



Research Article

QUALITY BY DESIGN (QBD) BASED OPTIMIZATION AND VALIDATION OF AN RP-HPLC METHOD FOR SIMULTANEOUS ANALYSIS OF MULTICOMPONENT TOPICAL CREAM

Om S. Bilone, Payal O. Bilone, Anil P. Dewani, Anil V. Chandewar

Department of Quality Assurance, P.Wadhwani College of Pharmacy, Yavatmal, M.S. India

Affiliated to Sant Gadge Baba Amravati University, Amravati

*Correspondence Author email: bilone.om@gmail.com

ARTICLE INFO

ABSTRACT

Article history:

Received: 14 July 2026

Revised: 13 Aug. 2026

Accepted: 13 Aug. 2026

Published: 20 Sept 2026

DOI:

[10.68007/rjmp.2026.v5.iss3.p1](https://doi.org/10.68007/rjmp.2026.v5.iss3.p1)



Copyright:© 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license(<https://creativecommons.org/licenses/by/4.0/>)

This study reports the development and validation of a rapid, specific, and robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous estimation of Ofloxacin (OFL), Ornidazole (ORN), Itraconazole (ITR), and Clobetasol (CLO) in a cream formulation. A systematic Quality by Design (QbD) approach, utilizing a three-factor, five-level Central Composite Design (CCD), was employed to optimize chromatographic conditions. The independent variables evaluated were the ratio of the polar phase (water), the pH of the polar phase, and the flow rate, while the response factors included peak tailing and total chromatographic run time. The optimized chromatographic conditions consisted of a Kinetex® 5µm Biphenyl 10 LC column (250×4.6 mm) with a mobile phase comprising Acetonitrile and Water (pH 4.0 adjusted with orthophosphoric acid) in a 60:40 v/v ratio, a flow rate of 1 mL/min, and a detection wavelength of 249 nm. The method demonstrated excellent linearity (correlation coefficient >0.9990), accuracy (mean recovery between 98.0% and 102.0%), and precision. System suitability parameters, including theoretical plates (>2000) and tailing factors (<2.5), were within acceptable limits. The validated method was successfully applied to the assay of a marketed cream formulation, yielding results consistent with label claims. Robustness studies confirmed the method's reliability under deliberate variations in flow rate, pH, and mobile phase composition.

Keywords:

RP-HPLC; Method Development; QbD Approach; Simultaneous Estimation; Central Composite Design; Ofloxacin; Ornidazole; Itraconazole; Clobetasol

How to cite this article: Om.S.Bilone Payal O.Bilone, Anil P. Dewani, Anil V. Chandewar. Quality By Design (QBD) based optimization and validation of an RP-HPLC method for simultaneous analysis of multicomponent topical cream. *Research Journal of Medicine & Pharmacy*. 2026 May, 5(3):92-106.

1. INTRODUCTION

Multidrug semisolid dosage forms, such as creams, represent a sophisticated approach in modern topical drug delivery. By incorporating two or more active pharmaceutical ingredients (APIs) into a single base, these formulations provide combined therapeutic actions, improve patient compliance, and enhance treatment efficacy for conditions such as skin infections, inflammation, and pain. The formulation of these creams requires rigorous attention to drug compatibility, stability, uniform distribution, and skin permeability.¹⁻²

The pharmaceutical industry operates under rigorous regulatory frameworks, necessitating the development of precise and robust analytical methodologies to ensure the identity, strength, quality, and purity of drug products. As combination therapies—specifically those incorporating

multiple active pharmaceutical ingredients (APIs) for localized dermatological treatments—become increasingly prevalent,³⁻⁴

This study addresses the simultaneous estimation of four chemically diverse drugs: Ofloxacin (OFL), chemically 7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid an antibacterial agent,⁵ Ornidazole (ORN), chemically 1-chloro-3-(2-methyl-5-nitroimidazol-1-yl)propan-2-ol an antiprotozoal,⁶ Itraconazole (ITR), chemically 2-butan-2-yl-4-[4-[4-[[[(2R,4S)-2-(2,4-dichlorophenyl)-2-[(1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one an antifungal⁷ and Clobetasol (CLO), (8S,9R,10S,11S,13S,14S,16S,17R)-17-(2-chloroacetyl)-9-fluoro-11,17-dihydroxy-10,13,16-

trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one a potent corticosteroid.⁸ The simultaneous determination of these specific analytes is particularly complex due to their disparate polarities and structural characteristics.

Quality by Design (QbD) is a concept originally introduced by renowned quality expert Joseph M. Juran in his influential publications, most notably Juran on Quality by Design. While these principles have been implemented across various industries to improve product and process quality—most notably in the automotive sector—they have recently been adopted by the U.S. Food and Drug Administration (FDA) to transform how drugs are discovered, developed, and manufactured.⁹⁻¹¹

Since the FDA first initiated this shift through its "Pharmaceutical cGMPs for the twenty-first century" program, QbD has become a cornerstone concept for the pharmaceutical industry. As further defined in the International Conference on Harmonisation (ICH) guidance on pharmaceutical development, QbD is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". Consequently, the scientific understanding gained during the method development process is now used to devise effective method control elements and manage identified risks.¹²⁻¹⁴

The aim of the analytical method is to separate and quantify the main compound while meeting the method performance criteria based on regulatory requirements, such as specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness.

The study employs a systematic Quality by Design (QbD) approach, utilizing a three-factor, five-level Central Composite Design (CCD) via Design-Expert 13.0 software to optimize chromatographic conditions. The study specifically evaluates how variations in the percentage of the polar phase (water), the flow rate of the mobile phase, and the pH of the polar phase influence critical responses, including the tailing factors of Ofloxacin (OFL), Ornidazole (ORN), Itraconazole (ITR), and Clobetasol (CLO) and the total chromatographic run time.

Following optimization and successful method finalization through various experimental trials, the

procedure was validated in accordance with ICH Q2B guidelines.

2. MATERIAL AND METHOD

Material and chemical

The materials, chemicals, and instruments used in the experiment are documented as follows. The drug samples—Ofloxacin, Ornidazole, Clobetasol, and Itraconazole—were obtained from Yarrowpharm, with purities of $\geq 98\%$, 99.93%, 98.09%, and 98%, respectively. Reagents and chemicals utilized included HPLC grade water from Millie Queue Water, methanol from SD Fine Chem. Limited, acetonitrile from Merck Lab, and orthophosphoric acid.

Equipment

The chromatographic analysis is performed using an HPLC system equipped with a Photodiode Array (PDA) detector. The system utilizes a Kinetex® 5 μ m Biphenyl 10 LC column, which measures 250 mm in length with an internal diameter of 4.6 mm. The experimental design model was developed on Design-Expert 13.0 (Stat-Ease Inc., Minneapolis, USA).

Design of Experiments (CCD)

To optimize the chromatographic conditions, a three-factor, five-level Central Composite Design (CCD) was employed during the method development phase. This experimental design evaluated three independent variables: the ratio of the polar phase (water) in percent, the flow rate of the mobile phase in mL/min, and the pH of the polar phase. The efficacy of these variables was assessed based on several dependent responses, specifically the tailing factors for Ofloxacin (OFL), Ornidazole (ORN), Itraconazole (ITR), and Clobetasol (CLO), as well as the total chromatographic run time. Following the optimization process, the conditions identified in Trial 7 were selected as the final method. These optimized parameters consist of a mobile phase composed of a 60:40 v/v ratio of acetonitrile to water (with the water pH adjusted to 4.0 using orthophosphoric acid), a flow rate of 1 mL/min, and a detection wavelength of 249 nm. Additionally, the method is conducted at a column temperature ranging between 37°C and 38°C, with a total run time of 20 minutes.

Table 1 Factors and their levels for experimental design

Variables	Levels				
	-1.68179	-1	0	+1	+1.68179
Ratio of polar phase (water) (%)	30	35	40	45	50
Flow rate of mobile phase (mL/min)	0.8	0.9	1.0	1.1	1.2
pH of polar phase	3.6	3.8	4.0	4.2	4.4

Preparation of Solution

Standard Stock Solutions: Accurately weighed amounts of OFL, ORN, ITR, and CLO were dissolved in methanol in

10 mL volumetric flasks, sonicated to dissolve, and made up to the mark with diluent. Working Standards: Prepared by diluting stock solutions with methanol to achieve

desired concentrations (e.g., 7.5 µg/mL for OFL, 20 µg/mL for ORN, 10 µg/mL for ITR, and 5 µg/mL for CLO). Sample Solution (Cream Formulation): 10 g of cream (containing 0.75% OFL, 2% ORN, 1% ITR, 0.05% CLO) was transferred to a 100 mL volumetric flask, 50 mL of methanol was added, and the mixture was sonicated for 15 minutes. The solution was filtered through a 0.45 µm nylon syringe filter and centrifuged at 3000 rpm for 10 minutes. A 1 mL aliquot was further diluted to 10 mL with methanol.

Method Validation

The method was validated according to ICH Q2B guidelines: ¹⁵⁻¹⁶

System Suitability

Assessed by six replicate injections of the standard solution; acceptance criteria included %RSD for retention time ≤ 2.5%, theoretical plates > 2000, and tailing factor ≤ 2.5.

Linearity:

Determined by analyzing five concentrations (10–50 µg/mL) of the analytes; calibration curves were plotted and R² values were evaluated.

Accuracy:

Performed via recovery studies at 80%, 100%, and 120% levels using the standard addition method.

Precision:

Evaluated through system precision (standard solution) and method precision (homogenous sample of a single batch analyzed six times).

Specificity:

Confirmed by injecting blank and placebo samples to ensure no interference at the analytes' retention times.

Robustness:

Assessed by making small, deliberate changes to flow rate (±0.1 mL/min), pH (±0.1 units), and organic phase composition (±10%).

Analysis of marketed formulation

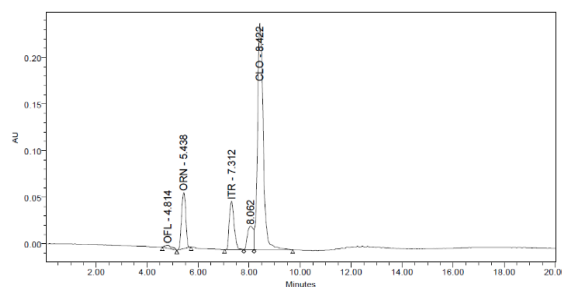
The analysis was conducted on the marketed cream formulation "Itromed-OC," manufactured by Leeford Healthcare Ltd., which has a label claim weight of 15 grams. To prepare the sample solution, 10 grams of the cream were transferred to a 50 mL volumetric flask, combined with 50 mL of methanol, and sonicated for 15 minutes to ensure complete dissolution. This solution was subsequently filtered through a 0.45 µm nylon syringe filter and centrifuged at 3000 rpm for 10 minutes. A 1 mL aliquot of the resulting clear supernatant was then transferred to a 10 mL volumetric flask and diluted to the mark with methanol.

3. RESULT AND DISCUSSION

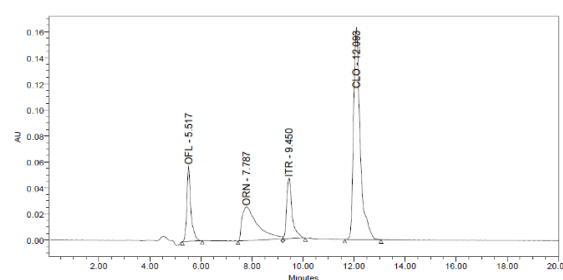
Design of Experiments (CCD)

A three-factor, five-level Central Composite Design (CCD) was employed to evaluate the influence of percentage of polar phase (water, X₁), flow rate (X₂), and pH of the polar phase (X₃) on the chromatographic performance. The selected response variables included the tailing factor (TF) of Ofloxacin (OFL), Ornidazole (ORD), Itraconazole (ITR), Clobetasol (CLO), and the total chromatographic run time (RT). A total of 19 experimental runs comprising factorial, axial, and centre points were performed, as presented in Table no. 2

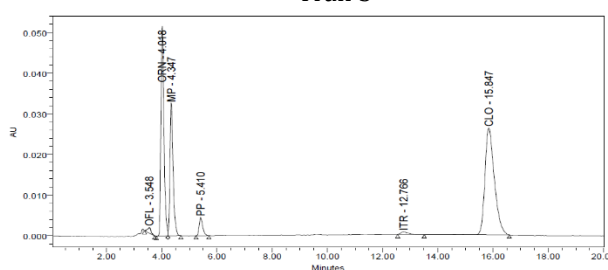
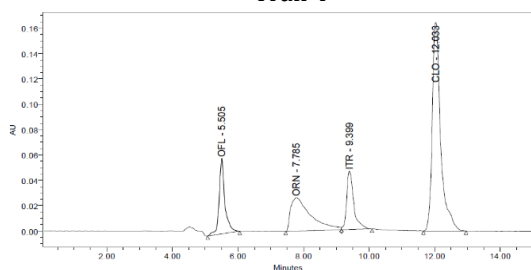
The centre point experiments (Runs 4, 5, 11, 13, and 19) demonstrated good reproducibility with minimal variation in the response values, confirming the reliability of the experimental design and indicating insignificant pure experimental error.



Trail 4



Trail 5



the statistics are given in Table 2. The contour (2D) plots and 3D plot of responses with respect to all factors are shown in Figure 01-05 for TF OFL, TF ORD, TF ITR, TFCLO and RT respectively. The optimum conditions were calculated using numerical optimization. To achieve the composite desirability (D), the response criteria were set as (lower-upper):

The three-dimensional response surfaces and contour plots demonstrated significant linear, interaction, and

quadratic effects of the selected factors. The optimized design space was identified near the center region of the experimental domain, where minimal tailing factors for all analytes and an acceptable chromatographic run time were simultaneously achieved. These findings confirmed that CCD is a suitable experimental design for systematic optimization of the RP-HPLC method, enabling robust separation with improved peak symmetry and reduced analysis time.

Table No.02. Response factors and independent variables fitted in central composite design CCD for analysis of OFL, ORD, ITR, CLO and Run Time

sr	%Polar phase (water)	Flow rate (mL/min)	pH of polar phase	Response factors				
				TF(OFL)	TF(ORD)	TF(ITR)	TF(CLO)	RT
1	1.68179	0	0	4.5	4.2	3.9	3.5	25
2	-1	1	1	3.5	2.9	3.1	4.2	26
3	0	0	-1.68179	3.9	3.2	2.9	3.5	20
4	0	0	0	1.5	1.6	1.2	1.8	21
5	0	0	0	1.6	1.6	1.5	1.8	20
6	0	1.68179	0	4.6	4.1	3.8	4.1	18
7	1	-1	-1	3.2	3.1	3.3	3.6	21
8	0	-1.68179	0	3.9	3.5	6.7	3.8	26
9	-1	-1	-1	3.8	3.6	4.1	3.5	29
10	0	0	1.68179	4.6	4.5	3.5	2.5	25
11	0	0	0	1.8	1.53	1.6	1.5	20
12	-1	1	-1	4.2	3.6	3.5	3.4	23
13	0	0	0	1.5	1.45	1.6	1.8	20
14	1	1	1	4.2	3.8	3.5	4.1	18
15	1	-1	1	4.4	3.8	3.6	4.5	25
16	-1	-1	1	3.5	3.1	3.5	3.6	24
17	-1.68179	0	0	3.6	3.45	3	3.6	28
18	1	1	-1	3.6	3.4	4.1	3.6	25
19	0	0	0	1.6	1.6	1.8	1.6	20

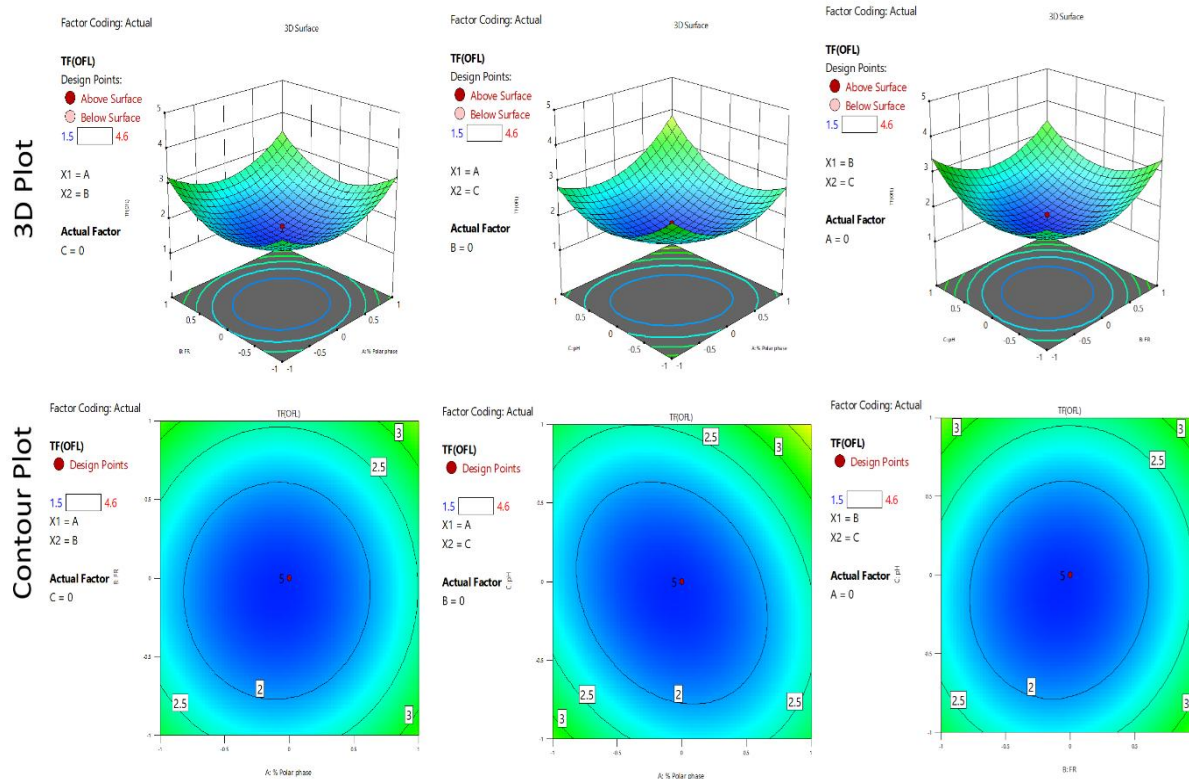


Figure No. 01 . Surface response plot outcome of CCD analysis (3D plot and Contour Plot) for ofloxacinThe tailing factor of Ofloxacin varied between 1.5 and 4.6

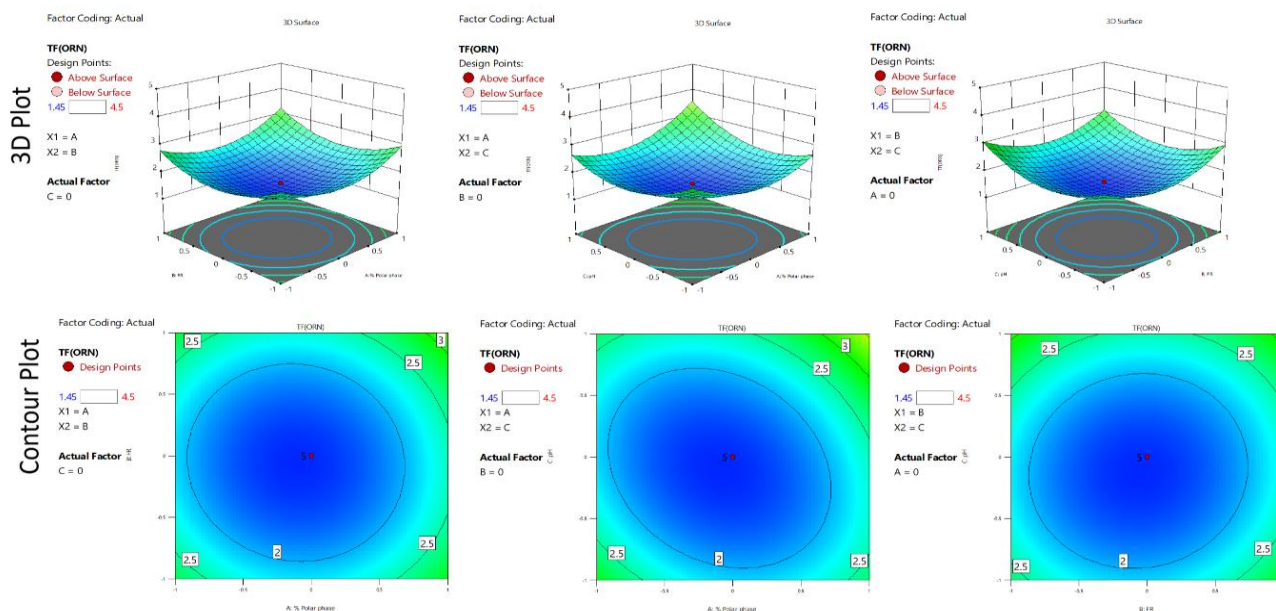


Figure No. 02 . Surface response plot outcome of CCD analysis (3D plot and Contour Plot) for Ornidazole the tailing factor ranged from 1.45 to 4.5. Similar to Ofloxacin

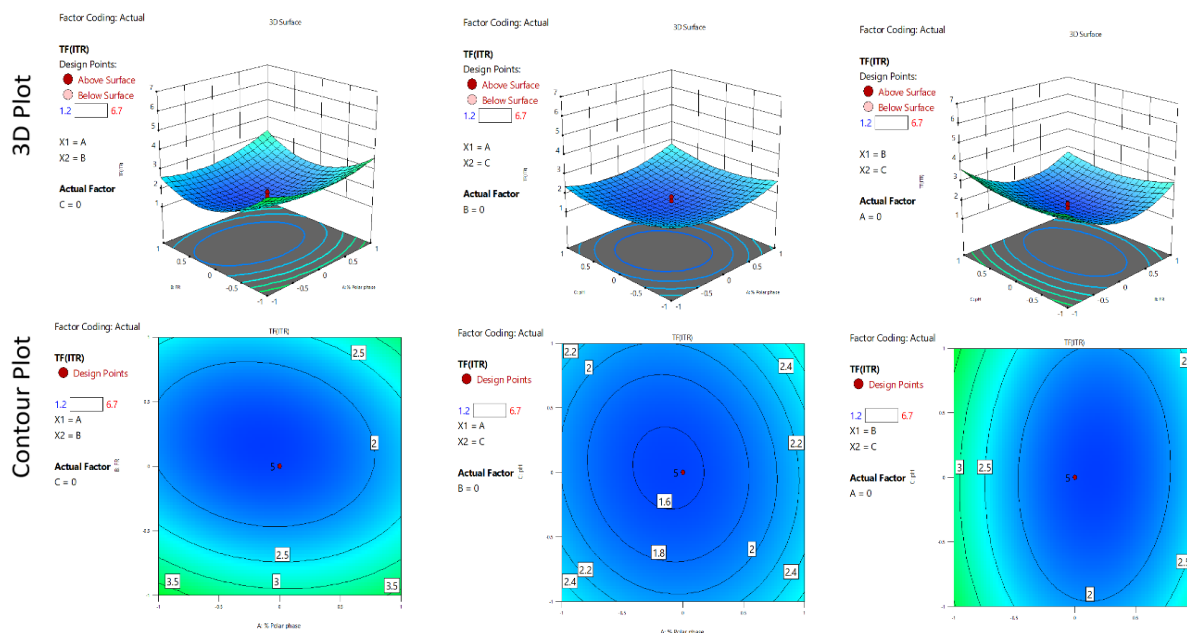


Figure No.03 .Surface response plot outcome of CCD analysis (3D plot and Contour Plot) for Itraconazole The tailing factor of Itraconazole varied from 1.2 to 6.7

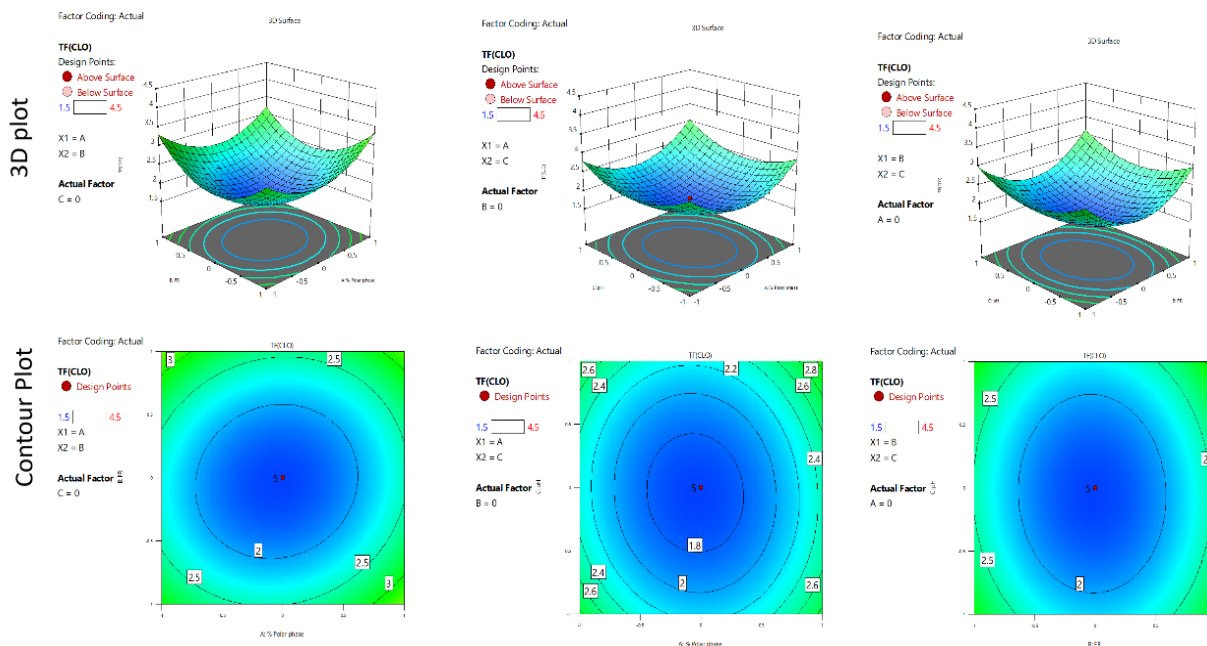


Figure No. 04 . Surface response plot outcome of CCD analysis (3D plot and Contour Plot) for clobetasol For Clobetasol, the tailing factor ranged between 1.5 and 4.5.

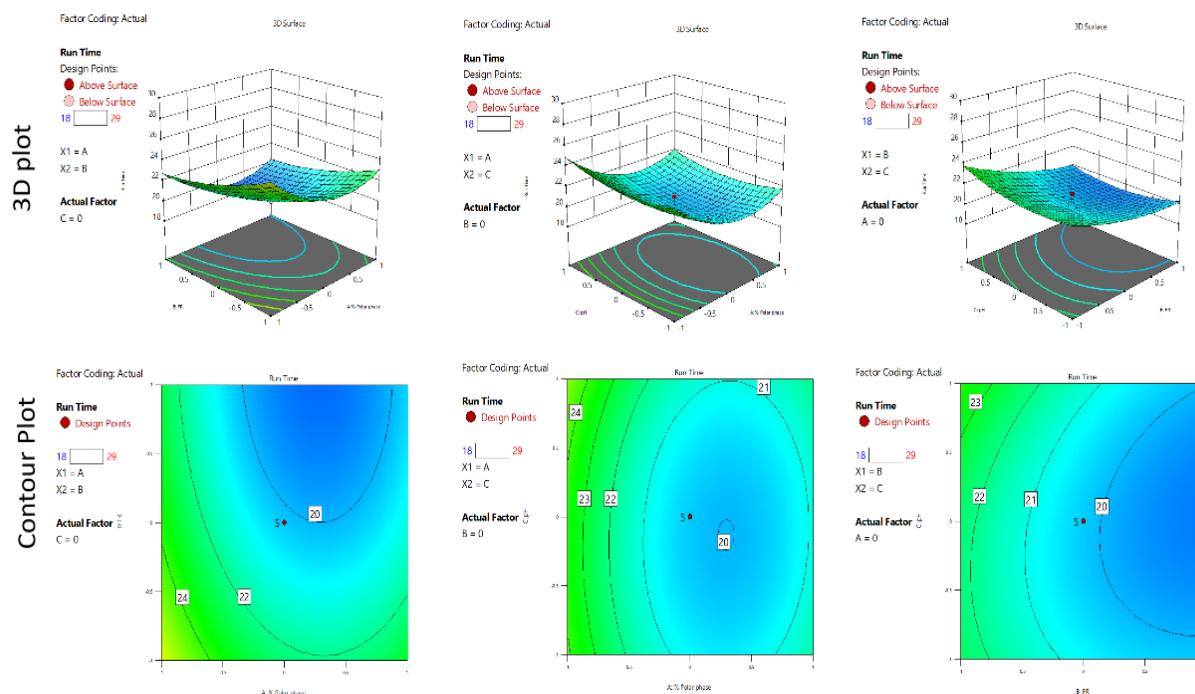


Figure No. 05.Surface response plot outcome of CCD analysis (3D plot and Contour Plot) for Run timeThe chromatographic run time ranged from 18 to 29 minutes

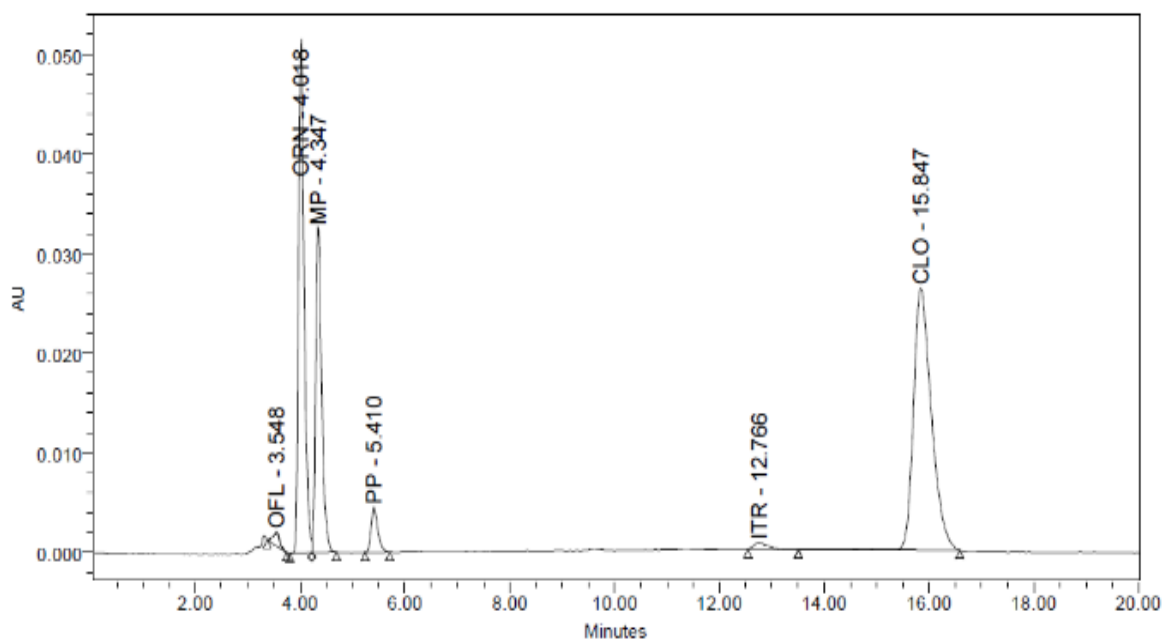


Figure No.6 Optimized chromatogram of standard solution mixture containing 7.5ug/ml of OFX, 20ug/ml of ORN, 10 ug/ml of ITR and 05 ug/ml of CLO

Method Validation

System suitability: -

All System suitability parameters (Retention Time, Peak Area, Tailing Factor) for OFL, ORD, ITR, CLO were

calculated for n=6 replicates to study the system suitability of HPLC method all the result were within acceptable limits, confirming the suitability of the instrument, reagents, and column. as shown in Table 3

Table No.03: Observation of System suitability Parameters

Parameter	compound	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Mean	SD	%RSD
Retention Time	OFL	3.039	3.061	3.057	3.041	3.036	3.066	3.058	0.0193	0.6322
	ORN	4.029	4.214	4.133	4.010	4.156	4.224	4.127	0.0907	2.1971
	ITR	7.365	7.405	7.447	7.412	7.298	7.304	7.371	0.0607	0.8243
	CLO	11.293	11.507	11.502	11.416	11.510	11.521	11.458	0.0894	0.7805
Peak area	OFL	15870	15848	15984	16014	15984	16030	15955	76.7619	0.4811
	ORN	540508	548619	540311	548469	542145	540516	543428	4018.1710	0.7394
	ITR	15863	15903	15873	15687	15658	15786	15795	102.8455	0.6511
	CLO	468630	468412	467972	468836	469004	468482	468630	361.0673	0.0770
Tailing Factor	OFL	1.76	1.72	1.71	1.69	1.74	1.73	1.725	0.0242	1.4081
	ORN	1.52	1.51	1.53	1.51	1.56	1.57	1.533	0.0258	1.6834
	ITR	1.64	1.69	1.63	1.69	1.61	1.64	1.653	0.0328	1.9917
	CLO	1.64	1.59	1.58	1.61	1.57	1.63	1.603	0.0280	1.7493

Linearity:-

The linearity of the HPLC detector response for determination of OFX, ORN, ITR and CLO was evaluated by analyzing a series of different concentrations of each compound. The calibration range was established with respect to the practical range necessary (according to content and ratio of each compound in the cream formulation), to give accurate, precise and linear results. Seven concentrations were chosen, ranging from 3.75-12.5

ug/mL OFX, 10-30 µg/mL ORN, 05-15 µg/mL ITR and 2.5-7.5 µg/mL CLO and the linearity was determined. Characteristic parameters for regression equations of the HPLC method are given in Table 04-.The R² values for all four drugs were found to be ≥ 0.9867, indicating a high level of linearity and a consistent detector response across the tested concentration ranges.

Table no. 04 Observation of linearity of OFL, ORD, ITR & CLO

% level	OFL Conc. (µg/ml)	Mean	ORD Conc. (µg/ml)	Mean	ITR Conc. (µg/ml)		CLO Conc. (µg/ml)	Mean
1	3.75	7977.5	10	281914	05	7780	2.5	
2	6	12764	16	423643	08	12436	4	224278
3	7.5	15955	20	543428	10	15795	5	374849
4	9	19146	24	644514	12	18854	6	468556
5	12.5	23932.5	30	827526	15	23690	7.5	562267
R ² Value	0.9984		0.9975		0.9999		0.9984	

Accuracy:-

Accuracy was determined by standard addition method at three levels Standard solution, Accuracy-80%, Accuracy-100% and Accuracy-120% solutions were injected in to HPLC system. Amount found and amount

added for OFL, ORN, ITR and CLO, individual recovery and mean recovery values were also calculated. The average % recovery of OFL, ORN, ITR, & CLO, was calculated and The excellent recoveries of standard addition method (Table 5) for HPLC suggested good accuracy of the proposed method.

Table No.05: Accuracy study of OFL, ORN, ITR and CLO by standard addition method at (80%, 100% and 120%)

Sr. no.		1	2	3
Level		80%	100%	120%
Amount added µg/ml	OFL	06	7.5	09
	ORN	16	20	24
	ITR	08	10	12
	CLO	04	05	06
Mean Area±%RSD	OFL	28902±1.2	32020±1.2	35295±1.8

(n=6)	ORN	954129±1.4	1074921±1.7	117558±1.3
	ITR	28507±1.7	31495±1.6	34686±1.1
	CLO	854748±1.1	949255±1.8	1046066±1.7
Mean Area Recovered (n=6)	OFL	12753	15871	19146
	ORN	422943	543735	644372
	ITR	12579	15567	18758
	CLO	374749	469756	566167
Amount Recovery (µg/ml) (n=6)	OFL	5.92	7.37	8.89
	ORN	15.93	20.47	24.26
	ITR	7.90	9.87	11.75
	CLO	3.90	4.89	5.89
%Recovery	OFL	98.66	98.26	98.77
	ORN	99.56	102.05	101.08
	ITR	98.75	98.70	98.00
	CLO	98.01	98.04	98.16

Precision:-

The precision of the method was determined by intraday studies. The peak areas of all four drugs were calculated for each trial. The experiment was repeated three times in a day for intra-day precision Prepare 20

µg/mL solutions from a standard solution and inject Six times in a day on to analytical column. The percentage relative standard deviation (%RSD) was calculated and lower % RSD indicates that there are less variation and there is high precision all % RSD value given in table no.6

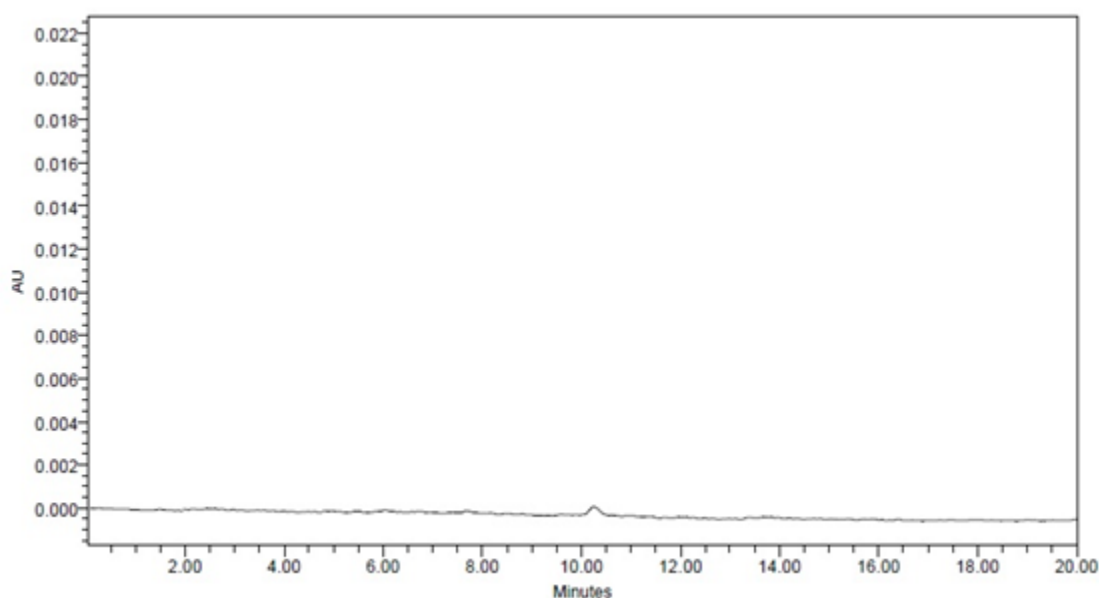
Table No.06: Observation for System precision

Parameter	compound	Run 1	Run 2	Run 3	Run4	Run 5	Run6	Mean	SD	%RSD
Peak area	OFL	15870	15848	15984	16014	15984	16030	15955	76.7619	0.4811
	ORN	540508	548619	540311	548469	542145	540516	543428	4018.1710	0.7394
	ITR	15863	15903	15873	15687	15658	15786	15795	102.8455	0.6511
	CLO	468630	468412	467972	468836	469004	468482	468630	361.0673	0.0770
Retention Time	OFL	3.039	3.061	3.057	3.041	3.036	3.066	3.058	0.0193	0.6322
	ORN	4.029	4.214	4.133	4.010	4.156	4.224	4.127	0.0907	2.1971
	ITR	7.365	7.405	7.447	7.412	7.298	7.304	7.371	0.0607	0.8243
	CLO	11.293	11.507	11.502	11.416	11.510	11.521	11.458	0.0894	0.7805

Specificity:-

Studies with blank and placebo solutions confirmed no interference at the retention times of the analytes,

indicating the method is specific. Shown in figure no.07(Blankchromatogram)



Robustness:-

Various factors were assessed to check the robustness of the method. Deliberate variations in flow rate (± 0.1 mL/min), pH (± 0.1 units), and organic phase composition

($\pm 10\%$) were tested. The method was also found to be robust for the factors thus studied and method remained robust within the tested ranges. the data of all factor given in table 7-9

Table No. 7. Robustness study for change in flow rate $\pm 10\%$ (± 0.1 ml/min)

Parameter	OFL	ORN	ITR	CLO
Flow Rate 0.9ml/min	15994	540560	15788	467972
	15838	543728	15864	469030
	16014	542140	15687	468482
	15890	549901	15795	468721
	15955	548322	15623	469821
Mean	15941	545495.5	15730	468974.5
SD	65.9393	3850.435	100.2916	741.3541
%RSD	0.414636	0.70586	0.637582	0.15808
Retention Time in Min.	3.13	3.72	11.86	14.17
	3.16	3.74	11.81	14.63
	3.09	3.70	11.76	14.02
	3.10	3.81	11.51	14.51
	3.16	3.76	11.69	14.23
Mean	3.13	3.74	11.72	14.29
SD	0.032042	0.038166	0.122638	0.227193
%RSD	1.022606	1.018221	1.046397	1.588948
Flow Rate 1.1ml/min	16793	550560	16128	477986
	16838	549989	16663	479125

	16714	551101	15975	478482
	16440	549901	16720	478747
	16999	556233	16421	479222
Mean	16797	552336	16388	478797
SD	208.635	3049.27	291.9575	495.6368
%RSD	1.242085	0.552068	1.781532	0.103517
Retention Time in Min.	2.50	2.98	9.40	11.37
	2.45	2.88	9.39	11.89
	2.56	2.85	9.63	11.54
	2.51	2.99	9.36	11.46
	2.56	2.89	9.31	11.71
Mean	2.52	2.91	9.40	11.61
SD	0.045019	0.057504	0.118996	0.191485
%RSD	1.784089	1.973809	1.265913	1.648841

Table No. 8: Robustness study for change in pH $\pm$ 0.1 units.

Parameter	OFL	ORN	ITR	CLO
pH 3.9 (-0.1 unit)	15954	548222	15523	469921
	15738	543928	15764	469030
	15994	542040	15687	468582
	15890	549901	15895	468721
	16114	540560	15888	467772
Mean	15941	545495	15730	468974.5
SD	65.9393	3850.435	100.2916	741.3541
%RSD	0.414636	0.70586	0.637582	0.15808
Retention Time in Min.	2.9	3.43	10.81	13.07
	2.96	3.48	10.92	13.10
	2.89	3.35	10.88	13.16
	2.81	3.41	10.90	13.01
	2.93	3.39	11.01	13.41
Mean	2.903333	3.408333	10.92167	13.19333
SD	0.052026	0.044008	0.077825	0.174662
%RSD	1.791928	1.291176	0.712571	1.323862

p H 4.1 (+0.1 unit)	15853	544222	15526	469826
	15738	547929	15764	469132
	15994	541041	15886	468682
	15990	549910	15798	468780
	15914	540560	15988	467792
Mean	15900.5	544037	15825	468667.3
SD	95.68647	4070.113	173.6145	787.8768
%RSD	0.601783	0.748132	1.09709	0.16811
Retention Time in Min.	2.94	3.44	10.67	12.90
	2.98	3.40	10.60	12.98
	2.89	3.56	10.74	12.78
	2.86	3.48	10.69	12.88
	2.98	3.43	10.71	12.91
Mean	2.938333	3.456667	10.68667	12.89333
SD	0.052313	0.056804	0.048442	0.065013
%RSD	1.780368	1.64331	0.453298	0.504236

Table No. 9: Robustness study for change in Organic Phase $\pm$ 10%.

Parameter	OFL	ORN	ITR	CLO
Organic Phase Change (-10%)	15857	548122	15427	469927
	15934	544020	15964	469130
	15798	542141	15783	468476
	15994	549900	15895	468622
	16110	540468	15688	467871
Mean	15941	545495	15730	468974.5
SD	65.9393	3850.435	100.2916	741.3541
%RSD	0.414636	0.70586	0.637582	0.15808
Retention Time in Min.	3.13	3.81	9.56	15.31
	3.09	3.78	9.49	15.40
	3.10	3.81	9.58	15.37
	3.13	3.89	9.53	15.31

	3.17	3.86	9.56	15.37
Mean	3.13	3.83	9.54	15.35
SD	0.033714	0.041352	0.03204164	0.036742346
%RSD	1.076567	1.078283	0.335631699	0.239285875
Organic Phase Change (+10%)	15958	544429	15664	469892
	15633	547722	15726	469232
	15998	541131	15986	468582
	15986	549820	15788	468726
	15914	540560	15998	467780
Mean	15900	544037	15825	468667
SD	95.68647	4070.113	173.6145	787.8768
%RSD	0.601783	0.748132	1.09709	0.16811
Retention Time in Min.	2.98	3.43	10.62	12.59
	2.94	3.40	10.60	12.78
	2.87	3.56	10.74	12.98
	2.88	3.48	10.69	12.88
	2.98	3.43	10.71	12.91
Mean	2.93	3.45	10.68	12.89
SD	0.052313	0.056804	0.048442	0.065013
%RSD	1.780368	1.64331	0.453298	0.504236

Application of validated method

The HPLC method was successfully applied to the determination of OFX, ORN, TBH and CBP in

cream formulation without the interference of excipients therein. The results of the assay are shown in Table 10

Table No.10: Result of marketed formulation OFL, ORN ITR, and CLO (Itromed-OC)

	OFL	ORN	ITR	CLO
Standard	N=6	N=6	N=6	N=6
Mean Area	15955	543428	15795	468556
Test Preparation Cream	N=6	N=6	N=6	N=6
Mean Area	16149	531186	15928	479899
Observed in mg	751.2mg	1965mg	997.3mg	48.2mg

Label claim in mg	750mg	2000mg	1000mg	50mg
% Assay	101.21	98.74	100.84	101.42

REFERENCE

- [1]. D.M. Dixon and Thomas J. Walsh. Medical microbiology 4th edition Baron S, editor. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
- [2]. G.R. Himalaya Chatwal Publishing ,S.K. Anand; House, Instrument Mumbai ;11 methods th edition of chemical, 2005,1.1-1.2, 2.108-;2.109,2.151-2.153.
- [3]. Settle F. A. "Handbook of Instrumental techniques for analytical chemistry", Prentice Hall PTR, New Jersey, 1997, 1st Edition, 17-19, 56-57.
- [4]. Skoog D.A., Holler F.J. and Crouch S.R. "Principle of Instrumental Analysis", Thomson Publications, India, 2007, 6th Edition, 1-3, 145-147, 180.
- [5]. <https://pubchem.ncbi.nlm.nih.gov/compound/Ofl oxacin>
- [6]. <https://pubchem.ncbi.nlm.nih.gov/compound/Orn idazole>
- [7]. <https://pubchem.ncbi.nlm.nih.gov/compound/Itra conazole>
- [8]. <https://pubchem.ncbi.nlm.nih.gov/compound/Clo betasol>
- [9]. Snyder LR, Kirkland JJ, Glajchl JI. Practical.HPLC Method Development. John Wiley & Sons, New York.1988; 3: 2 – 21.
- [10]. Validation of Chromatographic Methods, Reviewer Guidance, Center for Drug Evaluation and Research (CDER), November,1994; 17
- [11]. Yan Li,GeraldJ Terfloth, AlirezaS Kord "A Systematic Approach to RP-HPLC Method Development in a Pharmaceutical QbD Environment". American Pharmaceutical review, Chemical development, GSK, 2008.
- [12]. US Food and Drug Administration, Pharmaceutical CGMPs for the 21st Century A Risk Based Approach, 2004.
- [13]. Department of Health and Human Services, 1. U.S. Food and Drug Administration, Pharmaceutical cGMPs for the 21st century - A risk-based approach, Final report, September, 2004.
- [14]. Borman, P., Nethercote, P., Chatfield, M., Thompson, D. and Truman K. "The Application of Quality by Design to Analytical Methods". Pharm. Tech. 2007, 31(12) 142-152.
- [15]. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Quality Guideline Q8 Pharmaceutical development, 2006.
- [16]. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Quality Guideline Q2(R1) Validation of Analytical procedures: Text and Methodology, 2005.
- [17]. Schweitzer, M., Pohl, M., Hanna-Brown, M., Nethercote, P., Borman, P., Hansen, G., Smith, K. and Larew J. "Implications and Opportunities of Applying QbD Principles to Analytical Measurements". Pharm. Tech. 2010, 34 (2) 52-59.
- [18]. Vogt F.G. and Kord A.S. "Development of Quality-By-Design Analytical Methods". J. Pharm. Sci. 2011, 100(3), 797-812.
- [19]. Bhatt, D.A. and Rane, S.I. "QbD Approach to Analytical RP-HPLC Method Development and its Validation". Int. J. Pharm. and Pharm. Sci. 2011, 3 (1) 179-187.
- [20]. Krull I., Swartz, M., Turpin, J., Lukulay, P.H. and Verseput, R. "A Quality-by-Design Methodology for Rapid LC Method Development". Part I and Part II. LCGC N. Am. 2008, 26, 1190-1197.
- [21]. Meyer C., Soldo T. and Kettenring U. "Highlights of Analytical Chemistry in Switzerland". Chimia 2010, 64 (11), 825.
- [22]. Ling S. and McBrien, M. "A Quality by Design Approach to Chromatographic Method Development". LCGC: The Column, 2011, 7 (5), 16-20.
- [23]. Orlandini, S., Pinzauti S. and Furlanetto S. "Application of quality by design to the development of analytical separation methods". Analytical and Bioanalytical Chemistry. 2013, 405(2), 443– 450
- [24]. Musters, J., van den Bos L. and Kellenbach, E. "Applying QbD Principles to Develop a Generic UHPLC Method Which Facilitates Continual Improvement and Innovation throughout the Product Lifecycle for a Commercial API". Organic Process & Research Development, 2013, 17, 87–96.
- [25]. Rozet E., Lebrun, P., Debrus B., Boulanger, B. and Hubert P. "Design Spaces for analytical Methods". Trends in Analytical Chemistry, 2013, 42, 157-167.
- [26]. Raman, N.V., Mallu, U.R., and Bapatu, H.R. "Analytical quality by design approach to test method development and validation in drug substance manufacturing". Hindawi, Journal of Chemistry, 2015, 1-8.
- [27]. Schmidt A.H. and Molnár, I. "Using an innovative quality-by-design approach for development of a stability indicating UHPLC method for ebastine in the API and pharmaceutical formulations". Journal of Pharmaceutical and Biomedical Analysis. 2013, 78, 65–74.
- [28]. Dure N. I., Adeel A., Munawar I. and Arif N. "Analytical method development and validation of

- hydrocortisone and clotrimazole in topical dosage form using Reverse Phase high-performance liquid chromatography". *Future Journal of Pharmaceutical Sciences*, 2020, 6 (49), 01-07.
- [29]. Kamberi M., Katherine F., Jianmin L, Chemaly G.M. and Feder D. "A Sensitive High- Through put HPLC Assay for Simultaneous Determination of Everolimus and Clobetasol Propionate". *Saudi Journal of Chromatographic Science*, 2008, 46, 23-29.
- [30]. Geetha M., Rao P. V., Shakil S. and Palvai S. R. "A Validated RP-HPLC Method for Simultaneous Determination of Eberconazole, Mometasone Furoate and Methylparaben". *Oriental Journal of Chemistry*, 2013, 29 (2), 579-587.
- [31]. Majumder T., Hasan R., Roy P., Pramanik R and Hasan N. "Method development and validation of RP-HPLC method for estimation of luliconazole in marketed formulation (Cream)". *The Pharma Innovation Journal*, 2019, 8 (5), 103-108.
- [32]. Ramzia I. B., Fouada M. A., Manal A. S., and Tolba E. H. " Derivative, derivative of the ratio spectrophotometric and stability-indicating RP-HPLC methods for the determination of mometasone furoate and miconazole nitrate in cream". *Journal of Chemical and Pharmaceutical Research*, 2013, 5(11), 368-378.
- [33]. Ei1 Z. Z, Pimthon J., Vajragupta O., Leanpolchareanchai J. and Phechkrajang C.M. "Development and validation of high-performance liquid chromatography method for determination of miconazole, triamcinolone, methylparaben and propylparaben in cream". *Journal of Pharmaceutical Sciences*, 2016, 43 (4), 211-221.
- [34]. Shabir G. A. "A New Validated HPLC Method for the Simultaneous Determination of 2-phenoxyethanol, Methylparaben, Ethylparaben and Propylparaben in a Pharmaceutical Gel". *Indian Journal of Pharmaceutical Sciences*, 2010, 72(4), 421-425.
- [35]. Angel A. G., Garcia P. L., Maria S., Maria A. P., Santoro M. R., Maria E. R. and Hackman K. "Simultaneous determination of econazole nitrate, main impurities and preservative in cream formulation by high performance liquid chromatography". *Elsevier, Talanta*, 2008, 77, 673-678.
- [36]. Devaraj S. K., Sivaperuman A. L. and Nagarajan N. C. "RP-UPLC Method Development and Validation for Simultaneous Estimation of Mometasone Furoate and Miconazole Nitrate in Semisolid Dosage Form". *ACTA Pharmaceutica Scientia*, 2020, 58(3), 335-348.
- [37]. Bhamre P., Baghel M. and Rajput R. "QbD Approach for development and optimization of HPLC method for the simultaneous estimation of four component cream formulation. Application to permeability study". *Journal of Chemical and Pharmaceutical Research*, 2016, 8(4), 868-877.
- [38]. Sivaraman A. P. and Banga A. K. "Quality by design approaches for topical dermatological dosage forms". *Research and Reports in Transdermal Drug Delivery*, 2015, 4, 9-21.