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Research Article

QUALITY BY DESIGN-BASED OPTIMIZATION AND VALIDATION OF NEW REVERSE PHASE-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF LEVOFLOXACIN HEMIHYDRATE AND AMBROXOL HYDROCHLORIDE IN BULK AND ITS PHARMACEUTICAL DOSAGE FORM

SUMITHRA M[1]*, SHANMUGASUNDARAM P[2], RAVICHANDIRAN V[3]

[1]Director, NIPER, Kolkata, West Bengal, India. [2]Research Scholar, School of Pharmaceutical Sciences, Vels University, Chennai, Tamil Nadu, India. [3]Director, School of Pharmaceutical Sciences, Vels University, Chennai, Tamil Nadu, India. Email: sumithrapharmanalysis@gmail.com

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ABSTRACT

Objective: Innovative application of quality by design (QbD) technique for simultaneous estimation of levofloxacin and ambroxol hydrochloride (HCL) in bulk and its pharmaceutical dosage form using reverse phase-high-performance liquid chromatography (RP-HPLC) method.

Method: A method has been developed for the separation of levofloxacin and ambroxol HCL using RP-HPLC on C18 column (250 4.6 mm, 5 ml) with ultraviolet detection at 306 nm. Experimental designs were applied for multivariate optimization of the experimental conditions of RP-HPLC method. Three independent factors: Acetonitrile content in the mobile phase composition, buffer pH, and flow rate were used to design mathematical models. Here, central composite design (CCD) experimental design was used to study the response surface technique and to study in depth the effects of these independent factors. Derringer's desirability function was applied to simultaneously optimize the retention time of last eluting peak (ambroxol hydrochloride) and resolution between levofloxacin and ambroxol hydrochloride.

Result and Discussion: The predicted optimum assay condition consisted of acetonitrile, potassium dihydrogen phosphate buffer (pH 5.00; potassium dihydrogen phosphate), and methanol in a proportion of 20:70:10% v/v, respectively, as the mobile phase at a flow rate of 1.2 ml/minute. Using this optimum condition, baseline separation of both drugs with good resolution and a run time of <5 minutes were achieved. The optimized assay condition was validated according to the ICH guidelines to confirm specificity, linearity, accuracy, and precision.

Keywords: Levofloxacin, Ambroxol hydrochloride, Experimental design, Response surface methodology, Derringer's desirability, Quality by design approach.

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INTRODUCTION

Levofloxacin hemihydrate [1,2] is a synthetic broad-spectrum antibacterial agent. The chemical name is (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4benzoxazine-6-carboxylic acid hemihydrates Fig. 1. Levofloxacin is active against both Gram-positive and Gram-negative bacteria and acts as a bactericide by inhibiting the enzymes such as DNA gyrase and topoisomerase IV. Topoisomerase IV is necessary to separate DNA that has been replicated (doubled) before bacterial cell division. With the DNA not being separated, the process is stopped, and the bacterium cannot divide. DNA gyrase, on the other hand, is responsible for supercoiling the DNA so that it will fit in the newly formed cells. Both mechanisms explained the amount of killing the bacterium.

Ambroxol hydrochloride (HCL) [3,4] is a potent mucolytic and mucokinetic. Ambroxol HCL is chemically Trans-4-[(2-amino-3, 5-dibromobenzyl) amino]-cyclohexanol hydrochloride Fig. 2. Ambroxol is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. It promotes mucus clearance, facilitates expectoration, and eases productive cough, allowing patients to breathe freely and deeply."

Moreover, the extensive literature survey revealed that there is no reverse phase-high-performance liquid chromatography (RP-HPLC) method available for simultaneous estimation of levofloxacin or ambroxol combination in the pharmaceutical dosage forms using experimental design approach quality by design (QbD). A few analytical methods [5-8] have been reported in the literature for the determination of levofloxacin or ambroxol alone or combination with other drugs biological fluids and pharmaceutical dosage forms. They include derivative spectrophotometric [9,10] methods and other methods such as HPLC with ultraviolet (UV) detection, HPTLC [11,12], and liquid chromatography with tandem mass spectrometry were also reported for the determination of ambroxol from human body fluids and pharmaceutical dosage form. Some analytical procedure has been reported for quantitative determination of levofloxacin and norfloxacin by capillary electrophoresis with electrochemiluminescence detection [13]. Recently, one article is published for validated high-performance liquid chromatographic method for levofloxacin and ambroxol using Hypersil BDS C18 column (25 cm × 4.6 mm, 5 µm). The mobile phase constituted of Buffer:Acetonitrile:Methanol (650:250:100) at a flow rate of 1.0 ml/minutes. [14]. This method has a run time of more than 10 minutes as well as does not describe the design space (DS) [15] or interaction study of independent factors as per recent FDA guidelines [16].

Regulatory authorities such as FDA and ICH guidelines [17,18,19] are promoting and requesting the application of experimental design approach to understand chromatographic selectivity and support better method control, including method transfer. This prompted the researchers to adopt the experimental design in HPLC, and many papers were published related to this work [20-26]. The main objective of our work is to develop an improved RP-HPLC method suitable for the routine quality control of levofloxacin or ambroxol in a pharmaceutical industry and provide information on the sensitivity of chromatographic factors and their interaction effects on the separation characteristics. The optimization of chromatographic factors such as acetonitrile concentration in mobile phase, buffer pH, and flow rate are very complex that have a significant effect on chromatographic separation. All these independent factors can easily optimize using the design of

experiments that is called QbD approach. QbD is a systemic approach that includes multi-dimensional combinations and input variables using the design of experiment to obtain the optimum conditions with good assurance of quality. DS is generated through an experimental design that shows the flexible region, in which post-approval changes are not required during any of changes in the parameters (e.g., pH and % of organic modifier) (ICH Q8 (R2). When one needs to optimize more than one response (resolution, last retention time, and capacity factor of the drug peak) at a time, the use of derringer's desirability function [27,28] is the best choice. Derringer's desirability function was first used in chromatography by Deming to get better resolution and shorter analysis time as objective functions to get better separation quality. We have employed the same methodology for the development and optimization of a new HPLC method for the simultaneous estimation of levofloxacin or ambroxol from a bulk and tablet formulation.

EXPERIMENTAL

Materials

Levofloxacin and ambroxol working standards were received as gift samples from BAFNA Pharmaceuticals India. Disodium potassium hydrogen orthophosphate (analytical grade), methanol (HPLC grade), and acetonitrile (HPLC grade) (S.D Fine Chemical Pvt. Ltd., Mumbai, India) were used throughout these experiments. HPLC grade water was collected from the Milli-Q system. The marketed tablets (Livbest-AM) used containing 500 mg of levofloxacin and 75 mg of ambroxol per tablet were manufactured by Piramal Healthcare Pvt. Ltd., India, and it is procured from the local market.

Instrumentation and chromatographic conditions: A Shimadzu HPLC system consists of an LC-20AD solvent delivery system (pump), SPD-M20A photodiode array detector, Rheodyne injector with 20 µL loop volume, and LC-solution assisted for data collections and processing. The chromatographic separation was performed using phenomenex C_{18} 150×4.6 mm, 5 m column, detection wavelength is 306 nm and run time is 10 minutes.

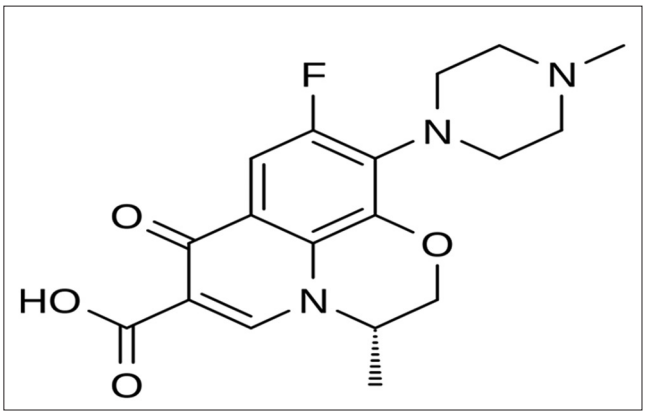


Fig. 1: Levofloxacin hemihydrate

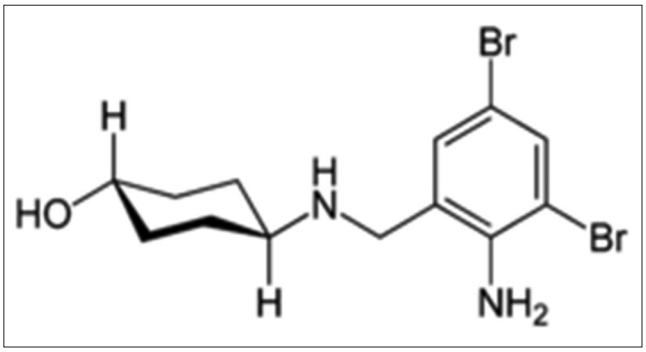


Fig. 2: Ambroxol hydrochloride

Software experimental design (rotatable central composite), desirability function, and data analysis calculations were performed using design expert (Version 7.0.1.0 Stat-Ease Inc., Minneapolis, MN, USA) trial version statistical software.

Preparation of phosphate buffer solution

About 6.8 g of potassium dihydrogen orthophosphate was dissolved in sufficient water (HPLC grade) with aid of sonicator. Then, add triethylamine (TEA) or orthophosphoric acid was used to adjusted the pH to 5.

Preparation of standard stock solution

About 100 mg of levofloxacin and 100 mg of ambroxol were accurately weighed and transferred into 100 ml volumetric flasks. The contents of the volumetric flask were dissolved in methanol (HPLC grade) to get 1 mg ml of both levofloxacin and ambroxol. Working standard solution was freshly obtained by diluting the standard stock solution with mobile phase during the analysis time.

Chromatographic procedure

Chromatographic separations were carried out on a phenomenex (C_{18} 150×4.6 mm, 5 m). A mixture of acetonitrile, potassium dihydrogen phosphate (pH-5, KH2PO4), and methanol (25:65:10) was used as the mobile phase. The pH of the buffer solution was adjusted by phosphoric acid (H_3PO_4) and TEA. The wavelength of 306 nm was used as detection at which both drugs gave good response.

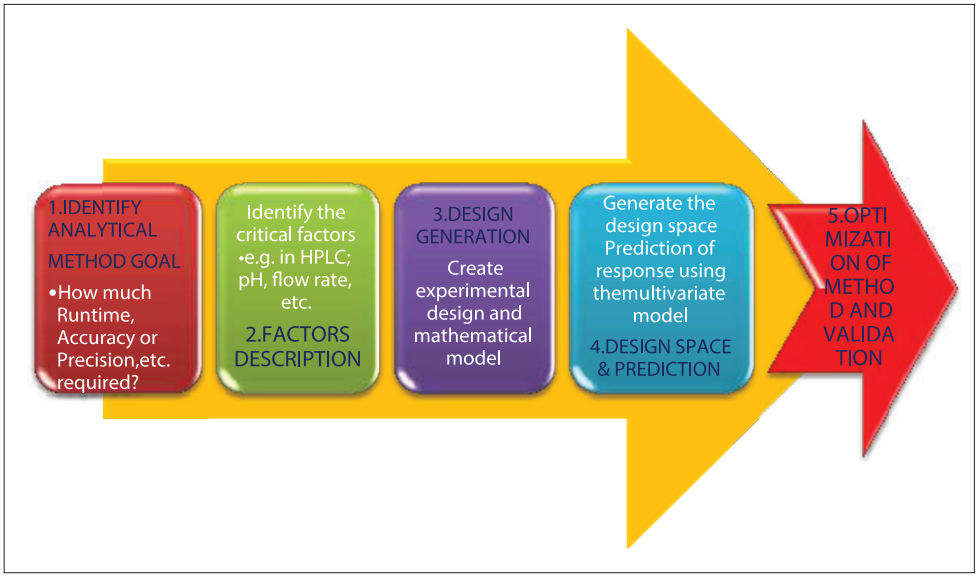
Experimental design and response surface methodology (RSM)

The central composite design approach can be useful to optimize the separation and to help out in the development of better understanding of the interaction of several chromatographic factors on separation quality. The development of a QbD for selected drugs and finally reach the definition of its DS and optimization of method, the process could be illustrated in four steps (Scheme 1) to complete the method. The first step is to identify the goal of the intended method that depends on the types of the method developed such as for routine quality control assay method should be fast, accurate, and specific. Hence, we set the same goal for our assay method. The second step is the assessment of critical factors that affect on the critical quality attributes such as in HPLC resolution, run time, and capacity factor of peak. The third step is the creation of experimental design and mathematical model that expresses the relation between the factors and response. In this work, the important chromatographic factors were selected based on preliminary experiments and prior knowledge from the literature and optimized by a central composite design (CCD) experiment. A CCD design was employed to locate the optimum flow rate, mobile phase pH, % of organic modifier for separation by mapping the chromatographic response surface. Table 1 shows three chromatographic factors and levels selected, in which experimental condition was optimized.

To provide a rotatable CCD for three independent variables, a partial factorial design was combined with five replicates of center points and five axial points at an extreme level. The qualities of the fitted polynomial models were examined on the basis of the coefficient of determination R2. The position of the true optimum condition was recognized by applying Derringer's desirability function, where responses were simultaneously optimized. The final step is to predict the response and DS from the polynomial equation. RSM is a mathematical and statistical technique valuable for analyzing problems where several independent

Table 1: The experimental factors and levels used in central composite design

Factor	Name	Level(-1)	Level (0)	Level(+1)
A	Acetonitrile (%v/v)	20	25	30
B	Buffer pH	4.5	5	5.5
C	Flow rate (mL min^{-1})	0.8	1.0	1.2



Scheme 1: Graphical representation of method development flow using quality by design technique

variables such as column temperature, pH, and flow rate affect dependent variables or responses (e.g., resolution, capacity factor, and run time). This technique is used to simultaneously optimize the levels of these variables to attain the best system performance. RSM enables definition of quadratic models that accurately explain the response for all values of the chromatographic conditions in the experimental region. For calculation of quadratic regression model coefficients, each design variable must be studied, at least at three distinct levels and consequently, a CCD was used in this optimization study.

RESULTS AND DISCUSSION

Method development and optimization of levofloxacin and ambroxol are medium-polar analytes because of the amino group and fluoroquinolone moiety. Hence, reverse phase mode is more preferable than normal phase. Initially, we tried different reverse phase columns such as C18, cyano, and C8 for separation of both analytes. However, cyano column showed poor separation of both analytes while in C8, levofloxacin was eluting early with a broader peak shape. Therefore, we considered only C18 column for optimization study. The overlay UV spectra Fig. 3 of both drugs, which indicates that 306 nm is the optimum wavelength to detect levofloxacin and ambroxol with good response as well as minimum baseline noise.

The mobile phase pH is an important factor that drives the selectivity of the method due to differences in the pKa of molecules. Initial method development was tried on three different pH 4.5, 5.0, and 5.5 based on the literature report [17]. However, high-tailing (>2) was observed with levofloxacin at pH 5 and 5.5 and mild tailing at pH 4.5 due to the interaction between a positively charged solute (amine of levofloxacin) and a negatively charged silanol on the surface of silica stationary phase at pH 4.5 and 5.5. It is observed most often when using HPLC columns packed with stationary phases that have significant silanol activity. It is usually worse in a basic pH mobile phase than in an acidic pH mobile phase because pKa of silanol groups is around 3.5, therefore, above pH 3.5 silanol groups are in ionized form and ready to interact with 1, 2 amines. Therefore, TEA was added to inhibit levofloxacin peak tailing due to the interaction of a free silanol group. Mainly organic modifiers for reversed-phase include acetonitrile, methanol, and in some cases, tetrahydrofuran. Due to the high UV cut off as well as presence of peroxide impurities in tetrahydrofuran that affect the stability of analytes, hence tetrahydrofuran was avoided for selection of organic modifier. We have used acetonitrile because of its cost, good solubility in all buffers, and it acts as Lewis acid by donating hydrogen that improves the only C18 column for optimization study. Fig. 3 shows the overlay UV spectra of both drugs, which indicates that 308 nm is the optimum wavelength to detect levofloxacin and ambroxol with good response as well as minimum baseline noise.

Design of experiment and DS: The design matrix generated for the rotatable central composite design is shown in Table 2, and the system was fully optimized using the 15 experiments. This design is composed of a three level factorial design with 15 experimental runs. In this study, the levels of each factor were selected based on prior scouting experiments. Many more experiments would have been required if this method was optimized with the standard univariate approach. Initially, it was found that at below 0.8 ml/minutes flow rate peaks became broad and above 1.2 ml/minutes proper separation was not observed. In the same way, ideal acetonitrile concentration was found in between 20% v/v and 30% v/v. If any C18 column works consistently at pH 5.0 that reduces the column shelf life, therefore, keeping this in mind, we tried to optimize the pH range in between 4.5 and 5.5. As can be seen in Table 2, the ranges of each factor were: Flow rate (0.8-1.2 ml/minute 1), buffer pH (4.5-5.5), and acetonitrile concentration (20-30% v/v). Here, our main goal is to develop the method with minimum run time as well as a good resolution between the peak of levofloxacin and ambroxol that facilitate the accurate quantification of drugs within a short period the results are shown in Fig. 4. Hence, retention time (Rt) of last eluting peak (ambroxol Rt), capacity factor, and resolution between two peaks (Rs) were taken as a response.

The statistical parameters obtained from analysis of variance for the regression models are listed in Table 3. Probability $p<0.05$ was obtained, implying that these models are significant. Adjusted R^2 was well within the acceptable limits ($R^2>0.8$) that show experimental model is good fit with polynomial equations. The adequate precision value is a measure of the "signal (response) to noise (deviation) ratio" that should be greater than four. In this study, the ratio was found to be greater than 15, which indicates an adequate signal, and therefore, the model is significant for the separation process. The reproducibility of the model depends on the coefficient of variation (C.V.) that is well within the limit of both responses (% C.V.<10) [28]. Table 3 illustrates the interaction term with the largest absolute coefficients among the fitted models is 0.61AC of Rt model. The positive interaction between A and C is statistically significant (p=0.001) for Rt.

The study reveals that changing the fraction of acetonitrile from low to high results in a rapid decline in Rt both at the low and high level of buffer pH. Further, at the low level of factor A and B, an increase in the flow rate results in a marginal decrease in the Rt of ambroxol Rt. Therefore, when acetonitrile concentration is set at its lowest level,

the buffer pH has to be at its lowest level to shorten the analysis time. Especially, this interaction is synergistic, as it led to a decrease in analysis time. The second response model T reveals that all factors affect moderately on the tailing of levofloxacin. To get a better understanding of the results, the perturbation plots are presented in Fig. 5a-c. For an optimization design, this graph shows how the response changes as each factor moves from a chosen reference point, with all other factors held constant at the reference value. A steep slope or curvature in a factor indicates that the response is sensitive to that factor. Fig. 5b reveals that as acetonitrile concentration in mobile phase decreases, resolution between levofloxacin and ambroxol reduces due to decline in the interaction with free silanol groups of column.

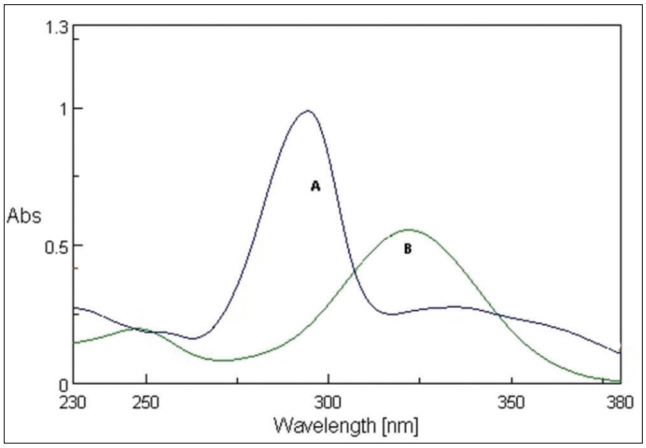


Fig. 3: Overlay ultraviolet spectra of levofloxacin and ambroxol

DS was generated after processing all data using the modeling software design expert@. From the constructed DS, the working point was selected by visual examination looking for the least Rt of ambroxol and symmetric peak of levofloxacin. As per our method's goal, at pH 4.5, % of acetonitrile 20% v/v, and flow rate of 1.2 ml/minute^{-1} satisfy faster separation (5.0) and optimum resolution between levofloxacin and ambroxol. Our objective was to maximize resolution with symmetric peak and to minimize analysis time.

Derringer's desirability function

Hence, when there are multiple responses to optimize with different targets, derringer's desirability function is a suitable technique. The derringer desirability (D) function is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions. Value of D different from zero implies that all responses are in a desirable range simultaneously and consequently, for a value of D close to

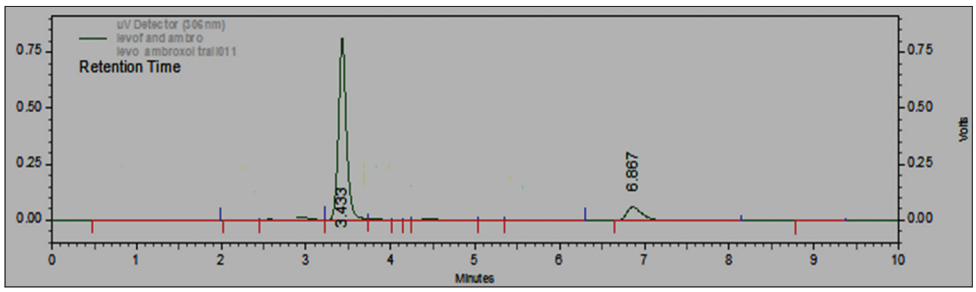


Fig. 4: Chromatogram of levofloxacin and ambroxol hydrochloride

Table 2: Central composite rotatable design arrangement and responses

S.N	Standard	Run	Type	Factor 1-A-acetonitrile	Factor-2-B-pH	Factor 3-C-flowrate	Response 1 capacity factor	Response 2 -resolution	Response 3-last retension time
1	10	1	Axial	25.00	5.00	1.28	2.07	7.24	5.375
2	12	2	Center	25.00	5.00	1.00	2.9	12.4	6.825
3	9	3	Axial	25.00	5.00	0.72	4.4	5.875	9.442
4	15	4	Center	25.00	5.00	1.00	2.9	12.71	6.825
5	4	5	Fact	20.00	4.50	0.80	3.2	5.15	7.56
6	11	6	Center	25.00	5.00	1.00	2.9	12.3	6.725
7	2	7	Fact	30.00	4.50	1.20	0.72	13.40	3.24
8	3	8	Fact	20.00	5.50	1.20	1.2	5.32	4.25
9	7	9	Axial	25.00	4.29	1.00	2.91	4.42	6.95
10	6	10	Axial	32.07	5.00	1.00	1.02	15.42	7.25
11	14	11	Center	25.00	5.00	1.00	2.91	12.4	6.825
12	1	12	Fact	30.00	5.50	0.80	1.8	6.24	5.34
13	13	13	Center	25.00	5.00	1.00	2.91	12.4	6.825
14	8	14	Axial	25.00	5.71	1.00	1.52	5.24	6.24
15	5	15	Axial	17.93	5.00	1.00	3.24	15.54	7.25

Table. 3: Regression model and statistical parameters obtained from ANOVA

Response	Reduced regression model	Adjusted R2	Model p	% CV	Adequate precision
Capacity factor	Capacity factor =−50.13640+0.96433 * A+19.37499 * B-3.9844*C-0.021796 *A^2-2.00964* B^2	0.9030	0.0049	13.57	16.287
Resolution	Resolution =−512.07167-2.04740* A+175.19159 * B+217.51185 * C+2.03891* A * C-23.36426 * B * C-15.12475 * B^2−72.93594* C^2	0.8025	0.0047	18.88	7.181
Last retension time	Last retension time=+6.46787-0.58223 * A-0.50205 *B+24.93415*C+0.55148* A*C-21.3396* C^2	0.8566	0.0020	6.46	7.374

1 (0.92), the combination of the different criteria is globally optimal so that the response values are near target values. The criteria for the optimization of each response are shown in Table 4. Criteria have been proposed for selecting an optimum experimental condition for analyzing routine quality control samples. In general, a short analysis time is usually preferred for routine analysis. Hence, high importance (value 4) was assigned to Rt of last eluting peak. Following the conditions and restrictions above, the optimization procedure was carried out using design expect. The response surface plot obtained for the maximum desirability function (D=0.92) is presented in Fig. 6a, which indicates our mathematical model is excellent. The coordinates produce the maximum desirability value at acetonitrile 20 v/v, buffer pH 4.5, and flow rate of 1.2 ml/minute. The predicted response values corresponding to the above optimum condition are given in Table 3.

Method validation

Specificity: The specificity of the method was evaluated after analyzing the placebo containing both levofloxacin and ambroxol at a concentration of 1.0 µg ml^{-1} each. The selectivity of the method is depicted by the sharp well-resolved peaks for levofloxacin and ambroxol. The specificity of the method was checked by comparing the chromatograms obtained from standard, sample, and the corresponding placebo. The Rt of the standard and the drugs from sample solution were identical. This confirmed the specificity of the method.

Linearity: The linearity was established at the concentration range of 1-10 µg ml^{-1} for levofloxacin and ambroxol. The standard stock solution was diluted with mobile phase to get 1, 2, 4, 6, 8, and 10 µg ml^{-1} of both levofloxacin and ambroxol. Each concentration was analyzed in 3 replicates. Peak areas (y) of levofloxacin and ambroxol were plotted versus their respective concentrations (x), and linear regression analysis performed on the resultant calibration curves. Linearity data are shown in Table 5.

Accuracy/recovery accuracy of the method was determined by performing the recovery experiment at 80%, 100%, and 120% of the expected assay value or label claim of the drugs in the commercial tablet dosage form. To the standard drug solution, 4 µg ml^{-1} (80%), 5 µg ml^{-1} (100%), and 6 µg ml^{-1} (120%) of both levofloxacin and ambroxol were added and analyzed by the proposed method in 3 replicates at each level. The % mean recovery of drugs at each level was determined. The recoveries of levofloxacin and ambroxol at each level were found to lie well within the acceptable criteria of bias ±2% [32]. The accuracy result is shown in Table 6.

Limit of detection (LOD) and limit of quantification (LOQ) for both levofloxacin and ambroxol were determined according to the ICH guideline Q2 (R1) . LOD was defined as 3.3 r/S and LOQ as 10 r/S based on, standard deviation of the response (r) and slope of the calibration

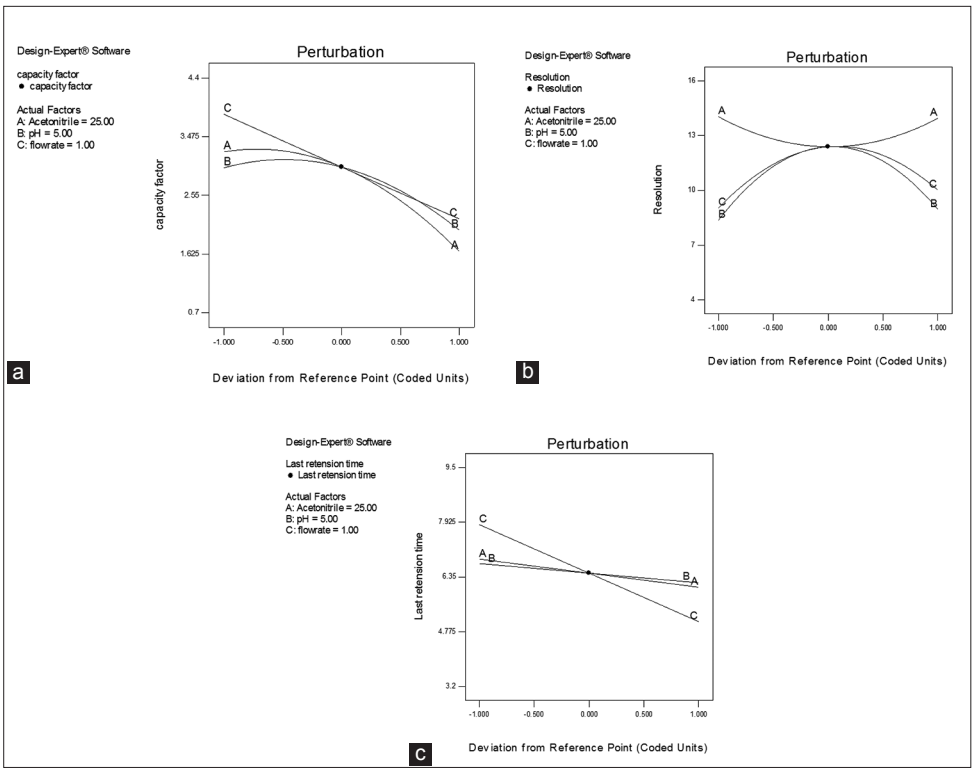


Fig. 5: Perturbation plot showing (a) the effect of each of the independent factors on capacity factor of both drugs, (b) the effect of each factor involved in resolution between two drugs, (c) the effect of each of the independent factors on last retention time of ambroxol, while keeping other factors at their respective mid-point levels

Table 4: Comparison of experimental and predictive values of different experimental runs under optimum conditions

Optimum Conditions 1	Selected optimized run					
	A: acetonitrile content (Ml)	**B: buffer pH**	**C: flowrate mL/minute**	**Response 1.Capacity factor**	**Response 2 resolution**	**Response 3 last retension time**
	20 ml	4.5	1.2	2.14	7.81	4.99
	Experimental value					
	Predictive value			2.33	7.59	5.14
	Predicted error			0.0102	0.0061	0.052

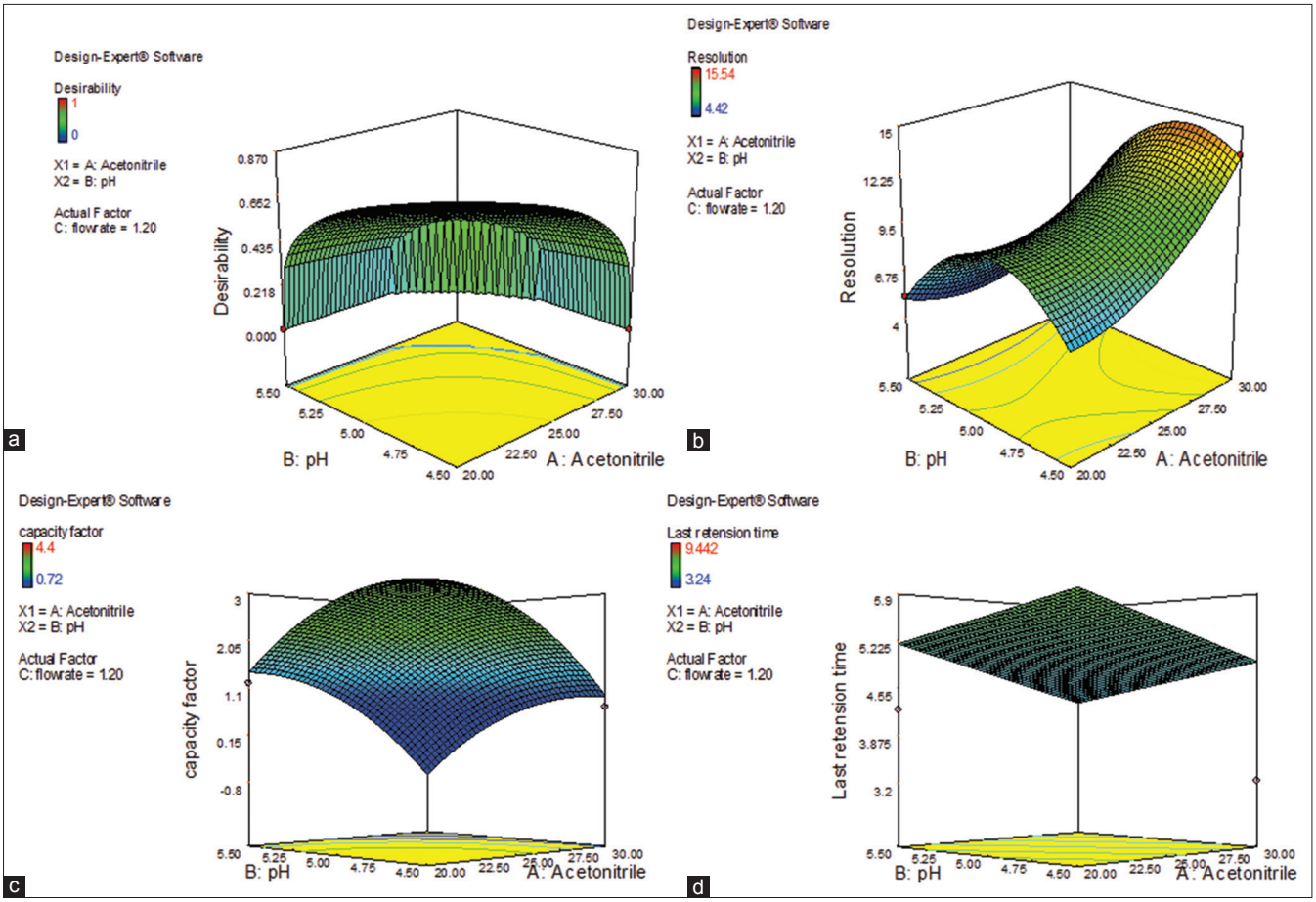


Fig. 6: 3-D models shows (a) design space for the maximum derringer's desirability function, (b) Design space for Resolution between Levofloxacin and ambroxol in pH_% of acetonitrile model, (c) Design space for capacity factor between levofloxacin and ambroxol in pH_Flow rate model, (d) Design space for last retension time of ambroxol drug peak in pH_% of acetonitrile model

Table 5: Parameters of linear regression equations for each drug compound

Parameters	Levofloxacin*	Ambroxol*
Calibration range (µg mL^{-1})	1-10	1-10
Correlation coefficient (r)	0.9998	0.9999
Slope	47922.21	64525.33
Intercept	−230.92	4633.00
S.D of slope	258.29	320.69
S.D of intercept	1567.58	1946.31
LOD (µg mL^{-1})	0.097	0.092
LOQ (µg mL^{-1})	0.330	0.310

S.D: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification *average of six determinations

Table 6: Accuracy study using the proposed method

% level	Amount added (µg mL^{-1})		Amount found* in mg mL^{-1} (Mean±S.D)		% recovery* (Mean±S.D)	
	Levofloxacin	Ambroxol	Levofloxacin	Ambroxol	Levofloxacin	Ambroxol
80	4	4	3.98±0.04	3.99±0.03	99.5±0.42	99.75±0.32
100	5	5	4.96±0.02	4.98±0.05	99.2±0.21	99.6±0.53
120	6	6	5.97±0.05	6.03±0.02	99.5±0.54	99.5±0.51

*Average of five determinations, SD: Standard deviation

curve (S) constructed at six levels ranging 1.0-10.0 µg ml^{-1} of both levofloxacin and ambroxol LOD and LOQ results are given in Table 5 are experimentally verified.

Precision: Precision was determined by studying the intermediate precision and repeatability. Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Intermediate precision expresses within-laboratories variations: Different days, different analysts, different equipment, etc. Intermediate precision is also termed inter-assay precision. The intra- and inter-day assay precision was studied at 2.0 µg ml^{-1} concentration level (n=5), and precision was confirmed as

Table 7: Intra- and Inter-day precision for levofloxacin and ambroxol

S.N	Intra day				Inter day			
	Levofloxacin*		Ambroxol*		Levofloxacin*		Ambroxol*	
	Area	%recovery	Area	%recovery	Area	%recovery	Area	%recovery
1	47387	99.37	67489	98.41	47497	98.37	68438	98.88
2	47271	99.12	69271	100.18	47167	99.52	68209	98.53
3	47522	99.65	68918	99.63	48003	99.05	68438	98.88
4	47198	98.97	68926	99.64	47391	98.90	67927	98.09
5	46987	98.53	68948	99.67	47281	99.45	67581	97.56
SD	201.51		698.59		323.4		366.7	
%RSD	0.426		1.01		0.681		0.538	

*Average of five determinations, SD: Standard deviation, RSD: Relative stratigraphic depth

the % relative stratigraphic depth was well within the target criterion (of the assay results was <2%, which indicates the method is precise. The precision data are shown in Table 7, and the values are complied the limits as per IP standard.

CONCLUSION

Statistically based experimental designs proved to be an important approach in optimizing selectivity-controlling parameters for the simultaneous determination of levofloxacin and ambroxol in commercial formulation. The significant factors were optimized by applying central composite design and RSM. The objective of responses is resolution, capacity factor, and the analysis time simultaneously optimized by applying (derringer's desirability function) a multi-criteria decision-making tool. This method has been evaluated for linearity, precision, accuracy, and selectivity and has proved to be convenient and effective for the quality control of levofloxacin and ambroxol in raw material and its formulations. Moreover, the previously reported method addresses only separation of both drugs with the traditional approach with longer run time of >10.0 minutes by using 150 mm length columns (5 µm particle size) while our proposed method is able to quantify levofloxacin and ambroxol within a run time of 5 minutes.

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