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Research Article

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QbD Approach for development and optimisation of HPLC method for the simultaneous estimation of four component cream formulation: Application to permeability study

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ABSTRACT

QbD based Design of Experiments (DoE) approach was explored to study the effect of various factors influencing the optimisation of HPLC method for the simultaneous estimation of the four drugs viz. Ofloxacin (OFX), Ornidazole (ORN), Terbinafine hydrochloride (TBH) and Clobetasol propionate (CBP) in bulk drug and in cream formulation. A full factorial design was employed to study the factors which may affect the chromatographic separation of the four drugs, such as pH of the buffer, initial percentage of organic content (%BI) and gradient time (Tg). The optimal conditions obtained after applying the principles of QbD with good system suitability parameters for all four drugs were found to be at pH 2.6, %BI as 24% of acetonitrile and gradient time of 4 min. The optimal conditions were found to be in a good agreement with the experimental results. The HPLC method thus developed was validated using ICH guidelines and was applied for the assay of cream formulation. The percentage recoveries were found to be 99.74±0.39 for OFX, 98.72± 0.71 fo r ORN, 98.19 ±0.23 for TBH and 99.05 ±0.76 for CBP. The HPLC method was successfully applied to study the in vitro permeability of cream formulation in rat skin using Franzch diffusion cell.

Keywords: QbD, HPLC, Ofloxacin, Ornidazole, Terbinafine hydrochloride, Clobetasol propionate,

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INTRODUCTION

OFX, chemically known as, 9-fluoro-3,7-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2H-[1,4]oxazino [2,3,4-ij]quinoline-6-carboxylic acid (Figure 1),is a quinolone antibiotic which prevents multiplication of bacteria by inhibiting supercoiling activity of DNA gyrase which further inhibits nucleic acid synthesis [1]. ORN, chemically known as, 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol (Figure 1), is a nitro imidazole which has broad spectrum cidal activity against protozoa and some anaerobic bacteria. It kills the bacteria that infect the inflamed skin and is also used in the systemic treatment of infections. [2].TBH, chemically known as, (E)-N,6,6-trimethyl-N-((naphthalen-5-yl)methyl)hept-2-en-4-yn-1-amine hydrochloride (Figure 1), is an allylamine antifungal agent, used to treat fungal infections of skin, fingernails and toes such as dermatophytoses, pityriasisversicolor, cutaneous candidiasis [3]. CBP, chemically known as,(1R,2S,10S,11S,13S,14R,15S,17S)-14-(2-chloroacetyl)-1-fluoro-14,17-dihydroxy-2,13,15-trimethyltetracyclo[8.7.0.0{2,7}.0{11,15}]heptadeca-3,6-dien-5-one(Figure 1), is a topical synthetic corticosteroid having anti-inflammatory, anti-pruritic and vasoconstrictive properties. CBP acts on the inflamed skin and reduces itching [4]. The cream formulation selected for the study was a combination of four drugs i.e. Ofloxacin (OFX), Ornidazole (ORN), Terbinafine hydrochloride (TBH) and Clobetasol propionate (CBP) The cream has antibacterial, antiprotozoal, corticosteroid and anti-inflammatory agents having a specific activity and hence the cream has a multipurpose range of being used in various skin disorders such as atopic dermatitis and capillaris dermatitis.

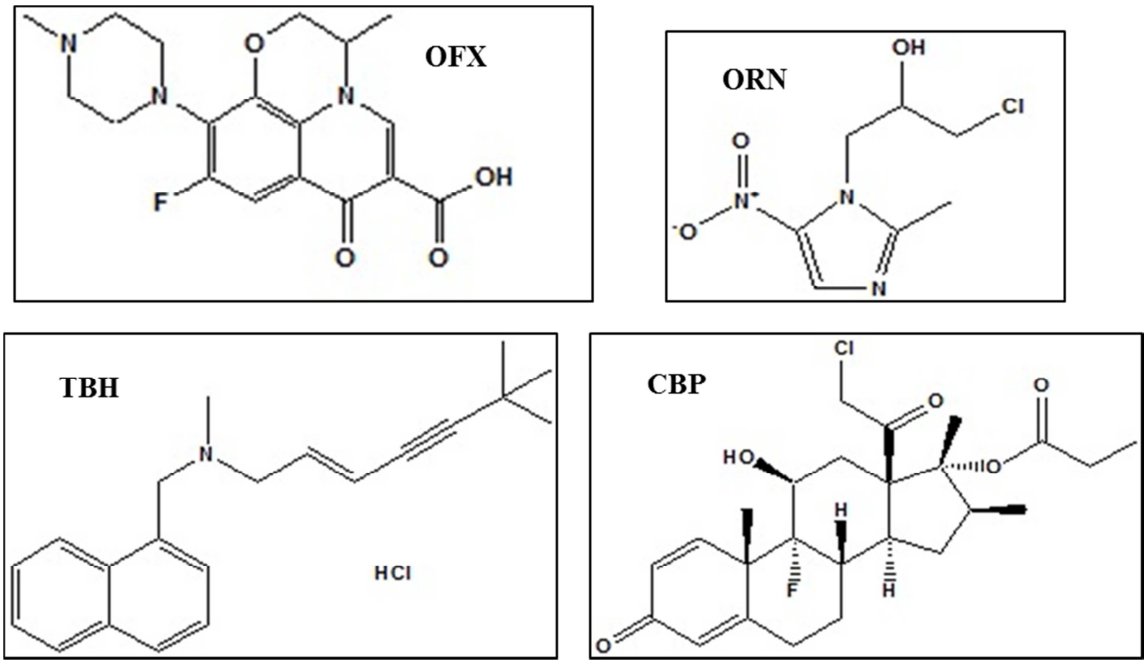

Figure1 Structure of OFX, ORN, TBH and CBP

Literature survey reveals various analytical methods for the quantitation of CBP, OFX, ORN and TBH either individually or in various combinations with other drugs; some of them are: HPLC methods [5-26], HPTLC methods [27, 28] and UV spectrophotometry methods [29-32]. Analytical methods for the same combination are available in the literature [33-35]but the method presented here deals with the QbD approach for the simultaneous estimation of the combination under study and hence explores application of analytical QbD (AQbD) for HPLC method development. The present study deals with exploring different factors influencing the HPLC method development for the simultaneous estimation of OFX, ORN, TBH and CBP and thus optimising the HPLC method with the help of QbD approach. The method thus optimised has been applied for the assay of cream formulation and *invitro* permeability study.

EXPERIMENTAL SECTION

2.1. Instrumentation

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AD pump (binary) and Shimadzu PDA-M20A Diode Array Detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 µL. Data acquisition and integration were performed using LC Solution software (Shimadzu Corporation, Kyoto, Japan). Separation and quantitation were made on a PhenomenexC18 column (5µm × 250mm × 4.6mm i.d.).The experimental design model was developed on Design Expert software® 7.1.

2.2. Materials and reagents

The API of Ofloxacin and Ornidazole were provided as gift samples from INTAS Pharmaceuticals, Terbinafine hydrochloride was purchased from Symbolic Pharma and Clobetasol propionate was provided by Sumit Laboratories. HPLC grade (Spectrochem) methanol and acetonitrile were used for the analysis. AR grade potassium dihydrogen phosphate (Loba Chemie), AR grade phosphoric acid (LobaChem) and HPLC grade triethylamine (Spectrochem) were used for preparation of buffer. The formulation, Panderm Plus cream (Mac cleods Pharma) was used for analysis.

2.3. Experimental conditions

Phosphate buffer (0.02 M) was prepared by dissolving 2.72 g of anhydrous potassium orthophosphate (KH_2PO_4) in 1 L of previously filtered double distilled water, 0.05% triethylamine was added and the pH was adjusted to 2.6 using phosphoric acid. The gradient elution was carried out with the mobile phase comprising of 0.02M phosphate buffer (pH 2.6) as solvent A and acetonitrile as solvent B. All determinations were performed at ambient temperature at the wavelength of 258 nm. The flow rate was 1 mL/min. The injection volume was 20 µL.

2.4. Preparation of standard solutions

The diluent used for the preparation of all the solutions was mixture of methanol, acetonitrile and 20mM phosphate buffer (pH 2.6) in the ratio of 50:25:25. All the solutions were prepared in amber coloured volumetric flasks. The standard solution mixtures were prepared from OFX, ORN, TBH and CBP stock solutions, in the range of 150-750 µg/mL of OFX, 400-2000 µg/mL of ORN, 200-1000 µg/mLof TBH and 10-50 µg/mL of CBP in the diluent, which were analysed by HPLC method under above mentioned chromatographic conditions.

2.5. Experimental Design

The DoE plan for the optimization of variables which affect the performance of developed method was based on a 3^3 full factorial design. The three key factors were: pH, initial percentage of organic (%BI) and gradient time (Tg). The design formed a chromatographic database, which was used to study the factors and enabled the selection of optimised conditions in order to get the best optimised HPLC conditions. The three parameters (or factors)and their levels are shown in Table 1 and accordingly a set of total 29 experiments was performed including two centre points. The responses (output) selected on the basis of performance were: TF1 (tailing factor of OFX), TF2 (tailing factor of ORN), TF3 (tailing factor of TBH), TF4 (tailing factor of CBP) and RS3 (resolution between pair of peaks i.e. TBH and CBP). The resolution between other pairs of drugs was found to be satisfied during the trials and hence only TBH and CBP resolution were taken into consideration.

Table 1 Factors and their levels for experimental design

Factors	Factor ID	low	middle	high
pH	A	2.5	4	5.5
%BI	B	15	20	25
Tg(min)	C	2	4	6

2.6. Method Validation

The validation of the HPLC method was carried out in accordance with the ICH guidelines [36]. The method was validated for various parameters like linearity, accuracy, precision, limit of detection, limit of quantification, sensitivity, selectivity and robustness.

2.7. Analysis of marketed formulation

The commercial cream product *i.e.* Panderm Plus cream, Macleods Pharma, containing 0.75% Ofloxacin, 2% Ornidazole, 1% Terbinafine hydrochloride and 0.05% Clobetasol propionate was procured from local pharmacy and used for analysis.500mg of cream was taken in 10ml of mixture of methanol, ACN and phosphate buffer (pH 2.6) in a ratio of 50:25:25 respectively. The mixture was shaken vigorously, sonicated for 10 min and was filtered through 0.2µ membrane filter. From this mixture 4mL aliquot was taken and diluted to 10 mL with mobile phase i.e. ACN and phosphate buffer of pH 2.6 (50:50 ratio) and injected into the HPLC.

2.8. Permeability study

Permeability study was carried out using Franzch diffusion cell for time period of 24 hrs. The receptor media used for the study was mixture of PBS (pH7.4): ethanol 70:30.The receptor media was kept at a constant temperature of 37°C and stirred using magnetic stirrer. The study was carried out on the rat skin mounted on the diffusion cell. The skin was allowed to stabilise with the receptor media for 30 min and 1gm of cream was loaded into the cell, 5 ml of aliquot was withdrawn from the receptor media and replaced by an equal volume of fresh receptor medium, at appropriate time intervals (1, 2, 3, 4, 5, 6, 7, 22 and 24 hours). The aliquot was diluted upto10ml with the mobile phase, filtered by 0.2µ membrane filter and analysed by HPLC.

RESULTS AND DISCUSSION

3.1. Screening of factors

Preliminary screening of several factors such as different columns (RPC8, RPC18), temperature (25º C, 40º C), various buffers (phosphate buffer, ammonium acetate buffer), concentration of buffer (20mM, 30mM, 40mM), organic solvent, column, etc. was carried out to find out significant factors affecting the HPLC separation of all the four drugs. The Phenomenex RPC18 column was found to give good elution as compared to RPC8 as TBH and CBP did not had symmetric peak shapes and CBP had long elution time. Temperature did not have a profound effect so it was kept ambient. Various buffers such as phosphate buffer, ammonium acetate buffer and ammonium formate buffer were tried out of which symmetric and sharp peak shapes were obtained in phosphate buffer. The study of pH revealed that at a pH greater than 5.5, the retention time of TBH increased up to more than 30 min and hence the pH range of 2.5-5.5 was considered as a prime factor for study. The organic solvents screening revealed that good results were obtained with acetonitrileas ACN gave desired peak shape and shorter run time. The four drugs eluted at different ratios of mobile phase and hence a gradient method had to be set up. The initial organic concentration

(%BI) range of 15-25%and the gradient time *i.e.* the time required to change the %BI from 25 to 70%, these two factors were found to be critical in setting the gradient. Thus the two factors i.e. initial percentage of organic phase (%BI) in the gradient and the gradient time Tg) were prime factors in optimising the gradient programme. Hence DoE was used, to study the effects of three specific factors such as pH, %BI and Tgin a systematic way and to optimise their values in such a way to obtain an HPLC method which is best suitable for the simultaneous estimation of the four drugs under study.

3.2. The design of experiments

The full factorial design including 27 runs (and 2 centre points) was worked out using three factors: (i) pH of buffer (2.5-5.5), (ii) %BI of acetonitrile (15-25) and (iii) gradient time, Tg (2-6). Few trials have been shown in Figure 2. In analysis window the model were chosen on the basis of ANOVA results which showed significant p-value, R^2 value and F value. It also showed reasonably good agreement between adjusted and predicted R^2 value. The model equations of various responses have been shown in Table 2 and the ANOVA results along with the statistics are given in Table 3**.**The contour (2D) plots of responses with respect to all factors are shown in Figure 3-7 for TF1, TF2, TF3, TF4 and RS3 respectively. The optimum conditions were calculated using numerical optimization. To achieve the composite desirability (D), the response criteria were set as (lower–upper): TF1 as (0.5-1.8), TF2 as (0.5-1.8), TF3 as (0.5-1.8), TF4 as (0.5-1.8) and RS3 as (>2). The Derringer's desirability was calculated for the set criterions, which indicated that maximum desirability was achieved at 0.84.

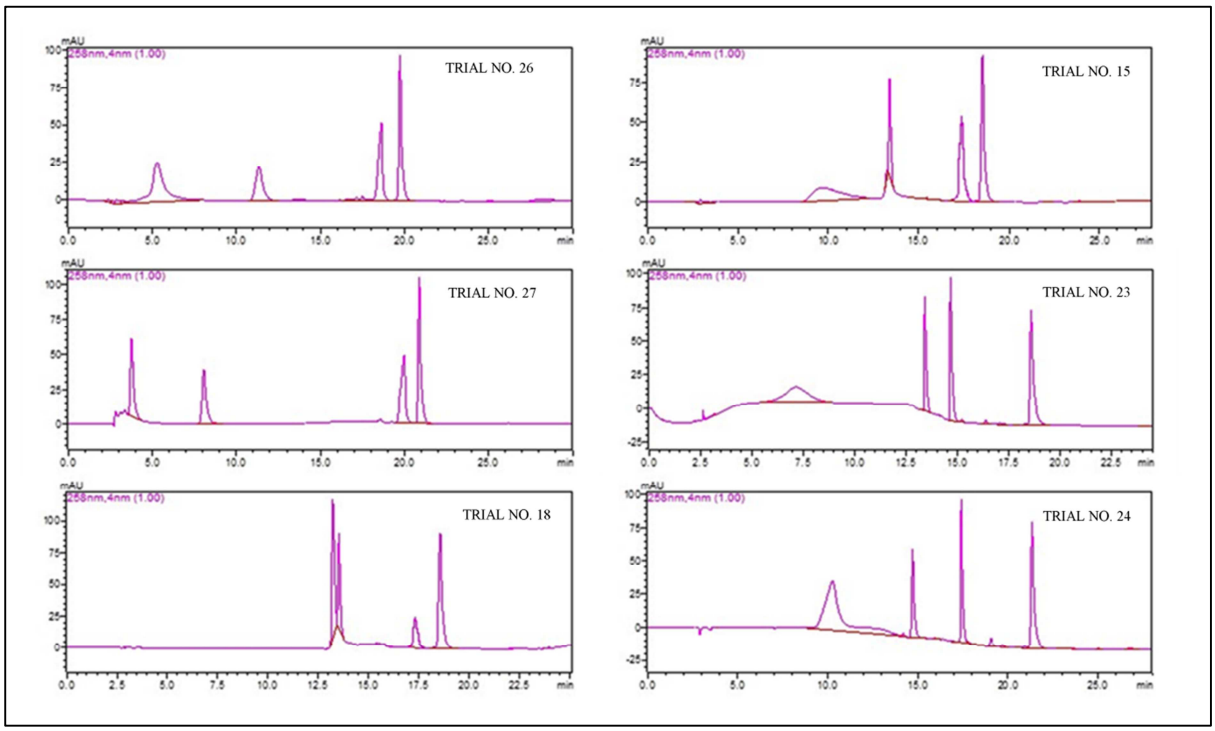

Figure 2 Chromatograms of few QbD trials

[Trial No. 26: pH=4, %BI=20, Tg=4; Trial No. 15: pH=5.5, %BI=20, Tg=4; Trial No. 27: pH=2.5, %BI=25, Tg=2; Trial No. 23: pH=4, %BI=20, Tg=6; Trial No. 18: pH=5.5, %BI=15, Tg=4; Trial No. 24: pH=5.5, %BI=25, Tg=4]

Table 2 Model Equations (in terms of coded values)

Responses	Equations
TF1	$TF1 = 1.325 + 0.744A + 0.423B + 0.101C + 0.913AB + 0.251AC - 0.053BC + 0.893A^2 + 0.269B^2 - 0.0616C^2$
TF2	$TF2 = 1.294 - 0.064A - 0.253B - 0.119C + 0.073AB + 0.006AC + 0.183BC - 0.117A^2 + 0.375B^2 + 0.0434C^2$
TF3	$TF3 = 0.919 - 0.496A - 0.0513B + 0.135C + 0.009AB - 0.117AC + 0.189BC + 0.973A^2 - 0.050B^2 + 0.040C^2$
TF4	$TF4 = 1.778 - 0.474A - 0.098B - 0.053C + 0.174AB - 0.032AC - 0.016BC - 0.364A^2 + 0.164B^2 - 0.044\ C^2$
RS3	$RS3 = 7.854 - 2.679A + 3.977B + 0.366C - 2.054AB + 0.232AC - 0.024BC - 0.696A^2 - 1.524B^2 - 0.352C^2$

A, B and C are the coded values for the factors pH, %BI and Tg

Table 3 Model Summary statistics

Statistical parameters	Responses				
	TF1	TF2	TF3	TF4	RS3
Std. Dev.	0.469	0.147	0.277	0.114	1.151
Mean	2.007	1.481	1.517	1.627	6.257
%C.V.	23.402	9.892	18.279	6.984	18.401
R-Squared	0.879	0.879	0.890	0.959	0.951
Adj R^2	0.821	0.821	0.838	0.939	0.928
Pred R^2	0.709	0.7	0.686	0.893	0.877
Adeq Precision	13.949	14.523	13.658	22.662	21.134
F value	15.3	15.3	17.15	48.71	41.13

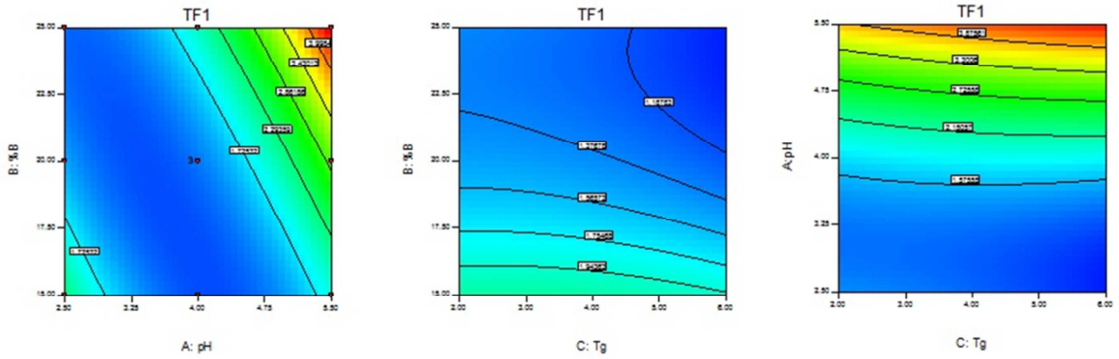

Figure 3 2D contour plots of response TF1 with respect to all the three factors

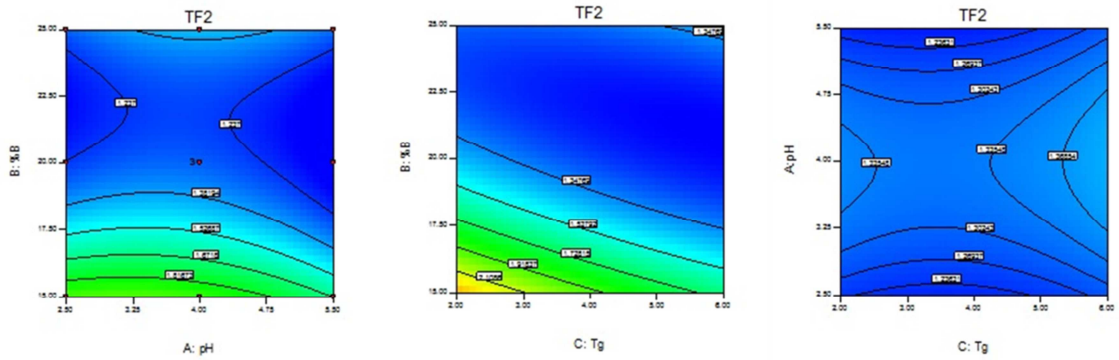

Figure 4 2D contour plots of response TF2 with respect to all the three factors

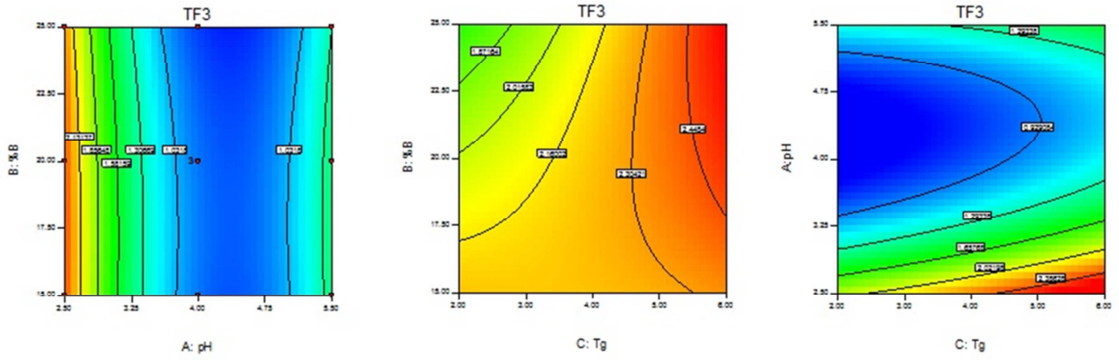

Figure 5 2D contour plots of response TF3 with respect to all the three factors

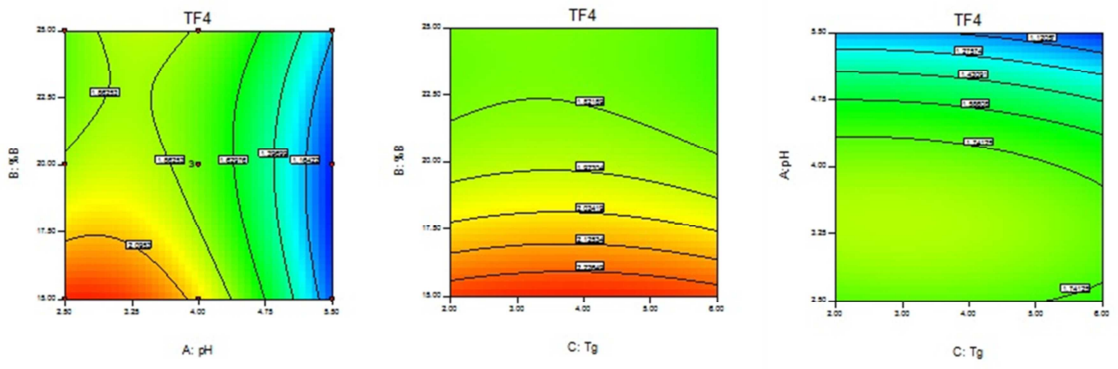

Figure 6 2D contour plots of response TF3 with respect to all the three factors

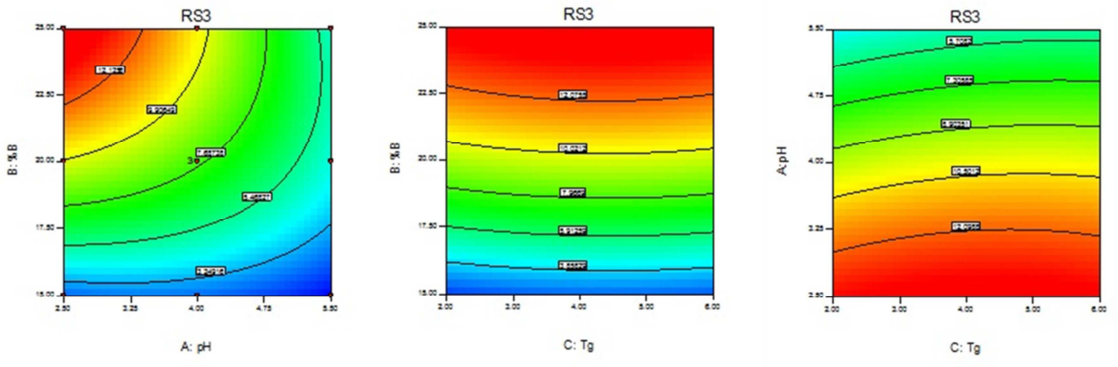

Figure 7 2D contour plots of response RS3 with respect to all the three factors

3.3. Robustness of model

To check the robustness of the model, four solutions among the generated solutions (8 solutions) were selected and chromatographed. To check the point prediction the experimental values were compared with the predicted values of responses. It was found that the experimental values lie within 95% confidence and predicted intervals (shown in Table 4). Finally solution with pH 2.6, %BI as 24 and Tgas 4 min, was chosen to record the chromatogram which allowed the complete separation of all the four compounds under study (as shown in the chromatogram i.e. Figure 8).

Table 4 Factors and targeted criteria used in Design expert

Solution	pH	%B	Tg	Response	Predicted value	Experimental value	95% CI Predicted		95% PI Predicted	
							low	high	low	high
1*	2.6	24	4	TF1	1.4	0.9	0.7	2.0	0.2	2.5
				TF2	1.3	1.0	1.1	1.5	0.9	1.7
				TF3	1.6	1.4	1.3	2.0	0.9	2.3
				TF4	1.8	1.7	1.7	2.0	1.5	2.1
				RS3	13.3	12.9	11.8	14.8	10.4	16.2
2	2.7	25	4	TF1	1.3	0.8	0.7	2.0	0.2	2.5
				TF2	1.3	1.3	1.1	1.5	0.9	1.7
				TF3	1.6	1.5	1.2	2.0	0.9	2.3
				TF4	1.8	1.7	1.7	2.0	1.5	2.1
				RS3	13.1	12.0	11.6	14.6	10.3	16.0
3	2.6	25	4	TF1	1.4	1.3	0.7	2.0	0.2	2.5
				TF2	1.3	1.3	1.1	1.5	0.9	1.7
				TF3	1.6	1.6	1.2	2.0	0.9	2.3
				TF4	1.8	1.8	1.7	2.0	1.5	2.1
				RS3	13.3	12.9	11.8	14.9	10.5	16.2
4	2.7	24	4	TF1	1.3	1.0	0.7	1.9	0.2	2.5
				TF2	1.3	1.2	1.1	1.5	0.9	1.6
				TF3	1.6	1.6	1.3	2.0	1.0	2.3
				TF4	1.8	1.8	1.7	2.0	1.5	2.1
				RS3	12.9	11.8	11.5	14.3	10.1	15.7

**Finally selected for chromatographic separation*

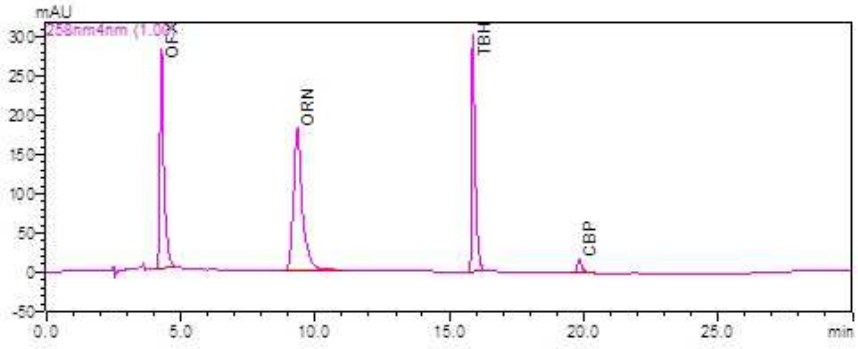

Figure 8 Optimised chromatogram of standard solution mixture containing 150 ppm of OFX, 400 ppm of ORN, 10 ppm of CBP and 200 ppm of TBH

3.4. Model validation

To validate the model, six check points were selected and the results of the experimental values obtained were compared with the predicted values. The results showed good agreement between the experimental and model generated values resulting in low residual values presented in terms of percentage bias (%bias) (Table 5). Figure 9 shows the 3D desirability contour plots and Figure 10 shows the design space for all the parameters. The final optimised gradient elution programme is shown in Table 5 and the final optimised gradient programme is given in Table 6.

Table 5 Results for validation of model (check point trials)

Trial No.	Factors			Responses	Experimental Value	Predicted value	% Bias
	pH	%BI	Tg				
1	3	20	3	TF1	1.2	1.2	0.08
				TF2	1.2	1.4	0.08
				TF3	1.6	1.6	0.01
				TF4	1.8	1.9	0.05
				RS3	9.4	9.1	-0.03
2	5	18	4.5	TF1	1.7	1.9	0.09
				TF2	1.1	1.3	0.19
				TF3	0.8	1.0	0.19
				TF4	0.8	1.3	0.42
				RS3	3.8	4.6	0.16
3	3.5	18	4	TF1	1.2	1.2	0.01
				TF2	1.4	1.5	0.08
				TF3	1.1	1.2	0.06
				TF4	1.8	2.0	0.10
				RS3	6.4	6.6	0.02
4	4.5	16	6	TF1	1.2	1.4	0.14
				TF2	1.4	1.5	0.07
				TF3	0.9	0.9	-0.10
				TF4	1.5	1.6	0.06
				RS3	3.1	3.4	0.08
5	4.8	17	5	TF1	1.5	1.6	0.06
				TF2	1.2	1.4	0.11
				TF3	0.8	0.9	0.10
				TF4	1.3	1.4	0.08
				RS3	3.9	4.1	0.05
6	2.8	19	5	TF1	1.2	1.3	0.07
				TF2	1.2	1.3	0.07
				TF3	2.2	2.1	-0.06
				TF4	2.0	2.0	-0.04
				RS3	8.4	8.4	-0.01

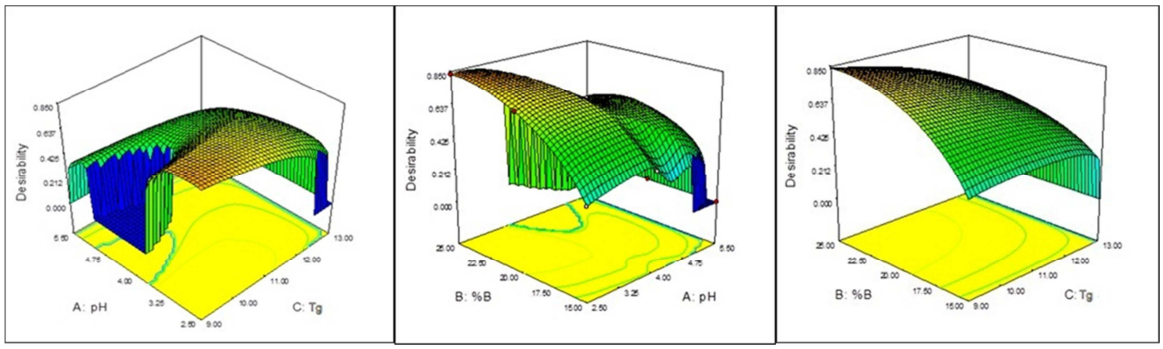

Figure 9 Desirability 3D plots for the three factors for optimised method

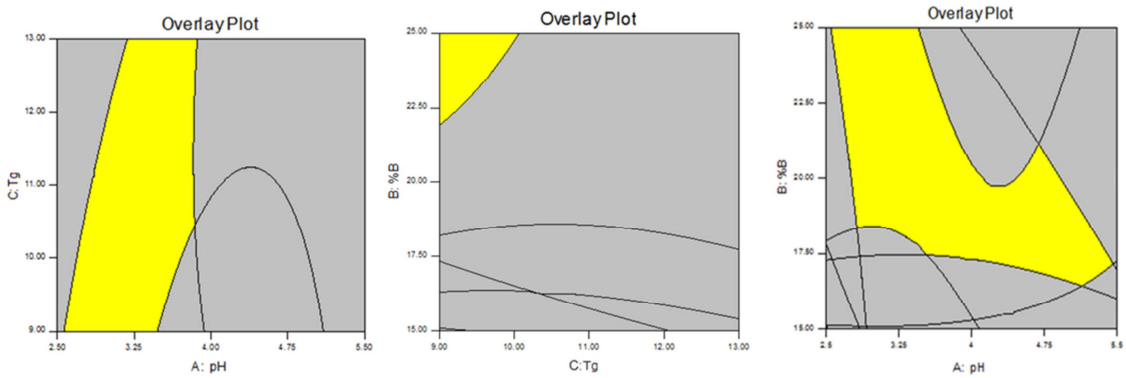

Figure 10 Plots for the design space

Table 6 Gradient Programme

Time(min)	%B(organic concentration)
0	24
7	24
11	70
18	70
20	50
22	24
24	Stop

3.4.Validation of HPLC method

3.4.1. Linearity

The linearity of the HPLC detector response for determination of OFX, ORN, TBH and CBP was evaluated by analysing 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 150-750µg/mL OFX, 400-2000µg/mL ORN, 200-1000 µg/mL TBH and 10-50 µg/mL CBP and the linearity was determined. Characteristic parameters for regression equations of the HPLC method are given in Table 7.

3.4.2. Precision

For evaluation of the precision estimates, intra and inter day precision studies were performed at three concentration levels in triplicates. 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 and on three different days for inter-day precision. The average percentage relative standard deviation (% RSD) of intra-day and inter-day measurements for OFX, ORN, TBH and CBP are given in Table 7

3.4.3. Accuracy

Accuracy was determined by standard addition method at three levels of standard addition *i.e.* 80%, 100%, and 120%. The standard addition was done with respect to 150, 400, 200 and 10µg/mL for OFX, ORN, TBH and CBP respectively, as 0% level. The resulting mixtures were analysed and results obtained were compared with the expected results. The excellent recoveries of standard addition method (Table 7) for HPLC suggested good accuracy of the proposed method.

3.4.4. Detection and quantitation limits

According to ICH recommendations [38], the approach based on the standard deviation (S.D.) of the y-intercept and the slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ) and values thus found are given in Table 7.

Table 7 Summary of validation parameters

Parameters	OFX	ORN	TBH	CBP
Calibration range (µg/mL)	150-750	400-2000	200-1000	10-50
LOD (µg/mL)	0.05	0.2	0.08	0.12
LOQ (µg/mL)	0.14	0.05	0.24	0.35
Regression equation	y = 21848.67x – 4356.67	y = 13685x – 1214	y = 15408.7x – 2857.3	y = 21466x + 8948
Correlation coefficient (r^2)	0.9989	0.9990	0.9994	0.9991
Accuracy (% recovery±SD)				
80%	98.0± 0.5	98.72± 0.75	99.32± 0.25	100.82±1.25
100%	98.84±0.80	99.56±0.46	98.56±1.11	98.22±1.30
120%	99.74±0.64	99.84±0.45	100.13±1.16	98.72±1.30
Precision(%RSD)*				
Intraday	1.08	1.089	0.76	1.18
Interday	1.09	1.32	1.11	1.33

3.4.5. Robustness

Various factors were assessed to check the robustness of the method. The factors such as: pH (2.4, 2.6, 2.8), Tg (3, 4, 5) and %BI (23, 24, 25) were varied in the region of design space, generated by applying QbD in method optimization. The method was also found to be robust for the factors thus studied and also for the change in flow rate of ±0.1 ml/min.

3.4.6. Stability

The standard solutions prepared in the mobile phase exhibited no chromatographic or absorbance changes for 24 h when kept at room temperature and for 48 h when stored in refrigerator (8-25 ºC). No additional peak was found in the chromatogram which indicated the stability of the standard solutions under study.

3.4.7. Specificity

The specificity of the method was assessed by analysing the commercial formulation. In the commercial formulation there were two labelled excipients *i.e.* propyl paraben and methyl paraben which gave resolved peaks without interfering the main drugs. This demonstrates the specificity of the method which can be confirmed by comparing the chromatograms of standard solution (Figure 8) and sample solution (Figure 11). The confirmation of the excipient peaks was done by analysing standard solutions of propyl paraben and methyl paraben by HPLC. The chromatograms of the same have been shown in Figure 12 and 13 respectively.

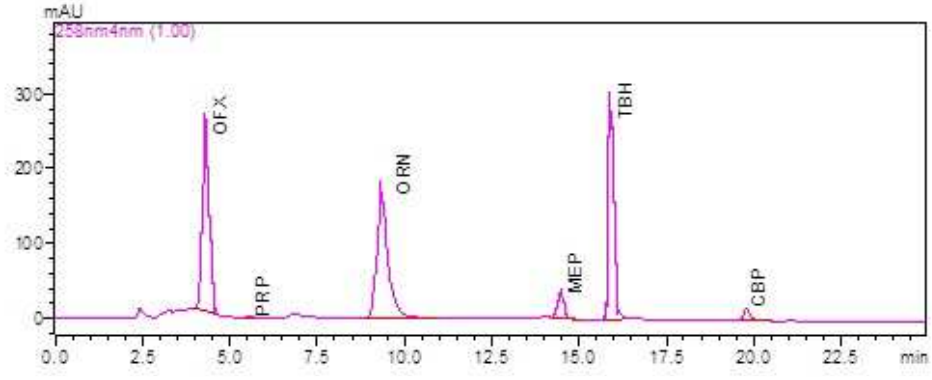

Figure 11 Chromatogram of sample solution mixture containing 150 ppm of OFX, 400 ppm of ORN, 10 ppm of CBP and 200 ppm of TBH. (PRP=propyl paraben and MEP=methyl paraben)

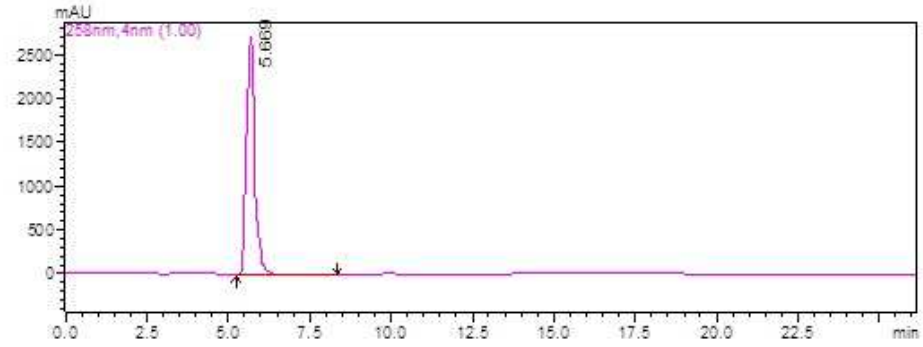

Figure 22 Chromatogram of standard sample of propyl paraben

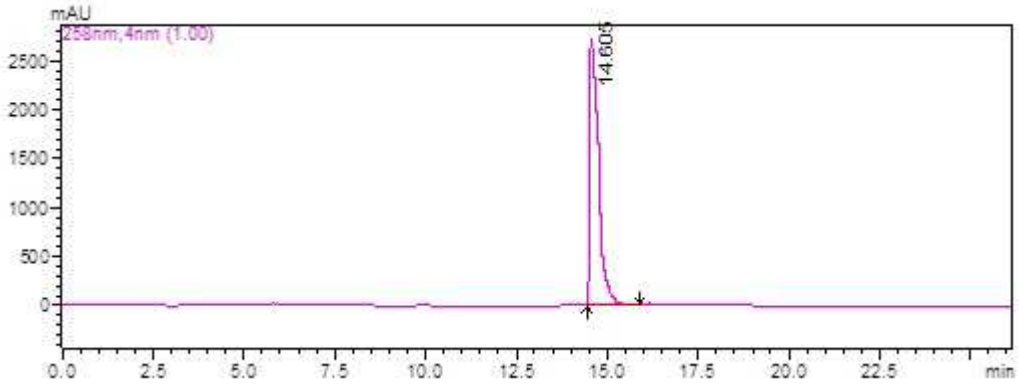

Figure 33 Chromatogram of standard sample of methyl paraben

3.4.8. System suitability

System suitability parameters such as theoretical plates, symmetry factor and resolution for OFX, ORN, TBH and BRM were calculated for n=6 replicates to study the system suitability of HPLC method. Satisfactory results were obtained as shown in Table 8.

Table 8 System Suitability Parameters for the developed HPLC method

Parameters	OFX	ORN	TBH	CBP
Retention Time	4.03±1.90	9.24±1.33	15.81±0.49	19.83±0.63
Tailing factor	1.37±1.82	1.39±1.18	1.78±1.44	1.69±1.42
Resolution	--	12.98±1.51	15.96±1.08	13.34±1.87
Theoretical Plates	4342.28±1.71	4792.34±1.95	49971.43±1.84	79732.02±1.75

Mean±standard deviation for n=6 replicates

3.5. Analysis of marketed formulation

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 9.

Table 9 Results for assay of cream formulation

	OFX	ORN	TBH	CBP
Label claim (%w/w)	0.75	2	1	0.05
% Assay±SD	99.74± 0.39	98.72± 0.71	98.19 ±0.23	99.05 ±0.76

Determination for n=6 replicates

3.6. Permeability study

Permeability study was carried out using franzch diffusion cell, in various media such as physiological buffer solution (PBS) and normal saline solution. The release for CBP was very less in these media. The reported literature suggested addition of ethanol to increase the release and hence with that reference [37, 38], ethanol was added to the PBS media upto 30% to enhance release of CBP. The permeability study was carried for upto 24 hrs and the cumulative percentage release was calculated. Figure 14 shows the plot of cumulative percentage release of the drugs versus time profile.

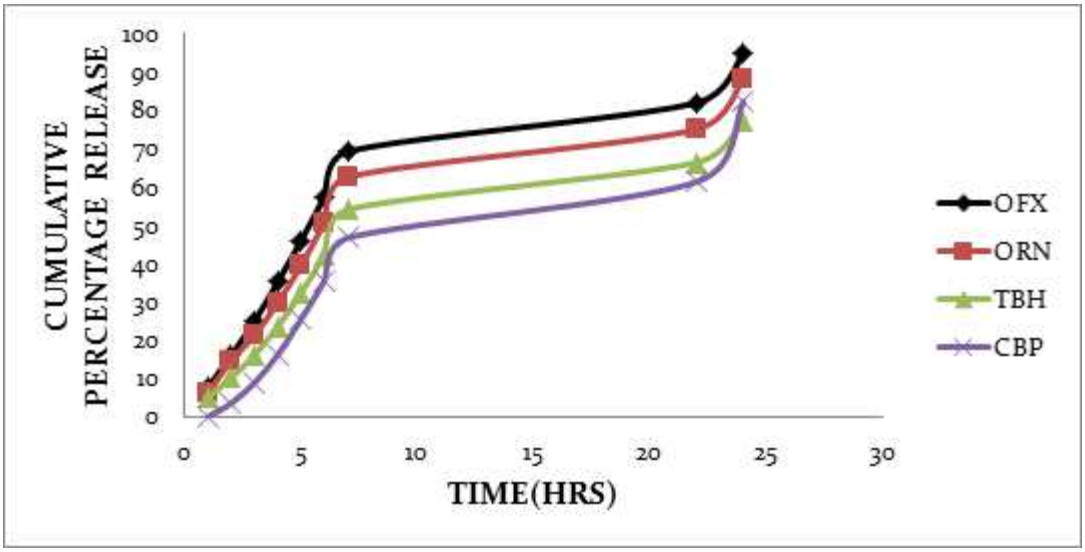

Figure 44 Plot of percentage permeability with respect to time in hour

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CONCLUSION

The QbD approach was successfully applied for the optimisation and development of HPLC method for the simultaneous estimation of Ofloxacin, Ornidazole, Terbinafinehydrochloride and Clobetasol propionate, in cream formulation, wherein full factorial design was used for finding out the most suitable conditions giving best separation of the four components within shortest possible time period and appropriate SST parameters. The optimised HPLC method has been applied for estimation of the four components in cream formulation and also to estimate their invitro permeability through rat skin.

REFERENCES

[1] http://www.drugbank.ca/drugs/DB01165.
[2] http://www.genericpedia.com/generic/ornidazole.
[3] http://www.rxlist.com/lamisil-drug/clinical-pharmacology.html.
[4] http://pubchem.ncbi.nlm.nih.gov/compound/clobetasol_propionate#section=Top.
[5] HKassem;MAAlmardini. *International Journal of Pharmaceutical Sciences Review & Research*, **2013**, 21(2), nº11,58-61.
[6] K Patel. *International Journal of Pharmacy & Technology*, **2012**, 4(3),4663-4669.
[7] SG Cardoso; EESSchapoval. *Journal of Pharmaceutical and Biomedical Analysis*, **1999**, 19,809–812.
[8] VVSNarayana Reddy K;R Suresh Kumar;E Hemnath;ST Venkata; KYamjala; RMalayand.*Int J Pharm PharmSci*, **2014**,6(5), 586-590.
[9] RR Raju;N BujjiBabu. *Pharmacophore*, **2011**, 2 (4), 232-238.
[10] VK Penmatsa; KBasavaiah. *Am. J. PharmTech Res.*, **2014**, 4(2), 899-916.
[11] S Ahmad;GK Jain; Md. Faiyazuddin;Z Iqbal;S Talegaonkar, Y Sultana; FJAhmad. *Acta Chromatographica*, **2009**,21(4), 631–639.
[12] MPTagliari; GKuminek; SHMBorgmann;CDBertol; SGCardoso. *Quim. Nova*,**2010**,33(8), 1790-1793.
[13] PMikus; I Valaskova; EHavranek. *Talanta*,**2005**, 65,1031–1037.
[14] PD Goswami. *International Journal of Pharmacy and Pharmaceutical Sciences*,**2013**,5(3), 536-540.
[15] MC Fontana;MO Bastos; RCR Beck. *Journal of Chromatographic Science*, **2010**, 48, 637-640.
[16] L Gagliardia;D De Orsi;F Manna;D Tonelli. *J. Liq. Chrom. & Rel. Technol*., **2000**, 23(3), 355–362.
[17] MS Ali; MS Alam; NAlam; MM Safhi.*Sci Pharm*., **2013**, 81, 1089–1100.
[18] AF Fauzee; RB Walker. *J Sep Sci*. **2013**, 36(5),849-56.
[19] L Gagliardi;D Orsi;MR Giudice;F Gatta;R Porrà;P Chimenti;DTonelli. *Anal. Chim. Acta*, **2002**,457,187–198.
[20] MN Shah; HU Patel; CN Patel. *Inventi Rapid: Pharm Analysis & Quality Assurance*, **2012**, Inventi:ppaqa/380/12 .

[21] R Siva Kumar; P Kumar Nallasivan; S Saravanakumar;CS Kandasamy;R Venkatnarayanan. *Asian J. Research* Chem, **2009**,2(1), 43-45.
[22] MPuranik;DV Bhawsar; PRathi;PG Yeole.*Indian J Pharm Sci*., **2010**,72(4), 513–517.
[23] JBurana-Osot; KSaowakul; C Charoensilpchai; NSurapeepong; WPongsiriwan; AKumsum;GZ Zivanovic; M Zecevic. *J. Liq. Chrom. & Rel. Technol*., **2012**,35, 1909–1919.
[24] SJ Ghosh; SDarbar; PP Chowdhury; SP Chattopadhyay; MR Chakraborty. *International Journal of PharmTech Research*, **2010**, 2(1), 367-374.
[25] JHuang; GCao; XHu; C Sun; J Zhang. *Chirality*, **2006**, 18, 587–591.
[26] FC Cheng; TR Tsai; YF Chen; LC Hung; TH Tsai. *J. Chromatogr*. A, **2002**, 961, 131-136.
[27] KK Patel; VV Karkhanis. *International Journal of Pharmaceutical Sciences and Research*, **2012**, 3(11), 4492-4495.
[28] BV Suma;K Kannan;V Madhavan;CR Nayar. *Int.J. ChemTech Res*., **2011**, 3(2), 742-748.
[29] PS Jain;AJChaudhari; SA Patel;ZN Patel;DT Patel. *Pharm Methods* **2011**; 2(3): 198–202.
[30] PD Goswami. *Der Pharmacia Lettre*, **2013**, 5(3),386-390.
[31] M Chennaiah;T Veeraiah; T Vinod Kumar; G Venkateshwarlu. *Indian Journal of Chemical Technology*, **2012**, 19, 218-221.
[32] AAbou-elkheir; HSaleh; MM El-henawee**.** *International Journal of Pharmaceutical, Chemical and Biological Sciences*, **2014**, 4(4), 931-943.
[33] AP Dewani;RL Bakal;PG Kokate;AV Chandewar;S Patra.*Journal of AOAC International*, **2015**, 98(4), 913.
[34] MAmipara; S Patel; S Pathan; J Patel; JKakadiya; N Shah. *Inventi Rapid: Pharm Analysis & Quality Assurance*, **2015**,Inventi:ppaqa/16221/15.
[35] P Bommadevara, SA Rahaman. *International Journal of Pharmacy and Analytical research* **2014**, 3(4), 301-318.
[36] ICH guidelines, validation of analytical procedure: Methodology Q2B, I.C.H. *Harmonized Tripartite Guidelines*, **1996**.
[37] JC Tsai. *Journal of Food and Drug Analysis*, **2012**,10(1), 7-12.
[38] UBadıllı; T Şen; NTarımcı. *AAPS PharmSciTech*, **2011**,12(3), 949-957.