ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.1.L67-78

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Pharmaceutical analysis for Novel drug formulation

Use Of Chemometrics For Development And Validation Of RP-HPLC Method For Simultaneous Determination Of Dimenhydrinate And Cinnarizine

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Abstract: A novel simple, sensitive, rapid and accurate isocratic RP-HPLC method was developed and validated for simultaneous determination of Dimenhydrinate and Cinnarizine applying statistical experimental design. Design of experiments (DoE) was applied for multivariate optimization of the experimental conditions of the RP-HPLC method. Three independent factors like; mobile phase composition, phosphate buffer strength and flow rate were employed to design mathematical models. Central composite design (CCD) was applied to study the response surface methodology. This study was used to find the deepness effects of these independent factors. Desirability function was used to simultaneously optimize the retention time and resolution of the analytes. Hence, our present study was aimed in finding the optimized and predicted data from the contour diagram. Mobile phase consisted of acetonitrile and ammonium acetate buffer (pH 7.0, strength 0.3 mM) in the ratio of 55:45%v/v respectively, at a flow rate of 1.7 mL/min was used. The present study objectives were to apply these optimum conditions baseline partition of both drugs with good resolution. The run time of less than 12.0 min was accomplished. The optimized assay conditions were validated according to ICH guidelines. The correlation coefficient value was found to be r^2 = 0.9990 and 0.9993 for Dimenhydrinate and Cinnarizine respectively. LOD and LOQ value was found to be least concentration for both drugs. It indicates the method was highly sensitive. The accuracy %RSD value was found to be less than 2%. Consequently the reports clearly showed that Quality by design approach could be triumphantly applied to optimize RP-HPLC method for simultaneous determination of Dimenhydrinate and Cinnarizine.

Keywords: Central Composite Design, Optimum Conditions, RP-HPLC, Dimenhydrinate, Cinnarizine and tablet dosage form.

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Received On 9 August, 2021
Revised On 7 December, 2021
Accepted On 20 December, 2021
Published On 7 January, 2022

Funding This work was not supported by any funding agencies

Citation Subramanian Jayaseelan, Nagappan kannappan and Vellichamy Ganesan , Use Of Chemometrics For Development And Validation Of RP-HPLC Method For imultaneous Determination Of Dimenhydrinate And Cinnarizine.(2022).Int. J. Life Sci. Pharma Res.12(1), P67-78 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.1.L67-78

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1. INTRODUCTION

Dimenhydrinate (figure 1a) is chemically known as 2-benzhydryloxy-N,N-dimethylethanamine;8-chloro-1,3-dimethyl-7H-purine-2,6-dione. It is commonly used to allay, nausea and vomiting from a variety of conditions. Cinnarizine is utilized for the treatment of vertigo and chemically known as 1-benzhydryl-4-[(*E*)-3-phenylprop-2-enyl] piperazine[1,2]. Literature survey revealed systematic analytical simultaneous estimation of Dimenhydrinate and Cinnarizine by HPTLC [3], stability indicating HPLC[4], RP-HPLC[5], TLC spectrodensiometric and RP-HPLC using binary mixture[6], Simultaneous determination of Cinnarizine, dimenhydrinate, Cinnarizine impurity determination by TLC and HPLC chromatographic methods [7]. A simple HPLC-UV method for the Determination of Dimenhydrinate and Related substances – Identification of an unknown impurity[8], UV Spectrophotometric method for Estimation of Diphenhydramine Hydrochloride in Soft Gelatin[9], UV Spectrophotometric Absorbance and Area under curve methods for the estimation of Dimenhydrinate in tablet[10] combined with other drugs by UV visible spectroscopy[11]has been reported. Determination of Cinnarizine by HPLC [12], LC and TLC determination of cinnarizine in pharmaceutical preparations and serum [13] has been reported. Determination of Cinnarizine combined with other drugs by reverse phase ion pair high performance liquid chromatography and RP-HPLC methods [14,15] has been reported. No method has been published for the estimation of Dimenhydrinate, Cinnarizine using response surface methodology. The main purpose of our research study is to develop RP-HPLC method suitable for the usual quality control of Dimenhydrinate and Cinnarizine in a pharmaceutical industry and produce guidance on the sensitivity of chromatographic factors and their interconnection consequence on the separation manner. The optimizations of chromatographic factors like acetonitrile concentration in mobile phase, buffer pH strength and flow rate are very complex and have important effects on chromatographic separation. All these independent factors can simply be optimized by applying the design of experiments that is called Quality by Design (QbD) approach. Quality by Design is a structured approaches that involve multi-dimensional mingling and input variables using Design of Experiment to apply the optimum conditions with good assurance of quality. When one needs to optimize more than one response (retention time and tailing factor of both the drug peaks) at a time, the use of Derringer's desirability function is the best option. Derringer's desirability function was initially applied in chromatography by Deming [16]; to get finer resolution and flying analysis time as objective functions to get finer separation quality. Therefore attempts were to develop and optimize the novel HPLC method for the simultaneous determination of Dimenhydrinate and Cinnarizine from tablet formulation.

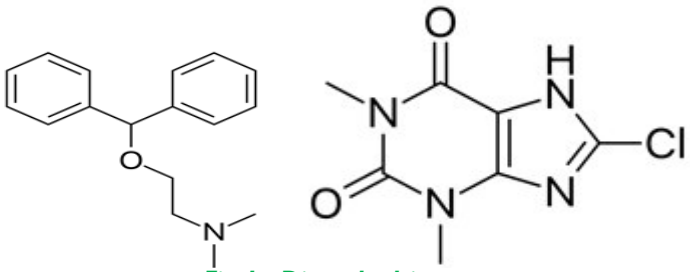


Fig 1a Dimenhydrinate

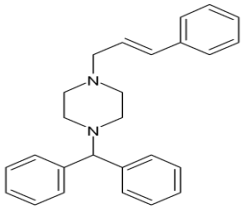


Fig 1b Cinnarizine

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pure Active pharmaceutical ingredients of Dimenhydrinate and Cinnarizine were obtained as gift samples from Nebulae Hi- Tech Laboratories, Chennai, Tamilnadu, India. Combination tablet of Diziron D (Cinnarizine 20mg + Dimenhydrinate 40mg) was procured from the local market. HPLC grade methanol, HPLC grade Acetonitrile, HPLC grade water and analytical grade ammonium acetate were purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

2.2 Instrumentation and Chromatographic Conditions

Analysis was performed with a Shimadzu LC2010 CHT separation module equipped with LC solution software, Pump LC2010 binary and UV detector set at 240 nm. Compounds were separated on an Intek chromasol column C_{18} (250 × 4.6 mm i.d., 5µm particle size) under reversed phase partition conditions. The mobile phase was Acetonitrile and ammonium acetate buffer. The flow rate was 1.0 ml/min and the run time was set as 12 minutes. Samples were injected by using Rheodyne injector with 10 µL loop and detection was carried out at 290 nm. Prior to analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45µ nylon filter. Chromatography was carried out in column temperature maintained at 30 ± 5°C[6].

2.3 Preparation of Mobile phase

About 500 mL of acetonitrile and 500 mL of ammonium acetate buffer pH (7.0, strength of buffer 0.5mm) was measured and taken in a beaker. Then it was placed in an ultrasonicator for degassing for about 5 min. The solution was then filtered thoroughly with a filter press below vacuum and then transferred to a standard flask of 1liter capacity[4].

2.4 Preparation of Standard stock solution

About 40 mg of Dimenhydrinate and 20 mg of cinnarizine were accurately weighed and then transferred to a 100 mL flask. 10 mL of the mobile phase was mixed to the contents and then sonicated for about 15 mins. The volumes were made up to 100 mL with a mobile phase solvent. It was then made up to the mark to attain the concentration 400 µg/mL for Dimenhydrinate and 200 µg/mL for Cinnarizine [5].

2.5 *Preparation of sample solution*

Ten tablets of (Diziron) were accurately weighed and crushed into fine powder. The tablet powder equivalent to 40 mg (20 mg of Cinnarizine and 40 mg of Dimenhydrinate) was taken in a 100 ml volumetric flask. About 50 mL of mobile phase was added, shaken for 5 minutes and then sonicated for 20 minutes with intermediate shaking. Following that, the volume was finally made up to the mark with 100 mL with mobile phase. 3.5 mL of the above solution was pipetted out and transferred into a 100 mL standard flask and made up to the volume with the same. Finally, it was filtered through a 0.45µ membrane filter. Hence, the final concentrations were attaining 7 µg/mL for Cinnarizine and 14 µg/mL for Dimenhydrinate [5].

2.6 Optimization using CCD

CCD can be used to optimize an HPLC partition by gaining a better understanding of the factor's main and interconnection effects. The CCD was constructed from the full factorial design 2k to which star and center points was added. The extent of the ordinance of the star estimated the number of levels and the shape of the experimental design. The CCD was finished by inclusion of center points. The total number N of experiments with k factors is: N = 2k +2k + c. The first term is related to the full factorial design, the second to the star points and the third to the center point. The extent of the arms of the star (α) played an important role for the aspect of the CCD. If $\alpha \neq 1$, each variable will conclude five levels are like $(-\alpha, -1, 0, +1, +\alpha)$[17]. In the present study, a rotatable CCD (RCCD) was used. In this type of design the star points are equal to ± (2k) 1/4 (α = 1.68). The detail is uniformly generated from all directions, i.e. the variance of the determined responses is the same at all points on a sphere centered at the origin. Six center point duplications were done to consider the experimental errors. Then, the 20 experiments (N = 8 + 6 + 6) were done in random order. The quadratic mathematical model for the three independent factors is given in the following equation:

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

where A, B and C are the factors examined, Y is the measured response, β_1, β_2 and β_3 represent the linear regression coefficients, β_{12}, β_{13} and β_{23} represent the interaction regression coefficients and β_{11}, β_{22} and β_{33} represent the quadratic regression coefficients. Surface plots were developed using the contoured quadratic polynomial equation and were used to detect the points of maximum HPLC response for each analyte in the examined domain. The optimal conditions were obtained by choosing the best optimum value for each HPLC response.

3. STATISTICAL TOOLS

Statistical calculations 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).

3.1 Method Validation [17,18]

System suitability tests constitute an essential part of the method and are used to ensure sufficient performance of the chromatographic system. The parameters like retention time, theoretical plates, tailing factor, peak symmetry and repeatability were evaluated. Five replicate injections of the drug solution at the concentration of 14 µg / mL for Dimenhydrinate for and 7 µg / mL for Cinnarizine were used. 20 µL standard solutions were injected. The system suitability tests ensured the validity of the analytical procedure as well as confirmed the resolution between different peaks of interest.

3.2 Linearity

The linearity of the proposed method was evaluated by analyzing a series of different concentrations of each compound. Five concentrations were chosen, ranging between 12-20 and 6-10 µg/mL for Dimenhydrinate and Cinnarizine respectively. 20 µL concentration solutions were injected and the chromatograms were recorded. Three replicate analyses of each of the concentrations were used to create the calibration curve [17].

3.3 Limit of detection & Limit of quantification

LOD is the lowest concentration in a sample that can be detected, but not definitely, quantitated, under the stated experimental conditions. The limit of detection is important for impurity tests and the assays of dosage containing low drug levels and placebos. LOQ is the lowest concentration in a sample that can be exposed and quantified. Preparation of calibration curve from the serial dilutions of standard was repeated for three times. LOD and LOQ were calculated by using the value of the slope and the standard deviation of intercept[17].

3.4 Quantification of pharmaceutical formulation

Assay (content estimation) was performed to determine the purity of Dimenhydrinate and Cinnarizine in tablet formulation. The nominal concentration from the calibration curve was selected and quantification of Dimenhydrinate and Cinnarizine performed. The tablet Diziron contains (Dimenhydrinate 40 mg and Cinnarizine 20 mg) were selected for the analysis. 14µg / mL of Dimenhydrinate and 7µg / mL of Cinnarizine standard and sample solution were prepared and 20 µL of each standard and sample solution were injected and chromatograms were recorded[17].

3.5 Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample in the same day. Aliquots of standard stock solution

of Dimenhydrinate and Cinnarizine (3.5mL of 400 µg / mL of Dimenhydrinate and 3.5 mL of 200 µg / mL of Cinnarizine) were transferred into a 100 mL standard flask and made up to the mark with mobile phase. 20 µL of the solution was injected and the chromatograms were recorded. The procedure was repeated five times on the same day [18].

3.6 Accuracy

The ICH defines the accuracy of an analytical procedure as the closeness of agreement between the values that are accepted as reference values and the values found. The accuracy of the method was checked by spiking the sample with a reference compound. It was evaluated in triplicate at the concentration levels (75%, 100% and 125%) of the target test concentrations (14 µg / mL of Dimenhydrinate and 7 µg / mL for Cinnarizine). 20 µL solutions of each concentration were injected and the chromatograms were recorded [18].

3.7 Robustness

The robustness was studied by evaluating the effect of small but deliberate variation in the chromatographic conditions. The conditions studied were flow rate (± 0.2 ml / min) and composition of mobile phase (± 2%). For each condition, 20 µl solutions were injected into the chromatographic system and chromatograms were recorded. The system suitability parameters were checked[18]

3.8 Ruggedness

The degree of reproducibility of test results by the proposed method of analytes was detected by analyzing the drug sample under the following variety of test conditions. 1. Different analyst 2. Different instruments [18].

4. RESULTS AND DISCUSSION

Reverse phase mode is more preferable than normal phase because drug substances are polar in nature. Solvent type (acetonitrile or methanol), Column chemistry (C_{18}), flow rate and solvent strength were then varied to determine the best chromatographic conditions that give quality separation. The mobile phase conditions are optimized such that the first eluting component does not interfere with the peaks of solvent and excipient. Other responses like analysis time, appropriate *k* range (1<*k*<10) for eluted peaks, tailing factor, assay sensitivity and noise were also observed. Hence Intek chromasol C_{18} column (250 mm X 4.6mm i.d., 5 µm) and mobile phase consisted of acetonitrile: ammonium acetate buffer (pH 7.0, strength of buffer 0.5mm) were tried to investigate first separation conditions. Prior to starting on optimization procedure it was important to explore the curvature term using factorial design with center points. ANOVA generated 2^Kfactorial design showed that curvature was important for all the responses (k_1, $Rs_{1,2}$ Rt_2) since p value was less than 0.05. This implied that a quadratic model should be considered to model the separation process. In order to obtain the second order predictive model, central composite design (CCD) a design type under response methodology was employed. CCD was chosen due to its flexibility and it could be used to optimize an HPLC separation by gaining finer interpretation of factors' main and interconnection effects. The selection of factors for optimization was established on initial experiment and preliminary knowledge from literature as well as certain instrumental limitations. From preliminary experiments, a C_{18} column stationary phase and mobile phase consisted of Acetonitrile: ammonium acetate buffer (pH 7.0, strength of buffer 0.5mm) was used. Phosphate buffer in the mobile phase volume was fixed at (50%) and only Acetonitrile content was varied. The mobile phase flow rate could also moderately influence selectivity in HPLC analysis. Therefore the key factors selected for the optimization process were Acetonitrile concentration (A), Buffer strength (B) and flow rate (C). The levels of each factors studied for finding out the optimum values and responses was shown in Table 1. The ranges of each factor used were acetonitrile concentration (45-55%v/v), buffer strength (0.3-0.7) and flow rate (1.3-1.7mL/min).

Table 1 Central composite arrangement and responses

Run	Space type	Factor A: ACN Con	Factor B: AAB strength	Factor C: Flow Rate ml/min	Response1 K_1	Response 2 $Rs_{1,2}$	Response 3 Rt_2
4	center	50	0.5	1.5	1.14	8.622	6.75
10	center	50	0.5	1.5	1.14	8.622	6.75
11	center	50	0.5	1.5	1.14	8.622	6.75
14	center	50	0.5	1.5	1.14	8.622	6.75
17	center	50	0.5	1.5	1.14	8.622	6.75
20	center	50	0.5	1.5	1.14	8.622	6.75
1	Axial	50	0.8363	1.5	1.15	8.444	6.28
2	Axial	50	0.5	1.8363	1.33	6.718	3.01
3	Axial	41.591	0.5	1.5	1.39	9.432	8.73
5	Axial	58.409	0.5	1.5	1.09	6.081	3.08
15	Axial	50	0.5	1.1636	1.35	9.237	7.88
16	Axial	50	0.1636	1.5	1.14	8.621	6.75
6	factorial	55	0.7	1.3	1.19	6.837	3.53
7	factorial	45	0.7	1.7	1.21	6.903	3.41
8	factorial	45	0.3	1.7	1.15	8.289	5.91
9	factorial	45	0.3	1.3	1.19	8.743	7.27
12	factorial	45	0.7	1.3	1.09	5.941	5.40
13	factorial	55	0.7	1.7	1.27	6.741	3.15
18	factorial	55	0.3	1.3	1.06	7.145	4.49

19	factorial	55	0.3	1.7	1.06	6.107	3.3

For Response, the capacity factor for the Ist eluted peak Dimenhydrinate (k_1), the resolution between two peaks dimenhydrinate and cinnarizine ($Rs_{1,2}$), the retention time of the last peak cinnarizine (Rt_2) were selected. For an experimental design with the three factors, including linear, quadratic and cross terms, the model can be expressed as $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32}$ where Y is the response to be modeled, β is the regression coefficient and X_1, X_2 and X_3 represent factors A, B and C respectively. Reduced models and statistical parameters were obtained from ANOVA. The reports were given in table 2.

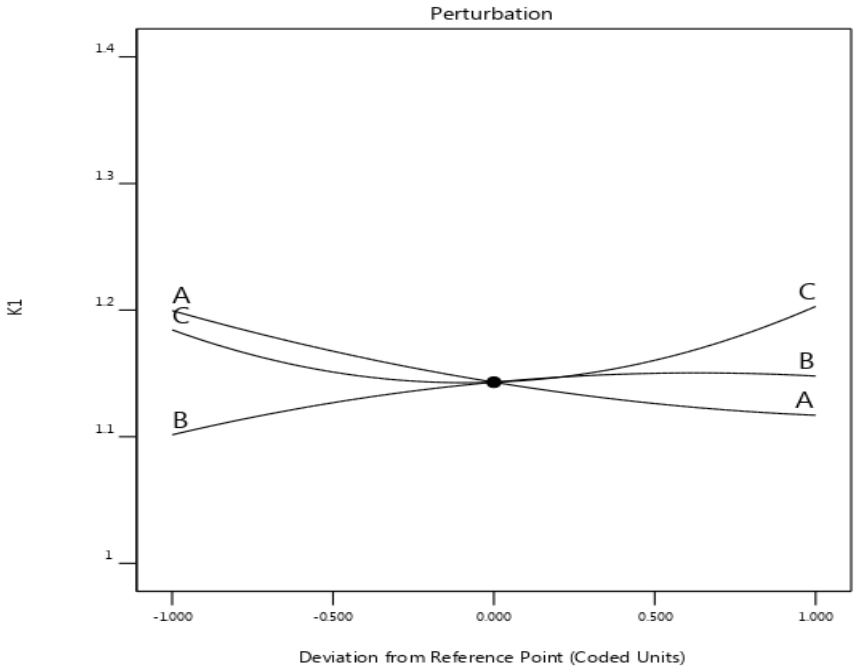
Table 2 Reduced response models and statistical parameters obtained from ANOVA for CCD

Responses	Regression model	Adjusted R^2	Model p value	C.V (%)	Adequate Precision
K_1	+1.14-0.041A+0.023B+0.009C+0.047AB+0.000AC+0.030BC+0.015A^2-0.018B^2+0.050C^2	0.9196	<0.0001	6.49	4.62
$Rs_{1,2}$	+8.65-0.635A-0.304B-0.356C+0.564AB-0.205AC+0.294BC-0.510A^2-0.236B^2-0.432C^2	0.9088	<0.0001	9.18	6.55
Rt_2	+6.79-1.25A-0.459B-0.960C+0.407AB+0.222AC+0.022BC-0.583A^2-0.367B^2-0.746C^2	0.9606	<0.0001	11.52	13.46

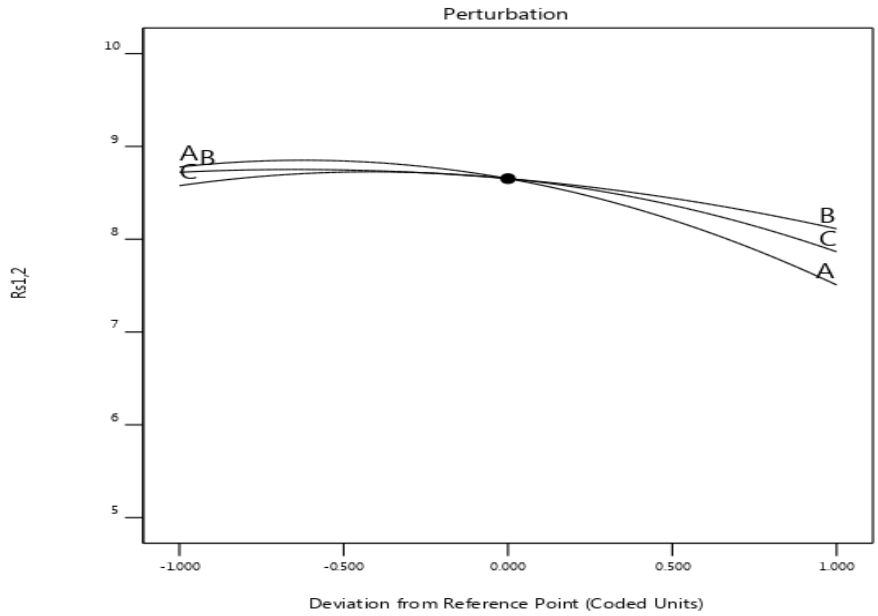
The insignificance of the terms of the study was avoided and removed using the model through the regression removal procedure to attain the easy and correct model. As the R^2 value reduces and the regressors variables are separate from the model, the statistical modeling of the R^2 value was diminished. This takes a large number of variables into account and is usually exposed [19,20]. The r^2 value that is adjusted for the study was within the normal limits of the acceptance of the r^2 greater than 0.8 [21,22], and this revealed that the data that were attained displayed a very good fitting in the 2nd order equations in the polynomial form. For all the above models that have reduced p value lower than 0.05 were acquired by implying the models which were significantly higher. The adequate precision value is a measure of the signal (response) to noise (deviation) ratio. The ratio above 4 was considered as desired [23,24]. The ratio was determined ranging from 4.62 to 13.46 which indicated an adequate signal and therefore it was called a model significant for the separation of the process. The coefficient of the variation CV is a measure of the reproducibility of the model and as a good rule of the designed model can be reasonably considered as reproducible it was found less than that of 10%. In table 2, the interconnection terms with the biggest term coefficient among the fitted model was AB (+ 0.564) of $Rs_{1,2}$ model. The positive interconnection between A and B was statistically significant (< 0.0001) for $Rs_{1,2}$. The existence of such interconnection highlights the necessity to carry out active multifactor experiments for the optimization of chromatographic separation. Concerning obtaining a finer interpretation of the results the predicted models were presented in the form of perturbation plot figure (2) and 3D response surface plot figure (3). Variables giving quadratic and interconnection terms with the biggest perfect coefficients in the fitted models were selected for the axes of the response surface plots. Accordingly, factors A and B were selected for the response plots of k_1, $Rs_{1,2}$ and Rt_2 with factor A held constant usually at the central value of acetonitrile concentration 50.00. All these three dimensional plots were beneficial to obtain an overall interpretation of the effect of acetonitrile concentration and ammonium acetate buffer strength on analysis time ($Rs_{1,2}$). Perturbation plots produce silhouette views of the response surface plots, where it shows how the response changes as each factor moves from a select reference point, with all other factors held constant at the reference value. The steepest slope or curvature shows the sensitiveness of the response to a specific factor. Figure 2b showed that ammonium acetate buffer pH (factor B) had most important effect on resolution between Dimenhydrinate and Cinnarizine $Rs_{1,2}$ followed by factor A and then factor C. The rest of the factors (acetonitrile concentration and flow rate) had significant effect on tR_2 and k_1. When k_1 and Rt_2 values were increased, the level of acetonitrile concentration (factor A) increased and when k_1 and Rt_2 values decreased, the level of flow rate (factor C) increased. Analysis of the perturbation plot and response surface plot of optimization models revealed that factor A and B had the significant effect on separation of analytes, whereas the factor C, flow rate was of less significance. The criteria for the optimization of each individual response were shown in table 3.

Table 3 Criteria for the Optimization of the Individual Responses

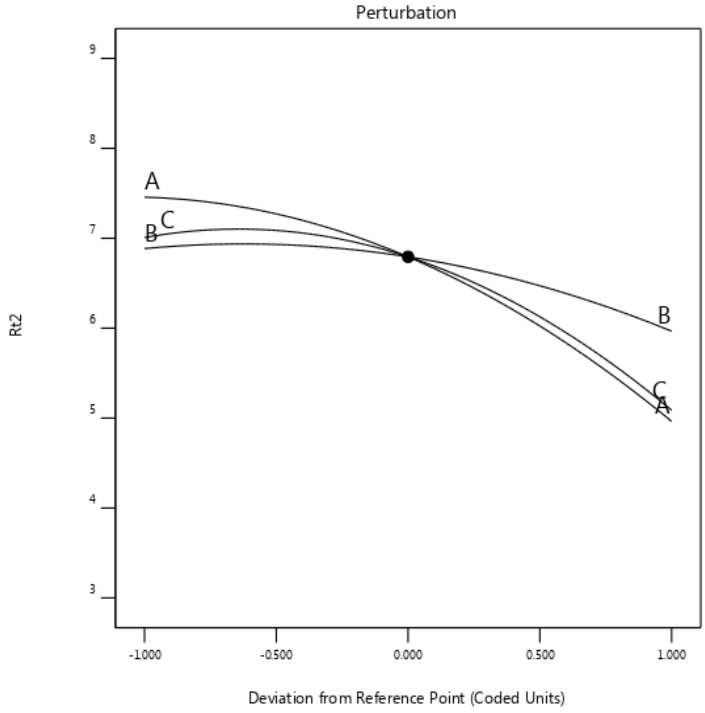
Response	Lower limit	Higher limit	Criteria / Goal
k_1	1.06	1.39	minimize
$Rs_{1,2}$	5.941	9.432	minimize
Rt_2	3.01	8.73	minimize



a) Capacity factor

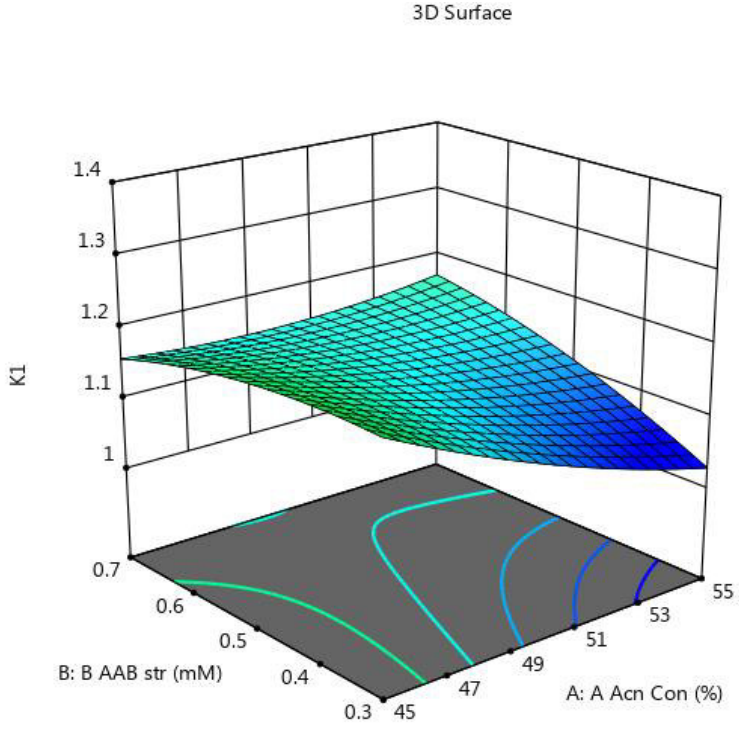


b) Resolution

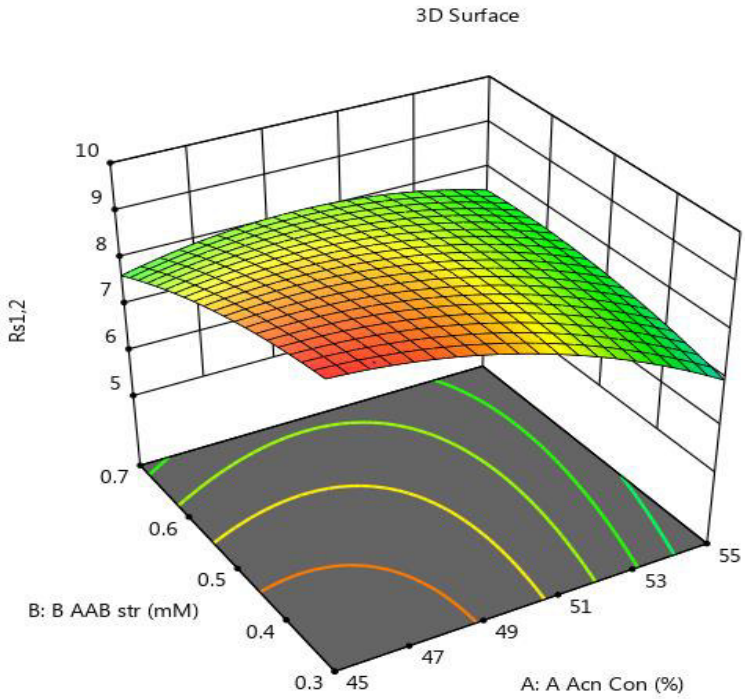


c) Retention time

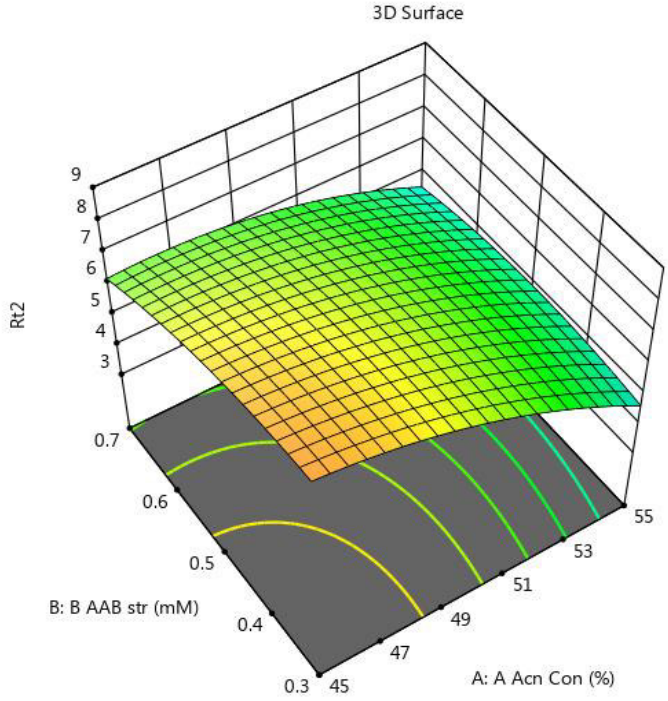
Fig 2. Pertubation plots for Responses



a) Capacity factor k_1



b) Resolution $Rs_{1,2}$



c) Retention time Rt_2

Fig 3. Response Surface plots for Responses

Table 3 reports that it could be seen under the column criteria that the response of Rt_2 was reduced in order to decrease the analysis time and the response of $Rs_{1,2}$ was reduced to allow the baseline separation of Dimenhydrinate and Cinnarizine. In order to separate the first eluting peak of Dimenhydrinate from the solvent front, K_1 was maximized. Importance could range from 1 to 5 which gave emphasis to a target value. Following the conditions and restrictions above, the optimization procedure was carried out. The response surface obtained for the global desirability function was presented in figure 4.

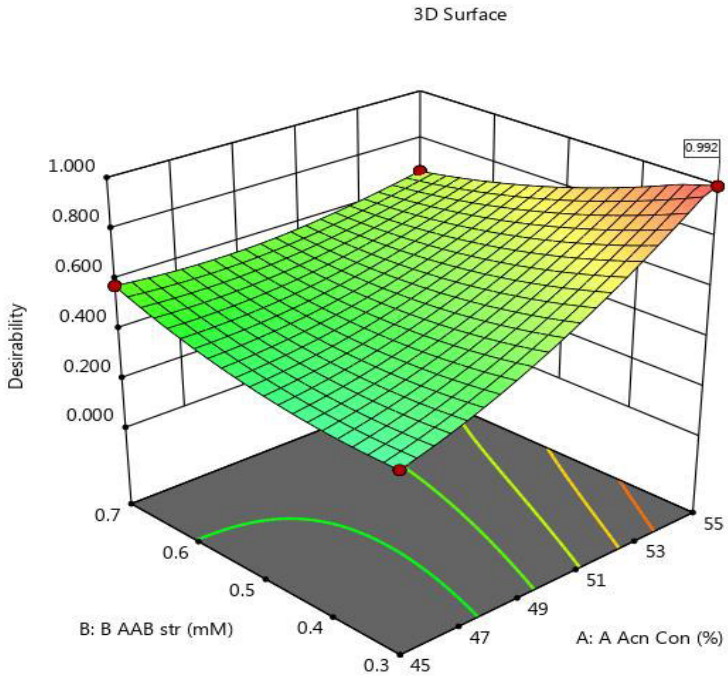


Fig 4. Graphical Representation of global desirability function (D=0.992)

Table 4 Comparison of experimental and predictive values of different objective functions under optimal conditions

Optimal conditions	ACN (%v/v)	Buffer strength (mM)	Flow rate (ml/min)	k_1	$Rs_{1,2}$	Rt_2
Predictive	55.00	0.30	1.7	1.12	7.432	9.89
Experimental	55.00	0.30	1.7	1.16	7.552	10.09
Average error				3.571	1.614	2.022
Desirability value (D) =0.992						

From Figure 4, it could be confirmed that there was a set of correlation providing high desirability value (D = 0.992), acetonitrile concentration 55 %, ammonium acetate buffer strength 0.30mM (pH7.0) and flow rate of 1.7 ml / min. The optimized formulation assay conditions were obtained from C_{18} column with acetonitrile concentration: ammonium acetate buffer strength 0.30mM (pH7.0) (55:45%v/v) as mobile phase at a flow rate of 1.7 ml /min and detection at 290 nm. The predicted response values corresponding to the latter value of D were k_1 = 1.12, $Rs_{1,2}$ = 7.43, Rt_2 = 9.89 minutes. The agreement between experimental and predicted responses under optimal conditions was shown in table 4 and the corresponding chromatograms were shown in figure 5.

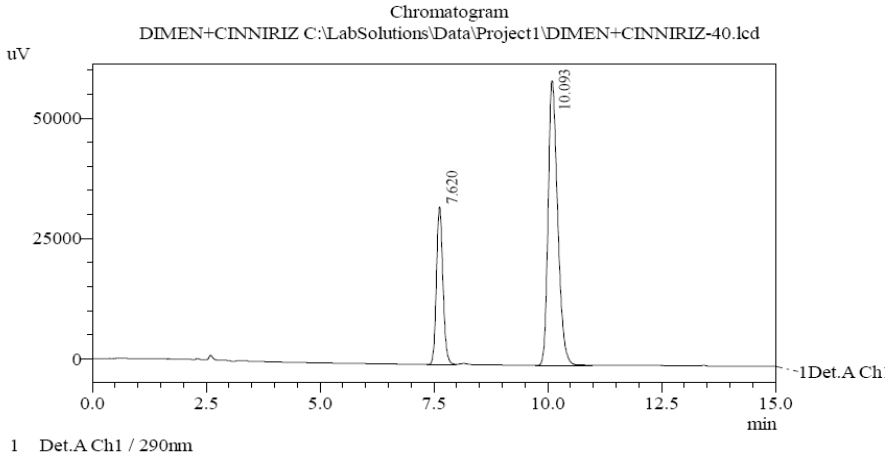


Fig 5. Optimal conditions corresponding Chromatogram

4.1 Validation of the method

Linearity: Linearity concentrations were in the range between 12-20 and 6-10 µg/ml for Dimenhydrinate and Cinnarizine respectively. The regression coefficients were found to be 0.9990 for Dimenhydrinate and 0.9993 for Cinnarizine. The equations for these are Y= 27627X + 1131.1 and Y= 164609X + 12713 for Dimenhydrinate and Cinnarizine respectively. The linearity of the proposed analytical method found R^2 values that were greater than 0.999 for both drugs used during validation. The linearity ranges of the reported RP-HPLC method[4,25,26] were more (DMH and CIN are 10-30 and 20-60 µg/ml respectively) when compared to the developed method. Hence, the

developed method can be applied for the estimation of Dimenhydrinate and Cinnarizine when least amount of drug was required.

4.2 Limit of detection and Limit of quantification

The LOD and LOQ of Dimenhydrinate were found to be 0.0040 and 0.0122 µg/ mL, respectively, while for Cinnarizine were 0.0050 and 0.0151µg/mL, respectively. The LOD and LOQ value for the reported stability indicating RP-HPLC[4,7,27]method was more (LOD for DMH0.866 and CIN 0.527, LOQ 2.860 for DMH and 1.742 for CIN[7])and LOD and LOQ for CIN is 0.067 and 0.221[27]) when compared to the developed method (LOD for DMH0.0040 and 0.0050 for CIN and LOQ for DMH 0.0122 and CIN 0.0151). Detection limit and Quantitation limit value was very less it indicate the sensitiveness of the method. Hence, the developed method was more sensitive to compare the reported method.

4.3 Precision

The percentage RSD value of the intraday analysis of analytes was found to be 0.3854 for Dimenhydrinate and 0.1541 for Cinnarizine which is lesser when compared to reported methods[5] (DMH is 0.55 and CIN 0.52). The %RSD value was found to be less than 2%. This indicated that the developed method had good precision with repeatability.

4.4 Accuracy

The percentage recovery of Dimenhydrinate and Cinnarizine were found to be 99.35 and 99.94% respectively. The percentage recovery is higher when compared to the reported methods[28, 29, 30] (DMH with 97.02 %[5] and CIN with 100.5%[28], 99.74%[29]). The % RSD value for Dimenhydrinate and Cinnarizine were found to be 0.9865 and 1.4315 % respectively. The % RSD value was found to be less than 2%.. The low percentage RSD value indicated that there was no interference due to the excipients used in formulation. Hence the accuracy of the method was confirmed.

4.5 Robustness

The robustness study indicated that the selected factors remain unaffected by small variation of flow rate and the organic composition of mobile phase. The system suitability results were within the limit. Hence the method was robust.

4.6 Ruggedness

The developed method was validated for ruggedness. It was confirmed by using different analysts. The percentage RSD values were found to be less than 2% for three analytes. Hence the precision of the method was further confirmed. The validation parameters reports were shown in table 5.

Table 5. Reports for Validation Parameters

Parameters	Dimenhydrinate	Cinnarizine
Range(µg/ml)	12-20	6-10
y = mx + c	y = 27627x + 1131.1	y = 164609x + 12713
r^2	0.9990	0.9993
Slope (m)	27627	164609
Intercept (c)	1131.1	12713
LOD (µg/ml)	0.0040	0.0050
LOQ(µg/ml)	0.0122	0.0151
Accuracy (%)	99.35	99.94
Precision (%RSD)	0.3854	0.1541
Ruggedness		
Analyst-I (%RSD)	0.9328	0.6343
Analyst-II (%RSD)	1.4939	0.7594

5. CONCLUSION

The analytes Cinnarizine and Dimenhydrinate had been simultaneously analysed in pharmaceutical formulations by using HPLC. Time of analysis, resolution and quality of the peaks were simultaneously optimized by applying useful tools of chemometrics: response surface design and Derringer's desirability function. The results of the study demonstrated the benefit of applying this approach in selecting optimum conditions for the determinations of drugs in pharmaceutical formulations. This method reduced overall assay development time and provided essential information regarding the sensitivity of various chromatographic variables on separation attributes. So, this method is implemented for routine quality routine quality control analysis in a pharmaceutical laboratory. The validation study supported the selection of the assay conditions by confirming that the assay was accurate, linear, precise and robust. Hence, it was concluded that experimental design approach is a suitable analytical tool to optimize and to develop a novel HPLC methods from the perspective of time of analysis, cost of analysis and laboratory resources.

6. ACKNOWLEDGEMENT

The authors are grateful to The Erode College of Pharmacy, Erode and Annamalai University, Chidambram, Tamil Nadu the facilities provided to complete the Research work completely.

7. FUNDING ACKNOWLEDGEMENT

This work was not supported by any funding agencies

8. CONFLICT OF INTEREST

The authors declare no conflict of interest.

9. AUTHOR CONTRIBUTION STATEMENT

Mr. Jambulingam conceptualized and gathered the data with regard to this work. Dr. T. Sudha and Mr S. Murugan

analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

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