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Original Article

APPLICATION OF QUALITY by DESIGN (CCD TECHNIQUE) FOR SIMULTANEOUS ESTIMATION OF CEFIXIME AND OFLOXACIN BY HPTLC METHOD

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ABSTRACT

Objective: The present manuscript describes about simultaneous multiple response optimizations using the Derringer's desirability function for the estimation of cefixime and ofloxacin in bulk and pharmaceutical dosage form by using HPTLC method.

Methods: Central composite design (CCD) was used for the optimization of chromatographic conditions in HPTLC. The independent variables used for the optimization were n-butanol content in the mobile phase, chamber saturation time and distance travel. HPTLC separation was performed on aluminum plates pre-coated with silica gel 60 F 254 as the stationary phase using n-butanol: Ammonia: water (8:3:1 % v/v/v) as the mobile phase. Quantification was achieved by densitometric analysis of cefixime and ofloxacin over the concentration range of 20-120ng/band at 297 nm.

Results: The method gave compact and well-resolved band at R_f of 0.43±0.02 and 0.73±0.02 respectively for cefixime trihydrate and ofloxacin hydrochloride. The linear regression analysis for the calibration plots showed r^2 = 0.99985 and r^2 = 0.9989 for cefixime and ofloxacin respectively. The optimized method complies validation parameters like precision, accuracy, robustness, specificity, limit of detection and limit of quantification as per ICH guidelines.

Conclusion: The mobile phase composition, chamber saturation time and solvent front factors are evaluated in the robustness test. The selected factors were found to have a significant effect on the R_f value of both drugs. The optimized method contains 7 ml of n-butanol, 75 mm solvent front and 33 min of saturation time was used. So the optimization process reduces the solvent usage and increases separation sensitivity for both drugs. The results indicate the CCD method is suitable for the routine quality control testing of marketed tablet formulation and bulk drugs.

Keywords: Cefixime, Ofloxacin, Central Composite Design (CCD), High-performance thin layer chromatography (HPTLC), Validation, Optimization

INTRODUCTION

Cefixime is a semi synthetic, cephalosporin antibacterial. Chemically, it is (6R,7R)-7-[2-(2-Amino-4-hiazolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo [4.2.0] oct-2ene-2-carboxylic acid, 7^2-(Z)-[O-(carboxy methyl) oxime] trihydrate fig. 1.

Ofloxacin is a synthetic broad-spectrum antimicrobial agent for oral administration. Chemically, ofloxacin, is fluorinated carboxy-quinolone, is the racemate, (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid fig. 2.

Cefixime (cephalosporin) and ofloxacin (fluoroquinolone) and has a unique dual mode of action i. e in this combination ofloxacin prevents nucleic acid synthesis of bacteria and cefixime inhibits bacterial cell wall synthesis. It is useful for killing the ESBL (Extended Spectrum Beta-lactamase) bacteria.

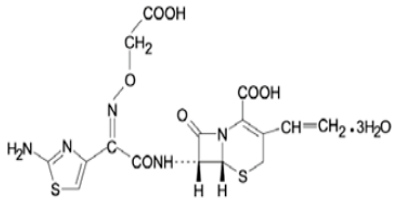


Fig. 1: Molecular structure of Cefixime trihydrate [3]

Both works instantly and synergistically and gives better patient compliance. Cefixime is highly stable in the presence of beta-lactamases enzymes. Cefixime is an effective treatment to stop the spread of several bacterial infections. It is often used as a treatment for gonorrhea, pharyngitis, typhoid fever and tonsillitis. Ofloxacin belongs to fluoroquinolones group of antimicrobials (i.e.) these are the quinolones having one or more fluorine substitution in their structure. Ofloxacin used to treat pneumonia and bronchitis caused by H. influenzae and S. pneumoniae. It is also used in treating skin infections caused by staphylococcus aureus and streptococcus pyogenes bacteria [1-2].

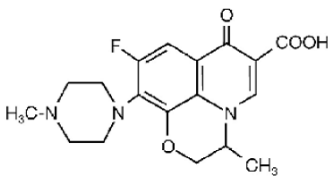


Fig. 2: Molecular structure of ofloxacin [3]

Literature survey states that cefixime and ofloxacin are official in IP, USP and BP individually [4] however, a combination of cefixime and ofloxacin is not official in any Pharmacopoeia. Various analytical methods like Spectrophotometry [8-13], HPLC [14-18], MASS Spectrometry [19], Spectroscopy [20] and Electrophoresis [21] methods were reported in the literature for the determination of cefixime and ofloxacin alone and in combination with other drugs in pharmaceutical dosage forms. However, development of a high-performance thin-layer chromatographic (HPTLC) method for simultaneous estimation of cefixime and ofloxacin in combined dosage form has not been reported till date.

The present manuscript is the first one that describes the method development and validation of HPTLC method as per ICH guidelines [5-7] ICH Q2 (R1) for simultaneous estimation of cefixime and ofloxacin. A multivariate approach using experimental design is used to study the simultaneous variation effect of the factors on the

responses. The best experimental design approach for the purpose of modeling and optimization is the response surface design. In the present study, central composite design (CCD) [22, 23] was used for optimization of chromatographic conditions of HPTLC method. CCD is chosen due to its flexibility and can be applied to optimize chromatographic conditions by gaining a better understanding of factor's main and interaction effects. Viewing the simplicity, economical, less time-consuming and few processing parameters [24-26]. The objective of this research work is, to develop a simple, rapid, precise and accurate HPTLC method using Design of experiment (DOE) approach for quantitative analysis of cefixime and ofloxacin and to validate the developed method as per ICH guidelines.

MATERIALS AND METHODS

Materials

The analytically pure cefixime and ofloxacin were obtained as gift samples from BAFNA pharmaceuticals Chennai. The Marketed tablet formulation used was Milixim-O, Glenmark, India, (Label claim: 200 mg cefixime and 200 mg ofloxacin per tablet) procured from the local market. All solvents and chemicals used were of analytical grade, purchased from Merck Specialities Pvt. Ltd., India.

Instrumentation

- Hamilton microlitre syringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz,
- Camag, Switzerland),
- Precoated silica gel aluminum plate 60 F254, (10 × 10 cm, 100 m thickness; E. Merck, Darmstadt, Germany),
- Linomat 5 sample applicator (Camag,Switzerland),
- Twin trough chamber (20 × 10 cm; Camag, Switzerland),
- UV chamber (Camag, Switzerland),
- TLC scanner 4 (Camag, Switzerland),
- Win CATS version 1.4.6 software (Camag, Switzerland) was used in the study.

All drugs and chemicals were weighed on an electronic balance (AUW 220, Shimadzu Corp., Japan).

Methods

Preparation of standard solutions

Weigh specified the quantity of standard cefixime and ofloxacin were freshly prepared by using methanol and got 1 mg/ml. Aliquots of a standard stock solution of cefixime and ofloxacin were diluted to obtain the mixed standard solution by using the methanol to contain 20 ng/µl of cefixime and ofloxacin.

Preparation of mobile phase: [n-butanol: ammonia: water] (8:3:1%v/v)

80 ml of N-butanol, 30 ml of ammonia and 10 ml of water were mixed and degassed in an ultrasonic water bath for 5 min. Then it was filtered through 0.45µ pore filter under vacuum and transferred into a 250 ml volumetric flask.

Chromatographic procedure

The standard solutions of cefixime and ofloxacin were spotted in the form of bands having a bandwidth of 6 mm using a Camag Linomat sample applicator. a pre-coated silica gel aluminum Plate 60F[25][4]are used as a stationary phase. Linear ascending development was carried out in a twin trough glass chamber. The mobile phase consists of n-butanol: ammonia: water (8:3:1, % v/v/v). The optimized chamber saturation time before chromatographic development was 30 min at room temperature (25±2 ◦C).

The length of chromatographic run was 8 cm. Subsequent to the development; HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag TLC scanner with winCATS software. All measurements were made in the reflectance–absorbance mode at 297 nm, slit dimension (6.00 x 0.30 mm, micro), scanning speed 20 mm/s, data resolution 100 µm/step. The source of radiation was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentration of both drugs was determined from the intensities of diffusely reflected lights, and evaluation was done by ordinary linear regression analysis of peak areas

Software aided method optimization

Central composite design (CCD) was used to optimize the compositional parameters and to evaluate the main effect, interaction effects and quadratic effects of the factors on the retardation factor (R_f) of both drug. CCD is useful in response surface methodology, for exploring quadratic response surfaces and constructing second order polynomial models without the need to use a complete three-level factorial experiment [27, 28].

The selection of critical factors and ranges examined for optimization was based on preliminary univariate studies of method development and chromatographic intuition. The composition of the mobile phase is the volume of n-butanol content with respect to total volume of the mobile phase. Totally fifteen experiments with five center points were conducted by selection of three factors, n-butanol content in mobile phase (A), chamber saturation time (B), distance travel (C) and R_f of cefixime and ofloxacin were the responses selected for both drugs depicted in the table: 1. The nominal value for these all three factors, A, B and C were 8 ml, 30 min, and 8 cm respectively. In context to this, n-butanol content (A) was kept between 6.59 and 9.41. Similarly, minimum and maximum values of chamber saturation time (B) were fixed as 22.93 min and 37.07 min respectively. Likewise, minimum and maximum values for distance travel(C) were fixed as 6.59 and 9.41 respectively. The coded value of α is 1.41. The data generated were analyzed using Design Expert (Version 7.0.1.0 Stat-Ease Inc., Minneapolis, MN, USA) trial version statistical software. The significance of the relevant factors was calculated using Fisher's statistical test for Analysis of Variance (ANOVA) model. All experiments were conducted in a randomized order to minimize the bias effects of uncontrolled variables. Replicates (n=5) of the center points were performed to estimate the experimental error.

Method validation

The developed HPTLC method was validated for accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), specificity, robustness, and ruggedness parameters, in accordance with ICH Q2 (R1).

Linearity and range

The mixed standard stock solution of 0.02 mg/ml of cefixime and 0.02 mg/ml of ofloxacin was used for linearity studies. The 1 to 6 µl stock solution was spotted on the TLC plate to obtain the final concentration 20-120 ng/spot for both cefixime and ofloxacin. Each concentration was applied three times to the TLC plate. The plate was developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curve. The measured peak areas versus corresponding concentration of both drugs were evaluated by ordinary linear regression analysis.

Sensitivity

Limit of detection (LOD) and Limit of quantitation (LOQ) of the developed method was calculated from the standard deviation of the response and slope of the calibration curve of drugs using the formula as per ICH guideline,

Limit of detection=$3.3 \times \sigma/S$

Limit of quantitation=$10 \times \sigma/S$

Where, "σ" is standard deviation of y-intercepts of regression lines, "S" is Slope of calibration curve.

Precision

The precision of the developed method was evaluated by performing Intra-day and Inter-day precision studies. Intra-day precision was carried out by performing three replicates of three different concentration (20, 40 and 60 ng/band for cefixime and ofloxacin) on same day and peak area measured was expressed in terms of

percent relative standard deviation (% RSD). The inter-day precision study was performed on three different days using mentioned concentrations of both drugs in triplicate

Accuracy

The accuracy of the method was ascertained in triplicates, at three concentration level of 50%, 75% and 100 %, by spiking known amount of cefixime and ofloxacin standard to the pre-quantified samples and calculating the recovery and % RSD for both the drugs. Recovery studies were carried out by spiking three different amount of cefixime and ofloxacin standard (20, 40, 60 ng/band) to the dosage form (40 ng/band) by standard addition method.

Specificity

The specificity of the method was ascertained by comparing the samples of tablet formulation with standard drugs. The band for cefixime and ofloxacin in the sample was confirmed by comparing the R_f and overlaying peak purity spectra with that of the standard. The peak purity of cefixime and ofloxacin was assessed by comparing the spectra at three different levels, i.e., peak start (S), peak apex(M) and peak end (E) positions of the band.

Robustness

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (20, 40, 60, ng/spot) for both drugs from the stock solution was applied at six times. The intra-day precision (RSD, %) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter-day precision (RSD %) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

The effect of small and deliberate variations on method parameters like a change in volume of n-butanol content in mobile phase composition, saturation time, distance travel and wavelength was evaluated. The effect of these changes on both the values and peak areas was examined by calculating the % RSD for each parameter

Recovery

Accuracy of the method was carried out by applying the method to the drug sample (cefixime and ofloxacin combination tablet) to which a known amount of cefixime and ofloxacin standard powder corresponding to 50, 75, 100, of label claim had been added (Standard addition method), mixed, and the powder was extracted and analyzed by running chromatogram in an optimized mobile phase. This process was done to check for the recovery of the drug at different levels in the formulation.

Analysis of marketed formulation

The content determination of cefixime and ofloxacin in a conventional tablet (Brand name: Milixim-O, Glenmark, India. (Label claim: 200 mg cefixime and 200 mg ofloxacin per tablet), the twenty tablets were weighed, and then the average weight was determined and finely powdered. The weight of the powdered tablet equivalent to 200 mg of cefixime and 200 mg of ofloxacin was transferred into a 100 ml volumetric flask. Then add 60 ml of methanol, sonicated for 30 min and diluted to 100 ml with methanol. The resulting solution was centrifuged at 3000 rpm for 15 min and the drug content of the supernatant liquid was determined (2 mg/ml for both cefixime and ofloxacin).

Then 1 ml of the filtered solution was diluted to 10 ml to produce a concentration of 0.2 mg/ml or 200µg/ml for both cefixime and ofloxacin. Again the 1 ml of the above solution was diluted to 10 ml the concentration was obtained 20ng/ml. The final concentration obtained was 20 ng/spot for both cefixime and ofloxacin which was developed in an optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipients interference with the analysis was examined.

RESULTS AND DISCUSSION

Selection of wavelength

The sensitivity of HPTLC method with ultraviolet detection depends on an appropriate wavelength. The developed plate was subjected to densitometric measurements in a scanning mode in the UV-Visible region of 200–700 nm, and the overlain spectrum was recorded on a CAMAG TLC Scanner 4. Both drugs absorbed appreciably at 297 nm, and selected as the detection wavelength fig. 3 overlain spectra.

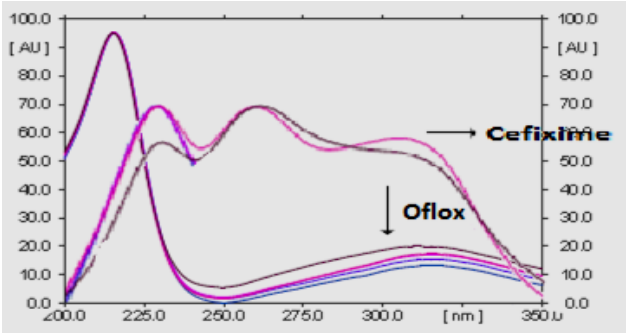


Fig. 3: Overlain absorption spectra of cefixime and ofloxacin at 297 nm

Method optimization

The optimizations of chromatographic conditions were done with a view to develop HPTLC method for simultaneous determination of cefixime and ofloxacin in bulk and in pharmaceutical dosage form.

Preliminary study

From the literature review, it is revealed that HPTLC method for cefixime and ofloxacin alone or with other drug combination had been reported, where selected mobile phase comprised of n-butanol, ammonia and water. Hence, various combinations of such components in different proportions such as n-butanol: ammonia: water (8:4:1 v/v); n-butanol: ammonia: :water (8:4:0.5, 7: 3: 0.5, 8: 1: 1, 9: 1: 1, 8: 2: 0.5, v/v/v) were tried at fixed 30 min chamber saturation time and 80 mm solvent migration distance. However, satisfactory resolution of the drugs was not achieved with acceptable R_f value. Generally, chamber saturation time and solvent migration distance were crucial to HPTLC chromatographic separation. Here, chamber saturation time of less than 25 min and solvent migration distances greater than 80 mm resulted in the diffusion of the analyte band. n-butanol: ammonia: water (8: 2: 0.5, v/v/v) was found to be a satisfactory mobile phase, giving good separation of cefixime and ofloxacin. But, R_f value of ofloxacin was found to near 0.8 and was also affected by chamber saturation time. Therefore, further chromatographic conditions were optimized to obtain well-defined, compact bands of cefixime and ofloxacin with acceptable R_f value (<0.8) of both drugs using CCD.

Optimization of chromatographic conditions using CCD

Central composite Design (CCD) is chosen due to its flexibility and can be applied to optimize HPTLC separation by gaining a better understanding of factor's main and interaction effects. A three-factorial, rotatable Central Composite statistical experimental design was performed using 15 experimental runs including five center points. The independent variables such as n-butanol content in mobile phase (A), chamber saturation time (B) and distance travel (C) and the responses for all 15 optimized trial experimental runs are summarized in table 1. During model selection, it was observed that the best-fitted model for R_f of cefixime and ofloxacin was linear and quadratic model respectively based on lowest PRESS value and adjusted R2 value nearer to 1.

Table 1: Central composite rotatable design arrangement and responses

Std	Run	Type of blocks	Factor 1 A: mobile phase in ml	Factor 2 B: saturation time in min	Factor 3 C: solvent front in mm	Response 1 R_f cefixime	Response 2 R_f ofloxacin
12	1	Center	8.00	30.00	80.00	0.41	0.64
2	2	Fact	9.00	25.00	90.00	0.17	0.53
6	3	Axial	9.41	30.00	80.00	0.25	0.62
1	4	Fact	9.00	35.00	70.00	0.58	0.75
8	5	Axial	8.00	37.07	80.00	0.47	0.77
13	6	Center	8.00	30.00	80.00	0.38	0.6
7	7	Axial	8.00	22.93	80.00	0.73	0.85
11	8	Center	8.00	30.00	80.00	0.46	0.74
14	9	Center	8.00	30.00	80.00	0.45	0.71
15	10	Center	8.00	30.00	80.00	0.43	0.67
10	11	Axial	8.00	30.00	94.14	0.24	0.64
9	12	Axial	8.00	30.00	65.86	0.68	0.97
3	13	Fact	7.00	35.00	90.00	0.56	0.81
4	14	Fact	7.00	25.00	70.00	0.57	0.88
5	15	Axial	6.59	30.00	80.00	0.39	0.7

The model was also validated by analysis of variance (ANOVA) using Design Expert software, and the results are as presented in table 2. Significant effects had P value less than 0.05. Adequate Precision, a measure of the signal (response) to noise ratio, greater than 4 is desirable, and the obtained ratio for both drugs indicated an adequate signal [21]. The coefficient of variation (% CV) that measures the reproducibility of the model less than 10% and high adjusted R-square values indicated a good relationship between the experimental data and those of the fitted models.

Here, the adjusted R2 were well within acceptable limits of R2≥ 0.80 which revealed the experimental data were a good fit to the polynomial equations [10]. The final equation, in terms of actual components and factors, is as shown in table 2.

Table 2: Predicted response models and statistical parameters obtained from ANOVA for CCD

Response(R_f)	Type of model	Polynomial equation for Y	Adjusted R2	Model P value	% CV	Adequate precision
Cefixime	2FI	R_f cefixime = -9.88269 +1.78927 *A -0.010287*B +0.11068*C -0.010113*AB -0.019192*AC +9.10051E-004*BC	0.5598	0.0384(limit less than 0.05)	3.18(less than 10%)	7.245(more than 4 is desirable)
Ofloxacin	Quadratic	R_f ofloxacin = +0.68 -0.028 * A -0.12 * C -0.044* A * B -0.038 * A * C +0.074 * B * C -0.021 * A2 +0.054 * B2 +0.051 * C2	0.7502	0.0191	8.09	8.724

*Result of predicted response are generated by Design Expert software

A positive value represents an effect that favours the optimization, while a negative value indicates an inverse relationship between the factor and the response Three-dimensional Response surface plots and perturbation plots were constructed to evaluate the effect of the factors on the retention factor of each drug. In fig. 4, perturbation plots were presented for predicted model in order to gain a better understanding of the investigated procedure. It gives the idea about how the response changes as each factor moves from its defined reference value, with all other factors held constant at a reference point, and steepest slope or curvature indicates sensitiveness to a specific factor. Fig. 4 (a) shows that distance travel (factor C) had the most significant effect on R_f value of cefixime as compared to other factors. While in fig. 4 (a) Solvent front (C) and chamber saturation time (B) had more significant effect on R_f value of cefixime followed by n-Butanol content (factor A). Fig. 4(b) represents a variation in R_f value of ofloxacin as a function of chamber saturation time and distance travel. The retention factor of ofloxacin decreases as travel distance increases but the saturation time increases the retention factor of ofloxacin also increases. Analysis of the perturbation plots and response plots of optimization model revealed that distance travel (C) and chamber saturation time (B) had a greater significant effect on responses of ofloxacin and cefixime as compared to factor A, i.e. n-butanol content.

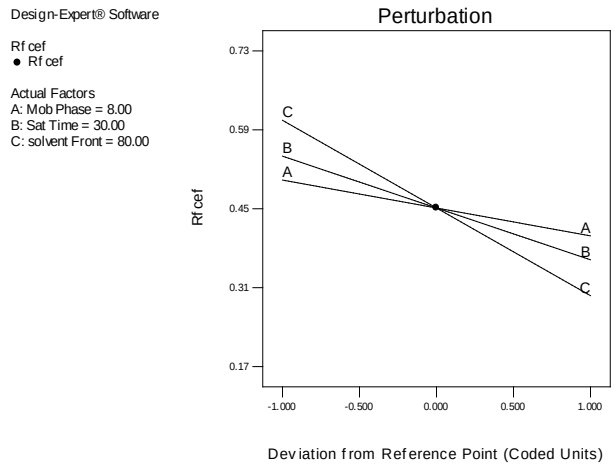


Fig. 4(a): Perturbation graph showing the effect of each factor A, B, and C on (a) R_f of cefixime

Table 3: Comparison of experimental and predictive values of different experimental runs under optimum conditions

Optimum conditions	Selected optimized run				
1	A: N-Butanol Content (Ml)	B: Chamber Saturation Time (Min)	C: Distance Travel (Cm)	R_f Value of Cefixime	R_f Value of Ofloxacin
	7 ml	33 min	7.6 cm		
	• Experimental Value			0.432	0.734
	• Predictive Value			0.454	0.752
	• Predicted Error			0.048	0.023
2	7 ml	32 min	7.6 cm		
	• Experimental Value			0.433	0.733
	• Predictive Value			0.454	0.750
	• Predicted Error			0.567	0.267

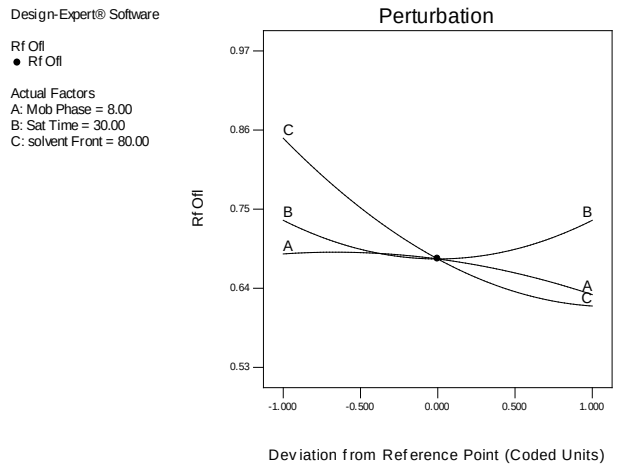


Fig. 4(b): Perturbation graph showing the effect of each factor A, B, and C on R_f value of ofloxacin

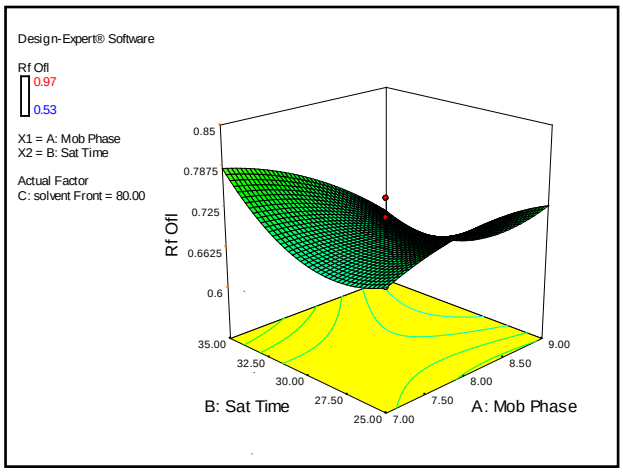
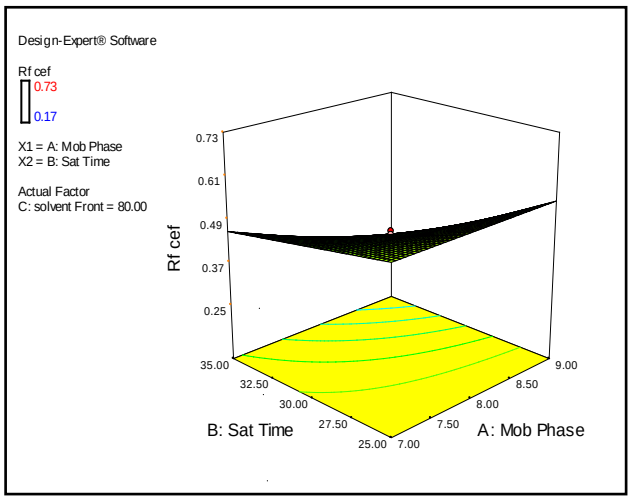


Fig. 5(b): Variation in R_f of ofloxacin as function of A and B while fixed factor C



Three-dimensional plots of the RSM for both responses

Fig. 5(a): Variation in R_f of Cefixime as function of B and C while fixed factor A;

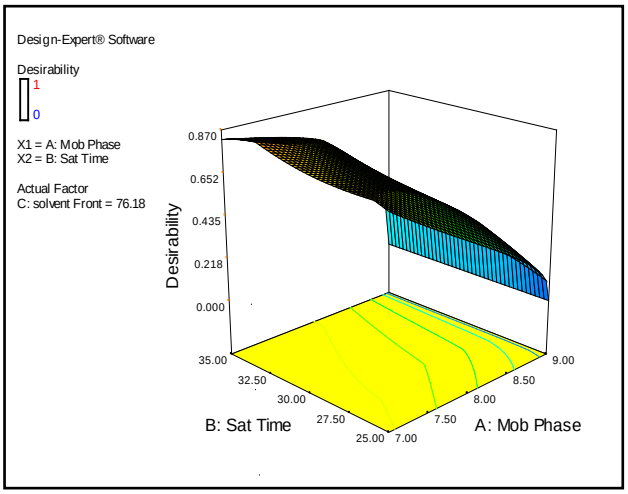


Fig. 5(c): Graphical representation of the maximum derringer's desirability function [D=0.870]

The optimum conditions of separation were estimated by Derringer's desirability function. During numerical optimization, firstly the target of individual factors and responses were fixed. Out of 15 different solutions of optimization provided by software two conditions were selected that have desirability near to 1.

The response surface obtained for the maximum Derringer's desirability function is presented in fig. 5(a, b, C).

In order to investigate the predictability of the proposed model, the agreement between experimental and predicted responses for both the predicted optimums 1 and 2 are shown in table 3. The Percentage of prediction error was calculated using formula,

Predicted Error = Experimental-Predicted/Predicted × 100.

From the table 3 and % predicted error, it is concluded that a set of coordinates producing high desirability value (D = 1) at optimum condition 1, hence proposed for selecting an optimum experimental

condition for analyzing cefixime and ofloxacin in combination. The optimized composition selected was n-butanol: ammonia: water (7:2:1 v/v/v), for the final HPTLC analysis. HPTLC dendrogram under optimized conditions showing R_f of 0.43 for cefixime (40 ng/band) and 0.73 for ofloxacin (40 ng/band) was depicted in fig. 6. The reported methods[17,20,21] shows the mobile phase composition was 8 ml of n-butanol and R_f value of ofloxacin was also more than 8 but the optimized method the mobile was composition was n-butanol: ammonia: water (7:2:1 v/v/v),distance travelled by solvent was 7.6 cm and R_f value ofloxacin was 0.73. So the optimized method required lesser analysis time and also produce the ideal, optimized densitogram for cefixime and ofloxacin.

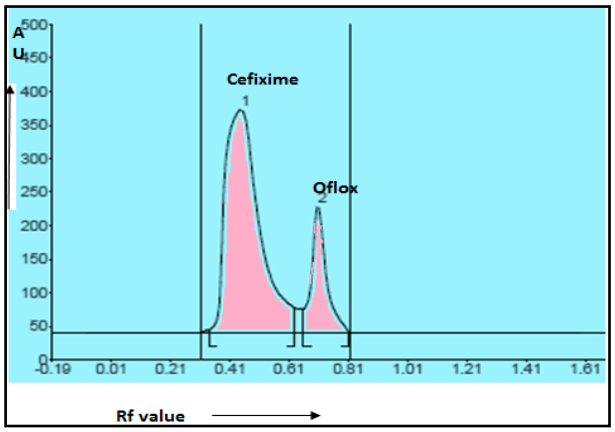


Fig. 6: HPTLC densitogram under optimized conditions showing Rf value 0.43 for cefixime (40 ng/band) and 0.72 for Ofloxacin (40 ng/band)

Method validation

Validation studies were conducted using the optimized assay conditions based on the principles of validation described in the ICH Q2A, QAB guidelines. Key analytical parameters including specificity, accuracy, precision, linearity, detection limit and quantification limit were evaluated.

Linearity

The linearity of an analytical method is its ability, within a given range, to provide results that are directly, or through a mathematical transformation, proportional to the concentration of the analyte. The cefixime and ofloxacin showed a good correlation coefficient (r2 = 0.99985 for cefixime and r2 = 0.9923 for ofloxacin) in the proposed concentration range of 20-120 ng/band for both cefixime and ofloxacin (table 4).

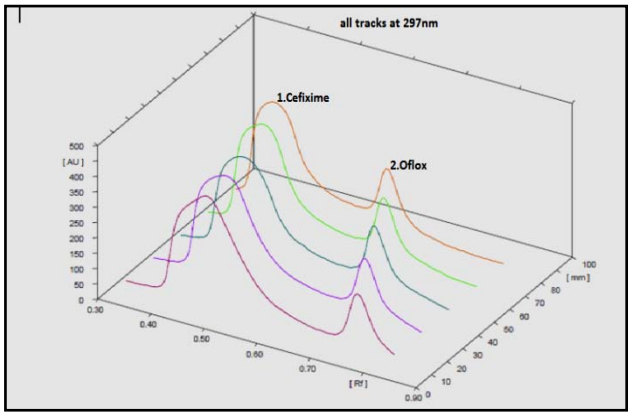


Fig. 7: Three-dimensional densitogram for linearity of Cefixime and Ofloxacin hydrochloride at 297 nm

Table 4: Linearity studies of Cefixime trihydrate

S. No.	Concentration in ng/spot	Mean peak area
1	20	203268
2	40	228623
3	60	254025
4	80	279328
5	100	304830
6	120	330440

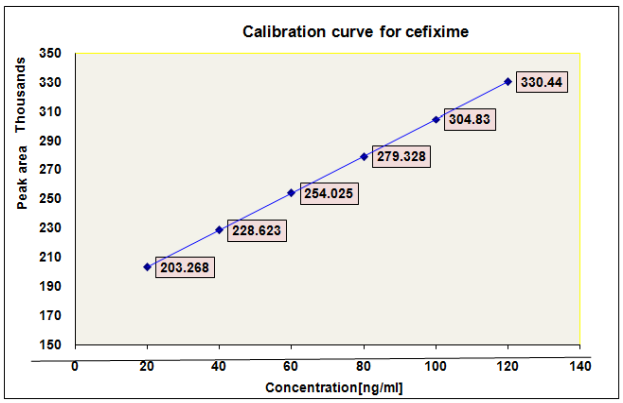


Fig. 8: Calibration curve of Cefixime (20-120ng/ml)

Table 5: Linearity studies of Ofloxacin

S. No.	Concentration in ng/spot	Mean peak area
1	20	244268
2	40	472330
3	60	709992
4	80	934354
5	100	1165716
6	120	1398051

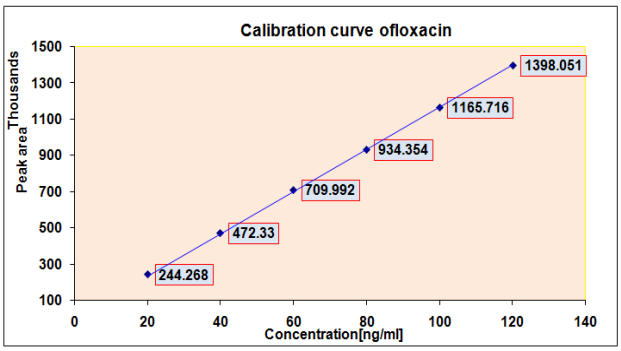


Fig. 9: Calibration curve of Ofloxacin (20-120ng/ml)

LOD and LOQ

LOD and LOQ of developed method were found to be 0.1076927 and 0.3263416 ng/band respectively for cefixime while 0.223097ng/ml and 0.676053 ng/band respectively for ofloxacin indicating the sensitivity of the proposed method (table 6).

Precision

The experiment was repeated for three times in a day (Intra-day precision) and the average % RSD values of the results were calculated. Similarly, the experiment was repeated on three different days (Inter-day precision), and the average % RSD values for peak area of cefixime and ofloxacin was calculated. Results of intra-day and inter-day precision expressed in terms of % RSD less than 2 confirm precision of the method (table 7).

Table 6: Analytical validation parameters for Cefixime and Ofloxacin by HPTLC method

S. No.	Parameter	Cefixime	Ofloxacin
1	Linearity range(ng/band)	20-120	20-120
2	Correlation coefficient (r2)*	0.99985	0.99923
3	Slope±SD	1258.73±1.156	77.5856
4	Confidence limit of slope*	1176.3-1287.63	22012.55556
5	Intercept±SD*	177760.87	22012.555
6	Confidence limit of intercept	176804.4-180226.33	21060.44-2341.32
7	Sensitivity-LOD(ng/band)*	0.1076927	0.223097
8	Sensitivity-LOQ(ng/band)*	0.3263416	0.676053
9	Average of standard error	702.4742	11650.53

*Average of five determinations

Table 7: Precision studies for cefixime and Ofloxacin

Drug name	%RSD for interday precision*	%RSD for intraday precision*	Limit
Cefixime	1.141-1.546	0.520-0.946	Less than 2
Ofloxacin	1.538-1.788	0.754-1.203	

*mean±SD of three determinations for each concentration

Accuracy

The proposed method when used for evaluation of recovery at three concentrations levels,

50%, 100% and 150% after spiking with standard, showed percentage recovery between 100.46 to 101.87 for cefixime and 100.46 to 101.1.832 for ofloxacin which was within acceptable ranges of 100±2 %.

Specificity

The chromatogram of the pharmaceutical formulation using the developed method showed only two peaks at R_f of 0.34 and 0.77 for cefixime and ofloxacin respectively; that was found to be at the same R_f for both standard drugs by comparison of chromatograms (fig. 7). The peak purity of both drugs in pharmaceutical dosage form was confirmed when evaluated by comparing the overlaid spectra at peak start, peak apex and peak end positions of the band. It was observed from results shown in table 4 that purity was more than 0.999 for all peaks, indicating the specificity of the method in the presence of various excipients (fig: 10 a and b).

Robustness

Deliberate change in different parameters like n-butanol content in mobile phase composition, chamber saturation time, distance travel and wavelength showed % Relative standard deviation of peak area less than 2%, indicating the robustness of method (table 9).

Table 8: Accuracy studies for cefixime and Ofloxacin

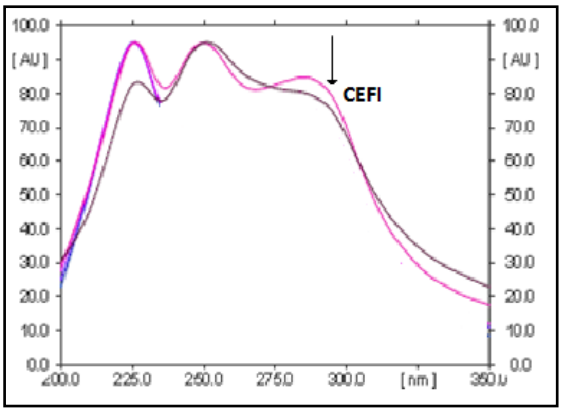
Spiked sample concentration at % level	Cefixime*	Ofloxacin*
50	100.46±0.316	101.54±0.129
75	101.87±1.832	101.43±1.689
100	101.46±0.259	100.41±0.339

*mean±SD of three determinations at each level

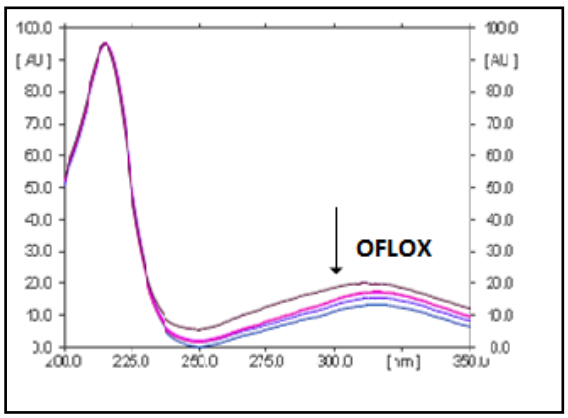
Table 9: Robustness study of cefixime and ofloxacin by HPTLC method

Change in mobile phase ratio (n-butanol: ammonia: Water, 8:2:0.5+0.25 in n-butanol content)*			
Drugs	**Ratio**	**R_f**	**% RSD**
Ofloxacin	8.25: 2:0.5	0.76+0.01	0.546
	7.75: 2:0.5	0.76+0.01	1.736
Cefixime	8.25: 2:0.5	0.46+0.01	0.510
	7.75: 2:0.5	0.46+0.01	0.668
Change in chamber saturation time (30 min+5)*			
Drugs	Saturation time (min)	R_f	% RSD
Ofloxacin	25	0.77+0.02	1.839
	35	0.77+0.02	1.004
Cefixime	25	0.44+0.02	1.492
	35	0.47+0.02	1.192
Change in Distance travel (8 cm+1)*			
Drugs	Distance travel (cm)	R_f	% RSD
Cefixime	7	0.77+0.02	1.283
	9	0.77+0.02	1.010
Ofloxacin	7	0.43+0.02	1.027
	9	0.44+0.02	0.893
Change in wavelength (297 nm+2)*			
Drugs	Wavelength (nm)	R_f	% RSD
Ofloxacin	299	0.76+0.02	0.506
	295	0.76+0.02	0.246
Cefixime	299	0.45+0.02	0.313
	295	0.45+0.02	0.809

mean±SD of three determinations of 40 ng/band for Cefixime and 40 ng/band for Ofloxacin, % RSD = relative standard deviation,



(a)



(b)

Fig. 10: Overlain peak purity spectra of (a) Cefixime and (b) Ofloxacin with the corresponding standard

Analysis of marketed dosage form

Analysis of tablet formulation containing 200 mg cefixime and 200 mg ofloxacin showed good recovery for both drugs. The %RSD value was found to be less than 2 indicating that the method can be applicable in routine quality control testing of the tablet dosage formulation.

Table 10: Assay of formulation (Cefixime 200 mg and Ofloxacin 200 mg)

Sample No	Cefixime amounts in %	Ofloxacin amount in %
I	101.46	100.54
II	100.87	102.43
III	99.46	101.41
IV	99.92	101.23
V	99.63	99.45
SD	0.86085	1.0044
%RSD	0.85855	1.0993

CONCLUSION

The CCD design provides essential information regarding the sensitivity of various chromatographic variables such as R_f value of drugs, mobile phase composition, chamber saturation time and distance travel. The CCD design and multi-criteria decision-making approach is a flexible procedure, able to reduce the number of the needed experiments for the development and optimization of HPTLC method and it is an economical method that can be used to generate a maximum amount of information in a lesser analysis time with a small number of experiments. The optimized method the mobile phase composition was n-butanol: ammonia: water (7:2:1 v/v/v), distance travelled by solvent was 7.6 cm and R_f value ofloxacin was 0.73. So the optimized method required only lesser analysis time compare to reported method and also produce the ideal, optimized densitogram for cefixime and ofloxacin. The established HPTLC method is simple, accurate, and reliable and suitable for rapid quantitative analysis of cefixime and ofloxacin. So the proposed HPTLC method can be successfully utilized for the simultaneous estimation of cefixime and ofloxacin in the pharmaceutical dosage form without interference and any prior separation of individual drugs.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. http://www.drugbank.ca/drugs/DB00671. [Last accessed on 10 Jan 2016].
2. https://en.wikipedia.org/wiki/Cefixime. [Last accessed on 10 Jan 2016].
3. https://en.wikipedia.org/wiki/Ofloxacin. [Last accessed on 10 Jan 2016].
4. Indian Pharmacopoeia: Ministry of Health and Family Welfare, Indian Pharmacopoeial commission, Ghaziabad; 2014. p. 254, 856.
5. ICH Topic Q8 (R2); "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '09): Pharmaceutical Development; 2009.
6. ICH Topic Q2 (R1); "ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '94): Validation of Analytical Procedures; 1994.
7. ICH Topic Q9;"ICH harmonised tripartite guideline," in Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '94) Quality Risk Management; 2005.
8. Attimarad M. Simultaneous determination of ofloxacin and flavoxate hydrochloride by first and ratio first derivative UV spectrophotometry. J Iranian Chem Soc 2012;9:1–7.
9. Gonzalez-Hernandez R, Nuevas-Paz L, Soto-Mulet L, Lopez-Lopez M, Hoogmartens J. Reversed-phase high-performance liquid chromatographic determination of cefixime in bulk drugs. J Liq Chromatogr Relat Technol 2001;24:2315–24.
10. Pawar SJ, Kale AP, Amrutkar MP, Jagade JJ, Pore NS, Bhosale AV. HPTLC estimation of cefixime and cloxacillin in tablet dosage form. Asian J Res Chem 2010;3:299–301.
11. Chaitanya Krishna A, Vignesh R, Chelladurai R, Rajaram S Patil. A highly specific, sensitive and high-throughput method for the determination of ofloxacin in human plasma by liquid chromatography coupled with tandem mass. Asian J Pharm Clin Res 2012;5:174-9.
12. Umamaheswari D, Jayakar B. Analytical method development, and validation for the simultaneous estimation of nimorazole and ofloxacin in pure and its pharmaceutical dosage form by RP-HPLC. Asian J Pharm Anal Med Chem 2014;2:268-75.
13. Prabhu S, Vijay Amirtharaj R, Senthilkumar N. Simultaneous RP-HPLC method development and validation of cefixime and ofloxacin in tablet dosage form. Asian J Res Chem 2010;3:367-9.
14. Khandagle KS, Gandhi SV, Deshpande PB, Kale AN, Deshmukh PR. High-performance liquid chromatographic determination of cefixime and ofloxacin in combined tablet dosage form. J Chem Pharm Res 2010;2:92-6.
15. Mahesh V Attimarad, Ahmed O Alnajjar. A conventional HPLC-MS method for the simultaneous determination of ofloxacin and cefixime in plasma development and validation. J Basic Clin Pharm 2013;4:36–41.
16. D Zendelovska, T Stafilov, P Milosevski. A high-performance liquid chromatographic method for determination of cefixime and cefotaxime in human plasma. Bull Chem Technol Maced 2003;22:39-45.
17. Rao J. Validated HPTLC method for simultaneous quantitation of cefixime and ofloxacin in bulk drug and in a pharmaceutical formulation. Pharmacie Globale (IJCP) 2011;4:1-5.
18. Patel Satish A, Patel Natavarlal J. Development and validation of RP-HPLC method for simultaneous estimation of cefixime

trihydrate and ofloxacin in tablets. Int J Pharm Res Biosci 2012;1:516-29.

19. Meng F, Chen X, Zeng Y, Zhong D. Sensitive liquid chromatography-tandem mass spectrometry method for the determination of cefixime in human plasma: application to a pharmacokinetic study. J Chromatogr B: Anal Technol Biomed Life Sci 2005;819:277-82.
20. Audumbar Digambar Mali, Ritesh Bathe, Ashpak Tamboli. Simultaneous determination of Cefixime trihydrate and Ofloxacin in pharmaceutical dosage form by second order derivative UV spectrophotometry. Int J Adv Pharm Anal 2015;(5 Suppl 2):42-6.
21. Ao Alnajjar**.** Simultaneous determination of ofloxacin and cefixime in tablet formulation using capillary electrophoresis. J Liq Chromatogr Relat Technol 2013;36:36-41.
22. P Borman, P Nethercote, M Chatfield. The application of quality by design to analytical methods. PharmTech; 2007.
23. MM Hendriks, JH De Boer, AK Smilde, DA Doornbos. Multicriteria decision making. Chemom Intell Lab Syst 1992;16:175-91.
24. T Sivakumar, R Manavalan, K Valliappan. Global optimization using derringer's desirability function: Enantioselective determination of ketoprofen in the formulation and in biological matrices. Acta Chromatogr 2007;19:29-47.
25. V Sree Janardhanan, R Manavalan, K Valliappan. Chemometric technique for the optimization of the chromatographic system. Simultaneous HPLC determination of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin used in combined cardiovascular therapy. Arabian J Chem 2012;1-10. Doi:10.1016/J.Arabjc.2012.03.001. [Article in Press]
26. TB Solanki, PA Shah, KG Patel. Central composite design for validation of HPTLC method for simultaneous estimation of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide in tablets. Indian J Pharm Sci 2014;76:179-87.
27. JH Zar. Biostatical analysis: Pearson Education Inc. New Jersey; 2010.
28. G Kleinschmidt. In: Ermer J, Miller JHM. Eds. Method validation in pharmaceutical analysis. A Guide to Best Practice, Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim; 2005.