www.sphinxsai.com

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.7, pp 3918-3926, Sept-Oct 2014

Development, optimization and validation of new LC Assay for the Quantification of cefixime, levofloxacin and moxifloxacin in bulk and Pharmaceutical Dosage Forms by applying Response surface methodology

Venkatesan.S*[1], Kannappan.N[2].

[1,2] Department of Pharmacy, Annamalai University, Tamilnadu.-608002, India.

***Corres.author: venkatmankala@gmail.com**
Mobile: 9789246126, Office: 04144-238282, Fax: 04144 238 080.

Abstract: Developed and optimized a validated isocratic reverse phase HPLC separation of Cefixime, Levofloxacin, and Moxifloxacin in pharmaceutical preparation using Response surface methodology. The separation was applied by using Phenomenex C18 column (15 cm × 4.6 mm id, 5 µm particle size) and UV detection at 283 nm. The ranges of the individual variables used for the optimization were acetonitrile: 30–35%, flow rate: 0.8-1.2 and buffer concentration 25-35mM. The influence of these individual variables on the output responses: capacity factor of the first peak(k_1), resolutions of the 2nd and 3rd peak ($Rs_{2,3}$) and Retention time of the third peak (tR_3) were evaluated. Using this strategy, a mathematical model was defined and a response surface was derived for the separation. The three responses were simultaneously optimized by using Derringer's desirability functions. The optimum conditions predicted for quality control samples were acetonitrile–potassium dihydrogen phosphate buffer (35 mM, pH 7.0)–triethylamine 32.43:67.52:0.05 (v/v) as mobile phase and 1.2 mL min^{-1} as flow rate. Total chromatographic analysis time per sample was approximately 4.7 min. The optimized assay condition was validated as per the ICH guidelines and applied for the quantitative analysis of commercial formulations. The developed method was simple, accurate and precise. Hence, it can be employed for the routine analysis in quality control laboratories.
Key words: Central composite design, Derringer desirability, Centre composite design and ICH.

Introduction

High performance liquid chromatography method development and optimization is a well-known procedure exceptionally for the simultaneous determination of pharmaceutical dosage forms. Since HPLC utilizes a wide selection of several chromatographic factors, viz., the sort and composition of the organic phase, column temperature, flow rate, buffer molarity, pH, type of the stationary phase, etc., optimization of the experimental conditions may be a sophisticated method. To achieve this objective, any one of the chemometric methods which incorporates the overlapping resolution maps, factorial design and response surface methodology can be applied.

In general, the chemometrics may be wont to accomplish a variety of goals in chromatography laboratory.The best experimental design approach for the purpose of modeling and optimization is the response surface design. However, for the HPLC method intended to be applied for the pharmaceutical or industrial environment, the analysis time is typically optimized while not losing resolution.

When one needs to optimize more than one response at a time the use of multicriteria decision making (MCDM), a chemometric technique is that the most suitable option. The various approaches of MCDM

embrace the path of steepest ascent, constrained optimization procedure, Among these the Derringer's method offers the user flexibility in the definition of desirability functions.

However, an intensive literature search exposed to the best of our knowledge that only few works describing the methods for the simultaneous determination of these drug combinations (CEF-MOX and CEF-LEV) in pharmaceutical formulations and plasma matrices by UV[1-2] HPLC[3-7] .Owing to the presence of interferences or time-consuming analysis, these analytical methods cannot be applied for the simultaneous estimation of samples containing mixtures of CEF, LEV and MOX. There seems to be no reports available concerning methods for the simultaneous determination of all these analytes (CEF, LEV and MOX) using HPLC in the commercial pharmaceutical mixtures.

To the best of our knowledge, currently there is no HPLC method employing optimization technique has been reported for the simultaneous estimation of CEF, MOX and LEV as well a single mobile phase is sufficient for quantification of these analytes either in combination (i.e., CEF-LEV, CEF-MOX) or in single dosage form as per availability of formulation. Many pharmaceutical industries manufacture their formulation of all mentioned drugs either in combination CEF-LEV, CEF-MOX or in single dosage form. Most of the pharmaceutical industries use time consuming LC method and different mobile phases for different dosage form of drugs. But with the proposed method developed, time and cost required for changing different mobile phases could be saved, because only one mobile phase can be used for all the drugs and their combinations. Therefore the simultaneous determination of these analytes becomes motivating and significant.

Hence, there is a need for the development and optimization of a new simultaneous HPLC method for CEF, LEV and MOX. Since HPLC utilizes a wide selection of chromatographic factors, viz., the type and concentration of organic modifier, pH, buffer molarity, temperature, flow rate, etc., optimization of the experimental conditions is a complicated process. Therefore, systematic approaches such as a CCD and Derringer's desirability function are more essential, in order to optimize chromatographic factors and to select optimal conditions for the analysis of formulation.

Materials and methods

Chemicals and reagents

Working standards of Cefixime, Levofloxacin and Moxifloxacin were donated by Dr.Reddy's Lab., Hyderabad, India. Acetonitrile (MeCN) was of HPLC grade, potassium dihydrogen phosphate, phosphoric acid and triethylamine were of analytical reagent grade supplied by SD Fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, and Bangalore, India. The pharmaceuticals Cefi-L (CEF 400 mg and LEV 500mg), Aelxim-M (CEF 400mg and MOX 400 mg) were purchased from the local market.

Standard solutions

Stock solutions of CEF, LEV and MOX were prepared in mobile phase. Working standard solutions were freshly obtained by diluting the stock standard solutions with mobile phase during the analysis day. The standard solution prepared for the optimization procedure constituted CEF, LEV and MOX at 40.0, 50.0 and 40.0 µg/ml, respectively.

Sample preparation

Twenty tablets were weighed and finely powdered. An amount of pharmaceutical products powder equivalent to 20mg of CEF, 25 mg of LEV with 20 mg of MOX were accurately weighed and transferred into a 50 ml volumetric flask; This mixture was subjected to sonication for 10 min for complete extraction of drugs. Pipette out 5ml of the above solution into 50ml volumetric flask .the solution was made up to the mark with a mobile phase to obtain a concentration as 40.0, 50.0 and 40.00 µg/ml of CEF,LEV,MOX respectively.

Chromatographic conditions

Chromatographic separations were carried out on a Phenomenex C18 analytical column (150 mm × 4.6 mm i.d., 5 µm) connected with a Phenomenex C18 guard cadridge (4 mm × 3 mm i.d., 5 µm). The mobile phase consisted of MeCN–potassium dihydrogen phosphate buffer (pH 3.2), adjusted with Phosphoric acid. Wavelength of 283 nm was selected for detection .An injection volume of the sample was 20 µl. The HPLC system was used in an air conditioned laboratory atmosphere.

Instrumentation

Chromatographic analysis was performed on a Shimadzu HPLC which contains SPD M20A PDA detector, a rheodyne injector valve with a 20 µl loop volume and Shimadzu chromatographic software LC Solution assisted for data collections and processing. The mobile phase was degassed using Branson sonicator (Branson Ultrasonic's Corporation, USA). The weighing was done on a Sartorius balance and all pH measurements were done on pH meter.

Design of experiments

Preliminary experiments indicated that the variables, such as MeCNconcentration, buffer concentration and flow rate were the main factors that affected the capacity factor of the first peak k_1, resolutions of the 2nd and 3rd peak and Retention time of the third peak tR_3. Thus, a central composite rotatable design–response surface methodology (CCRD–RSM) was used to methodically examine the influence of these three critical variables on the responses above said. The details of the design are listed in Table 1. For each factor, the experimental range was selected on the basis of the results of preliminary experiments. The value range of the variables was MeCN concentration (A) of 30% to 35% V/V, buffer concentration(B) of 25 to 35 mM and flow rate (C) of 0.80 to 1.20 mL/min A total of 20 tests were conducted. All the formulations in these experiments were prepared in duplicate.

Method validation parameters

Validation studies were conducted using the optimized assay conditions based on the principles of validation described in the ICH guidelines "Text on Validation of Analytical Procedures" and "Q2B, Validation of Analytical Procedures: Methodology". Key analytical parameters, including, specificity, accuracy, precision, linearity, detection limit and quantitation limit were evaluated. For specificity study**,** placebo containing starch, lactose monohydrate, hypromellose, Polyethylene glycol, Microcrystalline Cellulose, titanium dioxide and magnesium stearate was used.. Linearity was established in the range of 20-60, 25-75 and 20-60 for CEF, LEV and MOX respectively. Also, robustness of the proposed method was assessed with respect to small alterations in the MeCN concentration (35.00 ± 0.5%), the Flow rate (1.3 ± 0.2) and the buffer concentration (30 ± 2.0 mm).

Results and discussion

Optimization of formula

The central composite rotatable design–response surface methodology (CCRD–RSM) constitutes an alternative approach because it offers the possibility of investigating a high number of variables at different levels with only a limited number of experiments. The variables in Table 1 were chosen taking into account our preliminary experiments. Table 4 showed the experimental results concerning the tested variables on the capacity factor of the first peak k_1, resolutions of the 2nd and 3rd peak and Retention time of the third peak tR_3. The three dependent values ranged from 0.78 to 1.85, 3.11 to 6.33, and 3.53 to 10.99.A mathematical relationship between factors and parameters was generated by response surface regression analysis using Design- Expert® 7.0 software.

Table 1: Experimental responses and central composite rotatable design arrangements[a]

Design Points	Factor levels			Responses		
	MeCN (%v/v)	Flow rate (ml/min)	Buffer conc.	K_1	$Rs_{2,3}$	tR_3
1	30.00	0.80	25.00	1.44	6.18	10.32
2	35.00	0.80	25.00	1.07	4.01	5.61
3	30.00	1.20	25.00	1.44	6.06	6.21
4	35.00	1.20	25.00	1.05	3.99	4.01
5	30.00	0.80	35.00	1.42	5.45	9.78
6	35.00	0.80	35.00	1.06	3.65	5.09
7	30.00	1.20	35.00	1.43	5.39	5.96
8	35.00	1.20	35.00	1.06	3.11	3.53
9	28.30	1.00	30.00	1.95	6.33	10.99

10	36.70	1.00	30.00	0.89	3.55	3.81
11	32.50	0.66	30.00	1.21	4.16	10.89
12	32.50	1.34	30.00	1.19	4.02	4.14
13	32.50	1.00	21.59	1.18	4.12	6.92
14	32.50	1.00	38.41	1.19	4.12	5.25
15	32.50	1.00	30.00	1.22	4.12	5.91
16	32.50	1.00	30.00	1.21	4.11	5.99
17	32.50	1.00	30.00	1.22	4.14	5.97
18	32.50	1.00	30.00	1.19	4.12	5.94
19	32.50	1.00	30.00	1.22	4.09	5.91
20	32.50	1.00	30.00	1.18	4.14	5.96

Randomized[a]

It is momentus to scrutinize the curvature term utilizing CCD with centre points before starting the optimization procedure. ANNOVA generated for CCD exhibited the curvature is significant for all three responses. Since p value less than 0.05, quadratic model should be used. The mathematical equation of quadratic model[8] of the three independent factors is given in equation1.

$$\mathbf{Y = \beta_0 - \beta_1 X_1 + \beta_2 X_2 - \beta_3 X_3 - \beta_4 X_1 X_2 - \beta_5 X_1 X_3 + \beta_6 X_2 X_3 - \beta_7 X_1^2 + \beta_8 X_2^2 + \beta_9 X_3^2 \ldots} \text{ Eq. (1)}$$

The statistical analysis of the results generated the following polynomial equations:

Capacity factor of the first peak $k_1 = +1.19-0.24 \times A + 0.072 \times A2$

Resolutions of the 2nd and 3rd peak $Rs_{2,3} = + 4.21 – 0.95 \times A – 0.19 \times C + 0.35 \times A^2$

Retention time of the third peak $tR_3 = + 5.88 – 1.91 \times A - 1.64 \times B - 0.34 \times C + 0.60 \times A \times B + 0.37 \times A^2 +0.41 \times B^2$

Where A, B and C represent the coded values of the MeCN concentration, buffer concentration and flow rate respectively.

Statistical parameters obtained from ANOVA for the reduced models are given in Table 2. The insignificant terms (P >0.05) were eliminated from the model through backward elimination process to obtain a simple and realistic model. The adjusted R^2 values were well within the acceptable limits of $R^2 \geq 0.80$ revealing that the experimental data fits the second-order polynomial equation. P value of <0.05 is obtained for all the reduced models, implying the significance. The adequate precision was found to be in the range of 23.702-34.908 indicating an adequate signal and the model is significant for the separation process. The coefficient of variation (C.V) a measure of reproducibility of the model was found to less than 10% and could be considered reasonably reproducible.

Table 2: Response models and statistical parameters obtained from ANNOVA for CCD

Responses	Regression model	Adjusted R^2	Model Pvalue	% C.V	Adequate precision
K_1	$+1.19-0.24 \times A + 0.072 \times A^2$	0.9266	<0.0001	4.81	34.908
$Rs_{2,3}$	$+ 4.21 – 0.95 \times A – 0.19 \times C + 0.35 \times A^2$	0.9142	<0.0001	6.01	28.317
tR_3	$+ 5.88 – 1.91 \times A - 1.64 \times B - 0.34 \times C + 0.60 \times A \times B + 0.37 \times A^2 +0.41 \times B^2$	0.9410	<0.0001	8.66	23.702

The perturbation plots and three-dimensional (3D) response surface graphs for the most statistical significant variables on the evaluated parameters are shown in Figure 1 & 2.

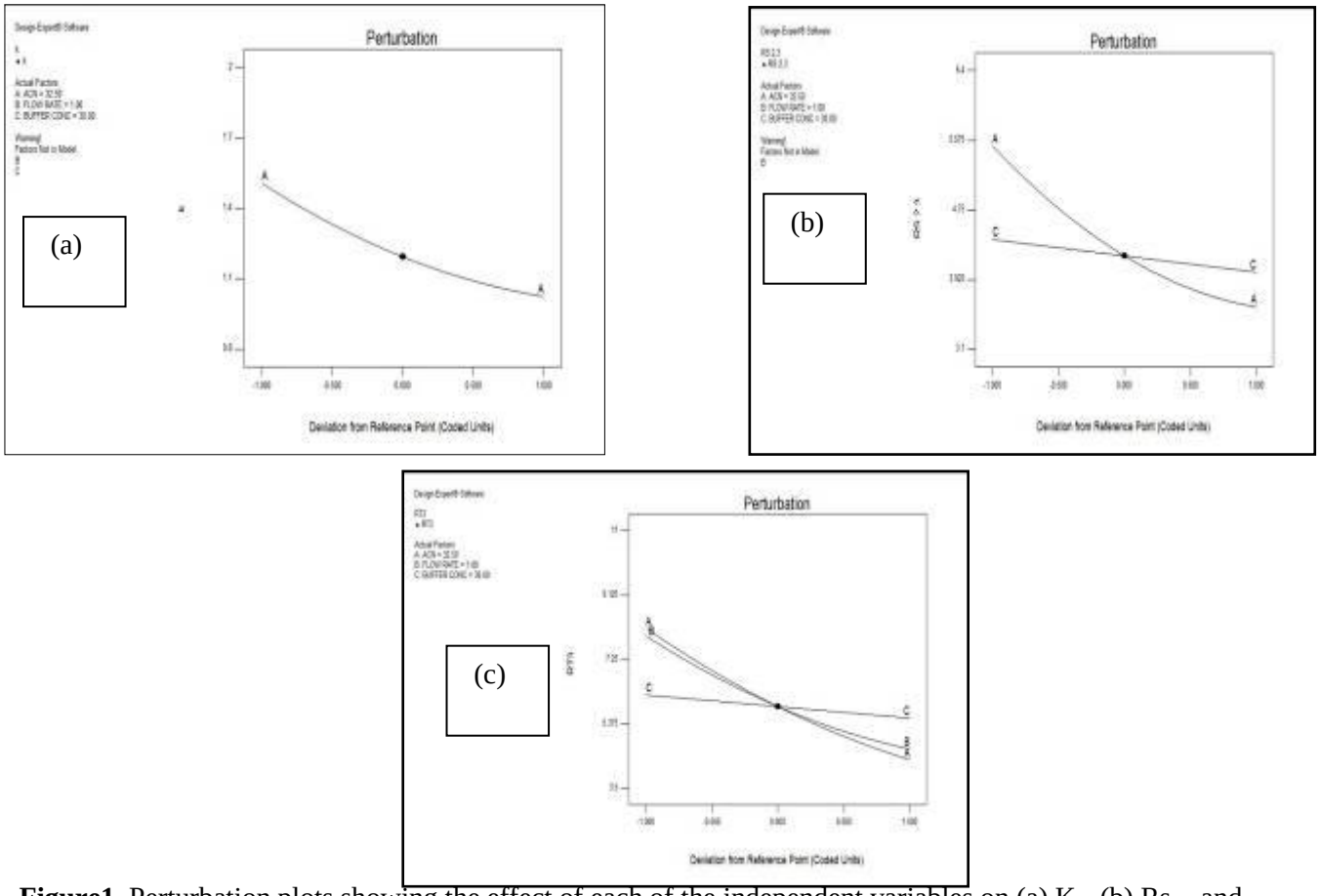
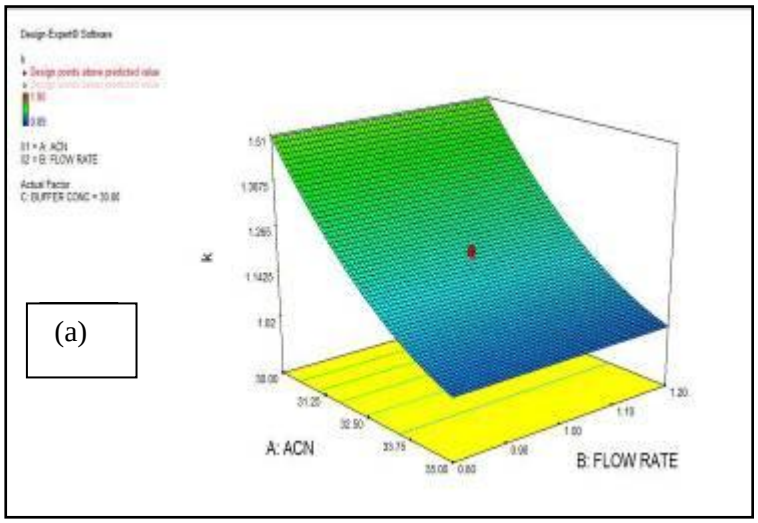


Figure1. Perturbation plots showing the effect of each of the independent variables on (a) K_1, (b) $Rs_{2,3}$ and (c) tR_3.Where A is the concentration of acetonitrile, B the buffer molarity and C the mobile phase flow rate.

The response surface diagrams showed that changing the fraction of MeCN from low to high results in a rapid decline in the retention time of RPV both at the low and high level of buffer molarity. An increase in the buffer molarity results in a marginal decrease in tR_3 at a low level of factor A. This may be due to reduced silanol effects as a result of higher buffer molarity used. Setting the MeCN concentration at its lowest level, the buffer concentration has to be at its highest level to shorten tR_3. Especially this interaction is synergistic, as it led to a decrease in run time. The existence of such interactions explains the need to carry out active multifactor experiments for optimization of chromatographic separations.



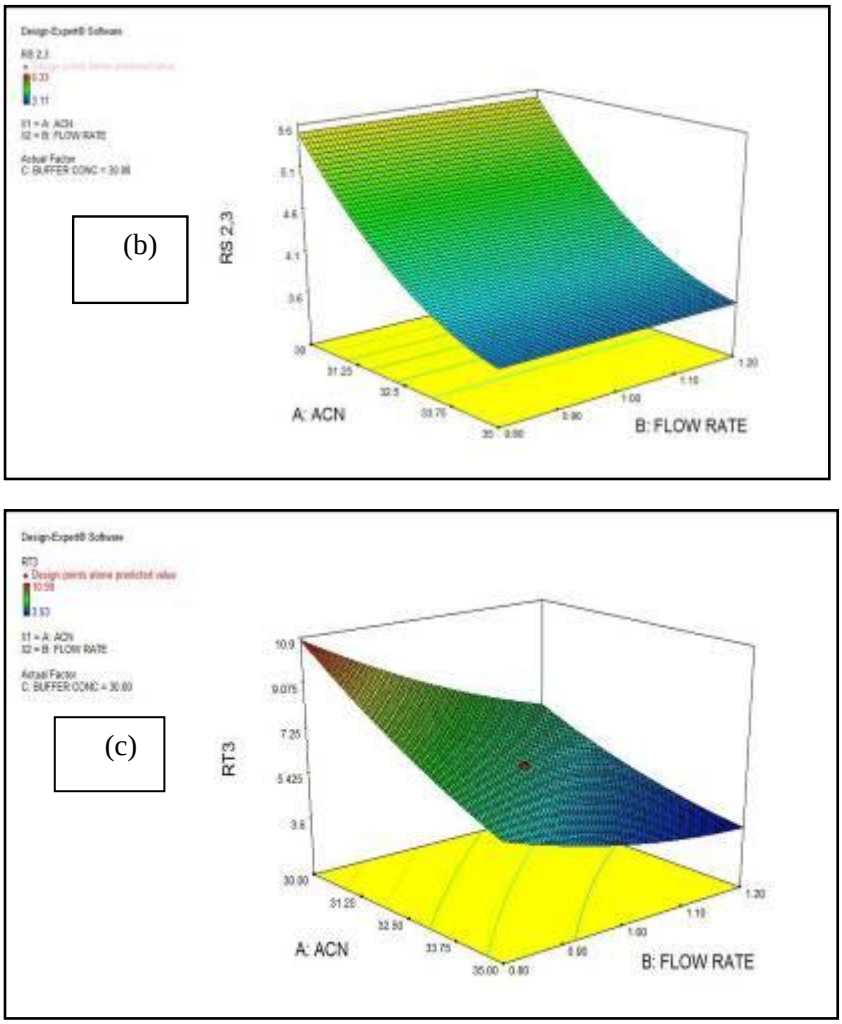


Figure 2. Response surfaces related to percentage acetonitrile concentration (a) and Flow rate (b): (a) capacity factor of the first peak (k_1), (b) resolution of the second and third peak ($Rs_{2,3}$), (c) retention time of third peak (tR_3).

Derringer's desirability function[9] was used to optimize three responses with distinct targets. In this study the known criteria for the optimization were: capacity factor of the first peak, resolution between the critical peaks $R_{S2,3}$ and elution time of third peak tR_3.The criteria for the optimization of each individual response are shown in Table 3.

Table 3: Criteria for the optimization of individual responses

Responses	Lower Limit	Upper Limit	Criteria	
Goal			Importance	
K_1	0.789	1.85	Target = 1.2	4
$Rs_{2,3}$	3.11	6.33	Minimize	2
Rt_3	3.53	10.99	Minimize	4

Criteria have been anticipated for selecting an optimum experimental condition for analyzing routine quality control samples. In order to separate the first eluting peak from the solvent front, K_1 was targeted at 1.2. The responses tR_3 was minimized, in order to shorten the analysis time while $Rs_{2,3}$ was minimized to allow baseline separation of LEV and MOX. Importance can range from 1 to 5, which gives prominence to a target value. The optimization procedure was carried out with the above said conditions and restrictions.

The response surface plots obtained for the global desirability function Fig.3 it can be concluded that there was a set of coordinates producing high desirability value (D = 0.892) were MeCN concentration of 32.43

% w/v, 35mM buffer concentration and flow rate of 1.20 ml/min. The predicted response values corresponding to the latter value of D were: K1 = 1.2, Rs 2,3 = 4.039and tR3 = 4.34 min.

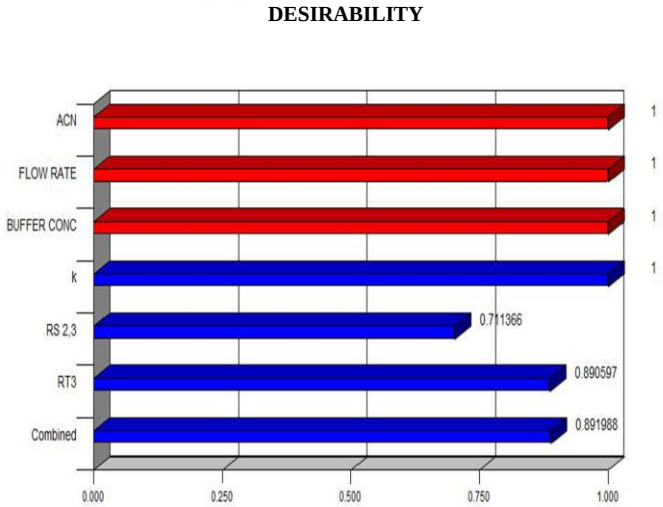


Figure 3. Response surface Bar graph showing for the global desirability function

The experiments could be performed under the optimal condition and the prediction efficiency of the model would be confirmed. The corresponding chromatogram is given in Fig.4.

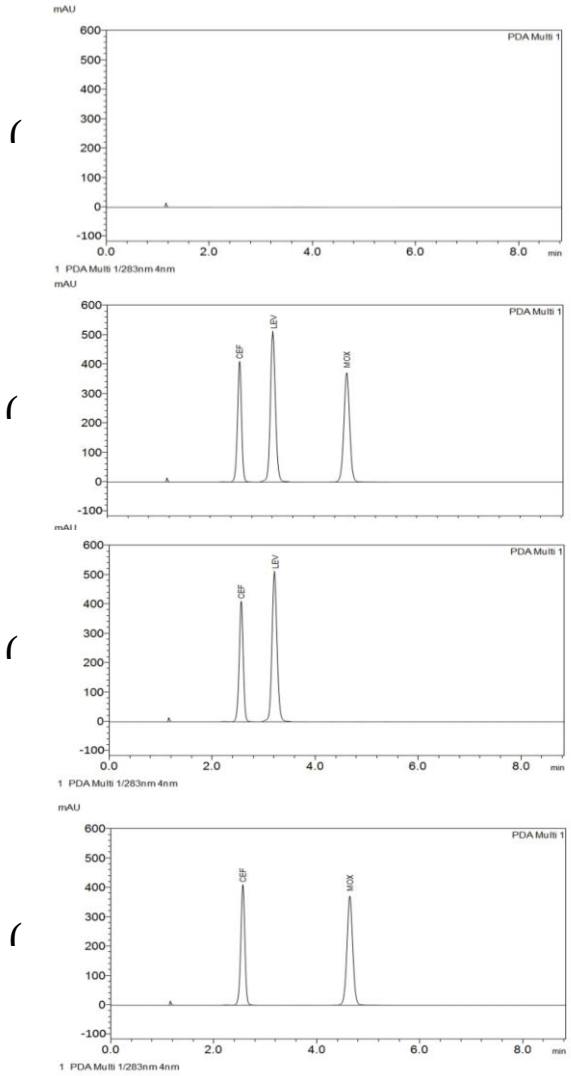


Fig.4. Chromatogram corresponding to (a) Placebo solution (b) Synthetic mixture of CEF, LEV & MOX (c) real sample of tablet containing CEF & LEV (d) a real sample of tablet containing CEF & MOX.

To investigate the predictability of the proposed model, the agreements between experimental and predicted responses for the predicted optimums are shown in Table 4.

Table 4: The comparison of observed and predictive values of different objective functions Under optimal conditions

Optimum condition	MecN	Flow rate	Buffer	K_1	$Rs_{2,3}$	Rt_3
1	Desirability (D) 0.892					
	32.43	1.2	35			
	Experimental value			1.265	4.165	4.602
	Predicted value			1.200	4.039	4.346
	Average % error			5.41	3.11	5.89

The Percentage of prediction error was calculated by Eq. (2). The average errors for K1, Rs2,3 and Rt3 were 5.41,3.11 and 5.89% respectively, indicating good correlation between the experimental and the predicted responses.

Percentage Error = Experimental - Predicted / Predicted x 100 Eq. (2)

Assay method validation

The last step of the present study was to check method's validation for specificity, linearity, accuracy, intra/inter-day precision, and robustness. The optimized HPLC method was specific in relation to the placebo used in this study. All placebo chromatograms showed no interference peaks. The linearity was established over the range of 20-60, 25-75 and 20-60 for CEF, LEV and MOX respectively. Correlation coefficients (R^2) were found to be more than 0.999 for all the analytes. Typically the mean of the regression equations were: y =28847x – 2910, y =38969x + 3862.6and y = 48818x +5257.2 for CEF, LEV and MOX. The LOD and LOQ were estimated as 88.6 and 268.6ng/ml for CEF, 14.16 and 42.9 ng/ml for LEV, 27.5 and 82.58 ng/ml for MOX respectively. Accuracy (n = 9) was in the range of 98 –102%; the values of standard deviation and % R.S.D. were found to be <2% shows the high accuracy of the method. The intra and inter-assay precision (n = 6) was confirmed since, the %RSD were well within the target criterion of ≤ 2%. Robustness study reveals that small changes did not alter the retention times, retention factor, and resolutions and therefore it would be concluded that the method conditions are robust.

Conclusion

An efficient isocratic reversed-phase LC method was developed, optimized and validated for the simultaneous estimation of the analytes CEF, LEV and MOX in pharmaceutical formulations in pure and tablets. This method reduces overall assay development time and provides essential information regarding the sensitivity of various chromatographic factors and their interaction effects on the attributes of separation.

Resolution time of analysis and quality of the peaks were simultaneously optimized by applying useful tools of chemometrics: Central composite design and Derringer's desirability function. Chromatographic techniques coupled with chemometrics tools can provide a complete picture of a separation process, making this combined technique a powerful and convenient analytical tool.

The validation study supported the selection of the assay conditions by confirming that the assay was specific, accurate, linear, precise, and robust. Therefore, this HPLC method can be used as a routine quality control analysis in a pharmaceutical environment. The results of the study demonstrate the benefit of applying this approach in selecting optimum conditions for the determination of drugs in pharmaceutical formulation. The validated assay condition was employed to determine the analytes in pharmaceutical formulations, commercially available on the Indian market.

References

1. Shah SR, Pradhan P, Dey S. Quantitative estimation of cefixime and moxifloxacin in Pharmaceutical Preparation by UV Spectrophotometric Method. International Journal of Pharm Tech Research,2004, 5;198-200.
2. Venkatesan S, Kannappan N. Development and validation of new spectrophotometric method using orthogonal polynomial method for simultaneous estimation of Moxifloxacin and Cefixime in tablet formulation.International journal of pharmacy and pharmaceutical science research,2013, 3; 130-145.
3. Devika G, Sudhakar M, Rao JV. Simultaneous Estimation of Cefixime and Moxifloxacin in Bulk and its Pharmaceutical Dosage form by RP-HPLC. Oriental Journal of Chemistry,2012, 28;1743-1750.
4. Chauhan R, Chabhadiya MB, Patel AK, Shah SA. Simultaneous estimation of cefixime trihydrate and moxifloxacin hydrochloride in their combined tablet dosage form by RP-HPLC. DerPharma Chemica, 2013, 5;197-201.
5. Patel HP, Akhta J.RP-HPLC Method for the Simultaneous Estimation of Moxifloxacin Hydrochloride and Cefixime Trihydrate in Synthetic Mixture. Asian Journal of Research In Chemistry,2013, 6; 498-501.
6. Schulte S, Ackermann T, Bertram N, Sauerbruch T, Paar WD. Determination of the newer quinolones levofloxacin and moxifloxacin in plasma by high-performance liquid chromatography with fluore scence detection. J Chromatogr Sci., 2013**,** 44;205-208.
7. Emirhan Nemutlu, DorukKatlan, ÖzgürÖzyüncü, SedefKır, SinanBeksaç M. Quantification of Moxifloxacin and Levofloxacin in Curettage Material by SPE–LC. Chromatographia,2012,71;1147-1150.
8. Ting HT, Abou-El-Hossein KA, and Chua HB. J.Scientific & Industrial Research, 2009,68; 11 .
9. Derringer G, and Suich R. Simultaneous optimization of several response variables. Journal of Qual. Technol,1980,12;214–219.

***** *****