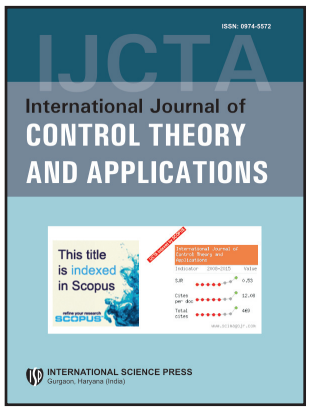


International Journal of Control Theory and Applications

ISSN : 0974-5572

Volume 10 • Number 24 • 2017

Quality by Design for Method Development and Validation of Simultaneous Estimation of Oflaxacin and Tinidazole in Marketed Formulation

K. Manikandan[a] and K.S. Lakshmi[b]

[a]*Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, Kattankulathur, Kancheepuram, Tamilnadu, India. Email: gurumani12@gmail.com*
[b]*Corresponding Author: Dean, SRM College of Pharmacy, SRM University, Kattankulathur Campus, TamilNadu – 603203. Email: kslakshmi13@gmail.com*

Abstract: The present work was estimated simultaneous multiple response optimization using Derringer's desirability function for the development of an High Performance Thin Layer Chromatography (HPTLC) method to detect Oflaxacin and Tinidazole in the marketed pharmaceutical formulation. Central composite design (CCD) was used to develop the chromatographic condition for HPTLC. The independent variables used for the optimization were the mobile phase content of acetonitrile, the saturation time and distance travelled. HPTLC separation was performed on pre coated silicagel $60F_{254}$ aluminium plate as the stationary phase using acetonitrile: water (5:5 v/v) as the mobile phase. Quantification was achieved based on a densitometric analysis of Ofloxacin and Tinidazole over the concentration range of 100-600ng/spot and 300-1800ng/spot, respectively, at 303nm. The method yielded compact and well resolved bands at R_f of 0.32 ± 0.02 and 0.53 ± 0.02 for Oflaxacin and Tinidazole respectively. The linear regression analysis for the calibration plots produced $r^2 = 0.9996$, $r^2 = 0.9998$ for Oflaxacin and Tinidazole respectively. The accuracy, precision, limit of detection, limit of quantification, specificity and robustness of the methods were validated as per the ICH guidelines. The factors evaluated were found to have an insignificant effect on the responses. The results indicated that the method is suitable for the routine quality control testing of marketed tablet formulations.

Keywords: Central Composite Design, High Performance Thin Layer Chromatography, Oflaxacin, Tinidazole, Validation.

1. INTRODUCTION

Oflaxacin (OFL) is a broad spectrum, fluorinated quinolone antibacterial drug, chemically it is a 9-fluro-2, 3 –dihydro-3 –methyl -10-(4-methyl-1-piperazinyl)-7-oxo-7H- pyrido [1, 2, 3-de]-1, 4 benzoxacine- 6- carboxylic acid[1]. Tinidazole (TNZ) is a antiprotozoal, antibiotic and antibacterial drug, chemically it is 1-[2-(ethyl sulphonyl) ethyl]-2-methyl-5-nitro-1H-imidazole (Figure 1). According to the literature, According to the literature, OFL and TNZ are official in IP, BP, USP when used individually, but the combination of OFL and TNZ is not official in any Pharmacopoeia. Various analytical methods such as spectrophotometric[1-3], HPLC[3-15], Spectrofluorimetry,

HPTLC methods were reported for the estimation of OFL and TNZ alone and combination with other drugs in tablet formulation[16-22]. Spectrophotometric method and HPLC method have been reported simultaneous estimation of OFL and TNZ in combined pharmaceutical formulation.

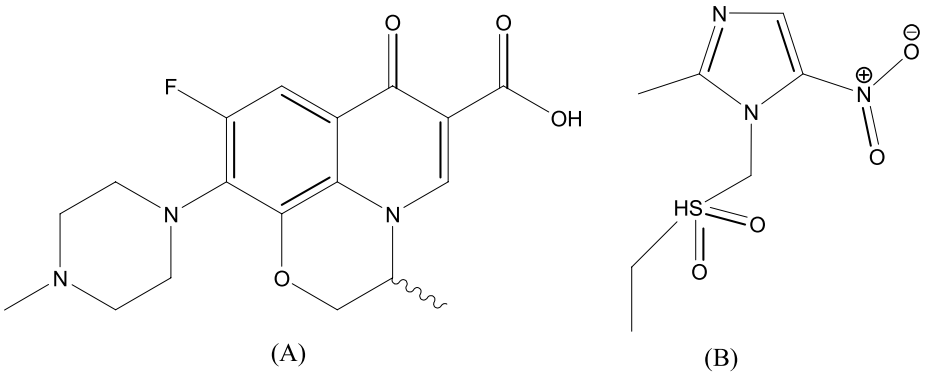


Figure 1: Chemical structure of (A) Ofloxacine and (B) Tinidazole

This investigation focused on development and validation of HPTLC method according to ICH guidelines (ICH (Q2) R1) for simultaneous estimation of OFL and TNZ[23-25]. The design of experiment was used to analyse the effect of several factors on the responses. Response surface methodology serves the purpose of this study viz modelling and optimisation of responses.

In the present study central composite designs to adopt validate and optimised chromatographic condition of HPTLC method. Due to flexibility ease in better understands of primary effect of factors and their interaction, the CCD was selected for the study. This research intended to develop a rapid and simple, precise, accurate HPTLC method using DoE approach for the quantitative estimation of Oflaxacin and Tinidazole, validate as per the ICH guidelines[26-32].

2. MATERIAL AND METHODS

Materials

Pure analytical drugs of OFL and TNZ were kindly donated by the pharmaceutical industries and were used without further purification. Marketed tablet formulations of Abiflox TN (Anant Pharmaceuticals), A Flox TZ (Psyco Remedies) and Viflox- TZ (Avalanche harmaceuticals) label claim 200mg of OFL and 600mg of TNZ respectively were purchased from the local market. All chemicals and solvent used were of analytical grade purchased from Sigma-Aldrich, India.

Instrumentation

Densidometric separation was achieved Linomat V Sample applicator (Camag, Switzerland). Hamilton microliter syringe (Bonaduz, Switzerland). Precoated aluminium plate with silica gel 60 F_{254} (10cm×10cm with 250mm thickness; E. Merck, Darmstadt, Germany). TLC scanner III (Camag, Switzerland). winCATS software Ver 4.4.1 (Camag, Switzerland), Camag Twin trough chamber (Camag, Switzerland) using this study, and All chemical were weighed using electronic balance (Shimadzu Corp., Japan).

Preparation of Standard Solution

Accurately 50mg OFL and 150mg TNZ were weighed and transferred to a 50ml standard flask, dissolved in 25ml of methanol and volume was makeup with the same. Each drug concentration was found to be 1000µg/ml of OFL and 3000 µg/ml TNZ respectively.

Chromatographic Procedure

The samples of 5mm band width were spotted on silica gel precoated aluminium plates with microlitre syringe using sample applicator. The mobile phase consisted of acetonitrile: water (5: 5v/v) and 10 ml of mobile phase were used for the chromatography. Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The optimised chamber saturation time for mobile phase was 30mins at room temperature (28 °C ± 2). The chromatogram was run for a distance of 8 cm. After the development; TLC plates were dried with the help of a Hair dryer. Densitometric scanning was executed on Camag TLC scanner III in the reflectance-absorbance mode at 303nm (Figure 2) and operated by CATS software. The evaluation was via peak areas with linear regression.

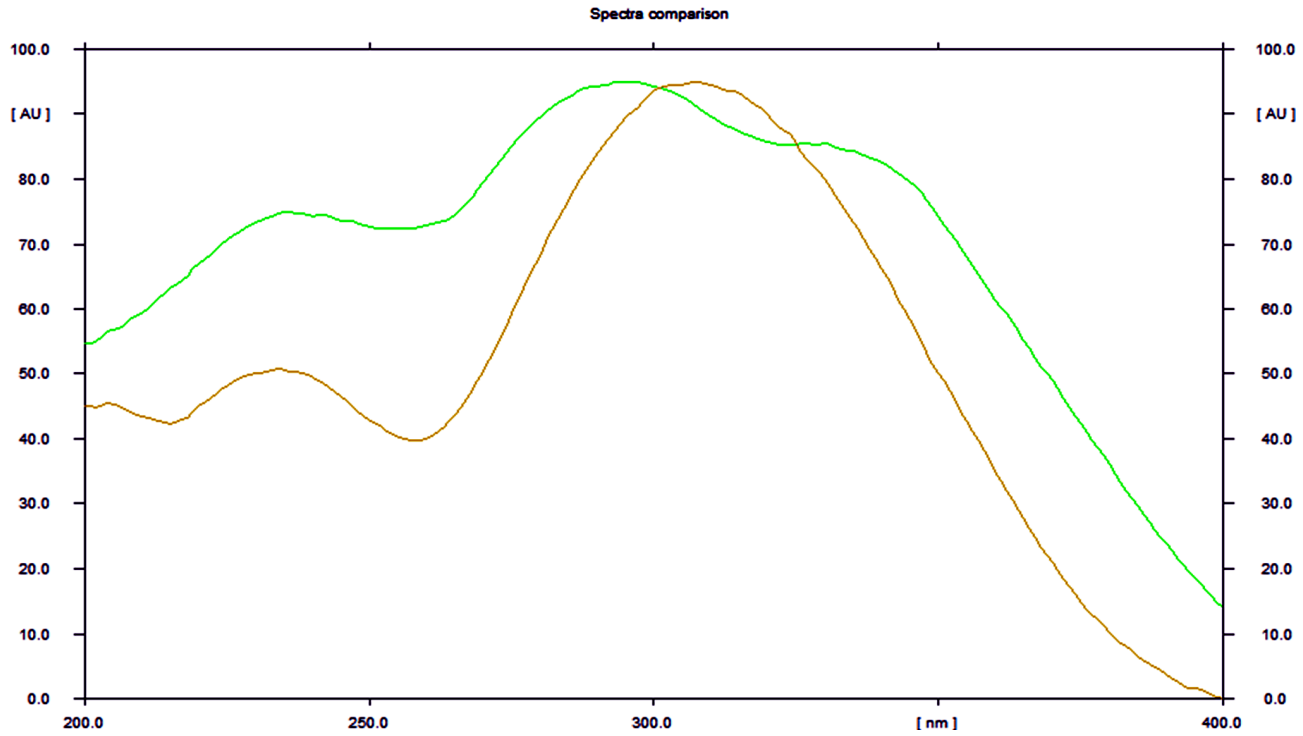


Figure 2: Overlain absorption spectra of Ofloxacine and Tinidazole at 303 nm

Software Aided Method Optimization

DoE is an effective tool which is used to optimize the compositional parameters and evaluate the principal effects and their interaction. CCD is subdivision of response surface methodology used to explore quadratic response surfaces without employing a three level factorial design.

The critical factors and their experimental levels investigated for optimization were estimated based on preliminary univariate studies of chromatographic method development.

A total of 20 experiments including five center points were conducted by selecting three factors; acetonitrile content in mobile phase (A), saturation time (B), developing distance(C) and the retardation factor (R_f) of OFL and TNZ were the response selected for both drugs represented in the Table 1. Acetonitrile concerned the total volume of the mobile phase. The suggested values for all three factors A, B and C were 5ml, 30mins and 8cm respectively. Accordingly, the Acetonitrile content (A) was maintained between 6.59 and 9.41. Similarly, the minimum and maximum values of the chamber saturation time (B) were fixed at 22.93 min and 37.07 min,

respectively. Likewise, the minimum and maximum values for the distance travelled (C) were fixed at 6.59 and 9.41, respectively. The coded value of α was 1.41. The data generated were analyzed using the trial version of the Design Expert (Version 10.0.0.1, Stat-Ease Inc, Minneapolis, MN, USA) statistical software. The significance of the relevant factors was calculated using Fisher's statistical test for the Analysis of Variance (ANOVA) model. All experiments were conducted in a randomized order to minimize the bias effects of uncontrolled variables. Centre points of the design were Replicated ($n = 5$) to estimate the experimental error.

Table 1
Central composite rotatable design arrangement and responses

Runs	*Type*	*Factors*			*Responses*	
		A: Acetonitrile content (ml)	*B: Chamber saturation time (min)*	*C: Distance travel (cm)*	*Rf of Ofloxacin*	*Rf of Tinidazole*
1	Factorial	4	35	9	0.37	0.59
2	Axial	6.68179	30	8	0.26	0.47
3	Factorial	4	25	9	0.36	0.58
4	Center	5	30	8	0.32	0.48
5	Axial	5	30	6.31821	0.3	0.5
6	Center	5	30	8	0.32	0.48
7	Factorial	6	25	9	0.27	0.48
8	Center	5	30	8	0.32	0.48
9	Factorial	6	35	7	0.28	0.49
10	Axial	3.31821	30	8	0.4	0.62
11	Factorial	6	25	7	0.27	0.48
12	Axial	5	30	9.68179	0.31	0.52
13	Factorial	4	35	7	0.36	0.58
14	Axial	5	38.409	8	0.33	0.55
15	Factorial	6	35	9	0.28	0.49
16	Center	5	30	8	0.32	0.48
17	Center	5	30	8	0.32	0.48
18	Center	5	30	8	0.32	0.48
19	Axial	5	21.591	8	0.3	0.52
20	Factorial	4	25	7	0.35	0.57

Validation of the Method

The linearity, limit of detection, limit of quantitation, accuracy, precision, specificity, and robustness of HPTLC method were validated by the ICH Q2 (R1) guideline.

Linearity

The standard solutions of both drugs were prepared to reach a concentration range of 100 to 600ng/spot of OFL and 300 t0 1800ng/ spot of TNZ respectively. One microlitre of each standard solution was spotted on the TLC plate. Linearity was repeated six times. The plate was developed on same mobile phase. The peak areas were plotted against the respective concentrations to obtain the calibration graphs.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

Estimation of LOD and LOQ was performed by spotting blank methanol six times by the same method as explained in chromatogram condition. The signal to noise ratio was determined. A LOD was considered as 3:1 and LOQ as 10:1. The LOD and LOQ were experimentally verified by diluting known concentrations of the standard solution of OFL and TNZ until the average responses were approximately 3 or 10 times the standard deviation of the responses for six replicate determinations.

Precision

The precision of method performed by repeatability and intermediate precision studies. Repeatability studies were performed by analyzing middle concentration of the linearity range of the drugs, six times on the same day. Intermediate precision of the method was performed by repeating studies on two different days.

Accuracy

The accuracy of the developed method was tested with three concentrations of drug corresponding to 80, 100 and 120% and determining the recovery of added drug. At each level of the amount, six determinations were performed.

Specificity

The specificity of the method was justified by analysing standard drug and sample. The spot for both drugs in the samples was confirmed by comparing the spectra and R_f of the spot regarding standard. The peak purity of OFL and TNZ was estimated by comparing the spectra position of the spot.

Robustness of the Method

The effect of small and deliberate variations in the process parameters, such as a change in the Acetonitrile content in the mobile phase by volume, saturation time, distance travelled and wavelength was studied. The effect of changes in both the R_f values and peak areas was examined by calculating the % RSD for each parameter

Analysis of Marketed Formulation

Estimation of the OFL and TNZ content of the tablets dosage form, ten tablets were accurately weighed, and their mean weight was determined. These tablets were then finely powdered in a glass mortar. A powder equivalent to 50 mg of OFL was accurately weighed and transferred into a 50ml volumetric flask with 5 ml methanol. The mixture was diluted to volume with methanol and sonicated for 15 min before being filtered through Whatman no. 42 filter paper wetted with methanol. The solutions were diluted to obtain a sample stock solution containing 1000µg/ml of OFL and 3000µg/ml of TNZ. 2microliter of the filtered solution (200ng/band of OFL and 600ng/band of TNZ) was applied to the HPTLC plate, followed by development and scanning. The analysis was repeated in triplicate.

3. RESULTS AND DISCUSSION

Selection of Wavelength

The sensitivity of the HPTLC method with ultraviolet detection depends on the use of an appropriate wavelength. The developed plate was subjected to densitometry measurements in the scanning mode in the UV–Vis region of 200–700 nm. The overlain spectrum was scanned on a CAMAG TLC Scanner 3, the both drugs appreciably absorbed light at 303nm, and this wavelength was selected as the detection wavelength.

Preliminary Study

An HPTLC method for OFL and TNZ alone or combined with another drug had previously been reported. Specifically, the selected mobile phase consisted of Acetonitrile: Water. Hence, various combinations of these components at different proportions, such as Acetonitrile: Water. (6:4, v/v) or Methanol: Water. (6:4, 7:3, 8:1, 9:1, 8:2v/v), were tested at a fixed chamber saturation time for 30 min and solvent migration distance of 80 mm. However, the drugs were not satisfactorily resolved with an acceptable R_f value. The chamber saturation time and solvent migration distance are crucial to HPTLC chromatographic separation. Here, the saturation time of the chamber was less than 25 min and the distance of the mobile phase front greater than 80 mm resulted in the diffusion of the analytic band. A solvent consisting of acetonitrile: water (5:5v/v) was found to be a satisfactory mobile phase that separated OFL and TNZ well. However, the R_f value of OFL was close to 0.8 and also affected by the chamber saturation time. Therefore, further chromatographic conditions were optimized to obtain well-defined, compact bands of OFL and TNZ with acceptable R_f values (<0.8) for both drugs using CCD

Optimization of Chromatographic Conditions using CCD

CCD was employed due to its flexibility and applied to optimize the HPTLC separation by gaining a better perception of the factor's main and interaction effects. A three-factorial, rotatable central composite statistical experimental design was employed using 15 experimental runs that included five centre points. The independent variables, such as the acetonitrile content in the mobile phase (A), chamber saturation time (B) and distance travelled (C), and the responses for all 15 optimised trial experimental runs were summarised in Table 1. During model selection, the best-fitted models for the Rf values of OFL and TNZ were a linear and quadratic model, respectively, based on the lowest PRESS value and the adjusted R^2 value closer to 1. The model was validated with an analysis of variance (ANOVA) using the Design Expert software and the results were presented in Table 2. Significant effects had a P value less than 0.05. An adequate precision greater than 4 is desirable, and the obtained ratio for both drugs indicated an adequate signal. The percentage coefficient of variation (% CV), a measure of reproducibility of the model, was less than 10%, and the adjusted R-square values were high, indicating a good relationship between the experimental data and those of the fitted models. Here, the adjusted R^2 values were well within the acceptable limit of $R^2 \geq 0.80$, which indicated that the experimental data fitted polynomial equations well. The final equation, regarding the actual components and factors, is shown in Table 2.

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

Response (Rf value)	*Type of Model*	*Polynomial equation model for Y*	*Adjusted R*	*Model P-value*	*% CV*	*Adequate precision*
Ofloxacin	Quadratic	$0.32 - 0.042A + 6.62B + 2.69C - 2.5AC + 3.754A^2 - 1.549B^2 - 5.085C^2$	0.9957	<0.0001	0.75	83.504
Tinidazole	Quadratic	$0.48 - 0.046A + 6.623B + 3.92C - 2.5AC + 0.023A^2 + 0.019B^2 + 0.010C^2$	0.9947	< 0.0001	1.12	63.810

A positive value represents the effect that favours optimization, whereas a negative value indicates an inverse relationship between the factor and the response. 3-d response surface plots and perturbation plots were constructed to evaluate the effect of the factors on the retention factor of each drug. In Figure 3, perturbation plots are presented for the predicted model to better understand the investigated procedure. This Figure 3 demonstrates how the response changes in response to perturbations in each factor from its defined reference value while all other factors are held constant at a reference point; the steepest slope or curvature indicates the sensitivity to a specific factor. Figure 3(a) shows that the distance travelled (factor C) had the most significant effect on the R_f value of OFL compared with other factors. Moreover, the Acetonitrile content (A) and chamber saturation time (B) had more significant effects on the R_f value of TNZ, followed by the distance travelled (factor C) (Figure 3(b)). Figure 4(a) represents a variation in the R_f value of OFL as a function of the chamber saturation time and

distance travelled while *n*-butanol concentration was constant. Specifically, the retention factor of OFL inversely correlated with the distance travelled. An analysis of the perturbation plots and response plots of the optimisation model revealed that the Acetonitrile content (A) and chamber saturation time (B) more significantly affected the responses than factor (C), i.e., the distance travelled.

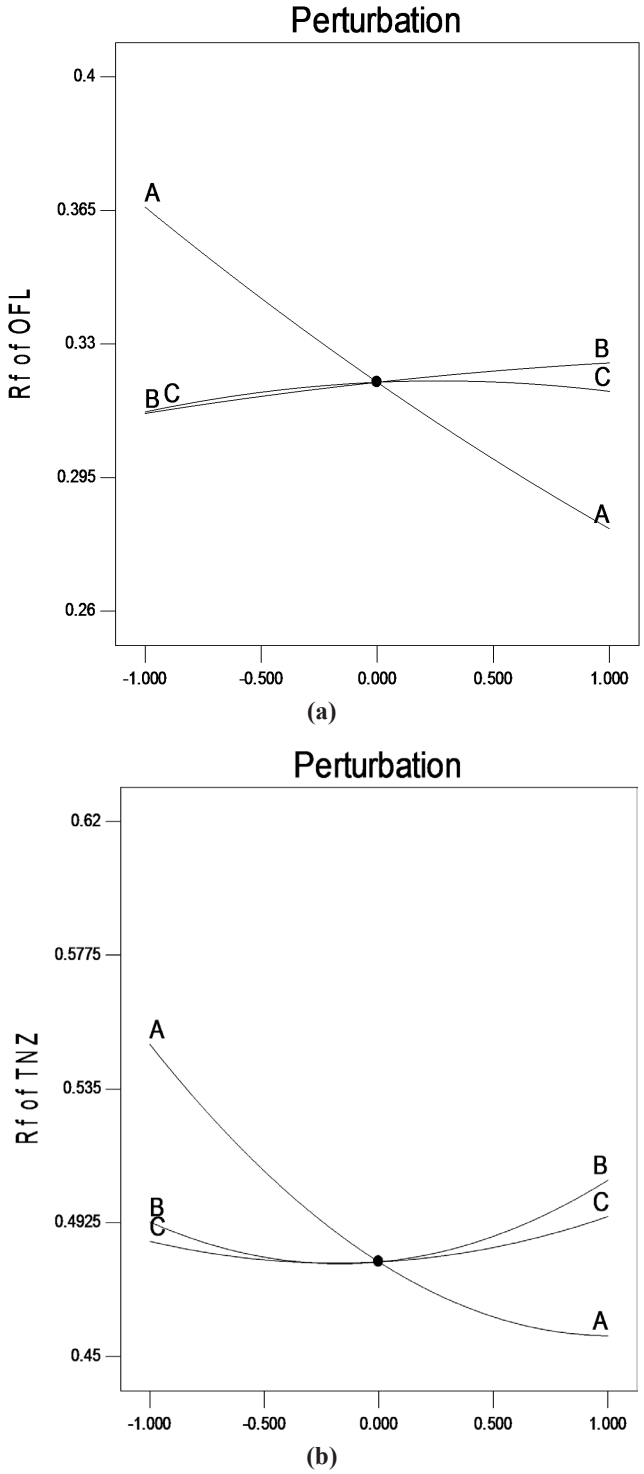
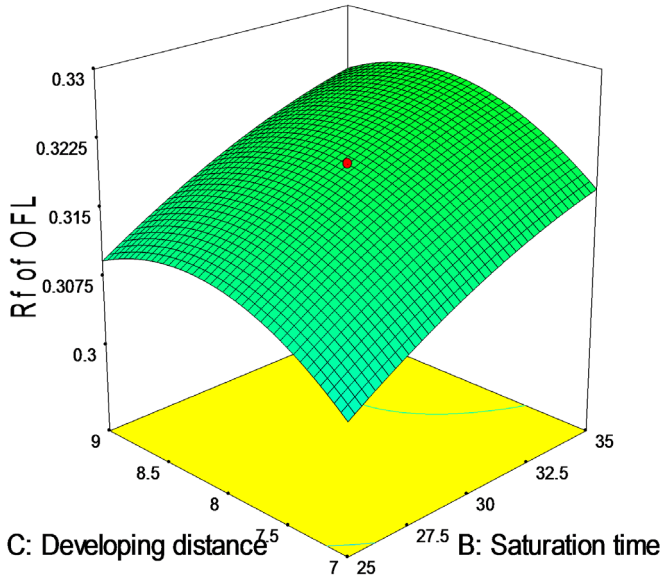
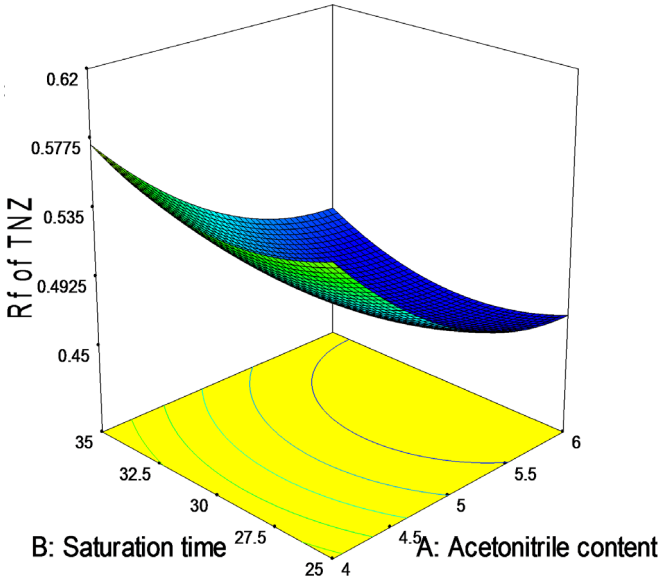


Figure 3: Perturbation graph showing the effect of each factor, A, B, and C, on the: (a) Rf value of Ofloxacine(OFL) and (b) the Rf value of Tinidazole (TNZ)

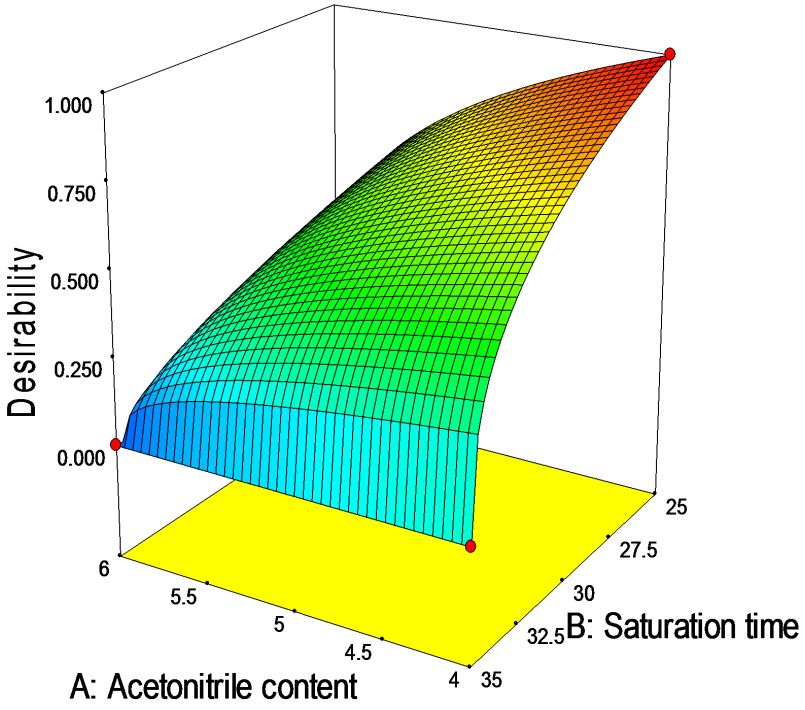
The optimum conditions of separation were estimated using Derringer's desirability function. During the numerical optimisation, the targets of individual factors and responses were fixed of the 15 different solutions of the optimisation provided by the software, two conditions that have a desirability near one were selected. The maximum Derringer's desirability function obtained for the response surface was presented in Figure 4(c). To investigate the predictability of the proposed model, the agreement between the experimental and predicted responses for both the predicted were shown in Table 3. The percentage of the prediction error was calculated using the following formula: predicted error = experimental-predicted/predicted × 100. Table 3 and the % predicted error identified a set of coordinates that produced a high desirability value (D = 1) at optimum condition 1. Thus, these coordinates were assessed to select an optimum experimental condition to analyse ZID and LAD in combination. The selected optimised composition for the final HPTLC analysis was Acetonitrile: Water (5: 5 v/v). Under the optimised conditions, the HPTLC densitogram showed a *Rf* of 0.24 for OFL (100 ng/band) and 0.54 for TNZ (300 ng/band) and was depicted in Figure 5.



(a)



(b)



(c)

Figure 4. Three-dimensional plots of the RSM for both responses (a) variation in the Rf of Ofloxacine as a function of B and C for a fixed value of A; (b) variation in the Rf of Tinidazole as a function of A and B for a fixed value of C; (c) graphical representation of the maximum of Derringer's desirability function.

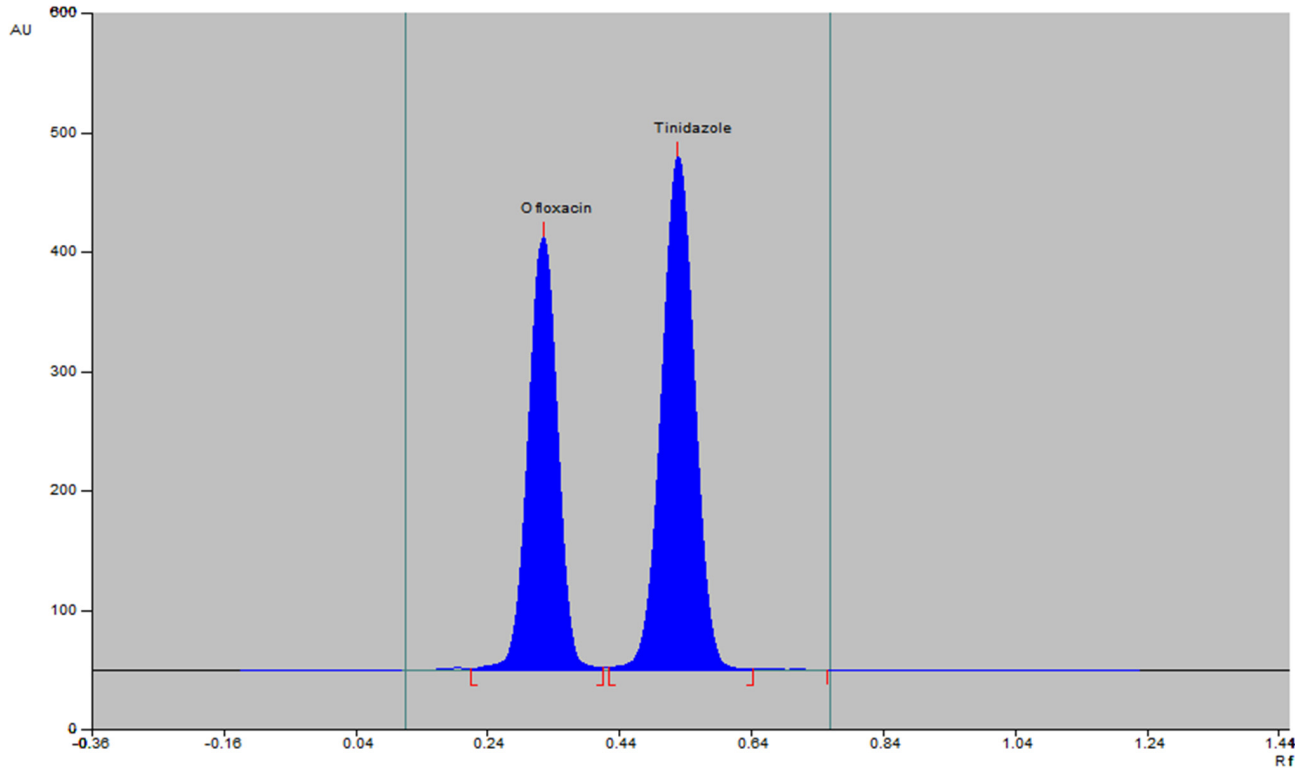


Figure 5: Densitogram of Ofloxacin and Tinidazole

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

Optimum conditions	*A: Acetonitrile content (ml)*	*B: Chamber saturation time (min)*	*C: Distance travel (cm)*	*Rf of Ofloxacin*	*Rf of Tinidazole*
1	4	25	9		
		Predicted		0.36	0.58
		Experimental		0.35	0.56
		Predicted Error %		1	2
2	6	35	7		
		Predicted		0.28	0.49
		Experimental		0.29	0.51
		Predicted Error %		1	2

3.3. Method Validation

3.3.1. Linearity

The linearity of an analytical method is its ability to provide results that are directly, or via a mathematical transformation, proportional to the concentration of the analyte within a given range. The OFL and TNZ showed a good correlation coefficient ($r2 = 0.9958$ for OFL and $r2 = 0.9989$ for TNZ) in the proposed concentration ranges of 100–600 ng/band for OFL and 300-1800 ng/band for TNZ (Table 4). The homoscedasticity of the variance was confirmed using Bartlett's test, and the responses of the peak area for both drugs exhibited homogenous variance, as indicated by a $\chi 2$ value less than the tabulated value (Table 4). Figure 6 shows a three-dimensional overlay of the HPTLC dendrograms for OFL and TNZ with calibration bands at 254nm.

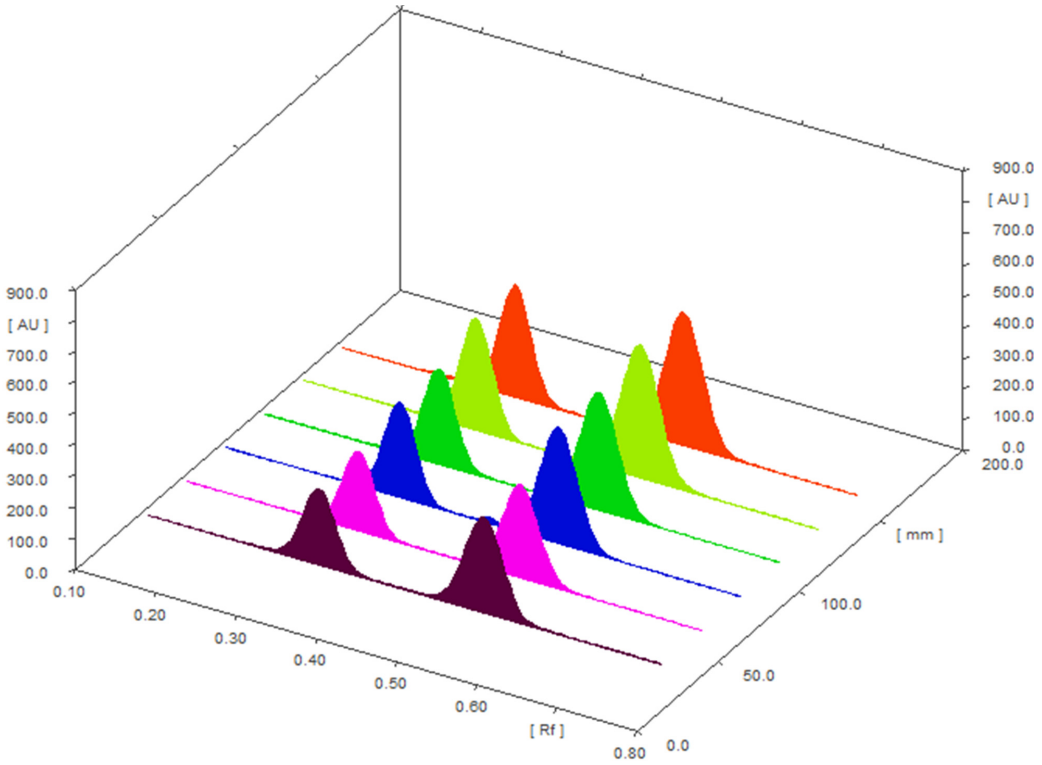


Figure 6: Linearity of Ofloxacin and Tinidazole

3.3.2. LOD and LOQ

The LOD and LOQ of the developed method were found to be 8.840 and 26.789 ng/band, respectively, for OFL and 63.416 and 195.260 ng/band, respectively, for TNZ, indicating the sensitivity of the proposed method (Table 4).

3.3.3. Precision

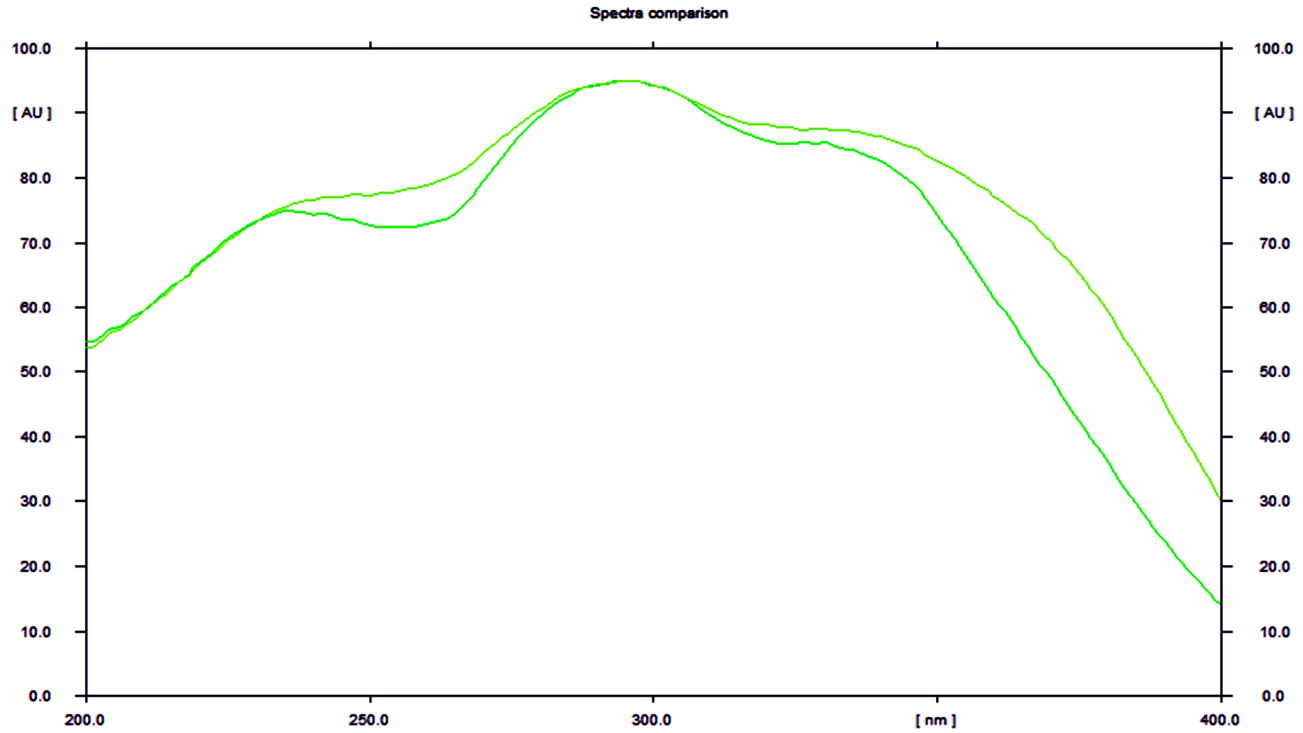
The experiment was repeated two times in one day (intra-day precision), and the average % RSD values of the results were calculated. Similarly, the study was conducted on two different days (inter-day precision), and the average % RSD values for the peak areas of OFL and TNZ were calculated. The Intra-day and inter-day precision is expressed regarding % RSD and was less than 2, confirming the precision of the method (Table 4).

3.3.4. Accuracy

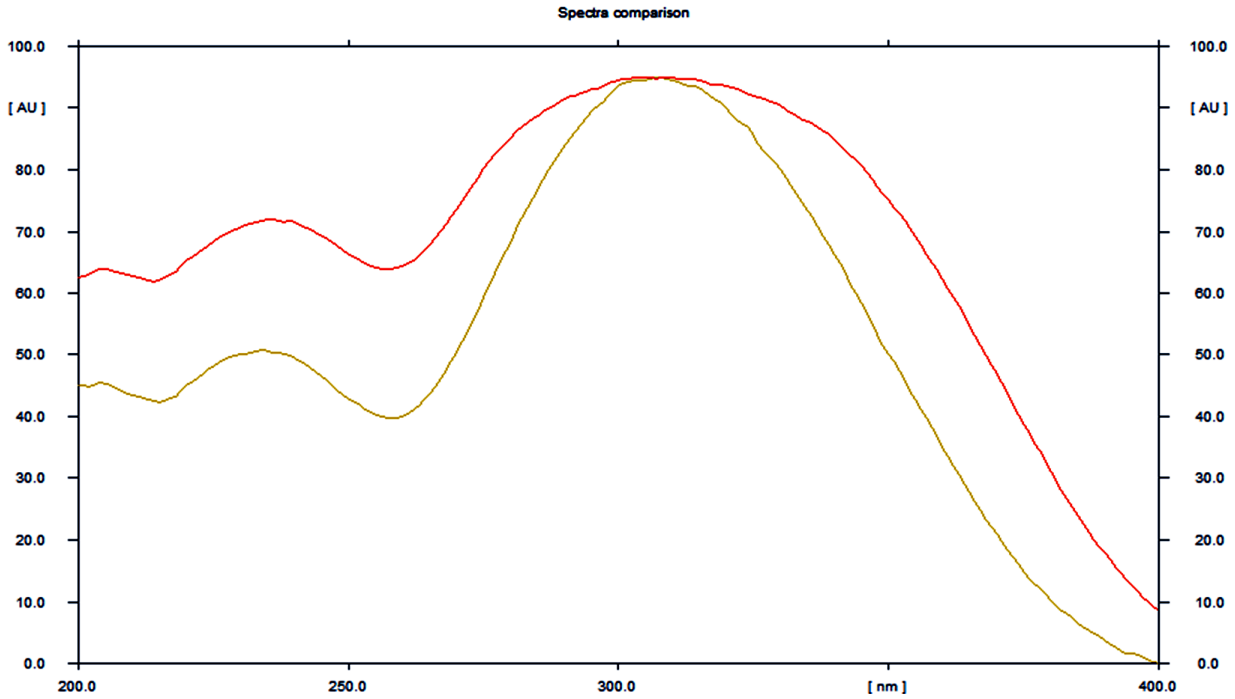
When used to evaluate the recovery after spiking with three concentrations of standard, 50%, 100% and 150%, the proposed method showed percentage recovery rates between 101.46–101.54 for OFL and 101.21–101.87 for TNZ, which were within the acceptable range of 100 ± 2%.

3.3.5. Specificity

The chromatogram of the pharmaceutical formulation obtained using the developed method showed only two peaks at R_f of 0.24 and 0.54 for OFL and TNZ, respectively, and was found to be at the same *Rf* for both standard drugs. The peak purity of both drugs in pharmaceutical dosage form was confirmed by comparing the overlaid spectra at the peak start, peak apex and peak end positions of the band. The results shown in Table 4 demonstrate that the purity exceeded 0.999 for all peaks, indicating the specificity of the method in the presence of various excipients (Figure 7).



(a)



(b)

Figure 7: Overlain peak purity spectra of (a) Ofloxacine (OFL) and Tinidazole (TNZ) with the corresponding standard

Table 4
Analytical validation parameters for CLO and PH using the HPTLC method

Parameters	*Ofloxacin*	*Tinidazole*
Linearity		
Linearity range (ng/spot)	100 - 600	300 - 1800
Correlation coefficient (r^2)	0.9997	0.9994
Slope	3.8927	5.2022
Intercepts	1.7916	23.0443
Sensitivity		
LOD (ng/spot)	1.2352	1.6529
LOQ (ng/spot)	3.7432	5.0089
Precision(%RSD)		
Intra- day Precision	0.07201	0.2668
Inter- day Precision	0.1226	0.1029
Accuracy		
50%	99.55	100.11
100%	99.90	99.97
150%	99.93	100.00

3.3.6. Robustness

A deliberate change in various parameters, such as the Acetonitrile content in the mobile phase, chamber saturation time, distance travelled and wavelength, produced %Relative standard deviations of the peak area of less than 2%, indicating the robustness of the method (Table 5).

Table 5
Robustness study of developed HPTLC method

Change in the mobile phase ratio (acetonitrile: water 5:5 v/v ± 0.5 in acetonitrile content)				
Drugs	*Ratio*	*Rf*	*Area ± SD (ng/band)*	*% RSD*
Ofloxacin	4.5:5.5	0.33 ± 0.02	1177.8 ± 0.7810	0.0663
	5.5:4.5	0.34 ± 0.02	1178.7± 0.7810	0.2979
Tinidazole	4.5:5.5	0.55 ± 0.02	4662.9 ± 0.2516	0.0055
	5.5:4.5	0.54 ± 0.02	4564.0 ± 0.5686	0.0124
Change in the chamber saturation time (30 min ± 5)				
Drugs	*Saturation time (min)*	*Rf*	*Area ± SD (ng/band)*	*% RSD*
Ofloxacin	25	0.33 ± 0.02	1176.1 ± 0.3785	0.0321
	35	0.34 ± 0.02	1178.5 ± 0.4041	0.0088
Tinidazole	25	0.55 ± 0.02	4562.2 ± 0.5033	0.0427
	35	0.54 ± 0.02	4564.0 ± 0.4163	0.0091
Change in distance travelled (8 cm ± 1)				
Drugs	*Distance travelled (cm)*	*Rf*	*Area ± SD (ng/band)*	*% RSD*
Ofloxacin	7	0.33 ± 0.02	1177.5 ± 0.2516	0.0213
	9	0.34 ± 0.02	1177 ± 0.5291	0.0449
Tinidazole	7	0.54 ± 0.02	4562.5 ± 0.1527	0.0033
	9	0.54 ± 0.02	4565.03 ± 0.2081	0.0045
Rf				
Drugs	*Wavelength (nm)*	*Rf*	*Area ± SD (ng/band)*	*% RSD*
Ofloxacin	301	0.33 ± 0.02	1178 ± 0.5291	0.04492
	305	0.33 ± 0.02	1178± 0.3511	0.01284
Tinidazole	301	0.55 ± 0.02	4563.1 ± 0.5859	0.02981
	305	0.54 ± 0.02	4563.7 ± 0.6245	0.01368

3.4. Analysis of Marketed Dosage Form

The analysis of the tablet formulation containing 200 mg OFL and 600 mg TNZ showed good recovery. Specifically, the percentages were 99.926% for OFL and 100.980% for TNZ, indicating that the method can be used for regular quality assessment when testing the tablet dosage formulation. The %RSD value was found to be less than 2.

4. CONCLUSIONS

The CCD design and response surface methodology help to obtain essential information on the sensitivity of the R_f values of OFL and TNZ to various chromatographic variables. The acetonitrile content, chamber saturation time and distance travelled were simultaneously optimised by applying a useful experimental design tool: response surface design and Derringer's desirability function. The obtained results indicated that the use of a CCD design is a variable procedure that can reduce the number of experiments for the development and optimisation of an HPTLC method. Furthermore, the method is economical and used to generate a maximum amount of information in less time with a small number of experiments. Methodological validation indicates that the investigated HPTLC method is simple, accurate\reliable and suitable for the rapid quantitative analysis of OFL and TNZ in routine tests. The proposed HPTLC method can be successfully utilised to simultaneously

estimate the amounts of OFL and TNZ in pharmaceutical dosage form without interference and the need to first separate individual drugs.

REFERENCES

[1] M Dasharath Patel, A Jignesh. Soneji, B Parth Patel, Chhagan N. Patel. "Development and validation of a method for simultaneous estimation of ofloxacin and ornidazole in different dissolution media", Pharm Methods. 2010, 3(2), pp.102–105.

[2] Jawed Akhtar, Birendra Shrivastava, Parth bhatt, Ankur Patel and Vikas Thakur. "Simultaneous Estimation of Ofloxacin and Ornidazole in Synthetic Mixture by Q Analysis UV-Spectrophotometric Method". Asian Journal of Pharmacy & Life Science, 2011, 1 (1), pp.71-75

[3] VR Alapati, G Divya Teja, G Sunitha, CH Rajesh, L Vamsikrishna and D Panikumar Anumolu "Validated Stability Indicating Liquid Chromatographic Method for Quantification of Ciprofloxacin HCL, Its Related Substance and Tinidazole In Tablet Dosage Form". International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(5), pp 611-615.

[4] B Anbarasi, R Seetharaman, V Suresh, Senthilkumar and N Parimaladevi. "Analytical method development and validation of Simultaneous RP-HPLC Estimation of Ornidazole and Ofloxacin in Tablet Dosage Forms" Journal of Pharmacy Research 2014 4(1), pp 272-273.

[5] Anju Goyal, Sweety Choudhary and Gaurav Deep Singh. "A Validated RP-HPLC Method for Estimation of Ciprofloxacin and Tinidazole in Tablet Dosage Form" International journal of Pharmaceutical Chemistry and Analysis, 2015 2(1) pp 22-27.

[6] B.Dhandapani, N.Thirumoorthy, Shaik Harun Rasheed, M.Rama kotaiah and N.Anjaneyalu. "Method development and Validation for the Simultaneous estimation of Ofloxacin and ornidazole in Tablet dosage form by RP-HPLC". International Journal of Pharma Sciences and Research 2010, 1(1), pp 78-83.

[7] D Boopathy, Prakash, Bibin Mathew, S Suresh Kumar, and P Perumal "Method development and validation of simultaneous determination of Ofloxacin and Satrinadazole in Pharmaceutical dosage form by RP-HPLC" .Scholars Research library. Der pharmacia letter 2010. 2(2): pp 22-26.

[8] CN Nalini, S Ramachandran, K Kavitha, Harikrishna. (2011). Simultaneous Determination of Ofloxacin and Ornidazole in Tablets by Spectrophotometry and Reverse Phase HPLC. 2(3); 693-708.

[9] J Dharuman. M Vasudevan, K.N. Somasekaran, B Dhandapani. D Prashant Ghode and M. Thiagarajan "RP-HPLC method development and validation for the simultaneous estimation of Ofloxacin and Tinidazole in tablets". International Journal of Pharm Tech Research. 2009; 1(2) pp.121-124.

[10] UP Halkar and PB Ankalkope "Reverse phase high performance liquid chromatographic determination of ofloxacin and tinidazole in tablets". Indian Drugs; 2000; 37:58 pp 5–8.

[11] C Immanuel and AK Kumar "Simple and rapid High Performance Liquid Chromatography method for the determination of Ofloxacin concentrations in plasma and urine". Journal of Chromatography and Biomed Science Application. 2001; 760: pp 91–5.

[12] J.Dharuman, M.Vasudevan, K.N.Somasekaran, B.Dhandapani, Prashant D.Ghode and M.Thiagarajan "RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ofloxacin and Tinidazole in Tablets". International Journal of PharmTech Research. 2009; 1(2), pp 121-124.

[13] RR Kalta, R Sharma, SC Chaturvedi "Simultaneous RP-HPLC determination of nitazoxanide and ofloxacin in combined dosage form". Indian Journal of Pharmaceutical Sciences.2008; 70:pp 491–494.

[14] NS Kamble and A Venkatachalam."High performance liquid chromatographic determination of ornidazole and ofloxacin in solid dosage form" Indian Drugs; 2009; 42: pp 723–725.

[15] Khaja Pasha, Asgar Ali, Shahana Bana and Syeda Humair.). "Reverse Phase – HPLC method for the analysis of Tinidazole in pharmaceutical Dosage form & Bulk drug." International Journal of Pharmacy and Pharmaceutical Sciences; 2010 2(2); pp 46-47.

[16] A. Madhuri , Hinge, A. Nikita, J. Desai, Rajvi, Mahida and P. Hitesh Dalvadi. "Spectrophotometric and High Performance Liquid Chromatographic Determination of Ofloxacin and Azithromycin in Pharmaceutical Tablets" Pharmaceutical Methods 2015; 6(1), pp 26-34.

[17] A Nooman Khalaf, K. Ashok. Shakya, Maher Shurabji and Diksha Goindi. "Development and validation of an RP-HPLC method for simultaneous analysis of Ofloxacin and Ornidazole in tablets". Jordan journal of Pharmaceutical Sciences. 2010; 3(2); pp87-98.

[18] M Rama Kotaiah, Shaik Harun Rasheed, Y Narasimha Rao, Y Venkateswarlu and Konda Ravi Kumar "Simultaneous estimation of ofloxacin and tinidazole in tablet dosage form by RP-HPLC" Research Journal of Pharmaceutical, Biological and Chemical Sciences; 2010; 1(4), pp 460-466.

[19] P. V. Rege and Ramesh mapari. "Simultaneous Quantification of Ofloxacin and Ornidazole from Combined pharmaceutical drug formulation by HPLC". International Journal of Pharma and Bio Sciences. 2011; 2(4); pp 51-58.

[20] V.V Pande, J.G. Chandorkar "A sensitive method HPLC method on determination of 2-methyl 5 nitro imidazole and reaction mass of intermediates of Ornidazole in Ornidazole bulk manufacturing". International Journal of Pharm Tech Research.2011 1(2): pp 310-312.

[21] M Rama Kotaiah, Shaik Harun Rasheed, Y Narasimha Rao, Y Venkateswarlu and Konda Ravi Kumar, "Simultaneous estimation of ofloxacin and tinidazole in tablet dosage form by RP-HPLC" Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010; 1(4), pp 460-466.

[22] Ranjit Singh, K Mukesh Maithani, Shailendra. Saraf, Shubhini Saraf and Ram C. Gupta. "Simultaneous Estimation of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole and Ornidazole by Reverse Phase – High Performance Liquid Chromatography". Eurasian Journal. Analytical. Chemistry. 2009 4(2): pp 161-167.

[23] V Rajan. Rele. and P Prathamesh, Tiwatane. "Simultaneous determination of ofloxacin and ornidazole in pharmaceutical dosage by reverse phase high performance liquid chromatography". Der Pharmacia Letter; 2015; 7 (6): pp 66-71

[24] R Shinde Sachin R, Bhoir Suvarna, S Pawar Namdev, B Yadav Suman and.M. Bhagwat Ashok "Simultaneous estimation of Satranidazole and Ofloxacin in tablet dosage form by HPLC". European Journal of chemistry. 2010;7(1); pp198-202.

[25] R.Sivakumar, P Kumar Nallasivan, S Saravana Kumar, CS Kandasamy, R. Venkatanarayan "Simultaneous RP-HPLC estimation of Nitazoxamide and Ofloxacin in tablet dosage form". Asian Journal of Research Chemistry. 2(1) pp 43-45.

[26] I. Suvarna I. V Bhoir, Poonam. S Gaikwad, Lavu. N Parab, Rohan. S Shringarpure, Sudha. Savant, and J. Pratibha Verma. "RP-HPLC Method Development and Validation for the Simultaneous Estimation of Satranidazole and Ofloxacin in Pharmaceutical Dosage Form" Journal of Chromatographic Science; 2011 49; pp 84-87.

[27] V. Bojaraju, D. Sireesha, V.V.L.N. Prasad and Prakash. V. Diwan. "Reverse Phase High Performance Liquid Chromatography Method for the Simultaneous Estimation of Amoxicillin Trihydrate and Tinidazole in the Tablet Dosage Form" World Journal of Chemistry 2012 7 (2): pp 47-52.

[28] P Vipul Rane, B Devanand, Shinde, "Simultaneous high performance liquid chromatographic determination of Nitazoxanide and Ofloxacin in tablet formulation". Eurasian Journal of analytical chemistry, 2009, 4(3), pp304-310.

[29] SB Wankhede, SS Prakash a Chitlange, "Simultaneous RP-HPLC estimation of Ofloxacin and Satranidazole in tablet dosage form". International Journal of Pharm Tech Research, 2009, 1(4), pp.1136-1138.

[30] XB Yin, J Kang, L Fang, X Yang and E Wang. "Short-capillary electrophoresis with electrochemiluminescence detection using porous etched joint for fast analysis of lidocaine and Ofloxacin". Journal of Chromatography A, 2004, 1055 pp.223–8.

[31] CN Nalini, S Ramachandran, K Kavitha, Harikrishna, "Simultaneous Determination of Ofloxacin and Ornidazole in Tablets by Spectrophotometry and Reverse Phase HPLC". 2(3) pp.693-708.