Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 3597 - 3615
Received 20 January 2021; Accepted 08 February 2021.

Simultaneous Optimization of the Resolution and Analysis time in RP-HPLC of Fexofenadine and Montelukast using Derringer's Desirability Function

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Abstract: For the simultaneous study of fexofenadine and montelukast using statistical experimental design, a new simple, sensitive, rapid and precise isocratic RP-HPLC was developed and validated. For multivariate optimization of the experimental conditions of the RP-HPLC process, Experiment Architecture (DoE) was applied. Mathematical models were constructed using three independent factors: mobile phase structure, phosphate buffer intensity and flow rate. In order to research the response surface technique and to study in detail the results of these independent variables, central composite design (CCD) was used. The desirability feature was used to maximize the retention time and resolution of the analytes simultaneously.The optimized and projected contour diagram data consisted of a 75:25 mixture of acetonitrile and phosphate buffer (pH 6.5, pressure 49 mM) at a flow rate of 1.4 ml/min,

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 3597 - 3615
Received 20 January 2021; Accepted 08 February 2021.

respectively. Baseline isolation of both drugs with a reasonable resolution and run time of less than 9.0 min was accomplished using these optimal conditions. According to ICH instructions, the optimized assay conditions were validated. The findings thus explicitly showed that the Consistency by methodology could be successfully extended in order to perform analyses the RP-HPLC process for simultaneous fexofenadine and montelukast estimation.

Keywords: Montelukast, Fexofenadine, Central Composite Design, Optimum conditions, RP-HPLC.

Introduction:

Montelukast (Figure 1a)chemically known as sodium;2-[1-[[(1*R*)-1-[3-[(*E*)-2-(7-chloroquinolin-2-yl)ethenyl] phenyl]-3-[2-(2-hydroxypropan-2-yl)phenyl]propyl]sulfanylmethyl]cyclopropyl]acetate [1].Montelukast is used to prevent and treat symptoms of asthma. Fexofenadine hydrochloride (Figure 1b) known as 2-(4-{1-hydroxy-4-[4-(hydroxyl diphenylmethyl)piperidin-1-yl]butyl}phenyl)-2-methylpropanoic acid hydrochloride. It is used to treat the symptoms of seasonal allergies [2]. Literature survey revealed that numerous analytical methods for the combination of montelukast and fexofenadine like HPTLC[3],HPLC[4], stability indicating RP –HPLC method [5], determination montelukast by UV [6], HPLC [7], stability indicating HPLC [8], montelukast combined with other drugs ratio derivative spectroscopy [9], HPLC and HPTLC [10]. Determination of fexofenadine by UV [11], stability-indicating HPLC [12],plasma concentration in LC –MS [13]combination with other drugs by RP-HPLC[14] was reported. However, the writing study uncovered that there is no distributed strategy for the synchronous chromatographic assurance of montelukast and fexofenadine bulk and in drug dosage forms utilizing Derringer's desirability function.

Creating and enhancing a HPLC method is an intricate technique that requires synchronous assurance of a few components (for example type and sythesis of the natural stage, section temperature, stream rate, pH and sort of the fixed stage). On the off chance that just one factor should be enhanced, a straightforward univariate methodology is performed. Be that as it may, normally at least two variables are contemplated. This should be possible utilizing either univariate or multivariate streamlining methodologies. The customary methodology was examining the impact of the relating factors by transforming each factor in turn (OVAT) approach, while keeping the others consistent. The OVAT methods were demonstrated wasteful in light of the fact that the worldwide ideal probably won't be found, and the discovered ideal

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conditions may rely upon the beginning conditions [15].Then again, a multivariate methodology shifts a few factors at the same time. An exploratory plan is a trial set-up that permits concentrating at the same time various components in a predefined number of analyses. Generally, test plans can be separated into screening plans, reaction criteria plans and blend plans. Evaluation plans permit screening a generally enormous amount elements in a moderately modest quantity of trials. They are utilized to recognize the most impacting factors. The components are assessed at two levels in these plans. Reaction plans are utilized to locate the ideal degrees of the main variables, which are chosen from a screening configuration approach. In these plans, factors are inspected at any rate at three levels. The methodology at last are applied relies upon the number and kind of components to be inspected, on the reason for their utilization, towards inclination of expert [16].

The point of this research is to build up an approved turned around stage elite fluid chromatography method for the concurrent division of montelukast and fexofenadine at the same time. The importance of the considered variables and ideal chromatographic methods that were assessed by a comprehensive composite plan (CCD) utilizing a numerical worldwide streamlining strategy (Derringer's-desirability function). At last, the indicatedproceudre was tried for linearity, specificity, precision, accuracy, robustness and ruggedness. Advertised drug dosage forms containing the isolated mixes were investigated to investigate the legitimacy of the planned method.

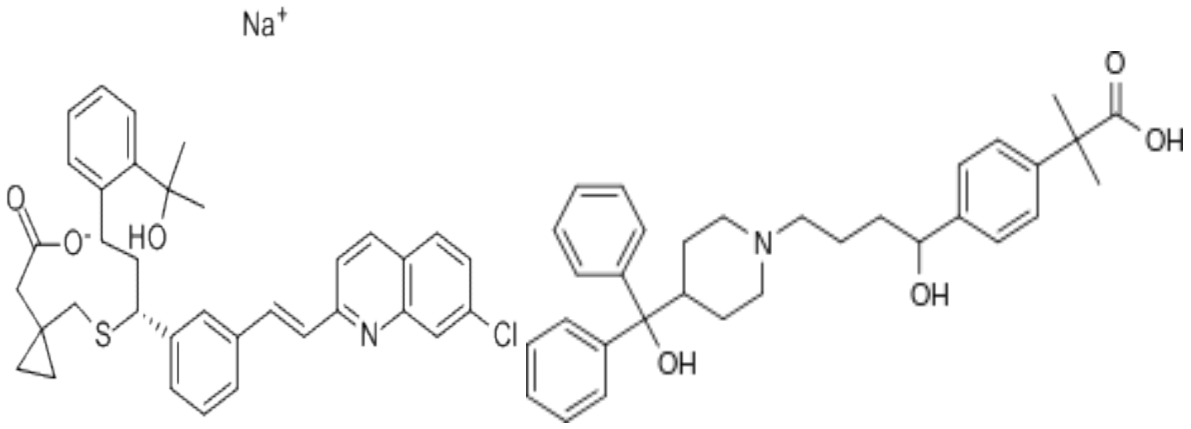

Figure 1(a) : Montelukast Sodium **Figure 1(b): Fexofenadine**

Figure 1: Structure for Analytes

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MATERIALS & METHODS

Chemicals & Reagents

Analytical grade pure samples of montelukast and fexofenadine were procured as a gift sample from Dr.Reddy's Labs, Hyderabad, India. A combination of montelukast and fexofenadine tablet formulations (Emlukast fx tablets) was procured from the local market. HPLC grade Acetonitrile, methanol,water and orthophosphoric acid buffer (AR grade) were purchased from Merck Chem India Pvt. Limited, India.

Instrumentation parameters & Chromatographic Conditions

Analysis was conducted with a Shimadzu LC2010 CHT separation module equipped with LC solution software, PumpLC2010 binary and UV detector set at 240 nm. Compounds were isolated on an Intek chromasol column (250×4.6 mm i.d., 5µm molecule size) under switched stage segment conditions. The versatile stage was Acetonitrile and phosphate cradle. The stream rate was 1ml/min and the run time was 8 minutes. Tests were infused utilizing Rheodyne injector with 10µL circle and discovery was done at 240 nm. Before investigation versatile stage was degassed with the utilization of anultra sonicator and sifted with a 0.45µ nylon channel. Experiment was conducted in column heat kept up at 30±0.5˚C.

Preparing of the mobile phase

700ml of pure acetonitrile along with 300ml of phosphate buffer (pH 6.5 Buffer quality 50mM) were blended and degassed in ultrasonic water bath for 5min. At that point the solution was separated through 0.45µm channel under significant vacuum after which it is moved into a1000ml standard flask.

Preparation of working standard stock solution

Around 20mg of montelukast and 240mg of fexofenadine were measured precisely and moved into a 100ml standard flask. 10ml of the versatile stage was added and mixed for 15 min. and the final volume was taken up to 100 ml with the versatile stage. From this, pipette out 2 ml of the arrangement and moved into 50ml volumetric flask. At that point it was created to the volume with portable stage to get a convergence of 8 µg/ml for montelukast and 96µg/ml for fexofenadine.

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Preparation of sample solution

Ten tablets of (Emlukast fx tablets) were precisely gauged and squashed into fine powder. The powder identical to 240 mg (10 mg of montelukast and 12 mg of fexofenadine) was placed in a 100ml standard flask. Around 50ml mobile phase was mixed, shaken for 5 min and then mixed for 20 minusing moderate shake. Then the final volume was at last done sufficient with 100ml with mobile phase. 2 ml of the above arrangement was pipetted out and moved into a 50ml standard flask and done up to the mark with the equivalent. At that point it was sifted through a 0.45µ layer channel. Along these lines, the last focuses were 8µg/ml for montelukast and 96µg/ml for fexofenadine.

Optimization using CCD

A CCD was assembled utilizing just the variations that are discovered critical. C.C.D will be adopted to improve a HPLC partition by increasing goodestimation of factor's fundamental and cooperation impacts. The C.C.D was worked along with the total factorial plan focuses are added. Total length of the hands of the start decided the quantity of volumes and the state of the test plan. The C.C.D was finished with expansion of focus focuses. The total number (N) of examinations with 'k' elements is: N=2k+2k+c. The initial variable is identified with the total factorial plan, the 2^{nd} to the focus and the 3^{rd} to the middle point. Total length of the arms of the circle (α) assumed a significant function for the presence of the C.C.D. In the event that $\alpha \neq 1$, every factor will expect 5 levels (−α,−1,0,+1,+α) [17]. Within the current examination, a revolving C.C.D (R.C.C.D) was utilized. In such sort of plan the star focuses are equivalent to±(2k)1/4 (α=1.68).The data is similarly created from all bearings, for example the change of the assessed reactions is the equivalent at all focuses on a circle focused at the root. 6 focus variables replications were performed to think about the exploratory blunders. At that point, the 20 trials (N = 8 + 6 + 6) were done in random request. The quadratic numerical assembly for the 3 free factors is given in the accompanying condition:

$$Y= \beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{12}AB+\beta_{13}AC+\beta_{23}BC+\beta_{11}A^2+\beta_{22}B^2+\beta_{33}C^2$$

where A,B,C are the variables analyzed, Y is a deliberate reaction, β1,β2,β3 equal to the straight relapse coefficient, β12,β13,β23 equals to the cooperation relapse coefficient and β11,β22,β33 speak to a quadratic relapse coefficient. Plots are created utilizing the perfect quadratic condition and were utilized to find the purposes of greatest HPLC reaction for every analyte in the thought

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about area. The ideal situations were acquired by picking the best ideal incentive for every HPLC reaction.

Statistical comparison

The procedural data was subjected to the HPLC response calculation and the fitting procedures were subjected tot eh statistical analysis using the Microsoft excel 2010 softwares. Design expert verison 12 was used to draw the response surface graphs, designs and contour diagrams.

The experimental data processing required for the HPLC response calculation, the fitting procedures and the corresponding statistical analysis were performed by using the Microsoft Excel 2010 software. Work on experimental design, response surfaces and contour diagrams, was performed by Design Expert Version 12 (Stat-Ease Inc., Minneapolis, MN).

Method Validation [18,19]

Test for Linearity:

The amount of linearity of a proposed method is capacity to find a straightforwardly corresponding interaction of a quantitative reaction to a particular volume of analyte inside a given indicated scope of relative concentration. The amount of linearity of both the comlecules has been achieved by sequentiallydiluting of the stock solution by utilizing the reasonable samples to yield the calibration graphstowardsthe concentration scope of 4-20µg/ml and 48-240µg/ml for montelukast and fexofenadine separately. 3 imitate investigations of every one of the dilutions were utilized to build up the calibration graphs.

Precision:

The method precision was valuated regarding repeatability. Around 8µg/ml for drug montelukast and 96µg/ml for drug fexofenadine concentration of tests were utilized for precision contemplates. The % RSD of the three examine values (n=3) was determined.

Test for Accuracy:

Exact Accuracy of the proposedmethod was controlled by treating the drugs at 3 levels with half, 100% and 150% of standard stock solutions. The combinations of both the drugs were broke down by the designed method. The examination was conducted and their recuperations and %-RSD were determined.

Limits of quantitation and detection (LOQ& LOD)

The boundaries of LOQ and LOD were resolved based on sign to clamor proportion, LOD and LOQ was determined by this method depended on the standard of deviation (SD) of the reaction

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and the slopes (S) of the calibration graphs at level ofusually the LOQ and LOD. LOD and LOQ were resolved as below.

LOD = 3.3xStandard of deviation of y block/Slope of the calibration graph

LOQ = 10xStandard of deviation of y block/Slope of the calibration graph

Analysis of pharmaceutical formulation:

8µg/ml of Montelukast and 96µg/ml of fexofenadine standard and test solution were readied and 20 µl of every standard and test solution were infused and chromatograms were recorded. The rate virtue was determined by utilizing the peak region.

Robustness:

The robustness for the created method was performed by fluctuating the chromatographic conditions which comparise: stream rate (±0.2 ml/min) and level of natural phase in the mobile phase (±2% acetonitrile, v/v).

Ruggedness

The level of reproducibility of test results by the proposed method of analytes was recognized by investigating the medication test under after assortment of test conditions. 1. Distinctive investigator 2.Different instruments.

Results and Discussion:

The focal composite plan can be applied to enhance the detachment and to help the advancement of better understanding of the connection of a few chromatographic components on division quality [20]. In this work, the significant chromatographic components were chosen and enhanced by a focal composite plan test. The determination for improvement depended on starter tests and earlier information from writing, just as certain instrumental impediments. The key variables chose for advancement measure were acetonitrile concentration (A), phosphate buffer quality (B) and stream rate(c). Table 1 show the degree of each components read for discovering the ideal qualities and reactions. In this table as can be seen the scopes of each variables were utilized acetonitrile concentration (65-75%), buffer quality (49-51mM) and stream rate (1.2-1.8ml/min). As reaction variables, the limit factor for the Ist eluted peak montelukast (k1), the

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resolution between two peaks montelukast and fexofenadine (Rs 1, 2), the retention time of the last peak fexofenadine (tR2) were chosen.For a test plan with the three variables, including direct, quadratic and cross terms, the model can be communicated as $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2$ where Y is the reaction to be demonstrated, β is the regression coefficient and X1, X2 and X3 speak to factors A, B and C separately. Measurable boundaries acquired from ANOVA for the decreased models are given in table 2. The unimportant terms ($p > 0.05$) were dispensed with from the model through in reverse disposal cycle to acquire a straightforward and practical model. Since R2 consistently diminishes when a regressor variable is killed from a regression model, in measurable displaying the changed R2 which considers the quantity of regressor variables, is generally chosen [21].The changed R2values were well inside the worthy furthest reaches of $R2 \geq 0.80$ [22], which uncovered that the trial information demonstrated a solid match with second request polynomial conditions. For all the decreased models p esteem < 0.05 was gotten, inferring these models were huge. The sufficient precision esteem is a proportion of the sign (reaction) to noise (deviation) ratio.A ratio more prominent than 4 is attractive [23]. The ratio was discovered to be in the reach from 13.0708 to 33.8949 which demonstrated a sufficient sign and accordingly the model was critical for the separation cycle. The coefficient of variety (C.V) is a proportion of reproducibility of the model and when in doubt a model can be considered sensibly reproducible on the off chance that it is under 10%. In table 45 the communication terms with the biggest term coefficient among the fitted model was AB (+ 0.208) of Rs1,2 model. The positive association among An and B was measurably huge (< 0.0001) for Rs1,2. The examination uncovers that changing the portion of acetonitrile from low to high outcomes in a diminishing in the resolution among montelukast and fexofenadine (Rs1,2) at the low and elevated level of buffer quality. This may due to decrease silanol impacts as result higher buffer quality utilized. Thus the most reduced part of acetonitrile and high quality of buffer level to abbreviate the resolution. The presence of such cooperations underlines the need to complete dynamic multifaceted tests for the improvement of chromatographic separation.

To increase a superior understanding of the outcomes the anticipated models were introduced as perturbation plot figure 1 and 3D reaction surface plot figure 2. Variables giving quadratic and collaboration terms with the biggest supreme coefficients in the fitted models, were picked for the tomahawks of the reaction surface plots. Therefore, factors An and C were

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chosen for the reaction plots of k1, Rs1,2 and tR2 with factor B held consistent. All these three dimensional plots were advantageous to increase a general understanding of the impact of phosphate buffer quality and stream rate on investigation time (Rs1, 2). Perturbation plots give outline perspectives on the reaction surface plots, where it shows how the reaction changes as each factor moves from a picked reference point, with all different elements held steady at the reference esteem.

The steepest slope or ebb and flow demonstrates the affectability of the reaction to a particular factor. Figure 1b indicated that phosphate buffer quality (factor B) had most significant impact on resolution among montelukast and fexofenadine Rs1,2 followed by factor C and then factor A. The remainder of the elements (acetonitrile concentration and stream rate) had critical impact on tR2 and k1. When k1 and tR2 values were expanded, the degree of acetonitrile concentration (factor A) expanded and when k1 and tR2 values diminished, the degree of stream rate (factor C) expanded. Investigation of the perturbation plot and reaction surface plot of enhancement models uncovered that factor B and C had the critical impact on separation of analytes, though the factor A, MeCN concentration was of little centrality. The standards for the advancement of every individual reaction were appeared in table 3.

From the above table it very well may be seen under the column measures that the reaction of tR2 was limited to abbreviate the examination time and the reaction of Rs1, 2 was limited to permit the benchmark separation of montelukast and fexofenadine. To isolate the first eluting peak of montelukast from the dissolvable front, k1 was in the reach. Significance could go from 1 to 5 which offered accentuation to an objective worth. Following the conditions and limitations over, the streamlining system was completed. The reaction surface acquired for the worldwide desirability function was introduced in figure 3.

From the figure it very well may be presumed that there was a bunch of directions creating high desirability esteem (D = 0.810), acetonitrile concentration 75 %, buffer quality 49mM (pH 6.5) and stream pace of 1.4 ml/min. The improved definition examine conditions were utilizing Intek chromasol C18 column with Acetonitrile: phosphate buffer (quality 49mM and pH 6.5) (75:25%v/v) as mobile phase at a stream pace of 1.4 ml/min and UV identification at 240 nm. The anticipated reaction esteems comparing to the last estimation of D were k1 = 1.14 , Rs1,2 = 15.453, tR2 = 7.654 minutes. The understanding among exploratory and anticipated

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reactions under ideal conditions was appeared in table 4 and the relating chromatograms were appeared in figure 4.

Validation of the proposed method:

Linearity result: The linearity of the designed method was assessed by breaking down a progression of various dilutions of each drug. Five volumes were picked, going from 4-20 and 48-240 μg/ml for montelukast and fexofenadine individually. The R-values were discovered to be 0.9991 for montelukast and 0.9994 for fexofenadine. The significant conditions for these are Y= 23408X + 1467 and Y= 11861X + 5972 for MOL and FEF individually. The tests for the linearity of the designed diagnostic methods yielded R2 value that is more prominent than 0.999 for the two medications utilized during approval.

Amount of Precision

Repeatability of the designed method was tried by picking 3volume levels for each rug and investigating both as portrayed under exploratory segment at ostensible conditions. The mean of relative standard of deviation esteems for montelukast and fexofenadine are discovered to be 0.8316 and 0.6069 individually.

Amount of Accuracy:

The exact accuracy of the drugswas determined from estimated dilutions of these drugsaremapped from a calibration graph explicitly produced for the assurance of the accuracy of his proposed method. The consequences of the accuracy reads for both the drugs montelukast and fexofenadine are summed up in table. % RSD values were discovered to be 0.4789 and 0.1648 for montelukast and fexofenadine separately. It is obviously clear from the outcome that, %-RSD of the two drugs was discovered to be under 2 consequently the procedure can be viewed as exact.

LOQ and LOD:

The LOQ and LOD of montelukast were discovered as 0.0023 and 0.0043μg/ml, individually, whereas for fexofenadine was 0.0070 to 0.0130μg/ml, separately. The qualities demonstrated that the method was delicate to evaluate both the medications.

Analysis of pharmaceutical formulation:

Measure (content assessment) was performed to decide the virtue of montelukast and fexofenadine in tablet plan. The ostensible concentration from calibration curve was chosen and

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measurement of montelukast and fexofenadine performed. The tablet detailing Emlukast fx tablets was chosen for examination and the rate virtue of analytes present in definition was discovered to be in the arrangement from 99.44 to 99.90 %. The % RSD values were discovered to be 1.2542 and 1.5186 for montelukast and fexofenadine individually.

Robustness

The robustness study demonstrated that the elements chose stayed unaffected by little variety of stream rate and the natural arrangement of mobile phase. The framework appropriateness results were inside the cutoff. Henceforth the method was good.

Ruggedness

Ruggedness is a proportion of reproducibility of test results under ordinary, anticipated that operational conditions from research facility should lab and from expert to examiner. The rate RSD esteem for expert I was discovered to be 1.4403 and 1.3881 % for Montelukast and fexofenadine separately. The rate RSD esteem for investigator II was discovered to be 1.5837 and 1.4232 % for Montelukast and fexofenadine separately. The method approval boundary reports were demonstrated table - 5.

Conclusion:

A straightforward, fast, touchy and prudent logical method has been effectively evolved utilizing the methodical methodology for measurement of montelukast and fexofenadine in bulk drug just as tablet dosage structure. The ideal standard of the chromatographic parameters was in the investigative plan work utilizing the desirability functions. Approval of this method certified amazing linearity and the method was accurate, precise, appropriate and specific and also robust. Furthermore, the tentatively noticed estimations of LOQ an dLOD of the two medications was additionally very narrow. The designed method showed a serious level of functional utility for assessment of mix compounds in drug dosaged forms.

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Table 1 Central composite design and responses

Std	Run	Space type	Factor-1 A:ACN con (%v/v)	Factor-2 B: PB strength (mM)	Factor-3 C: Flow rate ml/min	Response -1 K_1	Response-2 $Rs_{1,2}$	Response-3 Rt_2
16	5	center	70	50	1.5	1.16	14.215	10.92
19	9	center	70	50	1.5	1.16	14.215	10.92
15	13	center	70	50	1.5	1.16	14.215	10.92
17	14	center	70	50	1.5	1.16	14.215	10.92
18	17	center	70	50	1.5	1.16	14.215	10.92
20	18	center	70	50	1.5	1.16	14.215	10.92
14	3	Axial	70	50	2.004	1.15	12.48	7.56
13	4	Axial	70	50	0.995	1.14	14.655	14.62
12	6	Axial	70	51.68	1.5	1.14	13.105	10.63
9	8	Axial	61.59	50	1.5	1.14	14.361	13.78
11	11	Axial	70	48.13	1.5	1.14	11.927	7.10
10	15	Axial	78.409	50	1.5	1.14	12.318	7.19
4	1	Factorial	75	51	1.2	1.14	13.558	12.96
3	2	Factorial	65	51	1.2	1.13	14.357	16.59
8	7	Factorial	75	51	1.8	1.14	12.021	6.98
1	10	Factorial	65	49	1.2	1.13	15.509	17.94
2	12	Factorial	75	49	1.2	1.14	13.482	12.29
5	16	Factorial	65	49	1.8	1.15	12.988	8.27
7	19	Factorial	65	51	1.8	1.15	12.64	8.02
6	20	Factorial	75	49	1.8	1.14	11.927	7.1

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Table 2Reduced Response Surface Models and Statistical signficance obtained from the ANOVA

Response	Model of Regression	Adjustment of R^2	Model of P-value	(%) CV	Adequate amount ofprecision
k_1	+1.16+0.000*A+0.000*B+0.004*C+0.000 * AB-0.005*AC+0.000*BC-0.007*A^2-0.007*B^2-0.005*C^2	0.9865	< 0.0001	1.105	33.8949
$Rs_{1,2}$	+14.21-0.581*A+0.047*B-0.804*C+0.208*AB+0.143*AC+0.102*BC-0.260*A^2-0.551*B^2-0.179*C^2	0.8725	< 0.0001	2.751	13.0708
Rt_2	+10.83-1.65*A+0.357*B-3.02*C	0.8124	< 0.0001	5.801	16.4033

Table 3Data to optimize the responses individually

Responses	Low limit	Top limit	Criteria or Goal
k_1	1.13	1.16	Is in range
$Rs_{1,2}$	11.927	15.509	Minimize
Rt_2	6.68	17.94	Minimize

Table 4comparing the prediction and experimental data of various funcitons which are under normal conditions

Optimum conditions	ACN (%v/v)	Buffer strength(mM)	Flowing rate (ml/min)	k_1	$Rs_{1,2}$	tR_2
Expectation	75.00	49	1.37	1.14	15.453	7.654
Experimental	75.00	49	1.4	1.17	15.795	7.959
Average error				2.63	2.21	3.98
Desirability value (D) =0.810						

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Table-5 Reports for Validation Parameters

Parameters	Montelukast	Fexofenadine
Range(μgmL^{-1})	4-20	48-240
Y=mx + c	y = 23408x + 1467	y = 11861x + 5972
r^2	0.9991	0.9994
Slope (m)	23408	11861
Intercept (c)	1467	5972
LOD (μgmL^{-1})	0.0023	0.0043
LOQ(μgmL^{-1})	0.0070	0.0130
Accuracy (%)	101.17	100.08
Precision(%RSD)	0.8316	0.6069
Ruggedness		
Analyst-I (%RSD)	1.3764	1.4440
Analyst-II (%RSD)	1.5691	1.4145

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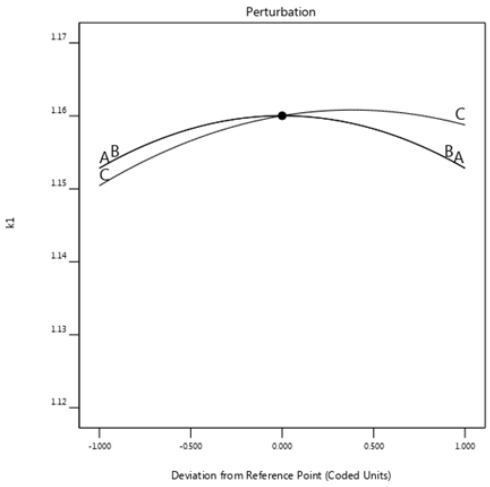

Fig.1(a) capacity factor

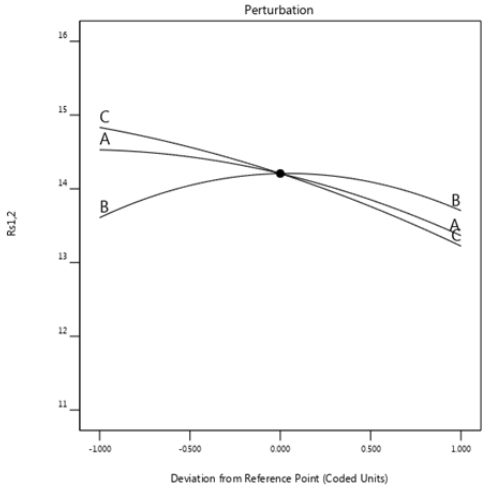

Fig.1 (b) Resolution

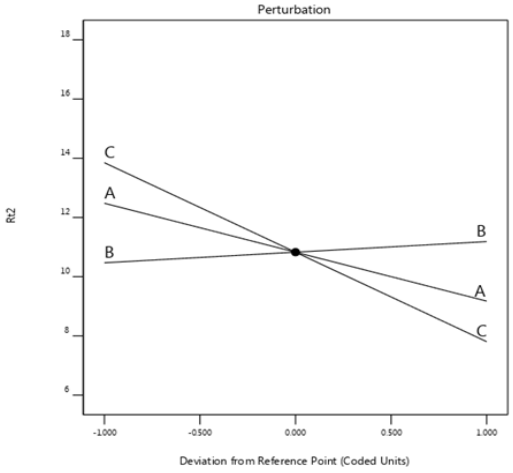

Fig.1(c) Retention time

Figure 1 Perturbation plots

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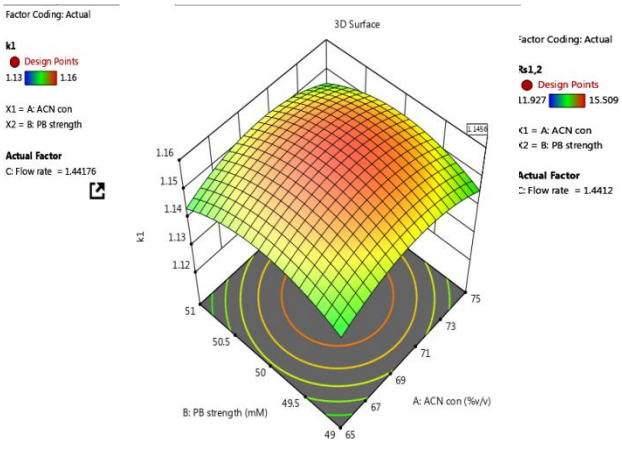
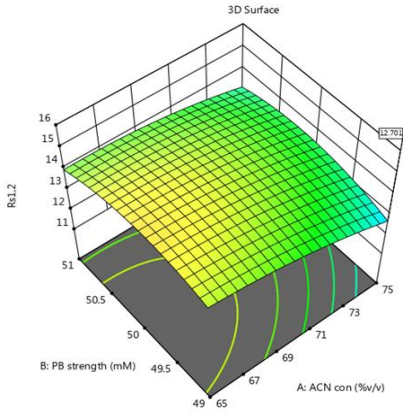
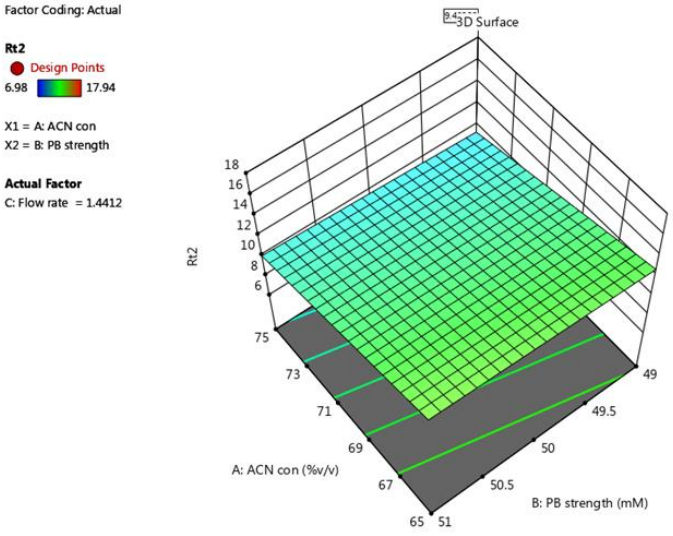


Capacity factor



Resolution



Retention time

Figure 2 Response Surface plots for Responses

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 2, 2021, Pages. 3597 - 3615
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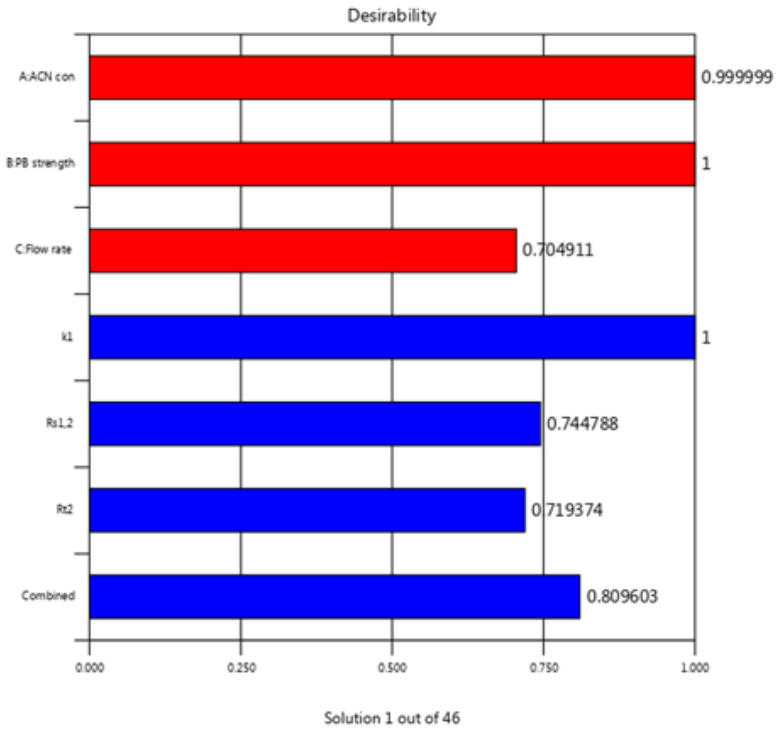

Figure 3Graph represented for the overall function of desirability

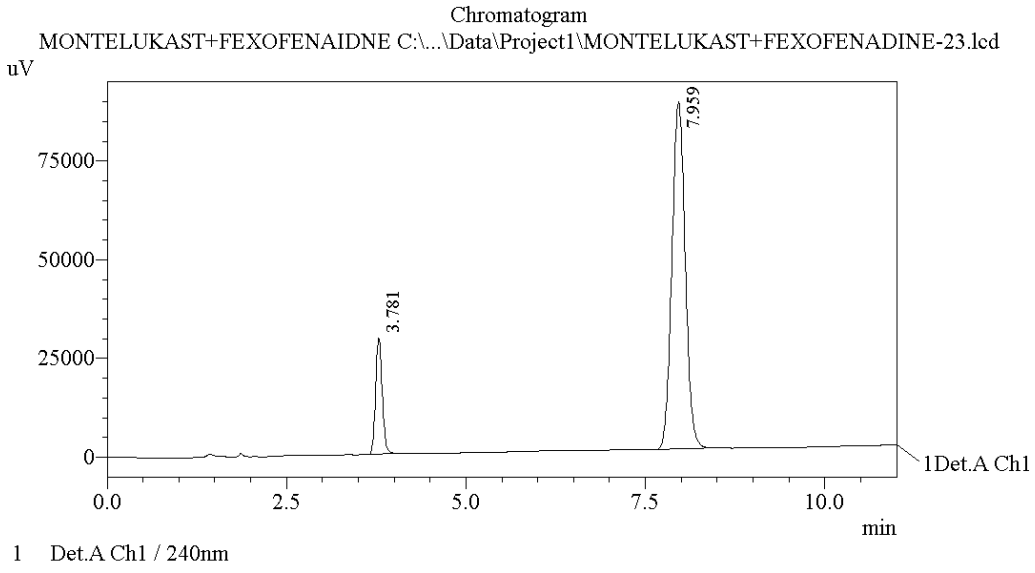

Figure 4Comparison of chromatograms for the experiments and predicted values in various functions