chemicals), HPLC Grade water (Rankem chemicals), HPLC Grade Glacial acetic acid (Rankem chemicals) are used in the study.

Standard Stock Solution

The stock solution of Clobetasol propionate was prepared by dissolving 12.5 mg in a 25 mL volumetric flask and then makes up the volume with Acetonitrile. The stock solution of Fusidic acid was prepared by dissolving 10 mg in a 25 mL volumetric flask and then makes up the volume with Acetonitrile. The stock solution of Methylparaben was prepared by dissolving 10 mg in a 10 mL volumetric flask and then makes up the volume with Acetonitrile.

A Binary Mixture of CP, FA, and MP Preparation

Aliquots of 1 mL from working solution of CP (50 µg/mL), 5 mL from the working solution of FA (400 µg/mL), and 1 mL from working solution of MP (100 µg/mL) were taken into a common volumetric flask and diluted up to 10 mL with mobile phase to make final concentration CP (5 µg/mL), FA (200 µg/mL), and MP (10 µg/mL).

Sample Preparation

Weigh 1 gram of cream, Dissolve it in 50 mL of methanol and sonicate it for 10 minutes. Heat at 60–65°C until the

Trial		
Reported HPLC		
Drug	*Clobetasol*	
Propionate	*Fusidic acid*	
Reported	5.5	7.4
Comparison	6.2	7.8

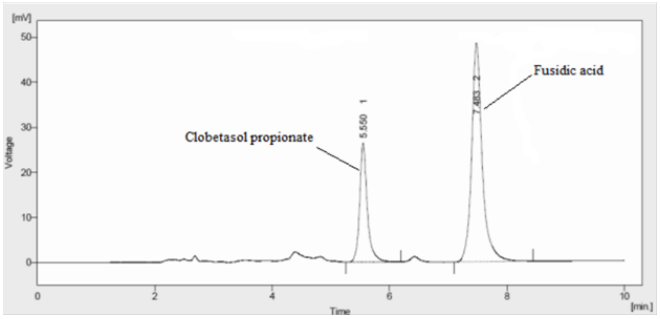

Fig. 2(a): Chromatogram of REPORTEDCP and FA in ACN: Water (80:20 %v/v)

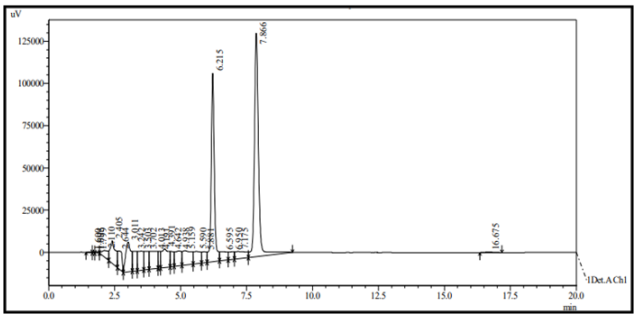

Fig.2(b): Chromatogram of CP and FA in ACN: Water (80:20 %v/v)

base is dissolved and cool it at room temperature. Filter the extract through Whatman filter paper no. 42 and make up the volume up to 25 mL with methanol. Final stock solution containing CP (10 µg/mL) + FA (400 µg/mL) + MP (20 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 CP (3–7 µg/mL), FA (200–280 µg/mL) and MP (6–14 µg/mL).

Method Development

The mobile phase consisting of Acetonitrile and Water in varying proportions and change in pH was tried. Finally, the ratio of 90:10 (pH-5.0 adjusted with diluted 10% Glacial acetic acid) was selected because it was found to give good separation for the peaks of Clobetasol propionate (Rt- 4.787 minutes), Fusidic acid (Rt- 6.006 minutes), and Methylparaben (Rt- 3.277 minutes) respectively as shown in Fig 3. 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 240 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Results

Method Development

There is one HPLC method reported. So I have performed using the same chromatographic conditions as reported ones and then compare them with the reported methods. In this comparison, the TRIAL gave a satisfactory result, so I decided to develop a new method by modifying the reported method (Fig.2 (a,b)). ACN: WATER (90:10%v/v) adjusted to pH 5.0 using Glacial acetic acid (10%) was selected because it was found to give good separation for the peaks of Clobetasol Propionate (Rt- 4.787 minutes), Fusidic acid (Rt- 6.006 minutes), and Methylparaben (Rt- 3.277 minutes) respectively as shown in Figure 4. In addition to this, 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 240 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Chromatographic Conditions

- *Stationary Phase:* Shim- pack solar C18 (250 × 4.6 mm, 5 µm)
- *Mobile phase:* Acetonitrile and Water (90:10 %v/v) adjusted to pH 5 using Glacial acetic acid (10%),
- *Flow rate:* 1 mL/min
- *Wavelength:* 240 nm

Preparation of Glacial acetic acid (10%):- was prepared by diluting 1 mL of concentrated Glacial acetic acid into 10 mL of HPLC grade water.

Method Validation

Specificity

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 was established by spiking of CP, FA, and MP in hypothetical placebo (i.e. might be expected to be present) and expressing that analytes peak did not interfere from excipients [Fig 3 (a,b,c)].

Linearity

Mixed standard solution of Clobetasol propionate and Fusidic acid were prepared with mobile phase in such a way that the final concentration of Clobetasol propionate and Fusidic acid and Methylparaben is in the range of 3–7 µg/mL, 120–280 µg/mL and 6–12 µg/mL, respectively. Overlay chromatogram of CP, FA, and MP as shown in Fig 5. The peak area was recorded for all the peaks as shown in Tables 3 and 4 for linearity of Clobetasol propionate, Fusidic acid, and Methylparaben. The plots of peak area versus the respective concentration were found to be linear with regression coefficient (R^2 =0.9998) for Clobetasol propionate, (R^2=0.9981) for Fusidic acid, and (R^2=0.9987) for Methylparaben as shown in Fig 6, 7, and 8.

System Suitability Studies

Evaluation of system suitability was done by analyzing six replicate of CP, FA, and MP in a mixture at a concentration of 5 µg/mL of CP, 200 µg/mL of FA, and 10 µg/mL of MP. The column efficiency, peak asymmetry, and resolution were calculated for each replicate. As shown in Table 2.

Accuracy

For accuracy study data from nine determinations over three concentrations at 80%, 100%, and 120% of expected sample concentration covering the specified range was determined & expressed as recovery values. The results were shown in Table 8.

Precision

The method Precision was established by carrying out the analysis of two drugs using the proposed analytical method in six replicates. It indicates the sample repeatability of the method. The results were shown in Tables 5, 6, and 7.

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 were shown in Tables 9, 10, and 11.

Assay

The validated HPLC method was applied to the simultaneous determination of Clobetasol propionate, Fusidic acid, and Methyl Paraben in marketed pharmaceutical dosage form, i.e., cream 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 is dissolved and cool it at room temperature. Filter the extract through Whatman filter paper no. 42 and make up the volume up to 25 mL with methanol. Final stock solution containing CP (10 µg/mL) + FA (400 µg/mL) + MP (20 µg/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 CP (5 µg/mL), FA (200 µg/mL), and MP (10 µg/mL). The results were shown in Table 12.

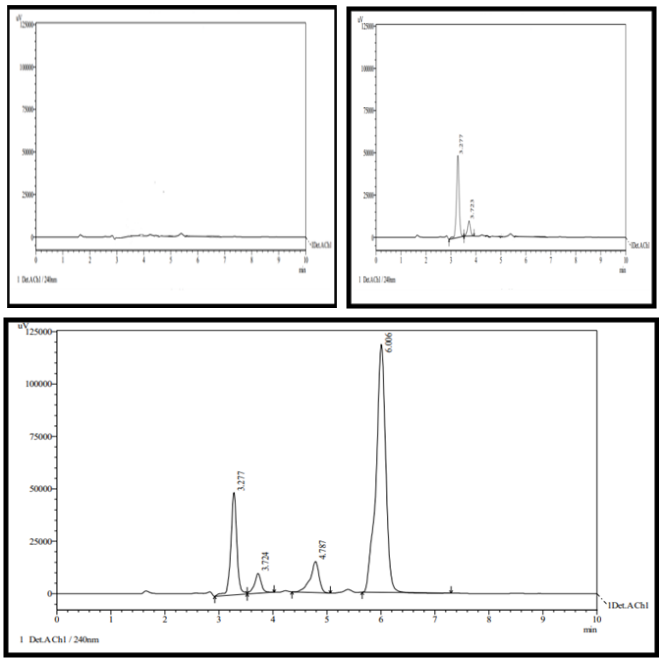

Fig. 3: Chromatograms of (A) Blank; (B) Placebo; (C) Formulation

Discussion

In the growing era of international competition for maintaining the standard of products in high commercial and market value, the development and validation

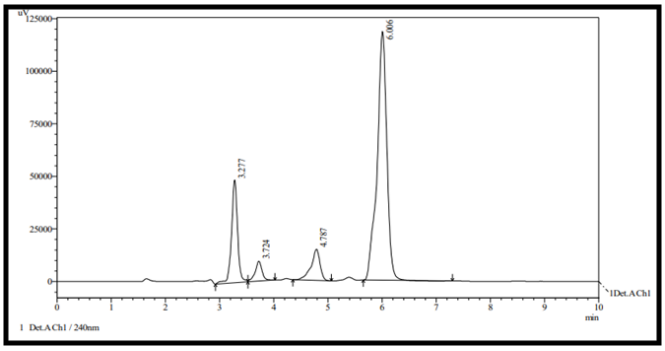

Fig.4: Chromatogram of Clobetasol Propionate (CP), FusidicAcid (FA), and Methylparaben (MP) in Acetonitrile: Water (90:10%v/v)pH adjusted to 5 with 10% Glacial acetic acid. Where MP and PP are Methyl and Propylparaben which are preservatives in a cream formulation.

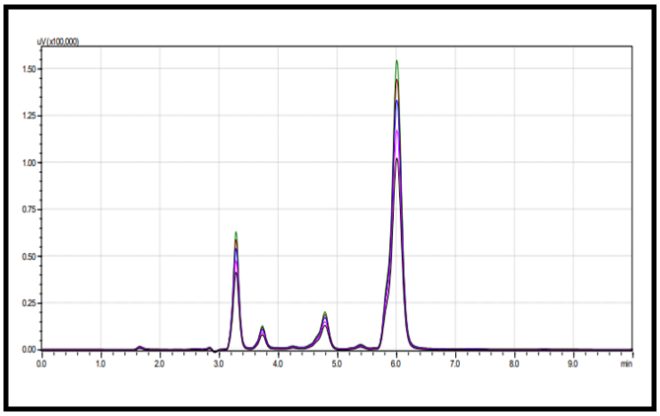

Fig. 5: Overlain Chromatogram of CP (3 - 7 µg/mL), FA (120 - 280 µg/mL) and MP (6 -14 µg/mL)

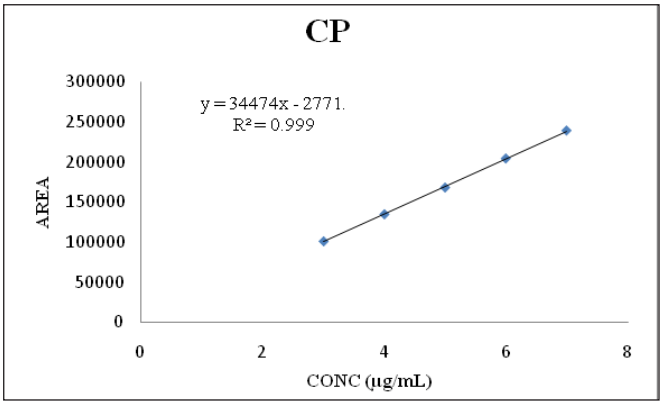

Fig 6: Calibration curve of CP

of analytical methods became obligatory. Analytical method development is the process of demonstrating whether an analytical method is acceptable for use in the workplace to quantify the concentration of a subsequent sample. All the methods were reported on the HPLC techniques with more retention time and run times in

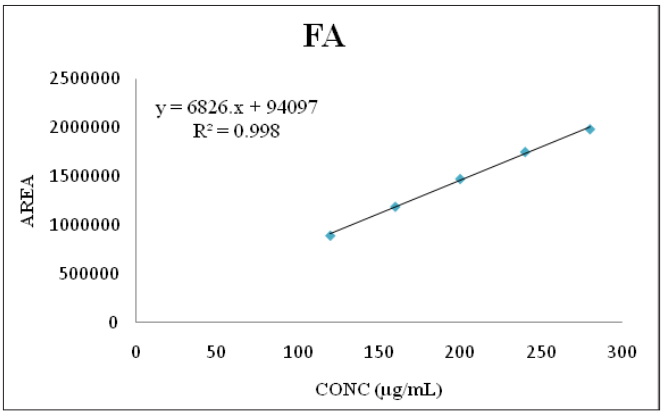

Fig 7: Calibration curve of FA

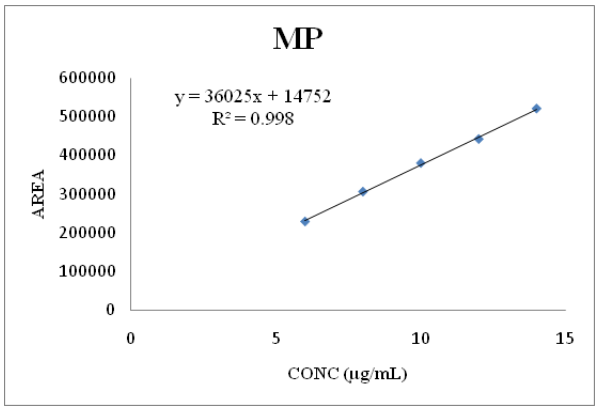

Fig. 8: Calibration curve of MP

Table 1: HPLC Trials for selection of mobile phase

No. of trials	*Mobile Phase*	*Ratio (%V/V)*	*pH*	*Observation*
Trial 1	Acetonitrile: Water	80:20	5	Same retention time as per reported method was observed
Trial 2	Acetonitrile:Methanol: Water	50:10:40	Water is adjusted to pH 5	Two peaks were separated but one peak is broad
Trial 3	Acetonitrile:Methanol: Water	50:15:35	Water is adjusted to pH 5	Two peaks were separated but one peak is broad
Trial 4	Acetonitrile:Methanol: Water	75:5:20	Water is adjusted to pH 5	Two peaks were separated but no good resolution
Trial 5	Acetonitrile: Water	80:20	-	Two peaks were separated but tailing was observed
Trial 6	Acetonitrile: Water	80:20	3.5	Two Peaks was Observed
Trial 7	Acetonitrile: Water	90:10	5	Two Peaks was Observed with good resolution

Table 2: System suitability data

Drugs	*Parameters*	*Mean ± SD (n=6)*	*% RSD*
MP	Retention Time	3.27 ± 0.0172	0.526
	Theoretical Plate	26905.52 ± 81.19	0.422
	Tailing Factor	0.921 ± 0.0065	0.687
PP	Retention Time	3.74 ± 0.0287	0.669
	Theoretical Plate	27524.13 ± 124.06	0.405
	Tailing Factor	0.9116 ± 0.0075	0.826
	Resolution	2.326 ± 0.0135	0.619
CP	Retention Time	4.75 ± 0.0463	0.974
	Theoretical Plate	31824.74 ± 167.27	0.522
	Tailing Factor	0.7816 ± 0.0075	0.963
	Resolution	4.210 ± 0.0377	0.895
FA	Retention Time	6.03 ± 0.0307	0.509
	Theoretical Plate	39764.51 ± 153.19	0.385
	Tailing Factor	0.8166 ± 0.0081	0.446
	Resolution	4.166 ± 0.0265	0.637

Table 3: Linearity data for CP and FA

Sr. No.	*CP Concentration (µg/mL)*	*FA Concentration (µg/mL)*	*CP Mean Peak Area ± S.D. (n=5)*	*FA Mean Peak Area ± S.D. (n=5)*	*CP % R.S.D.*	*FA % R.S.D.*
1.	3	120	101366.8 ± 447.3815	896572 ± 2278.778	0.441	0.254
2.	4	160	134961 ± 334.5445	1191641 ± 4376.962	0.244	0.367
3.	5	200	168277 ± 570.1929	1475468 ± 4203.394	0.338	0.284
4.	6	240	294357.4 ± 1923.747	1750798 ± 5380.699	0.541	0.307
5.	7	280	239040.2 ± 912.3268	1982219 ± 10256.66	0.481	0.517

Table 4: Linearity data for MP

Sr. No.	*MP Concentration (µg/mL)*	*MP Mean Peak Area ± S.D. (n=5)*	*MP % R.S.D.*
1.	6	228170 ± 1300.02	0.567
2.	8	305469 ± 1270.33	0.415
3.	10	379448 ± 2420.10	0.637
4.	12	441554 ± 3073.71	0.696
5.	14	520380 ± 4433.82	0.852

Table 5: Repeatability data for CP, FA, and MP

Drugs	*Concentration (µg/mL)*	*Mean Peak Area ± S.D. (n=6)*	*% R.S.D.*
CP	4	139295.7 ± 302.0872	0.216
FA	160	1189705 ± 4001.068	0.336
MP	8	305283 ± 1495.25	0.398

Table 6: Intraday precision data for CP,FA, and MP

Drugs	*Concentration (µg/mL)*	*Mean Peak Area ± SD (n=3)*	*% RSD.*
CP	4	135246.3 ± 464.7939	0.343
	5	168355.7 ± 614.0304	0.364
	6	202823.7 ± 577.3503	0.284
FA	160	1192264 ± 4338.237	0.405
	200	1476219 ± 5747.698	0.389
	240	1751743 ± 7155.65	0.408
MP	8	305292 ± 1663.64	0.445
	10	380646 ± 1166.07	0.307
	12	441548 ± 2165.64	0.490

Table 7: Interday precision data for CP, FA, and MP

Drugs	*Concentration (µg/mL)*	*Mean Peak Area ± S.D. (n=3)*	*% R.S.D.*
CP	4	135179.3 ± 686.4695	0.507
	5	168315.7 ± 1251.652	0.743
	6	204807 ± 1541.72	0.752
FA	160	1192234 ± 6675.735	0.559
	200	1484219 ± 9841.038	0.663
	240	1758519 ± 12139.66	0.690
MP	8	307359 ± 2158.11	0.702
	10	378201 ± 2424.72	0.641
	12	442458 ± 3975.97	0.892

Table 8: Accuracy data for CP, FA, and MP

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)*
CP	0	3	0	3	99840 ± 1506.687	2.980	99.36 ± 0.015
	80	3	2.4	5.4	184396 ± 6063.657	5.431	100.46 ± 0.202
	100	3	3	6	203706 ± 1114.785	5.990	99.84 ± 0.015
	120	3	3.6	6.6	224169 ± 643.021	6.583	99.79 ± 0.066
FA	0	120	0	120	742184 ± 32704.174	119.564	99.68 ± 0.075
	80	120	96	216	1403901 ± 59708.003	215.605	99.86 ± 0.020
	100	120	120	240	1572134 ± 13460.513	240.022	100.35 ± 0.277
	120	120	144	264	1734372 ± 6863.9153	263.568	99.89 ± 0.058
MP	0	6	0	6	198516 ± 35818.100	5.92	98.68 ± 0.065
	80	6	4.8	10.8	371534 ± 24073.350	10.73	99.28 ± 0.040
	100	6	6	12	419280 ± 564000.95	12.04	100.40 ± 0.297
	120	6	7.2	13.2	459506 ± 6371.83	13.16	99.73 ± 0.059

Table 9: Robustness data for CP

Parameters	*Level*	*Mean Peak Area ± SD (n=3)*	*% RSD.*	*Rt ± SD (n=3)*	*% RSD.*
Mobile Phase (90: 10 v/v)	88:8 v/v	168277 ± 509.996	0.303	4.728 ± 0.023	0.489
	92:12 v/v	168659 ± 726.772	0.430	4.75 ± 0.034	0.638
Flow rate (1.0 mL/min)	0.8 mL/min	169059 ± 779.872	0.461	4.950 ± 0.037	0.506
	1.2 mL/min	169287 ± 534.304	0.315	4.524 ± 0.024	0.785

Table 10: Robustness data for FA

Parameters	*Level*	*Mean Peak Area ± SD (n=3)*	*% RSD.*	*Rt ± SD (n=3)*	*% RSD.*
Mobile Phase (90: 10 v/v)	88:8 v/v	1477699 ± 4061.702	0.274	6.028 ± 0.019	0.319
	92:12 v/v	1479123 ± 6261.005	0.423	6.05 ± 0.031	0.522
Flow rate (1.0 mL/min)	0.8 mL/min	1481699 ± 9874.236	0.666	6.120 ± 0.039	0.401
	1.2 mL/min	1479179 ± 6730.084	0.454	5.830 ± 0.018	0.647

Table 11: Robustness data for MP

Parameters	*Level*	*Mean Peak Area ± SD (n=3)*	*% RSD.*	*Rt ± SD (n=3)*	*% RSD.*
Mobile Phase (90: 10 v/v)	88:8 v/v	226850 ± 1114.75	0.338	3.270 ± 0.018	0.419
	92:12 v/v	227450 ± 770.75	0.494	3.266 ± 0.020	0.632
Flow rate (1.0 mL/min)	0.8 mL/min	227670 ± 1121.96	0.476	3.073 ± 0.047	0.539
	1.2 mL/min	226690 ± 1085.64	0.359	3.470 ± 0.031	0.609

Table 12: Analysis of marketed formulation

Marketed Formulation	*Amount taken (µg/mL)*		*Amount Obtained Mean ± SD. (µg/mL)*	*% Amount Obtained Mean ± S.D. (n=5)*	
	CP	5	4.95 ± 0.030	CP	100.76 ± 1.031
	FA	200	199.95 ± 0.031	FA	99.95 ± 0.788
	MP	10	9.90 ± 0.042	MP	99.06 ± 0.642

Table 13: Summary of RP-HPLC method

Parameters	*CP*	*FA*	*MP*
Linearity (µg/mL) (n=5)	3–7 µg/mL	120–280 µg/mL	6–12 µg/mL
Regression Equation	y = 34474x - 2771.8	y = 6826.2x + 94097	y = 35688x + 16207
Regression coefficient (R^2)	0.9998	0.9981	0.9987
Correlation coefficient (r)	0.9999	0.9990	0.9989
Repeatability (%R.S.D.) (n=6)	0.216	0.336	0.398
Intraday precision (%R.S.D.) (n=3)	0.284–0.343	0.405–0.408	0.445–0.490
Interday precision (%R.S.D.) (n=3)	0.507–0.752	0.559–0.690	0.702–0.892
LOD (µg/mL) (n=5)	0.150	3.859	0.201
LOQ (µg/mL) (n=5)	0.456	11.69	0.619
% Recovery (n=3)	99.36 – 100.46	99.63 – 100.35	98.68 – 100.64
% Assay ± S.D. (n=3)	100.76 ± 1.031	99.95 ± 0.788	99.06 ± 0.642

the literature. In the present work, we selected RP-HPLC to reduce the total run time by modifying the reported method. Here, the quantity of Methylparaben is more than the quantity of Clobetasol propionate therefore, We included methylparaben and developed the method. Method development was executed with different columns and mobile phases. Finally, the method was optimized with a mobile phase of Acetonitrile and Water (90:10 %v/v) adjusted to pH 5.0 using Glacial acetic acid (10%) utilizing a Shim- pack solar C18 column, which has dimensions of 250 × 4.6 mm, 5.0 µm particle size, and the flow rate of 1 mL/min, following were detected by a UV detector.

Further, the developed method was subjected to validation. Validation was executed per the ICH Q2R1 guidelines for the parameters specificity, linearity, system suitability, LOD, LOQ, precision, accuracy, and robustness. All the parameters were within limits.

Conclusion

A sensitive, rapid, and accurate RP-HPLC method for the simultaneous estimation of Clobetasol Propionate, Fusidic acid, and Methylparaben in formulations was developed and validated as per the ICH guidelines. Retention times for Clobetasol Propionate (CP), Fusidic acid (FA), and Methylparaben (MP) were achieved at 4.787, 6.006, and 3.277 minutes, respectively. The mean percentage recovery of CP, FA, and MP were 100.46, 99.63, and 100.64%, respectively. LOD/LOQ values were obtained from regression equations of CP, FA, and MP and were found to be 0.15/0.45, 3.85/11.69, and 0.20/0.61 µg/mL, respectively. The regression equation of CP, FA, and MP were: y = 34474x - 2771.8, y = 6826.2x + 94097, and y = 35688x + 16207, respectively.Retention time and total run times of analytes were decreased. Hence, the developed method was rapid and economical that can be applied in the routine analysis of these drugs in the quality control department of pharmaceutical trades.

Acknowledgement

The authors are thankful to ROFEL Shri G.M. Bilakhia College of Pharmacy, Vapi, and Gujarat, India for providing all the facilities to carry out the work. The authors are thankful to Avik Pharmaceutical Ltd. Vapi, Gujarat, India and Aroma Remedies, Daman, India for providing Gift samples of Clobetasol propionate and Fusidic acid respectively.

References

1. Ahmed NR, Mohamad NS. Spectrophotometric determination of clobetasol propionate in pharmaceutical preparations and environmental samples. World Journal of Pharmacy and Pharmaceutical Sciences. 2018 Jul 25;7(10):167-173. Avaliable from: doi: 10.20959/wjpps201810-12386.
2. Devi N, Kumar S, Rajan S, Gegoria J, Mahant S, Rao R. Development and validation of UV spectrophotometric method for quantitative estimation of clobetasol 17-propionate. Asian Journal of Chemistry and Pharmaceutical Sciences. 2016 Nov 21;1(1):36-40. Avaliable from: doi: 10.18311/ajcps/2016/7726
3. Trivedi DG, Chaudhary AB, Patel BD. Method development and validation for estimation of clotrimazole, fusidic acid and mometasone furoate in cream by rp-hplc. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;6(5):1204-1219. Avaliable from: doi: 1020959/wjpps20175-9173
4. Rasool N, Kanwal Q, Waseem M, Khan MI. Analytical method development and determination of hydrocortisone acetate and fusidic acid simultaneously in cream formulation, by reversed-phase HPLC. Biomedical Chromatography. 2021 Mar;35(3):e4997. Avaliable from: doi:10.1002/bmc.4997
5. 5. Bhuyian MH, Rashid HA, Islam AA, Tareque MI. Development and validation of method for determination of clobetasol propionate and salicylic acid from pharmaceutical dosage form by HPLC. Journal of Pharmaceutical Research International. 2015 Jul 6:375-385. Avaliable from: doi : 10.9734/BJPR/2015/18494
6. Patel B, Raj H, Jain V, Sutariya V, Bhatt M. Development and validation of reversed phase—High performance liquid chromatography method for clobetasol propionate and salicylic acid in its pharmaceutical dosage froms. Pharma Science Monitor. 2014 Apr 2;5 (Suppl 1): 374-385.
7. Malani PS, Raj HA, Jain VC. Development and validation of analytical method for simultaneous estimation of miconazole nitrate and clobetasol propionate in cream by HPTLC method. International Journal of Pharmaceutical Sciences. 2014 Apr 2;5:386-399.
8. Jakasaniya MA, Shah JS, Maheswari DG. Simultaneous estimation of clobetasol propionate and fusidic acid in cream dosage form by reversed phase high performance liquid chromatographic method. Pharmacophore Journal. 2014 Mar 1;5(2):231-238. Avaliable from: Issn no: 2229-5402
9. Patel A, Shah B. RP-HPLC method development and validation using factorial design for simultaneous estimation of thiocolchicoside and etodolac with forced degradation studies. Journal of Pharmaceutical Science and Bioscientific Research. 2014;4(6):374-382. Avaliable from Issn no: 2271-3681
10. Guideline IH. Validation of analytical procedures: text and methodology. Q2 (R1). 2005 Nov;1(20):05.

HOW TO CITE THIS ARTICLE: Mohapatra DP, Patel A, Mohapatra TK. RP-HPLC Method Development and Validation for Simultaneous Estimation of Clobetasol Propionate and Fusidic Acid in Cream. Int. J. Pharm. Sci. Drug Res. 2021;13(4):402-409. **DOI:** 10.25004/IJPSDR.2021.130406